Journal of Chromatography B 1064 (2017) 92–99

Contents lists available at ScienceDirect

Journal of Chromatography B

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

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

Qiaoyuan Cheng[a,*], Linjun Shou[b], Cen Chen[a], Shi Shi[c], Minghao Zhou[a,*]

[a] Zhejiang Institute for Food and Drug Control, Hangzhou, Zhejiang, 310052, PR China
[b] Zhejiang University of Technology, Hangzhou, Zhejiang, 31000, PR China
[c] Zhejiang Chinese Medical University, Hangzhou, Zhejiang, 310053, PR China

ARTICLE INFO

Keywords:
Orbitrap
Illegal adulteration
Weight-loss drugs
Identification
Quantitation

ABSTRACT

In this paper, an ultra-high-performance liquid chromatography coupled with linear ion trap quadrupole Orbitrap high resolution mass spectrometry (UHPLC-LTQ-Orbitrap HRMS) method was developed and validated for identification, confirmation and quantitation of illegal adulterated weight-loss drugs in plant dietary supplements. 13 wt-loss drugs were well separated by the gradient elution of 10 mmol/L ammonium acetate − 0.05% formic acid H_2O and acetonitrile at a flow rate of 0.2 mL/min within 12 min. The MS analysis was operated under the positive ion and in full MS/dd-MS^2 (data-dependent MS^2) mode. The full MS scan with resolution at 60 000 FWHM and narrow mass windows at 5 ppm acquired data for identification and quantitation, and dd-MS^2 scan with resolution at 15 000 FWHM obtained product ions for confirmation. The method validation showed good linearity with coefficients of determination (r^2) higher than 0.9951 for all analytes. Meantime, all the LOD and LLOQ values were in the respective range of 0.3–2 and 1–9 ng/g. The accuracy, intra- and inter-day precision were in the ranges of −1.7 to 3.4%, 1.7–5.0% and 1.9–4.4%, respectively. The mean recoveries ranged from 85.4 to 107.1%, while the absolute and relative matrix effect were in the corresponding range of 98.2–108.6% and 2.6–8.7%. Among 120 batches of weight loss plant dietary supplements, sibutramine and fluoxertine or both were positive in 29 samples. In general, LTQ-Orbitrap HRMS technology was a powerful tool for the analysis of illegal ingredients in dietary supplements.

1. Introduction

Increased prevalence of overweight and obesity is a worldwide health concern [1]. According to the statistics of Chinese Center for Disease Control and Prevention, the Chinese obesity rate has risen to about 12%. Consequently, increasing numbers of people are searching for obesity treatments and weight loss products as they become more aware of the problems associated with obesity. Unfortunately, a lot of anti-obesity drugs such as amphetamine, sibutramine were withdrawn from the market because of concerns over increasing rates of severe side effects [2].

In recent years, plant 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 [3]. However, some manufacturers adulterate these products with synthetic drugs that have weight-loss effects or potentiate treatment efficacy; these adulterants include appetite suppressors, energy consuming/enhancing drugs, stimulants, antidepressants, anxiolytics, diuretics, and laxatives [4].

Since the adulterated products not only endanger consumers' long-term health, but also encourage illegal acts, it is necessary to develop a rapid screening, confirmation and quantitation method to detect the synthetic weight-loss drugs in adulterated plant dietary supplements to safeguard human health and safety.

Various studies have been reported on the detection of synthetic anti-obesity drugs including TLC [5], TLC-Micro IR [6], HPLC(UPLC) [7], GC–MS[8], NMR [9], immunochromatographic and radio-immunoassay methods[10]. During the last few years, LC–MS has become more and more common for screening illegal weight-loss drugs because of high selectivity and sensitivity. However, most LC–MS methods were liquid chromatography coupled to low-resolution mass

* Corresponding authors.
E-mail addresses: chengqiaoyuan@zjyj.org.cn (Q. Cheng), zhouminghao@zjyj.org.cn (M. Zhou).

http://dx.doi.org/10.1016/j.jchromb.2017.09.009
Received 17 July 2017; Received in revised form 1 September 2017; Accepted 7 September 2017
Available online 09 September 2017

analyzer such as quadrupole (Q) and triple quadrupole(QQQ) [11–16], while very few studies reported the application of quadrupole employed time of flight (TOF) or Orbitrap high resolution (HR) MS [17,18].

The Orbitrap™ mass analyser, developed by Makarov thirteen years ago and commercially introduced in 2005, implements the principles of Fourier transform (FT) through an electrostatic axially harmonic orbital trapping technique to yield high resolution (> 15,000 FMWH) and high mass accuracy (< 2 ppm) mass spectra [19]. The combination of a low resolution quadrupole or linear ion tap with the high resolution Orbitrap analyzer (Q- Orbitrap or LTQ-Orbitrap) has also been used for qualitative or quantitative analysis in various fields including metabolites, drug abusing, pesticide residues, lipolysis assay, bioactive compounds of Traditional Chinese Medicines, and etc. [20–28].

The objective of this study was to develop a rapid analytical method for the simultaneous identification, confirmation and quantification of 13 wt-loss drugs illegally added into plant dietary supplements by using UHPLC coupled with LTQ-Orbitrap MS. Additionally, monitoring was investigated in 120 batches of samples collected from the market in Zhejiang province in 2016. To the best of our knowledge, this is the first time for the dedicated application of LTQ-Orbitrap MS for the multiple screening of illegal adulterated weight-loss drugs in plant dietary supplements.

