ガスクロマトグラフ／質量分析計による食品中のジチオカルバメートおよびミルネブの残留分析

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Original

Determination of Dithiocarbamates and Milneb Residues in Foods by Gas Chromatography-Mass Spectrometry

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A highly sensitive gas chromatographic-mass spectrometric (GC-MS) method was developed for dithiocarbamates (DTCs) and milneb in foods. DTCs and milneb were extracted from foods with cysteine-EDTA solution as sodium salts, and methylated with methyl iodide. Methyl derivatives of DTCs and milneb were cleaned up on a neutral alumina mini column and determined by GC-MS. The mean recoveries of DTCs and milneb were in the range of 72-120%, except for methiram. The quantification limits were 0.01 mg/kg (as CS_2) in foods except tea (0.1 mg/kg as CS_2). The developed method was applied to 10 compounds (4 dimethyldithiocarbamates, 3 ethylenebisdithiocarbamates, polycarbamates, propineb and milneb).

Key words: dithiocarbamate; milneb; methylation; GC-MS; agricultural products; animal and fishery products

Introduction

Dithiocarbamates (DTCs) are widely used as fungicides for fruit trees, vegetables and ornamental plants because they exhibit low toxicity to humans and crops while acting against a broad spectrum of plant diseases. DTCs are also used as vulcanization accelerators and antioxidants in the rubber industry.

DTCs can be classified into three groups: dimethyldithiocarbamates (DMDC) such as ziram, thiram and ferbam, ethylenebisdithiocarbamates (EBDC) such as maneb, zineb and mancozeb, and propylenebisdithiocarbamates (PBDC) such as propineb.

The classical analytical methods for DTCs residues are based on the generation of CS_2 by heating with stannous chloride and hydrochloric acid[1]. In these methods, there is no clear differentiation between DMBC, EBDC and PBDC, and sample blanks interfere with precise determination in the measurement of some crops. Therefore, several attempts have been made to analyze DTCs by means of liquid chromatography (LC) for selective trace determination. Gustafsson *et al.* developed a method of liquid chromatography for methyl esters on reversed-phase columns[2),3)]. Kibune *et al.* improved the method with a column of macroporous diatomaceous earth[4)]. The problem with HPLC is that UV detection cannot achieve the detection limit of 0.01 mg/kg (as CS_2), which is the uniform limit in the positive list system for agricultural chemical residues in foods in Japan. Because of the capability for high selectivity and sensitivity, several analytical methods using LC-MS (/MS) have been reported[5)-7)].

However due to decomposition in contact with water[8)] and plant juice, samples cannot be mixed using a homogenizer and are usually cut in pieces. Therefore, sample preparation can result in variability of the analytical values.

For the purposes of preventing decomposition and improving sample homogeneity and sensitivity, a highly sensitive GC-MS method was developed for determination of DMBC, EBDC, PBDC and milneb in a wide range of foods.

Materials and Methods

Food samples

Ten agricultural products and 9 animal and fishery products were purchased from markets in Tokyo. Agricultural products were brown rice, soybean, potato, spinach, cabbage, apple, orange, pumpkin, cacao and green tea. Animal and fishery products were cattle muscle, cattle fat, cattle liver, salmon, eel, milk, chicken egg, honey and shrimp.

Chemicals and reagents

DTCs used as reference analytical standards were thiram (99.9% purity), and polycarbamate (98.2% purity), purchased from Kanto Chemical Co., Inc. (Tokyo, Japan); ziram (97.7% purity), ferbam (99.3%

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purity), zineb (80.5% purity), mancozeb (91.0% purity) and maneb (90.2% purity), purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan); nickel bis (dithiocarbamate) (97.9% purity), milneb (94.0% purity), methyl dimethyldithiocarbamate (99.9% purity), dimethyl ethylenebisdithiocarbamate (98.7% purity) and dimethyl propylenebisdithiocarbamate (99.8% purity), purchased from Hayashi Pure Chemical Ind., Ltd. (Osaka, Japan); propineb (100.0% purity) and methiram (100.0% purity), purchased from Sigma-Aldrich Co. (USA). Values of measured weight were corrected according to the purity of each DTC.

Acetone, dichloromethane and *n*-hexane (pesticide residue grade) were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Diethyleneglycol (DEG), sodium chloride, hydrochloric acid, L-cysteine hydrochloride monohydrate, sodium hydroxide, methyl iodide, disodium dihydrogen ethylenediamine tetraacetate dehydrate (EDTA-2Na) and tetrabutylammonium hydrogen sulfate (TBA) of analytical grade were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan).

The solid-phase materials used were Chem Elut (20 mL) purchased from Varian, Inc. (CA, USA) and Sep-Pak Plus Alumina N (1,710 mg) purchased from Waters, Co. (MA, USA).

