Screening and Quantitation of Drugs Illegally Added to Health Foods by UHPLC - Hybrid Quadrupole - Orbitrap Mass Spectrometry

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ABSTRACT

Purpose: Develop a comprehensive screening and quantitation method based on LC/Q-Orbitrap to determine the drugs illegally added to health foods.

Methods: The standards provided by Beijing Institute of Drug Control were dissolved in methanol. Commercially available health food samples are extracted with methanol and then centrifuged for 10 min. All experiments were carried out using a Thermo Scientific™ Vanquish™ Flex UHPLC system coupled to a Thermo Scientific™ Q Exactive™ Focus Hybrid Quadrupole-Orbitrap™ Mass Spectrometer. Separation was achieved using a Thermo Scientific™ Accucore™ RP-MS column (2.1 x 100 mm, 2.6 μm) and gradient elution with water (10mmol/L ammonium formate) and methanol. The runtime was 10 min and the injection volume was 1 uL. A heated-electrospray ionization(HESI) source was used and optimized MS conditions were: spray voltage 3.0 kV, vaporizer temperature 350°C, capillary temperatures 320°C, collision gas flow rate 1, sheath gas flow rate 40, auxiliary gas flow rate 10 and S-lens RF level 50. An accurate-mass based library with MS/MS information was constructed by using Thermo Scientific™ TraceFinder™ 4.1 software and Thermo Scientific™ mzVault™ 1.0 software. The collision voltages in the high energy collision induced dissociation (HCD) for MS/MS fragmentation of the 24 drugs is set at 5, 10 and 20eV.

Results: Full MS/data-dependent MS/MS (Full MS/dd-MS^2) scan mode was utilized for the simultaneous screening and quantitation method of 24 chemical drugs in the samples. The methodology of quantitative and qualitative analysis at the optimized LC and MS conditions was validated with the use of health food samples including plant-derived and soya bean lecithin products. Quantitative analysis is performed using the peak area of the extracted precursor ions. The study demonstrated that good linearity is obtained in the concentration range of 1-1000 ng/mL ($R2 > 0.990$) and the limits of detection (LODs) are from 0.5 to 2.0 ng/mL for each of the 24 synthesized drugs. The recoveries at 10 ng/mL spiked concentration are from 82% to 111% with relative standard deviations (RSDs) below 8.0% (n=6) for the targeted drugs. The established method is used to carry out fast simultaneous determination of 24 drugs in 5 health food products with 10 minute chromatographic run times. For the non-targeted approach, a MS/MS based library with accurate mass measurement is developed for the 24 drugs. By comparing the accurate mass of precursor ions with isotopic distribution, retention time and MS/MS library mirror-search, the potential illegal drugs in the real samples can be identified. The sensitivity and selectivity of screening proved to be satisfactory by achieving good matching results with the library at 5 ng/mL spiked concentration of all drugs in the real sample solutions. The established method can be effectively applied to simultaneously screening and quantitation of the illegally added chemical drugs in health food products with high-throughput results and high sensitivity.

INTRODUCTION

Interest in health food and supplements increases as a result of claims that these commodities can provide health benefits such as weight loss and a decrease in hypertension. In order to get faster and enhanced effects, some manufacturers illegally add synthetic drugs into their products, which can potentially represent a risk to health. Strictly monitoring the illegal addition of synthetic drugs is essential as the market for health foods and supplements is rapidly growing in China. In this study, we developed a MS/MS library-based screening, with simultaneous quantitation strategy, based on UHPLC tandem quadrupole-Orbitrap (Q-Orbit) mass spectrometry. This method has been tested for 24 drugs, which proved to deliver high throughput results and high sensitive with good repeatability.

