Forensic Science International 298 (2019) 10–19

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Screening of illegal sexual enhancement supplements and counterfeit drugs sold in the online and offline markets between 2014 and 2017

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ARTICLE INFO

Article history:
Received 30 July 2018
Received in revised form 9 February 2019
Accepted 11 February 2019
Available online 22 February 2019

Keywords:
Erectile dysfunction
Illegal products
Liquid chromatography–mass spectrometry
Method validation
Screening

ABSTRACT

The worldwide spread of illegal sexual enhancement products is posing a threat to public health. The aim of this study was to investigate illegal products claiming to be effective in improving sexual performance through the online or offline markets between 2014 and 2017; these products include foods, dietary supplements, counterfeit drugs, and herbal medicines. These samples were analysed by liquid chromatography-tandem mass spectrometry (LC–MS/MS) for the presence of 80 PDE-5 inhibitors (PDE-5i) and analogues. The developed method was validated as follows: LODs and LOQs spiked in solid- and liquid-type negative samples (0.03–3.33 ng/mL and 0.08–10.00 ng/mL), linearities ($R^2 > 0.997$), recoveries spiked negative samples (82.2–109.3 %), accuracies (81.6–118.9 %), precisions ($\geq$ 6.5%, RSD) of intra-day and inter-day, and stability ($\geq$10.0%, RSD). Out of 362 measured samples, 145 were adulterated samples mostly detected in food (51%). Sildenafil group (50%) was frequently observed, followed by tadalafil group (41%). Although sildenafil and tadalafil were mainly detected in adulterated samples, their analogues were also found. In particular, new analogues have appeared steadily on illicit erectile dysfunction (ED) products even after they were first discovered. The concentration of detected samples ranged from 0.1 to 826.0 mg/g, and sildenafil of them contained a considerable amount in illicit ED products in 2014, posing a potential toxicology risk of public health. The testing method is fast and reliable making it suitable for both routine screening and up-to-date quantitative analysis of PDE-5i and their analogues in suspicious foods, dietary supplements, and counterfeit drugs.

1. Introduction

With the increase in erectile dysfunction (ED), a condition frequently observed in males, the need to develop a treatment for ED also increases. According to the Massachusetts Male Aging Study, 52% of men aged 50–70 have suffered ED and more than 70% have been reported in 70 years or older [1]. Most of ED treatments are related to PDE-5i, blocking the degradative action of PDE-5 on cyclic guanosine monophosphate (cGMP) in the smooth muscle cells lining the blood vessels supplying the corpus cavernosum of the penis [2]. Since the approval of the first ED treatment, sildenafil (Viagra™, Pfizer), several PDE-5 inhibitors including tadalafil (Cialis®, Elli Lilly), vardenafil (Levitra® and Staxyn®, Bayer), and avanafil (Stendra® and Spedra®, Vivus Inc.) were approved by United State Food and Drug Safety Administration (USFDA) [3]. In other countries, udenafil (Zydena®, Dong-A Pharmaceutical Co. Ltd), mirodenafil (Mvix®,SK Chemicals Life Science) and lodenafil carbonate (Helleva®, Cristália Produtos Químicos Farmacêuticos) have also been prescribed [4]. Although these drugs were approved for medicinal purposes, misuse or abuse of ED drugs can cause adverse effects including headache, facial flushing, dyspepsia, muscle pain, and retinal disturbance [5,6].

Lately, illegal products containing PDE-5i and their analogues have been largely sold in international pharmaceuticals and dietary supplement markets [7,8] which are easily obtainable on the Internet and the open market. According to the FDA Tainted supplements, more than 95% of the 332 adulterated products were marketed as sexual enhancement products [9]. The survey conducted by the Korea Ministry of Food and Drug Safety reported that the use of ED medicine increased by five times between 2012 to 2016, making it the most frequently used online counterfeit drug. Despite regulations to protect the public from the toxic effects of adulterants in most countries, modification of the chemical structure in designer analogues are slowly emerging. Since the detection of the first synthetic PDE-5i analogue, homosildenafil, in beverages, approximately 80 new analogues

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https://doi.org/10.1016/j.forsciint.2019.02.014
0379-0738/

Table 1
Optimized multiple reaction monitoring (MRM) for the detection of PDE-5i and their analogues by LC–MS/MS.

Compound	Ion Mode	Precursor Ion (*m/z*)	DP (V)	Product Ion (*m/z*)	CE (V)	CXP (V)
Acetylvardenafil	+	467.2	146	151.1	63	12
				341.2	45	12
				127	41	14
Hydroxyvardenafil	+	505.2	176	151	57	12
				312.1	57	24
				299	57	12
Hydroxyhongdenafil	+	483.2	76	127	41	10
				297.2	55	14
				143.1	43	14
Hongdenafil	+	467.2	146	111	39	12
				127.1	41	8
				297.1	55	12
Lodenafilcarbonate	+	505.2	76	487.3	33	16
				283.1	57	10
				129.1	39	8
Aminotadalafil	+	391.1	86	269	29	16
				169.3	47	14
				262.1	47	11
epi-Aminotadalafil	+	391.1	80	269.1	19	15
				262.1	47	11
				169	42	14
Benzylsildenafil	+	551.2	91	377.2	39	16
				134.1	61	20
Mirodenafil	+	532.2	216	296	55	12
				312	59	16
Mutaprodenafil	+	630.2	156	141.9	35	14
				377.1	31	10
				312	47	32
Thiosildenafil	+	491.1	86	299	53	14
				341.2	41	10
				327	41	12
Dimethylthiosildenafil	+	505.2	26	299	51	22
				327	43	22
				113	39	10
Propoxyphenylthio homosildenafil	+	519.2	130	299.1	50	10
				327.1	40	13
				113.2	47	11
Cyclopentyl tadalafil	+	444.3	79	322.1	25	28
				169.1	60	11
				134.9	30	11
Nitrodenafil	+	358.2	71	284.2	43	16
				136.1	63	12
Norneosildenafil	+	460.2	71	166.1	69	12
				136	81	12
Vardenafil	+	489.2	186	151.1	75	14
				312	57	16
Carbodenafil	+	453.2	101	339.2	33	10
				311.1	47	12
				166	59	22
Dimethylacetildenafil	+	467.2	81	325.1	51	16
				127	41	14
				297.2	57	8
Avanafil	+	484.2	95	375.1	36	23
				155.1	53	12
				221.1	37	13
Sildenafil	+	475.1	176	100	37	14
				283	51	12
				311.1	41	12
Homosildenafil	+	489.2	116	112.9	39	12
				283	53	16
				311	43	16
Dimethylsildenafil	+	489.2	131	113.1	39	10
				283.1	55	18
				311.2	43	10
Homosildenafil	+	489.2	116	112.9	39	12
				283	53	16
				311	43	16
Dimethylsildenafil	+	489.2	131	113.1	39	10
				283.1	55	18
				311.2	43	10
Udenafil	+	517.2	71	283.2	61	14
				325.3	51	12
Cyclopentynafil	+	529.2	216	461.2	41	14
				283.1	61	20
Dapoxetine	+	306.1	70	261.2	20	10
				183.2	27	11

