APPLICATION NOTE 73301

Application of UHPLC-hybrid quadrupole-Orbitrap mass spectrometry for screening and quantification of synthetic drugs illegally added to health foods

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Goal

The objective of this application note is to describe a workflow that can achieve quantitation and identification results for targeted synthetic drugs illegally added to health foods, while providing a non-targeted screening analysis.

Introduction

In China, consumer interest in health foods and supplements has increased as a result of the recent improvement in people's living standards. Health foods that claim to promote weight loss or decrease hypertension and hyperlipidemia are popular products. However, due to the fiercely competitive marketplace, fraudulent manufacturers have been known to add synthetic drugs, such as sibutramine, bumetanide, and phenolphthalein, to their products to achieve faster and enhanced efficacy. This is a serious problem because of the potential adverse effects, especially for people exposed to these drugs unknowingly. In fact, many of these drugs are only available by prescription and some are even suspended by government. Although food safety laws and drug administration laws of the People's Republic of China

regulate the addition of synthetic drugs in food,[1] many manufacturers take risks for financial gain.

Therefore, comprehensive analysis of the illegal addition of synthetic drugs is essential. This is analytically challenging because many new analogs and other drugs are used to escape regulatory detection. Many analytical methods, including high performance liquid chromatography (HPLC),[2,3] gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS), have been developed for the detection of synthetic drugs in health foods. The spectrometric method with superior qualitative and quantitative ability is the most reliable.[4]

Experimental

Chemicals, apparatus, and consumables

- Water, Optima™ LC/MS Grade, Fisher Chemical
- Methanol, Optima™ LC/MS Grade, Fisher Chemical
- Ammonium formate, 99%, Fisher Chemical
- Weighing balance (analytical and precision)
- Vortex mixer (Thermo Scientific)
- Refrigerated centrifuge (Thermo Scientific™ Sorvall™ST8 ventilated benchtop centrifuge)
- Variable volume micropipettes (Thermo Scientific)
- All drug standards and health food samples were provided by Beijing Institute of Drug Control

Sample preparation

Commercially available health food samples that claimed functions of weight loss and a decrease in hypertension and hyperlipidemia were provided by Beijing Institute of Drug Control. These samples, including Chinese traditional plant-derived and soybean lecithin products, were presented in capsules. The capsules were cut open and a test portion (0.5 g) of the contents was accurately weighed and transferred to a 50 mL centrifuge tube. Methanol (25 mL) was added and the mixture was shaken vigorously for 1 min prior to sonication for 20 min. After centrifugation at 8000 rpm for 10 min, the supernatant was withdrawn and filtered through a syringe filter (0.22 µm). The filtrate was analyzed using an LC–high-resolution, accurate-mass (HRAM) spectrometer.

Liquid chromatography and mass spectrometer

The LC-MS system comprised a Thermo Scientific™ Vanquish™ Flex Binary 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 × 100 mm, 2.6 µm). Data acquisition was carried out using the full scan and data-dependent MS/MS (Full MS-ddMS^2) scan mode. To ensure the fragments of targeted analytes were acquired, the inclusion list was filled with information for 22 compounds. Common fragments were used for non-targeted screening to detect compounds whose structures were similar with known targeted analytes.

The detailed chromatography conditions and MS conditions are shown in Table 1 and Table 2, respectively. Table 3 shows the inclusion list used in the dd-MS^2 experiment for the targeted compounds.

Table 1. Liquid chromatography conditions

Property	Setting			
Instrumentation	Vanquish Flex Binary UHPLC			
Column	Accucore RP-MS column (2.1 × 100 mm, 2.6 µm)			
Column temperature	30 °C			
Injection volume	1 µL			
Mobile phase	A: Methanol B: 10 mmol/L Ammonium formate			
Flow rate	300 µL/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

Property	Setting
Full scan Resolution Mass range	 70,000 (FWHM) at *m/z* 200 100–1000 *m/z*
ddMS^2 Resolution Isolation windows	 17,500 (FWHM) at *m/z* 200 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	10, 20, 40 eV

Data processing

Data processing was performed using Thermo Scientific™ TraceFinder™ software version 4.1. For generation of the extracted ion chromatograms, an extraction window of 5 ppm was used. For targeted screening, a compound database including compound names, formulas, retention times, and fragment values was used for identification of the targeted compounds. For non-targeted screening, all fragments of targeted compounds were summarized and

analyzed to find the common fragments. Fragment Ion Search™ (FISh) was performed using Thermo Scientific™ Mass Frontier™ 8.0 software.

