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Development of a specific fragment pattern-based quadrupole-Orbitrap mass spectrometry method to screen adulterated products of phosphodiesterase-5 inhibitors and their analogues

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ABSTRACT

Recently, adulterated supplements with phosphodiesterase-5 inhibitors (PDE-5i) have frequently observed. New synthetic analogues obtained from the chemical modification of parent compounds are frequently found in illicit products despite continuous efforts to inspect for these adulterants. A rapid and accurate method based on quadrupole-Orbitrap mass spectrometry was developed for simultaneously confirming and quantifying 85 PDE-5i and derived analogues present in illicit products for erectile dysfunction (ED). Common ions of PDE-5i according to their similar structures were proposed based on MS/MS fragmentations. These common ions could be an important diagnosis of their presence targets or new emerging analogues in supplements. Several validation parameters were employed, resulting in a limit of detection and quantification of 0.09–8.55 ng/mL and 0.24–17.10 ng/mL, respectively. The linear correlation coefficient (r^2) was higher than 0.995, and mean recoveries of target compounds were in the range of 82–118%. A total of 187 illicit products, obtained from on/offline markets over a period of 3 years (2015–2017), were screened by the established method. Approximately 53% of them were adulterated with PDE-5i or derived analogues at concentrations of 0.1–726.0 mg/g in the illicit products. In the interests of public health, this study describes a rapid and accurate method to determine PDE-5i and new emerging analogues in adulterated products.

1. Introduction

Erectile dysfunction (ED) is a frequently occurring condition in men of various ages, resulting in mental or physical problems that have a negative impact on their quality of life. As the world population ages, the prevalence of ED is increasing, and the market for potential ED therapies has grown globally. The introduction of sildenafil (Viagra™), a phosphodiesterase-5 inhibitor (PDE-5i), in 1998 was followed by the approval of tadalafil (Cialis®) and vardenafil (Levitra®) by the US Food and Drug Administration (FDA) as first-generation ED drugs [1]. They are safe, effective, and easy to take orally, and they are rapidly absorbed after oral administration. A second generation of drugs has now emerged, including avanafil (Stendra® and Spedra®), mirodenafil (Mvix®), udenafil (Zydena®), and lodenafil (Helleva®), driving an ED market that is expected to reach $32 billion by 2022 [2,3].

Illicit products containing PDE-5i and their analogues create social problems such as increasing sexual crimes and black markets (both on the internet and offline) due to their high demand as an alternative to ED drugs that require prescriptions. In Europe, 35.8 million counterfeit sildenafil tablets were confiscated over 4 years beginning in 2004, while in France, PDE-5i and derived analogues were detected over 3 years in 150 herbal dietary supplements for improved sexual performance [4,5]. Moreover, the FDA Tainted Supplements Report from 2007 to 2012 showed that > 95% of the 332 adulterated products were marketed as sexual enhancement products [6]. In Korea, of the adulterants found in 188 samples over 5 years beginning in 2009, 43 were found in foods and 40 were in dietary supplements. Of those, tadalafil and its analogues (53%) were the most frequently detected, followed by sildenafil and its analogues (43%) [7]. Despite the beneficial pharmacological effects of ED drugs, their misuse and abuse should be of greater concern due to several side effects, including cardiovascular disorders, headaches, dyspepsia, and retinal disturbance [8]. Therefore, a number of analytical methods are being developed for detecting ED drugs in illicit products using high-performance liquid chromatography (HPLC) [9],

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Table 1
Identification of new sildenafil and tadalafil analogues in adulterated products in our laboratories (2011–2018).

PDE-5i	Analogue	Year of first report	Type of product	Ref
Sildenafil	Propoxyphenyl thiohomosildenafil	2013	Herbal product (capsule)	[16]
	Propoxyphenyl thiosildenafil	2013	Herbal product (capsule)	[24]
	Desmethylpiperazinyl propoxysildenafil	2018	Herbal product (Tablet)	[23]
Tadalafil	Acetaminotadalafil	2011	Dietary supplement (powder)	[17]
	Homotadalafil	2014	Herbal product (5 type forms)	[22]
	trans-Bisprehomotadalafil	2014	Dietary supplement (powder)	[21]
	Cyclopentyltadalafil	2014	Dietary supplement (powder)	[20]
	trans-Cyclopentyltadalafil	2014	Dietary supplement (powder)	[20]
	Bisprecyclopentyltadalafil	2014	Dietary supplement (powder)	[20]
	Bisprenortadalafil	2015	Health supplement (capsule)	[19]
	Isopropylnortadalafil	2016	Dietary supplement (pill)	[18]

liquid chromatography-mass spectrometry (LC-MS) [7,10], gas chromatography–mass spectrometry (GC–MS) [11], high-resolution mass spectrometry (HR-MS) [12], and nuclear magnetic resonance (NMR) spectroscopy [4,13].

New PDE-5i analogues are synthesised through minor chemical modifications of the parent structures in an attempt to avoid their detection by authorities. Since the first detection of homosildenafil in adulterated beverages, most synthetic PDE-5i analogues have been identified in Korea and Singapore [14,15]. As indicated in Table 1, a total of 11 new analogues were identified for the first time in our laboratory (starting with aminotadalafil in 2011), where tadalafil analogues accounted for 73% of the total [16–24]. The toxicity or pharmacological safety profile of these analogues is less specified and poses a significant risk to public health. Therefore, breakthrough analytical techniques that can detect potential PDE-5i analogues with updated simultaneous analysis and identification are needed.

