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# AVALIAÇÃO DAS FALSIFICAÇÕES E ADULTERAÇÕES DE MEDICAMENTOS E SUPLEMENTOS ALIMENTARES COM ESTEROIDES ANABOLIZANTES E CAFEÍNA, E DESENVOLVIMENTO DE MÉTODOS ANALÍTICOS

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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ciências Farmacêuticas da Faculdade de Ciências da Saúde, Universidade de Brasília, como requisito parcial à obtenção do título de Doutora em Ciências Farmacêuticas.

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## LISTA DE ABREVIATURAS E SIGLAS

| | |
|---|---|
| AAS | *Anabolic Androgenic Steroid* |
| ADAMS | *Anti-Doping Administraton and Management System* |
| AIDS | Síndrome da Imunodeficiência Adquirida |
| ANVISA | Agência Nacional de Vigilância Sanitária |
| API | *Active Pharmaceutical Ingredient* |
| ATR | Refletância Total Atenuada |
| BB | *Benzyl Benzoate* |
| BFP | *Brazilian Federal Police* |
| BU | *Boldenone Undecylenate* |
| CART | Árvores de Classificação e Regressão |
| CCDAE | Cromatografia em Camada Delgada de Alta Eficiência |
| CG | Cromatografia Gasosa |
| CL | Cromatografia Líquida |
| CLAE | Cromatografia Líquida de Alta Eficiência |
| CLS | Mínimos quadrados clássico |
| COI | Comitê Olímpico Internacional |
| DA | Análise Discriminante |
| DAD | Detector de Arranjo de Diodos |
| DART | Análise Direta com Ionização em Tempo Real |
| DHT | Dihidrotestosterona |
| DPF | Departamento de Polícia Federal |
| DS | *Dietary Supplement* |
| DSHEA | *Dietary Supplement Health and Education Act of 1994* |
| EAA | Esteroides Anabólicos Androgênicos |
| EM | Espectrometria de Massas |
| ES | *Stanozolol* |
| EUA | Estados Unidos da América |
| FDA | *Food and Drug Administration* |
| FTIR | Infravermelho com Transformada de Fourier |
| GC | *Gas Chromatography* |
| HPLC | *High Performance Liquid Chromatography* |
| IS | *Internal Standard* |
| k-NN | Regra do vizinho mais próximo |
| LOD | *Limit of Detection* |
| LOQ | *Limit of Quantification* |
| ME | *Methandrostenolone* |
| MCR-ALS | Resolução multivariada de curvas – Mínimos quadrados alternantes |
| MS | *Mass Spectrometry* |
| ND | *Nandrolone Decanoate* |
| NF | *Nandrolone Phenylpropionate* |
| NIR | Espectroscopia no Infravermelho Próximo |
| OMS | Organização Mundial da Saúde |

| | |
|---|---|
| OR | *Original* |
| OXA | *Oxandrolone* |
| PCA | Análise de Componentes Principais |
| PCs | Componentes Principais |
| PD | *Drostanolone Propionate* |
| PF | Polícia Federal |
| PH | Pró-hormônios |
| PLS-DA | *Partial Least Squares – Discriminant Analysis* |
| PR | *Prasterone* |
| RMSECV | *Root Mean Squared Error of Cross-Validation* |
| RSD | *Relative Standard Deviation* |
| $RSD_p$ | *Relative Standard Deviation for intermediate precision* |
| $RSD_r$ | *Relative Standard Deviation for repetability* |
| SA | Suplemento alimentar |
| SIMCA | *Soft Independent Modeling of Class Analogies* |
| SisCrim | Sistema Criminalística |
| SNC | Sistema Nervoso Central |
| SSFFC | *Substandard, spurious, falsely-labelled, falsified and counterfeit* |
| T | *Testosterone* |
| TC | *Testosterone Cypionate* |
| TE | *Testosterone Enanthate* |
| TF | *Testosterone Phenylpropionate* |
| TI | *Testosterone Isocaproate* |
| Tin | *Initial temperature* |
| Tinj | *Injector temperature* |
| Tf | *Final temperature* |
| TP | *Testosterone Propionate* |
| SVM | Máquina de vetor de suporte |
| UE | União Europeia |
| UPLC | Cromatografia Líquida de Ultra Eficiência |
| UV | Detector de Ultravioleta |
| Vinj | *Injection volume* |
| WADA | Agência Mundial Anti-Doping |
| WC | Correlação de comprimento de onda |
| WHO | *World Health Organization* |

## RESUMO

NEVES, Diana Brito da Justa. **Avaliação das falsificações e adulterações de medicamentos e suplementos alimentares com esteroides anabolizantes e cafeína, e desenvolvimento de métodos analíticos**. Brasília, 2016. Tese de Doutorado em Ciências Farmacêuticas – Faculdade de Ciências da Saúde, Universidade de Brasília, Brasília 2016.

A falsificação e adulteração de produtos farmacêuticos é um problema prevalente no mundo inteiro, que representa um grande risco potencial à saúde. Por se tratar de atividade criminosa, sua incidência não é exatamente conhecida, sendo apenas estimada por estudos pontuais. Os objetivos desse estudo foram identificar os principais medicamentos contendo anabolizantes e suplementos alimentares apreendidos pela Polícia Federal (PF) e a taxa de falsificações reportadas nos laudos periciais emitidos; avaliar a legislação sanitária relativa a suplementos alimentares no Brasil, Estados Unidos e Europa; desenvolver um método para identificar falsificações do medicamento Durateston®, empregando dados espectrométricos obtidos na região do infravermelho (FT-IR) e ferramentas quimiométricas; desenvolver métodos analíticos por CG-MS para a análise quantitativa de fármacos anabolizantes em medicamentos e suplementos alimentares e de cafeína em suplementos, e analisar estes compostos em produtos apreendidos pela PF. Dados oriundos de laudos periciais emitidos pela PF entre 2006 e 2011 mostraram que 31,7% dos medicamentos anabolizantes foram declarados falsos. Dados dos laudos de suplementos alimentares emitidos entre 2007 e 2013 apontaram que 6,2% dos produtos submetidos a análise química eram falsos. A legislação brasileira relacionada a suplementos alimentares mostrou ser mais restritiva que aquelas vigentes nos Estados Unidos e na União Europeia, porém, ao contrário dos outros países, o Brasil não dispõe de um mecanismo adequado para notificação e divulgação ao público da ocorrência de adulterações de suplementos alimentares. O método para avaliar amostras de Durateston® utilizando dados de FT-IR e quimiometria apresentou uma taxa de 100% de acerto na diferenciação entre amostras originais e falsas. Os métodos analíticos por CG-MS para análise quantitativa de esteroides anabolizantes em medicamentos e suplementos, e de cafeína em suplementos incluem apenas etapas de extração e diluição e foram satisfatoriamente validados. A maioria (53%) dos 328 medicamentos contendo esteroides anabolizantes eram falsos ou estavam abaixo do padrão esperado. Das 17 amostras de suplementos analisadas, cinco continuam anabolizantes não declarados, duas na dose terapêutica. Teores variados de cafeína foram encontrados nas 216 amostras de suplementos analisadas (entre 0,5 e 394 mg/cápsula), níveis muitas vezes diferentes daqueles descrito no rótulo. A ingestão de cafeína pelo consumo em 84,3% destes produtos foi superior à dose diária considerada segura (400 mg), considerando também a dieta como fonte de cafeína. Os resultados desse estudo indicam a necessidade de ações mais efetivas por parte das autoridades sanitárias e policiais no sentido de coibir a presença de produtos falsos e de baixa qualidade no mercado brasileiro e de alertar a população para o risco potencial envolvido no consumo desses produtos.


**Palavras chave:** anabolizantes, medicamentos, suplementos alimentares, CG-MS, falsificação/adulteração de medicamentos, adulteração de suplementos alimentares

## ABSTRACT

NEVES, Diana Brito da Justa. **Evaluation of counterfeiting and adulteration of medicines and dietary supplements with anabolic steroids and caffeine, and development of analytical methods.** Brasília, 2016. Doctoral Thesis in Pharmaceutical Sciences - Faculty of Health Sciences, University of Brasília, Brasília 2016.

The counterfeiting and adulteration of pharmaceutical products is a worldwide problem, representing a major potential health risk. As a criminal activity, its incidence is not exactly known, but only estimated by specific studies. The aims of the present study were to identify the main medicines containing anabolic steroids and dietary supplements seized by the Brazilian Federal Police (BFP) and the counterfeiting rates reported on forensic reports; evaluate the sanitary legislation concerning supplements in Brazil, United States and European Union; to develop a method to identify counterfeiting of the medicine Durateston®, using spectrometric data obtained at the infrared region (FT-IR) and chemometric tools; to develop GC-MS analytical methods for the quantitative analysis of anabolic steroids in medicines and supplements and of caffeine in supplements and quantify these compounds in products seized by the BFP. Data from forensic reports issued by the BFP from 2006 to 2011 showed that 31.7% of medicines containing anabolic steroids were counterfeits. Data from forensic reports on supplements issued from 2007 to 2013 indicated that 6.2% of chemically analyzed products were identified as counterfeits. Brazilian legislation concerning supplements was regarded as more restrictive than those at the United States and European Union, however, unlike these countries, Brazil does not have a suitable mechanism for notification and disclosure to the general public of the occurrence of supplement adulteration. The method for Durateston® using FT-IR data and chemometrics showed a 100% success rate to discriminate original and counterfeit samples. The quantitative GC-MS methods for the analysis of anabolic steroids in medicines and dietary supplements, and of caffeine in supplements, include only sample extraction and dilution and were satisfactorily validated. The majority (53%) of 328 medicine samples declaring the presence of anabolic steroids were counterfeit or substandard. Five of the 17 supplement samples analyzed contained undeclared anabolic steroids, two at therapeutic levels. A wide range of caffeine levels was found in the 216 supplement samples analyzed (between 0.5 and 394 mg/capsule), often different from what was stated on the labels. The caffeine daily intake from the consumption of 84.3% of the analyzed products exceeded the safe daily dose (400 mg), considering other caffeine sources from the diet. Results from this study indicate the need for more effective actions from sanitary and police authorities for preventing the presence of counterfeit and substandard products on the Brazilian market and in alerting the population for the potential risks involved from the consumption of these products.


**Keywords**: anabolic steroids, medicines, dietary supplements, GC-MS, medicine counterfeiting/adulteration, dietary supplement adulteration

## INTRODUÇÃO

Poucos crimes são tão potencialmente perigosos e ao mesmo tempo negligenciados como a falsificação de medicamentos (Chika et al., 2011). É um problema prevalente no mundo todo, relacionado a praticamente qualquer classe terapêutica e já levou milhares de pessoas a óbito (Chika et al., 2001; OMS, 2016a).

Segundo o Relatório Brasil Original, foram apreendidos no Brasil em 2010 18.150.578 unidades de medicamentos falsos/contrabandeados, cujo valor estimado foi de R$ 6.313.394,27 (Brasil, 2011). Ainda conforme o mesmo relatório, os medicamentos falsos ou contrabandeados mais frequentes no Brasil foram aqueles usados no tratamento da disfunção erétil, os anabolizantes e os indicados para o tratamento da obesidade. Dados oriundos de laudos periciais emitidos pela Polícia Federal (PF) entre 2006 e 2012 também indicam que a principal classe de medicamentos falsificados no Brasil são os produtos para disfunção erétil, seguidos dos anabolizantes (Marcheti, 2014).

Apesar da importância do tema, existem poucos trabalhos publicados relatando a investigação da falsificação de anabolizantes, e os que existem abordam um número limitado de amostras. Todos, entretanto, reportam taxas de falsificação superiores a 30% em produtos oriundos do mercado clandestino europeu, chegando a mais de 80% (Musshoff et al., 1997; Ritsch e Musshoff, 2000; Thevis et al., 2008; Coopman e Cordonnier, 2012; Pellegrini et al., 2012; Cho et al., 2015; Prokudina et al., 2015).

Perigo semelhante é representado pela adulteração de suplementos alimentares, produtos extensamente consumidos por atletas, mas também pela população fisicamente ativa em geral (Parra et al., 2011). Vários países não regulam a fabricação desse tipo de produto e nem obrigam os fabricantes a declarar integralmente sua composição, facilitando casos em que os ingredientes apresentados nos rótulos não correspondem integralmente àqueles presentes nos suplementos (Maughan, 2005; Parra et al., 2011). Isso pode acontecer tanto devido a contaminação cruzada entre diferentes produtos fabricados pela mesma empresa, quanto pela adição deliberada de esteroides, estimulantes do sistema nervoso central e outros fármacos ao produto visando garantir e aumentar sua eficácia (Geyer et al., 2011; Parra et al., 2011.

A adulteração de suplementos alimentares inclui casos onde a quantidade de determinada substância presente é superior ou inferior à descrita no rótulo e casos de adição de substâncias não declaradas no rótulo (Gurley et al., 2000; Maughan, 2005; Andrews et

al., 2007; Geyer et al., 2011). Não foram encontrados trabalhos científicos investigando a adulteração de suplementos alimentares comercializados no Brasil, apesar de a prevalência de uso desses produtos pela população brasileira ser expressiva, principalmente praticantes de atividades físicas (Nogueira et al., 2013). Isso não significa que esse tipo de crime não aconteça no país. Em 2010, uma operação conjunta da Agência Nacional de Vigilância Sanitária (ANVISA) e da PF culminou com a interdição temporária de uma empresa que adicionava sibutramina a produtos supostamente naturais, à base de quitosana (Brasil, 2010).

A falsificação e adulteração de medicamentos anabolizantes e suplementos alimentares representam riscos à saúde dos consumidores, que nem sempre estão conscientes desse risco ou de qual a sua magnitude. O objetivo desse trabalho é avaliar a incidência de falsificações e adulterações em medicamentos contendo substâncias anabolizantes e em suplementos alimentares apreendidos pela Polícia Federal brasileira.

# REVISÃO BIBLIOGRÁFICA

## 1. Esteroides Anabólicos Androgênicos (EAA)

### 1.1. Histórico

A constatação empírica da atividade da testosterona ocorreu há milhares de anos, quando fazendeiros notaram que animais castrados eram mais facilmente domesticados. Estudos envolvendo castração e reimplante testicular em galos se iniciaram ainda no século 18, e no século 19 começou-se a formar a ideia de que alguma substância proveniente dos testículos, e que estava na corrente sanguínea, era responsável pelos efeitos observados, como diminuição da crista e do comportamento agressivo típico dos machos (Freeman et al., 2001; Dotson e Brown, 2007).

Ainda no final do século 19, o pesquisador francês Charles Edouard Brown-Sequard publicou resultados de um estudo onde ele se aplicou extratos provenientes dos testículos de cães e porquinhos da índia. Ele reportou aumento de força física, das habilidades mentais e do apetite (Dotson e Brown, 2007). Seu experimento levou a uma série de pesquisas para investigar se essa substância testicular poderia curar doenças como diabetes, epilepsia, paralisia, gangrena, anemia e enxaqueca (Freeman et al., 2001; Silva et al., 2002; Dotson e Brown, 2007). Nessa mesma época, o fisiologista austríaco Oskar Zoth propôs pela primeira vez que se aplicasse esse extrato em atletas, uma vez que ele melhorava a força muscular e o aparato neuromuscular, melhorando o desempenho atlético. Zoth e Fritz Pregl se auto administraram extratos provenientes dos testículos de touros e mediram a força de seus dedos médios, e constataram que o extrato havia melhorado a força e condições dos seus músculos (Leneham, 2004; Dotson e Brown, 2007).

O isolamento das substâncias responsáveis pelos efeitos anteriormente descritos, porém, só aconteceu em 1929, quando o químico alemão Adolf Butenandt isolou, a partir de milhares de litros de urina, a estrona e a androsterona. Em 1939, Butenandt e Leopold Ružička receberam o Nobel de Química, o primeiro pelo isolamento do estrogênio, androsterona, progesterona e testosterona, e o segundo pela síntese de androsterona e testosterona a partir de um esterol neutro, como o colesterol, entre outras realizações (The Nobel Prize in Chemistry, 1939).

A experimentação da testosterona em humanos iniciou-se no final da década de 1930, focando no tratamento do hipogonadismo masculino. Uma vez descoberto o aumento nos processos proteicos anabólicos, foi aberta a porta para o tratamento de uma variedade de

desordens pelo estímulo ao crescimento tecidual (Dotson e Brown, 2007). Fisiculturistas e atletas começaram a usar a testosterona para aumentar a massa muscular nos Estados Unidos no final da década de 1940, e na década seguinte atletas olímpicos da União Soviética e Alemanha oriental já estavam usando EAA. Em 1974, o Comitê Olímpico Internacional (COI) baniu o uso da testosterona e seus derivados por atletas (Silva et al., 2002; Dotson e Brown, 2007).

### 1.2. Aspectos químicos

Um composto esteroidal é facilmente reconhecido pelo seu núcleo de quatro anéis de carbono fundidos (ciclopentanoperhidrofenantreno). Todos os compostos esteroidais, naturais e sintéticos, são derivados desse núcleo (Figura 1). O mais conhecido dos esteroides é o colesterol. As grandes famílias de esteroides incluem os gonanos, estranos, pregnanos, colanos, colestanos e androstanos, que incluem a testosterona (Figura 1). Todos os EAA são estruturalmente derivados da testosterona, quer seja naturalmente ou por vias sintéticas (Sturmi e Diorio, 1998; Kasal, 2010).

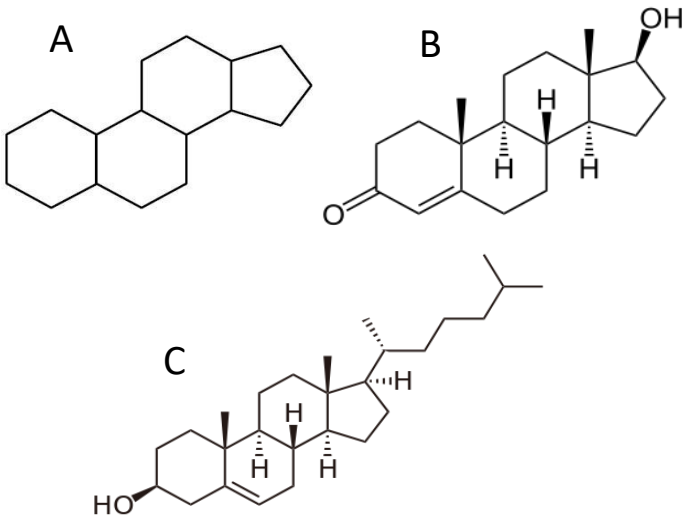



**Figura 1:** (A) ciclopentanoperhidrofenantreno, (B) testosterona, (C) colesterol.

Já foram sintetizados centenas de derivados diferentes da testosterona, visando a diminuição dos efeitos androgênicos ou evitar a detecção em testes anti-doping. A alquilação da testosterona na posição 17-alfa retarda a inativação hepática da substância, resultando em substâncias bastante ativas por via oral (Figura 2). A esterificação da testosterona na posição 17-beta aumenta as propriedades lipofílicas da substância e retarda sua absorção pela via intramuscular (Figura 2). Quanto mais longa a cadeia lateral, maior a lipossolubilidade e mais demorada a absorção e a depuração (Sturmi e Diorio, 1998; Shahidi, 2001).

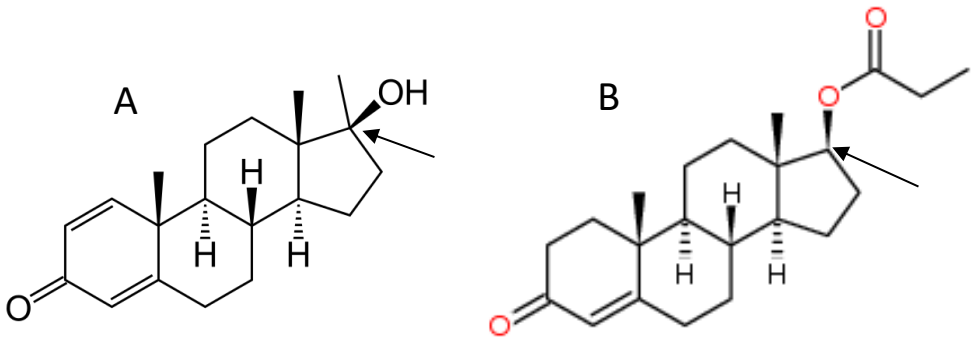



**Figura 2:** (A) metandrostenolona, EAA com grupo metila na posição 17-alfa (seta) e ativo por via oral; (B) propionato de testosterona, com o grupo éster em posição 17-beta. As setas indicam o carbono 17.

### 1.3. Efeitos farmacológicos e uso terapêutico

Uma vez disponível no organismo, parte da testosterona circulante é convertida enzimaticamente a dihidrotestosterona (DHT) e estradiol, pela ação respectiva das enzimas 5α-redutase e aromatase (Figura 3) que também são farmacologicamente ativas (Shahidi, 2001; Snyder, 2005; Kicman, 2008).

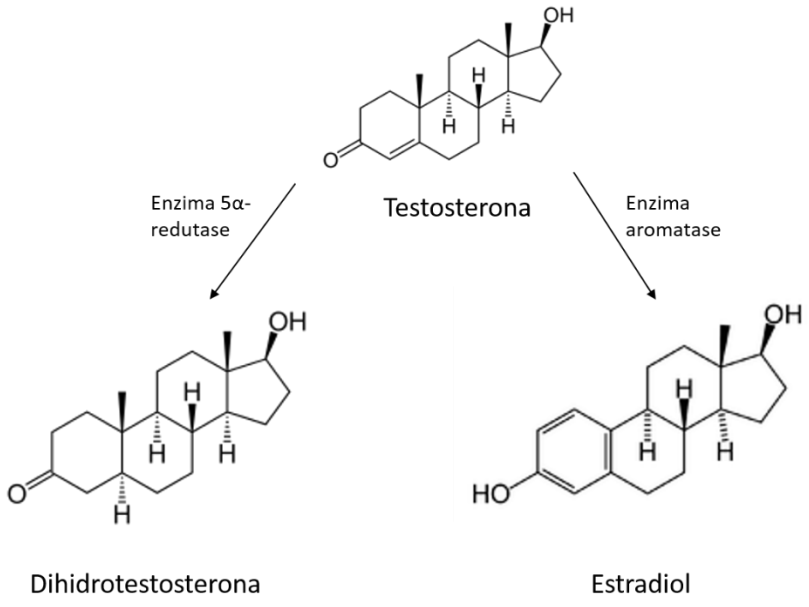



**Figura 3:** Testosterona e seus metabólitos ativos dihidrotestosterona e estradiol.

A testosterona, a DHT e os EAA sintéticos agem ligando-se a um receptor citosólico androgênico, o complexo hormônio-receptor formado entra no núcleo da célula, se liga a uma região específica do DNA e promove a transcrição / repressão de certos genes, para

produzir seus efeitos (Shahidi, 2001; Silva et al., 2002; Maravelias et al., 2005; Snyder, 2005). Enquanto a testosterona e a DHT se ligam ao mesmo receptor, a DHT se liga com maior afinidade e ativa a expressão do gene com mais eficiência (Snyder, 2005). O receptor androgênico está presente no trato reprodutivo e também nos ossos, músculos esqueléticos, cérebro, fígado, rins e adipócitos, enquanto o estradiol parece atuar em receptores localizados principalmente nas epífises ósseas, no tecido adiposo e em partes do cérebro (Shahidi, 2001; Snyder, 2005; Kicman, 2008).

Os efeitos da testosterona e da DHT podem ser divididos em anabólicos e androgênicos. Os efeitos androgênicos estão majoritariamente relacionados à atividade da DHT e se referem ao aparecimento de características masculinas, tais como maturação da genitália masculina, espessamento das cordas vocais, aumento da secreção das glândulas sebáceas e dos pelos e padrão masculino de distribuição dos pelos pubianos. Os efeitos anabólicos estão majoritariamente relacionados à própria testosterona e incluem o aumento de massa muscular e força, eritropoiese, aumento do hematócrito e da concentração de hemoglobina, aumento da retenção de nitrogênio, redução dos estoques de gordura corporal e aumento da deposição de cálcio nos ossos (Sturmi e Diorio, 1998; Silva et al., 2002; Snyder, 2005; Maravelias et al., 2005). Os esteroides possuem ainda efeito anti-catabólico, não relacionado ao receptor androgênio, mas provocado pela competição dos esteroides pelo receptor glucocorticosteroide, bloqueando a ação catabólica dos glucocorticosteroides (Maravelias et al., 2005; Kicman, 2008).

A testosterona possui várias aplicações clínicas, incluindo situações onde há grande necessidade de proliferação tecidual ou crescimento de massa muscular, como a caquexia, doenças debilitantes como a síndrome da imunodeficiência adquirida (AIDS) e estados pós-cirúrgicos ou queimaduras extensas. A testosterona provoca aumento do apetite, ganho de peso, aumento da massa muscular e força, levando a uma melhora geral no bem-estar do paciente (Shahidi, 2001; Silva et al., 2002; Snyder, 2005; NIDA, 2006; Dotson e Brown, 2007). Antes do uso da eritropoietina e transplantes de medula óssea, a testosterona era usada como auxílio no tratamento da anemia, e alguns EAA ainda são usados para esse fim (Shahidi, 2001; Dotson e Brown, 2007; Korolkovas e França, 2009). Alguns EAA, principalmente o estanozolol e a oximetolona, mostraram ser eficazes no tratamento do edema angioneurótico (Shahidi, 2001; Snyder, 2005). Existem ainda casos da utilização de EAA como terapia adjuvante no tratamento de alguns tipos de câncer de mama (Sturmi e Diorio, 1998; Korolkovas e França, 2009). A indicação terapêutica mais clara dos EAA,

entretanto, é no tratamento das deficiências androgênicas, como hipogonadismo, puberdade e crescimento retardados, deficiência androgênica parcial em homens idosos e deficiência androgênica secundária a doenças crônicas (Sturmi e Diorio, 1998; Snyder, 2005; Korolkovas e França, 2009).

**1.4. Uso abusivo**

Preparados de testosterona começaram a ser utilizados por fisiculturistas no final da década de 1940, nos Estados Unidos. A prática se difundiu rápido e o primeiro registro histórico do uso de hormônios sexuais no aumento do desempenho em campeonatos mundiais é de 1954, quando foram utilizados por atletas russos durante o Campeonato Mundial de Levantamento de Peso na Áustria (Silva et al., 2002).

Com o sucesso do uso da testosterona no esporte, começou-se uma busca pela síntese de derivados sintéticos com maior atividade anabólica e menor atividade androgênica. Em 1956 surgiu a metandrostenolona, comercializada pelo Laboratório Ciba como Dianabol. O sucesso da metandrostenolona foi rápido, surgiram outros derivados sintéticos e o uso dos EAA se espalhou por várias modalidades esportivas. A inclusão dos EAA nos testes de doping só ocorreu nas Olimpíadas de Montreal, em 1976. O caso mais conhecido de uso de EAA foi o do corredor canadense Ben Johnson, medalha de ouro nos 100m rasos nas Olimpíadas de Seul, em 1988, cujo exame detectou a presença dos metabólitos de estanozolol (Silva et al., 2002). Segundo o Instituto Nacional de Abuso de Drogas (*National Institute on Drug Abuse*), os EAA mais consumidos nos Estados Unidos são a oximetolona, oxandrolona, metandrostenolona, estanozolol, decanoato e fempropionato de nandrolona, cipionato de testosterona e undecilenato de boldenona (NIDA, 2006). Essas substâncias são usadas em doses que excedem amplamente aquelas empregadas no uso terapêutico (Lenehan, 2004; Kicman, 2008).

A Agência Mundial Anti-Doping (WADA) reporta, a cada dois anos, o total de análises de amostras biológicas de atletas realizadas naquele ano, a quantidade de resultados adversos, que identificaram a presença de substância proibida, seus metabólitos ou marcadores, ou evidências do uso de um método proibido, e de resultados atípicos, que requerem investigações adicionais para confirmação de resultados adversos. Dados reportados em 2010, 2012 e 2014 estão resumidos na Tabela 1.

**Tabela 1:** Dados reportados pela WADA relacionados aos exames realizados em amostras biológicas de atletas (WADA, 2010; WADA, 2012; WADA, 2014).

| **Ano de realização dos exames** | **2010** | **2012** | **2014** |
|---|---|---|---|
| Análises realizadas | 258.267 | 267.645 | 283.304 |
| Resultados adversos e/ou atípicos | 4.817 | 4.723 | 3.866 |
| Resultados adversos e/ou atípicos de EAA | 60,8% | 50,6% | 50,7% |
| Resultados adversos/atípicos de estimulantes | 10,3% | 15,5% | 13,3% |
| EAA mais frequentes (em ordem decrescente) | Testosterona, estanozolol, nandrolona, metandrostenolona | Testosterona, estanozolol, nandrolona, metandrostenolona | Estanozolol, nandrolona, metandrostenolona, metenolona, drostanolona, turinabol |

### 1.5. Efeitos adversos

EAA possuem uma ampla gama de efeitos adversos, a grande maioria dose-dependente, e nem todos reversíveis com a cessação da administração (Maravelias et al., 2005). Um dos principais objetivos das alterações estruturais da testosterona e consequente criação de novos EAA é a tentativa de se exacerbar os efeitos anabólicos da substância, e reduzir os efeitos androgênicos. Porém, este objetivo nem sempre é alcançado e efeitos virilizantes costumam ser observados em mulheres usuárias (Sturmi e Diorio, 1998; Kicman, 2008). Alguns dos efeitos adversos atribuídos aos EAA estão relacionados no Quadro 1.

**Quadro 1:** Possíveis efeitos adversos do uso de EAA (Shahidi, 2001; Silva et al., 2002; Maravelias et al., 2005; Dotson e Brown, 2007; Kicman, 2008;).

| **Alvo** | **Efeitos** |
|---|---|
| Fígado | Colestase hepática (obstrução do canal biliar), podendo causar icterícia, peliose hepática (bolsas de sangue no fígado), risco aumentado de tumores hepáticos |
| Sistema cardiovascular | Risco aumentado de eventos trombóticos como infarto do miocárdio, aumento do hematócrito, hipertensão, diminuição do fibrinogênio plasmático, dano cardíaco, morte súbita cardíaca, hipercolesterolemia com aumento das lipoproteínas de baixa densidade e diminuição das lipoproteínas de alta densidade, risco aumentado de acidente vascular cerebral |

| Alvo | Efeitos |
|---|---|
| Sistema nervoso central/efeitos psicológicos | Aumento da libido, autoestima, irritabilidade, agressividade e hostilidade, euforia, impulsos destrutivos e autodestrutivos |
| Sistema reprodutivo | Puberdade precoce, supressão da síntese endógena de testosterona, amenorreia, hipertrofia do clitóris (irreversível), atrofia testicular, diminuição na espermatogênese, infertilidade masculina, impotência masculina, crescimento desproporcional da próstata, carcinoma prostático, masculinização do feto feminino, teratogenicidade |
| Glândulas mamárias | Atrofia em mulheres, ginecomastia em homens |
| Sistema musculoesquelético | Fechamento precoce das epífises em crianças e adolescentes, com consequente parada no crescimento, risco aumentado de distensões e rupturas musculares e de tendinoses |
| Cabelos | Hirsutismo em mulheres (crescimento de pelos corporais e faciais com padrão masculino, desenvolvimento de calvície com padrão masculino), aceleramento da calvície em homens. O hirsutismo não é totalmente reversível com a cessação da administração de EAA |
| Pele | Acne Cística, aumento da oleosidade da pele |
| Cordas vocais | Se tornam mais espessas nas mulheres, levando a engrossamento irreversível da voz |
| Sintomas associados à abstinência | Depressão severa, insônia, diminuição da libido, cefaleia, artralgias, mialgias, fadiga, diminuição do apetite |

Uma prática comum entre os usuários abusivos é o consumo concomitante de outros medicamentos junto com os EAA, numa tentativa de prevenir o surgimento de determinados efeitos adversos. Um exemplo é o uso de tamoxifeno ou clomifeno, fármacos utilizados no tratamento do câncer de mama hormônio-dependente, numa tentativa de impedir o desenvolvimento de ginecomastia, ou o uso de gonadotrofina coriônica humana para bloquear efeitos testiculares (Sturmi e Diorio, 1998). Outra prática comum é o uso simultâneo de outras substâncias com efeitos anabólicos, o que pode potencializar efeitos adversos (Kicman, 2008).

## 2. Falsificação de medicamentos

### 2.1 Definições e prevalência no mundo

A falsificação de medicamentos é um problema crescente no mundo, e um dos obstáculos ao seu combate é justamente a falta de definição consensual entre os países do que seria um medicamento falso (Deisingh, 2005; Fernandez et al., 2008). A Organização Mundial da Saúde (OMS) reconhece que a ampla gama de produtos farmacêuticos irregulares não se restringe apenas a produtos "falsos", e passou a adotar a nomenclatura "Produtos médicos de baixo padrão, espúrios, falsamente rotulados, falsificados ou contrafeitos" (*Substandard, spurious, falsely labelled, falsified and counterfeit medical products,* SSFFC) (OMS, 2016a).

Entretanto, entende-se que enquanto produtos espúrios, falsamente rotulados, falsificados ou contrafeitos são naturalmente abaixo do padrão, nem todo medicamento abaixo do padrão é necessariamente espúrio, falsamente rotulado, falsificado ou contrafeito. Já o termo "contrafeito", por sua vez, costuma incluir produtos falsificados, fabricados sem licença, falsamente embalados, roubados ou abaixo do padrão. Assim, para melhorar o desenvolvimento de estratégias para combater cada tipo de produto, a OMS resolveu diferenciar "produtos abaixo do padrão" de "produtos contrafeitos". Produtos abaixo do padrão, também chamados de produtos fora da especificação, são medicamentos originais produzidos pelo fabricante autorizado pelas autoridades sanitárias, mas que não estão de acordo com as especificações estabelecidas. Produtos contrafeitos (ou falsos) são aqueles deliberadamente e fraudulentamente identificados de maneira errônea quanto à sua identidade e/ou origem. A falsificação pode se aplicar tanto a medicamentos de marca quanto a genéricos, e produtos falsos podem incluir produtos com os ingredientes certos ou errados, sem ingredientes ativos, com quantidade insuficiente de ingredientes ativos ou com embalagem falsa (OMS, 2016b).

Embora não seja possível determinar exatamente a incidência da falsificação de medicamentos, estima-se que em torno de 10% dos medicamentos circulando no mundo sejam falsos. Sabe-se, entretanto, que esse percentual pode variar drasticamente entre os países, de 1% em países desenvolvidos a 30% em alguns países em desenvolvimento da África, Ásia e América do Sul (Chika et al., 2001; Fernandez et al., 2008; Dégardin et al., 2014).

Medicamentos falsos podem conter desde misturas aleatórias de substâncias tóxicas a preparações inertes e ineficazes, cujo uso pode resultar em falha terapêutica ou até mesmo morte. Qualquer classe terapêutica pode ser alvo de falsificação, sendo as mais comuns no âmbito mundial os antibióticos, hormônios e esteroides, anti-histamínicos, antimaláricos,

analgésicos e antipiréticos (Deisingh, 2005; OMS, 2016a). Em países em desenvolvimento, os principais alvos são medicamentos para tratar condições que ameaçam a vida, como malária, tuberculose e AIDS. Em países industrializados, os alvos tendem a ser medicamentos de estilo de vida, como narcóticos e aqueles usados no tratamento da disfunção erétil ou obesidade. Entretanto, com a expansão do mercado, a falsificação tem incluído também medicamentos de alto custo para o câncer ou antivirais (Dégardin et al., 2014).

### 2.2 Prevalência no Brasil

Assim como no resto do mundo, não é possível determinar a prevalência de medicamentos falsos no Brasil, havendo apenas estimativas. O Relatório Brasil Original, de 2011, reportou que no período de 2008 a 2010 foram apreendidas quase 22 milhões de unidades de medicamentos falsos no país, havendo grande crescimento no período, de 496.663 unidades em 2008 para 18.150.578 em 2010 (Brasil, 2011).

Um trabalho feito com dados obtidos a partir de Laudos Periciais emitidos pela Polícia Federal (PF) entre 2007 e 2010 mostraram que dentre os 610 casos de medicamentos declarados falsos, 69% eram medicamentos para disfunção erétil, seguidos pelos esteroides anabolizantes (26%) (Ames e Souza, 2012). Conclusão semelhante foi atingida em trabalho posterior, que analisou dados oriundos de operações da ANVISA em parceria com a PF, mas se ateve ao mesmo período de 2007 a 2010 (Hurtado e Lasmar, 2014).

Talvez a melhor estimativa disponível de falsificação de medicamentos no Brasil seja aquela fornecida por Marchetti (2014), onde foram analisados dados oriundos de todos os laudos periciais emitidos pela PF no período de 2006 a 2012 relacionados a medicamentos. Foram considerados dados de 7.094 laudos periciais emitidos pelos estados da federação, relacionados ao exame de 30.452 medicamentos. Dentre eles, 25.833 eram medicamentos alopáticos de uso humano, e 2.586 (10%) foram declarados falsos após exames periciais (Marcheti, 2014), percentual esse semelhante ao aceito pela OMS como sendo a média global. Novamente, os medicamentos falsos mais frequentes foram os destinados ao tratamento da disfunção erétil (Cialis e Viagra), seguidos por medicamentos anabolizantes (Durateston, Deca Durabolin e Hemogenin) (Marcheti, 2014).

### 2.3 Falsificação de medicamentos contendo EAA

A preocupação com o mercado clandestino e o uso abusivo de EAA se manifestou, nos Estados Unidos em 1985 a partir de uma ação conjunta do *Food and Drug*

*Administration* (FDA), do Departamento de Justiça e da Agência Federal de Investigação (Burge, 1994). Em 1990, os Estados Unidos incluíram os EAA no Ato das Substâncias Controladas, por meio do Ato de Controle dos Esteroides Anabolizantes. Após esse ato, a simples posse sem receita médica apropriada de um dos 25 diferentes EAA listados passou a ser considerado crime (EUA, 1990).

Com o Ato de Controle, muitas empresas farmacêuticas pararam de produzir medicamentos contendo EAA, e com isso o mercado clandestino e as falsificações aumentaram (Dotson e Brown, 2007). Enquanto em 1985 estimava-se que 70% dos EAA no mercado clandestino norte-americano eram oriundos do fabricante legítimo, após o Ato de Controle, mais de dois terços dos produtos seriam originados do contrabando ou fabricados por laboratórios clandestinos (Burge, 1994). Um estudo mais recente conduzido no Reino Unido identificou laboratórios clandestinos localizados no próprio país, além de em outros como Tailândia, China, Chipre e Grécia (Antonopoulos e Hall, 2016).

A despeito do aumento da regulação e controle no comércio pelo mundo, o mercado clandestino de EAA continua aumentando drasticamente, especialmente pela internet, onde muitos dos produtos disponíveis são falsos ou de baixa qualidade (Donati, 2007; Cordaro et al., 2011; Coomber et al., 2014; Antonopoulos e Hall, 2016). Após a avaliação de vários estudos referentes à composição química de produtos contendo EAA oriundos do mercado clandestino, Coomber et al. (2014) concluíram que a variação na composição desses produtos é tão grande, que o usuário não tem como saber se o produto é mais ou menos potente do que descrito na embalagem, se contém a substância rotulada, ou se ao menos contém alguma substância ativa.

No Brasil, medicamentos contendo EAA foram identificados como a segunda classe terapêutica mais falsificada, atrás apenas daqueles destinados ao tratamento da disfunção erétil (Brasil, 2011; Ames e Souza, 2012; Hurtado e Lasmar, 2014; Marcheti, 2014). Alguns exemplos de produtos falsos apreendidos no país e enviados a perícia na Polícia Federal são mostrados nas Figuras 4 a 10.

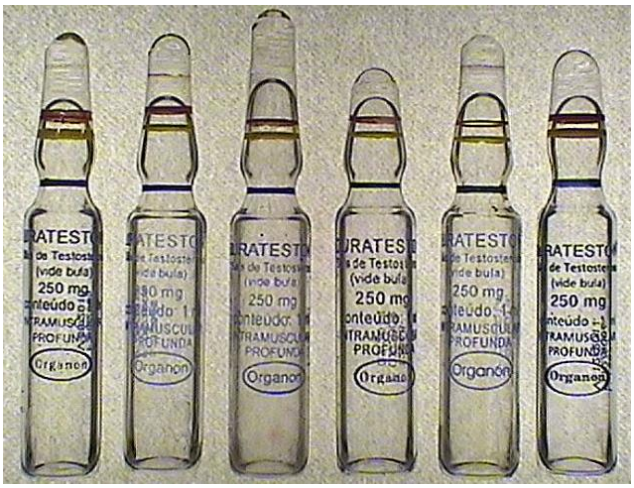



**Figura 4:** Ampolas do medicamento nacional Durateston®, um dos principais alvos de falsificação no Brasil (Ames e Souza, 2012; Marcheti, 2014), à base de quatro ésteres de testosterona. A ampola mais à esquerda é original, e todas as outras são falsas. Notar as diferenças de tamanho da ampola e qualidade da impressão dos escritos. Foto tirada com luz transmitida.

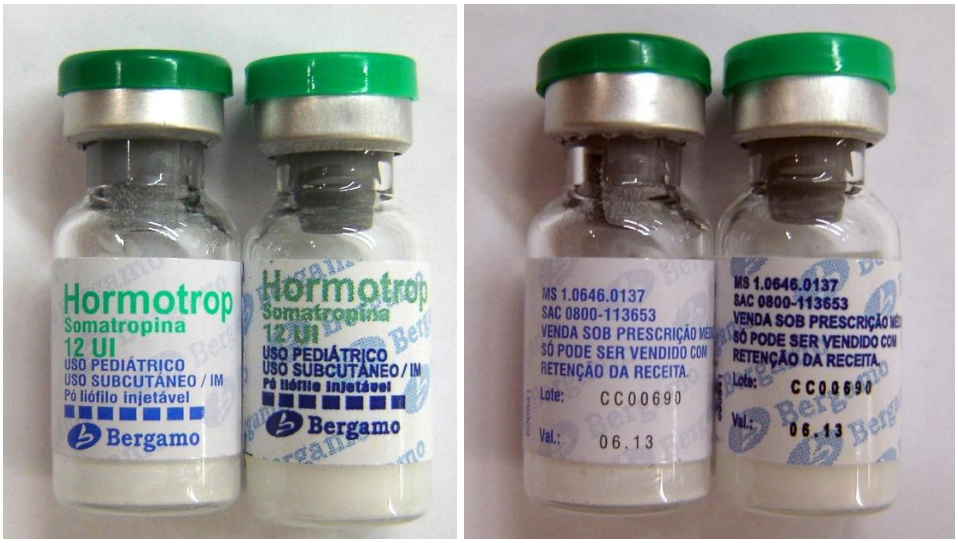



**Figura 5:** Frascos do medicamento nacional Hormotrop®, à base de somatropina (hormônio do crescimento). O original está à esquerda e o falso à direita. Notar as diferenças na qualidade de impressão do rótulo.


**Figura 6:** Frascos do medicamento de origem declarada Australiana Deca 50®, declarando conter Decanoato de Nandrolona. Os produtos ilustrados foram declarados falsos por não conter nenhuma substância ativa. Notar os erros de grafia no rótulo "Nandeolone Decandate" ao invés de "Nandrolone Decanoate" e "staroid" ao invés de "steroid" (destacados pelas setas).


**Figura 7:** Frascos do medicamento de origem declarada grega Nandrolone Decanoate®, declarando conter Decanoato de Nandrolona. Notar as diferenças nas dimensões do frasco, na rotulagem e no prazo de validade, apesar de ambos declararem o mesmo número de lote "17530". Os produtos ilustrados foram declarados falsos por não conter nenhuma substância ativa.


**Figura 8:** Frascos dos medicamentos "Winstrol CIII" e "Anavar SPa". Apesar de se tratarem de produtos diferentes e declararem fabricantes diferentes, ambos trazem endereços semelhantes, sendo um "Barceloneta, Puerto Rico 00617" e o outro "Barcelona Puerto Rico 617", e o mesmo número de lote "F417BOB". Os produtos ilustrados foram declarados falsos por conter substâncias anabolizantes diferentes daquelas descritas em suas embalagens.


**Figura 9:** Frascos do medicamento de origem declarada mexicana "Trenbo-Life". Notar que a concentração declarada na frente do rótulo é de 75 mg/mL, enquanto na parte de trás a concentração é de 50 mg/mL. Os produtos ilustrados foram considerados falsos por não conter nenhuma substância ativa.


**Figura 10:** Frasco de medicamento "underground", produto sem registro que não declara ser cópia de nenhum medicamento existente no mercado. O produto traz como responsável técnico um médico, constando número de inscrição no Conselho Regional de Medicina (indicado pela elipse), e não um farmacêutico; a numeração do código de barras não segue o padrão internacional vigente e a embalagem ainda traz a frase "Aplicação sob orientação de alguém especializado", estando totalmente fora dos padrões vigentes para embalagem de medicamentos. O produto ilustrado não continha nenhuma substância ativa.

## 2.4 Métodos analíticos empregados na análise de medicamentos contendo EAA

Existem poucos estudos disponíveis relatando a análise de medicamentos contendo EAA, muitas vezes realizados com um número limitado de amostras obtidas no mercado clandestino. As principais técnicas analíticas empregadas são cromatográficas, tanto em fase líquida (LC) quanto gasosa (CG), dando-se preferência a detectores por espectrometria de massas (MS) (Tabela 2).

A maior parte dos estudos publicados são qualitativos, e consideram como falsos medicamentos que não contenham o EAA declarado na embalagem, e/ou que contenham EAA não declarados nas embalagens. Dentre os poucos estudos quantitativos encontrados, apenas dois consideraram falsos também os produtos cuja concentração detectada diferia significativamente da concentração declarada (Shi et al., 2008; Pellegrini et al., 2012). O terceiro apenas menciona as concentrações detectadas, mas não cita quais as concentrações declaradas dos produtos e nem compara os resultados com as informações de rótulo (Cho et al., 2015).

**Tabela 2:** Resumo dos estudos publicados que se referem à análise de medicamentos contendo EAA suspeitos de falsificação.

| Referência | País | Nº amostras | Amostras falsas (%) | Técnicas | Análise |
|---|---|---|---|---|---|
| Musshoff et al., 1997 | Alemanha | 42 | 15 (35,7) | CG-MS | Qualitativa |
| Ritsch e Musshoff, 2000 | Alemanha | 40 | 15 (37,5) | CG-MS | Qualitativa |
| Thevis et al., 2008 | Alemanha | 48 | 17 (35,4) | LC-MS/MS | Qualitativa |
| Shi et al., 2008 | China | 9 | 2 (22,2) | HPLC-DAD | Quantitativa |
| Graham et al., 2009 | Reino Unido | 57 | 24 (42,1) | CG-MS | Qualitativa |
| Coopman e Cordonnier, 2012 | Bélgica | 74 | 25 (33,8) | HPLC-DAD<br>CG-MS | Qualitativa |
| Pellegrini et al., 2012 | Itália | 15 | 13 (86,7) | CG-MS | Quantitativa |
| Cho et al., 2015 | Coréia do Sul | 19 | 9 (47,3) | LC-MS/MS | Quantitativa |
| Prokudina et al., 2015 | República Tcheca | 7 | 5 (71,7) | DART-MS | Qualitativa |

CG = Cromatografia Gasosa; MS = Espectrometria de Massas; LC = Cromatografia Líquida; HPLC = Cromatografia Líquida de Alta Eficiência; DAD = Detector de Arranjo de Diodos; DART = Análise Direta com Ionização em Tempo Real

Vários dos estudos qualitativos encontraram uma taxa de falsificação semelhante, em torno de 35%. A quantidade de amostras dos estudos quantitativos é muito pequena para poder ser considerada significativa, podendo ter ocorrido viés de amostragem nos resultados obtidos. De qualquer forma, somando-se as amostras e resultados reportados por Shi et al. (2008), Pellegrini et al. (2012) e Cho et al. (2015), têm-se que dentre 43 amostras analisadas quantitativamente, 24 (55,8%) foram consideradas falsas, o que é um valor maior do que o reportado na maioria dos estudos qualitativos. Isso se deve ao fato de que, nos estudos qualitativos, não são detectadas falsificações onde o ingrediente ativo correto está presente, porém em concentrações muito discrepantes daquela descrita na embalagem.

Dentre os métodos que empregaram CG-MS, o metanol é o solvente de escolha e a etapa de derivatização do analito foi incluída em alguns casos, com o objetivo de aumentar a volatilidade e estabilidade térmica do EAA, bem como melhorar suas características

cromatográficas. Musshoff et al (1997) utilizaram extração com metanol, seguida apenas por banho em ultrassom e centrifugação para análise qualitativa de produtos farmacêuticos contendo EAA. Coopman e Cordonnier (2012) utilizaram metanol adicionado de difenilamina para extração de EAA em comprimidos e suspensões aquosas e metanol e hexano para produtos na forma de óleo, também em análise qualitativa. Pellegrini et al (2012), que analisaram quantitativamente os produtos, empregaram derivatização com N,O-bis-trimetilsilil-trifluoroacetamida e trimetilsilil ou com N-metil-N-trimetilsililtrifluoroacetamida, iodeto de amônio e ditioeritritol antes da análise cromatográfica. Os três estudos empregaram coluna com 5% de fenilmetilpolisiloxano (HP5-MS ou CPSil 8 CB). Graham et al. (2009) também empregaram derivatização no preparo de suas amostras, mas utilizaram uma coluna mais apolar, com 100% de dimetilpolisiloxano, e fizeram apenas análises qualitativas. Em matrizes biológicas, o uso de derivatização na análise de EAA por CG-MS é ainda mais frequente (Geyer et al., 2004; Mazzarino et al., 2007; Van Eenoo et al., 2011).

Outras técnicas analíticas empregadas na análise de produtos farmacêuticos incluem análises colorimétricas, cromatografia em camada delgada e espectroscopia na região do infravermelho próximo, infravermelho médio com ou sem transformada de Fourier ou Raman (Custers et al., 2015; Krakowska et al., 2016). Algumas dessas técnicas espectrométricas podem gerar até milhares de medidas para cada amostra, tornando complexo o processo de explorar e interpretar os dados obtidos. Ferramentas quimiométricas são bastante úteis para lidar com o excesso de variáveis e extrair informações relevantes dos dados coletados. Essas ferramentas são destinadas a simplificar dados complexos e a estudar as possíveis relações e/ou diferenças entre diferentes grupos de amostras (Rajalahti e Kvalheim, 2011; Krakowska et al., 2016).

## 2.5 Métodos quimiométricos empregados na análise de medicamentos sob suspeita de falsificação

O uso de ferramentas quimiométricas para o processamento de dados gerados por métodos espectrométricos segue uma sequência lógica: primeiro, os dados são pré-processados para se minimizar a variabilidade não desejada; em seguida, os dados são explorados para que possíveis padrões de resposta sejam revelados, podendo ser feita ainda seleção das variáveis relevantes para a melhor compreensão do fenômeno modelado e para reduzir o risco de calibração excessiva do método. Finalmente, podem ser empregadas técnicas supervisionadas para se propor modelos de classificação e discriminação entre

grupos de amostras, e então o método deve ser validado para que se possa verificar seu poder preditivo (Storme-Paris et al., 2010; Krakowska et al., 2016).

O pré-processamento de dados tem por objetivo corrigir ou suprimir efeitos indesejados, empregando-se transformações matemáticas que removem grande parte da variabilidade dos dados que não seja relacionada ao fenômeno estudado. O tipo de pré-processamento a ser empregado varia conforme os dados e a técnica analítica empregada, e incluem a centralização dos dados na média, colocar os dados na mesma escala, normalizar, suavizar e/ou derivar os dados, e alinhar sinais experimentais (Roggo et al., 2010; Dégardin et al., 2016; Krakowska et al., 2016).

Os métodos utilizados para a exploração inicial dos dados são chamados métodos não supervisionados, quando não há, ou não se informa, conhecimento prévio sobre as amostras (Storme-Paris et al., 2010; Krakowska et al., 2016). Esses métodos servem para avaliar tendências de agrupamento dos dados, similaridades entre amostras ou variáveis, identificar variáveis não relevantes e inspecionar amostras individualmente. Os métodos não supervisionados podem ser separados em métodos de projeção e agrupamento. Os métodos de projeção projetam as amostras, que antes eram descritas por uma série de parâmetros físico-químicos, em um novo sistema de coordenadas definido por poucas variáveis. Nesse novo sistema é possível visualizar estruturas ocultas nos dados, tais como grupos de amostras. Dentre os métodos de projeção destaca-se a análise de componentes principais (PCA), considerada a abordagem padrão para a compressão e visualização de dados multivariados (Rajalahti e Kvalheim, 2011; Krakowska et al., 2016). A PCA é uma técnica que diminui o número de variáveis fazendo combinações lineares ortogonais das variáveis originais; essas combinações são chamadas de componentes principais (PCs) e são definidas de tal maneira que elas explicam progressivamente a variabilidade residual dos dados (Sacré et al., 2010; Ortiz et al., 2013; Custers et al, 2015). As amostras são então posicionadas dentro do espaço multidimensional definido pelas PCs e, com base em suas coordenadas, é possível verificar a distâncias entre elas e a presença de tendências, agrupamentos e pontos aberrantes (Sacré et al., 2010; Storme-Paris et al., 2010; Rajalahti e Kvalheim, 2011; Ortiz et al., 2013;). Os métodos de agrupamento têm por objetivo construir grupos de amostras ou variáveis similares, ou seja, focam na similaridade entre amostras. Uma vez que na análise de medicamentos sob suspeita de falsificação o foco está nas diferenças entre produtos originais e falsos, métodos de agrupamento não são muito utilizados para esse fim.

Os métodos supervisionados são aqueles empregados quando há algum conhecimento prévio sobre as amostras, como quais as classes presentes no grupo de amostras ou informações sobre uma determinada característica da amostra, tal como a concentração de princípio ativo (Sacré et al., 2010; Storme-Paris et al., 2010; Krakowska et al., 2016). São métodos de discriminação, classificação ou regressão. Métodos classificatórios se baseiam na construção de regras de classificação para cada grupo separadamente e na definição dos limites de cada grupo (Custers et al., 2015; Krakowska et al., 2016). Um exemplo de método de classificação muito empregado na análise de medicamentos é a modelagem suave independente por analogia de classe (*soft independent modeling of class analogies*, SIMCA). No SIMCA, cada grupo de amostras é definido em um modelo de PCA, e as amostras desconhecidas são designadas para um determinado grupo com base na sua posição com relação aos espaços pré-definidos dos grupos de calibração (Custers et al., 2015; Krakowska et al., 2016).

Os métodos discriminantes, por sua vez, dividem o espaço das variáveis explanatórias em várias regiões mutuamente excludentes, uma para cada grupo pré-definido. Assim, uma amostra teste sempre será designada para algum grupo. Um método muito empregado na análise de medicamentos é o dos mínimos quadrados parciais – análise discriminante (*partial least squares – discriminant analyisis*, PLS-DA), onde se atribui uma variável dependente y para cada grupo pré-conhecido de amostras. O método é montado com um subconjunto das amostras (grupo de calibração) e é treinado para designar valores de y para as amostras; uma vez que o modelo é montado, uma nova amostra é designada para um ou outro grupo com base no seu valor de y calculado (Rajalahti e Kvalheim, 2011; Fernandes et al., 2012; Silva et al., 2014; Krakowska et al., 2016). As regras lógicas são montadas num espaço de fatores PLS, cuja definição é semelhante aos princípios da PCA. Entretanto, os fatores PLS são definidos de maneira a maximizar sua correlação com a variável resposta e não necessariamente com a maior variabilidade dos dados (Peinder et al., 2008; Sacré et al., 2010; Storme-Paris et al., 2010; Rajalahti e Kvalheim, 2011; Krakowska et al., 2016).

Existem vários estudos descrevendo o uso de ferramentas quimiométricas, associadas a diversas técnicas analíticas (principalmente espectroscópicas), para construção de modelos a serem empregados na detecção de falsificações de medicamentos. Até o momento, só foi encontrado um estudo se referindo a medicamentos contendo fármacos anabolizantes (Rebiere et al., 2015), descrevendo a análise de comprimidos de metandrostenolona. Esse e alguns outros estudos estão resumidos no Quadro 2.

**Quadro 2:** Resumo de estudos publicados que se referem ao uso de quimiometria na elaboração de modelos para detecção de falsificações de medicamentos.

| Referência | Técnica analítica empregada | Ferramenta quimiométrica empregada | Medicamentos investigados |
|---|---|---|---|
| Scafi e Pasquini, 2001 | NIRS | PCA, SIMCA | Várias classes |
| Vredenbregt et al., 2006 | NIRS | PCA, WC | Viagra |
| Dowell et al., 2008 | NIRS | PLS-DA | Comprimidos de artesunato |
| Peinder et al., 2008 | NIRS, Raman | PCA, PLS-DA | Lipitor |
| Roggo et al.; 2010 | Raman | SVM | Várias classes |
| Dorlo et al., 2012 | NIRS | PCA | Cápsulas de miltefosine |
| Ortiz et al., 2013 | ATR-FTIR | PCA | Viagra, Cialis |
| Li et al., 2014 | NIRS, Raman | PLS-DA | Comprimidos de anisodamina |
| Custers et al.,2015 | ATR-FTIR | k-NN, CART, SIMCA | Viagra, Cialis |
| Rebiere et al., 2015 | NIRS, Raman | PCA, MCR-ALS | Comprimidos de metandrostenolona |
| Zhao et al, 2015 | Raman | PCA, CLS | Doxofilina e Levofloxacino injetáveis (vários fabricantes) |
| Dégardin et al., 2016 | NIRS | PCA, SVM, k-NN, DA | Várias classes |

NIRS = Espectroscopia no Infravermelho próximo; PCA = Análise de Componentes Principais; SIMCA = Modelagem suave independente por analogia de classe; WC = Correlação de comprimento de onda; PLS-DA = Mínimos quadrados parciais – análise discriminante; SVM = Máquina de vetor de suporte; ATR = Refletância Total Atenuada; FTIR = Infravermelho com Transformada de Fourier; k-NN = Regra do vizinho mais próximo; CART = Árvores de Classificação e Regressão; MCR-ALS = Resolução multivariada de curvas – Mínimos quadrados alternantes; CLS = Mínimos quadrados clássico; DA = Análise discriminante.

## 3. Suplementos alimentares

### 3.1. Definições e questões legais relacionadas

Suplemento alimentar (SA) é uma expressão genérica usada para designar qualquer substância ingerida de forma oral, que contenha elementos com capacidade de complementar a dieta (Parra et al., 2011). Apesar de ser um termo muito comum, até mesmo na comunidade científica, a categoria "suplemento alimentar" não existe legalmente no Brasil. Dentre as categorias de alimentos previstas pela Agência Nacional de Vigilância Sanitária (ANVISA), aquelas que mais se assemelham ao que é popularmente chamado de SA são os alimentos para atletas; suplementos vitamínicos e/ou minerais; alimentos com alegações de propriedade funcional e/ou de saúde; novos alimentos e novos ingredientes; ou substâncias bioativas e probióticos isolados com alegação de propriedades funcional e/ou de saúde. Todas essas categorias são bem definidas e explicitam quais tipos de produtos podem ser comercializados como tal (Brasil, 1998; Brasil, 1999; Brasil, 1999b; Brasil, 2002; Brasil, 2010b).

Nos Estados Unidos, a gama de produtos que pode ser comercializada sob a categoria de *dietary supplements* ("suplementos dietéticos", aqui usado como sinônimo de SA) é muito maior. O *Dietary Supplement Health and Education Act of 1994*, conhecido como DSHEA, define SA como:

*"Um produto (que não o tabaco) destinado a suplementar a dieta que contenha um ou mais dos seguintes ingredientes dietéticos: (A) uma vitamina; (B) um mineral; (C) uma erva ou outro derivado de planta; (D) um aminoácido; (E) uma substância dietética para uso pelo homem para suplementar a dieta aumentando a ingestão diária total; (F) um concentrado, metabólito, constituinte, extrato ou combinação de qualquer ingrediente descrito nos itens (A), (B), (C), (D), ou (E) "* (EUA, 1994*).*

Segundo o DSHEA, um SA pode conter uma substância que tenha sido aprovada como nova droga, certificada como antibiótico ou licenciada para uso biológico desde que antes dessa aprovação, certificação ou licença ela tenha sido usada como suplemento dietético ou alimento, e não haja disposição específica em contrário (EUA, 1994). Dessa forma, o DSHEA garantiu o livre acesso da população norte-americana a uma grande variedade de produtos (Dickinson, 2011), e a *Food and Drug Agency* (FDA) tem que provar que determinado produto é nocivo à saúde para retirá-lo do mercado (EUA, 1994).

Outra diferença entre o mercado brasileiro e o norte-americano de SA se refere à rotulagem. Produtos considerados alimentos no Brasil não podem trazer em seus rótulos indicações de que ele possua propriedades medicinais ou terapêuticas, ou aconselhem seu consumo como estimulante, para melhorar a saúde, para prevenir doenças ou com ação

curativa (Brasil, 2002b). Mesmo aqueles que se enquadrem na categoria “Alimentos com alegações de propriedade funcional e/ou de saúde” só podem trazer em seus rótulos alegações pré-aprovadas pela ANVISA, que são específicas para a composição do produto em questão. Por exemplo, produtos contendo fitoesteróis, quando atendem aos requisitos necessários de quantidade, pureza e outros, podem trazer em seus rótulos a frase *“Os fitoesteróis auxiliam na redução da absorção de colesterol. Seu consumo deve estar associado a uma alimentação equilibrada e hábitos de vida saudáveis”* (Brasil, 2013).

A legislação norte-americana permite uma série de alegações nas embalagens dos SA, que podem ser feitas sem prévia autorização da FDA. Essas alegações incluem declarações de benefícios relacionados a doenças causadas por deficiências de nutrientes; declarações que descrevam o papel de um nutriente destinado a afetar a estrutura ou função em humanos; que caracterizem o mecanismo documentado pelo qual um nutriente ou ingrediente aja para manter a estrutura ou função; e que descrevam bem-estar geral pelo consumo de um nutriente ou ingrediente (EUA, 1994; Nesheim, 1998). Na prática, isso se traduz como uma série de eufemismos encontrados nos rótulos dos SA norte-americanos. Por exemplo, os rótulos não podem conter alegações como “reduz os níveis de colesterol”, mas podem trazer uma sentença genérica como “promove um nível saudável de colesterol” (Nestle, 1999).

### 3.2. Uso de suplementos alimentares

*Praticantes de atividade física e atletas*

Praticantes de atividade física são tidos como grandes consumidores de SA (Goston e Correia, 2010; Nogueira et al., 2013; Rovira et al., 2013), motivo pelo qual costumam ser a população de escolha quando se quer avaliar o consumo desses produtos. Nogueira et al., (2013) revisaram quinze estudos realizados no Brasil avaliando o uso de SA por praticantes de musculação. Foi identificado um elevado consumo de SA dentre a população estudada, com índices variando entre 20,5% dos entrevistados (estudo de Porto Alegre) e 94,0% (estudo de Belo Horizonte). É destacado o fato de que quanto mais recente era a pesquisa, maior o número de indivíduos que afirmaram utilizar algum SA, mostrando uma tendência de aumento no consumo. Os suplementos reportados como os mais consumidos eram produtos contendo aminoácidos ou concentrados proteicos, seguidos por aqueles contendo creatina e carboidratos.

