Detection and Identification of Unknown Contaminants in Food Using LC/QTOF MS

RAFA 2009 poster C-4

Jerry Zweigenbaum Agilent Technologies, Inc., Wilmington, Delaware, USA

Abstract

Screening for targeted and non-targeted compounds is accomplished using a hybrid quadrupole time-of-flight LC/MS (LC/QTOF-MS) and a database search. Data files can be examined with multiple database searches for different categories of contaminants (for example pesticides or toxins). Targeted compounds include retention times in the database whereas non-targeted do not. Screening the results through a pesticide database demonstrates the ability to detect target and non-target contaminants. Confirmation is done by accurate mass MS/MS with correlation of fragments to the structure of the identified compound or direct comparison of the MS/MS spectrum of a standard. The identification of unknowns (not found in any database) is investigated by taking replicate extracts of feed samples and analyzing them using single MS mode. All components found to be statistically consistent in all samples are characterized as endogenous and the results maintained as the "control." Control samples spiked with select pesticides are analyzed to demonstrate the capability of detecting compounds not in the control using principle component analysis.

Introduction

The detection and identification of unknown contaminants in food and feed is a major concern in today's global market. The ability to do so is complex because of the many factors involved. These include preparing the sample in a way that will lend to detection. For example an extraction procedure may be required or a direct analysis may be needed. Secondly an appropriate analytical technique must be used; a metal contaminant might require ICP/MS, an organic GC/MS, LC/MS or DART/DESI. Finally, there must be some process to distinguish contaminants from material that is normally in a complex matrix. Thus successful determination of contaminants in complex food samples requires a comprehensive strategy and process. The use of large databases to screen for contaminants using accurate mass measurement has been developed and can help the analyst determine if a contaminant in the database is present. However, the detection and identification of contaminant not in a database represents a difficult problem. Using LC/QTOF MS, a process to identify targeted compounds (a list of compounds being sought that have been shown to be detected by the LC/MS technique), non-targeted compounds (those in a large database) and unknowns (neither targeted or in a database) will be described. As part of the strategy to determine if unknown contaminants are present in a food sample, there must be a process to define what a "normal" sample contains thus allowing distinction between those materials that should be present and those that shouldn't.

Experimental

Agilent 6530 Q-TOF Mass Spectrometer

Ion Source ESI+Agilent Jet Stream

Source Parameters

Gas Temp (° C) 300	VCap 3500
Gas Flow (l/min) 8	Nozzle Voltage (V) 1000
Nebulizer (psi) 35	Fragmentor 175 Skimmer 65
Sheath Gas Temp (° C) 350	Octopole RF Peak 750
Sheath Gas Flow (l/min) 11	

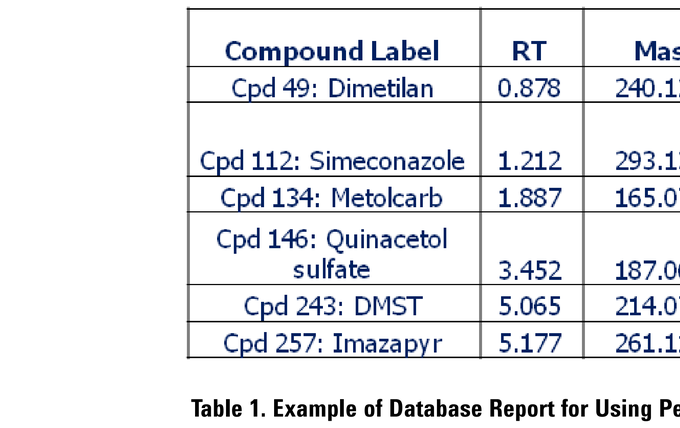
Acquisition Mode MS1
Min Range 110
Max Range 1000
Scan Rate 1.4

Agilent 1200 SL Wellplate Sampler Model G1367C **Injection Volume** 5

Agilent 1200 SL Binary Pump **Model** G1312B

Stop Time (min) 25
Flow (ml/min) 0.3
Solvent A 0.1 % formic acid
Solvent B 100 % acetonitirle
Solvent Ratio A 94 **Solvent Ratio B** 6

