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A novel vardenafil analog in a healthcare product: Preparation, characterization, and quantification

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ABSTRACT

Objective: This study is aimed to develop a qualitative and quantitative method to detect a novel vardenafil analogue from a healthcare product, which is claimed to enhance sexual function. ***Method*:** The unknown compound was detected by non-targeted screening using ultra-high performance liquid chromatography-quadrupole-time-of-flight mass spectrometry (UPLC-Q-TOF-MS). MS^2 spectra showed that the characteristic fragment ions of this unknown compound were highly similar to those of vardenafil. This compound was subsequently isolated by silica gel column chromatography and characterized by 1D (dimension) and 2D nuclear magnet resonance (NMR) specta, ultra-violet (UV) spectra, and fourier transform infrared (IR) spectra. A quantitative method for analyzing this identified compound in various healthcare product was developed based on high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS).

Results: The unknown compound was identified as 2-(5-((4-ethylpiperazin-1-yl)sulfonyl)-2-propoxyphenyl)-5-methyl-7-propylimidazo.[5,1-*f*] [1,2,4]triazin-4(3*H*)-one based on the spectroscopic data. Quantitative results revealed that the matrix calibration curves of this compound had a good linear ranges of 2 ~ 50 ng/mL in pressed candy (R^2 = 0.998), energy coffee (R^2 = 0.999), and health wine (R^2 = 0.997), respectively. The matrix effects, recoveries, and limit of quantitation (LOQ) of this compound all met the requirements of quantitative validation. Finally, the content of this compound in 5 batches of positive samples ranged from 1.24 to 7.20 g/kg. ***Conclusion*:** This study identified a novel vardenafil analog from a healthcare product and named it *O*-propyl vardenafil, and this compound was distinguished from vardenafil by the replacement of the ethyl group with a propyl group at the aryl alkyl ether moiety. Our developed quantitative method could meet practical needs. The high positive rate (16.67%) in 30 samples suggested that the related regulators should be alert to *O*-propyl vardenafil in routine test since it has not been detected ever before.

1. Introduction

Phosphodiesterase-5 (PDE-5) inhibitors, including sildenafil, vardenafil, tadalafil, and avanafil, have been mostly recommended as

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first-line prescription drugs for the treatment of male erectile dyfunction (ED) in many areas [1,2]. In the last few decades, the structurally modified PDE-5 analogs were persistently detected in powder [3–6], tablets [7,8], capsules [9–11], herbal supplement [12,13], beverages [14], and so on [1], either explicitly or implicitly claiming the effect of tonifying the kidney and anti-impotence. However, with the strengthening supervision, the conventional illegal additives have been gradually eliminated and replaced with some synthetic analogues or derivatives of existing drugs to evade national supervision. Since safety, efficacy, and metabolism of these novel analogues or derivatives have not been evaluated, there will be a serious security risk if customers who unknowingly consume those unapproved pharmaceutical ingredients [15,16]. Hence, identification and detection of PDE-5 inhibitor analogs from healthcare product are of great importance for regulatory agencies to provide technical means to combat the addition of new illegal additives in food.

Until today, more than 100 PDE-5 analogues have been identified, from which vardenafil analogs account for about 10% [17]. This study reported a new vardenafil analog isolated from a dietary supplement by routine screening. The structure of this unknown compound was characterized by UPLC-Q-TOF-MS, 1D and 2D NMR, UV, and IR spectra. The new illegally adulterant, simply named *O*-propyl vardenafil, bears a propoxy group at the aryl alkyl ether moiety of vardenafil, which may lead to miss detection since it has not been detected ever before. Furthermore, a method for quantitative analysis of *O*-propyl vardenafil in healthcare product was developed, and it was used to test 30 commercial foods available on Chinese online market.

2. Materials and methods

2.1. Materials and chemicals

Vardenafil standard (99.7% purity) was purchased from National Institutes for Food and Drug Control (Beijing, China), and its stock solution was prepared with methanol (MeOH) at a concentration of approximately 500 ng/mL and stored in a refrigerator at 4 °C. HPLC-grade acetonitrile (MeCN) and MeOH were purchased from Thermo Fisher Scientific (Shanghai, China), and 0.22 μm PTFE-membrane filter was obtained from FTSCI Science and Technology Co., Ltd (Hubei, China). Formic acid (for UPLC/LC-MS) was supplied by CNW Technologies GmbH (Düsseldorf, Germany). The ultrapure water was generated from a Milli-Q system (Billerica, MA, USA). Petroleum ether, ethyl acetate, dichloromethane, and triethylamine obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) in this study were of analytical grade. DMSO-d_6 with 99.8% deuteration degree was obtained from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA, USA). Pressed candy, energy coffee, and health wine without any information of authorized pharmaceutical ingredients, were purchased from Chinese online shopping and analyzed in the laboratory.