MATERIALS AND METHODS

Sample Preparation

The standards were dissolved in methanol. Commercially available health food samples are extracted with methanol

Instruments

Vanquish Flex UHPLC system coupled to a Q Exactive Focus Hybrid Quadrupole-Orbitrap Mass Spectrometer

Accucore RP-MS column(2.1 x 100 mm, 2.6 μm)

The detailed chromatography conditions and MS conditions were shown in Table 1 and Table 2

Data Analysis

TraceFinder 4.1 software

mzVault 1.0 software

Table 1. Liquid chromatography conditions

Instrumentation	Vanquish Flex UHPLC			
Column	Accucore RP-MS column (2.1 x 100 mm, 2.6 μm)			
Column temperature	30°C			
Injection Volume	1 μL			
Mobile Phase	A: methanol			
	B: 10mmol/L ammonium formate			
Flow Rate	300μL/min			
Gradient program	Time	Flow Rate	%B	Curve
	0.000	0.300	90	5
	1.000	0.300	55	5
	3.500	0.300	40	5
	6.500	0.300	0	5
	8.500	0.300	0	5
	8.600	0.300	90	5
	10.000	0.300	90	5

Table 2. Mass spectrometer conditions

Full Scan	
Resolution	70,000 (FWHM) at m/z 200
Mass range	100-1000m/z
ddMS2	
Resolution	17,500 (FWHM) at m/z 200
Isolation windows	2.0 m/z
Spray voltage	3000 V
Sheath gas flow rate	40 arb
Aux gas flow rate	10 arb
Sweep gas flow rate	1 arb
Capillary temperature	350°C
Aux gas Heater temperature	320°C
RF-lens level	50
HCD collision energy	30eV

Figure 1. Vanquish Flex UHPLC system coupled to a Q Exactive Focus hybrid quadrupole-Orbitrap Mass Spectrometer

Results

Data analysis was performed within TraceFinder software. Quantitative and qualitative analysis result could be obtained at the same time and the methodology at the optimized LC and MS conditions was validated with health food samples including plant-derived (Matrix 1) and soya bean lecithin (Matrix 2) products. The quantitative analysis was performed using the peak area of the extracted precursor ions and the detection results are shown in Table 3. Figure 2 shows a solvent standard curve for Homosibutramine. Figure 3 shows the extracted ion chromatograms for all analytes using a layout in Thermo Scientific™ Freestyle™ 1.3 software. For the screening approach, an accurate-mass based library with MS/MS information was constructed by using TraceFinder 4.1 and mzVault 1.0 software. The collision voltages in the high energy collision induced dissociation (HCD) for MS/MS fragmentation of the 24 drugs is set at 10, 20 and 40 eV. By comparing the accurate mass of precursor ions with isotopic distribution, retention time and MS/MS library mirror-search, the potential illegal drugs in the real samples can be identified. The sensitivity and selectivity of screening proved to be satisfactory by achieving good matching results with the library at 5 ng/mL spiked concentration of all drugs in the real sample solutions. Figure 4 shows the details of matching result at 5 ng/mL spiked concentration of Homosibutramine including the isotopic pattern and fragment match.

Table 3. The details result of quantitative analysis

Compound	r2	LODs	Matrix 1		Matrix 2	
			rsd	recovery	rsd	recovery
Amphetamine	0.9938	1.0	3.1%	101%	3.7%	100%
Methylamphetamine	0.9903	0.5	0.8%	111%	3.1%	106%
Lorcaserin	0.9987	0.5	1.3%	101%	2.3%	84%
Bupropion	0.9994	0.5	0.7%	88%	3.1%	86%
Fenfluramine	0.9956	0.5	0.8%	106%	1.7%	99%
Furosemide	0.9990	2.0	5.0%	88%	6.2%	102%
Indapamide	0.9983	0.5	1.6%	90%	5.9%	104%
Phenolphthalein	0.9978	0.5	2.0%	91%	1.4%	102%
Sibutramine	0.9999	0.5	1.4%	91%	2.2%	85%
Pravastatin	0.9992	2.0	4.5%	90%	4.8%	95%
11-Desisobutyl-11-benzyl Sibutramine	0.9995	0.5	1.2%	84%	1.4%	83%
Homosibutramine	0.9992	0.5	1.0%	88%	0.9%	83%
Fluoxetine	0.9938	0.5	2.3%	92%	1.5%	96%
N-monodesmethyl sibutramine	0.9981	0.5	1.5%	92%	0.8%	96%
Bisacodyl	0.9990	0.5	1.1%	94%	2.2%	95%
Bumetanide	0.9978	0.5	2.0%	87%	1.2%	111%
N-Didesmethyl Sibutramine	0.9965	0.5	1.7%	82%	1.0%	105%
Chloro Sibutramine	0.9995	0.5	1.4%	91%	0.8%	90%
Bezafibrate	0.9978	0.5	1.2%	97%	0.6%	111%
Lovastatin	0.9939	0.5	1.3%	103%	3.2%	110%
Fenofibrate	0.9972	0.5	2.2%	92%	1.2%	106%
Simvastatin	0.9946	0.5	1.1%	103%	1.8%	111%
Rimonabant	0.9962	0.5	1.8%	101%	2.3%	106%
Orlistat	0.9927	0.5	2.7%	105%	7.8%	106%

