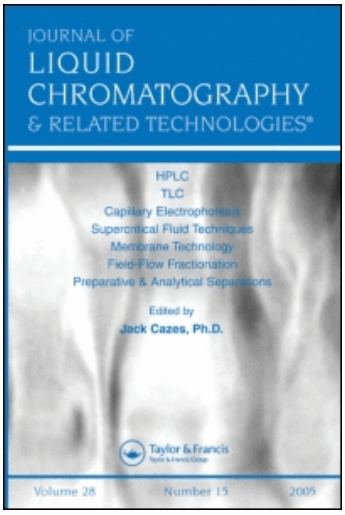

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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Online publication date: 20 August 2010

To cite this Article Cai, Yu , Cai, Tian-Ge , Shi, Yan , Cheng, Xian-Long , Ma, Ling-Yun , Ma, Shuang-Cheng , Lin, Rui-Chao and Feng, Wei(2010) 'SIMULTANEOUS DETERMINATION OF EIGHT PDE5-IS POTENTIALLY ADULTERATED IN HERBAL DIETARY SUPPLEMENTS WITH TLC AND HPLC-PDA-MS METHODS', Journal of Liquid Chromatography & Related Technologies, 33: 13, 1287 — 1306
To link to this Article: DOI: 10.1080/10826076.2010.488979
URL: http://dx.doi.org/10.1080/10826076.2010.488979

Journal of Liquid Chromatography & Related Technologies, 33:1287–1306, 2010

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826076.2010.488979

SIMULTANEOUS DETERMINATION OF EIGHT PDE5-IS POTENTIALLY ADULTERATED IN HERBAL DIETARY SUPPLEMENTS WITH TLC AND HPLC-PDA-MS METHODS

Yu Cai,[1] Tian-Ge Cai,[2] Yan Shi,[3] Xian-Long Cheng,[3] Ling-Yun Ma,[3] Shuang-Cheng Ma,[3] Rui-Chao Lin,[3] and Wei Feng[3]

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□ *A set of simple TLC and HPLC-PDA-MS methods were developed for detection of counterfeit herbal dietary supplements by the qualitative and quantitative analysis of eight phosphodiesterase type 5 inhibitors (PDE5-Is), sildenafil, hongdenafil, homosildenafil, hydroxyhomosildenafil, vardenafil, pseudovardenafil, tadalafil, and aminotadalafil. The analytical approach is simple for rapid screening with TLC and reliable with HPLC-PDA-MS. Good linearity* ($r^2 > 0.999$ *over the concentration range of 8.1 to 211.2 μg/ml), sensitivity (limits of quantification 0.04–0.09 μg/ml), accuracy (97.03–101.25%), and precision (RSD (%) < 1.32%) were achieved for all analytes. The methods were applied for detection of 36 commercial herbal dietary supplements suspected containing PDE5-Is. In addition, the characteristic MS fragmentations of three main typical structures, sildenafil, vardenafil, and tadalafil, were discussed and summarized, which are very helpful in rapid screening identification and determination of suspected chemicals not claimed in herbal products. Results have demonstrated that established TLC and HPLC-PDA-MS methods are good confirmation and quantification test schemes for the eight target compounds.*

Keywords DE5-Is, determination, herbal dietary supplements, HPLC-PDA-MS, PDE5-Is, TLC

INTRODUCTION

Herbal remedies and dietary supplements are increasing in popularity all over the world due to the widespread belief that natural products are safer and healthier than synthetic ingredients. Recently, along with the rise in health consciousness, the consumption of herbal dietary supplements

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has increased year by year. However, one of the major concerns related to the safety of these products is their adulteration with undeclared synthetic chemicals in order to enhance the claims stated on the label. In China, some of herbal dietary supplements are illegally advertised as effective for sexual enhancement. Consumers take these products without knowing that some of these "natural" products contain concealed substances, which are structurally modified analogues, such as phosphodiesterase type 5 inhibitors (PDE5-Is) and their analogues. Synthetic PDE5-Is are widely used for the treatment of erectile dysfunction (ED). Today, there are three such drugs approved by the U.S. Food and Drug Administration: sildenafil citrate (Viagra; Pfizer, NY, USA), vardenafil (Levitra; Bayer Pharmaceuticals Co., Wuppertal, Germany), and tadalafil (Cialis; Elli Lilly, IN, USA).[1,2] These drugs have documented numerous side effects such as headache, facial flushing, dyspepsia, visual disturbances, and muscle aches,[3,4] and they must be used under medical supervision.[5] However, unlike the parent pharmaceuticals, no formal studies have been performed to assure the safety and efficacy of their structurally modified analogues.

In the last few years, these ED drugs and structurally modified synthetic analogues have been found in "natural" herbal health supplements marketed as aphrodisiacs.[6–25] Herbal products have been adulterated not only with sildenafil, vardenafil, and tadalafil,[7–14] but also with analogues of these compounds. Analogues of sildenafil, including homosildenafil,[9–13] hydroxyhomosildenafil,[16–18] acetildenafil,[16–20] hydroxyacetildenafil,[21,22] and piperidino acetildenafil,[22] an analogue of tadalafil named aminotadalafil,[26] and an analogue of vardenafil, namely piperidenafil, were detected in herbal supplements.[22–25] Some new analogues like thiohomosildenafil,[27] thiosildenafil,[27,28] thiomethisosildenafil,[28] Cyclopentynafil and N-Octylnortadalafil[29] were also found recently.

Concerning the analysis of these compounds, PDE5-Is have been measured in herbal medicines or dietary supplements by several kinds of analytical methods, such as high performance liquid chromatography (HPLC), high performance liquid chromatography–mass spectrometry (HPLC–MS), and capillary electrophoresis (CE). There are a few studies in the literatures that introduced the strategy for the determination of sildenafil by the widely used HPLC technology.[30–36] HPLC–MS is the most common method currently utilized for the screening of adulterants in herbal dietary supplements. Various research has reported on the determination of PDE5-Is in herbal supplements[10,11,14–18,25–28,37–45] or in serum samples[46–50] by HPLC–MS methods. Li et al. reported a method for determining sildenafil with capillary electrophoresis.[51] J. J. Berzas reported a method for determination of sildenafil citrate and its main metabolite by sample stacking with polarity switching using micellar electrokinetic chromatography.[52] C. M. Gryniewicz et al. introduced an ion

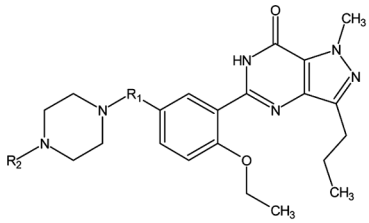
mobility spectrometry method for detection of undeclared erectile dysfunction drugs and analogues in dietary supplements.[53] Dong et al. reported a LC-MS-NMR method for determination of analogues of sildenafil and vardenafil in Foods.[54]

To the best of our knowledge, no methods have been reported for determination of PDE5-Is in herbal samples with TLC, and most of HPLC or HPLC-MS methods were focused on determination of 1–3 PDE5-Is,[8,10,34,45,52,53] and most of these studies were concerned with identification and structure elucidation of these compounds and their analogues adulterated in the herbals. There is no a rapid screening method with TLC and an HPLC-PAD-MS method with complete validation for the simultaneous determination of eight PDE5-Is, sildenafil, hongdenafil, homosildenafil, hydroxyhomosildenafil, vardenafil, pseudovardenafil, tadalafil, and aminotadalafil in herbal dietary supplements. In this work, a set of TLC and HPLC-PDA-MS methods were developed and validated for simultaneous identification and quantitative determination of eight PDE5-Is in herbal dietary supplements, which have been successfully applied to rapid screening and determination of 36 commercial products collected from Chinese markets suspected abusing of PDE5-Is. Furthermore, the HPLC-PDA-MS method established in this study achieved good sensitivity, precision, and accuracy, and the TLC method can significantly reduce analytical time and cost by simultaneously determination of eight compounds in one run.

