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SHORT COMMUNICATION

Analyzing food supplements to enhance erectile system function for the presence of undeclared sildenafil and tadalafil using HPLC-DAD

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Abstract: Phosphodiesterase-5 (PDE-5) inhibitors, such as sildenafil and tadalafil, are drugs commonly used to improve erectile function. However, some erectile function-enhancing supplements with claims of being entirely natural may be adulterated with these drugs, posing health risks. This study screened supplements for erectile dysfunction from the Republic of North Macedonia using a fast, specific and sensitive HPLC-DAD method (runtime: 15 min). No interference was observed at the retention times of sildenafil and tadalafil, allowing for their unambiguous identification and quantification. Tadalafil was absent, but sildenafil was found in two of eight samples (32.78 and 13.47 mg per dosage). The results demonstrated the applicability of the method to determine whether food supplements marked for the erectile function enhancement are free of sildenafil and tadalafil.

Keywords: adulteration; 5 phosphodiesterase inhibitors; sexual dysfunction; HPLC analysis.

INTRODUCTION

The therapeutic management of erectile dysfunction as a widespread condition affecting male sexual function, involves the use of several classes of drugs, with phosphodiesterase type-5 inhibitors (PDE-5 inhibitors) being the first-line treatment. Sildenafil, the first FDA-approved PDE-5 inhibitor for erectile dysfunction (1998), was later followed by vardenafil, tadalafil and avanafil.[1] How-

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ever, many men use alternative treatment approaches, including food supplements that contain natural aphrodisiacs extracted from *Panax ginseng*, *Lepidium meyenii* (maca), *Tribulus terrestris* (puncture vine), *Epimedium*, *Ginkgo biloba* and *Withania somnifera* (ashwagandha).[2] To achieve effects comparable to sildenafil in improving erectile function, some manufacturers illegally add pharmaceutical active substances and their analogs to these products.[3] This unethical practice, known as adulteration, allows manufacturers to profit from satisfied consumers, but poses health risks[4] due to weak supplement regulations.[5,6] FDA data shows many erectile dysfunction supplements contain undeclared sildenafil or its analogs,[7] which are dangerous for patients on nitrates, alpha blockers or CYP3A4 inhibitors.[8] Hence, the regulatory bodies have to increase regular inspections to ensure safety. To realize this, adequate spectroscopic and/or chromatographic methods[9–14] is required to enable simple and easy identification, detection and quantitative determination of adulterants in food supplements. Although there is growing interest in rapid and non-destructive spectroscopic methods,[14] the use of chromatography as confirmatory technique is unavoidable.[15,16] HPLC is widely recognized as a versatile and relevant technique for analyzing diverse samples, including multi-component dosage forms, due to its high sensitivity, speed and precision.[17] Thus, this study is aimed to propose a sensitive, specific and accurate HPLC-DAD approach that can be routinely applied for the simultaneous determination of sildenafil and tadalafil as adulterants in commercially available food supplements marketed for the improvement of erectile function.

EXPERIMENTAL

Materials and reagents

Tadalafil and sildenafil citrate reference standards (Ph. Eur) were obtained from Sigma–Aldrich. Eight erectile dysfunction supplements, marketed as natural products, were randomly selected from shops in North Macedonia. Besides herbal compounds, some supplements also included amino acids, vitamins and honey. Three supplements originated from Poland, two from Bulgaria, and one each from the UK, Hungary and India. The supplements were available in different forms: one as a tablet, one as a sachet and the rest as capsules. HPLC-grade acetonitrile (Thermo Fisher), sodium phosphate dibasic dehydrate (Sigma–Aldrich), and 85 % orthophosphoric acid (Park, UK) was used for the phosphate buffer. Ultrapure water from the Heal Force NW system was used throughout.

Instrumental and analytical conditions

A Waters Alliance e-2695 HPLC with a 2998 PDA detector was used. Separation was performed on a Symmetry C8 column (5 μm, 4.6 mm×250 mm) with a C18 guard column. The isocratic mobile phase consisted of acetonitrile and 50 mM phosphate buffer with pH 6.6 (45:55 volume ratio) flowed at 1 mL/min. A 20 μL injection was analyzed at 225 nm using Empower software, version 3.

