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CASE REPORT

CRIMINALISTICS

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Quantitative Analysis of Sildenafil and Tadalafil in Various Fake Drugs Recently Distributed in Korea

ABSTRACT: Illicit distribution of various illicit or counterfeit drugs containing sildenafil and tadalafil has increased and caused noticeable problems in Korea. This study has been performed to determine the content range of sildenafil and tadalafil in various fake drugs. Among the illicit or counterfeit drugs seized by Korean authorities, 105 exhibits were used for the quantification. HPLC–UV analysis of methanol extractions was used for separation and quantitation of the two target compounds. The most abundant type of fake drug was counterfeit Viagra® tablets. Sildenafil was found in 73 exhibits, and tadalafil was found in seven exhibits. Twenty-five exhibits out of the 105 contained both sildenafil and tadalafil. The contents of sildenafil ranged from 4.3 to 453.2 mg; for tadalafil, the range was 2.2–40.4 mg. The proportion of cases of having more than 100 mg of sildenafil was 50% and 78% had more than 20 mg of tadalafil.

KEYWORDS: forensic science, sildenafil, tadalafil, counterfeit drugs, quantitative analysis, high-performance liquid chromatography, Korea

The synthetic phosphodiesterase type-5 (PDE-5)-inhibitor drugs (viz., sildenafil, tadalafil, vardenafil, udenafil, and mirodenafil), which are the constituents of popular brands (viz., Viagra®, Cialis®, Levitra®, Zydena®, and Mvix®), have been used for the treatment for erectile dysfunction in males. There are widespread inclusions of counterfeit or adulterated versions of sildenafil analogues in dietary supplements (1). Recently, most of the illicit preparations or the counterfeits have been sold through illegal sales network and websites. These formulations can be easily bought in these ways. In many cases, counterfeit drugs look identical to the genuine products, but their ingredients are uncertain and carry a potential risk to public health (2–5). Fortunately, it has become possible to detect these drugs as counterfeits by using modern sensitive and selective analytical techniques such as liquid chromatography with tandem mass spectrometry (6), Fourier transforms (FT) with near infrared spectroscopy (7), and FT with Raman spectroscopy (8).

In most cases, illicit or counterfeit drugs are not equivalent in safety, efficacy, and quality to their genuine counterparts. Although the prevalence of counterfeit drugs appears to be increasing and it has been a noticeable problem in Korea, there have been no reports of the concentration of illegally manufactured sildenafil and tadalafil. To determine the content range of sildenafil and tadalafil in various illicit or counterfeit drugs, quantitative analysis of sildenafil and tadalafil was carried out.

Material and Methods

Chemicals

Sildenafil, tadalafil, and oxohongdenafil were provided by the Korea Food and Drug Administration (KFDA, Seoul, Korea). Methanol solutions of concentration 1 mg/mL were prepared for each standard and used as stock solutions for high-performance liquid chromatography (HPLC) analysis. All other chemicals used were of reagent grade and were locally and commercially available.

Seized Materials

All of the sample materials in this experiment were seized by Korean authorities. Each sample was seized from a different dealer or store. It is illegal to sell PDE-5-inhibitor drugs or analogues without permission or medical prescription in Korea, authorities usually request our laboratory to determine whether the seized materials contain PDE-5-inhibitor drugs or their analogues.

Extraction

Isolation of sildenafil and tadalafil in illicit preparations was carried out using a previously reported method (9,10). Samples were weighed, and 50 mg of homogenized powder was taken and extracted with 10 mL methanol by vortexing for 30 min and sonication for 10 min. They were then centrifuged and diluted with 10 mL methanol. An aliquot of this solution (200 μL) was taken and 20 μL of 100 μg/mL oxohongdenafil was added as an internal standard. This mixture was subjected to HPLC analysis. If the concentration of the target compound exceeded the calibration range, the sample was diluted with methanol and analyzed again. The 105 exhibits in this experiment were from different sources containing one or several tablets. The number of tablets seized and requested for the analysis ranged from one to five. If the exhibits contain more than three tablets, we analyzed three tablets to assess the sample's homogeneity. In all cases, each tablet was analyzed three times and the mean value was presented in the experiment.

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TABLE 1—*LOD, LOQ, linearity, precision, and recovery of sildenafil and tadalafil.*

			Linearity				Precision (CVs, %)		Recovery (Mean ± SD [%])	
	LOD (μg/mL)	LOQ (μg/mL)	Linearity Range (μg/mL)	Correlation Coefficient (r^2)	Slope (Mean ± SD)	Intercept (Mean ± SD)	Intra-Day (n = 5)	Inter-Day (n = 5)	Intra-Day (n = 5)	Inter-Day (n = 5)
Sildenafil	0.04	0.2	0.2–100	0.9962	0.0294 ± 0.0071	0.1933 ± 0.0402	5.32	5.69	97.8 ± 5.2	98.3 ± 5.6
Tadalafil	0.05	0.2	0.2–100	0.9976	0.0481 ± 0.0082	0.0968 ± 0.0535	3.86	5.32	98.5 ± 3.8	97.7 ± 5.2

LOD, limit of detection; LOQ, limit of quantitation; CVs, coefficient of variations; SD, standard deviation.