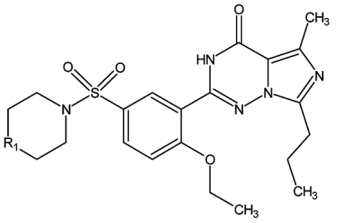
EXPERIMENTAL

Materials and Reagents

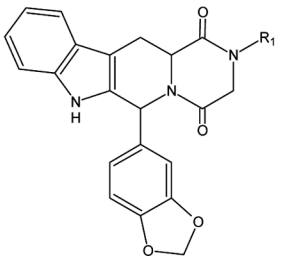
Standard references of sildenafil, tadalafil, and vardenafil were obtained from the National Institute for Control of Pharmaceutical and Biological Products (NICPBP), Beijing, China, hongdenafil, aminotadalafil, hydroxyhomosildenafil, homosildenafil, and pseudovardenafil were purchased from the Herbwide Sci. Tech. Co. Beijing, China. The structures of these compounds, **1–8**, are shown in Figure 1. All the reference standards were over 99% in purity. Solvents (methanol, acetonitrile) were HPLC grade and were purchased from Dima Technology Inc. (Richmond, Canada). Other reagents were of analytical grade, including formic acid. Ultra-pure water was prepared using a Millipore Milli-Q purification system (Millipore, Bedford, MA, USA). Mobiles used for HPLC were filtered (0.45 μm) and ultrasonically degassed before use. The 36 commercial products tested were purchased from markets in Beijing, China. All of these products examined are herbal medicines or natural dietary supplements for male sexual health, not for therapy of ED.



No.	Compound	R_1	R_2
1	sildenafil	$-SO_2-$	$-CH_3$
2	hongdenafil	$-CH_2CO-$	$-C_2H_5$
3	homosildenafil	$-SO_2-$	$-C_2H_5$
4	hydroxyhomosildenafil	$-SO_2-$	$-C_2H_5OH$



No.	Compound	R_1
5	vardenafil	$-NC_2H_5-$
6	pseudovardenafil	$-CH_2-$



No.	Compound	R_1
7	tadalafil	$-CH_3$
8	aminotadalafil	$-NH_2$

FIGURE 1 Chemical structures of eight standard references **1–8**.

Preparation of Solutions

Standard Solutions

A standard stock solution was prepared by dissolving all eight standard references in 50 ml methanol (10 mg of **1, 3, 4, 5, 7**; 8 mg of **2, 6, 8**). The stock solution was suitably diluted with the mobile phase solution to produce calibration solutions described in Section 2.4.

Sample Solutions

For the products in solid form (tablets, capsules, and pills), 5 g samples were taken, ground in a mortar and pestle, and the resulting fine powders of different commercial products were weighed accurately (0.100 g), and extracted three times with 10 ml methanol by sonication (10 min each, at ambient temperature) with an ultrasonic cleaner (TCQ-250, Beijing Medical Treatment Instrument Factory, China). After centrifugation at 3000 rpm for 5 min the extracts were combined in one 50 ml volumetric flask, which was then filled up to the final volume with extraction solvent.

For the products of soft gel capsules, 2 g of the contents were taken, homogenized and thoroughly mixed, weighed accurately (0.200 g) of the contents and transferred into a 50 ml volumetric flask and diluted to volume with methanol. For the products in liquid form, a volume of 2.00 ml was transferred into a 25 ml volumetric flask and diluted to volume with methanol. Prior to injection all solutions were filtered through a 0.45 μm nylon membrane filter (Phenex, Phenomenex, Torrance, CA, USA). Each sample solution was assayed in triplicate.

Chromatographic Conditions

TLC Conditions for Identification

The standard and sample solutions were applied in bands by means of an ATS4 auto-sampler (CAMAG, Muttenz, Switzerland) on a commercial 20 cm × 10 cm pre-coated HPTLC Silica gel 60F254-plate (Merck). The application conditions were: carrier gas, nitrogen; syringe delivery speed, 10 s/μl; application volume, 3 μl; bandwidth, 5 mm; space between two bands, 6 mm; distance from bottom, 1 cm. The sample-loaded plate was placed into a desiccator with phosphorus pentoxide and dried under vacuum for 2 hr before development. 15 ml of mobile phase consisting of chloroform–ethyl acetate–methanol–water (40:40:15:11, v/v/v/v) was added into a twin-trough chamber, to saturate it for 15 min. The plate in the chamber was developed upward over a path of 8 cm, and after being dried at room temperature, the fluorescent image of the plate was examined under UV 254 nm by using a UV viewer cabinet (CAMAG).

HPLC-PDA-MS Conditions for Identification

Analysis was performed with a Waters Alliance 2695 HPLC instrument (Milford, MA, USA) equipped with a Waters 996 photodiode array (PDA) detector and a Micromass ZQ2000 detector (Manchester, UK). MS detection was performed in a positive electrospray ion mode [(+)-ESI] on the instrument. The system was optimized for transmission of $[M+H]^+$ ions. Acquisitions were made in full scan (100–800 amu. one spectrum per second), and selected ion monitoring (SIM) mode was used for identification at m/z 475, m/z 467, m/z 489, m/z 505, m/z 460, m/z 390, and m/z 391 with a dwell time of 0.3 seconds and a span of 0.2 amu. Capillary voltage was set at 3.0 Kv and cone voltage was set at 50 V. The temperature of ESI probe was at 105°C, and desolvation temperature at 150°C. Nitrogen was used as the nebulizer gas as well as the dry gas. The desolvation gas flow was set at 300 L/hr. and cone gas flow at 80 L/hr. Masslynx V4.0 software was used for data acquisition and processing. An Agilent Eclipse Plus C18 column (4.6 mm × 250 mm, i.d., 5 μm, made in USA) was used for separation

and operated at room temperature. The column was equipped with a guard column (Supelco, Bellefone, PA). The mobile phase consisted of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B) at a flow rate of 1.0 ml/min for column and a flow rate of 0.2 mL/min for MS detector by a split ratio of 1:4. The gradient mobile phase elution procedure was set as follows: 0 min, 80% A/20% B, to 64% A/36% B in 25 min, to 58% A/42% B in 10 min, and held for 5 min, then to 50% A/50% B in 5 min. Each run was followed by a 5 min wash with 100% acetonitrile and equilibration period of 10 min with 80% A/20% B. The total run time for analysis was 45 min.

HPL-UV Conditions for Quantification

The HPLC-UV system consisted of a Waters Alliance 2695 HPLC instrument equipped with a Waters Model 2998 photodiode array detector, and a computerized data station equipped with Waters millennium software. The column and operation temperature, mobile phase, and gradient elution procedure were the same as the HPLC-PDA-MS condition mentioned in the TLC Conditions for Identification section. The wavelengths used for quantification with the photodiode array detector of **1, 3, 4, 6, 7**, and **8** were set at 226 nm, **4** at 234 nm, and **5** at 215 nm, respectively.

Calibration

Six additional calibration levels were prepared by diluting the standard stock solution (described in the Standard Solutions section) in 1:2 with the mobile phase solution. The calibration graph is described by the calibration equation $y = a + bx$, where "y" is the peak area, "x" is the concentration in μg/mL, "a" is the intercept and "b" is the slope. See Table 1 for calibration data. The limits of detection (LOD) and quantification

TABLE 1 Calibration Data of Standard References **1–8**, Including Regression Equation, Correlation Coefficient (R^2), Linear Range (in μg/mL), Determined Wavelength (nm), Limit of Detection (LOD; in μg/mL), and Limit of Quantification (LOQ; in μg/mL) for HPLC-UV and Limit of Quantification (LOQ^{MS}; in μg/mL) for HPLC-MS

	Regression Equation	R^2	Wavelength	Linear Range	LOD	LOQ	LOQ^{MS}
1	$Y = 73 + 1085x$	0.9991	226	7.2–230.4	0.01	0.05	0.004
2	$Y = 113 + 1097x$	0.9990	234	6.8–217.6	0.02	0.08	0.006
3	$Y = 87 + 1076x$	0.9992	226	7.4–236.8	0.01	0.04	0.005
4	$Y = 62 + 1083x$	0.9991	226	7.3–233.6	0.02	0.07	0.006
5	$Y = 115 + 1066x$	0.9993	215	8.1–259.2	0.01	0.04	0.003
6	$Y = 102 + 1079x$	0.9993	230	6.6–211.2	0.02	0.06	0.005
7	$Y = 95 + 1088x$	0.9991	226	7.7–246.4	0.02	0.09	0.007
8	$Y = 127 + 1075x$	0.9994	226	6.9–220.8	0.01	0.06	0.005

