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Determination of triazine herbicides in foods with liquid chromatography mass spectrometry

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Eight residual triazine herbicides and three metribuzin metabolites in foods were determined by liquid chromatography mass spectrometry (LC-MS) with an atmospheric pressure chemical ionization (APCI) interface, under both positive and negative ion modes. Herbicides were extracted with acetonitrile, and no cleanup procedure was adopted in this method. Four foods were spiked with eight herbicides and three metabolites at 0.05 ppm. The average recoveries of these herbicides usually ranged from 82 to 99% and the relative standard deviations were usually around 10%. These results suggest that LC-MS with APCI can be used to determine residues of triazine herbicides in foods.

Introduction

Triazine derivatives are widely used for control of grassy and broadleaf weeds in fields. Some of these compounds have been detected in natural soils and waters. Some triazines exhibit carcinogenic properties and their use is controversial. Especially, simazine, atrazine and metribuzin are listed among 67 chemicals that are suspected to be endocrine disrupters by the Japan Environment Agency in 1998 (Strategic Programs on Environmental Endocrine Disrupters '98). Therefore, data are needed to determine whether triazine residues are present in the food supply and to ascertain the extent of human exposure.

Several multi-residue methods have been developed for the analysis of triazine residues. Pardue published a methanol extraction procedure followed by a solid-phase extraction (SPE) cleanup for 19 triazines and 4 metabolites in foods.[1] Pensabene *et al*. reported a supercritical fluid extraction (SFE) method trapped with a Florisil cartridge for 10 triazines and 2 metabolites in eggs.[2] Both methods used gas chromatography (GC) equipped with a nitrogen–phosphorus (N/P) detector. Because plant materials contain many nitrogen compounds that can be determined by an N/P detector, a strict cleanup procedure or a special extraction method was needed. Other methods extract the residues with an organic solvent followed by cleanup with an SPE method using strong cation-exchange (SCX) or C18.[3,4] These methods used liquid chromatography (LC) equipped with a UV detector for 8 triazines in milk or 3 triazines in catfish. Some cleanup procedures were also needed to eliminate interferences in UV chromatograms.

Recent methods used mass spectrometry (MS) for the analysis of triazine residues in water samples. Panshin *et al*. used GC-MS for atrazine and 4 of its degradation products in water.[5] Curini *et al*. published a liquid chromatography MS (LC-MS) method using an ionspray interface for monitoring 52 herbicides in environmental water.[6] Ferrer *et al*. reported an LC-MS determination of cyanazine and 2 metabolites in ground water using atmospheric pressure chemical ionization (APCI).[7]

In the present study, 8 triazines and 3 metribuzin metabolites (metribuzin must be determined from the total of 3 metabolites and the parent according to Japanese regulation) were extracted from crops with acetonitrile, and determined using an LC-MS instrument equipped with APCI, without cleanup.

Experimental

Chemicals

Ametryn, simazine, desaminometribuzin (DA), diketometribuzin (DK), desaminodiketometribuzin (DADK) and prometryn were obtained from Wako (Osaka, Japan). Atrazine, cyanazine, dimethametryn and simetryn were obtained from Kanto (Tokyo, Japan). Metribuzin was purchased from Riedel de Haën (Seelze, Germany). The general structure of triazine and the chemical structures of metribuzin and its degradation products are shown in Fig. 1. Each compound was dissolved in acetone to make a 1000 μg ml^{-1} stock standard solution. The stock standard solutions were diluted to 50 μg ml^{-1} with acetone. A mixed solution of 4 μg ml^{-1} of each compound was used to fortify samples and also used for calculation after appropriate dilution. For LC-MS analysis, a mixed solution was dried under a warm air stream and dissolved in 0.5 ml of methanol and made up to 1 ml with water. Acetone, acetonitrile and hexane were of pesticide-analysis grade from Wako. Methanol was HPLC-grade from Wako. Distilled water was obtained by passing through the Milli-RX 12 and Milli-Q SP. TOC. apparatuses (Millipore, Bedford, MA, USA).