Instrumental Condition

The amount of sildenafil and tadalafil in seized drugs were measured using an HPLC system. The HPLC system was composed of a sample injector (Varian Prostar, Model 410, sample size 20 μL; Mulgrave, Australia), a UV photo-diode array detector (Varian Prostar Model 335), a liquid chromatograph pump (Varian Prostar, Model 230 I), a reverse phase column (Shiseido Capcell pack C_{18}, 250 × 4.6 mm, 5 μm; Tokyo, Japan), and a Galaxie Workstation Ver. 1.8 data processor (Varian Prostar). The HPLC method in this experiment was available in the literature (11).

The mobile phase consisted of acetonitrile and 0.1% phosphoric acid containing 0.1% sodium hexanesulfonic acid. The initial mobile phase composition was 30:70 of acetonitrile and 0.1% phosphoric acid containing 0.1% of sodium hexanesulfonic acid. This composition was maintained for 2 min. The gradient was programmed to linearly increase to 100% acetonitrile at 16 min and held there for 2 min. After that the mobile phase was programmed to return to the initial composition and held for 8 min to equilibrate the system. The flow rate of the mobile phase was set to 1.0 mL/min, and 10 μL of the sample solution was injected with partial loopfill. The analysis was monitored with a diode array detector at 294 and 285 nm for sildenafil and tadalafil, respectively. In all instances, the solutions and the mobile phases were filtered with 0.45 μm filters and degassed in an ultrasonic bath before use.

Validation Procedures

Limit of detection (LOD), limit of quantification (LOQ), linearity of calibration, intra- and inter-day accuracy, precision, and recovery were estimated for the validation of this method. Stock solutions of sildenafil and tadalafil (1 mg/mL) were used for analysis. Working solutions of 100 μg/mL of each compound were made by dilution. Calibration curves were prepared with seven sample concentrations ranging from LOQ to 100 μg/ml for sildenafil and tadalafil in five replicates. The seven concentrations used for calibration curves of each analyte were 0.2, 0.5, 1.0, 5, 10, 50, and 100 μg/mL. Standard curves were obtained by plotting the peak area ratios of sildenafil and tadalafil versus internal standard (oxohongdenafil) against the concentrations of the two drugs. LOD and LOQ were determined by analyzing methanol solution fortified with known drug concentrations. Each concentration was measured in five replicates. LOD was defined as the lowest concentration giving a response of three times the average baseline noise defined from five unfortified samples. LOQ was defined as the lowest observed concentration within 10% of the theoretical concentration for all five replicates. Ten blank drug samples were analyzed for chromatographic interference with each analyte. The extraction recovery and intra-day precision of this method were determined by analyzing 10 μg/mL of sildenafil and tadalafil in five replicates; they were extracted as previously described. Analyzing the target compounds in five different days assessed the inter-day precision and recovery.

Results and Discussion

Sildenafil (Viagra®) was first used for the treatment for erectile dysfunction. Related drugs—tadalafil, vardenafil, udenafil, and mirodenafil—are approved for similar use in Korea. Illegally manufactured counterfeit drugs are usually sold in an illegal market or through an online Internet network. Dosage variability and dosage mislabeling may lead to accidental overdose. An overdose of sildenafil and its analogues may result in hypotension, tachycardia, and cardiac arrest (12).

Several studies reported that using HPLC with UV is good for the determination of PDE-5-inhibitors in herbal dietary supplements, illicit drugs, or counterfeits (3,6). In the present study, HPLC was used for the identification and quantification of sildenafil and tadalafil in various exhibits of illicit drugs. Chromatographic separation was completed within 22.0 min. LOD of sildenafil and tadalafil were 0.04 and 0.05 μg/mL, respectively (Table 1). Calibration curves for the two target compound were prepared. Their correlation coefficients (r^2) were greater than 0.99. In the stability test, bias (%) was <5% in two target compounds (data not shown). Intra- and inter-day precisions are shown in Table 1. CV (%) was <6% in the sildenafil and tadalafil. The extraction recoveries were determined by analyzing 10 μg/mL of sildenafil and tadalafil. Recovery of these drugs was higher than 97% for the two compounds. The concentrations of sildenafil and tadalafil in samples were 0–24.4 and 0–5.5 ug/mL, respectively. If the concentration of the two drugs was below or exceeded the linearity range, it was less or more diluted to meet the range of linearity. The final content of them in the fake drugs was calculated by considering the dilution factor, amount of sampling, and weight of the drugs. These experiments satisfy validation requirements for quantitative methods.

