GCxGC-TOFMS of Pesticides in Tobacco

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Key Words: GCxGC-TOFMS, Deconvolution, Quantification, Pesticides, Tobacco

1. Introduction

The production of tobacco, a high-value crop for the United States, is increased by the use of pesticides that are specifically approved for use on tobacco by the Environmental Protection Agency (EPA). Even after the processing of tobacco, some pesticide residues remain on the product and under its pesticide registration program the EPA is charged with assessing risks to smokers from exposure to these residues. Because tobacco is such a complex matrix, the challenges associated with determining pesticides at trace levels are substantial. Often gas chromatography with selective detectors—for example the electron capture detector (ECD), the nitrogen-phosphorus detector (NPD), and the flame photometric detector (FPD)—are used. These detectors provide good sensitivity and some selectivity for pesticides that contain halogens, nitrogen, phosphorus, and sulfur. Unfortunately they do not provide unequivocal identification for pesticides, and due to the complexity of the matrix itself, coelutions (and thus interferences) can still occur. To combat this issue, analysts are turning to mass spectrometry (MS), which can provide a higher level of specificity to the analysis. Due to the trace levels of most pesticides in tobacco, selected ion recording (SIR) is used (in lieu of full scan MS), resulting in a vast loss of information, and the analysis is restricted to target pesticides. Even with SIR, interferences often occur, and add uncertainty to pesticide determinations in tobacco.

Time-of-flight mass spectrometry (TOFMS) provides a better solution because of its full mass range capability at low levels. In addition, TOFMS offers fast acquisition speeds (up to 500 spectra/second) and spectral continuity that allow incorporation of spectral deconvolution algorithms into processing software to add an extra degree of qualitative analysis to complex samples. Fast acquisition rates also support the use of comprehensive two-dimensional GC (GCxGC). GCxGC increases peak capacity by applying two independent separations to a sample in one analysis. Typically, GCxGC involves a serial column configuration (employing orthogonal phases) separated by a thermal modulator. Due to thermal modulation, most GCxGC peaks are on the order of 50 to 250 ms wide. When MS is used, only TOF has the necessary acquisition rates. The ability of the thermal modulator to narrow peaks (thereby increasing their height) prior to their detection also affords the ability to increase sensitivity for pesticides in tobacco, which is always desirable for health studies.

This note demonstrates the power of using GCxGC-TOFMS to determine pesticides in a complex tobacco extract. Interferences that would occur in a one-dimensional GC-MS analysis are eliminated when using GCxGC-TOFMS. Multi-point calibration curves were generated from matrix-matched standards for a select group of pesticides. The ability of TOFMS to automatically locate and identify target and non-target pesticides is presented.

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.

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
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, calibration, and quantify) was also accomplished with ChromaTOF.

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3. Results and Discussion

Figure 1 shows a contour plot for the tobacco extract as a total ion chromatogram (TIC). A contour plot is a way to display GCxGC data and visually indicates the peak capacity increase for a complex sample. The X-axis represents retention times for the first separation (the Rtx-1 column) and the Y-axis shows retention times for the second separation (the Rtx-200 column). A color scheme (with red being most intense) attempts to display peak height.

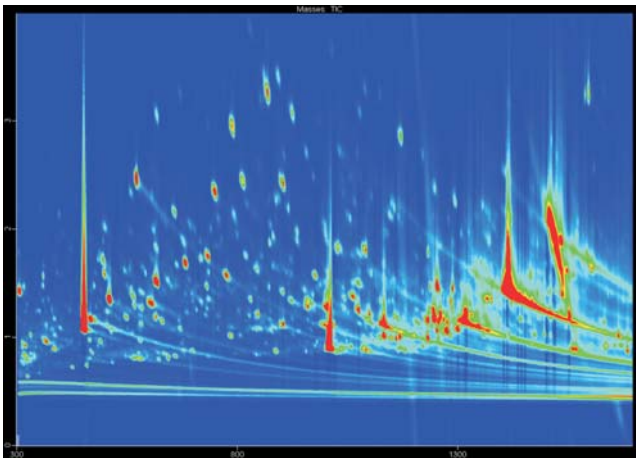

Figure 1. Contour plot of tobacco extract. Retention times for the Rtx-1 separation are plotted along the X-axis and retention times for the Rtx-200 separation are shown on the Y-axis.

