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Application of a high resolution benchtop quadrupole-Orbitrap mass spectrometry for the rapid screening, confirmation and quantification of illegal adulterated phosphodiesterase-5 inhibitors in herbal medicines and dietary supplements

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ABSTRACT

In this study, the applicability of high resolution quadrupole-Orbitrap (Q-Orbitrap) mass spectrometry for the simultaneous qualitative and quantitative analysis of illegal adulterated phosphodiesterase-5 inhibitors (PDE-5 inhibitors) in herbal medicines and dietary supplements was investigated. The mass spectrometer was operated in full MS scan/dd-MS^2 (data-dependent MS^2) mode. The use of 70 000 FWHM mass resolution and narrow mass windows (5 ppm) could effectively improve the selectivity of the method, increasing the signal-to-noise ratio for the analytes. The response showed good linear relationship with the analytes' concentrations over wide ranges (e.g., 0.05–10 μg/g for sildenafil) with all the coefficient of determinations (r^2) >0.9996. The detection limits (LODs) were in the range of 1.0–5.0 ng/g for different analytes. The recoveries ranged from 85.4% to 96.7%. The intra- and inter-day accuracies were in the range of −6.6 to 10.1%, while the intra- and inter-day precision ranged from 0.0039% to 13.2%. Among 68 batches of herbal medicines and 20 batches of dietary supplements (including 83 capsules, 3 pellets and 2 liquid) samples, sildenafil was detected in 8 dietary supplements, while noracetildenafil was detected in only one dietary supplement. The novel Q-Orbitrap mass spectrometry has been proved to be a very promising and powerful tool for routine screening of illegal adulterate in herbal medicines and dietary supplements, ensuring food safety and public health.

1. Introduction

In recent years, herbal medicines and dietary supplements have been booming all over the world because of being believed as safer and healthier than synthetic drugs and free of side effects [1]. However, illegal adulteration of herbal medicines and dietary supplements with undeclared synthetic chemicals in order to enhance the claims stated on the label is a practice which can lead to potentially serious health consequences and poses a global challenge to analysts. Phosphodiesterase-5 (PDE-5) inhibitors are a class of drugs used primarily in the treatment of erectile dysfunction (ED). There are three such drugs approved by both Europe and U.S. Food and Drug Administration (USFDA): sildenafil citrate (Viagra, manufactured by Pfizer), vardenafil hydrochloride (Levitra, manufactured by Bayer), and tadalafil (Cialis, manufactured by Lilly) [2]. In the past several years, such synthetic PDE-5 inhibitors such as sildenafil, tadalafil, thiohomosildenafil, noracetildenafil, piperidenafil, acetylvardenafil, etc., have been routinely identified in "all-natural" herbal medicines and dietary supplements as well as in counterfeit and other unapproved products, marketed as aphrodisiacs [2–12]. In many cases of herbal medicines and dietary supplements the illegally added drugs were not declared on the product label in an effort to conceal their addition from regulators and consumers. The presence of PDE-5 inhibitors in herbal supplements or in unapproved dosage forms could pose a significant risk to public health. PDE-5 inhibitors have a series of adverse effects such as headache, facial flushing, dyspepsia, visual disturbances, muscle aches and possibility of blindness and hearing loss [13,14], so that it is dangerous to take unwitting PDE-5 inhibitors from

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herbal products. Furthermore, patients taking nitrate medications should not use PDE-5 inhibitor, as this combination may provoke potentially life-threatening hypotension [15]. Therefore, it is urgent to develop an analytical method with high sensitivity and high selectivity to screen the presence of synthetic PDE-5 inhibitors in herbal medicines and dietary supplements.

A number of analytical techniques have been developed for the detection and determination of PDE-5 inhibitors such as immunoassay [16], atomic emission spectrometry [17], ion mobility spectrometry [18,19], micro-Raman spectroscopy [20], high performance liquid chromatography (HPLC) with ultraviolet or fluorescence detection [21–23], gas chromatography–mass spectrometry (GC–MS) [24,25], liquid chromatography–mass spectrometry (LC–MS) [26–31]. Several literatures have reported the detection of chemical substances in food using high-resolution mass spectrometry (HRMS) with quadrupole-time of flight (Q-TOF) or Fourier transform ion cyclotron resonance (FTICR) mass analyzers [32–35].

Orbitrap is the newest addition to the family of HRMS. With its revolutionarily design, the resolution is capable of resolving power in excess of 1 000 000 FWHM [36]. However, the powerful qualitative and quantitative potential of Orbitrap has not been previously adopted for the screening of illegal adulterants. In the present study, we demonstrate rapid screening, confirmation and quantification of illegal adulterated PDE-5 inhibitors in herbal medicines and dietary supplements employing an Q-Orbitrap. To the best of our knowledge, this is the first time to report the application of Q-Orbitrap in screening of illegal adulterated synthetic chemicals in herbal medicines or dietary supplements.

