Original Article

Simultaneous analysis of 23 illegal adulterated aphrodisiac chemical ingredients in health foods and Chinese traditional patent medicines by ultrahigh performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry

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ABSTRACT

This paper presents an application of ultra high-performance liquid-chromatography-quadrupole-TOF high resolution mass spectrometry (UHPLC-Q-TOF HRMS) for simultaneous analysis of 23 illegal adulterated aphrodisiac type chemical ingredients in health foods and Chinese Traditional Patent Medicines (CTPMs). The mass spectrometer was operated in Information Dependent Acquisition (IDA) mode, which provides crucial information for the elemental composition analysis, structure elucidation and quantitative analysis simultaneously. Quantitative analysis was performed using the peak areas of the precursor ions in the XICs. The method validation included assessment of selectivity, sensitivity, calibration curve, accuracy, precision, recovery, matrix effect and stability. The results show good linear relationship with the concentrations of the analytes over wide concentration ranges (e.g., 0.05–10 μg/g for sildenafil) as all the fitting coefficients of determination r^2 are >0.9984. The detection limits (LODs) were in the range of 0.002–0.1 μg/g. The recoveries were able to reach 82.5–103.6%, while the matrix effects ranged from 87.7 to 109.3%. The intra- and inter-day accuracies were in the range of 82.3–113.8%, while the intra- and inter-day precision ranged from 0.4 to 13.6%. Among 40 batches of health foods and 32 batches of CTPMs (including 28 capsules, 32 tablets, 10 liquid and 2 pills) samples, 28 batches of heath foods were positive. The detected chemical ingredients involved sildenafil, tadalafil, aildenafil and sulfoaildenafil. This method can be used for the screening,

identification and quantification of illegal adulterated aphrodisiac chemical ingredients in health foods and CTPMs. Moreover, the LC-Q-TOF MS is very useful to structural elucidation of unknown compound.

1. Introduction

In recent years, health foods and Chinese Traditional Patent Medicines (CTPMs) have been booming because of being believed as safer and healthier than synthetic drugs and free of side effects [1]. However, for some unscrupulous manufacturers, the deliberate addition of chemical ingredients into health foods and CTPMs is a profit-driven practice aiming to intensify the claimed natural health benefits of the products [2]. Over the past few years, many types of illegal adulterants are being detected in various forms of health foods and CTPMs without labeling, which can lead to potentially serious public health consequences [3–11].

Erectile dysfunction (ED) is a highly prevalent inability to achieve and maintain adequate erection and sexual performance. Synthetic phosphodiesterase type 5 enzyme (PDE-5) inhibitors (e.g. sildenafil, tadalafil, vardenafil) are a class of drugs clinically used for the treatment of ED. In addition, other drugs which mechanism of action is different from PDE-5 can also be used in clinic. These drugs include yohimbine (α-2 receptor antagonist), apomorphine (dopamine receptor agonist), phentolamine (α adrenergic receptor blocker), dapoxetine (selective serotonin reuptake inhibitor), testosterone (androgen), etc. However, clinically adverse side effects, such as headaches, gastrointestinal distress, tachycardia, facial flushing, hypertension, nasalcongestion, visual disorders, muscle aches, dizziness, and possibility of blindness and hearing loss have been reported [12–17]. Moreover, PDE-5 inhibitors may also cause potentially serious drug–drug interactions [18]. The patients taking nitrate medications should not use PDE-5 inhibitor, as this combination may result in severe hypotension and syncope [18,19]. Fatal cases caused by adulterated dietary supplements have been reported [20,21].

Therefore, it is dangerous for unknowing patients to be taking such health foods or CTPMs adulterated with PDE-5 inhibitors and other aphrodisiac chemical ingredients. Over the past few years, the approved PDE-5 inhibitors and their unapproved synthetic analogs have been routinely identified in "all-natural" health foods and CTPMs which claim to enhance sexual performance [22–31].

To escape regulatory detection and quality checking, the manufacturers of such illicit and adulterated sexual performance enhancement products are now using new analogs and other aphrodisiac drugs which are difficult to be detected by routine screening and inspection methods. A number of analytical methods have been developed for screening and confirmation of PDE-5 inhibitors in illicit sexual performance enhancer products, such as immunoassay [32], ion mobility spectrometry [33], thin-layer chromatography [34], high performance liquid chromatography (HPLC) with ultraviolet or fluorescence detection [35–38], gas chromatography–mass spectrometry (GC–MS) [39–42], liquid chromatography–mass spectrometry (LC–MS) [43–49]. Several literature have reported the detection of chemical substances in food or dietary supplements using high-resolution mass spectrometry (HRMS) with quadrupole-Orbitrap (Q-Orbitrap), atmospheric solids analysis probe (ASAP) or Fourier transform ion cyclotron resonance (FTICR) mass analyzers [8,50–54].

Time-of-flight (TOF) is one of the importance remembers of high resolution mass spectrometers analyzer. The development of TOF technology in high resolution mass spectrometers has enabled mass spectrometers to provide accurate mass up to 4–6 decimal places. This is importance for us to deduce elemental composition and the molecular formula of a compound [7,55]. In the previous reports, the methods based HRMS analyzers with TOF (such as LC-MS/TOF, LC-IT/TOF, LC-Q/TOF) have been adopted for screening, identification of PDE-5 inhibitors and deducing its fragmentation pathways [22,56–59].

However, these methods focus on PDE-5 inhibitors and its analogs. Since profit purpose and lack of test method, the possibility of other types of impotence drugs as adulterant in health foods and CTPMs is greatly increasing. The aim of the present study was to develop a rapid and effective multi-analyte method coupling ultrahigh-performance liquid chromatography (UHPLC) to Q-TOF HRMS for the detection of 23 illegal adulterated aphrodisiac chemical ingredients. This method was successfully applied to the screening, identification and quantification of 23 illegal adulterated aphrodisiac chemical ingredients. To the best of our knowledge, this is the first time to report the application of UPLC-Q-TOF/MS in screening of various types illegal adulterated synthetic chemicals in health foods and CTPMs.

2. Materials and methods

2.1. Chemicals and reagents

Noracetildenafil (99.8%), acetildenafil (99.7%), vardenafil hydrochloride (99.2%), hydroxyhomosildenafil (99.8%), sildenafil (99.6%), homosildenafil (99.2%), aminotadalafil (99.9%), tadalafil (99.5%), pseudovardenafil (99.5%) and norneosildenafil (99.9%) were purchased from TLC Pharmaceutical Standards Ltd. (Aurora, Canada). Sulfoaildenafil (98.0%), apomorphine hydrochloride hemihydrates (98.0%), yohimbine hydrochloride (98.0%), aildenafil (98.5%), avanafil (99.0%), N-desmethyltadalafil (98.5%), udenafil (96.1%), propoxyphenylthiohydroxyhomosildenafil (96.0%), dapoxetine hydrochloride (98.0%) and phentolamine methanesulfonate salt (98.3%)

were purchased from Toronto Research Chemicals Inc. (Toronto, Canada). Testosterone (99.5%) was purchased from Acros Organics Inc. (Geel, Belgium). Methyltestosterone (99.5%) and testosterone propionate (99.8%) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Their chemical structures are displayed in Fig. 1. Chinese traditional patent medicines and health foods which claimed functions of aphrodisiac, enhancement of sexual performance, physical fatigue relief or immunity enhancement were bought from the local drug shops or markets. The samples without the studied 23 illegal adulterants were used as blank matrices. LC-MS grade acetonitrile, acetic acid and ammonium acetate were purchased from Thermo Fisher Scientific Inc. (MA, USA). Ultrapure water (18.2 M) was obtained from a Milli-Q Advantage A10 ultrapure water purification system.