Another way to display GCxGC data is through the use of the surface plot, which in addition to the retention axes has a Z-axis for peak height (Figure 2).

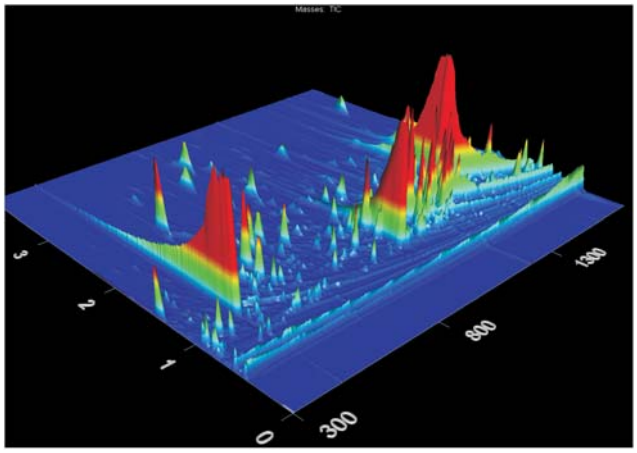

Figure 2. Surface plot of tobacco extract. Retention times for the Rtx-1 separation are plotted along the axis towards the right and retention times for the Rtx-200 separation are shown on the axis going left. A Z-axis represents peak height.

Contour plots for the pesticides illustrate how they are dispersed when using GCxGC-TOFMS (Figures 3 and 4). It should be noted that the primary goal of GCxGC-TOFMS is not to chromatographically separate the pesticides from each other in the standards, as that can often be accomplished with GC-MS, but instead to separate the pesticides from the often-overwhelming matrix components that are encountered when analyzing tobacco extracts. Specific examples of that will be demonstrated below.

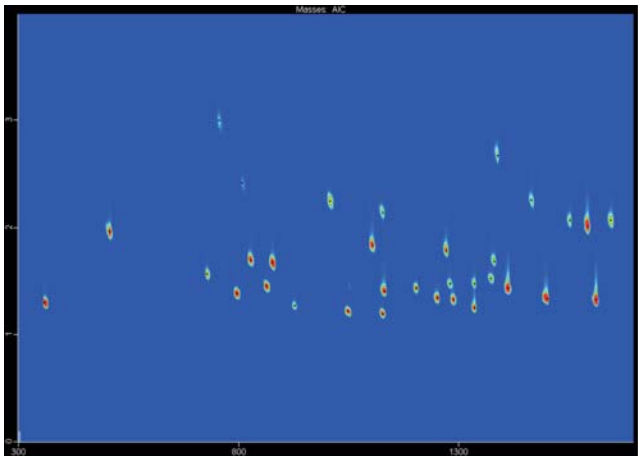

Figure 3. Contour plot of pesticide standard mix analyzed with GCxGC-TOFMS. Note how the pesticides are chromatographed in two dimensions when using the Rtx-1 x Rtx-200 column combination.