2. Materials and methods

2.1. Chemicals and reagents

Sildenafil (99.6%), vardenafil hydrochloride (99.2%), tadalafil (99.5%), homosildenafil (98.7%), hydroxyhomosildenafil (98.8%), noracetildenafil (99.9%), acetildenafil (99.1%), aminotadalafil (99.9%), pseudovardenafil (99.5%), norneosildenafil (99.9%) and thioaildenafil (98.0%) were purchased from TLC Pharma-Chem Inc. (Vaughan, Ontario, Canada). Their chemical structures are displayed in Fig. 1S. Herbal medicines and dietary supplements which claimed functions of physical fatigue relief, immunity enhancement or aphrodisiac were bought from the local drug shops. Herbal medicines and dietary supplements without the studied eleven illegal adulterants were used as blank matrices. HPLC grade methanol, acetonitrile and formic acid were purchased from TEDIA Inc. (USA). Ultrapure water (18.2 MΩ) was obtained from a Milli-Q Advantage A10 ultrapure water purification system.

2.2. Instrumentation

The UHPLC–HESI–Q-Orbitrap system consisted of an Accela 1250 LC pump and an Accela open autosampler coupled with a high resolution Q Exactive mass spectrometer (Thermo Fisher Scientific, Germany). XCalibur 2.2 software from Thermo Fisher Scientific (MA, USA) was used to control the instrument and for data processing. Q Exactive 2.1 (tune application) software from Thermo Fisher Scientific (MA, USA) was used to control the mass spectrometer. Chromatographic separation was achieved on a Hypersil GOLD aQ C18 column (100 mm × 2.1 mm, 1.9 μm particle size) (Thermo Fisher Scientific, 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 standard stock solutions were prepared in acetonitrile at 100 μg/mL. Mixture of standard solutions was prepared via dilution of the stock solutions in acetonitrile at six concentration levels (distinct for different drugs). Matrix-matched working solutions were freshly prepared in blank sample extracts, which were extracted from the commercial products purchased from the local market and confirmed not to contain any of the tested analytes. All of the standard stock solutions were stored at −20 °C in dark amber bottles for at most 5 days.

2.4. Sample preparation

Herbal medicines and dietary supplements which claimed functions of physical fatigue relief, immunity enhancement or aphrodisiac were presented in the form of pellets, capsules or oral liquid. For pellets, five pellets were smashed into a homogeneous powder, whereas for capsules, the shells of five capsules were removed and the powder was mixed. Afterward, a single oral dose of 0.2 g solid samples (for pellets and capsules) or 10 mL oral liquid was accurately transferred to a 50 mL centrifuge tube and extracted with 20 mL ACN–H_2O (1:1, v/v), followed by vortex for 1 min, ultrasonic treatment for 15 min, and centrifugation at 5000 rpm for 15 min, successively. Then the supernatant was filtrated through 0.22 μm microporous membrane and a portion of the solution was withdrawn for the UHPLC–MS analysis. Blank matrices samples were treated as samples described above. For screening, when the concentration was beyond the linear range, the sample solution was diluted to make the detection response within the linear ranges.

2.5. Chromatographic conditions

A binary mobile solvent was used: mobile solvent A was 0.1% formic acid–H_2O and mobile solvent B was 0.1% formic acid–acetonitrile. The mobile phase was delivered at a flow rate of 200 μL/min with a gradient elution profile. The gradient began at 35% B for 0.5 min, and then linearly ramped to 85% B over 7 min, held at 85% B for 1.0 min, then the column was re-equilibrated at 35% B for 1.5 min prior to the next injection. The total runtime for each injection was 10 min and the injection volume was 5 μL. The autosampler tray temperature was set to 20 °C, while the column temperature was 35 °C.

2.6. Mass spectrometry conditions

The Q Exactive mass spectrometer was equipped with a HESI and operated in the positive ionization mode. It was placed in an air-conditioned room with the temperature strictly controlled at 20 °C all the time. The spray voltage, capillary temperature and vaporizer temperature were set to 3.0 kV, 350 °C and 250 °C, respectively. The sheath gas, auxiliary gas, sweep gas and S-lens RF level were set to 35, 10, 0 (arbitrary units) and 50 V, respectively. Nitrogen was used for spray stabilization, for collision-induced dissociation experiments in the higher energy collision dissociation (HCD) cell, and as the damping gas in the C-trap. The instrument was calibrated in positive mode every 3 days using the manufacturer's calibration solutions (containing caffeine, the tetrapeptide MRFA, and a mixture of fluorinated phosphazines ultramark 1621). The analysis was performed in positive ion full MS/dd-MS^2 (Top N) mode. This mode comprised a full MS scan followed by a data-dependent scan (dd-MS^2) with a fragmentation energy applied. The mass spectrometer acquired a full MS scan at a resolution of 70 000 (FWHM at 200 m/z). The automatic gain control (AGC) target (the number of ions to fill C-Trap) was set at $1.0e^6$ with a maximum injection time (IT) of