Sample preparation

Chinese yam, Japanese radish, potato and rice were purchased at a local market in Osaka and the herbicide residues were confirmed to be below the detectable levels with the proposed method. About 500 g of food was chopped in a food processor MK-K3 (Matsushita, Japan) or a mill MX-X61 (Matsushita) for 5 min to obtain thoroughly mixed homogenates or powders. An aliquot of 20 g of sample homogenate was blended with 50 ml of acetonitrile in a homogenizer HG30 (Hitachi, Japan). In the case of rice, 20 ml of water were added to the powdered rice and the mixture was allowed to stand for 30 min before blending. The extract was filtered through a filter paper No. 5A (Advantec, Japan) into a separation funnel. The residue on the filter paper was washed with 10 ml of acetonitrile, and the washings were added to the filtrate. The extract was shaken vigorously with 5 g of NaCl for 10 min. The extract was left for about 30 min to salt out the water from the acetonitrile. After the

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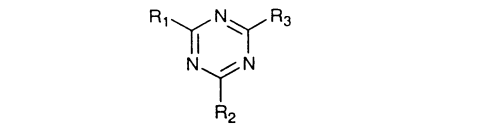
water portion was discarded, the extract was evaporated to dryness at 40 °C. The residue was dissolved in 1 ml of acetone and made up to 5 ml with hexane for GC analysis. An aliquot of 0.5 ml was dried under a warm air stream and dissolved in 0.5 ml of methanol and made up to 1 ml with water for LC-MS analysis.

Liquid chromatographic conditions

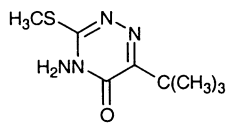
The LC system was compounded of two Jasco PU-980 LC pumps (Jasco, Tokyo, Japan), a Jasco DG-980-50 vacuum degasser, Jasco AS-950 auto sampler, and Jasco CO-965 column oven. Herbicides were chromatographed on a 150 mm × 4.6 mm id octadecylsilica column (Mightysil RP-18, Kanto) utilizing a linear gradient mobile phase of methanol–water (20 + 80) and acetonitrile at a flow rate of 1.0 ml min^{-1}. The acetonitrile percentage was as follows: 30% at 0–2 min, at 30–80% at 2–10 min, 80% at 10–18 min, 80–30% at 18–20 min, and 30% at 20–25 min. The analytical column was maintained at a constant temperature of 50 °C. The sample injection volume was 20 μl.

Mass spectrometric analysis

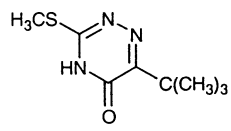
A Platform-II mass spectrometer (Micromass, UK) equipped with an APCI in both positive and negative modes of operation was employed for the determination of the compounds at low levels of concentration. The Microsoft Windows NT based software, Mass lynx, was used to control the instrument and for data acquisition and processing. The positive operating parameters were as follows: corona, 2.00 kV; HV lens, 0.20 kV; and skimmer lens offset, 5 V. The negative operating parameters were the same as the absolute values but negative. In either mode, the source temperature was 140 °C, and the APCI probe temperature was 550 °C. Cone voltages were set to 30 V for negative ions and 40 V for positive ions. The herbicides and their monitor ions are shown in Table 1.



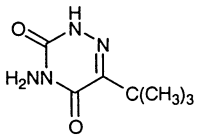
general structure of triazine



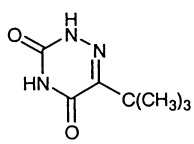
Metribuzin



DA



DK



DADK

compound	R1	R2	R3
cyanazine	-Cl	-NHC2H5	-NHC(CH3)2CN
simazine	-Cl	-NHC2H5	-NHC2H5
simetryn	-SCH3	-NHC2H5	-NHC2H5
atrazine	-Cl	-NHC2H5	-NHCH(CH3)2
ametryn	-SCH3	-NHC2H5	-NHCH(CH3)2
prometryn	-SCH3	-NHCH(CH3)2	-NHCH(CH3)2
dimethametryn	-SCH3	-NHC2H5	-NHCH(CH3)CH(CH3)2

Fig. 1 General structure of triazine and chemical structures of metribuzin and its degradation products, desaminometribuzin (DA), diketometribuzin (DK), and desaminodiketometribuzin (DADK).

