

Determination of *N*-methylcarbamate pesticides in foods using an accelerated solvent extraction with a mini-column cleanup

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Residual *N*-methylcarbamate pesticides in food were determined by accelerated solvent extraction (ASE) and HPLC with post-column fluorescence. Pesticides were extracted with acetonitrile at 100 °C under 2000 psi pressure in less than 20 min. Extracts were cleaned-up with a carboxylic acid mini-column eluted with 10% or 30% acetone in hexane. Eight foods were spiked with 17 pesticides at 0.2 ppm. The average recoveries of these pesticides were 70–100% and the relative standard deviations were <10%. These results suggested that ASE can be used to extract residues of *N*-methylcarbamate pesticides in foods.

Keywords: *N*-Methylcarbamate pesticides; food; accelerated solvent extraction; HPLC; post-column fluorescence; mini-column cleanup; residues

Laboratories that monitor pesticides have developed various means of measuring residual levels in food.^{1,2} Multiresidue methods are usually applied in pesticide monitoring because several compounds can be targeted simultaneously, which reduces labour and material costs. An introduction of automation in the analytical operation helps to monitor pesticides. Conventional manual liquid–liquid partition consumes time and it is labour intensive. Moreover, it sometimes results in an emulsion that can lower the reproducibility of the analytical results. A new solvent extraction system, which is called accelerated solvent extraction (ASE), has been developed and applied to residual analysis.³ ASE uses a heated solvent, usually more than 100 °C, that increases its penetration and solubility. The extraction is performed under high pressure to maintain a heated solvent in the liquid state. Fisher *et al.* reported that ASE could automatically and rapidly extract organochlorine pesticides and polycyclic aromatic hydrocarbons in soil.⁴ We reported that ASE could extract organophosphorus pesticides from food with good accuracy and precision.⁵ ASE achieved rapid extraction with small volumes of conventional organic solvents.

In this study, we extracted *N*-methylcarbamate pesticides from food using ASE to replace conventional manual extraction.

Experimental

Chemicals

Metolcarb, xylylcarb, XMC, pirimicarb, propoxur, carbofuran, carbaryl and isoprocarb were obtained from Wako (Osaka, Japan). Butoxycarboxim, methomyl, bendiocarb, trimethacarb, methiocarb, oxamyl, dioxacarb, fenobucarb and promecarb were purchased from Riedel de-Haën (Hannover, Germany). Each compound was dissolved in acetone to make a 1000 µg ml⁻¹ stock standard solution. The stock standard solutions were divided into two groups and diluted to 20 µg ml⁻¹ with methanol. Group A mixture contained butoxy-

carboxim, methomyl, metolcarb, bendiocarb, xylylcarb, XMC, pirimicarb, trimethacarb and methiocarb. Group B mixture contained oxamyl, dioxacarb, propoxur, carbofuran, carbaryl, isoprocarb, fenobucarb and promecarb (see Fig. 1 for classification).

Acetone, acetonitrile, hexane and anhydrous Na₂SO₄ were of pesticide analysis grade. Methanol was HPLC grade. Sodium hydroxide and boric acid were of amino acid analysis grade. Sodium chloride and β-mercaptopropionic acid and *o*-phthalaldehyde were of analytical-reagent grade. All reagents were purchased from Wako (Osaka, Japan). Particles of diatomaceous earth (Extrelut for refilling; particle size 160–800 µm) (Merck, Darmstadt, Germany) were heated at 550 °C for 15 h. A carboxylic acid mini-column (LC-WCX, 500 mg sorbent) was obtained from Supelco (Bellefonte, PA, USA).

Food preparation

Foods were purchased at a local market in Osaka and we confirmed that *N*-methylcarbamate pesticide residues were below detectable levels with the method described later. About 500 g of food was chopped in a conventional food processor (MK-K3, Matsushita, Japan) for 5 min to obtain thoroughly mixed homogenates. The operating manual for ASE recommended that wet samples should be mixed with a drying agent to facilitate solvent penetration into sample matrices.⁶ An aliquot of 5 g sample homogenate and 6 g Extrelut particles were ground in a mortar (12 cm id) with a pestle until the