A cysteine-EDTA solution was prepared by dissolving 50 g of EDTA-2Na and 50 g of L-cysteine hydrochloride monohydrate in 1,000 mL of water and adjusting to pH 9.6–10.0 with 12 mol/L sodium hydroxide solution.

A methyl iodide solution was prepared by adding 6 mL of methyl iodide to a mixture of 792 mL of *n*-hexane and 108 mL of acetone.

0.4 mol/L TBA solution was prepared by dissolving 27.16 g of TBA in 200 mL of water.

Apparatus and GC-MS conditions

The following equipment was used: Agilent 6890/5973 gas chromatograph/mass spectrometer (Agilent Technologies, Inc., USA) equipped with a DB-17 capillary column, 30 m×0.25 mm i.d., film thickness 0.25 μm (Agilent Technologies, Inc., USA).

The injection port was held at 240℃. The temperature program for the capillary column was started at 70℃, and raised after 2 min at 20℃/min to 280℃. The mass spectrometer was operated in electron-ionization mode with an ionization voltage of 70 eV. DTCs were detected by selected ion monitoring of the major ions at *m/z* 88 (DMDC), *m/z* 144 (EBDC) and *m/z* 158 (PBDC).

Standard solutions

The stock standard solutions were prepared as follows. Ziram, maneb and propineb (each 5 mg, as CS_2) were individually dissolved in 50 mL of cysteine-EDTA solution to prepare 100 mg/L (as CS_2) stock solutions.

The working solutions were prepared as follows: 1 mL of stock solution and 10 mL of 0.4 mol/L TBA solution were added to 150 mL of cysteine-EDTA solution. The solution was adjusted to pH 7.5–7.7 with 6 mol/L hydrochloric acid and made up to 200 mL with water. Twenty grams of sodium chloride was added to 20 mL of the diluted solution. The mixture was applied to a Chem Elut column and left to stand for 10 minutes. For methylation and elution, 60 mL of methyl iodide solution was applied to the column. The eluate was evaporated to 2 mL after adding 0.5 mL of 1% DEG in acetone. The concentrated solution was diluted to volume with acetone in a 200 mL volumetric flask. The working solutions for GC-MS were further diluted with acetone to the required concentration. Ziram was used as the reference analytical standard of DMDC, maneb as the reference analytical standard of EBDC, and propineb as the reference analytical standard of PBDC.

Sample preparation and extraction

In the case of grains, beans, nuts and seeds, the samples were pulverized into particles that could be passed through a 425 μm standard sieve. Animal and fishery products were homogenized with a blender. Ten grams of the homogenous sample was weighed into a centrifuge tube, and 100 mL of cysteine-EDTA solution and 50 mL of dichloromethane were added. The mixture was homogenized and centrifuged at 2,500 rpm for 5 min. The cysteine-EDTA solution layer (upper layer) was transferred to a volumetric flask with a komagome pipet, and 50 mL of cysteine-EDTA solution was added to the residue in the tube. The mixture was homogenized and centrifuged again. The resulting cysteine-EDTA solutions were combined.

In the case of fruits and vegetables, 500 g of cysteine-EDTA solution was added to 1 kg of sample, and the mixture was homogenized with a blender. The homogenous sample corresponding to 20 g was weighed into a centrifuge tube. Then 80 mL of cysteine-EDTA solution and 50 mL of dichloromethane were added, and the mixture was homogenized and centrifuged at 2,500 rpm for 5 min. The cysteine-EDTA solution layer (upper layer) was transferred to a volumetric flask with a komagome pipet, and 50 mL of cysteine-EDTA solution was added to the residue in the tube. The mixture was homogenized and centrifuged again. The resulting cysteine-EDTA solutions were combined.

In the case of tea, the sample was pulverized with a blender. Five grams of the powdered sample was weighed into a centrifuge tube, and 100 mL of cysteine-EDTA solution and 50 mL of dichloromethane were added. The mixture was homogenized and centrifuged at 2,500 rpm for 5 min. The cysteine-EDTA solution layer (upper layer) was transferred to a volumetric flask with a komagome pipet, and 50 mL of cysteine-EDTA solution was added to the residue in the tube. The mixture was homogenized and centrifuged again. The resulting cysteine-EDTA solutions were combined.

Methylation

An aliquot of 10 mL of TBA solution was added to the sample solution, and the mixture was adjusted to pH

7.5–7.7 with 6 mol/L hydrochloric acid and made up to 200 mL with water. Four grams of sodium chloride was added to 20 mL of the sample solution and dissolved in an ultrasonic bath. The solution was applied to a Chem Elut column, then the column was left to stand for 10 min and eluted with 60 mL of methyl iodide solution at the rate of 1 mL/min. The eluate was evaporated to dryness on a rotary evaporator at below 40℃ after adding 0.5 mL of 1% DEG in acetone. The residue was dissolved in acetone to 5 mL. DEG was effective in prevention of loss of DTCs by vaporization.