Goston e Correia (2010) avaliaram o consumo de SA dentre 1102 frequentadores de 50 diferentes academias de Belo Horizonte (MG). Quase 37% (N = 405) reportaram o uso de algum SA, sendo os mais frequentes os hiperproteicos, isotônicos, produtos à base de carboidratos, produtos naturais ("fitoterapêuticos"), multivitamínicos ou multiminerais, creatina e termogênicos (*fat-burners*). O estudo constatou que homens consumiam mais SA que mulheres, que os usuários eram mais novos do que os não-usuários, e que aqueles que se exercitavam há mais tempo e mais vezes na semana tendiam a usar mais SA do que os outros. Vários outros estudos foram publicados no Brasil abordando o consumo de SA, principalmente dentre frequentadores de academias de ginástica, alguns resumidos na Tabela 3.

Em outros países, o consumo de SA entre atletas e praticantes de atividade física também é muito frequente. Estudo realizado no estado da Virgínia, nos EUA avaliou o uso de SA entre 229 civis e militares matriculados em academias de ginástica, e constatou que apenas 35 declararam não usar nenhum SA. Dentre os civis, 57% declararam usar creatina, 67% proteínas, 48% cafeína e 15% declararam usar os hormônios dehidroepiandrosterona ou androstenediona (Sheppard et al., 2000). Outro estudo norte-americano aplicou questionários a 106.698 militares, dos quais 46,7% declararam usar ao menos um dos SA incluídos no questionário; 17,3% declararam o uso de hiperproteicos, aminoácidos ou creatina, 38% declararam o uso de bebidas energéticas e 19,4% o uso de algum SA para emagrecer (Jacobson et al., 2012). Um terceiro estudo norte-americano com 222 frequentadores de uma academia de ginástica mostrou que 84,7% declararam usar ao menos um SA, sendo vitaminas/minerais e proteínas consumidos mais de cinco vezes por semana. O consumo regular de carboidratos, aminoácidos, creatina e suplementos contendo efedrina foi citado por 9,5% dos entrevistados, e o uso frequente ou ocasional de dehidroepiandrosterona e androstenediona foi citado por 13,1% deles (Morrison et al., 2004).

**Tabela 3**: Resumo de estudos publicados abordando o uso de SA no Brasil.

| Ref. | População | Percentual de usuários | SA mais frequentes |
|---|---|---|---|
| Rocha e Pereira, 1998 | 160 alunos de academias em Niterói e São Gonçalo (RJ) | 32% consumiam algum SA | Hiperproteicos e aminoácidos e outros |
| Araújo et al., 2002 | 183 homens, frequentadores de academias de fisiculturismo de Goiânia | 34% reportaram o uso de algum SA | Hiperproteicos, aminoácidos e metabólitos de proteínas |
| Santos e Barros Filho, 2002 | 894 universitários de São Paulo | 30,4% haviam consumido nos últimos 3 meses | Estudo se restringia ao consumo de vitaminas |
| Pereira et al., 2003 | 309 alunos de academias de ginástica de São Paulo | 23,9% consumiam algum SA | Hiperproteicos e aminoácidos, Vitaminas, carboidratos, creatina |
| De Rose et al., 2006 | 234 atletas dos Jogos Desportivos Sul-Americanos selecionados para exames anti-doping | 50% declararam o uso de algum SA | Vitaminas, minerais e aminoácidos |
| Linhares e Lima, 2006 | 334 praticantes de musculação de Campos dos Goytacazes | 35% declararam usar algum SA | Hiperproteicos e aminoácidos |
| Hirschbruch et al., 2008 | 201 jovens entre 15 e 25 anos frequentadores de academias de ginástica de São Paulo | 61,2% consumiam algum SA | Isotônicos, Vitaminas, hipercalóricos, aminoácidos, proteínas e creatina |
| Goston e Correia, 2010 | 1102 frequentadores de academias de ginástica em Belo Horizonte | 36,8% estavam usando algum SA | Hiperproteicos, isotônicos, carboidratos, "produtos naturais", multivitamínicos/ multiminerais, creatina e termogênicos |

| Ref. | População | Percentual de usuários | SA mais frequentes |
|---|---|---|---|
| Andrade et al., 2012 | 60 clientes de uma clínica de nutrição esportiva de São Paulo | 43,3% declararam já ter consumido um SA antes de procurar a clínica de nutrição; 63% estavam atualmente consumindo por orientação de nutricionista da clínica | Hiperproteicos, creatina, termogênicos, aminoácidos (tanto antes quando depois da orientação) |
| Carvalho-Silva et al., 2012 | 204 alunos de academias de Alfenas-MG. | 52,4% consomem ao menos um SA | Aminoácidos, creatina, vitaminas |
| Brunacio et al., 2013 | 865 participantes do Inquérito de Saúde de São Paulo | 6,4% relataram o uso de algum SA (apenas 2,2% da população estudada foi considerada fisicamente ativa) | Não especificado |
| Fayh et al., 2013 | 316 frequentadores de academias de Porto Alegre | 63,3% relataram uso presente ou passado; 39,1% dos homens e 12,9% das mulheres os utilizavam no momento do estudo. | Hiperproteicos, vitaminas |
| Poll e Lima, 2013 | 302 universitários de cursos da área da saúde | 27,2% consumiam SA ou haviam consumido nos últimos seis meses | Isotônicos, vitaminas e minerais |
| Silva e Marins, 2013 | 351 jovens atletas praticantes de modalidades olímpicas residentes em Viçosa-MG. | 74% usavam ou conheciam algum usuário de SA. | Creatina, carboidratos, hiperproteicos. |

Estudo realizado em Sevilha, na Espanha com 415 frequentadores de academias indicou que 28% dos entrevistados consumiam ou já haviam consumido algum suplemento hiperproteico (Oliver et al., 2011). No Canadá foram aplicados questionários a 211 atletas de elite universitários, dos quais 98,6% reportaram o uso de algum SA, sendo os mais frequentes aqueles contendo carboidratos e cafeína, seguidos por produtos à base de vitaminas/minerais, proteínas e creatina (Kristiansen et al., 2005). Entre 1.138 atletas de elite alemães, com idade entre 14-18 anos, 91,1% declararam usar ao menos um SA, com frequência mínima mensal, e os mais consumidos foram produtos à base de vitaminas/minerais e carboidratos (Diehl et al., 2012). Questionários aplicados a 1.625 indivíduos matriculados em academias de ginástica em Teerã, no Irã, mostraram que 66,7% reportaram o uso de algum SA, principalmente vitaminas/minerais e creatina (Saeedi et al., 2013). Finalmente, na cidade de Palermo, na Itália, foram aplicados questionários a 207 frequentadores de academias de ginástica, dos quais 30,1% declararam usar algum SA, sendo os mais frequentes os hiperproteicos e a creatina (Bianco et al.; 2011).

*População geral*

O consumo de SA na população geral foi estudado em alguns países. Na Inglaterra, 15.465 adultos responderam a um questionário com perguntas relacionadas ao consumo de SA, características pessoais e de saúde, dos quais um terço declarou estar tomando ao menos um SA (dentre os que tomavam, 27,8% declararam tomar mais de um). O uso de SA era maior entre pessoas mais velhas, mulheres, não fumantes e pessoas fisicamente ativas (Harrison et al., 2004). Estudo semelhante realizado nos Estados Unidos no período entre 2003 e 2006 com 18.758 pessoas selecionadas aleatoriamente mostrou que 49% dos entrevistados usavam algum SA, principalmente multivitamínicos/minerais, produtos à base de extratos de plantas e aminoácidos. O uso entre as mulheres foi maior do que entre os homens (53% e 44% dos entrevistados, respectivamente), e o uso de mais de um SA simultaneamente foi declarado por mais de 40% dos usuários (Bailey et al., 2011). Na Espanha, estudo realizado com 6.352 adultos indicou o consumo de SA por apenas 9,3% dos entrevistados, 72% destes eram mulheres (Rovira et al., 2013). Um estudo realizado na Europa com 36.034 adultos que foram questionados quanto ao uso de algum SA nas últimas 24 horas mostrou uma grande variação entre os países, com um claro gradiente decrescente entre países do norte e países do sul. Por exemplo, 51,8% dos homens entrevistados na Dinamarca eram usuários enquanto este percentual foi de 2% na Grécia. Em quase todos os países avaliados, o percentual de mulheres usuárias foi maior que de homens. Os SA mais

frequentes foram vitaminas, minerais e os multivitamínicos/multiminerais (Skeie et al., 2009).

### 3.3. A cafeína como ingrediente de suplementos alimentares

A cafeína, ou 1,3,7-trimetilxantina (Figura 11), é um dos estimulantes mais consumidos e estudados no mundo, estando presente numa grande variedade de alimentos e bebidas e em cerca de 60 espécies de plantas (Schwenk and Costley, 2002; Gurley et al., 2015). A cafeína possui efeitos de estimulação do Sistema Nervoso Central (SNC), é diurética, estimulante do músculo cardíaco e provoca relaxamento dos músculos lisos. Provoca redução da fadiga, melhora na concentração, no desempenho em atividades motoras e em trabalhos mentais. Seu mecanismo de ação envolve o aumento da adenosina monofosfato cíclica intracelular e o antagonismo dos efeitos da adenosina (Rang et al., 1997). A cafeína também apresenta certo efeito termogênico, ou seja, aumenta o gasto energético do indivíduo, e parece ter efeito positivo na mobilização da gordura como fonte de energia, em detrimento do glicogênio (Greenway, 2001). Diversos estudos, resumidos por Westerterp-Plantega et al. (2006), demonstram aumento na taxa metabólica basal após o consumo de cafeína e potencial redução da ingestão calórica, mecanismos que poderiam contribuir para a redução do peso corpóreo. Os autores destacam, porém, que estudos de longo termo não mostram vantagens da cafeína sobre o placebo na perda e manutenção do peso (Westerterp-Plantega et al., 2006).

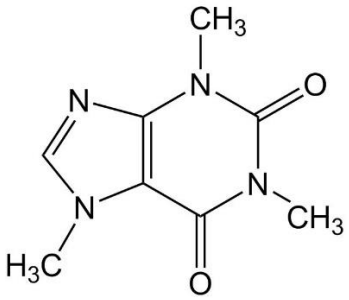



**Figura 11**: Estrutura química da cafeína (1,3,7-trimetilxantina).

Bebidas contendo cafeína, primariamente café (*Coffea arabica*) e chá (*Camellia sinensis*), são amplamente consumidas no mundo há centenas de anos. No início do século 20 surgiram ainda refrigerantes, que costumavam usar a semente de kola (*Cola acuminata*) como flavorizante e fonte de cafeína, e mais recentemente bebidas energéticas, contendo extratos de guaraná (*Paulina cupana*), chá e erva mate (*Ilex paraguariensis*) como fontes naturais de cafeína (Gurley et al., 2015). Em países com o costume de consumir café e chá,

estima-se que em média um adulto consuma 200 mg diário de cafeína (Rang et al., 1997). No Brasil, Sousa e Costa (2015) estimaram o consumo médio de café pela população em 163 $\pm$ 2,8 mL por dia, com base nos dados do módulo de consumo da Pesquisa de Orçamentos Familiares (POF) de 2008-9. Esse volume de café equivaleria à ingestão de 238 mg de cafeína/dia, nível que pode ser maior devido a outras fontes de cafeína comumente presentes na dieta, incluíndo chá, chocolate e refrigerante.

Quando consumida em doses moderadas (em torno de 200 mg/dia), a cafeína é uma substância com excelente perfil de segurança (Gurley et al., 2015). Alguns efeitos adversos do consumo da cafeína incluem diurese, desidratação, cefaleia, insônia, irritabilidade, taquicardia e tremores. Em doses elevadas (acima de 2000 mg) a cafeína pode causar efeitos tóxicos como taquicardia, hipertensão severa, arritmias, convulsões e até mesmo a morte. Indivíduos mais sensíveis podem apresentar efeitos adversos em doses inferiores (Schwenk and Costley, 2002; Holmgren et al., 2004; Kerrigan e Lindsey, 2005; Liddle e Connor, 2013; Gurley et al, 2015).

Após a aprovação do DSHEA em 1994, surgiram vários SA como uma nova fonte de cafeína. Dentre os mais populares estavam as formulações multi-ingredientes contendo extratos de plantas do gênero *Ephedra*, diversas fontes naturais de cafeína e outros extratos de plantas, que eram anunciadas como emagrecedores, energéticos e melhoradores do desempenho esportivo (Gurley et al, 2015). Esses SA eram legalmente comercializados apesar do FDA ter proibido em 1982 a venda de combinações sintéticas de efedrina e cafeína em medicamentos devido aos riscos de efeitos adversos. Vários estudos, alguns dos quais resumidos por Greenway (2001), demonstraram que a associação de efedrina e metilxantinas seria eficaz no auxílio na perda de peso, produzindo efeitos mais significativos do que a efedrina ou a cafeína isoladamente, inclusive em estudos de longo prazo (Greenway, 2001).

Em 1995 começaram a surgir relatos de efeitos adversos relacionados ao consumo de SA associando extratos herbais contendo efedrina e cafeína, atingindo o auge em 2003, até que em 2004 o FDA conseguiu retirar do mercado produtos contendo *Ephedra* ou outras fontes de efedrina, por apresentarem um risco inaceitável de efeitos adversos quando consumidos conforme recomendado na embalagem (EUA, 2004; Gurley et al., 2015).

Após o banimento da *Ephedra*, surgiu uma nova geração de SA, anunciados como "livres de *Ephedra*". Esses produtos continham várias fontes naturais de cafeína e outros extratos de plantas contendo substâncias com efeitos variados (tais como sinefrina e ioimbina). A quantidade de cafeína nesses produtos costuma exceder aquela presente em

bebidas e alimentos, e a maioria dos produtos não indica a concentração de cafeína. Esses produtos continuam sendo divulgados como auxiliares na perda de peso, energéticos e melhoradores do desempenho esportivo, com a recomendação de uso durante a prática de atividades físicas. A combinação de exercícios vigorosos com cafeína pode ser particularmente perigosa para alguns usuários, uma vez que a prática de exercícios diminui a excreção urinária da cafeína, e relatos de efeitos adversos relacionados a esses produtos têm sido reportados (Schwenk and Costley, 2002; Gurley et al., 2015).

A dose usualmente empregada de cafeína para melhora no desempenho esportivo é de 6 mg/kg peso corpóreo, equivalendo a 420 mg de cafeína para um adulto de 70 kg. Pesquisas estudando os efeitos da cafeína nesse sentido costumam testar doses variando entre 3 a 15 mg/kg peso corpóreo, equivalendo a 200 – 1000 mg de cafeína para um adulto de 70 kg (Schwenk and Costley, 2002; Liddle e Connor, 2013). A quantidade de cafeína presente nos alimentos varia bastante; enquanto refrigerantes costumam ter entre 50 a 70 mg por porção de 480 mL, o mesmo volume de café expresso teria cerca de 100 mg, bebidas à base de café poderia chegar a 300 mg e bebidas energéticas a 400 mg de cafeína na porção (Liddle e Connor, 2013).

A quantidade de cafeína presente em SA também é bastante variável. No Brasil, a cafeína só pode ser comercializada como SA sob a forma de “suplementos de cafeína para atletas”, e o produto deve indicar em sua embalagem a quantidade de cafeína presente, que deve estar entre 210 e 420 mg por porção, não podendo ser adicionada nenhuma outra substância (Brasil, 2010b). Nos Estados Unidos, entretanto, não é obrigatório listar no rótulo a quantidade de cafeína presente. Um estudo realizado com 56 amostras de SA contendo cafeína comercializadas nos Estados Unidos encontrou concentrações variando entre 0.60 mg e 828.7 mg de cafeína na porção diária recomendada; os autores destacaram que, no caso dos produtos que declaravam a quantidade de cafeína presente, em alguns houve divergência significativa entre a concentração declarada e aquela efetivamente detectada, chegando a 173% da dose indicada (Andrews et al., 2007).

### 3.4. Adulteração de suplementos alimentares

A adulteração de SA pode ser definida como a adição de substâncias não declaradas, a troca intencional de ingredientes ou a identificação errônea de espécies vegetais presentes no produto, feitos pelo próprio fabricante legítimo, visando o aumento do lucro e das vendas (Rocha et al., 2016). A inclusão de substâncias estranhas àquelas declaradas nos rótulos dos produtos pode tanto ser deliberada, com o objetivo de baratear seu custo de produção ou

"garantir" a eficácia do produto, quanto acidental, pela contaminação cruzada durante o processo de fabricação (Geyer et al., 2004; Parra et al., 2011; Rocha et al., 2016). A adulteração de SA representa um sério risco à saúde dos consumidores desses produtos, que podem estar sujeitos a interações medicamentosas e efeitos adversos por não saberem quais substâncias estão ingerindo (Gratz et al., 2004; Rocha et al., 2016).

Suplementos indicados como emagrecedores naturais e contendo sibutramina já foram detectados no mercado, sendo que em ao menos três casos, a sibutramina foi detectada em cápsulas emagrecedoras chinesas "puramente herbais" vendidas na internet (Geyer et al., 2008). Em um estudo realizado nos Estados Unidos, a maioria dos 40 SA indicados para melhorar o desempenho sexual declarava conter plantas com essa atividade, como casca de *Yohimbe, Cnidium monnier, Muira puama* e *Ginkgo biloba*. Em 19 deles (47,5%) foram encontrados inibidores da fosfodiesterase (sildenafil, tadalafil ou homosildenafil), em concentrações suficientes para produzir efeito terapêutico, chegando a 50,9 mg de sildenafil por comprimido (Gratz et al., 2004).

Uma revisão recente publicada por Rocha et al. (2016) sobre adulteração de suplementos herbais com fármacos não declarados mostrou que fármacos anoréticos são os mais frequentemente empregados na adulteração de suplementos emagrecedores, especialmente a sibutramina, além de estimulantes, laxantes, ansiolíticos e antidepressivos. Em suplementos para melhora do desempenho atlético, os EAA foram o grupo de adulterante mais frequente, além de estimulantes e anoréticos. Finalmente, em suplementos para melhora do desempenho sexual, foram detectados diversos casos de adulteração com fármacos inibidores da fosfodiesterase, como sildenafil e tadalafil, e análogos desses fármacos (como sulfosildenafil, dimetilsildenafil, aminotadalafil), muitos dos quais não têm estudos adequados indicando sua possível eficácia ou toxicidade (Rocha et al., 2016).

Uma situação específica ocorre com os chamados suplementos "hormonais", que desde 1996 estão legalmente disponíveis no mercado mundial contendo substâncias chamadas pró-hormônios (PH), que são precursores de alguns EAA (principalmente de testosterona e nandrolona) e são consideradas doping pela WADA (Geyer et al., 2008). Esses produtos costumam conter substâncias não declaradas em seus rótulos (Parra et al., 2011), ou então declarar nos rótulos substâncias como EAA ou PH por meio de eufemismos ou sinônimos não aprovados (Geyer et al., 2008). Por exemplo, um produto declara conter Nor19Dion, ao invés de 19-nor-4-androstene-3,17-diona ou norandrostenediona, que é um precursor do EAA nandrolona.

Baume et al. (2006) analisaram 103 produtos adquiridos na *internet* no final de 2002, que se dividiam em quatro categorias (PH, à base de creatina, à base de aminoácidos de cadeia ramificada ou emagrecedores). Em três produtos classificados como PH foi detectada a presença de metandrostenolona, sendo que em dois deles, o uso conforme orientação do rótulo resultaria em concentrações supraterapêuticas da substância. Em outros 18 produtos foram detectados metabólitos e/ou precursores de EAA, sendo 14 desses produtos da categoria dos PH, 3 emagrecedores e um de creatina, cujo uso continuado poderia ser suficiente para gerar um resultado positivo em exame antidoping.

Um estudo mais abrangente considerando apenas suplementos não hormonais obteve resultados ainda mais significativos (Geyer et al., 2004). Dos 634 produtos adquiridos em lojas de 13 países (Estados Unidos e Europa) e na *internet*, 14,8% continham PH não declarados no rótulo, sempre precursores de testosterona ou nandrolona. Mais de 20% das amostras de fabricantes que produziam suplementos hormonais estavam contaminadas, indicando contaminação cruzada no processo de fabricação do produto e da matéria prima, versus 9,6% de positivos das amostras de não-fabricantes.

Alguns estudos têm mostrado que o controle de qualidade na fabricação de SA é baixo, e desvios entre o que está descrito no rótulo e a real composição do produto são comuns (Gurley et al., 2000; Geyer et al., 2004; Haller et al., 2004; Baume et al., 2006; Geyer et al., 2008). Nos Estados Unidos, a análise de 20 amostras de produtos comerciais declarando conter extrato de *Ephedra* mostrou grandes discrepâncias entre a quantidade de alcaloides declarada (quando declarada) e a quantidade efetivamente presente, variando de 0% a 154% da quantidade declarada. Seis produtos apresentaram variação superior a 20% da quantidade declarada. Adicionalmente, foi detectada grande variabilidade entre dois lotes do mesmo produto, podendo chegar a 100% de diferença (Gurley et al., 2000).

### 3.5 Métodos analíticos empregados na detecção de estimulantes em suplementos alimentares

Não existem muitos trabalhos publicados relatando a quantificação de estimulantes em SA, e os disponíveis focam majoritariamente nos alcaloides de *Ephedra* e *Citrus aurantium* (laranja amarga). A quantidade de amostras costuma ser pequena e o foco principal está no método analítico desenvolvido, e não nos resultados e na sua relevância em termos legais e de saúde pública. Alguns trabalhos publicados estão resumidos na Tabela 4, e mostram a grande variação entre a concentração declarada nas embalagens dos produtos e

aquela efetivamente presente. Alguns produtos que não indicam a quantidade de cada substância, podem contê-las nas mais diversas concentrações, ou ainda não as conter.

**Tabela 4:** Resumo dos estudos publicados que se referem à quantificação de estimulantes em suplementos alimentares.

| Referência | País | Nº amostras | Analito | Resultados obtidos | Técnica |
|---|---|---|---|---|---|
| Gurley et al., 2000 | EUA | 20 | Alcaloides *Ephedra* | 0 – 23,5 mg alcaloides na dose. Dentre os produtos que declaravam a quantidade presente, foi detectado de 17 – 154% da dose declarada | HPLC-DAD |
| Abourashed e Mossa, 2004 | Arábia Saudita | 3 produtos herbais e 6 bebidas energéticas | Cafeína | 33,3 – 198,1 mg cafeína por porção de produto herbal, 26,9 – 79,8 mg cafeína por porção de bebida energética | CCDAE-UV |
| Haller et al., 2004 | EUA | 35 (33 declaravam o teor de alcaloides da *Ephedra* e 31 declaravam o teor de cafeína) | Cafeína | 24 produtos continham <90% do teor declarado. O maior teor foi 103%. | HPLC-DAD |
| | | | Alcaloides *Ephedra* | 11 produtos continham >110% do declarado, 2 continham < 90%. O maior teor foi 146%. | |
| Marchei et al., 2005 | Itália | 14 em cápsula, 2 em comprimido,2 em pó e 2 em líquido (apenas esses indicavam a quantidade de cafeína presente) | Cafeína | Quantidade compatível com o rótulo, no caso dos líquidos.<br>984,8 – 3098,1 μg/g (cápsulas)<br>438,8 e 1114,1 μg/g (comprimidos)<br>48,9 e 79509 μg/g (pós) | LC-MS |
| Marchei et al., 2006 | Itália | 4 (3 cápsulas e 1 líquido) | Sinefrina | 3,1; 7,1 e 60,3 μg/g (cápsulas)<br>480,2 μg/mL (líquido) | CG-MS |
| Marchei et al., 2006b | Itália | 18 (cápsulas) | Alcaloides *Ephedra* | 4,2 – 78,6 μg/g efedrina<br>0,1 – 1,3 μg/g pseudoefedrina | CG-MS |
| Seeram et al., 2006 | EUA | 19 (apenas 7 indicavam a quantidade de cafeína presente) | Cafeína | 0,4 a 17,4% da massa dos comprimidos, equivalente a 43 – 182% da quantidade de cafeína declarada (para aqueles que declaravam) | HPLC-DAD |

| Referência | País | Nº amostras | Analito | Resultados obtidos | Técnica |
|---|---|---|---|---|---|
| | | | Catequinas de chá verde | 12 – 143% da quantidade declarada | |
| Andrews et al., 2007 | EUA | 53 (28 indicavam a quantidade de cafeína presente) | Cafeína | 0,07 – 307 mg/comprimido, totalizando 1 – 829 mg cafeína /dia (se usado conforme uso máximo recomendado em rótulo). 27 continham <200 mg/dia e apenas 4 continham >600 mg/dia.<br>Dentre os 28 que indicavam a quantidade presente, 25 continham de -16 a +16% do indicado. Os outros 3 continham -56%, +24% e + 73%. | HPLC-UV |
| Evans e Siitonen, 2008 | EUA | 20 | Cafeína, alcaloides de Citrus aurantium e da *Ephedra* | 0 – 362 mg/g cafeína; 0 – 12,8 mg/g sinefrina; 4,2 – 25,5 mg/g efedrina. Reporta que as concentrações de cafeína encontradas são maiores que as declaradas | HPLC-DAD |
| Santana et al., 2008 | EUA | 6 | Para- e meta-sinefrina | 5421,1 – 14297,6 mg/kg p-sinefrina (a m-sinefrina não foi detectada) | HPLC-UV; LC-MS/MS |

HPLC = Cromatografia Líquida de Alta Eficiência; DAD = Detector de Arranjo de Diodos; MS = Espectrometria de Massas; CG = Cromatografia Gasosa; LC = Cromatografia Líquida; UV = Detector de Ultravioleta; CCDAE = Cromatografia em Camada Delgada de Alta Eficiência

Evans e Siitonen (2008) destacam que a quantidade de cafeína presente em suplementos emagrecedores costuma ser maior do que aquela declarada, uma vez que os rótulos dos produtos costumam especificar apenas a quantidade de cafeína anidra adicionada, negligenciando a cafeína oriunda dos extratos de plantas presentes nos produtos. Essa disparidade pode ser perigosa, devido às características farmacológicas das substâncias presentes e ao comprometimento da função cardiovascular comum em obesos (que seriam, em tese, a população alvo dos suplementos emagrecedores). Adicionalmente, uma vez que esses produtos também são empregados com objetivo de melhora no desempenho esportivo, é de se esperar que alguns indivíduos os consumam em quantidades superiores à máxima diária recomendada. O consumo de cafeína e outros estimulantes em doses muito superiores à recomendada, somado ao stress cardiovascular representado pelo exercício, representa um risco real à saúde desses consumidores (Evans e Siitonen, 2008).

## OBJETIVOS

***Objetivo geral:*** Avaliar a incidência de falsificações e adulterações em medicamentos contendo substâncias anabolizantes e em suplementos alimentares apreendidos pela Polícia Federal (PF).

***Objetivos Específicos:***

1. Identificar os principais medicamentos contendo anabolizantes apreendidos pela PF e a taxa de falsificações reportadas nos Laudos Periciais emitidos pela PF;
2. Identificar os principais suplementos alimentares apreendidos pela PF e a taxa de adulterações reportadas nos Laudos Periciais emitidos pela PF, comparando essas informações com as oriundas de outros países;
3. Avaliar a legislação sanitária vigente relativa a suplementos alimentares no Brasil, comparando-a com a legislação de outros países;
4. Desenvolver um método para a análise do medicamento Durateston®, empregando técnicas espectroscópicas e quimiométricas;
5. Desenvolver método analítico para determinar a concentração de fármacos anabolizantes em medicamentos e suplementos alimentares e analisar produtos apreendidos pela PF para identificar/confirmar falsificações;
6. Desenvolver método analítico para determinar a concentração de cafeína em amostras de suplementos alimentares e avaliar a conformidade dos resultados com as informações de rótulo.

**ESTRUTURA DA TESE**

Os métodos e resultados desse trabalho serão apresentados em cinco capítulos distintos, todos em formato de artigo. O primeiro capítulo (I. *Incidence of anabolic steroid counterfeiting in Brazil*) se refere a um levantamento de informações de laudos periciais do banco de dados Sistema Criminalística da PF (SisCrim), relacionadas a medicamentos contendo EAAs apreendidos e enviados a perícia na PF. Esse estudo atende ao objetivo 1 desse trabalho, e foi publicado no periódico *Forensic Science International* em 2013 (Neves et al., 2013).

O segundo capítulo (II. *Dietary supplements: International legal framework and adulteration profiles, and characteristics of products on the Brazilian clandestine market*) avalia a legislação nacional relacionada a suplementos alimentares, em comparação com a legislação vigente nos Estados Unidos e na União Europeia, além de reportar dados oriundos da Polícia Federal relacionados a suplementos alimentares e dados referentes à adulteração de suplementos alimentares nos Estados Unidos e na União Europeia. Esse artigo foi publicado no periódico *Regulatory Toxicology and Pharmacology* em 2015 (Neves e Caldas, 2015), e atende aos objetivos 2 e 3 desse trabalho.

O terceiro capítulo (III. *Detection of counterfeit Durateston® ampoules using Fourier Transform Infrared Spectroscopy and Partial Least Squares – Discriminant Analysis*) relata o desenvolvimento de método analítico por espectroscopia no infravermelho e quimiometria para diferenciação entre ampolas originais e falsas do medicamento anabolizante Durateston®, principal medicamento anabolizante falsificado no Brasil, e atende ao objetivo 4 desse trabalho.

O quarto capítulo (IV. *Quantitative analysis of pharmaceutical products containing anabolic androgenic steroids seized by the Brazilian Federal Police*) relata o desenvolvimento e validação de método analítico para análise quantitativa de EAAs em produtos farmacêuticos, bem como a aplicação desse método em produtos apreendidos pela PF e enviados a perícia. Esse estudo atende ao objetivo 5 desse trabalho.

O quinto capítulo (V. *Caffeine contents of dietary supplements seized by the Brazilian Federal Police*) relata a otimização e validação de método analítico para quantificação de cafeína em suplementos alimentares, e a sua aplicação em produtos apreendidos pela PF e enviados a perícia. Esse estudo atende ao objetivo 6 desse trabalho.

**I. Incidence of anabolic steroid counterfeiting in Brazil**

Esse artigo foi publicado no periódico Forensic Science International 228, 2013, e81-e83 (Anexo I).

Esse estudo retrospectivo reporta dados obtidos do Instituto Nacional de Criminalística do Departamento de Polícia Federal do Brasil (DPF) sobre 3676 produtos anabolizantes apreendidos entre 2006 e 2011. Esteroides Anabólicos Androgênicos (EAA) foram declarados nos rótulos de 96,2% dos produtos. Cerca de um terço dos produtos declarava ser do Paraguai, e 14,3% do Brasil. Estanozolol, testosterona e nandrolona foram as substâncias mais declaradas nos rótulos. Análises de embalagem e análises químicas qualitativas realizadas em 2818 produtos constataram que 31,7% dos produtos eram falsos, com um aumento na taxa de detecção de falsificações durante o período. Quase a metade dos produtos falsos não continham a substância declarada, e 28,3% continham apenas substâncias não declaradas. A testosterona e seus ésteres foram responsáveis por 45% dos 582 casos de detecção de substância não declarada. A análise de embalagem sozinha foi responsável pela identificação de 4,6% de todos os produtos falsos. Esses resultados indicam a necessidade de um esforço contínuo do governo no sentido de diminuir a disponibilidade desses produtos no país.

**II. Dietary supplements: International legal framework and adulteration profiles, and characteristics of products on the Brazilian clandestine market.**

Esse artigo foi publicado no periódico Regulatory Toxicology and Pharmacology 73, 2015, 93-104 (Anexo II).

Os objetivos desse trabalho foram avaliar a legislação vigente relacionada a suplementos alimentares nos Estados Unidos (EUA), na União Europeia (UE) e no Brasil, e o perfil dos produtos adulterados e/ou irregulares nesses mercados. Devido a um arcabouço legal menos restritivo, um suplemento livremente disponível nos EUA pode ser considerado um medicamento ou até mesmo ser proscrito na UE ou no Brasil, originando um mercado clandestino baseado no contrabando. De 2007 a 2014, o *Food and Drug Administration* dos EUA reportaram 572 casos de adulteração de suplementos no país, principalmente produtos para desempenho sexual (41,6%). Dados do sistema *Rapid Alert System for Food and Feed*, da UE, mostraram 929 adulterações durante o mesmo período, sendo mais de 40% devido à presença de ingredientes não autorizados ou fármacos não declarados. De 2007 a 2013, o Departamento de Polícia Federal brasileiro apreendeu 5470 suplementos alimentares, 92,2% dos quais com origem declarada norte-americana. Análises químicas qualitativas realizadas em 2898 produtos encontraram 180 adulterações, 41,1% devido à presença de fármacos não declarados, em sua maioria esteroides anabolizantes, anoréticos e produtos para disfunção erétil, todos considerados medicamentos no Brasil. Educar o público quanto aos riscos em potencial que estão correndo ao consumir produtos adulterados ou irregulares é necessário para proteger a saúde dos consumidores.

## III. Detection of counterfeit Durateston® ampoules using Fourier Transform Infrared Spectroscopy, Principal Component Analysis and Partial Least Squares – Discriminant Analysis

Diana Brito da Justa Neves, Márcio Talhavini, Jez Willian Batista Braga, Jorge Jardim Zacca, Eloísa Dutra Caldas.

**Abstract**

Medicines containing anabolic steroids are one of the main targets for counterfeiting worldwide, including Brazil. The aim of this work was to propose a method for discriminating original and counterfeit Durateston® ampoules by Fourier Transform Infrared Spectroscopy (FTIR) followed by chemometric analysis. Ninety-six ampoules of Durateston®, 49 originals and 47 counterfeits, were analyzed by Gas Chromatography with Mass Spectrometry (GC-MS) and by FTIR. Principal Component Analysis was applied to the infrared spectra to detected different clusters, corresponding to original samples and different types of counterfeits. A Partial Least Squares – Discriminant Analysis method was proposed to discriminate original samples from those counterfeits that were indistinguishable from the originals in the infrared analysis. A training subset comprised of one-third of the available spectra, was used to establish a suitable model that correctly discriminated all samples in the test subset, resulting in 0% of false positive or negative results and 100 % of efficiency rate, sensitivity and specificity. In addition to the low cost of the infrared technique, the proposed method is fast, reliable and suitable to replace GC-MS methods used in Durateston® ampoule analyses to detect counterfeiting.

### 1. Introduction

According to the World Health Organization (WHO), spurious/falsely-labeled/falsified/counterfeit medicines are those deliberately and fraudulently mislabeled with respect to their identity and/or source (OMS, 2016a). Counterfeit medicines pose a significant health risk to their consumers, as they can cause treatment failure, toxic reactions, and even death (Dégardin et al., 2014; OMS, 2016b). It is not possible to ascertain the actual incidence of counterfeiting worldwide and the only data available are estimates. These range from less than 1 % of the medicine market in some developed countries with well-established control policies, to 30 % in developing countries in

Africa, Asia and some regions of Latin America (OMS, 2008; Dégardin et al., 2014; OMS, 2016b). Up to 50 % of medicines sold on the internet are counterfeit, mostly from companies with no declared physical address (Fernandez et al., 2008; OMS, 2008; OMS, 2016b).

Counterfeit medicines may include products with the correct active pharmaceutical ingredients (API) but at concentrations different from those stated on the label, with incorrect API, no API, or only a mixture of toxic substances (Dégardin et al., 2014; OMS, 2016b). All therapeutic classes are potential targets for counterfeiting, and the most common worldwide are antibiotics, hormones and steroids, antihistaminics, antimalarials, analgesics, and those in the genito-urinary and central nervous system therapeutic categories (Dégardin et al., 2014; OMS, 2016b). A study conducted with data obtained from forensic reports issued by the Brazilian Federal Police (BFP) from 2007 to 2010 showed that the main seized counterfeit medicines of declared national origin were those for erectile dysfunction (69 %) and anabolic steroids (26 %) (Ames e Souza, 2012). Among the anabolic steroids, the most frequent was Durateston®, a Brazilian medicine currently manufactured by Aspen Pharma that accounted for 8.9 % of all seizures and was the third most frequent counterfeit medicine overall (behind Viagra® and Cialis® only) (Ames e Souza, 2012). A study conducted by our research group (not published) found that 11.9 % of the 25,833 medicines of different origins seized by the BFP from 2006 to 2012 were anabolic steroids, the second most prevalent class after medicines for erectile dysfunction. Approximately 10 % of all medicines were counterfeits, with Durateston® the third most frequent.

Another study showed that 31.7 % of the 3,537 medicines seized by the BFP between 2006 and 2011 that declared to contain anabolic steroids were counterfeits, with 48.6 % not containing any API and 28.3 % containing APIs different from those stated on their labels (Neves et al., 2013). The second most seized medicine of this dataset was Durateston® (N=264), of which 69.7 % were counterfeits (89 had no API and 57 had only some of the four testosterone esters they should have contained) [unpublished results].

Even though anabolic steroids are an important target for medicine counterfeiting, there are not many studies reporting the analysis of suspected medicines of this class. Those available relied mainly on gas or liquid chromatography analysis (Musshoff et al., 1997; Ritsch e Musshoff, 2000; Thevis et al., 2008; Coopman e

Cordonnier, 2012; Pellegrini et al., 2012). Spectroscopic techniques such as Raman, Near Infra-Red, and Fourier Transform Infra-Red (FTIR), together with chemometric tools, are being successfully used to analyze suspected counterfeit medicines (De Peinder et al., 2008; Storme-Paris et al., 2010; Fernandes et al., 2012; Kwok e Taylor, 2012; Sacré et al., 2012; Ortiz et al., 2013; Custers et al., 2015). They have the advantage of being less time-consuming than chromatographic methods, with little or no sample preparation (Kwok e Taylor, 2012; Sacré et al., 2012; Ortiz et al., 2013; Custers et al., 2015). However, to the best of our knowledge, only one work using spectroscopic methods to analyze anabolic steroid medicines has been published so far, describing the investigation of the composition of methandrostenolone-containing tablets by Near Infrared and Raman spectroscopy (Rebiere et al., 2016).

The aim of this work was to develop a simple, fast, low-cost and reliable method for the detection of Durateston® counterfeits using FTIR and Partial Least Squares – Discriminant Analysis (PLS-DA).

## 2. Material and methods

### *2.1. Samples*

A total of 96 ampoules of Durateston® were analyzed at the National Institute of Criminalistics (NIC) of the BPF in Brasilia, Federal District. Seventy-six ampoules were seized between 2009 to 2013 by the BPF in different regions of Brazil, mainly *Foz do Iguaçu* and other cities on the Parana state (border with Paraguay), 29 of which were originals and 47 counterfeits. After being seized, the samples were stored at room temperature, and analyzed within the expiring date reported in the label. In order to account for batch-to-batch variability, 20 additional original ampoules from 8 different batch numbers were purchased at local pharmacies in Brasilia in March 2014, adding up to 49 original ampoules.

### *2.2 Fourier Transform Infra-Red*

An IS10 FTIR Spectrometer (Nicolet Instrument Corp., Madison, USA) equipped with a room temperature DTGS detector and an ATR (attenuated total reflectance) accessory with a one-bounce diamond crystal was used for all experiments. Measurements were made with a single droplet of the ampoule content deposited directly on the ATR crystal, with no pressure applied to the droplet. Each spectrum consisted of

16 co-added scans measured at a resolution of 4 $cm^{-1}$ in the 4000 – 650 $cm^{-1}$ range. Spectra were collected and analyzed with OMNIC software, version 5.2.

After each measurement, the diamond crystal surface was cleaned with ethanol and dried. A spectrum preview was performed before adding a new sample to the diamond surface to ensure complete removal of the previous sample and the ethanol. Each spectrum yielded 6,949 variables (wavelengths).

*2.3 Gas Chromatography coupled with Mass Spectrometry*

All Durateston® ampoules were analyzed by GC-MS as a reference method, using a NIC screening method for forensic analysis of medicines and drugs. Analyses were qualitative and performed using a GC 6890N coupled with a MS 5973 Inert (Agilent Technologies, Palo Alto, CA, USA), with either Rxi-1MS (Restek Corporation, Bellefonte, PA, USA) or HP5-MS columns (Agilent Technologies). Samples were prepared by adding approximately five droplets of the sample to 1.0 mL of chloroform or methanol directly in a vial, which was homogenized and injected in the GC without further preparation. Results were analyzed with Enhanced MSD ChemStation D.02.00.275 (Agilent Technologies) and NIST MS Search 2.0 (distributed by Agilent Technologies).

*2.4 Chemometrics*

All data processing and modeling were performed using the Unscrambler X, v. 10.1 software (Camo software, Oslo).

*2.4.1 Principal Component Analysis (PCA)*

Principal Component Analysis (PCA) may be used as a first stage of chemometric processing, being applied as an exploratory data analysis tool, and for outlier detection (Storme-Paris et al., 2010; Sacré et al., 2012; Ortiz et al., 2013). It reduces the number of variables by making linear orthogonal combinations of the original variables, called the principal components (PCs), which progressively explain the remaining variability in the data (Sacré et al., 2012; Ortiz et al., 2013).

In this study, PCA was performed with all available spectra for exploratory analysis. Preprocessing included baseline offset correction, first derivative smoothing (Savitzky-Golay, fourth order polynomial, 5 smoothing points) and data mean centering. The NIPALS algorithm was used for the principal components computation. A first PCA

was performed using all variables in order to evaluate its loadings plot. Two spectral ranges (4000 to 3687 $cm^{-1}$, and from 2716 to 1810 cm-1) could be excluded due to their insignificant influence (loadings values around zero) to explain the variance. A new PCA was then performed with the remaining variables and a full cross validation.

*2.4.2 Partial Least Squares – Discriminant Analysis (PLS-DA)*

Despite the discriminating tendencies that may be observed in the score plot, PCA cannot be used alone for classification or discrimination problems, for which supervised models such as Partial Least Squares – Discriminant Analysis (PLS-DA) or Soft Independent Modelling of Class Analogies (SIMCA) are more appropriated (Storme-Paris et al., 2010; Rebiere et al.; 2016). PLS-DA is also based on the PCA decomposition, the difference being that the combinations of variables, called PLS factors or latent variables, are defined in such a way that the covariance of the instrumental data with the response variable (predefined classes) are maximized, leading to quantitative methods with low errors and high discrimination power between the different classes in discriminant analysis (De Peinder et al., 2008; Sacré et al., 2012; Ortiz et al., 2013; Rebiere et al.; 2016).

PLS-DA is performed using binary coding, in which a dummy discrete response vector **y** is attributed to the data set, such as 0 for counterfeit samples and 1 for original samples (Storme-Paris et al., 2010; Rajalahti e Kvalheim, 2011; Kwok e Taylor, 2012; Silva et al.; 2014). In the training stage, the method is trained to assign "membership values", one for each class; a test sample is then assigned to a specific class if its *y* value surpasses a specific prediction threshold that may be estimated by establishing confidence limits for each sample classified (Storme-Paris et al., 2010; Rajalahti e Kvalheim, 2011; Ferreira, 2015). Therefore, estimated values in *y* are approximations of 0 or 1, and a good discrimination is obtained when the distributions of the estimated values belonging to classes 1 and 0 are not overlapped (Silva et al., 2014).

In this study, PLS-DA was applied using the same spectral regions and pre-processing methods as with PCA. The number of latent variables for the model was defined using the smallest Root Mean Squared Error of Cross-Validation (RMSECV) determined by full cross-validation (leave-one-out approach) in the training set. The outlier identification was performed based on the Hotelling $T^2$ and residuals Q, both considering the confidence level of 95 % (Ferreira, 2015). The discrimination threshold for each class was defined as 0.5, and a confidence interval of 95% was estimated for

each test sample. Validation was performed by an independent test set comprised of a significant part of all samples available for this study and the figures of merit false positive and negative rates, specificity, sensitivity and efficiency rate estimated according to Botelho et al (2015).

## 3. Results and Discussion

### *3.1. Sample composition*

All ampoules were analyzed by FTIR and CG-MS as previously described. Some examples of counterfeit Durateston® seized by the BFP are shown in Figure 1. The declared Durateston® composition, found in the original products (OR), is testosterone propionate, decanoate, phenpropionate and isocaproate (Figure 2), with benzyl alcohol and peanut oil as excipients. The chemical analysis obtained for the OR products (four esters identified by GC-MS analysis, and the vegetable oils by FTIR) matched this formulation.

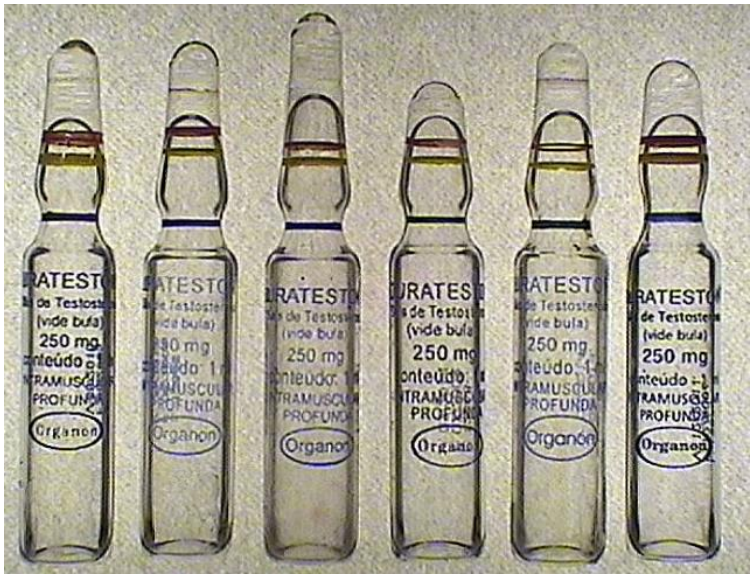



**Figure 1.** Different ampoules of counterfeit Durateston® compared with an original ampoule (far left). Picture taken with transmitted light.

Three different counterfeit types were identified in the samples: those containing only the excipient benzyl benzoate (BB), those containing testosterone propionate and prasterone (TP-PR), and those containing only testosterone propionate (TP). A total of 178 spectra of the ampoules were obtained: 93 from original samples and 85 from counterfeits (Table 1).

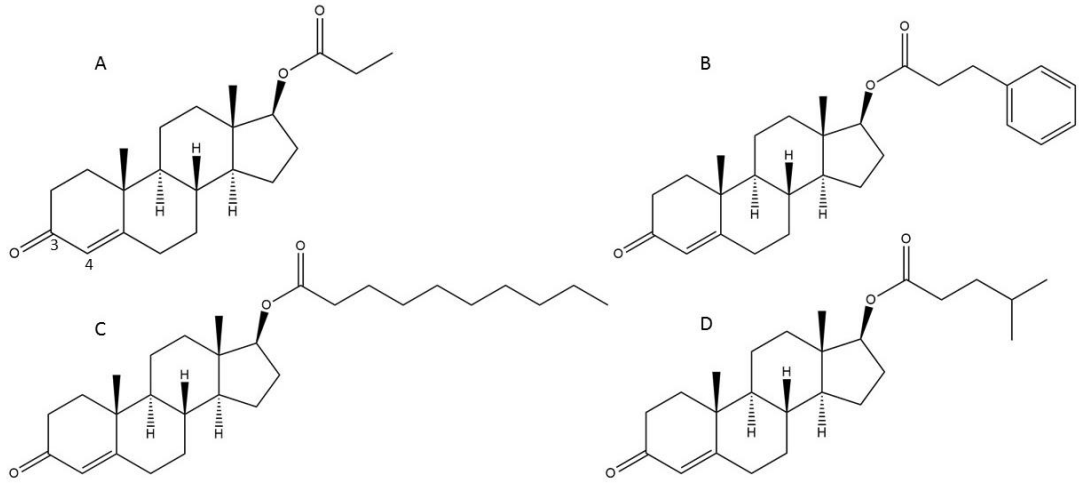



**Figure 2.** Testosterone esters: A: propionate, B: phenpropionate, C: decanoate and D: isocaproate. Numbers on (A) indicate the relevant carbons for FT-IR spectra.

**Table 1:** Number of ampoules and spectra and summary of the analysis results of the 96 Durateston® ampoules analyzed.

| Category | Number of ampoules and spectra | GC-MS and FTIR results |
|---|---|---|
| Original | 49 ampoules (27 with one spectrum each; 22 with three spectra each), total 93 spectra | Contain the testosterone esters propionate, decanoate, phenpropionate and isocaproate, main excipient is a vegetal oil |
| BB | 28 ampoules (16 with one spectrum each; 12 with three spectra each), total 52 spectra | Contain only benzyl benzoate, no API |
| TP-PR | One ampoule with 3 spectra | Contain testosterone propionate and prasterone, main excipient is propylene glycol |
| TP | 18 ampoules (12 with one spectrum each; 6 with three spectra each), total 30 spectra | Contain testosterone propionate and no other API, main excipient is either a vegetal oil, an ester (such as allyl octanoate) or propylene glycol |

OR: original product; TP: Only testosterone propionate; TP-PR: testosterone propionate and prasterone; BB: only the incipient benzyl benzoate; API = active pharmaceutical ingredient

Chemical analysis for the BB category showed a single peak in the CG-MS chromatogram corresponding to benzyl benzoate, and a match for benzyl benzoate in the FTIR. The ampoules classified as TP-PR showed two peaks in the CG-MS, corresponding to testosterone propionate and prasterone (another steroidal hormone), and a match for

propylene glycol in the FTIR. The TP was the most diverse category, with chromatograms of some samples showing one peak corresponding to testosterone propionate, and three or four others that yielded varying matches for different long-chain esters, such as allyl octanoate or allyl decanoate. Three ampoules in this category presented chromatograms that only exhibited the testosterone propionate peak. FTIR results of ampoules from the TP category were also dissimilar: samples not presenting the ester peaks had a match for propylene glycol, whereas the others had good matches for vegetable oils and for esters (allyl octanoate or methyl decanoate, whose spectra are very similar). Representative chromatograms of all categories are shown on Appendix A (Figures 1 to 4).

Representative FTIR spectra of the four categories are shown in Figure 3. Spectra shown in Figure 3A (OR ampoules) and 3B (TP ampoules) are similar, given that the vegetable oils present in OR ampoules and in some TP, and the long chain esters present in TP have similar spectra. One difference between these spectra is the presence of a narrow band of 1677 $cm^{-1}$, indicated by the arrow in Figure 3A, which is characteristic of 4-en, 3-one steroids, such as testosterone (Figure 2) (Kasal et al., 2010). This band, however, is not significant enough to be discriminated by the FTIR library search, which gave similar results for the spectra shown in Figs. 3A and 3B. The large, narrow band of around 1740 $cm^{-1}$, present in both the OR and TP spectra, is common to esters, and suffers mild dislocations according to the substituent group (Coates, 2006; Pretsch et al., 2009). The benzyl benzoate spectrum in Figure 3C stands out in relation to the others, mainly due to a large band of around 720 - 680 $cm^{-1}$ that refers to aromatic C-H bonds (Coates, 2006; Pretsch et al., 2009). The propylene glycol spectrum displayed in Figure 3D is the most distinguishable of the group, mainly due to the large –OH band at around 3300 $cm^{-1}$ (Pretsch et al., 2009).

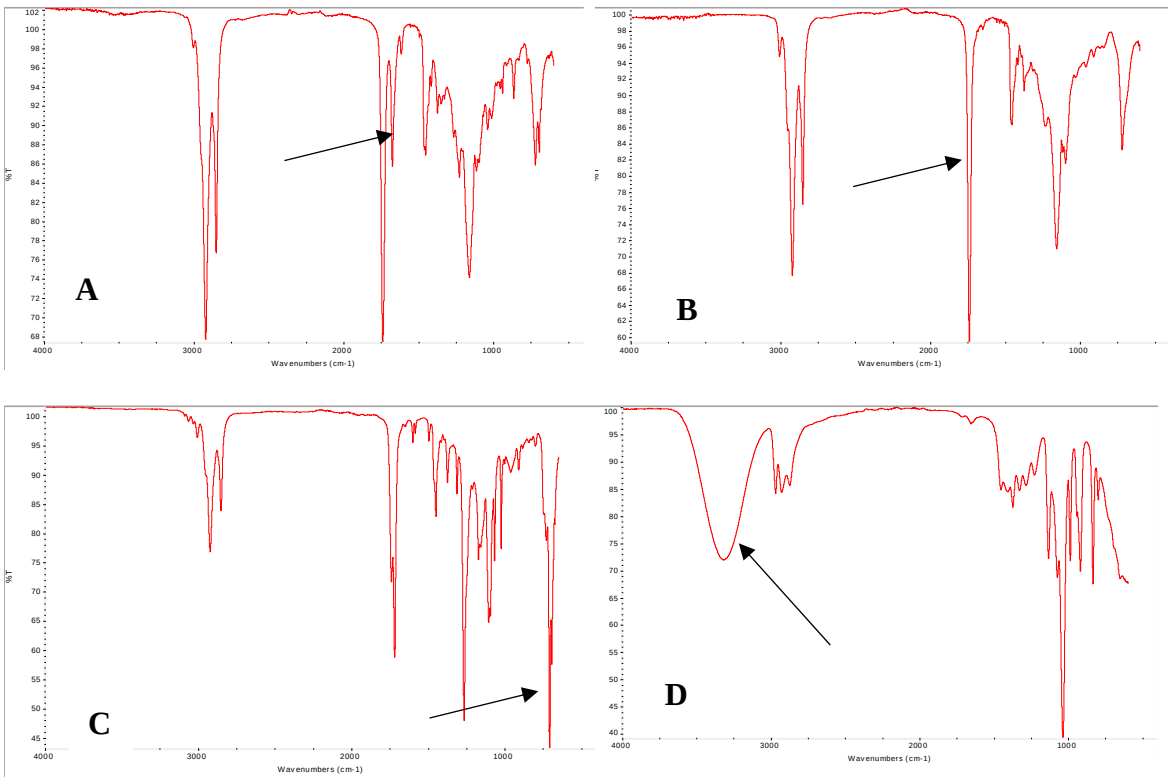



**Figure 3.** Representative FTIR spectra of seized Durateston® ampoules. A = OR (good matches for several vegetal oils); B = TP (good matches for vegetal oils and esters); C = BB (good match for benzyl benzoate and furfuryl benzoate); D = TP-PR (match for propylene glycol). The arrow on A indicates the band characteristic of 4-en, 3-one steroids; arrow on B indicates the band characteristic of esters; arrow on C indicates the band characteristic of C-H aromatic bonds and the arrow on D the band characteristic of –OH groups.

*3.2 PCA*

The PCA results for all the 178 spectra, after the exclusion of the two spectral regions (4000 – 3687 $cm^{-1}$ and from 2716 – 1810 $cm^{-1}$; Section 2.4.1) are shown by the score plots of PC1 x PC2 (Figure 4A) and PC1 x PC2 x PC3 (Figure 4B). No samples with simultaneous high leverage and high residual variance (outliers) were detected. The first two PCs describe 92% of the data variation, and allow a clear distinction to be made of three groups, based on their excipients. Group 1 is comprised only of BB ampoules, group 2 of the TP-PR ampoules (whose excipient was propylene glycol) and TP ampoules which also contain propylene glycol, and group 3 containing the OR and the remaining TP ampoules (Figure 4A). By using 3 PCs (Figure 4B), it was possible to explain 97% of the total variance, and to fully separate the OR from the TP in group 3, although each was split into two subgroups. It was not possible, however, to separate TP-PR from those TP ampoules that contained propylene glycol in group 2 (Table 1). Loadings plots for the

three PCs are displayed on Appendix A. These plots highlight that PC1 is responsible for separating BB ampoules from the rest (high loadings values at the 700 $cm^{-1}$ region, corresponding to the strong band of aromatic C-H bonds as shown in Figure 3C) (Appendix A, Figure 5). PC2 is responsible for separating samples containing propylene glycol from those with vegetable oils; its loading plots show several relevant regions around 2900 $cm^{-1}$, 1700 $cm^{-1}$ and a larger area from 800 – 1160 $cm^{-1}$ (Appendix A, Figure 6). Loadings plots for PC3 (Appendix A, Figure 7) have a significant region from 1690 - 1750 $cm^{-1}$, corresponding to the carbonyl groups from esters and to 4-en, 3-one steroids, illustrating that the third PC is responsible for differentiation based on API contents.

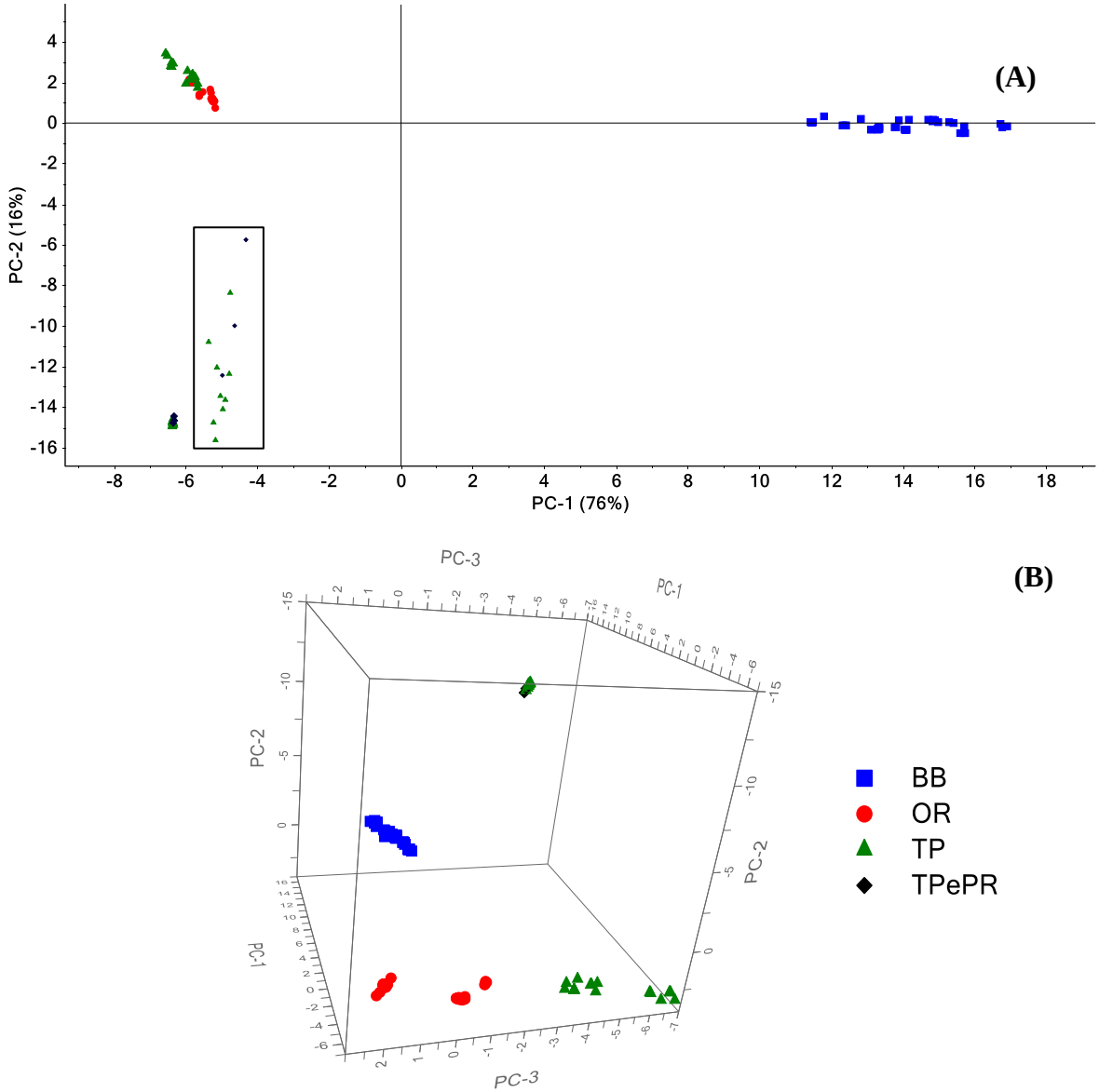



**Figure 4.** 2D score plot (A) and 3D score plot (B) of the 178 FTIR spectra. BB = benzyl benzoate; OR = original, TP = testosterone propionate, TPePR = testosterone propionate and prasterone. The inserted square on A shows the magnification of the relevant region.

Further analyses of the subgroups formed by the OR and TP categories (Figure 4B) showed that OR ampoules were subgrouped based on their "age". One of the subgroups (PC3 values around 2) was comprised of the new ampoules, purchased in 2014, and the other (PC3 values around -1 to 0) of ampoules seized by the BFP in 2009-2010. The difference in the spectral profile may be due to some variation in the formulation or in the manufacturing process, or even due to some small changes on the FTIR performance, since analysis were conducted within a five-year period. However, no clear explanation could be found for the two subgroups in the TP group. They might reflect two different illegal manufacturers, poor manufacturing practices inherent to counterfeit products, or differences in the API concentration. Since no quantitative analyses were performed, these hypotheses could not be verified.

As can be seen in the score plots (Figure 4), ampoules with benzyl benzoate (BB) or propylene glycol (TP-PR, some of the TP) are easily distinguishable from the ones containing vegetable oils or long chain esters as excipients. This classification could also be achieved by submitting the spectra to a search in the FTIR library, illustrating one great advantage of FTIR over Near Infra-Red (NIR), for which spectral libraries are not always available. As these counterfeits can be detected by FTIR, they were not included in the modeled classes for the multivariate data analysis and the PLS-DA was trained only with the OR ampoules and those in the TP group that clustered with them in the 2D score plot (Figure 4A). The other samples were used as a second test set to evaluate the efficiency of the PLS-DA method with unmodeled counterfeits.

*3.3 PLS-DA*

PCA is an exploratory tool to detect tendencies in a group of samples, showing which characteristics can be used for class separation (Custers et al., 2015), and it was possible to visualize all subgroups in this study using this tool (Figure 4B). However, since forensic results demand a high level of statistical certainty to be issued, and to avoid the visual/subjective interpretation of the PCA score plot, PLS-DA was used to develop a method that could be implemented as a routine analysis and yield results for which confidence limits could be estimated.

A PLS-DA model was constructed based on three latent variables (LV) to discriminate the OR ampoules (93 spectra; Table 1) from seized TP ampoules with vegetable oils or long-chain esters (21 spectra). Two-thirds of the spectra (62 OR and 14 TP) were used as a training subset and one-third (31 OR and 7 TP) as a test subset.

Training and test subsets were selected randomly, making sure that both included ampoules from the two OR and TP subgroups (Figure 4B). If replicates from a single ampoule were available, all replicates were assigned to the same subset. As the model obtained was successful in discriminating all spectra of the test subset, another model was built using half the spectra as a training set, which was also successful in discriminating the other half of the spectra. Finally, a third model was built using one-third of the spectra as a training subset and two-thirds of the spectra as a test subset. This third model was also developed with three LV, explaining 99.6% of the total variance, and it was also successful in discriminating all spectra in the test subset.

The analysis of the Hotelling $T^2$ and residuals Q statistics showed that no outliers were present on the training or test subsets, since no samples presented simultaneously values of Hotelling $T^2$ and residuals Q above the defined limits (Fig. 5A). On the other hand, all samples of the second test subset (unmodelled counterfeit samples from the BB and TP_PR groups, as well as those from the TP groups whose main excipient was propylene glycol), were considered outliers (Hotteling $T^2$ and Residuals Q far above the threshold, Fig. 5B). This result show that the Hotelling $T^2$ and residuals Q can identify samples belonging to classes not included in the training set and prevent discrimination errors for unmodeled classes. Recently, Rodionova et al. (2016) argued that PLS-DA or other discrimination models cannot be used for certification models due the risk of wrongly identify a sample belonging to an unmodeled class to the target/original class. However, the analysis of the Figure 5B shows that the Hotelling $T^2$ and residuals Q can prevent this kind of error.