The aim of the present study was to develop a new UPLC-Q-Orbitrap-MS-based method that can simultaneously screen 85 PDE-5i and their analogues to identify their specific fragmentation pathways and screen illegally manufactured products for ED. The Q-Orbitrap-MS, as an advanced analytical technique that is frequently applied for complex matrixes, not only allows screening for the presence of non-targeted compounds but also allows their accurate qualification and quantification [25]. Since most analogues were synthesised by chemical modification of specific function groups, common fragment ions of PDE-5i with similar structural moiety were generated to be distinguished from other ones. The advantage of specific fragmentation patterns for PDE-5i and their analogues is useful for the rapid screening of targeted adulterants and the detection of new emerging analogues. Recently, the fragmentation pathways for PDE-5i and their analogues have been reported, but these studies and reviews were limited to only one group of new analogues [15,26]. Therefore, we established a specific fragment patterns for the PDE-5i groups, developed and validated a method of analysis, and applied it to analyse several illicit ED products obtained from 2015 to 2017.

2. Materials and methods

2.1. Chemicals and reagents

Standard compounds (85 in total) were classified as analogues belonging to the sildenafil group, tadalafil group, vardenafil group, and others, as listed in Table S1. They were obtained from the Ministry of Food and Drug Safety (Osong, Korea), Sigma-Aldrich (St, Louis, MO, USA), TLC Pharmaceutical Standards Ltd. (Aurora, ON, Canada), US Pharmacopoeia (Rockville, MD, USA), and Toronto Research Chemicals (North York, ON, Canada). Stock solutions of all standards were prepared separately at 1000 μg/mL in methanol and stored at 4 °C. Acetonitrile (MeCN) and methanol (MeOH), both HPLC grade, were purchased from Merck (Darmstadt, Germany). Formic acid (LC-MS grade) was obtained from Sigma-Aldrich. Deionised water (DW) was prepared with a Milli-Q-water purification system (Millipore, Billerica, MA, USA) at 18.2 MΩ cm^{-1}. All solvents were filtered through a polyvinylidene difluoride filter (0.2 μm).

2.2. Sample preparation

The adulterated products (187 in total) for enhancing sexual performance were collected from various markets, including online sites, overseas direct purchases, international postal services, and various offline stores from 2015 to 2017. They were composed of foods, dietary supplements, counterfeit drugs, and herbal medicines in the form of capsules, tablets, powders, films, and liquids (Table 2). After blending the samples, approximately 1 g of homogenised sample was dissolved with 70% methanol by sonication for 30 min in a 50-mL volumetric flask. The extracts were diluted up to 50 mL and filtered through a polytetrafluoroethylene filter (0.2 μm), then diluted to appropriate concentrations with 70% methanol for instrumental analyses.

2.3. UHPLC-Q-Orbitrap-MS analysis

The UHPLC-Q-Orbitrap-MS analysis was conducted on a Q Exactive Orbitrap mass spectrometer (Thermo Scientific, San Jose, CA, USA) equipped with a Thermo Dionex UltiMate 3000 LC. The eluents were 1 mM ammonium acetate in distilled water (A) and MeCN (B), and the column was a Poroshell 120 EC-C_{18} (100 × 2.1 mm, i.d. 2.7 μm) maintained at 40 °C. The sample volume injected was 1 μL, and the flow rate was 0.4 mL/min. After eluent B was initially maintained at 20% for 2 min, the gradient profile was as follows: 2.0–15.0 min (A: 80–10%, B: 20–90%), 15.0–18.0 min (A: 10%, B: 90%), 18.0–18.1 min (A: 10–80%, B: 90–20%), and 18.1–20.0 min (A: 80%, B: 20%). The analysis was subjected to full MS/$ddMS^2$ (data-dependent MS^2), which was a full MS scan event followed by a data-dependent scan with certain fragmentation energy applied. The MS parameters were as follows: ion source, heated ESI (HESI); ion mode, positive; spray voltage, 3.5 kV; capillary temperature, 320 °C; sheath gas, 42 arbitrary units; auxiliary gas, 10 arbitrary units; probe heater temperature, 350 °C; S-lens RF level, 50; resolution, 70,000 (full scan), 17,500 (MS/MS); automatic gain control target, $3e^6$ (full scan), $1e^5$ (MS/MS); scan range, 50 to 1000 *m/z*; maximum infusion time (IT), 100 ms (full scan), 50 ms (MS/MS);

Table 2
Classification of the examined male enhancement products (n = 187).

Type	Form	Number of			
		2015	2016	2017	Total
Foods	Powder, Liquid	43	29	19	91
Dietary supplements	Tablet, Capsule	6	17	19	42
Counterfeit drugs	Tablet, Capsule, Film	21	6	17	44
Herbal medicines	Tablet, Capsule, Liquid	2	6	2	10
Total		72	58	57	187

microscans, 1; loop count, 5; MSX count, 1; Top N, 5; isolation window, 4 *m/z*; underfill ratio, 1.0%; intensity threshold, $2e^4$; exclude isotopes, on; and dynamic exclusion, 10.0 s. The mass spectrometer was calibrated to the manufacturer's specifications. Data were obtained using the Xcalibur 3.0 software (Thermo Scientific).