Table 1 (*Continued*)

Compound	Ion Mode	Precursor Ion (*m/z*)	DP (V)	Product Ion (*m/z*)	CE (V)	CXP (V)
				157	40	15
Tadalafil	+	390.1	61	268.1	19	10
				168.9	47	14
				134.9	31	12
Xanthoanthrafil	+	390.1	71	151.1	33	18
				107.2	83	12
Pseudovardenafil	+	460.2	96	151.1	63	12
				110.1	97	14
Propoxyphenylthiohydroxy homosildenafil	+	535.2	51	299.1	53	18
				128.9	41	16
				315	55	16
Gendenafil	+	355.1	46	285	43	24
				326.9	35	18
Thioquinapiperifil	+	449.2	126	204.1	35	8
				186.1	55	8
				118	71	16
Desmethylcarbodenafil	+	439.3	130	339.1	35	10
				166.1	60	20
				147.1	55	15
Norneovardenafil	+	357.1	121	151	43	12
				300.1	43	16
Piperidinohongdenafil	+	438.2	211	98.2	41	10
				166.1	67	20
Methylhydroxyhomo sildenafil	+	519.2	126	129.1	43	16
				297.2	53	10
				267.2	75	10
Desulfovardenafil	+	313.1	196	151.1	37	10
				256.1	41	8
Cinnamyldenafil	+	555.2	81	116.9	59	16
				437.2	31	12
				355.2	41	18
trans-Tadalafil	+	390.1	81	268.1	19	12
				169.1	57	14
				135.1	29	12
Hydroxythiohomo sildenafil	+	521.2	191	99.1	41	10
				299.1	55	12
Homotadalafil	+	404.2	11	282	19	10
				204	83	18
				169.1	47	11
Propoxyphenylthio aildenafil	+	519.1	151	299.1	49	10
				113	41	14
				315.1	51	12
Chlorodenafil	+	389.1	136	361.1	37	12
				285	43	12
N-Octylnortadalafil	+	488.3	86	366.3	25	12
				204.2	109	14
Yohimbine	+	355.2	156	144.2	43	10
				212.1	33	12
Demethylhongdenafil	+	453.2	130	113.2	41	12
				297.2	47	11
Oxohongdenafil	+	481.2	141	410.1	43	24
				297.1	57	10
				165	47	14
Icariin	–	675.1	95	513.2	16	15
				367.1	44	11
Hydroxyhomo sildenafil	+	505.1	86	487.2	37	18
				283.1	55	12
				112	39	8
Acetaminotadalafil	+	434.1	136	262.2	45	8
				134.9	27	10
				311	25	12
Demethyltadalafil	+	377.1	81	255	19	16
				204.9	56	14
Diethylaminopretadalafil	+	464.2	1	86.1	43	14
				274.1	57	8
				134.9	37	18
2-Hydroxypropyl nortadalafil	+	434.2	91	312.1	21	8
				135	37	10
				262.1	49	24
Acetil acid	+	357.2	84	329.2	42	11
				300.1	52	22
				256.2	53	9
Thiohomosildenafil	+	505.1	16	299	53	20
				327.1	45	16
				355.1	43	14
Propoxyphenylthio sildenafil	+	505.2	176	299.1	51	10
				315	57	12

Table 1 (*Continued*)

Compound	Ion Mode	Precursor Ion (*m/z*)	DP (V)	Product Ion (*m/z*)	CE (V)	CXP (V)
				271.1	59	12
Hydroxychlorodenafil	+	391.1	130	313.1	47	11
				285.1	47	11
N-Butyltadalafil	+	432.3	41	310	23	20
				134.9	35	16
Imidazosagatriazione	+	313.1	61	285	35	26
				256	43	20
Dichlorodenafil	+	407.1	71	378.9	39	24
				349.9	45	12
Desmethylpiperazinyl sildenafil	+	393.1	100	364.9	37	22
				256.1	49	12
Tadalafil Impurity A	+	350.1	71	264.1	25	16
				333	19	10
				206	47	22
Sildenafil Imputrity A	+	489.1	201	297.2	53	10
				325.1	43	10
				136.1	85	12
Dioxohongdenafil	+	495.3	176	127.1	47	10
				311.2	55	14
Tadalafil Impurity C	+	426.1	121	135.1	37	10
				395	17	20
				276	27	12
trans-Cyclopentyl tadalafil	+	444.2	1	322.2	23	14
				169.2	57	12
				204	87	22
N-Desmethylacetildenafil	+	439.2	146	99.1	39	8
				297.2	53	18
				166	65	10
N-Desethylvardenafil	+	461.1	96	151.1	51	14
				312.2	51	12
				284.1	51	14
Pyrazole *N*-Desmethylsildenafil	+	461.2	101	100.1	35	10
				269.1	53	10
				297.1	43	8
Apixaban	+	460.2	126	443.1	37	14
				199	51	16
Propoxyphenyl sildenafil	+	489.2	136	283.1	51	12
				299	55	18
				164.8	61	14
Sildenafil coupled	+	331.1	55	121.1	39	15
				149	30	12
				314.1	26	14
Bisprenortadalafil	+	766.2	130	360.2	45	11
				334	45	11
				262	60	20
Descarbonsildenafil	+	463.2	131	418.2	43	10
				311.2	53	10
				283.1	37	14
N-Desmethylsildenafil	+	461.2	61	283.1	51	18
				311	41	14
				85	65	10
Papaverine	+	340.1	101	324.1	43	12
				202	37	10
				171	51	14
Isopropylnortadalafil	+	418.1	105	296.2	30	10
				135.2	37	9
Desulfonylchlorosildenafil	+	347.2	130	319.1	38	13
				289.6	51	10
Chloropretadalafil	+	427.1	136	135.1	27	14
				274.2	43	14
Desmethylpiperazinyl propoxysildenafil	+	407.1	91	365.1	35	16
				256.1	49	14
				335.8	53	22

have been identified [10]. These new analogues could pose a significant risk to the public due to the lack of a pharmacological and toxicological profile [11].

