

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.

Key Words

- PolarisQ ion trap GC/MSn
- GC-MS/MS
- Fruit Extracts
- Food Safety
- Residual Pesticides



Instrument Parameters

POLARISQ 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
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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.

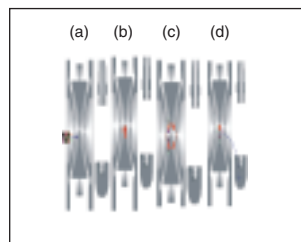


Figure 1: The process of Ion Trap MS/MS

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 μ L 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.

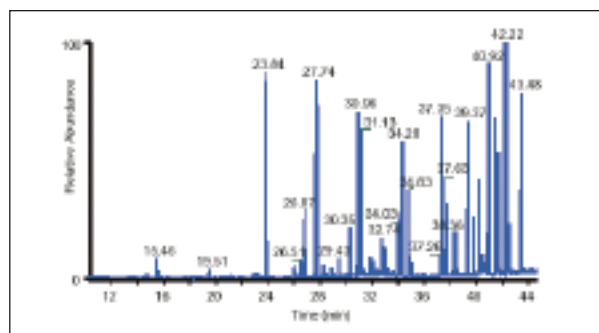


Figure 2: TIC of high standard - EI Full Scan

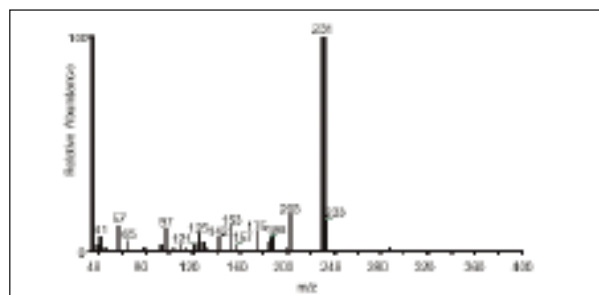


Figure 3: Terbufos EI Full Scan spectrum

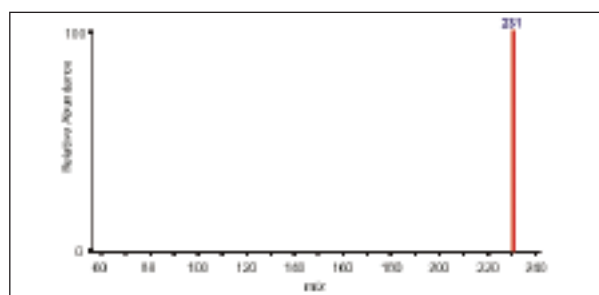


Figure 4: Terbufos precursor ion 231 m/z

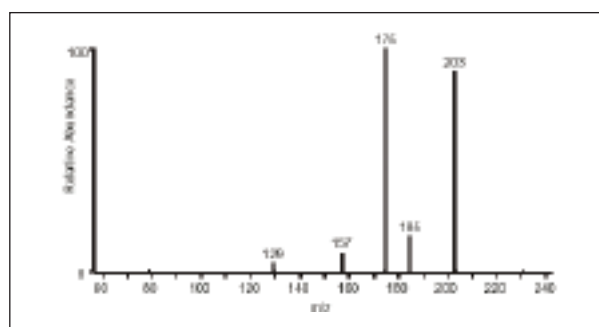


Figure 5: Optimization of collision energy for generation of terbufos product ions

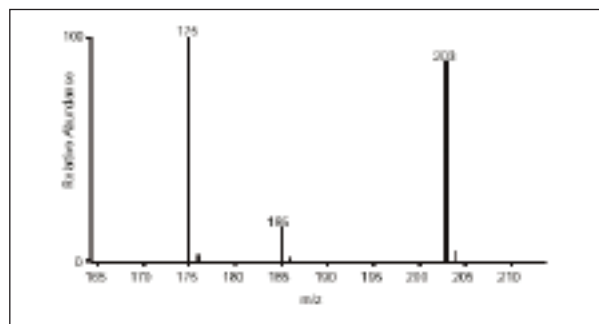


Figure 6: Defining scan range (165 to 213 m/z) for detection of terbufos MS/MS product ions

