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Research article

Isolation, characterization, identification and quantification of 6-F oxyphenisatin dipropionate, a novel illegal additive, from a fruit-flavored jelly

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ABSTRACT

Objective: This study is aimed to screen, identify and detect illegal additives from healthcare products which claim or imply to have weight-loss effects.
Method: Ultra-high performance liquid chromatography-quadruple-time-of-flight mass spectroscopy (UPLC-Q-TOF/MS) was employed to perform non-targeted screening of illegal additives from a total of 26 batches of healthcare products with weight-loss effects. A novel oxyphenisatin dipropionate analog was discovered in a fruit-flavored jelly that was not clearly labeled as containing added drugs. After being separated and purified by silica gel column chromatography, the analog was unambiguously characterized by one-dimensional (1D) and two-dimensional (2D) nuclear magnetic resonance (NMR) spectroscopies. The molecular structure of the analog was finally identified by comparing the spectra of the analog with those of suspected candidates prepared by *de novo* synthesis strategy. Thereafter, a sensitive and precise reversed phase ultra performance liquid chromatography coupled with photodiode array (UPLC-PDA) detection method was developed and verified for the determination of the analog in 15 batches of real samples.
Results: In the MS/MS spectra, the signal intensity of mass/charge ratios (*m/z*, 242 and 214) of the novel analog fragments was highly similar to that of mass/charge ratios (*m/z*, 224 and 196) of oxyphenisatin dipropionate fragments. Additionally, the 1D NMR spectrum of the analog was completely consistent with that of one of the suspected candidates prepared by the *de novo* synthesis strategy. Based on the above analysis, the structure of the analog was determined as 3,3-bis[4'-(propionyloxy)phenyl]-6-fluoro-2-oxoindoline, which was briefly named 6-F oxyphenisatin dipropionate. A developed quantitative method showed good linearity ($R^2 > 0.999$) in a concentration range of 1.0–100 μg/mL. The limits of detection (LOD) and quantification (LOQ) for the analog was 3 mg/kg and 10 mg/kg, respectively. The average recoveries of the analog from spiked three different matrix samples in low (1 time of LOQ), medium (2 times of LOQ), and high (10 times of LOQ) concentrations were varied from 93.9 % to 107.8 % with a precision of 0.03–1.56 %. Results of quantitative analysis in 15 batches of healthcare products revealed that

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the content of 6-F oxyphenisatin dipropionate in a fruit-flavored jelly and a solid beverage was 118 mg/kg and 330 mg/kg, respectively.

Conclusion: In terms of its structure, 6-F oxyphenisatin dipropionate replaces hydrogen atom by the fluorine atom at position 6 on the indolinone fragment in oxyphenisatin dipropionate. To our best knowledge, 6-F oxyphenisatin dipropionate has never been detected as an illegal additive in foods. Such illegal addition of the analog to foods is more concealing, thus the supervision and testing departments should attach great importance to its application in food markets.

1. Introduction

With the improvement of people's living standards, obesity has become a public health issue of common concern to adults and even adolescents [1,2], thus resulted in an immense sales of the weight-loss healthcare products on e-commerce platforms [3,4]. However, the events that some unlawful manufacturers added western medicine to healthcare foods to achieve the claimed efficacy of the products have been frequently exposed [5–8]. Currently, traditional weight-loss western medicines such as sibutramine [9,10], phenolphthalein [10] and metformin [11] are usually detected from food-related products. In recent years, it has been increasingly common to add chemical drugs other than traditional western medicine to healthcare products [12–15]. However, the toxicological and pharmacological data of these chemical drugs are unknown, and the intake of these chemical drugs by uninformed consumers may cause serious health consequence [16–18]. Therefore, the identification and quantification of these novel chemical ingredients is crucial for relevant departments to take measures to protect the health and safety of consumers.

In response to the common illegal additives in foods, China State Food and Drug Administration (SFDA) issued a series of national standard inspection and testing methods [19]. However, these methods only target limited number of compounds, making it difficult to detect massive new chemical additives. In addition, the concealment and complexity of food illegal additions have posed huge challenges to the inspection and testing of supervision departments.

From the perspective of medicinal properties of oxyphenisatin diacetate (Fig. 1a), it is a commonly used laxative drug in clinical practice. In the intestine, oxyphenisatin diacetate is subjected to alkaline decomposition by intestinal fluid to produce more irritating oxyphenisatin, thereby accelerating intestinal peristalsis and promoting cathartic effect. However, diesterified oxyphenisatin analogs [20] based on the modification of oxyphenisatin, such as oxyphenisatin diacetate, oxyphenisatin dipropionate (Fig. 1b), oxyphenisatin dihexylester, oxyphenisatin dienanthate and so on, have been frequently detected during the routine test of weight-loss healthcare products. Despite the supervision of oxyphenisatin diacetate to food via a specific standard by China SFDA, the effective strategy to identify and quantify diesterified oxyphenisatin analogs added to food is far from being explored. To strengthen the risk prevention and control of the illegal addition of novel chemicals to foods, a systematic technology roadmap is established for the screening, identification and quantification of diesterified oxyphenisatin analogs in healthcare products. The workflow of the roadmap is presented in Fig. 2. First, after proper pretreatment, each extract of the healthcare products to be inspected is injected twice for data acquisition under each of two MS acquisition modes of UPLC-Q-TOF/MS. Subsequently, in the screening scheme, algorithm parameters such as precursor ion mass, collision energy (CE), retention time (RT), and mass tolerance, confidence parameters such as mass error, RT error, isotope ratio difference, library score, and molecular formula score are set for the non-targeted screening of candidates through an in-house mass spectra library containing 400 illegally added chemicals. Next, whether candidates belong to a known compound is determined through MS^2 peak alignment. If the candidates belong to the known compound in the mass spectra library, they will be further identified by comparing their spectral properties with standards. In contrast, other strategies such as NMR characterization, X-ray diffraction (XRD) characterization, and *de novo* synthesis strategy will be used to determine the precise structure of these unknown compounds. Finally, a quantitative detection method will be developed and verified using the standard of the identified compound, and will be applied to test the actual food matrices.

