Application Note No. 082

Multi-Residue Analysis of Pesticides in Samples of Lettuce and Peas Using Large Volume-Difficult Matrix Introduction-Gas Chromatography-Mass Spectrometry (LV-DMI-GC-MS)

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Introduction

Ethyl acetate is widely used as a solvent for the multi-residue extraction of pesticides from foods because it provides acceptable recovery over a wide range of polarity. Prior to GC analysis of the extracts, some form of clean up (e.g. SPE, GPC) is usually necessary to remove the matrix co-extractives. These cause rapid contamination of the GC system (injector and column), and subsequent deterioration of chromatographic performance. The clean-up techniques increase the sample preparation time, solvent usage, and hence the cost of the analysis.

Difficult matrix introduction (DMI) is a relatively new technique that employs a microvial to hold the sample; this is then placed in a fritted liner and loaded into the Optic injector. Low sample volumes, and solids, can be placed in the microvial and the analytes directly transferred onto the column. A large sample volume may also be introduced and the solvent vented at a low temperature before transfer, as in a large volume injection. The injector is taken to a final temperature, calculated using *Selective Exclusion*, where the target analytes are transferred onto the column but the sample matrix is retained within the glass micro vial. The liner is then exchanged, manually or automatically, for a new liner/microvial containing the next sample. The liner can be re-used but the microvial is disposed of after use. Since contaminants are not able to build up in the system, the need for the clean up of crude extracts, and instrument maintenance, are reduced.

DMI for Lettuce and Pea Extracts

In this work DMI was employed to overcome chromatographic problems experienced with the determination of a range of pesticides [plus an internal standard (IS)] in ethyl acetate extracts of lettuce and fresh-podded peas.

The extracts analysed by DMI were aliquots of the same extracts that had been analysed previously using conventional methods. The conventional method included sample clean up using either HPGPC (lettuce) or SPE (peas), concentration, and measurement using splitless injection (3 µL) and GC-MSD (5973 for lettuce, 5972 for peas) operated in SIM mode. The DMI method was used to analyse the crude sample extracts (lettuce and peas) and the concentrated SPE cleaned-up extracts (peas). Large volume injections were made of the crude extracts (15 µl lettuce, 30 µl peas) and splitless injections of the cleanedup extracts (3 µl peas). Separation was achieved using a 30 m x 0.25 mm i.d. DB5 (film thickness 0.25 µm) capillary column.

All measurements (conventional and DMI) were made using matrix matched calibration standards bracketing the samples, and all calculations based on tetraphenylethylene (TPE) as an internal (syringe) standard.

DMI Instrumentation

- ATAS Optic 2-200 Programmable Injector
- Agilent 5890 GC with 5971 MSD

This analysis can be automated with the Focus-DTD sampler.

DMI Method

1) Extract sample with ethyl acetate
2) Place volume of extract in DMI microvial, place in fritted liner in Optic injector
3) Vent solvent (if large volume used)
4) Close split line & heat Optic to final temperature to transfer target analytes onto the column
5) Analyse target analytes by GC-MS
6) Dispose of microvial containing involatile matrix components

Lettuce results

The HPGPC clean-up step did not remove all of the coextractives in lettuce extracts, resulting in rapid contamination of the GC-MS system and consequent loss of sensitivity and peak shape for all pesticides sought, but was most noticeable for dimethoate as shown in Figure 1. This occurred after only 5-6 conventional (3 µL) splitless injections.