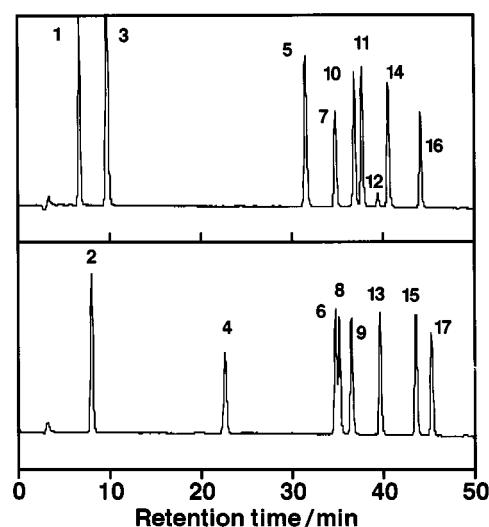


Fig. 1 Chromatograms of standard solutions (20 µl of 0.2 µg g⁻¹ solutions). 1, butoxycarboxim; 2, oxamyl; 3, methomyl; 4, dioxacarb; 5, metolcarb; 6, propoxur; 7, bendiocarb; 8, carbofuran; 9, carbaryl; 10, xylylcarb; 11, XMC; 12, pirimicarb; 13, isoprocarb; 14, trimethacarb; 15, fenobucarb; 16, methiocarb; and 17, promecarb. HPLC conditions given in text.

mixture became homogeneous. In the fortification study, 0.5 ml of each standard mixture at $2 \mu\text{g ml}^{-1}$ in methanol was added to each sample homogenate to a final concentration of 0.2 ppm on a sample mass basis and the mixture was left for 30 min before adding Extrelut. Mixtures of samples and Extrelut were placed in stainless-steel cells (33 ml; $11 \text{ cm} \times 1.9 \text{ cm id}$).

ASE

Accelerated solvent extraction was performed with an AS 200 system (Dionex, Sunnyvale, CA, USA). The conditions were as follows: extraction temperature, 100°C ; extraction pressure, 2000 psi; pre-heating period, 5 min; static extraction period, 5 min; extraction solvent, acetonitrile 60 ml; solvent flash, 19.8 ml; nitrogen purge, 60 s; and collection, in 60 ml glass vials with Teflon coated rubber caps (I-CHEM, New Castle, DE, USA). The extract was transferred into a separation funnel. The collecting vial was washed with 5 ml of acetonitrile twice, then the washings were added to the extract. Two grams of NaCl was also added, and the extract was shaken vigorously for 10 min. The extract was left for about 1 h to educe water from the acetonitrile. After the water portion was discarded, the extract was dried by adding anhydrous Na_2SO_4 and evaporated to dryness with warming at $<40^\circ\text{C}$. The residue was dissolved in acetone and made up to 5 ml.

Mini-column cleanup

The mini-column cleanup was performed automatically with a BenchMate Workstation (Zymark, Hopkinton, MA, USA). The BenchMate Workstation can make dilutions, filter, add reagents and internal standards, mix solutions, determine densities, and weigh samples. BenchMate parameters were as follows: conditioning flow, 0.25 ml s^{-1} ; sample load flow, 0.15 ml s^{-1} ; elute flow, 0.15 ml s^{-1} ; sample maximum size, 9.8 ml; collection tube volume, 10 ml.

A 500 mg carboxylic acid column was pre-rinsed with 10 ml of acetone and then 10 ml of acetone/hexane (3:7, v/v). A 1 ml (equivalent to 1 g sample) aliquot was transferred to a centrifuge tube and 2 ml of acetone was added, and diluted to 9.8 ml with hexane. In the citrus samples, a 1 ml aliquot was diluted to 9.8 ml with hexane. The diluted extracts were centrifuged and then subjected to the column and the eluate was collected in the first tube. Residual pesticides were then eluted with 10 ml of acetone/hexane (3:7) into the second tube. Since pesticides were mainly eluted into the first tube and the others were eluted into the second tube, both eluates were mixed and evaporated to dryness. The residue was dissolved in methanol and diluted to 1 ml with water. For the citrus fruits, the cartridge was pre-rinsed and eluted with 10 ml of acetone/hexane (1:9, v/v) instead of acetone/hexane (3:7, v/v).