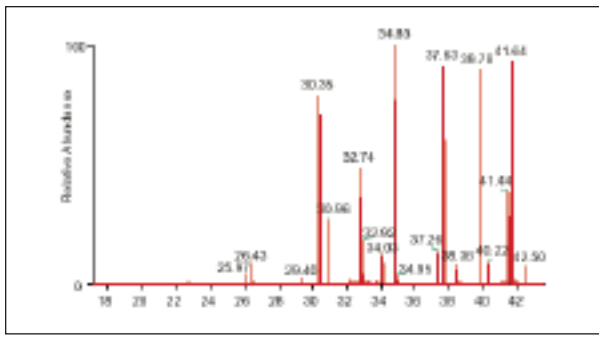


Figure 7: TIC run of high standard - MS/MS

Pesticides in Apple Extract

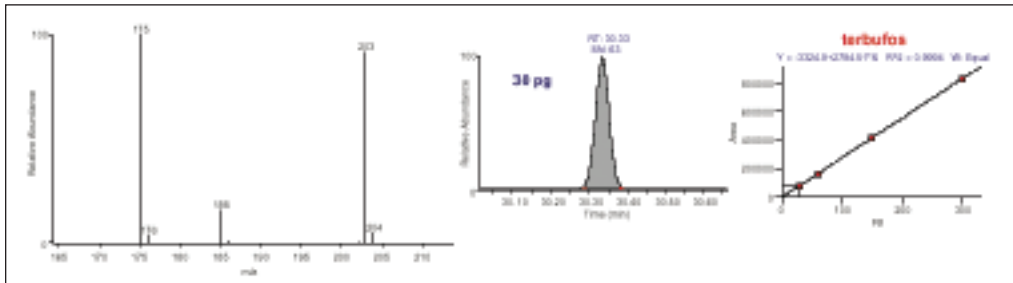


Figure 8: Terbufos MS/MS spectrum, quan ions and linearity (30,60,150,300 pg)

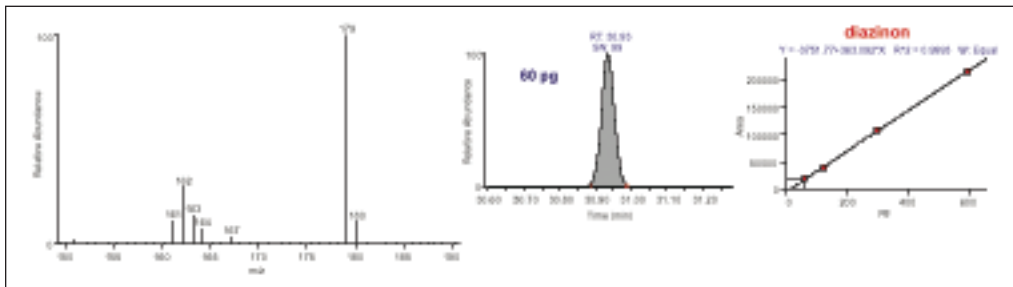


Figure 9: Diazinon MS/MS spectrum, quan ions and linearity (60,120,300,600 pg)

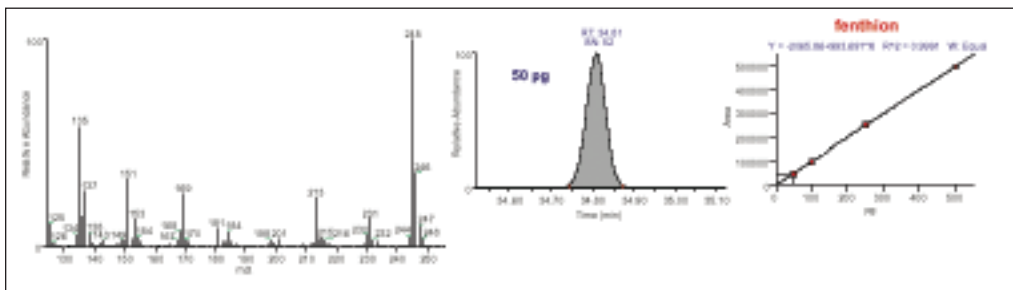


Figure 10: Fenthion MS/MS spectrum, quan ions and linearity (50,100,250,500 pg)

