Screening for Environmental Contaminants in Complex Matrices—Tobacco

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Key Words: GCxGC-TOFMS, Environmental, Tobacco, Pesticides

1. Introduction

Contamination of materials intended for consumer consumption may be difficult to determine in complex matrices. Tobacco in the finished product is a particularly challenging example, as it contains plant metabolites from the tobacco leaf as well as degradation products of the metabolites resulting from aging of tobacco and processing. While target analytical techniques can reliably identify many compounds, the reliability depends on sufficient separation of components in the mixture for the particular analytical technique. The use of GCxGC techniques greatly increases the peak capacity of a chromatographic system and, therefore, the capability of such analysis.[1] Target analyses are, however, limited to the target list of compounds. Environmental contamination can involve compounds from unexpected sources, such as application of unlabeled materials as pesticides or intentional adulteration of a product. Again, the ability to reliably separate identifiable chromatographic peaks becomes critical to the analysis. Additionally, spectra across a sufficient mass range are required to identify such analytes. With adequate separation and capability to rapidly evaluate the separated compounds, screening is possible - but automation is required in complex systems that may show thousands of compounds in each sample, such as tobacco. The application of GCxGC-TOFMS and automated spectral searching for spectral features associated with contamination combined with target analysis takes advantage of the high peak capacity afforded by GCxGC techniques, the use of full-mass range spectra from components at low concentration and automated searching of the peak table for suspect contaminants. This work shows the application of this technique to tobacco. Scripts were applied to a tobacco extract spiked at multiple levels to demonstrate not only the ability to locate pesticides with this technique, but also to demonstrate the utility of the technique at low concentrations.

Analytical Approach

GCxGC-TOFMS has been demonstrated to be applicable to the identification of pesticides in a tobacco extract. The automatic, non-target identification of pesticides and other environmental contaminants in tobacco is performed by examination of the acquired GCxGC-TOFMS data with scripts designed to identify compounds by specific spectral characteristics, such as easily recognized spectral characteristics indicating the presence of chlorine, sulfur, or dimethyl phosphate in the compounds present.

In previous work,[2,3] scripts have been used to identify chlorine and sulfur containing pesticides in citrus oils. These compounds can be identified by the isotope ratios found in the parent ions. Phosphorous containing compounds do not provide this advantage, but, because the positive charge tends to stay with the fragment containing the phosphorous atom as a phosphate or thiophosphate compound fragments, there are distinctive sets of ions that help to distinguish organophosphate pesticides.

For example, dimethyl phosphate compounds often show masses 127, 109, and 79 present, with 127 as the base peak or masses 109, 79, and 47 present with 109 as the base peak. Typical spectra are demonstrated with the spectra of Mevinphos and Dichlorvos (Figure 1).

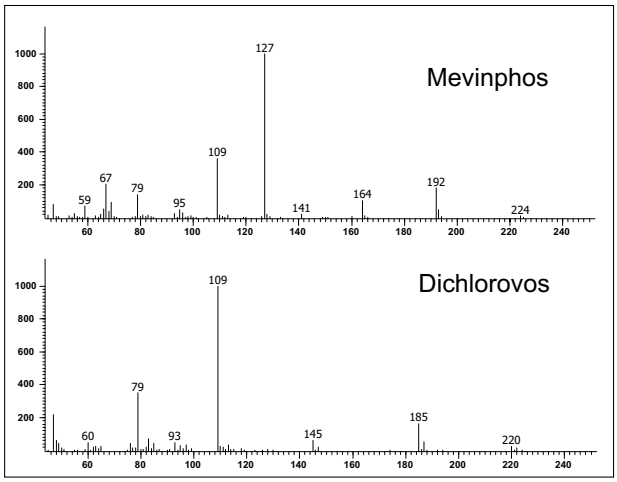

Figure 1. Spectra of two dimethyl phosphate pesticides showing characteristic patterns of masses 127, 109, and 79 or 109, 79 and 47.