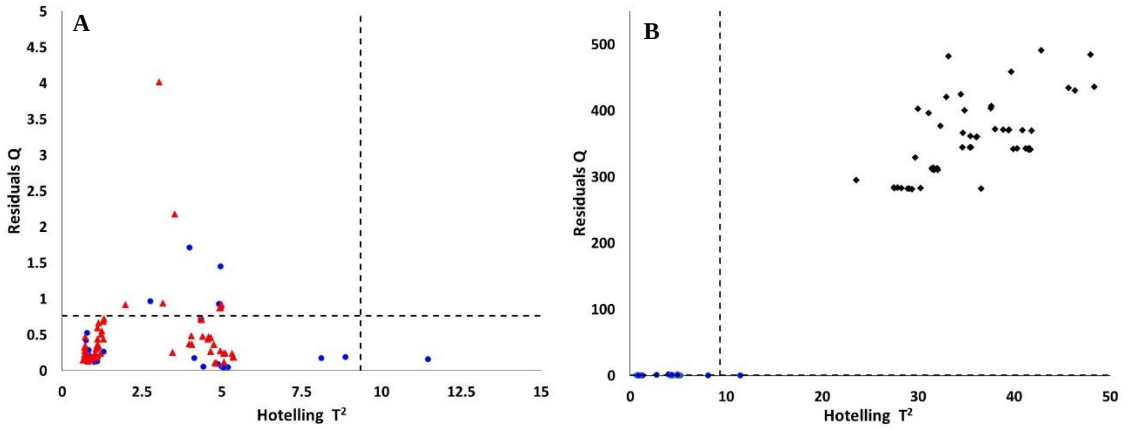



**Figure 5**: Dispersion plot of Hotelling $T^2$ versus residuals Q statistics for (A) the training subset (●) and test subset (▲) and (B) training subset (●) and unmodelled counterfeits

test subset (♦). Dashed lines indicate limit values for Hotelling $T^2$ and residuals Q with 95% confidence for 3 PLS factors.

The final model showed sensitivity and specificity equal to 100%, false positive and false negative rates equal to zero and efficiency rate equal to 100%, as illustrated in Figs. 6 and 7. Regression coefficients of the PLS-DA model (Appendix A, Figure 8) showed similar behavior when compared to the loadings plot for PC3 (Appendix A, Figure 7), indicating that the most relevant regions for discrimination were between 1770 and 1600 $cm^{-1}$, corresponding to the carbonyl groups of the esters and to the conjugated ketone of testosterone.

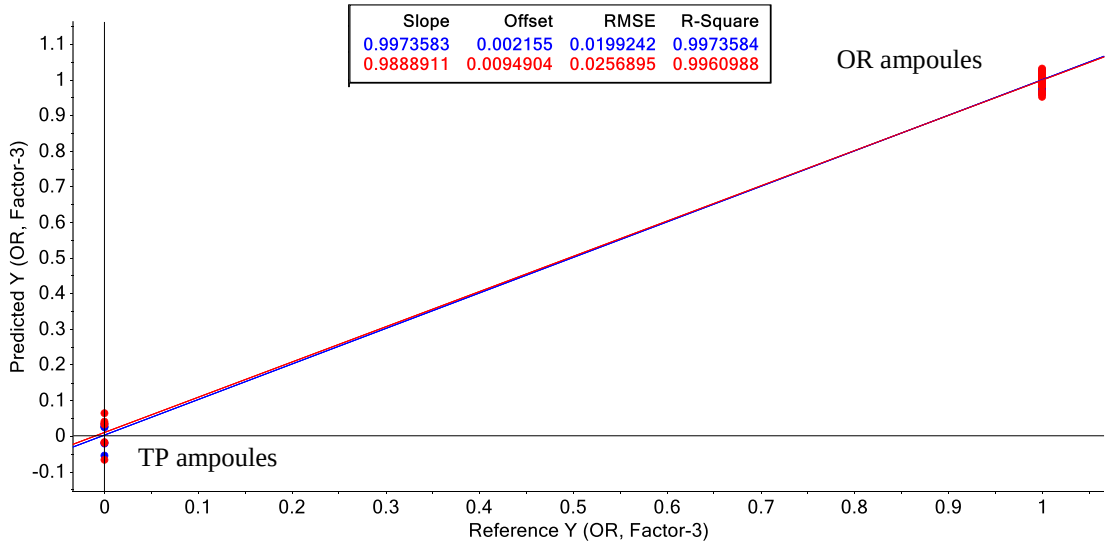



**Figure 6.** Class prediction plot of the PLS-DA model built to discriminate original (OR) and counterfeit (TP) ampoules. Estimated values for the training and test samples are shown in blue and red, respectively. TP ampoules have values between -0.07 and 0.06, whereas OR ampoules have values between 0.95 and 1.03.

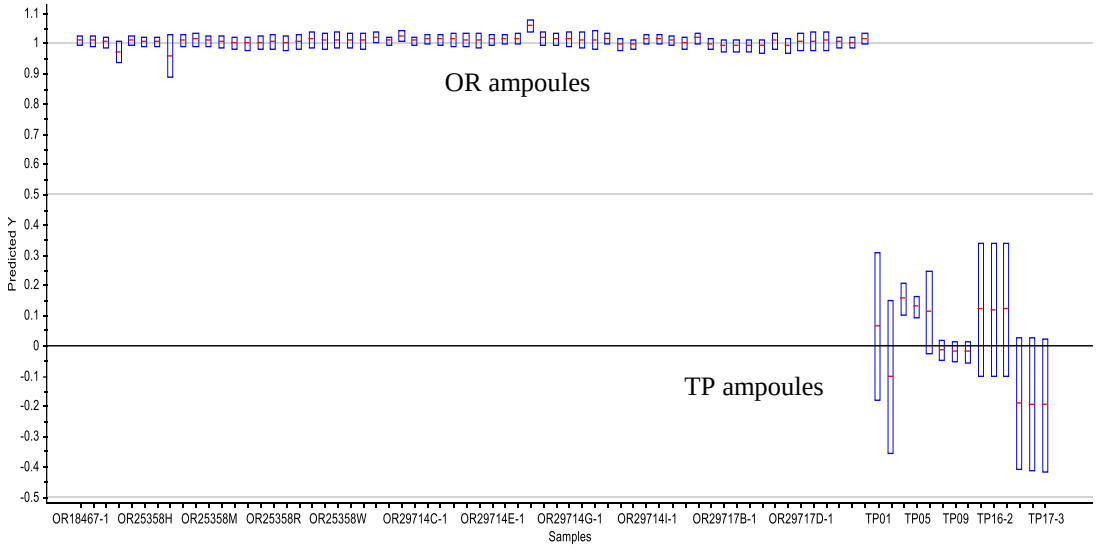



**Figure 7.** Class values of the test subset for original (OR) and counterfeit (TP) ampoules. The discrimination threshold was defined as 0.5.

Values of RMSECV obtained for training and test sets were low and similar (respectively, 0.020 and 0.026; Figure 6), indicating no overfitting of the model and a high discrimination capability. The variation of the estimated values for classes 0 (TP) and 1 (OR) in the training samples were -0.07 to 0.06, and 0.95 to 1.03, respectively (Figure 6), which indicate a high discrimination power between the classes. It is important to note that in PLS-DA, the discrimination threshold is more commonly determined by a Bayesian approach, which considers the distributions of the estimated class values of the training samples (Ferreira, 2015). For this particular application, as the variations for each class were extremely close, the Bayesian approach would result in a value very close to 0.5, therefore this value was adopted and the application of the Bayesian approach was not necessary.

Class values and confidence limits obtained for the test set were lower than the defined threshold (Figure 7). Values obtained for OR test samples ranged from 0.95 to 1.06 (maximum error 0.071) whereas values for TP test samples ranged from -0.20 to 0.15 (maximum error 0.218). There were no misclassifications and no unclassified samples (100% efficiency rate), confirming the applicability of the method in differentiating original from counterfeit samples of Durateston®.

A 100 % efficiency rate was also achieved by Fernandes et al. (2012) for discriminating original and counterfeit glibenclamide tablets by NIR and fluorescence spectroscopy along with SIMCA, PLS-DA and Unfolded PLS-DA. Sacré et al. (2012)

achieved similar result by combining FTIR and NIR or FTIR and Raman spectroscopy associated with PLS analysis for Viagra-like and Cialis-like samples, respectively. For the same products, Custers et al. (2015) achieved a 90.5% performance at discriminating genuine and counterfeit samples using FTIR and SIMCA.

## 4. Conclusion

Anabolic steroids are a frequent target for medicine counterfeiting, and Durateston® is the most frequent steroid medicine counterfeited in Brazil. Crude counterfeits, when the main excipient is drastically altered, could be detected by a simple FTIR analysis and a library search. However, FTIR followed by PLS-DA has proven to be a suitable tool for discriminating original samples from more elaborate counterfeits. The proposed PLS-DA method successfully classified all samples of the test subset, with a 100 % efficiency rate. It is a robust, cheaper and far less time-consuming alternative approach to the routine GC-MS analysis of suspect Durateston® samples, and can be easily implemented in all forensic laboratories from the BPF to standardize and improve Durateston® analysis.

## IV. Quantitative analysis of pharmaceutical products containing anabolic androgenic steroids seized by the Brazilian Federal Police

Diana Brito da Justa Neves, Eloísa Dutra Caldas

**Abstract**

The use of counterfeit or substandard medicines can have an important health impact as they can result in therapeutic failure, be toxic or even cause death. Anabolic steroids are a frequent target for counterfeiters and, in Brazil, they are the second most frequent counterfeited class, only behind medicines for erectile dysfunction. The aims of this work were to optimize and validate a GC-MS method for the quantitative analysis of suspected pharmaceutical products declaring the presence of anabolic steroids, and to analyze products sent to Brazilian Federal Police (BFP) for forensic analysis. Sample preparation included extraction with methanol in ultrasonic bath followed by centrifugation. Method development was conducted using the central composite design approach, with a total run time of 27.83 minutes. The method was successfully validated and 345 samples of pharmaceutical products were analyzed (328 medicines, in tablet, aqueous suspension and oily solution forms, and 17 dietary supplements). About 42% of the medicines were counterfeits and 11% were substandard. Five dietary supplements contained undeclared anabolic steroids, two of them at levels equivalent to those found in medicines. The proposed method is suitable for implementation in routine analysis for identification of counterfeits and substandard products.

### 1. Introduction

Substandard, spurious, falsely labelled, falsified and counterfeit (SSFFC) medical products are a serious concern worldwide, reaching any therapeutic class (WHO, 2016). SSFFC medicines can have no effect at all, result in therapeutic failure, be toxic or even cause death (Deisingh, 2005; WHO, 2016a). Substandard products, also known as out-of-specification products, are genuine medicines produced by authorized manufacturers that do not meet the quality specifications. Counterfeit (spurious, falsely labelled and falsified products, SFFC) are those deliberately and fraudulently mislabeled with respect to identity and/or source, both branded and generic products, with the wrong ingredients, without active ingredients, with insufficient quantities of correct ingredient(s) or with

fake packaging (WHO, 2016b). SFFC products are not easily identified, since they are designed to appear identical to genuine products.

Pharmaceutical products under suspicion of being SSFFC are routinely sent for forensic analysis at the Brazilian Federal Police (BFP). The National Health Surveillance Agency (ANVISA), in conjunction actions with the BFP, seized 115 thousand units of SSFFC products in the period of 2007 to 2011, mainly those for erectile dysfunction and anabolic androgenic steroids (AAS) (Hurtado and Lasmar, 2014). Data obtained from forensic reports issued by the BFP from 2007 to 2010 showed that 69% of 610 cases of seized counterfeit medicines were for erectile dysfunction and 26% declared the presence of AAS (Ames and Souza, 2012).

A total of 3537 AAS-declaring medicines, of national and foreign origin, were analyzed by the BFP in the period of 2006 to 2011, from which 1167 were considered counterfeit (Neves et al., 2013). Almost half of these products (48.6%) had no active ingredient, 28.3% had different ingredients from those stated on the label and 16.1% declared an inexistent manufacturer. Since not all products were chemically analyzed, and analysis were only qualitative, this counterfeit rate may be severely underestimated. Pellegrini et al. (2012) found that AAS concentration in counterfeit medicines could account for only 0.5% of the nominal value concentration. Therefore, a suitable method for the analysis of these medicines should be able to analyze several AAS simultaneously in a wide concentration range.

There are several proposed methods for the analysis of AAS in pharmaceutical products, some specific for SSFFC products. Most of these methods, however, are only qualitative or include several sample preparation steps, not being applicable for routine analysis in forensic laboratories (Musshoff et al., 1997; Ritsch and Musshoff, 2000; Thevis et al., 2008, Coopman and Cordonnier, 2012; Pellegrini et al., 2012). The aims of the present work were to develop and validate a Gas Chromatography – Mass Spectrometry (GC-MS) method suitable to quantify AAS in pharmaceutical products, with a simple sample preparation procedure, and apply this method to analyze pharmaceutical products seized by the BFP.

## 2. Material and methods

### *2.1. Reagents and standards*

HPLC-grade methanol was purchased from Merck (Darmstadt, Germany) and Tedia (Fairfield, OH, USA). Cellulose, lactose and starch, used as a blank tablet matrix, were purchased respectively from Merck (Darmstadt, Germany), Sigma-Aldrich (St. Louis, MO, USA) and J. T. Baker (Phillipsburg, NJ, USA). Manitol, also used as a blank tablet matrix, was a chemically characterized material sent for forensic analysis by the BFP. Water used as blank matrix for aqueous suspensions was produced by a Milli-Q Direct-Q system (Millipore, Bedford, MA, USA).

Reference standards of prasterone (PR; 99.9% purity), testosterone (T; 97.8% purity), methandrostenolone (ME; 96.7% purity), testosterone propionate (TP; 99.9% purity), stanozolol (ES; 98.4% purity), testosterone isocaproate (TI; 99.8% purity) and nandrolone decanoate (ND; 96.0% purity) were purchased from LGC Standards (Luckenwalde, Germany). Oxandrolone (OXA; 98.0% purity), boldenone undecylenate (BU; 98.0% purity) and nandrolone phenylpropionate (NF; 96.0% purity) standards were purchased from Toronto Research Chemicals (Toronto, Canada). Testosterone enanthate (TE; 99.0% purity, determined by Nuclear Magnetic Resonance in the context of this study) was purchased from the European Directorate for the Quality of Medicines & HealthCare (Strasbourg, France) and testosterone cypionate (TC; 100.0% purity) from the United States Pharmacopoeia.

Drostanolone propionate (PD) and testosterone phenylpropionate (TF), used as internal standards (IS), were prepared from bulk materials seized by the BFP that had their identity confirmed by Mass spectrometry and Infra-Red spectrometry. Spectrometric information did not indicate the presence of any substance that could interfere with the analysis.

*2.2 Standard solution preparation*

Methanol stock solutions at 1000 µg/mL of each AAS were used to prepare the methanol AAS working solutions at 50 µg/mL, with exception to ES (100 µg/mL), which showed a less intense signal in the GC-MS. Stock solutions at 1000 µg/mL of PD and TF in methanol were used to prepare the IS intermediate solutions at 200 µg/mL, granting a 9.52 µg/mL final concentration. PD was used as an internal standard for PR, T, ME, OXA, TP and ES, and TF as internal standard for TI, TE, TC, ND, NF and BU. Quantitation of each analyte was performed by determining the ratio between the AAS peak area and the respective IS peak area.

*2.3. Samples*

A total of 328 medicine and 17 dietary supplement samples seized by the BFP and for which qualitative analysis indicated the presence of any investigated AAS were retrieved for this study. The qualitative analysis was conducted during forensic exams of samples and was based on GC-MS; compound identification was performed by comparison of the mass spectra obtained with the National Institute of Standards and Technology (NIST) electronic library. Samples were seized from 2011 to 2016, with declared expiry date of at least 2012. The medicine samples included 87 tablets, 83 aqueous suspensions and 158 oily solutions. All dietary supplements were in tablet/capsule form. Samples had 17 different countries of declared origin, mainly Paraguay (N=154), Brazil (N=30), United States (N=24), Argentina (N=22), Australia (N=19) and Spain (N=13).

*2.4. Sample extraction method*

Sample preparation varied according to the pharmaceutical form, declared concentration and previous screening analysis. For tablets and capsules, their mean weight was determined and five tablets or the contents of five capsules were ground together and homogenized. In cases when there were fewer than five units available, all units where ground together and homogenized. An amount of $1/10^{th}$ the mean weight of the tablet/capsule was weighted in a falcon tube and 5 mL of methanol was added. Falcon tubes were vortexed for 10 seconds, sonicated for 10 minutes, centrifuged for 5 minutes in 3000 rpm, and the extracts diluted with methanol according to their nominal concentration and previous results, ranging from 20 µL:980 µL to 250 µL:750 µL.

Aqueous suspensions were homogenized, an aliquot of 50 or 100 µL transferred to a falcon tube and diluted to 5000 µL with methanol. Tubes were vortexed for 10 seconds, and if full dissolution was not obtained, sonicated for another 10 minutes. The extract was centrifuged for 5 minutes in 3000 rpm, and 50 µL diluted in methanol to a final volume of 1 mL.

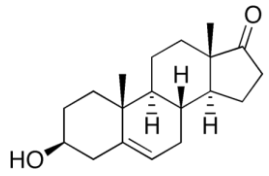
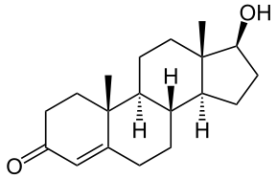
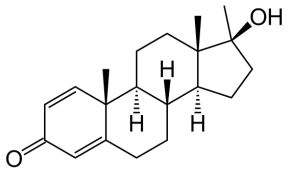
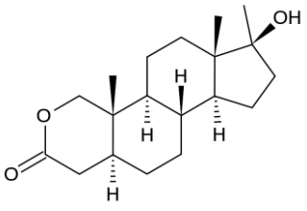
Oil forms were homogenized, 20 to 100 µL transferred to falcon tubes and diluted with methanol to a final volume of 5 mL. Tubes were vortexed for 10 seconds, sonicated for 10 minutes if needed, and centrifuged for 5 minutes in 3000 rpm. 20 to 100 µL of the extracts were diluted in methanol to a final volume of 1 mL.

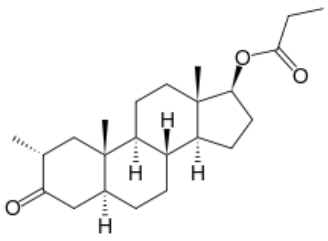
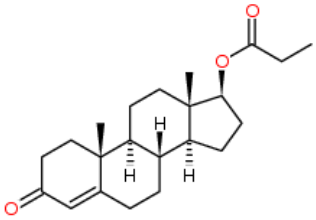
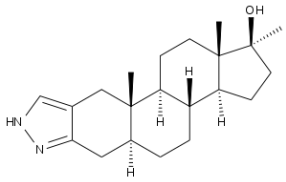
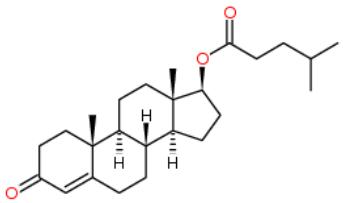
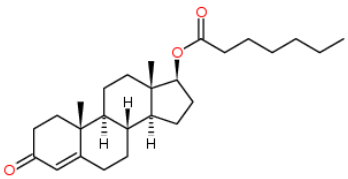
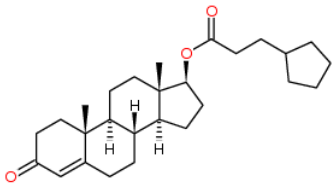
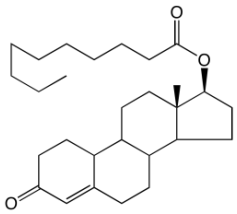
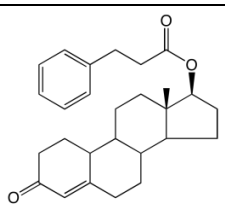
Aliquots of 50 µL of the IS working solutions were added to all sample and calibration solutions prior to GC-MS analysis.

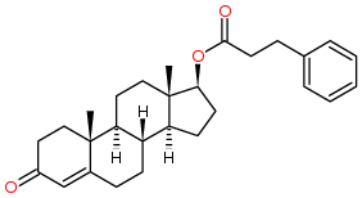
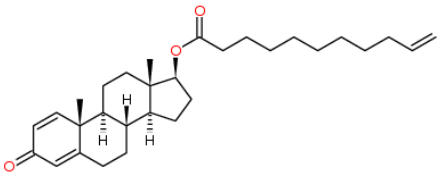
*2.5 Equipment*

GC-MS analysis was performed on a GC System 7890A, coupled with a 5975C Mass Spectrometer (operating at 70 eV) and an automated sample injector system CTC PAL G 6509-B (Agilent Technologies, Santa Clara, California, USA). Chromatography was performed on a HP5-MS capillary column (Agilent Technologies; 25 m x 0.20 mm i.d. x 0.33 µm film thickness). Temperatures of the MS ion source and GC-MS interface were 230 and 280 ºC, respectively. MS detector was used in Selected Ion Monitoring (SIM) mode. Two ions were monitored for each AAS and IS, based on their relative abundance and on their absence in neighbor peaks, in case they were too close. Table 1 shows the chemical structure, the molar mass and the monitored ions. Total response was the sum of both ion signals.

**Table 1**: Chemical structure of the anabolic androgenic steroids and internal standards with the respective monitored ions

| **Compound** | **Chemical structure** | **Molecular mass** | **Ions (m/z)** |
|---|---|---|---|
| Prasterone (PR) |  | 288.4 | 91; 105 |
| Testosterone (T) |  | 288.4 | 79; 124 |
| Methandrostenolone (ME) |  | 300.4 | 91; 122 |
| Oxandrolone (OXA) |  | 306.4 | 71; 291 |

| Compound | Chemical structure | Molecular mass | Ions (m/z) |
|---|---|---|---|
| Drostanolone Propionate (PD) |  | 358.5 | 149; 286 |
| Testosterone Propionate (TP) |  | 344.5 | 57; 124 |
| Stanozolol (ES) |  | 328.5 | 96; 328 |
| Testosterone Isocaproate (TI) |  | 386.6 | 81; 124 |
| Testosterone Enanthate (TE) |  | 400.6 | 113; 124 |
| Testosterone Cypionate (TC) |  | 412.6 | 124; 147 |
| Nandrolone Decanoate (ND) |  | 428.6 | 110; 155 |
| Nandrolone Phenylpropionate (NF) |  | 406.6 | 91; 257 |

| Compound | Chemical structure | Molecular mass | Ions (m/z) |
|---|---|---|---|
| Testosterone Phenylpropionate (TF) |  | 420.6 | 91; 271 |
| Boldenone Undecylenate (BU) |  | 452.7 | 122; 147 |

*2.6 GC-MS parameters optimization*

The GC-MS parameters were optimized using original medicines analyzed by the BFP. They were Duratestoland® (nominal concentration TP 30 mg/mL, TD 100 mg/mL, TI 60 mg/mL and TF 60 mg/mL), Testoland Depot® (TC 100 mg/mL), Decalant Depot ® (ND 200 mg/mL), Testenat Depot® (TE 250 mg/mL), Testosterone® (T 100 mg/mL), DHEA® (PR 25 mg/capsule), Metandrostenolona® (ME 10 mg/tablet), Oxandroland® (OXA 5 mg/tablet), Stanozoland Depot® (ES 50 mg/mL) and Boldenona® (BU 50 mg/mL).

The parameters were optimized using central composite design, where each parameter is tested at five levels (Neto et al., 2001). Inter-peak resolution, peak area and width and elution time were defined as response variables. Essays were conducted in duplicate with six replicates in the central point to estimate the variance of data, in random order. To evaluate the parameters related to the injector (temperature, Tinj; volume, Vinj; and split ratio), the oven was set at preliminary conditions (initial temperature 50ºC, hold for 2 minutes, temperature rate of 30ºC/min until 300ºC, hold for 15 minutes) and the mass spectrometer set to full scan mode. The three parameters were tested at five levels: Tinj from 260 to 300ºC, Vinj from 0.5 to 1.5 and split from 0 to 1:15. Response variables evaluated for each parameter combination were the smallest resolution between two peaks, the smallest and the mean peak areas, the largest and the mean peak width.

After determining the best injection conditions, the oven parameters were evaluated at five levels, namely initial temperature (Tin, from 50 to 80 ºC), temperature ramp (Ramp, from 10 to 30ºC/min) and final temperature (Tf, from 290 to 320 ºC). Initial and final hold times were set in 2 and 15 minutes, respectively. Since ES was the AAS

with the widest peak width, response variables investigated were ES-TI resolution (ES peak has a tail that could co-elute with TI), smallest area, mean area, largest peak width (except ES), mean peak width, ES peak width and ES area.

*2.7. Method validation*

Method validation was performed following ANVISA guidelines for medicines (Brasil, 2003) and MAPA guidelines for veterinary drugs (Brasil, 2011b). A mixture of lactose, cellulose, starch and mannitol in equal proportions was used as a tablet blank for all validation studies, and purified water as a suspension blank. A counterfeit medicine sample in oily form, for which previous forensic analysis showed to contain no AAS, was used as oily solution blank.

*Linearity* of the calibration curves was first evaluated for all AAS in methanol at concentrations ranging from 1.0 to 100 µg/mL (n=3 at each value; ES concentrations two times higher). As relative standard deviation (RSD) at the lowest level was higher than 30%, and peaks at 100 µg/mL were fronting (a sign of saturation), a calibration curve at five levels (2.5, 5.0, 10.0, 25.0 and 50.0 µg/mL, two times higher for ES) was built, preparing three sets of solutions and injecting each one two times (total of six replicates per level). Data were evaluated for a possible linear or quadratic relationship, and the quality of the regressions assessed considering the correlation coefficient, Cochran and F tests to detect heteroscedasticity, analysis of variance (ANOVA) to evaluate lack-of-fit; sum of relative errors; graphic evaluation of the randomness of the residuals; and residual standard deviation (measures their dispersion throughout the regression curve and evaluates their absolute value).

*Selectivity* was assessed by analyzing blank matrices and investigating any response at the AAS or IS retention times for possible interferences.

*Matrix effects* were evaluated by analyzing *in matrix* (higher matrix concentration predicted; 2.5, 10 and 50 µg/mL, in triplicates) and methanol solutions against a methanol calibration curve (Brasil, 2011b). The *in matrix* solutions were prepared by mixing 1.0 mL of the blank matrix with the appropriate amount of standard solution; vials were dried using a vacuum sample concentrator (Vacuum Concentrator Systems/LABCONCO) and ressuspended with 1.0 mL methanol prior to analysis. The same procedure was conducted for samples prepared in methanol.

*Precision and recovery* studies were conducted together. Aliquots of blank matrices were fortified at three levels (2.5 or 2.56, 10 and 50 µg/mL; four replicates for each level; doubled concentration for ES) with appropriate volumes of standard solutions prepared at 100 µg/mL (or 200 µg/mL for ES). A fourth level at 4.96 µg/mL was included for tablets and oily solutions. Falcon tubes were homogenized in a vortex, left uncapped under exhaustion for 2 hours, and any residual solvent dried using a vacuum sample concentrator. Fortified matrixes were diluted with methanol (1.25 mL for tablets and oily solutions, 1.0 mL for aqueous suspensions), homogenized for 10 seconds in a vortex, sonicated for 10 minutes and centrifuged for 5 minutes. Extracts were quantified using freshly prepared calibration curves prepared in methanol. Repeatability was accessed by the $RSD_r$ of the four intraday replicates and intermediate precision by the $RSD_p$ of eight replicates from two days. According to Brasil (2011b), $RSD_p$ should not be higher than 20% and $RSD_r$ not higher than 13.3% (2/3 of the 20 % intermediate precision). In this work, acceptable recovery range was from 80 to 120% and recovery was calculated as the mean result of the four intraday replicates. The method limit of quantification (LOQ) for each analyte was defined as the smallest concentration with acceptable repeatability, intermediate precision and recovery.

*Robustness* of the instrument parameters was evaluated by comparing the outcome of the methanol standard solutions (at 2.5, 10 and 50 µg/mL) after altering different parameters (injector temperature, gas flow, injector temperature, injection volume, initial temperature, temperature program), with a total of 14 altered final settings. Different ultrasound times during sample preparation were also tested (0, 5 or 10 minutes). Samples were quantitated and results from the altered methods were compared with the result obtained from the quantification with the validated method. The effect of the method change for each AAS was calculated as follows:

$$Effect = 100 * \frac{(Result\ in\ altered\ method - Result\ in\ optimized\ method)}{Result\ in\ optimized\ method} \quad \text{(Eq. 1)}$$

*Stability* of the standard solutions at 2.5 and 10 µg/mL in methanol and *in matrix* under different conditions was tested (n=3). Each solution was split in two: one kept at the GC tray and the other at the refrigerator. Both solutions were analyzed on the day they were prepared, then after three, seven, ten and fourteen days, every time with a freshly

prepared calibration curve (all vials septa were changed after each analysis to prevent solvent evaporation). Calibration curves used were stored in the freezer and were also analyzed on the subsequent days of analysis.

## 3. Results

### *3.1. GC-MS parameters*

Results obtained during the optimization of the GC-MS parameters indicated that only the smallest and the mean peak areas were affected by variations of the injector parameters. All resolution values were above the threshold of 1 (smallest value was 1.94), so this variable was not considered further. Tinj was not a significant parameter for variations related to peak area, which was largely due to Vinj and split ratio variations, as expected. To determine the optimal values for these two parameters, Tinj was set at the central value (280ºC) and a new experiment was conducted, with a full factorial planning of two parameters in three levels (Vinj = 0.8; 1.0 and 1.2 µL; split = 0; 1:3; 1:7), in triplicate and random order. Results indicated that increasing the Vinj (as long as the saturation volume of the liner, 1.3 µL, was not reached) and decreasing the split ratio led to larger peaks without compromising other variables. Therefore, the injection conditions were set at Vinj = 1.2 µL and splitless mode (split = 0).

None of the response variables (resolution, area and peak width) were affected by the oven initial temperature and ramps, but lower Tf increased the resolution, peak areas and peak widths. Since peak widths were not too large in the smallest Tf tested, this parameter was set at 290 ºC. Other parameters were set on their central level (Tin = 65ºC; Ramp = 20ºC/min). With the injector and oven conditions defined, punctual optimizations were performed aiming at decreasing the total run time to less than 30 minutes without compromising ES-TI resolution. An increase in Tin showed good results, as well as the inclusion of a second temperature ramp and a subsequent increase in Tf. After simple factorial experiments, the best helium flow was set at 2.5 mL/min.

Finally, the final optimized GC-MS conditions of the method were: Tinj= 280ºC, Vinj= 1.0 µL, splitless, helium flow of 2.5 mL/min. The oven temperature program was: Tin= 200ºC, 30ºC/min until 250ºC, hold for 16 minutes, 30ºC/min until 300ºC, hold for 8.5 minutes, with a total run time of 27.83 minutes. A chromatogram with all AAS included on the study and both internal standards is shown in Figure 1.

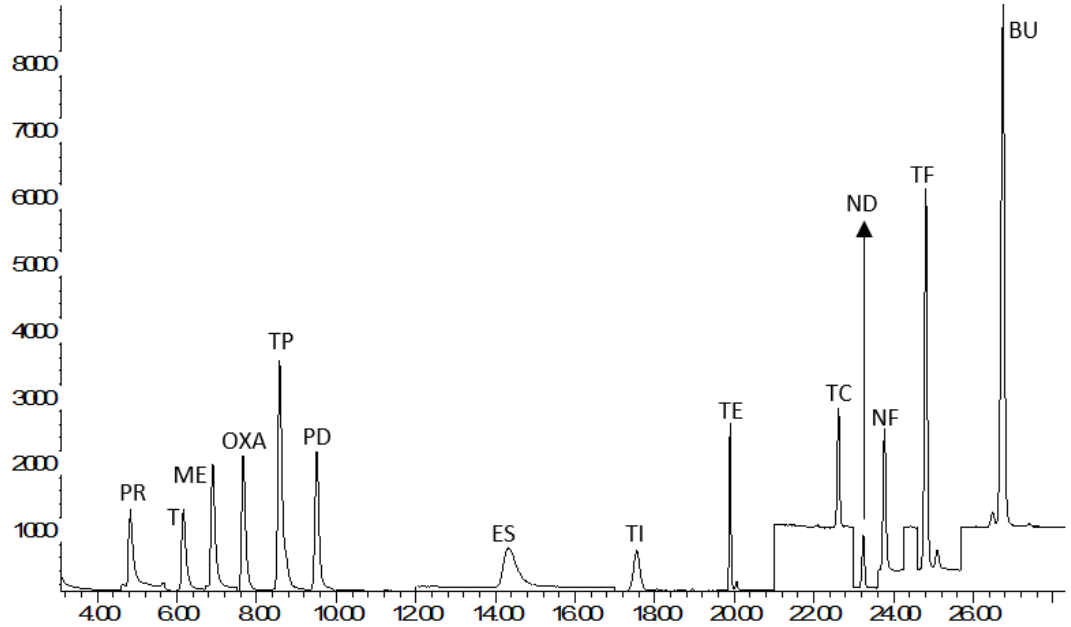



**Figure 1:** Cromatogram with all AAS and internal standards. PR = Prasterone; T = Testosterone, ME = Methandrostenolone; OXA = Oxandrolona, PD = Drostanolone Propionate (Internal Standard), TP = Testosterone Propionate, ES = Stanozolol, TI = Testosterone Isocaproate; TE = Testosterone Enanthate, TC = Testosterone Cypionate, ND = Nandrolone Decanoate, NF = Nandrolone Phenylpropionate, TF = Testosterone Phenylpropionate (Internal Standard), BU = Boldenone Undecylenate.

*3.2. Method Validation*

Residual plots from linear regressions were not random, showing a curved shape that indicates that a quadratic model was more adequate to describe the AAS calibration curves. Quadratic models also showed a lower lack-of-fit, lower dispersion of residuals and lower sum of percentual relative values. However, data were heteroscedastic, as shown by Cochran and F tests and by the shape of the residual plot. To address this issue, data transformation and weighted least squares regression were tested. Data transformation can normalize and linearize data, and stabilize variances; weighted regressions counterbalance the effect of heteroscedasticity by giving more importance to data points with low variance (Almeida et al., 2002; McDonald, 2014; Nakov et al., 2014).

A total of 36 regressions were proposed for each AAS, comprising all possible combinations between the three datasets for each curve (not transformed, square root and log10 transformation), two regression models (linear and quadratic) and six weighting factors (1; 1/variance; 1/X; $1/X^2$; 1/Y; $1/Y^2$). The best results were obtained for quadratic regressions, non-weighted (weighting factor = 1) and with data transformation. PR, T,

ME, OXA and TI had better results with square root transformation whereas TP, ES, TE, TC, ND, NF and BU with log10 transformation. However, log10 yielded smaller errors and was adopted for all AAS.

As an example, the results obtained for the 36 regressions for stanozolol (ES) are shown in Table 2. The final values of some of the parameters evaluated for all AAS, considering non-weighted quadratic regressions with log10 transformations are shown in Table 3.

**Table 2:** Values of the regression evaluation parameters obtained for the 36 stanozolol regressions.

| Data | Regression | Weighting factor | R | rsd | Lack of Fit (F value)* | Sum ER% (N=30) |
|---|---|---|---|---|---|---|
| Untransformed (heteroscedastic) | Linear | 1 | 0.9738 | 0.2223 | 21.542 | 372.65 |
| | | 1/Variance | 0.9804 | 0.5604 | 181.54 | 242.58 |
| | | 1/X | 0.9954 | 0.2718 | 36.324 | 260.39 |
| | | $1/X^2$ | 0.9827 | 0.5265 | 159.25 | 237.01 |
| | | 1/Y | 0.9938 | 0.3153 | 51.785 | 244.80 |
| | | $1/Y^2$ | 0.9590 | 0.8093 | 387.70 | 272.12 |
| | Quadratic | 1 | 0.9937 | 0.5474 | 19.642 | 570.95 |
| | | 1/Variance | 0.9856 | 0.8275 | 60.956 | 283.23 |
| | | 1/X | 0.9928 | 0.5855 | 24.276 | 302.99 |
| | | $1/X^2$ | 0.9902 | 0.6830 | 37.539 | 237.70 |
| | | 1/Y | 0.9904 | 0.6760 | 36.523 | 248.33 |
| | | $1/Y^2$ | 0.9880 | 0.7534 | 48.392 | 265.40 |
| Square root (heteroscedastic) | Linear | 1 | 0.9748 | 0.2309 | 182.49 | 263.93 |
| | | 1/Variance | 0.9338 | 0.3743 | 493.13 | 309.28 |
| | | 1/X | 0.9713 | 0.2466 | 209.28 | 236.77 |
| | | $1/X^2$ | 0.9585 | 0.2964 | 306.06 | 259.90 |
| | | 1/Y | 0.9636 | 0.2774 | 267.21 | 253.15 |
| | | $1/Y^2$ | 0.9044 | 0.4498 | 716.02 | 338.02 |
| | Quadratic | 1 | 0.9920 | 0.1303 | 78.675 | 135.37 |
| | | 1/Variance | 0.9894 | 0.1497 | 107.79 | 137.36 |
| | | 1/X | 0.9917 | 0.1323 | 81.541 | 123.58 |
| | | $1/X^2$ | 0.9913 | 0.1359 | 86.710 | 122.88 |
| | | 1/Y | 0.9911 | 0.1371 | 88.479 | 126.12 |
| | | $1/Y^2$ | 0.9904 | 0.1425 | 96.459 | 130.40 |
| Logarithm (homocesdastic) | Linear | 1 | 0.9876 | 0.0836 | 75.039 | 101.84 |
| | | 1/Variance | 0.9852 | 0.0914 | 91.392 | 130.59 |
| | | 1/X | 0.9873 | 0.0847 | 77.243 | 102.46 |
| | | $1/X^2$ | 0.9864 | 0.0875 | 82.992 | 106.79 |
| | | 1/Y | 0.9474 | 0.1722 | 345.68 | 273.14 |
| | | $1/Y^2$ | 0.9331 | 0.1943 | 442.08 | 200.40 |
| | Quadratic | 1 | 0.9981 | 0.0327 | 6.616 | 73.03 |
| | | 1/Variance | 0.9980 | 0.0337 | 7.799 | 76.39 |
| | | 1/X | 0.9981 | 0.0329 | 6.917 | 71.75 |
| | | $1/X^2$ | 0.9980 | 0.0334 | 7.441 | 71.52 |
| | | 1/Y | 0.9977 | 0.0358 | 10.404 | 70.93 |
| | | $1/Y^2$ | 0.9975 | 0.0372 | 12.291 | 73.61 |

R = Correlation coefficient; rsd = residual standard deviation; ER% = relative error; *tabulated Fvalue = 3.385

**Table 3:** Parameters for the non-weighted quadratic regressions (with log10 transformations) used for the anabolic androgenic steroids (AAS)

| AAS | Fvalue* (variances) | R | rsd | Lack of Fit (Fvalue)** | Sum ER% (N = 30) | Max ER% |
|---|---|---|---|---|---|---|
| PR | 0.008 | 0.9969 | 0.0330 | 0.819 | 88.62 | 15.5 |
| T | 0.06 | 0.9985 | 0.0244 | 0.716 | 54.92 | 14.1 |
| ME | 0.08 | 0.9980 | 0.0287 | 0.755 | 61.12 | 11.5 |
| OXA | 0.13 | 0.9984 | 0.0227 | 7.357 | 56.63 | 13.0 |
| TP | 0.102 | 0.9990 | 0.0205 | 1.979 | 46.61 | 9.8 |
| ES | 0.17 | 0.9981 | 0.0327 | 6.616 | 73.03 | 11.4 |
| TI | 0.096 | 0.9984 | 0.0232 | 1.610 | 54.09 | 17.9 |
| TE | 0.45 | 0.9991 | 0.0172 | 0.586 | 45.02 | 7.3 |
| TC | 0.78 | 0.9988 | 0.0204 | 6.451 | 42.08 | 8.4 |
| ND | 0.35 | 0.9986 | 0.0244 | 8.945 | 56.03 | 7.9 |
| NF | 1.3 | 0.9991 | 0.0194 | 7.286 | 38.95 | 5.6 |
| BU | 0.51 | 0.9992 | 0.0173 | 6.435 | 41.18 | 7.9 |

PR = Prasterone; T = Testosterone, ME = Methandrostenolone; OXA = Oxandrolone, TP = Testosterone Propionate, ES = Stanozolol, TI = Testosterone Isocaproate; TE = Testosterone Enanthate, TC = Testosterone Cypionate, ND = Nandrolone Decanoate, NF = Nandrolone Phenylpropionate, BU = Boldenone Undecylenate; R = Correlation coefficient; rsd = residual standard deviation; ER% = relative error; *tabulated Fvalue = 5.05; **tabulated Fvalue = 3.385

The method showed to be selective as no peaks were found near the AAS eluting times for tablet and suspension blanks. For the oily solutions some peaks near the retention times of T, ME and NF were detected, however only NF is found in oily preparations. Later it was demonstrated that this blank peak did not interfere with the NF signal. All results obtained *in matrix* were within ±10% of those obtained in solvent, and this effect was not considered significant.

Table 4 shows the data for recovery, repeatability, intermediate precision and LOQ. Threshold values were 13.3% for repeatability, 20% for intermediate precision (Brasil, 2011b) and 80-120% for recovery.

**Table 4:** Validation parameters for the analysis of anabolic androgenic steroids (AAS) in different formulations by GC-MS

| AAS | Conc. (µg/mL) | Recovery (%) (N=4) | Repeatability ($RSD_r$, %) (N=4) | Intermediate precision, $RSD_p$ %), (N=8) | LOQ** |
|---|---|---|---|---|---|
| ***Tablets*** | | | | | |
| PR | 2.56 | 103.0 | 10.5 | 11.6 | 0.5 mg/tablet |
| | 4.96 | 100.0 | 4.4 | 11.3 | |
| | 10 | 96.7 | 2.1 | 2.6 | |
| | 50 | 103.6 | 1.8 | 2.6 | |
| T | 2.56 | 131.5 | 21.9 | 27.4 | 2 mg/tablet |
| | 4.96 | 150.1 | 42.0 | 31.2 | |
| | 10 | 99.1 | 2.57 | 17.5* | |
| | 50 | 103.6 | 3.3 | 4.4 | |
| ME | 2.56 | 110.0 | 16.6 | 19.8 | 0.5 mg/tablet |
| | 4.96 | 96.7 | 11.0 | 12.8 | |
| | 10 | 95.3 | 1.6 | 6.1 | |
| | 50 | 107.3 | 2.5 | 4.3 | |
| OXA | 2.56 | 100.2 | 10.5 | 13.3 | 0.5 mg/tablet |
| | 4.96 | 82.3 | 8.5 | 20.3 | |
| | 10 | 91.6 | 2.0 | 2.8 | |
| | 50 | 102.6 | 3.2 | 3.5 | |
| TP | 2.56 | 108.4 | 3.0 | 13.4 | 0.5 mg/tablet |
| | 4.96 | 101.0 | 7.7 | 9.4 | |
| | 10 | 88.0 | 3.7 | 9.3 | |
| | 50 | 97.8 | 3.5 | 3.5 | |
| ES | 5.12 | 103.5 | 7.6 | 12.7 | 1 mg/tablet |
| | 9.96 | 91.0 | 6.0 | 13.6 | |
| | 20 | 94.9 | 1.4 | 5.3 | |
| | 100 | 102.5 | 3.1 | 3.9 | |
| ***Suspensions*** | | | | | |
| T | 2.56 | 105.2 | 5.7 | 17.2 | 2.56 mg/mL suspension |
| | 10 | 108.8 | 3.5 | 9.8 | |
| | 50 | 108.3 | 1.6 | 5.1 | |
| ES | 5.12 | 95.5 | 5.6 | 5.3 | 5.12 mg/mL suspension |
| | 20 | 104.9 | 3.1 | 2.4 | |
| | 100 | 96.6 | 0.9 | 4.1 | |
| ***Oil solutions*** | | | | | |
| TP | 2.56 | 122.0 | 4.2 | 9.4 | 2.48 mg/mL solution |
| | 4.96 | 111.1 | 4.7 | 9.6 | |
| | 10 | 105.8 | 3.0 | 6.0 | |
| | 50 | 92.3 | 2.4 | 5.9 | |
| TI | 2.56 | 97.5 | 2.2 | 10.1 | 1.28 mg/mL solution |
| | 4.96 | 91.8 | 7.1 | 8.6 | |
| | 10 | 94.0 | 2.7 | 6.0 | |
| | 50 | 87.1 | 4.2 | 5.9 | |
| TE | 2.56 | 109.8 | 5.6 | 15.6 | 1.28 mg/mL solution |
| | 4.96 | 97.9 | 6.3 | 11.4 | |
| | 10 | 96.8 | 4.5 | 7.8 | |
| | 50 | 87.1 | 5.3 | 5.7 | |
| TC | 2.56 | 93.7 | 6.3 | 11.9 | |

| AAS | Conc. (µg/mL) | Recovery (%) (N=4) | Repeatability ($RSD_r$, %) (N=4) | Intermediate precision, $RSD_p$ %), (N=8) | LOQ** |
|---|---|---|---|---|---|
| | 4.96 | 90.3 | 4.9 | 9.8 | 1.28 mg/mL solution |
| | 10 | 90.6 | 4.3 | 5.9 | |
| | 50 | 85.6 | 3.4 | 6.0 | |
| ND | 2.56 | 104.9 | 6.3 | 14.1 | 1.28 mg/mL solution |
| | 4.96 | 100.7 | 6.6 | 5.7 | |
| | 10 | 100.7 | 4.5 | 8.5 | |
| | 50 | 90.8 | 2.2 | 9.6 | |
| NF | 2.56 | 124.6 | 13.01 | 22.4 | 2.48 mg/mL solution |
| | 4.96 | 117.3 | 10.0 | 10.6 | |
| | 10 | 118.2 | 7.9 | 10.7 | |
| | 50 | 97.8 | 0.8 | 5.4 | |
| BU | 2.56 | 95.7 | 3.0 | 8.8 | 1.28 mg/mL solution |
| | 4.96 | 92.3 | 4.1 | 6.7 | |
| | 10 | 93.2 | 3.4 | 5.1 | |
| | 50 | 87.5 | 2.5 | 6.0 | |

* N=7; ** in the sample; PR = Prasterone; T = Testosterone, ME = Methandrostenolone; OXA = Oxandrolone, TP = Testosterone Propionate, ES = Stanozolol, TI = Testosterone Isocaproate; TE = Testosterone Enanthate, TC = Testosterone Cypionate, ND = Nandrolone Decanoate, NF = Nandrolone Phenylpropionate, BU = Boldenone Undecylenate;

The experiments showed that the method is robust regarding the instrumental conditions for most parameters, including the injector temperature (2.5-2.8% mean effect), helium flow (1.5% effect) and the initial oven temperature (2.1%). However, lowering or raising the final oven temperature had significant effects (42.4 and 48.6%, respectively) for some compounds or some compounds were not detected. To monitor for any change in retention time on different days, a calibration solution containing all AAS and IS to be analyzed was injected in the GC-MS prior to analysis to adjust for any change in retention time for each monitored ion. The use of sonication (10 min) during sample extraction provided a slight gain compared with no sonication (around 5%), so this step was kept in the sample preparation.

*3.3. Stability of the analytes under different conditions*

The results of the stability study are summarized in Figures 2 to 6, and can be seen in more detail on Appendix B. Overall, after 7 days all solutions tested still showed satisfactory results (between 90 – 110% of the initial value), both at room temperature (~22$^o$C) and refrigerated (~4$^o$C). After 10 days many were still adequate, but after 14 days most had at least one AAS with significantly altered levels (< 70%), with a large variation among the analytes (highlighted by the large error bars at 14 days, Figures 2-6). Solutions

kept refrigerated were less prone to degradation than those kept at the GC tray at room temperature, and more concentrated solutions were more stable than the diluted ones. These differences were more visible at 14 days (Figures 3 and 4). The less stable AAS was ES, which reached as low as 16.4% of its original concentration (suspension matrix; 2.5 µg/mL; room temperature; Appendix B, Figure 9). The stability in more than one matrix was investigated for T, ES and TP (Figures 3-5), with no apparent effect of the matrix (more detailed at Appendix B, Figures 5-16). In general, the stability of some AAS was slightly better in tablet and oil matrix than in methanol; suspension matrix and methanol had similar results, since the suspension matrix was only milli-Q water.

Since stock solutions of the AAS were kept frozen, it was expected that the frozen calibration curves would maintain their nominal concentrations. However, it was observed that at lower levels they were less stable, similarly to samples. After 14 days, the 2.5 µg/mL calibration solutions were no longer suitable (< 90% nominal value) for use, and even after 10 days some AAS at 2.5 µg/mL were already below the 90% threshold.

In conclusion, if analysis of a sample extract was not possible in the same day, samples and calibration solutions should be kept refrigerated and analyzed preferably within 7 days. As no significant losses occurred at room temperature after 3 or 7 days, the duration of each batch of analysis (sometimes up to 48 hours) was not a concern, preferably granting that the vials septa were unbroken.

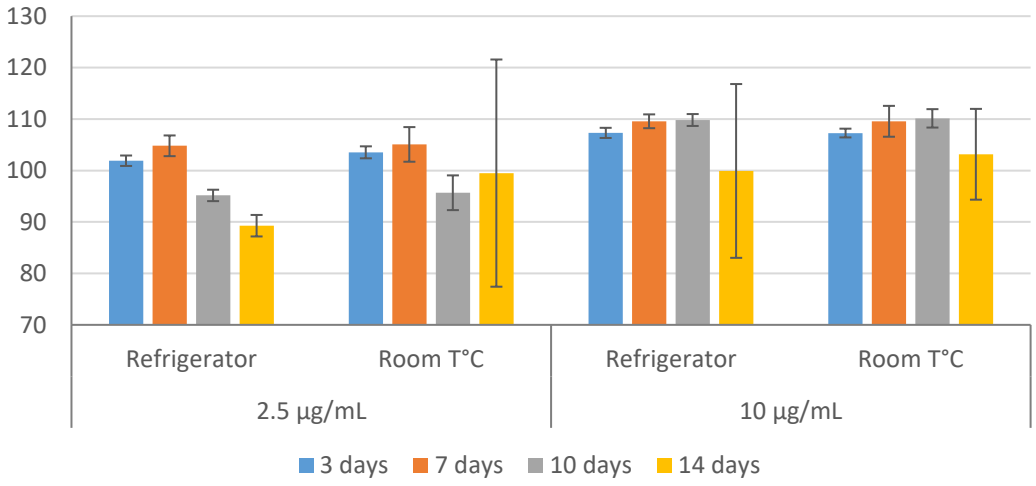



**Figure 2:** Stability of the 12 AAS in methanol kept under refrigeration or at room temperature for 3, 7, 10 and 14 days. N=3 samples for every concentration/storage condition. Results are expressed in percentage of the initial concentration (mean ± standard deviation)

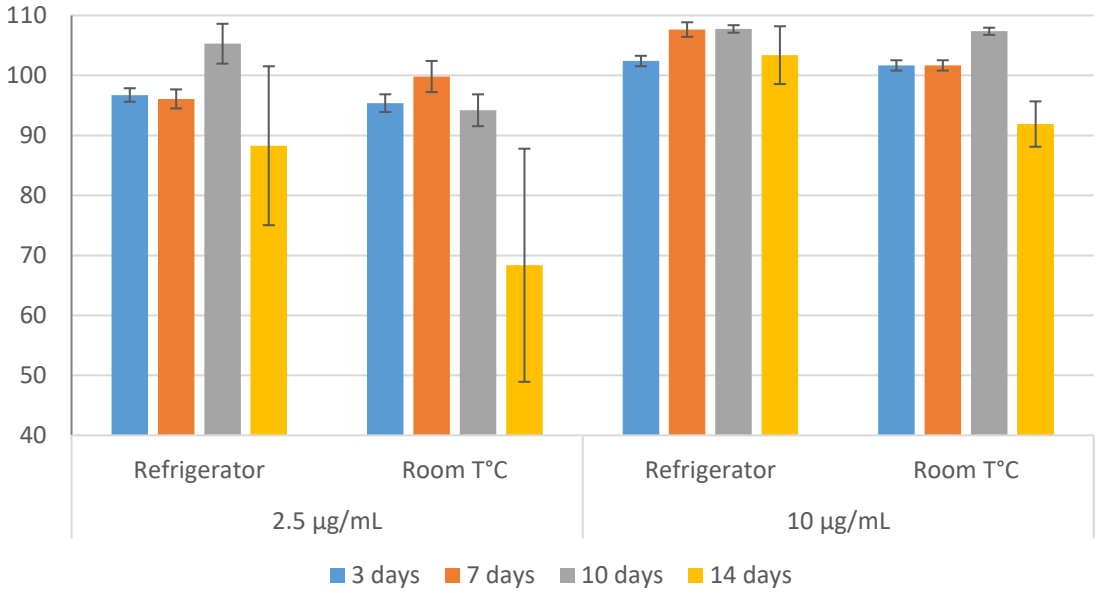



**Figure 3**: Stability of prasterone, testosterone, methandrostenolone, oxandrolone, testosterone propionate and stanozolol in tablet matrix kept under refrigeration or at room temperature for 3, 7, 10 and 14 days. N=3 samples for every concentration/storage condition. Results are expressed in percentage of the initial concentration (mean ± standard deviation)

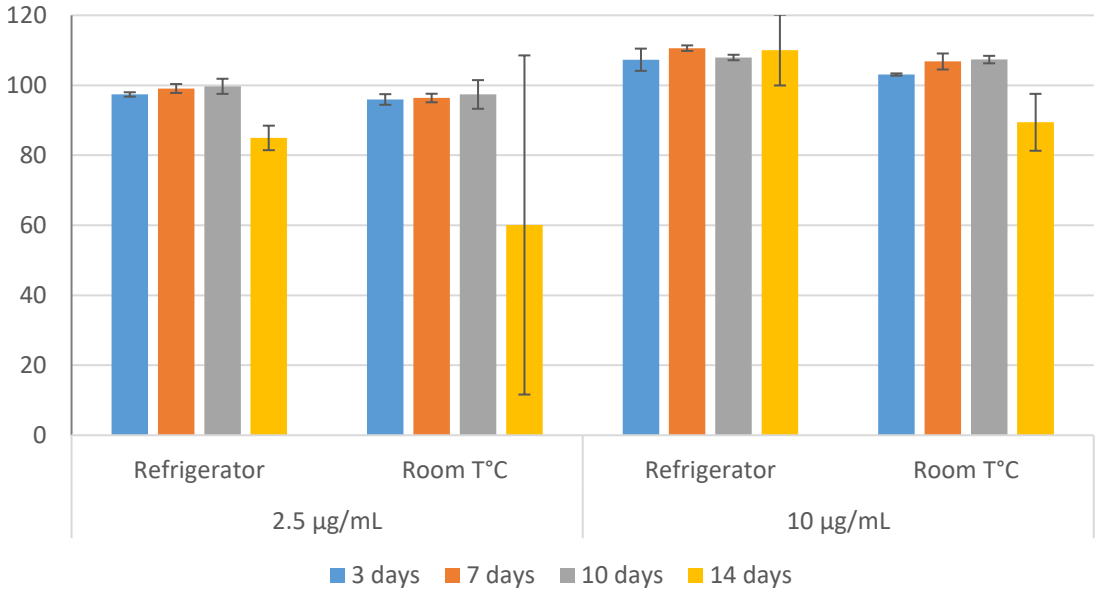



**Figure 4:** Stability of testosterone and stanozolol in suspension kept under refrigeration or at room temperature for 3, 7, 10 and 14 days. N=3 samples for every concentration/storage condition. Results are expressed in percentage of the initial concentration (mean ± standard deviation)

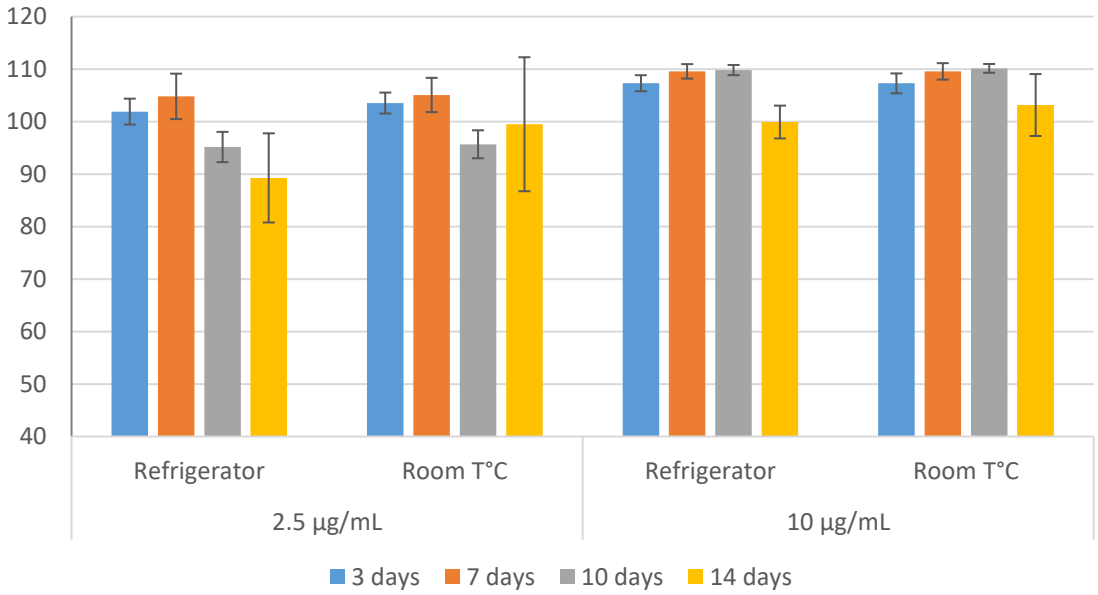



**Figure 5:** Stability of testosterone propionate, testosterone isocaproate, testosterone cypionate, testosterone enanthate, nandrolone decanoate, nandrolone phenylpropionate and boldenone undecylenate in oil matrix kept under refrigeration or at room temperature for 3, 7, 10 and 14 days. N=3 samples for every concentration/storage condition. Results are expressed in percentage of the initial concentration (mean ± standard deviation)

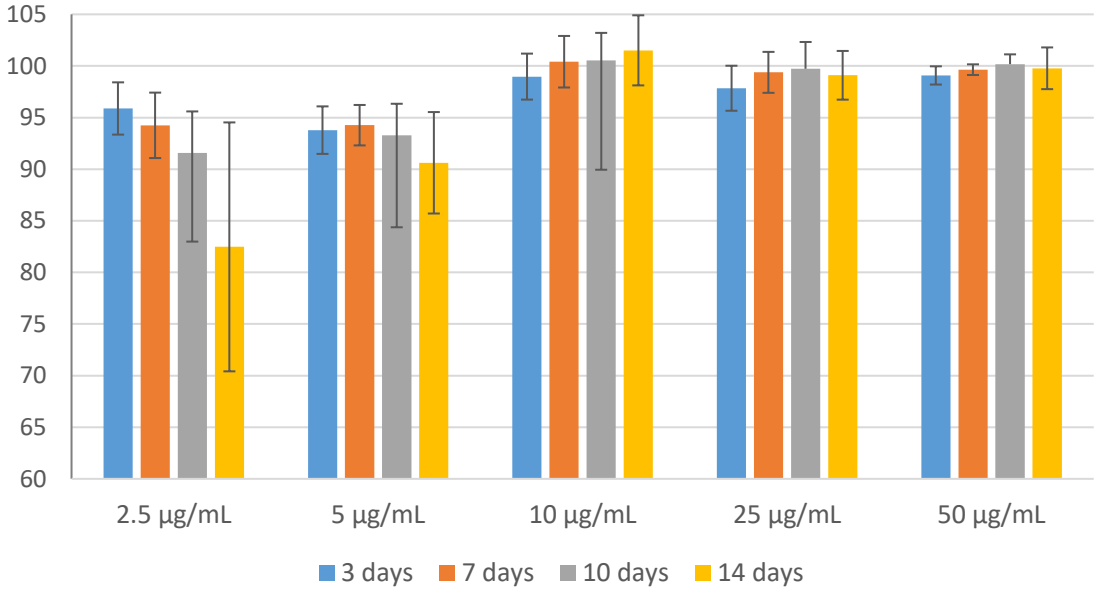



**Figure 6:** Stability of all 12 AAS in methanol solutions kept in the freezer for 3, 7, 10 and 14 days. N=4 for 3 days; N=3 for 7 days; N=2 for 10 days and N=1 for 14 days. Results are expressed in percentage of the initial concentration (mean ± standard deviation)

*3.4. Analysis of medicines and dietary supplements*

The validated method was used to analyze 345 samples of suspected medicines (328) and dietary supplements (17) sent for forensic analysis by the BFP. All samples were previously qualitatively analyzed by GC-MS for forensic purposes so their composition was known. Samples were prepared as previously described, and were analyzed in groups according to the pharmaceutical form and previous knowledge of their qualitative composition. For every batch of analysis, new calibration curves were prepared, and a fortified blank matrix at 10 μg/mL was prepared as previously described was analyzed as a quality control sample.

Quantitative results are presented in Appendix C for each sample. Samples were classified as original, counterfeit or substandard, following the criteria shown in Table 5. Most samples were analyzed after the stated expiry date, and such information was taken into account in the classification. Packaging analysis includes comparison with other similar products and evaluation of lot numbers and security codes (when such information was available). An example of package comparison is shown in Figure 7.

**Table 5:** Criteria adopted for medicine and dietary supplements classification according to the GC-MS analysis and packing characteristics

| **Classification** | **Criteria** |
|---|---|
| Original | • Qualitative formulation detected fully matches the one described*;<br>• Levels of active pharmaceutical ingredients (API) detected are between 80 – 130% of the declared formulation if product is not expired; if expired, levels detected are not below 50% of declared levels and/or are similar to levels detected in other products with the same expiry year. |
| Substandard | • Qualitative formulation detected fully matches the one described*;<br>• Levels of API detected are not on the range defined for original products, but packaging is authentic. |

| Classification | Criteria |
|---|---|
| Counterfeit | • Qualitative formulation detected does not match the one described (no API present; different API, additional API or not all API declared are present);<br>• Qualitative formulation detected matches the one described but at very low concentrations (< 50% of declared formulation) and/or differing significantly from similar products with the same expiry year;<br>• Fake packaging;<br>• Product presents significant differences from other products with the same lot number (such as the mean weight and tablet dimension);<br>• Product was declared as inexistent by ANVISA;<br>• Product declares an inexistent / unregistered manufacturer ("underground" products). |
| No specification | • Product was not sent in its original package or package did not state the contents of the product; no information available regarding the identity and concentration of AAS present. |

*Should the product declare an AAS not included on the study, identification by its mass spectrum on full scan mode was considered enough.


**Figure 7:** Original (left) and counterfeit (right) samples of Decaland Depot®. Counterfeit flask is slightly larger (black lines), the font used on the counterfeit is thinner and the overall label is of low quality.

The overall counterfeiting rate detected for medicines was 42.1% (138 of 328 samples): 28.7% of tablets, 12.0% of suspensions and 65.2% of oil solutions. Several kinds of counterfeit products were detected, as detailed in Table 6.

**Table 6:** Amount and kinds of counterfeit medicines detected.

| Matrix | Total N | Number of counterfeits (%) | Kinds of counterfeits |
|---|---|---|---|
| Tablet | 87 | 25 (28.7%) | 13 inexistent medicines;<br>5 had a different API;<br>4 had no API;<br>2 had lower doses of the correct API and different physical properties;<br>1 had an additional API. |
| Aqueous suspension | 83 | 10 (12.0%) | 6 had lower doses of the correct API;<br>3 had no API;<br>1 had a different API. |
| Oily solution | 158 | 103 (65.6%) | 65 had no API;<br>22 had a different API;<br>9 had lower doses of the correct API;<br>6 did not contain all API declared;<br>1 had an additional API |

API = Active Pharmaceutical Ingredient

One information concerning oil solutions must be emphasized. One of the most counterfeited Brazilian medicine is Durateston®, a product that contains TP, TI, TF and testosterone decanoate. In addition, there is a Paraguayan product with similar formulation, called Duratestoland®, which is very frequently seized in Brazil and sent to forensic analysis. In this study, 66 counterfeit Durateston® samples and 5 counterfeit Duratestoland® samples were analyzed (Appendix C, Table 03). However, since TF was adopted as an internal standard, 8 Durateston® original samples and 15 Duratestoland® original samples, that contained TF, were not included in this study.