2. Materials and methods

2.1. Chemicals and reagents

Ephedrine(100%), methamphetamine hydrochloride (100%), phenformin hydrochloride(99.7%), clenbuterol hydrochloride (100%), fenfluramine hydrochloride(100%), citalopram hydrbromide(100%), paroxetine hydrochloride(97.4%), fluoxertine hydrochloride(100%), sibutramine hydrochloride(100%) and bisacodyl (100%) were purchased from National Institutes for Food and Drug Control (Beijing, China), while amfepramone hydrochloride (97%), bupropion hydrochloride(100%) and rimonabant(95%) were provided by Shanghai Tao Su Biotechnology Co., Ltd (Shanghai, China), Shanghai Aladdin Biochemical Technology Co., Ltd (Shanghai, China) and J & K Technology Co., Ltd (Beijing, China), respectively. Plant dietary supplements claiming the function of weight loss were collected by Zhejiang Food and Drug Administration. HPLC grade methanol and acetonitrile were obtained from Merck Inc. (Germany), and HPLC grade formic acid was purchased from Sigma Inc.(USA). Ammonium acetate was supplied by Fluka Inc.(USA). Purified water (18.2 MΩ) was prepared using a Thermo Fisher apparatus (Thermo Fisher, GenPure UV/UF, USA).

2.2. Standard solutions

Stock standard solutions (100 μg/mL) were prepared by weighing and dissolving each chemical in methanol. Standard mixture working solutions were prepared via dilution of the stock solutions in methanol at five concentration levels, while matrix-matched working solutions for recovery experiment were freshly prepared at the same concentration levels with blank sample extracts, which were extracted from the market products and proved to be lack of the tested analytes. All standard solutions were stored in the dark at −18 °C.

2.3. Sample preparation

A dose of sample was accurately weighed: tablets and pellets were grinded into powder, whereas for capsules, the shells and the powder were mixed. Then, the sample was taken to a 50 mL volumetric flask and 25 mL methanol was added. After ultrasonic treatment (KQ-300GDV, Thermostat Ultrasonic Instrument, Kunshan, China) for 30 min, the mixture was diluted with methanol to the volume. After standing for 5 min, a portion of the supernatant was taken to filtrate through 0.22 μm microporous membrane. Blank samples were treated as samples described above. For screening, when the concentration was beyond the linear range, the sample solution was diluted to appropriate concentration.

2.4. Chromatographic conditions

The separation of the analytes was operated on a RS pump LC system (Ultimate 3000, DIONEX, Thermo Scientific, Bremen, Germany) equipped with a binary pump and an auto-sampler. An Agilent Eclipse C_{18} column (100 mm × 2.1 mm, 1.8 μm particle size) was used by the gradient elution of 10 mmol/L ammonium acetate − 0.05% formic acid H_2O(A) and acetonitrile (B): 0–2 min, 15% B; 2–5 min, 15–20% B; 5–8 min, 20–90% B; 8–12 min, 90%B; 12-12.1 min, 90-15% B; 12.1–16 min, 15% B. The mobile phase was delivered at a flow rate of 0.2 mL/min and the injection volume was 1 μL. The autosampler tray temperature was set to 15 °C, while the column temperature was 30 °C.

2.5. Mass spectrometry conditions

High-resolution ESI–MS and MS/MS spectral analysis was performed on a LTQ-Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) connected to the UHPLC instrument via ESI interface. The analysis was carried out under the positive mode using a heated electrospray ionization HESI II probe. The ion source conditions applied were set as follows: spray voltage 3.0 kV, capillary temperature 350 °C, vaporizer temperature 250 °C. The flow rate of sheath gas, auxiliary gas, sweep gas and S-lens RF level were set to 35, 10, 0 (arbitrary units) and 50 V, respectively. The scan mode was Full MS/dd-MS^2 which included a full MS scan and then followed a data-dependent MS^2 scan. For the full MS scan, it firstly scans the list of masses that are included in the inclusion list as shown in Table 1, column 5. Then, ions of the second scan event enter the HCD collision cell for fragmentation. The Full MS scan ranges and mass resolution were set at 100–500 *m/z* and 60 000 FWHM. For the dd-MS^2 scan, the mass resolution was 15 000 FWHM with minimum signal threshold 500.0 and isolation width 2.0 *m/z*. Data acquisition and processing were carried out using Xcalibur 2.1 software (Thermo Fisher Scientific, USA) with Qual and Quan browser.

2.6. Method validation

The validation of the method with respect to selectivity, linearity and sensitivity, accuracy and precision, recovery, matrix effect and stability was carried out after the optimization of UHPLC-LTQ-Orbitrap HRMS conditions according to FDA Draft Guidance for Industry: Bioanalytical Method Validation (2013) (U.S) [29].

2.6.1. Selectivity

The selectivity of the method was evaluated by determining the level of interfering components in six individual sources of blank matrix.

2.6.2. Linearity and sensitivity

The calibration curve was constructed using the peak area (Y-axis) of each standard compound versus its corresponding concentration (X-axis) and the regression equation was described as $y = a\ x + b$. The linearity was assessed by the coefficient of correlation (r^2). The limit of detection (LOD) and the lower limit of quantitation (LLOQ) for each compound were determined as the lowest concentration giving a signal-to-noise ratio of at least 3-fold (S/N > 3) and 10-fold (S/N > 10), respectively.

2.6.3. Accuracy and precision

The accuracy was calculated by comparing the mean values of the