Time	Flow	Solv Ratio B
0	0.3	6
0.5	No Change	6
14	No Change	95



Results and Discussion

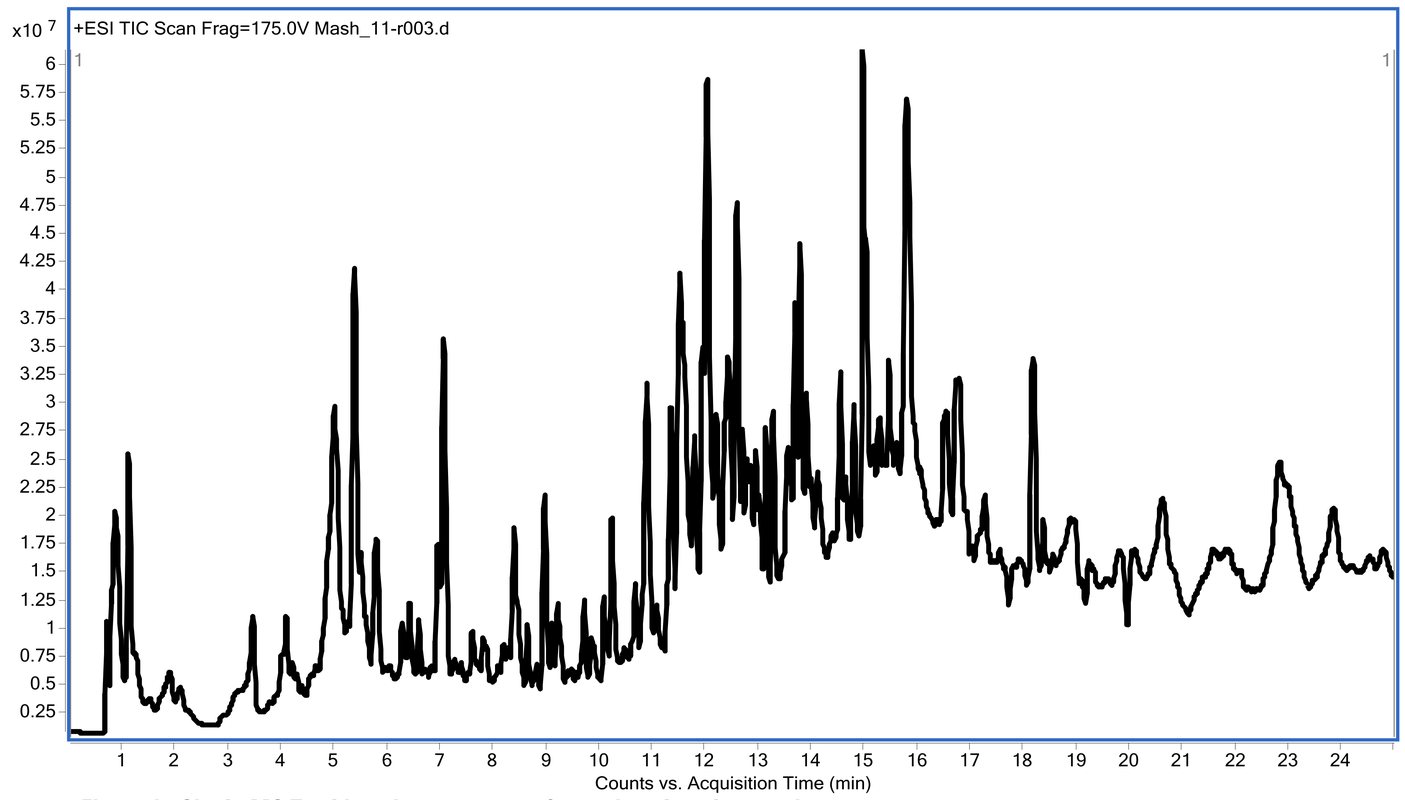


Figure 1. Single MS Total ion chromatogram of a methanol poultry mash extract.

