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 microvial. 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 cleaned up 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 3: Calibration line for dimethoate (0.01 – 1.0 µg/ml) in crude extracts of lettuce with 15 µl large volume DMI injection

Figure 4: Dimethoate 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

Figure 5: Vinclozolin 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

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Mean (%)</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethoate</td>
<td>97</td>
<td>12</td>
</tr>
<tr>
<td>Chlorothalonil</td>
<td>129</td>
<td>33</td>
</tr>
<tr>
<td>Furalaxyl</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>Oxadixyl</td>
<td>91</td>
<td>9</td>
</tr>
<tr>
<td>Pyrimethanil</td>
<td>105</td>
<td>4</td>
</tr>
<tr>
<td>Vinclozolin</td>
<td>96</td>
<td>7</td>
</tr>
</tbody>
</table>
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 extracts (Figure 7a). 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 and poor sensitivity for a 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 μg/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 30 μL splitless DMI injection, pesticide spike levels in the range 0.02-0.05 mg/kg, (n=6)

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Mean (%)</th>
<th>%CV</th>
<th>Pesticide</th>
<th>Mean (%)</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpyrifos</td>
<td>88</td>
<td>4</td>
<td>Proadine</td>
<td>93</td>
<td>18</td>
</tr>
<tr>
<td>Chlorpyrifos-methyl</td>
<td>88</td>
<td>4</td>
<td>Mecarbam</td>
<td>92</td>
<td>4</td>
</tr>
<tr>
<td>Lambda-cyhalothrin</td>
<td>96</td>
<td>14</td>
<td>Metalaxyl</td>
<td>103</td>
<td>5</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>-</td>
<td>-</td>
<td>Methidathion</td>
<td>103</td>
<td>5</td>
</tr>
<tr>
<td>Diazinon</td>
<td>90</td>
<td>3</td>
<td>Pirimiphos-methyl</td>
<td>93</td>
<td>4</td>
</tr>
<tr>
<td>Endosulfan</td>
<td>98</td>
<td>16</td>
<td>Permethrin</td>
<td>79</td>
<td>11</td>
</tr>
<tr>
<td>Endosulfan sulfate</td>
<td>99</td>
<td>16</td>
<td>Triazophos</td>
<td>92</td>
<td>7</td>
</tr>
<tr>
<td>Triazophos</td>
<td>101</td>
<td>4</td>
<td>Vinclozolin</td>
<td>89</td>
<td>5</td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Mean (%)</th>
<th>%CV</th>
<th>Pesticide</th>
<th>Mean (%)</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpyrifos</td>
<td>99</td>
<td>8</td>
<td>Proadine</td>
<td>105</td>
<td>32</td>
</tr>
<tr>
<td>Chlorpyrifos-methyl</td>
<td>95</td>
<td>7</td>
<td>Mecarbam</td>
<td>115</td>
<td>7</td>
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<tr>
<td>Lambda-cyhalothrin</td>
<td>109</td>
<td>11</td>
<td>Metalaxyl</td>
<td>80</td>
<td>8</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>-</td>
<td>-</td>
<td>Methidathion</td>
<td>140</td>
<td>9</td>
</tr>
<tr>
<td>Diazinon</td>
<td>72</td>
<td>14</td>
<td>Pirimiphos-methyl</td>
<td>84</td>
<td>8</td>
</tr>
<tr>
<td>Endosulfan</td>
<td>175</td>
<td>39</td>
<td>Triazophos</td>
<td>119</td>
<td>9</td>
</tr>
<tr>
<td>Endosulfan sulfate</td>
<td>89</td>
<td>72</td>
<td>Vinclozolin</td>
<td>69</td>
<td>6</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Volume injected:</th>
<th>15 µL</th>
</tr>
</thead>
</table>

#### 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/min
- **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)

#### 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
  - Vinlozinol: 187, 213, 212, 285
  - Furalaxyl: 242, 152, 301, 95
  - Oxadixyl: 163, 132, 120, 105
  - TPE: 332, 253

### Appendix II: Clean Pea Conditions

<table>
<thead>
<tr>
<th>Volume injected:</th>
<th>3 µL</th>
</tr>
</thead>
</table>

#### Optic Parameters:
- **Liner:** Fritted
- **Microvial:** 15 mm
- **Mode:** Splitless
- **Gas Flows:**
  - **Split:** 50 mL/min
- **Initial temperature:** 75 °C
- **Ramp rate:** 16 °C/min
- **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)
- **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
  - Vinlozinol: 187, 213, 285
  - Metanx: 206, 192, 249
  - Pirimiphos-methyl: 276, 290, 305
  - Chlorpyrifos: 199, 197, 97
  - Mecarbam: 131, 139, 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

<table>
<thead>
<tr>
<th>Volume injected:</th>
<th>30 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Optic Parameters:</strong></td>
<td></td>
</tr>
<tr>
<td>Liner:</td>
<td>Fritted</td>
</tr>
<tr>
<td>Microvial:</td>
<td>15 mm</td>
</tr>
<tr>
<td>Mode:</td>
<td>Large volume</td>
</tr>
<tr>
<td>Gas Flows:</td>
<td></td>
</tr>
<tr>
<td>Vent:</td>
<td>100 mL/min</td>
</tr>
<tr>
<td>Split:</td>
<td>50 mL/min</td>
</tr>
<tr>
<td>Initial temperature:</td>
<td>75 °C 5.5</td>
</tr>
<tr>
<td>Vent time:</td>
<td>mins</td>
</tr>
<tr>
<td>Ramp rate:</td>
<td>16 °C/s 280</td>
</tr>
<tr>
<td>Final temperature:</td>
<td>36.5</td>
</tr>
<tr>
<td>End time:</td>
<td>mins</td>
</tr>
<tr>
<td>Split open time:</td>
<td>3 mins</td>
</tr>
<tr>
<td>Purge pressure:</td>
<td>3.5 psi</td>
</tr>
<tr>
<td>Transfer pressure:</td>
<td>13.4 psi 3</td>
</tr>
<tr>
<td>Transfer time:</td>
<td>mins 8.2</td>
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<tr>
<td>Initial pressure:</td>
<td>psi 20.8</td>
</tr>
<tr>
<td>Final pressure:</td>
<td>psi</td>
</tr>
</tbody>
</table>

**GC & MS Parameters:**
Same as Appendix II