2.2.   Instrumentation

The UHPLC-Q-TOF-MS/MS system consisted of two ExionLC AD pumps and an ExionLC AD autosampler coupled with a high resolution X500 Q-TOF mass spectrometer (Sciex, USA). The SCIEX OS 1.0 software from Sciex (Sciex, USA) contains instrument control, data acquisition, data processing, and reporting functionality, all in the one package. Chromatographic separation was achieved on a Agilent SB-C18 RRHD column (100 mm × 3.0 mm, 1.8 μm) (Agilent Technologies, USA). All centrifugation were performed on a Sigma 3−30 K refrigerated centrifuge (Sigma, Germany). Ultrasonic process was operated on a KQ-300 GDV Thermostat Ultrasonic Instrument (Kunshan, China).

2.3.   Standard solutions

All individual standard stock solutions were prepared in acetonitrile at 1 mg/mL and stored at −20 °C. An intermediate standard mixture of the reference compounds was prepared by appropriate dilution of the individual stock solutions in acetonitrile. Matrix-matched working solutions were freshly prepared in blank sample extracts, which were extracted from the commercial products purchased from the local market. All of the stock and working solutions were stored at −20 °C in the dark when not in use.

2.4.   Sample preparation

Chinese traditional patent medicines and health foods in this study were presented in four different oral forms
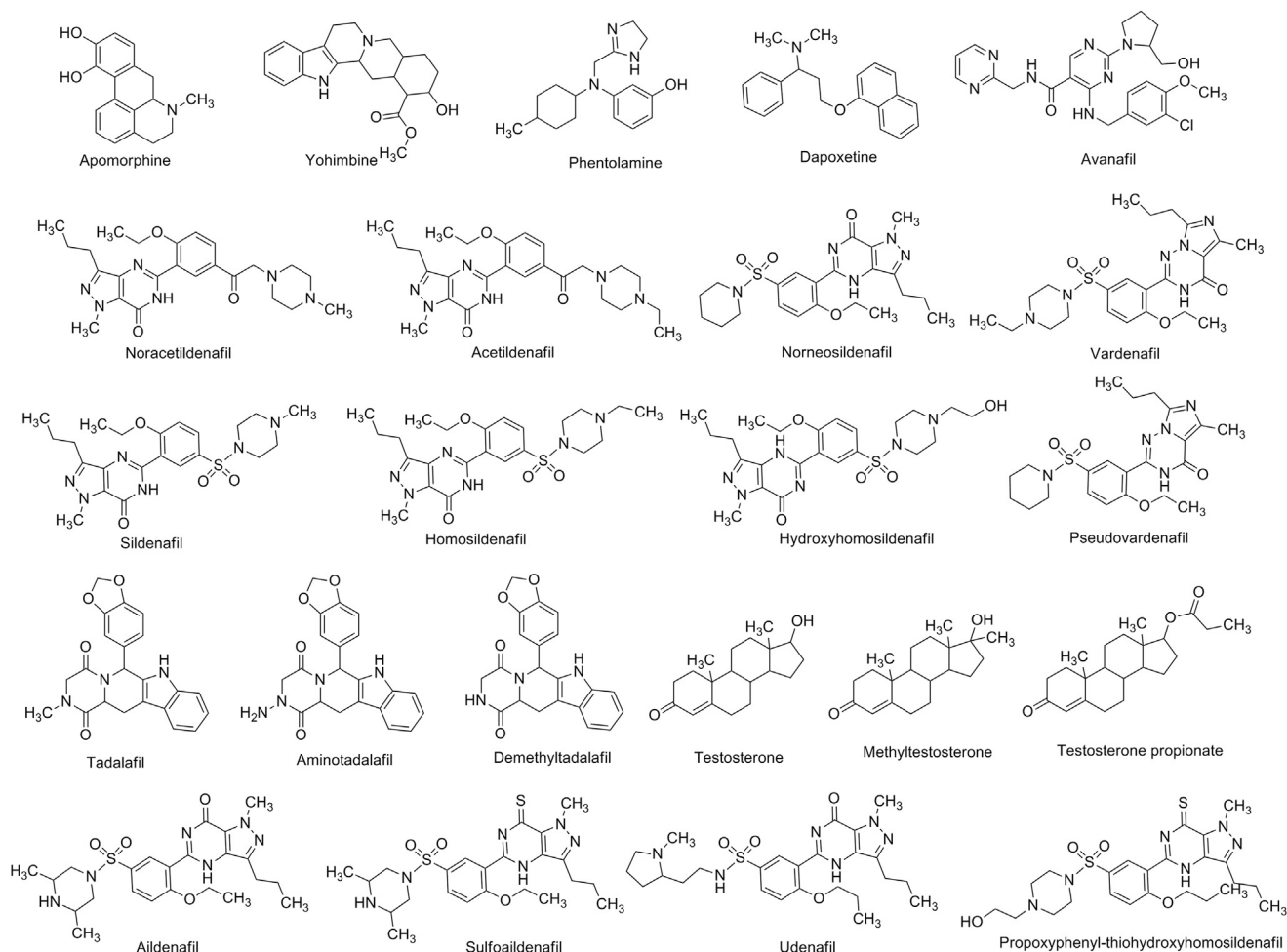

Fig. 1 − Chemical structures of studied 23 analytes.

including pellets, capsules, tablets or oral liquid. The tablets and pellets were smashed into fine powder; the capsules were cut open and the contents were mixed and then thoroughly homogenized. For oral liquids, three samples were evenly mixed and divided into fractions. Then a single oral dose of 0.2 g solid samples (for tablets, pills and capsules) or 2 mL oral liquid was accurately transferred to a 15 mL centrifuge tube and extracted with 10 mL ACN–H_2O (8:2, v/v), followed by vortex for 1 min, sonication for 15 min, and centrifugation at 5000 rpm for 15 min, successively. The upper phase was immediately withdrawn and filtered through a 0.22 μm pore PTFE syringe filter. The subsequent filtrate was used for the UHPLC–MS analysis. Blank matrices samples were treated as samples described above. When the sample concentration was beyond the linear range, the sample solution was diluted to make the detection response within the linear ranges.

2.5. Chromatographic conditions

Chromatographic separation was performed on a SB-C18 RRHD column of Agilent (100 mm × 3.0 mm, 1.8 μm). A binary mobile solvent was used: mobile solvent A was 5 mmol/L ammonium acetate solution (adjusted pH to 3.4 with acetic acid), and mobile solvent B was acetonitrile. The mobile phase was delivered at a flow rate of 0.4 mL/min with a gradient elution profile. The gradient began at 25% B for 2 min, and then linearly ramped to 55% B within 11 min, then ramped to 90% B in 1 min and held at 90% B for 2.0 min, then the column was re-equilibrated at 25% B for 2 min prior to the next injection. The autosampler tray temperature was set to 15 °C, while the column temperature was 40 °C. The injection volume was 5 μL.

2.6. Mass spectrometry conditions

The Q-TOF HRMS was equipped with a Turbo V™ ion source and the ESI⁺ mode was applied. The spray voltage and ion source temperature were set to 5.5 kV and 600 °C, respectively. The ion source gas 1, ion source gas 2, curtain gas and CAD gas were set to 55, 60, 35 and 7 psi, respectively. The analysis was executed in information dependent acquisition (IDA) mode. Under IDA mode, a TOF MS scan was performed firstly to generate "information" and a TOF MS/MS scan was then occurred based on predefined IDA criteria using information obtained in the TOF MS scan. The MS/MS spectra was generated in product ion scan mode at collision energies (CE) of 60 V with CE spread of 40 V.

2.7. Method validation

Quantitative analysis was performed using the peak areas of the precursor ions in the XICs. Method validation for assaying 23 aphrodisiac chemical drugs in traditional Chinese preparation and health food was done referring to the US Food and Drug Administration (FDA) guidelines on Bioanalytical Method Validation. The validation parameters included selectivity, sensitivity, calibration curve, accuracy and precision, recovery, matrix effect and stability.

2.7.1. Selectivity
The selectivity is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the matrix. The selectivity of the method was evaluated by determining the level of interfering components in six individual sources of blank matrix.

2.7.2. Calibration curve
Calibration curve was constructed by plotting the analyte peak area (Y-axis) vs a series of analyte concentrations (X-axis). The regression equations were described as Y = a + bX, which was used to calculate the concentrations of QCs and samples. Linearity was assessed by the coefficient of determination (r^2).