HPLC analysis

Pesticides were determined using HPLC and a post-column fluorescence system.⁷⁻¹⁰ The system utilized a Shimadzu (Kyoto, Japan) LC-10A *N*-methylcarbamate pesticides analysis system. Pesticides were chromatographed on a $150 \text{ mm} \times 4.6 \text{ mm id}$ octadecylsilica column (STR-ODS II, Shimadzu, Kyoto, Japan) utilizing a linear gradient mobile phase of methanol-water (18% at 0–10 min, 18–70% 10–40 min, 70–90% 40–43 min, 90% 43–60 min, 18% 60–75 min) flowing at 0.8 ml min^{-1} . The column was maintained at a constant temperature of 50°C . A post-column reactor utilizing 0.05 mol l^{-1} NaOH was maintained at 100°C . This reactor was coupled to a second reactor at 50°C that used 0.25 mmol l^{-1} *o*-phthalaldehyde and 0.25 mmol l^{-1} β -mercaptopropionic acid (boric acid buffer-methanol solution) to convert the compounds to a fluorescent derivative. All reactors were eluted at 0.4 ml min^{-1} flow rate. The resulting fluorescent derivative was analyzed using an

excitation wavelength of 340 nm and an emission wavelength of 445 nm. The sample injection volume was 20 μl . The limits of determination were 0.05 ppm (pirimicarb) and 0.01 ppm (others) in this method.

Methanol extraction

Conventional solvent extraction for *N*-methylcarbamate pesticides was performed according to Pesticide Analytical Manual² with slight modification. An aliquot of 10 g of sample homogenate was blended with 20 ml of methanol in a homogenizer (HG 30, Hitachi, Japan). The extract was filtered through a filter paper (No. 5A, Advantec, Japan) and evaporated to dryness. The extract was dissolved in 5 ml of methanol and diluted to 10 ml with water for HPLC analysis.

Results and discussion

Since vegetables and fruits, except citrus, usually do not have natural fluorescent matrices, the post-column HPLC method is widely used to detect *N*-methylcarbamate pesticides in foods. Chromatograms of broccoli extract had interfering peaks (data not shown). Thus, a carboxylic acid cartridge was used to remove such interference and 17 pesticides were eluted by acetone/hexane (3:7, v/v). A recovery test was performed with five foods. Five grams of food spiked with 0.2 ppm of each pesticide was extracted with acetonitrile. As shown in Table 1, extraction resulted in good recoveries: yields of most of the

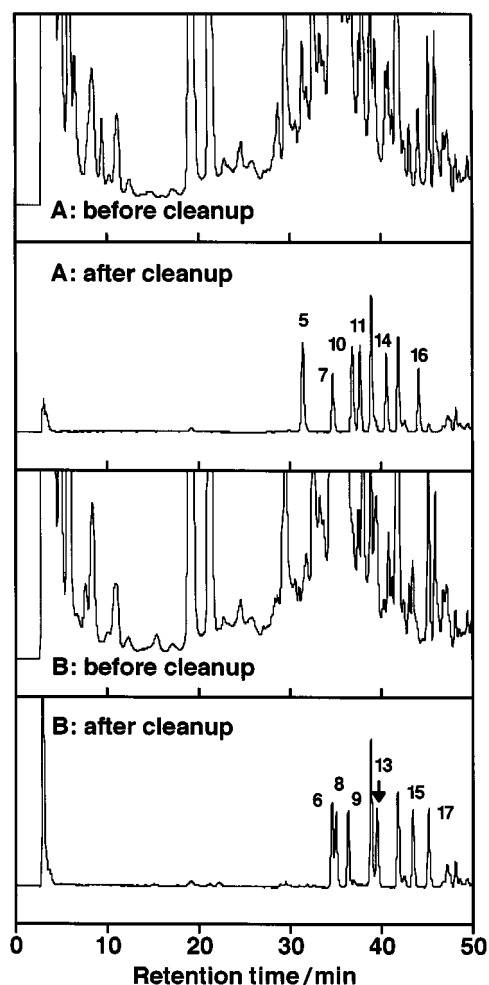


Fig. 2 Cleaned-up chromatograms of grapefruit fortified with $0.2 \mu\text{g g}^{-1}$ of each pesticide. A, grapefruit fortified group A mixture; B, grapefruit fortified group B mixture. See Fig. 1 for identification of peaks.

