Determination of Residues of Pirimicarb and Its Desmethyl and Desmethylformamido Metabolites in Fruits and Vegetables by Liquid Chromatography–Electrospray/Mass Spectrometry

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A method was developed for the simultaneous determination of residues of pirimicarb (I) and its desmethylformamido (II) and desmethyl (III) metabolites in plums, peas, green beans, broad beans, carrots, and swedes. The compounds were extracted with ethyl acetate and determined, without cleanup, by reversed-phase liquid chromatography and electrospray mass spectrometry (MS). MS and MS/MS were used concurrently to monitor the protonated molecules and their common collision-induced dissociation product. The limit of detection (signal-to-noise ratio of >3) was 1 ng/mL, corresponding to crop concentrations of <0.0015 mg/kg. All 3 compounds were determined in plums, broad beans, and green beans by MS without interference. Interferences which affected the determination of desmethylformamido-pirimicarb in peas, and to a lesser extent in carrots and swedes, were eliminated by MS/MS. Recoveries for all 3 compounds, at 0.05 mg/kg for plums and 0.005 mg/kg for other commodities, were in the range 83–124%. No interconversion of I, II, and III, occurred during extraction, and the compounds were stable in extracts for ≥7 days under appropriate conditions.

Pirimicarb is the International Standards Organization (ISO) common name for 2-dimethylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate (I), a widely used, fast-acting, aphid-selective, systemic carbamate insecticide. Degradation products of pirimicarb include 2 compounds in which the carbamate moiety is retained (1–3), and these are included in the residue definition associated with Codex Alimentarius maximum residue limits (MRLs) (4). MRLs are, thus, for the sum of the concentrations of pirimicarb and these 2 compounds, which are 2-[(methylformyl)amino]-5,6-dimethylpyrimidin-4-yl dimethylcarbamate (II; desmethylformamido-pirimicarb) and 2-methylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate (III; desmethyl-pirimicarb). The structures of I, II, and III are shown in Figure 1.

Recent research in our laboratory created a need for a robust and sensitive method for the separate determination of I, II, and III in a number of different crops. Residues of I and III can be determined readily by common generic or multiresidue extraction methods followed by gas chromatography (GC) (3, 5), but determination of II by GC requires its conversion to III, usually under acidic conditions (3, 6). Consequently II and III cannot be distinguished. I, II, and III can be separated by reversed-phase liquid chromatography (LC) (7), and LC with ultraviolet detection has been used to study degradation following the application of pirimicarb to lettuces (8), and to peaches and nectarines (9).

The post-column reaction and fluorescence detection method widely used with LC for the sensitive and highly selective determination of N-methylcarbamates (10–12) is not applicable to N,N-dimethylcarbamates, but a method using the inherent fluorescence of I and III for their determination in chicory, corn, and red beet, with a limit of determination of 0.002 mg/kg, has been reported (13).

The availability of robust and efficient atmospheric pressure ionization interfaces for LC–mass spectrometry (MS) has provided a powerful and versatile tool for sensitive and selective residues analysis. Previously we reported the use of atmospheric pressure chemical ionization (APCI)/MS for the determination of I, together with a number of other pesticides, in strawberries and plums (14). Pesticides were ex-Guest edited as a special report on “Chromatographic Pesticide Residue Analysis” by Antonio Valverde.
tracted with ethyl acetate and, without cleanup, determined by LC–MS, which afforded a limit of detection for I of 0.002 mg/kg. Electrospray (ES)/MS has also been shown to allow sensitive detection of a number of carbamate insecticides, including I, following extraction with methanol, cleanup with a graphitized carbon cartridge, and concentration of the extract (15).

We report here a simple, robust, and highly effective procedure for the determination of I, II, and III in plums, peas, green beans, broad beans, carrots, and swedes, using an ethyl acetate extraction and determination by LC–ES/MS or LC–ES/MS/MS, without cleanup.

Experimental

Materials and Reagents

(a) Solvent and reagents.—LC-grade ethyl acetate, acetonitrile, and water, and analytical reagent-grade ammonium acetate, acetic acid, sodium hydrogen carbonate, and anhydrous sodium sulfate were obtained from Fisher Scientific (Loughborough, UK).