TABLE 2 Accuracy of Developed HPLC-assay, Based on Recovery Experiments (Low, Medium and High Spike); Quantitative Values in μg/mL

		Low Spike		Medium Spike		High Spike	
	Amount in Sample (mg)	Added (mg)	Recovery (%)	Added (mg)	Recovery (%)	Added (mg)	Recovery (%)
1	1.66	0.53	101.25	2.12	99.16	4.24	100.86
2	2.12	0.49	97.85	1.96	98.44	3.92	97.31
3	4.06	0.43	98.92	1.72	100.26	3.44	98.11
4	1.82	0.56	97.21	2.24	97.35	4.48	97.45
5	1.56	0.47	99.02	1.88	99.86	3.76	99.82
6	1.62	0.58	97.35	2.32	97.76	4.64	97.03
7	1.54	0.51	97.89	2.04	96.94	4.08	97.19
8	2.08	0.44	98.27	1.76	98.33	3.52	98.35

(LOQ) for each analyte were determined as S/N of three and ten, RSD (%) $\leq$ 3%, respectively. The standard solutions were stable for at least 1 month if stored at 4°C (conformed by reassaying). The limit of detection (LOD) of MS in SIR mode was evaluated as the mass giving a signal equal to three times of noise (S/N = 3).

Validation

Accuracy was determined by spiking samples with three concentrations of standard references (low, medium, high spick). For this purpose, known amounts of standard references, **1–8**, were added to the solid or liquid commercial products material samples, which were then extracted and assayed as described previously. The actually found amounts in relation to the theoretically present ones were expressed as percentage of recovery (Table 2).

Precision of the method was deduced from repeatability of multiple injections as well as intra- and inter-day variances of the results. All the samples were extracted and assayed under optimized conditions on day 1; the same procedure was repeated on two more days. By comparing variations within same days, intra-day precision was deduced; by observing differences within the 3 days, inter-day precision was deduced (Table 3).

RESULTS AND DISCUSSION

TLC Method Development

Development of a TLC procedure on silica gel plates GF 254 with a particle size of 5–20 lm and with narrow particle size distribution has long been used in almost all researches for the identification of herbal raw materials.

TABLE 3 Intra- and Inter-day Precision of the Developed HPLC-assay Using Samples Adulterated PDE5-Is; Results are Based on Content in Percent, RSD (%) in Parenthesis

Sample No.	Compound Determined	Intra-day (n = 5)			Inter-day (n = 3)
		Day 1	Day 2	Day 3	
S1	**1**	13.55 (0.93)	13.53 (0.81)	13.50 (0.88)	13.53 (0.19)
S2	**2**	4.01 (1.12)	4.00 (1.25)	4.09 (1.13)	4.03 (1.22)
S3	**3**	9.11 (0.99)	9.14 (0.12)	9.19 (0.81)	9.15 (0.44)
S4	**5**	3.01 (1.55)	3.02 (1.57)	3.06 (1.14)	3.03 (0.87)
S5	**6**	3.10 (1.40)	3.16 (1.13)	3.10 (1.24)	3.12 (1.11)
S6	**4**	3.02 (0.95)	3.07 (0.84)	3.10 (0.99)	3.06 (1.32)
S12	**7**	8.10 (1.03)	8.13 (1.02)	8.17 (1.04)	8.13 (0.43)
S19	**8**	4.01 (1.25)	3.95 (1.14)	3.91 (1.12)	3.96 (1.27)

In the preliminary study, mixtures of ethyl acetate/methanol/water in different ratio (12:4:1 or 8:3:1, v/v/v), toluene/ethyl acetate/methanol/water (15:10:3:1, v/v/v/v), and dichloromethane/ethyl acetate/acetic acid/water (18:12:1:1, v/v/v/v/v) as mobile phase were investigated. There was no fine separation of all compounds at the mentioned conditions. Use of chloroform/ethyl acetate/methanol/water (40:40:15:11, v/v/v/v) solvent mixture as a mobile phase with migration for 8 cm allowed good separation of the eight compounds in the mixed standard references and sample extract (Figure 2). The comparison of Rf values of reference standard with those of sample extract components was the first step for identification of each of PDE5-Is. The identity of the bands of each of the PDE5-Is in the sample extracts was confirmed by (i) overlaying their UV absorption spectra

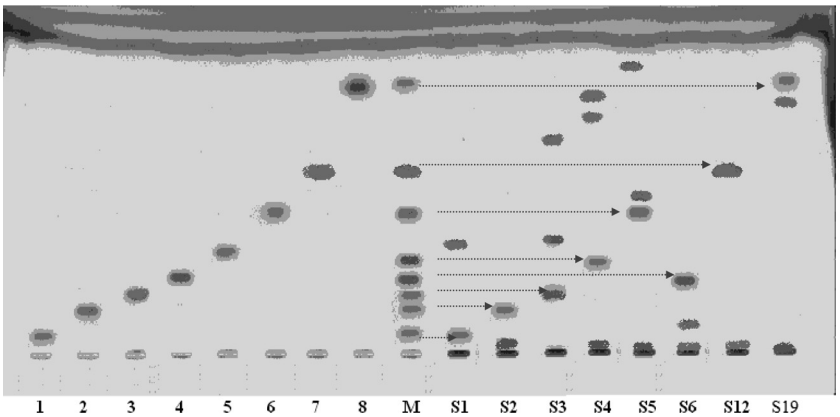


FIGURE 2 TLC profiles of the standard references (**1–8)** and eight commercial products (S1–S6, S12, and S18) showing positive results, which indicate the PDE5-Is under UV 254 nm with the conditions described in the TLC Conditions for Identification section. M is the mixture of eight standard references.

with those of the reference substance and (ii) coincidence of Rf values using a TLC Scanner 3. The peak-purity index is a numerical measure of the quality of coincidence between two datasets under consideration.

Thin layer chromatography is a globally accepted rational and practical solution to characterize the crude plant drug and pharmacologically active constituent enriched standardized extracts and their formulations; it also can be used for identification of herbal products counterfeited with chemical drugs. The established TLC procedure may be effectively used for the rapid screening analysis of herbal products that suspected added PDE5-Is.

HPLC-DAD-MS Method Development

The main disadvantage of all analytical methods reported on PDE5-Is, so far, is their limitation to single or 2–6 references. Most of these methods did not fully cover all three structural types of PDE5-Is, so that these methods cannot be effectively applied for rapid screening and determination of potential commercial products suspected adulterated chemical drugs. As the focus is on the simultaneous analysis of eight compounds **1–8**, and these compounds have very good solubility in methanol, methanol was chosen for the sample extraction in this work. Methanol is helpful in fully extracting all the analytes to ensure the accuracy of the quantification. Recoveries of all analytes were good and were consistent, precise, and reproducible.

The chromatographic conditions, especially the composition of mobile phase, were optimized through several attempts to achieve good resolution and symmetric peak shapes for eight analytes, as well as a relative short run time. It was found that an acetonitrile-water system was better than a methanol-water system for a good separation of eight peaks. This was consistent with published conclusions.[10] The pH of the mobile phase influences both the chromatographic elution of the compounds and the formation of the $[M+H]^+$ molecular ions and is strongly related to their degree of ionization. The use of slightly acidic solutions favors ionization of the analytes by protonization of their basic sites. Therefore, it was found that positive ionization of the compounds in the electrospray ion source increases in acidic mobile phases. Hence, the mobile phase was adjusted with formic acid (0.1% in content) to obtain high sensitivity and good peak shapes. The detector wavelengths for **1–8** in quantitative analysis were selected near the maximum wavelength of each compound, and no interference was observed in the chromatograms. Figure 3 shows the HPLC-UV and HPLC-MS chromatograms. It is possible to simultaneously analyze **1–8** within 45 min.