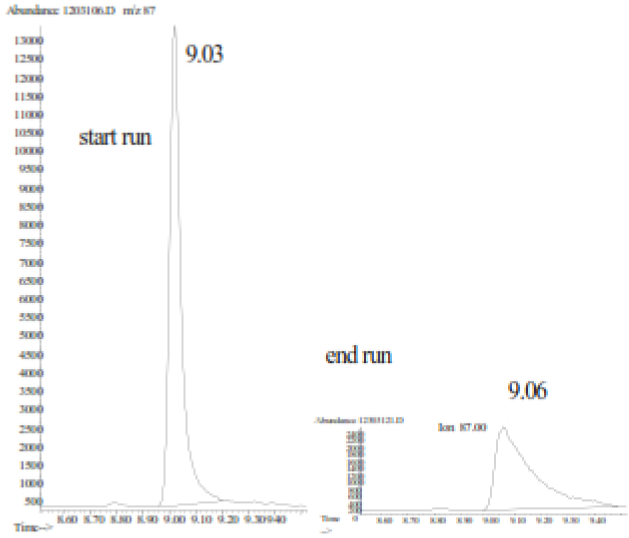


Figure 1: *Dimethoate (1.0 µg/ml) peak in post-HPGPC lettuce extracts (2.5g crop/ml) with 3µl splitless injection, showing deterioration from the start to the end of a series of 16 injections using an MSD 5973*

Even though the DMI method was not optimised the 15 µL large volume injections of the crude extract resolved the difficulties experienced with the conventional analysis. The stability and response for dimethoate were more stable over a similar series of

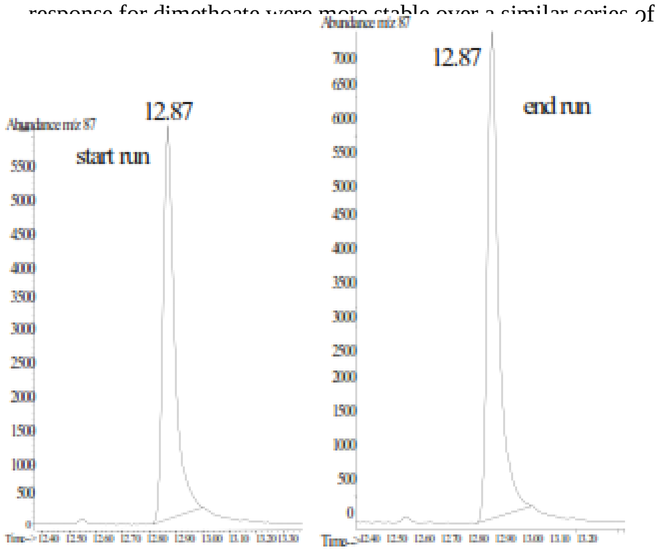
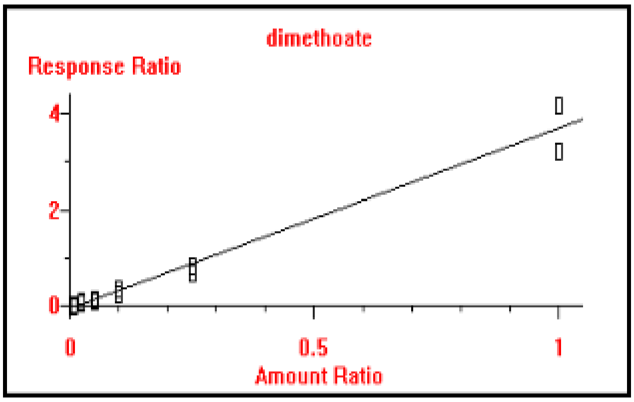


Figure 2: *Dimethoate (1.0 µg/ml) peak in a crude extract (0.5g crop/ml) of lettuce with 15 µl large volume DMI injection showing no deterioration after 10 injections using an MSD 5971*

In addition, good sensitivity and the linearity were achieved for all pesticides including, dimethoate (Figures 3 & 4) and vinclozolin (Figure 5), in crude extracts. The peaks in Figures 4 & 5 are equivalent to 0.02 mg/kg of each pesticide in the crop.



