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Assessing the adulteration of food supplements for improving sexual performance with PDE-5 inhibitors

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ABSTRACT

Plant food supplements (PFS) are considered as foods and are not submitted to any safety assessment prior to their commercialization. However, the adulteration with pharmaceutical drugs illegally added to PFS was already reported. PFS marketed for improving sexual performance are particularly prone to be adulterated with phosphodiesterase type-5 (PDE-5) inhibitors, which are prescription drugs used to treat erectile dysfunction that are known to have life-threatening side effects. Therefore, this work aimed to detect the presence of illegally added PDE-5 inhibitors in PFS marketed for improving sexual performance. Twelve samples were acquired and screened for the presence of sildenafil, acetildenafil, thiosildenafil, tadalafil, vardenafil and yohimbine by using a HPLC-DAD-FL method. The method was validated and showed suitable scores for the tested compounds. From the 12 analysed PFS, 6 were adulterated with PDE-5 inhibitors and 2 revealed the presence of two unknown compounds, whose UV-Vis spectra suggest a possible adulteration with PDE-5 analogues.

1. INTRODUCTION

Over the last decade, there has been a notorious growth in the consumption PFS, especially in developed countries [1]. According to the European Union legislation (Directive 2002/46/EC), PFS are considered as foods, thus not being submitted to any safety assessment prior to their commercialization [1]. Since this type of products include plants as ingredients, PFS are frequently advertised as "natural", leading to a false sense of security in consumers. Recent reports refer that PFS marketed for improving sexual performance are particularly prone to be adulterated by the addition of pharmaceutical drugs, namely, phosphodiesterase type-5 (PDE-5) inhibitors [2], which are prescription drugs used to treat erectile dysfunction. These drugs, including sildenafil (Viagra®), tadalafil (Cialis®) and vardenafil (Levitra®), can be added by unscrupulous producers to provide for quick effects to increase sales. However, PDE-5 inhibitors can have side effects such as headaches, flushing, dyspepsia, nasal congestion, and visual disorders, and can be life-threatening when concomitantly used with nitrates or α-blockers. Therefore, this type of adulteration is a

major public health concern. Additionally, beyond approved PDE-5 inhibitor drugs, PFS are frequently adulterated with their analogs, which are substances synthesized mainly based on the chemical structure of the approved pharmaceuticals. The use of unapproved analogs is even more difficult to detect in routine inspections using standard protocols and, from a human health perspective, raise additional safety concerns as their pharmacokinetics and safety profile are mostly unknown [3]. Therefore, this work aimed at assessing the presence of illegally added PDE-5 inhibitors in PFS marketed in Portugal for improving sexual performance.

2. MATERIAL AND METHODS

2.1 Samples and standards

A total of 12 samples acquired on the Portuguese market were kindly provided by ASAE, sealed and identified. After homogenization, the samples were extracted with 15 mL acetonitrile(MeCN)/methanol (MeOH) (50:50) in an ultrasound bath (5 min), followed by centrifuging, filtering (0.22 μm) and dilution (1:50). Stock solutions (2mg/mL) of the individual standards sildenafil citrate, acetildenafil, thiosildenafil, tadalafil, vardenafil hydrochloride and yohimbine hydrochloride were prepared in MeCN/MeOH (50:50).

2.2 Chromatographic analysis

The analyses were performed on HPLC system (Jasco, Japan) coupled to a DAD and FL detectors, using an YMC-Triart C18 analytical column (3 μm, 250×4.6 mm) and 50 mM ammonium acetate (A) and MeCN/MeOH (50:50) (B) as eluents. Elution was performed at 0.45 mL/min using the following gradient: 0 min – 70%B, 15 min – 80%B, 25 min – 98%B, 27.5 min – 98%B, 30 min – 70%B, 35 min – 70%B. The compounds were identified by comparison with authentic standards. Quantification was performed by the absorbance recorded relative to external standards, with detection at 290 nm.

2.3 Method Validation

The used methodology was validated regarding limits of detection (LOD) and quantification (LOQ), linearity range, intra- and inter-day precision and accuracy.

3. RESULTS AND DISCUSSION

3.1 Method Development and Validation

Firstly, using a mixture containing all standards, different chromatographic conditions were tested in order to achieve the best separation of compounds in the shortest elution time (Figure 1A). Each standard was also individually injected in the chromatographic system to obtain its retention time and UV-spectra. Additionally, considering the complexity of the matrix, the use of fluorescence detection was considered due to its higher sensibility and selectivity compared to UV detectors. For this purpose, each standard solution was also analysed by spectrofluorometry to obtain the wavelengths of excitation and emission for

each compound. Fluorescence was only evidenced by tadalafil (λex=307 nm; λem=328 nm), yohimbine (λex=299 nm; λem=333 nm), vardenafil (λex=347 nm; λem=469 nm) and sildenafil (λex=358 nm; λem=403 nm). However, in the concentration range used for the standard mixture, when it was injected on the chromatographic system fluorescence detection was only achieved for tadalafil, yohimbine and vardenafil (Figure 1B). Therefore, detection of compounds was accomplished using only the diode-array detector.

