Quantitation and Confirmation of Organophosphorus Pesticides in Apple Extract in a Single Injection by GC-MS/MS

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Introduction

The analysis of organophosphorus pesticides is routinely performed by injection on a selective GC detector, the Flame Photometric Detector (FPD) or Nitrogen Phosphorus Detector (NPD). Both the FPD and NPD are very sensitive because of their selectivity and show good linearity in the low picogram concentrations. Identification on the FPD or NPD is made by retention time. Then a second injection is made under identical GC conditions on a mass spectrometer in Full Scan for confirmation. When the matrix ions coelute with a target compound, the resulting spectrum is very complex, and the confirmation is not always clearly seen. The signal to noise of the primary ion used for quantitation is adversely affected by these coeluting matrix ions, resulting in poor sensitivity. Alternately, Single Ion Monitoring may be performed to enhance the sensitivity, but this does not provide a spectrum with sufficient ions for library matching.

The Thermo Scientific PolarisQ ion trap mass spectrometer uses a scanning method where a precursor ion is selected from the analyte spectrum. This ion is isolated in the ion trap and all other ions are expelled. Then sufficient energy is applied to the single ion for fragmentation into a unique spectrum of its product ions, generating response in the low picogram levels with a signal to noise or sensitivity similar to that of the FPD or NPD. This type of detection is called tandem MS/MS. The adverse effects of the sample matrix are minimized while generating confirmational data for quantitation. The PolarisQ ion trap GC/MS was used to develop a quantitative method for the analysis of organophosphorus pesticides in apple extract.

Instrument Parameters

**POLARIS Q ION TRAP**

- Source Temperature: 250 °C
- Ionization Mode: +EI, 70 eV
- AGC: 50
- Injection Waveform: 1 volt
- MS/MS Parameters: See Table 1

**TRACE GC Ultra**

- Column: Rtx™ 5 MS 0.25 mm x 30 meter, 0.25 µm
- Oven: 40 °C, 1.0 min; 5 °C/min, 200 °C, 2.0 min; 10 °C/min, 275 °C, 5.0 min
- PTV Inlet: Large Volume Mode
- Liner: 2 mm straight Silcosteel
- Carrier Flow: 1 mL/min helium, constant flow
- Split Flow: 50 mL/min
- Splitless Time: 0.75 min
- Solvent Valve Temperature: 100 °C
- Injection: 40 °C, 0.3 min at 10 psi
- Evaporation Phase: 10 °C/sec, 56 °C, 0.2 min
- Transfer: 10 °C/sec, 250 °C, 2.0 min at 15 psi
- Clean: 14 °C/sec, 275 °C, 38 min

**AUTOSAMPLER**

- Injection volume: 5 µL
MS/MS

The ion trap mass spectrometer is very sensitive and may be run in a tandem EI MS/MS mode. As the analyte enters the ion trap (Figure 1a), a precursor ion is isolated (Figure 1b). Then energy is applied to fragment the ion (Figure 1c) to form a complete spectrum of product ions (Figure 1d). The product spectrum is unique for each pesticide. The sample matrix ions are excluded from the ion trap during the isolation of the precursor ion and do not affect the product spectrum or sensitivity of the analysis.

Objective

A group of 20 organophosphorus pesticides listed in Table 1 was examined by tandem MS/MS to determine the optimum parameters in an apple extract matrix. A calibration curve in the low picogram levels was run. Then replicate injections were made to check the precision of the method. No internal standard was used and all calculations were done by external standardization.

First an injection was made in EI full scan to determine the retention times of each analyte (Figure 2). Then the spectrum of each analyte was reviewed for selection of a precursor ion with an abundant intensity and high mass for greater product ion yield. For terbufos, the typical EI full scan spectrum was reviewed in Figure 3. The second run was set up with defined segments for isolation of the precursor ion for each analyte. For terbufos, the precursor ion was 231 m/z as shown in Figure 4.

Multiple scan events were set up for each MS/MS segment for optimization of the appropriate collision energy for the fragmentation. Figure 5 shows the product ion spectrum for the optimum collision energy for terbufos at 1 volt. You can still see a small amount of 231 m/z. Then the product ion scan range was reduced to maximize the intensity of the product ions for quantitation. For terbufos, this was set at a scan range from 165 to 213 m/z (Figure 6). The MS/MS Total Ion Chromatogram (TIC) for the method is shown in Figure 7.

The injections were made using a temperature programmable injector with an injection volume of 5 µL of apple matrix spiked with the target list in acetone. The linearity was evaluated and replicates made to prove the robustness of the method in matrix. Ion ratios of the top two product ions were tested.
Pesticides in Apple Extract

Figure 7: TIC run of high standard - MS/MS

Figure 8: Terbufos MS/MS spectrum, quan ions and linearity (30,60,150,300 pg)

Figure 9: Diazinon MS/MS spectrum, quan ions and linearity (60,120,300,600 pg)

Figure 10: Fenthion MS/MS spectrum, quan ions and linearity (50,100,250,500 pg)

Figure 11: Methyl chlorpyrifos MS/MS spectrum, quan ions and linearity (25,50,125,250 pg)
Results
The MS/MS parameters for the pesticides are listed in Table 1. The standard was diluted in apple matrix extract for generation of a calibration curve in the low to high picogram ranges.

Some typical curves are shown for terbufos, diazinon, fenthion, and methyl chlorpyrifos. The mass chromatogram of the ions used for quantification for the low point and the product spectrum for each pesticide are shown in Figures 8-11 on page 3.

The precision for replicate injections and calibration curve linearity are shown in Table 2. The ion ratio confirmation data for the product ions was within the needed criterion of ±20 %.

Conclusion
A tandem MS/MS method was developed and validated on the Thermo Scientific PolarisQ ion trap GC/MS for the analysis of organophosphorus pesticides in apple matrix. The method provided good sensitivity at the low picogram range and excellent ion ratio confirmational data of the product ion spectra.

A linear working range was established at levels normally run on a GC specific detector like the NPD or FPD. A single injection generated a report for quantitation and confirmation of the presence of organophosphorus pesticides in apple matrix.

Acknowledgements:
We would like to thank John Meola of the New York State Department of Agriculture for providing the sample extracts.

Authors: Jessie Butler, Carl Feigel

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<tr>
<th>COMPOUND RETENTION TIME</th>
<th>PRECURSOR ION (M/Z)</th>
<th>COLLISION ENERGY (V)</th>
<th>PRODUCT ION SCAN RANGE</th>
<th>QUANTITATIVE ION (M/Z)</th>
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<tr>
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Table 1: MS/MS parameters for pesticides in apple matrix