A Turn-key System for Automated Detection of Organic Contaminants in Food Matrices and Economic Adulteration

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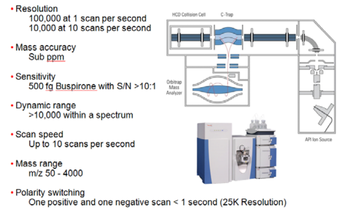
Overview

Purpose: To develop and validate a turn-key system for automated detection of organic contaminants in food matrices and economic adulteration.

Methods: A benchtop, high resolution Orbitrap (Exactive) mass spectrometer system (Figure 1) at 50,000 resolution (FWHM at *m/z* 200) coupled with ultra-high-pressure liquid chromatography (UHPLC), followed by SIEVE software analysis was used for screening unknown samples. The final results were searched against the ChemSpider database (Royal Society of Chemistry) using accurate mass data generated by the Exactive system.

FIGURE 1: Benchtop high resolution Orbitrap mass spectrometer



Results: Several individual and mixed pesticide standards, QuEChERS matrix blank samples (corn, orange, and spinach), and two unknown QuEChERS orange samples were used to evaluate the methodology using the UHPLC-Exactive system at 50,000 mass resolution. The data were processed using SIEVE differential analysis software for identification of compositional variations between the controls (blank and/or analytical standards) and experimental (unknown) samples. Library searches from ChemSpider generated potential candidates for compounds extracted from the samples that were previously analyzed by LC-MS/MS.

Introduction

The study of organic contaminants in food matrices includes "targeted" screening of known knowns, and "non-targeted" screening of known unknowns and unknown unknowns (Figure 2). Screening of food to protect the public is of great importance as demonstrated by the enactment of The Food Safety Modernization Act (FSMA) signed by President Obama in January 2011.[1] Multiresidue pesticide analysis of over 500 compounds within 12 minutes was earlier demonstrated (Figure 3) with great success.[2] The large number of residues is not a limiting factor since the resolving power is obtained from high resolution/accurate mass. The detection limit is approximately 1 ppb and the mass accuracy is less than 1 ppm. The future trend in food safety analysis and in the detection of economically adulterated foods is the implementation of "non-target" or full scan mass spectrometry utilizing both high mass resolution and high accuracy.

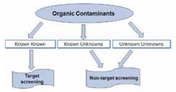


FIGURE 2: Definition of contaminants analysis

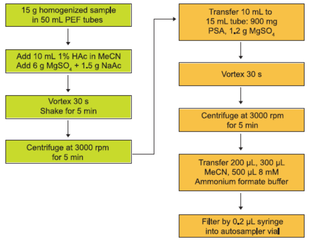
FIGURE 3: Analysis of 510 pesticides

Methods

Sample Preparation

Pesticide standards and mixtures were obtained from the U.S. Food and Drug Administration (FDA). All food commodities (blank and incurred samples) were prepared for analysis using a modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method (Figure 4). QuEChERS is an official (AOAC and CEN) sample preparation procedure used to extract pesticides from food matrices.

FIGURE 4 :Schematic of the modified QuEChERS workflow



Liquid Chromatography-Mass Spectrometry (LC-MS)

LC-MS analysis was performed using a Thermo Scientific Accela UHPLC system (LC parameters are listed in Table 1) and a CTC Analytics PAL™ autosampler coupled to a Thermo Scientific Exactive benchtop Orbitrap mass spectrometer (Figure 1).

TABLE 1: LC Parameters

Column:	Thermo Scientific Hypersil GOLD aQ C18 column (100 x 2.1 mm, 1.9 µm particle size)		
Mobile Phase:	A: Water with 0.1% formic acid and 4 mM ammonium formate B: Methanol with 0.1% formic acid and 4 mM ammonium formate		
Flow Rate:	300 µL/min		
Column Temperature:	ambient		
Sample Injection Volume:	10 µL		
Gradient:	Time (min)	%A	%B
	0	100	0
	1	100	0
	8	0	100
	12	0	100
	12.5	100	0
	14	100	0

Data Analysis

Data acquisition and analysis were performed using Thermo Scientific Xcalibur software. Thermo Scientific SIEVE differential analysis software was used to analyze and perform principal component analysis (PCA) of experimental and control samples.

The turn-key system was first validated by using a known spiked sample in a spinach matrix to demonstrate the effectiveness of the SIEVE™ software analysis. This is done by defining 980,000 "frames" of 0.35 min x 0.020 amu (Figure 5) for each data file according to the SIEVE software workflow (Figure 6) in a 10-min analysis. This "capacity" of 980,000 was used to define analytes that may exist in the sample by PCA analysis. The results showed a distinct difference between the spiked and controlled blank samples (Figure 7). The ChemSpider search returned with the correct identification of the compound methiocarb with 0.5 ppm mass accuracy.