Results and discussion

LC-MS/MS analysis

Different solvent compositions and a gradient elution procedure were investigated to obtain satisfactory chromatographic separation and ionization. The total ion chromatogram (TIC) is shown in Figure 1 for 22 compounds. Highly efficient separation of all compounds could be achieved within 10 min with excellent peak shapes, adequate retention, and reproducible retention times. Under the optimized MS conditions, the MS detector provided excellent sensitivity and repeatability.

Method validation

Using the optimized LC and MS parameters, quantitative and qualitative data could be obtained simultaneously, and stable mass accuracy (less than 5 ppm) was achieved. Table 3 shows the retention time and accurate mass of all compounds. Combining the retention time, mass accuracy, and MS/MS fragment ions, suitable qualitative results were obtained.

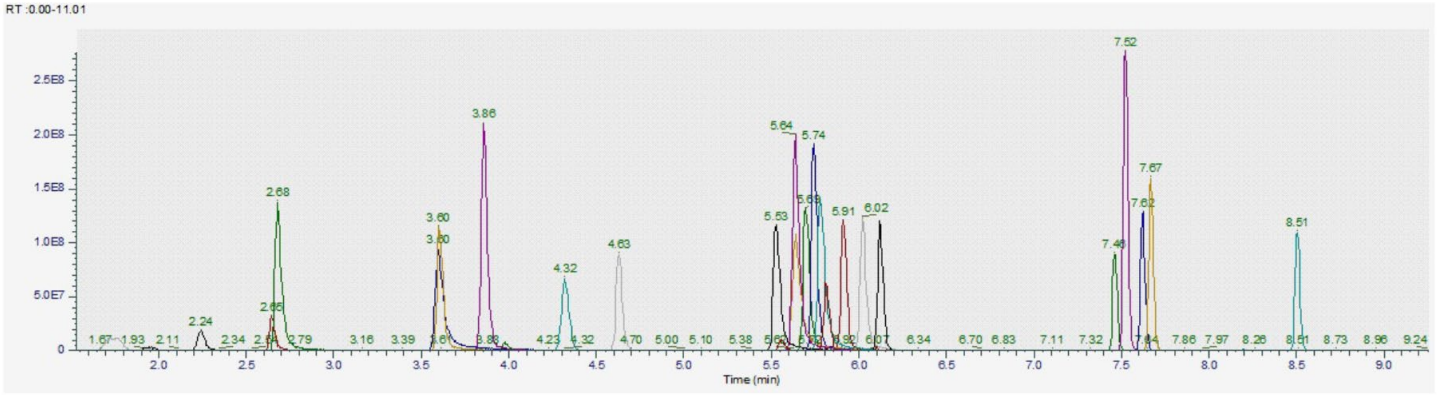

Figure 1. Total ion chromatograms with overlay of extracted ions (22 compounds)