2.2. Sample pretreatment and high-resolution mass (HRMS) screening

In the initial screening step, the analyte was extracted from 1 g pressed candy with 50 mL of MeOH through ultrasound-assisted extraction. The extracts were injected to HRMS system after filtrating through a 0.22 μm PTFE-membrane filters.

HRMS analysis was performed on Q-TOF (AB SCIEX, Framingham, MA, USA) coupled with an ExionLC Series UHPLC, with an ESI ion source in positive mode. The HRMS spectral data were acquired by Analyst version 1.8.1, recorded by PeakView@ version 2.2, and displayed by Library View. The sample separation was performed by an ACQUITY UPLC@BEH C18 column (2.1 × 75 mm, 2.1 μm; Waters Corporation) with column incubator maintained at 40 °C. Phase A was 0.1% formic acid in water, and phase B was MeOH with a flow rate of 300 μL/min. After 2-min equilibration, sample solution was injected to the column with a loading volume of 5 μL. The continuous gradient elution program was as follows: phase A 90%, 0–1.0 min; A 90–75%, 1.0–15.0 min; A 75%, 15.0–17.0 min; A 75–90%, 17.0–17.1 min; A 90%, 17.1–20.0 min.

The MS parameters were as follows: gas temperature, 500 °C; ion source temperature, 550 °C; curtain gas pressure, 241 316 Pa; auxiliary gas pressure, 344 738 Pa; nebulizer pressure, 344 738 Pa; capillary voltage, 5.5 kV; decluster voltage, 60 V; and fragmentor voltage, 10 V. The mass range (m/z) of both full MS scan and full MS/MS scan was from 50 to 1000 Da.

2.3. TLC and HPLC analysis

Thin Layer Chromatography (TLC) was performed on a 0.20 mm Kieselgel 60 F254 TLC plates (Merck, Darmstadt, Germany). The developed TLC plates were observed on ZF-20D ultra-violet analyzing equipment (Shanghai, China) under UV light at 254 nm.

HPLC analysis was performed using a 2695 series HPLC system with a diode-array detector (Waters Corporation, Milford, MA, USA). Separation and purification were conducted using an ACQUITY UPLC@BEH C18 (1.7 μm, 2.1 × 100 mm) column. The mobile phase A was water solution containing 0.7% phosphoric acid and 0.7% triethylamine, and mobile phase B was MeCN. The continuous gradient elution program was as follows: phase A 80%, 0–15.0 min; A 45%, 15.0–17.0 min; A 45%–80%, 17.0–17.1 min and A 80%, 17.1–19.0 min. The flow rate was 0.3 mL/min, and the injection volume was 5 μL. The column temperature was maintained at 35 °C. The wavelength of the ultraviolet ranged from 190 to 400 nm, and the peaks were monitored at 254 nm.

2.4. Isolation and preparation of reference material

About 100 g pressed candy containing this unknown compound was dissolved in 200 mL MeOH meanwhile stirring for 40 min at room temperature with a magnetic stirrer apparatus. After being centrifuged at 4000 rpm for 5 min, the extracts were filtered by a büchner funnel and evaporated by a rotary evaporator. The crude extracts were eluted from silica gel chromatography column with