Preparation of standard stock solutions and samples

Tadalafil and sildenafil citrate stock solutions (20 mg each) were prepared in 50 mL volumetric flasks using the mobile phase and sonication. Working solutions (0.2 mg/mL) and a combined standard solution (0.2 mg/mL each) were also prepared. A quantity of solid samples, corresponding to the homogenized average weight of a tablet or capsule (*Ph. Eur.* **10**, 2021), and the entire content of a sachet sample (9 g) were dissolved in 50 mL of the mobile phase, vortexed and sonicated for 15 min. Each sample was prepared in triplicate, filtered (0.2 μm nylon), and transferred to vials for analysis.

Method validation

The method was validated according to ICH guidelines, encompassing the validation parameters such as specificity, linearity, accuracy, precision, sensitivity and robustness.[21] Method specificity was assessed by analyzing the mobile phase (diluent), blank, tadalafil, and sildenafil citrate solutions at a concentration of 0.2 mg/mL, as well as mixed standard solution at the same concentration (0.2 mg/mL). The linearity was tested using mixed standard solutions at seven concentrations (0.0002–0.3 mg/mL corresponding to 0.1 to 150 % of the target concentration), with three injections per level. *LOD* and *LOQ* for tadalafil and sildenafil citrate were determined using the standard deviation (σ) and slope (S), calculated as $LOD = 3.3\sigma/S$ and $LOQ = 10\sigma/S$. The accuracy was evaluated at 50, 100 and 150 % of the target concentration (0.1, 0.2 and 0.3 mg/mL) with triplicate samples prepared by mixing of sildenafil citrate and tadalafil with a blank matrix and diluting in the mobile phase. The recovery of the added tadalafil and sildenafil citrate, as well as the relative standard deviation (*RSD*), was calculated for each set of replicates. Precision was confirmed through six same-day injections of a mixed standard solution at 100 % concentration. Robustness was assessed by varying flow rate, column temperature and concentration and pH of the phosphate buffer. Analytical solution stability was tested by analysing the standard solutions at 0, 2, 4, 6, 12 and 24 h in a single run and after 24, 36, 48 and 72 h under refrigeration. Six injections per solution were analysed, calculating the average recovery and *RSD*.

RESULTS AND DISCUSSION

HPLC-DAD method

The HPLC method for the determination of undeclared sildenafil and tadalafil in food supplements for erectile dysfunction was based on the method reported by Fidan and Bakirdere (2016)[19] with certain modification according to green chemistry principles.[21,22] To reduce acetonitrile content from 65 vol. % in the original method,[19] variations from 60 to 40 % were tested, with 45 vol. % providing optimal separation. Lowering the use of acetonitrile is crucial, as it was recognized as an environmental hazard,[24] aligning with modern efforts to minimize chemical pollution of the environment.[20,21]

The specificity of the method was confirmed by chromatograms showing no co-elution of tadalafil (7.79 min) and sildenafil citrate (9.08 min), Fig. 1. A strong linear correlation was observed ($R^2 = 0.9986$ for tadalafil, $R^2 = 1.0000$ for sildenafil citrate) across the concentration range of 0.0002 to 0.3 mg/mL. Sensitivity was high, with *LOD*s of 0.0022 mg/mL for tadalafil and 0.0005 mg/mL for sildenafil citrate and *LOQ*s of 0.0067 and 0.0014 mg/mL, respectively. The

accuracy ranged from 99.45–101.36 % for tadalafil and 99.06–100.34 % for sildenafil citrate, with RSDs below 2.0 % (Table I). The method proved robust under minor HPLC condition variations, including flow rate (0.8, 1 and 1.2 mL/min), column temperature (20, 25 and 30 °C), phosphate buffer concentration (40 50 and 60 mM), and pH of the phosphate buffer (6.3, 6.6 and 6.9). and stable over 72 h (*RSD*s of 0.16 for tadalafil and 0.10 for sildenafil citrate).