Table 3. Retention time and accurate mass of all compounds

Compound	Retention time (min)	Protonated ion mass $[M+H]^+$		Mass error (ppm)
		Theoretical	Experimental	
Amphetamine	2.64	136.11208	136.11191	1.26
Methylamphetamine	2.68	150.12773	150.12746	1.82
Lorcaserin	3.60	240.11497	240.11452	1.89
Bupropion	3.61	196.08875	196.08853	1.11
Fenfluramine	3.85	232.13076	232.13023	2.27
Indapamide	4.32	366.06737	366.06662	2.05
Phenolphthalein	4.63	319.09649	319.09573	2.37
Sibutramine	5.52	280.18265	280.18201	2.03
11-Desisobutyl-11-benzyl Sibutramine	5.63	294.19830	294.19760	2.37
Fluoxetine	5.63	310.14133	310.14091	1.39
Homosibutramine	5.64	314.16700	314.16632	2.16
N-monodesmethyl sibutramine	5.73	266.16700	266.16638	2.32
Bisacodyl	5.78	362.13868	362.13779	2.47
Bumetanide	5.81	365.11657	365.11575	2.24
N-Didesmethyl Sibutramine	5.90	252.15135	252.15079	2.23
Chloro Sibutramine	6.01	314.14368	314.14310	1.86
Bezafibrate	6.11	362.11536	362.11453	2.29
Lovastatin	7.46	405.26355	405.26266	2.18
Fenofibrate	7.52	361.12011	361.11935	2.09
Simvastatin	7.62	419.27920	419.27844	1.81
Rimonabant	7.67	463.08537	463.08475	1.34
Orlistat	8.52	496.39965	496.39816	2.98

A compound database, including compound name, retention time, precursor and fragment values, was constructed by using TraceFinder 4.1 software and utilized for the identification of targeted analytes. The fragment ions were obtained through the experimental MS/MS spectrum of reference standards. The collision voltages in the high-energy collision-induced dissociation (HCD) for MS/MS fragmentation were set at 10, 20, and 40 eV. The theoretical fragmentation of each fragment ion was interpreted by Mass Frontier 8.0 software. 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 literatures, allowing users to quickly search thousands of entries. As an example, Figure 2 shows the MS/MS spectrum of sibutramine acquired using reference standard solution. Three pathways of sibutramine interpreted by Mass Frontier software are exhibited in Figure 3 and match well with the experimental data.

