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Research Article

Determination of phosphodiesterase type 5 inhibitors in health supplements using liquid chromatography–tandem mass spectrometry (LC–MS/MS)

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Abstract

Phosphodiesterase type 5 inhibitors (PDE-5i) are the common oral medications prescribed for the treatment of erectile dysfunction (ED). In recent years, health supplements have gained popularity as alternative options to prescription PDE-5i. However, concerns have emerged regarding the adulteration of these products with undeclared synthetic PDE-5i or their analogues, posing significant health risks. In this study, a rapid, simple, and efficient analytical method for the determination of PDE-5i was developed and validated using a liquid chromatography–tandem mass spectrometry system (LC-MS/MS) for the rapid detection of these compounds in health supplement products collected in Hanoi. The method demonstrated excellent linearity ($R^2 \geq 0.999$). The limits of detection (LOD) and quantification (LOQ) were 1.5 µg/kg and 5.0 µg/kg for sildenafil, tadalafil, and vardenafil, and 15 µg/kg and 50 µg/kg for the remaining PDE-5i. Recoveries ranged from 80.9% to 113% with relative standard deviations (%RSD) below 15%. The validated procedure was applied to 24 real-world samples, and 13 of them were found to contain PDE-5i. Further comprehensive investigations involving larger sample sizes and additional PDE-5i compounds are necessary to better assess adulteration practices and potential health risks.

Keywords: *Phosphodiesterase type 5 inhibitors, Adulterants, Health supplements, LC-MS/MS.*

1. INTRODUCTION

Erectile dysfunction (ED) is the inability to obtain or sustain an erection adequate for sexual intercourse and affects hundreds of millions of men globally, with prevalence increasing with age and significantly impacting quality of life [1, 2]. Oral phosphodiesterase type 5 inhibitors (PDE-5i) are considered the first-line therapy for the treatment of ED [3]. To date, four PDE-5i have been approved by the U.S. Food and Drug Administration (FDA): sildenafil citrate, tadalafil, vardenafil hydrochloride, and avanafil. Among these, avanafil is a newer-generation drug, approved in 2012, with higher potency and a faster onset of action [4]. Other second-generation PDE-5i, such as udenafil, mirodenafil hydrochloride, and lodenafil carbonate, are also marketed in several countries outside the United States [2]. Despite their efficacy, PDE-5i may cause adverse effects including headache, facial flushing, and visual disturbances [5, 6]. In addition, these drugs are contraindicated in patients receiving nitrate therapy [5, 7]. Such undesirable effects, together with their high cost, have led many patients to seek cheaper and more tolerable alternatives [7]. Health supplements of natural origin are often perceived as safer, more widely accepted, and less expensive than PDE-5i drugs for improving sexual function [2, 7]. However, inadequate regulatory control and the widespread availability of online markets have facilitated intentional adulteration of these products with undeclared synthetic PDE-5i or

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structurally related analogues [8]. Many of these analogues can be readily synthesized based on information available in patent literature [8, 9]. According to Gilard *et al*., approximately 61% of 150 herbal sexual enhancement supplements surveyed contained undeclared active substances, including PDE-5i or their analogues [9]. Other studies have similarly reported widespread adulteration and documented numerous cases of serious adverse effects, including ataxia and even death [7, 10]. The detection of PDE-5i and their analogues in health supplements is particularly challenging due to their very low concentrations and the complexity of sample matrices [2, 8]. Therefore, analytical methods with high selectivity and sensitivity are required. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) stands out as an effective technique, enabling the simultaneous identification and quantification of multiple compounds at trace levels with high accuracy and specificity [2, 7, 8, 10–13]. Although LC–MS/MS methods have been widely applied worldwide for this purpose, their optimization, validation, and practical implementation for health supplements in Vietnam remain limited.

In this study, an LC–MS/MS method was developed and validated for the simultaneous quantification of 51 PDE-5i in various dietary supplement formulations, including hard capsules, soft capsules, and liquid preparations. After validation, the method was applied to investigate the presence of PDE-5i in dietary supplement products collected from the Vietnamese market. The results provide valuable scientific and practical information for the field of analytical chemistry and food quality control.