Four tablets, six suspensions and 26 oil solutions (almost 11% of all samples; 36 of 328) were considered substandard products. The quantitative analysis did not match the label, but no counterfeiting evidences were found on the packages. Two tablets, one suspension and 18 oils had much more API than the concentration stated (Up to 170% of the declared dose in tablets, 142% in suspension and 221% in oily solution), whereas two tablets, five suspensions and eight oils had lower API levels than declared (as low as 58% of the declared dose in tablets, 21.6% in suspensions and 16.4% in oily solution) (Appendix C, Tables 1, 2 and 3 respectively). This is probably due to poor quality control practices or substandard raw materials, but the possibilities of fraudulent package reutilization or deviation from the manufacturing line cannot be excluded.

From the 17 dietary supplement samples, 12 declared the presence of PR and were considered original after analysis (Appendix C, Table 4). The other five samples were

counterfeits, a conclusion that was already reached after qualitative analysis due to differences in the substances declared and effectively detected; quantitative analysis showed that two samples contained ME at 5.4 and 5.8 mg/capsule. The product label recommends the intake of one to two capsules a day, which corresponds to approximately 11 mg of ME per day, a dose similar to medicines declaring ME. Additionally, during the period of the study, one supplement containing oxymetholone and two containing oral turinabol were also detected, but since these AAS were not included in the study, no quantitative analysis was performed.

Counterfeits containing PR instead of methasterone and halodrol had an average of 5.6 mg of PR per capsule. Even though there are supplement products on the market declaring the presence of 5 mg and even 2 mg of PR per capsule, most declare the presence of 25 or 50 mg PR/capsule. The 10 products that stated the amount of PR present declared to contain 25 mg (N=3), 50 mg (N=6) or even 100 mg (N=1) per capsule (Appendix C, table 4).

The final classification of all samples is shown in Figure 8. Considering counterfeits and substandard products, 53% of the medicines were SSFFC products.

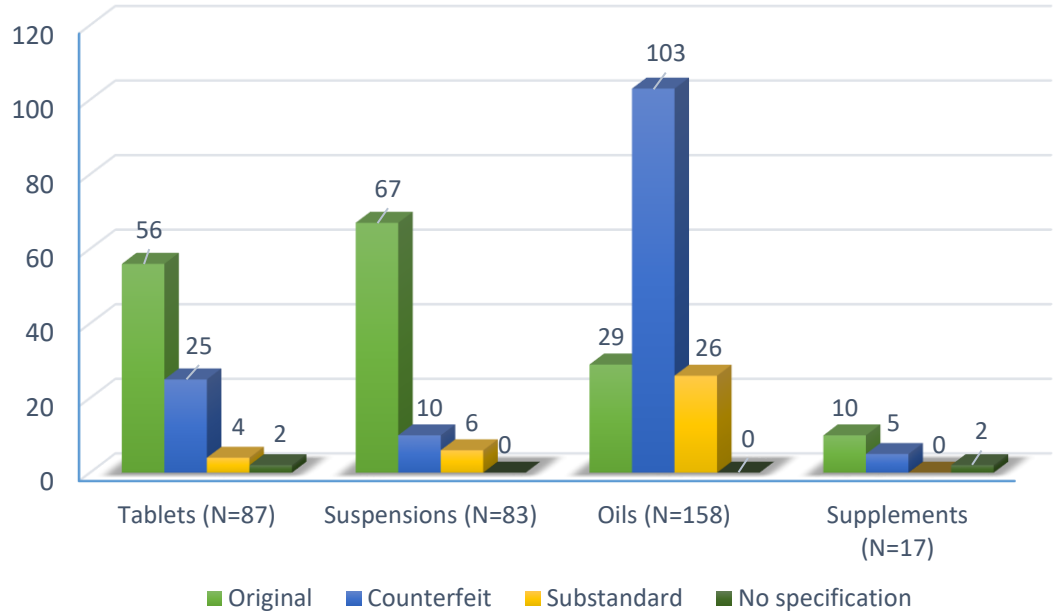



**Figure 8:** Final classification of samples after quantitative analysis. N=87 for tablets; N=83 for suspension, N=158 for oily solutions, N=17 for supplements.

## 4. Discussion

A GC-MS method for the quantitation of anabolic androgenic steroids in medicine and dietary supplement products using a simple sample preparation procedure was developed, validated and used to analyze 328 samples sent to forensic analysis by the BFP. The method is suitable to detect counterfeit and substandard products in forensic and quality control laboratories. The sample preparation was similar to that used by Musshoff et al. (1997) to analyze medicines containing anabolic steroids in oil, tablet and aqueous suspension pharmaceutical forms; the authors concluded that "an identification of anabolic steroid esters is possible without splitting or derivatization" (Musshoff et al., 1997), although the authors did not validate the method for quantitative analysis.

There are very few papers reporting the quantitative analysis of AAS in suspected medicines. Pellegrini et al. (2012) validated a GC-MS quantitative method using derivatization and analyzed fifteen medicine samples (including tablets, oily solutions and aqueous suspensions) seized by the Italian Anti-Adulteration and Safety Bureau. LOQ were reported as "below the first point of the calibration curves", which were 10 mg/g for tablets and 0.02 mg/mL for liquid preparations. A mean weight of 200 mg per tablet would yield a LOQ of "below 2 mg/tablet", which is similar to what was reached in this study. Their LOQs for liquid preparations, however, were lower than those obtained in the present study (ranging from 1.28 to 2.48 mg/mL). They found that 13 of the 15 samples analyzed did not contain what was declared, including two products without any API, three with lower doses and eight containing different API. The authors did not classified the analyzed samples either as counterfeits or as substandards. Cho et al. (2015) validated an UHPLC-MS/MS method and analyzed 3 tablets and 16 injectable medicines and dietary supplements obtained from the market or websites in South Korea. Instrumental LOQ ranged from 0.5 to 25 µg/L, but the lowest level evaluated during validation was 100 µg/L for recovery and 250 µg/L for repeatability and intermediate precision, levels that are still lower than those obtained in the present study. Nine medicines were considered "adulterated with AAS", implying that samples did not declare the presence of AAS.

The presence of undeclared anabolic steroids in dietary supplements is documented on the literature. Geyer et al. (2004) analyzed 634 non-hormonal supplements purchased in 13 different countries (United States and Europe) by GC-MS with derivatization. LOQ was reported at 0.01 µg/g. Ninety-four samples contained

undeclared prohormones at such low levels that they were considered to be due to cross-contamination during manufacturing. Van Poucke et al. (2007) analyzed by LC-MS/MS (no validation data available) 19 supplement samples intercepted by the Belgian pharmaceutical inspection at the post office, finding 11 samples containing AAS at low doses (highest at 2.54 mg/tablet). These samples declared the presence of prohormones, and the authors hypothesized that the AAS were, in fact, by-products formed during prohormone synthesis. More recently, Abbate et al. (2015) analyzed qualitative and semi-quantitatively 24 bodybuilding supplements sold in fitness equipment and online shops in the United Kingdom, using different techniques (GC-MS, LC-MS, HPLD-DAD and UV-Vis). They found that 23 of the 24 samples contained steroids, 16 of which were different from what was declared on the products label. In Italy, Odoardi et al. (2015) analyzed 30 supplements using a LC-HRMS validated method for quantification of AAS and clenbuterol. They found that 12 samples contained PR, 11 contained androstenedione, 3 contained ME, one contained ES and seven contained T. Although the levels of ME, ES and T found in the samples were below those required for biological activity, their consumption could lead to a positive anti-doping exam, and be a health hazard if consumed at high amounts or continually. Two products without identification contained TP, TF, TI and testosterone decanoate (oily solution) and oxymetholone (tablet), but no quantitative information was available.

It is important to emphasize that the number of samples analyzed in the published studies is much lower than the present study, what makes incidence comparison difficult. The large number of samples analyzed in the present study, mainly medicines, and the fact that they were collected during a five-year period (2011 – 2016) most likely reflects the real situation of clandestine medicines circulating in Brazil. The data complements a previous study conducted by our research group based on package analysis and GC-MS qualitative data for AAS-containing medicines seized by BFP from 2006 to 2011 (Neves et al., 2013). In this study, a counterfeiting rate of 31.7% was detected, but since all analysis were qualitative, the hypothesis of under counterfeit detection was raised. This has proven true since quantitative information was decisive on the detection of the 17 low dose counterfeits (12.3% of 138 counterfeits) and all 36 substandard products, which would not be identified without a quantitative analysis, leading to a rate of 53% of SSFFC products.

Some limitations of the present study must be pointed out. Most of the products were seized on Brazilian borders (such as Foz do Iguaçu, border with Paraguay and Argentina), which means that they may be representative of clandestine products entering the country, but not necessarily of products that may be manufactured in Brazil for local distribution. Some samples were intercepted by post offices and they might account for the "internal market" products, but they may also be foreign products that successfully entered the country and are just being distributed. Some relevant AAS were not included in this study, such as oxymetholone, methyltestosterone, trembolone and methenolone, due to difficulties during the standard importing process in Brazil. Qualitative data of samples seized during the period of the study showed 46 samples containing oxymetholone, 12 with methyltestosterone, 10 with trembolone acetate and 4 with methenolone enanthate.

Additionally, most samples were evaluated after their declared expiry date, and their quality specifications regarding acceptable contents were not available. In an attempt to take this into account, since information on the long-term stability of the AAS in the matrices was not available, large concentration ranges were adopted as classification criteria, to ensure that no original samples would be considered substandard. This means that some substandard samples might have been regarded as originals.

## 5. Conclusion

This is the largest study concerning analysis of clandestine medicine samples containing or suspected to contain AAS worldwide. Comprehensive work like this should be conducted in other countries, in collaboration with polices and health surveillance agencies to grant access to samples, so better estimates of the rate of AAS counterfeiting in the world can be made.

The results of this study show the need to raise awareness of consumers of clandestine market-originated AAS to the real risk of purchasing counterfeit products and health risks associated to SSFFC products. It also indicates the need for more incisive actions from government agencies aiming at decreasing the availability of SSFFC medical products on the Brazilian and worldwide market.

## V. Caffeine contents in dietary supplements seized by the Brazilian Federal Police

Diana Brito da Justa Neves, Eloísa Dutra Caldas

### 1. Introduction

Caffeine, or 1,3,7-trimethylxanthine (Figure 1), is one of the most consumed and studied stimulants in the world. It is present in a wide variety of foods and beverages, as well as in about 60 plant species (Schwenk and Costley, 2002; Gurley et al., 2015). Caffeine has Central Nervous System stimulating properties, is diuretic, decreases fatigue, enhances mental focus and enhances athletic performance, and presents thermogenic effects (Rang et al., 1997; Greenway, 2001). Moreover, there is evidence suggesting that the consumption of caffeine also seems to reduce caloric intake, reasons for which it could contribute for weight loss (Westerterp-Plantega et al., 2006).

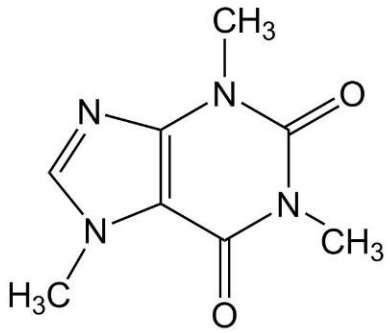



**Figure 1:** Chemical structure of caffeine.

Caffeine is a major component found in dietary supplements, referred in this work as supplements, mainly in products for weight loss, energetics and athletic performance enhancers. Caffeine was frequently associated with herbal extracts from the *Ephedra* family that contain ephedrine alkaloids (Gurley et al, 2015), since this association was more efficient for weight loss than any of the substances isolated (Greenway, 2001). In 2004, however, the US Food and Drug Administration (FDA) removed from the market all products containing *Ephedra* extracts or ephedrine, since they presented an unreasonable risk illness or injury under the conditions of use recommended or suggested in the product label (EUA, 2004; Gurley et al., 2015).

After the banning of ephedra, a new generation of "ephedra-free" supplements came to the market, containing several natural sources of caffeine and other herbal extracts containing numerous substances with pharmacologic action (such as synephrine and yohimbine). The amount of caffeine in these supplements usually exceeds that found in beverages and foods, but most products do not declare the caffeine content at all (Schwenk and Costley, 2002; Gurley et al., 2015).

In Brazil, caffeine can only be commercialized as a supplement under the "caffeine supplements for athletes" category; products cannot contain any other substances and must declare the caffeine amount present, which must be between 210 and 420 mg per dose (Brasil, 2010b). On the US, however, products do not need to state their caffeine content. A study conducted in the US with 56 samples of supplements declaring caffeine found levels ranging from 0.60 mg to 828.7 mg on the daily recommended dose. In products whose caffeine content was declared, the authors found significant discrepancies between declared and detected doses, reaching 173% of the declared content (Andrews et al., 2007).

There are few papers reporting the quantification of stimulants in supplements, focusing mainly on *Ephedra* and *Citrus aurantium* alkaloids, and the information is frequently limited to a low number of samples. Studies that did report quantification of caffeine in supplements found major variations between declared and detected contents; in products that did not declare caffeine content, the compound was present at varying levels or even absent (Haller et al., 2004; Marchei et al., 2005; Seeram et al., 2006; Evans and Siitonen, 2008). It was not found, however, studies conducted in Brazil concerning the caffeine contents in supplements.

The aims of this work are to develop and validate a GC-MS method for the quantification of caffeine and identification of other substances present in weight loss supplements, and to apply it to real samples seized by the Brazilian Federal Police (BFP) and sent to forensic analysis on the National Institute of Criminalistics.

## 2. Material and methods

### *2.1. Standards and reagents*

Caffeine standard (98.5% purity, confirmed by Nuclear Magnetic Ressonance) and dipentyl phthalate, used as an internal standard (IS; 97% purity), were from Acros

Organics (Geel, Belgium). HPLC grade chloroform was purchased from Tedia (Fairfield, OH, USA) and water was produced by a Milli-Q Direct-Q system (Millipore, Bedford, MA, USA). Hexane used for capsule cleaning was purchased from J. T. Baker (Phillipsburg, NJ, USA).

A mixture of cellulose (Merck - Darmstadt, Germany), lactose (Sigma-Aldrich - St. Louis, MO, USA), starch (J. T. Baker Phillipsburg, NJ, USA) and mannitol (bulk material sent to forensic analysis by the BFP and chemically characterized prior to use) was used as blank matrix for tablets/capsules; a supplement containing *Tribulus terrestris* extract (GC-MS analysis showed they contained no caffeine) was used as blank matrix for herbal extract tablets/capsules, and glycerin (Cinética – Jandira, SP, Brazil) as a blank matrix for capsules with liquid content.

*2.2. Standard solution preparation*

All standard solutions and sample extractions were done using a solution of the IS dipentyl phthalate in chloroform at 50 μg/mL (hereby called "IS solution").

A stock solution of 250 μg/mL of caffeine was prepared by weighting 12.5 mg of the caffeine standard and solubilizing it in 50 mL of the IS solution. Every time a new IS solution was prepared, a new caffeine stock solution was also prepared. Caffeine stock solutions and IS solutions were kept at room temperature and usually consumed within one week.

Calibration points were prepared by diluting the stock solution with IS solution; the highest calibration point was the undiluted stock solution itself.

*2.3. Samples*

Usually, dietary supplements are seized and sent to forensic analysis by the BFP whenever there is a suspicion that they may be counterfeited, adulterated, smuggled into the country, contain any proscribed or controlled substances or cannot, for any reason, be commercialized in Brazil. This study focused on supplements claiming to aid in weight loss, but also included other kinds of supplements that not necessarily declared caffeine on their labels (such as pro-hormones), but in which caffeine was detected during forensic analysis. The 216 samples included in this study were seized from 2010 to 2016, and include tablet and capsule with powder content ("solid capsules") and capsule with liquid content ("liquid capsules") forms. All samples were stored at room temperature before

analysis. The expiry date, when declared, varied from 2007 to 2020, and the samples were analyzed in September 2016; 82.4% of the samples were analyzed after the expiry date.

*2.4. Sample preparation*

First, the mean weight of five tablets/capsules of each sample was determined. Tablets were weighted together and the total weight divided by five. Capsules were individually weighted, their contents removed and the capsule shells were thoroughly cleaned with cotton swabs and hexane. After drying, the shells were individually weighted, the weight of their contents calculated and the mean weight of contents was determined. The contents of solid capsules were homogenized and stored for analysis at room temperature. New liquid capsules were used for analysis.

For the analysis, three tablets or the contents of three liquid capsules were ground and/or homogenized. An amount equivalent to 1/10 of the mean weight was transferred to a 15 mL falcon tube, 1 mL of milli-Q water and 5 mL of the IS solution were added. Tubes were shaken manually, vortexed for 10 seconds, sonicated for 10 minutes, centrifuged for 5 minutes at 3000 rpm and a 50 µL aliquot of the organic layer was added to a vial containing 950 µL of IS solution, to a final volume of 1 mL. In case the concentration felt below the lowest calibration point, the organic layer was analyzed at a 500:500 dilution or undiluted; if the result was higher than the highest calibration point, a 25:975 dilution of the organic layer was made in IS solution and analyzed.

*2.5. Equipment*

GC-MS analyses were performed on a GC System 7890A, coupled with a 5975C Mass Spectrometer (operating at 70 eV) and an automated sample injector system CTC PAL G 6509-B (all Agilent Technologies, Santa Clara, California, USA). A HP5-MS (Agilent Technologies) capillary column was used (25 m x 0.20 mm i.d. x 0.33 µm film thickness). Temperatures of the MS ion source and GC/MS interface were 230 and 280 ºC, respectively. MS detector was used in Full Scan mode, with two different sets of parameters. In the beginning and the end of the run, a mass range of 20 to 400 m/z was monitored, operating with 1.95 sweeps per second (sampling rate of 3), to detect substances other than caffeine potentially present in the supplements, including anorectics. During a 2-minute interval in the middle of the chromatogram, in the region where caffeine and the IS elute (from 6.3 to 8.3 minutes), the detector was set to monitor from 40 to 200 m/z, operating with 4.32 sweeps per second (sampling rate of 3), a range

that comprises all expected fragments of caffeine and internal standard (IS) mass spectra. This alteration lead to a lower baseline on this region of the chromatogram, as illustrated in Figure 2.

The injection port temperature was 280ºC, injection volume was 0.5 µL and split injection mode (50:1) was used. The oven temperature was programmed at 70ºC for two minutes, increased to 250ºC at 40ºC/minutes, held at 250ºC for 2 minutes, raised to 315ºC at 40ºC/minute and held at 315ºC for 3.875 minutes, with a total run time of 14 minutes.

Quantification was performed considering the ratio between the caffeine peak area and the IS peak area. Peak areas were calculated from the Total Ion Chromatogram. Qualitative identification of other substances was conducted by comparison of the mass spectrum obtained with the National Institute of Standards and Technology (NIST) electronic library. A chromatogram of caffeine and the internal standard is shown in Figure 2.

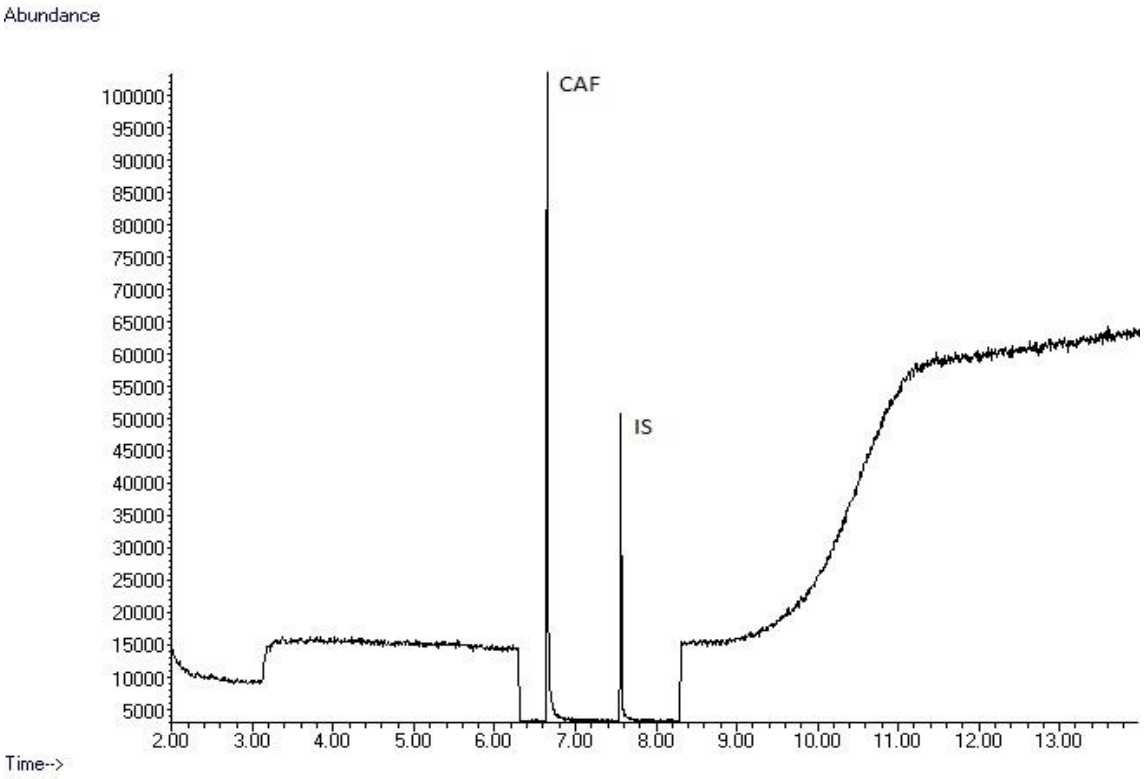



**Figure 2:** Total ion chromatogram of caffeine (CAF) at 100 µg/mL and internal standard (IS) at 50 µg/mL.

*2.6. Method Validation*

Method validation was performed following ANVISA guidelines for medicines (Brasil, 2003) and the MAPA guidelines for drugs in veterinary products (Brasil, 2011b).

Linearity of the calibration curve was assessed by preparing and analyzing six replicates of each calibration level (25, 50, 100, 175 and 250 µg/mL) in IS solution. Data was evaluated for linear or quadratic relationships, using untransformed data, square root transformation and logarithm transformation. The quality of the regressions obtained was evaluated considering the correlation coefficient, Cochran tests and F tests for variances (to detect heteroscedasticity); analysis of variance (ANOVA) to evaluate lack-of-fit; sum of relative errors; graphic evaluation of the randomness of the residuals; and residual standard deviation (measures their dispersion throughout the regression curve and evaluates their absolute value).

Selectivity of the method was evaluated by analyzing blanks of the three matrices (pharmaceutical, herbal and glycerin) and the IS solution alone, investigating the presence of any interfering peaks at the caffeine or IS retention times. Matrix effects were evaluated by using extracts of the blank matrices, prepared accordingly to the proposed sample preparation method. A calibration curve was built in IS solution and four sets of samples were prepared by dissolving the adequate amount of caffeine standard in the three matrices extracts and in IS solution (three replicates at three levels each; each solution was injected three times). Samples were quantified with the calibration curve and the results obtained for the *in matrix* samples were compared with those obtained for the IS solution samples using a t-test (Brasil, 2011b).

Precision and recovery studies were conducted together. Twelve aliquots of each blank matrix were weighted in 15 mL falcon tubes (approximately 70 mg) and four different caffeine solutions were prepared in chloroform. The solutions were used to fortify the aliquots at five different levels, three aliquots per level. The fortified blank samples were homogenized, left to dry for 48 hours, extracted following the proposed method and the analytes quantified with a freshly prepared calibration curve (each solution was injected two times). The caffeine solutions were prepared at concentrations that would yield final levels of 0, 25, 50, 100 and 250 µg/mL.

Recovery was the mean value (in % of the fortified level) and repeatability as the Relative Standard Deviation (RSD) obtained in the three replicates (two injections for

each replicate) analyzed in the same day; intermediate precision was defined as the RSD of the six replicates considering the two days of analysis. Limits of quantification (LOQ) were defined as the smallest concentration with acceptable repeatability, intermediate precision and recovery; threshold values adopted were 10% for repeatability, 15% for intermediate precision (Brasil, 2011b) and 80-120% for recovery.

## 3. Results and discussion

### *3.1. Method Validation*

Data obtained in the linearity study showed that the calibration curves were homoscedastic. The shape of the residual plot from the linear regression had a characteristic curved shape that indicates a quadratic model was adequate; residuals from the quadratic regression were random and the quadratic term of the regression was significant according to its confidence interval. Since the quadratic regression still presented some lack-of-fit (Experimental F = 11.63; tabulated F value = 3.385) and the sum of the residual errors was still considered large (145.27; N=30), data transformation was tested. Square root transformation did not yield good results but logarithm transformation yielded significant improvements on the quadratic regression (Experimental F for lack-of-fit = 5.82; sum of residual errors 24.8; N=30). Correlation coefficient and residual standard deviation were satisfactory so this regression was chosen (log10 transformed, quadratic).

The method showed to be selective, as no peaks were detected on the blank matrix chromatograms. No matrix effects were observed, since t-tests showed there was no significant difference between results in the three matrixes compared with results obtained in IS solution.

Table 1 shows the results obtained for recovery, repeatability and intermediate precision. Adequate results were obtained at 25 µg/mL for all matrixes, so the LOQ was calculated as 25 mg caffeine/capsule or tablet.

**Table 1:** Validation parameters for the analysis of caffeine in different kinds of dietary supplements by GC-MS

| Matrix | Conc. (µg/mL) | Recovery (%) (N=6) | Repeatability ($RSD_r$, %) (N=6) | Intermediate Precision ($RSD_p$, %) (N=12) |
|---|---|---|---|---|
| Glycerin | 25 | 84.4 | 4.7 | 8.2 |
| | 50 | 95.1 | 6.1 | 6.3 |
| | 100 | 101.1 | 4.4 | 5.6 |
| | 250 | 93.9 | 3.9 | 3.2 |
| Herbal | 25 | 84.6 | 5.5 | 7.9 |
| | 50 | 94.3 | 1.8 | 3.4 |
| | 100 | 100.6 | 3.6 | 3.6 |
| | 250 | 92.4 | 3.5 | 2.9 |
| Pharmaceutical | 25 | 87.5 | 10.8 | 10.8 |
| | 50 | 94.6 | 3.3 | 3.8 |
| | 100 | 98.6 | 2.7 | 3.3 |
| | 250 | 93.5 | 3.2 | 2.3 |

*3.2. Analysis of dietary supplements*

The proposed method was used to determine the caffeine content of 216 supplement samples sent to forensic analysis by the BFP. 204 samples were weight loss supplements (one did not declare the presence of caffeine), 9 were body building supplements that did not declare the presence of caffeine, two declared to have diuretic action and indicated the presence of caffeine, and one had no identification whatsoever. All samples declared to be manufactured in/for the United States, except the product without identification and the weight loss supplement that did not declare caffeine in the label, which stated to be of Brazilian origin.

Samples were prepared as described and analyzed in batches of 36 samples. A fortified sample was prepared by the addition of 100 mg of caffeine standard to the content of one of the blank herbal matrix capsule; the mixture was thoroughly homogenized and an aliquot of one-tenth of the final mass of the mixture was analyzed with each batch as a quality control sample.

Individual quantitative results are presented in Appendix D. Caffeine levels detected ranged from 49.6 to 276.8 mg per tablet, 0.5 to 389.4 mg per solid capsule and from 12.8 to 255.7 mg per liquid capsule, with seven occurrences of absence of caffeine (<LOD) in solid capsules that declared its presence. For samples that declared the amount of caffeine present (13 tablets, 68 solid capsules and 27 liquid capsules), the ratio of caffeine detected/declared was calculated and samples were classified in five categories, and a value between 80-120% of the declared amount was considered according with the

specification. Samples that declared the presence of caffeine but did not specified the amount present (1 tablet, 18 solid capsules and 78 liquid capsules) were classified according to the amount of caffeine detected. The final classification of these samples is shown in Figures 3 and 4.

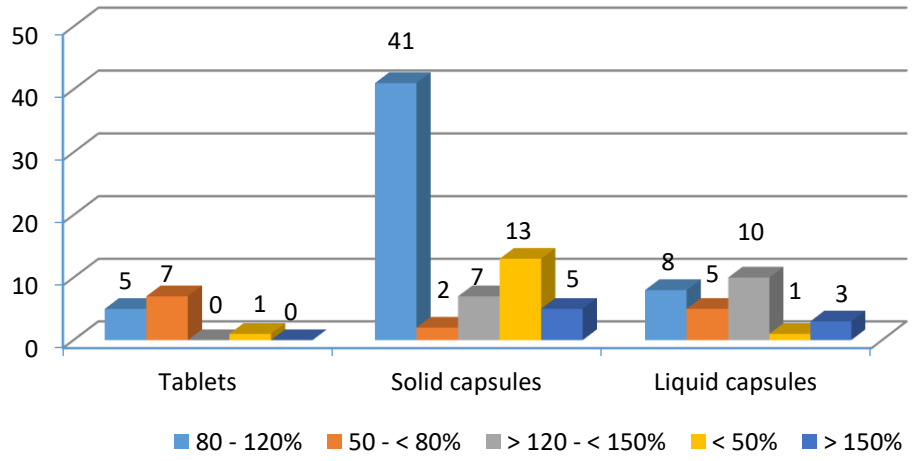



**Figure 3:** Classification of supplement samples seized by the Brazilian Federal Police that declared the amount of caffeine present, based on their detected/declared ratio.

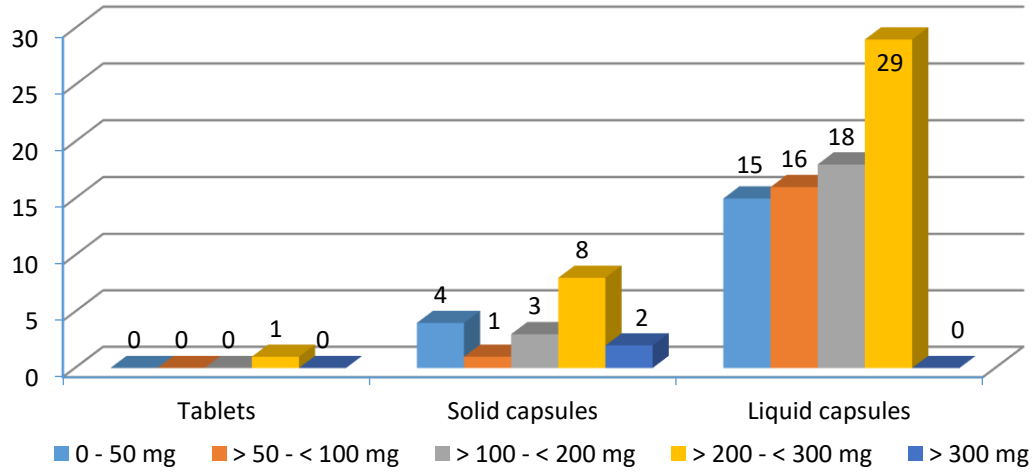



**Figure 4:** Classification of supplement samples seized by the Brazilian Federal Police that did not declared the amount of caffeine present, based on the amount of caffeine detected.

Most tablets (13 of 14) declared the amount of caffeine present, and from those, 61.5 % contained less than 80% of the declared amount (lowest value 49.6%) (Figure 3). However, only one of these tablets was analyzed before its expiry date (and contained 72.2% of the declared caffeine amount), so caffeine degradation cannot be ruled out. The

highest relative amount of caffeine found on a tablet was 114.9% of the declared value, within the defined acceptable variation. Most of the solid capsules (68 of 86) declared the amount of caffeine present, from which 22% contained less than 80% of the stated amount (reaching <LOQ, regardless of the expiry date) and 17.6% contained more than the stated amount (up to 382.2%). About 26% of the liquid capsules declared the caffeine amount (27 of 105), from which 22.2% contained less than 80% of the stated amount (lowest value 47%, all expired) and 48.1% contained more than 120% of the stated amount (up to 197%). Individual values are shown in Appendix D.

Eleven samples did not declare the presence of caffeine, all solid capsules. Nine were bodybuilding supplements, in which the amount of caffeine present ranged from 0.5 mg/capsule to 294.8 mg/capsule, with an average of 115.6 mg/capsule. The presence of caffeine at 0.5 mg/capsule might be due to cross contamination during the manufacturing process; the second lowest caffeine concentration detected in these products was 18.9 mg/capsule, which is already a relevant amount that may denote intentional adulteration. The Brazilian weight loss supplement sample with undeclared caffeine contained 0.7 mg caffeine/capsule, a low concentration that also might be attributed to cross contamination. Finally, the product without any identification contained 49.3 mg caffeine/capsule.

Six solid capsule samples contained undeclared compounds: four weight loss supplement contained sibutramine (with no or only traces of caffeine), one weight loss supplements contained amphepramone and femproporex (plus 47.6 mg caffeine/capsule) and one supplement that declared to contain dehydroepiandrosterone (DHEA) contained, in fact, 88.1 mg caffeine/capsule, ketamine and clobenzorex. The levels of these compounds were not determined under the scope of this study.

*3.3. Caffeine intake from the consumption of dietary supplements*

The Brazilian legislation states that caffeine supplements for athletes should provide between 210 and 420 mg caffeine per serving, added only as anhydrous caffeine (at least 98.5% purity; Brasil, 2010b). Six liquid capsule samples analyzed in this study (all Hydroxycut Hardcore®) provided more than 420 mg/serving (max. of 590.7 mg).

All except two samples analyzed were from the USA market, a country that does not require the amount of caffeine present to be stated in the products, which may also contain other sources of caffeine such as botanical extracts (EUA, 1994). Most products analyzed recommended the consumption of more than one tablet/capsule per day (up to

9). Using the maximum recommended dose in the product label and the caffeine concentration determined in this study, the daily caffeine intake for each product was estimated and shown in Figure 5.

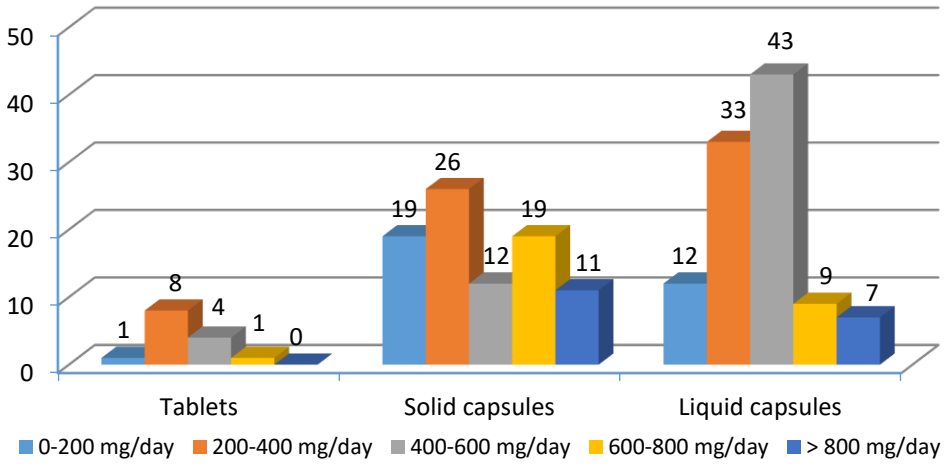



**Figure 5:** Intake of caffeine from the consumption of the maximum daily dose recommended for the supplements analyzed in this study.

The safe daily intake of caffeine for non-pregnant adults is estimated to be 400 mg (EFSA, 2015), which would be equivalent to 6.7 cups of Brazilian expresso coffee (average of 59.8 mg caffeine/60 mL; Camargo and Toledo, 1998). The caffeine intake above the safe daily dose might lead to adverse effects such as tachycardia, insomnia, nervousness, headaches, abdominal pain, nausea, vomiting, diarrhea and diuresis (Nawrot et al., 2003; Higdon and Frei, 2006). Specific populations such as pregnant women, elderly people or hypertensive individuals may present adverse events at lower doses, and pregnant women should not ingest more than 200 mg/day (EFSA, 2015).

The daily intake from the consumption of the supplements analyzed exceeded 400 mg caffeine for 106 products: 36% of the tablets, 48.3% of the solid capsules and 56.7% of liquid capsules (Figure 5). Daily intake varied from 198.4 to 647.4 mg/ day for tablets (mean of 375.8 mg/day), from 0.7 to 1101.3 mg caffeine/day for solid capsules (mean of 449.4 mg/day) and from 76.8 to 1181.4 mg/day for liquid capsules (mean of 433.3 mg/day).

Additionally, supplement consumers should be aware that caffeine is present in various food that may be consumed daily and contribute to the total intake. Sousa and Costa (2015) estimated the mean usual coffee consumption in Brazil as 163 mL/day,

which according to the authors would be equivalent to 238 mg of caffeine. Previously, Camargo et al. (1999) had estimated that, in average, Brazilians ingest 171 mg caffeine/day from several dietary sources, including coffee, tea, chocolate and soft drinks. Considering a mean caffeine daily intake of 200 mg from dietary sources, and the consumption of caffeine containing supplements, the total intake would exceed the safe dose (400 mg/day) for most of the supplements analyzed in this work (84.4%; Figure 5), and may represent a health concern.

The findings in this study are according to Andrews et al. (2007), who evaluated the caffeine content of 53 supplements purchased in USA. They found concentrations ranging from 0.07 to 307 mg caffeine per tablet, equivalent to up to 829 mg caffeine per day if the maximum dosage recommended was consumed. The authors also found discrepancies between the stated caffeine amount and what was actually detected (up to 173%). In the present study, even higher levels of caffeine and higher discrepancies were detected, reaching 197% of the declared dose for liquid capsules and 384% for solid capsules. Haller et al. (2004) quantified caffeine and several *Ephedra* alkaloids in 35 samples of supplements, finding that 86% of samples contained less than 90% of the declared caffeine content, with one sample containing levels below 80% of the declared dose.

There are two main limitations in this study. The first concerns the origin of the samples, since nearly all products declared to be manufactured in/for the United States, and the data reflect the profile of the products of this market only. Second, most samples were evaluated after its declared expiry date, what might impair the evaluation as to whether the detected amount is compatible to what was declared on the label. Caffeine is considered to be stable for 4 years at room temperature (Sigma-Aldrich, USA) and many products whose expiry date was 2012, 2011 or even 2007 had detected/declared ratios of 100% or higher. It is not possible to confirm, however, if this is due to caffeine stability on the product or if, originally, it contained much more than the declared amount. It should be noted that some expired tablet samples, remarkably Lipodrene®, showed visible signs of degradation (tablet coating changed color; tablet contents were moist), what may have contributed to their low detected/declared ratio (Appendix D; Table 1).

## 4. Conclusion

In this study, caffeine was quantified in 216 samples of supplements, most with declared USA origin, and large discrepancies between the stated and detected amounts of caffeine were found. In products that did not specified the present amount of caffeine, concentrations ranging from 0.6 mg/capsule to 367.1 mg/capsule were found. The consumption of some of these products may exceed the safe daily intake, especially if we consider other caffeine sources, such as coffee and tea, even though some labels have warnings for the consumer to limit the consumption of other caffeine sources while consuming the product. Additionally, cases of adulteration of these products with anorectic drugs such as sibutramine and amphepramone were found, what represents a health risk for inadvertent consumers.

The commercialization of foreign supplements in which caffeine is associated with other substances is not allowed in Brazil; nonetheless, they are easily acquired on the clandestine market. Since they are advertised as foods, they tend to be perceived as safe and devoid of adverse effects. Findings from this study should be used to raise awareness of government agencies and consumers to the risks implied on the consumption of foreign weight loss supplements.

# CONCLUSÕES

A falsificação de medicamentos e outros produtos farmacêuticos é um crime prevalente no mundo todo, cuja real incidência não é exatamente conhecida, sendo apenas estimada por meio de estudos pontuais. Dados de diferentes fontes indicam que o principal alvo de falsificação no Brasil são os medicamentos para disfunção erétil, seguidos dos esteroides anabolizantes. Ambos são classificados como medicamentos "de estilo de vida", em oposição a medicamentos utilizados para curar doenças e salvar vidas, o que coloca o Brasil no perfil de falsificação de medicamentos encontrado em países desenvolvidos.

Por meio da análise de dados oriundos de laudos periciais emitidos pela PF no período de 2006 a 2011 relacionados a medicamentos anabolizantes, foi possível estimar que 31,7% dos produtos enviados à perícia se tratam de falsificações. Durateston® foi identificado como o principal medicamento anabolizante nacional alvo de falsificações e o segundo mais frequentemente apreendido pela PF dentre todos os medicamentos anabolizantes avaliados. Dados de espectroscopia na região do infravermelho associados a ferramentas quimiométricas foram utilizados para desenvolver um método capaz de diferenciar amostras originais e falsas deste medicamento, com uma taxa de 100% de acerto. Este método se mostra como uma opção interessante para ser utilizada por todas as unidades de perícia da PF, harmonizando o atendimento pericial a essa demanda e os resultados obtidos.

Dados oriundos de laudos periciais emitidos pela PF entre 2007 e 2013 relacionados a suplementos alimentares também foram avaliados neste trabalho. A maioria dos produtos periciados se enquadra na categoria de suplementos emagrecedores, seguido de suplementos que declaram modificar a quantidade de algum hormônio e por suplementos que declaram ter ao mesmo tempo ação vasodilatadora e estimulante. Foram identificados 180 casos de adulterações (incluindo presença de substâncias não declaradas ou ausência de substâncias declaradas em rótulo), principalmente em suplementos hormonais e emagrecedores. Uma comparação do arcabouço legal relacionado a suplementos alimentares vigente no Brasil com aquele dos Estados Unidos e da União Europeia mostrou que a legislação brasileira é a mais restritiva, limitando quais formulações podem ser comercializadas como alimentos e quais devem obrigatoriamente ser registradas como medicamentos. Os Estados Unidos e a União Europeia dispõem de

mecanismos adequados para notificação e divulgação ao público da detecção de suplementos irregulares, incluindo a consolidação anual de ocorrências e sua estratificação por tipo de produto. No Brasil, porém, não dispões de sistema semelhante, tendo sidos encontrados apenas dois boletins isolados da ANVISA reportando a detecção de sibutramina em suplementos emagrecedores. Esses achados indicam que as autoridades brasileiras não estão tratando o assunto com a mesma prioridade que outros países, a despeito dos potenciais riscos à saúde implicados.

O método analítico utilizando CG-MS desenvolvido e validado no âmbito deste trabalho para quantificação de 12 esteroides anabolizantes em medicamentos e suplementos alimentares se mostrou de simples execução com potencial aplicação em laboratórios forenses e de controle de qualidade. Constatou-se que 42,1% das 328 amostras de medicamentos analisadas eram falsas e 11% estavam fora das especificações, totalizando 53% de produtos irregulares, uma incidência maior que a reportada no estudo anterior que incluiu apenas análise qualitativa. A comparação com estudos de outros países é limitada, uma vez que se referem a um número de amostras bastante inferior ao analisado no presente estudo. De qualquer forma, a taxa de irregularidade encontrada foi bastante expressiva e indica a necessidade de ações dos órgãos de fiscalização e controle visando um combate mais eficaz à entrada e comercialização desses produtos no Brasil.

O método por CG-MS desenvolvido e validado para quantificação de cafeína em suplementos alimentares emagrecedores no âmbito deste estudo foi utilizado para analisar 216 amostras apreendidas pela PF entre 2010 e 2016. Os resultados indicaram teores variados de cafeína nesses produtos, muitas vezes superando significativamente os níveis reportados na embalagem. O consumo recomendado pelo fabricante desses produtos resultaria na ingestão de cafeína em dose superior àquela considerada segura, mesmo sem considerar a ingestão adicional pela dieta. Muitas vezes o consumidor não tem como saber a quantidade de cafeína efetivamente presente nesses produtos, que não costumam trazer avisos referentes a possíveis efeitos adversos que podem decorrer do seu uso.

Foram detectados ainda fármacos emagrecedores em alguns suplementos analisados, incluindo sibutramina e anfepramona, cuja ingestão não intencional pode representar risco sério à saúde dos consumidores. Posteriormente, estes produtos serão reanalisados para se quantificar os níveis destes fármacos presentes, e caracterizar melhor o perfil dos suplementos estrangeiros que circulam de maneira clandestina no Brasil.

## REFERÊNCIAS BIBLIOGRÁFICAS


Abbate, V.; Kicman, A.T.; Evans-Brown, M.; McVeigh, J.; Cowan,D.A.; Wilson,C.; Coles,S.J.; Walker, C.J. *Anabolic steroids detected in bodybuilding dietary supplements – a significant risk to public health*. **Drug Testing and Analysis** 7(7), 2015, p. 609-618

Abourashed, E.A.; Mossa, J.S. *HPTLC determination of caffeine in stimulant herbal products and power drinks*. **Journal of Pharmaceutical and Biomedical Analysis** 36, 2004, p. 617-620

Agência Mundial Anti-Doping. *2010 Adverse Analytical Findings and Atypical Findings.* Disponível em:
<https://wada-main-prod.s3.amazonaws.com/resources/files/WADA_2010_Laboratory_Statistics_Report.pdf> Acesso em 13/10/2016

Agência Mundial Anti-Doping. *2012 Anti-Doping Testing Figures Report.* Disponível em:
<https://wada-main-prod.s3.amazonaws.com/resources/files/WADA-2012-Anti-Doping-Testing-Figures-Report-EN.pdf> Acesso em 13/10/2016.

Agência Mundial Anti-Doping. *2014 Anti-Doping Testing Figures*. Disponível em: <https://wada-main-prod.s3.amazonaws.com/wada_2014_anti-doping-testing-figures_full-report_en.pdf> Acesso em 13/10/2016.

Ames, J.; Souza, D. Z. *Falsificação de medicamentos no Brasil.* **Revista de Saúde Pública**, 46 (1), 2012, p. 154-159

Andrade, L.A.; Braz, V.G.; Nunes, A.P.O.; Velutto, J.N.; Mendes, R.R. *Consumo de suplementos alimentares por clientes de uma clínica de nutrição esportiva de São Paulo*. **Revista Brasileira de Ciência e Movimento** 20(3), 2012, p. 27-36

Andrews, K.W.; Schweitzer, A.; Zhao, C.; Holden, J.M.; Roseland, J.M.; Brandt, M.; Dwyer, J.T.; Picciano, M.F.; Saldanha, L.G.; Fisher, K.D.; Yetley, E.; Betz, J.M.; Douglass, L. *The caffeine contents of dietary supplements commonly purchased in the US: analysis of 53 products with caffeine-containing ingredients.* **Analytical and Bioanalytical Chemistry** 389, 2007, p. 231-239

Antonopoulos, G.A.; Hall, A. '*Gain with no pain': Anabolic-androgenic steroids trafficking in the UK*. **European Journal of Criminology** 2016, publicado online antes da versão impressa, DOI: 10.1177/1477370816633261.

Araújo, L.R.; Andreolo, J.; Silva, M.S. *Utilização de suplemento alimentar e anabolizante por praticantes de musculação nas academias de Goiânia-GO*. **Revista Brasileira de Ciência e Movimento** 10(3), 2002, p. 13-18

Bailey, R.L.; Gahche, J.J.; Lentino, C.V.; Dwyer, J.T.; Engel, J.S.; Thomas, P.R., Betz, J.M.; Sempos, C.T.; Picciano, M.F. *Dietary Supplement Use in the United States, 2003-2006.* **The Journal of Nutrition** 141, 2011, p. 261–266

Baume, N.; Mahler, N.; Kamber, M.; Mangin, P.; Saugy, M. *Research of stimulants and anabolic steroids in dietary supplements.* **Scandinavian Journal of Medicine & Science in Sports** 16, 2006, p. 41-48

Bianco, A.; Mammina, C.; Paoli, A.; Bellafiore, M.; Battaglia, G.; Caramazza, G.; Palma, A.; Jemni, M. *Protein supplementation in strength and conditioning adepts: knowledge, dietary behavior and practice in Palermo, Italy*. **Journal of the International Society of Sports Nutrition** 8:25, 2011

Botelho, B.G.; Reis, N.; Oliveira, L.S.; Sena, M.M. *Development and analytical validation of a screening method for simultaneous detection of five adulterants in*

*rawmilk using mid-infrared spectroscopy and PLS-DA.* **Food Chemistry** 181, 2015, p. 31-37

BRASIL. Secretaria de Vigilância Sanitária - SVS (1998). *Portaria nº 32, de 13/01/1998*. Disponível em: <http://portal.anvisa.gov.br/documents/33916/394219/PORTARIA_32_1998.pdf/551775c4-9fc2-4f62-bb62-c7ceea757476 > Acesso em 13/10/2016

BRASIL. Agência Nacional de Vigilância Sanitária – ANVISA (1999). *Resolução nº 16, de 30/04/1999*. Disponível em: <http://portal.anvisa.gov.br/documents/33916/394219/RESOLUCAO_16_1999.pdf/66b77435-cde3-43ce-839f-f468f480e5e5> Acesso em 13/10/2016

BRASIL. Agência Nacional de Vigilância Sanitária – ANVISA (1999b). *Resolução nº 18, de 30/04/1999.* Disponível em: <http://portal.anvisa.gov.br/documents/33916/388845/RESOLUCAO_18_1999.pdf/d2c5f6d0-f87f-4bb6-a65f-8e63d3dedc61> Acesso em 13/10/2016

BRASIL. Agência Nacional de Vigilância Sanitária – ANVISA (2002). *Resolução da Diretoria Colegiada nº 02, de 07/01/2002*. Disponível em: <http://portal.anvisa.gov.br/documents/33916/394219/RDC_02_2002.pdf/eea25458-6317-4c28-9f57-1982ee32623c> Acesso em 13/10/2016

BRASIL. Agência Nacional de Vigilância Sanitária – ANVISA (2002b). *Resolução da Diretoria Colegiada nº 259, de 20/09/2002.* Disponível em: <http://www.ibravin.org.br/admin/arquivos/informes/1455824267-1ed.pdf> Acesso em 13/10/2016

BRASIL. Agência Nacional de Vigilância Sanitária – ANVISA (2003). *Resolução da Diretoria Colegiada nº 899, de 29/05/2003.* Disponível em: <http://redsang.ial.sp.gov.br/site/docs_leis/vm/vm1.pdf> Acesso em 13/10/2016

BRASIL. Agência Nacional de Vigilância Sanitária – ANVISA (2010). *Anvisa interdita empresa que adicionava a sibutramina a alimentos*. Notícia publicada em 11/03/2010. Disponível em: <http://oglobo.globo.com/sociedade/saude/anvisa-interdita-empresa-que-adicionava-sibutramina-em-alimentos-3041813 > Acesso em 13/10/2016

BRASIL. Agência Nacional de Vigilância Sanitária – ANVISA (2010b). *Resolução da Diretoria Colegiada RDC nº 18, de 27/04/2010* (b). Disponível em: <http://portal.anvisa.gov.br/documents/33916/394219/RDC%2B18_2010.pdf/d6815465-e99a-477f-bb35-48b1432b380e > Acesso em 13/10/2016

BRASIL. Ministério da Justiça (2011). **Brasil Original – Compre essa Atitude**. Relatório de atividades com ações consolidadas de 2009 e 2010. Conselho Nacional de Combate à Pirataria e Delitos Contra a Propriedade Intelectual, Brasília, 2011

BRASIL. Ministério da Agricultura Pecuária e Abastecimento (2011b). **Guia de Validação e Controle de Qualidade Analítica – Fármacos em Produtos para Alimentação Animal e Medicamentos Veterinários**, Ministério da Agricultura Pecuária e Abastecimento, Secretaria de Defesa Agropecuária, Brasília, 2011

BRASIL. Agência Nacional de Vigilância Sanitária – ANVISA (2013). *Alegações de propriedade funcional aprovadas.* Disponível em: <http://portal.anvisa.gov.br/alimentos/alegacoes> Acesso em 13/10/2016.

Brunacio, K.H.; Verly-Jr, E.; Cesar, C.L.G.; Fisberg, R.M.; Marchioni, D.M. *Uso de suplementos dietéticos entre residentes do Município de São Paulo, Brasil*. **Cadernos de Saúde Pública** 29(7), 2013, p. 1467-1472

Burge, J. *Legalize and Regulate: A prescription for reforming anabolic steroid legislation*. 15 Loy. L.A. Ent. L. Rev. 33, 1994. Disponível em: <http://digitalcommons.lmu.edu/elr/vol15/iss1/2> Acesso em 13/10/2016

Camargo, M.C.R.; Toledo, M.C.F. *Teor de cafeína em cafés brasileiros*. **Food Science and Technology** 18(4), 1998, p. 421-424

Camargo, M.C.R.; Toledo, M.C.F.; Farah, H.G. *Caffeine daily intake from dietary sources in Brazil*. **Food Additives and Contaminants** 16(2), 1999, p. 79 - 87

Carvalho-Silva, L.B.; Braga, G.G.; Lollo, P.C.B. *Utilização de recursos ergogênicos e suplementos alimentares por praticantes de musculação*. **Revista Brasileira de Nutrição Clínica** 27(3), 2012, p. 158-163

Chika, A.; Bello, S.O.; Jimoh, A.O.; Umar, M.T. *The Menace of Fake Drugs: Consequences, Causes and Possible Solutions.* **Research Journal of Medical Sciences** 5(5), 2001, p. 257-261

Cho, S-H; Park, H.J.; Lee, J.H.; Do, J-A; Heo, S.; Jo, J.H.; Cho,S. *Determination of anabolic-androgenic steroid adulterants in counterfeit drugs by UHPLC-MS/MS*. **Journal of Pharmaceutical and Biomedical Analysis** 111, 2015, p. 138-146

Coates, J. Interpretation of Infrared Spectra, a practical approach. **Encyclopedia of Analytical Chemistry** (2006)

Coomber, R.; Pavlidis, A.; Santos, G.H.; Wilde, M.; Schmidt, W.; Redshaw, C. *The supply of steroids and other performance and image enhancing drugs (PIEDs) in on English city: Fakes, counterfeits, supplier trust, common beliefs and access.* **Performance Enhancement & Health** 3, 2014, p. 135-144

Coopman, V.; Cordonnier, J. *Counterfeit drugs and pharmaceutical preparations seized from the black market among bodybuilders.* ***Annales de Toxicologie Analytique*** 24(2), 2012, p. 73 – 80

Cordaro, F.G.; Lombardo, S.; Cosentino, M. *Selling androgenic anabolic steroids by the pound: identification and analysis of popular websites on the Internet.* **Scandinavian Journal of Medicine & Science in Sports** 21, 2011, e247-e259

Custers, D.; Cauwenbergh, T.; Bothy, J.L.; Courselle, P.; De Beer, J.O.; Apers, S.; Deconinck, E. *ATR-FTIR spectroscopy and chemometrics: An interesting tool to discriminate and characterize counterfeit medicines***. Journal of Pharmaceutical and Biomedical Analysis** 112, 2015, p. 181-189

Dégardin, K.; Roggo, Y.; Margot, P. *Understanding and fighting the medicine counterfeit market.* **Journal of Pharmaceutical and Biomedical Analysis** 87, 2014, p. 167-175

Dégardin, K.; Guillemain, A.; Guerreiro, N.V.; Roggo, Y. *Near infreared spectroscopy for counterfeit detection using a large database of pharmaceutical tablets*. **Journal of Pharmaceutical and Biomedical Analysis** 128, 2016, p. 89-97

Deisingh, A.K. *Pharmaceutical counterfeiting.* **Analyst** 130, 2005, p. 271 – 279

De Peinder, P.; Vredenbregt, M.J.; Kaste, D. *Detection of Lipitor® counterfeits: A comparison of NIR and Raman spectroscopy in combination with chemometrics.* **Journal of Pharmaceutical and Biomedical Analysis** 47, 2008, p. 688-694

De Rose, E.H.; Feder, M.G.; Pedroso, P.R.; Guimarães, A.Z. *Uso referido de medicamentos e suplementos alimentares nos atletas selecionados para controle de doping nos Jogos Sul-Americanos*. **Revista Brasileira de Medicina do Esporte** 12(5), 2006, p. 239-242

Dickinson, A. *History and overview of DSHEA.* **Fitoterapia** 82, 2011, p. 5 – 10

Diehl, K.; Thiel, A.; Zipfel, S.; Mayer, J.; Schnell, A.; Schneider, S. *Elite Adolescent Athletes' Use of Dietary Supplements: Characteristics, Opinions, and Sources of Supply and Information.* **International Journal of Sport Nutrition and Exercise Metabolism** *22*, 2012, p. 165 – 174

Donati, A. *World traffic in doping substances.* Disponível em: < https://www.wada-ama.org/en/resources/world-anti-doping-program/donati-report-on-trafficking> Acesso em 13/10/2016

Dorlo, T.P.C., Eggelte, T.A., de Vries, P.J., Beijnen, J.H. *Characterization and identification of suspected counterfeit miltefosine capsules*. **The Analyst** 137, 2012, p. 1265-1274.

Dotson, J.L.; Brown, R.T. *The History of the Development of Anabolic-Androgenic Steroids.* **Pediatric Clinics of North America** 54, 2007, p. 761-769

Dowell, F.E.; Maghirang, E.B.; Fernandez, F.M.; Newton, P.N.; Green, M.D. *Detecting counterfeit antimalarial tablets by near-infrared spectroscopy*. **Journal of Pharmaceutical and Biomedical Analysis** 48, 2008, p. 1011-1014

EFSA. European Food Safety Authority. Scientific Opinion on the safety of caffeine. **The EFSA Journal**. 13(5), 2015, p. 4102

EUA (Estados Unidos da América), 1990. *Anabolic Steroid Control Act of 1990*. Public Law 101-647, 104. Stat. 4789 – 4968; 29 de novembro de 1990

EUA (Estados Unidos da América). Food and Drug Administration (1994). *Ato de Saúde e Educação de Suplementos Dietéticos de 1994*. *Dietary Supplement Health and Education Act of 1994*. 25 de outubro de 1994. Disponível em: <https://ods.od.nih.gov/About/DSHEA_Wording.aspx> Acesso em 13/10/2016

Evans, R.L.; Siitonen, P.H. *Determination of caffeine and sympathomimetic alkaloids in weight loss supplements by high-performance liquid chromatography*. **Journal of Chromatographic Science** 46, 2008, p. 61-67

Fayh, A.P.T.; Silva, C.V.; Jesus, F.R.D.; Costa, G.K. *Consumo de suplementos nutricionais por frequentadores de academias da cidade de Porto Alegre*. **Revista Brasileira de Ciências do Esporte** 35(1), 2013, p. 27-37

Fernandes, R.S.; Costa, F.S.L.; Valderrama, P.; Março, P.H.; Lima, K.M.G. *Non-destructive detection of adulterated tablets of glibenclamide using NIR and solid-phase fluorescence spectroscopy and chemometric methods*. **Journal of Pharmaceutical and Biomedical Analysis** 66, 2012, p. 85-90

Fernandez, F.M.; Green, M.D.; Newton, P.N. *Prevalence and Detection of Counterfeit Pharmaceuticals: A Mini Review.* **Industrial & Engineering Chemistry Research** 47, 2008, p. 585 – 590

Ferreira, M.M.C. **Quimiometria – Conceitos, Métodos e Aplicações**. 1ª Ed., Editora Unicamp, Campinas, 2015.

Freeman, E.R.; Bloom, D.A.; McGuire, E.J. *A brief history of testosterone.* **The Journal of Urology** 165, 2001, p. 371 – 373

Geyer, H.; Braun, H.; Burke, L.M.; Stear, S.J.; Castell, L.M. *A-Z of nutritional supplements: dietary supplements, sports nutrition foods and ergogenic aids for health and performance – Part 22*. **British Journal of Sports Medicine** 45, 2011, p. 752-754

Geyer, H.; Parr, M.K.; Koehler, K.; Mareck, U.; Schänzer, W.; Thevis, M. *Nutritional supplements cross-contaminated and faked with doping substances.* **Journal of Mass Spectrometry** 43, 2008, p. 892-902

Geyer, H.; Parr, M.K.; Mareck, U.; Reinhart, U.; Schrader, Y.; Schänzer, W. *Analysis of Non-Hormonal Nutritional Supplements for Anabolic-Androgenic Steroids – Results of an International Study.* **International Journal of Sports Medicine** 25, 2004, p. 124-129

Goston, J.L.; Correia, M.I.T.D. *Intake of nutritional supplements among people exercising in gyms and influencing factors.* **Nutrition** 26, 2010, p. 604-611

Graham, M.R.; Ryan, P.; Baker, J.S.; Davies, B.; Thomas, N-E; Cooper, S-M; Evans, P.; Easmon,S.; Walker, C.J.; Cowan, D.; Kicman, A.T. *Counterfeiting in performance- and image-enhancing drugs*. **Drug Testing and Analysis** 1, 2009, p. 135-142

Gratz, S.R.; Flurer, C.L.; Wolnik, K.A. *Analysis of undeclared synthetic phosphodiesterase-5 inhibitors in dietary supplements and herbal matrices by LC-ESI-MS and LC-UV.* **Journal of Pharmaceutical and Biomedical Analysis** 36, 2004, p. 525-533

Greenway, F.L. *The safety and efficacy of pharmaceutical and herbal caffeine and ephedrine use as a weight loss agen*t. **Obesity Reviews** 2, 2001, p. 199-211

Gurley, B.J.; Gardner, S.F.; Hubbard, M.A. *Content versus label claims in ephedra-containing dietary supplements.* **American Journal of Health-System Pharmacy** 57 (10), 2000, p. 963-969

Gurley, B.J.; Steelman, S.C.; Thomas, S.L. *Multi-ingredient, caffeine-containing dietary supplements: History, Safety, and Efficacy*. **Clinical Therapeutics** 37(2), 2015, p. 275-301

Harrison, R.A.; Holt, D.; Pattison, D.J.; Elton, P.J. *Are those in need taking dietary supplements? A survey of 21 923 adults.* **British Journal of Nutrition** 91, 2004, p. 617-623

Haller, C.A.; Duan, M.; Benowitz, N.L.; Jacob III, P. *Concentrations of Ephedra Alkaloids and Caffeine in Commercial Dietary Supplements.* **Journal of Analytical Toxicology** 28, 2004, p. 145-151

Higdon, J.V.; Frei, B. *Coffee and Health: A Review of Recent Human Research*. **Critical Reviews in Food Science and Nutrition** 46(2), 2006, p. 101-123

Hirschbruch, M.D.; Fisbergm M.; Mochizuki, L. *Consumo de Suplementos por Jovens Frequentadores de Academias de Ginástica em São Paulo*. **Revista Brasileira de Medicina do Esporte** 14(6), 2008, p. 539-543

Holmgren, P.; Nordén-Pettersson, L.; Ahlner, J. Caffeine fatalities – four case reports. Forensic Science International 139(1), 2004, p. 71 - 73

Hurtado, R.L.; Lasmar, M.C. *Medicamentos falsificados e contrabandeados no Brasil: panorama geral e perspectivas de combate ao seu consumo*. **Cadernos de Saúde Pública** 30(4), 2014, p. 891-895

Jacobson, I.G.; Horton, J.L.; Smith, B.; Wells, T.S.; Bouko, E.J.; Lieberman, H.R.; Ryan, M.A.K.; Smith, T.C. *Bodybuilding, Energy, and Weight-Loss Supplements Are Associated With Deployment and Physical Activity in U.S. Military Personnel*. **Annals of Epidemiology** 22 (5), 2012, p. 318 – 330

Kasal, A. *Structure and Nomenclature of Steroids*. In: Makin, H.L.J.; Gower, D.B., eds. **Steroid Analysis.** 2. ed. Londres: Springer, 2010. Cap. 1, p. 1-25

Kasal, A.; Budesinsky, M.; Griffiths, W.J. *Spectroscopic Methods of Steroid Analysis*. In: Makin, H.L.J.; Gower, D.B., eds. **Steroid Analysis.** 2. ed. Londres: Springer, 2010. Cap. 2, p. 27-161

Kerrigan, S.; Lindsey, T. Fatal caffeine overdose: Two case reports. Forensic Science International 153(1), 2005, p. 67 - 69

Kicman, A.T. *Pharmacology of anabolic steroids.* **British Journal of Pharmacology** 154, 2008, p. 502 – 521

Korolkovas, A.; França, F.F.A.C. **Dicionário Terapêutico Guanabara**. 16. Ed. Rio de Janeiro: Guanabara Koogan, 2009.

