‘‘CAROL DAVILA” UNIVERSITY OF MEDICINE AND PHARMACY, BUCHAREST
SCHOOL FOR DOCTORAL STUDIES
FIELD OF PHARMACY

“ANALYTICAL - TOXICOLOGICAL STUDIES ON THE ADULTERATION OF CERTAIN DIETARY SUPPLEMENTS WITH SYNTHETIC CHEMICAL COMPOUNDS”

SUMMARY OF THE DOCTORAL THESIS

Scientific Coordinator:
PROFESSOR BACONI DANIELA LUIZA, PhD

Doctoral candidate:
GHEORGHIU OANA-RAMONA-CĂTĂLINA

2025

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Table of Contents

Published Scientific Papers

1 Molecules, 2023, volumul 28, numărul 10, paginile 4116;

Indexare: ISI, factor de impact 4,2 pentru 2023;

Link către publicație: https://doi.org/10.3390/molecules28104116

Gheorghiu ORC, Ciobanu AM, Guțu CM, Chițescu CL, Costea GV, Anghel DM, Vlăsceanu AM, Baconi DL (2023). Determination of Phosphodiesterase Type-5 Inhibitors (PDE-5) in Dietary Supplements. *Molecules*. 28(10):4116; (chapter 6) (Attachement nr. 1).

2 Journal of Mind and Medical Sciences, 2025, volumul *12*, numărul 1, paginile 23;

Indexare: ISI, factor de impact 1,6 pentru 2025;

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Oana Ramona Cătălina Gheorghiu, Anne Marie Ciobanu, Claudia Maria Guțu, George Mădălin Dănilă, Viorela Gabriela Nițescu, Ștefan Rohnean, Daniela Luiza Baconi (2025). Detection of adulterants in herbal weight loss supplements, *Journal of Mind and Medical Sciences*: 12(1):23; (chapter7) (Attachement nr. 2).

Introduction

The main working hypothesis of this research was that, due to the increasing demand and the desire for effective and rapid results, a significant proportion of dietary supplements available on the market—especially herbal ones—are unsafe, lack proven efficacy, or are adulterated through the illegal addition of synthetic chemical compounds or other undeclared ingredients on the label.The doctoral research focused on dietary supplements used for weight control and for enhancing physical, sports, and sexual performance. The choice of these types of supplements was driven by the high public interest in such products, correlated with users' pressure to achieve fast and effective outcomes—factors that make these categories the most likely to be adulterated.Therefore, the research addressed the detection and quantitative determination of synthetic chemical compounds in dietary supplements marketed as natural or "herbal", with the goal of providing specialists and authorities with reliable, objective, rapid, and cost-effective tools to prevent such harmful practices as adulteration.

I. Current State of Knowledge

1. Dietary Supplements: Overview, Definition, Classification, Legislative/Regulatory Aspects

1.1. Overview and Definition

In the context of a general trend toward self-medication, one major factor contributing to the surge in demand for supplements is the wide variety of information channels (especially online—news, articles, advertisements, social media posts, and forum reviews) and the ease of access from various sources, especially via the internet (Lam M. et al., 2022).At the same time, the significant increase in the use of dietary supplements for weight loss, and to improve sexual, physical, and sports performance reflects current societal concerns related to health, aesthetics, and overall performance (Hys K., 2020; Fahmideh F. et al., 2022).

1.2. Classification of Dietary Supplements

Dietary supplements can be classified according to various criteria, such as their ingredient composition, function/role, origin/source, or base material, as outlined in Table I.1.

1.3. Legislative and Regulatory Aspects

In the European Union, dietary supplements fall under the legislative framework of Directive 2002/46/EC, which harmonizes national regulations across member states regarding dietary supplements. Considered food products, they must comply with strict safety and labeling requirements, including providing information on nutrients, the recommended daily intake, and warnings about not exceeding the indicated dose. Furthermore, the inclusion of claims about a product's ability to prevent or treat diseases is strictly prohibited.

2. Types of Dietary Supplements. Therapeutic Uses

2.1. Herbal Supplements for Erectile Dysfunction

In addition to authorized medications containing PDE-5 inhibitors, the management of erectile dysfunction includes dietary supplements based on herbal extracts, whose natural origin is often emphasized by manufacturers. They claim that the efficacy of these products is due to the bioactive compounds present in the used plants. Recent animal and human studies suggest that certain plants (such as *Panax ginseng*, *Lepidium meyenii* (maca), *Tribulus terrestris*, *Epimedium* spp., and *Ginkgo biloba*) and their components may act as effective natural aphrodisiacs and possess inherent PDE-5 inhibitory properties (Akuamoa F. et al., 2021).

2.2. Herbal Supplements for Weight Control

Most herbal weight loss supplements contain plants or plant extracts. Caffeine and naturally caffeine-containing plants—such as guarana (*Paullinia cupana*), kola nut (*Cola nitida*), and yerba maté (*Ilex paraguariensis*)—stimulate the central nervous system, heart, and skeletal muscles. These plants also act as diuretics and enhance the activity of the colon and stomach.