Mini-column purification

The sample solution in acetone was applied to a Sep-Pak Alumina N mini-column preconditioned with 5 mL of acetone. Methyl derivatives of DTCs were eluted with 20 mL of acetone. The eluate from the column was evaporated to dryness on a rotary evaporator at below 40℃ after addition of 0.5 mL of 1% DEG in acetone. The residue was dissolved in acetone to 2 mL in the case of grains, beans, nuts, seeds and teas and animal and fishery products, and 4 mL in the case of fruits and vegetables. An aliquot of 2 μL of the solution was injected into the GC-MS.

Results and Discussion

GC-MS conditions

In the determination of DTCs, HPLC with UV detection is widely used. However, this approach is insuffiently sensitive. As previously reported, the quantification limit of DTCs is 0.05 mg/kg by HPLC[4]. To improve the selectivity and sensitivity, analytical methods using LC-MS (/MS) were proposed,[5)-7)] but we found that ion suppression was an issue with some of our samples. Therefore, we adopted GC/MS.

Mass spectra of DMDC, EBDC and PBDC are shown in Fig. 1. As there were several interfering peaks on the DMDC ion chromatogram at *m/z* 135, the ion of *m/z* 88 was selected as the monitoring ion. The ions of *m/z* 144 of EBDC and *m/z* 158 of PBDC provided good selectivity.

DB-1, DB-5 and DB-17 capillary GC columns were investigated. The best separation between DTCs and other compounds was observed using DB-17. Chromatograms of the DTCs are shown in Figs. 2 and 3.

Linearity of calibration plots

The calibration curves for reference standard solutions at 5 concentrations were obtained by the linear least-squares regression procedure, plotting peak height data against weight as CS_2. Satisfactory linearity was obtained in the range from 0.005 to 0.1 ng for DMDC, EBDC and PBDC. The correlation coefficients (r^2) were over 0.999.

Sample preparation

Because DTCs are degraded by contact with plant juice or water, fruits and vegetables should not be homogenized. Therefore, sample homogeneity was

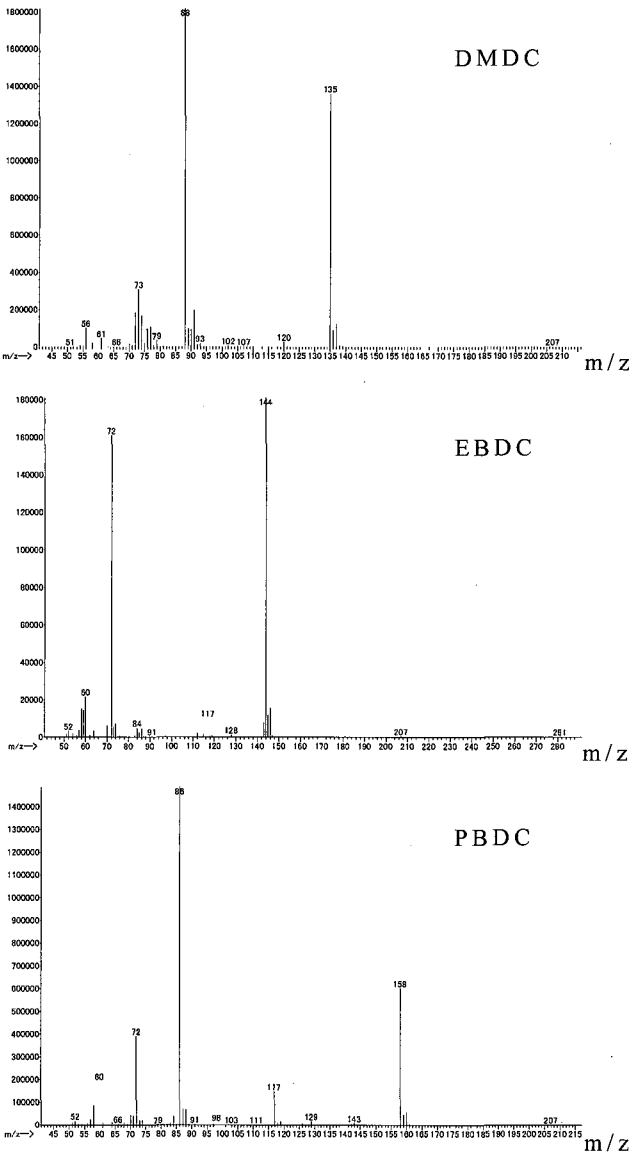

Fig. 1. Mass spectra of DTCs obtained by GC-MS

poor. We investigated the effect of adding cysteine–EDTA solution to the sample prior to homogenizing with a blender. Higher recoveries were obtained than those without cysteine–EDTA solution (Fig. 4). In the case of DTCs from dry samples and animal and fishery products, sufficient recoveries were obtained, so the addition of cysteine–EDTA solution during sample preparation was not necessary.