2. MATERIALS AND METHODS

2.1. Chemicals and standards

In this study, 12 PDE-5i standards supplied by LGC Standards Ltd. were used, including acetildenafil (95.9%), aminotadalafil (99.5%), carbodenafil (99.1%), chloropretadalafil (99.7%), sildenafil-descarbon (99.2%), desmethyl carbodenafil (99.7%), homosildenafil (99.26%), hydroxychlorodenafil (98.7%), mirodenafil (99.1%), sildenafil (98.67%), tadalafil (99.51%), and udenafil (98.05%). In addition, 39 PDE-5i reference standards supplied by TRC were employed, including 2-hydroxypropyl-nortadalafil (98%), acetaminotadalafil (98.86%), acetyl acid (97%), acetylvardenafil (98%), aildenafil (98%), avanafil (>95%), benzamidenafil (98%), cyclopentynafil (96%), desmethylthiosidenafil (99.8%), dithio-desmethyl-carbodenafil (98%), gendenafil (98%), hydroxythiovardenafil (98%), hydroxyacetildenafil (97%), imidazosagatriazinone (98%), lodenafil carbonate (96%), mutaprodenafil (98%), N-butyl tadalafil (98%), N-desethyl vardenafil (97%), N-desmethyl sildenafil (98%), N-desmethyl tadalafil (95%), N-desmethyl vardenafil (95%), N-desmethyl-N-benzylsildenafil (99.45%), nitrodenafil (98%), norneosildenafil (98%), nor-acetildenafil (>95%), norneovardenafil (96%), octylnortadalafil (96%), piperadino vardenafil (98%), piperazonifil (95%), piperiacetildenafil (98%), propoxyphenylhomohydroxysildenafil (98.7%), propoxyphenyl thioaildenafil (>95%), propoxyphenyl thiohydroxyhomosildenafil (98%), propoxyphenyl aildenafil (98%), propoxyphenylsildenafil (98%), sildenafil N-oxide (99.5%), sulfohydroxyhomosildenafil (99.41%), thiosildenafil (98%), and vardenafil (98%). Stock standard solutions at a concentration of 1000 µg/mL for each compound were prepared by diluting 10 mg of the analytical reference standard to 10 mL of methanol. These solutions were stored at 2–8°C in airtight amber glass vials. Working standard solutions were prepared by diluting the stock solutions with methanol to concentrations of 1.5, 3, 6, 12, and 15 ng/mL for sildenafil, tadalafil, and vardenafil, and 10, 20, 40, 80, and 100 ng/mL for the remaining 48 PDE-5i compounds. Chromatography-grade solvents used in the study included methanol (MeOH), acetonitrile (ACN), formic acid, ammonium formate, and deionized water (Merck KGaA).

The chromatographic analysis system consisted of a Shimadzu HPLC-20AXL coupled to a Sciex Triple Quad 5500 mass spectrometer for the separation and quantification of PDE-5i. A reversed-phase C18 column (100 mm × 2.1 mm, 1.7 µm; Waters) equipped with a corresponding guard column (2.1 × 5 mm, 1.7 µm; Waters) was used. Sample preparation equipment included analytical and precision balances (Mettler Toledo), a sample homogenizer (Philips), an ultrasonic bath (Elma), a vortex mixer (IKA), and a centrifuge (Hettich).

2.2. Sample preparation

Based on the literature [14], the sample preparation procedure was evaluated using spiked blank samples, in which known amounts of the target analytes were added to representative blank matrices, including hard capsules, soft capsules, and liquid samples (all of which had been previously analyzed by LC–MS/MS to

confirm the absence of PDE-5i). For hard capsule samples, the contents were finely ground and homogenized using a sample homogenizer. For soft capsules, the outer shell was removed and the inner contents were thoroughly mixed. Liquid samples were vortex-mixed to ensure homogeneity prior to analysis. In addition, 24 dietary supplement samples were collected in Hanoi to evaluate the applicability of the developed analytical method, including hard capsules (*n = 19*), soft capsules (*n = 3*), and liquid samples (*n = 2*).