The limit of detection (LOD) was determined as the lowest concentration giving a signal-to-noise ratio of at least threefold (S/N ≥ 3). The lower limit of quantitation (LLOQ) was defined as the lowest concentration of the calibration curve, giving a signal-to-noise ratio of at least 10-fold (S/N ≥ 10), acceptable accuracy (80–120%) and sufficient precision (within 20%).

2.7.3. Accuracy and precision
The accuracy was calculated from the ratio of the mean values of the detected concentration (C_{det}) and the nominal concentration (C_{nom}) as following: (C_{det}/C_{nom}) × 100. The precision was expressed by relative standard deviation (RSD), which was calculated as RSD% = [standard deviation (SD)/C_{det}] × 100.

2.7.4. Recovery
The recoveries of 23 aphrodisiac chemical drugs from matrices were investigated by comparing the response of 23 aphrodisiac chemical drugs after extraction from matrices with the response of the same concentration analytes spiked into the solution extracted from blank matrices.

2.7.5. Matrix effect
The matrix effect was evaluated by analyzing the response of analytes prepared in solvent and in extracted blank matrix at the same concentrations for three levels (low, medium and high). The value of matrix effect can be calculated as (Eq. (1)):

$$ME(\%) = B/A \times 100 \tag{1}$$

A refers to the peak areas obtained from neat solution standards, while B refers to the corresponding peak areas of standards spiked after extraction from matrix [60,61].

2.7.6. Stability
The post-preparative stability was conducted by repeatedly determining the processed QC samples which were kept in the autosampler (15 °C) for 24 h. The concentrations of the samples were calculated on the basis of original calibration standards.

2.8. Calculation

The chromatograms were processed using SCIEX OS 1.0 software developed by SCIEX. All calculations were completed in Microsoft Excel 2013 (Microsoft Co., Redmond, USA). The structures of chemicals were drawn in Chem & Bio Draw Ultra 12.0 (PerkinElmer, Inc., MA, USA).

2.9. Library-based qualitative screening and confirmation

The compound database could be imported and named by the Library Importer. Then, the exact mass library was generated in which the MS/MS spectra of each target parent ion were included.

Qualitative analysis is the identification of a target or untarget compound. In qualitative analysis, a sample can be processed with the searching library for screening out suspected positive samples and further reliable confirmation. Comparing acquired MS/MS spectra from unknown samples to a database of compounds with reference spectra is one of the most powerful tools in qualitative analysis. Library search algorithms compare the unknown spectra from the sample and then try to match the spectra to the known compounds and spectra in the database. The closer the match and the higher the reported score are, the more likely it is that the compound was identified.

In mass spectrometry, determining which compound is present is accomplished using mass accuracy, retention time, isotope pattern, library searching, and formula finding. Using all of these tools together can increase the confidence in identifying both targeted and non-targeted compounds in unknown samples. In general, the confidence levels for the qualitative rules were configured as: mass error less than 2 ppm, error in retention time less than 0.5 min, difference isotope ratio less than 5%, library hit score more than 80. If the confidence levels above were reached, the traffic light was green. Their weights are 30%, 30%, 10%, 30%, respectively. The combined score was obtained by synthesizing above four factors. The higher the score, the greater the possibility of positive was. The library search results, formula finding results, and other qualitative analysis results are available in the *Results Table. Results Tables* also include the calibration curves, the calculated concentration of each analyte, as well as statistics for the results.

3. Results and discussion

3.1. UHPLC-HRMS optimization

The peak shape and the chromatographic resolution were the main criteria of evaluation during optimization of the UHPLC method. Chromatographic conditions, such as the composition of mobile phase and the gradient condition, played a critical role in achieving good chromatographic behavior and appropriate ionization [8]. Different solvent compositions were investigated for the mobile phase consisting of methanol or acetonitrile as organic phase and water with formic acid, acetic acid, ammonium formate, ammonium acetate as aqueous phase. The pH of the mobile phase was optimized because most analytes contained one or more basic groups. Accordingly, water (containing 5 mM ammonium acetate and adjusted pH 3.4 with acetic acid)—acetonitrile was applied as the binary mobile solvents system. The gradient elution procedure was optimized and described in Section 2.5. Satisfactory results were obtained using the binary mobile solvents system and the gradient

elution procedure described above, giving better peak shapes and excellent chromatographic separation of the 23 analytes within 18 min. Furthermore, no carryover could be detected.

Before the formal experiment started, a positive quick status check was performed to quickly verify the mass accuracy and resolution in TOF MS and MS/MS modes. If the mass accuracy did not meet the specification, the steps above could be repeated. If the resolution did not meet the specification, the TOF Tuning procedure could be performed to optimize the system. Thus, the satisfactory results were obtained with the mass errorless than 2 ppm and the resolution greater than 30,000.

Under the optimized chromatographic conditions, acceptable chromatographic separation of 23 chemicals was achieved expect for aildenafil (7.87 min, m/z 489.2278) and avanafil (7.89 min, m/z 484.1858) (Fig. 2B). But this couple can be identified because of their m/z distinction. Furthermore, it can be noted that the baseline separation can be reached for two groups of isomers, as was the case of vardenafil, aildenafil and homosildenafil (m/z 489.2278); and pseudovardenafil and nor-neosildenafil (m/z 460.2013). Therefore, all 23 compounds did not interfere with each other for qualitation and quantitation using the optimized methods in this assay. Therefore, the combination of RT, mass accuracy and MS^2 fragment ions (Fig. 3) provide a suitable detection of the compounds. As showed in Table 1, all the mass errors (ppm) between theoretical and experimental m/z of analytes were below 2, indicating the highly reliable mass accuracy of Q-TOF.

3.2. Method validation

3.2.1. Selectivity
Representative XICs of 23 aphrodisiac chemicals in blank matrices and matrices spiked with 23 analytes were shown in Fig. 2A and B, respectively. The Q-TOF HRMS provided sufficient resolving power to distinguish analytes from the isobaric co-eluting sample matrix compounds. No interfering peaks were detected at the RT of all analytes in the matrix.

3.2.2. Calibration curve
The linear relationship between the chromatographic peak area and the concentration of the analytes was investigated and exhibited in Table 1. The linearity of calibration curve was assessed by the coefficient of determination (r^2). As can be seen in Table 1, the calibration curves showed good linearity with r^2 values higher than 0.9984 for all 23 analytes.

The LODs and LLOQs were determined as the analyte concentrations giving peak heights at least 3 and 10 times higher than the baseline noise, respectively. All the LOD and LLOQ values were in ranges of 0.002—0.1 μg/g and 0.005—0.25 μg/g, respectively.