Extraction

As previously reported, DTCs were extracted as sodium salts[2)-4)]. Dichloromethane was added for defatting. As the cysteine–EDTA solution layer was the upper layer after centrifugation, it was easy to transfer the extract. Because the environmental quality standard for water pollution of dichloromethane is 0.02 mg/L, care is needed in the disposal of waste water.

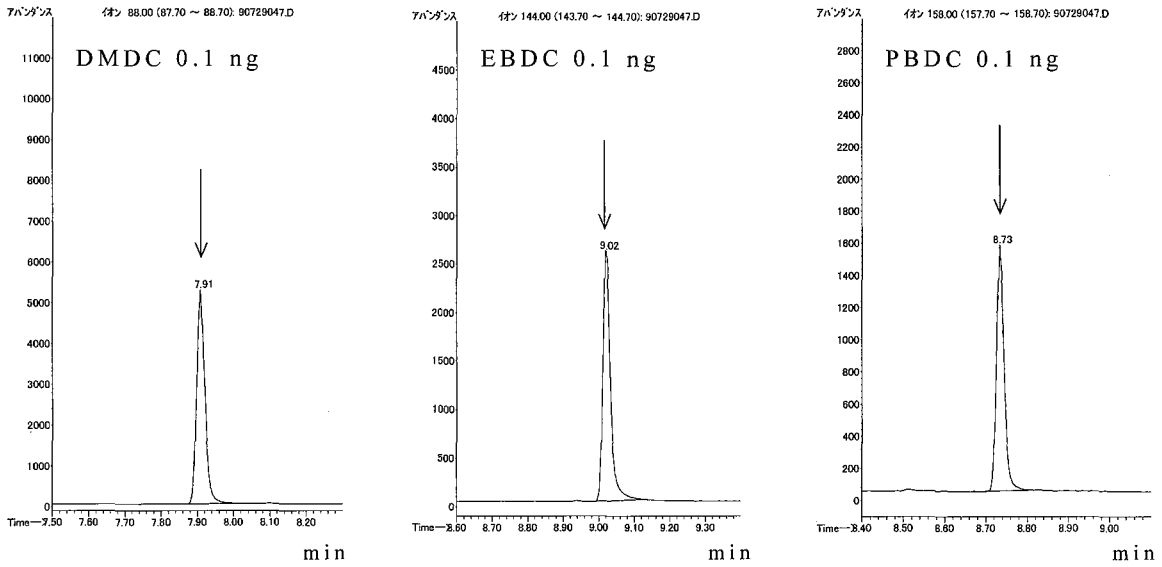

Fig. 2. GC-MS chromatograms of DTCs

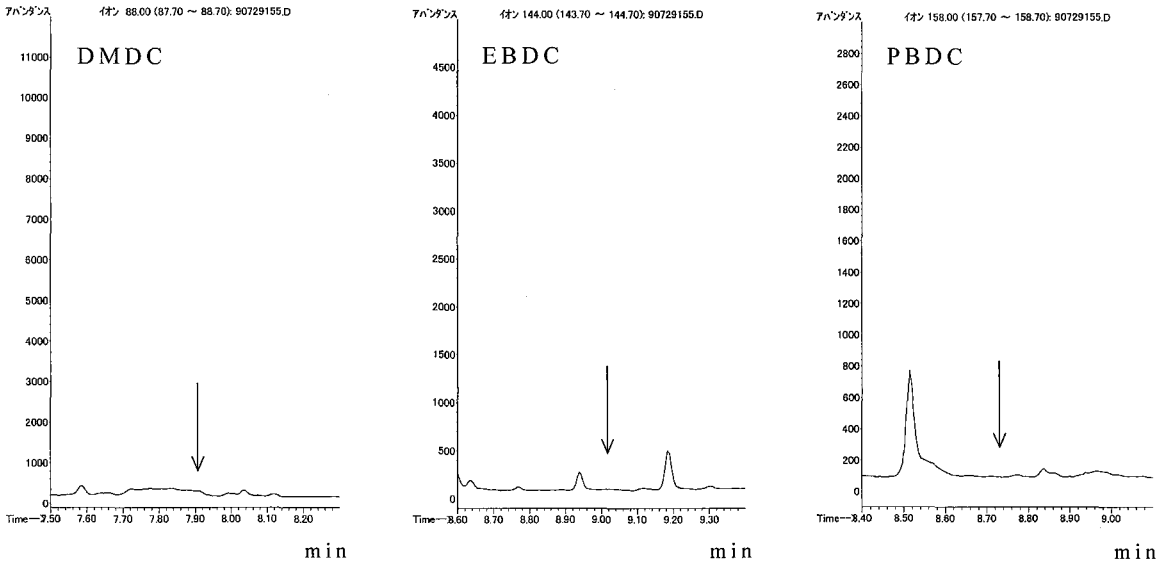

Fig. 3. GC-MS chromatograms of blank sample of spinach

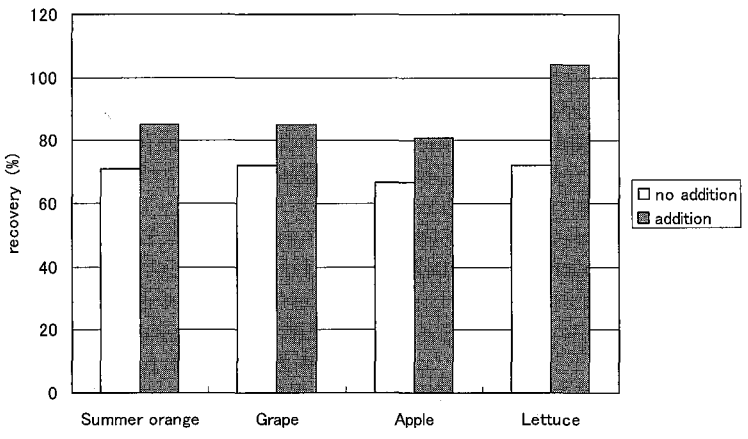

Fig. 4. Effect of addition of cysteine–EDTA solution

Methylation

Methylation was carried out in the Chem Elut column to prevent emulsion formation[4]. TBA was used as an ion pair reagent and methyl iodide was used as a methylation reagent. The rates of methylation at methyl iodide concentrations of 0.01, 0.05, 0.1, 0.2 and 0.5 mol/L are shown in Table 1. The highest levels of methylation were observed at 0.1 mol/L. Methyl derivatives of DTCs were extracted with a mixture of hexane and acetone (Table 2). When the proportion of *n*-hexane to acetone was 85 to 15, water was eluted slightly. On

Table 1. Effect of methyl iodide concentration on degree of methylation* (%)

	Methyl iodide concentration (mol/L)				
	0.01	0.05	0.1	0.2	0.5
Ziram	67	97	100	102	99
Maneb	59	95	100	90	72
Propineb	68	98	100	91	78

* Defined as 100% when methyl iodide concentration was 0.1 mol/L.

Table 2. Effect of elution solvent on degree of methylation (%)

	n-Hexane–acetone containing 0.1 mol/L methyl iodide					
	98 : 2	95 : 5	93 : 7	90 : 10	88 : 12	85 : 15
Ziram	94	96	98	104	103	107
Maneb	25	79	82	87	91	93
Propineb	58	73	73	76	81	81