2.3. Optimization of LC-MS/MS conditions

The analytical conditions for the detection of PDE-5i on the tandem mass spectrometer were optimized and automatically selected by directly injecting a mixed standard solution (20 ng/mL) into the detector without passing through the chromatographic column. The optimization was carried out under positive electrospray ionization (ESI+) mode and monitored in multiple reaction monitoring (MRM) mode. The optimized parameters using Analyst software included ion spray voltage, gas temperature, gas flow rate, nebulizer pressure, and quantitative and qualitative ion transitions of each analyte. In addition, the composition of the mobile phase was investigated to achieve optimal separation efficiency and signal intensity. Two solvent systems were evaluated: (1) 10 mM ammonium formate containing 0.1% formic acid in water (mobile phase A) and methanol (mobile phase B); and (2) 10 mM ammonium formate containing 0.1% formic acid in water (mobile phase A) and acetonitrile (mobile phase B).

2.4. Optimization of extraction conditions

The extraction efficiency of PDE-5i from hard capsule, soft capsule, and liquid sample was evaluated using four different solvents: methanol (MeOH), acetonitrile (ACN), MeOH:H_2O (1:1, v/v), and ACN:H_2O (1:1, v/v). Spiked samples were prepared at a concentration level of 1.5 ng/g for sildenafil, tadalafil, and vardenafil (corresponding to an extract concentration of 1.5 ng/mL), and 10 ng/g for the remaining 48 PDE-5i compounds (corresponding to an extract concentration of 10 ng/mL). The extraction conditions were selected based on achieving the highest recoveries for each analyte in each sample matrix.

2.5. Calculation of analyte concentration in samples and recovery

Quantification of PDE-5i in health supplements was calculated using an external calibration approach according to the equation:

$$A = C \times V \div m$$

where C is the analyte concentration in the sample extract (ng/mL), V is the final extract volume (mL), and m is the sample mass (g).

The recovery of PDE-5i was determined using spiked samples and calculated using the formula: $H = (C_1 \div C_0) \times 100\%$ where C_1 is the measured concentration and C_0 is the spiked concentration of the analyte. All data were processed using Microsoft® Excel® (Microsoft Office Home and Student 2019) and Minitab® 21.3 Statistical Software (Minitab, LLC).

3. RESULTS AND DISCUSSION

3.1. MS/MS conditions

The optimized MS/MS parameters for the analysis of PDE-5i were established based on previous studies and direct optimization on the instrument [14]. All measurements were performed in positive electrospray ionization mode (ESI+). Key source parameters included a curtain gas pressure of 35 psi, a collision gas pressure of 7 psi, an ion spray voltage of 5500 V, and an ion source temperature of 550°C. The flow rates of source gas 1 and source gas 2 were set at 50 psi and 60 psi, respectively. The entrance potential was maintained at 10 V, while the collision energy was optimized at approximately 35 eV. The collision cell exit potential was adjusted to 26 V to ensure stable ion transmission and achieve high analytical sensitivity. The optimized parameters are shown in **Table 1**.

In addition to ion source optimization, the precursor-to-product ion transitions for all 51 PDE-5i compounds were established based on reference data from previous studies. For each compound, one parent ion and two fragment ions were selected, with one product ion used for quantification and the other for confirmation. The combination of optimized MS/MS conditions and selected MRM transitions ensured high selectivity and sensitivity for the simultaneous determination of multiple PDE-5i, fulfilling the identification point (IP ≥ 5)

requirements specified in EC Regulation 2021/808, in which LC contributes 1 point, the precursor ion contributes 1 point, and each product ion contributes 1.5 points [15].