2. Experimental Conditions

Samples

A laboratory that does independent testing of tobacco provided a tobacco extract in ethyl acetate. Organochlorine and organophosphorus pesticide standards were obtained from Restek Corporation. Dilutions of the standards were made in ethyl acetate. For the matrix-matched standards, spike levels were 2.5, 5, 10, 20, and 50 pg/µL respectively.

Pegasus 4D GCxGC-TOFMS

Primary Column:
30 m x 0.25 mm x 0.25 µm Rtx-1 (Restek Corp.)
Secondary Column:
1 m x 0.18 mm x 0.18 µm Rtx-200 (Restek Corp.)
Primary Oven:
40°C (1 minute), 40°C/minute to 120°C, 5°C/minute to 290°C
Secondary Oven:
5°C positive offset from the primary oven
Modulation:
Quad-jet, dual-stage

Life Science and Chemical Analysis Solutions

Modulation Time:
4 seconds
Carrier Gas:
Helium at 1.0 mL/minute constant flow
Injection:
1 µL direction injection with a Uniliner (Restek Corp.)

TOFMS Conditions

Ionization:	EI at 70 eV
Source Temp.:	225°C
Stored Mass Range:	50 to 500u
Acquisition Rate:	100 spectra/second

Instrument Control and Data Processing

The autosampler, the GC, the thermal modulator, and the TOFMS were all fully controlled through LECO ChromaTOF software. In addition, all data processing (including Automated Peak Find, Spectral Deconvolution, GCxGC slice combine, and application of scripts) was also accomplished with ChromaTOF. Scripts to detect various groups of phosphorous containing pesticides were established to locate patterns as shown in Table 1. A typical script is shown in Figure 2.

Scripts for the detection of chlorinated and sulfur-containing compounds have already been described.[3]

Table 1. Masses used to filter for specific phosphate compounds.

Group	Base Peak	Second Mass		Third Mass		Fourth Mass	
		Mass	Abundance Range	Mass	Abundance Range	Mass	Abundance Range
Dimethyl Phosphates (1)	127	109	> 20%	79	> 10%		
Dimethyl Phosphates (2)	109	79	> 20%	47	> 5%		
Dimethylphosphodithioicacid thioester (1)	93	125	> 80%				
Dimethylphosphodit hioicacid thioester (2)	125	93	> 80%				
Dethylphosphodithioicacid thioester (1)		109	> 80%	97	> 80%	65	> 1%
Dethylphosphodithioicacid thioester (2)		88	> 95%	60	> 25 %	47	> 10%
Diethylphosphorothioic ester	97	47	> 20%	65	> 20%		

```
function dimethylphosphate()
    dimethylphosphate = (rank(1) = 127 and
    abundance(79) > 100 _
        and abundance(109) >200) _
        or (rank(1) = 109 and
        abundance(79)>200 _
        and abundance(47)>50)
end function
```

Figure 2. Typical script for identifying organophosphate compounds. The script is automatically applied to the spectrum by the data processing method. A true/false value is returned.

3. Results

Application of the scripts located pesticides in the tobacco extract with a low false hit rate for other compounds in the tobacco extract. Figure 3 shows the peaks identified as showing the characteristics of dimethyl phosphate compounds. All six dimethyl phosphate compounds found in the standard mixture were located in the extract and only six extraneous peaks were identified even though the peak table for the sample shows about 9300 peaks.