(b) Reference standard materials.—A stock solution of I (Qmx Laboratories, Saffron Walden, UK) was prepared at 1 mg/mL in ethyl acetate. A working solution at 2 µg/mL was prepared by sequential dilution with acetonitrile. II and III were obtained as 10 µg/mL solutions in toluene (Qmx Laboratories). To prepare working solutions at 2 µg/mL, a aliquot was decreased to 0.05–0.1 mL by evaporation in a nitrogen stream. To remove the remaining toluene, 0.2 mL acetonitrile was added, and the solution was again evaporated to a small volume. This step was repeated twice, and finally the small volume was diluted to 10 mL with acetonitrile.

(c) Fruit and vegetable test samples.—Plums, peas, green (French) beans, broad beans, carrots, and swedes for use as analytical blanks, in recovery determinations, and for preparation of matrix-matched calibration solutions were organically produced and were shown to contain no detectable residues of I, II, or III.

(d) LC mobile phase components.—(A) 0.05M ammonium acetate containing 0.1%, v/v, acetic acid and (B) acetonitrile containing 0.1%, v/v, acetic acid.

Apparatus

(a) Sample mill.—Stephan (Hameln, Germany) UM12.

(b) Homogenizer.—Ultra-Turrax (Janke & Kunkel, Staufen, Germany) TK-2.

(c) Syringe filter.—HPLC Technology (Macclesfield, Cheshire, UK) Type PRO-6154 25 mm dia. nylon-membrane syringe filter of 0.45 µm pore size.

(d) LC–MS system.—Micromass (Manchester, UK) Quattro I mass spectrometer fitted with an ES interface, a Waters (Watford, UK) 600 solvent delivery system, and a Gilson 231/401 autosampler (Anachem, Luton, UK) with a 10 µL injection loop. 

Sample Processing

Plums, peas and beans.—Plums were halved, and the stones were removed. A minimum of 10 fruits or 1 kg was taken for processing. Fruits or vegetables were sealed in polyethylene bags and frozen at −18 ± 2°C, overnight. Without thawing, dry ice, about 10% by weight, was added to the sample, which was then comminuted in a mill until finely divided. If the dry ice appeared likely to dissipate completely during this procedure, further small quantities were added. Before extraction, the remaining dry ice was allowed to dissipate while the sample material was kept, in unsealed polyethylene bags, at −18 ± 2°C for a minimum of 16 h. The bags were sealed before further storage.

Carrots and swedes.—Whole vegetables were comminuted in a mill at room temperature to give a finely divided, homogeneous, friable product.

Extraction

A 40 g (plums) or 30 g (other commodities) portion of milled sample material was placed in a 250 mL borosilicate glass stoppered flask, and 80 mL of the aqueous portion of the mobile phase was added. The stopper was removed, and the mixture was agitated vigorously overnight. Excess dry ice was removed by evaporation with a nitrogen stream, and the remaining dry ice was allowed to dissipate while the sample material was kept, in unsealed polyethylene bags, at −18 ± 2°C for a minimum of 16 h. The bags were sealed before further storage.

(e) LC column.—A 150 × 2.1 mm Shandon Hypersil 5 µm Carbamate column (Anachem, Luton, UK), protected by a 10 × 2 mm Techsphere 5 µm C18 guard column (HPLC Technology.)

Figure 2. ES mass spectra of (from top to bottom) pirimicarb (I; M = 238), desmethyformamidopirimicarb (II; M = 252), and desmethy-pirimicarb (III; M = 224).
glass bottle. For recovery determinations, working standard solution was added to give a concentration of each analyte of 0.05 mg/kg for plums and 0.005 mg/kg for other sample types. While the bottle was swirled, 5 g sodium hydrogen carbonate and 35 g anhydrous sodium sulfate were added slowly, followed by 60 mL ethyl acetate. The mixture was warmed to 30°C in a water bath for 20 min, and then homogenized for 0.5 min at 10,000–12,000 rpm. After the solids were allowed to settle, the supernatant was decanted. A 1 mL aliquot was decreased to 0.05–0.1 mL by evaporation in a nitrogen stream. Then, to remove the remaining ethyl acetate, three 100 µL portions of acetonitrile were added, with evaporation to a small volume after each addition. Finally 0.7 mL water was added, and the extract was diluted to 1 mL with acetonitrile. Before analysis, the extract was passed through a syringe filter.