Table 3. Degree of methylation of various compounds with 0.1 mol/L methyl iodide solution

Compounds	Rate of methylation (%)
Ziram	94
Thiram	90
Nickel bis(dithiocarbamate)	87
Ferbam	93
Propineb	78
Zineb	89
Mancozeb	87
Maneb	89
Methiram	0
Milneb	71
Polycarbamate	87

Table 4. Elution recovery of DMDC, EBDC and PBDC from Alumina N mini-column

	Acetone (mL)			
	0–15	15–20	20–25	25–30
DMDC	76	7	0	0
EBDC	87	18	0	0
PBDC	81	27	5	0

Table 5. Recoveries of DTCs from spiked agricultural products

Sample	MRL (ppm)	Spiked level (mg/kg)		Ziram	Thiram	Ferbam	Nickel bis(dithiocarbamate)	Propineb	Maneb	Zineb	Mancozeb	Polycarbamate	Milneb
Brown rice	0.3	0.1	Mean (%)	93	100	87	99	94	92	106	84	86	74
			RSD (%)	2.1	4.8	9.0	1.3	4.8	6.4	5.2	3.2	2.7	7.3
Soybean	3	0.1	Mean (%)	93	85	85	87	81	86	93	75	80	77
			RSD (%)	5.6	5.3	7.4	4.3	10.6	1.3	2.6	3.7	3.4	7.9
Potato	0.2	0.1	Mean (%)	90	92	90	93	94	96	120	117	110	81
			RSD (%)	4.3	2.4	8.0	12.1	4.9	4.7	2.8	3.2	2.2	4.6
Spinach	0.2	0.1	Mean (%)	87	95	102	98	82	87	119	109	95	85
			RSD (%)	7.7	2.4	7.3	9.5	3.3	2.4	3.9	3.7	2.4	1.9
Cabbage	5	0.1	Mean (%)	87	91	86	96	72	86	82	99	98	76
			RSD (%)	2.7	4.4	2.5	4.5	3.3	3.4	10.8	6.9	3.8	3.0
Apple	5	0.1	Mean (%)	88	84	87	93	86	96	106	93	92	77
			RSD (%)	3.0	4.4	10.9	8.9	2.4	2.6	9.3	7.4	4.0	8.9
Orange	2	0.1	Mean (%)	88	85	95	82	83	91	116	95	101	88
			RSD (%)	1.1	3.4	2.0	10.7	8.4	7.4	2.0	3.1	1.1	6.3
Green tea	5	0.2	Mean (%)	94	94	105	105	83	94	100	96	116	81
			RSD (%)	5.3	4.9	1.7	6.2	7.1	6.2	4.2	5.9	6.2	4.2
Pumpkin	1	0.1	Mean (%)	86	90	94	90	104	109	115	105	103	83
			RSD (%)	4.5	12.0	6.7	10.6	5.6	4.8	4.4	13.5	5.1	8.1
Cacao	0.02	0.02	Mean (%)	91	104	90	86	90	109	116	110	89	105
			RSD (%)	3.4	7.9	9.9	9.5	6.0	9.6	6.3	6.9	3.6	10.5

