Analytical Method for Dithiocarbamates
(Agricultural, Animal and Fishery Products)

1. Analyte

Zineb
Ziram
Thiram
Nickel bis (Dithiocarbamates)
Ferbam
Propineb
Polycarbamate
Mancozeb
Maneb

2. Applicable foods

Agricultural, Animal and Fishery Products

3. Instrument

Gas chromatograph-mass spectrometer (GC-MS)

4. Reagents

Use reagents listed in Section 3 of the General Rules, except the following.

Cysteine-ethylenediaminetetraacetic acid solution (hereinafter referred to as "cysteine-EDTA solution"): Dissolve 50 g of L-cysteine hydrochloride monohydrate and 50 g of disodium dihydrogen ethylenediamine tetraacetic acid dihydrate in approximately 500 mL of water, adjust pH to 9.6-10.0 with 12 mol/L of sodium hydroxide solution, and add water to make 1,000 mL (prepare at time of use).

Methylated solution: Add 6 mL of methyl iodide to a mixture of 792 mL of *n*-hexane and 108 mL of acetone.

Tetrabutylammonium hydrogen sulfate solution: Dissolve 27.2 g of tetrabutylammonium hydrogen sulfate in water to make 200 mL.

Reference standard of ziram: Contains not less than 96% of ziram.

Reference standard of propineb: Contains not less than 75% of propineb.

Reference standard of maneb: Contains not less than 75% of maneb.

5. Procedure

1) Extraction

i) Grains, legumes, nuts, seeds, animal and fishery products

Weigh 10.0g of sample (when an individual sample is small in size such as freshwater clam, weigh exactly the sample, add the same amount of cysteine-EDTA solution in weight ratio, grind and homogenize, and weigh the sample equivalent to 10.0 g). Add

100 mL of cysteine-EDTA solution (90 mL for such as freshwater clam) and 50 mL of dichloromethane, homogenize, and centrifuge at 2,500 rpm for 5 min. Remove a cysteine-EDTA layer, add 50 mL of cysteine-EDTA solution to the residue, homogenize, centrifuge as the same condition described above. And combine the cysteine-EDTA layer. Add 10 mL of tetrabutylammonium hydrogen sulfate solution, adjust pH to 7.5-7.7 with 6 mol/L of hydrochloric acid, and add water to make exactly 200 mL.

ii) Fruits and vegetables

Weigh approximately 1 kg of sample accurately, add 1 kg of cysteine-EDTA solution, homogenize, and then weigh the sample equivalent to 20.0 g.

Add 80 mL of cysteine-EDTA solution and 50 mL of dichloromethane, homogenize, and centrifuge at 2,500 rpm for 5 min. Remove a cysteine-EDTA layer, add 50 mL of cysteine-EDTA solution to the residue, homogenize, centrifuge as the same condition described above, and combine the cysteine-EDTA layer. Add 10 mL of tetrabutylammonium hydrogen sulfate solution, adjust pH to 7.5-7.7 with 6 mol/L of hydrochloric acid, and add water to make exactly 200 mL.

iii) Tea leaves

Add 100 mL of cysteine-EDTA solution and 50 mL of dichloromethane to 5.00 g of sample, homogenize, and centrifuge at 2,500 rpm for 5 min. Remove a cysteine-EDTA layer, add 50 mL of cysteine-EDTA solution to the residue, homogenize, centrifuge as the same condition described above, and combine the cysteine-EDTA layer. Add cysteine-ETDA solution to make exactly 200 mL. Take exactly a 40 mL aliquot of the solution, add 110 mL of cysteine-ETDA solution and 10 mL of tetrabutylammonium hydrogen sulfate solution, adjust pH to 7.5-7.7 with 6 mol/L of hydrochloric acid, and add water to make exactly 200 mL.