2.3. Supplements for Enhancing Physical and Sports Performance

From the perspective of the desired effect on the body, dietary supplements intended for athletes aim to increase muscle mass, energy levels, and weight loss. This has led to the widespread use of pharmacological agents such as anabolic steroids and amphetamines. Due to their adverse effects and ethical concerns—especially in a field where fair

competition is crucial—anti-doping legislation and strict testing protocols have been implemented to discourage their use.

3. Adulteration of Dietary Supplements. Analytical Methods Used to Detect Adulterants

3.1.1. Common Adulterants in Herbal Supplements for Erectile Dysfunction

The practice of adulterating dietary supplements is a widespread phenomenon globally (Žuntar I. et al., 2018). The sustained efficacy of herbal supplements marketed for sexual performance enhancement may be the result of intentionally added pharmaceutical ingredients, with phosphodiesterase-5 (PDE-5) inhibitors being frequently reported in this context (Patel D.N. et al., 2014).

3.1.2. Common Adulterants in Herbal Supplements for Weight Control

The most common adulterants belong to the class of pharmaceutical substances that act on the central nervous system (dopaminergic and serotonergic anorexigens). Laxatives and diuretics have also been reported, and, less frequently, substances that inhibit lipid digestion and absorption, such as orlistat.

3.1.3. Common Adulterants in Supplements for Enhancing Physical and Sports Performance

The most frequent adulterants found in dietary supplements intended to improve physical performance are anabolic agents and stimulants.

3.2. Analytical Methods Used to Detect Adulterants

A review of the specialized literature highlights numerous analytical techniques used to detect adulterants, including:Gas Chromatography–Mass Spectrometry (GC-MS), Liquid Chromatography–Mass Spectrometry (LC-MS), Nuclear Magnetic Resonance (NMR), Fourier-Transform Infrared Spectroscopy (FTIR) , Ion Mobility Spectrometry (IMS), Capillary Electrophoresis (CE) . These methods have been described by various authors (Hachem R. et al., 2016; Popescu A.M. et al., 2015; Odoardi S. et al., 2015; Pratiwi R. et

al., 2021; Dunn J.D. et al., 2012; Cianchino V. et al., 2008; Kowalska T. et al., 2022; Komsta L. et al., 2013).

II. Personal Contributions

4. Working Hypothesis and General Objectives

The experimental part of this thesis focuses on the analytical investigation of possible adulteration in herbal supplements used for erectile dysfunction, herbal weight loss supplements, and those intended to support sports performance. It also evaluates supplement use among athletes and includes the development of an online platform dedicated to reporting adverse reactions caused by potentially adulterated supplements, to enable targeted laboratory analysis. The working hypothesis is based on the premise that many dietary supplements sold on both Romanian and international markets may contain synthetic chemical compounds or active pharmaceutical ingredients not listed on the label, which may produce pharmacological effects and adverse reactions. Given this context, the **general objective** of the thesis was to investigate, through analytical methods, the presence of adulterants in various types of dietary supplements, and to provide specialists and authorities with reliable, objective, fast, and accessible tools for preventing such harmful practices, avoiding the use of adulterated products, and identifying possible adverse effects associated with their use.

Specific objectives included:

- Analytical research into adulteration of supplements for erectile dysfunction;
- Investigation of adulterants in herbal weight loss supplements;
- Evaluation of dietary supplement use among athletes via a survey;
- Research on adulteration in performance-enhancing supplements for athletes;
- Development of an online platform for reporting adverse reactions related to supplement use.

5. General Research Methodology

The methodology applied in this research is complex and interdisciplinary, integrating aspects of analytical toxicology and drug control in order to identify and quantify synthetic

chemical compounds fraudulently added to dietary supplements. It also includes risk assessment and the development of preventive tools.

A detailed description of the analytical methods used is presented in the specific chapters on personal contributions (Chapters 6, 7, and 9). To evaluate supplement use among athletes, a questionnaire-based survey study was conducted, which is detailed in Chapter 8.

6. Analytical Research on the Adulteration of Herbal Supplements Used for Erectile Dysfunction

6.1. Introduction

The studies presented in this chapter were dedicated to establishing the experimental conditions necessary to detect adulterants in herbal dietary supplement samples marketed as sexual stimulants, using the HPTLC method. Accordingly, we investigated whether the analyzed products were adulterated with authorized pharmaceutical active substances such as PDE-5 inhibitors—sildenafil, tadalafil—or other analogs. The results obtained through the HPTLC method were confirmed by applying an ultra-high-performance liquid chromatography method coupled with high-resolution tandem mass spectrometry (UHPLC-HRMS-MS), which offers higher sensitivity and specificity.