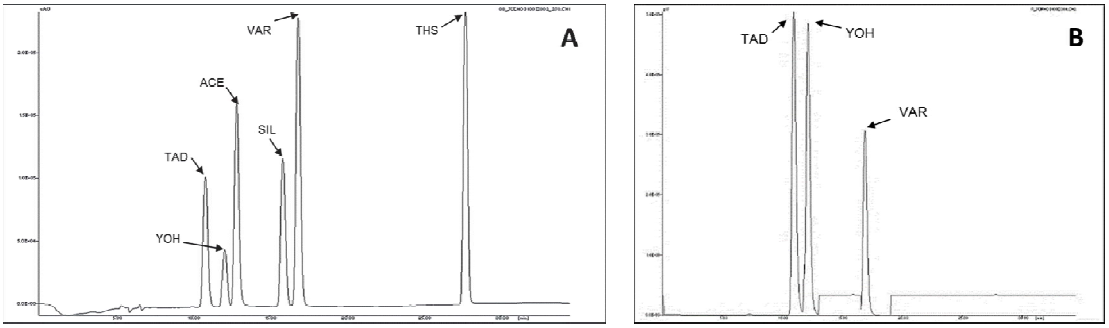

Figure 1. Chromatogram obtained using HPLC with DAD (A) and with FL detection (B). TAD: tadalafil, YOH: yohimbine, ACE: acetildenafil, SIL: sildenafil, VAR: vardenafil, THS: thiosildenafil.

Considering the chemical characteristics of the compounds under study, their extraction is generally accomplished by using organic solvents, such as methanol and/or acetonitrile, or aqueous mixtures of these solvents. In this work, re-extraction with different volumes of acetonitrile/methanol (50:50) and different sonication periods were tested, with better recovery (%) of compounds being achieved when using a single extraction with 15 mL of acetonitrile/methanol (50:50) and sonication during 5 min.

After optimization, the method was submitted to in-house validation. Calibration curves were obtained for each standard based on the linear regression analysis of the peak area versus concentration. Table 1 shows the linearity range, correlation coefficients, LODs and LOQs obtained for each standard.

The method precision was determined based on the coefficient of variation (%) obtained for the analysis of a standard solution mixture at different concentration levels. Recoveries were calculated as the ratio between the concentrations obtained in the sample and the spiking levels. Table 2 shows the results obtained for intra-day precision (repeatability), inter-day precision and recoveries for each compound.

Table 1. Calibration parameters, LODs and LOQs obtained for each standard.

Standard	Linearity range (µg/ml)	Calibration curve	R^2	LOD (µg/mL)	LOQ (µg/mL)
Tadalafil	5.0 – 200.0	y = 25566x + 96611	0.993	0.017	0.052
Yohimbine	5.0 – 200.0	y = 12316x + 34087	0.992	0.031	0.094
Vardenafil	5.0 – 200.0	y = 10551x + 54868	0.992	0.047	0.143
Sildenafil	5.0 – 200.0	y = 16323x + 47477	0.991	0.044	0.132
Acetildenafil	5.0 – 200.0	y = 37932x + 107194	0.991	0.013	0.038
Thiosildenafil	5.0 – 200.0	y = 17347x + 61789	0.991	0.025	0.076

3.2 Application to PFS samples

The optimized validated methodology was applied for the analysis of 12 PFS marketed for improving sexual performance. A total of 7 samples were found to be adulterated with PDE-5 inhibitors, namely sildenafil, thiosildenafil and tadalafil, with some samples presenting more than one drug simultaneously. Additionally, 2 samples evidenced the presence of an unidentified compound showing UV-spectra similar to that of sildenafil, which can possibly suggest the presence of a sildenafil analogue. The identified compounds were quantified showing identical levels to the dosages used in prescribed medicines. The identified compounds and respective quantification are shown in Table 3.

Table 2. Repeatability, inter-day precision and recoveries obtained for each standard.

Standard	Intra-day precision (CV%)			Inter-day precision (CV%)			Recovery	
	10 µg/mL	50 µg/mL	150 µg/mL	10 µg/mL	50 µg/mL	150 µg/mL	Mean* (%)	CV%
Tadalafil	1.70	2.32	1.66	8.84	6.69	6.95	81.5	8.0
Yohimbine	2.95	2.69	3.04	9.57	5.13	6.92	76.2	10.6
Acetildenafil	2.54	2.34	2.96	9.78	8.53	6.79	82.2	4.1
Sildenafil	2.33	2.20	2.90	8.93	7.17	6.64	87.0	9.0
Vardenafil	1.87	2.66	5.89	9.23	8.66	6.57	76.0	11.6
Thiosildenafil	1.02	2.44	2.85	9.85	5.85	6.71	91.0	23.1

* Recoveries were determined by spiking 3 different samples, each at two spiking levels (150 µg/ml e 25 µg/mL); CV: coefficient of variation.

Table 3. Adulterant drugs identified in PFS samples.

Sample	SA1		SA4	SA5		SA8	SA9	SA10	SA11	
PDE-5 inhibitor	SIL	THS	Possible analogue of SIL	SIL	THS	TAD	TAD	TAD	THS	Possible analogue of SIL
mg/ g sample	32.7±0.7	71.7±0.8	90.5±2.9	27.3±0.7	61.5±1.4	2.7±0.2	24.0±0.8	22.5±0.7	77.2±0.4	33.4±0.4

*TAD: tadalafil, YOH: yohimbine, ACE: acetildenafil, SIL: sildenafil, VAR: vardenafil, THS: thiosildenafil.

4. CONCLUSIONS

The proposed methodology proved to be fast, simple and suitable for the screening of PFS adulteration with PDE-5 drugs. From the 12 analysed PFS, 7 were found to be adulterated with one, or more, PDE-5 inhibitors, corresponding to a high incidence of adulteration (58%). In the future, more samples should be included in the study for the purpose of monitoring the Portuguese market respecting the adulteration of these products. Moreover, for positive and inconclusive samples, the use of mass spectrometry is advisable for the unequivocal identification of compounds.

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