2.4. Method validation

The method was validated based on triplicate measurements for specificity, limit of detection (LOD), limit of quantification (LOQ), linearity, and recovery. Specificity was defined as the ability to assess unequivocally the analyte in the presence of other components in the sample. The specificity was identified using a spiked target compound in both solid and liquid-type matrixes; blank samples contained no target compounds. LODs and LOQs were defined as the lowest concentrations of an analyte detected (signal-to-noise ratio of > 3) and quantified (signal-to-noise ratio of > 10) in samples, respectively. Linearity was determined using a series of peak areas for 6 points, which were proportional to the concentration of the analyte within a given range. The effectiveness of sample preparation (evaluated by recovery) was assessed by comparing the response of a target compound dissolved in matrix-blank samples and pure solvent.

3. Results and discussion

3.1. Fragmentation of PDE-5i analogues

Sildenafil, tadalafil, and vardenafil have received considerable attention over the last decade. We studied the specific fragmentation of PDE-5i analogues by interpretation of their MS/MS spectra, thereby finding characteristic common structural moieties in each group of PDE-5i analogues, except for some analogues with special functional groups.

3.1.1. Sildenafil analogues

Sildenafil and its analogues comprised 60% of the PDE-5i that have been identified in adulterated products. Major backbone of sildenafil is pyrazolo pyrimidin-7-one, which improves erections through selective inhibition on type 5 cyclic guanosine monophosphate (cGMP) PDE hydrolysing activity [27]. As shown in Fig. 1, sildenafil analogues were classified based on X, R^1, and R^2 groups. First, sildenafil analogues were divided into groups with pyrazolo pyrimidin-7-one and pyrimidine-7-thione groups at X and then subcategorised by ethoxy and propyl groups at R^1. Sildenafil analogues with pyrazolo pyrimidin-7-ones and ethoxy were grouped with sulfonamide, acetyl, and carbonyl, indicating sildenafil, acetildenafil (hongdenafil), and carbodenafil as representative examples. Sildenafil analogues were mostly fragmented at the R^1 and R^2 positions, reflecting the presence of the characteristic phenyl pyrazolopyrimidine moiety [28]. Specific common ions of sildenafil analogues with the sulfonamide group were generated at *m/z* 377, 311, and 283 through inductive cleavage of the methyl piperazine, sulfonyl (R^2) and ethoxy groups (R^1). However, desmethylpiperazinyl sildenafil showed unique fragmentation with large peaks at *m/z* 365, 336 and 284 due to sulfonic acid at R^1. If a propoxy group was located at R^2, the *m/z* was 325 due to exclusion of the ethoxy group (14 Da). Sildenafil analogue with acetyl groups produced characteristic ions at *m/z* 311, 341, and 297, eliminating an acetyl moiety and an ethoxy group, whereas sildenafil analogues with a carbonyl group showed an *m/z* of only 311. Both groups were found to produce several protonated fragment ions because the positive charge could be delocalised to increase their stability. Otherwise, thiosildenafil analogues with pyrimidine-7-thione were mostly binding a sulfonamide group, which generated common ions *m/z* 327 and 299 due to the mass difference (16 Da) between oxygen and sulphur. However, the propoxythio-linked analogue generated common ions at *m/z* 341 and 299. In particular, thiosildenafil analogues with a thione at R^2 produced fragment ions at *m/z* 371 and 343 by eliminating piperazine and ethoxy groups.

3.1.2. Tadalafil analogues

Tadalafil, largely used as an ED drug following sildenafil, is a cGMP-specific PDE-5i with an improved PDE-5/PDE-6 selectivity compared with that of sildenafil and vardenafil. Synthesis of tadalafil starts from (D)-tryptophan methyl ester and piperonal by Pictet-Spengler reaction, and both tadalafil and its trans-isomers are generated [29]. Tadalafil analogues are subcategorised into monomeric and dimeric structures. The fragment ion at *m/z* 135 is the benzylic cation that was originally a part of the starting material, piperonal. Most of them are structurally modified at the *N*-methyl group of the diketopiperazine ring. Their common ions are at *m/z* 197, 169, and 135, indicating sequential breaks of 2,5-diketopiperazine and tetrahydro-b(symbol font)-carboline, respectively [30]. Pre-tadalafil analogues, with an opened diketopiperazine ring, were synthesised without the condensation of methylamine. As shown in Fig. 2, common ions were indicated at *m/z* 334, 274, and 135. Recently, our laboratory identified dimeric tadalafil analogues composed of 2 pre-tadalafil moieties, surmising that these analogues were generated from either stereoisomer of chloroacetamide during the synthesis of their monomeric analogues, being by-products that were not properly removed during purification [31]. While bisprenortadalafil, replacing the *N*-methyl group of tadalafil with the tadalafil precursor moiety, has *m/z* 334, dimeric tadalafil analogues with any group generated one common ion at *m/z* 336.