6.2. Materials and Methods

6.2.1. Analyzed Herbal Dietary Supplements

Fifteen herbal dietary supplements (in capsule or tablet form), purchased online or from specialized stores, were analyzed. Product selection was based on the label-declared composition and user-reported satisfaction, assessed through a rating score (on a scale from 1 to 5). The samples were coded using the prefix SP and numbered from 1 to 15. Information on the country of origin, pharmaceutical form, composition, and product price is detailed in Table VI.1 (Appendix 2).

6.3. Results

6.3.1. HPTLC Method Development

A mobile phase consisting of a mixture of ethyl acetate:toluene:methanol:ammonia in the ratio of 50:30:20:0.5 (v/v/v/v) was selected, providing optimal separation of the two compounds.

6.3.2. Analysis of Herbal Supplements

6.3.2.1. Product Characterization and Qualitative HPTLC Analysis

Analysis of supplements SP1, SP2, SP3, SP4, SP6, SP7, SP8, SP9, SP10, and SP14 revealed the presence of sildenafil, which was not declared on the product label. Sample SP6 tested positive for both sildenafil and tadalafil, while sample SP11 tested positive only for tadalafil. Samples SP12, SP13, and SP14 did not show the presence of any adulterants, including undeclared PDE-5 inhibitors (Table VI.1., Appendix 2). The presence of sildenafil in herbal supplement samples was confirmed by comparing the UV spectra of the standards and the samples, which were perfectly superimposable (Figure 6.5). Similar results were obtained for the other samples that tested positive for sildenafil. Sample SP5 tested negative for sildenafil (Figure 6.6, Appendix 3), but tested positive for caffeine due to the identification of a spot with Rf = 0.78 and a characteristic UV spectrum with a maximum absorbance at 275 nm (Figure 6.8). Analysis of sample SP6 showed the presence of both undeclared sildenafil and tadalafil (Figure 6.6, Appendix 3). Sample SP10 tested positive for sildenafil, and analysis of sample SP11 showed a peak in the 3D chromatogram at an Rf value corresponding to the tadalafil standard (Figure 6.10); neither of these pharmaceutical substances was declared in the product composition.

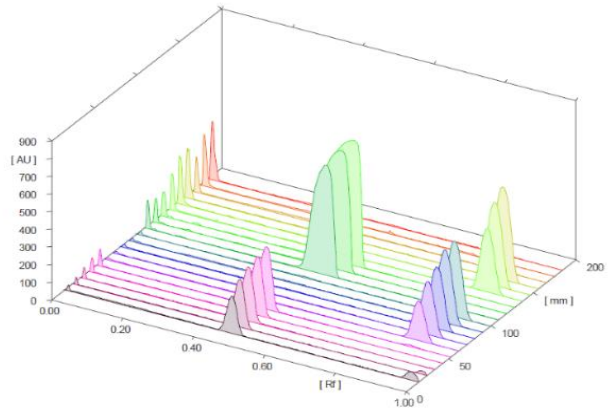

Fig. 6.10. 3D chromatogram for sildenafil standard (migration lanes 1–5), tadalafil standard (migration lanes 6–10), and samples SP10 (migration lanes 11–13), SP11 (migration lanes 14–16), and SP12 (migration lanes 17–20); detection at $\lambda = 254$ nm. Samples SP10 and SP11 tested positive for sildenafil and tadalafil, respectively; sample SP12 tested negative.

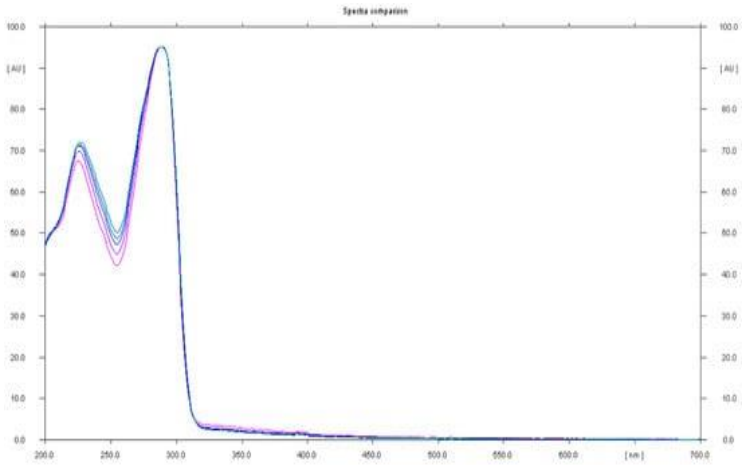

Fig. 6.11. In situ UV spectra in absorbance–reflectance mode for tadalafil standards and sample SP11

6.3.2.2. Quantitative Determination of Sildenafil and Tadalafil in Herbal Supplement Samples Using HPTLC

Quantitative analysis revealed sildenafil concentrations ranging widely from 15 mg/capsule to 116 mg/capsule. For most samples, the determined sildenafil concentration was similar to that found in authorized medicinal products (Table VI.2). In the case of tadalafil, both positive samples showed higher concentrations (approximately 24 mg and 34 mg/capsule) than the maximum authorized concentration in medicinal products (20 mg/unit dose).