To check our designed scheme, UPLC-Q-TOF/MS [21] was used to screen illegal additives from a total of 26 batches of weight-loss health products (10 batches of fruit-flavored jelly, 8 batches of pressed candy and 8 batches of solid beverage) by in-house database matching, but the matching was not successful. Unexpectedly，a novel suspected oxyphenisatin dipropionate analog was discovered in a fruit-flavored jelly. After being separated, purified, and enriched, the analog was characterized by nuclear magnetic resonance (NMR) spectroscopy. And we compared the spectra of the analog with that of suspected candidates prepared by the *de novo* synthesis

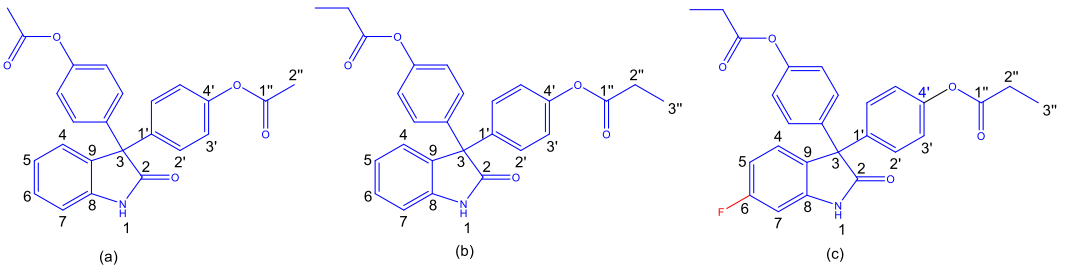

Fig. 1. The structure of oxyphenisatin diacetate (a), oxyphenisatin dipropionate (b) and the novel analog, 6-F oxyphenisatin propionate (c).

approaches, and determined the structure of this analog as 3,3-bis[4'-(propionyloxy)phenyl]-6-fluoro-2-oxoindoline, which was briefly named 6-F oxyphenisatin propionate. The specific structure of the novel analog was shown in Fig. 1c. An UPLC-PDA quantitative detection method for the analysis of 6-F oxyphenisatin dipropionate in weight-loss healthcare products was also developed and verified. The results showed that the accuracy, repeatability, and sensitivity of this method were desirable. During the detection of 15 batches of real samples, 2 batches of positive samples were found, with a positive rate of 13.3 %. The result indicated that the illegal addition of chemicals to healthcare products should be attached importance to relevant market supervision authorities. This study provided an efficient strategy for screening, identification, and quantification of oxyphenisatin and their derivatives illegally added to weight-loss healthcare foods, and our scheme would be very helpful for addressing food safety-related cases.

2. Experiment

2.1. Materials and chemicals

41 Batches of weight-loss healthcare products were purchased from e-commerce platforms. Oxyphenisatin dipropionate standard (99.9 % HPLC purity) was obtained from Alta Scientific. Ltd. (Tianjin, China). 6-F oxyphenisatin dipropionate standard (98 % HPLC purity) was prepared by *de novo* synthesis pathway. HPLC-grade acetonitrile (MeCN) and ammonium acetate were provided by Fisher Scientific (New Jersey, USA) and Macklin Biochemical Co., Ltd (Shanghai, China), respectively. UPLC/MS-grade formic acid was obtained from CNW Technologies GmbH (Düsseldorf, Germany). Analytical-grade petroleum ether and ethyl acetate used in this study were supplied by Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Dimethyl sulfoxide-d_6 (DMSO-d_6) with 99.8 % deuteration degree was provided by Cambridge Isotope Laboratories Inc.(Tewksbury, MA, USA). Ultrapure water (18.2 MΩ cm) was prepared on a Milli-Q water purification system (Billerica, MA, USA). The 0.22 μm PTFE membrane filters were obtained from FTSCI Science and Technology Co., Ltd (Hubei, China).

2.2. Sample preparation

The oxyphenisatin dipropionate standard (10 mg) was dissolved in 50 mL MeCN, and the solution was further diluted to 1 μg/mL for direct infusion. Stock solutions of 6-F oxyphenisatin dipropionate standard (100.0 μg/mL) were prepared in MeCN and stored in a freezer at 4 °C in the dark. Standard solution of 6-F oxyphenisatin dipropionate for calibration curves (1.0, 2.0, 5.0, 10.0, 20.0, 50.0, 80.0, 100.0 μg/mL) and for spiking (1.0, 5.0, 10.0 μg/mL) were prepared by diluting the stock solution with MeCN. The analyst in each homogenized weight-loss healthcare product was extracted by ultrasound-assisted extraction. After centrifugation at 3000 rpm for 5 min, the supernatants were filtered through 0.22 μm PTFE membrane filters, and 1 mL of extract was used for UPLC-Q-TOF/MS screening.

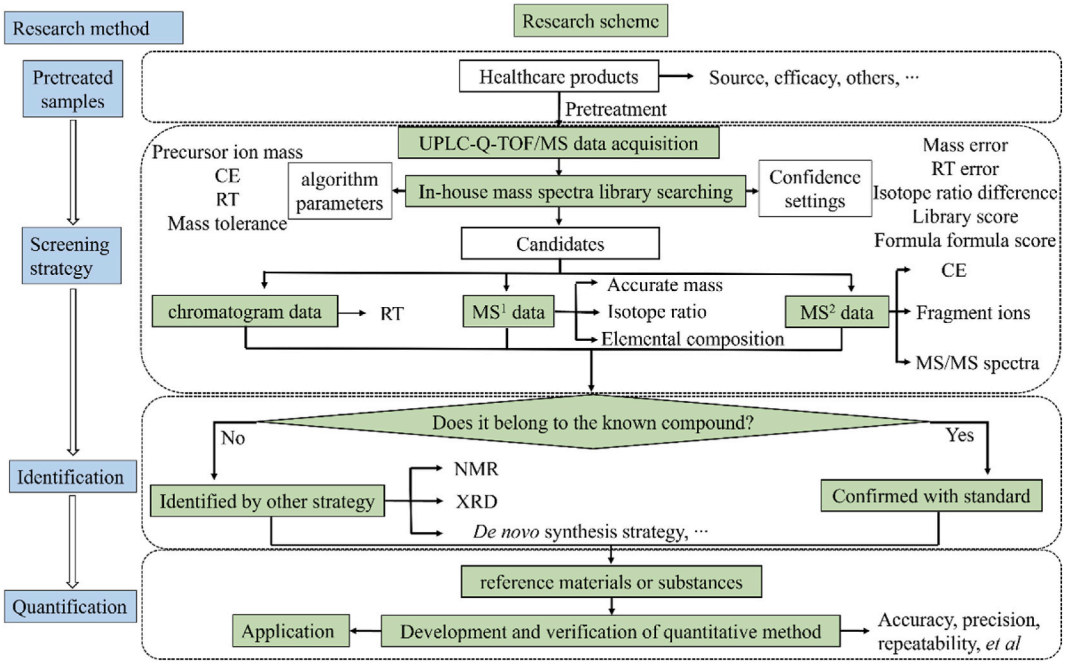

Fig. 2. The systematic technology roadmap for the test of illegally added chemicals in healthcare products.