3.2.3. Accuracy and precision
The results of intra- and inter-day accuracy and precision analyses performed at three spiking levels (low, medium and high) are presented in Table 2. The intra-day and inter-day accuracy of 23 analytes ranged from 82.8% to 113.0% and 80.0%—113.8%, respectively. The intra- and inter-day precision was in the range of 0.4—9.0% and 0.7—13.6%, respectively.
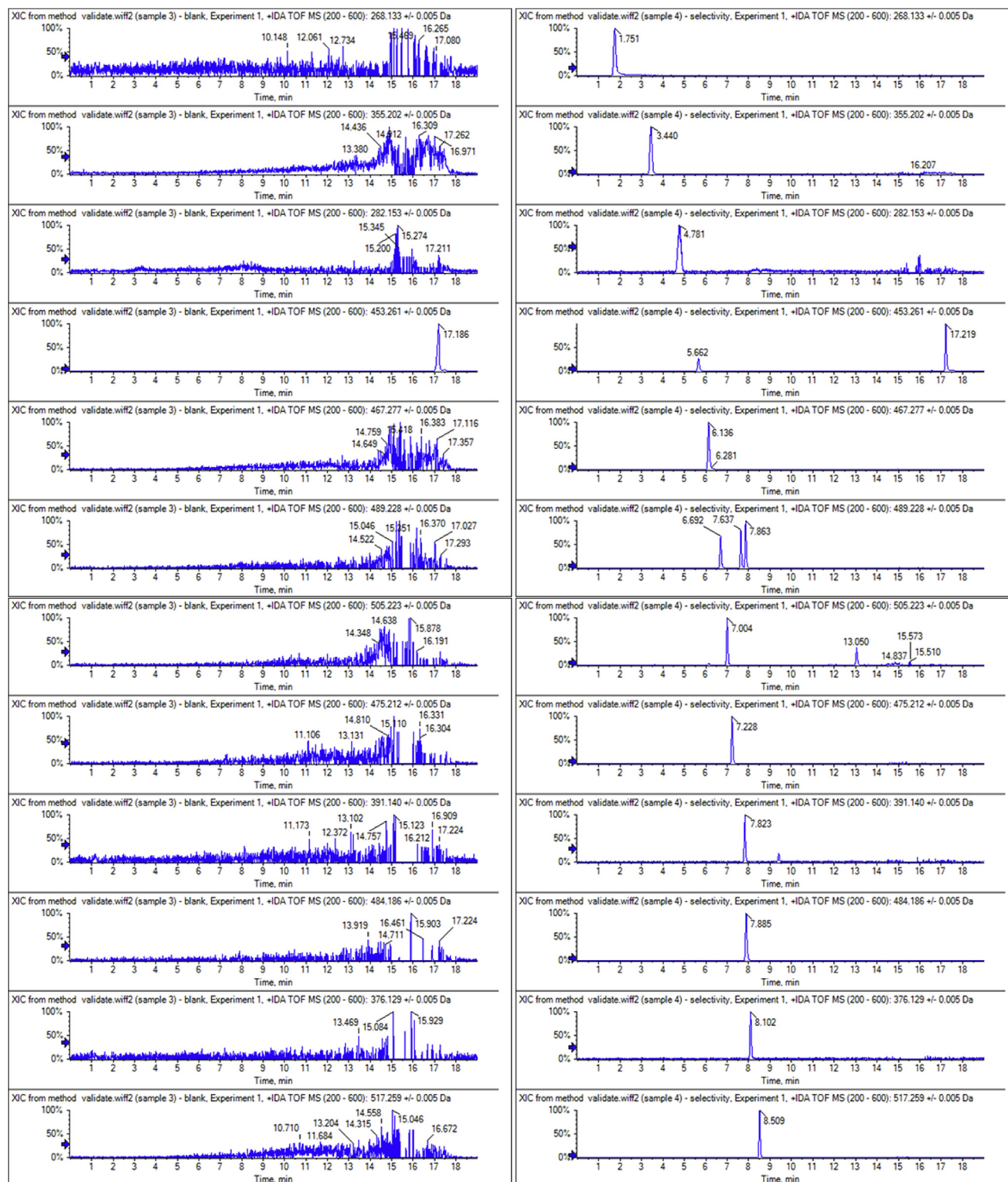
Fig. 2 – **Extracted ion chromatograms of 23 analytes in (A) blank matrices and (B) matrices spiked with 0.1 μg/g 23 analytes standards.**

These accuracy and precision results were within the acceptable criteria, showing that the method was reliable for the quantitative analysis of the 23 analytes in Chinese traditional patent medicines and health foods.

3.2.4. Recovery and matrix effect

As shown in Table 2, the extraction recoveries of the 23 analytes at three concentrations (low, medium and high) were over the range of 82.5–103.6%, which indicated that the
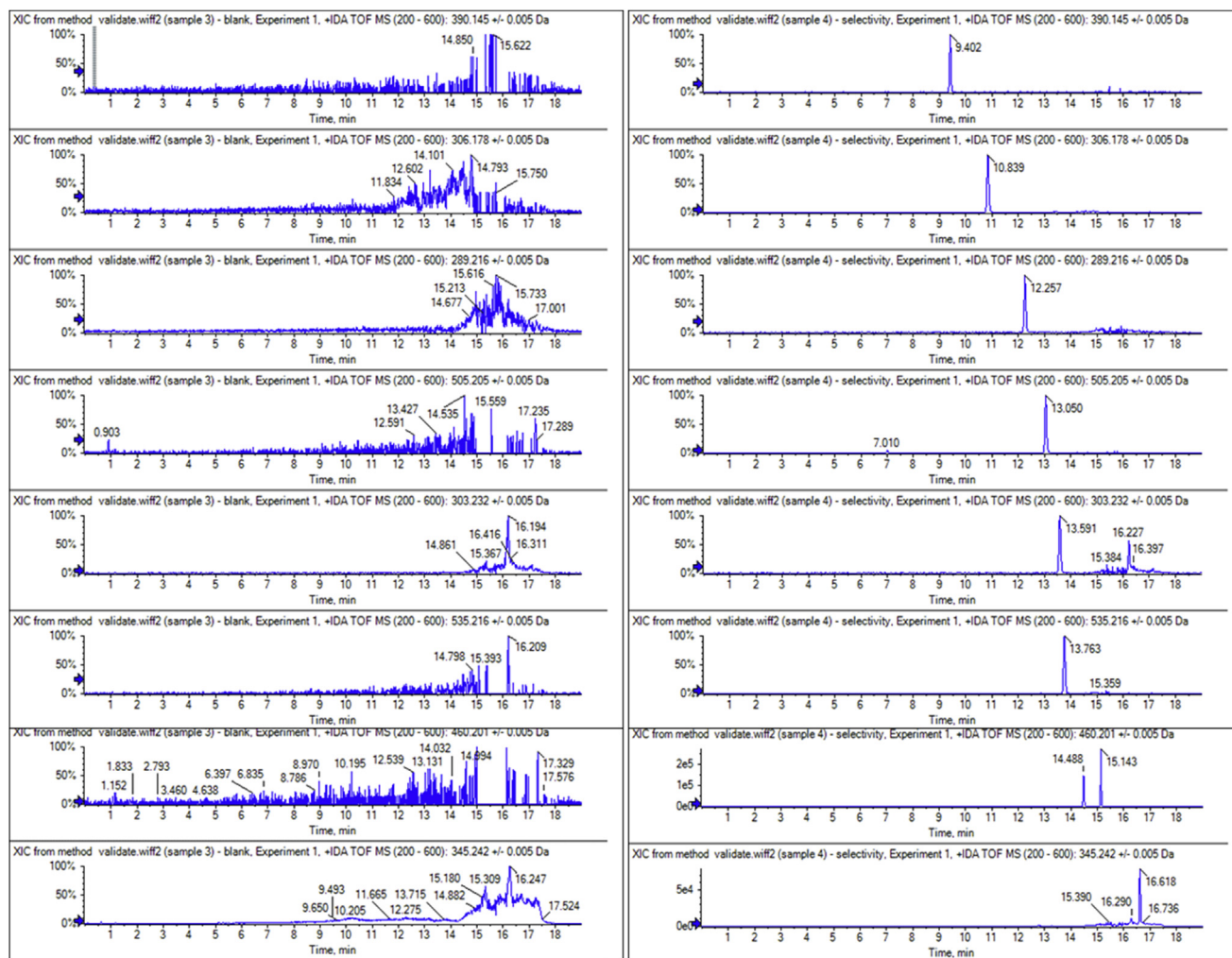
Fig. 2 — (continued).

selected extraction solvent ACN–H2O (8:2, v/v) could provide excellent extraction efficiency for all the 23 analytes from matrix.

The results of matrix effects evaluation are shown in Table 2. Signal suppression or enhancement effect was considered tolerable if the matrix effect value was in the range of 80%–120%. As shown in Table 2, the matrix effects of analytes ranged from 87.7% to 109.0% with SDs less than 8.8%. With such low level of matrix effects, this assay would be reliable for analysis in matrix.

3.2.5. Stability

As summarized in Table 2, the post-preparative was expressed as a percentage of the initial concentration (first analyzed batch) of the analytes in QC samples. The stability of all analytes in matrix was over the range of 80.3–111.0%, which indicated that all 23 analytes were stable in autosampler (24 h) at 15 °C. The results suggested that the developed method was reliable and suited for large scale sample screening.

