Determination of Seventeen Pesticide Residues in Agricultural Products by LC/MS

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Residues of 17 pesticides in agricultural products were determined by LC/MS with an atmospheric pressure chemical ionization (APCI) interface in both positive and negative ion modes. Pesticides were extracted with acetonitrile, and the extracts were cleaned-up with a primary and secondary amine (PSA) mini-column eluted with acetone–hexane (1 : 1). Rice, orange and potato were spiked with the 17 pesticides at 0.1 μg/g and analyzed by the proposed method. The average recoveries of these pesticides usually ranged from 70 to 98% and the relative standard deviations were usually around 10%. These results suggested that LC/MS with APCI could be used to determine the residue levels of the 17 pesticides in these crops.

Key words: agricultural products; pesticide residue; benzoylurea insecticide; LC/MS; atmospheric pressure chemical ionization (APCI)

Introduction

In Japan, the maximum residue levels (MRLs) have been set for about 200 pesticides in the last decade. It is very difficult to analyze over 200 pesticide residues in routine work. Several methods for multi-residue analysis have been developed using gas chromatography (GC) for detection. Some kinds of pesticides could not be analyzed by GC because of thermal decomposition or low volatility. Therefore, HPLC was used to analyze such pesticides. These methods usually adopted UV for detection, but because of the lower selectivity of UV detection, extensive cleanup procedures were sometimes required and the peaks of pesticides sometimes overlapped or were subject to interference by sample matrices. To solve this problem, supercritical fluid extraction (SFE) or LC/MS has been used. We focused on some pesticides listed below which could not readily be analyzed by GC/MS. Etoxazone, daimuron and cumyluron are herbicides for control of weeds in rice. Inabenfide is a growth regulator to reduce the damage from typhoons by making the rice stalk shorter. Diclomezine is a fungicide for rice. These 6 pesticides are herbicides for control of weeds in rice. Inabenfide is a growth regulator to reduce the damage from typhoons by making the rice stalk shorter. Diclomezine is a fungicide for rice. These 6 pesticides are herbicides for control of weeds in rice. Diflubenzuron, hexafluorobenzuron, teflubenzuron, diflubenzuron and teflubenzuron were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Diclomezine, pencycuron and tebufenozide were obtained from Kanto Kagaku (Tokyo, Japan). Dimethomorph, chlorfluazuron, diflubenzuron and lufenuron were obtained from Riedel de Haën (Seelze, Germany). Flufenoxuron was purchased from Hayashi Pure Chemical Industries, Ltd. (Osaka). Flufenoxuron was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Each compound was dissolved in acetone to make a 1,000 μg/mL stock solution.

Experimental

Chemicals

Acibenzolar-S-methyl, etobenzanid, inabenfide, cumyluron, daimuron, pentoaxzone, methabenzthiazuron and diflubenzuron were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Diclomezine, pencycuron and tebufenozide were obtained from Kanto Kagaku (Tokyo, Japan). Dimethomorph, chlorfluazuron, diflubenzuron and lufenuron were obtained from Riedel de Haën (Seelze, Germany). Flufenoxuron was purchased from Hayashi Pure Chemical Industries, Ltd. (Osaka). Hexafluorobenzuron was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Each compound was dissolved in acetone to make a 1,000 μg/mL stock solution.

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standard solution. The stock standard solutions were diluted to 50 µg/mL with acetone. A mixed solution of the 17 compounds, 5 µg/mL of each, was used to fortify the samples as appropriate. For LC/MS analysis, a mixed solution was dried under a gentle warm air stream and the residue was dissolved in 0.5 mL of acetonitrile and made up to 1 mL with water to exchange the solvent. Acetone, acetonitrile and hexane were of pesticide analysis grade from Wako. Acetic acid was for HPLC grade from Wako. Distilled water was obtained by using the Milli-RX 12 and Milli-Q SP. TOC apparatuses (Millipore, Bedford, MA, USA). All mini-columns, primary and secondary amine (PSA), silica (SIL), quaternary amine (SAX) and diol (2OH) were obtained from Varian Inc. (CA, USA).