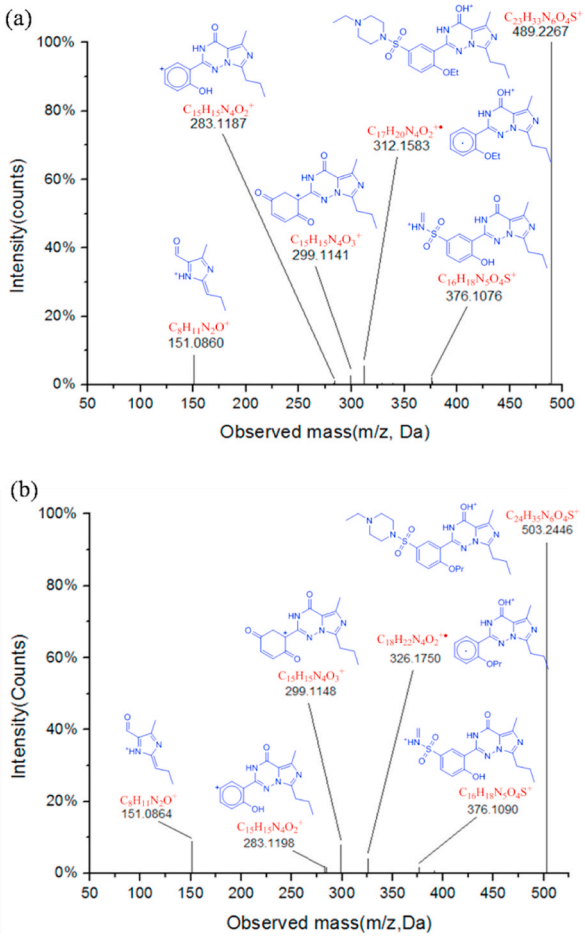

Fig. 1. Q-TOF/MS^2 spectra of (a) vardenafil and (b) *O*-propyl vardenafil.

dichloromethane-triethylamine (100:1, v/v) to obtain the unknown compound. Further recrystallisation was conducted to prepare the purified compound (about 500 mg, white powder).

2.5. IR and NMR analysis

IR analysis was performed on a vertex70 IR fourier spectrometer (Bruker, German). The IR data were acquired and recorded over the spectral range of 4000 cm^{-1} to 400 cm^{-1} with an optical resolution of 4 cm^{-1}.

Vardenafil and this unknown compound were dissolved in DMSO-d_6 and analyzed by ^{1}H NMR, ^{13}C NMR spectra, distortionless enhancement by polarization transfer (DEPT), heteronuclear multiple-bond correlation (HMBC), heteronuclear single quantum coherence (HSQC), and ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY) on a Bruker AVIII 600 MHz FT-NMR spectrometer. Chemical shift (δ) was expressed as ppm and coupling constant (J) was expressed as Hz with solvent peak used as a reference.

2.6. Quantitative analysis

2.6.1. Preparation of standard solution

Ten mg of the reference material (section 2.4) was dissolved in 10 mL MeCN to prepare the standard stock solution (1000 μg/mL) of the identified compound. The standard stock solution was then diluted to 1 μg/mL with MeCN for spiking. Standard solutions (2, 5, 10, 20, and 50 ng/mL) were prepared by diluting the standard stock solution with MeCN for plotting external solvent-calibration curves.

2.6.2. Conditions of LC-MS/MS

The mass spectrometric analysis for *O*-propyl vardenafil was performed at multiple reaction monitoring (MRM) mode on a triple quadrupole mass spectrometer (Waters Corporation, Milford, MA, USA) equipped with an electrospray ionization source running in positive mode. The chromatographic separation was conducted with an ACQUITY UPLC (Waters Corporation, Milford, MA, USA) C18 column (2.1 mm × 50 mm , 1.7 μm). The column temperature was maintained at 35 °C with a flow rate of 0.3 mL/min. The mobile