6.3.3. Analysis of Herbal Supplements by UHPLC-HRMS-MS

The results obtained from HPTLC analysis were confirmed by ultra-high-performance liquid chromatography coupled with high-resolution tandem mass spectrometry (UHPLC-HRMS-MS). This confirmed the presence of the adulterants sildenafil and tadalafil in the tested samples, consistent with the HPTLC findings. Additionally, some samples were found to contain avanafil, vardenafil, and two vardenafil analogues (pseudovardenafil and vardenafil oxopiperazine).

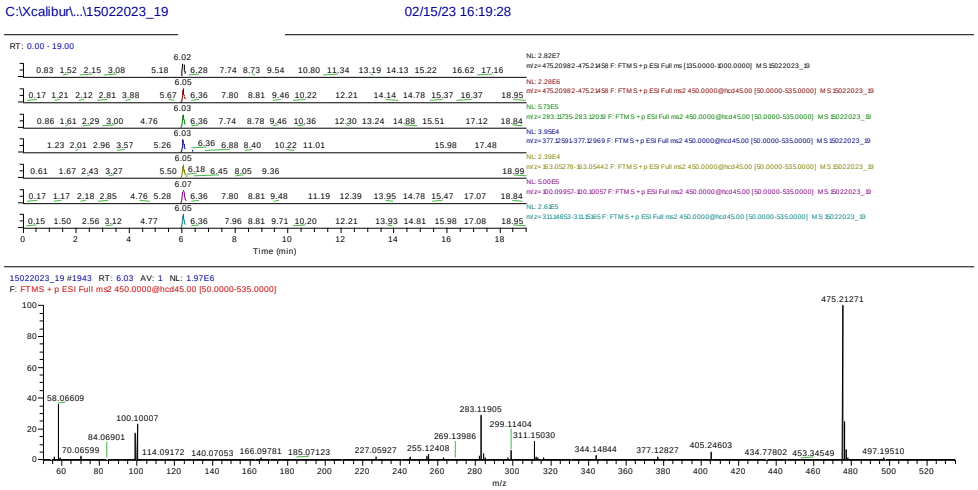

Fig. 6.12. Identification of sildenafil in sample SP10 (MS-MS)

7. Investigation of Adulterants in Herbal Supplements for Weight Loss

7.1. Introduction

This study investigates the potential adulteration of dietary supplements marketed for weight control with synthetic compounds. A series of products advertised as natural and plant-based were selected for screening. These were subjected to analytical screening using a High-Performance Thin-Layer Chromatography (HPTLC) method, followed by confirmatory analyses via Gas Chromatography–Mass Spectrometry (GC-MS) and High-Performance Liquid Chromatography (HPLC).

7.2.Materials and Methods

7.2.1. Analyzed Herbal Dietary Supplements

A total of 34 herbal dietary supplements (capsules, tablets, or powder sachets for oral suspension) were analyzed. These products were purchased online or from specialized stores.

7.3. Results

7.3.2. HPTLC, HPLC and GC-MS Analysis of Herbal Supplements

7.3.2.1. Qualitative Analysis

The results indicate the presence of caffeine in 47% of the analyzed herbal supplements (16 out of 34 products) (Table VII.1, Appendix 2; Figures 7.1 and 7.2).

The presence of caffeine in all 16 products, except SS19, correlates with their declared composition, as caffeine may originate from natural sources (green tea, green coffee, and guarana are listed as ingredients) or is explicitly added, according to the label (as in products SS12, SS14, and SS20). The presence of caffeine was confirmed by in situ UV spectral recordings, which showed a characteristic absorption maximum at 275 nm for caffeine.

It was demonstrated that three supplements—SS21, SS24, and SS27—were adulterated with sibutramine. In the absence of a reference standard, sibutramine was identified by comparing its in situ UV spectrum with spectra reported in the literature.