The ability to quickly screen complex samples for low level targeted contaminants is accomplished by putting those compounds in the analytical method. However, compounds not being sought (non-targeted and unknowns) is much more difficult. Figure 1 shows the single MS full spectrum TIC a complex extract with over 10,000 natural components detected. Figure 2 shows a snapshot of the contents of a pesticide database that allow fast screening of targeted (retention times added) and non-targeted pesticides (those not being sought with no standards but that are in the database). Table 1 gives an example report from the results of the database search. Accurate mass measurement is required for a database search because of the many possible nominal mass isobaric compounds. Screening with a database can provide valuable information on the presence of these compounds, but does not indicate they are not present.

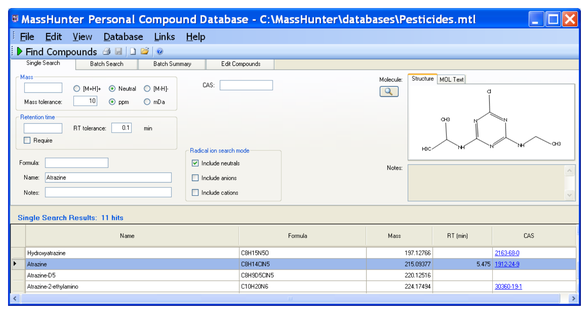


Figure 2. Database software showing contents of pesticide database. Database contains 1591 entries.

Compound Label	RT	Mass	Name	DB Formula	DB Diff (ppm)
Cpd 49: Dimetilan	0.878	240.1225	Dimetilan	C10 H16 N4 O3	-1.17
Cpd 112: Simeconazole	1.212	293.1363	Simeconazole	C14 H20 F N3 O Si	-1
Cpd 134: Metolcarb	1.887	165.0791	Metolcarb	C9 H11 N O2	-0.75
Cpd 146: Quinacetol sulfate	3.452	187.0631	Quinacetol sulfate	C11 H9 N O2	0.96
Cpd 243: DMST	5.065	214.0773	DMST	C9 H14 N2 O2 S	1.33
Cpd 257: Imazapyr	5.177	261.1109	Imazapyr	C13 H15 N3 O3	1.59

Table 1. Example of Database Report for Using Pesticide Database

The identification of "true" unknowns is extremely difficult. If a contaminant has been extracted and detected by the LC/MS technique it would be almost impossible to determine its presence manually in an extract as complex as shown in Figure 1. The problem is if you don't know what you are looking for how do you determine whether a component present belongs there or is extraneous to a "typical" sample. Using Agilent's Mass Profiler Professional software, this type of data can be statistically analyzed to determine how samples compare. Figure 3 shows the preliminary results of a principal component analysis of 10 extracts. Two of the extracts were spiked with pesticides and are shown as outside the group of others. This statistical process needs to be developed further but shows the feasibility of determining if true unknown contaminants are present.

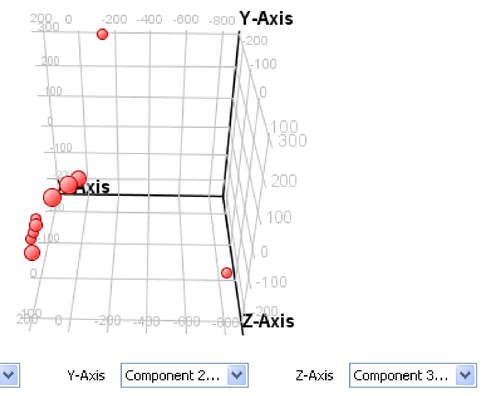


Figure 3. PCA of 10 extracts (2 spiked with pesticides).

Conclusion

- **Screening complex samples for targeted compounds can be accomplished with a accurate mass databases and QTOF LC/MS by running standards and adding retention times to the database**
- **Screening non-targeted compounds is readily done with large databases where no standards are available**
- **Screening for unknowns in complex samples at low levels is very difficult but statistical analysis offers one approach. This process needs to be developed further along with a strategy for identification of discriminated unknowns using accurate mass MS/MS.**