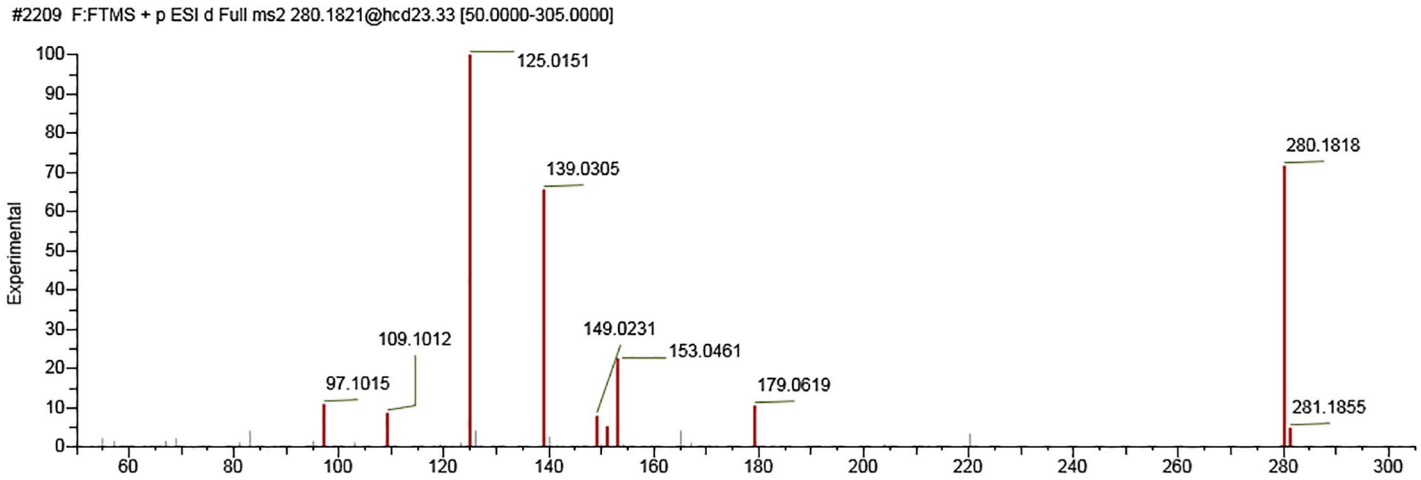

Figure 2. The MS/MS spectrum of sibutramine

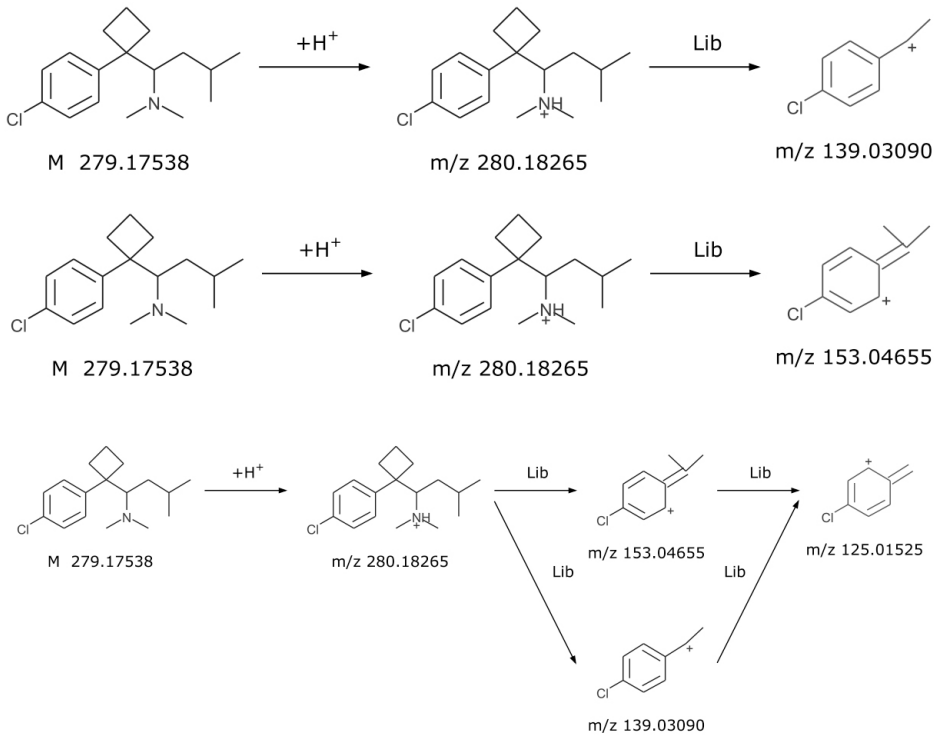

Figure 3. The fragmentation pathways of sibutramine

Figure 4 demonstrates the compound details in the Quan Peak, Fragment Matching, Isotopes Matching, and Calibration Curve views of TraceFinder software, which can assist the analyst in quickly viewing the data set for confirmation.

The method was validated using plant-derived (Matrix 1) and soybean lecithin (Matrix 2) products. The quantitative analysis was performed using the peak area of the extracted precursor ions from the full scan data acquisition and the results are shown in Table 5. The response was linear over the range of 0.5–500 µg/L with R^2 values higher than 0.990 for all analytes. The limits of detection (LODs) and limits of quantitation (LOQs) were determined as the signal-to-noise ratio of more than 3 and 10, respectively. For each of the 22 synthesized drugs the LOD and LOQ values were 25–100 µg/kg and 75–250 µg/kg, respectively. The recoveries at 250 µg/kg spiked concentration are from 82% to 111% with relative standard deviations (RSDs) below 8.0% (n=6) for the targeted drugs in each matrix.

Based on the review of the MS/MS fragments of targeted analytes, product ions frequently occurring in records of certain families were found. Fragment ion exact masses *m/z* 91.0544 and 119.0894 were present in fragmentation spectra of amphetamine and its analogs. Fragment ion exact masses *m/z* 125.0153, 139.0309, and 153.0460 were present in fragmentation spectra of sibutramine and its analogs. Fragment ion exact masses *m/z* 121.0648 and 138.9945 were present in fragmentation spectra of bezafibrate and its analogs. Fragment ion exact masses *m/z* 199.1481 and 285.1849 were present in fragmentation spectra of lovastatin and its analogs.