Many analytical techniques have been previously reported using high-performance liquid chromatography (HPLC) [12], liquid chromatography–mass spectrometry (LC–MS) [13–15], gas chromatography–mass spectrometry (GC–MS) [16], and nuclear magnetic resonance (NMR) [14,17] for inspection. Among them, LC–MS is widely applied for qualitative confirmation and quantitative determination of PDE-5i and their analogues because of the higher sensitivity and selective separation of target molecules in a complex matrix without an extensive sample preparation [4]. Due to their similarity in chemical structures, most PDE-5i analogues need to be clearly distinguished by their MS/MS spectra. Not only have the new PDE-5i analogues been identified in recent years, but they are also steadily emerging in a variety of

Table 2
LOD, LOQ, linearity and recovery of PDE-5i and their analogues spiked in solid and liquid type samples by LC–MS/MS (n = 3).

No.	Compound	Correlation coefficients (r^2)	LOD (ng/mL)		LOQ (ng/mL)		Conc. (ng/mL)	Recovery (% ± RSD%)	
			Solid	Liquid	Solid	Liquid		Solid	Liquid
1	Acetylvardenafil	0.999	3.33	3.33	10	10	10	98.1 ± 2.2	103.8 ± 3.1
2	Hydroxyvardenafil	0.999	3.33	3.33	10	10	10	91.8 ± 4.2	96.7 ± 1.7
3	Hydroxyhongdenafil	0.999	3.33	3.33	10	10	10	98.9 ± 2.9	98.2 ± 1.1
4	Hongdenafil	0.999	3.33	3.33	10	10	10	99.3 ± 2.7	98.1 ± 0.6
5	Lodenafilcarbonate	0.999	2.5	1	7.5	3	10	99.4 ± 3.4	98.1 ± 2.0
6	Aminotadalafil	0.999	2	2	6	6	10	97.1 ± 3.1	97.5 ± 2.3
7	*epi*-Aminotadalafil	0.999	2	2	6	6	10	100.2 ± 1.8	97.4 ± 2.0
8	Benzylsildenafil	0.999	1	1	3	3	10	99.8 ± 0.2	98.2 ± 1.8
9	Mirodenafil	0.999	1	1	3	3	10	98.7 ± 3.3	99.2 ± 2.5
10	Mutaprodenafil	0.999	0.1	0.1	0.3	0.3	10.2	101.6 ± 1.7	98.7 ± 0.8
11	Thiosildenafil	0.999	0.5	0.5	1.5	1.5	10	85.2 ± 2.2	93.1 ± 0.3
12	Dimethylthiosildenafil	0.999	3.33	3.33	10	10	10	97.9 ± 1.6	98.2 ± 1.2
13	Propoxyphenylthiohomosildenafil	0.999	0.2	0.2	0.6	0.6	10	101.1 ± 2.1	99.0 ± 2.4
14	Cyclopentyltadalafil	0.999	0.1	0.1	0.3	0.3	10.5	87.5 ± 0.8	94.3 ± 0.7
15	Nitrodenafil	0.999	0.1	0.1	0.3	0.3	10	89.0 ± 1.2	94.5 ± 1.3
16	Norneosildenafil	0.998	0.5	0.5	0.15	0.15	10	98.0 ± 3.4	101.1 ± 6.2
17	Vardenafil HCl	0.999	1	1	3	3	10	95.2 ± 1.3	100.2 ± 0.9
18	Carbodenafil	0.999	0.2	0.2	0.6	0.6	10	97.6 ± 0.5	98.7 ± 0.7
19	Dimethylacetildenafil	0.999	0.8	0.3	2.4	0.9	10.1	95.1 ± 0.6	94.3 ± 0.5
20	Avanafil	0.999	0.1	0.1	0.3	0.3	10	95.8 ± 0.3	94.8 ± 0.8
21	Sildenafil	0.999	3.33	3.33	10	10	10	96.1 ± 0.3	95.9 ± 0.7
22	Homosildenafil	0.999	1	1	3	3	10	96.0 ± 0.6	102.0 ± 1.1
23	Dimethylsildenafil	0.999	0.5	0.5	1.5	1.5	10	96.7 ± 0.9	97.3 ± 0.9
24	Udenafil	0.999	0.25	0.2	0.75	0.6	10	97.4 ± 0.7	97.3 ± 0.9
25	Cyclopentylnafil	0.999	0.5	0.5	1.5	1.5	10	96.0 ± 0.6	98.5 ± 0.5
26	Dapoxetine HCl	0.999	0.08	0.05	0.24	0.15	10.6	94.1 ± 0.6	95.0 ± 0.2
27	Tadalafil	0.999	0.5	0.5	1.5	1.5	10	97.1 ± 0.6	96.6 ± 0.3
28	Xanthoanthrafil	0.999	0.5	0.2	1.5	0.6	10	93.6 ± 2.2	94.0 ± 0.9
29	Pseudovadenafil	0.999	0.1	0.1	0.3	0.3	10	96.1 ± 0.5	96.3 ± 1.2
30	Propoxyphenylthiohydrohomosildenafil	0.999	0.2	0.2	0.6	0.6	10	85.6 ± 0.6	91.2 ± 0.7
31	Gendenafil	0.999	0.5	0.5	1.5	1.5	10	96.5 ± 0.7	96.0 ± 0.5
32	Thioquinapiperifil	0.999	1	0.5	3	1.5	10	96.5 ± 2.3	106.5 ± 4.4
33	Desmethylcarbodenafil	0.999	0.1	0.1	0.3	0.3	9.5	102.7 ± 2.3	105.4 ± 9.1
34	Norneovardenafil	0.999	1	1	3	3	10	97.6 ± 1.4	98.8 ± 7.8
35	Piperidinohongdenafil	0.999	0.5	0.5	1.5	1.5	10	100.0 ± 2.1	99.4 ± 6.9
36	Methylhydroxyhomosildenafil	0.999	1	1	3	3	10	99.1 ± 2.2	97.5 ± 8.7
37	Desulfovardenafil	0.999	0.05	0.05	0.15	0.15	10	97.7 ± 2.4	97.7 ± 8.9
38	Cinnamyldenafil	0.999	3.33	3.33	10	10	10	94.8 ± 2.4	94.0 ± 2.6
39	*trans*-Tadalafil	0.999	0.5	0.5	1.5	1.5	10	101.8 ± 2.0	95.4 ± 9.2
40	Hydroxythiohomosildenafil	0.999	3.33	3.33	10	10	10	87.6 ± 2.7	94.8 ± 9.8
41	Homotadalafil	0.999	0.1	0.1	0.3	0.3	10.1	91.5 ± 1.8	97.0 ± 9.0
42	Propoxyphenylthioaildenafil	0.999	0.4	0.4	1.2	1.2	10	92.5 ± 1.9	95.6 ± 9.0
43	Chlorodenafil	0.999	0.1	0.10.	0.3	0.3	10	97.8 ± 1.6	98.3 ± 5.1
44	*N*-Octylnortadalafil	0.999	0.2	0.2	0.6	0.6	10	95.8 ± 1.4	95.9 ± 7.0
45	Yohimbine HCl	0.999	0.2	0.2	0.6	0.6	10	100.6 ± 1.1	102.8 ± 2.0
46	Demethylhongdenafil	0.999	3.33	3.33	10	10	10	92.4 ± 1.0	101.5 ± 0.7
47	Oxohongdenafil	0.999	1	1	3	3	10	93.7 ± 0.4	97.2 ± 0.4
48	Icariin	0.999	0.1	0.1	0.3	0.3	10	97.7 ± 1.6	97.7 ± 0.4
49	Hydroxyhomosildenafil	0.999	3.33	3.33	10	10	10	96.1 ± 0.3	97.7 ± 0.2
50	Acetaminotadalafil	0.999	2	2	6	6	10	94.6 ± 2.3	97.0 ± 0.2
51	Demethyltadalafil	0.999	2	2	6	6	10	95.5 ± 1.1	97.5 ± 0.3
52	Diethylaminopretadalafil	0.999	0.1	0.1	0.3	0.3	10	98.4 ± 0.9	98.7 ± 0.4
53	2-Hydroxypropylnortadalafil	0.999	0.1	0.1	0.3	0.3	10.5	95.3 ± 0.6	97.7 ± 0.4
54	Acetil acid	0.999	0.1	0.1	0.3	0.3	10	101.3 ± 0.9	97.4 ± 0.6
55	Thiohomosildenafil	0.999	3.33	3.33	10	10	10	82.2 ± 0.9	96.8 ± 0.2
56	Propoxyphenylthionsildenafil	0.999	0.4	0.4	1.2	1.2	10	84.1 ± 1.2	98.1 ± 1.0
57	Hydroxychlorodenafil	0.999	0.1	0.1	0.3	0.3	10	95.9 ± 1.1	97.6 ± 0.3
58	*N*-Butyltadalafil	0.999	0.5	0.2	1.5	0.6	10	96.0 ± 1.5	98.1 ± 0.4
59	Imidazosagatriazinone	0.998	0.05	0.05	0.15	0.15	10	96.1 ± 1.1	98.1 ± 2.1
60	Dichlorodenafil	0.999	0.5	0.5	1.5	1.5	10	97.6 ± 0.9	101.8 ± 4.3
61	Desmethylpiperazinyl sildenafil	1	0.4	0.5	1.2	1.5	10.4	103.3 ± 0.1	105.8 ± 0.2
62	Tadalafil impurity A	1	0.1	0.1	0.3	0.3	9.7	107.3 ± 0.1	108.6 ± 0.1
63	Sildenafil impurity A	1	1	1	3	3	10	106.3 ± 0.2	109.3 ± 0.3
64	Dioxohongdenafil	1	0.1	0.1	0.3	0.3	10.5	102.4 ± 0.2	108.2 ± 0.1
65	Tadalafil impurity C	1	0.3	0.5	0.9	1.5	10	102.8 ± 0.1	95.6 ± 0.3
66	trans-Cyclopentyltadalafil	1	0.1	0.1	0.3	0.3	10	95.3 ± 0.7	94.5 ± 0.2
67	*N*-Desmethylacetildenafil	1	2.5	1	7.5	3	9.3	102.9 ± 0.4	94.9 ± 0.4
68	*N*-Desethylvardenafil	1	1	1	3	3	10.1	103.6 ± 0.5	102.3 ± 0.7
69	Pyrazole *N*-Desmethylsildenafil	1	1	1.5	3	4.5	50	105.0 ± 0.5	100.5 ± 0.2
70	Apixaban	1	0.3	0.1	0.8	0.3	9.6	100.7 ± 0.2	98.6 ± 0.0
71	Propoxyphenylsildenafil	1	0.4	0.5	1.2	1.5	9.6	100.6 ± 0.2	98.6 ± 0.2
72	Sildenafil coupled	1	0.1	0.1	0.3	0.3	10.5	103.7 ± 0.4	102.2 ± 0.7
73	Bisprenortadalafil	1	0.5	0.5	1.5	1.5	11	100.3 ± 0.7	100.0 ± 0.5
74	Descarbonsildenafil	1	1	0.5	3	1.5	10.5	96.0 ± 0.6	98.1 ± 1.2

