Determination of Ethephon in an Apple Extract Using GCxGC-TOFMS

LECO Corporation; Saint Joseph, Michigan USA

Key Words: GCxGC-TOFMS, Quantification, Food and Flavors

1. Introduction

Ethephon, or 2-chloroethylphosphonic acid, is a plant growth regulator used for the accelerated ripening of fruits and vegetables. Its residues are monitored in food, but due to its two hydroxyl groups it must be derivatized prior to gas chromatography analysis. To increase selectivity and sensitivity for Ethephon in fruit and vegetable extracts, Selected Ion Monitoring (SIM) is often used with Gas Chromatography—Quadrupole Mass Spectrometry (GC-QMS). Even then, a GC-QMS may not meet the sensitivity and selectivity needs for the most complex samples.

Comprehensive two-dimensional GC with Time-of-Flight Mass Spectrometry (GCxGC-TOFMS) is one way to increase selectivity for compounds like Ethephon in complex samples. GCxGC increases peak capacity by applying two independent separations to a sample in one analysis. When GCxGC is combined with TOFMS, where a full mass spectrum is always acquired, selectivity can be very high. In addition, sensitivity is increased with GCxGC because thermal modulation close to the detector dramatically sharpens (and increases the peak height of) chromatographic peaks. In GCxGC, the peak widths are on the order of 100 to 300 ms, and TOFMS is the only MS capable of defining these peaks due to its acquisition rates of up to 500 spectra/second.

This application note compares GC-QMS with SIM and GCxGC-TOFMS for the analysis of derivatized Ethephon in an apple extract. Selectivity of both methods is discussed and approximate detection limits are given.

2. Experimental Conditions

Sample Extraction—Apples

Apple samples were extracted by the Minnesota Department of Agriculture as follows.

- Freeze dry apple with liquid nitrogen
- Grind finely
- Weigh 10 g sample in 50 mL PTFE centrifuge tube
- Adjust sample pH to 1.5 with 1N HCl
- Vortex sample for 1 min with 1% HCl in methanol
- Centrifuge for 5 min at 3000 rpm
- Transfer 4.5 mL extract to another 50 mL PTFE centrifuge tube containing 35 mL ethyl acetate
- Shake by hand for 1 min
- Centrifuge for 1 min at 3000 rpm
- Pipette and discard bottom layer
- Rotary evaporate ethyl acetate at 50°C
- Add 3 x 5 mL acetone to remove water and evaporate to dryness after each addition
- Finish with 1 mL methanol addition

Sample Extraction—Ethephon Methylation

Ethephon in the apple extract was methylated as follows.

- Vortex flask contents (1 mL methanol) from extraction
- Transfer extract to 15 mL polypropylene centrifuge tube containing 4 mL MTBE
- Add 50 µL 2M trimethylsilyldiazomethane (TMSD), cap tightly
- Place tube in 50°C water bath for 1 hour
- Remove from bath; allow cooling to room temperature
- Add 100 µL of 2M acetic acid in methanol
- Centrifuge for 1 min at 3000 rpm
- Transfer extract to 50 mL evaporation flask
- Rotary evaporate at 50°C
- Add 3 x 5 mL acetone to remove water and evaporate to dryness after each addition
- Finish with 1 mL ethyl acetate addition
- Transfer extract to concentration tube
- Adjust final volume to 1 mL ethyl acetate

Instrumentation

GC-QMS

Column:
15 m x 0.25 mm x 0.25 µm SUPELCOWAX 10 (Supelco)

Carrier:
Helium at 1 mL/minute, constant flow

Injection:
2 µL splitless at 250°C

Oven Program:
56°C (2 minutes), 10°/minute to 180°, 30°/minute to 250° (13 minutes)

MS: Quadrupole

Ionization:	Electron ionization at 70 eV
Source Temp:	280°C
Stored Mass Range:	Selected ion monitoring of 109, 110, 137, 145, 172
Dwell Time:	100 ms

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 µm DB-5 (J&W Scientific)

Column 2:
1 m x 0.10 mm x 0.10 µm Rtx-CLPesticidesII (Restek)

