codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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Agenda Item 7(b) **CX/PR 07/39/6-Add. 1**
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JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON PESTICIDE RESIDUES
Thirty-ninth Session
Beijing, China, 7 - 12 May 2007

COMMENTS on Proposed Draft Revision of the List of Methods for Pesticide Residue Analysis at Step 4, submitted by Kenya, Korea

KENYA

In Kenya the commonly used methods for Pesticide analysis are GC-MS and HPLC mainly in the analysis of organochlorine, organophosphorous and carbamates. However, Kenya has no objection with the ten methods of Analysis proposed by Canada.
European standards elaborated by CEN/TC275 Food analysis-Horizontal methods proposed by German delegation.

Determination by PCBs
Kenya proposes that those methods of 1998 to be updated or to be confirmed so that their validity for use can be continued.

KOREA

Republic of Korea submitted suggestion of analytical methods for dithiocarbamate residues at the 38th CCPR.
The contents of this suggestion are somewhat changed. In the experimental section, conversion factors are changed from 1.125 to 1.78 for thiram derivation and from 0.938 to 1.07 for nabam derivation. Including these corrections, a revised version of method is attached as follows;

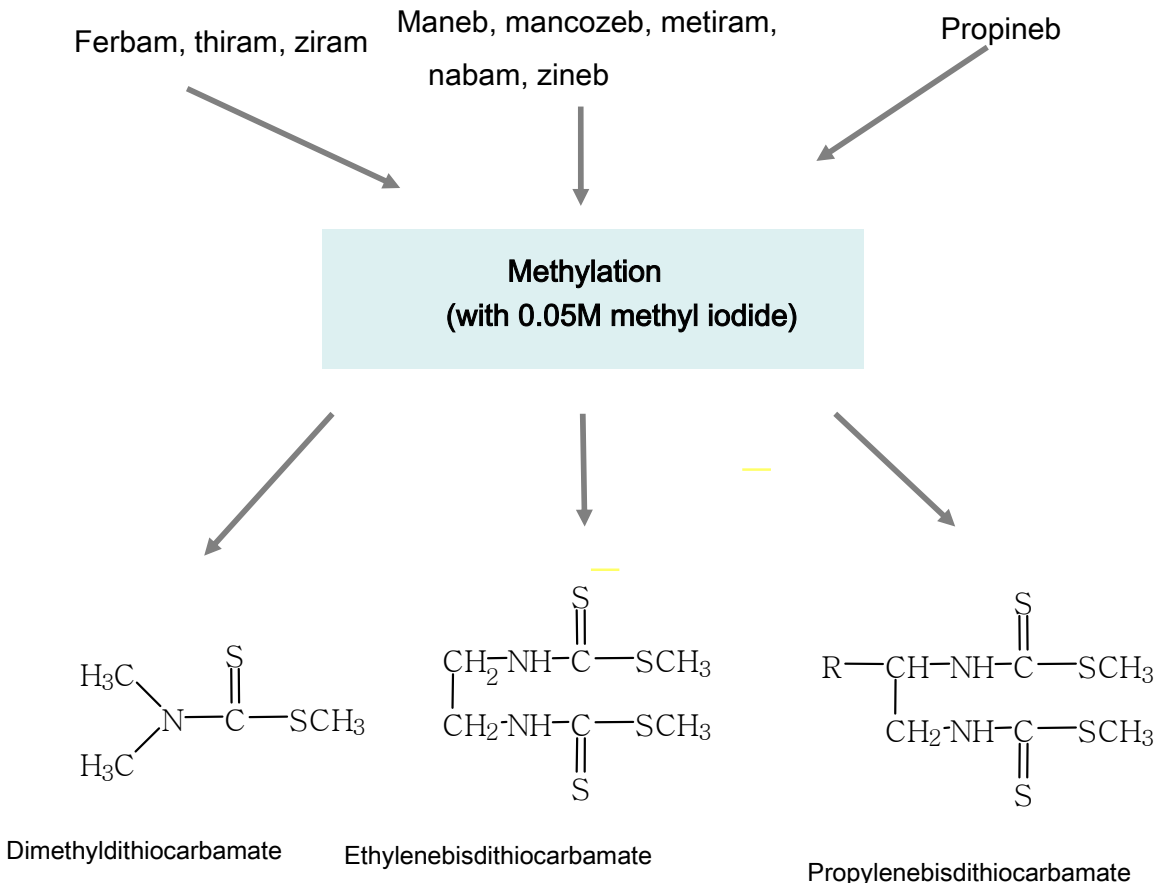
Analytical Methods for Dithiocarbamate Residues