Table 1 Mean recoveries of 17 *N*-methylcarbamate pesticides in foods with ASE

Pesticide	Banana		Green beans		Broccoli		Melon		Carrot	
	Recovery (%) [*]	RSD (%)	Recovery (%) [*]	RSD (%)	Recovery (%) [*]	RSD (%)	Recovery (%) [†]	RSD (%)	Recovery (%) [†]	RSD (%)
1 Butoxycarboxim	85.0	3.4	67.1	10.4	88.8	1.1	84.4	3.8	87.7	14.3
2 Oxamyl	87.4	2.6	82.6	6.5	76.9	1.3	90.6	5.7	91.7	10.4
3 Methomyl	89.0	2.0	78.4	6.0	100.5	0.5	78.4	9.2	85.7	5.1
4 Dioxacarb	90.1	1.7	98.6	6.1	87.4	0.3	85.8	6.9	71.8	18.5
5 Metolcarb	86.0	1.7	70.7	16.7	89.4	0.8	61.5	25.6	62.1	24.6
6 Propoxur	92.5	1.3	84.4	6.9	74.9	2.5	78.1	6.6	80.1	4.4
7 Bendiocarb	91.8	1.3	76.3	5.7	88.2	0.9	80.5	4.1	71.3	17.1
8 Carbofuran	94.5	1.1	87.0	6.5	78.5	9.1	87.2	4.3	88.1	5.8
9 Carbaryl	100.5	1.5	90.6	6.5	80.9	2.3	93.8	6.9	97.7	10.1
10 Xylcarb	92.1	1.2	78.5	10.0	96.4	2.3	78.4	9.1	78.9	10.2
11 XMC	90.6	1.3	77.0	10.7	89.7	0.4	74.7	11.0	76.7	11.6
12 Pirimicarb	88.6	2.9	70.0	11.3	77.5	0.5	82.2	2.9	93.1	7.8
13 Isoproc carb	86.2	1.5	78.6	6.4	79.5	0.5	68.2	9.7	70.2	7.8
14 Trimethacarb	95.8	1.2	82.6	7.9	90.8	0.5	82.6	5.7	85.2	6.3
15 Fenobucarb	89.9	1.3	83.7	6.4	79.8	0.1	76.0	7.3	79.6	4.1
16 Methiocarb	99.4	1.1	80.4	9.1	88.7	0.3	94.3	2.6	87.2	8.3
17 Promecarb	97.5	1.3	88.9	7.8	80.6	0.2	83.3	14.9	88.7	5.3

* Mean of three experiments. † Mean of five experiments.

Table 2 Mean recoveries of 13 *N*-methylcarbamate pesticides in citrus fruit with ASE

Pesticide	Grapefruit		Lemon		Orange	
	Recovery (%) [*]	RSD (%)	Recovery (%) [*]	RSD (%)	Recovery (%) [†]	RSD (%)
1 Butoxycarboxim	—	—	—	—	—	—
2 Oxamyl	—	—	—	—	—	—
3 Methomyl	—	—	—	—	—	—
4 Dioxacarb	—	—	—	—	—	—
5 Metolcarb	90.4	1.8	71.6	1.7	85.5	8.2
6 Propoxur	95.2	0.2	83.6	1.6	89.8	8.7
7 Bendiocarb	102.3	4.7	75.4	0.3	88.3	9.6
8 Carbofuran	87.8	0.4	84.5	1.2	89.8	8.9
9 Carbaryl	93.1	1.1	86.3	1.5	93.8	8.6
10 Xylcarb	100.4	1.6	‡	—	90.5	8.5
11 XMC	93.8	2.0	‡	—	89.4	8.7
12 Pirimicarb	‡	—	27.6	5.6	91.4	10.1
13 Isoproc carb	92.2	0.6	85.1	1.7	89.0	10.0
14 Trimethacarb	95.1	2.1	80.5	1.0	91.4	9.3
15 Fenobucarb	90.7	0.6	85.1	1.6	90.6	9.8
16 Methiocarb	101.7	2.0	88.7	0.8	101.8	14.1
17 Promecarb	106.0	0.8	94.2	1.8	96.3	8.8

* Mean of three experiments. † Mean of five experiments. ‡ Interference. — Not eluted.

