High-Performance Liquid Chromatography Multiresidue Method for the Determination of N-Methyl Carbamates in Fruit and Vegetable Juices

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ABSTRACT

A rapid multiresidue method has been developed for the analysis of N-methylcarbamate insecticides (oxamyl, methomyl, propoxur, carbofuran, carbaryl, and methiocarb) in fruit and vegetable juices. The method is based on the adsorption of the N-methyl carbamates in Florisil and the subsequent extraction of pesticides using a low volume of acetone. Residue levels in juice were determined by reversed-phase high-performance liquid chromatography with fluorescence detection after postcolumn derivatization. The separation of carbamates is performed on a C8 column with water-methanol as mobile phase. Recovery studies were performed at 500-, 100-, and 10-ng/ml fortification levels, and average recoveries obtained for carbamates ranged from 79 to 109%, with relative standard deviations between 1.4 and 9.9%. The method was found to be linear over the range assayed from 10 to 1,000 ng/ml, and the detection limits for carbamates varied from 0.8 to 1.9 ng/ml.

N-methylcarbamates are an important class of insecticides widely used in plant treatments due to their high efficacy in protecting crops from pests. As a consequence, their residues may be found in crops, where these compounds persist during a certain time according to their properties and the environmental conditions. Residues left after pesticide treatment may penetrate plant tissues and can be found in the pulp and juice of fruit and vegetables, although their concentrations are, in general, lower than those observed in whole fruit (3, 4, 23). These residues may constitute a hazard for consumers and, therefore, they are regulated by international organizations, which determine the maximum residue limits allowed in food. The presence of pesticide residues in food is a concern for consumers due to their possible long adverse health effects. This is especially important for children, not only because they are consuming a higher proportion of processed fruits and vegetables in relation to their body weight but also because they are more susceptible to chemicals during their development.

Different methods have been published for the analysis of carbamate residues in fruits and vegetables (5, 6, 9, 10), although very few have been reported for the analysis of these compounds in juices (13–16). Direct gas chromatographic determination of carbamate pesticides is difficult, due to the thermal instability of these compounds, which have a tendency to break down under common gas chromatography conditions. Therefore, high-performance liquid chromatography (HPLC) has become the preferred choice for the determination of carbamates, because in this case the thermal lability problem is obviated (20). Although normal-phase separations were successful in many cases, reversed-phase separations have been used more often, mainly due to the better performance (19, 22). Carbamates can be determined by HPLC with UV (10, 12) or diode array (16) detection, but fluorescence detection gives at least one order of magnitude improvement in sensitivity regarding UV detection (8). With this aim, various HPLC methods have been developed based on a postcolumn derivatization procedure to increase sensitivity (11, 17, 18, 21). The postcolumn derivatization is necessary to convert compounds with unfavorable detection properties into derivatives, with a higher sensitivity and selectivity for specific detectors such as fluorescence. Liquid chromatography with mass spectrometry detection has also been used lately for the identification and quantification of low concentration levels of carbamate insecticides in fruits (7).

Sample preparation is an important step in pesticide residue analysis. Miniaturized procedures present several advantages in sample extraction, such as a decrease in the use of toxic solvents and glassware. Among the various techniques used recently, matrix solid-phase dispersion has been used in the analysis of several pesticide classes in juice samples with good results (1, 2).

The aim of this work was to develop a simple and fast miniaturized procedure, based on column adsorption, for the analysis of N-methylcarbamate insecticides in fruit and vegetable juices. N-methylcarbamates were determined by HPLC, with subsequent hydrolysis by sodium hydroxide, and derivatization of the methylamines obtained using ortho-phthalaldehyde (OPA) and Thiofluor at high pH to produce the highly fluorescent isoidole. The developed method was applied to the determination of carbamate insecticides in several juices commercialized in Spain.

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MATERIALS AND METHODS

Chemicals and material. Carbamate standards were obtained from commercial sources: oxamyl and methomyl from DuPont (Paris, France), propoxur and methiocarb from Riedel de Haën (Seelze, Germany), carbofuran from Mitsubishi Chemicals Industries (Tokyo, Japan), and carbaryl from Cequisa (Barcelona, Spain). The chemical structures of all the carbamates studied are shown in Figure 1. Methanol and acetone, HPLC grade, were from Scharlau (Barcelona, Spain). All the solvents were passed through a 0.45-μm filter (Scharlau) before use. Ultrapure water was prepared using a Milli-Q water purification system. OPA, dimethyl-2-mercaptoethylamine hydrochloride (Thiofluor), and the hydrolysis reagent (NaOH) were purchased from Pickering Labs (Mountain View, Calif.). The OPA solution was prepared by dissolving 100 mg of OPA in a premixed solution of 2 g of Thiofluor in 1 liter of OPA diluent. Florisil, a magnesium silicate adsorbent, 150 to 250 μm (60 to 100 mesh) research grade, was obtained from Merck (Darmstadt, Germany). The Florisil adsorbent was heated for 12 h at 140°C before placing it in glass columns (2 g per column).

Various fruit and vegetable juices (peach, grape, and carrot) used in this study were purchased in local supermarkets. Juice samples used for fortification assays were previously analyzed to ensure the absence of carbamate residues.