Table 1. *MS/MS conditions*

Parameter	Abbreviation	Optimized value ESI (+)
Curtain gas	CUR	35 psi
Collision gas	CAD	7 psi
Ion spray Voltage	IS	5500 V
Temperature	TEM	550°C
Ion source gas 1	GS1	50 psi
Ion source gas 2	GS2	60 psi
Entrance Potential	EP	10 V
Collision Energy	CE	35 eV
Collision Cell Exit Potential	CXP	26 V

3.2. LC conditions

Two mobile-phase systems were evaluated in this study, including: (1) 10 mM ammonium formate containing 0.1% formic acid in water (mobile phase A) and methanol (mobile phase B); and (2) 10 mM ammonium formate containing 0.1% formic acid in water (mobile phase A) and acetonitrile (mobile phase B). The chromatographic results shown in **Figure 1** indicate that the PDE-5i peaks obtained with mobile-phase system (2) exhibited sharper peak shapes and higher signal intensities compared to system (1). Therefore, the mobile-phase combination of 10 mM ammonium formate with 0.1% formic acid in water (A) and acetonitrile (B) was selected for further analyses.

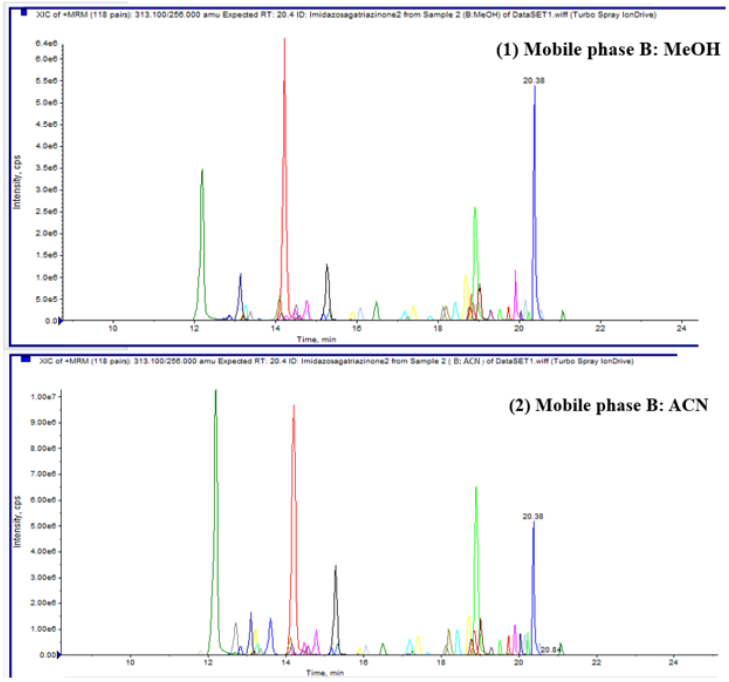

Figure 1. *Chromatograms of PDE-5i obtained using different mobile-phase systems*
(1) 10 mM ammonium formate containing 0.1% formic acid in water (mobile phase A) and methanol (mobile phase B)
(2) 10 mM ammonium formate containing 0.1% formic acid in water (mobile phase A) and acetonitrile (mobile phase B)

The mobile-phase flow rate was 0.4 mL/min with a 10 µL injection volume. The gradient elution program was established as follows: 0–1 min (99% A), 1–18 min (decrease from 99% A to 55% A), 18–20 min (decrease from 55% A to 5% A), 20–24 min (5% A), 24–25.5 min (increase from 5% A to 99% A), and 25.5–30 min (99% A). The retention times of the PDE-5i ranged from 12.0 to 21.0 min, demonstrating good

chromatographic separation and a well-distributed elution profile across the gradient. Overall, retention time tended to increase with decreasing polarity or increasing molecular mass of the analytes.

3.3. Extraction conditions

The selection of an appropriate extraction solvent is a critical factor influencing the efficiency of releasing PDE-5i from health supplement matrices and directly affects the overall accuracy of the analytical method. In this study, ultrasound-assisted extraction was evaluated using four solvent systems: (1) MeOH; (2) ACN; (3) MeOH:H_2O (1:1, v/v); and (4) ACN:H_2O (1:1, v/v). The recoveries of five common PDE-5i (sildenafil, tadalafil, vardenafil, avanafil, and udenafil) obtained under these conditions are shown in **Figure 2**.