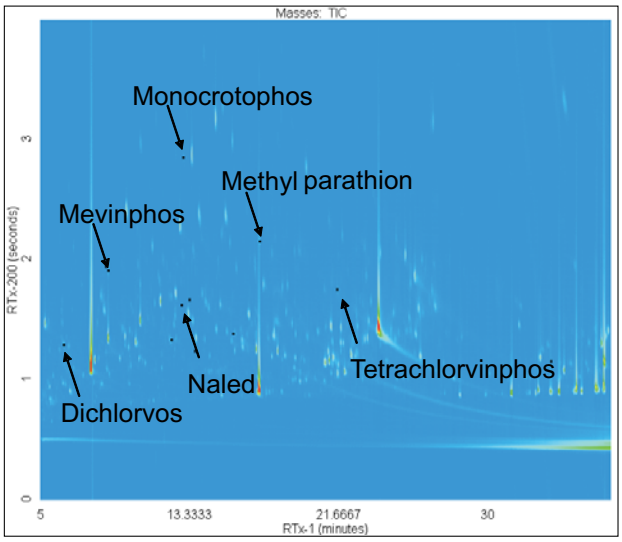

Figure 3. Peaks identified as showing spectral characteristics consistent with dimethyl phosphate compounds. In this example, the peak table of 9300 peaks was reduced to a list of 12 peaks for review.

When peaks identified as possible organophosphates, chlorinated, or sulfur containing pesticides were included in the peak table, 79 peaks were identified (Figure 4). Of these 40 were clearly identified as such compounds (Figure 5). Of the remaining 39 compounds identified, some, such as diphenyl sulfide, were identified correctly by the script, but are compounds that are not of interest.

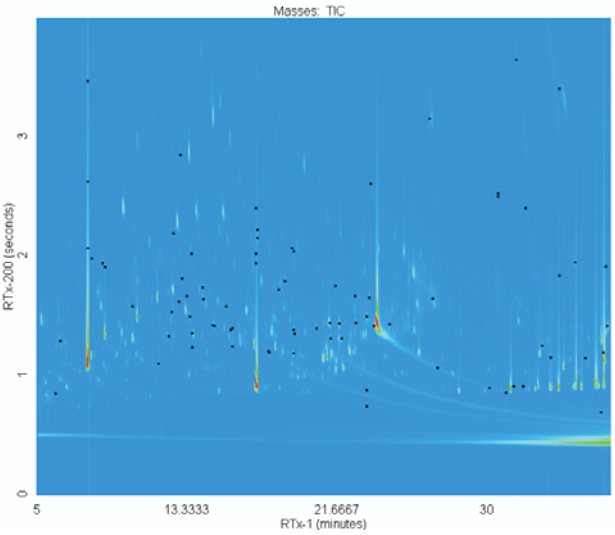

Figure 4. Peaks showing spectral characteristics consistent with phosphorous containing pesticides, sulfur containing pesticides, or chlorine containing pesticides.

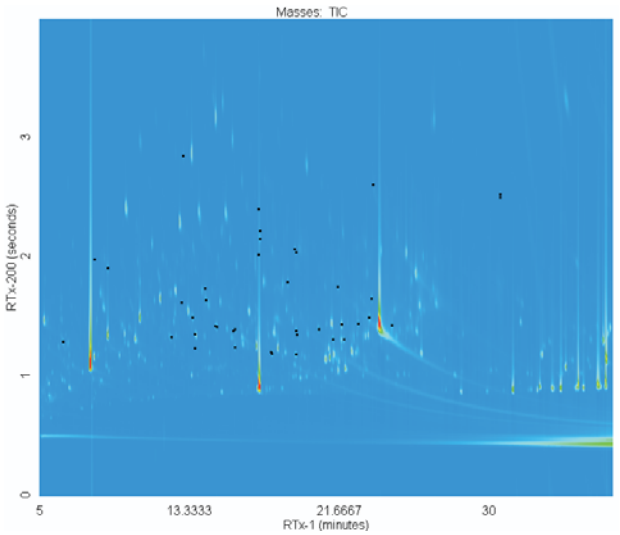

Figure 5. Peaks for which identification by scripts is supported either by spectral match or identification of a halogen cluster in the spectrum.

Examination of the samples containing the pesticides at lower levels showed that often the script would identify a compound as belonging to a particular class to a concentration close to the lowest concentration at which the compound could be located as a target analyte. This is particularly true when the ions selected by the script are some of the stronger ions in the spectrum. When the ions selected by the script are of fairly low intensity relative to the rest of the spectrum, the script is not able to detect the compounds at concentrations as low as the compounds can be detected as target analytes. Endrin and related compounds are an example. The results of all pesticides identified by scripts at any level are shown in Table 2.