3.1.3. Vardenafil analogues

Although vardenafil is structurally similar to sildenafil, vardenafil and its analogues possess an imidazo[1,5-*f*][1,2,4]triazin-4(3*H*)-one ring instead of a 1*H*-pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one ring found in sildenafil and represent a new class of potent cGMP-PDE-5i that proved to be better than other purine-isosteric inhibitors [32]. We categorised 8 vardenafil analogues in the sildenafil group (Fig. 3). Vardenafil analogues have common ions at *m/z* 312 and 151, indicating the elimination of sulfonamide or acetyl moieties, then broken to imidazo[1,5-*f*][1,2,4]triazin-4(3*H*)-ones. If *m/z* 377 was shown in the MS spectrum, presentation of the sulfonamide group was confirmed. The thiovardenafil group generated 3 ions at *m/z* 393, 328, and 167 with differences of sulphur and oxide (16 Da).

3.2. Optimisation of instrument conditions

To optimise the chromatographic conditions, we surveyed 2 solvents consisting of DW and MeCN with 0.1% formic acid or 10 mM ammonium acetate. Of them, 0.1% formic acid in both DW and MeCN were selected as suitable solvents, resulting in good peak shape and sensitivity. In reversed-phase columns, Poroshell 120 EC-C_{18} (2.1 × 100 mm, i.d, 2.7 μm) more effectively separated all peaks than BEH C_{18} (2.1 × 100 mm, 1.7 μm, Waters) and HSS T_3 (2.1 × 100 mm, 1.8 μm, Waters). The gradient programme was optimised for analysing all target compounds within 20 min under these conditions.

Data were acquired in full scan and data-dependent MS/MS (full MS/ddMS^2) owing to its advantages for the obvious identification of isomeric or untargeted compounds. For example, the same protonated values ($C_{23}H_{32}N_6O_4S$, *m/z* 489.23221) of homosildenafil and dimethylsildenafil were distinguished using the characteristic fragment ions homosildenafil (*m/z* 461.19665) and dimethylsildenafil (*m/z* 432.14743), respectively, caused by the different piperazinyl group of sildenafil. Moreover, isopropylnortadalafil was recently identified as a new tadalafil analogue by Orbitrap-MS analysis during the inspection of an adulterated dietary supplement [18]. Several MS parameters were manually controlled to obtain a good response. In particular, MS resolution was set at 70,000 FWHM in full MS and 17,500 FWHM in dd-MS^2 considering both selectivity and sensitivity. All protonated compounds were extracted with a mass tolerance window within 10 ppm and up to 5 decimal places in *m/z*. Target compounds were identified by