The type of formulation and logo in the 105 seized fake drugs are presented in Table 2. The most abundant type of fake drug was counterfeit Viagra® (VGR100/Pfizer) tablet and the next was C100

TABLE 2—*Formulation, logo, and number of detected ingredients in 105 exhibits of seized drugs.*

Formulation	Tablet					Capsule	Pill	Total
Logo	VGR 100 / Pfizer	C100	C50	C20	C200		–	–
Number of type	47	24	18	8	1	5	2	105
Detected ingredient								
Sildenafil	35	16	13	2	1	4	2	73
Tadalafil	0	0	0	6	0	1	0	7
Sildenafil and tadalafil	12	8	5	0	0	0	0	25

TABLE 3—*Detected number in the upper range of prescription amount: >100 mg of sildenafil and >20 mg of tadalafil.*

Sildenafil		Tadalafil		Sildenafil and Tadalafil			
				Sildenafil		Tadalafil	
Range (mg)	Detected number	Range (mg)	Detected number	Range (mg)	Detected number	Range (mg)	Detected number
>100	36	>20	4	>100	13	>20	21
50–100	27	10–20	3	50–100	10	10–20	2
<50	10	<10	0	<50	2	<10	2
Total	73	Total	7	Total	25	Total	25

The left-side group represents 100 exhibits that had either sildenafil or tadalafil. The right-side group represents 25 exhibits that contained both sildenafil and tadalafil.

TABLE 4—*Concentrations of sildenafil and tadalafil in 25 seized exhibits in which both of them were detected.*

Sample No	Logo	Sildenafil (mg)	Tadalafil (mg)	Sample No.	Logo	Sildenafil (mg)	Tadalafil (mg)
1	VGR100	179.3	40.1	13	C100	126.6	8.6
2	VGR100	178.1	27.5	14	C100	88.0	31.4
3	VGR100	166.1	33.0	15	C100	80.2	30.0
4	VGR100	155.1	39.5	16	C100	63.1	24.2
5	VGR100	151.1	42.2	17	C100	62.3	21.9
6	VGR100	145.9	24.3	18	C100	54.7	20.1
7	VGR100	138.8	20.1	19	C100	52.6	22.9
8	VGR100	128.2	25.5	20	C100	62.3	21.9
9	VGR100	118.5	28.3	21	C50	105.2	21.6
10	VGR100	110.1	27.3	22	C50	58.3	25.6
11	VGR100	103.8	22.0	23	C50	56.7	18.1
12	VGR100	96.3	16.3	24	C50	50.9	20.7
				25	C50	30.3	5.8

and C50. These Cialis® shaped tablets' ingredients were sildenafil alone or sildenafil with tadalafil. In Korea, C100 and C50 are not produced and sold with commercial formulation. Genuine Viagra® tablets contain 100, 50, or 25 mg of sildenafil and Cialis® 20, 10, or 5 mg of tadalafil. Sildenafil was found in 73 exhibits and tadalafil was found in seven exhibits. Twenty-five exhibits contain both sildenafil and tadalafil. Except for the C20 tablets, sildenafil was more frequently found than tadalafil.

The contents of sildenafil and tadalafil in 105 seized exhibits showed large variations in amount. Sildenafil ranged from 4.3 to 453.2 mg per sample with a mean content of 113.2 mg. Tadalafil ranged from 2.2 to 40.4 mg per sample with a mean content of 21.1 mg. The number of exhibits containing the upper range of prescription amount of sildenafil (100 mg) and tadalafil (20 mg) is shown in Table 3. The proportion of cases of having more than 100 mg of sildenafil was 50% and 78% had more than 20 mg of tadalafil. In the present study, only 10.1% of samples labeled "Viagra® 100 mg" was within 10% of the advertised tablet strength. In case of "Cialis® 20 mg," only one of eight tablets was within 10%.

It is noteworthy that 25 exhibits of fake drugs had both sildenafil and tadalafil. The concentrations of both drugs in these 25 cases were shown in Table 4. The maximum content of sildenafil and tadalafil was 179.3 and 40.1 mg, whereas the minimum content of them was 30.3 and 5.8 mg.

Other drugs were also found as ingredients in a few samples including yohimbine, hydroxyhomosildenafil, thiosildenafil, dimethylsildenafil, hydroxyvardenafil, and aminotadalafil. Yohimbine is the principal alkaloid of the bark of the African yohimbe tree and primarily selective for the presynaptic alpha-2 receptor that enhances the central release of norepinephrine, which increases sexual arousal (13). Although the efficacy of yohimbine in sexual function has been questioned, it has been previously used for illegal sexual enhancement drugs. In Korea, 36 PDE-5-inhibitor compounds including five approved drugs have been illegally used for the treatment for erectile dysfunction. In the present study, hydroxyhomosildenafil, thiosildenafil, dimethylsildenafil, hydroxyvardenafil, and aminotadalafil were detected as adulterants.

Recently, illegal distribution of various illicit or counterfeit drugs containing sildenafil and its analogues has increased in Korea. In the present study, we performed the quantification of sildenafil and tadalafil in 105 seized exhibits to evaluate the concentration range of the two drugs in illegal counterfeit drugs. As a result, about 25% of seized counterfeits have both of sildenafil and tadalafil and more than half of the seized counterfeits have larger than the prescription amount of sildenafil and tadalafil.

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