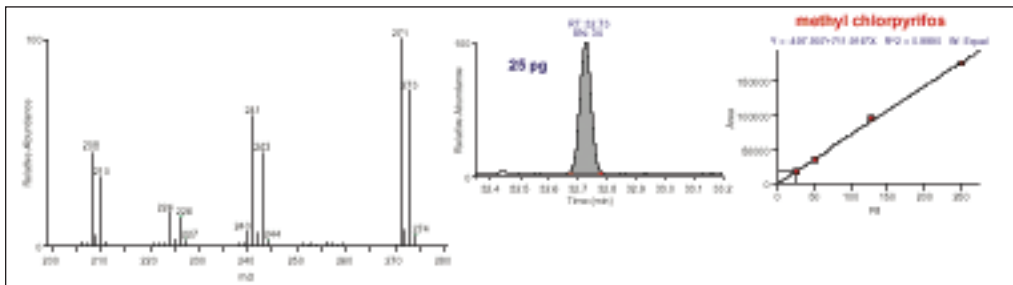


Figure 11: Methyl chlorpyrifos MS/MS spectrum, quan ions and linearity (25,50,125,250 pg)

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 quantitation for the low point and the product spectrum for each pesticide are also 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 $\pm 20\%$.

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.

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Authors: Jessie Butler, Carl Feigel

COMPOUND	RETENTION TIME	PRECURSOR ION (M/Z)	COLLISION ENERGY (V)	PRODUCT ION SCAN RANGE	QUANTITATIVE IONS (M/Z)
methamidophos	18.72	94	1	60-104	64,80
acephate	22.98	136	0.5	35-100	42,94
TEPP	26.11	263	1	200-245	207,235
omethoate	26.58	156	0.5	100-150	110,141
dimethoate	29.43	125	0.5	55-90	62,79
terbufos	30.34	231	1	165-213	175,203
diazinon	30.94	304	0.75	150-190	162,179
methyl chlorpyrifos	32.71	286	1	200-280	210,271
malathion O analog	32.91	127	1	90-110	99,109
pirimiphos	34.02	305	1	170-300	180,290
fenthion	34.8	278	1.5	125-255	135,245
parathion	34.92	291	0.5	132-270	142,263
methidathion	37.26	145	0.5	50-95	58,85
tetrachlorofenvinphos	37.63	331	2.5	165-341	109,316
DEF	38.36	202	0.5	80-160	89,147
ethion	39.7	231	1	170-213	175,203
carbophenothion	40.22	342	1	190-300	199,276
fenamiphos sulfoxide	41.45	304	0.5	186-286	196,276
phosmet	41.65	160	1.5	95-145	105,133
azinphosmethyl	42.49	160	0.5	94-142	104,132

Table 1: MS/MS parameters for pesticides in apple matrix

COMPOUND	AVG. RF	LINEARITY		PRECISION FOR 6 REPLICATE INJECTIONS OF HIGH SPIKE		
		STD. DEV.	%RSD	AVG. AREA CTS.	STD. DEV. - CTS.	% RSD
methamidophos	75	6.1	8	25634	1210	5
acephate	71	5.7	8	31768	3910	12
TEPP	75	11.5	15	35207	3461	10
omethoate	123	8.0	7	80956	7095	9
dimethoate	183	22.6	12	62358	5023	8
terbufos	2237	88.8	4	565996	45090	8
diazinon	259	18.5	7	144968	5099	4
methyl chlorpyrifos	546	33.8	6	116779	5200	4
malathion O analog	391	88.7	13	171177	22477	13
pirimiphos	824	123.2	15	146692	16456	11
fenthion	726	77.7	11	308088	24343	8
parathion	87	12.5	14	25485	2656	10
methidathion	924	104.1	11	294206	31105	11
tetrachlorofenvinphos	239	21.9	9	107317	6256	6
DEF	672	83.9	13	146192	14092	10
ethion	7315	603.4	8	1121116	166329	15
carbophenothion	51	8.1	16	18648	1518	8
fenamiphos sulfoxide	569	90.0	16	347068	67890	20
phosmet	844	181.0	19	921658	66839	7
azinphosmethyl	57	32.9	22	90594	8132	9

Table 2: Linearity and precision of the MS/MS Method

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