Gas chromatographic analysis

GC analysis was carried out with a GC-17A (Shimadzu, Kyoto, Japan) equipped with a flame thermionic detector (FTD) which can detect N and P. A C-R7A (Shimadzu) integrator was used to control the instrument and for data acquisition. A DB-1HT column (J & W Scientific, Folsom, CA), 20 m × 0.25 mm id was programmed from 80 to 100 °C at 20 °C min^{-1}, from 100 to 200 °C at 4 °C min^{-1} and from 200 to 280 °C at 20 °C min^{-1}; a temperature of 80 °C was held for 1 min, and a temperature of 280 °C was held for 5 min. The FTD current was 5 pA. The injector temperature was held at 250 °C and detector temperature at 300 °C. Helium was the carrier gas at 71 kPa and nitrogen was the make-up gas at 75 kPa. The hydrogen and air pressure were 70 kPa and 40 kPa, respectively. The injection volume was 2.0 μl by the splitless mode.

Results and discussion

A comparison of the use of APCI under positive and negative ion mode of operation was carried out. Atrazine, ametryn, simazine, simetryn, prometryn and dimethametryn had no response under negative ion mode but were sensitive under positive ion mode. The sensitivity of cyanazine obtained under positive ion mode was higher than that under negative ion mode. Whereas metribuzin and DA were sensitive under both positive and negative ion mode, DK and DADK responded only under negative ion mode. Cyanazine showed maximum sensitivity in the fragment ion at *m/z* 214, which corresponded to the loss of the cyanogen.[7] Others were measured with the molecular ions that were the most sensitive. Thus, metribuzin metabolites were measured in negative ion mode, while other triazines were measured under positive ion mode. As shown in Fig. 2, most herbicides and metribuzin metabolites were separated under the proposed gradient condition: the tail of simetryn owed its origin to the sulfur isotope and atrazine peaks having overlapped. Metribuzin was also observed at *m/z* 198 under negative ion mode.

Calibration curves of all herbicides and metabolites were constructed from 0.02, 0.1, 0.2, 0.5, 1.0, and 2.0 ppm solutions. The curve of cyanazin was linear in the concentration range from 0.02 to 1.0 ppm; others were linear in the concentration range studied and the correlation coefficients were higher than 0.993 for all the compounds. The limits of detection (LODs) were calculated to be 1–5 ppb for this method, based on a signal-to-noise ratio of 3.

Table 1 Monitor ions of 8 triazine herbicides and 3 metribuzin metabolites

No.	Compound	Time/min	Monitor ion (*m/z*)	Molecular weight	Ion mode	Cone/V
1	DK	3.08	183	184	negative	30
2	DADK	3.35	168	169	negative	30
3	DA	4.10	198	199	negative	30
4	Cyanazine	4.80	214	240	positive	40
5	Simazine	5.18	202	201	positive	40
6	Metribuzin	5.48	215	214	positive	40
7	Simetryn	6.93	214	213	positive	40
8	Atrazine	7.10	216	215	positive	40
9	Ametryn	8.48	228	227	positive	40
10	Prometryn	9.81	242	241	positive	40
11	Dimethametryn	10.58	256	255	positive	40

After the acetonitrile extract was evaporated, many sediments and dissolved NaCl were found in the round bottom flask. To avoid staining the MS detector, acetone and hexane were used to dissolve the residue, because acetone–hexane (1 : 4 v/v) could dissolve herbicides but dissolved only a little of the sediment and no NaCl.

A recovery test was performed with four foods. All the crops have established tolerances for at least some of the herbicides on which the fortification studies were conducted. In the fortification study, 0.25 ml of mixed solution at 4 μg ml^{-1} in acetone was spiked with 20 g of sample homogenate to a final concentration of 0.05 ppm on a sample mass basis and the mixture was left for 30 min before extraction.