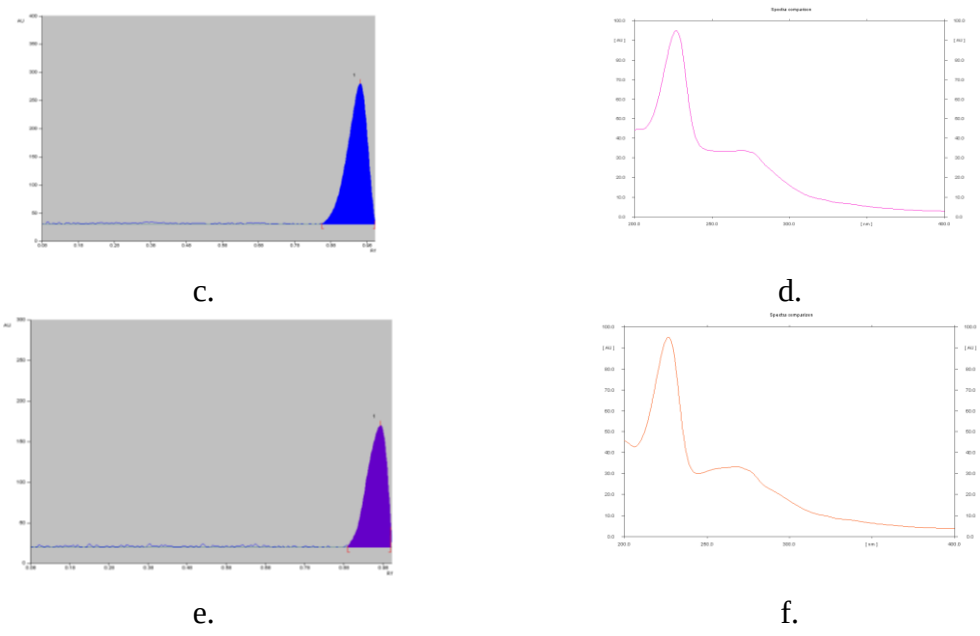

Fig. 7.4. Chromatograms and UV spectra obtained during the identification of sibutramine in supplements SS24 (c, d) and SS27 (e, f) by HPTLC

The presence of sibutramine was also indicated by a rapid screening test reported in the literature (Liang, Q. et al., 2021), based on a precipitation reaction with ammonium reineckate. A positive result is indicated by the formation of a pink precipitate (Fig. 7.5, Appendix 3).

Positive results for the presence of sibutramine were confirmed using the GC-MS method (Fig. 7.6). The base peak of sibutramine in the mass spectrum is **m/z 114**.

In four of the samples (SS3, SS6, SS17, and SS19), phenolphthalein was detected using an HPLC method with diode array detection (Fig. 7.10).

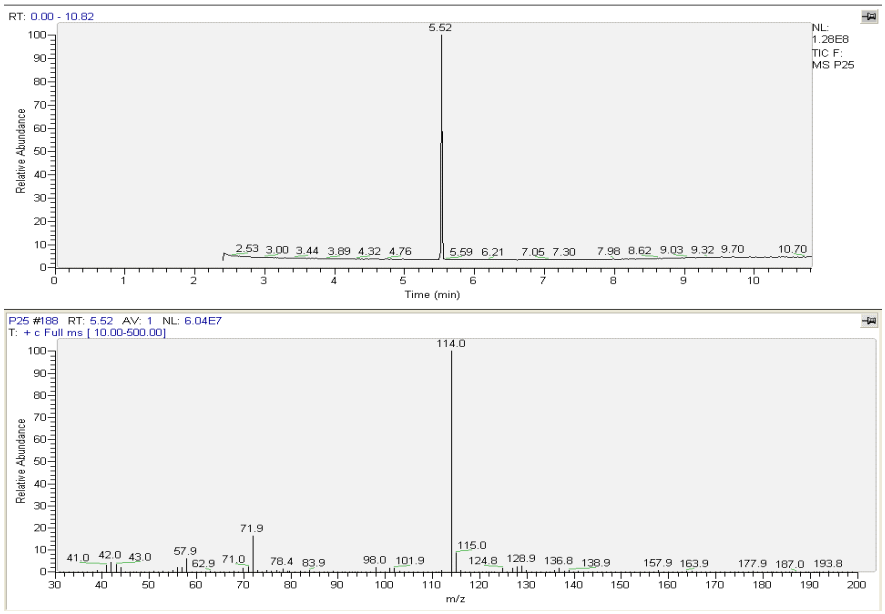

Fig. 7.6. Chromatogram and mass spectrum for sibutramine (sample SS24, Tr = 5.52 min, m/z = 114)

7.3.2.2. Quantitative Analysis

The results of the qualitative analysis suggested high levels of caffeine in most of the analyzed products. Given that many supplements contain one or more caffeine-containing plant products, and some also have added caffeine, caffeine content was evaluated using the HPTLC method. For phenolphthalein, quantitative analysis was performed using the HPLC method described in section 7.2 "Materials and Methods." The amount of caffeine ranged from 2.5 mg to 302 mg per unit dose. The highest concentrations were found in supplements with added caffeine (SS12, SS14, and SS20). The quantity of phenolphthalein determined by HPLC in supplements SS3, SS6, SS10, and SS12 ranged from 104 to 293 micrograms per unit dose (Table VII.3, Appendix 2).

8. Evaluation of Dietary Supplement Use for Enhancing Physical and Sports Performance – Survey Study

8.1. Introduction

To assess athletes' knowledge and use of dietary supplements, as well as the risk of adverse reactions, we developed a survey questionnaire. This tool gathered information regarding athletes' age, place of residence, sex, types of supplements used, consumption frequency, and declared adverse effects. The findings of this study help guide the selection of products for analytical investigation to detect the possible presence of adulterants.