2.3. UPLC-PDA test

UPLC-PDA analysis was conducted on a 2695 series HPLC system with a photodiode-array detector (Waters Corporation, Milford, MA, USA). The samples were separated using an ACQUITY UPLC@BEH C18 column (1.7 μm, 2.1 × 100 mm) with column temperature maintained at 35 °C. The mobile phase contained 0.1 % ammonium acetate in water (phase A) and MeCN (phase B). The gradient elution program was as follows: 95 % A for 0∼1.0 min, 95∼5 % A for 1.0∼6.0 min, 5 % A for 6.0∼9.0 min, 5∼95 % A for 9.0∼9.1 min, and 95 % A for 9.1∼12.0 min. The elution flow rate was 0.3 mL/min, and 2 μL extract was injected to the column for sample separation. The wavelength of the ultraviolet visible spectroscopy ranged from 190 nm to 400 nm, and the peak at 254 nm was monitored.

2.3.1. UPLC-Q-TOF/MS screening

High resolution mass (HRMS) analysis was performed on the Q-TOF mass spectrometry (5600+ series, AB SCIEX, Framingham, MA, USA) coupled with an UPLC system (ExionLC series, AB SCIEX, Framingham, MA, USA), employing an electrospray ion (ESI) source. The sample was separated using an ACQUITY UPLC@BEH C18 column (2.1 μm, 2.1 × 75 mm; Waters Corporation) with column temperature maintained at 40 °C. Phase A was 0.1 % formic acid in water, and phase B was MeCN. The continuous gradient elution was performed as follows: 0∼1.5 min, A 95 %; 1.5∼8.0 min, A 95∼5 %; 8.0∼12.0 min, A 5 %; 12.0∼12.1 min, A 5∼95 %; 12.1∼15.0 min, A 95 %. The flow rate was set as 300 μL/min, and 5 μL sample solution was loaded to the column for subsequent analysis.

The ESI source was operated in positive ion mode, and data were acquired by information dependent acquisition (IDA) mode. MS parameters were set as follows: ion source temperature (TEM), 550 °C; curtain gas pressure (CUR), 35 psi; nebulizer pressure (GS1), 50 psi; auxiliary gas pressure (GS2), 50 psi; ion spray voltage floating (ISVF), 5.5 kV; declustering potential (DP), 60 V; and collision energy (CE), 35 ± 15 V. The mass/charge ratios (m/z) of MS scan and MS/MS scan ranged from 50 to 1100 Da.

2.4. Sample isolation and purification

About 100 g fruit-flavored jelly containing the novel analog was homogenized in 300 mL MeCN for 5 min, ultrasound-assisted extracted for 10 min, and centrifuged at 4000 rpm for 5 min. Immediately afterwards, the extracts were filtered and evaporated to produce a crude product. Finally, a white high-purity powder was obtained by silica gel column chromatography technique with ethyl acetate/n-hexane (4:1, v/v) as eluent, and completely characterized by 1D and 2D NMR.

2.5. De novo synthesis of all suspected candidates

The fluorinated oxyphenisatin were synthesized using Lambert salt catalyst by previously reported method [22] and they were subsequently esterified using propionic anhydride to prepare fluorinated oxyphenisatin dipropionates (Fig. 3, details are provided in supplementary materials). Specifically, the fluorinated oxyphenisatin (2 mmol, 670 mg) was mixed with 2.0 mL propionic anhydride, and the mixture changed from turbid to clear immediately after the addition of 0.3 mL of pyridine. At 2 min post pyridine addition, a white precipitate was observed. After the reaction completion monitored by thin layer chromatography (TLC), the precipitate was filtered to obtain the crude products, and then the resultant crude products were reslurried to obtain a high-purity fluorinated oxyphenisatin dipropionates.

2.6. NMR test

High-resolution NMR spectra of oxyphenisatin dipropionate standard, the novel analog and all suspected candidates dissolved in DMSO-d_6 were obtained on a Bruker AVIII FT-NMR spectrometer at working frequency 600 MHz for ^{1}H spectra, 151 MHz for ^{13}C spectra and 565 MHz for ^{19}F spectra and analyzed by Mestrenova software. The structure of the novel analog was further analyzed by distortionless enhancement by polarisation transfer (DEPT), heteronuclear multiple-bond correlation (HMBC), and heteronuclear single quantum coherence (HSQC) in 2D NMR. Chemical shifts (δ) in NMR spectra were reported in parts per million (ppm) relative to the residual solvent signals as reference (DMSO-d_6, 2.50 ppm for ^{1}H and 39.5 for ^{13}C). ^{1}H and ^{13}C multiplicities were reported as following abbreviations: s for singlet, d for doublet, t for triplet, q for quartet, dd for doublet of doublets, td for triplet of doublets, m for complex pattern (multiplet).

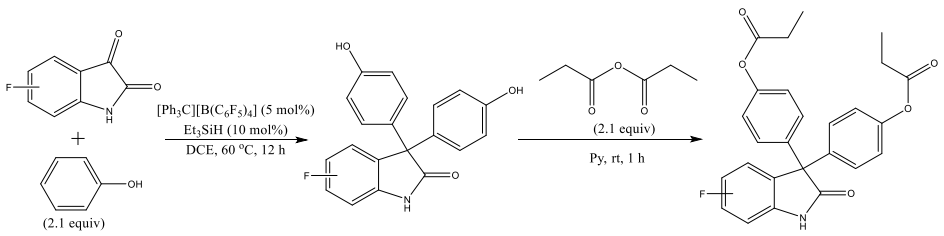

Fig. 3. A roadmap for the synthesis of fluorined oxyphenisatin dipropionates.

3. Results and discussion

3.1. UPLC-PDA analysis

In the UV spectra, the maximum absorption wavelength of oxyphenisatin dipropionate standard at 204.1 nm (Fig. 4) was highly similar to that of the novel analog, suggesting a highly similar structure of the two compounds. Notably, the two compounds had a retention time of 6.42 min (Figs. 4a) and 6.49 min (Fig. 4b), respectively, at the UPLC-PDA chromatogram, suggesting that the difference of their structures. Therefore, we further conducted HRMS and NMR analysis of the compounds.

3.2. UPLC-Q-TOF/MS analysis

The total ion chromatogram (TIC) spectra showed a strong intensity of the novel analog at 7.59 min (Fig. 5a), and the MS spectra displayed a quasi-molecular ion with the mass of *m/z* 448.1557 (Fig. 5b), suggesting that the molecular formula of the novel analog was matched to $C_{26}H_{22}FNO_5$ with the mass error of 0.45 ppm. Several product ions of the novel analog such as *m/z* 392.1312, 298.0877, 242.0627, and 214.0661 (Fig. 5c) generated from fragmentation of the precursor ion in the MS/MS spectra attached to our attention. Comparing the distinguishable product ions (*m/z* 242.0627 and 214.0661) with strong response signal of the novel analog with that (*m/z* 224.0706 and 196.0757) of oxyphenisatin dipropionate (Fig. 5d), we found the novel analog exhibited an additional molecular weight of 17.99, indicating that a hydrogen atom in oxyphenisatin dipropionate might have been replaced by the fluorine atom in the novel analog. Based on the information acquired from mass spectrometry, we further explored the specific position of fluorine atom in the molecular skeleton of the novel analog by comparing NMR spectra of this analog with that of the suspected compounds sourced from *de novo* synthesis strategy.