As shown in Table 2, the extraction resulted in good recoveries; yields of most of the herbicides were 82–99%, and the relative standard deviations (RSDs) were usually around the 10% levels. Only in Japanese radish was an interference peak found near the peak of DK and the recovery value was affected.

The usefulness of LC-MS was confirmed by comparison with GC-FTD using the same samples. As shown in Fig. 3, GC-FTD could not detect 0.2 ppm of triazines in potato extract. In the chromatogram of the standard, 3 metribuzin metabolites showed lower intensity than other triazines. If an additional strict cleanup were adopted, the LODs of triazines with GC-FTD should be around 20 ppb and the LODs of parent metribuzin and its metabolites should be much higher. On the

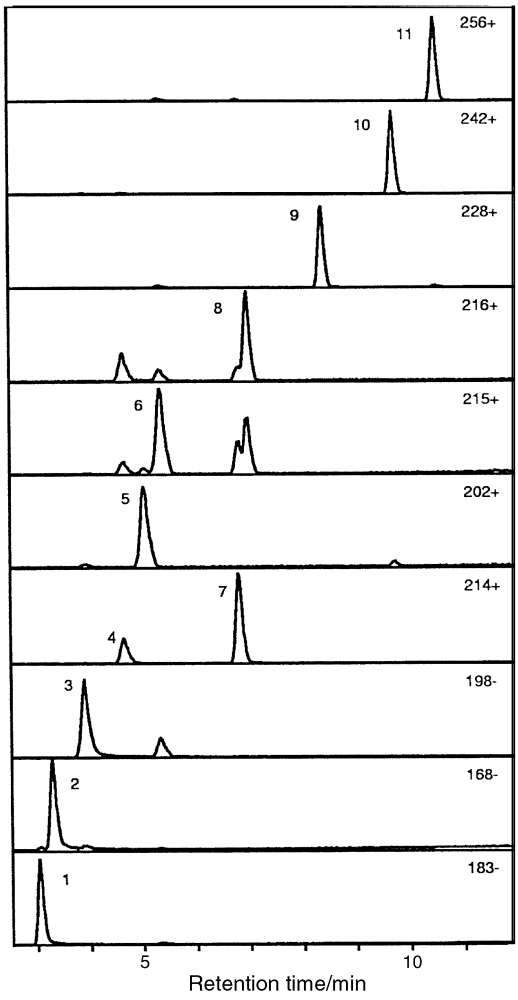


Fig. 2 LC-MS chromatograms of a standard solution (20 μl of 0.2 μg ml^{-1} solution). See Table 1 for identification of peaks. LC-MS conditions are given in the text.

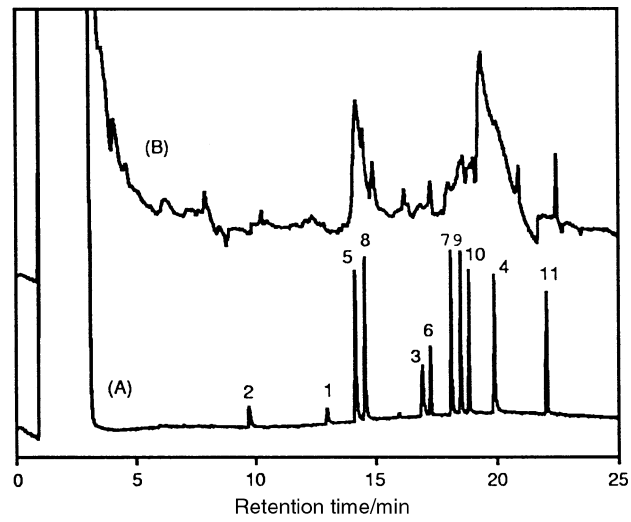


Fig. 3 GC-FTD chromatograms of samples fortified with 0.2 μg g^{-1} of each herbicide. A, standard solution (2 μl of 0.2 μg ml^{-1} solution); see Table 1 for identification of peaks. B, fortified potato.