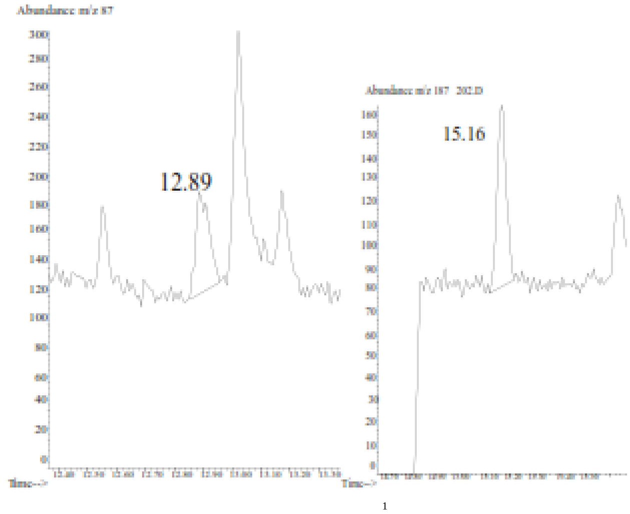


Figure 4: *Dimethoate* ***Figure 5:*** *Vinclozolin*
Both in crude extracts of lettuce (0.5 g crop/ml), with 15 µl large volume DMI injection, at 0.01 µg/ml, equivalent to 0.02 mg/kg

Table 1 summarises the results of the analysis of the crude (non-cleaned-up) lettuce extracts by DMI. With the exception of chlorothalonil the sensitivity, linearity, recovery and repeatability were excellent. The chlorothalonil results may be explained by the fact that it can be unstable in lettuce extracts, these had been stored for several weeks before analysis.

Table 1: Recovery of pesticides from crude extracts of lettuce using DMIGC-MS, spiking level = 0.1 mg/kg, n =7

Pesticide	Mean (%)	%CV
Dimethoate	97	12
Chlorothalonil	129	33
Furalaxyl	100	6
Oxadixyl	91	9
Pyrimethanil	105	4
Vinclozolin	96	7

Pea results

In the case of the SPE cleaned-up extracts (fresh peas) the peak shape of the internal standard, TPE, was inconsistent using conventional splitless injection, as shown in Figure 7. Although, peak splitting did not occur for all fresh pea extracts, (re Figure 7b) the peak shape is still poor compared to the consistent peak shape observed with frozen pea

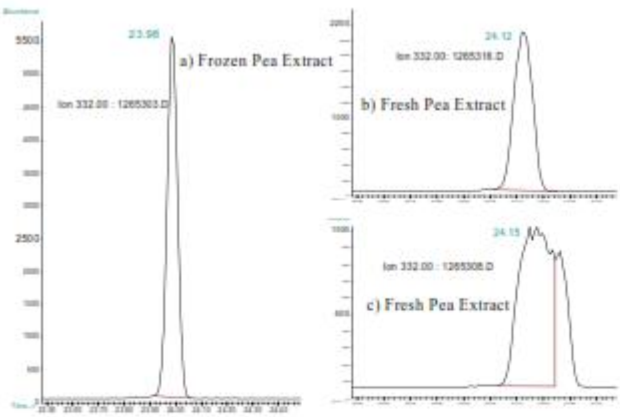
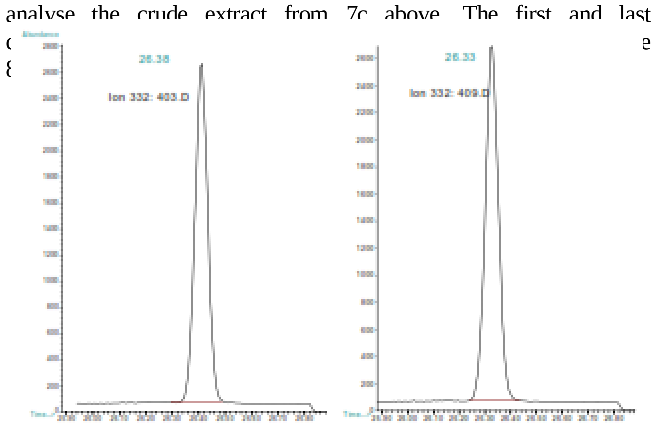


