Fragmentation Pathways of Synthetic Drugs Added in Health Food Based on Higher Energy Collisional Dissociation in High-resolution Quadrupole-Orbitrap Mass Spectrometry

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ABSTRACT

Purpose: To provide a robust and fast strategy for identifying and quantifying drugs illegally added to health food products.

Methods: The food samples were extracted with methanol followed with centrifugation 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 LC Column and gradient elution with water (10mmol/L ammonium formate) and methanol as mobile phases. A heated-electrospray ionization(HESI) source was used and MS conditions were optimized. The collision energy in HCD is set at 10, 20, 40 eV for the MS/MS fragmentation. Thermo Scientific™ Mass Frontier™ Spectral Interpretation Software to facilitate structural elucidation.

Results: Fragmentation pathway mapping in mass spectrometry plays an essential role in reliable interpretation of the physical and chemical properties of a compound's structure. Due to the similarity of the fragmentation mechanism for structural analogues, the fragmentation pathway allows elucidation of the structure of new derivatives of a drug added to food samples for the purpose to evade the regulations. High-resolution MS/MS spectra of chemical drugs were produced on a Quadrupole-Orbitrap mass spectrometer. Mass Frontier Spectral Interpretation Software was used to automatically calculate the neutral loss, elucidate the structures of product ions and interpret the structural relationship between precursor ion and product ions, thus establishing the fragmentation pathway map. Using this fragmentation pathway analysis, the characteristic fragments of a structural drug analogue can be used as markers for the detection and identification of its unknown derivative variants in food samples and bio-samples. The study demonstrates that high-quality MS/MS spectra at 1-5 μg/ml of drugs in real samples can be used for high-sensitive screening in complex samples. The parallel reaction monitoring (PRM) mode was successfully used for the quantitation of chemical drugs in the health food products with good linearity in the range of 0.5-200 ng/mL (R^2>0.99) and high sensitivity in the limits of detection (LODs) from 0.2 to 5 ng/mL.

INTRODUCTION

The illegal addition of synthetic drugs in health food is strictly prohibited due to serious risk implications to health. New synthesized derivatives of known drugs are often used to evade detection, a practice which creates serious analytical challenges. In this study, a high-resolution Quadrupole-Orbitrap mass spectrometer with higher energy collisional dissociation (HCD) was used to study the fragmentation pathways of chemical drugs having different therapeutic categories. Based on the high-quality, MS/MS spectra with accurate mass measurement, the fragmentation pathways were mapped by interpretation of the fragmentation relationship between precursor and product ions and structural elucidation. The established strategy proved to be fast and robust for identifying and quantifying the drugs illegally added to health products, their derivatives, and relevant metabolites.

MATERIALS AND METHODS

Sample Preparation

The standards provided by Beijing Institute of Drug Control were dissolved in methanol. Commercially available health food samples were bought from a supermarket in Beijing. The samples were homogenized, extracted with methanol and centrifuged at 8000 rpm for 8 minutes.

Instruments

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

Spray voltage 3.0 kV, Vaporizer temperature 350°C, Aux gas heater temperatures 320°C, Aux gas flow 10, Sweep gas flow rate 1, sheath gas flow rate 40, and S-lens RF level 50. The initial high resolution acquisition obtained full MS1 data at a resolution of 70,000 (FWHM @ m/z200). Data-dependent MS^2 was triggered using higher energy collisional dissociation (HCD) at a resolution of 35,000.

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

The detailed chromatography conditions was shown in Table 1.

Data Analysis

Thermo Scientific™ TraceFinder™ 4.1 software. Mass Frontier 8.0 software.

Thermo Scientific™ Freestyle™ 1.5 software.

Table 1. Liquid chromatography conditions

Instrumentation	Vanquish Flex UHPLC System
Column	Accucore RP-MS LC 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

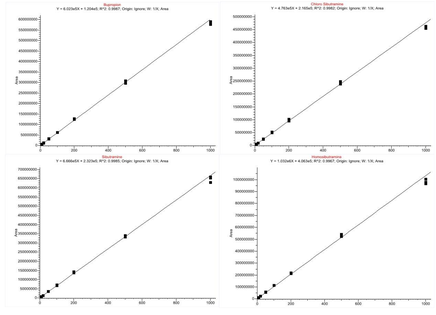
RESULTS

Data analysis was performed within TraceFinder software. Quantitative and qualitative analysis results could be obtained at the same time and the methodology at the optimized LC and MS conditions was validated with the use of health food samples. The quantitative analysis was performed using the peak area of the extracted product ions and the detection results are shown in Table 2.