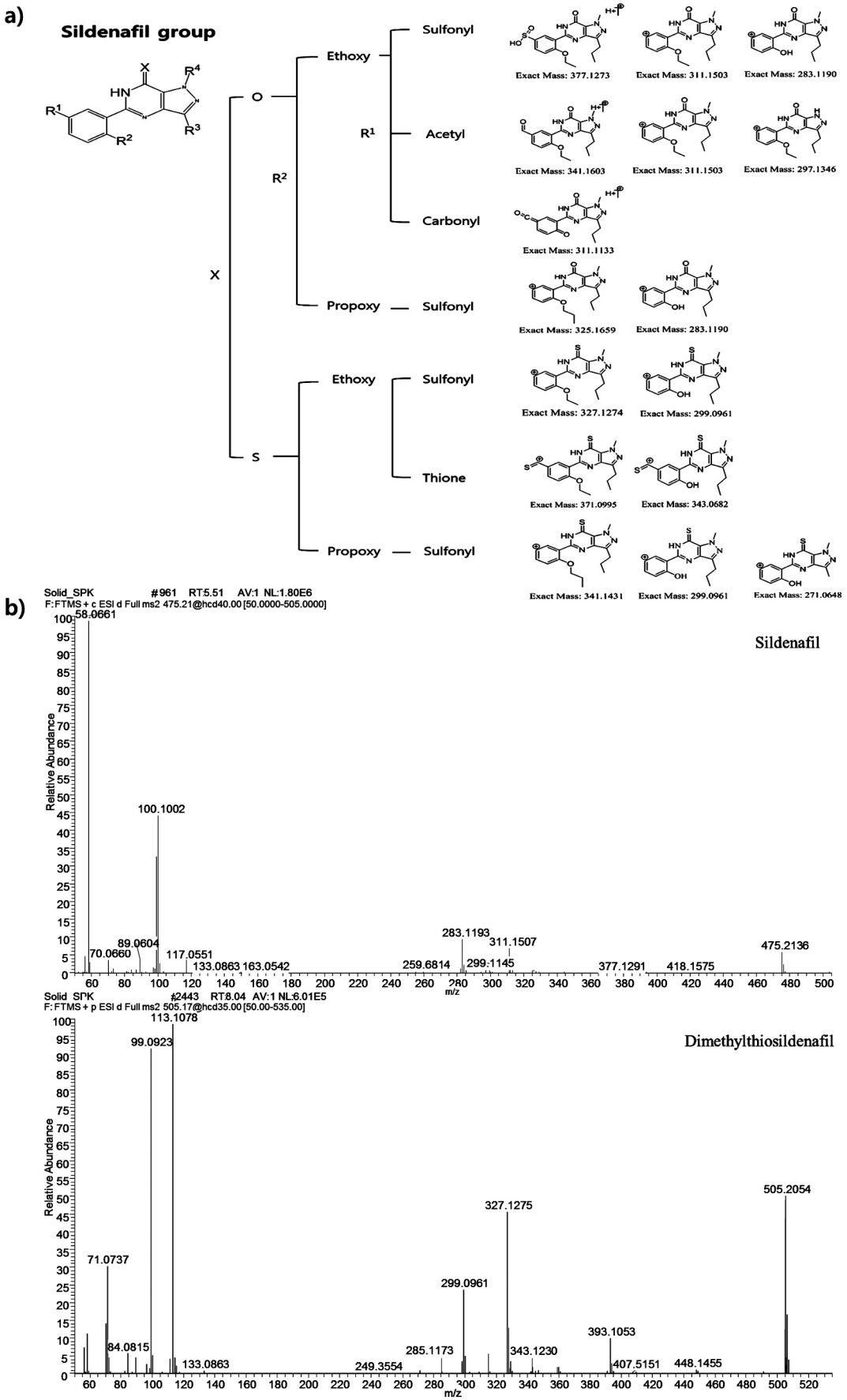

Fig. 1. Proposed fragmentation for analogues of the sildenafil group (a) and LC-Orbitrap-MS/MS spectra of sildenafil at *m/z* 475.2 and dimethylthiosildenafil at *m/z* 505.2 (b).

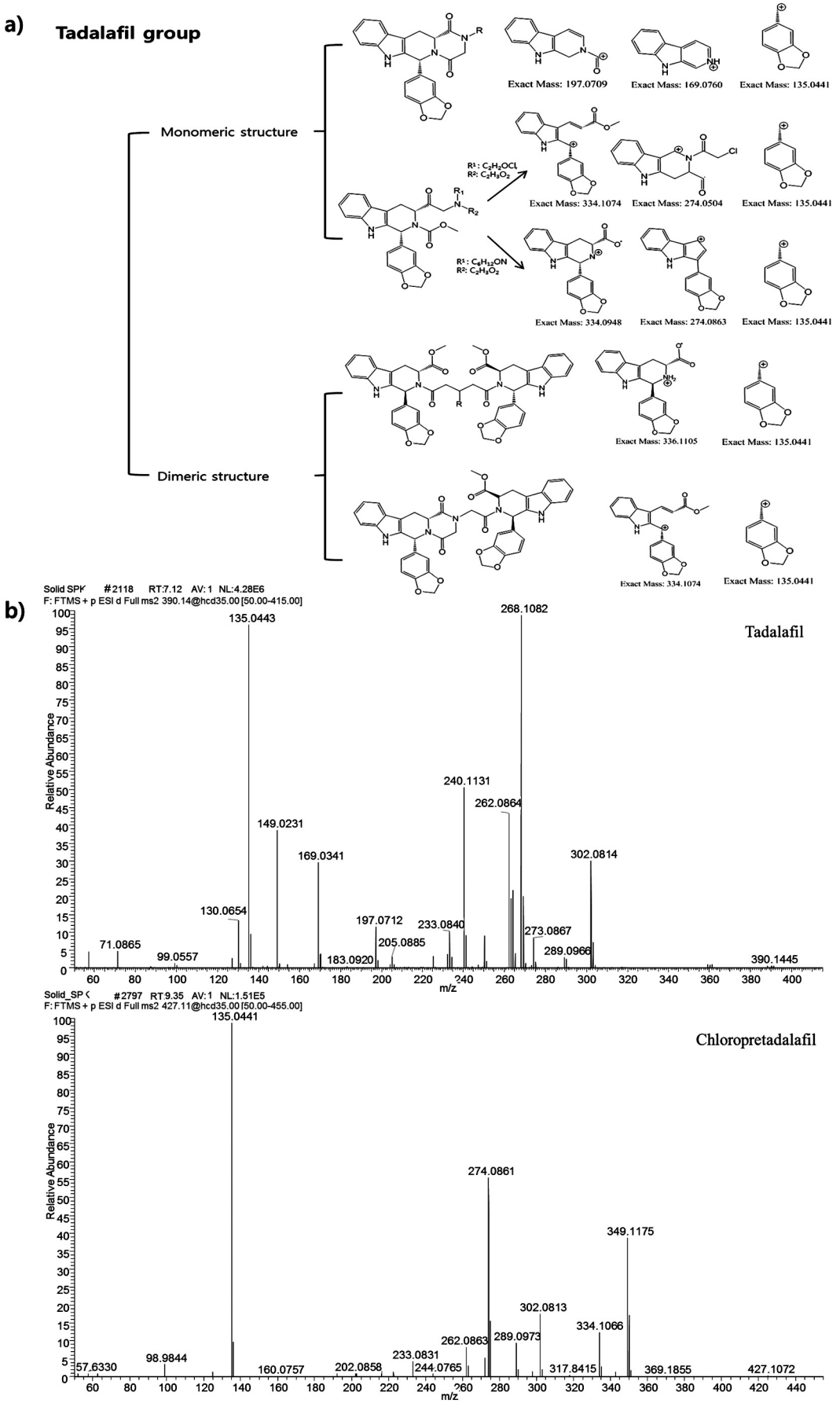

Fig. 2. Proposed fragmentation for analogues of the tadalafil group (a) and LC-Orbitrap-MS/MS spectra of tadalafil at *m/z* 390.1 and chloropretadalafil at *m/z* 427.1 (b).