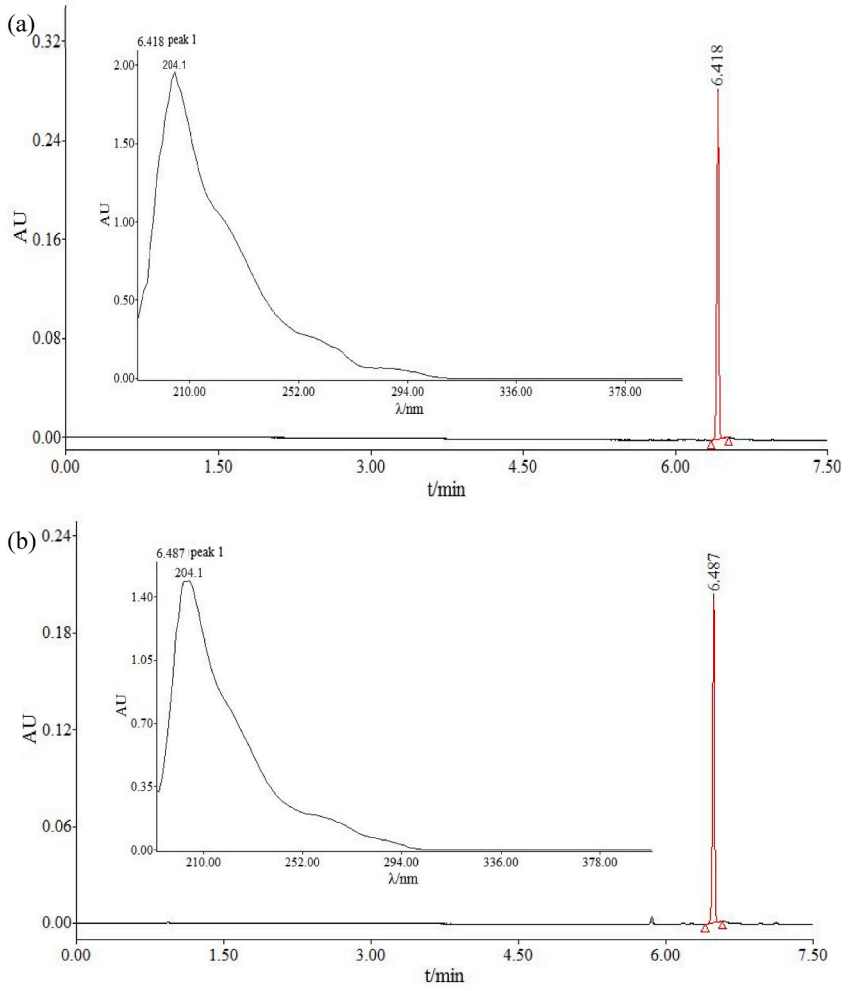

Fig. 4. Ultra-performance liquid chromatogram recorded at 254 nm and UV spectra in the region of 190~400 nm of oxyphenisatin dipropionate standard (a) and the novel analog (b).

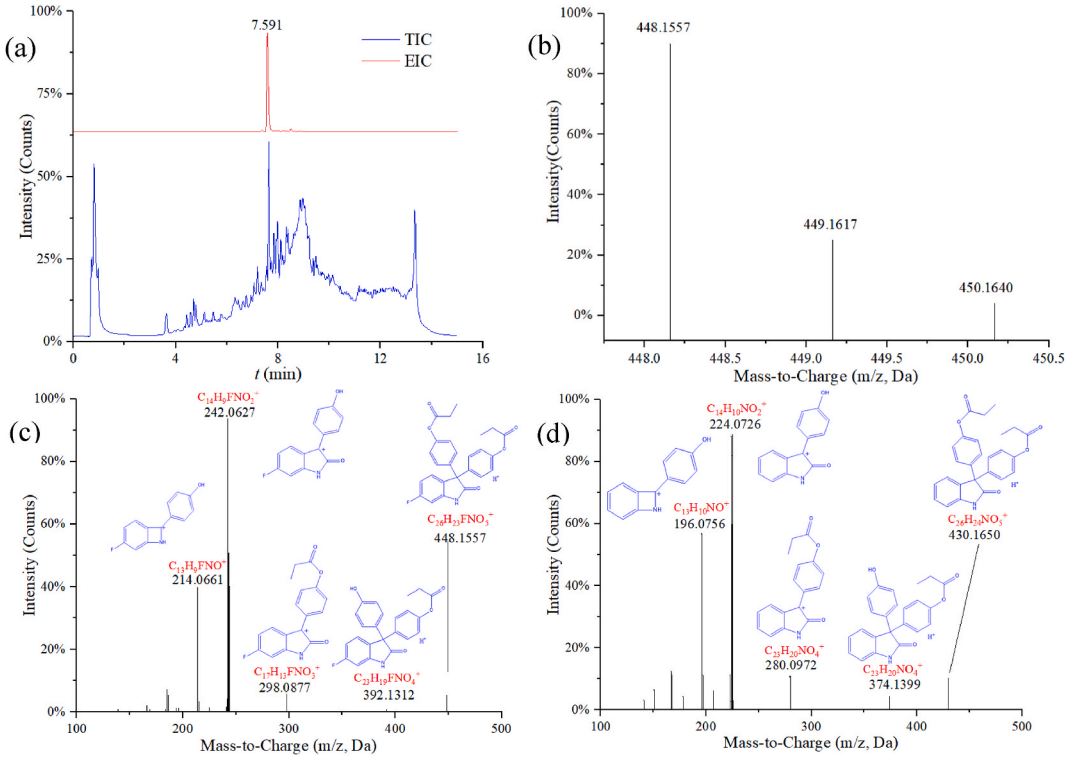

Fig. 5. The TIC and EIC chromatogram for the fruit-flavored jelly (a); The MS (b) and MS/MS (c) spectra of the novel analog; The MS/MS of oxyphenisatin dipropionate (d).