Mass Frontier 8.0 software features an enhanced 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. Figure 5 shows the common fragment ions of sibutramine and its analogs used as FISh filter and corresponding results in the Fish detection. The triangles indicate the detected compound has similar structure.

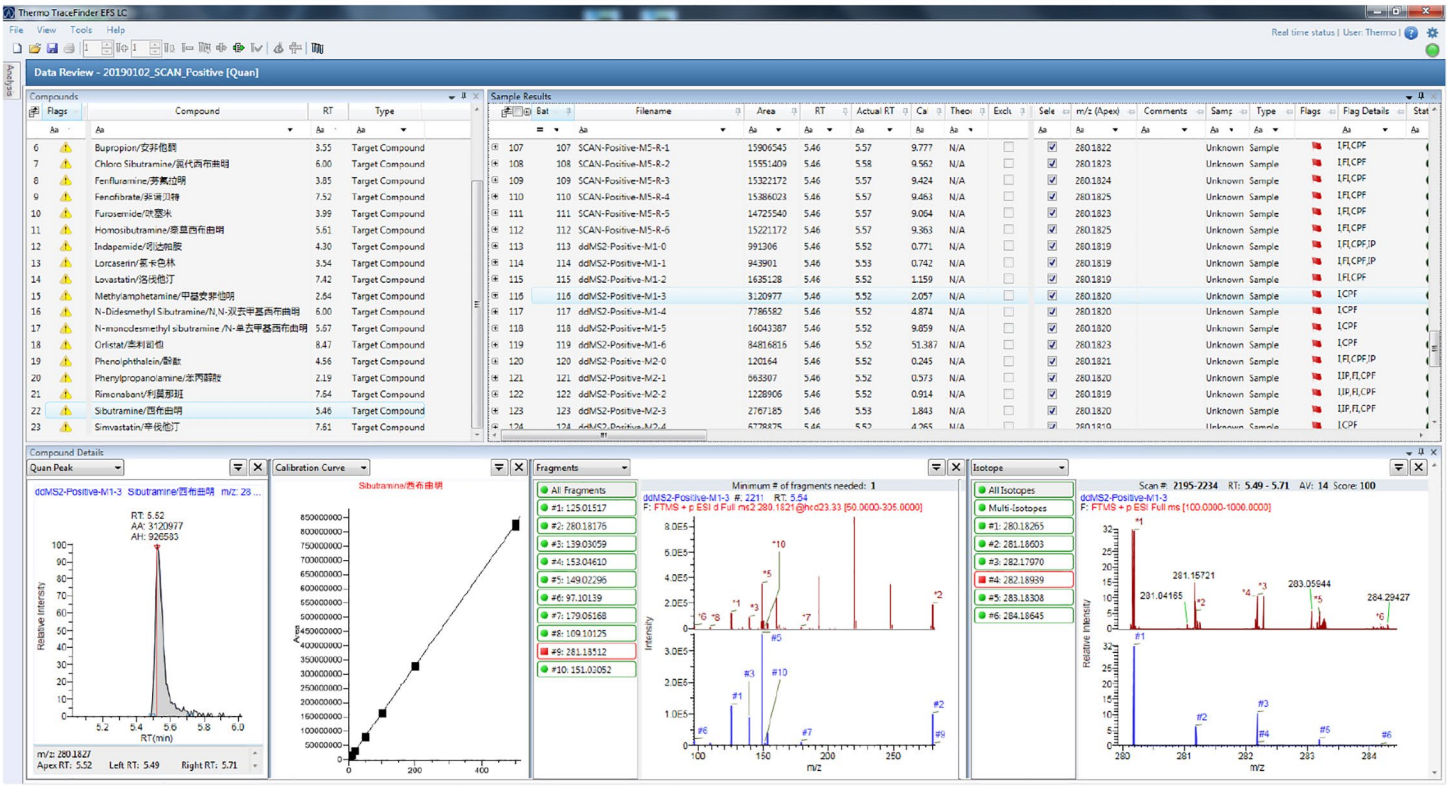

Figure 4. The details of sibutramine in the Quan Peak, Fragment Matching, Isotopes Matching, and Calibration Curve views of TraceFinder software