3.3. Application of the Q-TOF/MSMS method to real samples

The application of the developed method for identifying the 23 aphrodisiac chemical ingredients in health foods or CTPMs was evaluated. Considering the fact that aphrodisiac chemical ingredients have been frequently detected in health foods or CTPMs in recent years, the significance of a novel high sensitive and high selective assay in this field is obvious.

Qualitative analysis is the identification of a target or unknown compound. In mass spectrometry, determining which compound is present is accomplished using mass accuracy, retention time, isotope pattern, library searching, and formula finding. Using all of these tools together can increase the confidence in identifying both targeted and non-targeted compounds in unknown samples. In general, the confidence levels for the qualitative rules were configured as: mass error less than 5 ppm, error in retention time less than 0.5 min, difference isotope ratio less than 5%, library hit score
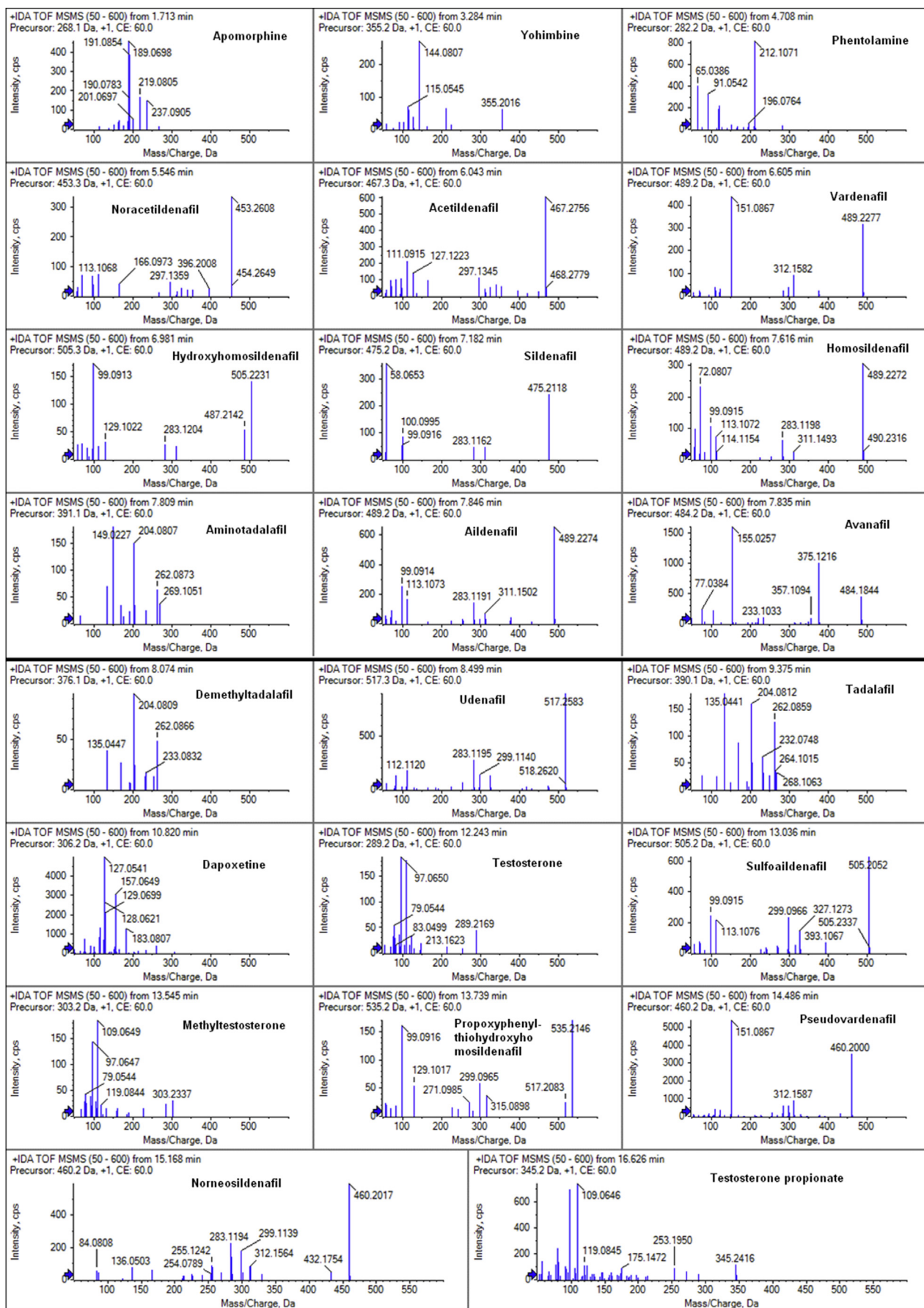
Fig. 3 — MS² fragment ions of 23 analytes.

Table 1 – Retention time, accurate mass, correlation coefficients, linear ranges, LODs and LLOQs of 23 analytes.

Analyte	Retention time (min)	Protonated ion mass [M + H]+		Mass error (ppm)	Correlation coefficient (r²)	Linear range (μg/g)	LOD (μg/g)	LLOQ (μg/g)
		Theoretical	Experimental					
Apomorphine	1.72	268.1332	268.1331	−0.4	0.9995	0.15–10	0.05	0.15
Yohimbine	3.42	355.2016	355.2013	−0.8	0.9994	0.15–10	0.05	0.15
Phentolamine	4.78	282.1601	282.1603	0.7	0.9991	0.05–8	0.02	0.05
Noracetildenafil	5.65	453.2609	453.2608	−0.2	0.9991	0.05–10	0.02	0.05
Acetildenafil	6.12	467.2765	467.2761	−0.9	0.9986	0.05–10	0.02	0.05
Vardenafil	6.70	489.2279	489.2274	−1.0	0.9998	0.05–10	0.02	0.05
Hydroxyhomosildenafil	7.00	505.2228	505.2227	−0.2	1.0000	0.05–10	0.02	0.05
Sildenafil	7.22	475.2122	475.2118	−0.8	1.0000	0.05–10	0.02	0.05
Homosildenafil	7.63	489.2279	489.2274	−1.0	1.0000	0.05–10	0.02	0.05
Aminotadalafil	7.83	391.1401	391.1403	0.5	0.9996	0.25–10	0.10	0.25
Aildenafil	7.87	489.2278	489.2274	−0.8	1.0000	0.05–10	0.02	0.05
Avanafil	7.89	484.1858	484.1855	−0.6	0.9993	0.05–10	0.02	0.05
Demethyltadalafil	8.10	376.1298	376.1294	−1.1	0.9996	0.25–10	0.10	0.25
Udenafil	8.52	517.2592	517.2587	−1.0	0.9992	0.05–10	0.02	0.05
Tadalafil	9.41	390.1448	390.145	0.5	0.9996	0.15–10	0.05	0.15
Dapoxetine	10.84	306.1852	306.1849	−1.0	0.9984	0.005–2.5	0.002	0.005
Testosterone	12.25	289.2162	289.2161	−0.3	0.9997	0.15–10	0.05	0.15
Sulfoaildenafil	13.05	505.2050	505.2048	−0.4	0.9994	0.05–10	0.02	0.05
Methyltestosterone	13.58	303.2319	303.2321	0.7	0.9991	0.15–10	0.05	0.15
Propoxyphenyl-thiohydroxyhomosildenafil	13.76	535.2156	535.2152	−0.7	0.9995	0.15–10	0.05	0.15
Pseudovardenafil	14.48	460.2013	460.2009	−0.9	0.9991	0.05–10	0.02	0.05
Norneosildenafil	15.14	460.2013	460.2007	−1.3	0.9994	0.05–10	0.02	0.05
Testosterone propionate	16.28	345.2424	345.2423	−0.3	0.9998	0.25–10	0.10	0.25

more than 80. If the confidence levels above were reached, the traffic light was green. Their weights are 30%, 30%, 10%, 30%, respectively. The combined score was obtained by synthesizing above four factors. The higher the score, the greater the possibility of positive was. Quantitative analysis was performed using the peak areas of the precursor ions in the XICs.