8.2. Materials and Methods

The study was conducted using the Google Forms platform, and the questionnaire was distributed in Romanian. The survey, titled *"The Use of Dietary Supplements by Athletes and the Potential Presence of Adulterants,"* received ethical approval from the Ethics Committee of the Romanian National Anti-Doping Agency (ANAD) (Appendix 1).

Study Design

The questionnaire aimed to gather information on the types of supplements used by athletes and to assess the risk of adverse reactions, in order to explore possible correlations with the presence of synthetic adulterants in such supplements.

8.3. Results

8.3.1. Socio-demographic Characterization of the Study Group

The research included 87 athlete participants. Socio-demographic analysis revealed a predominance of female respondents (68.96%), possibly due to a higher willingness to complete the questionnaire (Fig. 8.1, Appendix 3). Most responses were from participants living in urban areas (80%) (Fig. 8.2, Appendix 3). The dominant age group was 14–20 years, representing 55.17% of participants. In terms of education, the majority of respondents had completed high school, totaling 44.2%.

8.3.2. Prevalence and Characteristics of Supplement Use

Responses regarding prevalence and use patterns showed that most athletes consume supplements (79.32%) (Fig. 8.5, Appendix 3), and the majority began using them between the ages of 14 and 18 (71.4%) (Fig. 8.6, Appendix 3). More than half (58.1%) reported using only one supplement (Fig. 8.7, Appendix 3), and among those who used more than one, 97.3% stated they did not combine them with steroids or hormones. As for reasons for use, most users (86.5%) cited a desire for fast results and endurance during training, 10.8% cited curiosity, and the remaining 2.7% mentioned peer and media influence (Fig. 8.9, Appendix 3).

8.3.3. Assessment of Athletes' Knowledge of Dietary Supplements

Regarding the level of knowledge, based on responses related to types of supplements consumed, sources of information, ease of access, and awareness of potential risks, the following results were noted: the most frequently used supplements are those containing vitamins and minerals (74.57%). Supplements with amino acids and proteins are used by 30.50% of respondents. Creatine-based supplements are used by 13.55% of respondents (Fig. 8.11, Appendix 3).

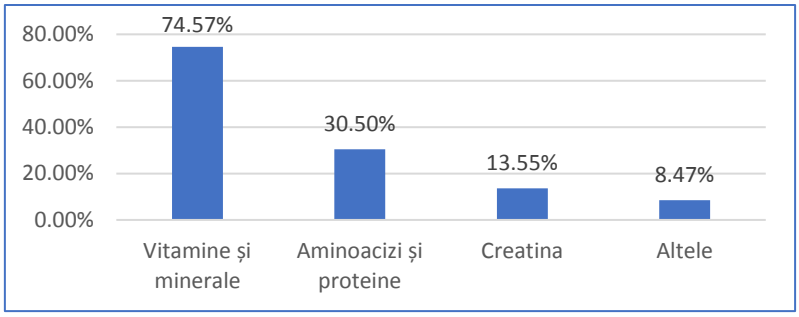

Fig. 8.11. Distribution of study participants based on the type of supplement used

8.3.4. Characterization of the study group based on desired effects and reported adverse effects

Regarding the desired effects of users (affirmative responses to questions about each type of effect), more than half (60.5%) reported increased energy (Fig. 8.14., Appendix 3), while 51% reported muscle mass gain. The time required to achieve the effect (e.g., muscle mass gain and weight loss) was most frequently between 1 and 3 months (64.7% of

consumers) (Fig. 8.17., Appendix 3). The characterization of the study group based on reported adverse reactions shows that, at the cardiovascular level, palpitations and increased heart rate predominated (48.1%), while at the respiratory level, cough was reported by 63.5% of consumers, and shortness of breath by 36.5%. Among neurological effects, headaches were the most frequent, reported by 55.8% of users, followed by dizziness (21.2%) and bruxism (9.6%) (Fig. 8.22., Appendix 3). The predominant effects in the ENT sphere were dry mouth, experienced by 75% of users, and tinnitus (9.6%) (Fig. 8.19., Appendix 3).

8.3.5. Statistical analysis of data

8.3.5.1. Chi-square test

Correlation between education level (primary/middle/high school/higher education) and: Consumption of dietary supplements (question: "Have you ever consumed dietary supplements?"). According to the results obtained, the group of respondents with primary education (Chi-square contribution 1.72) showed a tendency to use dietary supplements more frequently. This may be explained by the type of supplements used, mainly vitamins and minerals.

8.3.5.2. Pearson test

The following possible correlations were analyzed:

a. *Consumption of creatine supplements or amino acid-based products in relation to the observed time until weight loss and muscle mass gain.*

A moderate negative correlation was observed, meaning that higher scores of variable X (type of supplement, i.e., amino acid-based products) were correlated with lower scores of variable Y (observed time until weight loss) (and vice versa). Thus, the consumption of amino acid-based products correlates with a shorter time until the desired effects (weight loss and muscle mass gain) appear.

d. Headache and dry mouth

A highly significant positive correlation was observed between the occurrence of headaches (neurological adverse effect) and dry mouth (ENT adverse effect). These may be adverse reactions to the same type of supplement, suggesting potential adulteration.