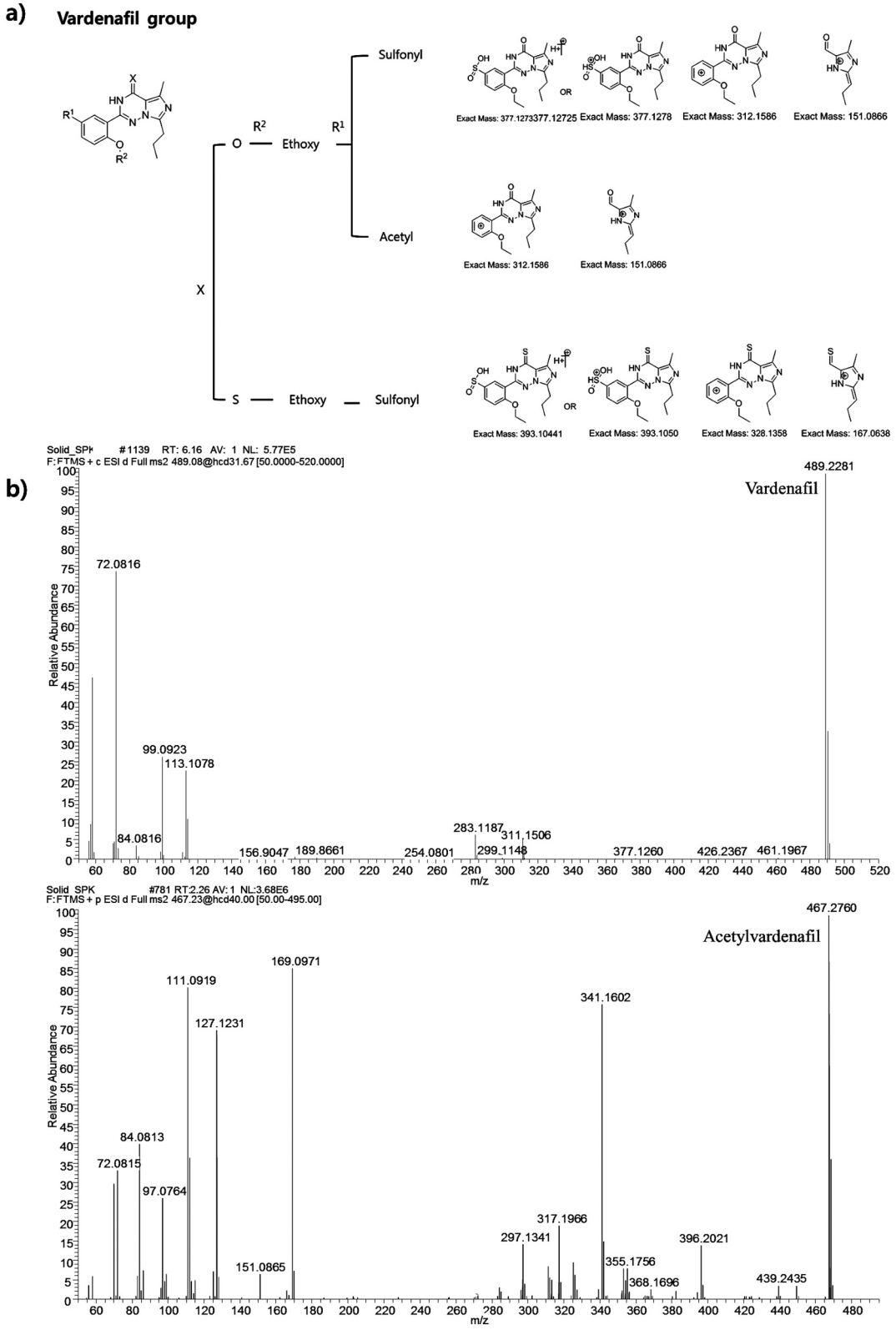

Fig. 3. Proposed fragmentation for analogues of the vardenafil group (a) and LC-Orbitrap-MS/MS spectra of vardenafil at *m/z* 489.2 and acetylvardenafil at *m/z* 467.2 (b).

the Q-Orbitrap-MS method using a combination of retention time, mass accuracy, and fragmentation. The mass accuracies between the theoretical and experimental masses of all precursor ions were from −3.8 to 4.7 ppm (Table S2), indicating a high level of confidence in accurate masses provided by the Q-Orbitrap-MS.

3.3. Method validation

The established method was validated based on the specificity, LOD, LOQ, linearity, and recovery. The mass tolerance window was within 10 ppm, considering the detection capability, signal intensity, and

matrix interferences. Specificity was defined by a lack of interference peaks between 2 type-matrix blanks and matrix spikes for the target compounds. Fig. S1 shows the chromatograms of representative compounds. We concluded that our method provides a resolution high enough to distinguish the analyte from their isobaric ions. LODs and LOQs were in ranges of 0.09–5.52 ng/g and 0.24–16.57 ng/g (solid), 0.26–8.55 ng/mL and 0.78–17.10 ng/mL (liquid), respectively (Table S3). The correlation coefficients (r^2) were higher than 0.995, indicating excellent linear relationships from 10 to 1000 ng/mL. Mean recoveries of 3 different concentrations in solid and liquid-type samples ranged from 83 to 116% and from 82 to 118%, respectively.