Preparation of Calibration Solutions

For use in our normal application of the method, matrix-matched calibration solutions with concentrations of I, II, and III of 0.01–0.5 µg/mL (equivalent to 0.015–0.75 mg/kg) for plums, and 0.0025–0.5 µg/mL (equivalent to 0.005–1 mg/kg) for the other commodities, were prepared by using pooled extracts of blank sample material. For use in some experiments, solutions with a wider range of concentrations were also prepared, using the same procedures. To prepare the 0.5 µg/mL calibration solution, a 1 mL aliquot of the blank ethyl acetate extract was solvent exchanged to water–acetonitrile, as described above, but with the incorporation of 0.25 mL working standard solution. To provide lower concentrations, aliquots (0.05–0.25 mL) of the 0.5 µg/mL solution were further diluted to 1 mL with blank extract that had been solvent exchanged to water–acetonitrile, and further sequential dilutions were also made in a similar manner. Solvent-based calibration solutions were prepared by serial dilution of stock solutions with water–acetonitrile (70+30, v/v).

LC–MS

The mobile phase was degassed continuously by helium sparging. The flow rate was 0.2 mL/min. Filled-loop injections (10 µL) were made. After injection, the solvent composition was changed linearly from 20 to 90% B over 9 min, held at 90% B for 4 min, and then returned to initial conditions over 1 min. A 9 min column equilibration period was allowed before the subsequent injection, so that the total time for each determination, including column equilibration, was 23 min.

The mass spectrometer was operated in the positive ion ES mode without splitting the solvent flow from the column. The source temperature was 120°C, the nebulizer gas flow was 20 L/h, and the bath gas flow was 400 L/h. After initial
tuning using background ions, instrument operating conditions were optimized while repeated injections of standards (10 ng injected) were made with the LC column removed. Typical settings of capillary and HV lens voltages were 3 and 0.3 kV, respectively. After initial optimization, the sampling-cone potential used was 25 V. Collision-induced dissociation (CID) for MS/MS used argon as the collision gas at $1 \times 10^{-3}$ mbar pressure and a collision energy of 30 eV.

For initial recording of complete mass spectra, MS-1 was scanned from m/z 50 to 600 with a scan time of 1.5 s. CID product ion spectra were recorded by selecting the protonated molecules at m/z 239 (I), m/z 253 (II), or m/z 225 (III) with MS-1, and scanning MS-2 from m/z 50 to 300 with a scan time of 1 s. To identify and quantify residues of I, II, and III, MS and MS/MS were employed concurrently, using selected-ion monitoring (SIM) at the detector of MS-1, and selected-reaction monitoring (SRM), respectively, for the protonated molecules and the CID transitions of each of these precursors to the common product ion at m/z 72. The dwell time for each of the 6 channels was 0.1 s, the interchannel delay was 0.02 s, and the mass span was 0.2.

Concentrations of I, II, and III were measured by using matrix-matched calibrants covering the range 0.01–0.5 µg/mL (5 concentrations) for plums, whereas for other sample types the calibration range was 0.0025–0.5 µg/mL (6 concentrations). Calibration solutions were injected both before and after each batch of sample extracts (typically consisting of 10 extracts). In addition, a solution containing all 3 compounds (0.05 µg/mL) in solvent only was injected at intervals during each batch, to monitor possible drift in MS response.