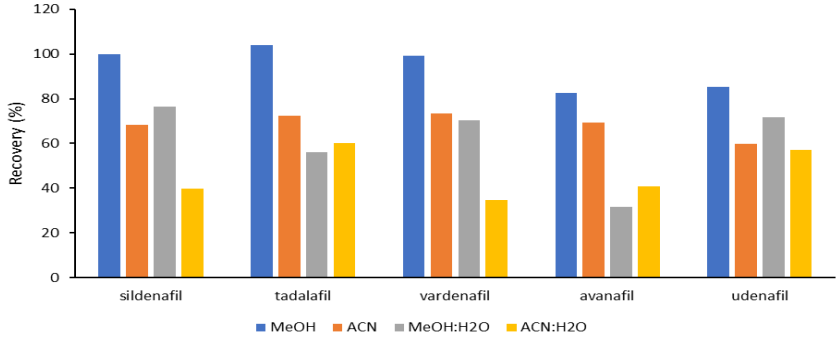

***Figure 2**. Extraction solvent evaluation for PDE-5i*

The results showed that methanol (MeOH) provided the highest extraction efficiency compared with acetonitrile (ACN) and mixed solvent systems. This can be explained by the semi-polar and hydrophobic nature of most PDE-5i, which are readily soluble in MeOH but poorly soluble in ACN and even less soluble in water. In addition, MeOH is more effective at wetting and disrupting the complex matrices of health supplements, thereby facilitating the release of PDE-5i from excipient-rich matrices containing polymers, starches, soluble fibers, or oil-based components. In contrast, ACN tends to precipitate proteins and polysaccharides, causing part of the analytes to be retained in the residue and thus reducing extraction recovery [14]. MeOH also minimizes emulsion formation in oil-rich products, making filtration and sample preparation easier. Therefore, MeOH was selected as the most suitable and efficient extraction solvent for the analysis of PDE-5i in health supplements. The choice of extraction solvent plays a critical role in the sample preparation and analytical procedure for PDE-5i in health supplements, ensuring the accuracy of the method.

Based on the optimization results, the final extraction procedure was established as follows: 5 g of sample was extracted twice, each time with 10 mL of MeOH, vortex-mixed for 1 min, followed by ultrasonic extraction for 30 min. The extract was then centrifuged at 6000 rpm for 5 min, filtered through a 0.22 µm membrane filter, and injected into the LC–MS/MS system for analysis.

3.4. Method validation

Selectivity is a critical parameter that determines the accuracy and reliability of an analytical method. In this study, each analyte was monitored using one parent ion and two fragment ions, corresponding to two MRM transitions, yielding a total of five identification points (IP), which meets the requirements of EC Regulation 2021/808 for chromatographic methods coupled with mass spectrometric detection [15]. Chromatograms of the standard solution, spiked blank samples, and blank samples are shown in **Figure 3**. The results indicated that no analytical signals were observed in the blank samples, whereas clear peaks were observed in both the standard and spiked samples at identical retention times, with deviations not exceeding 2%, demonstrating excellent method selectivity. The ion ratios for all analytes showed deviations R_{diff} of less than 10%, fully complying with the criteria specified in EC Regulation 2021/808 (<40%) [15].

Calibration curves were constructed over the concentration range of 1.5–15 ng/mL for sildenafil, tadalafil, and vardenafil, and 10–100 ng/mL for the remaining 48 PDE-5i compounds. All analytes exhibited good linearity with correlation coefficients (R^2) ≥ 0.999 and calibration curve deviations of less than 15%. The limits of detection (LOD) and limits of quantification (LOQ) for sildenafil, tadalafil, and vardenafil were 1.5 µg/kg and 5.0 µg/kg, respectively. For the other 48 PDE-5i compounds, the LOD and LOQ were 15 µg/kg and 50

µg/kg, respectively. These detection limits fully meet the requirements for trace-level analysis of PDE-5i in health supplementmatrices.