Principle

Dithiocarbamates are agricultural fungicides that can be classified into 3 groups depending on the chemical structures such as dimethyldithiocarbamates, ethylenebisdithiocarbamates and propylenebis-dithiocarbamate. Ferbam, thiram and ziram belong to dimethyldithio-carbamate, mancozeb, maneb, metiram, nabam and zineb belong to ethylenebisdithiocarbamate, and propineb belongs to propylenebisdithio-carbamate, respectively. The analytical method for dithiocarbamates

in food has been improved to detect them as 3 different groups. Targets are decomposed in alkaline median, followed by methylation, and then analyzed by HPLC.

- **Dimethyldithiocarbamates : ferbam, thiram, ziram**
- **Ethylenebisdithiocarbamates: mancozeb, maneb, metiram, nabam, zineb**
- **Propylenebisdithiocarbamate : Propineb**



Experimental section

1. Apparatus & Equipment

Instrument : HPLC(Agilent 1100 series)

Column : C18 column(250*4.6mm i.d., 5 ㎛, Shiseido, Japan)

Mobile phase : acetonitrile-water-methanol(25:65:35)

Flow rate : 1.0ml/min.

Detector : UV 272nm(Agilent G1314A VWD)

Centrifuge : Multipurpose Refrigerated Centrifuge LX-130(TOMY KOGYO, TOKYO, Japan)

Glass fiber filter : PYREX 17G – 1(ID 65mm)

2. Reagents

Thiram(99% Dr. Erenstorfer GmbH , Germany)

Nabam(67% Dr. Erenstorfer GmbH , Germany)

Propineb(75% Riedel - de Haen, Germany)

Methyl iodide(99% : Lancaster, England)

Tetrabutyl ammonium hydrogen sulfate(97%, Sigma-Aldrich, USA)

Ethylenediaminetetraacetic acid tetrasodium salt dihydrate(minimum 99% titration , Sigma-Aldrich, USA)

L- Cysteine hydrochloride anhydrous(minimum 98%, Sigma-Aldrich, USA)

1,2-propanediol(99%, Sigma-Aldrich, USA)

Dichloromethane(99.8%, J.T.Baker, USA)

Methanol(99.9%, Burdick & Jackson, USA)

Hexane(99.9%, Burdick & Jackson, USA)

Hydrochloric acid(35~37%, Wako, Japan)

Sodium hydroxide(96%, Wako, Japan)

Sodium chloride(99%, Wako, Japan)

EDTA solution

- **pH 9.5-9.6 :** 0.5g of L-cysteine and 100ml of 0.25M EDTA in 0.45M **sodium hydroxide**
- **pH 7.0 : 0.5g of L-cysteine and 100ml of 0.25M EDTA in 0.45M sodium hydroxide(adjusted to pH 7.0 with 2 M hydrochloric acid).**

Standard solution

- **Thiram** was prepared in methanol(100mg/mL).
- **Nabam and propineb** were prepared in EDTA solution (pH 9.5-9.6). And then the pH was immediately adjusted to ca 7.0 with 2 M hydrochloric acid(100mg/mL). These solutions were to use immediately after preparation.
- The stock solutions were diluted with EDTA solution(adjust pH 7.0).

- The concentrations of final standard solutions were multiplied by conversion factor 1.78 for thiram derivative and 1.07 for nabam derivative.

Analytical Procedure

1. Extraction

An aliquot(20g) of sample was cut into 10-15 pieces and analyzed immediately. The outer pieces of grapes, ginseng, and Chinese matrimony vine were analyzed. Cabbage, red pepper, onion and Korean cabbage were not chopped. The aliquot was shaken in 0.5g of L-cysteine and 100ml of EDTA solution(pH 9.5-9.6) for 5 min in a closed glass bottle. The extract was filtered through a glass fiber filter. Extracts which were too viscous to be filtered directly (e.g., those of strawberries) were centrifuged for 10 min at 1,700g before filtering. The bottle and the filter were rinsed with 10 mL(*3) of the EDTA solution, and combined into the extracts. Five mL of 0.41M tetrabutylammonium hydrogen sulfate and 10g of sodium chloride was added into the extracts while stirring. The pH of the extracts was cautiously adjusted to ca 7.0 with 2 M hydrochloric acid(*This extraction time must be within 15 min.)