4. Conclusions

The application of GCxGC-TOFMS analysis and automatic filtering of the peak table based on spectral characteristics provides the possibility of detecting both anticipated and unanticipated contaminants in the sample.

Scripts can provide detection capability at or close to that afforded by a target analytical method. However, because the ability of the script to detect a compound is dependent on the quality the spectrum, identification of compounds trough a target analytical method is recommended as well. With GCxGC-TOFMS, this can be accomplished with a single injection.

Table 2. Peaks identifiable by quantitation and by the application of scripts at various levels of spiking in the tobacco extract.

Spike Level (pg/uL)	200		100		50		20		10		2	
	Detectable in Chromatogram	Detectable by Script	Detectable in Chromatogram	Detectable by Script	Detectable in Chromatogram	Detectable by Script	Detectable in Chromatogram	Detectable by Script	Detectable in Chromatogram	Detectable by Script	Detectable in Chromatogram	Detectable by Script
Dichlorvos	X	X	X	X	X	X	X	X	X	X	X	
Mevinphos	X	X	X	X	X	X	X	X	X	X	X	
Ethoprop	X	X	X	X	X	X	X	X	X	X		
Naled	X	X	X	X								
Monocrotophos	X	X	X	X	X		X		X	X		
Sulfotepp	X	X	X	X	X	X	X	X	X			
à-Lindane	X	X	X	X	X	X	X	X	X	X		
Demeton-s	X	X										
beta lindane	X	X	X	X	X	X	X	X	X			
delta lindane	X	X	X	X	X	X	X	X	X			
Diazinon	X	X	X		X	X	X		X		X	
Disulfoton	X	X										
Methyl parathion	X	X	X	X	X	X	X	X	X		X	
Heptachlor	X	X	X	X	X	X	X	X	X			
Ronnel	X		X	X	X		X	X	X			
Malathion	X	X	X	X	X		X	X	X	X	X	X
Fenthion	X		X	X	X	X	X					
Parathion	X	X	X	X	X	X	X	X	X		X	
Aldrin	X	X	X	X	X	X	X	X	X	X		
Chlorpyrifos	X	X	X	X	X	X	X	X	X	X	X	
Heptachlor epoxide	X	X	X	X	X	X	X	X	X	X		
Chlordane, alpha	X	X	X	X	X		X	X	X			
Stirofos	X	X	X	X	X	X	X	X	X	X		
Endosulfan I	X	X	X	X	X		X		X		X	
Chlordane, gamma	X	X	X	X	X	X	X		X			
p,p'-DDE	X		X	X	X	X	X	X	X			
Dieldrin	X	X	X	X	X	X	X		X	X		
Endrin ketone	X	X	X		X		X	X				
Endrin	X	X	X		X		X	X	X			
Endosulfan II	X	X	X		X		X		X			
p,p'-DDD	X		X	X	X		X		X			
Bolstar	X	X	X	X	X							
Endosulfan sulfate	X		X		X		X					
Coumaphos	X	X	X		X	X	X		X			

5. References

[1]Jack Cochran. "Evaluation of comprehensive two-dimensional gas chromatography—time-of-flight mass spectrometry for the determination of pesticides in tobacco." J. Chromatography A 1186.1-2 (2008): 202-210.

[2]D.C. Hilton, "Automated Screening For Hazardous Components in Complex Mixtures Based on Functional Characteristics Identifiable in GCxGC-TOF-MS Data," Current Trends in Mass Spectrometry, July 2007, 28-34.

[3]LECO Applications note: "Automated Screening of GC-TOFMS Chromatograms with Specific Detection of Chlorine, Bromine, and Sulfur Containing Compounds" (form no. 203-821-341).

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