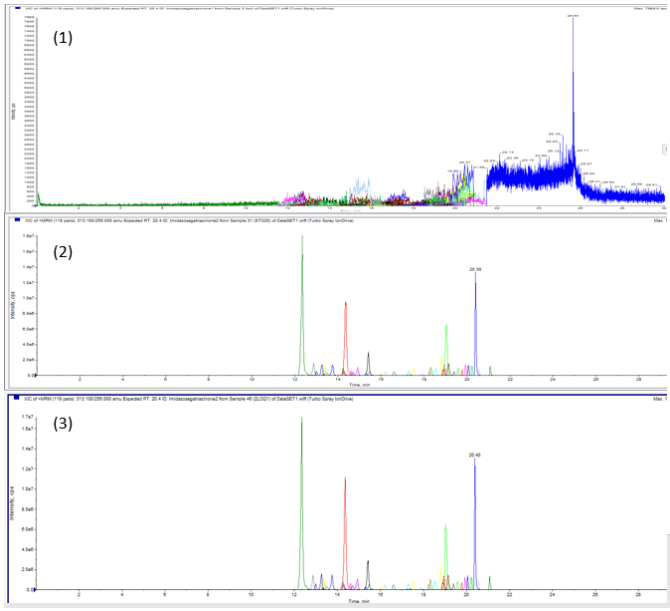

Figure 3. *Results of selectivity evaluation*

(1) blank, (2) standard solution, and (3) spiked blank sample

Recovery (R%) and relative standard deviation (RSD%) were evaluated by analyzing spiked blank samples at three concentration levels (1.5, 3, and 12 ng/mL for sildenafil, tadalafil, and vardenafil; and 10, 20, and 80 ng/mL for the remaining 48 analytes), with triplicate at each level (n = 3). The results showed that the recoveries of the 51 PDE-5i compounds in the three matrices ranged from 80.9% to 113%, with %RSD values between 1.0% and 9.5%. These results comply with the criteria of AOAC and EC Regulation 2021/808, which specify acceptable recovery ranges of 70–120% and RSD% ≤ 20% at trace concentration levels [15, 16], thereby confirming the reliability and accuracy of the developed method.

3.5. Analysis results of collected samples

Based on the validated analytical procedure, 24 health supplement samples marketed for sexual enhancement or with no declared sexual enhancement ingredients on the label were collected in Hanoi between February and July 2024. The contents of PDE-5i in these samples were determined and are presented in **Table 2**.

Table 2. *Concentrations of PDE-5i detected in positive health supplement samples*

Sample	Sample matrix	Concentrations of PDE-5i (mg/g)		
		Sildenafil	Tadalafil	Homosildenafil
M1	Hard capsule	2×10^{-4}	-	-
M2	Hard capsule	7×10^{-3}	-	-
M3	Hard capsule	7.6×10^{-3}	-	-
M4	Hard capsule	1.0×10^{-4}	-	-
M5	Hard capsule	1.21	9.2×10^{-4}	-
M6	Hard capsule	1.39	5.8×10^{-4}	-
M7	Hard capsule	7.2×10^{-3}	-	-
M8	Hard capsule	1.35	8.6×10^{-4}	-
M9	Hard capsule	1.28	6.0×10^{-4}	-
M10	Hard capsule	1.38	7.4×10^{-4}	-
M11	Hard capsule	1.29	8.2×10^{-4}	1.3×10^{-3}

M12	Hard capsule	1.4×10^{-2}	-	7.0×10^{-5}

–: Not detected (<LOD)

The analytical results showed that only 3 of the 51 targeted PDE-5i were detected in the 24 analyzed samples, and these compounds are also the ones most frequently reported in previous studies. In total, 13 out of 24 samples (54.2%) tested positive for at least one PDE-5i. Among the positive samples, sildenafil was the most frequently detected compound (12/13 samples, 92.3%). Seven samples (accounting for 29.2% of all samples and 53.8% of the positive samples) contained two or more PDE-5i. In most cases, the detected levels of PDE-5i were substantially lower than the therapeutic doses found in pharmaceutical products.