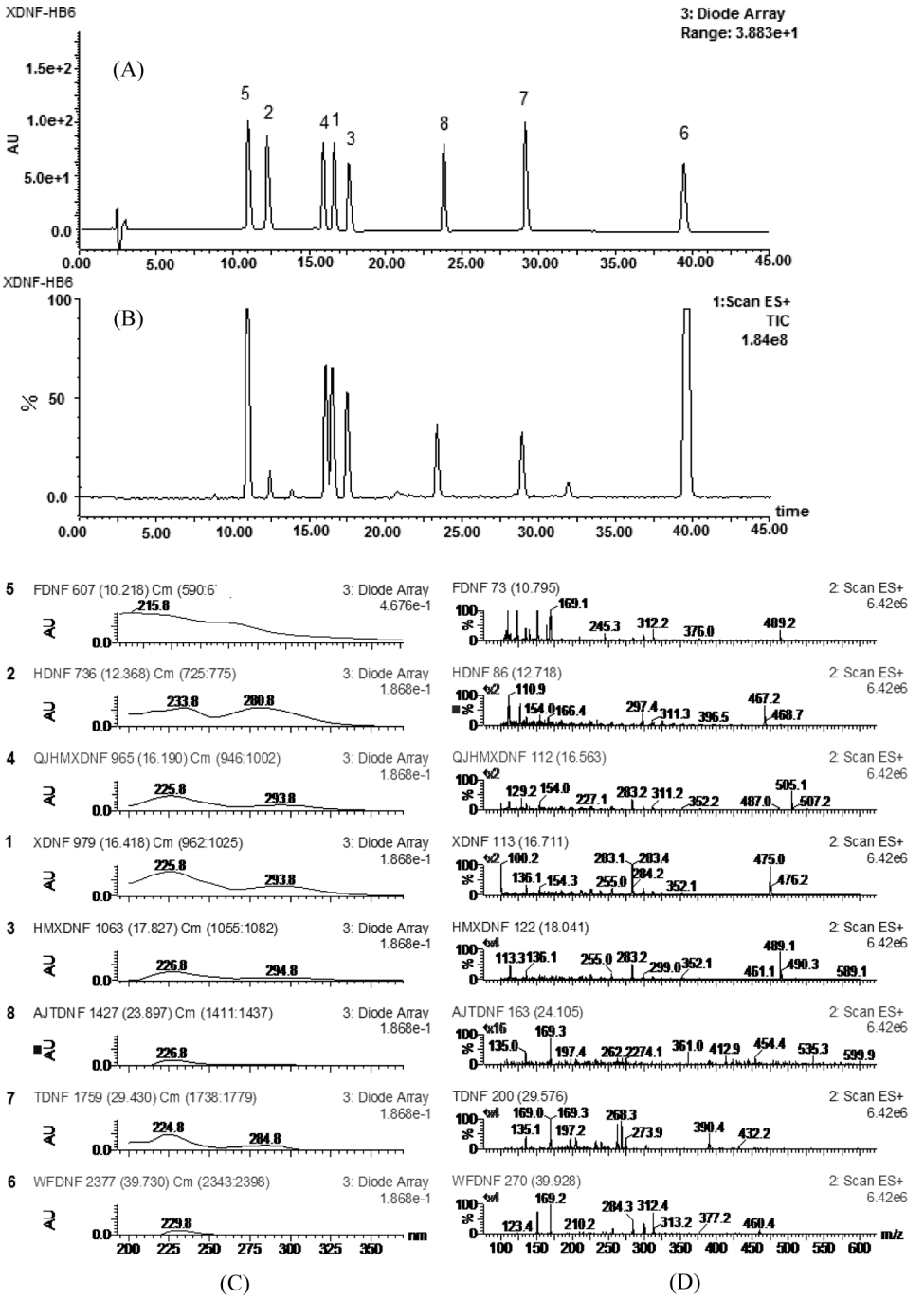


FIGURE 3 Representative HPLC-UV (A) chromatogram, HPLC-ESI-MS total ion chromatogram (B), UV spectra (C), and Mass spectra (D) of eight standard references **1–8** using the conditions described in the Chromatographic conditions section with a gradient mobile phase.

Identification of 1–8 and Three Structural Types of PDE5-Is with HPLC-PDA-MS

HPLC-PDA-MS is a powerful qualitative technique for quick and accurate determination of the molecular mass of an analyte. The HPLC-PDA system is capable of providing a more definite identification than the retention index. Compounds **1–8** adulterated in herbal products were identified by comparing the retention times, UV spectra, and MS spectra of authentic standards with those obtained from the samples. Figure 3 shows HPLC chromatogram and total ion current (TIC) trace of the separation of eight reference standards. Combined with UV spectrum and MS spectrum, the eight compounds can be easily specified, and the selectivity and accuracy are highly improved. For example, homosildenafil (**3**) and vardenafil (**5**) present the same $[M+H]^+$ at 489 in their MS spectrum, but their retention times in HPLC are very different, and compared with their UV spectrum, the two compounds show big differences (see Table 4), so that the two compounds can been easily identified from multi-herbal products. In order to increase the selectivity and sensitivity of the developed method, qualitative determination of eight compounds were performed by selected ion monitoring (SIM) of $[M+H]^+$. As a result, peaks in the samples were easily identified.

Furthermore, from the MS spectrum of three typical standards, sildenafil, vardenafil, and tadalafil, each of them exhibited characteristic ion peaks that are very useful for structural type conformation. For example, the MS spectrum of sildenafil and its analogues usually exhibited major characteristic ion peaks at m/z 475, 311, 283, and 255, respectively, in the positive scan mode, whereas, vardenafil and its analogues exhibited peaks at m/z 489, 312 or 311,[25,43] 283, and 186, and tadalafil and its analogues exhibited peaks at m/z 390, 268, 197, and 169, respectively. These data strongly suggested the structural types to which detected peaks should belong. The main characteristic MS fragmentations of three typical standards are showed in Figure 4. The results are consistent with the published data.[14,25,28,43]

TABLE 4 HPLC Retention Times (tR, min), Character UV Spectrums (nm), and $[M+H]^+$ (m/z) of Eight Standard References

No.	Name	tR (min)	UV, λmax/nm	$[M+H]^+$
1	sildenafil	16.4	225, 293	475
2	hongdenafil	12.3	234, 280	467
3	homosildenafil	17.8	226, 294	489
4	hydroxyhomosildenafil	16.1	225, 293	505
5	vardenafil	10.2	215	489
6	pseudovardenafil	39.7	229	460
7	tadalafil	29.4	224, 284	390
8	aminotadalafil	23.9	226	391