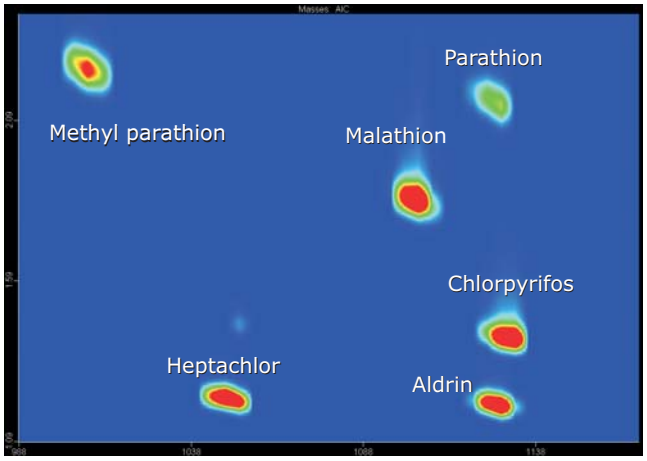

Figure 4. Zoomed in region of contour plot for pesticides standard mix analyzed using GCxGC-TOFMS. The Rtx-200 (the Y-axis separation) provides selectivity to separate Aldrin, Chlorpyrifos, Malathion, and Parathion in the second dimension.

Calibration curves were prepared from matrix-matched standards (using the tobacco extract) at levels of 2.5, 5, 10, 20, and 50 pg/μL. Tetrabromothiophene was employed as an internal standard. Example curves are shown for gamma-hexachlorocylohexane (Lindane) and Tetrachlorvinphos (Stirophos) as Figures 5 and 6. Linearity is very good. The benefit of TOFMS is that a full mass spectrum is used as a Reference Spectrum to locate (match to) the pesticide of interest in a sample (within retention time windows for the GCxGC chromatogram). A significant, typically higher m/z, ion is used for the quantification mass. Although it will not be discussed in detail in this note, chromatographic peaks are "sliced" during the thermal modulation process and must have their areas recombined for calibration and quantification purposes. This is done automatically through ChromaTOF.

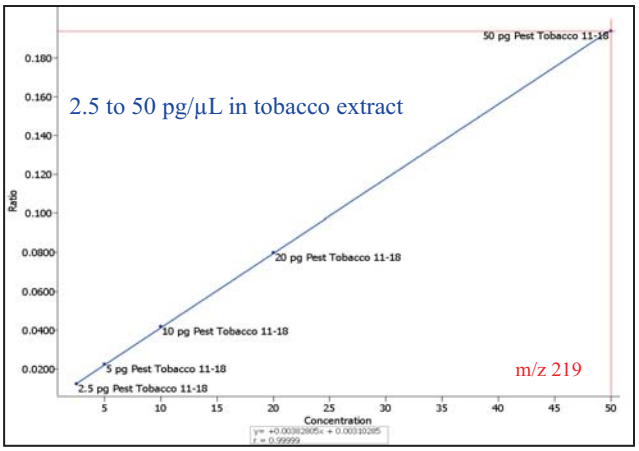

Figure 5. GCxGC-TOFMS calibration curve for gamma-hexachlorocyclohexane (Lindane). The curve was prepared from matrix-matched standards from a tobacco extract.

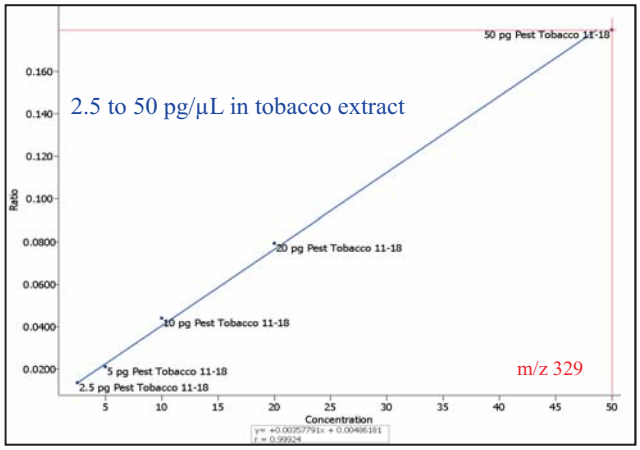

Figure 6. GCxGC-TOFMS calibration curve for Tetrachlorvinphos (Stirophos). The curve was prepared from matrix-matched standards from a tobacco extract.