Figure 7: Efect of diferent pea extracts (post SPE) on the internal standard (TPE)

Poor peak shape was not dependent on the build up of contamination from a series of injections but occurred intermittently for individual samples within a sequence. It is possible that an interfering component present in some samples of fresh peas was eliminated during the blanching process used in the production of frozen peas. Also, the response for several pesticides decreased during a series of approximately 20 injections. For mecarbam and triazophos the peak was lost completely. The problem of inconsistent peak shape for TPE was eliminated when DMI (30 µL large volume injection) was used to analyse the crude extract from 7c above. The first and last



As in the case of lettuce, the DMI methods used were not optimised. A summary of the results for the DMI analysis of SPE cleaned-up pea extracts (3 µL splitless DMI injection), and crude pea extracts (30 µL large volume DMI injection) is presented in Tables 2 and 3 respectively. The majority of pesticides, including mecarbam and triazophos, gave good sensitivity, linearity, repeatability and recovery. The relatively high %CVs for the endosulfan was due to the low response of the GC-MSD 5971 at 0.01 ug/ml. Further work is needed to optimise this method and improve sensitivity for deltamethrin.

Table 2: Recovery of pesticides from post-SPE extracts: (5g crop/ml) of fresh peas using a 3 µL splitless DMI injection, pesticide spike levels in the range 0.02-0.05 mg/kg, (n=6)

Pesticide	Mean (%)	%CV	Pesticide	Mean (%)	%CV
Chlorpyrifos	88	4	Iprodione	93	18
Chlorpyrifos-methyl	88	4	Mecarbam	92	4
Lambda-cyhalothrin	96	14	Metalaxyl	103	5
Deltamethrin	-	-	Methidathion	103	5
Diazinon	90	3	Permethrin	79	11
á-Endosulfan	98	16	Pirimiphos-methyl	93	4
â-Endosulfan	99	16	Triazophos	92	7
Endosulfan sulfate	101	4	Vinclozolin	89	5

Table 3: Recovery of pesticides from crude extracts: (0.5g crop/ml) of fresh peas using 30 µL large volume DMI injection, spike levels in the range 0.02-0.05 mg/kg, (n=7)

Pesticide	Mean (%)	%CV	Pesticide	Mean (%)	%CV
Chlorpyrifos	99	8	Iprodione	105	32
Chlorpyrifos-methyl	95	7	Mecarbam	115	7
Lambda-cyhalothrin	109	11	Metalaxyl	80	8
Deltamethrin	-	-	Methidathion	140	9
Diazinon	72	14	Permethrin	93	10
á-Endosulfan	175	39	Pirimiphos-methyl	84	8
â-Endosulfan	89	72	Triazophos	119	9
Endosulfan sulfate	75	51	Vinclozolin	69	6

Conclusions

This preliminary work on the analysis of pesticides in lettuce and peas demonstrates the capability of DMI-GC-MS to reduce the need for time consuming and expensive clean-up techniques, and to improve chromatographic performance by overcoming common matrix effects. In the analysis of the lettuce extracts, DMI reduced the matrix contamination of the GC system, thus maintaining sensitivity and repeatability, especially for dimethoate. Similarly for peas, the problems of poor peak shape for TPE and poor sensitivity for a number of pesticides, particularly mecarbam and triazophos, were eliminated. DMI-GC-MS has the potential to provide rapid, sensitive and cost effective multi-residue analysis of pesticides, and other trace contaminants in food.