2. Derivatization

Fourty mL of 0.05 M methyl iodide in dichloromethane-hexane (1:1) was added into the extracts. The mixture was shaken vigorously for 10min. And then the upper layer was centrifuged for 5min at 800 rpm. 20mL of the extracts was taken and 5 mL of 20% 1, 2-propanediol in dichloromethane(keeping solution) was added. The solvent and the excess of methyl iodide were stripped off at 30 in a rotatory evaporator. The residue was diluted with 1.0 mL of methanol and 10 μℓ was analyzed by HPLC using UV detection at 272 nm.

Results

1. HPLC Method

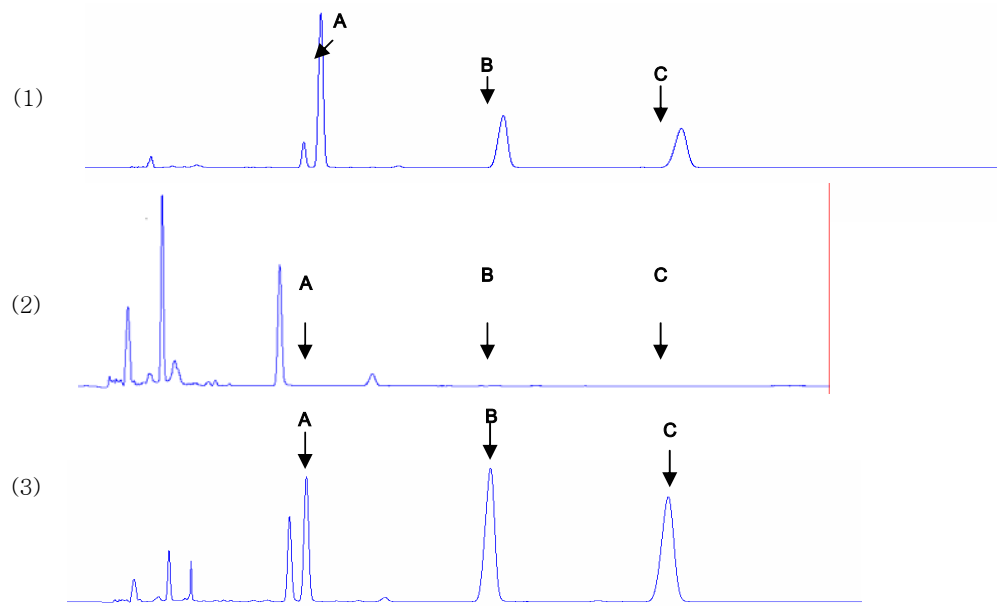


Fig. 1 Typical chromatogram of dithiocarbamate residues

(1) Standard mixture of thiram, nbam and propineb
(2) Sample blank
(3) Grape sample spiked with thiram, nbam and propineb

A : Dimethyldithiocarbamate (ferbam, thiram and ziram)
B : Ethylenebis(dithiocarbamate) (maneb, mancozeb, metiram, nabam and zineb)
C : Propylenebisdithiocarbamate(propineb)

Limit of determination

- Dimethyldithiocarbamates 0.005mg/kg,
- Ethylenebisdithiocarbamates 0.01mg/kg,
- Propylenebisdithiocarbamate 0.02mg/kg