Krakowska, B.; Custers, D.; Deconinck, E.; Daszykowski, M. *Chemometrics and the identification of counterfeit medicines – A review*. **Journal of Pharmaceutical and Biomedical Analysis** 127, 2016, p. 112-122

Kristiansen, M.; Levy-Milne, R.; Barr, S., Flint, A. *Dietary Supplement Use by Varsity Athletes at a Canadian University*. **International Journal of Sport Nutrition and Exercise Metabolism** 15, 2005, p. 195 – 210

Kwok, K.; Taylor, L.S. *Analysis of countefeir Cialis® tablets using Raman microscopy and multivariate curve resolution*. **Journal of Pharmaceutical and Biomedical Analysis** 66, 2012, p. 126-135

Lenehan, P. **Anabolic Steroids and other performance enhancing drugs.** Taylor & Francis e-Library, 2004

Li, L.; Zang, H.; Li, J.; Chen, D.; Wang, F. *Identification of anisodamine tablets by Raman and near-infrared spectroscopy with chemometrics*. **Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy** 127, 2014, p. 91-97

Liddle, D.G.; Connor, D.J. *Nutritional supplements and ergogenic aids*. **Primary Care: Clinics in Office Practice** 40, 2013, p. 587-505

Linhares, T.C.; Lima, R.M. *Prevalência do uso de suplementos alimentares por praticantes de musculação nas academias de Campos dos Goytacazes/RJ, Brasil*. **Vértices** 8(1/3), 2006, p. 101-122

Maravelias, C.; Dona, A.; Stefanidou, M.; Spiliopoulou, C. *Adverse effects of anabolic steroids in athletes A constant threat.* **Toxicology Letters** 158, 2005, p. 167 – 175

Marchei, E.; Pellegrini, M.; Pacifici, R.; Palmi, I.; Pichini, S. *Development and validation of a high-performance liquid chromatography-mass spectrometry assay for methylxanthines and taurine in dietary supplements.* **Journal of Pharmaceutical and Biomedical Analysis** 37, 2005, p. 499-507

Marchei, M.; Pichini, S.; Pacifici, R.; Pellegrini, M. ; Zuccaro, P. *A rapid and simple procedure for the determination of synephrine in dietary supplements by gas chromatography-mass spectrometry.* **Journal of Pharmaceutical and Biomedical Analysis** 41, 2006, p. 1468-1472

Marchei, E.; Pellegrini, M.; Pacifici, R; Zuccaro, P.; Pichini, S. *A rapid and simple procedure for the determination of ephedrine alkaloids in dietary supplements by gas chromatography-mass spectrometry.* **Journal of Pharmaceutical and Biomedical Analysis** 41, 2006(b), p. 1633-1641

Marcheti, R.G.A. *Avaliação da falsificação de medicamentos a partir dos dados de laudos periciais do Departamento de Polícia Federal no Brasil no período de 2006 a 2012*. 2014. 89 f. Dissertação (Mestrado em Ciências Farmacêuticas) – Faculdade de Ciências da Saúde, Universidade de Brasília, Brasília. 2014.

Maughan, R.J. *Contamination of dietary supplements and positive drug tests in Sport*. **Journal of Sports Nutrition** 23(9), 2005, p. 883-889

Mazzarino,M.; Orengia, M.; Botrè, F. *Application of fast gas chromatography/mass spectrometry for the rapid screening of synthetic anabolic steroids and other drugs in anti-doping analysis*. **Rapid Communications in Mass Spectrometry** 21, 2007, p. 4117-4124

Morrison, L.J., Gizis, F.; Shorter, B. *Prevalent Use of Dietary Supplements Among People Who Exercise At a Commercial Gym*. **International Journal of Sport Nutrition and Exercise Metabolism** 14, 2004, p. 481 – 492

Musshoff, F.; Daldrup, T.; Ritsch, M. *Black Market in Anabolic Steroids – Analysis of Illegally Distributed Products.* **Journal of Forensic Sciences** 42 (6), 1997, p. 1119-1125

National Institute on Drug Abuse. *Research Report Series - Anabolic Steroid Abuse.* Disponível em <http://www.drugabuse.gov/sites/default/files/rrsteroids_0.pdf> Acesso em 13/10/2016

Nawrot, P.; Jordan, S.; Eastwood, J.; Rotstein, J.; Hugenholtz, A.; Feeley, M. *Effects of caffeine on human health*. **Food Additives and Contaminants** 20(1), 2003, p. 1 - 30

Nesheim, M.C. *Dietary Supplements.* **Nutrition** 14 (9), 1998, p. 729-730

Nestle, M. *Dietary Supplement Advertising: Policies Based on Politics, Not Science.* **Journal of Nutrition Education** 31 (5), 1999, p. 278 – 282

Neto, B.B.; Scarmínio, I.S.; Bruns, R.E. **Como fazer experimentos**. 2. Ed. Campinas: Editora da Unicamp, 2001

Nogueira, F.R.S.; Souza, A.A.; Brito, A.F. *Prevalência do uso e efeitos de recursos ergogênicos por praticantes de musculação nas academias brasileiras: uma revisão sistematizada.* **Revista Brasileira de Atividade Física & Saúde** 18 (1), 2013, p. 16-30

Odoardi, S.; Castrignanò, E.; Martello, S.; Chiarotti, M.; Strano-Rossi, S. Determination of anabolic agents in dietary supplements by liquid chromatography-high-resolution mass spectrometry. Food Additives & Contaminants: Part A 32(5), 2015, p. 635-647

Oliver, A.S.; León, M.T.M.; Guerra-Hernández, E. *Prevalence of protein supplement use at gyms*. **Nutrición Hospitalaria** 26(5), 2011, p. 1168 – 1174

Organização Mundial da Saúde. *International Medical Products Anti-Counterfeiting Taskforce – IMPACT Brochure*. Atualizada em maio/2008. Disponível em: <http://apps.who.int/medicinedocs/documents/s14149e/s14149e.pdf> Acesso em 13/10/2016.

Organização Mundial da Saúde. *Fact Sheet – Substandard, spurious, falsely labelled, falsified and counterfeit (SSFFC) medical products - January/2016*. Disponível em: <http://www.who.int/mediacentre/factsheets/fs275/en/> Acesso em 13/10/2016 (2016a)

Organização Mundial da Saúde. *Definitions on SSFFC Medical Products*. Disponível em: <http://www.who.int/medicines/regulation/ssffc/definitions/en/> Acesso em 13/10/2016 (2016b)

Ortiz, R.S.; Mariotti, K.C.; Fank, B.; Limberger, R.P.; Anzanello, M.J.; Mayorga, P. *Counterfeit Cialis and Viagra fingerprinting by ATR-FTIR spectroscopy with chemometry: Can the same pharmaceutical poder mixture be used to falsify two medicines?* **Forensic Science International** 226, 2013, p. 282-289

Parra, R.M.T.; Palma, A.; Pierucci, A.P.T.R. *Contaminação de suplementos dietéticos usados para prática esportiva: uma revisão de literatura.* **Revista Brasileira de Ciências do Esporte** 33 (4), 2011, p. 1071 – 1084

Pellegrini, M.; Rotolo, M.C.; Di Giovannadrea, R.; Pacifici, R.; Pichini, S. *A simple toxicological analysis of anabolic steroid preparations from the black market.* **Annales de Toxicologie Analytique**, 24 (2), 2012, p. 67 – 72Shahidi, N.T. *A Review of the Chemistry, Biological Action, and Clinical Applications of Anabolic-Androgenic Steroids.* **Clinical Therapeutics** 23 (9), 2001, p. 1355 – 1390

Pereira, R.F.; Lajolo, F.M.; Hirschbruch, M.D. *Consumo de suplementos por alunos de academias de ginástica em São Paulo*. **Revista de Nutrição** 16(3), 2003, p. 265-272

Poll, F.A.; Lima, A.P. *Consumo de suplementos alimentares por universitários da área da saúde*. **Cinergis** 1(1), 2013, p. 33-37

Pretsch,E.; Bühlmann, P.; Badertscher, M. **Structure determination of organic compounds**. 4th Ed., Springer-Verlag, Berlin, 2009.

Prokudina, E.A.; Prchalová, J.; Vysatová, E.; Kuchai, M.; Rajchl, A.; Lapcik, O. *Analysis of anabolic androgenic steroids by direct analysis in real time ionization with time-of-flight mass spectrometry*. **International Journal of Mass Spectrometry** 392, 2015, p. 28-33

Rajalahti, T.; Kvalheim, O.M. *Multivariate data analysis in pharmaceutics: a tutorial review.* **International Journal of Pharmaceutics** 417, 2011, p. 280-290

Rang., H.P.; Dale, M.M.; Ritter, J.M. **Farmacologia**. 3. Ed. Guanabara Koogan, 1997, Rio de Janeiro.

Rebiere, H.; Ghyselinck, C.; Lempereur, L.; Brenier,C. *Investigation of the composition of anabolic tablets using near infrared spectroscopy and Raman chemical imaging*. **Drug Testing and Analysis** 8, 2016, p. 370-377

Ritsch, M.; Musshoff, F. *Dangers and risks of black market anabolic steroid abuse in sports – gas chromatography-mass spectrometry analyses.* **Sportverletz Sportschaden** 14(1), 2000, p. 1–11

Rocha, T.; Amaral, J.S.; Oliveira, M.B.P.P. *Adulteration of dietary supplements by the illegal addition of synthetic drugs: A review*. **Comprehensive Reviews in Food Science and Food Safety** 15, 2016, p. 43-62

Rocha, L.P.; Pereira, M.V.L. *Consumo de suplementos nutricionais por praticantes de exercícios físicos em academias*. **Revista de nutrição** 11(1), 1998, p. 76-82

Rodionova, O.Y.; Titova, A.V.; Pomerantsev, A.L. *Discriminant analysis is an inappropriate method of authentication.* **Trends in Analytical Chemistry** 78(4), 2016, p. 17-22

Roggo, Y.; Dégardin,K.; Margot, P. *Identification of pharmaceutical tablets by Raman spectroscopy and chemometrics*. **Talanta** 81, 2010, p. 988-995

Rovira, M-A; Grau, M.; Castañer, O.; Covas, M-I; Schröder, H. *Dietary Supplement Use and Health-Related Behaviours in a Mediterranean Population.* **Journal of Nutrition Education and Behaviour** 45 (5), 2013, p. 386 - 391

Sacré, P.-Y., Deconinck, E., De Beer, T., Courselle, P., Vancauwenberghe, R., Chiap, P., Crommen, J., De Beer, J.O. *Comparison and combination of spectroscopic techniques for the detection of counterfeit medicines*. **Journal of Pharmaceutical and Biomedical Analysis** 53, 2010, p. 445-453

Saeedi, P.; Nasir, M.T.M.; Hazizi, A.S.; Vafa, M.R.; Foroushani, A.R. *Nutritional supplement use among fitness club participans in Tehran, Iran*. **Appetite** 60, 2013, p. 20 – 26.

Santana, J.; Sharpless, E.; Nelson, B.C. *Determination of para-synephrine and meta-synephrine positional isomers in bitter orange-containing dietary supplements by LC/UV and LC/MS/MS.* **Food Chemistry** 109, 2008, p. 675-682

Santos, K.M.O.; Barros Filho, A.A. *Consumo de produtos vitamínicos entre universitários de São Paulo, SP*. **Revista de Saúde Pública** 36(2), 2002, p. 250-253

Scafi, S.H.F. e Pasquini, C. *Identification of counterfeit drugs using near-infrared Spectroscopy*. **The Analyst** 126, 2001, p. 2218-2224

Schwenk, T.L.; Costley, C.F. *When food becomes a drug: Nonanabolic nutritional supplement use in athletes*. **American Journal of Sports Medicine** 30(6), 2002, p 907-916

Seeram, N.P.; Henning, S.M.; Niu, Y.; Lee, R.; Scheuller, H.S.; Heber,D. *Catechin and caffeine content of green tea dietary supplements and correlation with antioxidant capacity*. **Journal of Agricultural and Food Chemistry** 54, 2006, p. 1599-1603

Shahidi, N.T. *A Review of the Chemistry, Biological Action, and Clinical Applications of Anabolic-Androgenic Steroids.* **Clinical Therapeutics** 23 (9), 2001, p. 1355 – 1390.

Sheppard, H.L.; Raichada, S.M.; Kouri, K.M.; Stenson-Bar-Maor, L.; Branch, J.D. *Use of Creatine and Other Supplements by Members of Civilian and Military Health Clubs: A Cross-Sectional Survey*. **International Journal of Sport Nutrition and Exercise Metabolism** 10, 2000, p. 245-256

Silva, P.R.P.; Danielski, R.; Czepielewski, M.A. *Esteroides anabolizantes no esporte.* **Revista Brasileira de Medicina do Esporte** 8 (6), 2002, p. 235 – 243

Silva, A.A.; Marins, J.C.B. *Consumo e nível de conhecimento sobre recursos ergogênicos nutricionais em atletas*. **Bioscience Journal** 29(4), 2013, p. 1038-1048

Silva, V.A.G.; Talhavini, M.; Zacca, J.J.; Trindade, B.R.; Braga, J.W.B. Discrimination of black pen inks on writing documents using Visible Reflectance Spectroscopy and PLS-DA. Journal of the Brazilian Chemical Society 25(9), 2014, p. 1552-1564

Skeie, G.; Braaten, T.; Hjartaker, A.; Lentjes, M.; Amiano, P.; Jakszyn, P.; Pala, V.; Palanca, A.; Niekerk, E.M.; Verhagen, H.; Avloniti, K.; Psaltopoulou, T.; Niravong, M.; Touvier, M.; et al. *Use of dietary supplements in the European Prospective Investigation into Cancer and Nutrition calibration study*. **European Journal of Clinical Nutrition** 63, 2009, S226-S238

Snyder, P.J. *Androgênios*. In: Hardman, J.G.; Limbird, L.E.; Gilman, A.G.,eds. **Goodman & Gilman, As Bases Farmacológicas da Terapêutica.** 10. ed. Rio de Janeiro: McGraw-Hill Interamericana do Brasil, 2005. Cap. 59, p. 1231 – 1240

Sousa, A.G.; Costa, T.H.M. *Usual coffee intake in Brazil: results from the National Dietary Survey 2008-9*. **British Journal of Nutrition** 113, 2015, p. 1615 – 1620

Storme-Paris, I.; Rebiere, H.; Matoga, M.; Ciaved, C.; Bonnet, P.-A.; Tissier, M.H.; Chaminade, P. *Challenging Near InfraRed spectroscopy discriminating ability for counterfeit pharmaceuticals detection*. **Analytica Chimica Acta** 658, 2010, p. 163-174

Sturmi, J.E.; Diorio, D.J. *Anabolic Agents*. **Sports Pharmacology** 17 (2), 1998, p. 261 – 282

The Nobel Prize in Chemistry 1939. *Nobelprize.org.* Nobel Media AB 2014. Web. 3 Sep 2016. <http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1939/>

Thevis, M.; Schrader, Y.; Thomas, A.; Sigmund, G.; Geyer, H.; Schänzen, W. *Analysis of Confiscates Black Market Drugs Using Chromatographic and Mass Spectrometric Approaches.* **Journal of Analytical Toxicology** 32, 2008, p. 232 – 240

Van Eenoo, P.; Van Gansbeke, W.; De Brabanter, N.; Deventer, K.; Delbeke, F.T. *A fast, comprehensive screening method for doping agents in urine by gas chromatography-triple quadrupole mass spectrometry*. **Journal of Chromatography A** 1218, 2011, p.3306-3316

Van Poucke, C.; Detavernier, C.; Van Cauwenberghe, R.; Van Peteghem, C. *Determination of anabolic steroids in dietary supplements by liquid chromatography-tandem mass spectrometry*. **Analytica Chimica Acta** 586, 2007, p. 35-42

Vredenbregt, M.J.; Blok-Tip, L.; Hoogerbrugge, R.; Barends, D.M.; de Kaste, D. *Screening suspected counterfeit Viagra® and imitations of Viagra® with near-infrared spectroscopy*. **Journal of Pharmaceutical and Biomedical Analysis** 40, 2006, p. 840-849

Westerterp-Plantega, M.; Diepvens, K.; Joosen, A.M.C.P.; Bérubé-Parent, S.; Tremblay, A. *Metabolic effects of spices, teas, and caffeine*. **Physiology & Behavior** 89, 2006, p. 85-91

Zhao, Y.; Ji, N.; Yin, L.; Wang, J. *A non-invasive method for the determination of liquid injectables by Raman Spectroscopy*. **AAPS PharmSciTech** 16(4), 2015, p. 914-921

## Apêndice A

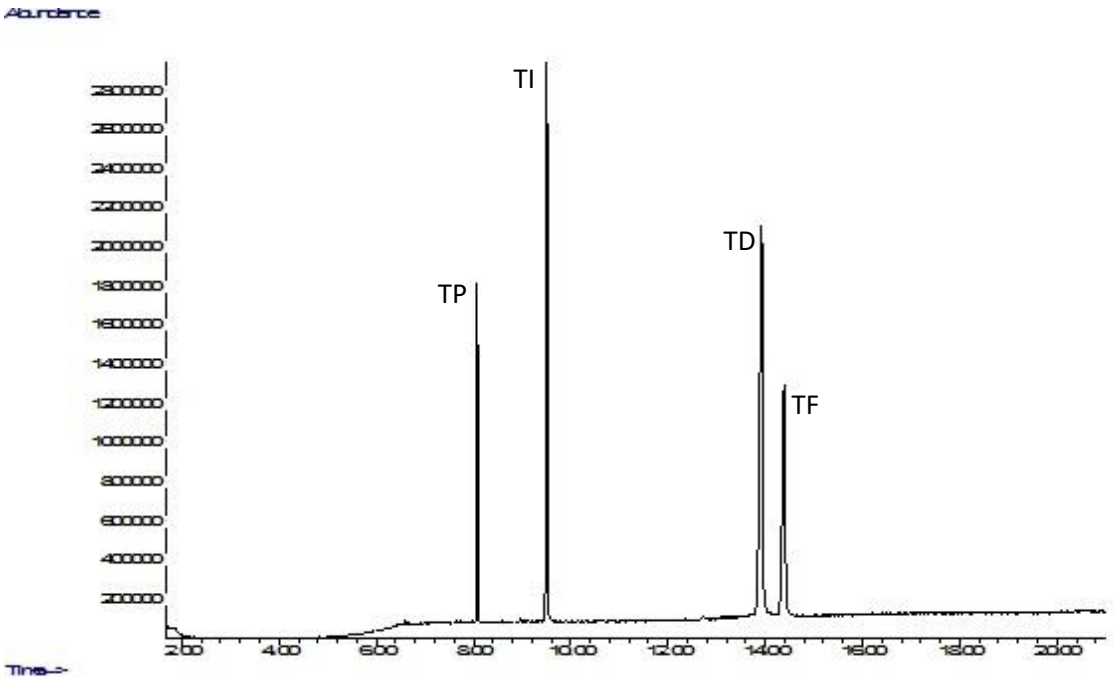



Figure 1: Representative Total Ion Chromatogram of an original Durateston sample. The four peaks refer to testosterone propionate (TP), isocaproate (TI), decanoate (TD) and phenpropionate (TF).

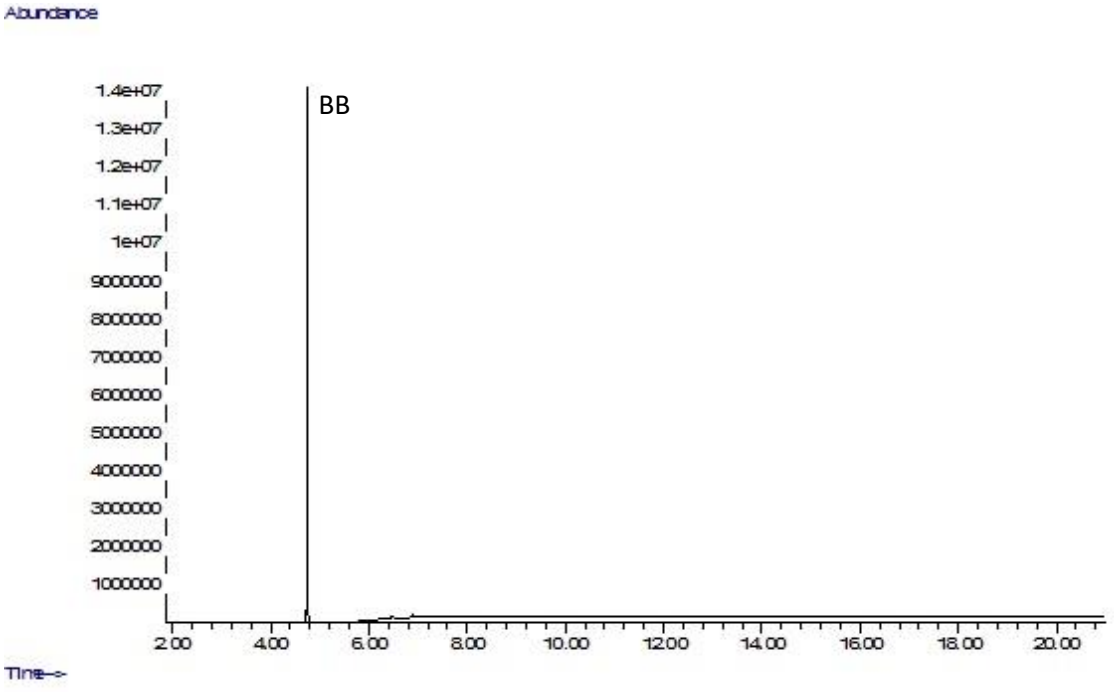



Figure 2: Representative Total Ion Chromatogram of a BB counterfeit. The large peak at approximately 5 minutes refers to benzyl benzoate (BB).

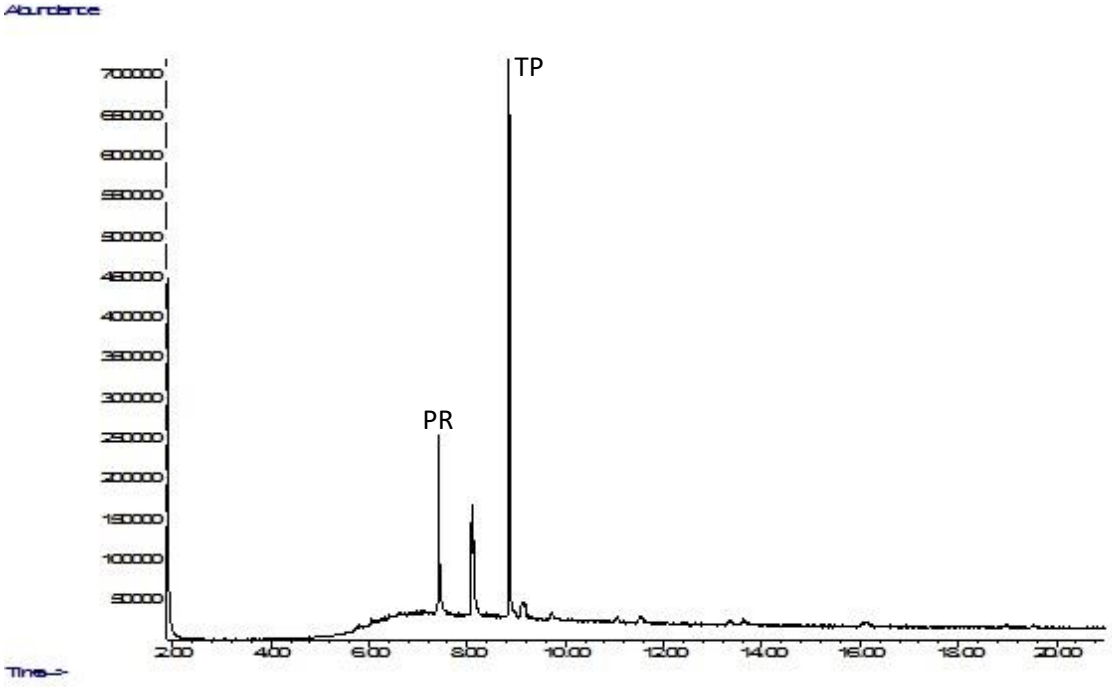



Figure 3: Representative Total Ion Chromatogram of a TP_PR counterfeit. PR indicates the prasterone peak and TP the testosterone propionate peak. The visible peak between them could not be identified, but seems to be another steroidal molecule.

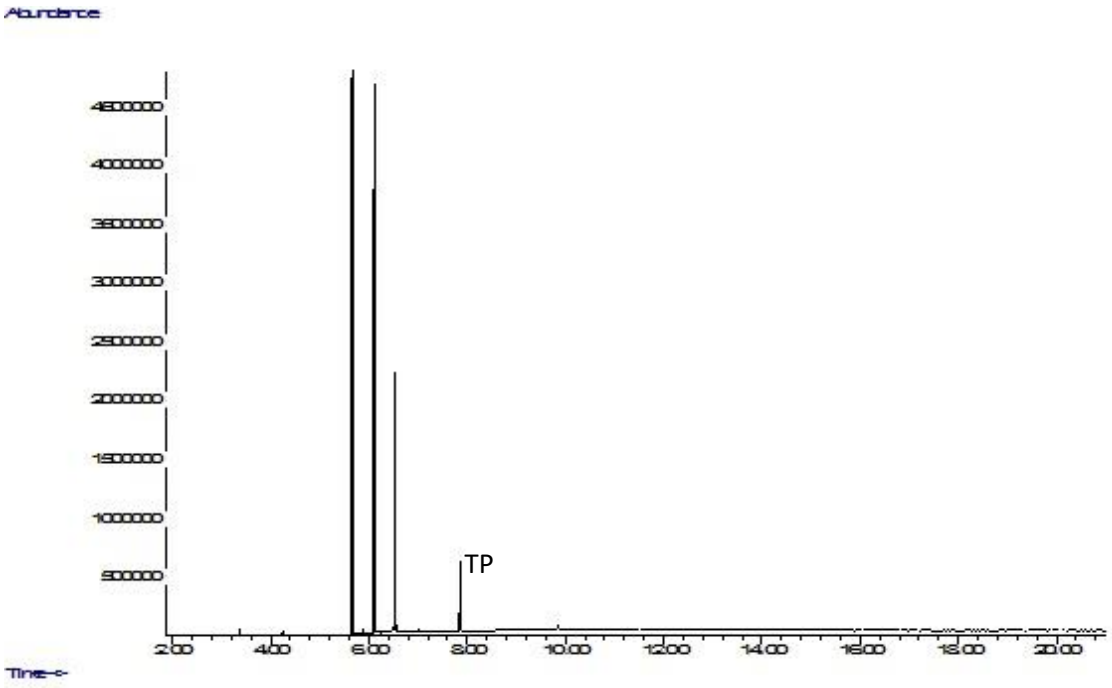



Figure 4: Representative Total Ion Chromatogram of a TP counterfeit. TP indicates the testosterone propionate peak. The three large peaks around 6 minutes refer to long chain esters such as allyl caprylate.

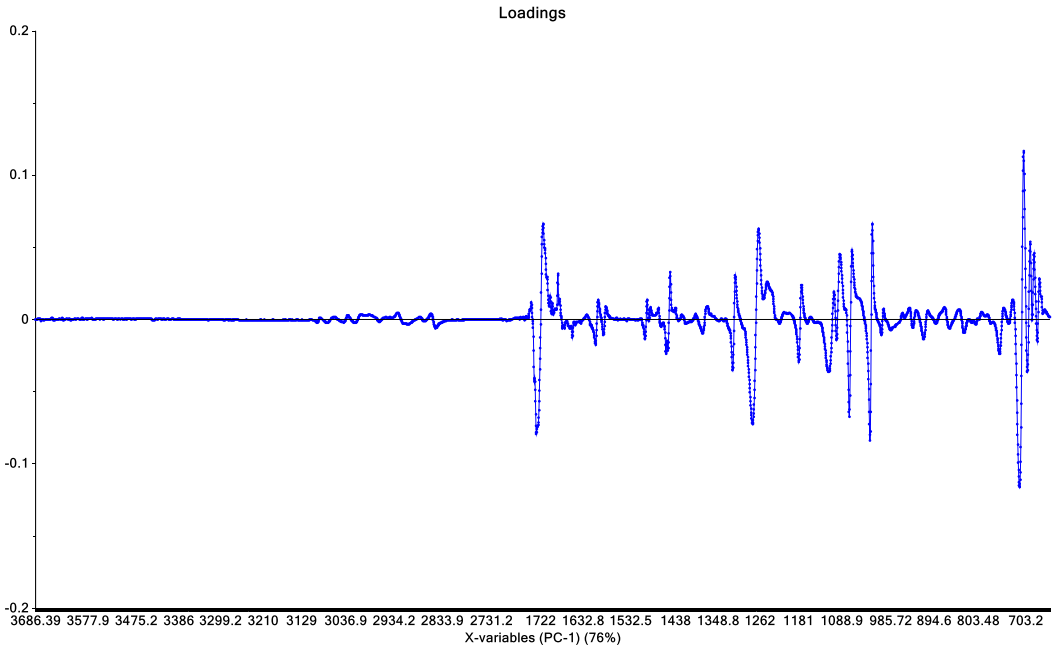



Figure 5: Loadings plot of the first Principal Component.

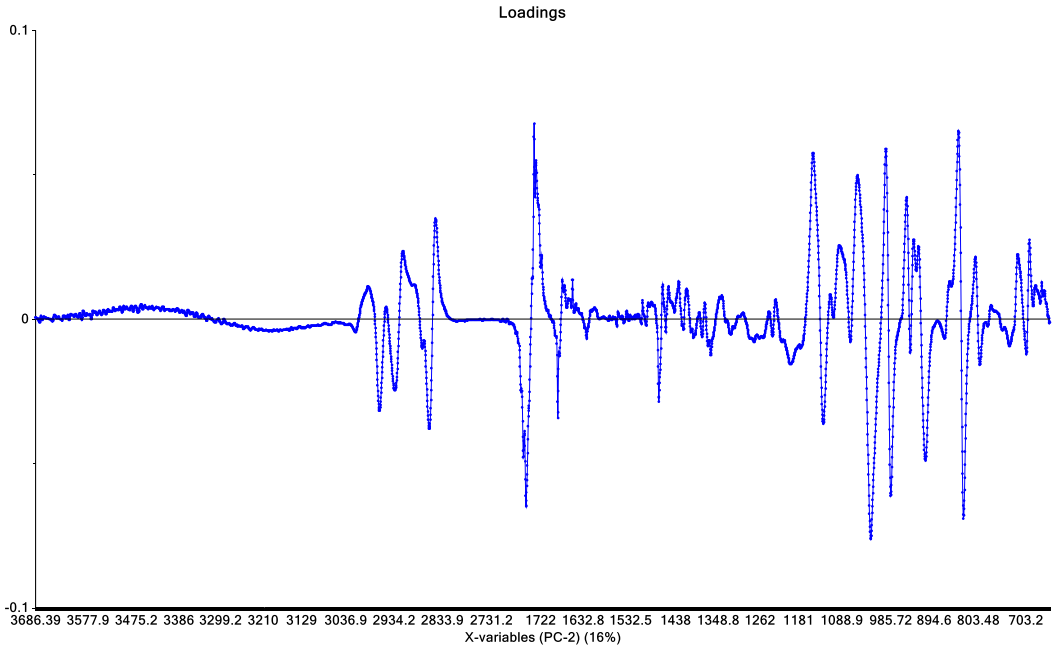



Figure 6: Loadings plot of the second Principal Component.

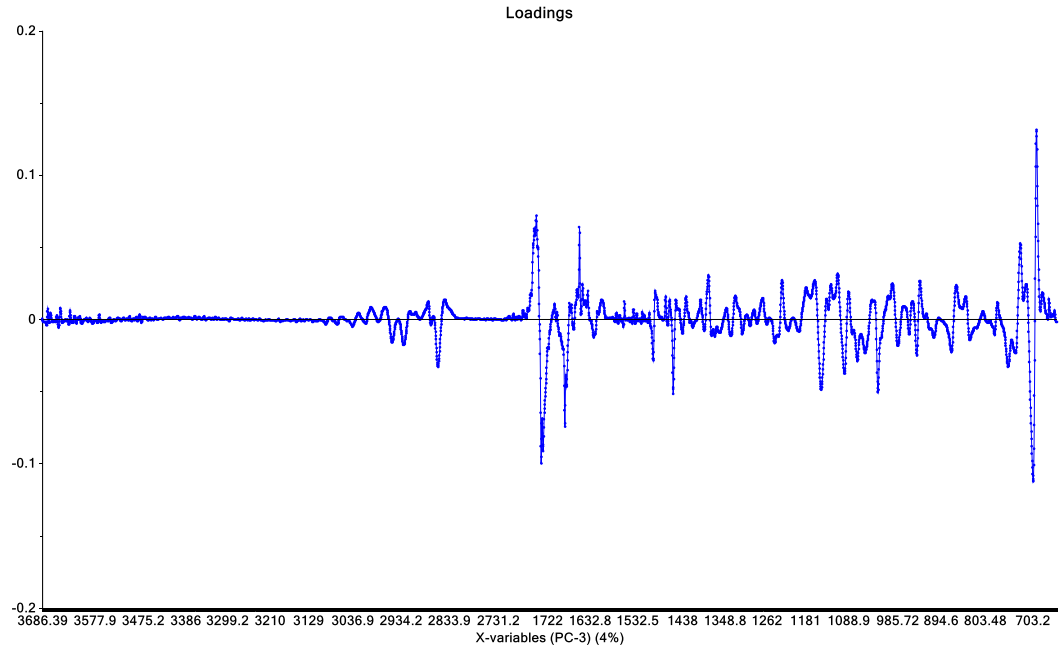



Figure 7: Loadings plot of the third Principal Component.

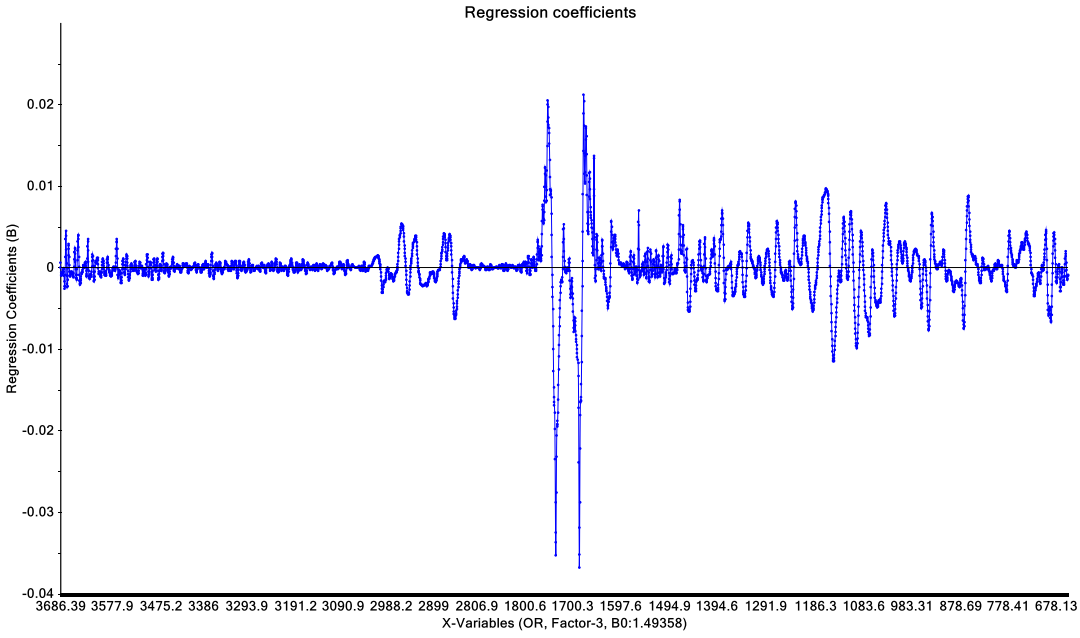



Figure 8: Regression Coefficients of the X variables of the PLS-DA model.

## Apêndice B

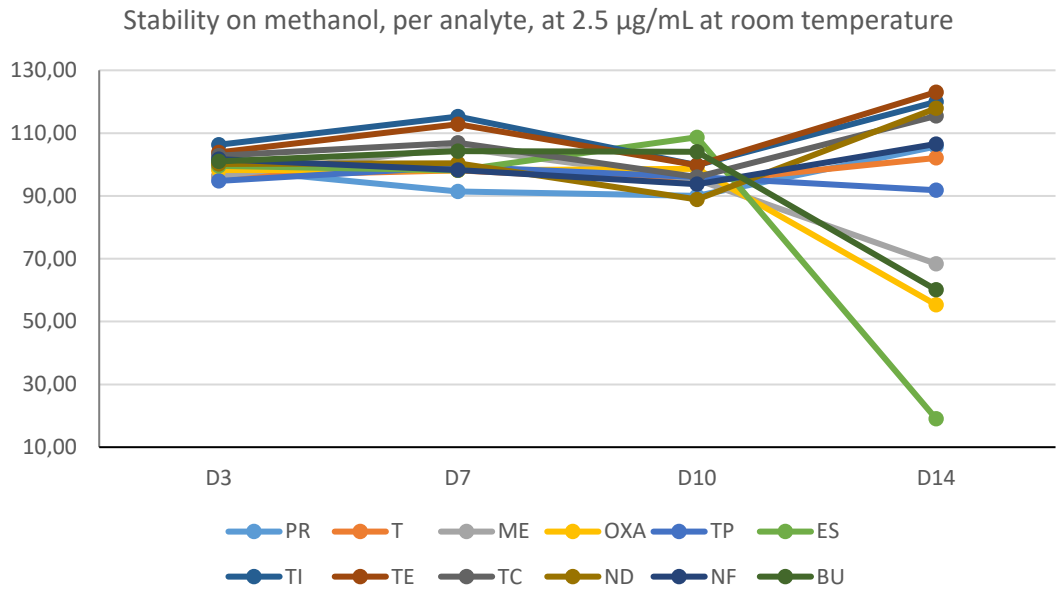



Figure 1: Stability of the AAS Prasterone (PR), Testosterone (T), Methandrostenolone (ME), Oxandrolone (OXA), Testosterone Propionate (TP), Testosterone Isocaproate (TI), Testosterone Enantate (TE), Testosterone Cypionate (TC), Nandrolone Decanoate (ND), Nandrolone Phenylpropionate (NF) and Boldenone Undecylenate (BU) in metanol, per analyte, at 2.5 µg/mL, kept at room temperature for 3, 7, 10 and 14 days. N=3 samples.

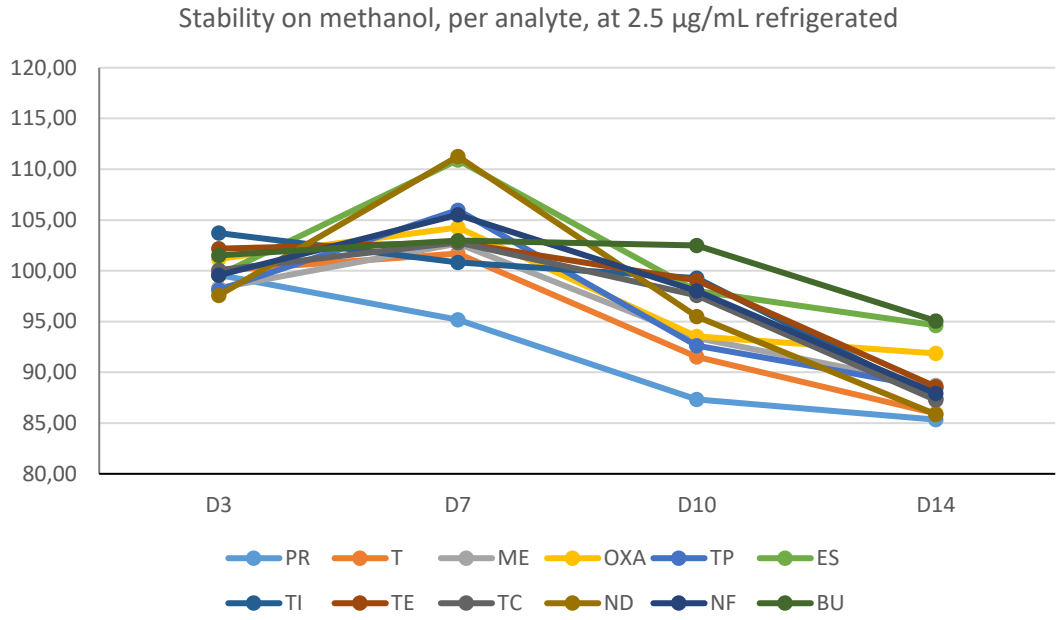



Figure 2: Stability of the AAS Prasterone (PR), Testosterone (T), Methandrostenolone (ME), Oxandrolone (OXA), Testosterone Propionate (TP), Testosterone Isocaproate (TI), Testosterone Enantate (TE), Testosterone Cypionate (TC), Nandrolone Decanoate (ND), Nandrolone Phenylpropionate (NF) and Boldenone Undecylenate (BU) in metanol, per analyte, at 2.5 µg/mL, kept refrigerated for 3, 7, 10 and 14 days. N=3 samples.

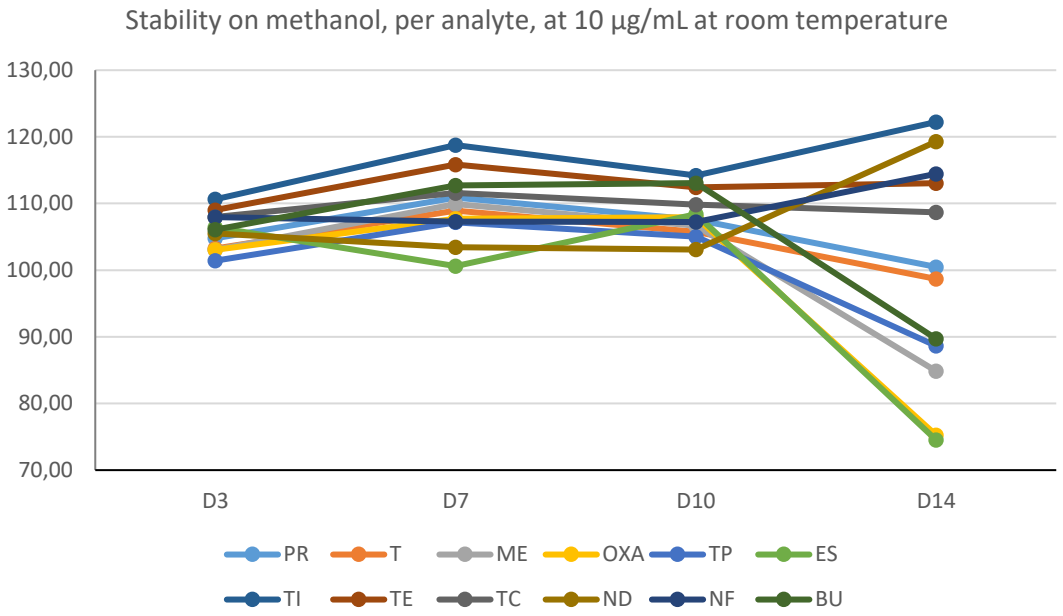



Figure 3: Stability of the AAS Prasterone (PR), Testosterone (T), Methandrostenolone (ME), Oxandrolone (OXA), Testosterone Propionate (TP), Testosterone Isocaproate (TI), Testosterone Enantate (TE), Testosterone Cypionate (TC), Nandrolone Decanoate (ND), Nandrolone Phenylpropionate (NF) and Boldenone Undecylenate (BU) in metanol, per analyte, at 10 µg/mL, kept at room temperature for 3, 7, 10 and 14 days. N=3 samples.

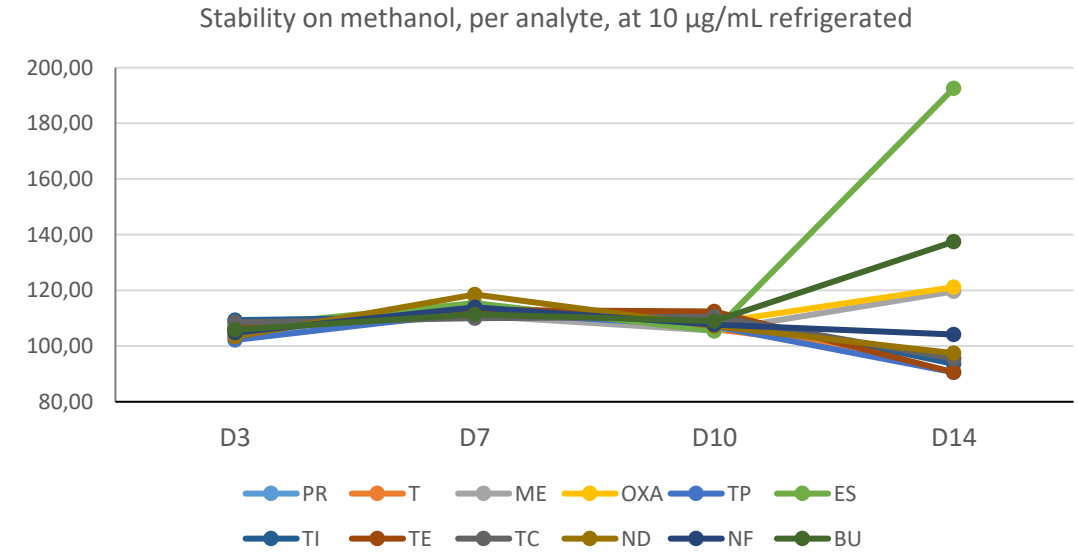



Figure 4: Stability of the AAS Prasterone (PR), Testosterone (T), Methandrostenolone (ME), Oxandrolone (OXA), Testosterone Propionate (TP), Testosterone Isocaproate (TI), Testosterone Enantate (TE), Testosterone Cypionate (TC), Nandrolone Decanoate (ND), Nandrolone Phenylpropionate (NF) and Boldenone Undecylenate (BU) in metanol, per analyte, at 10 µg/mL, kept refrigerated for 3, 7, 10 and 14 days. N=3 samples.

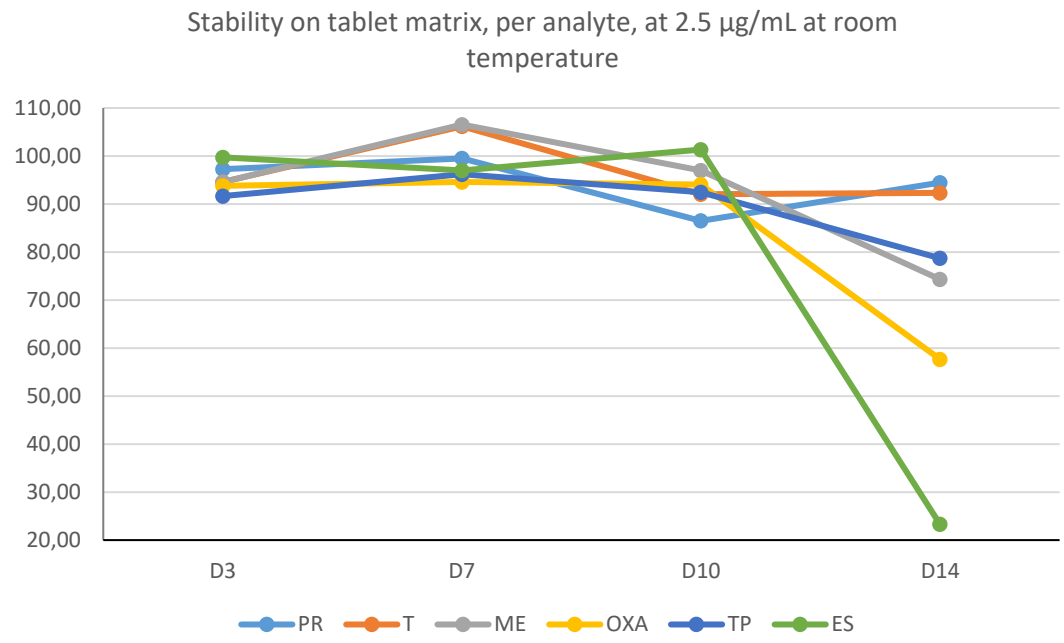



Figure 5: Stability of the AAS Prasterone (PR), Testosterone (T), Methandrostenolone (ME), Oxandrolone (OXA), Testosterone Propionate (TP) and Stanozolol (ES) in tablet matrix, per analyte, at 2.5 µg/mL, kept at room temperature for 3, 7, 10 and 14 days. N=3 samples.

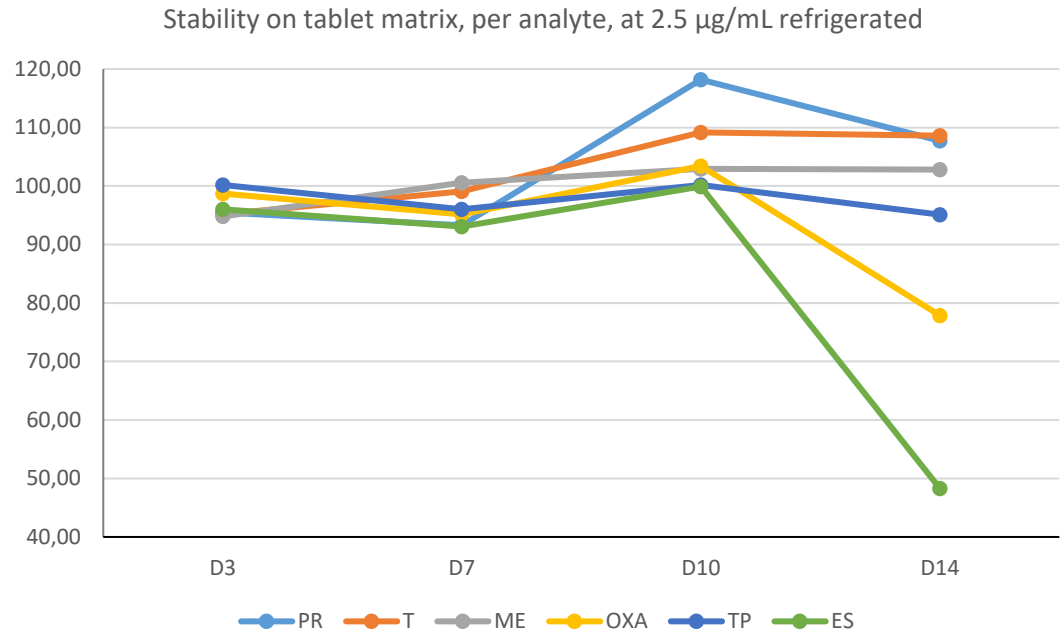



Figure 6: Stability of the AAS Prasterone (PR), Testosterone (T), Methandrostenolone (ME), Oxandrolone (OXA), Testosterone Propionate (TP) and Stanozolol (ES) in tablet matrix, per analyte, at 2.5 µg/mL, kept refrigerated for 3, 7, 10 and 14 days. N=3 samples.

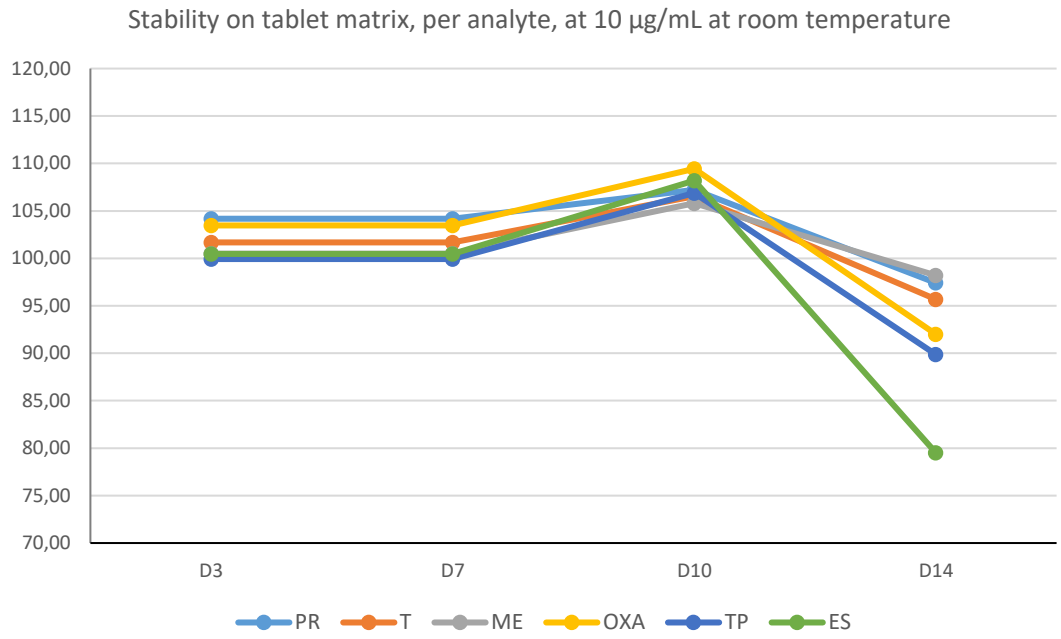



Figure 7: Stability of the AAS Prasterone (PR), Testosterone (T), Methandrostenolone (ME), Oxandrolone (OXA), Testosterone Propionate (TP) and Stanozolol (ES) in tablet matrix, per analyte, at 10 µg/mL, kept at room temperature for 3, 7, 10 and 14 days. N=3 samples.

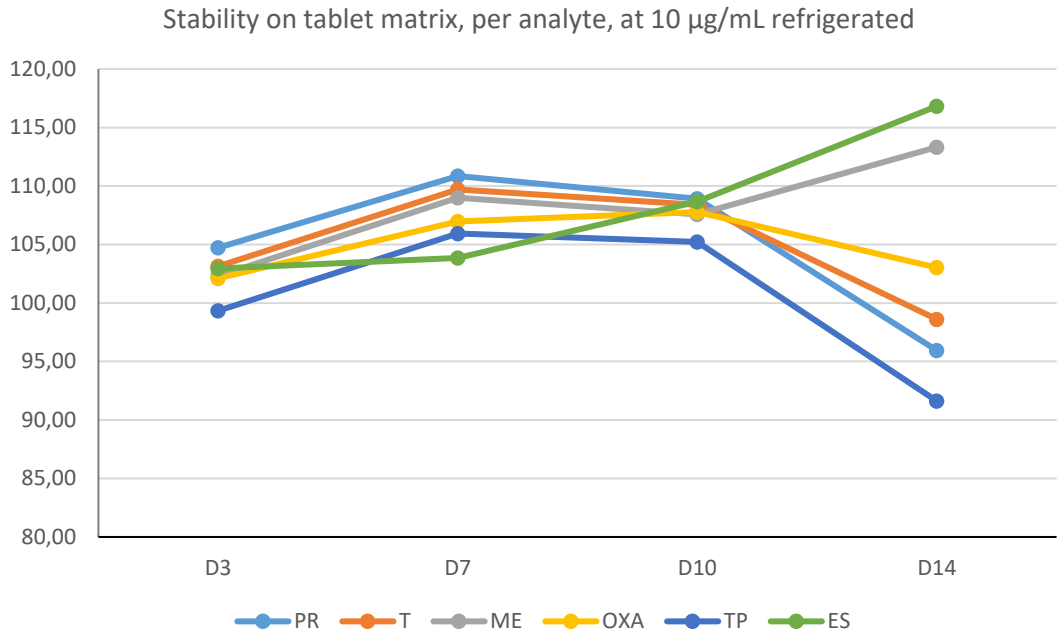



Figure 8: Stability of the AAS Prasterone (PR), Testosterone (T), Methandrostenolone (ME), Oxandrolone (OXA), Testosterone Propionate (TP) and Stanozolol (ES) in tablet matrix, per analyte, at 10 µg/mL, kept refrigerated for 3, 7, 10 and 14 days. N=3 samples.

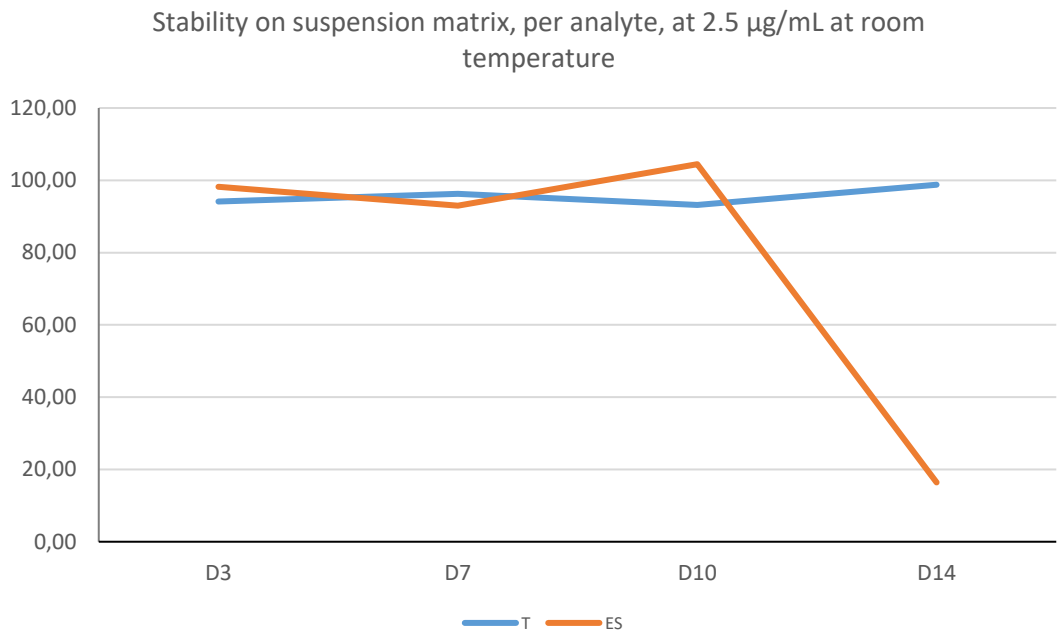



Figure 9: Stability of the AAS Testosterone (T) and Stanozolol (ES) in suspension matrix, per analyte, at 2.5 µg/mL, kept at room temperature for 3, 7, 10 and 14 days. N=3 samples.

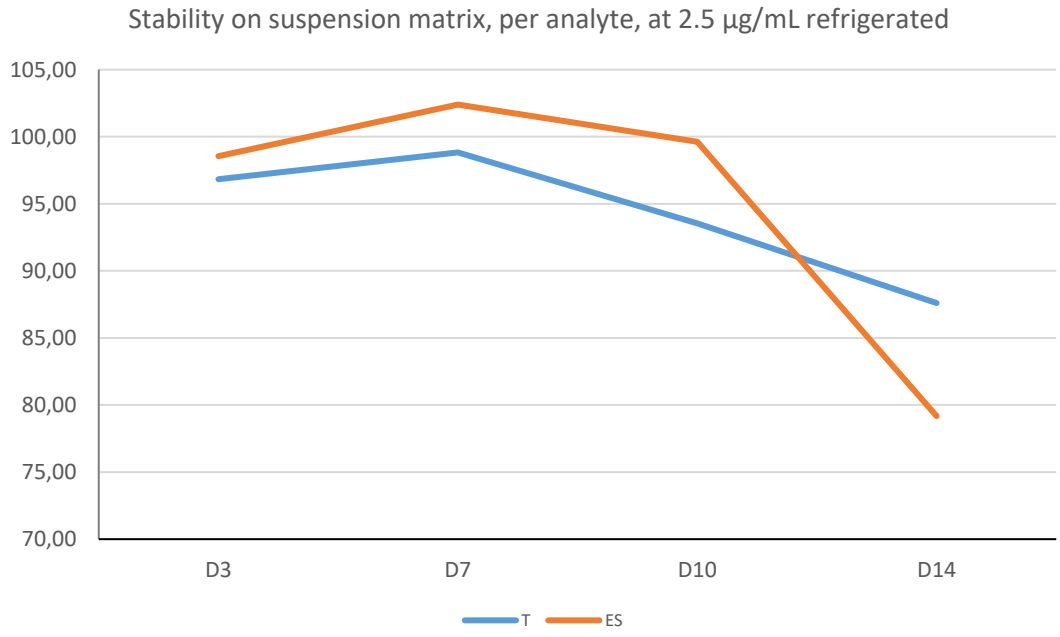



Figure 10: Stability of the AAS Testosterone (T) and Stanozolol (ES) in suspension matrix, per analyte, at 2.5 µg/mL, kept refrigerated for 3, 7, 10 and 14 days. N=3 samples.

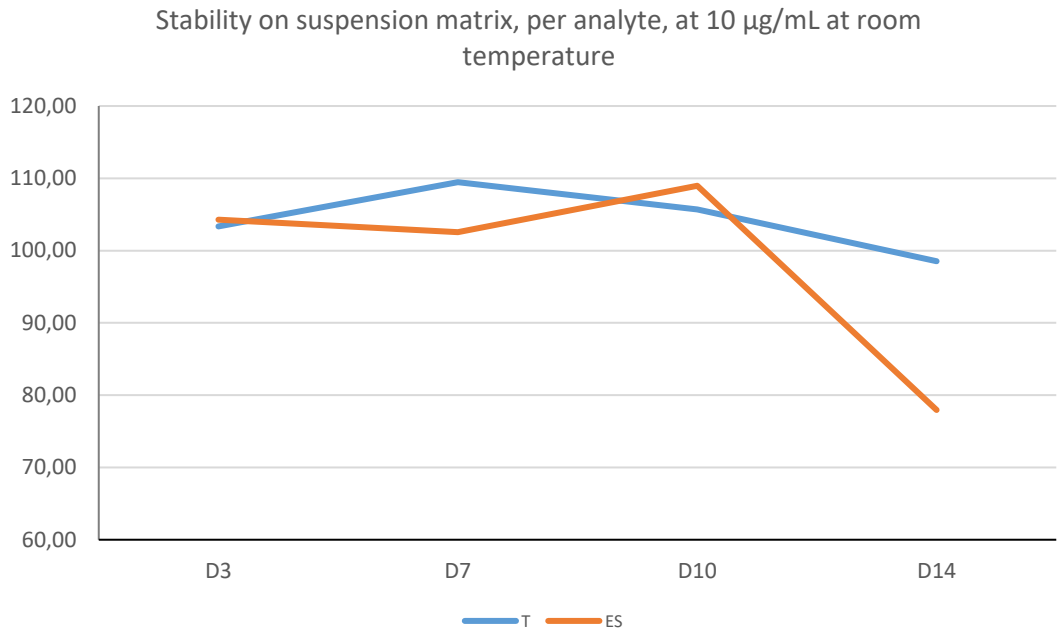



Figure 11: Stability of the AAS Testosterone (T) and Stanozolol (ES) in suspension matrix, per analyte, at 10 µg/mL, kept at room temperature for 3, 7, 10 and 14 days. N=3 samples.