Table 2 (*Continued*)

No.	Compound	Correlation coefficients (r^2)	LOD (ng/mL)		LOQ (ng/mL)		Conc. (ng/mL)	Recovery (% ± RSD%)	
			Solid	Liquid	Solid	Liquid		Solid	Liquid
75	*N*-Desmethylsildenafil	1	1	1	3	3	9.7	100.0 ± 0.1	99.7 ± 0.1
76	Papaverine	0.997	0.1	0.05	0.3	0.15	10.5	110.6 ± 5.5	114.1 ± 3.1
77	Isopropylnortadalafil	0.999	0.1	0.3	0.3	0.9	10.3	92.0 ± 2.7	91.6 ± 3.5
78	Desulfonylchlorosildenafil	0.999	0.03	0.05	0.08	0.15	10.1	95.5 ± 0.9	93.4 ± 3.1
79	Chloropretadalafil	0.999	0.5	0.5	1.5	1.5	10	95.6 ± 0.1	98.9 ± 0.4
80	Desmethylpiperazinylpropoxysildenafil	0.999	0.1	0.3	0.1	0.3	9.2	104.6 ± 3.8	108.2 ± 0.6

illegal ED products. Jeong et al. reported that a new analogue, the propoxyphenyl-linked sildenafil analogue, was detected in illegal products even after they were first identified in 2013 and 2014 [18]. Thus, an LC–MS method should be developed to detect PDE-5i and its analogs in illegal ED products, including new PDE-5i analogs identified up until recently.

In this study, 80 PDE-5i and their analogues, including 11 new analogues identified in our laboratory until 2017, were analyzed by LC–MS [19–27]. The established method was applied to 362 samples from foods, dietary supplements, and counterfeit drugs collected between 2014 to 2017 claiming to be effective in improving sexual performance. Based on our laboratory results from 2009 to 2013 [18,29,30], continuous screening to detect illegal ED analogues in these products is necessary to protect the public from harm.