9. Research on the adulteration of dietary supplements for improving physical and sports performance

9.1. Introduction

This chapter presents a qualitative analysis of dietary supplements for athletes to detect possible adulterants. Identification was performed using the HPTLC method, and confirmation was done using the GC-MS method. Quantitative analysis was applied to sports supplements containing caffeine.

9.2. Materials and methods 9.2.1. Analyzed dietary supplements A total of 35 dietary supplements for athletes were analyzed, mostly in the form of oral solutions (shots), but also in capsule, tablet, and powder form. The selection of products used in the study was based on the composition declared on the label, correlated with data obtained from athlete survey responses conducted in collaboration with ANAD through the athlete questionnaire (Appendix 1).

9.3. Results 9.3.1. Qualitative analysis of dietary supplements for athletes using HPTLC

Following qualitative analysis using the HPTLC technique, it was observed that most samples contained a compound with an Rf value between 0.12 and 0.14, but without indications of the presence of synthetic steroidal active substances or pharmacologically active compounds added as adulterants. These results support the conclusion that the analyzed samples do not contain compounds classified as doping agents from the steroid, beta-blocker, or stimulant classes. Additionally, the method proved suitable for qualitative screening of supplements, allowing precise identification of target substances by comparing Rf values and UV absorption spectra.

9.3.2. Qualitative analysis of dietary supplements for athletes using GC-MS The presence of caffeine was confirmed in supplements SSP12, SSP14, SSP15, SSP19, SSP20, SSP22, and SSP23. Pregnenolone was identified in supplement SSP8 (Fig. 9.6., Appendix

3). This is a precursor/metabolic intermediate for the biosynthesis of steroid hormones but is not included in the list of substances banned by the World Anti-Doping Agency. No synthetic adulterants, such as steroids or other doping substances, were identified.

9.3.3. Quantitative analysis of caffeine in dietary supplements for athletes using HPTLC

For the quantitative analysis of caffeine using HPTLC, seven dietary supplements that tested positive for caffeine in the qualitative analysis were selected. The analyzed products covered different functions: energy boosters, pre-workout supplements, and amino acid mixes. The chromatogram obtained from the quantitative analysis is shown in Fig. 9.8.

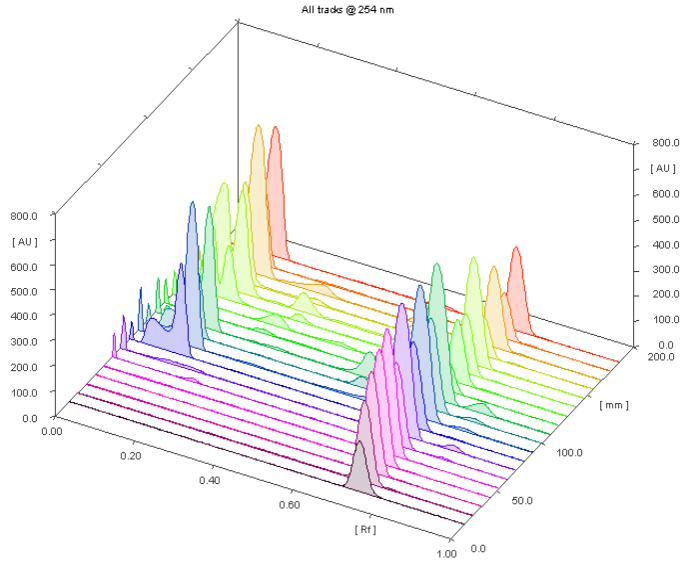

Fig. 9.8. 3D Chromatogram obtained from the quantitative HPTLC analysis of standard caffeine in dietary supplements for athletes

(Solvent system: ethyl acetate:toluene:methanol:ammonia 50:30:20:0.5; detection at 254 nm; standard caffeine - migration lanes 1–5; SSP22 - migration lanes 6–7; SSP20 - migration lanes 8–9; SSP19 - migration lanes 10–11; SSP23 - migration lanes 12–13; SSP12 - migration lanes 14–15; SSP14 - migration lanes 16–17; SSP15 - migration lanes 18–19).

Following the quantitative analysis, it was observed that SSP19, SSP20, and SSP22 products were overdosed (Table IX.3.). In the case of SSP22, the deviation exceeded acceptable limits, with an overdose of **119.5%**, while SSP19 and SSP20 fell within the

acceptable limits established for medicinal products (**±10%** of the declared amount). For SSP12, SSP14, SSP15, and SSP23, caffeine concentrations were below those declared on the label. These values were outside the acceptable limit, as they deviated by more than **10%** from the declared content.

10. Online Platform for Reporting Adverse Reactions Associated with Dietary Supplements

10.1. Introduction

This chapter explores the concept and implementation of an online platform dedicated to reporting adverse reactions associated with dietary supplements, highlighting its importance, benefits, and challenges.