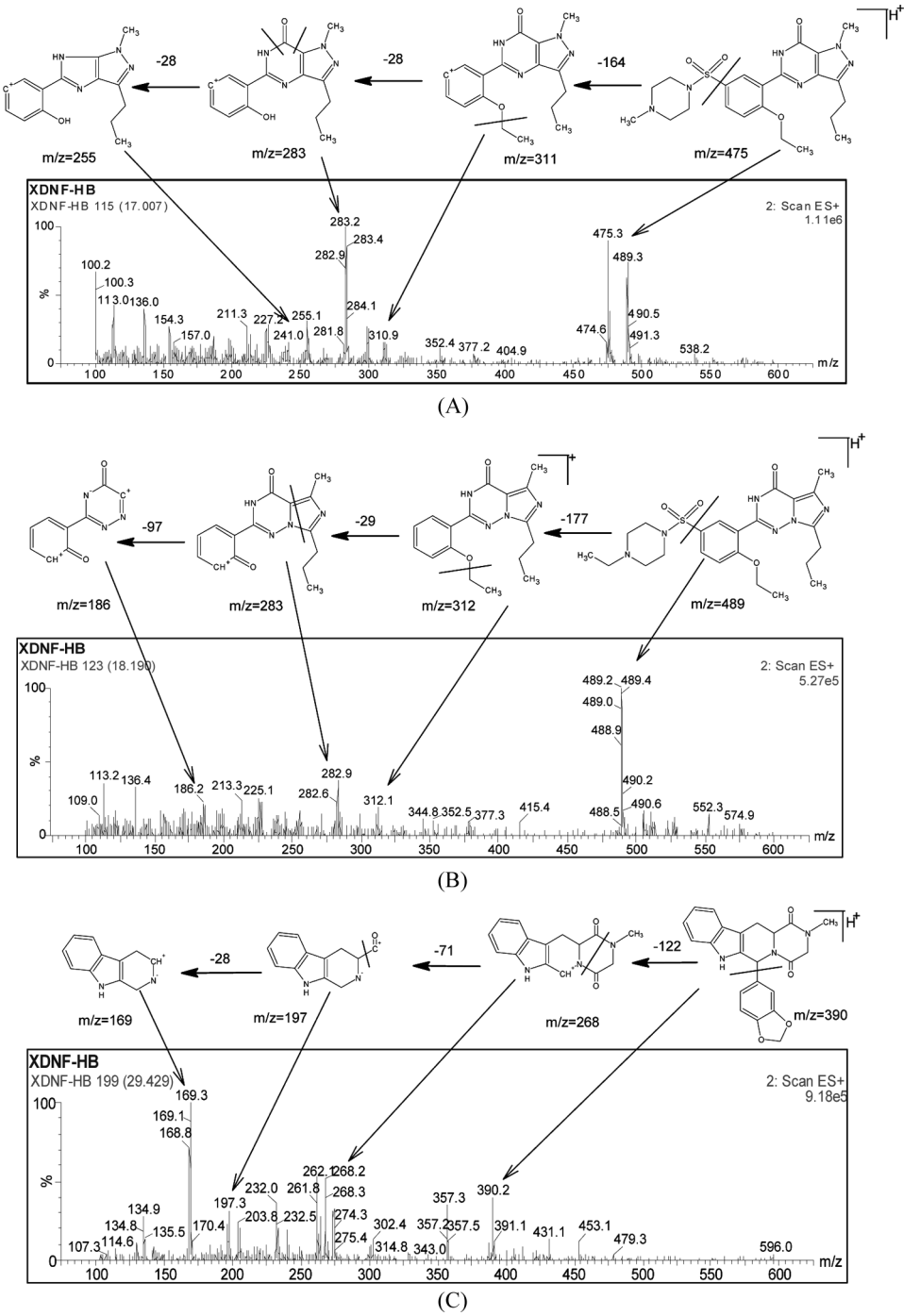


FIGURE 4 MS spectra and characteristic ion fragmentations of sildenafil type (A), vardenafil type (B), and tadalafil type (C).

Reepmeyer et al. had proposed that the hydrolysis product m/z 312 would be produced from the cleavage of vardenafil at the nitrogen–sulphur bond, followed by the subsequent loss of SO_3 molecule from the sulphonic acid compound.[25] The MS spectrum on the protonated molecular ion of vardenafil recorded on an ESI mode spectrometer in our laboratory, which presented a m/z 312 peak, differs from the one recorded on a EI mode spectrometer, which presented a m/z 311 peak in research by Dong et al.,[54] but it is practically identical to the spectrum recorded on an ion trap instrument by Venhuis et al.[44] Usually, most of fragment ions of sildenafil and vardenafil are the same due to their similar structures. When compared with their UV spectrums, they showed big differences, where sildenafil and its analogues showed λmax at 226 nm and 294 nm, whereas, vardenafil and its analogues only had one absorbent peak at approximately 220 nm (see Table 4).

Hence, the UV detection method can be used for conventional analysis of these compounds even without mass spectrometry. However, on line analysis displays that the proposed HPLC–PDA-MS method is advantageous in rapid screening analysis of these compounds and can provide structure information for identification when no standards are available.

Method Validation of HPLC-PDA for Quantification

Linearity, LOD, and LOQ

The calibration curves of **1–8** in the concentration range showed good linearity with a maximum detection wavelength for each (Table 1). The correlation coefficients were better than 0.999. The instrumental detection limits based on a signal-to-noise ratio of about 3 for standard solutions of **1–8** were also showed in Table 1 respectively. The LOQ for each analyte was determined by six replicate repeated analyses at the lowest quantitative concentration levels by RSD (%) $< 3.0\%$. Thus, it is acceptable to analyze **1–8** in adulterated dietary supplements.

Precision and Accuracy

The accuracy of the method was studied by calculating the mean recovery of the target compounds after adding standards to samples at low, medium, and high levels. Each level was repeated three times to obtain three samples, and each sample of the same concentration was injected at least three times. The results are summarized in Table 2. From this table, it can be seen that the mean recovery for each of the eight compounds was between 96.94–101.3%. These results regarding accuracy met the acceptable criteria.

Precision of the method was deduced from repeatability of multiple injections as well as intra- and inter-day variances of the results. All the samples were extracted and assayed under optimized conditions on day 1; the same procedure was repeated on two more days. By comparing variations within same days, intra-day precision was deduced; by observing differences within the 3 days, inter-day precision was deduced. The resulting RSD (%) varied from 0.12 to 1.55%. See Table 3 for relative standard deviations, which were below 2% for all compounds.

Sample Analysis

The herbal samples examined in the present work had complicated formulations and compositions. These samples included *Radix et rhizoma ginseng, Radix morindae officinalis, Herba epimedii, Echinacea purpurea, Radix et rhizoma ginseng rubra, Cordyceps, Serenoa repe*ns (saw palmetto), *Cornu cervi pantotrichum, Herba taraxaci, Radix puerariae lobate, Fructus lycii, Semen ziziphi spinosae, Fructus schisandrae chinensis, Colla corrii asini, Ganoderma*, and *Carapax et plastrum testudinis* as showed in products labels. The formulations are varied from solid form (tablets, capsules, pills, soft gel capsules) to liquid form (oral liquids). However, under the aforementioned conditions, no interference from these herbs was observed. Figure 5 shows the chromatograms of eight standard references and a typical sample (S12). Figure 6 shows representative HPLC-ESI-MS total ion chromatograms (TIC) of samples, and the identified PDE5-Is adulterated in commercial products are indicated.

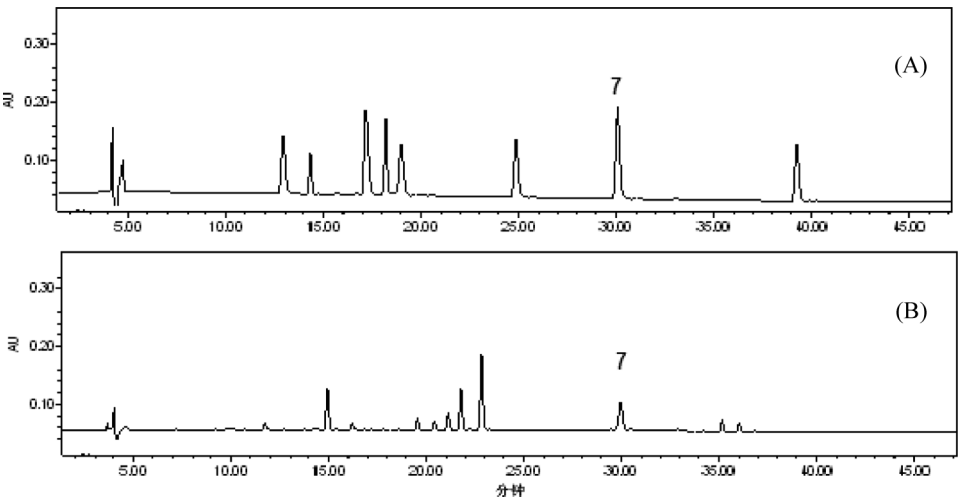


FIGURE 5 Representative HPLC-UV chromatograms for eight standard references (A) and sample 12 (B) using the conditions described in the Chromatographic Conditions section with a gradient mobile phase, and detector wavelength at 226 nm.

