Sample Preparation by Solid-Phase Extraction with Zinc Acetate Coagulation for the Determination of Pesticides in Crops

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Several cleanup techniques for pesticide-residue analysis have been investigated in order to reduce interferents, e.g., fatty acids and pigments, in the extract.1,2 The coagulation of fatty acids and pigments by adding zinc acetate3, column chromatography4, solid-phase extraction5,6 and gel-permeation chromatography7-10, has been introduced.

Although dichloromethane was commonly used as a solvent because of its high extractability of pesticides, the effluent of this solvent became regulated in 1995. Newly developed solid-phase extraction has been applied for pesticide-residue analysis in water, and has reduced solvent consumption. Unfortunately, solid-phase extraction has seldom been used in pesticide-residue analysis, except for liquid foods11, because the retention capacity was extremely small and a large amount of matrix existed in the crude extract.

In this study, a multiresidue analysis using a zinc acetate treatment, followed by solid phase-extraction, was developed, and applied to pesticide-residue analyses of crop samples.

Experimental

Material's and apparatus

All of the solvents and chemicals used were of pesticide grade and were purchased from Wako (Osaka, Japan). Acephate, dimethoate, dichlorvos, ethoprophos, chlorpyrifos, ethiofencarb, and bendiocarb were purchased from Wako; methacrifos, triazophos, tetrachlorvinphos, profenofos, oxamyl, aldicarb, and carbaryl were purchased from Hayashi (Osaka, Japan). The stock solution (250 ppm) of each pesticide was prepared in either acetone or methanol.

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An octadecylsilica (ODS) column, 1 g/6 ml Mega Bond Elute, was purchased from Valian (CA, USA). Conditioning of the ODS column was attained by percolating methanol, followed by water, prior to applying a sample.

Sample preparation

First, 20 g of a ground sample and 50 ml of acetonitrile were placed into a 500 ml Erlenmeyer flask and occasionally shaken for 3 h. In the case of high-moisture products, like vegetables or fresh fruits, 50 ml of pure acetonitrile was used for extraction; however, in the case of dry or low-moisture products, they were soaked by adding 20 ml water before extraction.

The homogenate was filtered using a GF/A filter (Whatman) under a vacuum, and the residue was rinsed by a small amount of 70% acetonitrile solution. Fatty acids and pigments in the filtrate were coagulated by adding 2 g of zinc acetate and 100 ml of distilled water. Around 3 h later, the insoluble zinc salts of the fatty acids and the coagulated pigments were isolated by filtration (No. 5A filter paper, Toyo).

The filtrates were diluted using 200 ml of distilled water, until their acetonitrile content became 15 - 20%. The filtrate was then percolated through the ODS column by applying a low vacuum.

After the column was washed with 10 ml water and dried under a gentle stream of dry air for 2 - 3 h, the pesticides were eluted with 5 ml ethylether/hexane (1/1). The elute was then dried by adding sodium sulfate anhydrous, followed by the addition of 100 µl of internal standard solution.

The sample extract was then transferred into a vial and capped with a Teflon-lined septum for a GC analysis.

Gas chromatography

A GC analysis was carried out on a Hewlett Packard HP 5890 equipped with an NP-detector operated under the following conditions.

The fused-silica column used was 30 m×0.32 mm i.d., 0.25 µm thick (DB-5 capillary column (J&W Scientific)). The carrier gas was helium regulated at 1.75 ml/min by electronic pressure control. After the oven temperature was held at 50°C for 1 min following injection, it was programmed to increase 30°C/min up to 170°C, which
was maintained for 1 min; the temperature was then increased 10°C/min up to 250°C, and finally held for 8 min. The injection port and the detector temperature were both 250°C. The sample (2 µl) was injected with an HP7673 auto sampler using splitless injection.

Results and Discussion

Gas chromatography

The chromatogram shown in Fig. 1 demonstrates the separation quality for 14 compounds of the standard mixture and 2 internal standards. Although all of the compounds gave clear signals, the peaks of aldicarb and oxamyl were their thermal cracking products. By using two varieties of internal standards, the daily variation of all retention times was compensated by their retention indexes. The programmed temperature retention indexes for each pesticide to the two internal standards are listed in parentheses in Fig. 1.

Adsorption of pesticide to the ODS column

The retention of the targetted compound on the ODS column depends on the hydrophilic solvent/water ratio in the solution. Although pesticides in fresh water are easily retained to the ODS column, their retention volume decreases according to the increase in the solvent ratio in the sample water.

Figure 2 shows the pesticides adsorption efficiency on the ODS column (0.5 g) at various solvent concentrations from 5 to 30%. According to increase in the acetonitrile concentration to 20%, tetrachlorvinphos (which has a high octanol/water partition coefficient (log PoW>3)) could maintain 80% recovery; however, the pesticides whose log PoW values were low (log PoW<2) rapidly decreased their percent recoveries.

The retention volume for homologous pesticides on the ODS column are related to both their log PoW and their solubility. It is possible to roughly estimate the recovery using the log PoW value or solubility of the pesticide.

Recoveries of pesticides from fortified agricultural products

The recoveries of pesticides from fortified samples are listed in Table 1. Water-soluble pesticides, like acephate and oxamyl, could not be retained on the ODS column. Dimethoate, dichlorvos, and aldicarb have low log PoW(<2), and their recoveries were lower than 50%. Although pesticides having high log PoW(>2) were well recovered (>70%), the percent of the recoveries depended on the type of crops.

