

GC-TOFMS and GCxGC-TOFMS of Acephate in Bird Gizzard Extracts

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1. Introduction

Acephate is an organophosphate insecticide with a wide variety of approved uses on ornamental and agricultural plants. Recently in Tennessee it was suspected that Acephate was used to intentionally (and illegally) poison a large number of grackles and starlings, bird species that are considered pests by some farmers. Extracts of the gizzards of some of the dead birds were produced, and Gas Chromatography—Time-of-Flight Mass Spectrometry (GC-TOFMS) and comprehensive two-dimensional GC—Time-of-Flight Mass Spectrometry (GCxGC-TOFMS) were used to investigate for the presence of Acephate.

2. Experimental Conditions

Sample Extraction

Bird gizzard extracts were prepared by the Tennessee Department of Agriculture in Nashville, Tennessee.

GC-TOFMS

Column:

10 m x 0.18 mm x 0.18 μ m DB-5 (J&W Scientific)

Carrier:

Helium at 0.5 mL/minute, constant flow

Injection:

1 μ L splitless at 250°C, valve time 60 seconds

Oven Program:

40°C (1 minute), 20°/minute to 340°

MS: LECO Pegasus® TOFMS

Ionization: Electron ionization at 70 eV

Source Temp: 200°C

Stored Mass Range: 45 to 550 u

Acquisition Rate: 20 spectra/second

GCxGC-TOFMS

GCxGC:

Agilent 6890 Gas Chromatograph equipped with a LECO GCxGC Thermal Modulator and Secondary Oven

Column 1:

20 m x 0.18 mm x 0.18 μ m DB-1 (J&W Scientific)

Column 2:

1 m x 0.10 mm x 0.10 μ m Rtx-CLPesticidesII (Restek)

Carrier:

Helium at 1.5 mL/minute, constant flow

Injection:

1 μ L splitless at 250°C, valve time 60 seconds

Oven 1 Program:

40°C (1 minute), 10°/minute to 320°

Oven 2 Program:

30°C offset from oven 1

Modulation Time:

3 seconds

MS: LECO Pegasus TOFMS
Ionization: Electron ionization at 70 eV
Source Temp: 225°C
Stored Mass Range: 45 to 550 u
Acquisition Rate: 100 spectra/second

Data Processing

LECO ChromaTOF® software with Automated Peak Find and Spectral Deconvolution.

3. Results and Discussion

Figure 1 shows the results from a GC-TOFMS analysis of an Acephate-free bird gizzard extract spiked with 500 pg/ μ L of Acephate. The Acephate, seen as extracted ions 94 (red) and 136 (green) in the figure, is buried in the presence of overwhelming matrix. Yet the deconvoluted spectrum produced by LECO ChromaTOF software matches well to the NIST library mass spectrum. It is important to note that with TOFMS, a full mass spectrum is always available. One distinction that can be made between TOFMS and an ion trap MS system, which can also produce a full mass spectrum, is that due to the automatic gain control used to avoid space charging in the ion trap (ionization time is reduced), the population of 94 and 136 ions could be suppressed to the point of making the Acephate undetectable in this coelution situation. This does not occur with TOFMS.

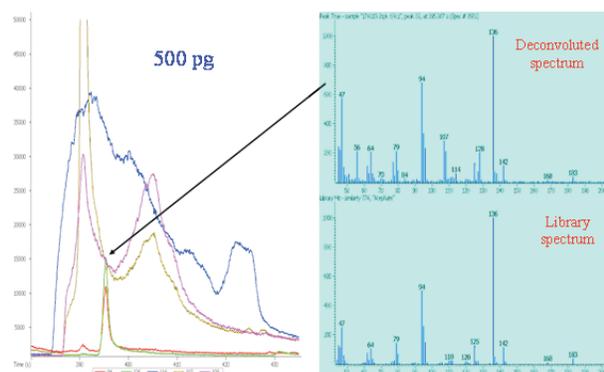


Figure 1. Chromatogram of Acephate in a bird gizzard extract. Ions 94 (red) and 136 (green), representative of Acephate, are plotted in the presence of a huge, coeluting matrix background from the bird gizzard. The deconvoluted TOF mass spectrum matches well with the NIST library spectrum.

Figure 2 shows the GC-TOFMS results when there is only 50 pg/ μ L of Acephate in the bird gizzard extract. The 94 and 136 ions of Acephate are easily seen in the chromatogram. Although the deconvoluted spectrum does not lead to as high a match with the library spectrum as seen for the 500 pg/ μ L spike of Figure 1, it is a great improvement over the caliper (raw) spectrum, which is obviously representing mainly the matrix background.

