A Rapid and Specific Method for the Detection of Spiked Toxins Into the Food Supply

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Overview

Purpose: Fast and accurate screening of unknown toxic substances in food supply

Methods: Toxins containing samples were analyzed by UHPLC-HR/AM MS/MS on a Q Exactive benchtop Orbitrap mass spectrometer with full scan at 70,000 resolution and data dependent MS/MS at 17,500 resolution. Chromatograms were analyzed via a data mining program, SIEVE software, using a three-step method including 1) chromatogram alignment, 2) component detection, and 3) identification through online or customized libraries.

Results: All three spiked toxins in acetonitrile sample and one toxin in an apple juice sample, were successfully and fairly easily identified as the spiked toxic unknowns. The second toxin in apple juice sample has in-source fragmentation under the ion source condition. Therefore, the target toxin and its co-eluted fragment products were identified from SIEVE software. In addition, two overlapped toxins in LC chromatograms with short gradient were accurately identified through the workflow.

Introduction

Developing a fast and accurate screening method for detecting a wide range of toxic compounds is an important task for food safety. Recently, there has been a trend toward the use of full scan high-resolution, accurate mass (HR/AM) spectrometry for this purpose. HR/AM spectrometry overcomes the screening limitation via selected reaction monitoring (SRM) on triple stage quadrupoles, because specific compounds need not be selected before analysis. The entire mass range is essentially "selected". HR/AM measurement provides the specificity. Highly confident identification is achieved by accurate mass measurement of both precursor and fragment ions. A novel UHPLC-MS/MS method employing the Thermo Scientific Q Exactive benchtop Orbitrap™ mass spectrometer (Figure 1) is proposed here for the study of possible spiked toxic agents into apple juice.

Methods

Sample Preparation: Two sets of samples (acetonitrile and apple juice matrix) were prepared by spiking 10 ppm level of toxin compounds into 10 ml of the matrix and then adding 10 ml of acetonitrile. Spiked toxins in the acetonitrile sample were Colchicine, Strychnine and Aconitine; Lobeline and Solanine were spiked in the apple juice sample. The mixtures were shaken for 30 minutes and stored at 4 ^{0}C until further analysis. Solvent and matrix blanks were prepared in the same way without spiking any compounds. Samples were analyzed by ultrahigh pressure liquid chromatography-mass spectrometry (UHPLC-MS) with the Thermo Scientific Accela 1250 pump and the Q Exactive™ benchtop mass spectrometer with full scan and data dependent MS/MS with a 6.5-minute gradient. The identity of the spiked compounds was not known by the analyst prior to the analysis. The spiked compounds were thus "unknown" to the analyst.

Liquid Chromatography:

Column:	C18 column (2.1 x 50 mm, 1.8 µm)
Injection Volume:	2 µL
LC:	Accela™ 1250 pump
Solvent A:	Water, 0.1% Formic Acid
Solvent B:	Methanol
Flow Rate:	350 µL/min

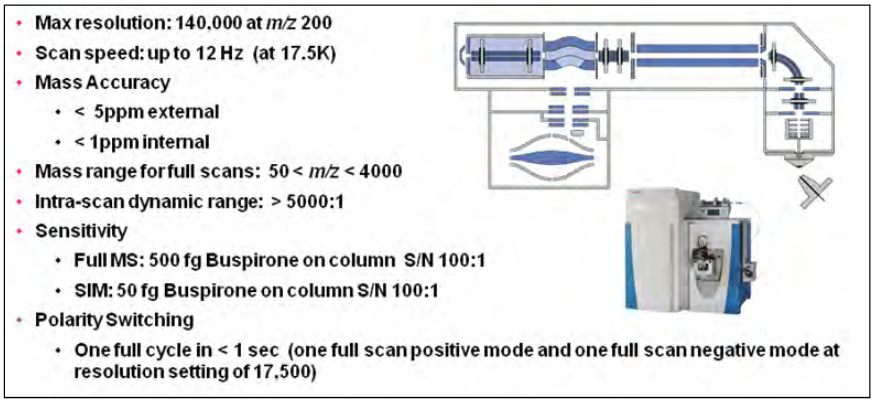
Gradient:

Time	A%	B%
0.0	95	5
3.5	5	95
6.5	5	95
6.6	95	5
9.0	95	5

Mass Spectrometry:

Spray Voltage (+)	3800 kV
Capillary Temperature (+)	320 ºC
Sheath Gas (+)	50
Aux Gas (+)	15
Sweep Gas (+)	0
Heater Temperature (+)	450 ºC
S-lens	50
Positive MS Scan	1 microscan
Full Scan	R = 70,000; AGC = 1e6; Inject = 250 ms; Lock Mass = off
MS/MS	R = 17,500; AGC = 2e5; Inject = 120 ms; HCD = 35±20%

FIGURE 1. Q Exactive Benchtop Orbitrap Mass Spectrometer



Data Analysis: Data acquisition was performed using Thermo Scientific Xcalibur 2.1 software. Differential analysis in Thermo Scientific SIEVE 1.3 software was used to analyze spiked and control samples with principal component analysis(PCA).

Figure 2 shows the overall SIEVE workflow for the unknown screening. Briefly, acquired LC chromatograms are first aligned based on the selected reference raw file to correct the retention time variance from LC runs. Then all peaks found above given intensity threshold will be ordered based on intensity. Frames of 0.30 min x 0.010 amu (see Figure 3) are defined based on the orders from the most intense to second highest peak and so on, without overlapping with previous frames. The capacity of 2,700,000 frames in 9-minute gradient are used to define analytes that may exist in the sample by PCA analysis. The ions found from differential analysis is identified through ChemSpider™ online database search and confirmed with MS/MS spectra.

FIGURE 2. SIEVE Workflow

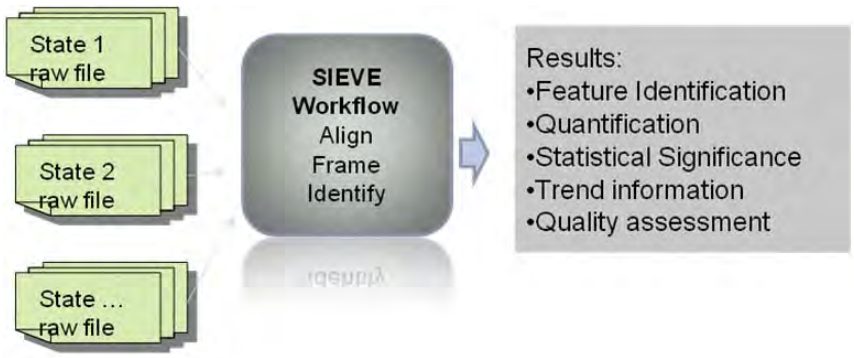
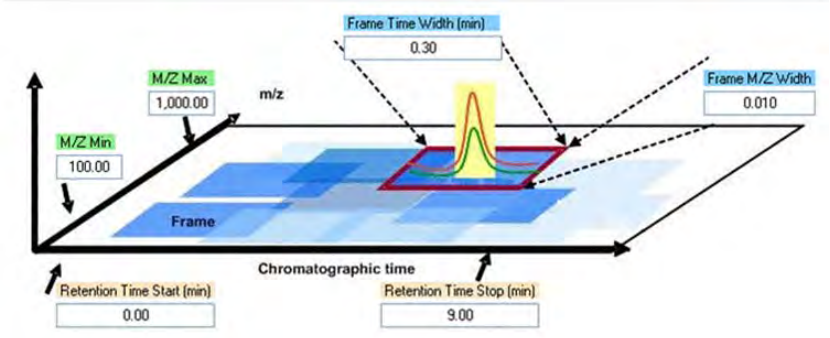


FIGURE 3. Frame Parameters



Results

Solvent Sample Analysis: The results showed a distinct difference between the spiked and controlled blank samples (Figure 4). The ChemSpider search returned with the correct identification of three compounds: Strychnine, Colchicine and Aconitine with the mass tolerance of 2 ppm with external calibration (Figures 5 & 6). Notice that two of the spiked toxins, Colchicine and Aconitine (Figure 6), overlapped with each other in UHPLC chromatograms with a short gradient but were accurately identified by SIEVE software via a differential analysis between samples versus the control blank when searched against KEGG and Sigma-Aldrich online databases in ChemSpider.