The contents of PDE-5i vary markedly among countries. For sildenafil, the concentrations detected in samples from Vietnam in this study (2×10^{-4}–1.39 mg/g) were much lower than those reported in Saudi Arabia (0.0201–37.5 mg/g) and dramatically lower than those found in some adulterated products in the Netherlands (2–322 mg/g) [11,12]. A similar trend was observed for tadalafil. In Saudi Arabia, tadalafil was detected in seven products at concentrations ranging from 0.00446 to 19.986 mg/g, which are considerably higher than the levels observed in Vietnam (5.8×10^{-4}–9.2×10^{-4} mg/g) and also exceed those reported in products from the Netherlands (0.2–2 mg/g) [11,12]. These comparisons indicate that the extent of PDE-5i tor adulteration in Vietnam remains significantly lower than in the other two countries. In contrast, products from Saudi Arabia contained very high levels of PDE-5i, suggesting insufficient regulatory oversight. Although the degree of adulteration in the Netherlands is less severe than in Saudi Arabia, some products still contained sildenafil and tadalafil at concentrations hundreds to thousands of times higher than those found in Vietnam, underscoring the need for stricter surveillance of health supplements at risk of illegal adulteration. When considering dosage forms, differences in PDE-5i adulteration were also observed. For tablet and hard capsule, active ingredient levels tend to be more consistent and easier to control, as solid manufacturing processes allow manufacturers (legal or illegal) to directly blend PDE-5i into powders or granules. Soft capsule and liquid samples are more limited in their ability to incorporate large amounts of PDE-5i due to the limited solubility of these compounds in lipid-rich matrices, which may contribute to the lower levels detected in some products of these formulations.

4. CONCLUSION

This is one of the first studies in Vietnam to simultaneously analyze 51 phosphodiesterase type 5 inhibitors (PDE-5i) in health supplementproducts collected from the Hanoi market. A simple and effective analytical procedure was developed, validated, and applied to 24 real samples. The results showed that only 3 of the 51 target compounds-sildenafil, tadalafil, and homosildenafil-were detected, with sildenafil and tadalafil also being the most commonly reported adulterants in international studies. The detected concentrations were relatively low compared with those reported in illegally adulterated products from the Netherlands and Saudi Arabia. Although the number of samples and analytes investigated remains limited, the present findings indicate that the extent of adulteration in Vietnam is still low. Nevertheless, further studies with a larger number of samples and the inclusion of newly emerging PDE-5i are necessary to provide a more comprehensive assessment of potential risks and to support more effective regulatory surveillance of health supplementproducts.

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REFERENCES

[1]. National Institute of Diabetes and Digestive and Kidney Diseases, "Erectile Dysfunction (ED)" [Online]: https://www.niddk.nih.gov/health-information/urologic-diseases/erectile-dysfunction. Accessed: 28/7/2025.

[2]. C.L. Kee, X. Ge, V. Gilard, M. and M. Y. Low, "A review of synthetic phosphodiesterase type 5 inhibitors (PDE-5i) found as adulterants in health supplements," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 147, pp. 250-277, 2018.

[3]. M. Jiru, M. Stranska-Zachariasova, Z. Dzuman, K. Hurkova, M. Tomaniova, R. Stepan, P. Cuhra, and J. Hajslova, "Analysis of phosphodiesterase type 5 inhibitors as possible adulterants of botanical-based

health supplements: extensive survey of preparations available at the Czech market," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 164, pp. 713-724, 2019.

[4]. J. L. Zurawin, C. A. Stewart, J. E. Anaissie, F. A. Yafi, and W. J. G. Hellstrom, "Avanafil for the treatment of erectile dysfunction," *Expert Review of Clinical Pharmacology*, vol. 9, no. 9, pp. 1163-1170, 2016.