Mean of 5 replicates
RSD=relative standard deviation
MRL and spiked level: concentration as CS_2

the basis of this result, we decided to use a mixture of hexane and acetone (88 : 12) containing 0.1 mol/L methyl iodide.

The rates of methylation for 11 compounds methylated with 0.1 mol/L methyl iodide are shown in Table 3. Methiram was not methylated. The methyl derivative of milneb gave the same mass spectrum as that of EBDC and showed the same retention time in GC-MS. Therefore, the methyl derivative of milneb was the same as EBDC. The rates of methylation of propineb and milneb were under 80%. Therefore, commercial methyl derivatives were not used as reference analytical standards. Ziram, maneb and propineb were used as reference analytical standards and these compounds were methylated in the same manner as the samples. The repeatability of the methylation was investigated. Three replicate of methylations of the three compounds were tested in a day and the tests were repeated on another day. Coefficients of variation of methylation of ziram, maneb and propineb were 2.0, 2.5 and 2.3%, respectively.

Clean-up

Clean-up using a Sep-Pak Alumina N mini-column was investigated to remove interfering components. Clean-up procedure was necessary for a wide range of foods. The methyl derivatives of DTCs were eluted with acetone. The elution recoveries from the mini-column are shown in Table 4. DMDC, EBDC and PBDC were eluted with 20 mL of acetone.

Recovery test from foods

Recovery tests were carried out using 19 samples that had been confirmed to contain no DTCs. These samples were analyzed by the proposed method. The mean recoveries from agricultural products in five experiments are shown in Table 5, and the recoveries from animal and fishery products are shown in Table 6. Mean recoveries were in the range of 72–120%, and the relative standard deviations were in the range of 1.1–14.3%. The S/N ratios were 10 or higher in all samples, and the quantification limits were 0.01 mg/kg (as CS_2), except for tea (0.1 mg/kg as CS_2).

Conclusion

A reliable method using GC-MS has been developed for the determination of DTCs and milneb in foods. The method consists of extraction with cysteine–EDTA solution, methylation with methyl iodide in a Chem-Elut column, clean-up on a Sep-pak Alumina N mini-column, and determination using GC-MS. Because methiram was not methylated by methyl iodide, this method could not be applied to it. The methyl derivative of milneb is the same as that of EBDC and the concentration of EBDC was the sum of EBDC and milneb.

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Table 6. Recoveries of DTCs from spiked animal and fishery products

Sample	MRL (ppm)	Spiked level (mg/kg)		Ziram	Thiram	Ferbam	Nickel bis(dithiocarbamate)	Propineb	Maneb	Zineb	Mancozeb	Polycarbamate	Milneb
Cattle muscle	0.05	0.05	Mean (%)	103	85	89	89	94	91	97	90	101	86
			RSD (%)	3.2	6.8	6.1	7.2	5.9	10.1	6.7	10.4	5.0	13.2
Cattle fat	0.05	0.05	Mean (%)	99	106	91	80	91	91	105	96	98	92
			RSD (%)	4.0	2.2	8.9	6.4	9.4	7.9	5.8	2.4	8.3	7.3
Cattle liver	0.1	0.1	Mean (%)	89	83	109	84	88	84	77	88	75	71
			RSD (%)	3.5	3.3	6.9	9.9	6.6	5.2	7.3	1.9	5.1	8.0
Salmon	—	0.02	Mean (%)	88	101	97	105	113	112	105	93	115	108
			RSD (%)	5.5	10.2	2.4	4.3	6.8	3.9	9.9	3.6	8.0	9.8
Eel	—	0.02	Mean (%)	95	90	93	108	91	89	101	87	113	96
			RSD (%)	4.6	5.8	3.2	10.2	1.6	3.7	6.3	4.8	4.1	4.6
Milk	0.05	0.05	Mean (%)	94	94	73	94	92	92	100	83	101	93
			RSD (%)	4.3	5.9	11.6	7.5	5.3	11.9	5.1	9.8	5.9	9.2
Chicken egg	0.05	0.05	Mean (%)	80	93	79	74	82	87	91	83	85	90
			RSD (%)	6.2	4.6	9.9	11.5	4.0	4.3	5.4	10.4	4.4	6.9
Honey	—	0.02	Mean (%)	103	105	98	111	103	113	120	89	109	104
			RSD (%)	3.4	8.1	3.3	3.2	9.1	7.9	1.9	8.9	2.9	7.5
Shrimp	—	0.02	Mean (%)	88	95	74	101	78	92	96	90	109	109
			RSD (%)	2.5	8.2	5.8	4.2	6.2	2.5	8.7	5.8	7.1	10.7