FIGURE 4. Chromatographic Alignment, Frame and PCA Analysis of Solvent Sample1 Spiked with 3 Toxins

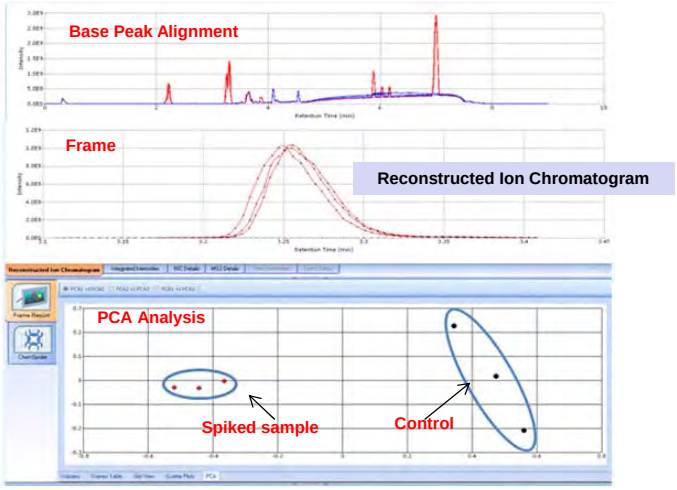


FIGURE 5. Identified Toxin1-Strychnine(*m/z* 335.1751) in Solvent Sample

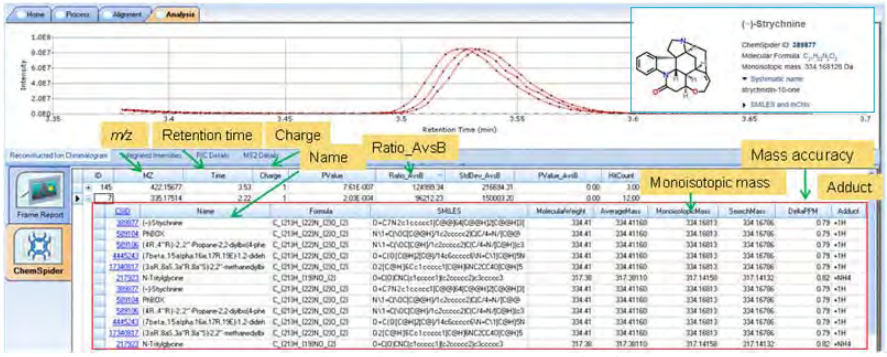
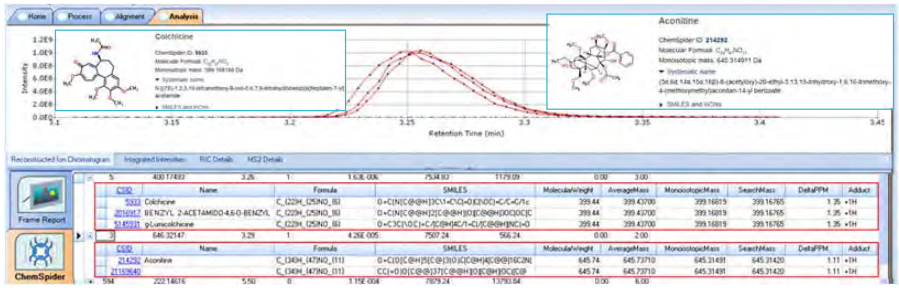


FIGURE 6. Identified Toxin2-Colchicine(*m/z* 400.1749) and Toxin3-Aconitine (*m/z* 646.3215) in Solvent Sample



Apple Juice Sample Analysis: Two toxins were spiked in the apple juice sample. Three replicates were analyzed against a clean apple juice matrix. Results are shown in Figures 7,8 & 10. Lobeline was fairly easily identified as the first spiked toxin (Figure 8). The identity of Lobeline was confirmed by MSMS interpretation as shown in Figure 9.