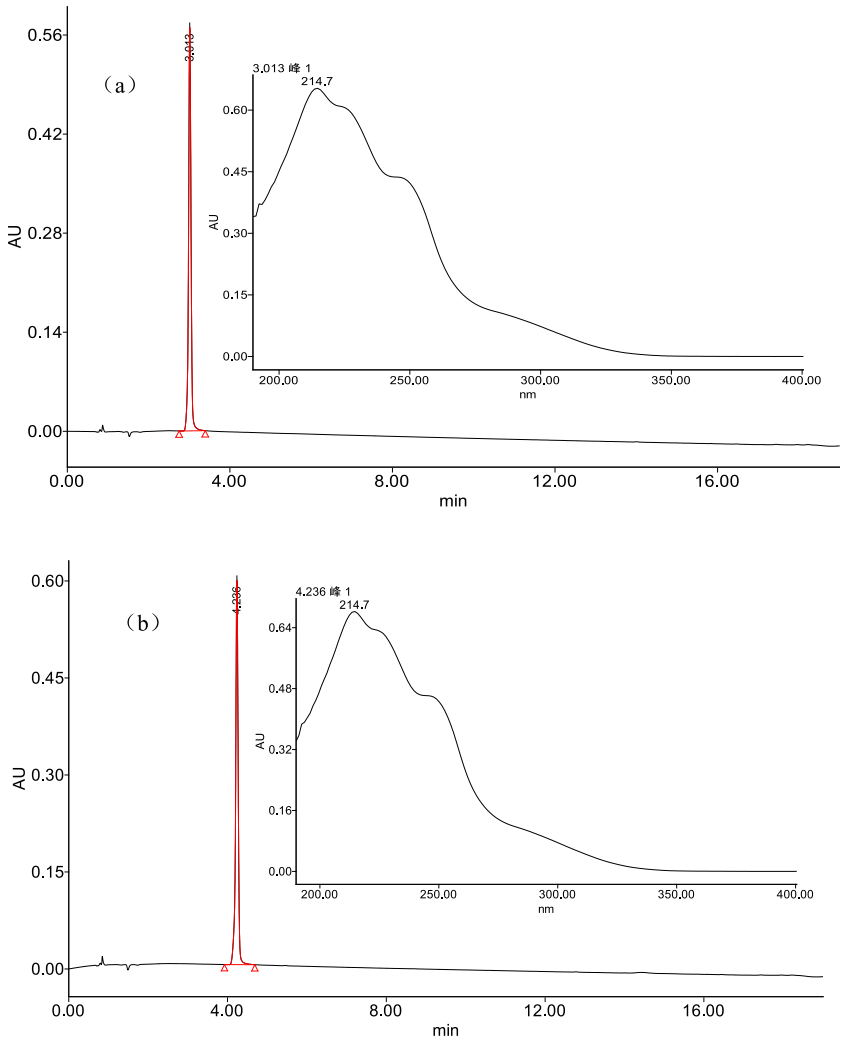

Fig. 2. Ultra-performance liquid chromatogram recorded at 230 nm and UV spectra in the region of 200∼400 nm of (a) vardenafil and (b) *O*-propyl vardenafil.

phase A was water solution containing 0.1% formic acid and phase B was MeCN. The continuous gradient elution program was as follows: 90% phase A for 0.5 min, decrease to 5% phase A from 0.5 min to 1.5 min and maintaining it at the status for 1.5 min, then increase to 90% phase A within 0.1 min and maintaining it for 1.9 min, followed by a 2 min equilibration. Sample loading volume was 2 μL. The optimized mass spectroscopy parameters were as follows: capillary voltage, 2.5 kV; ion-source temperature, 150 °C; gas temperature, 450 °C; nebulizer flow rate, 650 L/h; cone voltage, 100 V; and collision energy, 44 eV.

2.6.3. Calibration curves and matrix effects

Quantification values of *O*-propyl vardenafil in healthcare product were calculated from external solvent-calibration curve. Blank solutions were obtained by filtering 3 kinds of product (pressed candy, energy coffee, and health wine) used in the addition recovery test in which *O*-propyl vardenafil was not detected. Matrix-matched standards at 5 concentrations (2, 5, 10, 20, and 50 ng/mL) were prepared by adding 500 μL of standard solution (4, 10, 20, 40, and 100 ng/mL) into 500 μL of blank solutions. The matrix effects were calculated as the ratio of the matrix-matched standard calibration slope to the external solvent-calibration slope.

2.6.4. Validation of quantitative method

The quantitative method for the identified compound was validated by examining the repeatability, recovery, limit of quantification (LOQ), and accuracy. Representative matrices including pressed candy, energy coffee, and health wine, were used to test the applicability of the proposed method by evaluating the recoveries and relative standard deviation of repeatability (RSD) of the identified compound in these healthcare product. The LOQ of the method were evaluated by signal versus noise (S/N) values of 10:1.