Table 3 Concentration of metribuzins in potatoes (ppb)

Compound	Boiled	Mashed	Crisps
DK	1	4	ND[b]
DADK	ND[b]	7	ND[b]
DA	ND[b]	ND[b]	ND[b]
Metribuzin	Tr[c]	22	8
Total metribuzin[a]	—	35	8

[a] Total metribuzin = metribuzin + DK × 1.17 + DA × 1.08 + DADK × 1.27. [b] ND = not detected. [c] Tr = trace level detected.

Table 2 Recoveries of 8 triazine herbicides and 3 metribuzin metabolites in foods; n = 5

		Chinese yam		Japanese radish		Potato		Rice		All	
No.	Compound	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
1	DK	78	4	135	22	84	14	83	6	95	30
2	DADK	100	9	80	5	81	12	95	4	89	12
3	DA	89	9	76	6	90	8	83	7	84	10
4	Cyanazine	105	8	84	12	94	7	90	7	93	12
5	Simazine	106	8	89	6	96	3	104	4	99	9
6	Metribuzin	83	6	79	19	91	6	75	4	82	12
7	Simetryn	88	3	88	3	99	3	83	7	89	7
8	Atrazine	93	8	101	11	96	5	89	5	95	9
9	Ametryn	84	3	90	4	96	5	74	8	86	11
10	Prometryn	85	6	88	6	96	6	76	7	86	10
11	Dimethametryn	85	4	86	2	96	3	72	7	85	11

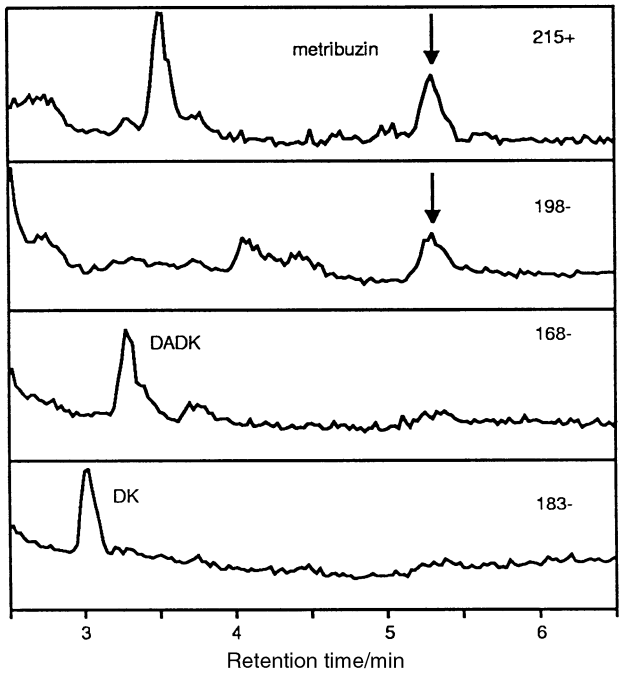


Fig. 4 LC-MS chromatograms of dried mashed potato; see Table 3.

other hand, LC-MS chromatograms of potato extract were almost the same as those of the standard.

Some proposed root crops and potatoes were analyzed by LC-MS. No triazines were found in the fresh food but metribuzin and its metabolites were found in processed potato samples, such as crisps, dried mash and boiled and frozen potatoes (Table 3). They were analyzed without cleanup and serious interferences were not found around their peaks in the chromatograms (Fig. 4). Although metribuzin was detected at a higher level in dried mash than other potato products, it is diluted 5 times with water when served. The levels of metribuzin contamination in processed potatoes were under 10 ppb on the whole.

In this study, 8 triazine herbicides and 3 metribuzin metabolites can be effectively extracted from crops. The LC chromatography is completed within 25 min, and this method includes only extraction and evaporation. Six samples can be analyzed in a day. The sensitivity and accuracy of this method are satisfactory. This method could be used for monitoring triazine herbicides in crops and their products.

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