Figure 2. Example calibration curves for Homosibutramine

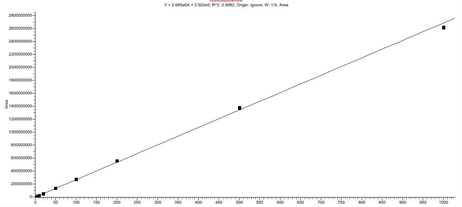


Figure 3. Extracted ion chromatograms for all 24 analytes,(Mass tolerance set to ± 5 ppm)

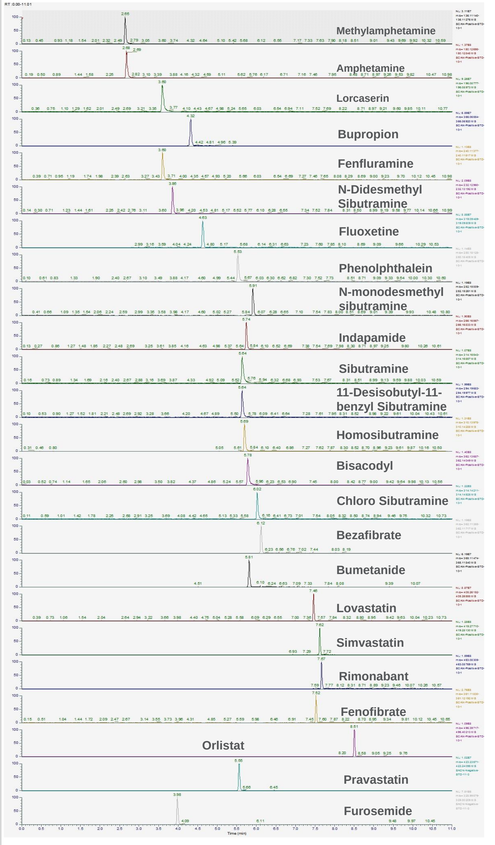
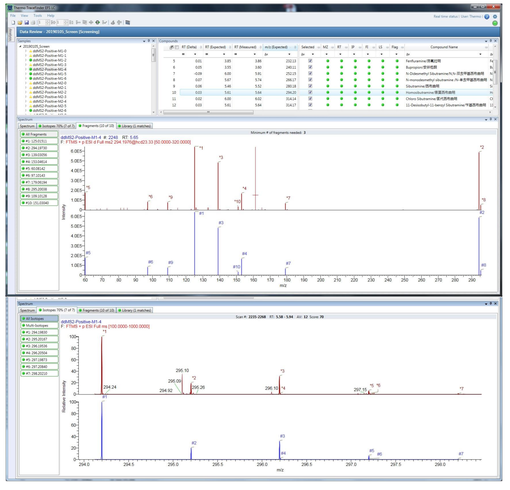


Figure 4. The details of matching result at 5 ng/mL spiked concentration of Homosibutramine



CONCLUSIONS

A library-based screening and simultaneous quantitation method by UHPLC/Q-Orbitrap is established for 24 chemical drugs that can be potentially illegally added to health food. Full MS-ddMS^2 method is employed which can provided quantitative and qualitative information in a single injection. This method has been tested for 24 drugs, which proved to deliver high throughput results and highly sensitive with good repeatability.

TRADEMARKS/LICENSING

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