Table 3 Comparison between ASE and methanol extraction in pesticide-containing samples

Food	Pesticide	Extraction			
		ASE		Methanol	
		Mean* (ppm)	RSD (%)	Mean* (ppm)	RSD (%)
Grape	Methomyl	1.33	3.6	1.49	1.5
Green beans	Methomyl	0.12	8.7	0.12	21.2
	Carbaryl	0.02	11.3	0.02	2.7

* Mean of three experiments.

pesticides were 70–100%, and the relative standard deviations (RSDs) were acceptable, usually < 10%.

An extract from the citrus fruit had a substantial natural fluorescence which interfered with the detection of pesticides. At first, a citrus extract was applied to the cartridge and eluted

with acetone/hexane (3:7, v/v) to elute 17 pesticides; this reduced interference without eliminating it. A citrus extract was therefore eluted by less polar acetone/hexane (1:9, v/v) which could elute 13 pesticides, but butoxycarboxim, oxamyl, methomyl and dioxacarb were retained in the cartridge.

Grapefruit contained a substantial interference after cleanup, so pirimicarb could not be quantified (Fig. 2). In orange, no interference was overlapped to pesticides. In lemon, xylcarb and XMC were interfered with and pirimicarb showed low recovery for an unknown reason. Thus, 11–13 pesticides were determined in the three spiked citrus fruits. As shown in Table 2, the average recoveries of the 10–13 pesticides in three samples were acceptable. Most RSD values were below 10%.

ASE extraction was compared with methanol extraction in pesticide-containing samples to evaluate the performance of ASE (Table 3). (Mini-column cleanup was omitted.) These samples were obtained during our routine monitoring and stored at –20 °C until analysis. Residual values extracted by the two methods produced similar results in green beans. ASE showed slightly smaller values than methanol extracts for methomyl in

grapes. Methomyl is a hydrophilic pesticide and grape samples are very juicy, so some deleterious effects may have occurred during ASE.

In this study, extraction and cleanup were performed automatically. The ASE procedure is simple, requiring only mixing of the samples with drying agent and transferring the mixture to an extraction cell. After ASE, the BenchMate II cleaned-up the samples sequentially. Only dehydration, evaporation and dilution were performed manually. ASE used high temperature and high pressure, *N*-methylcarbamate pesticides were stable under these conditions.

This study demonstrated that ASE automatically and rapidly extracted *N*-methylcarbamate pesticides from foods with good accuracy and precision. ASE could be introduced to determine residual levels of *N*-methylcarbamate pesticides in foods. We have shown that organophosphorus pesticides can be determined using ASE.⁵ We are studying its applicability to multiresidual analysis of other agrochemicals.

References

- 1 Kadenczki, L., Arpad, Z., Gardi, I., Ambrus, A., Gyorft, L., Reese, G., and Ebing, W., *J. Assoc. Off. Anal. Chem.*, 1992, **75**, 53.
- 2 *Pesticide Analytical Manual*, ed. McMahon, B. M., and Wagner, R. F., Food and Drug Administration, Washington, DC, 3rd edn., revised, 1996, vol. 1, pp. 401.
- 3 Richter, B. E., Jones, B. A., Ezzell, J. L., Porter, N. L., Avdalovic, N., and Pohl, C., *Anal. Chem.*, 1996, **68**, 1033.
- 4 Fisher, A. J., Scarlett, J. M., and Stott, D. A., *Environ. Sci. Technol.*, 1997, **31**, 1120.
- 5 Obana, H., Kikuchi, K., Okihashi, M., and Hori, S., *Analyst*, 1997, **122**, 217.
- 6 Dionex Corporation, *ASE 200 Accelerated Solvent Extractor Operator's Manual*, Document N. 031149 Revision 01, Dionex Corporation, Sunnyvale, CA, 1995, Section 3–5.
- 7 Yoshihide, Y., Morimasa, H., and Hirohisa, M., *Shimadzu Hyoron*, 1995, **52**, 113.
- 8 Krause, R. T., *J. Assoc. Off. Anal. Chem.*, 1980, **63**, 1114.
- 9 Nagayama, T., Kobayashi, M., Shioda, H., Morino, M., Ito, M., and Tamura, Y., *J. Food Hyg. Soc. Jpn.*, 1994, **35**, 470.
- 10 Brooks, M. W., and Barros, A., *Analyst*, 1995, **120**, 2479.

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