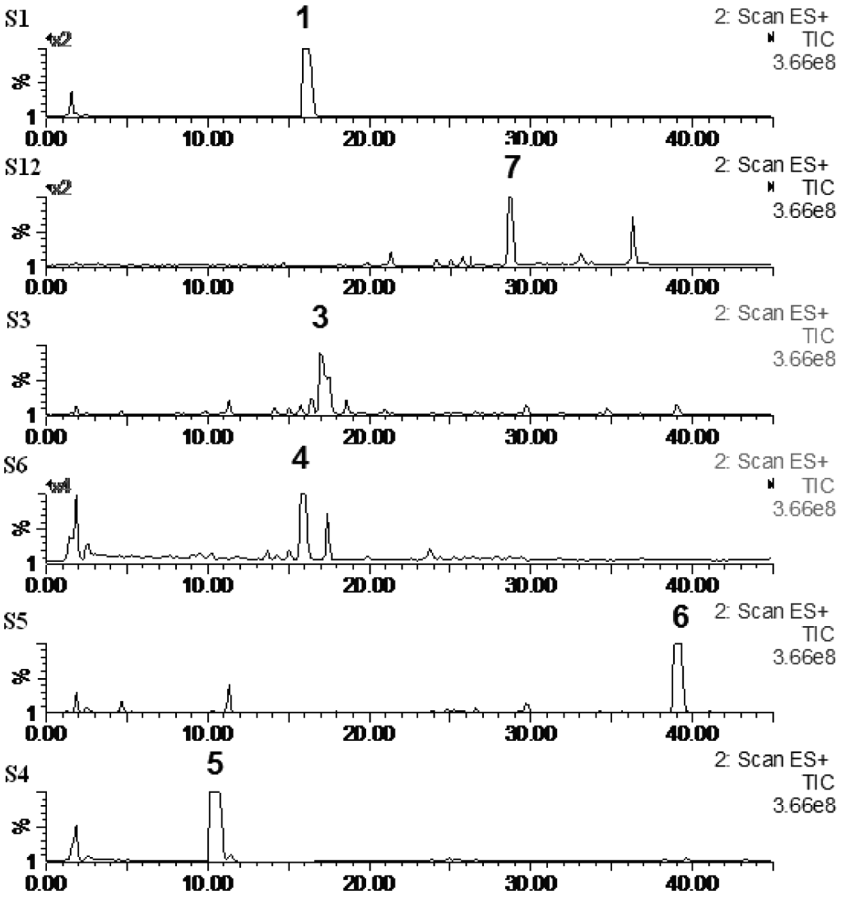


FIGURE 6 Representative HPLC-ESI-MS total ion chromatograms of samples and identified PDE5-Is adulterated in commercial products indicated as **1, 7, 3, 4, 6**, and **5**, respectively.

Thirty-six commercial herbal dietary supplements marketed as aphrodisiacs were surveyed, and ten commercial products (28%) were found containing one of eight compounds **1–8** by using a combination of HPLC-PDA-MS system; and their contents were separately determined by HPLC-UV system. As summarized in Table 5, Sample 1–6, Sample 8, Sample 12, Sample 19, and Sample 26 were found containing PDE5-Is. Sample 1 (S1) and Sample 26 (S26) were both determined to contain sildenafil, and the contents of sildenafil in Sample 1 was as high as 13.52%, which amounted to 70 mg of sildenafil per tablet. The therapeutic dose of sildenafil citrate (Viagra®) is typically 50 mg. At the same time, Sample 3 (S3) and Sample 12 (S12) were also found to contain adulterated high contents of homosildenafil (9.15%) and tadalafil (8.13%), separately. These herbal dietary supplements are especially worrying and can frequently lead to

TABLE 5 Information and Quantitative Results for Ten Positive Samples Based on Content in Percent, RSD (%) in Parenthesis (n = 3)

Sample No.	Brand Name	Formulation	Labeled Ingredients	Unclaimed Detected	Content RSD (%)
S1	Herb power	Tablets	Radix et rhizoma ginseng, Radix morindae officinalis, Herba epimedii	sildenafil	13.52 (0.89)
S2	HUOLONG	Capsules	Echinacea purpurea, Radix et rhizoma ginseng rubra, Cordyceps	hongdenafil	4.02 (1.19)
S3	LQSH	Capsules	Saw palmetto, Herba epimedii, Cornu cervi pantotrichum, Herba taraxaci	homosildenafil	9.15 (0.86)
S4	Holexum	Soft gel capsules	Radix et rhizoma ginseng, Echinacea purpurea, Radix puerariae lobate, Cornu cervi pantotrichum, Fructus lycii	vardenafil	3.03 (1.17)
S5	Bodlii	Tablets	Semen ziziphi spinosae, Fructus schisandrae chinensis, Fructus lycii	Pseudovardenafil	3.12 (1.23)
S6	SANBIAN	liquid	Fructus schisandrae chinensis, Fructus lycii, Semen cuscutae, Colla corrii asini, Ganoderma,Carapax et plastrum testudinis	hydroxyhomosildenafil	3.06 (0.93)*
S8	Enhanix	Tablets	Radix et rhizoma ginseng rubra, Herba epimedii, Cordyceps, Carapax et plastrum testudinis	homosildenafil	5.45 (1.09)
S12	Herbal stron	Soft gel capsules	Cordyceps, Herba cistanches, Radix et rhizoma rhodiolae crenulatae, Radix puerariae lobate, Piper methysticum (kava)	tadalafil	8.13 (1.08)
S19	Power 9	Capsules	Cornu cervi pantotrichum, Herba taraxaci, Semen ziziphi spinosae, Fructus lycii, Semen cuscutae, Colla corrii asini, Ganoderma	aminotadalafil	3.96 (1.15)
S26	Herbal max	Pills	Echinacea purpurea, Radix et rhizoma ginseng rubra, Herba epimedii, Fructus lycii, Ganoderma	sildenafil	5.38 (1.12)

*Content in g/100 mL.

overuse. Without prior knowledge of the addition of the chemical drugs, the potential hazards to the health cannot be evaluated and heeded.

The relative standard deviations measured by sample analysis were <2%. The assay results indicated that the proposed analytical method is adequate for simultaneous identification and determination of **1–8** in commercial herbal dietary supplements.

CONCLUSIONS

A simple TLC method was developed for rapid screening and identification of eight PDE5-Is: sildenafil, hongdenafil, homosildenafil, hydroxyhomosildenafil, vardenafil, pseudovardenafil, tadalafil, and aminotadalafil, and a sensitive, applicable. A reliable HPLC-PDA-MS method was developed and validated for simultaneous identification and quantification of eight compounds that are potentially added to herbal dietary supplements. Good linearity, LOD, LOQ, accuracy, precision, and recovery were demonstrated. This is the first reported case of the simultaneous identification/determination of eight PDE5-Is as adulterants in herbal dietary supplements. Identification of adulterants in dietary supplements poses an even greater challenge to the laboratories that are required to conduct routine surveillance programs. The procedure described here is considered to be available for routine determinations and rapid screening applications.

It is important to keep in mind that there can be risks associated with adulterated chemical drugs in herbals that one is taking. The chemical drugs are forbidden to be added into herbals; therefore, these chemicals would never be listed on a product label, and the consumer has no way of knowing what the risks are. This can make some herbal dietary supplements dangerous. Literature has discussed the safety issues of herbal dietary supplements relating to chemical drugs adulteration.[5,55,56] With the improvement in production technology of PDE5-Is drugs and its analogous compounds, the quantity of these compounds is becoming bigger and bigger; hence, even more of these compounds are being added illegally to dietary supplements. The method presented in this paper is useful for simultaneous identification/determination of eight PDE5-Is. It can be employed to inspect those herbal dietary supplements that may contain these chemicals in order to ensure people's safety.

REFERENCES

1. Porst, H. IC351 (tadalafil, Cialis): Update on Clinical Experience. *Int. J. Impot. Res.* 2002, 14 (1), 57–64.
2. Kuan, J.; Brock, G. Selective Phosphodiesterase Type 5 Inhibition Using Tadalafilfor the Treatment of Erectile Dysfunction. *Expert Opin. Investig. Drugs.* 2002, 11 (11), 1605–1613.