3.3. NMR analysis

The ^{1}H NMR spectra of the novel analog and oxyphenisatin dipropionate showed their structural similarity. The response signals of two aromatic protons with chemical shifts of δ_H 7.23–7.15 (4H, m) and 7.14–7.05 (4H, m) in the ^{1}H NMR spectra (Fig. 6a) and those of carbon atoms with chemical shifts of δ_C 129.03 and 121.87 in the ^{13}C NMR spectra (Fig. 6b) indicated the presence of two symmetrical 1,4-disubstituted aromatic rings in the novel analog. Therefore, we speculated that the novel analog was highly likely to be a compound with hydrogen atom on the indolinone benzene ring skeleton of oxyphenisatin dipropionate being substituted. Our speculation was confirmed by three C–H signals at δ_C 127.5 (d, 9.81), 108.47 (d, 21.75), 98.32 (d, 26.66) in the DEPT135 spectra (Fig. 6c). In the ^{13}C NMR spectra, the coupling constants (J) of the chemical shifts at δ_C 162.19, 108.47, 98.32, 142.89, and 127.5 were 241.82, 21.75, 26.66, 12.00, and 9.81 Hz, respectively, suggesting the presence of a fluorine atom in the molecular structure of the novel analog, which was verified by the ^{19}F NMR spectra (Fig. 6d). Additionally, the splitting signals of ^{19}F to ^{1}H nuclei at δ_H 7.34 (1H, dd, 8.40, 5.52), 6.85 (1H, td, 10.68, 2.40), and 6.80 (1H, dd, 9.00, 2.34) in the ^{1}H NMR spectra indicated that the novel analog might be one of 5-F or 6-F oxyphenisatin dipropionate, but it was difficult to accurately identify its structure by 2D NMR. Therefore, we prepared 5-F and 6-F oxyphenisatin dipropionate through *de novo* synthesis strategy for further comparison and identification.

3.4. Data characterization of suspected candidates

The suspected candidates, that was 5-F and 6-F oxyphenisatin dipropionate, were prepared by *de novo* synthesis strategy and characterized by NMR and UPLC-Q-TOF/MS (details are provided in supplementary materials). The results were as follows: 5-F oxyphenisatin dipropionate, ^{1}H NMR (DMSO-d_6) δ 10.88 (s, 1H), 7.30 (dd, J = 8.5, 2.6 Hz, 1H), 7.26–7.17 (m, 4H), 7.17–7.03 (m, 5H), 6.98 (dd, J = 8.6, 4.5 Hz, 1H), 2.59 (q, J = 7.5 Hz, 4H), 1.11 (t, J = 7.5 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 177.72, 172.47, 158.22 (d, J = 237.0 Hz), 149.69, 138.64, 137.55 (d, J = 1.5 Hz), 134.40 (d, J = 7.5 Hz), 129.10, 121.89, 115.11 (d, J = 22.5 Hz), 113.70 (d, J = 24.0 Hz), 111.10 (d, J = 7.5 Hz), 61.72, 26.84, 8.79; ^{19}F NMR (DMSO-d_6) δ −120.67; HRMS (ESI): calculated for $C_{26}H_{23}NO_5F$ $[M+H]^+$: 448.1555; found:448.1537. 6-F oxyphenisatin dipropionate, ^{1}H NMR (DMSO-d_6) δ 11.00 (s, 1H), 7.34 (dd, J = 8.3, 5.4 Hz, 1H), 7.22–7.15 (m, 4H), 7.14–7.05 (m, 4H), 6.85 (td, J = 9.9, 2.4 Hz, 1H), 6.80 (dd, J = 9.0, 2.3 Hz, 1H), 2.58 (q, J = 7.5 Hz, 4H), 1.11 (t, J = 7.5 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 178.17, 172.47, 162.19 (d, J = 241.8 Hz), 149.64, 142.89 (d, J = 12.0 Hz), 138.86, 129.03, 128.64 (d, J = 3.0 Hz), 127.50 (d, J = 10.6 Hz), 121.87, 108.47 (d, J = 21.8 Hz), 98.32 (d, J = 26.7 Hz), 60.81, 26.84, 8.80; ^{19}F NMR (DMSO-d_6) δ −112.19. HRMS (ESI): calculated for $C_{26}H_{23}NO_5F$ $[M+H]^+$: 448.1555; found:448.1573. Comparing ^{1}H NMR (Fig. 7) and ^{13}C NMR of illegal additive extracted from fruit-flavored jelly with those of candidates prepared by *de novo* synthesis strategy, we determined the illegal additive to be 6-F oxyphenisatin dipropionate. In addition, in ^{19}F NMR spectrum, the chemical shifts of 5-F