FIGURE 7. Apple Juice Sample-PCA Analysis

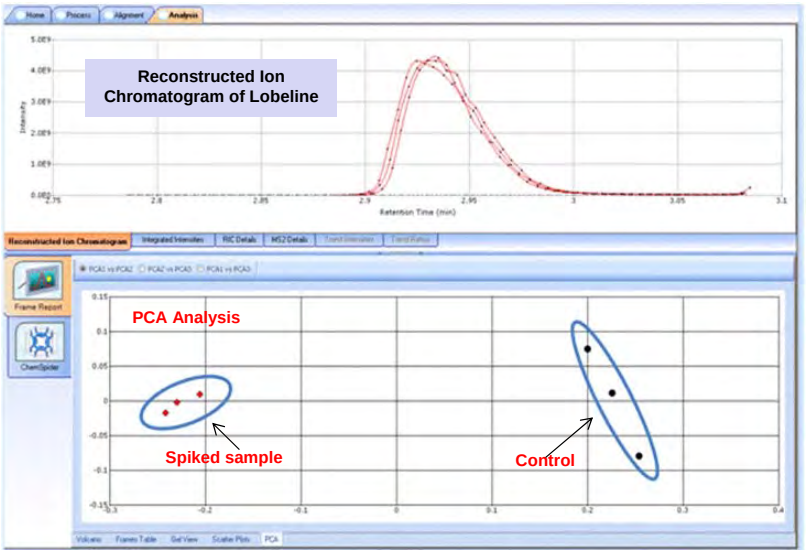


FIGURE 8. Identified Toxin1-Lobeline(*m/z* 338.2113) in Apple Juice Sample

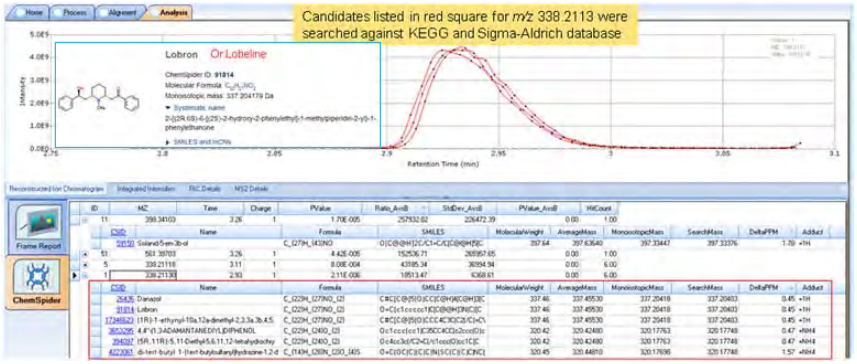
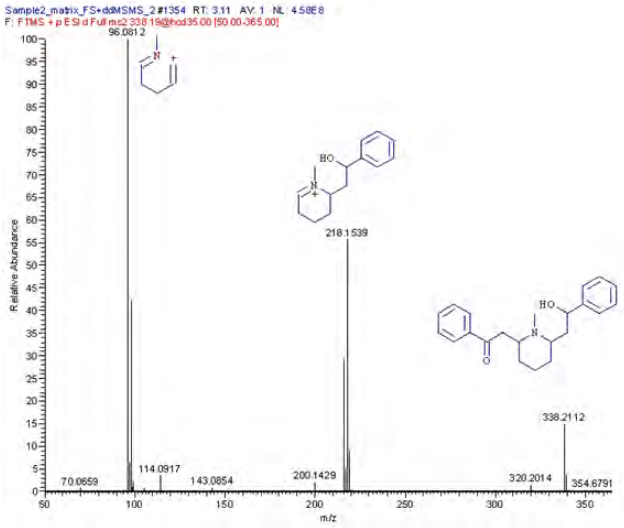