2. Materials and methods

2.1. Chemical and reagents

The 80 standard compounds 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 Pharmacopeia (Rockville, MD, USA), and Toronto Research Chemicals (North York, ON, Canada). They were classified as the sildenafil group (sildenafil, acetil acid, benzylsildenafil, carbodenafil, cinnamyldenafil, desmethylcarbodenafil, chlorodenafil, dichlorodenafil, cyclopentynafil, demethylhongdenafil, *N*-desmethylacetildenafil, *N*-desmetnylsildenafil, pyrazole *N*-desmethylsildenafil, dimethylacetildenafil, dimethylsildenafil, dioxohongdenafil, gendenafil, homosildenafil, hongdenafil, hydroxychlorodenafil, hydroxyhomosildenafil, imidezosagatriazinone, hydroxyhongdenafil, descarbonsildenafil, lodenafilcarbonate, methylhydroxyhomosildenafil, mirodenafil, mutaprodenafil, nitrodenafil, norneosildenafil, oxohongdenafil, piperidinohongdenafil, desulfonylchlorosildenafil, propoxyphenylsildenafil, sildenafil coupled, sildenafil impurity A, udenafil, desmethylpiperazinyl sildenafil, thiosildenafil, dimethylthiosildenafil, hydroxylthiohomosildenafil, thiohomosildenafil, propoxyphenylthioaildenafil, propoxyphenylthiohomosildenafil, propoxyphenylthiohydroxyhomosildenafil, propoxyphenylthiosildenafil, desmethylpiperazinyl propoxysildenafil), the tadalafil group (tadalafil, *trans*-tadalafil, acetaminotadalafil, aminotadalafil, *epi*-aminotadalafil, *N*-butyltadalafil, chloropretadalafil, demethyltadalafil, cyclopentyltadalafil, *trans*-cyclopentyltadalafil, bisprenortadalafil, diethylaminopretadalafil, homotadalafil, isopropylnortadalafil, 2-hydroxypropylnortadalafil, *N*-octhylnortadalafil, tadalafil impurity A, tadalafil impurity C), the vardenafil group (vardenafil, pseudovardenafil, acetylvardenafil, desulfovardenafil, hydroxyvardenafil, norneovardenafil, *N*-desethylvardenafil) and other groups (icariin, thioquinapiperifil, avanafil, xanthoanthrafil, dapoxetine, yohimbine, papaverine, apixaban). Each standard compound (10 mg) was dissolved in Methanol (10 mL) and appropriately diluted. Methanol (MeOH) and acetonitrile (MeCN) were HPLC grade and supplied by Merck KGaA (Darmstadt, Germany). Formic acid for LC–MS grade was purchased from Sigma-Aldrich (St, Louis, MO, USA). Deionized water (DW) was prepared using 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) before use.

2.2. Sample preparation

A total of 362 samples advertised as enhancing sexual performance between 2014 to 2017 were obtained from the Criminal Investigation Division of Ministry of Food and Drug Safety (MFDS). This division investigates illegal products in Korea collected from various markets including online sites, overseas direct purchases, international postal services, and odd offline stores. The samples consisted of foods, dietary supplements, counterfeit drugs, and herbal medicines in the form of capsules, tablets, powders, films, and liquids. For sample preparation, the samples were first homogenized with a blender. About 1 g of sample content was dissolved in 70% aqueous MeOH (50 mL) and sonicated for 30 min. The extracts were filtered through a polytetrafluoroethylene filter (0.22 μm). Each sample was appropriately diluted with MeOH for LC–MS/MS analysis.

2.3. LC–MS/MS conditions

The experiment was performed using API Triple Quad™ 5500 triple quadrupole mass spectroscopy system (AB Sciex, Framingham, USA) equipped with a Shiseido SP3133 liquid chromatograph (Shiseido, Tokyo, Japan). The flow rate was 0.2 mL min^{-1} and injection volume was 2 μL. The column was a Capcell Pak C_{18} (2.0 × 150 mm, 3 μm, Shishido, Tokyo, Japan) and kept at 40 °C. The mobile phase consisted of 0.1% formic acid in both DW (A) and MeCN (B). The gradient elution profile was programmed as followed: 0.0–2.0 min (A: 90%, B: 10%), 2.0–2.5 min (A: 90-80%, B: 10–20%), 2.5–5.0 min (A: 80%, B: 20%), 5.0–15.0 min (A: 80-50%, B: 20–50%), 15.0–23.0 min (A: 50-10%, B: 50–90%), 23.0–26.0 min (A: 10%, B: 90%) 26.0–26.5 min (A: 10–90%, B: 90–10%), 26.5–30.0 min (A: 90%, B: 10%). The electrospray ionization (ESI) source was operated in positive mode except for Icariin. The MS parameters were as followed: curtain gas (CUR), 30 psi; collision gas (CAD), 9 psi; spray voltage, 5000 V (+) or 4500 V (−); source temperature (TEM), 450 °C (+) or 500 °C (−); ion source gas 1 (GS1), 50 psi (+) or 55 psi (−); ion source gas (GS2), 50 psi (+, −). Each standard solution was separately infused to optimize multiple reaction monitoring (MRM). Their MRM settings are listed in Table 1.

2.4. Method validation

The method was validated with several parameters including the limit of detection (LOD) and limit of quantification (LOQ),

Table 3
Intra- and Inter day and stability assay precision and accuracy for PDE-5i and their analogues by LC–MS/MS (n = 3).