FIGURE 5. Frame selection

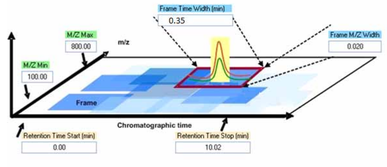
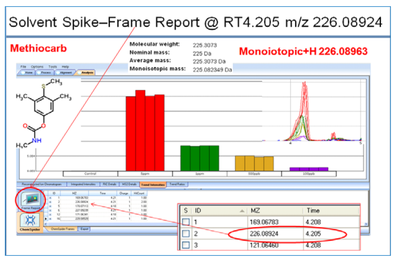


FIGURE 6. SIEVE workflow

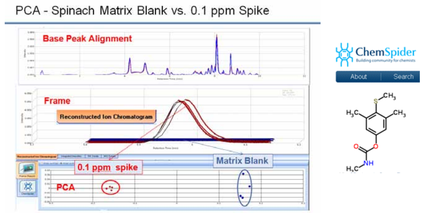
The typical limit of detection (LOD) of this workflow was evaluated by methiocarb-spiked spinach samples at concentrations of 0.01, 0.1,1.0 and 5.0 ppm. Trend analysis of all spiked samples is shown in Figure 7 and indicates a positive identification of methiocarb at the time frame of 4.205 minutes, *m/z* 226.08924, utilizing the ChemSpider database searches.

FIGURE 7. Trend analysis on all spiked levels



The workflow was demonstrated to have the ability to distinguish signature differences between the spiked and control samples analyzed at these four levels of concentration. Figure 8 shows results obtained from the 0.1 ppm level with 0.5 ppm mass accuracy. Additional samples analyzed showed the LOD of the SIEVE differential is 10 to 50 ppb, analytes pending.

FIGURE 8. Alignment, frame, PCA analysis and library search result



Incurred Orange Sample Analysis

Two incurred samples, navel and clementine orange QuEChERS extracts, were provided as double blind samples. Four replicates were analyzed against a clean orange matrix. The results of the navel orange sample are shown in Figure 9. Results from the clementine analysis are shown in Figure 10, and the comparison of navel and clementine oranges is presented in Figure 11.

Figure 9. Navel orange sample and ChemSpider search result

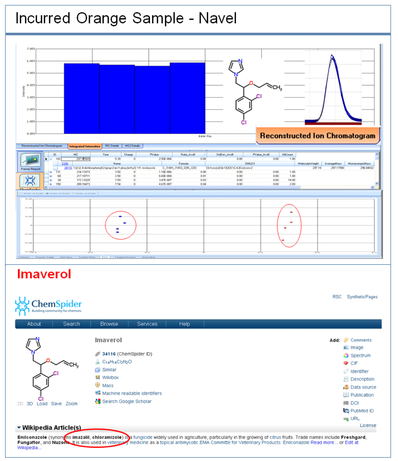


FIGURE 10. Clementine orange sample and ChemSpider search result

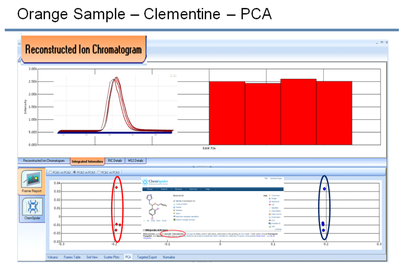
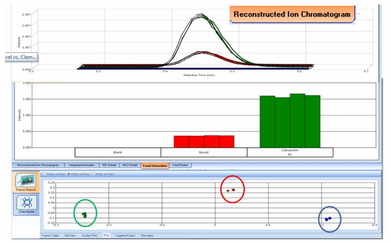


FIGURE 11. Comparison between navel and clementine orange samples



Conclusion

- Current study showed the turn-key system can effectively use the 980,000 "capacity" for the identification of analytes in various food sample matrices.
- Depending on analytes, typical LODs of this turn-key system are 10 to 50 ppb.
- Accuracy in mass measurements greatly increase the selectivity and confidence in the data quality. The Exactive™ mass spectrometer achieved a better than 1 ppm mass accuracy and remained stable for the entire experiment.
- UHPLC and small particle columns coupled with HR/AM afford fast analysis time, provide high system "capacity" of 980,000, and allow for the analysis and segregation of more than 500 compounds in 12 minutes.
- SIEVE differential expression software enables the statistical evaluation of multiple complex PCA "fingerprinting" analysis of 980,000 theoretical analytes for adulteration and authenticity of a given product.
- More than 50,000 mass resolution is required to prevent the isobaric interferences in the sample matrices.

References

1. Food Safety Modernization Act of 2011, H.R. 2751, 111th Congress (2011).
2. A. Zhang, J. S. Chang, C. Gu, M. Sanders. *Thermo Scientific Application Note* 51878, 2010.