Table 5. Quantitative analysis results

Compound	R^2	LOD (µg/kg)	LOQ (µg/kg)	250 µg/kg spike (Matrix 1)		250 µg/kg spike (Matrix 2)	
				RSD	Recovery	RSD	Recovery
Amphetamine	0.9938	50	150	3.1%	101%	3.7%	100%
Methylamphetamine	0.9903	25	75	0.8%	111%	3.1%	106%
Lorcaserin	0.9987	25	75	1.3%	101%	2.3%	84%
Bupropion	0.9994	25	75	0.7%	88%	3.1%	86%
Fenfluramine	0.9956	25	75	0.8%	106%	1.7%	99%
Indapamide	0.9983	25	75	1.6%	90%	5.9%	104%
Phenolphthalein	0.9978	25	75	2.0%	91%	1.4%	102%
Sibutramine	0.9999	25	75	1.4%	91%	2.2%	85%
11-Desisobutyl-11-benzyl Sibutramine	0.9995	25	75	1.2%	84%	1.4%	83%
Homosibutramine	0.9992	25	75	1.0%	88%	0.9%	83%
Fluoxetine	0.9938	25	75	2.3%	92%	1.5%	96%
N-monodesmethyl sibu-tramine	0.9981	25	75	1.5%	92%	0.8%	96%
Bisacodyl	0.9990	25	75	1.1%	94%	2.2%	95%
Bumetanide	0.9978	25	75	2.0%	87%	1.2%	111%
N-Didesmethyl Sibu-tramine	0.9965	25	75	1.7%	82%	1.0%	105%
Chloro Sibutramine	0.9995	25	75	1.4%	91%	0.8%	90%
Bezafibrate	0.9978	25	75	1.2%	97%	0.6%	111%
Lovastatin	0.9939	25	75	1.3%	103%	3.2%	110%
Fenofibrate	0.9972	25	75	2.2%	92%	1.2%	106%
Simvastatin	0.9946	25	75	1.1%	103%	1.8%	111%
Rimonabant	0.9962	25	75	1.8%	101%	2.3%	106%
Orlistat	0.9927	25	75	2.7%	105%	7.8%	106%