[5]. S. Shi, Y. Wu, M. Zhou, and Q. Cheng, "Simultaneous analysis of 31 anti-impotence compounds potentially illegally added to herbal-based health supplements by ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry," *Journal of Chromatography B*, vol. 1144, p. 122077, 2020.

[6]. W. Lee, H. J. Kim, M. E. Lee, B. H. Kim, S. Park, J. H. Lee, Y. M. Lee, H. B. Oh, and J. Hong, "Reliable screening and classification of phosphodiesterase type 5 inhibitors in health supplements using gas chromatography / mass spectrometry combined with specific common ions," *Journal of Chromatography A*, vol. 1623, p. 461210, 2020.

[7]. A. Mostafa, "Analysis of phosphodiesterase type 5 inhibitors and analogues as adulterants in herbal dietary food supplements in Saudi market using green UPLC-MS/MS method: Assessment of the greenness and whiteness profiles," *Microchemical Journal*, vol. 203, p. 110867, 2024.

[8]. B. J. Venhuis and D. de Kaste, "Towards a decade of detecting new analogues of sildenafil, tadalafil and vardenafil in food supplements: A history, analytical aspects and health risks," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 69, pp. 196-208, 2012.

[9]. V. Gilard, S. Balayssac, A. Tinaugus, N. Martins, R. Martino, and M. Malet-Martino, "Detection, identification and quantification by 1H NMR of adulterants in 150 herbal health supplements marketed for improving sexual performance," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 102, pp. 476-493, 2015.

[10]. F. Akuamoa, T. F. H. Bovee, R. van Dam, L. Maro, S. Wesseling, J. Vervoort, I. M. C. M. Rietjens, and R. L. A. P. Hoogenboom, "Identification of phosphodiesterase type-5 (PDE-5) inhibitors in herbal supplements using a tiered approach and associated consumer risk," *Food Additives & Contaminants: Part A*, vol. 39, no. 6, pp. 1021-1032, 2022.

[11]. C. L. Chan, H. K. F. Wai, P. Wu, S. W. Lai, O. S. K. Chan, and H. M. Tun, "A Universal LC-MS/MS Method for Simultaneous Detection of Antibiotic Residues in Animal and Environmental Samples," *Antibiotics*, vol. 11, no. 7, 2022.

[12]. M. Dušek, M. Sanda, P. Cuhra, and S. Baršová, Simultaneous LC-MS/MS Determination of Sildenafil and Related Analogues Added Illegally to Herbal Products Intended for the Treatment of Erectile Dysfunction Methods and Materials XX cc Results and Discussion. 2007.

[13]. J. H. Lee, N. S. Kim, K. M. Han, S. H. Kim, S. Cho, and W. S. Kim, "Monitoring by LC-MS/MS of 48 compounds of sildenafil, tadalafil, vardenafil and their analogues in illicit health food products in the Korean market advertised as enhancing male sexual performance," *Food additives & contaminants. Part A, Chemistry, analysis, control, exposure & risk assessment*, vol. 30, no. 11, pp. 1849-1857, 2013.

[14]. T. O. Nguyen, S. C. Tran, T. T. H. Do, T. M. H. Nguyen, Q. D. Bui, C. T. Bui, H. N. Nguyen, T. H. Dang, V. C. Dinh, T. A. H. Nguyen, and T. H. H. Le, "Rapid Screening and Quantitative Determination of Illegal Phosphodiesterase Type 5 Inhibitors (PDE-5i) in Herbal Health supplements," *Journal of Analytical Methods in Chemistry* , vol. 2021, p. 5579500, 2021.

[15]. Commission European, "Commission Implementing Regulation (EU) 2021/808 on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results as well as on the methods to be used for sampling and repealing Decisions 2002/657/EC and 98/179/EC.," 2021.

[16]. AOAC, "Appendix F: Guidelines for standard method performance requirements," AOAC Official Methods of Analysis, vol. 9, 2012.