Mean of 5 replicates
RSD=relative standard deviation
MRL and spiked level: concentration as CS_2

References

1) Keppel, G. E. Collaborative study of the determination of dithiocarbamate residues by a modified carbon disulfide evolution method. J. AOAC, **54**, 528–532 (1971).
2) Gustafsson, K. H., Thompson, R. A. High-pressure liquid chromatographic determination of fungicidal dithiocarbamates. J. Agric. Food Chem., **29**, 729–732 (1981).
3) Gustafsson, K. H., Fahlgren, C. H. Determination of dithiocarbamate fungicides in vegetable foodstuffs by high-performance liquid chromatography. J. Agric. Food Chem., **31**, 461–463 (1983).
4) Kibune, N., Higasisaka, N., Nakamura, M., Maekawa, Y. Rapid analysis method of dithiocarbamate pesticides in agricultural products by high performance liquid chromatography. Shokuhin Eiseigaku Zasshi (J. Food Hyg. Soc. Japan), **36**, 244–251 (1995).
5) Crnogorac, G., Schwack, W. Determination of dithiocarbamate fungicide residues by liquid chromatography/mass spectrometry and stable isotope dilution assay. Rapid Commun. Mass Spectrom., **21**, 4009–4016 (2007).
6) Crnogorac, G., Schmauder, S., Schwack, W. Trace analysis of dithiocarbamate fungicide residues on fruits and vegetables by hydrophilic interaction liquid chromatography/tandem mass spectrometry. Rapid Commun. Mass Spectrom., **22**, 2539–2546 (2008).
7) Hayama, T., Takada, M. Simple and rapid method for the determination of ethylenebisdithiocarbamate fungicides in fruits and vegetables using liquid chromatography with tandem mass spectrometry. Analyt. Bioanalyt. Chem., **392**, 969–976 (2008).
8) Lyman, W. R., Lacoste, R. J. New developments in the chemistry and fate of ethylenebis(dithiocarbamate) fungicides. Environ. Qual. Saf., Suppl. **3**, 67–74 (1975).

定量 NMR に基づく既存添加物中のクエルセチンおよびクエルセチン配糖体の絶対定量（報文）

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われわれは，国際単位系 (SI) にトレーサブルな有機化合物の絶対定量法として，定量 NMR (quantitative NMR: qNMR) の開発を行っている．本研究では qNMR を応用し，既存添加物ルチン（抽出物），ルチン酵素分解物およびクエルセチンの各添加物製品中のルチン，イソクエルシトリンおよびクエルセチンや，これら化合物の市販試薬の絶対定量を行った．今回新たに，計量学的に正確に値付けされた1,4-ビストリメチルシリルベンゼン-d_4 (1,4-BTMSB-d_4) を qNMR 基準物質として用い，そのメチル基と測定化合物の各 2′ 位プロトンとのシグナル積分値比から含量を算出し，より簡便な 1 段階の qNMR 測定を行った．その結果，qNMR を用いることにより，分離操作を行うことなく，かつ，測定対象化合物と同一の標準品を必要とせずに，ルチン，イソクエルシトリンおよびクエルセチンの定量が可能であることを見いだした．

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ガスクロマトグラフ/質量分析計による食品中のジチオカルバメートおよびミルネブの残留分析（報文・英文）

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食品中のジチオカルバメートおよびミルネブの高感度な GC-MS 分析法を開発した．ジチオカルバメートおよびミルネブを農作物および畜水産物からシステイン–EDTA 溶液でナトリウム塩として抽出し，ヨウ化メチルでメチル化した．ジチオカルバメートおよびミルネブのメチル化物を中性アルミナを用いて精製した後，GC-MS で測定した．メチラムを除くジチオカルバメートおよびミルネブの平均回収率は 72～120% の範囲であった．食品における定量限界は二硫化炭素として 0.01 mg/kg であった（茶は 0.1 mg/kg）．今回開発した方法は 10 物質（ジメチルジチオカルバメート 4 物質，エチレンビスジチオカルバメート 3 物質，ポリカーバメート，プロピネブおよびミルネブ）に適用可能であった．