No.	Compound	Conc. (ng/mL)	Intra-day (% ± RSD%)	Inter-day (% ± RSD%)	Stability (RSD%)		
					0h	24 h	48 h
1	Acetylvardenafil	10	113.6 ± 0.4	112.6 ± 1.9	2	0.8	2.6
2	Hydroxyvardenafil	10	110.5 ± 0.7	111.1 ± 1.0	3.6	0.8	5.8
3	Hydroxyhongdenafil	10	110.2 ± 0.1	110.5 ± 0.4	0.1	0.2	0.9
4	Hongdenafil	10	111.3 ± 0.4	111.5 ± 0.8	1.5	1.7	0.1
5	Lodenafilcarbonate	10	107.8 ± 0.6	108.3 ± 0.6	1.6	6.1	4.3
6	Aminotadalafil	10	107.6 ± 0.7	107.7 ± 0.2	1	1.8	2.5
7	*epi*-Aminotadalafil	10	112.3 ± 0.3	112.5 ± 0.6	0.4	0.1	2.8
8	Benzylsildenafil	10	109.4 ± 0.2	109.8 ± 0.3	0.4	0.7	0.3
9	Mirodenafil	10	110.5 ± 1.8	111.0 ± 1.2	0.7	0.8	1.5
10	Mutaprodenafil	10.2	104.4 ± 0.6	105.2 ± 1.3	0.8	3.2	1.5
11	Thiosildenafil	10	109.6 ± 0.6	110.0 ± 0.8	0.4	3.4	0.2
12	Dimethylthiosildenafil	10	111.6 ± 1.0	111.5 ± 0.2	0.8	1.3	0.3
13	Propoxyphenylthiohomosildenafil	10	110.3 ± 1.2	109.8 ± 0.8	0.4	1.8	0.1
14	Cyclopentyltadalafil	10.5	108.8 ± 1.8	108.9 ± 0.6	0.1	0	0
15	Nitrodenafil	10	99.2 ± 0.5	103.1 ± 3.3	0.1	0.6	0.5
16	Norneosildenafil	10	94.0 ± 0.2	93.8 ± 1.6	0	3.6	7.4
17	Vardenafil HCl	10	103.4 ± 0.7	104.3 ± 2.1	0.4	1.8	5.4
18	Carbodenafil	10	103.3 ± 0.4	105.2 ± 2.4	0	3.2	7.2
19	Dimethylacetildenafil	10.1	101.6 ± 0.7	102.9 ± 2.0	0.6	1.5	4.9
20	Avanafil	10	102.3 ± 1.5	103.8 ± 1.9	0.6	2.1	5.2
21	Sildenafil	10	99.8 ± 1.6	94.6 ± 1.9	0.2	2.4	5.9
22	Homosildenafil	10	101.0 ± 0.7	102.7 ± 2.0	0.4	2.4	5.4
23	Dimethylsildenafil	10	98.4 ± 0.3	111.6 ± 2.0	0.1	2.4	5.6
24	Udenafil	10	104.8±0.4	106.3 ± 2.0	0.8	2.2	5.7
25	Cyclopentylnafil	10	100.7 ± 1.0	102.4 ± 2.4	0.1	2.4	5.7
26	Dapoxetine HCl	10.6	106.9 ± 0.6	109.3 ± 2.7	0.6	4.2	7.4
27	Tadalafil	10	97.8 ± 0.8	98.8 ± 1.9	1.3	0.1	2.4
28	Xanthoanthrafil	10	99.9 ± 1.0	101.5 ± 2.5		3.3	5.6
29	Pseudovadenafil	10	108.8 ± 1.1	110.8 ± 2.2	0.9	1.2	3.8
30	Propoxyphenylthiohydrohomosildenafil	10	100.1 ± 0.6	102.0 ± 2.2	0.9	0.4	3.8
31	Gendenafil	10	100.8 ± 1.2	102.9 ± 2.6	0.9	1.6	2
32	Thioquinapiperifil	10	95.4 ± 2.0	93.1 ± 3.4	0.9	1	7
33	Desmethylcarbodenafil	9.5	118.9 ± 2.3	115.2 ± 3.0	1.8	3.5	1.4
34	Norneovardenafil	10	110.4 ± 2.1	106.5 ± 3.9	3.1	3.1	2.4
35	Piperidinohongdenafil	10	108.6 ± 1.4	104.9 ± 3.0	1	2.1	1.4
36	Methylhydroxyhomosildenafil	10	111.1 ± 1.8	106.8 ± 3.5	0.3	1.9	1.6
37	Desulfovardenafil	10	108.3 ± 1.1	104.6 ± 3.2	2.3	1	3
38	Cinnamyldenafil	10	112.2 ± 1.6	107.7 ± 3.6	0.4	0.2	0.2
39	*trans*-Tadalafil	10	110.6 ± 0.4	106.2 ± 3.7	1	0.4	1
40	Hydroxythiohomosildenafil	10	110.3 ± 0.7	107.1 ± 2.6	2	2.2	2.7
41	Homotadalafil	10.1	107.0 ± 1.1	103.0 ± 3.6	2.3	4.5	3.4
42	Propoxyphenylthioaildenafil	10	108.1 ± 0.7	104.6 ± 2.9	0.8	1.5	1.5
43	Chlorodenafil	10	103.8 ± 5.3	97.6 ± 5.7	0.2	3.9	4
44	*N*-Octylnortadalafil	10	99.8 ± 2.2	98.6 ± 1.3	0.3	1	0.1
45	Yohimbine HCl	10	94.0 ± 2.8	103.2 ± 7.8	1.9	0.4	0.2
46	Demethylhongdenafil	10	94.6 ± 2.4	100.7 ± 7.6	0.2	1.1	1
47	Oxohongdenafil	10	92.1 ± 1.0	95.4 ± 3.8	0.2	0.2	0.1
48	Icariin	10	95.8 ± 2.6	99.6 ± 4.1	0.1	1.8	0.3
49	Hydroxyhomosildenafil	10	96.0 ± 0.6	99.8 ± 4.3	0.1	1.8	0.3
50	Acetaminotadalafil	10	100.5 ± 0.2	103.6 ± 3.8	0.1	0.3	0
51	Demethyltadalafil	10	97.5 ± 0.7	100.9 ± 3.7	0.3	0.5	0.6
52	Diethylaminopretadalafil	10	99.0 ± 0.4	102.6 ± 3.9	0.3	0.1	0.3
53	2-Hydroxypropylnortadalafil	10.5	94.8 ± 0.8	98.3 ± 3.4	0.5	0.2	0.3
54	Acetil acid	10	100.7 ± 0.7	104.4 ± 3.3	0.3	0.1	0.1
55	Thiohomosildenafil	10	97.1 ± 0.5	99.9 ± 3.1	1	1.7	1.1
56	Propoxyphenylthionsildenafil	10	94.9 ± 0.8	98.3 ± 4.2	0.7	2.6	1.5
57	Hydroxychlorodenafil	10	97.6 ± 0.5	100.6 ± 3.6	0.1	0.1	0.6
58	*N*-Butyltadalafil	10	94.2 ± 3.0	97.7 ± 3.3	6	6.7	6.6
59	Imidazosagatriazinone	10	99.0 ± 1.0	103.7 ± 6.5	10	0.1	1.9
60	Dichlorodenafil	10	100.4 ± 3.6	100.3 ± 4.3	6	5.3	6.4
61	Desmethylpiperazinyl sildenafil	10.4	92.4 ± 0.3	93.8 ± 1.8	0.3	0.9	0.1
62	Tadalafil impurity A	9.7	90.3 ± 0.1	90.6 ± 1.0	0.2	1.2	1.2
63	Sildenafil impurity A	10	92.7 ± 0.6	93.7 ± 1.2	0.8	1.1	0.8
64	Dioxohongdenafil	10.5	91.3 ± 0.4	92.5 ± 1.5	0	1	0.1
65	Tadalafil impurity C	10	109.0 ± 1.4	112.0 ± 2.9	0.2	1.5	0.5
66	*trans*-Cyclopentyltadalafil	10	82.0 ± 1.9	81.3 ± 1.6	1	0.1	0
67	*N*-Desmethylacetildenafil	9.3	104.8 ± 0.2	106.7 ± 2.2	0.3	1.7	0.3
68	*N*-Desethylvardenafil	10.1	109.3 ± 0.6	112.3 ± 2.4	0.3	1.1	1
69	Pyrazole *N*-Desmethylsildenafil	10	107.6 ± 4.4	111.0 ± 3.6	0.1	2.4	2.9
70	Apixaban	9.6	84.9 ± 0.6	88.7 ± 4.4	1.4	2.4	2.9
71	Propoxyphenylsildenafil	9.6	87.9 ± 0.6	91.3 ± 4.1	1.4	2.2	2.7
72	Sildenafil coupled	10.5	85.5 ± 0.5	89.2 ± 4.5	1.2	2.3	2.4
73	Bisprenortadalafil	11	85.0 ± 0.3	88.6 ± 4.4	1.4	2.2	2.5
74	Descarbonsildenafil	10.5	86.3 ± 2.1	88.5 ± 2.3	0.4	0.1	0.3