Carrier:
Helium at 1.5 mL/minute, constant flow

Injection:
1 µL splitless at 250°C, valve time 60 seconds

Oven 1 Program:
50°C (1 minute), 10°/minute to 300°

Oven 2 Program:
20°C offset from oven 1

Modulation Time:
2.5 seconds

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

3. Results and Discussion

Figure 1 shows the mass spectrum and structure for underivatized Ethephon. After methylation, important ions in the "Ethephon" mass spectrum (and those chosen for GC-QMS SIM), are 109, 110 (base peak), 137, 145, and 172. Figure 2 is a chromatogram generated from the SIM ions, and not only shows the derivatized Ethephon peak, but also indicates how SIM is often not that selective in a complex sample like an apple extract, especially when only lower m/z ions are available. Based on work done by the Minnesota Department of Agriculture, it was estimated that a detection limit of 70 ppb of Ethephon in an apple could be achieved with GC-QMS SIM.

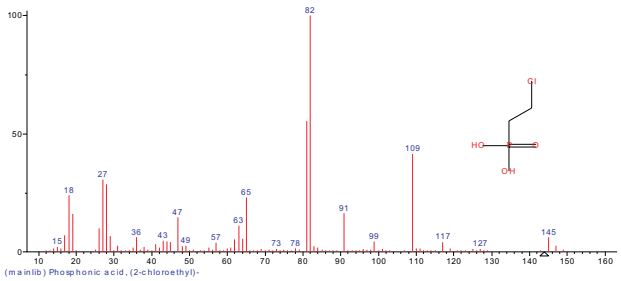

Figure 1. Mass spectrum and structure of Ethephon. Due to its hydroxyl groups, Ethephon must be derivatized prior to GC analysis.

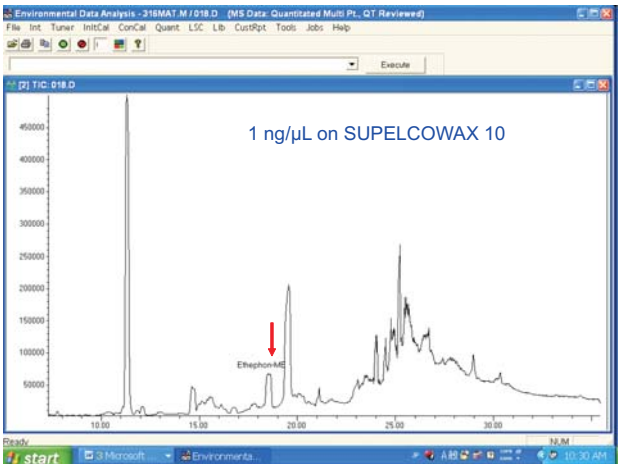

Figure 2. SIM chromatogram on SUPELCOWAX 10 of 1 ng/µL Ethephon (red arrow) in an apple extract.

Figure 3 is a GCxGC-TOFMS contour plot, a way to visualize GCxGC data, of an apple extract. Note how separations are occurring in two dimensions, one along the X-axis (the DB-5) and another along a Y-axis (the Rtx-CLPesticidesII). The white box denotes the area of the contour plot where derivatized Ethephon elutes, and it is this area that is displayed in Figure 4 along with the Ethephon TOF mass spectrum pulled from the apple extract. (NOTE: Hereafter in this application note, "Ethephon" refers to derivatized Ethephon.)

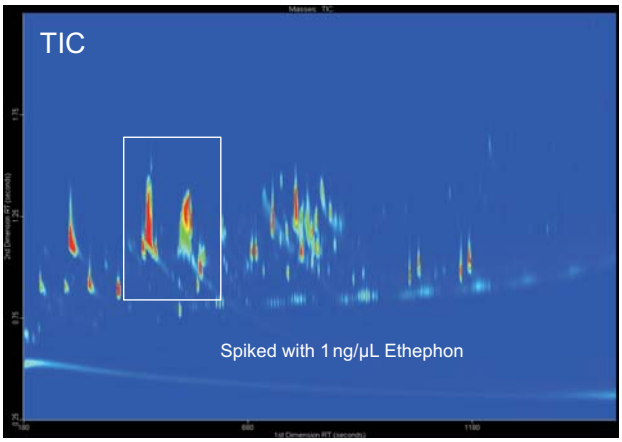

Figure 3. GCxGC-TOFMS contour plot (TIC = total ion chromatogram) of an apple extract spiked with 1 ng/µL Ethephon. Separations occur in two dimensions with the X-axis representing retention times on DB-5, and the Y-axis showing retention times on Rtx-CLPesticidesII. The white box outlines the area where Ethephon elutes.