FIGURE 9. Annotated MS/MS spectrum of Lobeline(*m/z* 338.2113)



In apple juice sample, Solanine *m/z* 868.5031 and its in-source fragmentation ions, Solanadine *m/z* 398.3410, 560.3936, 561.3970 (n+1 isotope of 560.3936) co-eluted at 3.26 min and were identified as the 2nd toxic compound (Figures10 & 11).

FIGURE 10. Identification of Toxin2-Solanine(*m/z* 868.5031) and its In-Source Fragment Solanadine(*m/z* 398.3410) in Apple Juice Sample

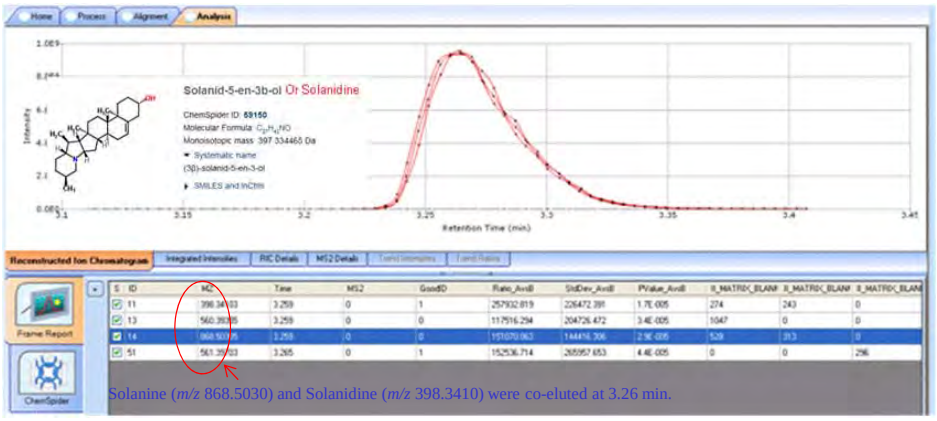
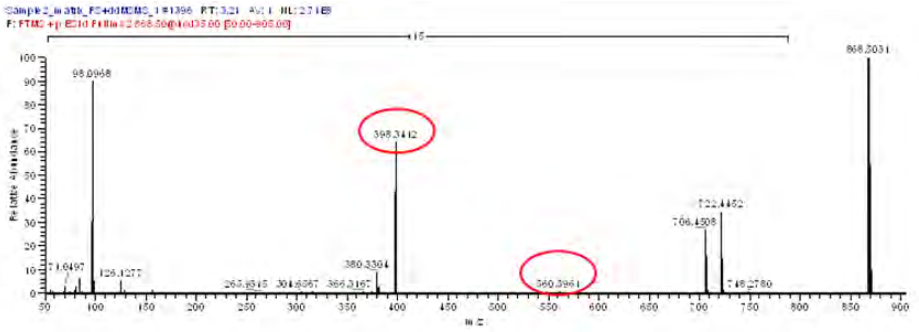


FIGURE 11. MS/MS Spectrum of *m/z* 868.5031



Conclusions

- Acetonitrile solvent sample spiked with 3 toxins, apple juice sample with 2 toxins and matrix blanks were analyzed in triplicate by the Q Exactive MS with Full Scan at 70,000 and Top1 MS/MS at 17500 resolution.
- Mass accuracy of all identified toxins is within 2 ppm with external mass calibration.
- Spiked toxins were screened through SIEVE1.3 software. Four out of five unknowns were easily and successfully targeted. The other toxin was found to co-eluted with its in-source fragmentation products.
- With the function of precursor ion selection for MS/MS and the HR/AM data acquired from the Q Exactive MS, unknown compounds can be screened out at high confidence with the structure elucidation within one injection.

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