The developed UHPLC-Q-TOF/MSMS method was adopted for the routine screening of the 23 aphrodisiac chemical ingredients in 40 batches of health foods and 32 batches of CTPMs (including 28 capsules, 32 tablets, 10 liquid and 2 pills). These samples were collected from the local markets by Shandong Food and Drug Administration. The results showed that all the CTPMs were negative, while 28 batches of heath foods were positive. Sildenafil and tadalafil were detected simultaneously in 4 health foods, while aildenafil and sulfoaildenafil was detected simultaneously in 1 batch sample. Sildenafil was detected in 26 batches oral solid preparation with contents of 2.8–272.0 mg/g. Tadalafil was detected in 5 batches oral solid preparation with contents of 0.78–80.9 mg/g. Aildenafil and sulfoaildenafil was detected simultaneously in 1 batch liquid sample with contents of 102.3 μg/mL and 185.7 μg/mL respectively. As an example, Fig. 4 showed the extracted ion chromatograms of a representative positive sample which is used as a health wine product for tonifying kidney and improving sexual performance. This mass chromatography in Fig. 4 indicates that at least three main peaks (I, II, and III) appeared around 8–13 min, besides many other peaks from matrix interference.

After processing the chromatograms using the method based on the qualitative and quantitative work flow of target compound, the results were shown in Results Table. As can be seen from the Results Table, the traffic lights of peaks I and III were green. Also, library research results show that the MS/MS spectra agree well with the library (purity 93.6 and 100, see Fig. 5.). Therefore, the compound-I and compound-III were assigned to aildenafil (m/z 489.2274, 7.87 min) and sulfoaildenafil (m/z 505.2048, 13.05 min).

For compound-II (m/z 517.2579, 13.00 min), despite the accurate mass matched well with udenafil (m/z 517.2587, 8.52 min), a false positive identification was produced because its retention time and MS/MS spectra were totally different from udenafil. By analyzing the MS/MS spectrum of compound-II, it is very likely to belong to the family of sildenafil [50,57,62,63] because of the presence of fragment ions including m/z 489.2, 377.1, 311.1 and 283.1 (see Fig. 6). Among these fragment ions, the major product ion (m/z 489.2299) matched well with aildenafil (m/z 489.2274) with minor mass error (5 ppm). Moreover, we observed that the peak-II would disappear after a sample solution was diluted and placed for about one month, and no new peak arose. Therefore, it indicates that compound-II is a precursor of aildenafil ($C_{23}H_{32}N_6O_4S$), which may be generated by loss of C_2H_4 from the unknown molecule. Since the structural modifications of sildenafil analogs are commonly performed on the piperazine ring [5], it is a good indication of the insertion of one ethyl group attached to the nitrogen in the piperazine ring of aildenafil. These indicate that the compound-II is very likely to be a new synthetic analog of aildenafil, in which the 2,6-dimethylpiperazine moiety is replaced with N-ethyl-2,6-dimethyl piperazine. The chemical structure and the proposed fragmentation pathways forits main fragment ions are outlined in Fig. 6, which matches the findings reported by other authors [5,50,57,62,63].

Analyte	QC concentration (µg/g)	Accuracy (%)		Precision (RSD, %)		Recovery (Mean ± SD, %)	Matrix effect (Mean ± SD, %)	Stability (Mean ± SD, %)
		Intra-day	Inter-day	Intra-day	Inter-day			
Apomorphine	0.2	107.5	94.6	5.3	3.5	82.5 ± 7.6	95.7 ± 8.8	86 ± 5.3
	2.5	96.1	95.7	3.4	4.5	94.1 ± 2.4	99.5 ± 3.1	94.7 ± 3.9
	8	99.6	99.1	5.6	2.8	94.3 ± 6.9	97.1 ± 1.2	97.2 ± 6.9
Yohimbine	0.2	109.0	91.3	1.7	1.5	88.6 ± 5.4	100.8 ± 7.3	87.9 ± 7.6
	2.5	100.0	99.2	1.1	2.0	97.1 ± 1.2	103.2 ± 1.4	94.2 ± 2.1
	8	99.3	93.9	1.6	2.1	93.9 ± 3.3	102.6 ± 2.3	91.7 ± 2.8
Phentolamine	0.3	113.0	96.1	2.1	1.2	97.6 ± 3.4	109 ± 5.8	91.9 ± 2.2
	2.5	98.7	90.7	1.3	6.7	97.2 ± 0.8	98.6 ± 0.7	100.9 ± 2.2
	8	99.4	89.1	0.6	1.6	97.5 ± 1.9	97.9 ± 1.7	101.1 ± 1.8
Noracetildenafil	0.06	91.4	84.2	2.3	2.9	94.2 ± 4.3	102.4 ± 1.9	92.1 ± 2.1
	0.5	106.8	102.4	1.4	1.1	98.7 ± 1.2	96.6 ± 1.5	96.7 ± 1.6
	8	101.7	100.0	0.7	1.6	98.7 ± 1.3	105.9 ± 0.8	98.4 ± 2.4
Acetildenafil	0.06	83.0	83.4	1.6	1.9	99.2 ± 2.4	95 ± 1.1	100.4 ± 1
	0.5	110.8	106.8	1.5	1.2	98.4 ± 1.5	101.2 ± 1.4	96.4 ± 1.9
	8	100.8	99.0	1.8	1.5	100 ± 2.8	106.3 ± 2.0	98.2 ± 2.1
Vardenafil	0.06	91.9	90.7	2.6	4.4	101.3 ± 3.4	93.6 ± 2.2	98.7 ± 4.2
	0.5	96.9	92.1	1.2	2.5	97.7 ± 1.7	95.2 ± 1.2	95.1 ± 2.6
	8	95.6	98.7	1.4	5.0	98.4 ± 1.2	96.7 ± 1.3	103.3 ± 6.2
Hydroxyhomosildenafil	0.06	105.0	102.4	2.7	2.5	100.3 ± 4.3	103.2 ± 2.6	97.6 ± 3.6
	0.5	97.9	91.4	2.1	0.8	96.3 ± 3.1	99.4 ± 2.1	93.4 ± 1.8
	8	97.1	94.7	1.1	2.9	98.4 ± 0.7	97.1 ± 1.0	97.6 ± 3.7
Sildenafil	0.06	92.4	87.5	7.8	4.2	96.6 ± 8.6	94.4 ± 5.7	91.7 ± 6.4
	0.5	97.5	92.7	1.5	2.0	96.5 ± 0.8	97.1 ± 1.4	95.1 ± 2.7
	8	96.9	94.5	0.6	1.2	97.2 ± 1.2	96.7 ± 0.7	97.5 ± 1.7
Homosildenafil	0.06	94.0	90.6	1.8	1.0	99.1 ± 3.8	95.9 ± 1.5	96.4 ± 1.8
	0.5	98.2	94.4	1.3	1.4	97.6 ± 2.0	96.8 ± 1.3	96.1 ± 1.0
	8	97.0	96.5	1.2	3.5	98.1 ± 1.7	97.3 ± 1.1	99.5 ± 4.2
Aminotadalafil	0.3	86.0	86.5	4.9	3.5	98 ± 12.4	109.3 ± 11	100.8 ± 7.5
	2.5	95.1	92.3	2.2	1.7	96.7 ± 1.7	97.8 ± 4.6	97.1 ± 3.2
	8	96.0	92.8	3.2	1.1	94.3 ± 1.7	100.1 ± 0.8	96.8 ± 3.5
Aildenafil	0.06	96.9	97.7	5.9	1.0	103.6 ± 5.8	99.5 ± 5.4	101.1 ± 5.8
	0.5	99.7	96.5	1.1	0.7	98.2 ± 1.2	97 ± 1.0	96.9 ± 0.7
	8	96.3	95.3	0.6	3.2	97.8 ± 0.7	99.5 ± 0.7	99 ± 3.5
Avanafil	0.06	82.8	80.0	1.5	1.4	96.6 ± 1.0	98.1 ± 1.1	96.6 ± 1.7
	0.5	107.2	105.0	0.4	1.2	99.1 ± 0.7	92.4 ± 0.4	97.9 ± 1.4
	8	96.5	99.7	0.9	3.3	99.9 ± 1.2	102.8 ± 1.0	103.3 ± 3.9
Demethyltadalafil	0.3	92.8	88.3	6.3	5.2	97.4 ± 4.7	104.4 ± 6.2	95.3 ± 3.6
	2.5	96.0	91.8	1.9	1.5	95.9 ± 2.2	99.3 ± 2.3	95.7 ± 2.4
	8	96.6	93.0	1.5	1.8	96.3 ± 1.8	100.8 ± 0.8	96.3 ± 2.1
Udenafil	0.06	95.1	91.8	1.2	1.2	95.3 ± 2.2	100.8 ± 1.2	96.6 ± 2.1
	0.5	103.8	98.4	0.4	1.3	98 ± 1.3	99.2 ± 0.4	94.8 ± 1.4
	8	99.9	100	0.4	1.5	99.6 ± 1.0	103.1 ± 0.6	100.1 ± 1.4