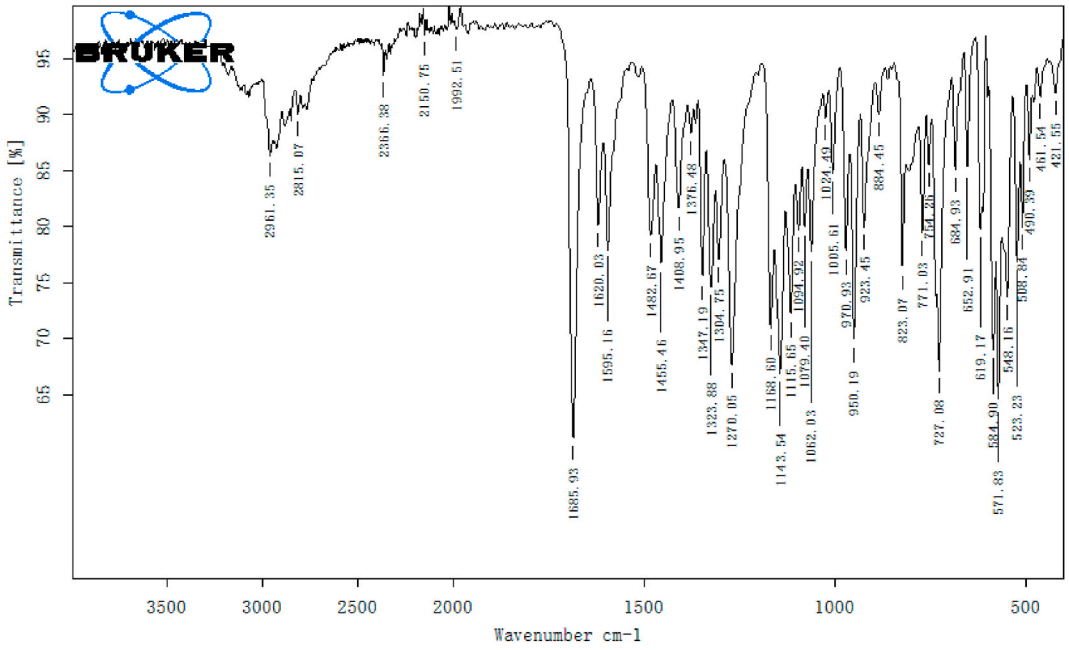

Fig. 3. IR spectra of *O*-propyl vardenafil.

3. Results and discussion

3.1. HRMS analysis

The accurate mass of this unknown compound was determined as *m/z* 503.2446 ($[M+H]^+$) by HPLC-Q-TOF/MS, while that of vardenafil was *m/z* 489.2267 ($[M+H]^+$). As shown in Fig. 1a, the characteristic fragment ions of vardenafil showed *m/z* ($[M+H]^+$) values of 376.1076 , 299.1141, 283.1187, and 151.0860, and these results agreed well with those reported by Zou [18]. The fragmentation ions of this unknown compound showed the *m/z* ($[M+H]^+$) values such as 376.1090, 299.1148, 283.1198, and 151.0864, which were extremely similar to those of vardenafil (Fig. 1b). The molecular formula of this unknown compound was matched to $C_{24}H_{34}N_6O_4S$ with a matching score of 99.5%. Therefore, this unknown compound could be expected to have one more methylene group than vardenafil ($C_{23}H_{32}N_6O_4S$). Further 1D and 2D NMR analysis were performed to determine the position of the methylene group.

3.2. TLC and HPLC analysis

Since this unknown compound had the same R_f value as vardenafil on a TLC plate, we firstly tested this compound using the HPLC. The results showed that the UV spectra had λ_{max} at 214.7 nm (Fig. 2), and exhibited a similar trend to that of vardenafil in the region of 200∼400 nm. Notably, different retention time of 3.01 min (for vardenafil, Fig. 2a) and 4.24 min (for unknown compound, Fig. 2b) was observed from HPLC spectra, suggesting that the difference of this unknown compound from vardenafil.

3.3. IR analysis

The IR spectra of this compound were presented in Fig. 3. The absorption wavenumber at 2961 cm^{-1} and 2815 cm^{-1} for C–H stretching vibration and at 1482 cm^{-1}, 1455 cm^{-1}, 1408 cm^{-1}, and 1376 $cm^{-1}$for C–H bending vibration indicated the presence of alkyl groups, while those at 1270 cm^{-1} and 1024 cm^{-1} indicated the presence of ether bond (C–O). The absorption bands at 1685 cm^{-1} arose from the carbonyl group (C=O) in the imidazotriazinone ring, while absorption wavenumber at 1347 cm^{-1}, 1324 cm^{-1}, 1305 cm^{-1}, 1270 cm^{-1}, 1167 cm^{-1}, 1144 cm^{-1}, 1144 cm^{-1}, and 1116 cm^{-1}were attributed to $–SO_2$-, –C=N- and -C-O bonds. The characteristic wavenumber at 1620 cm^{-1} and 1596 cm^{-1} revealed the existence of unsaturated C=C and C=N bonds, respectively. The three absorption bands at 823 cm^{-1}, 771 cm^{-1}, 727 cm^{-1} was assigned to *m*-disubstitution of benzene ring. These results indicated that the structure of this unknown compound was analog of vardenafil.