Table 2. The detail results of quantitative analysis

Compounds	R^2	LOD (ng/mL)	Quantitative ion
Phenylpropanolamine	0.9971	2.0	152.10699→134.09180
Methylamphetamine	0.9971	0.2	150.12773→91.05460
Lorcaserin	0.9981	0.5	196.08875→129.06982
Bupropion	0.9909	0.2	240.11497→184.05911
Fenfluramine	0.9987	0.2	232.13076→159.04134
Indapamide	0.9943	0.5	366.06737→132.08066
Phenolphthalein	0.9954	0.5	319.09649→225.05408
Sibutramine	0.9946	0.5	280.18265→125.01517
11-Desisobutyl-11-benzyl Sibutramine	0.9985	0.5	314.16700→91.05458
Homosibutramine	0.9931	0.5	294.19830→125.01523
Fluoxetine	0.9967	0.2	310.14133→91.05419
N-monodesmethyl sibutramine	0.9938	0.5	266.16703→125.01517
Bisacodyl	0.9929	0.5	362.13868→184.07545
Bumetanide	0.9983	0.5	365.11657→240.13776
N-Didesmethyl Sibutramine	0.9913	0.5	252.15135→125.01517
Chloro Sibutramine	0.9949	0.5	314.14368→158.97601
Bezafibrate	0.9982	0.5	362.11536→138.99419
Lovastatin	0.9947	0.5	405.26355→199.14752
Fenofibrate	0.9932	0.5	419.27920→199.14752
Simvastatin	0.9929	0.5	463.08537→380.99435
Rimonabant	0.9963	0.2	361.12011→233.03590
Orlistat	0.9926	0.5	496.39965→319.29861

Figure 1. Example calibration curves.



For the screening approach, an accurate-mass based library with MS/MS information was constructed by using TraceFinder 4.1. 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 2 shows the details of matching result at 5 ng/mL spiked concentration of bezafibrate including the isotopic pattern and fragment match. Figure 3 shows the extracted product ion chromatograms for analytes using a layout in Freestyle 1.5 software.

Figure 2. The details of matching result at 5 ng/mL spiked concentration of bezafibrate including the isotopic pattern and fragment match.

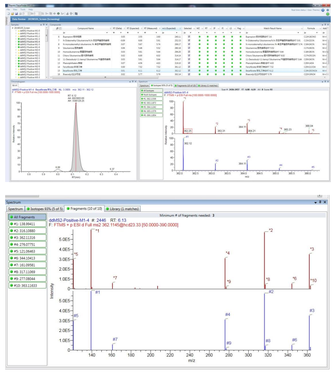
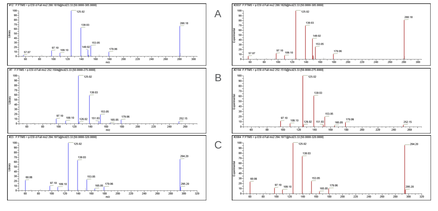


Figure 3. Extracted ion chromatograms for analytes (Mass tolerance set to ± 5 ppm).

Fig 4 shows the MS/MS spectra of Sibutramine, N-Didesmethyl sibutramine and Homosibutramine in Library and Experiment. Good matching result can be observed. As sibutramine, N-Didesmethyl sibutramine and Homosibutramine are the family, they can product the same fragment ions including m/z 125.0, 139.0 and 153.0.

Figure 4. MS/MS spectra of Sibutramine (A), N-monodesmethyl sibutramine (B) and Homosibutramine (C) in Library (blue) and Experiment (red).



Mass Frontier 8.0 is the latest release of the small molecule structural elucidation software product. The software is enhanced with chemically intelligent tools that accelerate the interpretation of mass spectral data. The predictive fragmentation capabilities of the HighChem Fragmentation Library contain fragmentation mechanisms for small molecules collated from published literature, allowing users to quickly search thousands of entries. The fragmentation pathways of sibutramine are exhibited in Fig 5 and Fig 6. These characteristic ions can be used to estimate whether a compound belongs to a corresponding family.

Figure 5. Fragment ions list and possible structures of each fragment ion.

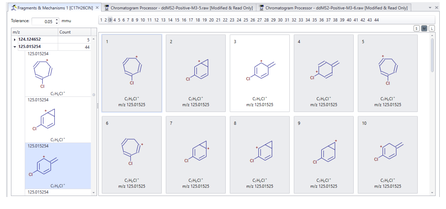
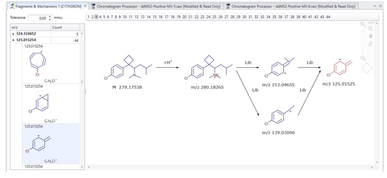
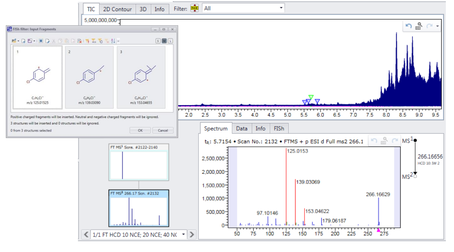


Fig 6. The fragmentation pathways of sibutramine



Mass Frontier 8.0 features an enhanced fragment ion search (FISh) screening tool, which is a powerful tool for the screening of structurally similar compounds based on the fragmentation pattern of the parent compound acquired, either by theoretical fragment prediction or experimental MSn spectral trees.

Fig 7. Fragment structures used as FISh filter and corresponding results in the Fish detection. The triangles indicate the detected component belongs to the same family.



CONCLUSIONS

- A screening and simultaneous quantitation method by UHPLC/Quadrupole-Orbitrap MS is established for drugs that can be potentially illegally added to health food. This method has been proved to deliver high throughput results and high sensitivity with good repeatability
- Mass Frontier is a cutting-edge software package for the management, evaluation and interpretation of mass spectral and chromatographic data. With its advanced algorithms, extensive libraries and superior graphical user interface, Mass Frontier is capable of solving analytical problems in a wide range of applications including: metabolomics, forensics, environmental, natural products, impurities, and degradants research.

TRADEMARKS/LICENSING

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