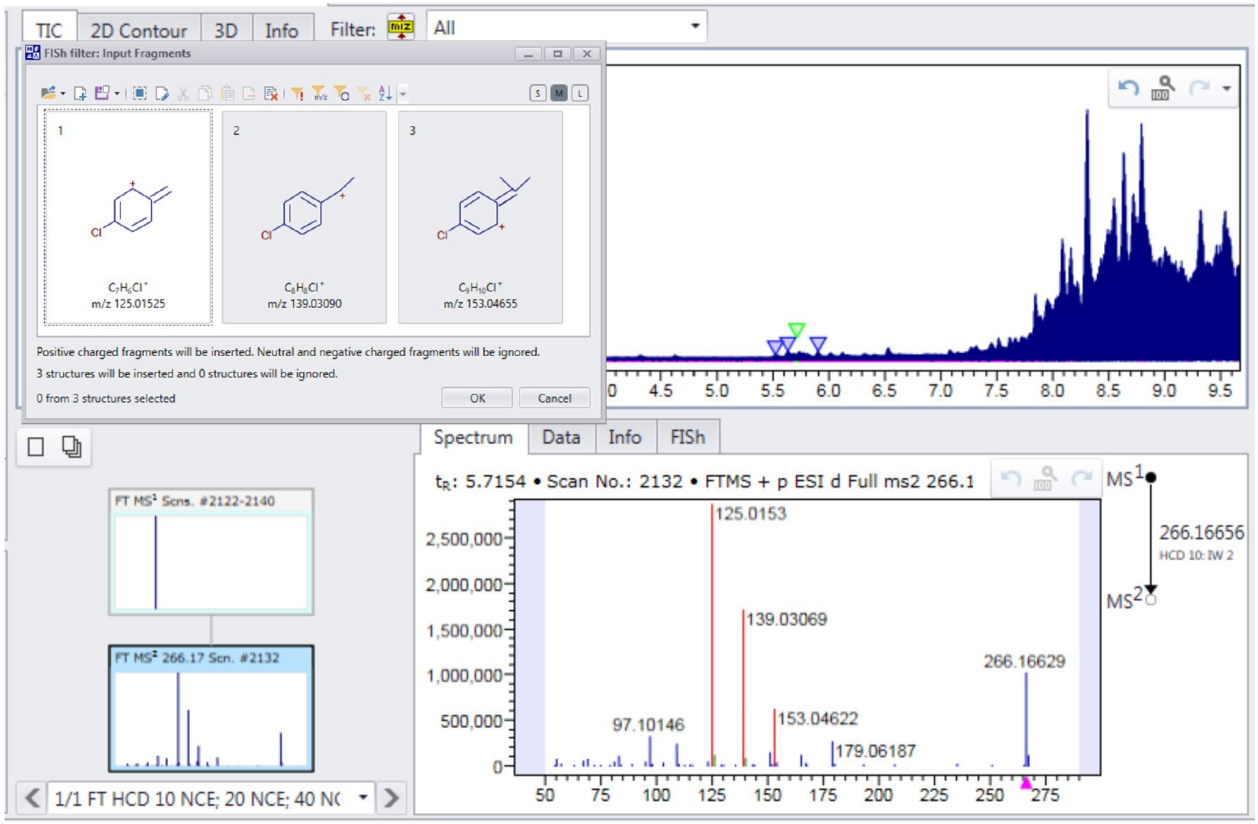

Figure 5. Fragment structures used as FISh filter and corresponding results in the FISh detection

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Conclusion

A screening and simultaneous quantitation method by UHPLC/Q-Orbitrap MS was established for 22 synthetic drugs that can be potentially and illegally added to health foods. The Full MS-ddMS^2 method was employed, which can provide quantitative and qualitative information in a single injection. The study demonstrated that good linearity is obtained in the concentration range of 0.5–500 ng/mL (R^2 > 0.990). The LOD and LOQ values were from 25 to 100 µg/kg and 75 to 250 µg/kg, respectively, for each of the 22 synthetic drugs. The recoveries at 250 µg/kg spiked concentration are from 82% to 111% with relative standard deviations (RSDs) below 8.0% (n=6). A non-targeted screening strategy was also included in the method. The characteristic fragments of a structural drug analogue can be used as markers for the detection and identification of its unknown derivative variants in health food samples.

References

1. State Food and Drug Administration of China. The notice on the cessation of production, sale and use of fenfluramine hydrochloride in both forms of bulk drug and formulations. Chinese *Pharmaceutical Affairs*, **2009,** *2,* 136–136.
2. Kakali, S.; Sharma, S.P.; Lahiri, S.C. Detection and quantitation of trace phenolphthalein (in pharmaceutical preparations and in forensic exhibits) by liquid chromatography-tandem mass spectrometry, a sensitive and accurate method. *Journal of Forensic Sciences,* **2013,** *58,* S208–S214.
3. Ma, W.; Ma, Q.; FU, I. et al. Simultaneous determination of rimonabant and orlistat illegally added in the weight-loss functional foods by high performance liquid chromatography-tandem mass spectrometry. *Chin. J. Chromatogr.*, **2010,** *28*(1), 43.
4. Hird, S.J.; Lau, B.P.Y.; Schuhmacher, R.; Krska, R. Liquid chromatography-mass spectrometry for the determination of chemical contaminants in food. T*rends Anal. Chem.* **2014,** *59,* 59–72.

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