3.4. NMR analysis

The ^1H NMR spectra showed a total of 32 protons (Fig. 4a), including a broad N–H signal at 11.66 (1H, brs), ABX-type aromatic proton signals at 7.83 (1H, d, $J = 2.4$ Hz), 7.85 (1H, dd, $J = 8.76$, 2.4 Hz), and 7.39 (1H, d, $J = 8.76$ Hz), 5 methylene signals at 4.11

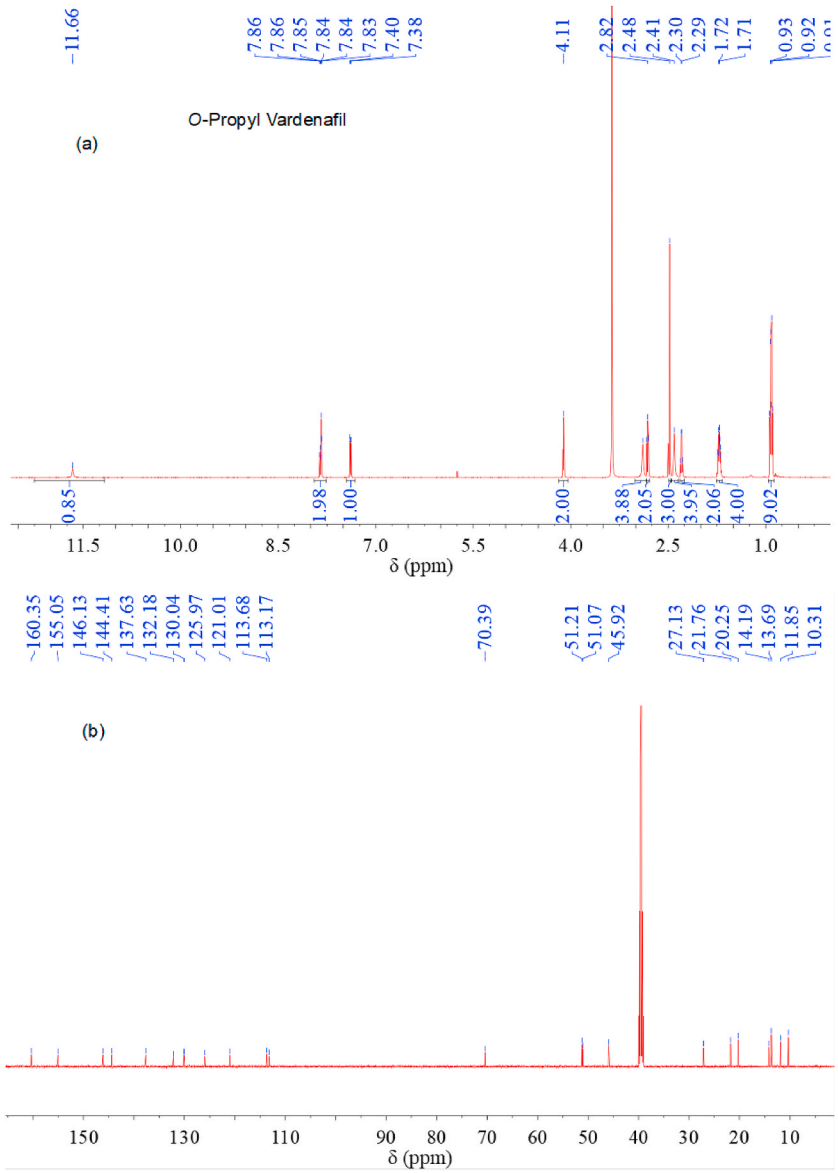

Fig. 4. ^{1}H NMR (a) and ^{13}C NMR (b) of *O*-propyl vardenafil.

(2H, t, $J = 6.30$ Hz), 2.82 (2H, t, $J = 7.44$ Hz), 2.30 (2H, q, $J = 7.14$ Hz), and 1.74–1.69 (2 signals overlapped, 4H, m), 4 methyl signals at 2.48 (3H, s), 0.92 (3H, t, $J = 7.4$ Hz), 0.94–0.90 (3H, m) and 0.90 (3H, t, $J = 7.2$ Hz), and 2 piperazine signals at 2.89 (4H, brs) and 2.41 (4H, brs). The ^{13}C NMR spectra showed 22 carbon atoms, of which 11 carbon atoms were in alkyl region, and the remaining 11 carbon atoms were in aryl region (Fig. 4b). These signals were similar to those of vardenafil, except for the slight difference in signals at position 21 and 22, suggesting that this compound was highly structurally similar to vardenafil. The triplet signal at 4.11 (2H, t, $J =$ 6.30 Hz) and multiple signal at 1.74–1.69 (2H, m) showed that H-21 might lie between H-20 and H-22. Another evidence supporting that the *n*-propyl group was linked to the phenoxyl group was the correlation between H-20 and H-21 and that between H-21 and H-22 in the ^{1}H–^{1}H COSY spectra (Table 1). Furthermore, the correlation between H-22 and C-20 or C-21 in HMBC spectra was also a favorable support (Table 1). The DEPT135 spectra of this analyte showed that 3 aromatic CH carbons (signals at 132.18, 130.04, and 113.17), 7 methylene carbons (signals at 70.39, 51.21, 51.07, 45.92, 27.13, 21.76, and 20.25), and 4 methylated carbons (signals at 14.19, 13.69, 11.85, and 10.31) were presented in the molecular structure (Table 1), which was completely in line with the predicted structural formula.