Chlorpyrifos has an extremely high log PoW(>5), and is essentially retainable on the ODS column, though its recovery ranged from 14% to 75%. It is considered that pesticides were adsorbed on the coagulates or glass wall in the analytical process because of the low solubility to the aqueous acetonitrile.

References

Table 1  Relation between the recoveries of pesticides from fortified samples and their log $P_{ow}$ on 1 g ODS

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>log $P_{ow}$</th>
<th>Beet</th>
<th>Onion</th>
<th>Carrot</th>
<th>Lettuce</th>
<th>Wheat</th>
<th>Rice</th>
<th>Soybean</th>
<th>Spinach</th>
<th>Apple</th>
<th>Grapefruit</th>
<th>Lemon</th>
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<tr>
<td>Acephate</td>
<td>0.50</td>
<td>4.5±0.2</td>
<td>5.4±0.7</td>
<td>5.5±0.9</td>
<td>4.7±0.5</td>
<td>4.5±1.0</td>
<td>2.6</td>
<td>1.1</td>
<td>5.9</td>
<td>3.8</td>
<td>3.4</td>
<td>4.2</td>
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<td>Dimethoate</td>
<td>1.47</td>
<td>29.6±5.2</td>
<td>—</td>
<td>26.3±3.5</td>
<td>—</td>
<td>21.0±12.5</td>
<td>50.0</td>
<td>30.6</td>
<td>29.9</td>
<td>11.3</td>
<td>16.5</td>
<td>21.6</td>
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<td>Dichlorvos</td>
<td>108.3±3.7</td>
<td>95.1±2.4</td>
<td>86.6±3.6</td>
<td>98.5±3.2</td>
<td>91.0±7.7</td>
<td>76.6</td>
<td>86.7</td>
<td>88.5</td>
<td>70.6</td>
<td>91.0</td>
<td>100</td>
<td>—</td>
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<td>Methacetin</td>
<td>99.4±0.1</td>
<td>94.5±1.6</td>
<td>85.3±4.5</td>
<td>97.0±2.6</td>
<td>94.9±5.1</td>
<td>77.5</td>
<td>77.6</td>
<td>82.2</td>
<td>98.0</td>
<td>124</td>
<td>71.0</td>
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<td>Ethoprophos</td>
<td>3.55</td>
<td>92.9±2.3</td>
<td>87.7±2.3</td>
<td>83.3±6.4</td>
<td>100.4±2.2</td>
<td>83.4±12.5</td>
<td>68.2</td>
<td>70.0</td>
<td>74.4</td>
<td>85.6</td>
<td>84.7</td>
<td>78.5</td>
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<td>Tetrachlorvinphos</td>
<td>3.53</td>
<td>119.5±17.9</td>
<td>98.1±0.6</td>
<td>88.0±15.6</td>
<td>95.8±1.4</td>
<td>92.8±9.4</td>
<td>76.3</td>
<td>75.4</td>
<td>89.9</td>
<td>89.2</td>
<td>89.3</td>
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<td>Profenofos</td>
<td>4.70</td>
<td>88.1±3.2</td>
<td>66.7±2.5</td>
<td>52.9±5.5</td>
<td>90.3±2.7</td>
<td>68.3±11.9</td>
<td>40.9</td>
<td>56.5</td>
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<td>Chlorpyrifos</td>
<td>5.11</td>
<td>53.1±9.9</td>
<td>72.8±1.9</td>
<td>59.3±4.3</td>
<td>74.7±2.0</td>
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<td>Oxamyl</td>
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<td>0</td>
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<td>Aldicarb</td>
<td>1.08</td>
<td>11.1±1.9</td>
<td>17.0±2.0</td>
<td>14.9±1.6</td>
<td>7.5±0.5</td>
<td>8.0±1.3</td>
<td>23.5</td>
<td>3.6</td>
<td>9.5</td>
<td>11.4</td>
<td>13.6</td>
<td>9.2</td>
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<td>Ethiofencarb</td>
<td>3.0±9.2</td>
<td>102.3±12.1</td>
<td>81.1±5.7</td>
<td>47.5±6.6</td>
<td>47.5±17.8</td>
<td>93.7</td>
<td>30.0</td>
<td>36.8</td>
<td>27.0</td>
<td>56.3</td>
<td>79.8</td>
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<tr>
<td>Carbaryl</td>
<td>2.34</td>
<td>68.2±8.4</td>
<td>109.4±12.1</td>
<td>103.9±10.5</td>
<td>58.6±0.7</td>
<td>66.6±23.7</td>
<td>89.5</td>
<td>49.1</td>
<td>57.5</td>
<td>78.0</td>
<td>92.0</td>
<td>99.1</td>
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<td>Bendiocarb</td>
<td>3.58±2.9</td>
<td>50.4±6.7</td>
<td>49.7±5.7</td>
<td>32.2±1.6</td>
<td>31.3±7.9</td>
<td>75.6</td>
<td>25.5</td>
<td>32.5</td>
<td>30.2</td>
<td>37.5</td>
<td>35.9</td>
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</table>

a. Mean recovery±standard deviation for triplicate determinations.  b. Determination was not possible because of the presence of interferences by NP-detector.  Spiking level: 0.1 ppm for each pesticide.