2) Methylation

Take exactly a 20 mL aliquot from the solution obtained in 1), dissolve 4 g of sodium chloride. Transfer the solution to a porous diatomaceous earth cartridge (for holding 20 mL of solution), let stand for 10 minutes, attach a cock in an open state at one end of the cartridge, and add 60 mL of methylated solution to the cartridge. Adjust the cock on dropping the solution so that the flow rate at 1 mL/min. Add 50 mL of acetone/diethylene glycol (99:1, v/v), concentrate to approximately 2 mL at 40℃ or below, and add 5 mL of acetone.

3) Clean-up

Add 5 mL of acetone to a neutral alumina cartridge (1,710 mg), discard the effluent. Transfer the solution obtained in 2) to the cartridge, elute with 20 mL of acetone, add 0.5 mL of acetone/diethylene glycol (99:1, v/v) to the eluate, concentrate to approximately 2 mL at 40℃ or below under spray nitrogen, and remove the solvent. Dissolve the residue in acetone to make exactly 2 mL for grains, legumes, nuts, seeds, animal and fishery products, 4 mL for fruits and vegetables, 2 mL for tea leaves, and use these solutions as the test solutions.

6. Calibration curve

Weigh 5 mg each of reference standard (as carbon disulfide) of ziram, propineb, and maneb, dissolve respectively in cysteine-EDTA solutions to make exactly 50 mL each as stock standard solutions (100 mg/L). Add 1 mL each of the stock standard solutions and 10 mL of tetrabutylammonium hydrogen sulfate solutions respectively to 150 mL of cysteine-EDTA solutions, adjust pH to 7.5-7.7 with 6 mol/L of hydrochloric acid, and add water to make exactly 200 mL. Take exactly a 20 mL aliquot, dilute the solution obtained by the same operation described in 2) of 5. with acetone, and prepare each agricultural chemical solution (acetone) of several concentrations. Inject each standard solution to GC-MS, make calibration curves by peak-height or peak-area method.

For ziram, thiram, nickel bis (dithiocarbamate) and ferbam, which are converted into methyl dimethyldithiocarbamate (hereinafter referred to as "DMDC") for measurement, quantify ziram as a reference standard. For zineb, mancozeb and maneb, which are converted into dimethyl ethylenebisdithiocarbamate (hereinafter referred to as "EBDC") for measurement, quantify maneb as a reference standard. For propineb, which is converted into dimethyl propylenebisdithiocarbamate (hereinafter referred to as "PBDC") for measurement, quantify propineb as a reference standard. For polycarbamate, which is converted into DMDC or EBDC for measurement, quantify ziram or maneb respectively as a reference standard. When the test solutions are prepared following the above procedure, the samples containing 0.01 mg/kg each (as carbon disulfide) of materials other than tea leaves give the test solutions of 0.005 mg/L each (as carbon disulfide) in concentration, and the sample containing 0.1 mg/kg (as carbon disulfide) of tea leaves gives the test solution of 0.005 mL (as carbon disulfide).

7. Quantification

Inject the test solution to GC-MS, and calculate the concentrations of each agricultural chemical as carbon disulfide from the calibration curves made in **6**.

8. Confirmation

Confirm using GC-MS.

9. Measurement conditions

(Example)

1) For quantification

Column: 50% phenyl-methyl silicone, inside diameter 0.25 mm, 30 m in length, 0.25 μm in film thickness

Colum temperature: 70℃ (2 min) -20℃/min-280℃

Inlet temperature: 240℃

Carrier gas: Helium

Ionization mode (Ionized energy): EI (70eV)

Major monitoring ions (*m/z*): DMDC 135, 88

PBDC 158

EBDC 144

Injection amount: 2 μL

Expected retention time: DMDC 8 min
PBDC 9 min
EBDC 9 min

2) For confirmation

i) DMDC or EBDC

Column: methyl silicone, inside diameter 0.25 mm, 30 m in length, 0.25 μm in film thickness

Colum temperature: 60℃ (2 min) - 20℃/min heating - 120℃ - 10℃/min heating - 160℃ - 20℃/min heating - 280℃