3. Wespes, E.; Amar, E.; Hatzichristou, D.; Montorsi, F.; Pryor, J.; Vardi, Y. Guidelines on Erectile Dysfunction. *Eur. Urol.* 2002, 41 (1), 1–5.
4. Hicklin, L. A.; Ryan, C.; Wong, D. K.; Hinton, A. E. Nose-bleeds After Sildenafil (Viagra). *J. R. Soc. Med.* 2002, 95 (8), 402–403.
5. Weinmann, W.; Bohnert, M.; Wiedemann, A.; Renz, M.; Lehmann, N.; Pollak, S. Post-mortem Detection and Identification of Sildenafil (Viagra) and its Metabolites by LC/MS and LC/MS/MS. *Int. J. Legal. Med.* 2001, 114 (4–5), 252–258.
6. Xueyi, Z.; Zhonghu, Z.; Feng, L.; Yutian, W.; Yunpeng, Q. Modified Local Straight-Line Screening to Detect Synthetic Drugs in Adulterated Herbal Medicines. *Appl. Spectrosc.* 2009, 63 (4), 471–476.
7. Tseng, M. C.; Li, J. H. Determination of Sildenafil Citrate Adulterated in a Dietary Supplements. *J. Food Drug Anal.* 2002, 10 (2), 112–119.
8. Mikami, E.; Ohno, T.; Matsumoto, H. Simultaneous Identification/Determination System for Phentolamine and Sildenafil as Adulterants in Soft Drinks Advertising Roborant Nutrition. *Forensic Sci. Int.* 2002, 130 (2–3), 140–146.
9. Fleshner, N.; Harvey, M.; Adomat, H.; Wood, C.; Eberding, A.; Hersey, K.; Guns, E. Evidence for Contamination of Herbal Erectile Dysfunction Products with Phosphodiesterase Type 5 Inhibitors. *J. Urol.* 2005, 174 (2), 636–641.
10. Zhu, X.; Xiao, S.; Chen, B.; Zhang, F.; Yao, S.; Wan, Z.; Yang, D.; Han, H. Simultaneous Determination of Sildenafil, Vardenafil and Tadalafil as Forbidden Components in Natural Dietary Supplements for Male Sexual Potency by High-Performance Liquid Chromatography-Electrospray Ionization Mass Spectrometry. *J. Chromatogr. A.* 2005, 1066 (1–2), 89–95.
11. Liang, Q.; Qu, J.; Luo, G.; Wang, Y. Rapid and Reliable Determination of Illegal Adulterant in Herbal Medicines and Dietary Supplements by LC/MS/MS. *J. Pharm. Biomed. Anal.* 2006, 40 (2), 305–311.
12. Bogusz, M. J.; Hassan, H.; Al-Enazi, E.; Ibrahim, Z.; Al-Tufail, M. Application of LC-ESI-MS-MS for Detection of Synthetic Adulterants in Herbal Remedies. *J. Pharm. Biomed. Anal.* 2006, 41 (2), 554–564.
13. Abdel, H. M. Determination of Sildenafil, Tadalafil, and Vardenafil in Tablets and Adulterated Herbal Products by ESI-MS-MS. *J. Liquid Chromatogr.* 2006, 29, 591–603.
14. 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. *J. Pharm. Biomed. Anal.* 2004, 36 (3), 525–533.
15. Shin, M. H.; Hong, M. K.; Kim, W. S.; Lee, Y. J.; Jeoung, Y. C. Identification of a New Analogue of Sildenafil Added Illegally to a Functional Food Marketed for Penile Erectile Dysfunction. *Food Addit. Contam.* 2003, 20 (9), 793–796.
16. Blok-Tip, L.; Zomer, B.; Bakker, F.; Hartog, K. D.; Hamzink, M.; Hove, J.; Vredenbregt, M.; De, Kaste, D. Structure Elucidation of Sildenafil Analogues in Herbal Products. *Food Addit. Contam.* 2004, 21 (8), 737–748.
17. Zou, P.; Oh, S. S.; Hou, P.; Low, M. Y.; Koh, H. L. Simultaneous Determination of Synthetic Phosphodiesterase-5 Inhibitors Found in a Dietary Supplement and Pre-Mixed Bulk Powders for Dietary Supplements Using High-Performance Liquid Chromatography with Diode Array Detection and Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry. *J. Chromatogr. A.* 2006, 1104 (1–2), 113–122.
18. Oh, S. S.; Zou, P.; Low, M. Y.; Koh, H. L. Detection of Sildenafil Analogues in Herbal Products for Erectile Dysfunction. *J. Toxicol. Environ. Health A.* 2006, 69 (21), 1951–1958.
19. Shin, C.; Hong, M.; Kim, D.; Lim, Y. Structure Determination of a Sildenafil Analogue Contained in Commercial Herb Drinks. *Magn. Reson. Chem.* 2004, 42 (12), 1060–1062.
20. Lai, K. C.; Liu, Y. C.; Tseng, M. C.; Lin, J. H. Isolation and Identification of a Sildenafil Analogue Illegally Added in Dietary Supplements. *J. Food Drug Anal.* 2006, 14, 19–23.
21. Hou, P.; Zou, P.; Low, M. Y.; Chan, E.; Koh, H. L. Structural Identification of a New Acetildenafil Analogue from Pre-Mixed Bulk Powder Intended as a Dietary Supplement. *Food Addit. Contam.* 2006, 23 (9), 870–875.
22. Gratz, S. R.; Gamble, B. M.; Flurer, R. A. Accurate Mass Measurement Using Fourier Transform Ion Cyclotron Resonance Mass Spectrometry for Structure Elucidation of Designer Drug Analogs of Tadalafil, Vardenafil and Sildenafil in Herbal and Pharmaceutical Matrices. *Rapid Commun. Mass Spectrom.* 2006, 20 (15), 2317–2327.