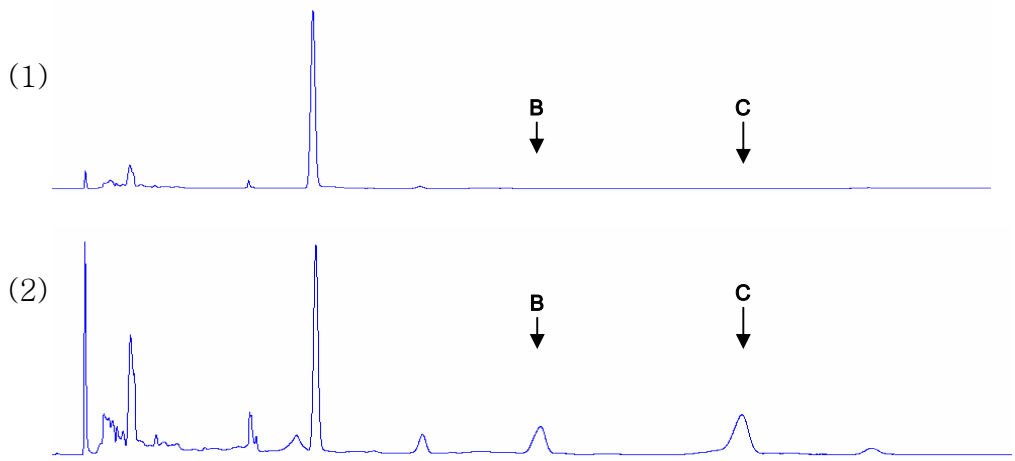


Fig. 2 Typical chromatogram of dithiocarbamate residues
(1) Sample blank
(2) onion sample spiked with nbam and propineb
B : Ethylenebis(dithiocarbamate) (maneb, mancozeb, metiram, nabam and zineb)
C : Propylenebisdithiocarbamate(propineb)

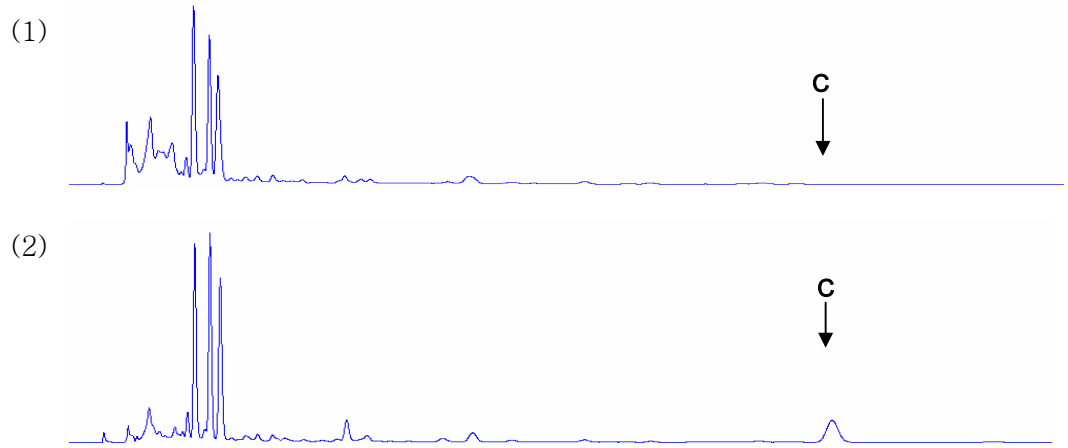


Fig. 3 Typical chromatogram of dithiocarbamate residues

(1) Sample blank
(2) Chinese matrimony vine sample spiked with nbam and propineb
C : Propylenebisdithiocarbamate(propineb)