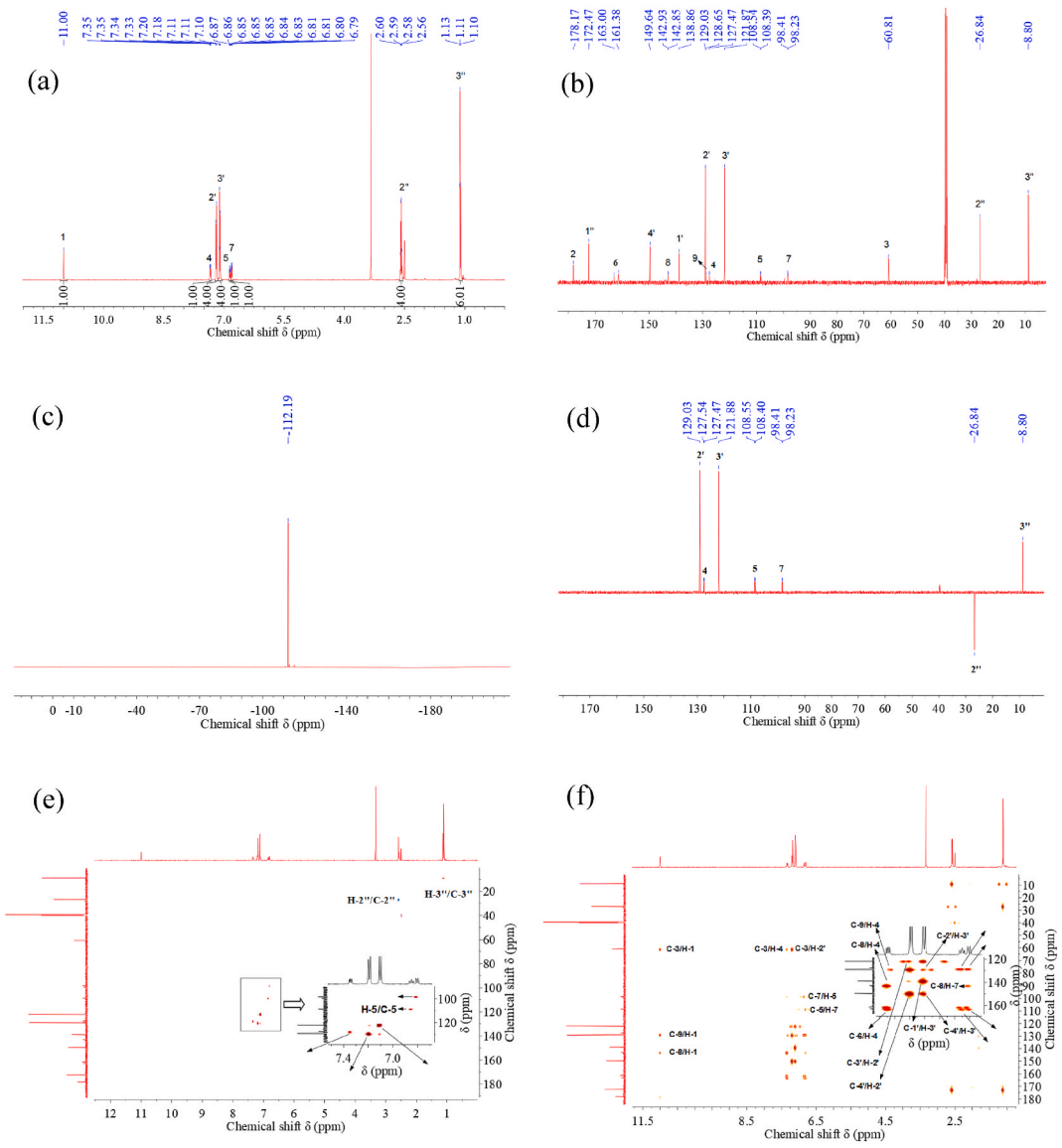

Fig. 6. ^{1}H NMR spectrum (a), ^{13}H NMR spectrum (b), ^{19}F NMR spectrum (c), DEPT 135 spectrum (d), HSQC spectrum (e), and HMBC spectrum (f) of the novel analog.

oxyphenisatin dipropionate and 6-F oxyphenisatin dipropionate were −120.67 ppm and −113.22 ppm, respectively, suggesting the slight differences in structure of the two isomers. The peak assignments of the ^{1}H NMR, ^{13}C NMR, ^{19}F NMR, DEPT135, HSQC (Fig. 6e), and HMBC (Fig. 6f) of 6-F oxyphenisatin dipropionate and the ^{1}H NMR, ^{13}C NMR of oxyphenisatin dipropionate for comparison were presented in Table 1.

3.5. Quantitative analysis

An UPLC-PDA method for quantitative analysis of 6-F oxyphenisatin dipropionate in weight-loss health products was found to be sensitive with LOQ of 10 mg/kg. The linear equation for range 1~100 μg/mL was $y = 5800\,x+1210$ with linear regression coeffcient $R^2 > 0.99$. The RSD values for intra-day precision (n = 6) and inter-day precision (n = 6) was 0.7 % and 1.1 %, respectively. The average recoveries rate of the analog from spiked three different blank matrix samples in low (1 time of LOQ), medium (5 times of LOQ), and high (10 times of LOQ) concentrations were varied from 93.9 % to 107.8 % with a precision of 0.03–1.56 % (Table 2). The above results indicated that accuracy and repeatability of this method fell within the acceptable criteria for analyte. The applicability of this method was proven by analyzing 15 batches of healthcare foods (fruit-flavored jelly, pressed candy and solid beverage), and the content of 6-F oxyphenisatin dipropionate in a batch of fruit-flavored jelly and a batch of solid beverage was 118 mg/kg and 330 mg/kg, respectively.

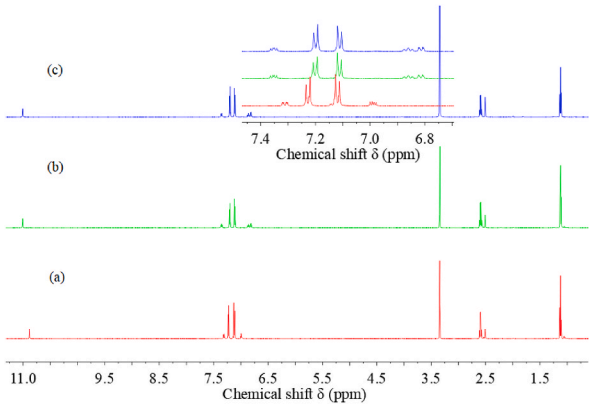

Fig. 7. Comparison of the ^{1}H NMR spectra of 5-F oxyphenisatin dipropionate (a) and 6-F oxyphenisatin dipropionate (b) obtained from *de novo* synthesis pathway with novel analog extracted from fruit-flavored jelly (c).

Table 1
The NMR data of oxyphenisatin dipropionate and the novel analog in DMSO-d_6 (δ in ppm, J in Hz).

Carbon No.	oxyphenisatin dipropionate			the novel analog (6-F oxyphenisatin dipropionate)				
	^{1}H (δ_H)	^{13}C (δ_C)	^{1}H (δ_H)	^{13}C (δ_C)	^{19}F (δ_F)	DEPT 135[a]	HSQC	HMBC
1	10.84(1H, s)	–	11.00(1H, s)	–	−112.19	0	–	–
2	–	177.86	–	178.17		0	–	H-1
3	–	61.21	–	60.81		0	–	H-1/H-4/H-2′
4	7.31 (1H, d, 7.44)	125.99	7.34(1H, dd, 8.40, 5.52)	127.5 (d, 9.81)		1	H-4/C-4	H-5
5	7.04 (1H, td, 7.56, 0.84)	122.17	6.85 (1H, td, 10.68, 2.40)	108.47 (d, 21.75)		1	H-5/C-5	H-7
6	7.27 (1H, td, 7.68, 1.08)	128.55	–	162.19 (d, 241.82)		0	–	H-4/H-5/H-7
7	6.99 (1H, d, 7.68)	110.19	6.80 (1H, dd, 9.00, 2.34)	98.32 (d, 26.66)		1	H-7/C-7	H-5
8	–	141.30	–	142.89 (d, 12.00)		0	–	H-1/H-4/H-7
9	–	132.76	–	128.64 (d, 2.37)		0	–	H-1/H-4/H-5/H-7
1′	–	139.11	–	138.86		0	–	H-3′
2′	7.23–7.16 (4H, m)	129.05	7.23–7.15 (4H, m)	129.03		1	H-2’/C-2′	H-3′
3′	7.13–7.07 (4H, m)	121.79	7.14–7.05 (4H, m)	121.87		1	H-3’/C-3′	H-2′
4′	–	149.55	–	149.64		0	–	H-2’/H-3′
1’’	–	172.46	–	172.47		0	–	H-2’’/H-3’’
2’’	2.58 (4H, q, 7.5)	26.83	2.58 (4H, q, 7.5)	26.84		2	H-2’’/C-2’’	H-3’’
3’’	1.11 (6H, t, 7.5)	8.79	1.11 (6H, t, 7.5)	8.80		3	H-3’’/C-3’’	H-2’’

[a] Number in DEPT 135 is the number of attached protons.