Table 1 summarizes the calibration data from the other pesticides analyzed in this GCxGC-TOFMS experiment, while also listing retention times (each pesticide has two!) from GCxGC analysis. Pesticides that were present in the tobacco extract that was used for matrix-matched calibration standards do not have correlation coefficient (CC) data shown in this table. In addition, where a pesticide was found in the tobacco extract, any analogs of that pesticide do not have their calibration CCs included in this table. For example, Endosulfan II was found in the tobacco extract, so Endosulfan I and Endosulfan sulfate CCs are not included in the calibration summary table. Dimethoate, p,p'-DDT, and Methoxychlor were calibrated down to 5 pg/µL.

Table 1. GCxGC-TOFMS calibration summary data, including correlation coefficients (Corr Coeff) for a select group of organochlorine and organophosphorus pesticides. Data was compiled from matrix-matched standards. Where NS is listed, the Corr Coeff is not shown due to incurred pesticide in the tobacco extract that skews the curve.

Pesticide Name	RT 1 (sec)	RT 2 (sec)	Quant Mass	Corr Coeff
Dichlorvos	360	1.31	185	0.992
Mevinphos	508	1.97	192	0.992
Ethoprop	728	1.57	200	0.998
Monocrotophos	756	3.00	192	0.997
alpha-HCH	796	1.39	219	1.00
Dimethoate	808	2.42	229	0.998
gamma-HCH	828	1.70	219	1.00
beta-HCH	864	1.46	219	1.00
delta-HCH	876	1.68	181	1.00
Diazinon	928	1.28	304	NS
Methyl parathion	1008	2.25	263	NS
Heptachlor	1048	1.23	272	1.00
Malathion	1104	1.84	173	NS
Aldrin	1128	1.20	263	1.00
Parathion	1128	2.14	291	1.00
Heptachlor epoxide	1204	1.44	353	1.00
gamma-Chlordane	1252	1.35	373	1.00
Tetrachlorvinphos	1272	1.79	329	1.00
Endosulfan I	1280	1.48	195	NS
alpha-Chlordane	1288	1.33	373	1.00
p,p'-DDE	1336	1.26	318	1.00
Dieldrin	1336	1.48	263	1.00
Endrin	1372	1.53	263	0.993
Endosulfan II	1380	1.69	195	NS
Fensulfothion	1388	2.67	293	0.998
p,p'-DDD	1412	1.44	235	0.994
Endosulfan sulfate	1464	2.26	272	NS
p,p'-DDT	1500	1.34	235	0.999
Endrin ketone	1552	2.07	317	1.00
EPN	1592	2.02	157	0.997
Methoxychlor	1612	1.33	227	0.999
Azinphos methyl	1644	2.07	160	0.995

The advantage of GCxGC for eliminating potential quantification bias, even when a mass spectrometer is being used as the detector, is seen in Figure 7 where Methyl parathion is nicely resolved in the second dimension from a large tobacco-matrix interference that contains the same m/z ion used to quantify Methyl parathion.

Another even more dramatic example of this same occurrence is shown in Figure 8 for Chlorpyrifos in the tobacco extract where at least three interferences that contain m/z 197, the Chlorpyrifos quantification mass, would be present in a one-dimensional GC-MS analysis. Although GCxGC eliminated the potential for quantification bias here, spectral deconvolution was still necessary to provide the unequivocal identification of Chlorpyrifos in this complex tobacco extract (also Figure 8).

This theme, the one represented by Figures 7 and 8, the potential for quantification bias in one-dimensional GC-MS, was seen over and over for the pesticides-in-tobacco work done for this note. Repeatedly, employing GCxGC-TOFMS eliminated this problem.