3.4. Application of real samples

A total of 187 samples were collected from online and offline markets over a period of 3 years (2015–2017) and screened using our established method. These samples consisted of food, dietary supplements, herbal medicines, and counterfeit drugs that were advertised as being effective in enhancing sexual performance. Samples were rapidly screened within a mass tolerance window of 10 ppm. Target compounds in samples were easily confirmed based on retention time, mass accuracies, and further MS^2 fragment ions. For example, sildenafil (m/z 475.2115) and tadalafil (m/z 390.1440), were observed in samples at 5.51 and 7.12 min. Their mass accuracies were < 2 ppm, indicating positive confirmation results. Fig. 4 shows that specific fragment ions of samples corresponded to those of standard solutions. Considering the presence of adulterants in samples, the established method proved that the risk of false positive could be significantly minimized using Quadrupole-Orbitrap-MS. The number of screened products for each year is shown in Fig. 5 along with the adulterants they contained. Although the ratio of adulterants detected for all samples over 3 years was > 50%, the rate of detection decreases every year. These results may imply that both analytical method development to detect PDE-5i in adulterated products and identification of new PDE-5i prevent the spread of these adulterated products. As shown in Table 3, the overall number of detected compounds was 23, where most were in the group of sildenafil or tadalafil as follows: 12 sildenafil analogues (sildenafil, desulfonylchlorosildenafil, descarbonsildenafil, desmethylpiperazinylsildenafil, desmethylpiperazinylpropoxysildenafil, dimethylsildenafil, dimethylthiosildenafil, homosildenafil, hydroxyhomosildenafil, hydroxythiohomosildenafil, imidazosagatriazinone, thiosildenafil), 9 tadalafil analogues (tadalafil, *trans*-tadalafil, bispernortadalafil, chloropretadalafil, cyclopentyltadalafil, demethyltadalafil, dimethyltadalafil, homotadalafil, isopropylnortadalafil), icariin, and yohimbine. Over the past 3 years, sildenafil and tadalafil (65%) were most frequently adulterated in adulterated products, followed by their analogues (30%), showing a pattern similar to the previous reports [33,34]. However the tadalafil analogue of 2016 and sildenafil analogues of 2017 were found more frequently than the original compounds. PDE-5i and their analogues were detected in the adulterated products at concentration ranges of 0.1–726.0 mg/g, 0.1–382.0 mg/g, and 0.1–373.0 mg/g in 2015, 2016, and 2017, respectively.

4. Conclusion

We have developed and validated a UPLC-Q-Orbitrap-MS method for the simultaneous detection of 85 PDE-5i and their analogues in illicit male enhancement products. Specific fragmentation pathways were proposed for sildenafil, tadalafil, and vardenafil analogues that were classified based on common fragment ions. A total of 187 samples, which were advertised to enhance sexual performances, were assessed by combining this analytical method and the proposed fragmentation pathways, with 53% of the adulterants identified in the concentration range of 0.1–726.0 mg/g. Sildenafil were the most frequently adulterated (57%), followed by tadalafil (36%), and others (7%). These results suggest that potential adulterants should be regularly screened to protect public health. This approach offers rapidity and accuracy, which will be useful in the screening of illicit ED products. Moreover, new PDE-5i analogues, which are obtained by chemical modifications of the parent compound, could be identified in adulterated products using specific common ions. These results might be used to allow the reliable and steady monitoring of adulterants in various sample matrixes, thereby promoting food safety and public health.

Conflicts of interest

The authors declare no conflicts of interest.

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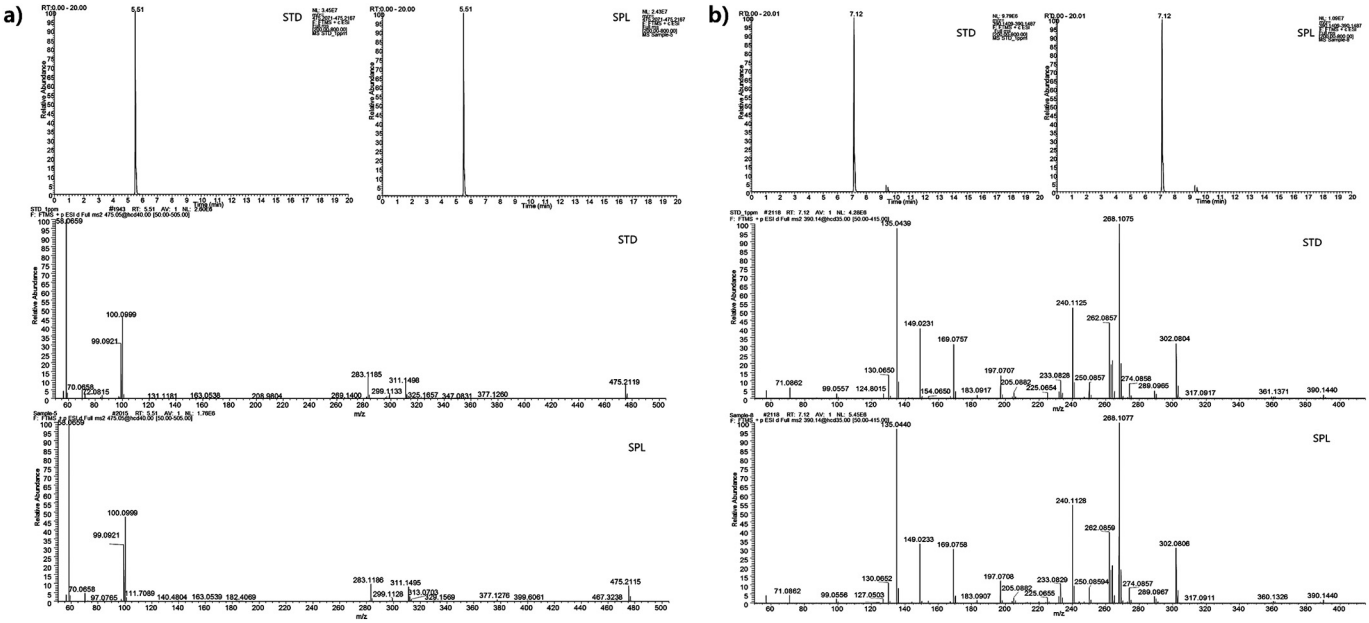