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ポリ乳酸製器具・容器包装の含有物質の検討および溶出液の変異原性（報文）

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ポリ乳酸製器具・容器包装 7 検体について食品衛生法における規格試験を実施した．さらに，その他の含有物質や溶出物質の検討を ICP-AES および GC/MS を用いて行い，溶出液について 2 種類の変異原性試験を実施した．その結果，すべての試料が食品衛生法における規格基準を満たしており，金属の溶出もほとんど見られなかった．溶出液の GC/MS によるピーク検索の結果，大きなピークは見られず，レックアッセイおよび *umu*-テストの両方の試験においてすべての試料が陰性を示した．*umu*-テストにおいて汁椀の溶出液が β-ガラクトシダーゼ活性を若干増加させたが，素地であるポリ乳酸からの溶出物によるものではなく，塗装面のポリウレタンによるものと推測された．

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ナイロン製品からのモノマーおよび芳香族第一級アミン類の溶出（報文）

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おたま，フライ返し，ラップフィルムなどナイロン製品 21 試料について，熱分解ガスクロマトグラフィー (Py-GC/MS) を用いてそのナイロンの種類を判別するとともに，モノマー 2 種類および芳香族第一級アミン類 (PAAs) 21 種類の溶出量を LC/MS/MS により測定した．試料の材質はナイロン 6 が 1 検体，ナイロン 66 が 15 検体，ナイロン 6/66 共重合体が 3 検体，ナイロンと PE，PP のラミネートが 2 検体であった．ただし，ナイロン 66 製品はナイロン 6 のモノマーである ε-カプロラクタム (CPL) も含有していた．また，20% エタノール 60℃ 30 分間でのモノマーおよび PAAs の溶出量は，ラップフィルム 1 検体を除くすべての検体から CPL が 0.015～38 μg/mL，すべてのナイロン 66 製品とナイロン 6/66 製品 1 検体から 1,6-ヘキサメチレンジアミンが 0.002～0.013 μg/mL 検出された．また，4,4′-ジアミノジフェニルメタンが 3 検体から 0.006～4.3 μg/mL，アニリンが 4 検体から 0.032～0.23 μg/mL，その他 4-クロロアニリンが 2 検体から各 0.001 μg/mL，2-トルイジンおよび 1-ナフチルアミンがそれぞれ 1 検体ずつから 0.002 および 0.066 μg/mL 検出された．さらに，95℃ および 121℃ 30 分間では各溶出量が 95℃ では 60℃ の約 3 倍，121℃ では約 10 倍に増加した．

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細菌培養処理法（A3T 法）による二枚貝からのノロウイルス遺伝子の検出（ノート）

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RT-PCR やリアルタイム PCR などの高感度な遺伝子検査法を用いても，食品からノロウイルス (NV) が検出される事例は非常に少ない．われわれは，食品成分由来の夾雑物が検査に与える影響に着目し，その除去方法として細菌を利用した処理方法（A3T 法）を考案した．今回，実際の食品検査における同法の有用性を検証することに加え，二枚貝の NV 汚染状況をより明らかにするため，市場に流通する二枚貝や NV を原因とする食中毒事件との関連が疑われた生食用カキを対象に NV 検出を試みた．二枚貝 111 検体の検査では，A3T 法では 20 検体（18.0%）から NV が検出されたが，厚生労働省通知による検査法で NV が検出されたのは 1 検体（0.9%）のみであった．また，食中毒事件関連の生食用カキ 35 検体を用いた検査では，A3T 法により 10 検体が NV 陽性となったが，通知法では検出されなかった．A3T 法は簡易な操作を加えるだけで NV 検出率の向上が図れることから，日常検査に適した手法と考えられた．

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遺伝子組換え (GM) ダイズ新系統 MON89788 の系統特異的定量検知法の開発および性能指標の評価（ノート・英文）

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GM ダイズ新系統 MON89788 の系統特異的定量分析法を開発し，その性能指標を評価した．内標比とは，各 GM 系統に固有であり，混入率算出の際に必要となる係数であるが，本研究によって MON89788 の内標比が実験的に決定された．さらに，さまざまな濃度の MON89788 を含む疑似混入試料を調製し，単一試験室あるいは複数試験室において性能指標を明らかにしたところ，本分析法の定量下限値は 0.1% 以下と見積もられ，偏差，室間再現性ともに 20% を下回る結果が得られた．以上の結果から，本分析法は検査に適用可能であることが示された．

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