Table 2
Average recoveries and RSD of the HPLC-PDA method for the determination of 6-F oxyphenisatin dipropionate in different matrices.

Spiking level (mg/kg)	10 (Low)		50 (Middle)		100 (High)	
Matrix	Recovery/%	RSD/%	Recovery/%	RSD/%	Recovery/%	RSD/%
Solid beverage	100.2	1.50	98.9	0.32	100.8	0.03
Pressed candy	94.4	1.56	93.9	0.25	107.8	0.08
fruit-flavored jelly	94.4	1.56	94.8	0.16	94.4	0.10

In the past two years, the frequency of detection of oxyphenisatine diacetate and oxyphenisatin dipropionate [20] from weight-loss healthcare foods has been increasing. Recently, 6-F oxyphenisatin dipropionate has been detected from weight-loss healthcare foods. Considering this, it is reasonable to assume that criminals will continue to use different acid anhydrides and/or indolinones with substituent groups on the aromatic ring as raw materials to prepare diesterified oxyphenisatin and their analogs and add them into foods, thus claiming the efficacy of the products and evading existing standard supervision. Fortunately, in our regulation and supervision system, non-targeted screening strategies based on mass spectrometry databases are effective in identifying diesterified oxyphenisatin and their analogs with characteristic mass spectrometry fragmentation patterns. It should be noted that compared with traditional methods [23–25] for identifying the structure of the new illegal additives, this study innovatively proposes to prepare suspected candidates through *de novo* synthesis strategy and then conduct a matching so as to precisely determine the molecular structure of suspected compounds, when HRMS and NMR spectroscopies fail to completely confirm the molecular structure.

This study provides a reference for the identification of new illegal additives in foods. In addition, 6-F oxyphenisatin dipropionate

prepared through chemical synthesis strategy provides a material basis for the development of standard samples or substances, which promotes the implementation of inspection- and testing-related standards or supplementary inspection methods, meanwhile satisfies the needs for laboratory expansion.

Up to now, there have been no relevant reports on toxicology and pharmacology of oxyphenisatin and their analogs. The recommended dosage of oxyphenisatin diacetate for adults is 5–10 mg/day [20]. Overdosing may cause abdominal pain. In this study, the contents of 6-F oxyphenisatin dipropionate detected from a batch of fruit-flavored jelly and a batch of solid beverage was 118 mg/kg and 330 mg/kg, respectively. Referring to the dosage of oxyphenisatin diacetate, the daily consumption of this two healthcare products should be below 30 g/day and 85 g/day respectively. Although the trademark recommendation dosages for this two healthcare products is 15 g/day and 20 g/day, respectively, uninformed consumers may not realize that these new compounds that have not been clinically studied may have potential health threats to the human health.

4. Conclusion

In conclusion , a novel oxyphenisatin dipropionate analog was detected in a batch of fruit-flavored jelly by UPLC-Q-TOF/MS during the inspection of 26 batches of healthcare products for weight loss purpose. The novel analog was isolated and purified by silica gel chromatography column, and its structure was characterized by HRMS and NMR spectra. Spectral analysis showed that the structure of the novel analog exhibited an additional fluorine atom at the indolinone benzene ring skeleton of oxyphenisatin dipropionate. Further comparison with suspected candidates prepared through *de novo* synthesis strategy revealed that the fluorine atom was located at the sixth position of oxyphenisatin dipropionate, and thus the novel analog was named 6-F oxyphenisatin dipropionate. An UPLC-PDA quantitative method was subsequently established for analyzing 6-F oxyphenisatin dipropionate, and the reliability of this method was confirmed by the methodological parameters. This method was applied to the detection of 15 batches of weight-loss healthcare foods, and the contents of 6-F oxyphenisatin dipropionate in a batch of fruity jelly and a batch of solid beverage was detected to be 118 mg/kg and 330 mg/kg, respectively. Like many other novel illegal additives, the toxicity and pharmacology profiles of this analog remain unknown. Therefore, strengthening the development of test methods or standards and increasing public's alertness to this health-related products are of great importance.

Data availability

Data will be made available on the request.

CRediT authorship contribution statement

Jintao Xia: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Wanqin Wu:** Validation, Resources, Investigation, Data curation, Conceptualization. **Xiuxiu Huang:** Resources, Methodology, Investigation. **Feng Jiang:** Supervision, Funding acquisition, Conceptualization. **Songsong Zhu:** Resources. **Li Chen:** Resources. **Xiaolong Fan:** Resources.

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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Appendix A. Supplementary data

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References

[1] R.M. Puhl, C.A. Heuer, Obesity stigma: important considerations for public health, Am. J. Public. Health 100 (2010) 1019–1028, https://doi.org/10.2105/ajph.2009.159491.

[2] N.B. Lister, L.A. Baur, J.F. Felix, A.J. Hill, C. Marcus, T. Reinehr, C. Summerbell, M. Wabitsch, Child and adolescent obesity, Nat. Rev. Dis. Primers. 9 (2023) 24, https://doi.org/10.1038/s41572-023-00435-4.