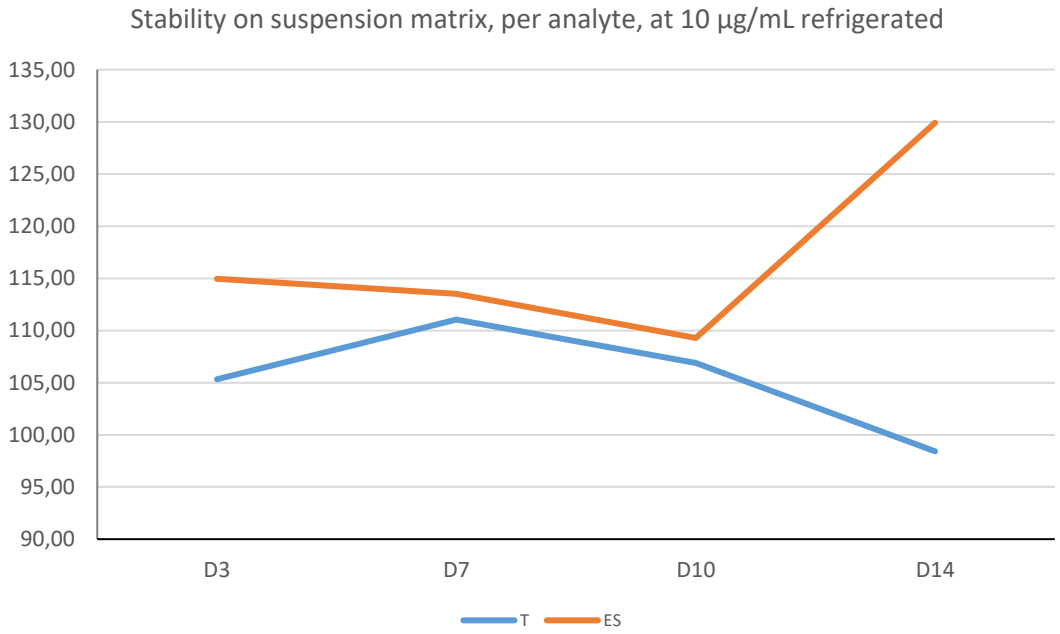



Figure 12: Stability of the AAS Testosterone (T) and Stanozolol (ES) in suspension matrix, per analyte, at 10 µg/mL, kept refrigerated for 3, 7, 10 and 14 days. N=3 samples.

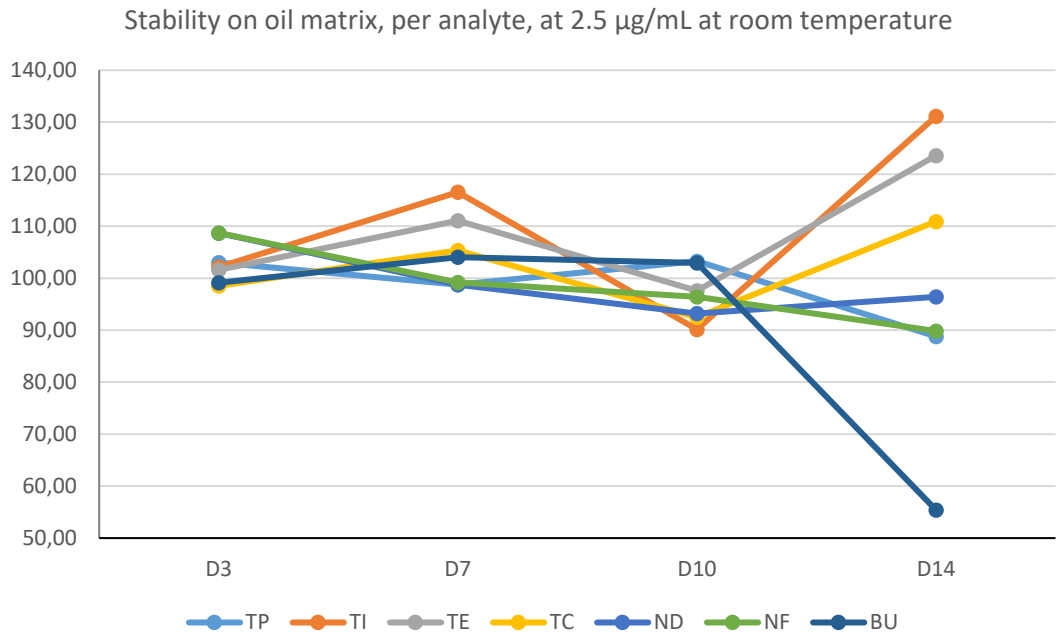



Figure 13: Stability of the AAS Testosterone Propionate (TP), Testosterone Isocaproate (TI), Testosterone Enantate (TE), Testosterone Cypionate (TC), Nandrolone Decanoate (ND), Nandrolone Phenylpropionate (NF) and Boldenone Undecylenate (BU) in oil matrix, per analyte, at 2.5µg/mL, kept at room temperature for 3, 7, 10 and 14 days. N=3 samples.

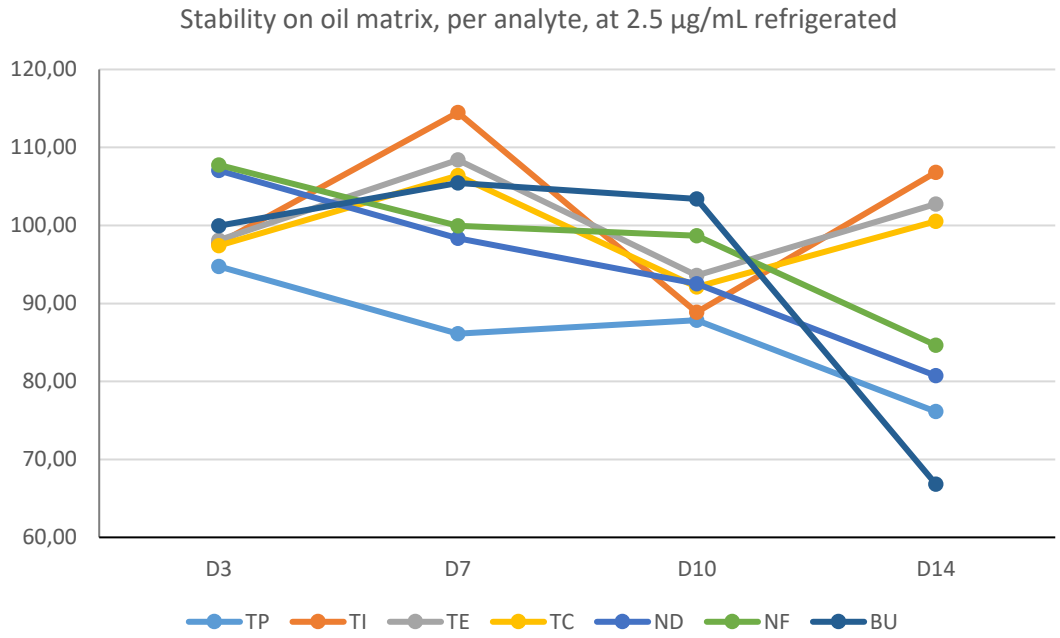



Figure 14: Stability of the AAS Testosterone Propionate (TP), Testosterone Isocaproate (TI), Testosterone Enantate (TE), Testosterone Cypionate (TC), Nandrolone Decanoate (ND), Nandrolone Phenylpropionate (NF) and Boldenone Undecylenate (BU) in oil matrix, per analyte, at 2.5µg/mL, kept refrigerated for 3, 7, 10 and 14 days. N=3 samples.

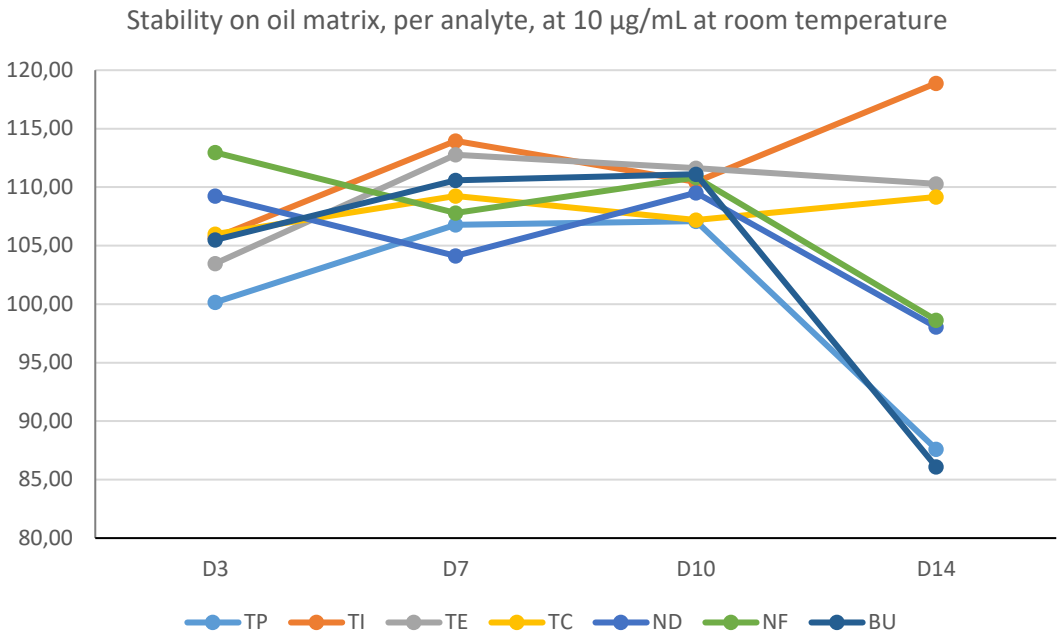



Figure 15: Stability of the AAS Testosterone Propionate (TP), Testosterone Isocaproate (TI), Testosterone Enantate (TE), Testosterone Cypionate (TC), Nandrolone Decanoate (ND), Nandrolone Phenylpropionate (NF) and Boldenone Undecylenate (BU) in oil matrix, per analyte, at 10 µg/mL, kept at room temperature for 3, 7, 10 and 14 days. N=3 samples.

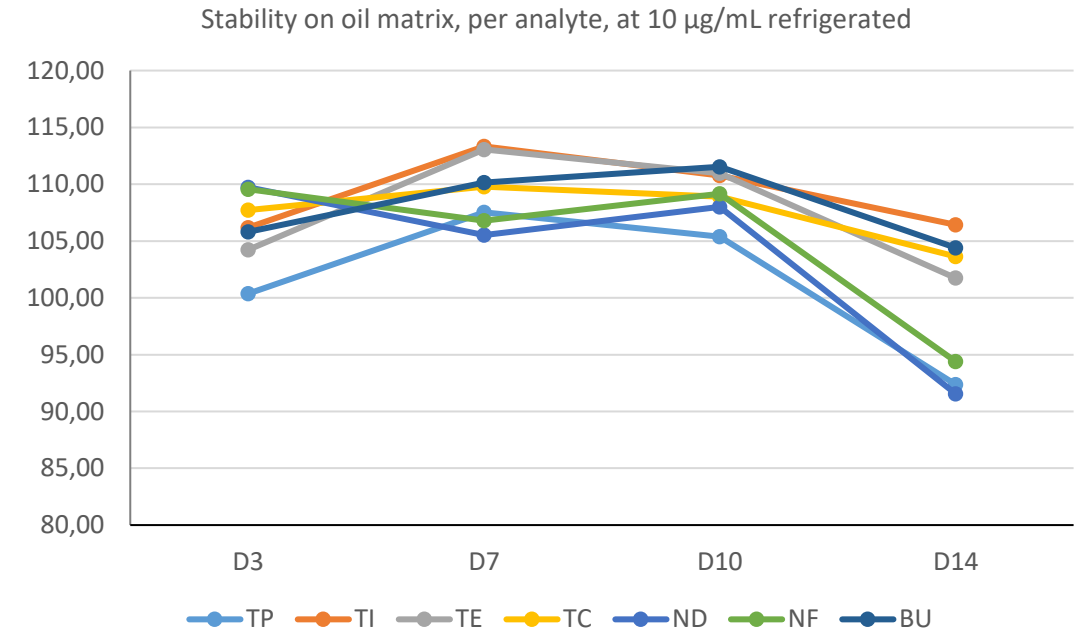



Figure 16: Stability of the AAS Testosterone Propionate (TP), Testosterone Isocaproate (TI), Testosterone Enantate (TE), Testosterone Cypionate (TC), Nandrolone Decanoate (ND), Nandrolone Phenylpropionate (NF) and Boldenone Undecylenate (BU) in oil matrix, per analyte, at 10 µg/mL, kept refrigerated for 3, 7, 10 and 14 days. N=3 samples.

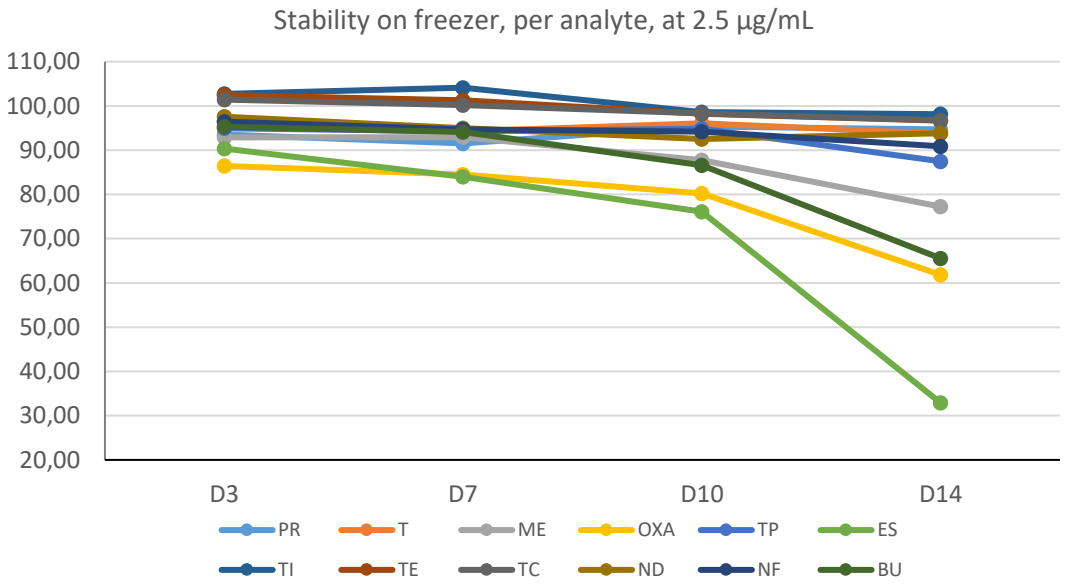



Figure 17: Stability of the AAS Prasterone (PR), Testosterone (T), Methandrostenolone (ME), Oxandrolone (OXA), Testosterone Propionate (TP), Testosterone Isocaproate (TI), Testosterone Enantate (TE), Testosterone Cypionate (TC), Nandrolone Decanoate (ND), Nandrolone Phenylpropionate (NF) and Boldenone Undecylenate (BU) in metanol, per analyte, at 2.5 µg/mL, kept frozen for 3, 7, 10 and 14 days. N=4 samples for D3, 3 samples for D7, 2 samples for D10 and one sample for D14.

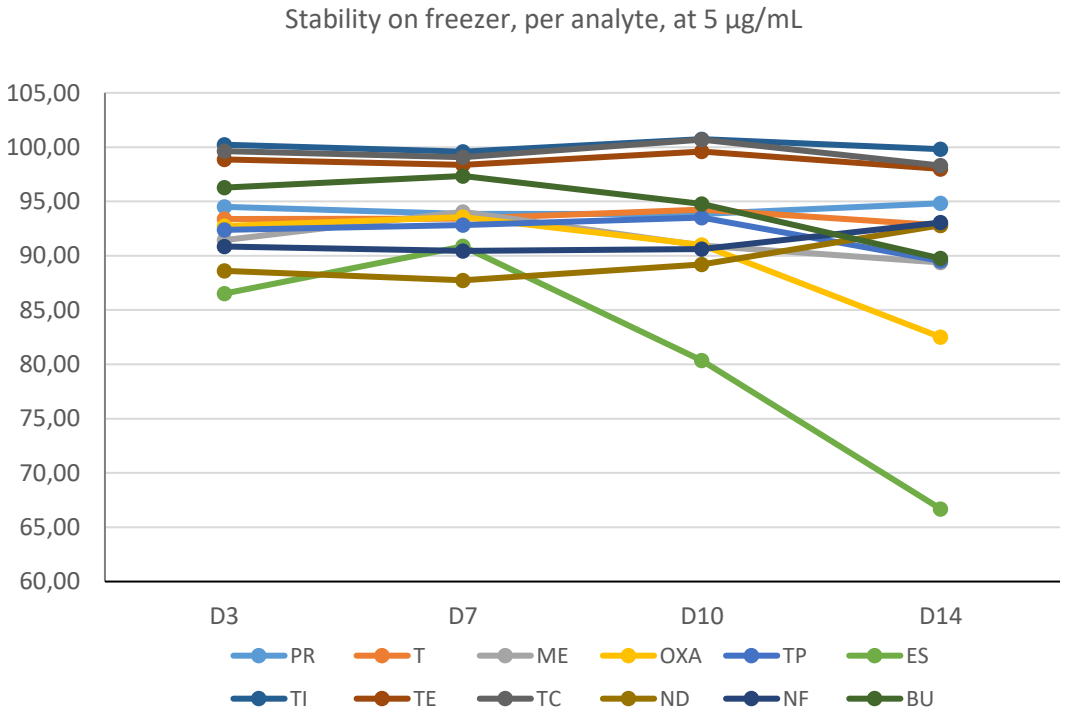



Figure 18: Stability of the AAS Prasterone (PR), Testosterone (T), Methandrostenolone (ME), Oxandrolone (OXA), Testosterone Propionate (TP), Testosterone Isocaproate (TI), Testosterone Enantate (TE), Testosterone Cypionate (TC), Nandrolone Decanoate (ND), Nandrolone Phenylpropionate (NF) and Boldenone Undecylenate (BU) in metanol, per analyte, at 5 µg/mL, kept frozen for 3, 7, 10 and 14 days. N=4 samples for D3, 3 samples for D7, 2 samples for D10 and one sample for D14.

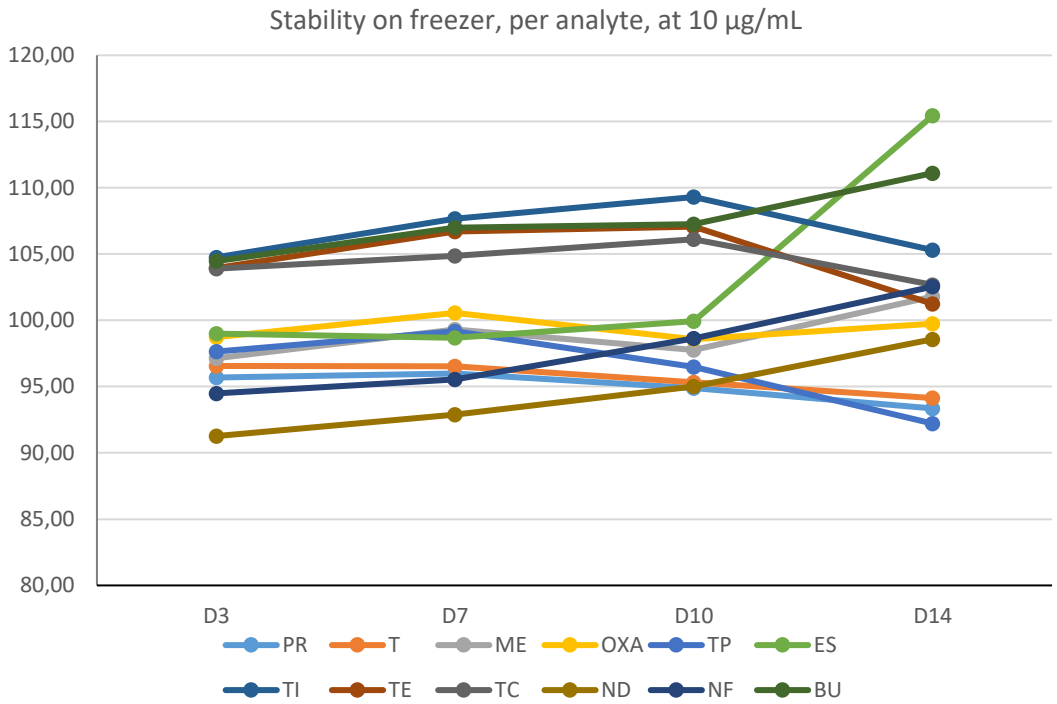



Figure 19: Stability of the AAS Prasterone (PR), Testosterone (T), Methandrostenolone (ME), Oxandrolone (OXA), Testosterone Propionate (TP), Testosterone Isocaproate (TI), Testosterone Enantate (TE), Testosterone Cypionate (TC), Nandrolone Decanoate (ND), Nandrolone Phenylpropionate (NF) and Boldenone Undecylenate (BU) in metanol, per analyte, at 10 µg/mL, kept frozen for 3, 7, 10 and 14 days. N=4 samples for D3, 3 samples for D7, 2 samples for D10 and one sample for D14.

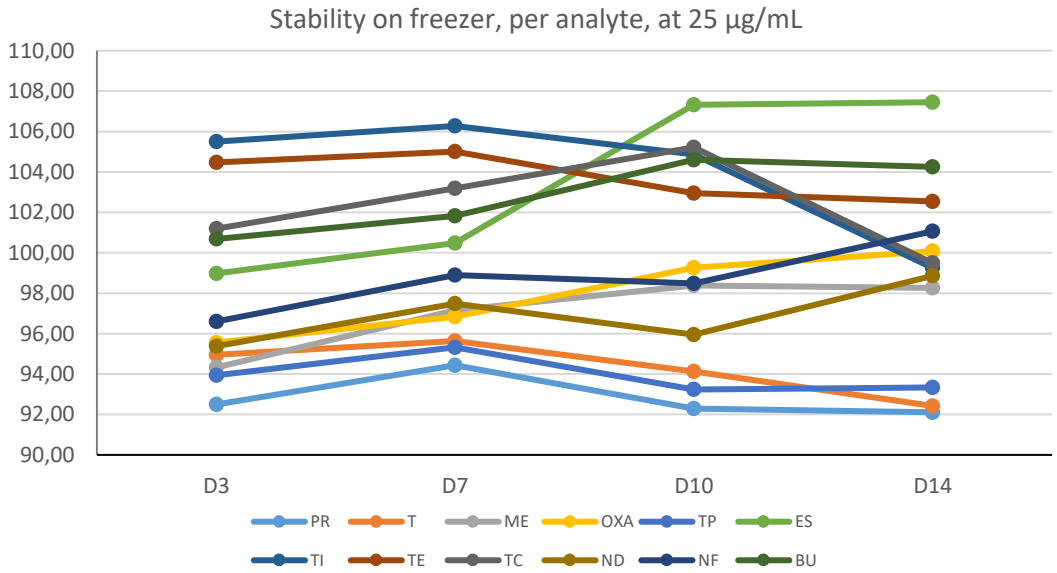



Figure 20: Stability of the AAS Prasterone (PR), Testosterone (T), Methandrostenolone (ME), Oxandrolone (OXA), Testosterone Propionate (TP), Testosterone Isocaproate (TI), Testosterone Enantate (TE), Testosterone Cypionate (TC), Nandrolone Decanoate (ND), Nandrolone Phenylpropionate (NF) and Boldenone Undecylenate (BU) in metanol, per analyte, at 25 µg/mL, kept frozen for 3, 7, 10 and 14 days. N=4 samples for D3, 3 samples for D7, 2 samples for D10 and one sample for D14.

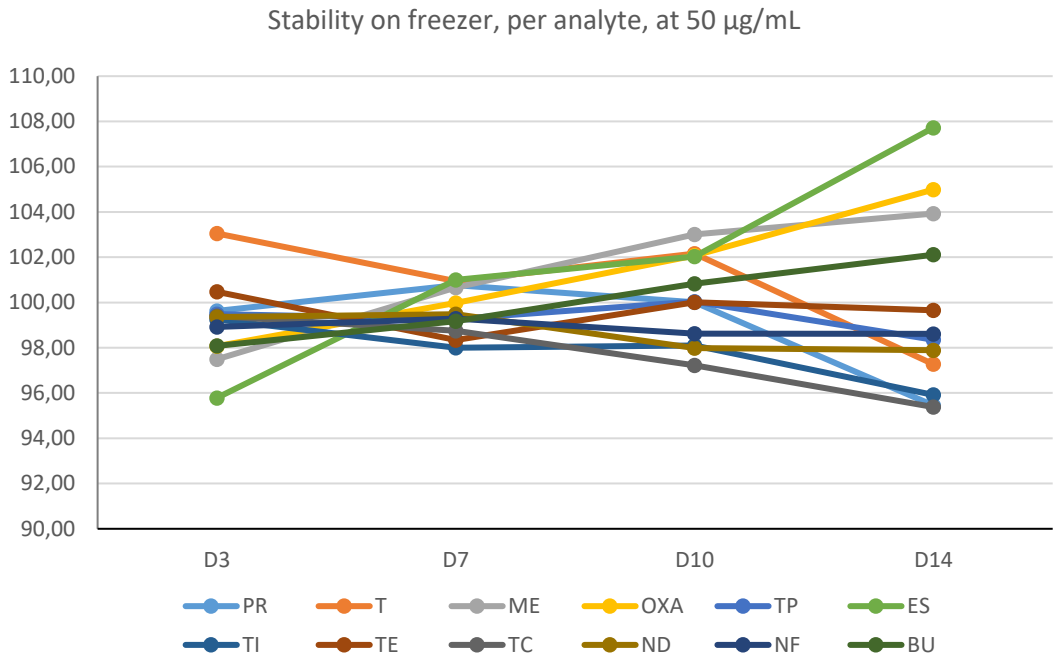



Figure 21: Stability of the AAS Prasterone (PR), Testosterone (T), Methandrostenolone (ME), Oxandrolone (OXA), Testosterone Propionate (TP), Testosterone Isocaproate (TI), Testosterone Enantate (TE), Testosterone Cypionate (TC), Nandrolone Decanoate (ND), Nandrolone Phenylpropionate (NF) and Boldenone Undecylenate (BU) in metanol, per analyte, at 50 µg/mL, kept frozen for 3, 7, 10 and 14 days. N=4 samples for D3, 3 samples for D7, 2 samples for D10 and one sample for D14.

## Apêndice C

Table 1: Quantitative results for tablets (Total N = 87).

| Formulation declared | Product name and declared manufacturer | Lot number Expiry year | Result (mg/tablet) | Conclusions |
|---|---|---|---|---|
| ES 5 mg/tablet | Estanozolol (USPLabs) | EZO111003 2014 | ES 2.7 | Regular |
| ES 10 mg/tablet | Stanozoland (Landerlan) | 08-477/6 2012 | ES 7.1 | Regular |
| ES 10 mg/tablet | Stanozoland (Landerlan) | 08-477/8 2012 | ES 6.4 | Regular |
| ES 10 mg/tablet | Stanozoland (Landerlan) | 09-477/8 2012 | ES 7.3 | Regular |
| ES 10 mg/tablet | Stanozoland (Landerlan) | 08-477/8 2012 | ES 7.6 | Regular |
| ES 10 mg/tablet | Stanozoland (Landerlan) | 08-477/8 2012 | ES 9.1 | Regular |
| ES 10 mg/tablet | Stanozoland (Landerlan) | 08-477/9 2012 | ES 5.9 | Counterfeit (low dose, tablet size differed significantly from others of same batch) |
| ES 10 mg/tablet | Stanozoland (Landerlan) | 08-477/9 2012 | ES 8.2 | Regular |
| ES 10 mg/tablet | Stanozoland (Landerlan) | 08-477/12 2012 | ES 7.2 | Regular |
| ES 10 mg/tablet | Stanozoland (Landerlan) | 08-477/14 2012 | ES 7.9 | Regular |
| ES 10 mg/tablet | Stanozoland (Landerlan) | 08-477/21 2012 | ES 9.6 | Regular |
| ES 10 mg/tablet | Stanozoland (Landerlan) | 08-477/22 2012 | ES 8.7 | Regular |
| ES 10 mg/tablet | Stanozoland (Landerlan) | 08-477/23 2012 | ES 7.6 | Regular |

| Formulation declared | Product name and declared manufacturer | Lot number Expiry year | Result (mg/tablet) | Conclusions |
|---|---|---|---|---|
| ES 10 mg/tablet | Stanozoland (Landerlan) | 08-477/23 2012 | ES 8.3 | Regular |
| ES 10 mg/tablet | Stanozoland (Landerlan) | 08-477/25 2013 | ES 9.2 | Regular |
| ES 10 mg/tablet | Stanozoland (Landerlan) | 08-477/28 2013 | ES 8.9 | Regular |
| ES 10 mg/tablet | Stanozoland (Landerlan) | 04812 2014 | ES 9.3 | Regular |
| ES 10 mg/tablet | Stanozoland (Landerlan) | 04912 2014 | ES 9.7 | Regular |
| ES 10 mg/tablet | Stanozoland (Landerlan) | 06012 2014 | ES 9.6 | Regular |
| ES 10 mg/tablet | Stanozoland (Landerlan) | 06012 2014 | ES 10.7 | Regular |
| ES 10 mg/tablet | Stanozoland (Landerlan) | 08012 2014 | ES 10.2 | Regular |
| ES 10 mg/tablet | Stanozoland (Landerlan) | 08012 2014 | ES 10.2 | Regular |
| ES 10 mg/tablet | Stanozoland (Landerlan) | 09312 2014 | ES 10.3 | Regular |
| ES 10 mg/tablet | Stanozoland (Landerlan) | 09412 2014 | ES 9.6 | Regular |
| ES 10 mg/tablet | Stanozoland (Landerlan) | 10112 2014 | ES 9.0 | Regular |
| ES 10 mg/tablet | Stanozoland (Landerlan) | 15023 2017 | ES 10.7 | Regular |
| ES 10 mg/tablet | Stanozoland (Landerlan) | 15042 2017 | ES 10.0 | Regular |
| ES 10 mg/tablet | Stanazol (RWR) | 9229 2012 | ES 7.1 | Regular |

| Formulation declared | Product name and declared manufacturer | Lot number Expiry year | Result (mg/tablet) | Conclusions |
|---|---|---|---|---|
| ES 10 mg/tablet | Stanazol (RWR) | 012622 2014 | ES 9.4 | Regular |
| ES 10 mg/tablet | Neurabol (Muscle Labs) | NEU 081094 2015 | ES 15.5 | Out of Specification (high dose) |
| ME 5 mg/tablet | Methandrostenolone (Akrikhin) | 448 2012 | TP 2.1 | Counterfeit (different API) |
| ME 10 mg/tablet | Dianabol (Muscle Labs) | DI 081073 2015 | ME 17.0 | Out of Specification (high dose) |
| ME 10 mg/tablet | Metandrostenolona (Landerlan) | 09-197/6 2012 | ME 8.9 | Regular |
| ME 10 mg/tablet | Metandrostenolona (Landerlan) | 09-197/8 2012 | ME 11.0 | Regular |
| ME 10 mg/tablet | Metandrostenolona (Landerlan) | 09-197/8 2012 | ME 11.1 | Regular |
| ME 10 mg/tablet | Metandrostenolona (Landerlan) | 09-197/8 2012 | ME 6.8 | Counterfeit (low dose, tablet size differed significantly from others of same batch) |
| ME 10 mg/tablet | Metandrostenolona (Landerlan) | 03811 2013 | ME 9.3 | Regular |
| ME 10 mg/tablet | Metandrostenolona (Landerlan) | 08212 2014 | ME 9.6 | Regular |
| ME 10 mg/tablet | Metandrostenolona (Landerlan) | 08312 2014 | ME 9.6 | Regular |
| ME 10 mg/tablet | Metandrostenolona (Landerlan) | 08512 2014 | ME 10.2 | Regular |
| ME 10 mg/tablet | Metandrostenolona (Landerlan) | 11612 2014 | ME 9.7 | Regular |
| ME 10 mg/tablet | Metandrostenolona (Landerlan) | 13622 2014 | ME 9.7 | Regular |
| ME 10 mg/tablet | Metandrostenolona (Landerlan) | 98 2015 | ME 8.6 | Regular |

| Formulation declared | Product name and declared manufacturer | Lot number Expiry year | Result (mg/tablet) | Conclusions |
|---|---|---|---|---|
| ME 10 mg/tablet | Metandrostenolona (Landerlan) | 13622 2015 | ME 9.7 | Regular |
| ME 10 mg/tablet | Metandrostenolona (Landerlan) | 13645 2015 | ME 10.1 | Regular |
| ME 10 mg/tablet | Metandrostenolona (Landerlan) | 13646 2015 | ME 10.6 | Regular |
| ME 10 mg/tablet | Metandrostenolona (Landerlan) | 15009 2017 | ME 9.9 | Regular |
| ME 10 mg/tablet | Metandrostenolona (Landerlan) | 15065 2017 | ME 9.4 | Regular |
| ME 10 mg/tablet | Metandrostenolona (Landerlan) | 15602 2017 | ME 9.5 | Regular |
| ME 10 mg/tablet | Methandrostenolone (RG) | 487 2012 | TP 0.2* | Counterfeit (different API) |
| ME 10 mg/tablet | Methandrostenolone (RG) | 787 2012 | ME 1.5 / TP 1.5 | Counterfeit (additional API, low dose) |
| OXA 5mg/tablet | Anavar (Lab Pharmaceutical) | F417BOB 2013 | PR 1.6 / TP 1.2 | Counterfeit (different API) |
| OXA 5mg/tablet | Anavar (Spa) | 448 2012 | < LD | Counterfeit (no API) |
| OXA 5mg/tablet | Anavar (Spa) | 462 2013 | PR 1.1 / TP 0.6 | Counterfeit (different API) |
| OXA 5mg/tablet | Anavar (Spa) | 462 2013 | TP 1.0 | Counterfeit (different API) |
| OXA 5mg/tablet | Oxandrolona (Landerlan) | 08-033/7 2012 | OXA 3.5 | Regular |
| OXA 5mg/tablet | Oxandrolona (Landerlan) | 08-033/7 2012 | OXA 3.3 | Regular |
| OXA 5mg/tablet | Oxandrolona (Landerlan) | 08-033/8 2012 | OXA 4.2 | Regular |

| Formulation declared | Product name and declared manufacturer | Lot number Expiry year | Result (mg/tablet) | Conclusions |
|---|---|---|---|---|
| OXA 5mg/tablet | Oxandrolona (Landerlan) | 08-033/8 2012 | OXA 3.6 | Regular |
| OXA 5mg/tablet | Oxandrolona (Landerlan) | 08033/12 2012 | OXA 3.4 | Regular |
| OXA 5mg/tablet | Oxandrolona (Landerlan) | 08033/13 2013 | < LD | Counterfeit (no API) |
| OXA 5mg/tablet | Oxandrolona (Landerlan) | 08033/13 2013 | < LD | Counterfeit (no API) |
| OXA 5mg/tablet | Oxandrolona (Landerlan) | 08033/13 2013 | < LD | Counterfeit (no API) |
| OXA 5mg/tablet | Oxandrolona (Landerlan) | 08033/15 2013 | OXA 3.6 | Regular |
| OXA 5mg/tablet | Oxandrolona (Landerlan) | 08-033/16 2013 | OXA 3.4 | Regular |
| OXA 5mg/tablet | Oxandrolona (Landerlan) | 07712 2014 | OXA 2.9 | Out of Specification (low dose) |
| OXA 5mg/tablet | Oxandrolona (Landerlan) | 07812 2014 | OXA 3.5 | Regular |
| OXA 5mg/tablet | Oxandrolona (Landerlan) | 07812 2014 | OXA 2.7 | Out of Specification (low dose) |
| OXA 5mg/tablet | Oxandrolona (Landerlan) | 10912 2014 | OXA 3.9 | Regular |
| OXA 5mg/tablet | Oxandrolona (Landerlan) | 13639 2015 | OXA 3.5 | Regular |
| OXA 5mg/tablet | Oxandrolona (Landerlan) | 14006 2016 | OXA3.7 | Regular |
| OXA 10 mg/tablet | Anavar (Muscle Labs) | ANA 081062 2015 | OXA 8.1 | Regular |
| Absent information | Hemogenin (Sarsa) | 439 2012 | TP 4.2 | Counterfeit (inexistent medicine) |

| Formulation declared | Product name and declared manufacturer | Lot number Expiry year | Result (mg/tablet) | Conclusions |
|---|---|---|---|---|
| Absent information | Hemogenin (Sarsa) | 448 2012 | TP 2.6 | Counterfeit (inexistent medicine) |
| Absent information | Hemogenin (Sarsa) | 448 2012 | TP 1.8 | Counterfeit (inexistent medicine) |
| Absent information | Hemogenin (Sarsa) | 448 2012 | TP 2.4 | Counterfeit (inexistent medicine) |
| Absent information | Hemogenin (Sarsa) | 448 2012 | TP 3.1 | Counterfeit (inexistent medicine) |
| Absent information | Hemogenin (Sarsa) | 448 2012 | PR 2.9 / TP 0.6 | Counterfeit (inexistent medicine) |
| Absent information | Hemogenin (Sarsa) | 448 2012 | PR 2.3 / TP 0.6 | Counterfeit (inexistent medicine) |
| Absent information | Hemogenin (Sarsa) | 910 2012 | TP 0.14* | Counterfeit (inexistent medicine) |
| Absent information | Hemogenin (Sarsa) | 482 2013 | TP 0.9 | Counterfeit (inexistent medicine) |
| Absent information | Hemogenin (Sarsa) | 13621 2015 | < LD | Counterfeit (inexistent medicine) |
| Absent information | Hemogenin (Sarsa) | 13621 2015 | < LD | Counterfeit (inexistent medicine) |
| Absent information | Hemogenin (Sarsa) | 13621 2015 | < LD | Counterfeit (inexistent medicine) |
| Absent information | Hemogenin (Sarsa) | AV 2234-13 No expiry year | OXA 0.8 | Counterfeit (inexistent medicine) |
| Absent information | Absent information | Absent information | ES 10.0 | No identification |
| Absent information | Absent information | Absent information | ES 9.0 | No identification |

* Value obtained below the method's quantitation limit; therefore just an estimate.
ES = Stanozolol; ME = Methandrostenolone; OXA = Oxandrolone; TP = Testosterone Propionate; PR = Prasterone, API = Active Pharmaceutical Ingredient; LD = Limit of Detection

Table 2: Quantitative results for aqueous suspensions (Total N = 83).

| Formulation declared | Product name and declared manufacturer | Lot number Expiry year | Results (mg/mL suspension) | Conclusions |
|---|---|---|---|---|
| T 100 mg/mL | Testosterone (RWR) | 009842 2012 | T 78.4 | Regular |
| T 100 mg/mL | Testosterone (RWR) | 009842 2012 | T 85.0 | Regular |
| T 100 mg/mL | Testosterone (RWR) | 011415 2013 | T 88.7 | Regular |
| T 100 mg/mL | Testosterone (RWR) | 012576 2014 | T 90.3 | Regular |
| ES 50 mg/mL | Estanozolol (FM) | Illegible | ES 53.3 | Regular |
| ES 50 mg/mL | Stanazol (RWR) | 009125 2012 | ES 39.6 | Regular |
| ES 50 mg/mL | Stanazol (RWR) | 008009 2013 | ES 21.1 | Counterfeit (Low dose, inconsistent lot number) |
| ES 50 mg/mL | Stanazol (RWR) | 009744 2013 | ES 60.4 | Regular |
| ES 50 mg/mL | Stanazol (RWR) | 010046 2013 | ES 39.6 | Regular |
| ES 50 mg/mL | Stanazol (RWR) | 010046 2013 | ES 50.3 | Regular |
| ES 50 mg/mL | Stanazol (RWR) | 010046 2013 | ES 41.8 | Regular |
| ES 50 mg/mL | Stanazol (RWR) | 010611 2013 | ES 44.6 | Regular |
| ES 50 mg/mL | Stanazol (RWR) | 010667 2013 | ES 42.7 | Regular |
| ES 50 mg/mL | Stanazol (RWR) | 010997 2014 | ES 14.5 | Counterfeit (Low dose, inexistent secutiry code) |

| Formulation declared | Product name and declared manufacturer | Lot number Expiry year | Results (mg/mL suspension) | Conclusions |
|---|---|---|---|---|
| ES 50 mg/mL | Stanazol (RWR) | 011843 2014 | ES 61.6 | Regular |
| ES 50 mg/mL | Stanazol (RWR) | 011857 2014 | ES 41.0 | Regular |
| ES 50 mg/mL | Stanazol (RWR) | 012442 2015 | ES 46.5 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 07-0626 2012 | < LD | Counterfeit (no API) |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 10-002 2012 | ES 35.5 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 10-002 2012 | ES 46.6 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 10-189 2012 | ES 63.3 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 10-207 2012 | ES 42.5 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 10-216 2012 | ES 39.9 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 10-348 2012 | ES 56.5 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 10-435 2012 | ES 35.3 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 10-462 2012 | ES 50.0 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 10-472 2012 | ES 34.9 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 10-650 2012 | ES 51.8 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 10-650 2012 | ES 48.3 | Regular |

| Formulation declared | Product name and declared manufacturer | Lot number Expiry year | Results (mg/mL suspension) | Conclusions |
|---|---|---|---|---|
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 10-11-45 2012 | ES 49.3 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 10-11-45 2012 | ES 46.8 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 10-11-52 2012 | ES 54.0 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 09-500 2013 | ES 51.4 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 09-501 2013 | ES 51.1 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 09-507 2013 | ES 55.1 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 09-508 2013 | ES 47.5 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 09-509 2013 | ES 50.8 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 09-510 2013 | ES 49.2 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 09-510 2013 | ES 71.7 | Out of specification (high dose) |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 09-512 2013 | ES 54.1 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 09-518 2013 | ES 49.1 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 09-519 2013 | ES 48.0 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 11-493 2013 | ES 56.0 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 11-540 2013 | ES 54.2 | Regular |

| Formulation declared | Product name and declared manufacturer | Lot number Expiry year | Results (mg/mL suspension) | Conclusions |
|---|---|---|---|---|
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 11-572 2013 | ES 50.0 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 11-630 2013 | ES 46.2 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 11-632 2013 | ES 54.1 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 11-01-02 2013 | ES 47.9 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | Illegible 2014 | ES 48.6 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 235 2014 | ES 52.4 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 09-531 2014 | ES 61.3 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 09-535 2014 | ES 52.5 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 09-536 2014 | ES 54.4 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 09-548 2014 | ES 48.9 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 09-554 2014 | ES 43.1 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 09-555 2014 | ES 55.2 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 09-557 2014 | ES 49.1 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 413-014 2015 | ES 49.4 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 413-017 2015 | ES 50.1 | Regular |

| Formulation declared | Product name and declared manufacturer | Lot number Expiry year | Results (mg/mL suspension) | Conclusions |
|---|---|---|---|---|
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 413-044 2015 | ES 14.5 | Out of specification (low dose) |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 415-051 2017 | ES 55.2 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 413-069 2015 | ES 53.6 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 413-069 2015 | ES 56.3 | Regular |
| ES 50 mg/mL | Stanozoland Depot (Landerlan) | 413-070 2015 | ES 59.3 | Regular |
| ES 50 mg/mL | Winstrol Depot (Zambom) | T080 2012 | ES 50.1 | Regular |
| ES 50 mg/mL | Winstrol Depot (Zambom) | U012 2012 | ES 48.5 | Regular |
| ES 50 mg/mL | Winstrol Depot (Zambom) | U081 2012 | ES 45.9 | Regular |
| ES 50 mg/mL | Winstrol Depot (Zambom) | U082 2012 | ES 49.9 | Regular |
| ES 50 mg/mL | Winstrol Depot (Zambom) | W023 2013 | ES 13.9 | Counterfeit (Low dose) |
| ES 50 mg/mL | Winstrol Depot (Zambom) | W023 2013 | ES 12.7 | Counterfeit (Low dose) |
| ES 50 mg/mL | Winstrol Depot (Zambom) | W30 2013 | ES 47.0 | Regular |
| ES 50 mg/mL | Winstrol Depot (Zambom) | W032 2013 | ES 56.2 | Regular |
| ES 50 mg/mL | Winstrol Depot (Zambom) | W055 2013 | ES 52.3 | Regular |
| ES 50 mg/mL | Winstrol V (The Upjohn Company) | 8989/6 2013 | <LD | Counterfeit (no API) |

| Formulation declared | Product name and declared manufacturer | Lot number Expiry year | Results (mg/mL suspension) | Conclusions |
|---|---|---|---|---|
| ES 50 mg/mL | Winstrol V (The Upjohn Company) | 8989/6 2013 | <LD | Counterfeit (no API) |
| ES 50 mg/mL | Winstrol V (The Upjohn Company) | 9585/6 2013 | T 0.26* | Counterfeit (different API) |
| ES 50 mg/mL | Winstrol V (The Upjohn Company) | 9585/6 2013 | ES 21.9 | Counterfeit (Low dose, fake packaging) |
| ES 100 mg/mL | Estanozolol (USP Labs) | Illegible | ES 107.2 | Regular |
| ES 100 mg/mL | Estanozolol (USP Labs) | ENX424221 2014 | ES 38.3 | Out of specification (low dose) |
| ES 100 mg/mL | Estanozolol (USP Labs) | ENX424223 2015 | ES 21.6 | Out of specification (low dose) |
| ES 100 mg/mL | Estanozolol (USP Labs) | ENX424224 2014 | ES 47.7 | Out of specification (low dose) |
| ES 100 mg/mL | Estanozolol (USP Labs) | ENX424225 2014 | ES 37.4 | Out of specification (low dose) |
| ES 100 mg/mL | Stanozolol (Burn Lab) | No lot number 2012 | ES 41.2 | Counterfeit (Low dose, underground manufacturer) |

* Value obtained below the method's LQ; therefore just an estimate.
T = Testosterone; ES = Stanozolol; API = Active Pharmaceutical Ingredient; LD = Limit of Detection

Table 3: Quantitative results for oily solutions (Total N = 158).

| Formulation declared | Product name and declared manufacturer | Lot number Expiry year | Results (mg/mL solution) | Conclusions |
|---|---|---|---|---|
| BU 250 mg/mL | Boldenone Undecylenate (Cook Labs) | Absent information | ND 105.8 | Counterfeit (different API) |
| BU 50 mg/mL | Equipoise (Solvay) | 7EFA048 2012 | < LD | Counterfeit (no API) |
| BU 50 mg/mL | Equipoise (Solvay) | 9F43328 2013 | < LD | Counterfeit (no API) |
| BU 50 mg/mL | Maxigan (Inpel Quality) | UOG 024 2012 | BU 15.6 | Counterfeit (low dose) |
| ME 50 mg/mL | Methandrostenolone (Gedeon Richter) | E1AF06 2012 | < LD | Counterfeit (no API) |
| Methen 300 mg/mL | Enantato 300 (USP Labs) | ENA671538 2016 | < LD | Counterfeit (no API, inexistent secutiry code) |
| Methen (no dose declared) | Gold pH (Gold Pharma) | Illegible 2016 | BU 10.3 | Counterfeit (different API) |
| Methen 100 mg/mL | Primobolan (USP Labs) | NDA662015 2016 | < LD | Counterfeit (no API, inexistent secutiry code) |
| Methen 100 mg/mL | Primobolan Depot (Schering) | N-326 2013 | < LD | Counterfeit (no API) |
| Methen 100 mg/mL | Primobolan Depot (Schering) | N-326 2013 | TP 3.2 | Counterfeit (different API) |
| Methen 100 mg/mL | Primobolan Depot (Schering) | N-822 2016 | < LD | Counterfeit (no API) |
| ND 50 mg/mL | Deca 50 (RWR) | 49855/0498 2012 | < LD | Counterfeit (no API) |
| ND 50 mg/mL | Deca Durabolin (Organon) | 11873 2012 | < LD | Counterfeit (no API) |
| ND 50 mg/mL | Deca Durabolin (Organon) | 11873 2012 | < LD | Counterfeit (no API) |

| Formulation declared | Product name and declared manufacturer | Lot number Expiry year | Results (mg/mL solution) | Conclusions |
|---|---|---|---|---|
| ND 50 mg/mL | Deca Durabolin (Organon) | 13382 2014 | < LD | Counterfeit (no API) |
| ND 50 mg/mL | Deca Durabolin (Organon) | 14328 2014 | TP 8.7 | Counterfeit (different API) |
| ND 50 mg/mL | Deca Durabolin (Organon) | 14328 2014 | TP 8.4 | Counterfeit (different API) |
| ND 50 mg/mL | Deca Durabolin (Organon) | 14642 2014 | TP 1.5* | Counterfeit (different API) |
| ND 50 mg/mL | Deca Durabolin (Organon) | 20385 2014 | < LD | Counterfeit (no API) |
| ND 250 mg/mL | Deca Durabolin (Organon) | 12953 2012 | TP 39.5 | Counterfeit (different API) |
| ND 250 mg/mL | Deca Durabolin (Organon) | 12047 2014 | < LD | Counterfeit (no API) |
| ND 250 mg/mL | Deca Durabolin (Organon) | 13127 2014 | < LD | Counterfeit (no API) |
| ND 250 mg/mL | Deca Durabolin (Organon) | 13127 2014 | TP 2.5 | Counterfeit (different API) |
| ND 250 mg/mL | Deca Durabolin (Organon) | 13382 2014 | < LD | Counterfeit (no API) |
| ND 250 mg/mL | Deca Durabolin (Organon) | 13382 2014 | < LD | Counterfeit (no API) |
| ND 200 mg/mL | Decaland Depot (Landerlan) | Absent information | NF 2.4* | Counterfeit (different API) |
| ND 200 mg/mL | Decaland Depot (Landerlan) | No lot number 2012 | ND 29.7 | Counterfeit (low dose, fake packaging) |
| ND 200 mg/mL | Decaland Depot (Landerlan) | No lot number 2012 | ND 15.2 | Counterfeit (low dose, fake packaging) |
| ND 200 mg/mL | Decaland Depot (Landerlan) | 10-180 2012 | ND 96.7 | Regular |

| Formulation declared | Product name and declared manufacturer | Lot number Expiry year | Results (mg/mL solution) | Conclusions |
|---|---|---|---|---|
| ND 200 mg/mL | Decaland Depot (Landerlan) | 10-180 2012 | ND 105.5 | Regular |
| ND 200 mg/mL | Decaland Depot (Landerlan) | 10-180 2012 | ND 111.3 | Regular |
| ND 200 mg/mL | Decaland Depot (Landerlan) | 10-11-37 2012 | ND 108.3 | Regular |
| ND 200 mg/mL | Decaland Depot (Landerlan) | 10-12-54 2012 | ND 108.2 | Regular |
| ND 200 mg/mL | Decaland Depot (Landerlan) | 10-12-54 2012 | ND 103.7 | Regular |
| ND 200 mg/mL | Decaland Depot (Landerlan) | 09-543 2014 | ND 117.1 | Regular |
| ND 200 mg/mL | Decaland Depot (Landerlan) | 09-547 2014 | ND 106.7 | Regular |
| ND 200 mg/mL | Decaland Depot (Landerlan) | 11-550 2013 | ND 128.9 | Regular |
| ND 200 mg/mL | Decaland Depot (Landerlan) | 09-554 2014 | ND 104.7 | Regular |
| ND 200 mg/mL | Decaland Depot (Landerlan) | 09-574 2014 | ND 118.6 | Regular |
| ND 200 mg/mL | Decaland Depot (Landerlan) | 09-575 2014 | ND 121.4 | Regular |
| ND 200 mg/mL | Decaland Depot (Landerlan) | 09-578 2014 | ND 127.7 | Regular |
| ND 200 mg/mL | Decaland Depot (Landerlan) | Illegible 2015 | ND 121.7 | Regular |
| ND 200 mg/mL | Decaland Depot (Landerlan) | 413-019 2015 | ND 139.7 | Regular |
| ND 200 mg/mL | Decaland Depot (Landerlan) | 413-020 2015 | ND 113.8 | Regular |

| Formulation declared | Product name and declared manufacturer | Lot number Expiry year | Results (mg/mL solution) | Conclusions |
|---|---|---|---|---|
| ND 200 mg/mL | Decaland Depot (Landerlan) | 413-055 2015 | ND 105.7 | Regular |
| ND 200 mg/mL | Decaland Depot (Landerlan) | 413-092 2015 | ND 125.3 | Regular |
| ND 200 mg/mL | Decaland Depot (Landerlan) | 413-105 2015 | ND 111.6 | Regular |
| ND 200 mg/mL | Decaland Depot (Landerlan) | 415-002 2017 | ND 118.7 | Out of specification (low dose) |
| ND 200 mg/mL | Decaland Depot (Landerlan) | 415-034 2017 | ND 116.3 | Out of specification (low dose) |
| ND 200 mg/mL | Decaland Depot (Landerlan) | 415-035 2017 | ND 105.7 | Out of specification (low dose) |
| ND 200 mg/mL | Decaland Depot (Landerlan) | 415-052 2017 | ND 117.8 | Out of specification (low dose) |
| ND 200 mg/mL | Decaland Depot (Landerlan) | 415-084 2017 | ND 105.9 | Out of specification (low dose) |
| ND 100 mg/mL | Nandrolone Decanoate (Norma Hellas) | 17530 2012 | < LD | Counterfeit (no API) |
| ND 100 mg/mL | Nandrolone Decanoate (Norma Hellas) | 19740 2012 | < LD | Counterfeit (no API) |
| ND 100 mg/mL | Nandrolone Decanoate (Norma Hellas) | 19740 2012 | < LD | Counterfeit (no API) |
| ND 100 mg/mL | Nandrolone Decanoate (Norma Hellas) | 17530 2013 | < LD | Counterfeit (no API) |
| ND 100 mg/mL | Nandrolone Decanoate (Norma Hellas) | 17530 2013 | < LD | Counterfeit (no API) |
| ND 100 mg/mL | Nandrolone Decanoate (Norma Hellas) | 1730 2013 | < LD | Counterfeit (no API) |
| ND 100 mg/mL | Nandrolone Decanoate (Norma Hellas) | 19740 2013 | < LD | Counterfeit (no API) |

| Formulation declared | Product name and declared manufacturer | Lot number Expiry year | Results (mg/mL solution) | Conclusions |
|---|---|---|---|---|
| ND 200 mg/mL | Nandrolone Decanoate (Cook Labs) | Absent information | ND 113.8 | Counterfeit (underground manufacturer) |
| ND and NF (100 mg/mL each) | Deca Drobol (Mager Pharmazeutischer) | 006.10.11 2012 | < LD | Counterfeit (no API) |
| ND and NF (100 mg/mL each) | Deca Drobol (Mager Pharmazeutischer) | 112.41.08 2012 | < LD | Counterfeit (no API) |
| ND and NF (100 mg/mL each) | Deca Drobol (Mager Pharmazeutischer) | 112.41.08 2012 | ND 1.0* / NF 12.2 | Counterfeit (low dose) |
| ND and NF (100 mg/mL each) | Deca Drobol (Mager Pharmazeutischer) | 112.41.08 2012 | ND 1.0* / NF 11.6 | Counterfeit (low dose) |
| ND and NF (100 mg/mL each) | Deca Drobol (Mager Pharmazeutischer) | 125.84.10 2013 | < LD | Counterfeit (no API) |
| ND and NF (100 mg/mL each) | Deca Drobol (Mager Pharmazeutischer) | 125.84.10 2013 | NF 1.4* | Counterfeit (low dose, incomplete formulation) |
| ND and NF (100 mg/mL each) | Deca Drobol (Mager Pharmazeutischer) | 125.84.10 No expiry date | < LD | Counterfeit (no API) |
| NF 100 mg/mL | Nandrorapid (Alpha Pharma) | 1\|81Q5TR 2013 | NF 54.3 | Regular |
| PD 100 mg/mL | Drostanolon (USP Labs) | DRO150736 2014 | TE 121.8 | Counterfeit (different API, inexistent security code) |
| TP, TF, TI and TD (30, 60, 60 and 100 mg/mL) | Durateston (Organon) | 12656 2012 | < LD | Counterfeit (no API) |
| TP, TF, TI and TD (30, 60, 60 and 100 mg/mL) | Durateston (Organon) | 12656 2012 | < LD | Counterfeit (no API) |
| TP, TF, TI and TD (30, 60, 60 and 100 mg/mL) | Durateston (Organon) | 12656 2012 | < LD | Counterfeit (no API) |
| TP, TF, TI and TD (30, 60, 60 and 100 mg/mL) | Durateston (Organon) | 12656 2012 | < LD | Counterfeit (no API) |

| Formulation declared | Product name and declared manufacturer | Lot number Expiry year | Results (mg/mL solution) | Conclusions |
|---|---|---|---|---|
| TP, TF, TI and TD (30, 60, 60 and 100 mg/mL) | Durateston (Organon) | 12656 2012 | < LD | Counterfeit (no API) |
| TP, TF, TI and TD (30, 60, 60 and 100 mg/mL) | Durateston (Organon) | 12656 2012 | < LD | Counterfeit (no API) |
| TP, TF, TI and TD (30, 60, 60 and 100 mg/mL) | Durateston (Organon) | 12856 2012 | < LD | Counterfeit (no API) |
| TP, TF, TI and TD (30, 60, 60 and 100 mg/mL) | Durateston (Organon) | 12824 2014 | < LD | Counterfeit (no API) |
| TP, TF, TI and TD (30, 60, 60 and 100 mg/mL) | Durateston (Organon) | 12824 2014 | < LD | Counterfeit (no API) |
| TP, TF, TI and TD (30, 60, 60 and 100 mg/mL) | Durateston (Organon) | 12824 2014 | < LD | Counterfeit (no API) |
| TP, TF, TI and TD (30, 60, 60 and 100 mg/mL) | Durateston (Organon) | 12868 2014 | TP 2.9 | Counterfeit (incomplete formulation, low dose) |
| TP, TF, TI and TD (30, 60, 60 and 100 mg/mL) | Durateston (Organon) | 12868 2014 | TP 3.2 | Counterfeit (incomplete formulation, low dose) |
| TP, TF, TI and TD (30, 60, 60 and 100 mg/mL) | Durateston (Organon) | 12868 2014 | TP 3.7 | Counterfeit (incomplete formulation, low dose) |
| TP, TF, TI and TD (30, 60, 60 and 100 mg/mL) | Durateston (Organon) | 12868 2014 | TP 3.4 | Counterfeit (incomplete formulation, low dose) |
| TP, TF, TI and TD (30, 60, 60 and 100 mg/mL) | Durateston (Organon) | 13257 2015 | < LD | Counterfeit (no API) |
| TP, TF, TI and TD (30, 60, 60 and 100 mg/mL) | Durateston (Organon) | 82012 2015 | TE 260.5 | Counterfeit (different API) |

| Formulation declared | Product name and declared manufacturer | Lot number Expiry year | Results (mg/mL solution) | Conclusions |
|---|---|---|---|---|
| TP, TF, TI and TD (30, 60, 60 and 100 mg/mL) | Duratestoland (Landerlan) | 11-357 2012 | TP 2.2* | Counterfeit (incomplete formulation) |
| TP, TF, TI and TD (30, 60, 60 and 100 mg/mL) | Duratestoland (Landerlan) | 14-823 2015 | < LD | Counterfeit (no API) |
| TC 200 mg/mL | Depo-testosterone (The Upjohn Company) | B1AD50 2012 | < LD | Counterfeit (no API) |
| TC 250 mg/mL | Testex (Muscle Labs) | No lot number 2018 | TE 147.1 | Counterfeit (different API) |
| TC 125 mg/mL | Testex Elmu Prolongatum (Byk Elmu) | S26 2012 | < LD | Counterfeit (no API) |
| TC 125 mg/mL | Testex Elmu Prolongatum (Byk Elmu) | S27 2013 | TP 3.3 | Counterfeit (different API) |
| TC 125 mg/mL | Testex Elmu Prolongatum (Byk Elmu) | S27 2013 | TP 2.0* | Counterfeit (different API) |
| TC 110 mg/mL | Testoland Depot (Landerlan) | 09-837 2012 | TC 111.8 | Regular |
| TC 110 mg/mL | Testoland Depot (Landerlan) | 09-837 2012 | TC 111.2 | Regular |
| TC 110 mg/mL | Testoland Depot (Landerlan) | 10-515 2012 | TC 127.8 | Regular |
| TC 110 mg/mL | Testoland Depot (Landerlan) | 10-10-29 2012 | TC 121.0 | Regular |
| TC 110 mg/mL | Testoland Depot (Landerlan) | F10-10-29 2012 | < LD | Counterfeit (no API) |
| TC 110 mg/mL | Testoland Depot (Landerlan) | 11-01-08 2013 | TC 120.5 | Regular |

| Formulation declared | Product name and declared manufacturer | Lot number Expiry year | Results (mg/mL solution) | Conclusions |
|---|---|---|---|---|
| TC 110 mg/mL | Testoland Depot (Landerlan) | 09100 2014 | TC 130.4 | Regular |
| TC 110 mg/mL | Testoland Depot (Landerlan) | 1501530 2017 | TC 111.7 | Regular |
| TC 200 mg/mL | Testosterona Ultra Lenta Fuerte (Dispert) | 7474 2017 | TC 30.8 | Counterfeit (low dose) |
| TC 200 mg/mL | Testosterona Ultra Lenta Fuerte (Dispert) | 7530 2017 | TC 218.3 | Regular |
| TE 300 mg/mL | Ciclo 6 (Drag Pharma) | No lot number 2012 | < LD | Counterfeit (no API) |
| TE 300 mg/mL | Ciclo 6 (Drag Pharma) | 71057 2012 | TP 7.7 | Counterfeit (different API) |
| TE 300 mg/mL | Ciclo 6 (Drag Pharma) | 90511 2012 | TE 459.1 | Out of specification (high dose) |
| TE 300 mg/mL | Ciclo 6 (Drag Pharma) | 091092 2012 | < LD | Counterfeit (no API) |
| TE 300 mg/mL | Ciclo 6 (Drag Pharma) | 091092 2012 | NF 0.6* | Counterfeit (different API) |
| TE 300 mg/mL | Ciclo 6 (Drag Pharma) | 091194 2012 | TE 526.7 | Out of specification (high dose) |
| TE 300 mg/mL | Ciclo 6 (Drag Pharma) | 101052 2013 | TE 480.4 | Out of specification (high dose) |
| TE 300 mg/mL | Ciclo 6 (Drag Pharma) | 101052 2013 | TE 402.4 | Out of specification (high dose) |
| TE 300 mg/mL | Ciclo 6 (Drag Pharma) | 101052 2013 | < LD | Counterfeit (no API) |
| TE 300 mg/mL | Ciclo 6 (Drag Pharma) | 1104016 2014 | TE 439.6 | Out of specification (high dose) |
| TE 300 mg/mL | Ciclo 6 (Drag Pharma) | No lot number 2015 | < LD | Counterfeit (no API) |

| Formulation declared | Product name and declared manufacturer | Lot number Expiry year | Results (mg/mL solution) | Conclusions |
|---|---|---|---|---|
| TE 300 mg/mL | Ciclo 6 (Drag Pharma) | 1201042 2015 | < LD | Counterfeit (no API) |
| TE 300 mg/mL | Ciclo 6 (Drag Pharma) | S1201042 2015 | < LD | Counterfeit (no API) |
| TE 300 mg/mL | Ciclo 6 (Drag Pharma) | 1304043 2016 | TE 416.2 | Out of specification (high dose) |
| TE 300 mg/mL | Ciclo 6 (Drag Pharma) | Absent information | < LD | Counterfeit (no API) |
| TE 300 mg/mL | Ciclo 6 (Drag Pharma) | Absent information | NF 4.4 | Counterfeit (different API) |
| TE 250 mg/mL | Enanthate de Testosterone (Adrian Mariner Labs) | A0C143 2012 | < LD | Counterfeit (no API) |
| TE 250 mg/mL | Testenat Depot (Landerlan) | 09-429 2012 | TE 424.8 | Out of specification (high dose) |
| TE 250 mg/mL | Testenat Depot (Landerlan) | 10-151 2012 | TE 426.0 | Out of specification (high dose) |
| TE 250 mg/mL | Testenat Depot (Landerlan) | 11-04-41 2013 | TE 543.1 | Out of specification (high dose) |
| TE 250 mg/mL | Testenat Depot (Landerlan) | 09-549 2014 | TE 495.8 | Out of specification (high dose) |
| TE 250 mg/mL | Testenat Depot (Landerlan) | 09-579 2014 | TE 552.1 | Out of specification (high dose) |
| TE 250 mg/mL | Testenat Depot (Landerlan) | 09-579 2014 | TE 552.2 | Out of specification (high dose) |
| TE 250 mg/mL | Testenat Depot (Landerlan) | 413-078 2015 | TE 374.0 | Out of specification (high dose) |
| TE 250 mg/mL | Testenat Depot (Landerlan) | 414-088 2016 | < LD | Counterfeit (no API) |
| TE 250 mg/mL | Testenat Depot (Landerlan) | 415-023 2017 | TE 457.4 | Out of specification (high dose) |

| Formulation declared | Product name and declared manufacturer | Lot number Expiry year | Results (mg/mL solution) | Conclusions |
|---|---|---|---|---|
| TE 250 mg/mL | Testenat Depot (Landerlan) | 415-062 2017 | TE 460.5 | Out of specification (high dose) |
| TE 250 mg/mL | Testenat Depot (Landerlan) | 415-074 2017 | TE 410.8 | Out of specification (high dose) |
| TE 250 mg/mL | Testobolin (Alpha Pharma) | 1378W913 2014 | TE 415.3 | Out of specification (high dose) |
| TE 250 mg/mL | Testoviron (Muscle Pharma) | No lot number 2016 | TE 349.3 | Out of specification (high dose) |
| TE 250 mg/mL | Testoviron (Muscle Pharma) | No lot number 2018 | TP 81.5 | Counterfeit (different API) |
| TP, TE, TC, Tren (30, 130, 110, 30 mg/mL) | Androgenon (USP Labs) | AGX550367 2014 | < LD | Counterfeit (no API) |
| TP, TE, TC, Tren (30, 130, 110, 30 mg/mL) | Androgenon (USP Labs) | AGX550369 2014 | TP 20.3/TE 73.6/TC 18.0 (and Tren) | Out of specification (low dose) |
| TP, TE, TC, Tren (30, 130, 110, 30 mg/mL) | Androgenon (USP Labs) | AGX550371 2014 | TP 9.9/TE 22.3/TC 19.7 (no Tren) | Out of specification (low dose, incomplete formulation, package is authentic) |
| TP 100 mg/mL | Propionato (Biopharma Underground) | 2011.02 2014 | < LD | Counterfeit (no API) |
| TP 200 mg/mL | Testogar (Mager Pharmazeutischer) | 002.09.18 2012 | TP 8.1 | Counterfeit (low dose) |
| TP 200 mg/mL | Testogar (Mager Pharmazeutischer) | 014.07.25 2013 | NF 8.8 | Counterfeit (different API) |
| TP 200 mg/mL | Testogar (Mager Pharmazeutischer) | 073.16.42 2014 | < LD | Counterfeit (no API) |
| TP 200 mg/mL | Testogar (Mager Pharmazeutischer) | 073.16.42 2014 | < LD | Counterfeit (no API) |
| TP 200 mg/mL | Testogar (Mager Pharmazeutischer) | 014.17.25 2015 | < LD | Counterfeit (no API) |

| Formulation declared | Product name and declared manufacturer | Lot number Expiry year | Results (mg/mL solution) | Conclusions |
|---|---|---|---|---|
| TP 50 mg/mL | Testosterona (Calastreme) | 25<br>2012 | < LD | Counterfeit (no API) |
| TP 50 mg/mL | Testosterona (Calastreme) | 25<br>2012 | < LD | Counterfeit (no API) |
| TP 50 mg/mL | Testosterona (Calastreme) | No lot number<br>2016 | NF 3.4 | Counterfeit (different API) |
| TP 100 mg/mL | Testosterona Propionato (USP Labs) | TEP100921<br>2014 | TP 25.1 | Out of specification (low dose) |
| TP 100 mg/mL | Testosterona Propionato (USP Labs) | TEP100925<br>2014 | TP 39.0 | Out of specification (low dose) |
| TP 200 mg/mL | Testosterona Propionato (Landerlan) | 414-106<br>2016 | TP 174.0 | Regular |
| TP 100 mg/mL | Testosterone Propionate (Gedeon Richter) | F0DA22<br>2012 | < LD | Counterfeit (no API) |
| Tren 100 mg/mL | Parabolan (Muscle Pharma) | No lot number<br>2016 | TE 7.0 (and Tren) | Counterfeit (additional API) |
| Tren 100 mg/mL | Parabolan (Muscle Pharma) | No lot number<br>2018 | TP 55.2 | Counterfeit (different API) |
| Tren 50 mg/mL | Trenbo-Life (VT Life) | C143<br>2012 | < LD | Counterfeit (no API) |
| Tren 75 mg/mL | Trenbo-Life (VT Life) | C143<br>2013 | < LD | Counterfeit (no API) |
| Tren 75 mg/mL | Trenbo-Life (VT Life) | E41D<br>2013 | < LD | Counterfeit (no API) |
| Tren 75 mg/mL | Trenbo-Life (VT Life) | E410<br>2014 | < LD | Counterfeit (no API) |
| Tren 75 mg/mL | Trenbo-Life (VT Life) | 46164<br>2015 | < LD | Counterfeit (no API) |
| | | | | |

| Formulation declared | Product name and declared manufacturer | Lot number Expiry year | Results (mg/mL solution) | Conclusions |
|---|---|---|---|---|
| Tren 75 mg/mL | Trenbolona Acetato (USP Labs) | TRE081094 2015 | TE 7.5/ND 0.5* | Counterfeit (different API) |
| Tren Enant 150 mg/mL | Trenbolona Enantato (USP Labs) | TXE300201 2015 | < LD | Counterfeit (no API) |