Glass columns (20 ml) from Afora (Barcelona, Spain) with Whatman no. 1 filter paper circles of 2 cm diameter (Whatman, Maidstone, UK) at the end were used in the extraction step. A 12-port vacuum manifold (Visiprep, Supelco, Madrid, Spain) was used for the filtration of the extraction solvent. Durapore membrane filters 0.45 μm Millex-HV from Millipore (Watford, Ireland) were used in the final filtration step.

| TABLE 1. Effect of the elution solvent on the carbamate residues recovery (%) from juice samples fortified at 100 ng/ml a |
|---------------------------------|------------------|------------------|------------------|
| Compound           | Acetone-hexane (25:75) | Acetone-hexane (50:50) | Acetone |
|                    | Peach | Carrot | Peach | Carrot | Peach | Carrot |
| Oxamyl             | 32.7 ± 4.9 | 29.2 ± 2.5 | 60.4 ± 6.6 | 69.5 ± 1.5 | 89.4 ± 3.8 | 79.6 ± 7.2 |
| Methomyl           | 50.3 ± 7.4 | 55.2 ± 2.0 | 79.7 ± 9.8 | 81.2 ± 1.4 | 86.4 ± 4.2 | 87.0 ± 2.0 |
| Propoxur           | 74.1 ± 5.3 | 78.3 ± 2.0 | 85.0 ± 7.1 | 98.8 ± 0.9 | 89.1 ± 3.2 | 91.6 ± 1.3 |
| Carbofuran         | 80.0 ± 6.3 | 78.5 ± 1.2 | 81.3 ± 5.8 | 92.4 ± 0.4 | 93.5 ± 3.4 | 92.6 ± 3.0 |
| Carbaryl           | 57.6 ± 7.2 | 59.3 ± 2.9 | 65.3 ± 5.0 | 82.0 ± 1.0 | 88.6 ± 4.9 | 90.2 ± 7.9 |
| Methiocarb         | 42.2 ± 2.5 | 50.2 ± 4.2 | 52.0 ± 3.4 | 53.2 ± 2.7 | 79.0 ± 4.8 | 91.4 ± 6.2 |

a Results are the mean of five replicates ± standard deviation.
### TABLE 2. Carbamate recoveries from juice samples

<table>
<thead>
<tr>
<th>Compound</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>500 ng/ml</td>
</tr>
<tr>
<td>Oxamyl</td>
<td>88.3 ± 8.6</td>
</tr>
<tr>
<td>Methomyl</td>
<td>103.7 ± 5.5</td>
</tr>
<tr>
<td>Propoxur</td>
<td>102.3 ± 5.6</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>106.7 ± 9.9</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>93.3 ± 3.0</td>
</tr>
<tr>
<td>Methiocarb</td>
<td>90.5 ± 1.4</td>
</tr>
</tbody>
</table>

- Results are the mean of five replicates ± standard deviation.

**FIGURE 2.** HPLC with fluorescence detection chromatograms of (A) a blank extract of a grape juice sample and (B) a grape juice sample fortified at 100 ng/ml.

- Methanol was used to provide column cleanup before returning to initial conditions (15% methanol) for injection of a subsequent sample. The step time was fixed at 60 min. A HP-ChemStation data system was used for data collection.

**RESULTS AND DISCUSSION**

**Optimization of the extraction procedure.** Florisil and aluminium oxide, previously used for the determination of pesticide residues in fruit juices in our laboratory (1, 2),
TABLE 3. Retention times (tR), limits of detection (LOD), limits of quantification (LOQ), maximum residue limits (MRL), and calibration data of the studied carbamates

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>tR (min)</th>
<th>LOD (ng/ml)</th>
<th>LOQ (ng/ml)</th>
<th>MRL (fruit) (mg/kg)</th>
<th>Calibration data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxamyl</td>
<td>13.25</td>
<td>1.8</td>
<td>5.9</td>
<td>0.05</td>
<td>r = 0.999</td>
</tr>
<tr>
<td>Methomyl</td>
<td>14.71</td>
<td>1.0</td>
<td>3.3</td>
<td>0.05–3</td>
<td>9.87·10^5 × −2.39·10^4</td>
</tr>
<tr>
<td>Propoxur</td>
<td>33.10</td>
<td>1.7</td>
<td>5.6</td>
<td>3</td>
<td>0.999</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>33.70</td>
<td>1.7</td>
<td>5.6</td>
<td>0.1–1</td>
<td>1.51·10^6 × −1.95·10^4</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>35.54</td>
<td>0.8</td>
<td>2.6</td>
<td>1–3</td>
<td>7.43·10^5 × −9.50·10^3</td>
</tr>
<tr>
<td>Methiocarb</td>
<td>43.71</td>
<td>1.9</td>
<td>6.3</td>
<td>0.05</td>
<td>1.000</td>
</tr>
</tbody>
</table>

The spectra of the carbamates, after postcolumn derivatization, were obtained in the experimental conditions described above. A good correspondence of the spectra at peak maxima was obtained for fortified samples in comparison with those recorded for standards. The developed method can therefore be used for the control of N-methylcarbamate residues in juice samples. A total of six commercial juices (grape, peach, and carrot) produced by different companies in Spain were analyzed, and no residues of the carbamate insecticides were found at levels above the LODs in those samples.

The method used for the determination of these compounds allows their extraction and the cleanup of extracts in a single step and requires only a low volume of organic solvent. Results obtained show that the reversed-phase HPLC method, with fluorescence detection after postcolumn derivatization, enables the analysis of N-methylcarbamates in juice at low levels. The proposed method, based on column adsorption using acetonitrile as elution solvent, is rapid and sensitive and provides good response linearity, high precision, and low detection limits.

REFERENCES
5. de Kok, A., and M. Hiemstra. 1992. Optimization, automation and