| Analyte	QC concentration (µg/g)	Accuracy (%)		Precision (RSD, %)		Recovery (Mean ± SD, %)	Matrix effect (Mean ± SD, %)	Stability (Mean ± SD, %)
		Intra-day	Inter-day	Intra-day	Inter-day			
Tadalafil	0.18	102.4	113.8	1.9	12.1	87.3 ± 7.3	87.7 ± 8.4	111 ± 11.8
	2.5	100.3	96.8	3.4	2.6	98.6 ± 1.4	94.3 ± 5.1	96.5 ± 4.7
	8	97.7	89.3	1.7	1.7	98.3 ± 1.0	99.1 ± 1.6	91.4 ± 3.0
Dapoxetine	0.02	107.9	99.4	3.8	1.9	101 ± 7.4	100.6 ± 4.9	97.8 ± 8.4
	0.25	108.2	112.7	1.4	1.8	98.2 ± 1.6	99.4 ± 1.1	101.6 ± 1.9
	2.5	93.5	95	1.7	3.6	97.4 ± 2.3	93.1 ± 1.9	106.4 ± 1.9
Testosterone	0.2	103	91.8	6.4	7.8	94.4 ± 11.3	104.1 ± 6.3	87 ± 16.4
	2.5	100.9	104.6	3.3	1.6	100.6 ± 2.5	104.3 ± 5.5	96.5 ± 7
	8	101.7	96.1	4.1	1.3	94.4 ± 4.1	105.6 ± 4.5	89.3 ± 3.7
Sulfoaildenafil	0.06	96.1	94.4	6.6	7.6	101.3 ± 12.7	104.8 ± 4.4	98.6 ± 11.0
	0.5	105.2	82.3	4.6	12.2	86.7 ± 4.4	96.2 ± 4.2	80.3 ± 6.0
	8	93.9	89.3	1.5	2.5	96.8 ± 1.3	96.7 ± 1.6	95.1 ± 3.5
Methyltestosterone	0.2	101.6	95.9	6.4	5.3	99.4 ± 7.6	101.3 ± 2.2	96 ± 11.5
	2.5	101.3	106.1	1.7	1.6	99.9 ± 3.5	100 ± 3.8	100 ± 3.8
	8	98.9	97.9	2.6	3.4	99.6 ± 2.5	101.2 ± 2.3	98.4 ± 3
Propoxyphenyl-thiohydroxyhomosildenafil	0.2	105.4	91.6	8.2	13.6	97.3 ± 8.2	100.4 ± 5	87.8 ± 17.6
	2.5	95.3	91.5	9	6.6	92.3 ± 9.4	97.1 ± 8.3	96.7 ± 11.1
	8	99	91.1	1.2	1.9	95.6 ± 2.9	101 ± 1.2	92.1 ± 2.5
Pseudovardenafil	0.06	99.5	89.7	6.4	5.2	95.8 ± 5.2	108.9 ± 5.7	90.3 ± 5.8
	0.5	102.4	97.3	2.2	2.7	97.9 ± 2.1	93.9 ± 1.9	95.1 ± 1.7
	8	91.6	94.9	2.6	4.2	98.6 ± 2.7	92.6 ± 2.4	103.6 ± 4.7
Norneosildenafil	0.06	105.2	90.8	5.9	5.4	89.8 ± 6.4	105.8 ± 5.7	86.7 ± 9.4
	0.5	100.9	95.3	1.5	3.1	95.7 ± 1.7	100.7 ± 1.5	94.4 ± 4.0
	8	98.5	95.2	1.9	1.3	97.9 ± 1.2	96.9 ± 1.9	96.7 ± 2.6
Testosterone propionate	0.2	97.1	95.2	5.9	5.1	99.8 ± 9.8	95.4 ± 7.5	104.6 ± 5.5
	2.5	103	96.8	1.7	3.1	99.6 ± 5.2	98.1 ± 3.7	96.5 ± 4.8
	8	103.4	96	1.8	3.3	101.5 ± 3.6	105.7 ± 2.8	97.2 ± 1.9

Table 2 – (continued)
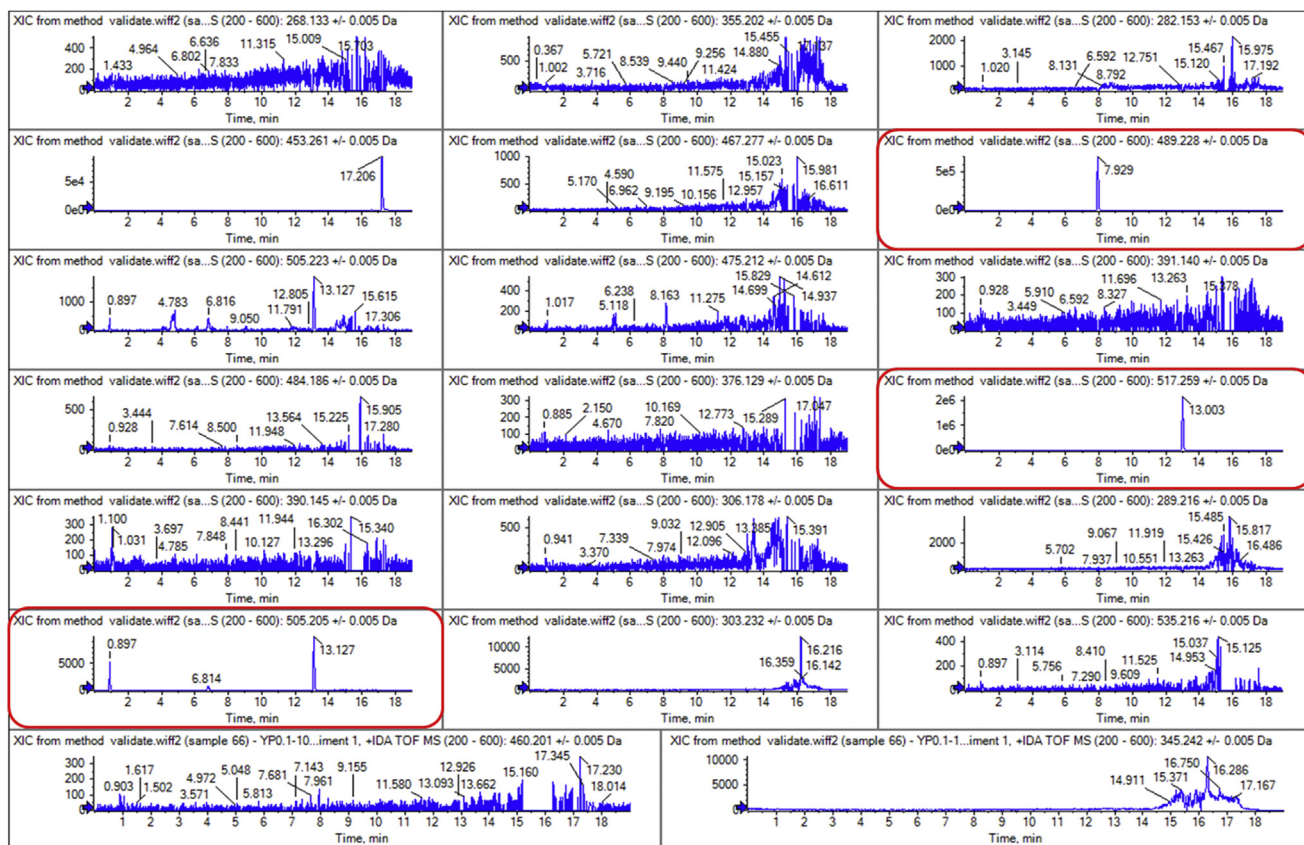
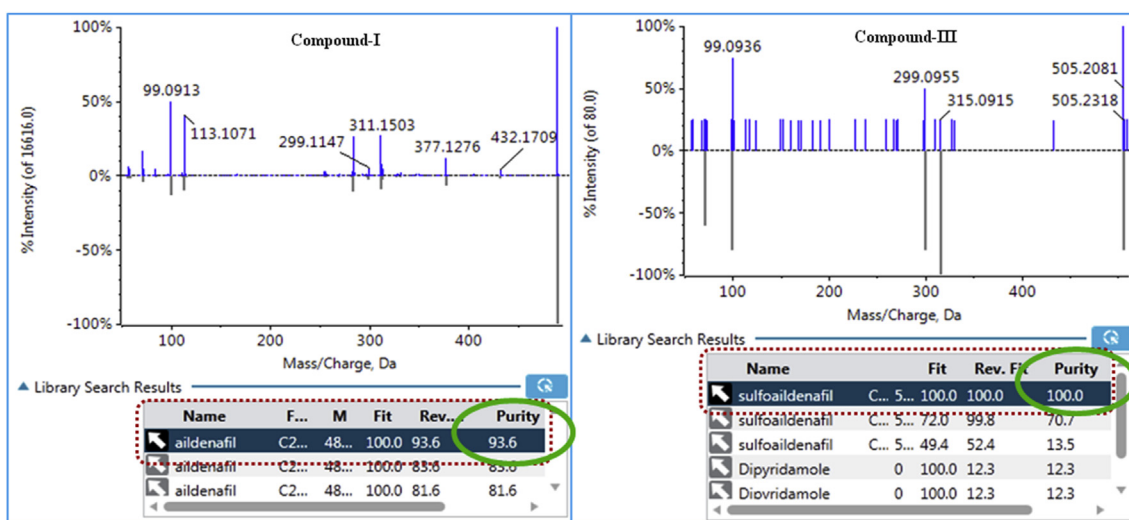Fig. 4 − **Extracted ion chromatograms of the representative positive sample.**