* Value obtained below the LOQ; therefore just an estimate.
BU = Boldenone Undecylenate; ME = Methandrostenolone; Methen = Methenolone Enanthate; ND = Nandrolone Decanoate; NF = Nandrolone Phenylpropionate; TP = Testosterone Propionate; TD = Testosterone Decanoate; TI = Testosterone Isocaproate; TF = Testosterone Phenylpropionate; PD = Drostanolone Propionate; TE = Testosterone Enanthate; TC = Testosterone Cypionate; Tren = Trenbolone Acetate, Tren Enant = Trenbolone Enanthate; API = Active Pharmaceutical Ingredient; LD = Limit of Detection

Table 04: Quantitative results for dietary supplements (Total N = 17).

| Formulation declared | Product name and declared manufacturer | Expiry year | Results (mg/tablet or capsule) | Conclusion |
|---|---|---|---|---|
| PR 25 mg/tablet | DHEA (Ultimate Nutrition) | 9010732 2012 | PR 16.3 | Regular |
| PR 25 mg/tablet | DHEA (Sun Naturals) | 904515 2012 | PR 19.6 | Regular |
| PR 25 mg/tablet | DHEA (GNC) | 5051JJ5436 2012 | PR 19.0 | Regular |
| PR 50 mg/tablet | DHEA (Ultimate Nutrition) | 9111822 2012 | PR 44.0 | Regular |
| PR 50 mg/tablet | DHEA (Ultimate Nutrition) | 911822 2012 | PR 36.9 | Regular |
| PR 50 mg/tablet | DHEA (Sun Naturals) | 907490 2012 | PR 38.7 | Regular |
| PR 50 mg/tablet | DHEA (Sun Naturals) | 907608 2012 | PR 50.7 | Regular |
| PR 50 mg/tablet | DHEA (Sun Naturals) | 4555 2013 | PR 34.5 | Regular |

| Formulation declared | Product name and declared manufacturer | Expiry year | Results (mg/tablet or capsule) | Conclusion |
|---|---|---|---|---|
| PR 50 mg/tablet | DHEA (Sun Naturals) | 010501<br>2013 | PR 30.7 | Regular |
| PR 100 mg/tablet | DHEA (Chosen Vitamins) | 2955208<br>2014 | PR 80.8 | Regular |
| PR (amount not specified) | Anavar (Hi-Tech Pharmaceuticals) | 10637037<br>2015 | PR 78.1 | Regular |
| PR (amount not specified) | Dianabol (Hi-Tech Pharmaceuticals) | 92201031<br>2015 | PR 54.5 | Regular |
| Methasterone 10 mg | M-Drol (Competitive Edge Labs) | 54443<br>2013 | ME 5.4 | Counterfeit (different API) |
| Methasterone 10 mg | M-Drol (Competitive Edge Labs) | 54445<br>2013 | ME 5.8 | Counterfeit (different API) |
| Halodrol 25 mg +<br>Methasterone 15 mg | Reign (Dark Cyde) | Absent information | PR 4.3 | Counterfeit (different API) |
| Halodrol 25 mg +<br>Methasterone 15 mg | Reign (Dark Cyde) | Absent information | PR 6.1 | Counterfeit (different API) |
| Halodrol 25 mg +<br>Methasterone 15 mg | Reign (Dark Cyde) | Absent information | PR 6.5 | Counterfeit (different API) |

PR = Prasterone; ME = Methandrostenolone

**Apêndice D**

Table 1: Quantitative results for caffeine (CAF) in dietary supplements in tablet form

| **Product name (manufacturer)** | **Lot number Expiry year** | **Declared CAF concentration (mg/tablet)** | **Detected CAF concentration (mg/tablet)** | **Detected / Declared CAF ratio (%)** |
|---|---|---|---|---|
| Hydroxycut (Inovate Health Sciences) | 3464007 2010 | 200 | 107.9 | 54.0 |
| Lipodrene (Hi-Tech Pharmaceuticals) | 9121086 2014 | 100 | 64.6 | 64.6 |
| Lipodrene (Hi-Tech Pharmaceuticals) | 9121096 2014 | 100 | 49.6 | 49.6 |
| Lipodrene (Hi-Tech Pharmaceuticals) | 10121011 2015 | 100 | 97.6 | 97.6 |
| Lipodrene (Hi-Tech Pharmaceuticals) | 10121011 2015 | 100 | 65.5 | 65.5 |
| Lipodrene (Hi-Tech Pharmaceuticals) | 10121011 2015 | 100 | 80.1 | 80.1 |
| Lipodrene (Hi-Tech Pharmaceuticals) | 151210943 2020 | 100 | 111.6 | 111.6 |
| Lipodrene (Hi-Tech Pharmaceuticals) | Absent information | 100 | 93.0 | 93.0 |
| Lipodrene-SR (Hi-Tech Pharmaceuticals) | 0195163 2012 | 75 | 86.2 | 114.9 |
| Stimerex-ES (Hi-Tech Pharmaceuticals) | 1017S01 2012 | 150 | 113.9 | 75.9 |
| Stimerex-ES (Hi-Tech Pharmaceuticals) | 9175113 2014 | 150 | 87.6 | 58.4 |
| Stimerex-ES (Hi-Tech Pharmaceuticals) | 9175113 2014 | 150 | 110.2 | 73.5 |

| Product name (manufacturer) | Lot number Expiry year | Declared CAF concentration (mg/tablet) | Detected CAF concentration (mg/tablet) | Detected / Declared CAF ratio (%) |
|---|---|---|---|---|
| Viper (Dymatize) | 9021-1 2912 | unspecified | 276.8 | unspecified |
| Vivadrein (Natural Sport) | 180112 2018 | 152.5 | 110.1 | 72.2 |

Table 2: Quantitative results for caffeine (CAF) in dietary supplements in capsule (solid contents) form

| Product name (manufacturer) | Lot number Expiry year | Declared CAF concentration (mg/capsule) | Detected CAF concentration (mg/capsule) | Detected / Declared CAF ratio (%) | Additional data |
|---|---|---|---|---|---|
| Amp´D (Dymatize) | 6284-2 2009 | Unspecified | 152.9 | Unspecified | |
| Amp´D (Dymatize) | 7193-3 2010 | Unspecified | 145.5 | Unspecified | |
| B4 (BPI) | 1304010 2016 | Unspecified | 271.8 | Unspecified | |
| B4 (BPI) | Illegible | Unspecified | 268.1 | Unspecified | |
| Black Mamba (Innovative Bio-Laboratories) | 12120 2015 | 200 | 349.0 | 174.5 | |
| Black Mamba (Innovative Bio-Laboratories) | 13JN9 2017 | 200 | 1.0* | 0.5 | |
| Black Spider (Cloma Pharma Laboratories) | 12947431 2015 | Unspecified | 265.7 | Unspecified | |
| Black Widow (Cloma Pharma Laboratories) | 60802 2013 | Unspecified | 224.6 | Unspecified | |

| Product name (manufacturer) | Lot number Expiry year | Declared CAF concentration (mg/capsule) | Detected CAF concentration (mg/capsule) | Detected / Declared CAF ratio (%) | Additional data |
|---|---|---|---|---|---|
| Black Widow (Cloma Pharma Laboratories) | 9110302 2012 | Unspecified | 288.0 | Unspecified | |
| D4 Thermal Shock (Cellucor) | Absent information | 145 | 157.9 | 108.9 | |
| D-Drol (4everfit) | 8130 2010 | Undeclared | 294.8 | Undeclared | Product declares to contain only prohormones |
| D-Drol (4everfit) | 8130 2012 | Undeclared | 205.7 | Undeclared | Product declares to contain only prohormones |
| D-Drol (4everfit) | 8130 2012 | Undeclared | 147.7 | Undeclared | Product declares to contain only prohormones |
| D-Drol (4everfit) | 8130 2013 | Undeclared | 74.8 | Undeclared | Product declares to contain only prohormones |
| D-Drol (4everfit) | 8130 2013 | Undeclared | 125.5 | Undeclared | Product declares to contain only prohormones |
| DHEA (Sun Naturals) | 603513 2009 | Undeclared | 88.1 | Undeclared | Product declares to contain only DHEA. Also detected ketamine and clobenzorex |
| Diablos (Innovative Bio-Laboratories) | 12111 2015 | 225 | 356.8 | 158.6 | |
| Diablos (Innovative Diet Labs) | 14J5V6 2017 | 225 | 0.8* | 0.4 | Sibutramine was detected |
| Diablos (Innovative Diet Labs) | 14O25B 2017 | 225 | 389.4 | 173.1 | |
| DiurX (Max Muscle Sport Nutrition) | Illegible | Unspecified | 2.0 | Unspecified | |
| Dyma-Burn Xtreme (Dymatize) | 10169-2 2013 | 165 | 178.2 | 108.0 | |

| Product name (manufacturer) | Lot number Expiry year | Declared CAF concentration (mg/capsule) | Detected CAF concentration (mg/capsule) | Detected / Declared CAF ratio (%) | Additional data |
|---|---|---|---|---|---|
| Dyma-Burn Xtreme (Dymatize) | 8077-5 2011 | 165 | 187.1 | 113.4 | |
| Dyma-Burn Xtreme (Dymatize) | 8077-5 2011 | 165 | 179.9 | 109.0 | |
| Dyma-Burn Xtreme (Dymatize) | 9233-4 2011 | 165 | 188.3 | 114.1 | |
| Dyma-Burn Xtreme (Dymatize) | 9192-2 2012 | 165 | 193.3 | 117.2 | |
| Dyma-Burn Xtreme (Dymatize) | 9192-2 2012 | 165 | 192.8 | 116.8 | |
| Dyma-Burn Xtreme (Dymatize) | 9262-1 2012 | 165 | 181.1 | 109.8 | |
| Dyma-Burn Xtreme (Dymatize) | 9330-2 2012 | 165 | 159.7 | 96.8 | |
| Dyma-Burn Xtreme (Dymatize) | 9330-2 2013 | 165 | 161.9 | 98.1 | |
| Dyma-Burn Xtreme (Dymatize) | 9330-2 2013 | 165 | 198.4 | 120.2 | |
| ECA Fuel (RFD) | Absent information | Unspecified | 47.6 | Unspecified | Amfepramone and femproporex were also detected |
| Energy Fuel (Ideasphere) | 209455341 2012 | 88 | 96.3 | 109.4 | |
| Epiburn Pro (USPLabs) | Illegible | 100 | 128.2 | 128.2 | |
| H57 (JEC Nutrition) | 0138B6 2012 | Unspecified | 0.6* | Unspecified | |

| Product name (manufacturer) | Lot number Expiry year | Declared CAF concentration (mg/capsule) | Detected CAF concentration (mg/capsule) | Detected / Declared CAF ratio (%) | Additional data |
|---|---|---|---|---|---|
| Halovar (Purus Labs) | 52730312 2014 | Undeclared | 18.9 | Undeclared | |
| Hydroxyelite (Hi-Tech Pharmaceuticals) | 14433434 2019 | 100 | 119.8 | 119.8 | |
| Hydroxystim (Muscletech) | 99732 2013 | 100 | 127.1 | 127.1 | |
| Hydroxystim (Muscletech) | 99782 2013 | 100 | 111.9 | 111.9 | |
| Methyldrene (Cloma Pharma Laboratories) | 9040409 2012 | 250 | 304.3 | 121.7 | |
| Methyldrene (Cloma Pharma Laboratories) | 9101403 2012 | 250 | 269.1 | 107.6 | |
| Methyldrene (Cloma Pharma Laboratories) | 2091003 2015 | 250 | 267.1 | 106.8 | |
| Methyldrene (Cloma Pharma Laboratories) | Illegible | 250 | 224.4 | 89.8 | |
| Oxyelite (USPLabs) | 216732 2017 | 100 | <LD | <LD | |
| Oxyelite (USPLabs) | 216732 2017 | 100 | <LD | <LD | |
| Oxyelite (USPLabs) | 216732 2017 | 100 | <LD | <LD | |
| Oxyelite (USPLabs) | 416294 2015 | 100 | 101.0 | 101.0 | |
| Oxyelite (USPLabs) | 416460 2015 | 100 | <LD | <LD | Sibutamine was detected |

| Product name (manufacturer) | Lot number Expiry year | Declared CAF concentration (mg/capsule) | Detected CAF concentration (mg/capsule) | Detected / Declared CAF ratio (%) | Additional data |
|---|---|---|---|---|---|
| Oxyelite (USPLabs) | 417203 2015 | 100 | 110 | 110.0 | |
| Oxyelite (USPLabs) | 419358 2015 | 135 | 8.8 | 6.5 | |
| Oxyelite (USPLabs) | 413700 2014 | 100 | 121.0 | 121.0 | |
| Oxyelite (USPLabs) | 414984 2014 | 100 | 106.6 | 106.6 | |
| Oxyelite (USPLabs) | 415103 2014 | 100 | 104.6 | 104.6 | |
| Oxyelite (USPLabs) | 415111 2014 | 100 | 117.0 | 117.0 | |
| Oxyelite (USPLabs) | 415111 2014 | 100 | 116.4 | 116.4 | |
| Oxyelite (USPLabs) | 416185 2015 | 100 | 114.8 | 114.8 | |
| Oxyelite (USPLabs) | 416185 2015 | 100 | <LD | <LD | |
| Oxyelite (USPLabs) | 416185 2015 | 100 | <LD | <LD | |
| Oxyelite (USPLabs) | 416185 2015 | 100 | 15.4 | 15.4 | |
| Oxyelite (USPLabs) | 416233 2015 | 100 | 116.4 | 116.4 | |
| Oxyelite (USPLabs) | 417025 2015 | 100 | 114.00 | 114.0 | |

| Product name (manufacturer) | Lot number Expiry year | Declared CAF concentration (mg/capsule) | Detected CAF concentration (mg/capsule) | Detected / Declared CAF ratio (%) | Additional data |
|---|---|---|---|---|---|
| Oxyelite (USPLabs) | 417186 2015 | 100 | 103.00 | 103.0 | |
| Oxyelite (USPLabs) | 417186 2015 | 100 | 119.00 | 119.0 | |
| Oxyelite (USPLabs) | 417186 2015 | 100 | 119.6 | 119.6 | |
| Oxyelite (USPLabs) | 417186 2015 | 100 | 113.2 | 113.2 | |
| Oxyelite (USPLabs) | 418460 2015 | 100 | <LD | <LD | Sibutramine was detected |
| Oxyelite (USPLabs) | 418824 2015 | 100 | 110.1 | 110.1 | |
| Oxyelite (USPLabs) | 419368 2015 | 135 | 148.7 | 110.1 | |
| Oxyelite (USPLabs) | 419652 2015 | 100 | 110.5 | 110.5 | |
| Oxyelite (USPLabs) | 420129 2016 | 100 | 134.0 | 134.0 | |
| Oxyelite (USPLabs) | 420210 2016 | 135 | 171.2 | 126.8 | |
| PowerFULL (USPLabs) | 30030110A 2013 | Undeclared | 0.5* | Undeclared | Product declares to “support healthy growth hormone and sleep” |
| Product without identification | Absent information | Undeclared | 49.3 | Undeclared | |
| Pyroburn (GE Pharma) | 0701IHC 2014 | Unspecified | 232.8 | Unspecified | |

| Product name (manufacturer) | Lot number Expiry year | Declared CAF concentration (mg/capsule) | Detected CAF concentration (mg/capsule) | Detected / Declared CAF ratio (%) | Additional data |
|---|---|---|---|---|---|
| Ramped (Dark Cyde) | 688212013 2015 | Unspecified | 240.1 | Unspecified | |
| Ripped Fast (Universal Nutrition) | 175309 2013 | 31.25 | 38.3 | 122.6 | |
| Ripped Fast (Universal Nutrition) | 176190 2014 | 31.25 | 36.2 | 115.8 | |
| Ripped Fast (Universal Nutrition) | 181884 2014 | 31.25 | 28.3 | 90.6 | |
| Roxylean (BPI) | 1402113 2017 | Unspecified | 331.9 | Unspecified | |
| Sledge (Dark Cyde) | Absent information | Undeclared | 84.2 | Undeclared | |
| Stack Xtreme (Medstar Nutriceuticals) | Absent lot number 2012 | 200 | 223.3 | 111.7 | |
| Stack Xtreme (Medstar Nutriceuticals) | Absent information | 200 | 0.5* | 0.3 | Sibutramine also detected |
| Stacker 3 (NVE Pharmaceuticals) | 105970 Absent lot number | 300 | 224.0 | 74.7 | |
| Super HD (Cellucor) | 0239F2F 2014 | 53.3 | 204.8 | 384.2 | |
| Super HD (Cellucor) | 0535E2 2014 | 53.3 | 167.9 | 315.0 | |
| Super HD (Cellucor) | 0072K4A 2016 | 53.3 | 0.8* | 1.5 | |
| Synedrex (Metabolic Nutrition) | 5391 2016 | Unspecified | 8.0 | Unspecified | |
| Thermalean (Nutrition Global) | 70140 2010 | Unspecified | 181.9 | Unspecified | |

| Product name (manufacturer) | Lot number Expiry year | Declared CAF concentration (mg/capsule) | Detected CAF concentration (mg/capsule) | Detected / Declared CAF ratio (%) | Additional data |
|---|---|---|---|---|---|
| Thermocore (Dynamic Formulas) | 1898 2017 | Unspecified | 367.1 | Unspecified | |
| Viventus (Sinérgika) | 1 2013 | Undeclared | 0.7* | Undeclared | Product of Brazilian origin. Declares to contain chitosan. aubergine extract and vitamin C |
| Xenadrine (Phoenix Laboratories) | 83411 Absent lot number | Unspecified | 87.2 | Unspecified | |
| Xpel (MHP) | 0566H4B 2017 | 18.75 | 19.0 | 101.3 | |
| Yellow Hornet (NVE Pharmaceuticals) | 0557165 Absent lot number | 300 | 309.8 | 103.3 | |
| Yellow Hornet (NVE Pharmaceuticals) | 0557165 Absent lot number | 300 | 267.5 | 89.2 | |
| Yellow Hornet (NVE Pharmaceuticals) | 0557165 Absent lot number | 300 | 319.3 | 106.4 | |
| Yellow Hornet (NVE Pharmaceuticals) | Absent information | 300 | 331.0 | 110.3 | |
| Yellow Swarm (Yellow Hornet (NVE Pharmaceuticals)) | 484054 2007 | 300 | 330.7 | 110.2 | |
| Zenalean Stack (Nutracoastal) | 1807057 2011 | Unspecified | 229.0 | Unspecified | |

*Values obtained below the methods LOQ, therefore just an estimate

Table 3: Quantitative results for caffeine (CAF) in dietary supplements in capsule (liquid contents) form

| **Product name (manufacturer)** | **Lot number Expiry year** | **Declared CAF concentration (mg/capsule)** | **Detected CAF concentration (mg/capsule)** | **Detected / Declared CAF ratio (%)** |
|---|---|---|---|---|
| Hydroxycut Hardcore (Muscletech) | 6203608 2010 | 100 | 110.5 | 110.5 |
| Hydroxycut Hardcore (Muscletech) | 7565409 2011 | 100 | 142.0 | 142.0 |
| Hydroxycut Hardcore (Muscletech) | 7970409 2011 | 100 | 127.5 | 127.5 |
| Hydroxycut Hardcore (Muscletech) | 8110109 2011 | 100 | 167.6 | 167.6 |
| Hydroxycut Hardcore (Muscletech) | 8252710 2012 | 100 | 136.0 | 136.0 |
| Hydroxycut Hardcore (Muscletech) | 8926910 2012 | 100 | 144.3 | 144.3 |
| Hydroxycut Hardcore (Muscletech) | 9451510 2912 | 100 | 196.9 | 196.9 |
| Hydroxycut Hardcore X (Muscletech) | 1687011 2013 | 100 | 185.0 | 185.0 |
| Hydroxycut Hardcore X (Muscletech) | Illegible | 100 | 144.0 | 144.0 |
| Leanfire v.2 (Instone) | LI000819 2008 | 100 | 81.8 | 81.8 |
| Lipo 6 (Nutrex Research) | 0967902 2012 | 100 | 143.1 | 143.1 |
| Lipo 6 (Nutrex Research) | 1115905C 2012 | 100 | 117.6 | 117.6 |
| Lipo 6 (Nutrex Research) | 1237906C 2012 | 100 | 145.0 | 145.0 |

| Product name (manufacturer) | Lot number<br>Expiry year | Declared CAF concentration (mg/capsule) | Detected CAF concentration (mg/capsule) | Detected / Declared CAF ratio (%) |
|---|---|---|---|---|
| Lipo 6 (Nutrex Research) | 1266908C<br>2012 | 100 | 138.0 | 138.0 |
| Lipo 6 (Nutrex Research) | 2322003C<br>2012 | 100 | 113.6 | 113.6 |
| Lipo 6 (Nutrex Research) | C001214-001<br>2013 | 100 | 145.3 | 145.3 |
| Lipo 6 Black (Nutrex Research) | 10009594<br>2012 | Unspecified | 44.8 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 10011011<br>2012 | Unspecified | 37.3 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 100015100<br>2013 | Unspecified | 59.7 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 10012961<br>2013 | Unspecified | 36.8 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 10012961<br>2013 | Unspecified | 38.4 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 10013251<br>2013 | Unspecified | 57.7 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 10013251<br>2013 | Unspecified | 60.5 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 10015100<br>2013 | Unspecified | 40.6 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 10015353<br>2013 | Unspecified | 18.0 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 10015353<br>2013 | Unspecified | 77.4 | Unspecified |

| Product name (manufacturer) | Lot number<br>Expiry year | Declared CAF concentration (mg/capsule) | Detected CAF concentration (mg/capsule) | Detected / Declared CAF ratio (%) |
|---|---|---|---|---|
| Lipo 6 Black (Nutrex Research) | 10016073<br>2013 | Unspecified | 43.6 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 10016081-P<br>2013 | Unspecified | 17.6 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 10016081-P<br>2013 | Unspecified | 18.9 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 10016680<br>2013 | Unspecified | 12.9 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 10016680<br>2013 | Unspecified | 25.8 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 10016831<br>2013 | Unspecified | 12.8 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 10016831<br>2013 | Unspecified | 51.9 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 3891012C<br>2013 | Unspecified | 211.7 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 50004246<br>2013 | Unspecified | 240.4 | Unspecified |
| Lipo 6 Black (Nutrex Research) | Illegible<br>2013 | Unspecified | 18.6 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 10017931<br>2014 | Unspecified | 63.3 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 10018618<br>2014 | Unspecified | 82.0 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 10019557<br>2014 | Unspecified | 81.4 | Unspecified |

| Product name (manufacturer) | Lot number<br>Expiry year | Declared CAF concentration (mg/capsule) | Detected CAF concentration (mg/capsule) | Detected / Declared CAF ratio (%) |
|---|---|---|---|---|
| Lipo 6 Black (Nutrex Research) | 10019557<br>2014 | Unspecified | 80.4 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 10022246<br>2014 | Unspecified | 205.2 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 10022246<br>2014 | Unspecified | 193.8 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 10022617<br>2014 | Unspecified | 214.9 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 10023978<br>2014 | Unspecified | 223.7 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 4352104C<br>2014 | Unspecified | 195.5 | Unspecified |
| Lipo 6 Black (Nutrex Research) | C001423-001<br>2014 | Unspecified | 203.0 | Unspecified |
| Lipo 6 Black (Nutrex Research) | C001423-001<br>2014 | Unspecified | 176.8 | Unspecified |
| Lipo 6 Black (Nutrex Research) | C001425-001<br>2014 | Unspecified | 193.8 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 5003040<br>2015 | Unspecified | 223.5 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 50000003<br>2015 | Unspecified | 255.7 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 50000005<br>2015 | Unspecified | 50.7 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 50000005<br>2015 | Unspecified | 73.4 | Unspecified |

| Product name (manufacturer) | Lot number<br>Expiry year | Declared CAF concentration (mg/capsule) | Detected CAF concentration (mg/capsule) | Detected / Declared CAF ratio (%) |
|---|---|---|---|---|
| Lipo 6 Black (Nutrex Research) | 50002011<br>2015 | Unspecified | 231.8 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 50000412<br>2015 | Unspecified | 211.2 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 50000412<br>2015 | Unspecified | 223.1 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 50000413<br>2015 | Unspecified | 216.1 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 50002332<br>2015 | Unspecified | 212.7 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 50002332<br>2015 | Unspecified | 199.6 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 50002332<br>2015 | Unspecified | 199.6 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 50002332<br>2015 | Unspecified | 187.4 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 50002947<br>2015 | Unspecified | 247.7 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 50002947<br>2015 | Unspecified | 225.8 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 60030171<br>2015 | Unspecified | 212.7 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 5003232<br>2016 | Unspecified | 207.3 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 50004418<br>2016 | Unspecified | 196.1 | Unspecified |

| Product name (manufacturer) | Lot number<br>Expiry year | Declared CAF concentration (mg/capsule) | Detected CAF concentration (mg/capsule) | Detected / Declared CAF ratio (%) |
|---|---|---|---|---|
| Lipo 6 Black (Nutrex Research) | 50009803<br>2016 | Unspecified | 200.8 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 50016933<br>2017 | Unspecified | 239.0 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 50029966<br>2017 | Unspecified | 190.4 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 50030960<br>2017 | Unspecified | 211.1 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 50030960<br>2017 | Unspecified | 195.7 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 50030961<br>2017 | Unspecified | 204.2 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 50013801<br>2017 | Unspecified | 203.4 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 50026218<br>2017 | Unspecified | 251.9 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 50029037<br>2017 | Unspecified | 200.6 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 50031139<br>2017 | Unspecified | 222.5 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 50031139<br>2017 | Unspecified | 190.1 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 50039041<br>2017 | Unspecified | 188.4 | Unspecified |
| Lipo 6 Black (Nutrex Research) | 50069477<br>2019 | Unspecified | 202.1 | Unspecified |

| Product name (manufacturer) | Lot number<br>Expiry year | Declared CAF concentration (mg/capsule) | Detected CAF concentration (mg/capsule) | Detected / Declared CAF ratio (%) |
|---|---|---|---|---|
| Lipo 6 Black (Nutrex Research) | Product came without package | Unspecified | 35.7 | Unspecified |
| Lipo 6 Black Hers (Nutrex Research) | 10013614<br>2013 | Unspecified | 95.7 | Unspecified |
| Lipo 6 Black Hers (Nutrex Research) | 10017375<br>2013 | Unspecified | 79.3 | Unspecified |
| Lipo 6 Black Hers (Nutrex Research) | 10017738<br>2013 | Unspecified | 55.5 | Unspecified |
| Lipo 6 Black Hers (Nutrex Research) | Illegible<br>2013 | Unspecified | 42.4 | Unspecified |
| Lipo 6 Black Hers (Nutrex Research) | 4359104C<br>2014 | Unspecified | 235.9 | Unspecified |
| Lipo 6 Black Hers (Nutrex Research) | 50000155<br>2015 | Unspecified | 61.7 | Unspecified |
| Lipo 6 Black Hers (Nutrex Research) | 60030151-1<br>2015 | Unspecified | 211.3 | Unspecified |
| Lipo 6 Black Hers (Nutrex Research) | 50013167<br>2017 | Unspecified | 66.2 | Unspecified |
| Lipo 6 Black Hers (Nutrex Research) | 50037728<br>2018 | Unspecified | 216.1 | Unspecified |
| Lipo 6 Unlimited (Nutrex Research) | 50022111<br>2017 | Unspecified | 166.2 | Unspecified |
| Lipo 6x (Nutrex Research) | 1111906C<br>2010 | 100 | 59.8 | 59.8 |
| Lipo 6x (Nutrex Research) | 961902<br>2011 | 100 | 60.9 | 60.9 |

| Product name (manufacturer) | Lot number<br>Expiry year | Declared CAF concentration (mg/capsule) | Detected CAF concentration (mg/capsule) | Detected / Declared CAF ratio (%) |
|---|---|---|---|---|
| Lipo 6x (Nutrex Research) | 1112906C<br>2012 | 100 | 71.4 | 71.4 |
| Lipo 6x (Nutrex Research) | 1205906C<br>2012 | 100 | 74.4 | 74.4 |
| Lipo 6x (Nutrex Research) | 1205906C<br>2012 | 100 | 47.0 | 47.0 |
| Lipo 6x (Nutrex Research) | 1205906C<br>2012 | 100 | 72.6 | 72.6 |
| Lipo 6x (Nutrex Research) | 1205906C<br>2012 | 100 | 99.5 | 99.5 |
| Lipo 6x (Nutrex Research) | 1868912C<br>2012 | 100 | 113.7 | 113.7 |
| Lipo 6x (Nutrex Research) | 1870912C<br>2012 | 100 | 94.8 | 94.8 |
| Lipo 6x (Nutrex Research) | 1885912C<br>2012 | 100 | 100.7 | 100.7 |
| Redline (Vital Pharmaceuticals) | I00002<br>2012 | Unspecified | 100.3 | Unspecified |
| Redline (Vital Pharmaceuticals) | 10032381-48<br>2013 | Unspecified | 120.5 | Unspecified |
| Redline (Vital Pharmaceuticals) | 11003238148<br>2013 | Unspecified | 100.6 | Unspecified |
| Redline (Vital Pharmaceuticals) | 12000538148<br>2014 | Unspecified | 128.2 | Unspecified |
| Redline (Vital Pharmaceuticals) | Absent information | Unspecified | 121.4 | Unspecified |
| Xenadrine (Inovate Health Sciences) | 7744408<br>2011 | 66.67 | 86.8 | 130.2 |

# ANEXO I

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Short communication

# Incidence of anabolic steroid counterfeiting in Brazil

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ABSTRACT

This retrospective study reports data obtained from the National Institute of Criminalistics of the Brazilian Federal Police Department (DPF) on 3676 anabolic products seized between 2006 and 2011. Anabolic androgenic steroids (AAS) were declared on the labels of 96.2% of the products. About one third of the products declared to be from Paraguay, and 14.3% from Brazil. Stanozolol, testosterone and nandrolone were the substances most declared on the labels. Package and qualitative chemical analyses (performed on 2818 products) found that 31.7% of the seized products were counterfeit, with an increase in the counterfeit detection rate during the period. Almost half of the fake products did not contain the declared substances, and 28.3% had only non-declared substances. Testosterone and its esters were responsible for 45% of the 582 cases of non-declared drug detection. Package analysis alone was responsible for the identification of 4.6% of all counterfeit products. These results indicate the need for a continuous effort by the government aimed at decreasing the availability of these products in the country.



## 1. Introduction

The presence of counterfeit medicines on the international market was first mentioned at a World Health Organization (WHO) convention held in 1985 in Kenya [1]. A counterfeit medicine may be defined as a product that is deliberately and fraudulently mislabeled with respect to its identity and/or source, and includes products with the wrong ingredients, without active ingredients, with insufficient active ingredients, with fake packaging and/or high levels of impurities and contaminants [1].

The WHO once estimated that fake medicines were responsible for 1% of all medicine sales in developed countries and could reach 30% in some regions of Eastern Europe, Africa, Asia and Latin America [2]. Worldwide sales of counterfeit medicines could top US$ 75 billion in 2009, a 90% rise in five years, but most studies only give a snapshot of the situation as counterfeiters are extremely flexible in the way they mimic products and avoid detection [3]. Any kind of medicine can be counterfeited, but common targets include antibiotics, hormones, analgesics, steroids, and antihistamines [1]. Data obtained from forensic reports issued by the Brazilian Federal Police Department (DPF) between 2007 and 2010 have shown that 69% of the counterfeits were phosphodiesterase type 5 inhibitors (used for erectile dysfunction), and 26% were anabolic androgenic steroids (AAS) [4].

The AAS testosterone was first used in humans in 1937 to treat hypogonadism and associated conditions, and currently its main uses are for hormonal dysfunctions and aplastic anemia [5,6]. In the United States, there are around 3 million AAS users, and about 3% of young adults have admitted the non-medical use at least once in their lives of medicines containing AAS, mainly for esthetic and strength gain purposes [7]. In Brazil, the non-medical use of AAS has been declared by 9% of interviewees in Goiânia [8], 11.1% in Porto Alegre [9], and 19% in São Paulo [10].

The aim of the present study was to investigate the data collected on medicine products said to contain drugs with anabolic action seized by the DPF from 2006 to 2011.

## 2. Material and methods

This is a retrospective study describing the data obtained from the Brazilian Federal Police Department's (DPF) Criminalistics System database on pharmaceutical product reports issued between January 1, 2006 and December 31, 2011 by forensic experts at all DPF offices nationwide. These products were seized in criminal situations, mainly from smuggling or in raids on places where they were being stored to be sold.

First, a search using "anabolic" as the keyword was conducted in the database to identify the most frequent products and the most frequent AAS declared to be present in the products. The search was then repeated using the nine most reported AAS and the fifteen most reported products as keywords.

Information obtained from the DPF reports include the year and state where they were issued, the name of the products, the ingredients and country of origin stated on the labels, and final conclusions. When available, results of the chemical analyses and the analytical techniques used were also obtained. Qualitative chemical

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analyses were usually performed by gas chromatography coupled with mass spectrometer (CG/MS), infrared or ultraviolet spectrophotometry, and/or liquid chromatography coupled with a time-of-flight mass spectrometer (LC/TOF).

## 3. Results

Of the 6348 reports in the Criminalistics System database on pharmaceutical products issued by the DPF during the period of study, 923 (14.5%) contained at least one of the keywords considered in this study. The reports were issued in 21 of the 26 Brazilian states and in the Federal District, covering all five Brazilian regions. Almost one-fifth (18.5%) of the reports were issued in the state of Parana', located on the border with Paraguay, an area with major smuggling problems. The state of Sa∼o Paulo, where the country's main airport is located, issued 13.5% of the reports.

The 923 DPF reports investigated in this study contained information on 3676 products that declared containing drugs with anabolic action, of which 3537 declared containing AAS, 99 declared containing clenbuterol, a β-agonist originally used as bronchodilator, 38 declared containing growth hormone, and 2 products declared containing gonadotropin. The number of anabolic products seized by the DPF increased sharply during the period under study, from 282 products in 2007 to 1468 in 2011 (Table 1). The DPF Criminalistics database was implemented in 2006, so the number reported for 2006 might not reflect the total number of anabolic products seized that year.

About one-third of the products were declared as coming from Paraguay and 14.3% from Brazil. Other common declared origins were Spain (9.6%) and the United States (7.6%) and 7.5% of the products had undeclared origin. Of the 145 different anabolic products found in the reports, ten of them (Stanozoland Depot, Durateston, Winstrol Depot, Winstrol V, Deca Durabolin, Stanozoland, Hemogenin, Nandrolone Decanoate and Ciclo-6) accounted for more than a half (52%) of all products seized. The most frequent AAS declared on the labels were stanozolol, testosterone and its esters, and nandrolone and its esters, accounting for, respectively, 29.6, 27.5 and 18.5% of the 3537 products from this class.

Qualitative chemical analyses were performed on 2818 products (76.7%), with almost all products seized in 2011 being analyzed (Table 1). Different drugs had different detection rates (detected/declared), ranging from 98.6% (clenbuterol) to 12.2% (boldenone and its esters); stanozolol was detected in 94% of the products declaring this compound (Fig. 1). Almost one-third of the 3676 anabolic products investigated were considered to be fake (31.7%), with an increase in the counterfeit detection rate during the period, reaching 38.8% in 2011 (Table 1). This increase was strongly correlated with the increase in chemical analyses ($r = 0.956$, $p = 0.003$).

**Table 1**
Products seized, products chemically analyzed and counterfeit detections during 2006–2011 by the Brazilian Federal Police Department.

| Year | Seized | Chemically analyzed | | Counterfeit detections | |
|---|---|---|---|---|---|
| | | *n* | % | *n* | % |
| 2006 | 41 | 1 | 2.4 | 1 | 2.4 |
| 2007 | 282 | 127 | 45.0 | 32 | 11.3 |
| 2008 | 474 | 180 | 38.0 | 99 | 20.9 |
| 2009 | 558 | 366 | 65.6 | 165 | 29.6 |
| 2010 | 853 | 748 | 87.7 | 300 | 35.2 |
| 2011 | 1468 | 1396 | 95.1 | 570 | 38.8 |
| Total | 3676 | 2818 | 76.7 | 1167 | 31.7 |

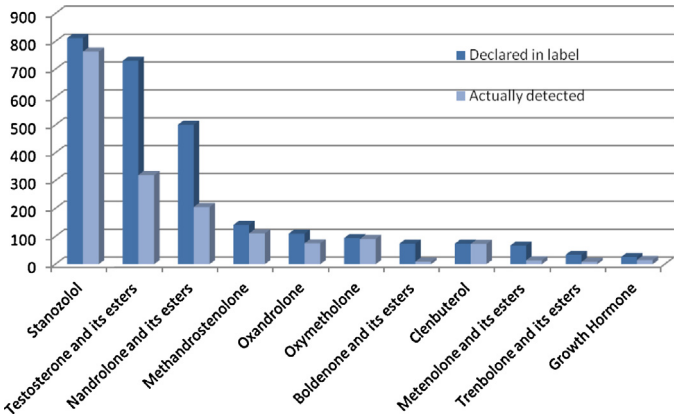


**Fig. 1.** Active ingredients declared on the product label and actually detected by CG/MS or LC/TOF (growth hormone).

Considering the 1167 fake products found in this study, 567 (48.6%) did not contain any active ingredient (for example, 11 "stanozolol" suspension products contained nothing but talc), 330 (28.3%) contained drugs that were different from those stated on the label, 188 (16.1%) declared an inexistent manufacturer or had fake packages, 66 (5.7%) did not contain all the drugs stated on the labels, and 16 (1.4%) contained additional drugs from those declared. In cases of non-declared drug detection ($N = 582$), the most frequent AAS involved were testosterone and its esters (45%), followed by nandrolone and its esters (18%) and prasterone (12%).

About 16.1% of the counterfeit products declared an inexistent manufacturer and/or had a fake package, and in these cases counterfeiting was easily identified by package analysis only. For

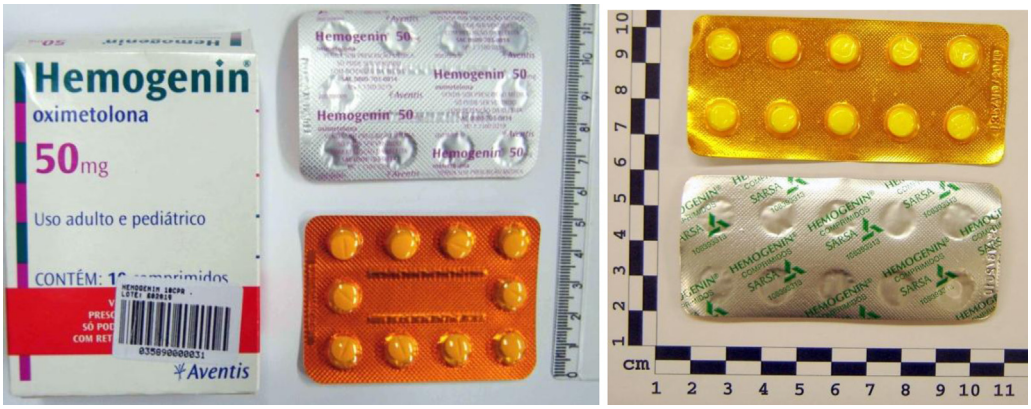


**Fig. 2.** Actual regular Hemogenin® package (left) and a fraudulent "Sarsa" package (right).


**Fig. 3.** (A) Original (left) and a fake (right) Hormotrop® flask, showing significant differences in the printing quality of the labels. (B) Fake Deca-drobol® flasks, with misspellings (Dacanoat instead of Decanoat, Proplonat instead of Propionat). (C) Stanazol® package with erasured label.

example, the product Hemogenin® was fraudulently commercialized under manufacturer names such as "Sarsa" with the appearance of the previous version of the original product (Fig. 2). Package analysis allowed the identification of 54 counterfeits among the 858 products that were not chemically analyzed, and which were replicas of an original product (and therefore could not be classified as "inexistent manufacturer"). Some examples are shown in Fig. 3.

## 4. Discussion and conclusions

The main strength of this study is the number of products investigated (3676), far larger than the number evaluated in most studies. Nevertheless, the percentage of counterfeit anabolic products found (31.7%) is similar to what has been described for the German and Belgian clandestine markets. In Germany, Musshoff et al. [11] found 35.7% of the 42 AAS products analyzed containing substances different than those stated on the label, or had no active ingredients. Similar results were obtained by Ritch and Musshoff [12], who evaluated 40 AAS-containing products (37.5% were fake), and Thevis et al. (35.4% of 48 products analyzed) [13]. Coopman and Cordonnier [14] found 33.8% of the 74 products analyzed in Belgium to be fake. Pellegrini et al. [15] found only two of the 15 AAS-containing products seized by the Italian Anti-Adulteration and Safety Bureau to contain what was declared on the label.

It should be noted that the counterfeit rate found in this study may be underestimated, as almost one-fourth of the products (23.3%) were not chemically analyzed. Additionally, the chemical analysis performed by the DPF was only qualitative, and counterfeiting due to the presence of the active ingredient at a different concentration from what was declared was not detected. Furthermore, as most of these products are foreign, neither the original packages nor information on them were available to aid in the package analysis.

In most countries, including Brazil, AAS are drugs that can only be sold with the presentation of a controlled prescription. However, a clandestine market at gyms and on the Internet has emerged to supply these products to those wishing to make non-medical use of AAS [11]. The users may assume that these products are weaker than the original and take a larger dose than recommended, which is a dangerous practice, as some fake products may contain as much as twice the stated content. Furthermore, the product may contain undeclared substances, which may lead to additional side effects. For example, women consuming a product that contains an undeclared AAS with high androgenic characteristics might have irreversible virilizing effects [16]. In addition to the health concerns, counterfeiting may have major economic impacts on health systems and legal manufacturers, which suffer with the loss of confidence by the public [1].

The results found in this study indicate that the total amount of anabolic products available on the clandestine market is rising in Brazil, requiring continuous efforts by the government to decrease the availability of these products in the country.

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## References

[1] WHO, General information on counterfeit medicines [online]. Available from: www.who.int/medicines/services/counterfeit/overview/en/index.html, 2010 (accessed on 21.01.2013).
[2] WHO, WHO-led anti-counterfeiting coalition examines technologies to prevent fake drugs [online]. Available from: www.who.int/mediacentre/news/releases/2007/pr07/en/index.html, 2007 (accessed on 21.01.2013).
[3] Growing threat from counterfeit medicines, Bull. World Health Organ 88 (2010) 247–248.
[4] J. Ames, D.Z. Souza, Counterfeiting of drugs in Brazil, Rev. Saude Publica 46 (2012) 154–159.
[5] P. Lenehan, Anabolic Steroids and Other Performance Enhancing Drugs, Taylor and Francis, London, 2004 (e-library).
[6] J.E. Sturmi, D.J. Diorio, Anabolic agents, Clin. Sports Med. 17 (1998) 261–282.
[7] J.A.B. Iriart, J.C. Chaves, R.G. Orleans, Body cult and use of anabolic steroids by bodybuilders, Cad. Saude Publica 25 (2009) 773–782.
[8] L.R. Araújo, J. Andreolo, M.S. Silva, Use of alimentary supplement and anabolizante for apprentices of muscular activity in the academies of Goiânia-GO, Rev. Bras. Ciênc. Mov. 10 (2002) 13–18.
[9] P.R.P. Silva, L.C.M. Júnior, V.C. Figueiredo, A.P. Cioffi, M.C. Prestes, M.A. Czepielewski, Prevalence of the use of anabolic agents among strength training apprentices in Porto Alegre, RS, Arq. Bras. Endocrinol. Metabol. 51 (2007) 104–110.
[10] L.S.M.F. Silva, R.L.M. Moreau, Use of anabolic-androgenic steroids among body builders in major gym centers in São Paulo, Brazil, Braz. J. Pharm. Sci. 39 (2003) 327–333.
[11] F. Musshoff, T. Daldrup, M. Ritsch, Black market in anabolic steroids—analysis of illegally distributed products, J. Forensic Sci. 42 (1997) 1119–1125.
[12] M. Ritsch, F. Musshoff, Dangers and risks of black market anabolic steroid abuse in sports—gas chromatography–mass spectrometry analyses, Sportverletz. Sportschaden 14 (2000) 1–11.
[13] M. Thevis, Y. Scharder, A. Thomas, G. Sigmund, H. Geyer, W. Schänzer, Analysis of confiscated black market drugs using chromatographic and mass spectrometric approaches, J. Anal. Toxicol. 32 (2008) 232–240.
[14] V. Coopman, J. Cordonnier, Counterfeit drugs and pharmaceutical preparations seized from the black market among bodybuilders, Ann. Toxicol. Anal. 24 (2012) 73–80.
[15] M. Pellegrini, M.C. Rotolo, R. Di Giovannadrea, R. Pacifici, S.A. Pichini, Simple toxicological analysis of anabolic steroid preparations from the Black market, Ann. Toxicol. Anal. 24 (2012) 67–72.
[16] M.S. Bahrke, C.E. Yesalis, Abuse of anabolic androgenic steroids and related substances in sport and exercise, Curr. Opin. Pharmacol. 4 (2004) 614–620.

# ANEXO II

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# Dietary supplements: International legal framework and adulteration profiles, and characteristics of products on the Brazilian clandestine market

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ABSTRACT

The objectives of this work were to evaluate current legislation on dietary supplements in the United States, the European Union and Brazil, and the profile of adulterated and/or irregular products on these markets. Due to a less restrictive legal framework, a supplement product that is freely available in the US may be considered a drug or even be proscribed in the EU and Brazil, thus giving rise to a clandestine market based on smuggling. From 2007 to 2014, the United States Food and Drug Administration reported 572 cases of supplement adulterations in the country, mainly products for sexual enhancement (41.6%). Data from the European Union Rapid Alert System for Food and Feed showed 929 adulterations during the same period, over 40% due to unauthorized ingredients or undeclared medicines. From 2007 to 2013, the Brazilian Federal Police Department seized 5470 supplement products, 92.2% with an American-declared origin. Qualitative chemical analyses performed on 2898 products found 180 adulterations, 41.1% due to undeclared drugs, mainly anabolic steroids, anorectics and products for erectile dysfunction, all considered medicines in Brazil. Educating the public regarding the potential risks they are taking when consuming adulterated or irregular products is necessary to protect the health of consumers.



## 1. Introduction

Dietary, food or nutrient supplements, referred to in this work as supplements, may be defined as concentrated sources of nutrients or other substances with a nutritional or physiological effect, marketed in dose form, with the purpose of supplementing the normal diet (EC, 2002). The use of supplements has been increasing worldwide in the last decades, even though the efficacy and safety of some of these products are still under discussion in the scientific community (Petroczi et al., 2011; Eudy et al., 2013; Cohen, 2012; Sepkowitz, 2013; Lachenmeier et al., 2013; Finley et al., 2014).

Most studies addressing the consumption of supplements worldwide involve athletes or physically active people, who are the main consumers of these products. Consumption rates for these populations in Brazil range from 20 to 94%, with an increase in recent years (De Rose et al., 2006; Goston and Correia, 2010; Silva and Marins, 2013; Carvalho-Silva et al., 2012; Fayh et al., 2013; Nogueira et al., 2013). Similar results were reported in Spain (28%; Oliver et al., 2011), Iran (66.7%; Saeedi et al., 2013), Germany (91.1%; Diehl et al., 2012), Canada (98.6%; Kristiansen et al., 2005), and the USA (46.7%; Jacobson et al., 2012).

The legal framework for supplements varies among countries. In Brazil, the category “dietary supplement” does not exist, and these products are placed in other food categories such as food for athletes, vitamins and/or mineral supplements, and foodstuffs with functional properties or health claims (SVS, 1998; ANVISA, 1999a,b; ANVISA, 2010a,b,c). Substances with therapeutic functions cannot be included in these products, as they are classified as medicines and are specifically regulated (BRAZIL, 1976). The distinction between foodstuffs and medicinal products is also clear in the European Union (EC, 2001), although there are the so-called “borderline products”, which contain substances that may have pharmacological effects at a given dose (Lachenmeier et al., 2012). In the United States, legislation allows a wider range of products to be marketed as supplements, which may contain a substance that has been

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0273-2300/

approved as a new drug, certified as an antibiotic, or licensed for biological use if, prior to such approval, it has been marketed as a supplement or food, unless stated otherwise by specific regulation (USA, 1994). Thus, many products that are legally commercialized in the United States as supplements are considered medicines in Brazil and in Europe and, as such, need to comply with all the obligatory requirements for a medicine product.

In addition to the legal issues, another potential problem related to supplements is the risk of adulteration. Supplements can be adulterated either unintentionally due to cross contamination, or intentionally with drugs to ensure or enhance the product's results. The substances reported to be most frequently used in supplement adulteration are steroids, stimulants, anorectics and phosphodiesterase inhibitors, used for erectile dysfunction (Geyer et al., 2004, 2008; Petroczi et al., 2011; Damiano et al., 2014).

The aims of this work were to overview the legislation related to supplements in Brazil, the European Union and the United States, the international scenario of supplement adulteration, and to evaluate supplements seized and analyzed by the Brazilian Federal Police Department (DPF) from 2007 to 2013.

## 2. Legal framework for dietary supplements

### 2.1. United States legislation

The first attempts made by the United States Food and Drug Administration (FDA) to regulate dietary supplements as drugs occurred in the 1960s and 1970s, which met strong resistance from consumers, including protests, and from manufacturers. In 1994, the US Congress approved the Dietary Supplement Health Education Act (DSHEA), which established that dietary supplements be treated as foods (USA, 1994), not drugs, which are regulated more stringently (USA, 1938). This Act effectively assured the public unrestricted access to dietary supplements (Brownie, 2005).

According to the DSHEA, dietary supplements may contain a vitamin, mineral, herb, botanical, amino acid, or a dietary substance to supplement the diet by increasing the total dietary intake, or a concentrate, metabolite, constituent, extract or combination of any of these ingredients. In addition, they may include substances that have been approved as a new drug or certified as an antibiotic if they were, prior to such approval, marketed as a dietary supplement or food (USA, 1994). This means that many substances with pharmacological actions can be regularly sold as food supplements in the US.

Under the DSHEA, supplement manufacturers are not required to notify, gain approval, or register their products with the FDA, nor are they obliged to obtain FDA approval to release the product on the market (USFDA, 2011a,b; Brownie, 2005; USA, 1994). They must comply with specific dietary supplement Good Manufacturing Practices (GMPs), which were established by the FDA in 2007 (USFDA, 2007). These GMPs include quality control procedures and recording requirements for each step in the manufacturing process, to ensure that the final product contains the appropriate ingredients at the right dose, without the presence of contaminants, such as toxins, bacteria, pesticides, glass, and heavy metals, or improper packaging and labeling.

Also according to the DSHEA, the FDA must prove – at its own expense – that a supplement presents an unreasonable risk of illness or injury before acting to remove it from the market as being unsafe. Contrary to what is required for drugs, manufacturers are not legally required to provide evidence that their product is safe or effective. Structure or function claims can be made on the supplement's label as long as the manufacturer has substantiation that such claims are "truthful and not misleading" and declares that "This statement has not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease" (Brownie, 2005; USA, 1994). The supplement label can also contain health claims, which must be authorized by the FDA and meet a significant scientific agreement (SSA), based on evidence from well-designed studies and agreement among experts (USFDA, 2009). Additionally, health claims can be used when they are based on authoritative statements from federal scientific bodies, within the FDA Modernization Act (USFDA, 1997), or when there are qualified health claims based on less scientific evidence but approved by the FDA, using standardized qualifying language (USFDA, 2003; Corby-Edwards, 2013).

The only case of necessary notification to the FDA is when manufacturers intend to include a new dietary ingredient in their products, meaning an ingredient that was not marketed as food in the US before October 15, 1994 (USDA, 1994). In this situation, manufacturers are required to notify the FDA of their plans 75 days before the product goes to market, and to submit evidence that the dietary ingredient would be reasonably expected to be safe under the conditions of use recommended or suggested in the supplement labeling (USFDA, 2013a,b; USA, 1994). Many manufacturers fail to report their intention to include new dietary ingredients, which has led to the withdrawal of some well-known products from the market (USFDA, 2013).

Only in 2011 did the US government introduce slightly more stringent measures to regulate the supplement market. The Food Safety Modernization Act (FSMA), which went into effect on January 4, 2011, changed part of the Federal Food, Drug and Cosmetic Act (USA, 1938), declaring that an officer or a qualified FDA employee may order the withdraw of any food item if they have reason to believe that it is adulterated or misbranded. If there is a reasonable doubt that consumption of a food item will cause serious adverse health consequences to humans and animals, the Agency may require that its distribution or sale be immediately ceased (USA, 2011; USFDA, 2013b). It was based on this new Act that the FDA managed to take OxyElite Pro off the market, due to the reasonable probability that it was related to several cases of liver failure caused by a new ingredient, aegeline, whose safety for consumers had not been demonstrated (USFDA, 2013b).

### 2.2. European Union legislation

In the European Union (EU), food is defined as "any substance or product, whether processed, partially processed or unprocessed, intended to be, or reasonably expected to be ingested by humans". 'Food' includes drink, chewing gum and any substance, including water, intentionally incorporated into the food during its manufacture, preparation or treatment". It is clearly stated in Regulation (EC) 178/2002 that "food" shall not include medicinal products (EC, 2002b). There are also several norms to regulate food products, such as Regulation 1925/2006 (which refers to fortified foods; EC, 2006b), Directive 2002/46/EC (which refers to food supplements, specifically vitamins and minerals; EC, 2002), and Regulation 1924/2006 (which refers to nutrition and health claims; EC, 2006). Some of these norms already foresee the need for establishing additional guidelines to cover a wider range of products already available on the market. The area is deemed well-regulated, although the norms may be difficult to interpret (Petroczi et al., 2011).

Medicinal products are defined as "(a) Any substance or combination of substances presented as having properties for treating or preventing disease in human beings; or (b) Any substance or combination of substances which may be used in or administered to human beings either with a view to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action, or to making a medical diagnosis" (EC, 2004). These products may not be placed on the market

without a prior authorization issued by the competent authorities of the Member State; requests for such authorization must be accompanied by an evaluation of potential environmental risks posed by the product, adverse reactions and results of pharmaceutical tests, pre-clinical tests and clinical trials, among others (EC, 2001).

In addition to these definitions and the requirement that foods may not contain medicinal products, the European Union legislation also includes the so-called "borderline products", referring to products containing substances that may, or may not, have pharmacological action depending on their dosage (Lachenmeier et al., 2012; Coppens et al., 2006). Such products were marketed as medicines until 1965, when Directive 65/65/EEC (EEC, 1965) stated that quality, efficacy and safety data were required for medicinal products. When these data could not be provided for a borderline product, the manufacturer simply changed its label from "medicine" to "dietary supplement" and continued to market it (Lachenmeier et al., 2012).

In the European Union, it is up to each Member State to decide whether a herbal or botanical product falls within the definition of a medicinal product. Herbs and botanical extracts may be present in functional foods, dietary supplements, and also in medicines, and a product would be considered a medicine when presented as having therapeutic or prophylactic properties, or used for medical diagnoses (EC, 2004; Eussen et al., 2011; Coppens et al., 2006). This may lead to a situation in which a product containing a bioactive ingredient at a certain dosage could be considered a dietary supplement in some Member States, but registered as a medicine in others. It is also possible that in a single Member State a given herb or botanical extract is sold both as a medicine and as a supplement, depending on its dosage and form (Eussen et al., 2011; Coppens et al., 2006).

EU legislation establishes that in cases of doubt regarding the "food × drug" nature of a product, the product should be regarded as a medicine, complying with the jurisprudence that has already been established by the European Court of Justice in borderline cases (EC, 2004; Coppens et al., 2006b).

The criterion of "possessing pharmacological action" is currently seen by EU courts as the most important indication to classify borderline products (Lachenmeier et al., 2012). A partial agreement was reached in 2008, suggesting that food and medical products could be distinguished based on the homeostasis of the body. Products intended to support, maintain or optimize normal physiological processes (without altering or blocking them) would be considered as foods, whereas medicines would be those intended to prevent disease or to correct these physiological processes when they are beyond normality, and therefore pathological (CE, 2008).

An important point is that any pharmacological action depends on the concentration of the substance in the body, and therefore a numerical threshold for each compound should be defined above which pharmacological action can be assumed (Lachenmeier et al., 2012). The Council of Europe (2008) reinforced the importance of evaluating a minimal therapeutic dosage. If a product contains a substance at levels below its minimal therapeutic dosage, it is no longer considered a medicine (CE, 2008; Lachenmeier et al., 2012; Coppens et al., 2006).

### 2.3. Brazilian legislation

In Brazil, the legal definition of medicine is "a pharmaceutical product, technically obtained or manufactured, with prophylactic, curative or palliative purposes, or destined to diagnose" (BRAZIL, 1973). Foodstuff is defined in Decree-Law 986/1969 as any substance or mixture of substances, in solid, liquid, paste or any other suitable form, aimed at providing the human organism with the normal elements required for its formation, maintenance and development (BRAZIL, 1969). This Decree clearly states that products with medicinal or therapeutic properties, regardless of how they are presented or consumed, cannot be considered as food.

There are many products, however, that are still classified as food according to Brazilian legislation but may nevertheless resemble medicines, either because they were "technically obtained or manufactured", are presented in tablet or capsule form and sold in pharmacies, or because they seem to have therapeutic properties. These products are classified under several categories, all under the jurisdiction of the National Health Surveillance Agency (ANVISA), with each having its specific regulations. Some examples include food products for athletes, vitamins and/or mineral supplements, and foodstuffs with functional properties or health claims. None of these products can contain substances with medicinal or therapeutic properties and, to avoid confusion between "functional properties" or "health claims" and therapeutic purposes, the norms specify what products can be sold under which category and what claims can be made on their labels. A summary of the main categories of food that are similar to dietary supplements, their legal definitions, and regulatory norms are shown in Table 1.