**Results and Discussion**

ES spectra obtained from flow injections of I, II, or III (10 ng) in water–acetonitrile were dominated by [M+H]$^+$ with some higher-mass ions evident, e.g., [M+Na]$^+$, [M+H+MeCN]$^+$, and [M+Na+MeCN]$^+$ (Figure 2). Because the MS/MS capability of a triple quadrupole instrument was available, we did not seek to induce fragmentation by increasing the sampling-cone voltage. It is noteworthy that a cone voltage setting of 25 V on our instrument did not produce the fragment at m/z 182 that Di Corcia et al. (15) observed from I at a voltage as low as 20 V. However, this fragment was observed in the product ion spectrum (Figure 3) produced by CID of [M+H]$^+$ (collision energy, 30 eV), and an analogous ion was present in the product ion spectrum of III (m/z 168). The main product ion from CID of all 3 compounds was at m/z 72, which we attribute to the N,N-dimethylisocyanate cation, [(CH$_3$)$_2$NCO]$^+$. Following these
initial investigations, detection and quantification were based on concurrent SIM and SRM as described above. As demonstrated by Cabras et al. (7), separation of pirimicarb and its metabolites can be achieved on standard reversed-phase LC columns, although the conditions used by these workers involved a phosphate buffer that is not compatible with LC–ES/MS on our instrument. We employed a Hypersil Carbamate column, used routinely by us for a variety of carbamate pesticide separations and found to couple selectivity with robust long-term performance. Symmetrical peak shapes were obtained with little evidence of peak tailing (Figure 4). Retention times for I, II, and III were about 12, 10.5, and 10 min, respectively.

Under the conditions described, a mixed solution of I, II, and III in water–acetonitrile (70 + 30, v/v) gave detectable responses (unsmoothed signal-to-noise ratio [S/N] of ≥2) by both SIM (MS) and SRM (MS/MS) for each compound at injected concentrations as low as 0.5 ng/mL. Although the peak areas produced by MS/MS were 10- to 20-fold smaller than by MS, baseline drift due to chemical noise was evident in SIM chromatograms obtained from solutions in water–acetonitrile but not in those for SRM, and the S/N performance for the 2 modes was comparable.

SIM chromatograms for the [M+H]+ ions from extracts of untreated plums, broad beans, and green beans were free of interferences at the retention times of the analytes and contained few extraneous peaks. Extracts of plums spiked with I, II, or III at 1 ng/mL (equivalent to a crop concentration of 0.0015 mg/kg) and extracts of broad beans and green beans spiked with I, II, or III at 0.5 ng/mL (0.001 mg/kg) gave S/N performance similar to that of solutions of similar concentra-

Figure 6. MS/MS (SRM) chromatograms from analysis of an extract of vining peas. The left-hand column contains chromatograms from an unspiked blank extract, and the right-hand column contains chromatograms from an extract spiked with I, II, and III at the equivalent of 0.0005 mg/kg. Traces are (from top to bottom) m/z 239→72, m/z 253→72, and m/z 225→72.

Figure 7. Calibration curve for ES determination of pirimicarb by LC–MS (SIM of m/z 239). The equation for the fitted line is \( y = -12.769x^2 + 32.518x \), giving a coefficient of determination, \( r^2 \), of 1.0.
tions in solvent only. However, from extracts of untreated vining peas there was a peak in the SIM chromatogram for m/z 253 (Figure 5) with a retention time about 6 s shorter than that of II, constituting a potential interference and giving a response equivalent to 0.001 mg/kg. The interference was eliminated by MS/MS (Figure 6). Peaks interfering with the determination of II at concentrations of ≤0.001 mg/kg were also observed in chromatograms obtained from extracts of edible-podded peas, swedes, and carrots, but they were eliminated by MS/MS.

The short-term precision of the LC–MS determination was established from 10 injections of a single extract of plums spiked with all 3 compounds at a concentration of 0.1 µg/mL. Peak areas for I, II, and III each had relative standard deviations (RSDs) of about 5% for MS and 10% for MS/MS detection. Solvent-based check solutions were injected at intervals (n = 6) within each LC–MS run used to generate the method validation data for each matrix, and these had similar RSDs.

In accord with Euporean Union guidelines for pesticide residue monitoring (16), matrix-matched calibration solutions are used routinely in our laboratory. However, during development of this method, matrix-matched calibration and solvent-based calibration were compared for several sample types (carrots, swedes, vining peas, and broad beans), keeping the solutions paired but injecting in random concentration order. The resulting calibration curves were essentially superimposable, showing that neither suppression nor enhancement of response occurred in the presence of matrix coextractives.