Inlet temperature: 240℃

Carrier gas: Helium

Ionization mode (Ionized energy): EI (70eV)

Major monitoring ions (*m/z*): DMDC 135, 88
EBDC 144

Injection amount: 2 μL

Expected retention time: DMDC 8 min
EBDC 9 min

ii) PBDC

Column: 5% phenyl-methyl silicone, inside diameter 0.25 mm, 30 m in length, 0.25 μm in film thickness

Colum temperature: 60℃ (3 min) - 20℃/min heating - 120℃ - 10℃/min heating - 160℃ - 20℃/min heating - 280℃

Inlet temperature: 240℃

Carrier gas: Helium

Ionization mode (Ionized energy): EI (70eV)

Major monitoring ions (*m/z*): PBDC 158

Injection amount: 2 μL

Expected retention time: PBDC 11 min

10. Limit of quantification

Zineb, ziram, thiram, nickel bis (dithiocarbamate), ferbam, propineb, polycarbamate, mancozeb, maneb

0.01 mg/kg each as carbon disulfide (0.1 mg/kg each for tea leaves)

11. Explanatory note

1) Outline of analytical method

The method consists of extraction of dithiocarbamate from samples using cysteine-EDTA solution under dichloromethane, transfer of the extract to a porous diatomaceous earth cartridge, flow through of methylated solution into the cartridge, conversion of each

compound for analysis into DMDC, EBDC or PBDC in the cartridge, quantification and confirmation using GC-MC. For mixture of reference standards of ziram, propineb or maneb, methylate the reference standards as in the same way with the test solutions, make calibration curves, quantify, obtain a sum as an analysis value, which is calculated as contents of the carbon disulfide in the samples. The following table shows a relationship between each analytes and analyte to assay.

Analyte	Analyte to assay
Ziram	DMDC
Thiram	
Nickel bis (dithiocarbamate)	
Ferbam	
Zineb	EBDC
Mancozeb	
Maneb	
Propineb	PBDC
Polycarbamate	DMDC and EBDC

2) Notes

i) As for a solid sample of animal products, an extracting solvent and the sample may not be fully mixed in extracting operation using the cysteine-EDTA solution only. Accordingly, dichloromethane is added at the same time in extraction and wash.

ii) Compounds similar to dithiocarbamate-based agricultural chemicals are used as rubber vulcanization accelerators. Therefore, take care in operations such as sample preparation or analysis not to mix those compounds accidentally.

iii) Ions used in the development of analytical method in the GC-MS measurement for DMDC, EBDC and PBDC are as follows;
Quantification ion (m/z): DMDC 88, EBDC 144, PBDC 158
Confirmation ion (m/z): DMDC 135

iv) The above analytical method is not applicable for metiram because metiram is difficult to be methylated (converted to EBDC). However, when the analytical method is performed for metiram, and an obtained value is exceeded reference values of dithiocarbamate for each food product, it may not be determined that the food with metiram should conform to the regulation standards under the Food Sanitation Act.

v) In “4. Reagents”, there are the description such as “a reference standard of propineb: contains not less than 75% of propineb” and “a reference standard of maneb: contains not less than 75% of maneb” because highly pure reference standards of propineb or maneb were not available at the time of development for the analytical method. However, it is desirable to use purer reference standards with not less than 95% of propineb or maneb for analysis if these are available.

vi) Food items considered when the analytical method was developed:
Agricultural products: brown rice, soya beans, cabbages, spinaches, oranges, apples, pumpkins, cacao beans and tea leaves

Animal and fishery products: cattle muscle, cattle fat, cattle liver, milk, chicken eggs, salmon, eel, freshwater clam, shrimp and honey

12. References

Nobuyuki Kifune, Journal of the Food Hygienics Society of Japan 36, 244-251, 1995 Japanese Society for Food Hygiene and Safety

13. Type

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