The analysis of these spectroscopic data further identified the unknown compound as *O*-propyl vardenafil (Fig. 5). Structural formula showed that this identified compound replaced the ethyl group of vardenafil with a propyl group at the alkyl ether moiety.

Table 1
NMR data of vardenafil and *O*-propyl vardenafil in DMSO-d_6 (δ in ppm, J in Hz).

Carbon No.	Vardenafil		*O*-Propyl vardenafil				
	^{1}H (δ_H)[a]	^{13}C (δ_C)	^{1}H (δ_H)[a]	^{13}C (δ_C)	DEPT135[b]	HMBC	COSY
1	–	144.43	–	144.41	0	H-11/H-12	–
3	–	137.65	–	137.63	0	H-10	–
4	–	155.10	–	155.05	0	H-10 (weak)	–
5	11.69 (1H, brs)	–	11.66 (1H, brs)	–	–	–	–
6	–	146.15	–	146.13	0	H-15/H-18 (weak)	–
9	–	113.69	–	113.68	0	H-10	–
10	2.47 (3H, s)	14.18	2.48 (3H, s)	14.19	3	–	–
11	2.83 (2H, t, J = 7.44 Hz)	27.16	2.82 (2H, t, J = 7.44 Hz)	27.13	2	H-12/H-13	H-12
12	1.72(2H, qt, J = 7.44 Hz)	20.26	1.74–1.69 (2H, m)	20.25	2	H-11/H-13	H-11/H-13
13	0.92 (3H, t, J = 7.4 Hz)	14.27	0.92 (3H, t, J = 7.4 Hz)	13.69	3	H-11/H-12	H-12
14	–	126.02	–	121.01	0	H-17 (weak)/H-18	–
15	7.84 (1H, d, J = 2.4 Hz)	130.12	7.83 (1H, d, J = 2.4 Hz)	130.04	1	H-17	H-18
16	–	120.90	–	125.97	0	H-18	–
17	7.86 (1H, dd, J = 8.7, 2.4 Hz)	132.21	7.85 (1H, dd, J = 8.76, 2.4 Hz)	132.18	1	H-15	H-18
18	7.39 (1H, d, J = 8.70 Hz)	113.69	7.39 (1H, d, J = 8.76 Hz)	113.17	1	–	H-17
19	–	160.28	–	160.35	0	H-17/H-18 (weak)/H-15/H-20	–
20	4.22 (2H, q, J = 6.90 Hz)	64.98	4.11 (2H, q, J = 6.30 Hz)	70.39	2	H-21/H-22	H-21
21	1.33 (3H, t, J = 6.90 Hz)	13.70	1.74–1.69 (2H, m)	21.76	2	H-20/H-22	H-20/H-22
22	–	–	0.94–0.90 (3H, m)	10.31	3	H-20/H-21	H-23
24/28	2.90 (4H, brs)	45.83	2.89 (4H, brs)	45.92	2	–	H-25/H-27
25/27	2.43 (4H, brs)	51.07	2.41 (4H, brs)	51.21	2	H-29 (weak)/H-30	H-24/H-28
29	2.32–2.31 (2H, m)	51.16	2.30 (2H, q, J = 7.14 Hz)	51.07	2	H-29 (weak)/H-30	H-30
30	0.90 (3H, t, J = 7.2 Hz)	11.75	0.90 (3H, t, J = 7.2 Hz)	11.85	3	–	H-29

[a] d: doublet; t: triplet; q: quartet; m: multiplet; brs: broad signal.
[b] Number in DEPT is the number of attached protons.

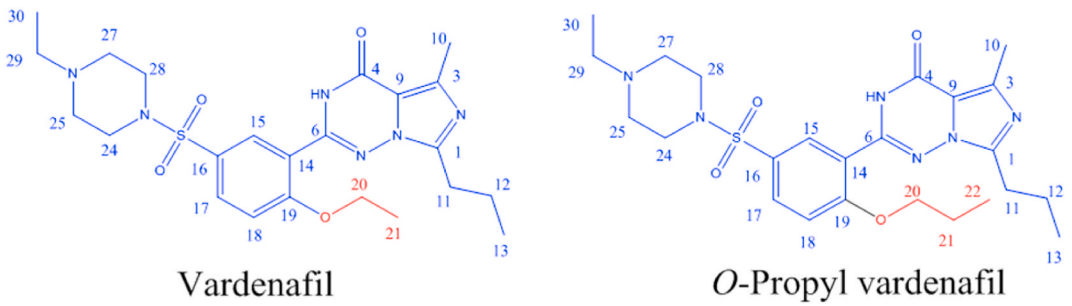

Fig. 5. The structure of vardenafil and *O*-propyl vardenafil.