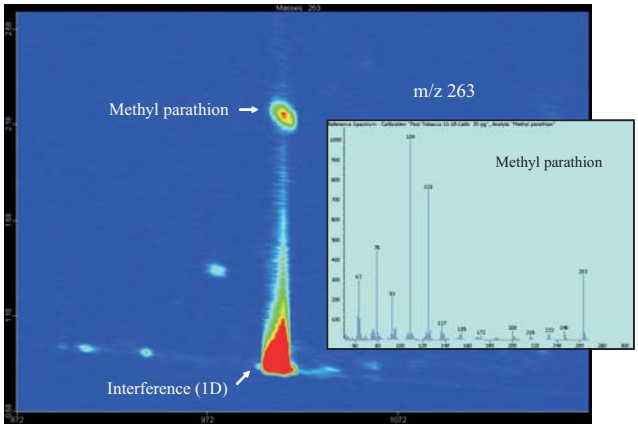

Figure 7. The GCxGC contour plot shows methyl parathion resolved in the second dimension on the Rtx-200 column. The quantification mass 263 is plotted. Note the large peak at the bottom of the contour plot that would bias any one-dimensional GC-MS results.

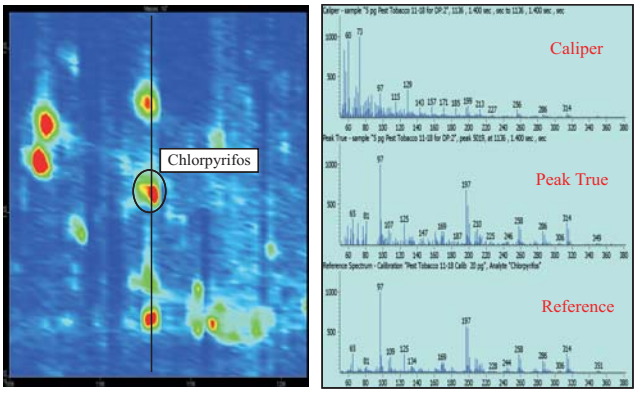

Figure 8. The GCxGC contour plot on the left represents m/z 197 for a tobacco extract that contains the pesticide Chlorpyrifos (black circle). The two "spots" below, and the one above Chlorpyrifos would be interferences causing quantification bias when using one-dimensional GC-MS. Even though GCxGC can separate the quantification interferences from Chlorpyrifos, the qualitative identification is provided through a combination of GCxGC and spectral deconvolution (Peak True), as seen from the spectra on the right.

As mentioned above, GCxGC, while indeed powerful, does not always provide the separation power to perfectly qualitatively identify pesticides in such a complex matrix as tobacco. TOFMS allows an elegant solution to this problem by supporting the use of automated peak find and spectral deconvolution algorithms that are integral to ChromaTOF software. Examples are shown in Figures 9 and 10 where matrix interferences would thwart the identification of pesticides without spectral deconvolution. In these examples, the caliper spectrum was taken at the peak apex of the pesticide. Even so, the caliper spectrum is not representative of the pesticide due to coelutions with other components in the tobacco extract. Simple background subtraction is not a practical alternative to this automated spectral deconvolution routine available with ChromaTOF due to the time it would take and the likelihood that an analyst could not produce a clean spectrum for the pesticide of interest in such a complex matrix as tobacco. As with the quantification bias relief provided through the strength of GCxGC, many other examples could be shown for spectral deconvolution alleviating problems in identification of compounds that coeluted with larger matrix interferences.

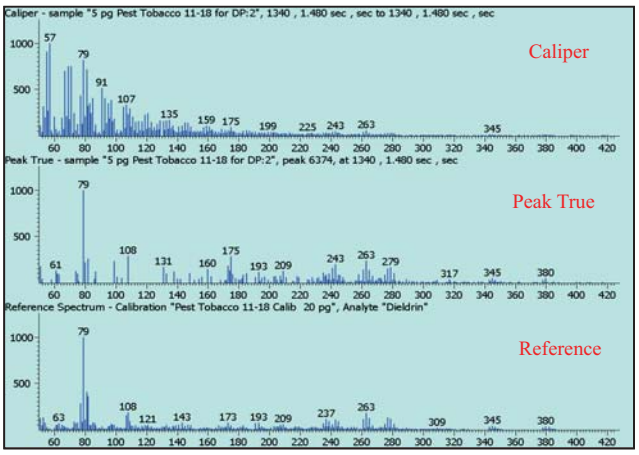

Figure 9. Coeluted (Caliper) and deconvolved (Peak True) spectra for the pesticide Dieldrin in a tobacco extract, compared to a Reference Spectrum. The Caliper spectrum represents mainly the coeluting compound or compounds, but the Peak True has a good match of 804 against the Reference Spectrum. Dieldrin is at only 5 pg/µL in the tobacco extract.