23. Zou, P.; Hou, P.; Low, M. Y.; Koh, H. L. Structural Elucidation of a Tadalafil Analogue Found as an Adulterant of an Herbal Product. *Food Addit. Contam.* 2006, 23 (5), 446–451.
24. Zou, P.; Hou, P.; Oh, S. S.; Low, M. Y.; Koh, H. L. Electrospray Tandem Mass Spectrometric Investigations of Tadalafil and its Analogue. *Rapid Commun. Mass Spectrom.* 2006, 20 (22), 3488–3490.
25. Reepmeyer, J. C.; Woodruff, J. T. Use of Liquid Chromatography-Mass Spectrometry and a Hydrolytic Technique for the Detection and Structure Elucidation of a Novel Synthetic Vardenafil Designer Drug Added Illegally to a "Natural" Herbal Dietary Supplement. *J. Chromatogr. A.* 2006, 1125 (1), 67–75.
26. Gao, Q.; Zhang, Z.; Guo, H. Z.; Dai, H.; Che, B. Q.; Wang, Z. B. Study on PDE_5 Inhibitors and Its Unknown Derivatives in Traditional Chinese Medicine and Health Food Discovered. *Chin. Pharm. J.* 2008, 43, 142–146.
27. Zou, P.; Hou, P.; Oh, S. S.; Chong, Y. M.; Bloodworth, B. C.; Low, M. Y.; Koh, H. L. Isolation and Identification of Thiohomosildenafil and Thiosildenafil in Health Supplements. *J. Pharm. Biomed. Anal.* 2008, 47 (2), 279–284.
28. Reepmeyer, J. C.; d'Avignon, D. A. Structure Elucidation of Thioketone Analogues of Sildenafil Detected as Adulterants in Herbal Aphrodisiacs. *J. Pharm. Biomed. Anal.* 2009, 49 (1), 145–150.
29. Takashi, H.; Kazunaga, T.; Masaaki, S.; Toshiyasu, I.; Tomoko, N.; Masaaki, K.; Yuji, H.; Yukihiro, G.; Nobuo, K. Isolation and Structural Elucidation of Cyclopentynafil and N-Octylnortadalafil Found in a Dietary Supplement. *Chem. Pharm. Bull.* 2009, 57 (2), 185–189.
30. Nagaraju, V.; Sreenath, D.; Rao, J. T.; Rao, R. N. Separation and Determination of Synthetic Impurities of Sildenafil (Viagra) by Reversed-Phase High-Performance Liquid Chromatography. *Anal. Sci.* 2003, 19 (7), 1007–1011.
31. Joo, Y. C.; Hyeong, S. L.; Kyung, S. Y.; Hyun, J. S.; In, J. J.; Sang, G. S. Sensitive Liquid Chromatography Assay with Ultraviolet Detection for a New Phosphodiesterase V Inhibitor, DA-8159, in Human Plasma and Urine. *J. Chromatogr. B*, 2003, 795 (2), 179–186.
32. Cooper, J. D.; Muirhead, D. C.; Taylor, J. E.; Baker, P. R. Development of an Assay for the Simultaneous Determination of Sildenafil (Viagra) and its Metabolite (UK-103,320) Using Automated Sequential Trace Enrichment of Dialysates and High-Performance Liquid Chromatography. *J. Chromatogr. B.* 1997, 701, 87–95.
33. Lia, J.; Chang, T. W. Determination of Transdermal Sildenafil in Nude Mouse Skin by Reversed-Phase High-Performance Liquid Chromatography. *J. Chromatogr. B.* 2001, 765, 161–166.
34. Ming, T. S.; An, B. W.; Geng, C. Y.; Angel, H.; Hsiu, O. H. Development of a Liquid Chromatographic Method for Bioanalytical Applications with Sildenafil. *J. Chromatogr. B.* 2003, 791 (1–2), 255–262.
35. Dinesh, N. D.; Vishukumar, B. K.; Nagaraja, P.; Gowda, N. M.; Rangappa, K. S. Stability Indicating RP-LC Determination of Sildenafil Citrate (Viagra) in Pure Form and in Pharmaceutical Samples. *J. Pharm. Biomed. Anal.* 2002, 29, 743.
36. Angela, E.; Tom, A.; Weng, N. D. Simultaneous Assay of Sildenafil and Desmethylsildenafil in Human Plasma using Liquid Chromatography–Tandem Mass Spectrometry on Silica Column with Aqueous–Organic Mobile Phase. *J. Chromatogr. B.* 2002, 768 (2), 277–284.
37. Tracqui, A.; Ludes, B. HPLC-MS for the Determination of Sildenafil Citrate (Viagra) in Biological Fluids. Application to the Salivary Excretion of Sildenafil After Oral Intake. *J. Anal. Toxicol.* 2003, 27 (2), 88–94.
38. Fleshner, N.; Harvey, M.; Adomat, H.; Wood, C.; Eberding, A.; Hersey, K.; Guns, E. Evidence for Contamination of Herbal Erectile Dysfunction Products with Phosphodiesterase Type 5 Inhibitors. *J. Urol.* 2005, 174 (2), 636–641.
39. Reepmeyer, J. C.; Woodruff, J. T. Use of Liquid Chromatography-Mass Spectrometry and a Chemical Cleavage Reaction for the Structure Elucidation of a New Sildenafil Analogue Detected as an Adulterant in an Herbal Dietary Supplement. *J. Pharm. Biomed. Anal.* 2007, 44 (4), 887–893.
40. Venhuis, B. J.; Blok, T. L.; de Kaste, D. Designer Drugs in Herbal Aphrodisiacs. *Forensic Sci. Int.* 2008, 177 (2–3), 25–27.
41. Reepmeyer, J. C.; Woodruff, J. T.; d'Avignon, D. A. Structure Elucidation of a Novel Analogue of Sildenafil Detected as an Adulterant in an Herbal Dietary Supplement. *J. Pharm. Biomed. Anal.* 2007, 43 (5), 1615–1621.
42. Park, H. J.; Jeong, H. K.; Chang, M. I.; Im, M. H.; Jeong, J. Y.; Choi, D. M.; Park, K.; Hong, M. K.; Youm, J.; Han, S. B.; Kim, D. J.; Park, J. H.; Kwon, S. W. Structure Determination of New Analogues of Vardenafil and Sildenafil in Dietary Supplements. *Food Addit. Contam.* 2007, 24 (2), 122–129.

43. Lam, Y. H.; Poon, W. T.; Lai, C. K.; Chan, A. Y.; Mak, T. W. Identification of a Novel Vardenafil Analogue in Herbal Product. *J. Pharm. Biomed. Anal.* 2008, 46 (4), 804–807.
44. Venhuis, B. J.; Zomer, G.; de Kaste, D. Structure Elucidation of a Novel Synthetic Thiono Analogue of Sildenafil Detected in an Alleged Herbal Aphrodisiac. *J. Pharm. Biomed. Anal.* 2008, 46 (4), 814–817.
45. Miller, G. M.; Stripp, R. A. Study of Western Pharmaceuticals Contained within Samples of Chinese Herbal/Patent Medicines Collected from New York City's Chinatown. *Leg. Med. (Tokyo).* 2007, 9 (5), 258–264.
46. Vos, R. M.; Chahbouni, A.; Sinjewel, A.; Swart, E. L. Quantitative Analysis of Sildenafil and Desmethylsildenafil in Human Serum by Liquid Chromatography–Mass Spectrometry with Minimal Sample Pretreatment. *J. Chromatogr. B.* 2008, 876 (2), 283–287.
47. Bae, S. K.; Kang, M. J.; Yeo, C. W.; Kim, M. J.; Shon, J. H.; Liu, K. H.; Shin, J. G. Simultaneous Determination of Udenafil and Its Active Metabolite, DA-8164, IN Human Plasma and Urine Using Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry: Application to a Pharmacokinetic Study. *Biomed. Chromatogr.* 2008, 22 (9), 939–946.
48. Guermouche, M. H.; Bensalah, K. Solid Phase Extraction and Liquid Chromatographic Determination of Sildenafil and N-Demethylsildenafil in Rat Serum with Basic Mobile Phase. *J. Pharm. Biomed. Anal.* 2006, 40 (4), 952–957.
49. Pistos, C.; Papoutsis, I.; Dona, A.; Stefanidou, M.; Athanaselis, S.; Maravelias, C.; Spiliopoulou, C. Off-line HPLC Method Combined to LC-MS for the Determination of Sildenafil and its Active Metabolite in Post-Mortem Human Blood According to Confirmation Criteria. *Forensic Sci. Int.* 2008, 178 (2–3), 192–198.
50. Hei, Y. K.; Ji, H. S.; Kwang, H. L.; Jae, G. S.; Soo, K. B. Liquid Chromatography/Tandem Mass Spectrometry Method for the Simultaneous Determination of Vardenafil and Its Major Metabolite, N-Desethylvardenafil, in Human Plasma: Application to a Pharmacokinetic Study. *J. Chromatogr. B.* 2009, 877 (1–2), 95–100.
51. Li, R. K.; Bo, T.; Liu, H. W.; Li, K. A. Determination of Sildenafil in Medicines for Erectile Dysfunction by Capillary Electrophoresis. *Se Pu.* 2002, 4, 49–51.
52. Berzas, N. J.; Flores, J. R.; Peñalvo, G. C.; Rodríguez, F. N. Determination of Sildenafil Citrate and its Main Metabolite by Sample Stacking with Polarity Switching Using Micellar Electrokinetic Chromatography. *J. Chromatogr. A.* 2002, 953 (1–2), 279–286.
53. Gryniewicz, C. M.; Reepmeyer, J. C.; Kauffman, J. F.; Buhse, L. F. Detection of Undeclared Erectile Dysfunction Drugs and Analogues in Dietary Supplements by Ion Mobility Spectrometry. *J. Pharm. Biomed. Anal.* 2009, 49 (3), 601–606.
54. Dong, M.; Sangaeh, P.; Tae, H. Y.; Hye, K. J.; Jae, S. P.; Janghyun, P.; Deukjoon, K.; Sung, W. K. Determination of Analogs of Sildenafil and Vardenafil in Foods by Column Liquid Chromatography with a Photodiode Array Detector, Mass Spectrometry, and Nuclear Magnetic Resonance Spectrometry. *J. AOAC Inter.* 2008, 91 (3), 580–588.
55. Poon, W. T.; Lam, Y. H.; Lai, C. K.; Albert, Y. W.; Chan, A. Y.; Tony, W. M. Analogues of Erectile Dysfunction Drugs: An Under-Recognized Threat. *Hong Kong Med. J.* 2007, 13 (5), 359–363.
56. Cockburn, R.; Newton, P. N.; Agyarko, E. K.; Akunyili, D.; White, N. J. The global Threat of Counterfeit Drugs: Why Industry and Governments Must Communicate the Dangers. *PLoS Med.* 2005, 2 (4), 100.