Table 2
Matrix effects and linearity determined using optimal LC–MS/MS conditions for three healthcare product.

Analyte	Matrixes	Slope	R^2	Matrix Effects
O-Propyl vardenafil	Blank	3770.21	0.997	/
	Pressed candy	3864.6	0.998	1.02
	Energy coffee	14161.53	0.999	1.10
	Health wine	4259.85	0.997	1.13

3.5. Quantitative analysis

3.5.1. Method validation

The analysis results of linearity and matrix effects indicated that this method was suitable for the analysis of *O*-propyl vardenafil in healthcare product. The correlation coefficients (R^2) of external solvent-calibration curve and matrix-matched standard calibration

Table 3
Recoveries and RSDs determined using optimal LC–MS/MS conditions for matrixes.

Analyte	Matrixes	Spiking level (mg/kg)	Recoveries (%)						RSDs (%)	Estimated LOQ (mg/kg)
			1	2	3	4	5	6		
O-Propyl vardenfil	Pressed candy	0.1	100.0	93.5	97.5	96.5	93.0	96.0	2.7	0.1
		0.2	101.0	98.5	99.5	102.0	99.5	102.0	1.5	
		1.0	86.5	82.5	85.0	85.5	77.0	83.5	4.1	
	Energy coffee	0.1	94.0	92.0	94.5	95.5	98.0	94.0	2.1	0.1
		0.2	102.0	103.0	99.5	101.0	104.0	98.0	2.2	
		1.0	97.5	103.0	93.0	95.5	90.0	92.5	4.8	
	Health wine	0.1	103.0	98.0	94.0	93.5	96.5	89.5	4.8	0.1
		0.2	85.0	99.5	85.0	86.0	102.0	100.0	9.1	
		1.0	96.0	99.0	102.0	100.0	107.0	106.0	4.2	

Table 4
Assessment of *O*-propyl vardenafil in healthcare product.

Analyte	Entry	Sample type	Content (g/kg)
O-Propyl vardenafil	1	Pressed candy	7.20
	2	Energy coffee	1.24
	3	Pressed candy	6.20
	4	Pressed candy	3.02
	5	Pressed candy	5.55

curves were not lower than 0.990 (Table 2). The obtained ratio of three matrix-matched curve slopes to solvent-calibration curve slope ranged from 1.02 to 1.13, which was considered as acceptable according to the criteria. The recoveries at 3 spiking levels ranged from 77% to 107%, and RSD values of the three different matrixes were less than 10%, meeting the acceptance criteria (Table 3). Furthermore, the LOQ of three different matrixes was all estimated as 0.1 mg/kg.

3.5.2. Application of quantitative method

The applicability of our developed method for the determination of *O*-propyl vardenafil was assessed by testing 30 samples purchased on Chinese online market. The results showed that 5 samples were tested as positive with a positive rate of 16.67%, and the contents of *O*-propyl vardenafil ranged from 1.24 g/kg to 7.2 g/kg (Table 4). High positive rate and high content of this compound in healthcare product indicated a potential public health risk.

4. Conclusion

In this study, a novel vardenafil analog was isolated from healthcare product and its structure was characterized by HPLC, UV, FT-IR, HPLC-Q-TOF/MS, and NMR. The 1D and 2D NMR data of this analog were compared to those of reported vardenafil, and the results indicated their structural high similarity. A rapid and accurate LC–MS/MS method was developed for quantitative analysis of this compound. Although this compound has been found by Sun [19] recently, to the best of our knowledge, this is the first time to conduct both qualitative and quantitative systematic analysis of *O*-propyl vardenafil. Considering the unknown safety and toxicity profile of this compound, we suggest that the regulators should strengthen the screening efforts and be alert to this health-related illegal product.

Declarations

Author contribution statement

Jintao Xia：Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Wanqin Wu：Performed the experiments; Contributed reagents, materials, analysis tools or data.

Feng Jiang; Songsong Zhu：Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Additional information

No additional information is available for this paper.

Data availability

Data included in article/supplementary material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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