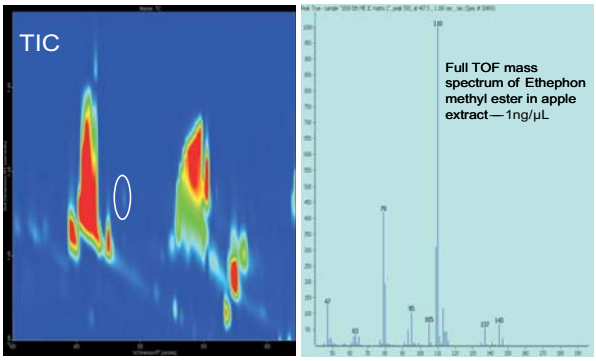

Figure 4. Zoom of Figure 3 GCxGC-TOFMS contour plot where Ethephon elutes in an apple extract. The white oval marks the Ethephon. The TOF mass spectrum for the 1 ng/µL Ethephon is clean and unbiased.

Due to the selectivity and sensitivity of the GCxGC-TOFMS method, it was possible to construct an external standard calibration curve for Ethephon in the apple extract down to 6.25 pg/µL (Figure 5). An extracted ion chromatogram (representing the base slice in a linear chromatogram) for m/z 110, the quantification ion used for Ethephon, at 100 pg/µL in the apple extract is shown in Figure 6, along with its TOF mass spectrum. For 25 pg/µL, the TOF mass spectrum matches up well with a Reference Spectrum for Ethephon (Figure 7). Even 12.5 pg/µL Ethephon in the apple extract had a recognizable mass spectrum (Figure 8).

Based on the good TOF mass spectrum for Ethephon at 12.5 pg/µL in the apple extract, the detection limit can be estimated to be around 5 ppb in an apple, an order of magnitude better than GC-QMS SIM, with a full mass spectrum! A full mass spectrum is also available for all other compounds in the apple extract, including pesticides and other potential contaminants.

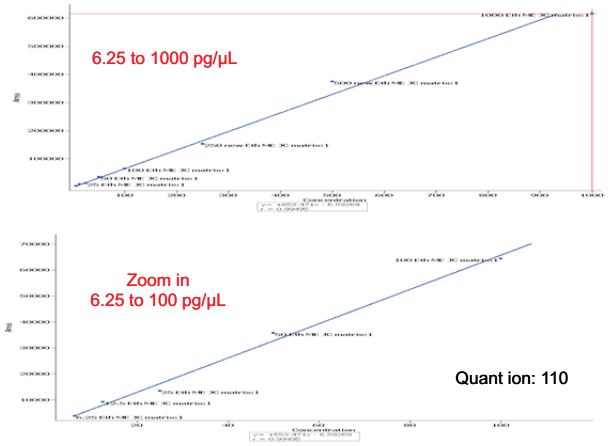

Figure 5. GCxGC-TOFMS calibration curve of Ethephon in an apple extract from 6.25 pg/µL to 1 ng/µL. Linearity is very good even at the low end.

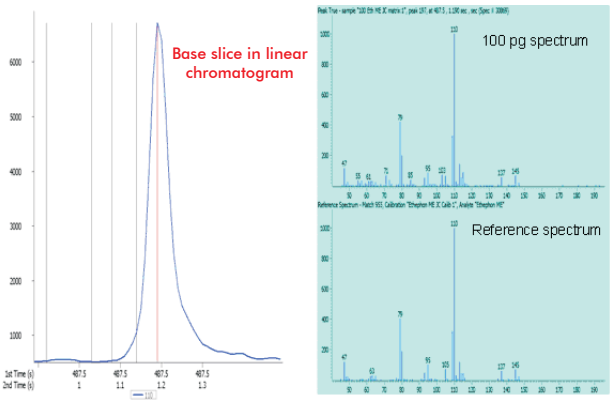

Figure 6. Linear extracted ion chromatogram (m/z 110), base slice, of 100 pg/µL of Ethephon in an apple extract, and its TOF mass spectrum compared to a Reference Spectrum. The chromatographic peak is only 200 ms wide at the base, requiring a fast detector to record it.