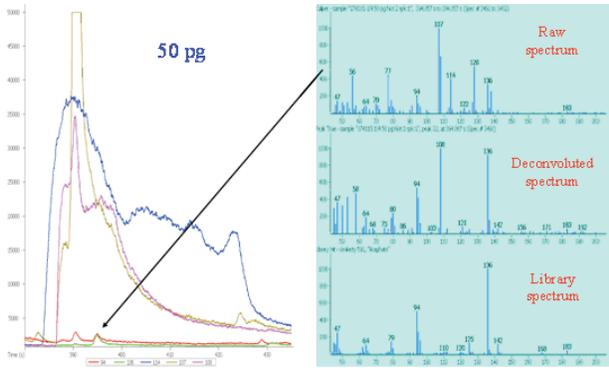


Figure 2. Chromatogram of only 50 pg/ μ L of Acephate in a bird gizzard extract. Ions 94 (red) and 136 (green) are again plotted in the presence of a huge, coeluting matrix background from the bird gizzard. The deconvoluted TOF mass spectrum (middle), while not perfect, contains the major ions of Acephate and is a substantial improvement over a caliper, or "raw" mass spectrum (top), which shows mainly interfering matrix ions.

After it was determined that GC-TOFMS could be applied successfully for relatively low levels of Acephate in bird gizzards, extracts from two of the birds that died in the incident were analyzed. As can be seen in Figure 3, no Acephate was located in these extracts.

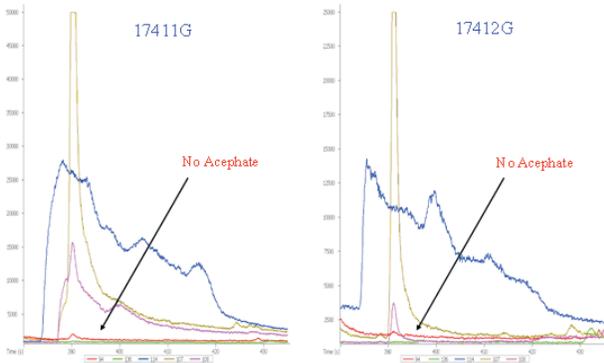


Figure 3. GC-TOFMS chromatograms of two gizzard extracts from birds that died in a suspected pesticide poisoning incident. No Acephate was found in these extracts.

GCxGC-TOFMS has some advantages for analysis of compounds like Acephate in complex samples. The most obvious advantage is the extra peak capacity available with GCxGC, where separations are conducted in two dimensions. This is easily illustrated in the form of a contour plot, a GCxGC chromatogram (Figure 4). Another advantage is that peaks are sharpened close to the detector by the thermal focusing aspect of GCxGC. For example, in the work for this note, the Acephate peaks generated during GCxGC are approximately 150 ms wide at the base! This mandates a fast recording detector and explains the 100 spectra/second acquisition rate used for GCxGC. In fact, TOFMS is the only mass spectrometer that can adequately define GCxGC peaks.

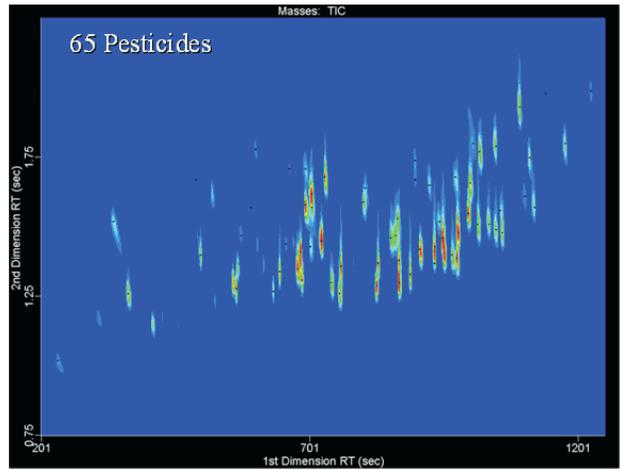


Figure 4. Contour plot, or GCxGC-TOFMS chromatogram, of a pesticide standard mix. Note how separations are occurring along two dimensions.

Figure 5 shows a contour plot for the bird gizzard extract that was spiked with 500 pg/ μ L of Acephate. The white box in this figure shows the general area of elution for Acephate. The contour plot in Figure 6 focuses in on this area of elution, points out the Acephate peak, and shows deconvoluted and library mass spectra.

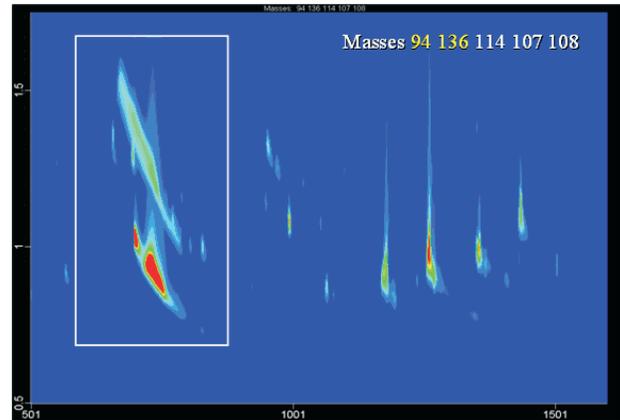


Figure 5. Contour plot of an Acephate-spiked bird gizzard extract. Extracted ions for Acephate and matrix components are plotted, with the white box indicating the area of elution for Acephate.