10.2. Materials and Methods

Xojo is a cross-platform programming environment that allows the development of applications for multiple operating systems, including Windows, macOS, Linux, iOS, and web, using a single source code.

10.3. Results

Creation of the Reporting Forum The platform is designed for dietary supplement users, with the primary objective of identifying and analyzing possible adverse reactions associated with their consumption. The reporting form can be accessed at: http://datacore.go.ro:8100/ (Appendix 4).

Database Configuration The database is created as users access the platform and involves recording each individual report.

Administration Interface The administration interface of an online platform is essential for efficiently managing content, users, and functionalities.

Creating an Administration Page Application testing: The application was run, and the platform's functionality was demonstrated. The graphs recorded during platform testing are shown in **Fig. 10.2 – 10.5** (Appendix 4).

III. Conclusions and Personal Contributions

Conclusions

Extent to which research objectives were achieved

The literature review (Chapters 1–3) highlights major challenges in the field of dietary supplements, particularly regarding regulation, quality assurance, and safety, as well as the interpretation of study results. This analysis underscores the importance of adopting a rigorous scientific methodology for verifying supplement quality and safety, based on advanced analytical techniques and a standardized testing framework.

The research conducted in this doctoral thesis confirms the presence of synthetic chemical compounds in supplements marketed as "100% natural," demonstrating a high frequency of adulteration, particularly in products intended for **sexual performance enhancement** and **weight control**, as well as those used to improve **physical capacity and sports performance**.

Regarding the adulteration of supplements for sexual performance enhancement (Chapter 6) and weight loss (Chapter 7), the identification of **PDE-5 inhibitors** such as **sildenafil and tadalafil**, and **sibutramine and phenolphthalein**, respectively, highlights significant risks for consumers. Additionally, discrepancies between the actual caffeine content and the declared amount on labels of supplements used for physical performance emphasize the risks of excessive consumption (Chapter 9).

The similarity of chromatographic profiles observed for multiple products suggests the presence of common ingredients used by various brands or supplement lines. However, variations in product composition and different adulterants were identified throughout the research, without consistency in the use of the same adulterant for a particular product. Moreover, not all tested batches of the same product showed adulteration.

Another research direction in this thesis was the **evaluation of knowledge and use of supplements among athletes**, as well as the risk of adverse reactions. A **survey-based study** was conducted, targeting athletes in Romania (Chapter 8). The questionnaire correlated various information, including **socio-demographic characteristics, types of supplements used, consumption frequency, and adverse reactions experienced**.

The results, statistically interpreted using **Chi-square and Pearson tests**, provided valuable insights into the relationships between studied variables. For example, **amino acid-based products** were associated with a **shorter time** to achieve desired effects, such as **weight loss and muscle mass gain**. Additionally, the results highlighted a **high frequency of dietary supplement consumption among young athletes**, often **without prior medical consultation**. Although the general perception of the beneficial effects of these products is predominantly positive, **adverse reactions are frequently reported**, necessitating a **responsible approach**. In this context, **educational initiatives** and the **promotion of reliable sources of information** become essential to **reduce self-medication risks**. The research led to the **development of an online platform** for reporting adverse reactions associated with dietary supplements (Chapter 10), which is **easy to use** for all supplement consumers. This tool enables the **creation of a useful database** for identifying and predicting the presence of adulterants, facilitating subsequent verification through **laboratory analytical methods**. The collected information supports **health authorities**, providing them with a **solid basis** for analyzing trends and identifying emerging issues related to dietary supplement safety.

Personal Contributions

The main personal contributions to the issue of **adulterants in dietary supplements** consist of the **development and validation of high-performance analytical methods** for detecting synthetic compounds fraudulently introduced into such products. Thus, the **HPTLC methods** developed for **identifying and quantifying synthetic adulterants** provide a **reliable, cost-effective, and accessible tool** for **screening products and detecting falsifications**. The **complex analytical approach**, which includes **HPTLC screening followed by confirmation using GC-MS, HPLC, or LC-HRMS-MS methods**, reflects a **rigorous and multidimensional effort** in the **qualitative and quantitative analysis** of potential adulterants in various types of dietary supplements. Additionally, the thesis makes a **valuable personal contribution** by **creating an online platform** for **reporting adverse reactions to dietary supplements**. This represents an **important step** in **monitoring adverse reactions** and **building a relevant database** for **predicting adulterant presence**. This initiative facilitates **collaboration between consumers, specialists, and authorities**, contributing to **greater transparency and safety** in dietary supplement use.

Finally, the **survey-based research method** allows for a **detailed and well-founded data analysis**, facilitating the **formulation of relevant conclusions** for **improving sports performance** and **promoting a balanced lifestyle**. Through these efforts, the thesis makes a **significant contribution** to both **analytical and toxicological research** and the **implementation of practical solutions** for **consumer protection** and **improving dietary supplement regulations**.

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