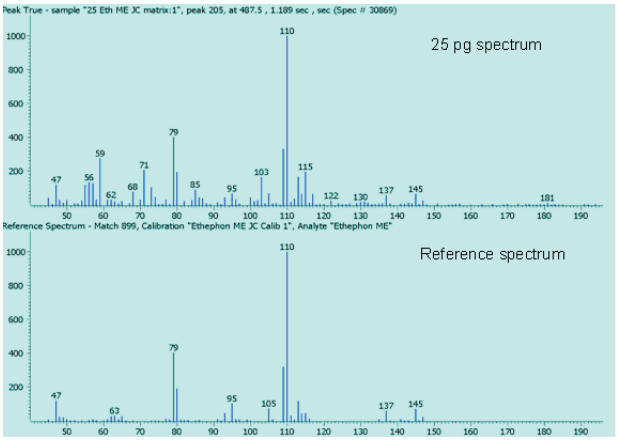

Figure 7. TOF mass spectrum of 25 pg/µL of Ethephon in an apple extract compared to a Reference Spectrum.

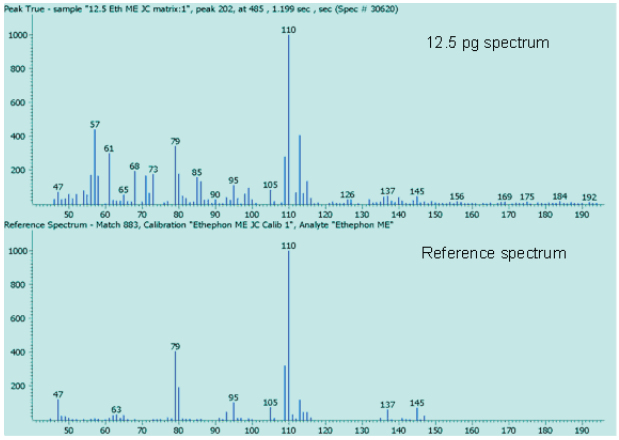

Figure 8. TOF mass spectrum of 12.5 pg/µL of Ethephon in an apple extract compared to a Reference Spectrum.

Delivering the Right Results

4. Conclusions

GC-QMS SIM had approximately a 70 ppb detection limit for Ethephon in an apple while GCxGC-TOFMS with full mass range acquisition had a detection limit of 5 ppb. The high selectivity of GCxGC-TOFMS allowed for good quality mass spectra for the Ethephon derivative even at low levels.

5. References

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6. Acknowledgment

Yoko S. Johnson at the Minnesota Department of Agriculture in St. Paul, Minnesota provided the apple extracts and performed the GC-QMS work.

7. Addendum

The main goal of this work was to compare GC-MS (quadrupole, selected ion monitoring) and GCxGC-TOFMS for the determination of Ethephon methyl ester, the analyte generated under the current Minnesota Department of Agriculture derivatization procedure. The most intense ion in its mass spectrum is 110, a low m/z ion that is easily biased by matrix interferences in food extracts.

Derivatization of Ethephon with (N-methyl-N-[tert-butyldimethylsilyl]trifluoroacetamide), or MTBSTFA, produces strong ions at 245, 287, and 315, and could be a better choice for interference-free determination of Ethephon in food extracts (Figure 9).

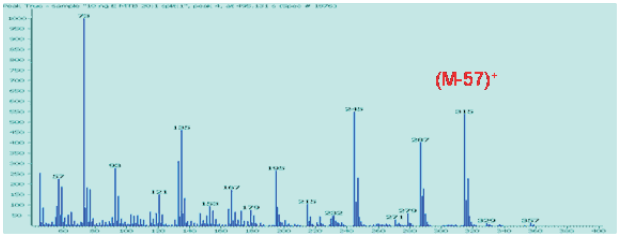

Figure 9. TOF mass spectrum of Ethephon derivative from MTBSTFA.

LECO Corporation • 3000 Lakeview Avenue • St. Joseph, MI 49085 • Phone: 800-292-6141 • Fax: 269-982-8977

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