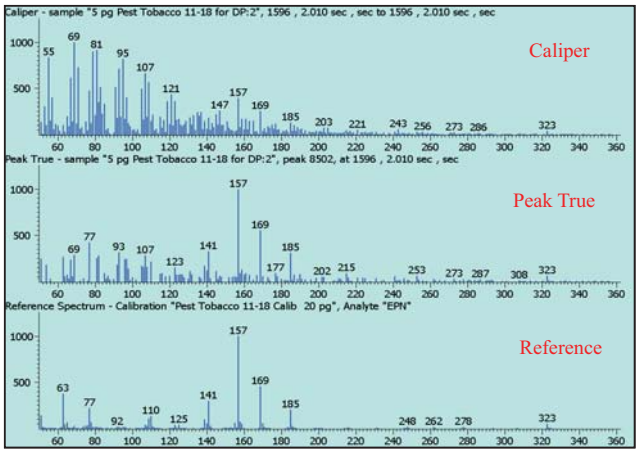

Figure 10. Coeluted (Caliper) and deconvolved (Peak True) spectra for the pesticide EPN in a tobacco extract, compared to a Reference Spectrum. The Caliper spectrum represents mainly the coeluting compound or compounds, but the Peak True has an excellent match of 914 against the Reference Spectrum. EPN is at only 5 pg/µL in the tobacco extract.

GCxGC-TOFMS is excellent at doing target pesticide analysis as has been demonstrated above, but because a full mass spectrum is always available with TOFMS the technique can also simultaneously do non-target pesticide analysis. Again, it is the automated peak find, spectral deconvolution, and library searching capabilities of ChromaTOF software that quickly accomplish non-target pesticide location in complex samples. Table 2 lists all of the pesticides found in the unspiked tobacco extract by automated routines, including those that were not specifically targeted through calibration. In addition, manual review of the data (because a full mass spectrum is always available with TOFMS) indicated the likely presence of the herbicides Trifluralin and Pendimethalin.

Table 2. Pesticides located in a tobacco extract using GCxGC-TOFMS with automated peak find, spectral deconvolution, and library searching.

Pesticide Name	Action/Use	RT 1 (sec)	RT 2 (sec)	Ion (m/z)
2,4-D methyl ester	Herbicide	732	1.54	234
2,4-D ethyl ester	Herbicide	808	1.53	248
Diazinon	Insecticide, nematicide	936	1.23	304
Methyl parathion	Insecticide	1016	2.21	263
Chlorpyrifos methyl	Insecticide	1024	1.46	286
Carbaryl	Insecticide	1024	2.09	144
Metalaxyl	Fungicide	1056	1.84	206
Pirimiphos methyl	Insecticide	1100	1.35	290
Malathion	Insecticide	1108	1.83	173
Chlorpyrifos	Insecticide	1136	1.41	197
Flumetralin	Plant-growth regulator	1312	1.94	143
Ethion	Acaricide, insecticide	1432	1.56	231
Bromopropylate	Acaricide	1616	1.47	341

4. Conclusions

GCxGC-TOFMS is a viable way to determine pesticides in tobacco extracts. GCxGC provides the selectivity necessary to operate in such complex matrices while the thermal modulation process of GCxGC enhances the innate, full mass range sensitivity of TOFMS. Full mass spectra provide powerful confirmation of the pesticide in the sample, including non-target pesticides. This capability is supplemented by the automated peak find and spectral deconvolution software of ChromaTOF. Archived data will always contain full mass range spectral information should it be necessary to go back and check for other pesticides in a sample that may have degraded or been discarded.

5. References

Government Accounting Office Report, Pesticides on Tobacco, March 2003.

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