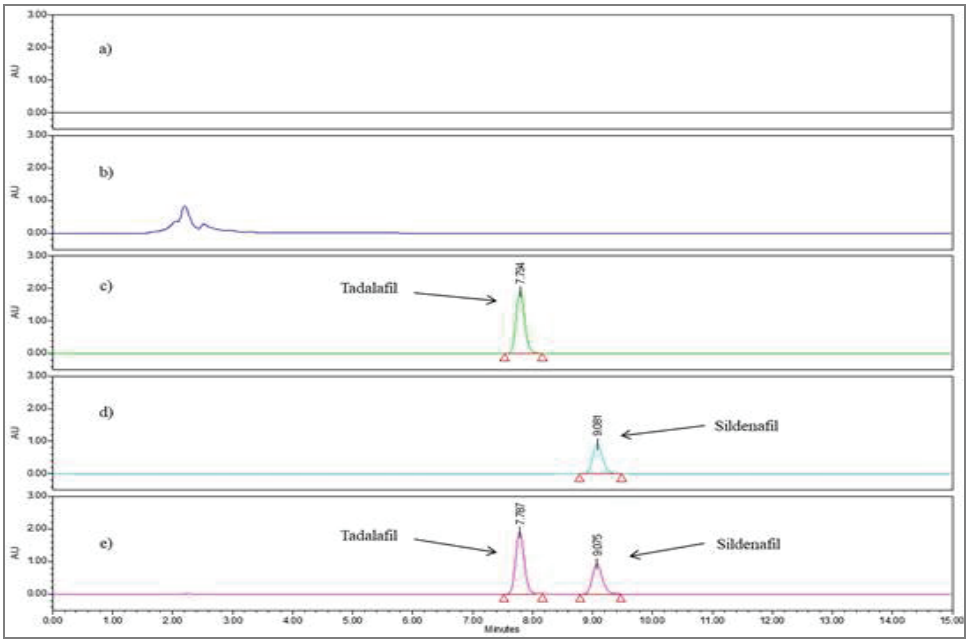

Fig.1. HPLC chromatograms: a) mobile phase (diluent); b) blank; c) standard solution of tadalafil (0.2 mg/mL); d) standard solution of sildenafil citrate (0.2 mg/mL); e) standard solution of both tadalafil and sildenafil citrate (0.2 mg/mL).

TABLE I. Accuracy and precision of HPLC-DAD method for determination of sildenafil citrate and tadalafil as adulterants in food supplements for improving erectile function

Parameter	Accuracy, recovery + *RSD*, %			Precision *RSD* / %
Concentration unit (concentration level)	mg/mL (50 %)	mg/mL (100 %)	mg/mL (150 %)	
Tadalafil	100.27 + 0.89	101.02 + 0.43	100.22 + 0.13	0.13
Sildenafil citrate	99.65 + 0.27	99.45 + 0.37	99.80 + 0.37	0.16

Determination of sildenafil and tadalafil in real samples

Two of the eight analysed food supplements for erectile dysfunction contained undeclared sildenafil, representing 25 % of samples. The sachet sample, manufactured in Bulgaria, and the capsule sample, manufactured in India, matched the UV spectra and retention time of the standard (Fig. 2). The sachet

contained 18.92 mg of sildenafil citrate (equivalent to 13.47 mg of sildenafil), while the capsule contained 46.02 mg of sildenafil citrate (equivalent to 32.78 mg of sildenafil). Both samples did not comply with the claim of being natural. These products were found to be adulterated with a medium-low and moderate doses of sildenafil, considering that prescription drugs contain sildenafil as the active component at a dose of 5, 20, 25, 50 and 100 mg. The undisclosed sildenafil adulteration poses health risks, especially for consumers avoiding PDE-5 inhibitors or taking other medications increasing the potential for severe complications.