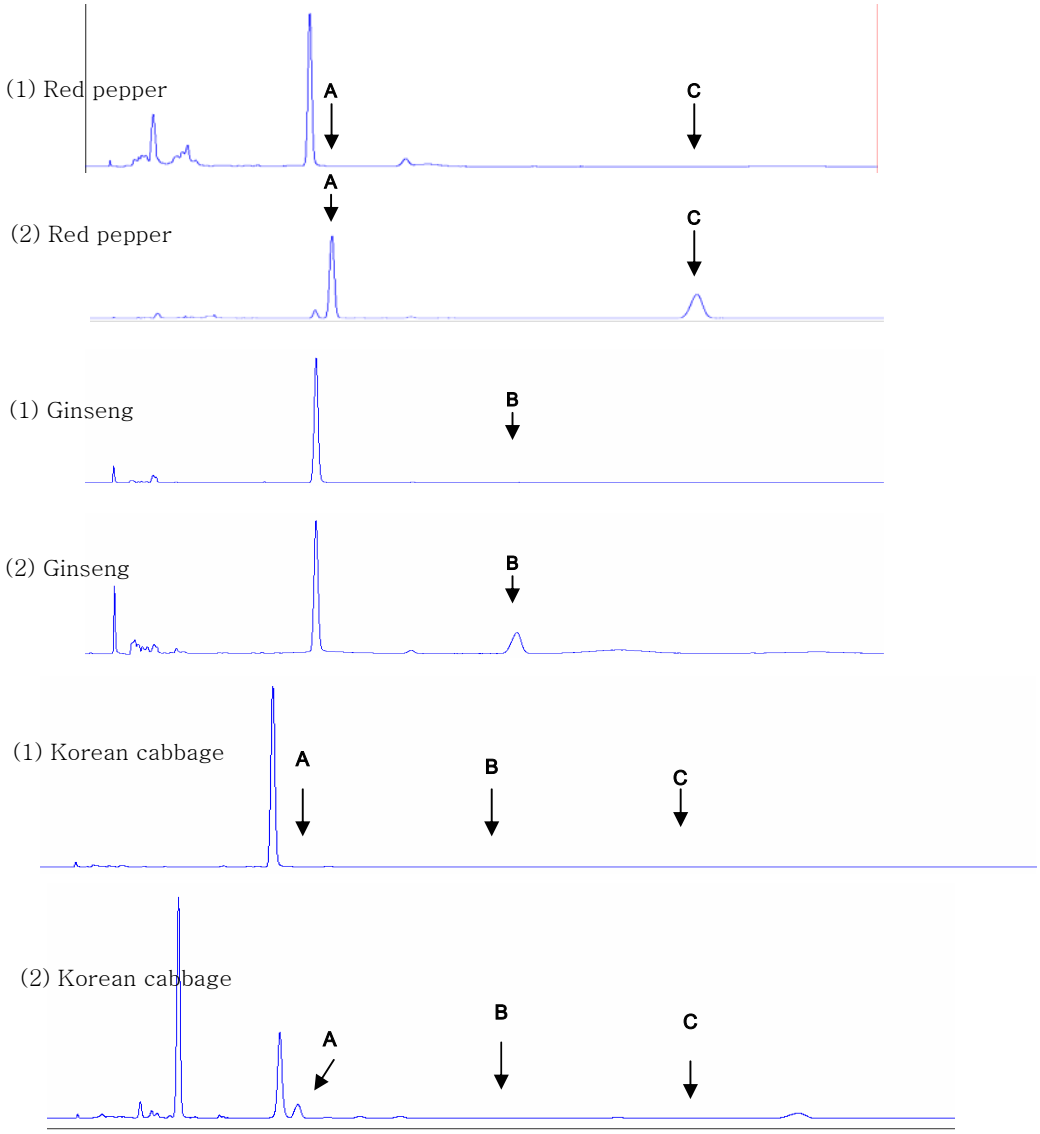


Fig. 4 Typical chromatogram of dithiocarbamate residues

(1) Sample blank

(2) Sample spiked with thiram, nbam and propineb

A : Dimethyldithiocarbamate (ferbam, thiram and ziram)

B : Ethylenebis(dithiocarbamate) (maneb, mancozeb, metiram, nabam and zineb)

C : Propylenebisdithiocarbamate(propineb)