Appendix I: Crude Lettuce Conditions

Volume injected:	15 µL

Optic Parameters:

Liner:		Fritted
Microvial:		15 mm
Mode:		Large volume
Gas Flows:	Vent:	100 mL/min
	Split:	50 mL/min
Initial temperature:		75 °C
Vent time:		4 mins
Ramp rate:		8 °C/s
Final temperature:		280 °C
End time:		33.5 mins
Split open time:		2 mins
Purge pressure:		3.5 psi
Transfer pressure:		8.2 psi
Transfer time:		3 mins
Initial pressure:		8.2 psi
Final pressure:		20.8 psi

GC Parameters:

Column: DB5 30m x 0.25 mm i.d. x 0.25 µm film

Initial temperature:	60 °C (hold 3 mins)
Ramp rate 1:	20 °C/min
Final temperature 1:	180 °C (hold 1 min)
Ramp rate 2:	4 °C/min
Final temperature 2:	230 °C (hold 3 mins)
Ramp rate 2:	4 °C/min
Final temperature 2:	230 °C (hold 3 mins)

MS Parameters:

Acquisition mode:		SIM
Transfer line:		280 °C
Solvent delay:		8 mins
Dwell time:		80-100
Ions:	Dimethoate:	87, 93, 125, 229
	Pyrimethanil:	198, 199, 200, 77
	Chlorothalonil:	266, 264, 268, 109
	Vinclozolin:	187, 213, 212, 285
	Furalaxyl:	242, 152, 301, 95
	Oxadixyl:	163, 132, 120, 105
	TPE:	332, 253

Appendix II: Clean Pea Conditions

Volume injected:	3 µL

Optic Parameters:

Liner:		Fritted
Microvial:		15 mm
Mode:		Splitless
Gas Flows:	Split:	50 mL/min
Initial temperature:		75 °C
Ramp rate:		16 °C/s
Final temperature:		280 °C
End time:		36.5 mins
Split open time:		3 mins
Transfer pressure:		13.4 psi
Transfer time:		3 mins
Initial pressure:		8.2 psi
Final pressure:		20.8 psi

GC Parameters:

Column: DB5 30m x 0.25 mm i.d. x 0.25 µm film

Initial temperature:	60 °C (hold 3 mins)
Ramp rate 1:	20 °C/min
Final temperature 1:	180 °C (hold 1 min)
Ramp rate 2:	4 °C/min
Final temperature 2:	230 °C (hold 3 mins)
Ramp rate 2:	4 °C/min
Final temperature 2:	230 °C (hold 6 mins)

MS Parameters:

Acquisition mode:		SIM
Transfer line:		280 °C
Solvent delay:		8 mins
Dwell time:		80-100
Ions:	Diazinon:	179,152, 137
	Chlorpyrifos-Me	286,288, 125
	Vinclozolin:	187,213, 285
	Metalaxyl:	206,192, 249
	Pirimiphos-methyl:	276,290, 305
	Chlorpyrifos:	199,197, 97
	Mecarbam:	131,159, 97
	Methidathion:	145,85, 125
	Endosulfan (I):	195,197, 241
	Endosulfan (II):	195,197, 241
	Triazophos:	161,162, 172
	Endosulfan sulfate:	272,274, 229
	Iprodione:	314,316, 187
	Lambda-cyhalothrin:	181, 197, 208
	Permethrin pk1:	183,163, 165
	Permethrin pk2:	183,163, 165
	Deltamethrin:	253,181, 251
	TPE:	332,253

Appendix III: Crude Pea Conditions

Volume injected: 30 µL

Optic Parameters:

Liner:		Fritted
Microvial:		15 mm
Mode:		Large volume
Gas Flows:	Vent:	100 mL/min
	Split:	50 mL/min
Initial temperature:		75 °C 5.5
Vent time:		mins
Ramp rate:		16 °C/s 280
Final temperature:		°C 36.5
End time:		mins
Split open time:		3 mins
Purge pressure:		3.5 psi
Transfer pressure:		13.4 psi 3
Transfer time:		mins 8.2
Initial pressure:		psi 20.8
Final pressure:		psi

GC & MS Parameters:
Same as Appendix II