Fig. 4. Extraction chromatogram and MS/MS spectra of sildenafil (a) and tadalafil (b) in real samples.

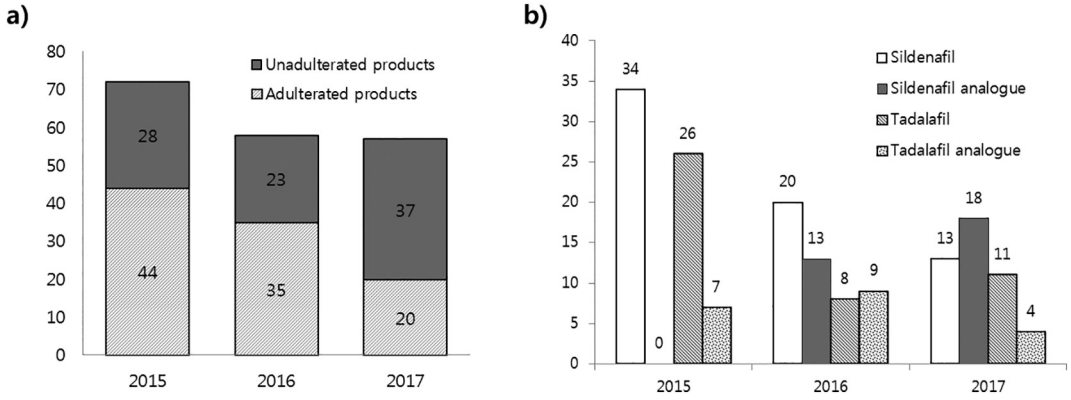

Fig. 5. Number of adulterated and unadulterated male enhancement products among the examined products from 2015 to 2017 (a) and number of detected analogues of the sildenafil and tadalafil groups in adulterated samples (b).

Table 3
Concentration range (mg/g) of PDE-5i and their analogues in adulterated products during 2015–2017.

Group	PDE-5i analogues	2015		2016		2017		Total
		Number of	Conc. (mg/g)	Number of	Conc. (mg/g)	Number of	Conc. (mg/g)	
Sildenafil	Sildenafil	34	0.4–726.0	20	0.1–382.0	13	4.1–373.0	67
	Desulfonylchlorosildenafil	0	–	0	–	2	0.1–0.2	2
	Descarbonsildenafil	0	–	3	0.9–2.7	0	–	3
	Desmethylpiperazinylsildenafil	0	–	0	–	1	1.38	1
	Desmethylpiperazinylpropoxysildenafil	0	–	0	–	1	8.0	1
	Dimethylsildenafil	0	–	3	1.0–1.3	3	0.2–0.3	6
	Dimethylthiosildenafil	0	–	3	55.2–135.0	3	3.1–4.3	6
	Homosildenafil	0	–	0	–	2	0.2–0.3	2
	Hydroxyhomosildenafil	0	–	0	–	3	0.3–0.4	3
	Hydroxythiohomosildenafil	0	–	0	–	3	34.3–75.0	3
	Imidazosagatriazinone	0	–	1	0.1	0	–	1
	Thiosildenafil	0	–	3	2.7–28.1	0	–	3
Tadalafil	Tadalafil	26	0.1–60.0	8	0.2–99.9	11	16.2–23.7	45
	tans-Tadalafil	0	–	0	–	1	0.2	1
	Bisprenortadalafil	1	0.5	0	–	0	–	1
	Chloropretadalafil	2	0.1–2.1	1	0.1	2	0.1–0.2	4
	Cyclopentyltadalafil	0	–	1	1.8	0	–	1
	Demethyltadalafil	1	36.4	1	0.1	0	–	2
	Dimethyltadalafil	0	–	1	6.0	1	0.7	2
	Homotadalafil	0	–	1	3.6	0	–	1
	Isopropylnortadalafil	0	–	4	1.4–7.9	0	–	4
Etc.	Icarrin	1	15.8	9	0.4–16.0	2	1.1–2.3	12
	Yohimbine	0	–	1	6.6	0	–	1
Total		65	0.1–726.0	59	0.1–382.0	48	0.1–373.0	172

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scijus.2019.02.006.

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