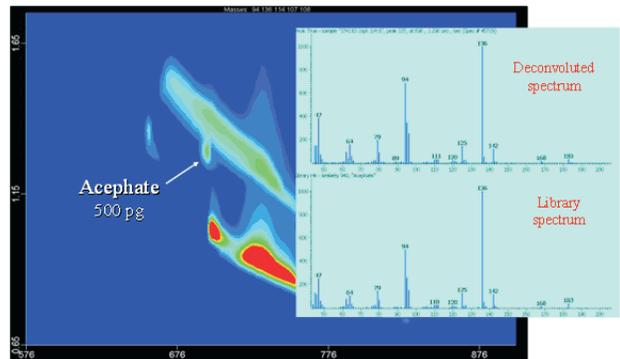


Figure 6. Contour plot zoom of Acephate elution region from Figure 5 with Acephate peak marked. The Acephate elutes in the second dimensional space between two large matrix interference regions. Deconvoluted and library spectra show an excellent match of almost 950 (out of 999).

Figures 7 and 8 continue to demonstrate the GCxGC-TOFMS edge in terms of separation and sensitivity, where 50 and 10 pg/ μ L of Acephate are easily determined in the complex bird gizzard extract. And, as always, a full mass spectrum is produced with TOFMS.

As mentioned previously, GCxGC promotes a sensitivity enhancement, and this was the case for Acephate in this work. The signal-to-noise ratios on the 136 ions for 50 pg/ μ L of Acephate in gizzard extracts were 30:1 and 500:1 for GC-TOFMS and GCxGC-TOFMS, respectively.

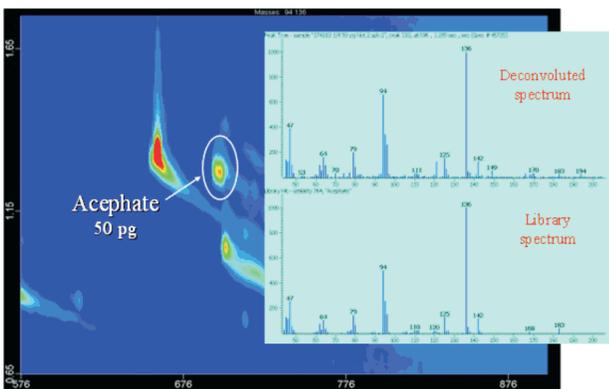


Figure 7. Contour plot of 50 pg/ μ L of Acephate in a bird gizzard extract. Only ions 94 and 136, representative of Acephate, are plotted here. The deconvoluted spectrum matches well with the library spectrum.

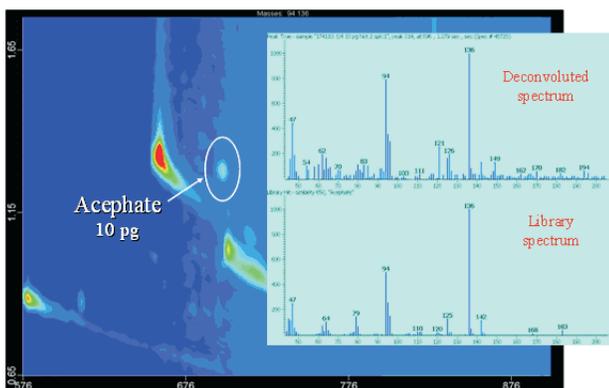


Figure 8. Contour plot of 10 pg/ μ L of Acephate in a bird gizzard extract. Only ions 94 and 136, from Acephate, are plotted here. The deconvoluted spectrum, while containing some ions that can be attributed to the bird gizzard matrix, is representative of a library spectrum for Acephate.

4. Conclusions

GC-TOFMS and GCxGC-TOFMS are both powerful techniques for determining pesticides in a matrix due to automated peak find and spectral deconvolution, and because of the peak capacity increase afforded with GCxGC. GCxGC-TOFMS produced better spectra for Acephate in the complex matrix, and showed a sensitivity enhancement. Neither Acephate nor any other pesticide was located in the bird gizzard extracts. Searching for all pesticides that can be analyzed by GC in complex samples, at low pg levels, is only possible in one analysis with the full mass range acquisition power of TOFMS.

5. Acknowledgment

Douglas Hite at the Tennessee Department of Agriculture in Nashville, Tennessee provided the bird gizzard extracts.