Sample preparation

Rice, orange and potato were purchased at a local market in Osaka and we confirmed that pesticide residues were below detectable levels with the proposed method. About 500 g of food was chopped in a food processor MK-K3 (Matsushita Electric Industrial Co., Ltd., Osaka) or a mill MX-X61 (Matsushita) for 5 min to obtain thoroughly mixed homogenates or powder. An aliquot of 20 g of sample homogenate was blended with 50 mL of acetonitrile with a homogenizer HG30 (Hi-tech Co., Ltd., Osaka) or a mill MX-X61 (Matsushita) for 5 min to obtain thoroughly mixed homogenates or powder. An aliquot of 20 g of sample homogenate was blended with 50 mL of acetonitrile with a homogenizer HG30 (Hitachi Koki Co., Ltd., Tokyo). In the case of rice, 20 mL of water was added to 20 g of powdered rice and the mixture was left to stand for 30 min before blending. The extract was filtered through a filter paper No. 5A (Advantec Toyo Kaisya, Ltd., Tokyo) into a separation funnel. The sample residue on the filter paper was washed with 10 mL of acetonitrile, then the washing was added to the filtrate. The extract was shaken vigorously with 5 g of NaCl for 10 min and was left for about 30 min to salt out water from the acetonitrile. The water portion was discarded, and the extract was evaporated to near-dryness at 40 °C. The residue was dissolved in 5 mL of acetone and made up to 10 mL with hexane.

SPE cleanup

A 500 mg PSA column was pre-rinsed with 10 mL of acetone and then 10 mL of acetonitrile, hexane (1:1). A 0.5 mL (equivalent to 1 g of sample) aliquot was applied to the column and pesticides were eluted with 10 mL of acetonitrile-hexane (1:1) at the flow rate of 2.5 mL/min. The eluate was dried under a gentle warm air stream and the residue was dissolved in 0.5 mL of acetonitrile and made up to 1 mL with water for LC/MS analysis.

LC conditions

The LC system was composed of two JASCO PU-980 LC pumps (Jasco Corporation, Tokyo), a JASCO DG-980-50 vacuum degasser, a JASCO AS-950 auto sampler, and a JASCO CO-965 column oven. The Microsoft Windows NT based software, Masslynx was used to control the instrument. Pesticides were chromatographed on a 150 mm×2.0 mm id octadecylsilica gel column (Wakosi-II 3C18HG, Wako) utilizing a linear gradient mobile phase of 0.1 mol/L acetic acid (pH 3.3) and acetonitrile at a flow rate of 0.2 mL/min. The acetonitrile concentration was as follows: 50% in 0–3 min, 50–80% in 3–8 min, 80% in 8–18 min, 80–50% in 18–20 min, and 50% in 20–25 min. The analytical column was maintained at 50 °C. The sample injection volume was 5 µL.

MS conditions

A Platform-II mass spectrometer (Micromass Ltd., Manchester, UK) equipped with an APCI available in both positive and negative modes was employed for the determination of the compounds. The Microsoft Windows NT based software, Masslynx was used to control the instrument and for data acquisition and processing. The operating parameters for the positive mode were as follows: corona 3.00 kV; HV lens 0.00 kV; skimmer lens 30 V. In either mode, source temperature was 140 °C, and APCI probe temperature was 550 °C. Cone voltages were set to 30 V. Acquisition times and monitor ions of all pesticides are shown in Table 1.

LC-UV conditions

The LC system was composed of two Shimadzu LC-10AD LC pumps (Shimadzu, Kyoto, Japan), a Shimadzu DGU-12A vacuum degasser, a Shimadzu SIL-10A auto sampler, a Shimadzu CTO-10A column oven, a Shimadzu SPD-M10Avp diode-array detector and a Shimadzu CBM-10A communications bus module. The Microsoft Windows 95 based software, CLASS-LC10 was used to control the instrument and for data acquisition.
were chromatographed on a 75 mm × 4.6 mm id octadecylsilica gel column (Cadenza CD-C18, Imtakt, Kyoto, Japan) utilizing a linear gradient mobile phase of 0.1 mol/L acetic acid (pH 3.3) and acetonitrile at a flow rate of 1.0 mL/min. The acetonitrile concentration was as follows: 30% in 0–2 min, 30–90% in 2–22 min, 90% in 22–27 min, 90–30% in 27–30 min, 30% in 30–35 min. The analytical column was maintained at 50°C. The eluates were detected at 250 nm. The sample injection volume was 20 μL.

Results and Discussion

Several SPE sorbents such as SI, SAX, 2OH and PSA were examined. Mixed standard solutions were loaded on the columns, and eluted with acetone–hexane (1:1). Inabenfide showed a low recovery on SAX, and hexaflumuron showed a low recovery on SAX, SI and 2OH. The PSA column gave good recovery, over 70%.

A comparison of the use of APCI in the positive and negative ion modes was made. Dimethomorph gave no response in the negative ion mode, while lufenuron, pentoxazone and chlorfluazuron could not be detected in the positive ion mode. The others could be detected in both modes, so the monitor ions were set to get maximum responses. Methabenzthiazuron, inabenfide, diclomezine, dimethomorph, cumyluron, acibenzolar-S-methyl, daimuron, etobenzanid and pencycuron were detected at 250 nm. The sample injection volume was 20 μL.