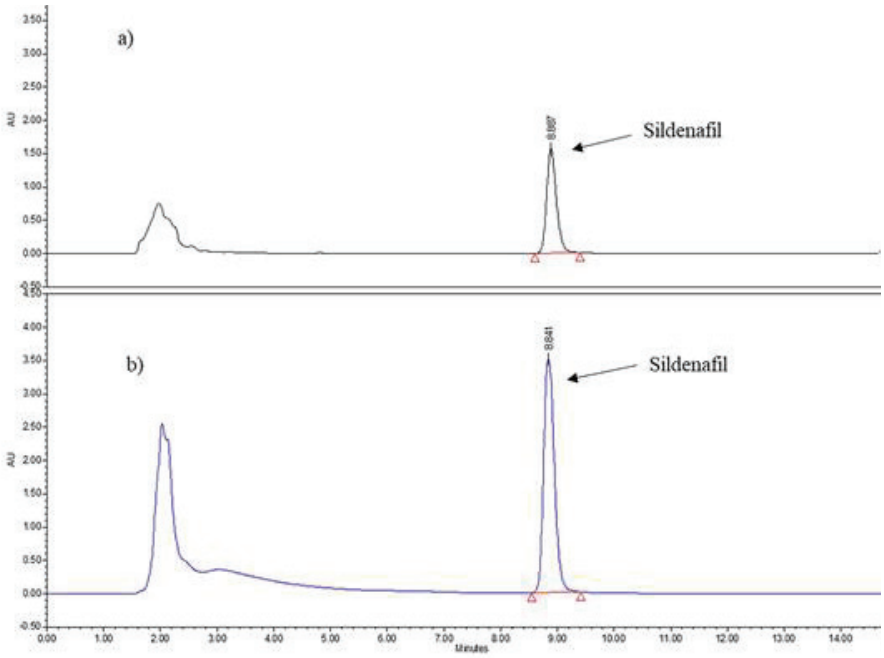

Fig. 2. HPLC chromatograms: a) supplement containing sildenafil 13.47 mg/sachet; b) supplement containing sildenafil 32.78 mg/capsule.

Certain studies confirm the global issue of PDE-5 inhibitor adulteration in "natural" supplements for erectile dysfunction,[22–24] aligning with our findings. In Nairobi, 32.5 % of 80 tested herbal products contained undeclared sildenafil.[22] Similar adulteration rates were found in Malaysia, Romania and Bangladesh, with supplements originating from China, the USA, Europe, India and other regions.[10,12,22–24] These studies highlight the global trend of using sildenafil to adulterate supplements marketed as natural products for improving erectile function. Efficient screening methods are crucial for regulatory enforcement. Our proposed HPLC method offers a simple sample preparation, a 15-min runtime, and moderate use of organic solvents, making it efficient for the routine sildenafil and tadalafil detection.

CONCLUSION

This study investigated eight erectile dysfunction supplements in the Republic of North Macedonia for undeclared sildenafil and tadalafil using HPLC--DAD. The method ensured good separation within 15 min with minimal sample preparation. Two supplements from Bulgaria and India contained sildenafil at medium-low and moderate doses. The authorities must address adulteration and warn the public that "natural" supplements are not always safe. Given the potential health risks, thorough screening is essential, and our cost-effective, sensitive HPLC method offers a practical solution.

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И З В О Д

АНАЛИЗА СУПЛЕМЕНАТА ЗА ПОБОЉШАЊЕ ФУНКЦИЈЕ ЕРЕКТИЛНОГ СИСТЕМА НА ПРИСУСТВО НЕПРИЈАВЉЕНОГ СИЛДЕНАФИЛА И ТАДАЛАФИЛА КОРИШЋЕЊЕМ HPLC-DAD

ZORICA BOZHINOSKA[1], ZORAN ZHIVIKJ[2], STEFANIJA UBAVKOVA[1], MARIJA BUJAROSKA PERKOVIKJ[1], VERICA POPOSKA[1], ALEKSANDAR STANKOV[1] и TANJA PETRESKA IVANOVSKA[2]

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Инхибитори фосфодиестеразе-5 (PDE-5), као што су силденафил и тадалафил, су често коришћени лекови за побољшање еректилне функције. Поједини суплементи за побољшање еректилне функције, који су декларисани као потпуно природни могу садржавати ове лекове, што представља ризик по здравље. У овом раду су испитани суплементи за еректилну дисфункцију из Републике Северне Македоније применом брзе, специфичне и осетљиве HPLC-DAD методе (трајање анализе је 15 min). Утврђено је да нема интерференција на ретенционим временима сиденафила и тадалафила што омогућава њихову идентификацију и квантификацију. У испитиваним узорцима није утврђено присуство тадалафила, док је силденафил идентификован и квантификован у два од осам узорака (32,78 и 13,47 mg по дози). Добијени резултати указују на применљивост ове методе за утврђивање да ли су природни суплементи за побољшање еректилне функције, доступни на тржишту, без додатака сиденафила и тадалафила.

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