Table 3 (*Continued*)

No.	Compound	Conc. (ng/mL)	Intra-day (% ± RSD%)	Inter-day (% ± RSD%)	Stability (RSD%)		
					0h	24 h	48 h
75	*N*-Desmethylsildenafil	9.7	92.7±0.4	93.3±0.6	1.3	2.1	2.8
76	Papaverine	10.5	97.6 ± 0.3	98.7 ± 1.1	2.2	1.9	2.7
77	Isopropylnortadalafil	10.3	81.6 ± 1.4	82.4 ± 1.9	0.6	0.7	1
78	Desulfonylchlorosildenafil	10.1	84.7 ± 2.3	88.5 ± 4.7	1.9	3.6	1.7
79	Chloropretadalafil	10	95.1±1.0	99.3±3.9	0.1	2.3	6.3
80	Desmethylpiperazinylpropoxysildenafil	9.2	108.3 ± 1.8	107.9 ± 1.7	0.2	0.2	0.2

linearity, recovery, precision, accuracy, and stability. Each validation factor was investigated based on triplicate measurements. The quantitative ion (most sensitive daughter ion) of compounds was used for validation. LODs and LOQs in solid- and liquid-type negative samples spiked into 80 compounds, determined by the signal-to-noise ratio (S/N), were more than approximately 3 and 10, respectively. Linearity was evaluated using a series of peak areas of 6 points with LOQ set to the lowest concentration. Recovery was calculated by comparing the response of the extract of solid- and liquid-type negative samples with the response of the target compounds dissolved in a pure solvent. Precision was determined by calculating the relative standard deviation (RSD). Accuracy was evaluated by comparing the average concentration calculated using linear equation with theoretical concentration. Inter-day precision was confirmed with three replicates on three different days, while intra-day precision was done in single day. Stability test was performed by storing for 6 h at room temperature and then 24 h and 48 h at 4 °C.

3. Results and discussion

3.1. Method validation

LOD and LOQ, shown in Table 2, were in ranges of 0.03–3.33 ng/mL (LOD) and 0.08–10.00 ng/mL (LOQ) in solid- and liquid-type negative samples. For linearity factors, also shown in Table 2, all correlation coefficients (r^2) were higher than 0.997 which showed a good linear relationship. The recoveries in solid- and liquid-type negative samples ranged from 82.2 to 105.0% and from 91.2 to 109.3%, respectively, as shown in Table 3. These results provided excellent extraction efficiency and low matrix effect in the presented method. Accuracy, precision, and stability were shown in Table 3. In intra-day, the accuracy was 81.6–118.9% and precision was less than 5.3%. In inter-day, the accuracy was 82.4–115.2% and precision was within 6.5%. These results indicated acceptance values for method validation. Stability was observed at the different storage times and temperatures in triplicate. After storing at room temperature for 6 h, RSD was within 10.0%. After 24 h and 48 h at 4 °C, RSDs were within 7.4% indicating no significant degradation.

3.2. Analysis of samples

A total of 362 illegal products, advertised as enhancing sexual performance, were screened using our validated LC–MS/MS method. Samples were collected from online and offline markets between 2014 and 2017. They consisted of foods (241), dietary supplements (58), counterfeit drugs (52), and herbal medicines (11). As seen in Fig. 1, about 40% of all samples were adulterated, and two years (2015 and 2016) were frequently adulterated over 60%. Of adulterated samples, foods (51%) were mostly detected followed by the counterfeit drugs (32%), dietary supplements (12%), and herbal medicines (6%) in Table 4. However, detected to undetected ratios of each type were accounted for in foods (31%; n = 74/241), dietary

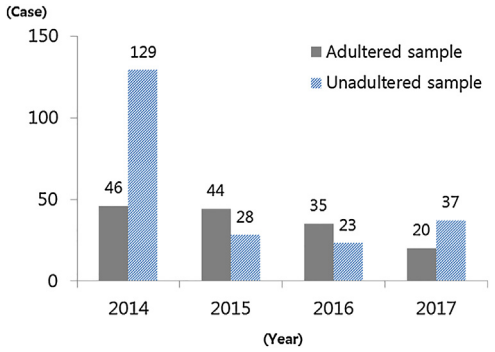

Fig. 1. Number of adulterated and unadulterated samples from 2014 to 2017 (n = 362).

supplements (29%; n = 17/58), counterfeit drugs (88%; n = 46/52), and herbal medicines (72%; n = 6/11). It implied that pharmaceutics and herbal medicines related on ED treatments are likely to be significantly counterfeited. Sildenafil group constituted a half of the adulterants followed by tadalafil group (41%). Similarly, recent reports in Malaysia (2014–2016) and France (2014) showed that the presence of sildenafil group was much higher than any other group [17,28]. Although majority of detected compounds are therapeutic ingredients, sildenafil (68%) and tadalafil (58%) as shown in Fig. 2a and b, their analogues were also detected which includes sildenafil analogues (acetil acid, carbodenafil, desulfonylchlorosildeilafil, desmethylpiperazinylsildenafil, dimethylsildenafil, dimethylthiosildemafil, descarbonsildenafil, desmethylpiperazinylpropoxysildenafil, hongdenafil, homosildenafil, hydroxyhomosildenafil, hydroxythiohomosildenafil, imidazosagatriazinone, thiosildenafil) and tadalafil analogues (tadalafil, aminotadalafil, bisprenortadalafil, *trans*-tadalafil, chloropretadalafil, cyclopentyltadalafil, *trans*-cyclopentyltadalafil, demethyltadalafil, dimethyltadalafil, homotadalafil, isopropylnortoadalafil), implying that modification of designer analogues from the original structure was purposefully adulterated in a variety of products to avoid inspection of authorities. These results are similar to a previous report by Lee et al. stating that from 2009 to 2012, sildenafil and tadalafil were primarily detected in comparison to their analogues [30]. Six analogues (homotadalafil, cyclopentyltadalafil, *trans*-cyclopentyltadalafil, bisprenortadalafil, isopropylnortadalafil, desmethylpiperazinyl propoxysildenafil) were detected in illicit ED products even after they were first identified from 2014 to 2017. Of them, homotadalafil (13%) were the most detected analogue in tadalafil group. Fig. 2c showed that Icariin (8%), a type of flavonoid which should not be used as food materials, was the third of the detected compounds, indicating that natural ED ingredients in illegal products should be screened. All compounds detected in the adulterated samples have diverse concentrations in ranges of 0.1–826.0 mg/g (2014), 0.1–726.0 mg/g (2015), 0.1–382.0 mg/g (2016), and 0.1–373.0 mg/g (2017). The amounts ranged from 0.1 to 826.0 mg/g in sildenafil group, 0.1–139.0 mg/g in tadalafil group, 0.6–0.8 mg/g in vardenafil group, and 0.1–16.0 mg/g in others. Among these, sildenafil had the highest

Table 4
Detected number of PDE-5i and their analogues in four-type samples advertised enhancing sexual performance during 2014–2017.