2. GC Method

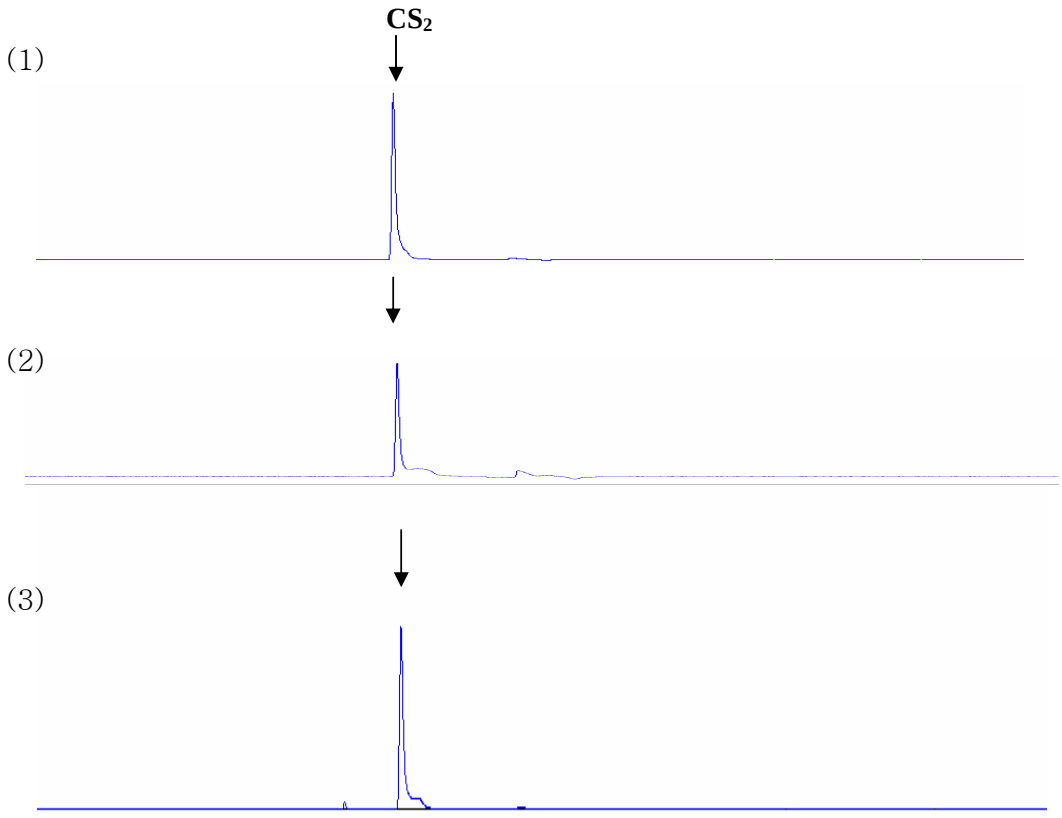


Fig. 5 Typical chromatogram of dithiocarbamate residues by using CS_2 method
(1) Standard of nabam
(2) Cabbage sample blank
(3) Cabbage sample spiked with nabam

Limit of determination(LOD) : 0.005mg/kg

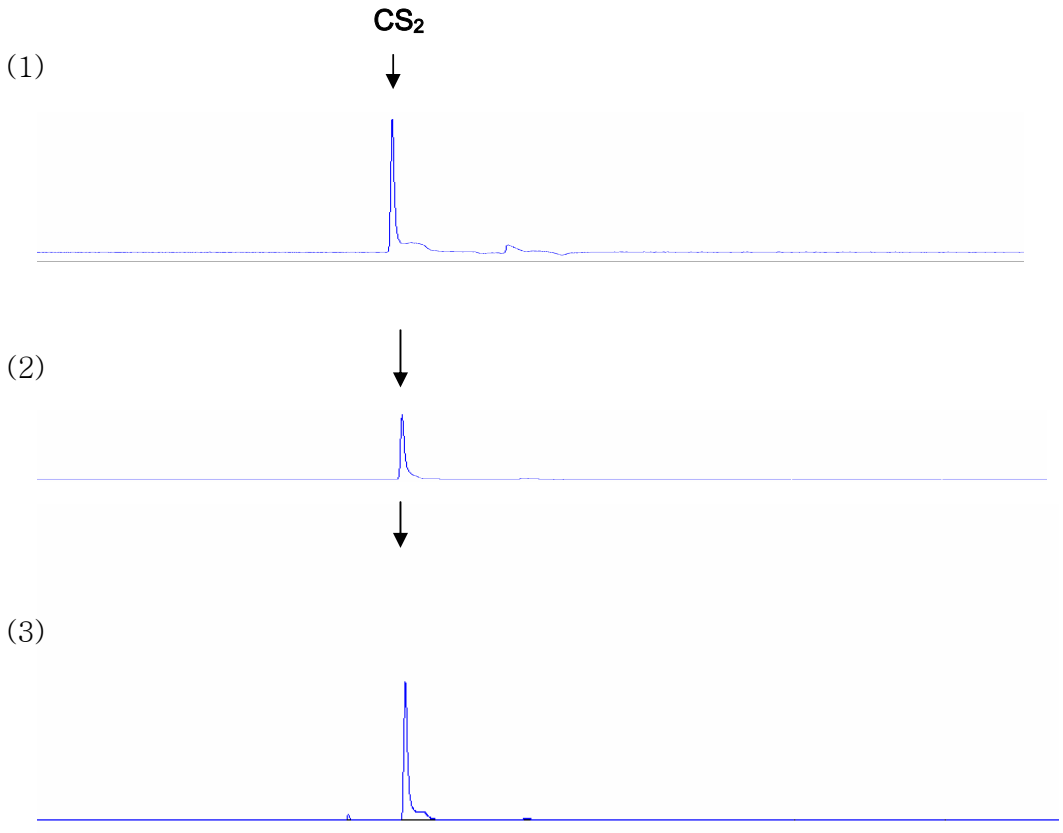


Fig. 6 Typical chromatogram of dithiocarbamate residues by using CS_2 method
(1) Standard of nabam
(2) Korean cabbage sample blank
(3) Korean cabbage sample spiked with nabam