Table 2. Recoveries and MRLs of 17 Pesticides

<table>
<thead>
<tr>
<th>No.</th>
<th>Pesticide</th>
<th>Rice Recovery (%)</th>
<th>MRL#2 (ppm)</th>
<th>Orange Recovery (%)</th>
<th>MRL#2 (ppm)</th>
<th>Potato Recovery (%)</th>
<th>MRL#2 (ppm)</th>
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<td>1</td>
<td>Methabenzthiazuron</td>
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<td>87</td>
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<td>Diclofop</td>
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<td>74</td>
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<td>Daimuron</td>
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#1 Spiked levels of pesticides were 0.1 μg/g. Mean of five experiments.
#2 MRLs are set by the Japanese Food Sanitation Law.

Fig. 1. LC/MS chromatograms of a standard solution (5 μL of 0.2 μg/mL solution) See Table 1 for the identification of peaks.

Fig. 2. LC/MS chromatograms of orange fortified with 0.1 μg/g each pesticide. See Table 1 for the identification of peaks.
determined in the positive ion mode. Diflubenzuron, tebufenozide, hexafluorobenzonitrile, lufenuron, pentoxazone, flufenoxuron and chlorfluazuron were done in the negative ion mode. As shown in Fig. 1, all pesticides could be identified in individual channels except tebufenozide and lufenuron. Dimethomorph showed two isomer peaks in the +388 chromatogram, and these peaks were summed for quantification.

Calibration curves of all pesticides were constructed as follows: acibenzolar-S-methyl: 0.1, 0.5, 1.0, and 2.0 µg/mL; diclomezine and pentoxazone: 0.05, 0.1, 0.5, 1.0, and 2.0 µg/mL; others: 0.02, 0.05, 0.1, 0.5, 1.0, and 2.0 µg/mL. The calibration curves were linear in the concentration range studied, and the correlation coefficients were higher than 0.994 for all the compounds. The limits of detection (LODs), calculated based on a signal-to-noise ratio of 3, were as follows: acibenzolar-S-methyl: 0.1 µg/mL, methabenzthiazuron, diclomezine and pentoxazone: 0.05 µg/mL, others: 0.02 µg/mL.

A recovery test was performed with rice, orange, and potato, for which MRLs have been established for at least some of the pesticides. In the recovery test, 0.4 mL of mixed standard solution at 5 µg/mL in acetone was spiked into 20 g of sample homogenate (equivalent to 0.1 µg/g on a sample) and the sample was left for 30 min before extraction. The results are shown in Table 2. The recoveries of most of the pesticides were 70 to 97%, and the relative standard deviations (RSDs) were usually acceptable at around 10%. The high RSDs of methabenzthiazuron recovery in rice and orange were caused by drift of the chromatograms owing to the change of acetonitrile concentration. As shown in Fig. 2, LC/MS chromatograms of orange extract were almost the same as those of the standard solution, but one peak of dimethomorph isomer showed interference. Thus, in the case of orange, dimethomorph was determined by using the other peak. Though the chromatograms showed no interference, cymyluron, daimuron and pencurion gave low recoveries. However, this is not a problem because these pesticides are mainly used for rice and MRLs have not set for orange in Japan. Recovery of acibenzolar-S-methyl showed high RSDs in the case of potato. Acibenzolar-S-methyl is applied only to grain, therefore further cleanup for potato samples was not examined. Except for these cases, the proposed method resulted in good recoveries.

The usefulness of the LC/MS was confirmed by comparison with LC-UV using orange fortified with 0.5 µg/g of pesticides. This was a five times higher concentration than in the LC/MS study, because it was difficult to detect low levels of pesticides by LC-UV. To get an appropriate separation, the gradient program was extended from 25 to 35 minutes. Nevertheless, as shown in Fig. 3, three groups of pesticides could not be resolved with this gradient program. Diclofop-methyl and one isomer of dimethomorph, lufenuron and pentoxazone were very close, hexafluorobenzonitrile and tebufenozide could not be divided and pencurion was also very close to them. The chromatogram of orange extract showed extensive interference, and some pesticides could not be identified. Even if further cleanup were adopted, the LODs would obviously have been higher than in LC/MS.

In this study, 17 pesticides were effectively extracted from crops and determined by LC/MS. The LC/MS determination could be completed within 25 min, and the LODs and accuracy of this method are satisfactory in relation to the MRLs in Japan. This method could be used for monitoring residues of these pesticides in crops.

References

8) Anastassiades, M., Scherbaum, E., Schwack, W., Analysis