Sample type	2014		2015		2016		2017		Total
	Detected	Not detected	Detected	Not detected	Detected	Not detected	Detected	Not detected	
Food	38	112	21	22	14	15	1	18	241
Dietary supplement	2	14	0	6	11	6	4	15	58
Counterfeit drug	6	2	21	0	4	2	15	2	52
Herbal medicine	0	1	2	0	6	0	0	2	11
Total	46	129	44	28	35	23	20	37	362

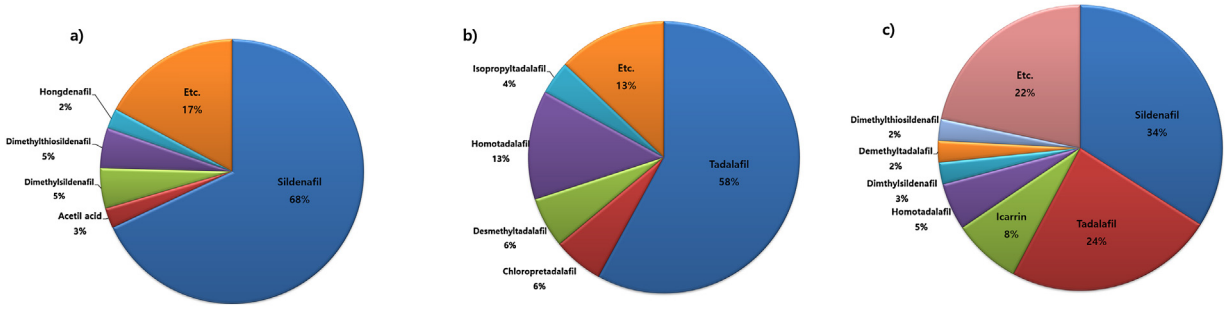

Fig. 2. Classification of erectile dysfunction (ED) compounds detected as adulterated samples: (a) sildenafil group, (b) tadalafil group, and (c) total compounds.

content (826.0 mg/g) in 2014. The results from high concentration ranges in samples, shown in Table 5, suggest that adulterated products should be regularly screened to protect public from potential toxicology risk.

4. Conclusion

In this study, detection of 80 PDE-5i analogues in 362 illegal ED products was performed through LC–MS analysis. Out of the 145 studied samples, a significant level of adulteration was found in food. The major adulterated compounds found in illicit ED products are sildenafil followed by tadalafil with traces of PDE-5i analogues and natural ED ingredients in various samples. Detection of the new PDE-5i analogues in illicit ED products continues to increase even after they were first discovered from 2014 to 2017 indicating the use of these analogues in ED products as a measure to avoid inspection from authorities.

Table 5
Concentration range (mg/g) of PDE-5i and their analogues in adulterated products during 2014–2017.

PDE-5i analogues	2014		2015		2016		2017		Total
	Number of	Conc. (mg/g)	Number of	Conc. (mg/g)	Number of	Conc. (mg/g)	Number of	Conc. (mg/g)	
Sildenafil	16	1.0–826.0	34	0.4–726.0	20	0.1–382.0	13	4.1–373.0	83
Acetil acid	3	0.3–1.3	0	–	0	–	0	–	3
Carbodenafil	2	0.1	0	–	0	–	0	–	2
Desulfonylchlorosildenafil	0	–	0	–	0	–	2	0.1–0.2	2
Desmethylpiperazinylsildenafil	0	–	0	–	0	–	1	1.38	1
Desmethylpiperazinylpropoxysildenafil	0	–	0	–	0	–	1	8.0	1
Descarbonsildenafil	0	–	0	–	3	0.9–2.7	0	–	3
Dimethylsildenafil	0	–	0	–	3	1.0–1.3	3	0.2–0.3	6
Dimethylthiosildenafil	0	–	0	–	3	55.2–135.0	3	3.1–4.3	6
Hongdenafil	3	33.0–39.0	0	–	0	–	0	–	3
Homosildenafil	0	–	0	–	0	–	2	0.2–0.3	2
Hydroxyhomosildenafil	0	–	0	–	0	–	3	0.3–0.4	3
Hydroxythiohomosildenafil	0	–	0	–	0	–	3	34.3–75.0	3
Imidazosagatriazinone	0	–	0	–	1	0.1	0	–	1
Thiosildenafil	0	–	0	–	3	2.7–28.1	0	–	3
Tadalafil	13	0.3–34.5	26	0.1–60.0	8	0.2–99.9	11	16.2–23.7	58
tans-Tadalafil	0	–	0	–	0	–	1	0.2	1
Aminotadalafil	2	3.3–4.9	0	–	0	–	0	–	2
Bisprenortadalafil	0	–	1	0.5	0	–	0	–	1
Chloropretadalafil	2	0.1–9.2	2	0.1–2.1	0	–	2	0.1–0.2	6
Cyclopentyltadalafil	3	136.0–139.0	0	–	1	1.8	0	–	4
trans-Cyclopentyltadalafil	3	4.0	0	–	0	–	0	–	3
Demethyltadalafil	4	0.1–9.2	1	36.4	1	0.1	0	–	6
Dimethyltadalafil	0	–	0	–	1	6.0	1	0.7	2
Homotadalafil	12	0.1–128.0	0	–	1	3.6	0	–	13
Isopropylnortadalafil	0	–	0	–	4	1.4–7.9	0	–	4
Vardenafil	2	0.6-0.8	0	–	0	–	0	–	2
Yohimbine	0	–	0	–	1	6.6	0	–	1
Icarrin	7	0.1-5.4	1	15.8	9	0.4–16.0	2	1.1–2.3	19
Total	72	0.1–826.0	65	0.1–726.0	59	0.1–382.0	48	0.1–373.0	244

This study demonstrates that adulterated samples containing considerable amounts of PDE-5i and their analogues pose a significant risk to consumers' health due to the lack of labelling on packages and increased exposure to their side effects. Therefore, routine monitoring for adulterated products by the current LC–MS/MS method will potentially help with the protection of public health.

Acknowledgements

This research was supported by research grants (15181MFDS521&MFDSAAT2018) from the Ministry of Food and Drug Safety (MFDS) in Korea.

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