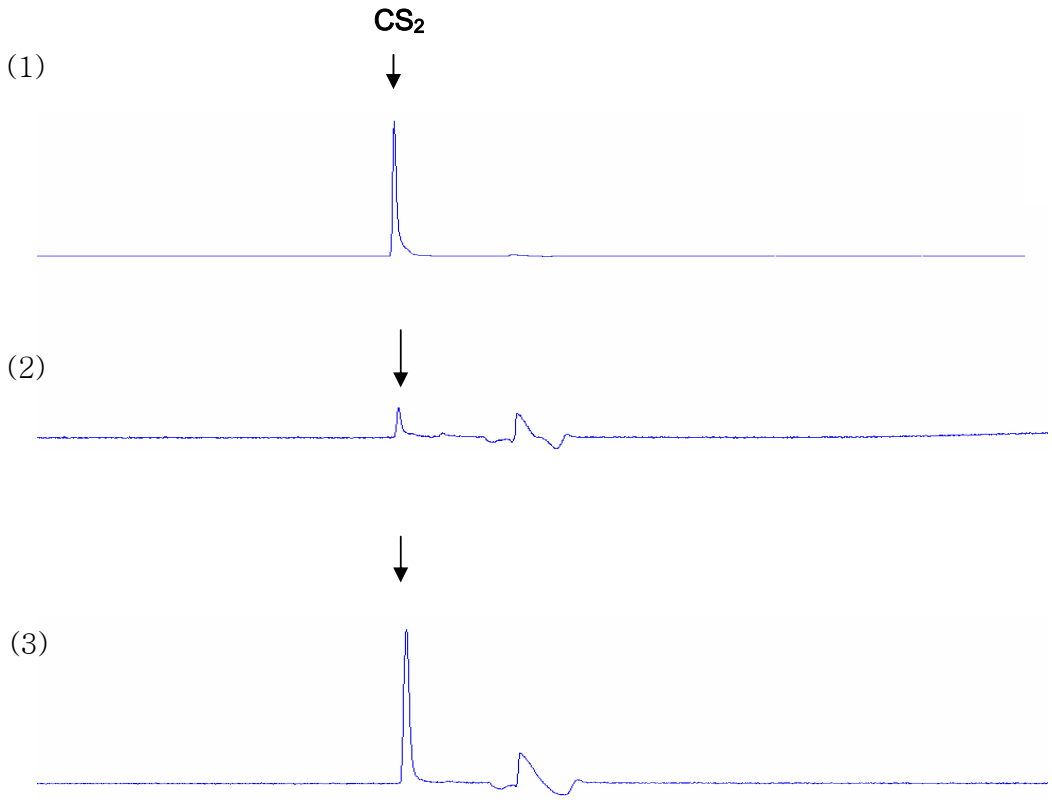


Fig. 7 Typical chromatogram of dithiocarbamate residues by using CS_2 method
(1) Standard of nabam
(2) Red pepper sample blank
(3) Red pepper sample spiked with nabam

Table 1. Comparison of Recoveries

	HPLC method			GC method(CS_2)
	A	B	C	nabam
Onion	-	81.2±3.2	74.4±1.2	-
Red pepper(dried)	89.0±2.5	-	80.7±0.8	106.2±15.3
Cabbage	-	81.2±1.8	-	122.2±16.9
Korean Cabbage	-	85.9±2.6	-	112.5±17.1
Grape	76.9±1.6	108.8±3.4	112.5±3.2	77.7±8.5
Ginseng	-	-	95.9±2.5	83.5±10.4
Chinese matrimony vine	-	-	76.9±1.8	90.6±14.2

A : Dimethyldithiocarbamate

B : Ethylenebis(dithiocarbamate),

C : Propylenebisdithiocarbamate

Table2. the Condition of CS2 method

Instrument	**HP-6890 Series**
Detector	**Flame Photometric Detector(FPD)**
Column	**DB-WAXETR Capillary column,** **0.53mm i.d. X 30m, 1.00 ㎛ film thickness**
Temperature	**Column oven : 80 (3min)** **Detector : 250 , Injector : 250**
Flow rate	**Carrier N_2 0.4mL/min** **Fuel gases H_2 80mL/min** **Air 110mL/min** **makeup 45mL/min**
Injection volume	**1 ㎕**