It is important to emphasize that some restrictions that are stated in a specific norm may apply to all other categories of food. For example, Ordinance n° 32/1998 states that vitamin and/or mineral supplements cannot contain more than 100% of the Recommended Daily Intake (RDI) of any vitamin and/or mineral (SVS, 1998). ANVISA Resolution RDC 18/2010 states that food for athletes shall not contain stimulants (with exception of caffeine), hormones, or other substances considered doping by the World Anti-Doping Agency (WADA) or related legislations, nor substances with therapeutic properties, including herbal drugs, or their association with nutrients or non-nutrients (ANVISA, 2010a,b,c). These and other restrictions stated in other sanitary norms are valid for all foods, once they are merely ratifications or exemplifications of what is stated in Decree-Law 986/1969.

Another peculiarity of Brazilian legislation is that herbal products, in general, are not considered food. Some plant extracts/derivatives may be commercialized under the "new foods and new ingredients" category that demands pre-market registration at ANVISA, such as *Plantago ovata* (a fiber supplement). The full list of approved new foods and new ingredients is available at the ANVISA website. Products containing other herb extracts must be registered as phytotherapic medicines, and require safety and efficacy data (ANVISA, 2014a,b,c,d).

An additional relevant aspect is the differentiation between medicines and foods on labeling. Food labels or packaging may not imply that the food has medicinal or therapeutic purposes, or indicate its consumption as a stimulant, to improve health, to prevent diseases, or as having curative action (ANVISA, 2002). One can conclude, therefore, that a product cannot be classified as food if its label states something like "lowering cholesterol levels", for that is the therapeutic action of hypolipidemic agents. However, one of the functional properties and health claims approved by ANVISA for phytosterols is "*Phytosterols help reduce cholesterol absorption*". Clearly there is a subtle difference between these two phrases, but to the general consumer they may seem the same.

The problem escalates when foreign products are introduced in the country and legal authorities must determine whether they should be classified as food, medicines or neither. To clarify matters, after being officially requested by the DPF, the ANVISA General Office of Medicines issued the Technical Note 04/2011 containing guidelines to differentiate medicines from foods. It states that any product, regardless of its nature, that presents therapeutic claims in its label or package, or that contains substances that are

**Table 1**
Categories of food similar to dietary supplements in the Brazilian sanitary legislation.

| Category | Product legal definition and examples | Legislation |
|---|---|---|
| Food for athletes | Specially formulated to achieve specific nutritional needs and improve performance. Examples: whey protein, creatine, Branched-chain amino acids, caffeine. | RDC 18/2010 |
| Vitamin and/or mineral supplement | Foods for supplying the daily intake of vitamins and/or minerals of a healthy person. | Ordinance n° 32/1998 |
| Foods with functional properties or health claims[a] | Functional properties claims: metabolic or physiologic role on growth, development, maintenance and other functions. Health claims: state, suggest or imply a relationship between the food or ingredient with a disease or a health-related condition. Examples: products containing phytosterols, omega-3, lutein and lycopene, inulin, chitosan. | RDC 18/1999 |
| New foods and new ingredients[a] | With no history of consumption in the country, or foods already consumed but containing substances in much higher levels than what is normally present in the diet. Examples: fish oil, soy lecithin, guaraná extract in capsules | RDC 16/1999 |
| Bioactive substances and probiotics with functional properties or health claims[a] | Bioactive substances: nutrients or non-nutrients with specific metabolic or physiologic action. Probiotics: live microorganisms that improve the intestinal microbiologic balance, such as *Bifidobacterium* sp. | RDC 02/2002 |

[a] Must be registered in the National Health Surveillance Agency prior to marketing (RDC 27/2010). RDC = Resolution of the Executive Board.

recognizably used for medicinal purposes due to their pharmacological properties, shall be considered as medicines. For example, products containing *Tribulus terrestris* extract "are classified as herbal medicines, for there are medicines registered at ANVISA with this composition". Melatonin, pro-hormones, ephedrine, synephrine, yohimbine and phenethylamines all have pharmacological activities and therefore, a product containing any of these substances should be classified as a medicine. Vasodilation is a pharmacological action and therefore products that claim to increase nitric oxide levels or, by any other means, declare to have vasodilatory properties should also be classified as medicines (ANVISA, 2011a,b).

Products considered supplements elsewhere, but classified as medicines in Brazil, must comply with medicinal laws and norms. According to Law 6.360/1976, updated by Law 10.742/2003, no medicine, including "medicine-supplements", may be sold in Brazil before being registered at ANVISA (BRAZIL, 1976; BRAZIL, 2003); the illegal trade of unregistered medicines is considered a crime against public health. According to the Penal Code (Art. 273), the counterfeiting, adulteration, corrupting or altering of a product intended for therapeutic or medicinal purposes, the sale or distribution of these products, and the sale or distribution of unregistered products are all considered crimes (BRAZIL, 1998).

It is legal to import unregistered products if they are not intended for sale, in amounts compatible with personal use, and if they do not contain proscribed or controlled substances (ANVISA, 2008; ANVISA, 2011b) listed in Ordinance 344/98 (SVS, 1998b) and its updates. However, most individuals are not fully aware of this legislation and its details. For example, dehydroepiandrosterone (DHEA), which is freely marketed in the US, is a controlled substance in Brazil. Dimethylamylamine (DMAA) was proscribed in Brazil in 2012, but buyers may not be aware that euphemisms, such as "geranamine" or "geranium oil", may be used to declare the substance in labels. Consumers may thus end up buying and bringing into the country a proscribed product which has the same legal status as cocaine (ANVISA, 2012).

The full spectrum of the Brazilian legislation is so large that it is not always fully known or understood by law enforcement professionals at national borders and customs offices throughout the country. This leads to the unnecessary seizure of legal products, such as creatine or whey protein, and of products that fall under the category of "unregistered medicines, which are neither controlled nor proscribed, and intended for personal use". To further complicate things, there is no norm stating what amount is considered "compatible with personal use". On the other hand, a single unit of an unregistered medicine may not be brought into the country if it is intended for sale. It is usually not possible for the immigration officer to evaluate on site what the intended use of the product is, especially if small amounts are involved. Consequently, the products may end up being seized, and the involved individual submitted to legal/sanitary sanctions.

## 3. Adulteration of dietary supplements

The adulteration of dietary supplements with undeclared classic drugs was first mentioned in a FDA's "Safety Alerts for Human Medical Products" in 2002 (USFDA, 2014). Since then, the health authorities of several countries, such as the National Institute for Public Health and the Environment of the Netherlands, Health Canada, and Swiss Medic have reported an increasing number of adulterations (USFDA, 2014; Geyer et al., 2011; Rebiere et al., 2012). The main targets include products indicated for weight loss, body building, and sexual performance enhancement (USFDA, 2011a,b). These adulterated products may contain approved drugs, analogs or other compounds (such as novel synthetic steroids), and can be found on the internet, and in retail and dietary supplement stores (USFDA, 2011a,b; Rebiere et al., 2012; Tang et al., 2011; Vaysse et al., 2010).

The presence of an undeclared substance in a supplement may be due to cross-contamination related to poor manufacturing practices and to the use of the same production line for several products. This is usually characterized by the presence of substances that are not necessarily related to the supplement claim (such as traces of steroids in vitamins and minerals), and are present at levels that might not be sufficient for pharmacological action, but may nevertheless lead to a positive result in anti-doping exams (Baume et al., 2006; Geyer et al., 2008, 2011).

The majority of adulteration cases, however, are intentional and aimed at increasing the efficacy of the supplement (Tang et al., 2011; Rebiere et al., 2012). These undeclared drugs may be present at levels that are much higher than those found in approved medicines, representing a health hazard for consumers (USFDA, 2011a; Geyer et al., 2008, 2011). Furthermore, it is not unusual for combinations of up to four or five active substances to be detected in adulterated supplements, which is of particular concern since interaction effects between these substances are not always known (Li et al., 2012; Rebiere et al., 2012).

Fraudulent supplements can cause serious adverse effects in humans, including strokes, acute liver injury, kidney failure, pulmonary embolisms, heart palpitations and death (USFDA, 2011a; Vaysse et al., 2010; USFDA, 2013a,b; Tang et al., 2011; Rebiere et al., 2012). Consumers may not be aware of the presence of drugs and the risk they are taking when consuming these products (USFDA, 2011b).

Banned or controlled anorectics such as sibutramine, fenfluramine and diethylpropione can be found in slimming products

(Geyer et al., 2011; Tang et al., 2011), and phosphodiesterase-5 inhibitors have been detected in supplements that claim to enhance sexual performance (Gratz et al., 2004). Designer steroids, which are not listed as ingredients in any currently available medication, are now produced exclusively for the nutritional supplement market, even though there is limited or no data regarding their effects and adverse reactions in humans (Geyer et al., 2011).

On its website, the FDA summarizes data on undeclared drug detection in dietary supplements in the US (USFDA, 2014b). The list (first entry in March, 2007) comprised 572 cases up to December 30, 2014, the main product categories being sexual enhancement (238 entries), weight loss (228) and muscle building (90). Sexual enhancement products contained sildenafil, tadalafil, vardenafil and their analogs, alone or in combination. Weight loss products contained mainly sibutramine, associated or not with its analogs or with phenolphthalein, which was also detected alone. Other weight loss drugs included DMAA, fenproporex, furosemide, rimonabant, fenfluramine, cetilistat and phenytoin, among others. Muscle building products contained either an anabolic steroid (not specified) or an aromatase inhibitor (not specified) (USFDA, 2014b).

Another view of the supplement adulteration situation in the US is given by the analysis of data produced by the FDA's MedWatch system, which is responsible for issuing safety alerts on human drugs, medical devices, vaccines and other biologics, dietary supplements and cosmetics. Regarding dietary supplements, these alerts may refer to the risk of adverse effects or drug interactions, bacterial contamination, excessive amounts of toxic substances (such as lead), and undeclared drugs. Data are available on the FDA website from the year 2000 and a summary of alerts issued since 2007 is shown in Table 2. It should be noted that one alert may refer to several different products, and that in nearly all cases the undeclared drug matched the supplement claim (e.g., weight loss products containing anorectic drugs). Until 2009, all supplement alerts were issued in the "Special nutritional and cosmetic products" section. In 2010, the FDA created the specific category of "Products with undeclared drug ingredients: products marketed as dietary supplements, but containing one or more unlisted drug ingredients" (USFDA, 2014).

In the European Union, notifications regarding dietary supplements are made through the Rapid Alert System for Food and Feed (RASFF), in operation since 1979, and available on the RASFF website (European Commission, 2014). From January 1st, 2007 to December 30th, 2014, a total of 929 notification records were found in the system for "dietetic foods, food supplements and fortified foods" (excluding baby and infant food products and notifications related to an industry, but not specifically to a product) (European Commission, 2014). These records are summarized in Table 3.

Over 60% of the RASFF notifications regarded the presence of an unauthorized ingredient, undeclared medicinal drug, and an unauthorized new ingredient (Table 3). The most frequent unauthorized ingredient was DMAA, followed by a variety of herbal extracts, yohimbine, and synephrine. The most frequent medicinal drugs were those related to erectile dysfunction (83 cases; mainly sildenafil, tadalafil and their analogs), and weight management (78 cases; mainly sibutramine). There were also 16 cases of products containing anabolic steroids, such as dehydroepiandrosterone, progesterone and androstenedione. Most of the novel ingredients were herbal extracts, such as *Hoodia gordonii*, *Eurycoma longifolia* (tongkat ali), *Stevia rebaudiana* and noni (European Commission, 2014).

Information regarding the adulteration of foods with drugs in Brazil is limited. Of the 63 Technical Reports issued by ANVISA from 2002 to October, 2014 regarding foodstuffs (ANVISA, 2014b), only two concerned the adulteration of food products with drugs, both cases being sibutramine present in products classified as "new foods and new ingredients" and in "foods for athletes". Searches on the internet found just one other case of adulteration reported by ANVISA, another sibutramine detection in a "new food and/or new ingredient" product. Adverse reactions to medicines, cosmetics and other products, technical complaints and intoxications may be reported by citizens, hospitals, universities, companies and others on the ANVISA Health Surveillance Notification System website (NOTIVISA), (ANVISA, 2014c). However, foodstuffs are not included in the system, hampering the communication between the general public and ANVISA regarding food product adulteration.

Several studies have been published worldwide investigating the presence of synthetic adulterants in dietary supplements, although most were conducted with a limited number of samples. Three studies of "natural slimming products" from Brazil tested 12 to 20 samples and found 30–90% were adulterated with prescription drugs, such as fenproporex, amfepramone, benzodiazepines or furosemide (Almeida et al., 2000; Carvalho et al., 2010; Doménech-Carbó et al., 2013). Studies conducted with samples originating from Japan, China, Syria, USA,UK, Hong Kong, France or "the internet" also found several cases of so-called natural slimming products or dietary supplements adulterated with sibutramine, phenolphthalein, fenfluramine, and other drugs (Vaysse et al., 2010; Tang et al., 2011; Li et al., 2012; Rebiere et al., 2012; Song et al., 2014). Gratz et al. (2004) found that 19 out of 40 samples of supplements for sexual performance originating from different

**Table 2**
Summary of United States Food and Drug Administration (FDA) MedWatch Safety Alerts regarding dietary supplements from 2007 to December 2014 (USFDA, 2014).

| Year (total) | Alerts |
|---|---|
| 2007 (12) | Possible contamination with *Salmonella*, undeclared sildenafil, tadalafil or their analogs, lovastatine, sibutramine |
| 2008 (9) | Excessive chromium and selenium, presence of ephedra alkaloids, aristolochic acid (carcinogenic and nephrotoxic) or human placenta (cannot be sold as a supplement), undeclared sildenafil analogs, fenproporex, fluoxetine, furosemide, cetilistat. |
| 2009 (4) | Possible contamination with *Salmonella*, risk of side effects, undeclared sildenafil analogs |
| 2010 (24) | Excessive lead, undeclared sildenafil, tadalafil and their analogs, sibutramine and its analogs, steroids, fenfluramine, propranolol and ephedrine |
| 2011 (19) | Possible contamination with *Salmonella*, package identical to a known antibiotic package, undeclared sildenafil, tadalafil and their analogs, sibutramine, superdrol (designer steroid), terazosin (used to treat benign prostatic hyperplasia, in a supplement to "support prostate health") |
| 2012 (16) | Possible contamination with *Salmonella*, undeclared sildenafil, tadalafil and sildenafil analogs, sibutramine, ephedra alkaloids, dexamethasone, diclofenac and methocarbamol (the last three associated in a supplement for arthritis, muscle pain, osteoporosis or bone cancer) |
| 2013 (45) | Undeclared allergens (soy or milk), risk of side effects, presence of DMAA (already forbidden at the time), traces of cloranfenicol (not related to the supplement's alleged purpose), undeclared sibutramine, fluoxetine, sildenafil, tadalafil, vardenafil, dapoxetine, phenolphthalein, methasterone, dimethazine, dimethyltestosterone, non-specified steroids and diuretics, methocarbamol, diclofenac, chlorpromazine, doxepin. |
| 2014 (45) | Possible contamination with *Salmonella* and other microorganisms, undeclared allergen (milk), presence of DMAA, undeclared sildenafil, tadalafil and sildenafil analogs, sibutramine, desmethyl sibrutamine, phenolphthalein, chlorzoxazone, dexamethasone, nefopam, cyproheptadine, diclofenac, ibuprofen, lorcaserin, naproxen, indomethacin, lovastatin, fluoxetine |

DMAA: dimethylamylamine.

**Table 3**
Summary of Rapid Alert System for Food and Feed (RASFF) notifications regarding dietary supplements from 2007 to December 2014 (European Commission, 2014).

| Year (total) | UNA ingredient | UND drug | UNA new ingredient | UNA irradiation | High levels of metals | Irregular product | Others |
|---|---|---|---|---|---|---|---|
| 2007 (100) | 21 | 11 | 24 | 13 | 7 | 5 | 19 |
| 2008 (56) | 6 | 9 | 4 | 16 | 4 | 1 | 16 |
| 2009 (91) | 9 | 19 | 21 | 7 | 8 | 1 | 26 |
| 2010 (114) | 14 | 35 | 18 | 12 | 8 | 2 | 25 |
| 2011 (116) | 27 | 39 | 13 | 5 | 5 | 10 | 17 |
| 2012 (143) | 40 | 42 | 20 | 15 | 9 | 3 | 14 |
| 2013 (126) | 36 | 28 | 9 | 7 | 18 | 16 | 12 |
| 2014 (161) | 65 | 33 | 32 | 5 | 10 | 17 | 21 |
| TOTAL | 218 | 216 | 141 | 80 | 69 | 55 | 150 |

UNA = unauthorized; UND = undeclared.

sources in the USA contained a synthetic phosphodiesterase inhibitor at therapeutic levels.

Geyer et al. (2004) published one of the most comprehensive studies investigating the presence of undeclared substances in dietary supplements. They analyzed 634 non-hormonal supplements originating from 215 companies in 13 different countries, and found that 94 samples (covering nearly all kinds of supplements) contained low levels of prohormones. The authors assumed that, since levels were low, they might have been due to cross-contamination. Other studies detected the presence of anabolic steroids as adulterants in supplements from Switzerland (Baume et al., 2006) and Belgium (Van Poucke et al., 2007), sometimes at levels high enough to be detected in anti-doping exams.

## 4. The clandestine market in Brazil – products seized by the Brazilian Federal Police Department (DPF)

Information on supplements seized by the DPF was obtained from the DPF Criminalistics System (SisCrim), which was implemented in 2007. The main objectives of the system are the registering of documents and materials related to forensic exams, and to archive forensic reports issued by the criminalistics units. Access to the SisCrim system is restricted to the DPF forensic experts and it allows word searches of its contents. In the present study, a search was conducted to retrieve information on supplements sent for forensic analysis by the DPF between January 1, 2007 and December 31, 2013. The search was first conducted using the keywords *suplemento*, *suplementos*, as well as dietary and supplement. In addition, based on a preliminary search, the keywords *lipo*, *creatina*, creatine, jack3d, pak, nutrition, dymatize, nutrex, dyma, naNO, whey, tribulus, fat and drol were included. All 18 keywords were searched simultaneously using the logical operator "or".

Data obtained from the reports included the year and state where they were issued, name and brand of the products, country of declared origin, and conclusions. More than one product may be included in a given report, and one product may be comprised of several identical units. When available, results of chemical analyses were also obtained from the reports. All analyses were qualitative, performed by forensic experts using screening methods by Gas Chromatography–Mass Spectrometry (Agilent Technologies, GC 6890N coupled with MS 5973 Inert or GC 7890A coupled with MS 5975C) and/or Infrared Spectrometry with Fourier Transform (Thermo Scientific, FT-IR Spectrometer Nicolet iS10, Nicolet 380 or Nicolet Nexus 470). In certain cases, it was also necessary to perform a specific DMAA-confirmation analysis by Liquid Chromatography–Time-of-Flight Mass Spectrometry (LC-MSD TOF, Agilent Technologies 1100 Series). These methods are routinely used by the DPF laboratories in forensic analyses.

The SisCrim search produced 1222 forensic reports, which included the results of 5470 products classified as supplements. There was an upward trend in the number of reports that included supplements and of products over the years, increasing from 13 reports (118 products) in 2007 to 402 reports (1590 products) in 2013. Considering the total number of forensic reports issued by the DPF (including other forensic fields), reports containing supplement data ranged from 0.35‰ of all reports in 2007 to 9.02‰ in 2013.

The reports were issued by the criminalistics units of 20 of the 26 Brazilian states and of the Federal District. The majority of products was seized in the states of *Paraná* and *São Paulo* (34.6 and 22.4%, respectively), followed by *Mato Grosso do Sul* (10.9%) and *Ceará* (9.6%). These results were expected given that 94.8% of products were of declared foreign origin (92.5% declared to be manufactured in/by the US): *Paraná* and *Mato Grosso do Sul* are states bordering Paraguay, from which most illegal products enter the country, and *São Paulo* is where the main Brazilian international airport is located.

### 4.1. Supplement categories and products

Once individualized by name and brand, the search revealed 1535 different supplement products, which were classified into categories according to their declared composition and information on the packaging. Some of these products were further divided into subcategories. The categories, their characteristics, and the number of products seized are shown in Table 4. In cases where the claims of the product did not reflect the composition shown on the label, the classification was made based on the declared composition. For example, a product containing only amino acids, but that claimed to "increase the amount of circulating testosterone", was classified as an amino acid.

The most frequent products found in the reports were slimming/energy (SLIM) items, also known as fat burners, accounting for 23.5% of the products (Table 4). These were followed by hormone modulators (MOD; 17.1%), and by those in the vasodilator/volumizer category (VASO; 16.1%), also known as pre-workout products. Among the products classified as having a defined therapeutic action (N = 297, Table 4), the most frequent were those recommended to promote better sleep (N = 105) and joint rebuilders (N = 78), followed by those claiming to "improve the circulatory system" and diuretics (N = 19 and 18, respectively). Among products classified as herbal (N = 125), most claimed to have a positive effect on concentration and mental focus (N = 26), followed by those recommended for prostatic hyperplasia (N = 15), immune system adjuvants (N = 14), and antioxidants (N = 12).

Fig. 1 shows the percentages of the main supplement categories seized over the period of the study. SLIM accounted for 19–34% of all products over the entire period. MOD and VASO supplements have "gained importance" over the years (from 7.6% of all products in 2007 to 43% in 2013), while amino acids and vitamins/minerals and proteins showed a tendency of "losing importance" with time.

The 5470 products analyzed were individualized by name,

**Table 4**
Dietary supplement products seized by the Brazilian Federal Police Department (DPF) from 2007 to 2013, classified by category.

| Category (example) | Characteristics of the products | N (% of total) |
|---|---|---|
| SLIM (Lipo 6 Black®) | Indicated for weight loss (*fat burners*), usually containing central nervous system stimulants | 1285 (23.5%) |
| MOD (DHEA®, M-Drol®) | Anabolic-androgenic steroid precursors (pro-hormones), products claiming to increase endogenous steroids | 938 (17.1%) |
| VASO (Jack3d®) | "Pre-workout", claim to provide energy and promote vasodilation and/or increase muscle cell volume | 880 (16.1%) |
| Amino acid (BCAA, creatine) | One or few amino acids, or amino acid products | 665 (12.2%) |
| Vitamin/mineral (Centrum®) | Containing vitamins and/or minerals | 460 (8.4%) |
| Protein (*Whey protein*) | Containing only or mainly proteins | 366 (6.7%) |
| Therapeutic Action (Osteo Bi-Flex®, Melatonin®) | Claiming specific therapeutic action, excluding weight loss or anabolism, such as joint repair, diuretic or sleep improvement. | 297 (5.4%) |
| Multifunctional (Animal Pak®) | Small plastic bag with several pills/capsules, a complex formulation and several claims | 202 (3.7%) |
| Herbal (Ginkgo Plus®, Green Tea®) | Plant extracts (with exception of *Tribulus terrestris*); usually with well defined therapeutic claims. | 125 (2.3%) |
| Oil (Omega 3-6-9®) | Containing only fatty acids | 41 (0.7%) |
| Meal replacements (Lean Mass®) | Containing carbohydrates, proteins and sometimes fat; controlled calories | 38 (0.7%) |
| Hypercaloric (Mega Gainer®) | Containing carbohydrates, proteins and fat; high calories | 37 (0.7%) |
| Carbohydrate (Carb Up®) | Containing simple carbohydrates, such as maltodextrin | 33 (0.6%) |
| Nutrients for hair, skin and nails (Natural Gelatin®) | Indicated to nourish the hair, skin or nails | 20 (0.4%) |
| Antioxidant (Cell Guard®) | Non-herbal products with purported anti-oxidant activity | 19 (0.3%) |
| Probiotic (AcidoPhilus®) | Containing microorganisms that are believed to improve health | 10 (0.2%) |
| Recovery (After FX®) | Supposedly improve recovery after exercising | 10 (0.2%) |
| Others | None of the previous categories, include soy lecithin and chitosan | 44 (0.8%) |

SLIM: slimming/energy; MOD: hormone modulators; VASO: vasodilator/volumizer.

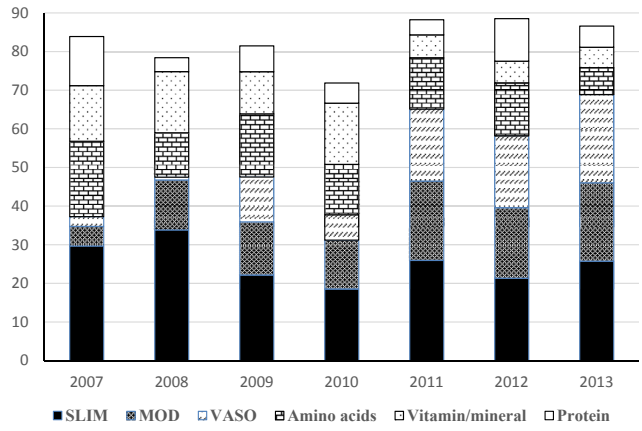


**Fig. 1.** Percentages of the main supplement categories seized by the Brazilian Federal Police Department from 2007 to 2013 (SLIM = slimming/energy; VASO = vasodilator/volumizer; MOD = hormone modulator).

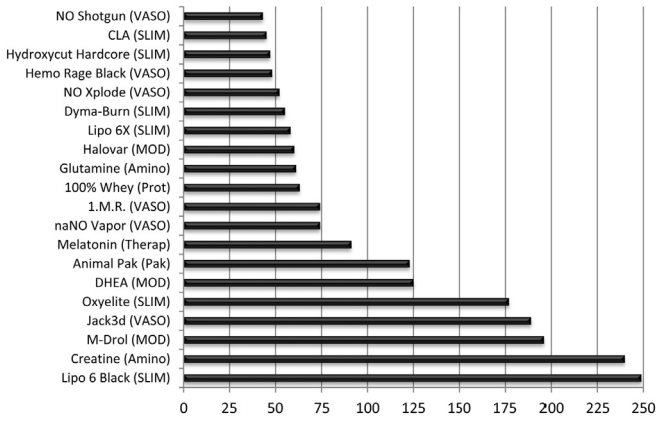


**Fig. 2.** Most frequent products seized by the Brazilian Federal Police Department from 2007 to 2013 individualized by name, independent of the brand. SLIM = slimming/energy; VASO = vasodilator/volumizer; MOD = hormone modulator; Amino = amino acid; Pak = multifunctional pak; Therap = therapeutic action; Prot = protein.

regardless of the brand. Of the twenty most frequent products, seven were VASO, six SLIM, and three MOD (Fig. 2). The most frequent products were Lipo 6 Black (SLIM, N = 249), and Creatine (Amino acid, N = 240).

### 4.2. Chemical analyses and adulterations

The DPF has no standard procedure for the forensic evaluation of dietary supplements. When deemed necessary by the forensic expert, chemical analyses of the seized products were performed. Between 2007 and 2013, most products (N = 2898, 53%) were chemically analyzed, a percentage that has increased over the years (from 17.8% in 2007 to 58.7% in 2013), reaching a maximum in 2011 (75.3%). All analyses were qualitative, and most were non-specific screenings (except DMAA-confirming analysis) aimed mainly at the detection of undeclared synthetic drugs. Hence, products could only be considered adulterated when an undeclared substance was detected or when the product did not contain a declared substance and such substance was known to be detectable by the techniques used. In total, 180 adulteration cases were detected (6.2% of analyzed products), with a steady increase in the number of adulterations detected over the years, reaching almost 10% of all products chemically analyzed in 2013. The origin of adulterated products was related to the origin of all products (92.2% of adulterated products with declared north-American origin; 5% of adulterated products were of declared Brazilian origin).

The types of adulteration, their definitions, and the number of products are listed in Table 5. In 41.1% of the cases, the product contained an undeclared drug related to what was claimed on the label, mainly anabolic steroids, anorectics or phosphodiesterase inhibitors. Undeclared drugs from other therapeutic classes (mainly caffeine and anti-inflammatory drugs in MOD products) accounted for 21.7% of the cases. Since all analyses were qualitative, it was not possible to determine the concentration of the substances detected. In most cases, the detected substance was shown as a significant peak in the GC–MS analysis, and no forensic report mentioned needing to concentrate the sample in order to detect undeclared drugs.

About 60% of the MOD and SLIM products were chemically analyzed, accounting for 95.6% of the adulterated products (132 and 40 products, respectively). Among the MOD, 39.4% had an undeclared related drug (such as turinabol, oxymetholone and

**Table 5**
Types of adulterations found in the supplements chemically analyzed by the Brazilian Federal Police Department (DPF) from 2007 to 2013 (total of 180 adulterated products).

| Type | Examples | N (%) |
|---|---|---|
| Undeclared pharmacologically related drugs | Presence of anabolic steroids in MOD (oxymetholone, metandienone or oral turinabol), presence of anorectics in SLIM (sibutramine, fenproporex), presence of phosphodiesterase inhibitors in herbal products indicated to enhance masculine sexual performance (tadalafil) | 74 (41.1) |
| Undeclared drug of another therapeutic class | MOD or anti-aging product that only contained caffeine | 39 (21.7) |
| Incomplete formulation | Suppression of listed ingredient, such as Oxyelite Pro containing only yohimbine, but not the declared caffeine and DMAA. | 29 (16.1) |
| Absence of active ingredients | Amino acid product containing only starch | 24 (13.3) |
| Replacement by structurally similar substances | Replacement of pro-hormones such as halodrol, methasterone and dienedione by dehydroepiandrosterone, 16,17-epoxyprogesterone or 16-dehydroprogesterone | 14 (7.8) |

SLIM: slimming/energy; MOD: Hormone modulators; DMAA: dimethylamylamine.

metandienone), 28.0% contained undeclared substances of another therapeutic class (mainly caffeine, with five dipyrone and two aminopyrine detections), and 16.7% had no active substance whatsoever. Of the 40 SLIM adulterated products, 55.0% did not contain all the substances declared on the package and 42.5% had an undeclared related medicine (such as sibutramine, fenproporex, phenolphthalein or amfepramone). The other eight adulterated products were: three therapeutic action products (all for sexual enhancement, containing phosphodiesterase inhibitors), two multifunctional paks that claimed to increase muscles and contained undeclared pro-hormones, one protein product containing sibutramine, one amino acid product containing only starch, and one product classified in the "others" category, with purported anti-aging properties, but containing only undeclared caffeine (Table 5).

The adulteration rate for the MOD category was 22.1% (597 products, 132 adulterations), whereas SLIM products had a far lower rate of 5.2% (766 products, 40 adulterations). Out of the six analyzed products with a declared therapeutic action of male sexual enhancement, three were adulterated.

Most of the adulterations (68.9%) concerned only five products: Halovar (MOD; N = 39), M-Drol (MOD; N = 31), Oxyelite Pro (SLIM; N = 26), Reign (MOD; N = 17) and D-Drol (MOD, N = 11). It was not possible to determine if these adulterated products were from the original manufacturer, or whether they were counterfeits made in clandestine facilities as the original packaging was not available at the forensic laboratory.

## 5. Discussion

In most part of the world medicines tend to be more strictly regulated than food products (McCann, 2005; Brownie, 2005; Eussen et al., 2011; Lachenmeier et al., 2012; Coppens et al., 2006). There is no simple way of regulating dietary supplements, and determining whether dietary supplements are food, medicines, or fall in an "in-between" category is an issue of the utmost importance to decide which legal norms apply to these products.

Brazilian legislation is similar to the EU in several aspects, but both are more restrictive in comparison with US legislation for requiring pre-market registration for some supplements freely marketed in US. In Brazil, "herbal supplements" must be registered as phytotherapic medicines (such as those containing St John's Wort or *T. terrestris* extract) or, in some cases, as "new foods and new ingredients". In both situations, the safety of the product must be attested to by the registration authority. One example of a Brazilian legislation restriction regards green tea extract in capsules, which is not allowed in Brazil, although green tea is freely available as a food. According to ANVISA, "capsule" is a new presentation of green tea, and must be registered as a new food, requiring evidence of its safety (ANVISA, 2010c).

Brazil, United States and the European Union forbid the presence of medical claims (stating that the product can prevent, treat or cure a disease) in food product packaging or labeling, but allow such claims for medical products (EC, 2000; USA, 1994; Eussen et al., 2011). Food products may present nutrition or health claims, such as "Phytosterol esters have been shown to lower/reduce blood cholesterol. High cholesterol is a risk factor in the development of coronary heart disease". It is not allowed, however, to state "phytosterol esters reduce the risk of coronary heart disease", a subtle distinction, even though this conclusion is easily reached by most consumers who read the approved health claim (Eussen et al., 2011).

The vitamins and/or mineral supplements category is apparently well-defined in Brazil and the European Union. While in Brazil these products must contain between 25 and 100% of the Recommended Daily Intake (SVS, 1998), in Europe the maximum amount should take into account the upper safe levels and intakes from other dietary sources. US legislation, on the other hand, does not mention any maximum amount of vitamins and/or minerals in supplements.

In Brazil, certain categories of food, such as those with functional properties or health claims, have positive lists of substances that can be legally present. On the other hand, the permissive nature of the US DSHEA and the non-publication of additional regulation established by EU Directive 2002/46/EC may produce borderline products on their respective markets. Brazil and the EU explicitly forbid the presence of substances with therapeutic action in products sold as food. However, legislation in certain European countries may grant a different status to a given product. Determining whether a product has or does not have therapeutic action may be difficult in some cases, and it is an issue that EU is trying to address by adopting the homeostasis model. The US allows several products with therapeutic action to be marketed as dietary supplements, as long as they have been in use before the enactment of the DSHEA in 1994 and bear a sentence stating that such products are not intended to diagnose, treat, cure or prevent diseases.

Data on supplement products seized by the DPF from 2007 to 2013 showed that most were slimming products (SLIM), hormone modulators (MOD) and vasodilators/volumizers (VASO). Practically no product in these categories may be commercialized in Brazil as a supplement, as they contain substances with therapeutic purposes, stimulants, and/or contain more than 100% of the RDI of a vitamin or mineral. Differences in legislation between countries mean that many of these products are legally available abroad, but because Brazilian legislation is confusing and scattered, Brazilian travelers may not be aware that some products are considered medicines or even proscribed in Brazil.

SLIM and MOD products make similar claims, and sometimes have similar composition as medicines with anabolic and anorectic actions. Both therapeutic classes are subject to control by ANVISA, and cannot be obtained in Brazil without a prescription. The fact that these were the two most frequently seized categories by the

DPF suggests that consumers are replacing anabolic steroids and anorectic medicaments with dietary supplements that have allegedly similar functions.

Results from the DPF suggest that the market reacts positively to regulation, when new products are approved. This phenomenon became very evident with products containing only creatine, whose seizures diminished significantly after the approval of creatine as food for athletes by RDC 18/2010 (6% of all products in 2009 and 2.1% in 2013). On the other hand, DMAA was banned in Brazil in 2012 (ANVISA, 2012), but the two most frequently seized products in 2013 were Oxyelite Pro and Lipo 6 Black, both containing DMAA (15% of the total seized that year). While the creatine case illustrates the positive effect of regulation on the clandestine market, the case of DMMA and others indicate that this market is not inhibited by regulation nor even by proscription as long as there is a consumer demand for the product.

The fact that supplements are commercialized as "food" leads consumers to perceive that they are harmless and devoid of adverse effects (McCann, 2005; Vaysse et al., 2010; Tang et al., 2011), and the adverse effects are rarely mentioned on the product label. Even ingredients common in supplements, such as caffeine, creatine or Ginkgo Biloba, can lead to serious adverse effects (Bove, 2002; Eudy et al., 2013; Sepkowitz, 2013). Herbal extracts in supplements have been implicated in cases of liver injury, allergic reactions, toxic reactions and drug interactions, sometimes resulting in death (Ernst, 1998; Navarro and Seeff, 2013; Timcheh-Hariri et al., 2012). MOD products may be associated with all adverse effects related to anabolic steroid consumption, including masculinization in women, hepatotoxicity, and alteration of blood lipid levels and coagulation factors (Shahidi, 2001; Kicman, 2008). DMAA was banned in Brazil and other countries due to, among others, cases of cerebral hemorrhage and deaths associated with its intake (Eliason et al., 2012; Gee et al., 2012; Health Canada, 2011; USFDA, 2013b).

However, due to certain restrictive aspects of Brazilian legislation, products with otherwise "harmless" formulations may be considered irregular or classified as medicines. For example, according to RDC 18/2010, caffeine supplements for athletes must not contain "nutrients or other non-nutrients", and therefore a product containing a mixture of creatine and caffeine is considered an "unauthorized association". Likewise, although protein and branched-chain amino acid (BCAAs) supplements are legal, adding BCAAs to a protein supplement is not allowed. This was the case with Isofast-MHP, whose distribution and sale was forbidden in the country (ANVISA, 2014d).

The adulteration of dietary supplements, mainly by the inclusion of undeclared drugs or other non-approved ingredients, is another point of concern. Its occurrence has been well documented abroad over the past decade, and both the US and EU sanitary authorities have systems informing consumers of the detection of adulterated products (Tables 2 and 3). Brazil does not have a comparable notification system, since the NOTIVISA does not include foodstuffs. Data reported by the FDA and the RASFF indicate that the number of adulterations detected over the past years has remained stable or increased slightly. As the total number of products evaluated was not available, it was not possible to determine if the adulteration rates were actually increasing.

In general, investigations on supplement adulterations published in the literature have focused on target products to detect undeclared drugs, mainly anorectics, anabolic steroids or drugs for erectile dysfunctions. Few studies have been published in Brazil in this area, and they usually refer to the investigation of anorectics in herbal formulations. Considering the nine adulterated products found in this study whose declared origin was Brazil, five were SLIM products containing sibutramine (3 cases), femproporex or an association of chlordiazepam and fluoxetine, one was a protein product also containing sibutramine, and the three others contained caffeine, being two MOD and one in the "others" category.

The overall adulteration rate of the supplement products analyzed by the DPF from 2007 to 2013 was 6.2% (180 cases), with 78 cases of undeclared medicinal drugs, a number much lower than that notified to the FDA (474) and to the RASFF (183) for the same period. It was not possible to ascertain whether these higher numbers are due to a higher adulteration rate, a larger total supplement market or to a more efficient adulteration detection and reporting system. It is possible that the Brazilian figure is underestimated due to the nature of the chemical analyses performed by the DPF (discretionary, qualitative, mainly general-screening). It is reasonable to hypothesize, however, that a less restrictive legal framework could lead to more adulteration cases, since there are fewer control mechanisms, a case in point being the US, where the registry or even the reporting of the intention of placing a product on the market is not required, except for those with a new dietary ingredient.

The major targets for adulteration differ between countries. While in the US and the EU muscle-building products were the least frequent targets for adulteration (9.1 and 4.4% of adulterated products, respectively), in Brazil these products accounted for 73% of all adulterations. The adulteration rate for MOD products found in this study was 22.1%. Products for sexual enhancement accounted for 42.6% of all notifications in the US and 39.3% in the EU, while in Brazil they represented only 4% of adulterated products (only 9 seized products had declared sexual enhancement properties). It is possible that the low incidence of these supplements in Brazil is a consequence of the Brazilian clandestine market of medicines containing substances for erectile dysfunction. These medicines were the most frequently analyzed by DPF forensic experts, and were also the main targets for counterfeiting, representing 46.1% of all counterfeits detected by the DPF from 2006 to 2012 (Marcheti, 2014). Drugs for erectile dysfunction require prescriptions to be purchased in Brazil, the US and the EU. Nevertheless, the product is generally freely sold at Brazilian pharmacies. Therefore, as these products are widely available both on the regular and the clandestine market in Brazil, there is no significant demand for supplements with these characteristics.

The consumption of an adulterated product poses an additional health risk since consumers do not know what substances they are ingesting. The unrecognized use of drugs such as tadalafil (for erectile dysfunction), sibutramine (an anorectic), or oral turinabol (an anabolic steroid), all of which were detected in some of the products analyzed by the DPF, may not only lead to adverse effects inherent to these substances, but also to more unforeseeable effects due to association with other drugs a given individual may be consuming. Unfortunately, a quantitative analysis was not performed by the DPF which would allow a more precise evaluation of the potential adverse effects.

The main limitations of the DPF data investigated in this study were that not all the seized samples were chemically analyzed, that the analyses performed were mostly general-screening, and were all qualitative. Adulterations due to the presence of an active ingredient at a different concentration from what was declared could not be detected, although this information was not always stated in the label. The complexity of some supplements was also a great challenge, since analytical methods that could detect every substance declared were not available at the DPF forensic laboratories, and therefore cases of supplements lacking declared substances could not always be detected. Furthermore, data originating from the DPF refer mainly to products seized at the country's borders or at post offices, and cannot be extrapolated to fully represent the overall Brazilian situation. On the other hand, to the best of our knowledge, the information provided in this study

reflects the largest data available on detection of adulterated supplements in the literature, including products classified as medicines according to current legislation, as most studies were restricted to a much smaller number of samples.

## 6. Conclusion

Dietary supplements comprise a wide variety of products that fall under different regulatory frameworks worldwide. Although US legislation may be considered more permissive, Brazil's more restrictive, with the EU lying in between, the respective legal frameworks have not proven to be very effective in dealing with all the products available on the market or with preventing the distribution and sale of adulterated or irregular products. The US had the highest number of supplement adulteration notifications during the period from 2007 to 2013, which may be a consequence of a larger market when compared with other countries, an aspect that was not evaluated in this study. Brazil does not have a notification system, so the present study, based on products sent to forensic analysis by the DPF, is the first to estimate the incidence and profiles of adulterated and irregular supplements on the Brazilian market.

In this study, we could not establish the adulteration rates for dietary supplements in Europe (RASFF) and the USA (FDA) due to the lack of information on the total number of samples tested in each dataset. Most likely, because of the limitations mentioned earlier, the calculated Brazilian adulteration rate of 6.2% is underestimated. Hence, any quantitative comparison of adulteration rates between Europe, USA and Brazil is not possible with the available data. However, the rising numbers of notifications from the FDA and RASFF, as well as the rising trend observed in data coming from the DPF, suggest that either the adulteration problem is increasing or that detection mechanisms are improving. Educating the public regarding the potential risks they are taking when consuming adulterated or irregular products is essential to protect human health. Public education could also decrease the demand for adulterated and irregular products with a significant impact on the illegal market.

## Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.yrtph.2015.06.013.

## References


Almeida, A.E., Ribeiro, M.L., Polese, L., 2000. Determination of Amfepramone Hydrochloride, Fenproporex, and Diazepam in so-called "Natural" Capsules used in the Treatment of Obesity, vol. 23. Rel. Tech, pp. 1109–1118 (Accessed 29.09.14.).

ANVISA (Brazilian Sanitary Surveillance Agency), 1999a. Resolução – RDC n° 16, de 30 de abril de 1999 – Aprova o Regulamento Técnico de procedimentos para registro de alimentos e ou novos ingredientes, constante do anexo desta Portaria.

ANVISA (Brazilian Sanitary Surveillance Agency), 1999b. Resolução – RDC n° 18, de 30 de abril de 1999 – Aprova o Regulamento Técnico que estabelece as diretrizes básicas para análise e comprovação de propriedades funcionais e ou de saúde alegadas em rotulagem de alimentos.

ANVISA (Brazilian Sanitary Surveillance Agency), 2002. Resolução – RDC n° 259, de 20 de setembro de 2002 – Aprova o Regulamento sobre rotulagem de alimentos embalados.

ANVISA (Brazilian Sanitary Surveillance Agency), 2008. Resolução – RDC n° 81, de 05 de novembro de 2008 – Dispõe sobre o Regulamento Técnico de Bens e Produtos Importados para fins de Vigilância Sanitária.

ANVISA (Brazilian Sanitary Surveillance Agency), 2010a. Resolução – RDC n° 18, de 27 de abril de 2010 – Dispõe sobre alimentos para atletas.

ANVISA (Brazilian Sanitary Surveillance Agency), 2010b. Resolução – RDC n° 27, de 06 de agosto de 2010 – Dispõe sobre as categorias de alimentos e embalagens isentos e com obrigatoriedade de registro sanitário.

ANVISA (Brazilian Sanitary Surveillance Agency), 2010c. Informe Técnico n° 45, de 28 de dezembro de 2010 – Esclarecimentos sobre a regulamentação de chás.

ANVISA (Brazilian Sanitary Surveillance Agency), 2011a. Gerência Geral de Medicamentos. Nota Técnica n° 4, de 07 de janeiro de 2011.

ANVISA (Brazilian Sanitary Surveillance Agency), 2011b. Resolução – RDC n° 28, de 28 de junho de 2011 – Altera dispositivos da Resolução de Diretoria Colegiada – RDC n° 81, de 5 de novembro de 2008, que aprovou o Regulamento Técnico de Bens e Produtos Importados para fins de Vigilância Sanitária.

ANVISA (Brazilian Sanitary Surveillance Agency), 2012. Resolução – RDC n° 37, de 02 de julho de 2012-Dispõe sobre a atualização do Anexo I, Listas de Substâncias Entorpecentes, Psicotrópicas, Precursoras e Outras sob Controle Especial, da Portaria SVS/MS n° 344, de 12 de maio de 1998 e dá outras providências.

ANVISA (Brazilian Sanitary Surveillance Agency), 2014a. Resolução – RDC n° 26, de 13 de maio de 2014 – Dispõe sobre o registro de medicamentos fitoterápicos e o registro e a notificação de produtos tradicionais fitoterápicos.

ANVISA (Brazilian Sanitary Surveillance Agency), 2014b. Alimentos/Assuntos de Interesse/Informes Técnicos. Updated on October 2, 2014. http://portal.anvisa.gov.br/wps/content/Anvisa+Portal/Anvisa/Inicio/Alimentos/Assuntos+de+Interesse/Informes+Tecnicos (Accessed 30.06.15).

ANVISA (Brazilian Sanitary Surveillance Agency), 2014c. NOTIVISA – Sistema de Notificações em Vigilância Sanitária. Updated on May 26, 2014. http://www.anvisa.gov.br/hotsite/notivisa/relatorios/index.htm (Accessed 30.06.15).

ANVISA (Brazilian Sanitary Surveillance Agency), 2014d. Resolução – RE n° 572, de 14 de fevereiro de 2014.

Baume, N., Mahler, N., Kamber, M., Mangin, P., Saugy, M., 2006. Research of stimulants and anabolic steroids in dietary supplements. Scand. J. Med. Sci. Sports 16, 41–48.

Bove, A.A., 2002. Dietary supplements in athletes. ACC Curr. J. Rev. 11, 18–20.

BRAZIL, 1969. Decreto-Lei n. 986, de 21 de outubro de 1969. Institui normas básicas sobre alimentos. Diário Oficial da União, p. 8935 retificação em 11 de novembro de 1969, p. 9737.

BRAZIL, 1973. Lei n. 5.991, de 17 de dezembro de 1973. Dispõe sobre o controle sanitário do comércio de drogas, medicamentos, insumos farmacêuticos e correlatos, e dá outras providências. Diário Oficial da União, p. 13049 retificação em 21 de dezembro de 1973, p. 13182.

BRAZIL, 1976. Lei n. 6.360, de 23 de setembro de 1976. Dispõe sobre a Vigilância Sanitária a que ficam sujeitos os medicamentos, as drogas, os insumos farmacêuticos e correlatos, cosméticos, saneantes e outros produtos, e dá outras providências. Diário Oficial da União, p. 12647.

BRAZIL, 1998. Lei n. 9.677, de 02 de julho de 1998. Altera dispositivos do Capítulo III do Título VIII do Código Penal, incluindo na classificação dos delitos considerados hediondos crimes contra a saúde pública, e dá outras providências, vol. 1. Diário Oficial da União, Seção, p. 1 (03/07/1998).

BRAZIL, 2003. Lei n. 10.742, de 06 de outubro de 2003. Define normas de regulação para o setor farmacêutico, cria a Câmara de Regulação do Mercado de Medicamentos – CMED e altera a Lei n° 6.360, de 23 de setembro de 1976, e dá outras providências, vol. 1. Diário Oficial da União, Seção, p. 1 (07/10/2003).

Brownie, S., 2005. The development of the US and Australian dietary supplement regulations – what are the implications for product quality? Comp. Ther. Med. 13, 191–198.

Carvalho, L.M., Correia, D., Garcia, S.C., Bairros, A.V., Nascimento, P.C., Bohrer, D., 2010. A new method for the simultaneous determination of 1,4-benzodiazepines and amfepramone as adulterants in phytotherapeutic formulations by voltammetry. For. Sci. Int. 202, 75–81.

Carvalho-Silva, L.B., Braga, G.G., Lollo, P.C.B., 2012. Utilização de suplemento alimentar e anabolizante por praticantes de musculação nas academias de Goiânia-GO. Rev. Bras. Nutr. Clin. 27, 158–163.

Cohen, P.A., 2012. DMAA as a dietary supplement ingredient. Arch. Int. Med. 9 (172), 1038–1039.

Coppens, P., Delmulle, L., Gulati, O., Richardson, D., Ruthsatz, M., Sievers, H., Sidani, S., 2006. Use of botanicals in food supplements. Ann. Nutr. Met. 50, 538–554.

Coppens, P., Silva, M.F., Pettman, S., 2006b. European regulations on nutraceuticals, dietary supplements and functional foods: a framework based on safety. Toxicology 221, 59–74.

Corby-Edwards, A.K., 2013. Regulation of Dietary Supplements. Congressional Research Service, p. R43062. http://www.law.umaryland.edu/marshall/crsreports/crsdocuments/R43062_11132012.pdf (Accessed 30.06.15).

CE (Council of Europe), 2008. Homeostasis, a Model to Distinguish between Foods (Including Food Supplements) and Medicinal Products. Partial Agreement in the Social and Public Health Field. Council of Europe, Strasbourg, France.

Damiano, F., Silva, C., Gregori, A., Vacondio, F., Mor, M., Menozzi, M., Di Giorgio, D., 2014. Analysis of illicit dietary supplements sold in the Italian market: identification of a sildenafil thioderivative as adulterant using UPLC-TOF/MS and GC/MS. Sci. Justice 54, 228–237.

De Rose, E.H., Feder, M.G., Pedroso, P.R., Guimarães, A.Z., 2006. Referred use of medication and dietary supplements in athletes selected for doping control in the South-American games. Rev. Bras. Med. Esporte 12, 239–242.

Diehl, K., Thiel, A., Zipfel, S., Mayer, J., Schnell, A., Schneider, S., 2012. Elite adolescent athletes' use of dietary supplements: characteristics, opinions, and sources of supply and information. Int. J. Sport Nutr. Exerc. Met. 22, 165–174.

Doménech-Carbó, A., Martini, M., Carvalho, L.M., Viana, C., Doménech-Carbó, M.T., Silva, M., 2013. Screening of pharmacologic adulterant classes in herbal formulations using voltammetry of microparticles. J. Pharm. Biomed. Anal. 74, 194–204.

Eliason, M.J., Eichner, A., Cancio, A., Bestervelt, L., Adams, B.D., Deuster, P.A., 2012.

Case reports: death of active duty soldiers following ingestion of dietary supplements containing 1,3-dimethylamylamine (DMAA). Mil. Med. 177, 1455–1459.
Ernst, E., 1998. Harmless herbs? A review of the recent literature. Am. J. Med. 104, 170–178.
Eudy, A.E., Gordon, L.L., Hockaday, B.C., Lee, D.A., Lee, V., Luu, D., Martinez, C.A., Ambrose, P.J., 2013. Efficacy and safety of ingredients found in preworkout supplements. Am. J. Health Sys. Pharm. 70, 577–588.
European Commission, 2014. Rapid Alert System for Food and Feed (RASFF) Portal. https://webgate.ec.europa.eu/rasffwindow/portal/?event=SearchForm&cleanSearch=1 (Access on December 30, 2014).
EEC (European Economic Community), 1965. Council Directive 65/65/EEC of 26 January 1965 on the approximation of provisions laid down by law, regulation or administrative action relating to medicinal products. Off. J. 022, 0369–0373.
EC (European Parliament and European Council), 2000. Directive 2000/13/EC of the European Parliament and of the Council of 20 March 2000 on the approximation of the laws of the member states relating to the labelling, presentation and advertising of foodstuffs. Off. J. Eur. Comm. L109, 29–42.
EC (European Parliament and European Council), 2001. Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the community code relating to medicinal products for human use. Off. J. Eur. Comm. L311, 67–128.
EC (European Parliament and European Council), 2002. Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the member states relating to food supplements. Off. J. Eur. Comm. L183, 51–57.
EC (European Parliament and European Council), 2002b. Regulation 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European food safety authority and laying down procedures in matters of food safety. Off. J. Eur. Comm. L31, 1–24.
EC (European Parliament and European Council), 2004. Directive 2004/27/EC of the European Parliament and of the Council of 31 March 2004 amending Directive 2001/83/EC on the community code relating to medicinal products for human use. Off. J. Eur. Comm. L136, 34–57.
EC (European Parliament and European Council), 2006. Regulation 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. Off. J. Eur. Comm. L404, 9–25.
EC (European Parliament and European Council), 2006b. Regulation 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods. Off. J. Eur. Comm. L404, 26–38.
Eussen, S.R.B.M., Verhagen, H., Klungel, O.H., Garssen, J., van Loveren, H., van Kranen, H.J., Rompelberg, C.J.M., 2011. Functional foods and dietary supplements: products at the interface between pharma and nutrition. Eur. J. Pharm. 668, S2–S9.
Fayh, A.P.T., Silva, C.V., Jesus, F.R.D., Costa, G.K., 2013. Consumption of nutritional supplements among individuals in Porto Alegre's fitness centers. Rev. Bras. Cien. Esporte 35, 27–37.
Finley, J.W., Finley, J.W., Ellwood, K., Hoadley, J., 2014. Launching a new food product or dietary supplement in the United States: industrial, regulatory, and nutritional considerations. Ann. Rev. Nutr. 34, 421–447.
Gee, P., Tallon, C., Long, N., Moore, G., Boet, R., Jackson, S., 2012. Use of recreational drug 1,3-dimethylethylamine (DMAA) associated with cerebreal hemorrhage. Ann. Emerg. Med. 60, 431–444.
Geyer, H., Braun, H., Burke, L.M., Stear, S.J., Castell, L.M., 2011. A–Z of nutritional supplements: dietary supplements, sports nutrition foods and ergogenic aids for health and performance—part 22. Br. J. Sports Med. 45, 752–754.
Geyer, H., Parr, M.K., Mareck, U., Reinhart, U., Schrader, Y., Schänzer, W., 2004. Analysis of non-hormonal nutritional supplements for anabolic-androgenic steroids - results of an international study. Int. J. Sports Med. 25, 124–129.
Geyer, H., Parr, M.K., Koehler, K., Mareck, U., Schänzer, W., Thevis, M., 2008. Nutritional supplements cross-contaminated and faked with doping substances. J. Mass Spectr. 43, 892–902.
Goston, J.L., Correia, M.I.T.D., 2010. Intake of nutritional supplements among people exercising in gyms and influencing factors. Nutrition 26, 604–611.
Gratz, S.R., Flurer, C.L., Wolnik, K.A., 2004. Analysis of undeclared synthetic phosphodiesterase-5 inhibitors in dietary supplements and herbal matrices by LC–ESI–MS and LC–UV. J. Pharm. Biomed. Anal. 36, 525–533.
Health Canada, Health Products and Food Branch, 2011. Classification of 1,3 Dimethylamylamine (DMAA). http://pt.scribd.com/doc/82744576/DMAA-Health-Canada-2011.
Jacobson, I.G., Horton, J.L., Smith, B., Wells, T.S., Bouko, E.J., Lieberman, H.R., Ryan, M.A.K., Smith, T.C., 2012. Bodybuilding, energy, and weight-loss supplements are associated with deployment and physical activity in U.S. military personnel. Ann. Epidem. 22 (5), 318–330.
Kicman, A.T., 2008. Pharmacology of anabolic steroids. Br. J. Pharmacol. 154, 502–521.
Kristiansen, M., Levy-Milne, R., Barr, S., Flint, A., 2005. Dietary supplement use by varsity athletes at a Canadian university. Int. J. Sport Nutr. Exerc. Met. 15, 195–210.
Lachenmeier, D.W., Steffen, C., El-Atma, O., Maixner, S., Löbell-Behrends, S., Kohl-Himmelseher, M., 2012. What is a food and what is a medicinal product in the European Union? Use of the benchmark dose (BMD) methodology to define a threshold for "pharmacological action". Regul. Toxicol. Pharmacol. 64, 286–295.
Lachenmeier, D.W., Löbell-Behrends, S., Böse, W., Marx, G., 2013. Does European Union food policy privilege the internet market? Suggestions for a specialized regulatory framework. Food Control 30, 705–713.
Li, Y., Zhang, H., Hu, J., Xue, F., Li, Y., Sun, C., 2012. A GC–EI-MS-MS method for simultaneous determination of seven adulterants in slimming functional foods. J. Chromatogr. Sci. 50, 928–933.
Marcheti, R.G.A., 2014. Evaluation of medicine counterfeiting in Brazil from forensic reports issued by the Brazilian Federal Police Department from 2006 to 2012 (Master dissertation). Universidade de Brasília, Brasília.
McCann, M., 2005. Dietary supplement labeling: cognitive biases, market manipulation & consumer choice. Am. J. Law Med. 31, 215–268.
Navarro, V.J., Seeff, L.B., 2013. Liver injury induced by herbal complementary and alternative medicine. Clin. Liver Dis. 17, 715–735.
Nogueira, F.R.S., Souza, A.A., Brito, A.F., 2013. Prevalence of the use and ergogenic resources effects by body builders in Brazilian academies: a systematic review. Rev. Bras. Ativ. Fís. Saúde 18, 16–30.
Oliver, A.S., León, M.T.M., Guerra-Hernández, E., 2011. Prevalence of protein supplement use at gyms. Nutr. Hosp. 26, 1168–1174.
Petroczi, A., Taylor, G., Naughton, D.P., 2011. Mission impossible? Regulatory and enforcement issues to ensure safety of dietary supplements. Food Chem. Toxicol. 49, 393–402.
Rebiere, H., Guinot, P., Civade, C., Bonnet, P.-A., Nicolas, A., 2012. Detection of hazardous weight-loss substances in adulterated slimming formulations using ultra-high-pressure liquid chromatography with diode-array detection. Food Addit. Cont. 29, 161–171.
Saeedi, P., Nasir, M.T.M., Hazizi, A.S., Vafa, M.R., Foroushani, A.R., 2013. Nutritional supplement use among fitness club participants in Tehran, Iran. Apetite 60, 20–26.
SVS (Brazilian Secretary of Health Surveillance), 1998. Portaria n° 32, de 13 de janeiro de 1998. – Aprova o regulamento técnico para suplementos vitamínicos e ou de minerais.
SVS (Brazilian Secretary of Health Surveillance), 1998b. Portaria n° 344, de 12 de maio de 1998 – Aprova o Regulamento Técnico sobre substâncias e medicamentos sujeitos a controle especial.
Sepkowitz, K.A., 2013. Energy drinks and caffeine-related adverse effects. J. Am. Med. Assoc. 309, 243–244.
Shahidi, N.T., 2001. A review of the chemistry, biological action, and clinical applications of anabolic-androgenic steroids. Clin. Ther. 23, 1355–1390.
Silva, A.A., Marins, J.C.B., 2013. Consumption and level of knowledge about nutritional ergogenic resources in athletes. Biosci. J. 29, 1038–1048.
Song, F., Monroe, D., El-Demerdash, A., Palmer, C., 2014. Screening for multiple weight loss and related drugs in dietary supplement materials by flow injection tandem mass spectrometry and their confirmation by liquid chromatography tandem mass spectrometry. J. Pharm. Biomed. Anal. 88, 136–143.
Tang, M.H.Y., Chen, S.P.L., Ng, S.W., Chan, A.Y.W., Mak, T.W.L., 2011. Case series on a diversity of illicit weight-reducing agents: from the well known to the unexpected. Br. J. Clin. Pharmacol. 71, 250–253.
Timcheh-Hariri, A., Balali-Mood, M., Aruan, E., Sadeghi, M., Riahi-Zanjani, B., 2012. Toxic hepatitis in a group of 20 male body-builders taking dietary supplements. Food Chem. Toxicol. 50, 3826–3832.
USA (United States of America), 1938. Federal food, drug, and cosmetic act. Public Law 75–717, 52. Stat. 1040 – 1059; June 25 1938. http://www.fda.gov/regulatoryinformation/legislation/FederalFoodDrugandCosmeticActFDCAct/default.htm (last updated in December 5, 2011).
USA (United States of America), 1994. Dietary supplement health and education act of 1994. Public Law 103–417, 108. Stat. 4325 – 4335; October 25 1994.
USA (United States of America), 2011. FDA food safety modernization act. Public Law 111–353, 124. Stat. 3885 – 3973; January 4 2011.
USFDA (U. S. Food and Drug Administration), 1997. Food and drug administration modernization act. Public Law 105–115. Section 303.
USFDA (U. S. Food and Drug Administration), 2003. Guidance for Industry: Interim Procedures for Qualified Health Claims in the Labeling of Conventional Human Food and Human Dietary Supplements. http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm053832.htm.
USFDA (U. S. Food and Drug Administration), 2007. Current Good Manufacturing Practice in Manufacturing, Packaging, Labeling, or Holding Operations for Dietary Supplements; Final Rule, 72 Federal Register 34752–34958, June 25, 2007.
USFDA (U. S. Food and Drug Administration), 2009. Guidance for Industry: Evidence Based Review System for the Scientific Evaluation of Health Claims - Final. http://www.fda.gov/food/guidanceregulation/ucm073332.htm (Accessed 30.06.15).
USFDA (U. S. Food and Drug Administration), 2011a. Beware of Fraudulent Dietary Supplements. http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm246744.htm (Accessed 30.06.15).
USFDA (U. S. Food and Drug Administration), 2011b. Questions and Answers on Tainted Products Marketed as Dietary Supplements. Updated on March 23, 2011. http://www.fda.gov/Drugs/ResourcesForYou/Consumers/BuyingUsingMedicineSafely/MedicationHealthFraud/ucm247094.htm (Accessed 30.06.15).
USFDA (U. S. Food and Drug Administration), 2013a. OxyElite Pro Supplements Recalled. http://www.fda.gov/forconsumers/consumerupdates/ucm374742.htm (Accessed 30.06.15).
USFDA (U. S. Food and Drug Administration), 2013b. FDA Uses New Authorities to Get OxyElite Pro off the Market. http://blogs.fda.gov/fdavoice/index.php/2013/

11/fda-uses-new-authorities-to-get-oxyelite-pro-off-the-market/ (Accessed 30.06.15).
USFDA (U. S. Food and Drug Administration), 2014. MedWatch Safety Alerts for Human Medical Products. http://www.fda.gov/Safety/MedWatch/SafetyInformation/SafetyAlertsforHumanMedicalProducts/default.htm (Accessed 30.12.14.).
USFDA (U. S. Food and Drug Administration), 2014b. Tainted Supplements CDER. http://www.accessdata.fda.gov/scripts/sda/sdNavigation.cfm?sd=tainted_supplements_cder (Accessed 30.06.15).
Van Poucke, C., Detavernier, C., Van Cauwenberghe, R., Van Peteghem, C., 2007. Determination of anabolic steroids in dietary supplements by liquid chromatography–tandem mass spectrometry. Anal. Chim. Acta 586, 35–42.
Vaysse, J., Balayssac, S., Gilard, V., Desoubdzanne, D., Malet-Martino, M., Martino, R., 2010. Analysis of adulterated herbal medicines and dietary supplements marketed for weight loss by DOSY $^{1}$H-NMR. Food Addit. Cont. 27, 903–916.