[3] Y.B. Monakhova, T. Kuballa, S. Löbell-Behrends, S. Maixner, M. Kohl-Himmelseher, W. Ruge, D.W. Lachenmeier, Standardless ^{1}H NMR determination of pharmacologically active substances in dietary supplements and medicines that have been illegally traded over the Internet, Drug, Test. Anal. 5 (2013) 400–411, https://doi.org/10.1002/dta.1367.
[4] R. Hachem, G. Assemat, N. Martins, S. Balayssac, V. Gilard, R. Martino, M. Malet-Martino, Proton NMR for detection, identification and quantification of adulterants in 160 herbal food supplements marketed for weight loss, J. Pharm. Biomed. Anal. 124 (2016) 34–47, https://doi.org/10.1016/j.jpba.2016.02.022.
[5] J.H. Lee, S.J. Kim, M.K. Seo, H.J. Ham, E.J. Jung, N.S. Kim, H. Kim, S.Y. Baek, Application of liquid chromatography-high resolution mass spectrometry and liquid chromatography-tandem mass spectrometry methods to 45 weight loss compounds in health functional food, food, and illegal drug, J. Sep. Sci. 45 (2022) 2795–2803, https://doi.org/10.1002/jssc.202101030.
[6] M. Wang, S. Haider, A.G. Chittiboyina, J.F. Parcher, I.A. Khan, 1,5-Dimethylhexylamine (octodrine) in sports and weight loss supplements: natural constituent or synthetic chemical? J. Pharm. Biomed. Anal. 152 (2018) 298–305, https://doi.org/10.1016/j.jpba.2018.02.008.
[7] R. Hachem, M. Malet-Martino, V. Gilard, First identification and quantification of lorcaserin in an herbal slimming dietary supplement, J. Pharm. Biomed. Anal. 98 (2014) 94–99, https://doi.org/10.1016/j.jpba.2014.05.003.
[8] M. Khazan, M. Hedayati, S. Askari, F. Azizi, Adulteration of products sold as Chinese Herbal medicines for weight loss with thyroid hormones and PCP, J. Her. Med 3 (2013) 39–43, https://doi.org/10.1016/j.hermed.2012.11.003.
[9] J.W. Kim, S.J. Kweon, S.K. Park, J.Y. Kim, J.H. Lee, K.M. Han, S. Cho, J. Kim, S.Y. Han, H.J. Kim, W.S. Kim, Isolation and identification of a sibutramine analogue adulterated in slimming dietary supplements, Food Addit. Contam. A. 30 (2013) 1221–1229, https://doi.org/10.1080/19440049.2013.793826.
[10] F.H. Song, D. Monroe, A. El-Demerdash, C. Palmer, Screening for multiple weight loss and related drugs in dietary supplement materials by flow injection tandem mass spectrometry and their confirmation by liquid chromatography tandem mass spectrometry, J. Pharm. Biomed. Anal. 88 (2014) 136–143, https://doi.org/10.1016/j.jpba.2013.08.031.
[11] X.M. Wu, B.H. Zhu, L. Lu, W.X. Huang, D.W. Pan, Optimization of a solid phase extraction and hydrophilic interaction liquid chromatography–tandem mass spectrometry method for the determination of metformin in dietary supplements and herbal medicines, Food Chem. 133 (2012) 482–488, https://doi.org/10.1016/j.foodchem.2012.01.005.
[12] Q.Y. Cheng, L.J. Shou, C. Chen, S. Shi, M.H. Zhou, Application of ultra-high-performance liquid chromatography coupled with LTQ-Orbitrap mass spectrometry for identification, confirmation and quantitation of illegal adulterated weight-loss drugs in plant dietary supplements, J. Chromatogr. B. 1064 (2017) 92–99, https://doi.org/10.1016/j.jchromb.2017.09.009.
[13] A.A. Jairoun, S.S. Al-Hemyari, M. Shahwan, S.H. Zyoud, Adulteration of weight loss supplements by the illegal addition of synthetic pharmaceuticals, Molecules 26 (2021) 6903–6914, https://doi.org/10.3390/molecules26226903.
[14] S. Eichner, M. Maguire, L.A. Shea, M.G. Fete, Banned and discouraged-use ingredients found in weight loss supplements, J. Am. Pharm. Assoc. 56 (2016) 538–543, https://doi.org/10.1016/j.japh.2016.03.013.
[15] T. Rocha, J.S. Amaral, M.B.P.P. Oliveira, Adulteration of dietary supplements by the illegal addition of synthetic drugs: a review, Compr. Rev. Food. Sci. F. 15 (2016) 43–62, https://doi.org/10.1111/1541-4337.12173.
[16] D. Joshi, T.J.S. Cross, V.S. Wong, Acute drug induced hepatitis secondary to a weight loss product purchased over the internet, Nutr. J. 6 (2007) 13, https://doi.org/10.1186/1475-2891-6-13.
[17] M.H.Y. Tang, S.P.L. Chen, S.W. Ng, A.Y.W. Chan, T.W.L. Mak, Case series on a diversity of illicit weight-reducing agents: from the well known to the unexpected, Br. J. Clin. Pharmacol. 71 (2011) 250–253, https://doi.org/10.1111/j.1365-2125.2010.03822.x.
[18] D. Shin, J. Kwon, H.-S. Kang, J. Suh, E. Lee, The presence of unauthorized ingredients in dietary supplements: an analysis of the risk warning data in Korea, J. Food Compos. Anal. 108 (2022) 104462, https://doi.org/10.1016/j.jfca.2022.104462.
[19] The State Food and Drug Administration, Chinese supplementary inspection methods in foods (BJS 201701, BJS 201911, BJS 201916, BJS 201917, BJS 202209, BJS 202213).
[20] J.X. Zhang, H. Yu, J. Sun, Y.Y. Shen, Y.Y. Ran, X.H. Mao, Q. Hu, S. Ji, Isolation and characterization of a new oxyphenisatin analogue, oxyphenisatin propionate, from a processed plum intended as a weight loss product, J. Pharm. Biomed. Anal. 230 (2023) 115391, https://doi.org/10.1016/j.jpba.2023.115391.
[21] N.S. Kim, H.S. Choi, N.Y. Lim, J.H. Lee, H. Kim, S.Y. Baek, Intercomparison study of fragmentation pathways and fragment ion structures for screening of illegal drugs and their novel analogues used to adulterate dietary supplements using liquid chromatography/quadrupole time-of-flight mass spectrometry, Rapid. Commun. Mass. Sp. 36 (2022) e9353, https://doi.org/10.1002/rcm.9353.
[22] J. Khan, A. Tyagi, N. Yadva, R. Mahato, C.K. Hazra, Lambert salt-initiated development of friedel-crafts reaction on isatin to access distinct derivatives of oxindoles, J. Org. Chem. 86 (2021) 17833–17847, https://doi.org/10.1021/acs.joc.1c02058.
[23] J.T. Xia, W.Q. Wu, F. Jiang, S.S. Zhu, A novel vardenafil analog in a healthcare product: preparation, characterization, and quantification, Heliyon 9 (2023) e19456, https://doi.org/10.1016/j.heliyon.2023.e19456.
[24] H.C. Lee, Y.L. Lin, Y.C. Huang, C.F. Tsai, D.Y. Wang, Identification of a new tadalafil analogue, N-3-hydroxypropylnortadalafil, in a supplement product, J. Pharm. Biomed. Anal. 158 (2018) 257–261, https://doi.org/10.1016/j.jpba.2018.05.049.
[25] T. Doi, K. Takahashi, M. Yamazaki, A. Asada, A. Takeda, K. Kiyota, T. Tagami, Y. Sawabe, T. Yamano, Characterization of a new illicit phosphodiesterase-type-5 inhibitor identified in the softgel shell of a dietary supplement, J. Pharm. Biomed. Anal. 161 (2018) 61–65, https://doi.org/10.1016/j.jpba.2018.08.031.