The relationship between peak area and concentration over the range 0.001–0.1 µg/mL, when either matrix-matched or solvent-based standards were used, was linear (correlation coefficient, r, >0.999). Calibration routinely covered the range 0.01–0.5 µg/mL. For concentrations of >0.1 µg/mL the relationship was found to be slightly curved, with the slope decreasing at the higher concentrations (Figure 7); the data were fitted with a quadratic equation.

The accuracy and precision of the complete method for plums were assessed separately for I, II, and III by analysis of test batches of 7 replicate samples, each spiked before extraction with I of the analytes at 0.05 mg/kg (equivalent to 0.033 µg/mL for 100% recovery). For other commodities, I, II, and III were added simultaneously at 0.005 mg/kg (0.0025 µg/mL). Recovery data are given in Table 1. As noted above, in the analysis of vining peas, an interferent in the blank contributed significantly to the response obtained for II by MS. The contribution made by interferences from other commodities was small or negligible at the spiking level employed.

To establish whether further oxidation of I to II, or conversion of II to III, could occur during extraction and analysis, recovery experiments with plums were performed separately for each analyte. In every case, only the compound that had been added before extraction was detected, demonstrating that no measurable degradation occurred by those routes. In addition, some of the extracts were kept for 7 d, at either 4°C in the dark or at room temperature without exclusion of light, and reanalyzed with freshly prepared matrix-matched calibrants. Although extracts containing II were found to contain traces of III after storage under ambient conditions, no such changes were observed after storage in the dark at 4°C, and measured concentrations of II were in excellent agreement with those obtained initially.

The method was applied to the analysis of over 300 plum samples, analyzed in 21 batches over a period of several weeks. The method was found to be robust and the LC–MS system, capable of sustained high throughput. No deterioration of the chromatographic peak shape was observed, and it was not necessary to replace the LC precolumn. Three sets of 5-point calibration data were obtained during each run, and these showed excellent agreement. Each batch included at least 1 recovery determination for each analyte at 0.05 mg/kg, and recoveries were between 91 and 97% with RSDs <10%.

Table 1. Recovery* of pirimicarb (I), desmethyIformamido-pirimicarb (II), and desmethyI-pirimicarb (III)

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Concentration, mg/kg</th>
<th>I MS</th>
<th>MS/MS</th>
<th>II MS</th>
<th>MS/MS</th>
<th>III MS</th>
<th>MS/MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plums</td>
<td>0.05</td>
<td>124 (9)</td>
<td>124 (9)</td>
<td>110 (6)</td>
<td>110 (5)</td>
<td>105 (9)</td>
<td>103 (8)</td>
</tr>
<tr>
<td>Broad beans</td>
<td>0.005</td>
<td>87 (5)</td>
<td>89 (6)</td>
<td>95 (12)</td>
<td>85 (5)</td>
<td>85 (13)</td>
<td>91 (6)</td>
</tr>
<tr>
<td>Green beans</td>
<td>0.005</td>
<td>113 (6)</td>
<td>115 (7)</td>
<td>102 (14)</td>
<td>109 (8)</td>
<td>115 (10)</td>
<td>106 (14)</td>
</tr>
<tr>
<td>Edible-podded peas</td>
<td>0.005</td>
<td>89 (3)</td>
<td>88 (8)</td>
<td>95 (11)</td>
<td>86 (7)</td>
<td>84 (4)</td>
<td>83 (9)</td>
</tr>
<tr>
<td>Vining peas</td>
<td>0.005</td>
<td>96 (6)</td>
<td>99 (6)</td>
<td>111b (18)</td>
<td>98 (6)</td>
<td>89 (19)</td>
<td>89 (17)</td>
</tr>
<tr>
<td>Swedes</td>
<td>0.005</td>
<td>98 (6)</td>
<td>93 (6)</td>
<td>110 (10)</td>
<td>93 (8)</td>
<td>90 (6)</td>
<td>84 (10)</td>
</tr>
<tr>
<td>Carrots</td>
<td>0.005</td>
<td>96 (5)</td>
<td>102 (7)</td>
<td>90 (14)</td>
<td>109 (8)</td>
<td>104 (13)</td>
<td>93 (8)</td>
</tr>
</tbody>
</table>

* Percentage relative standard deviations (n = 7) are given in parentheses.

b Significant interference identified.
Acknowledgments

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References