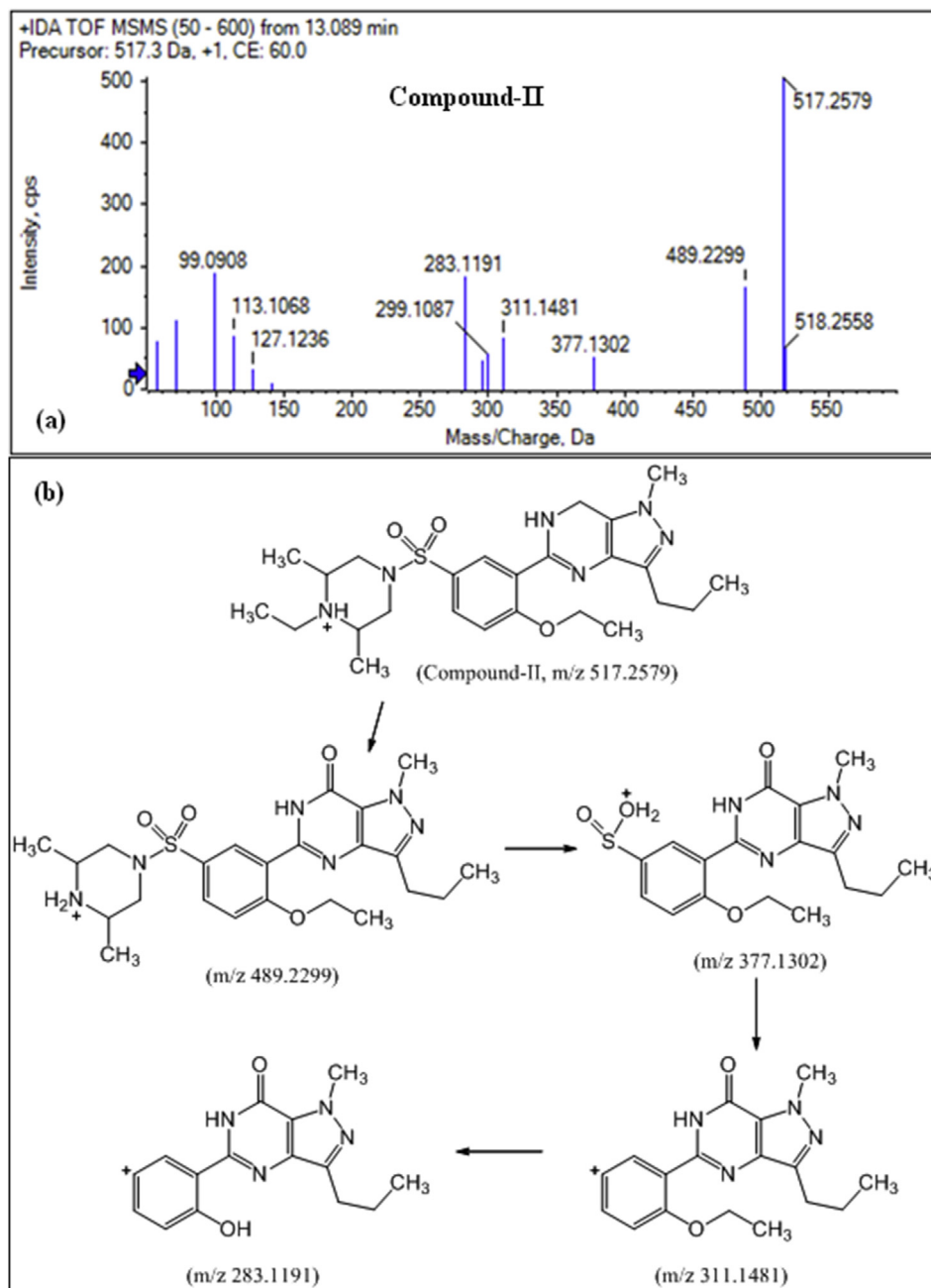Fig. 5 − **The library research results of compound-I and III.**

Fig. 6 − (a) MS^2 fragment ions of compound-II and (b) the proposed fragmentation pathways forits main fragment ions.

4.   Conclusions

A rapid, sensitive and selective Q-TOF HRMS method was established and employed to screen, confirm, and quantitate 23 illegal adulterated aphrodisiac chemical ingredients in health foods and CTPMs. To the best of our knowledge, this is the first time to report the application of UPLC-Q-TOF/MS in screening of various types illegal adulterated synthetic chemicals in health foods and CTPMs. Simultaneous identification, confirmation and quantitation of analytes were achieved based on IDA mode of the Q-TOF/MS analyzer. The response showed good linear relationship with the analytes' concentrations over wide ranges (e.g., 0.05–10 µg/g for

sildenafil) with most the coefficient of determinations (r^2) >0.9991. The detection limits (LODs) were in the range of 0.002–0.1 µg/g for different analytes. The recoveries ranged from 82.5% to 103.6%. The intra- and inter-day accuracies were in the range of 80.0%–113.8%, while the intra- and inter-day precision ranged from 0.4% to 13.6%.

Among 40 batches of health foods and 32 batches of CTPMs (including 28 capsules, 32 tablets, 10 liquid and 2 pills) samples, 28 batches of heath foods were positive. Sildenafil and tadalafil were detected simultaneously in 4 health foods, while aildenafil and sulfoaildenafil was detected simultaneously in 1 batch sample. The Q-TOF HRMS spectrometry has been proved to be a powerful tool for routine screening and quantitating of illegal adulterate in health foods and CTPMs.

Moreover, the LC-Q-TOF method is very useful to structural elucidation of unknown compound.

Acknowledgement

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