J. Pesticide Sci. **7**, 357–362 (1982)

Original Article

A Method for Determination of Dazomet Residues in Crops by High Performance Liquid Chromatography

Naofumi SHIGA, Osami MATANO and Shinko GOTO

The Institute of Environmental Toxicology, Suzuki-cho, Kodaira 187, Japan

(Received February 6, 1982)

A procedure was developed for the analysis of dazomet residues in tomato, cucumber and cabbage employing high performance liquid chromatography (HPLC). The crops were homogenized with silver diethyldithiocarbamate (DDTC-Ag) and extracted with dichloromethane. The addition of DDTC-Ag was necessary for all samples to obtain a good recovery of dazomet. The extract was concentrated and cleaned up using a Florisil column. The concentrated eluate was analyzed by HPLC employing a Nucreosil 5 CN column and a 2,2,4-trimethylpentane-ethyl acetate solvent system. Dazomet was detected by ultraviolet absorption (285 nm) and 0.5 ng per injection was detectable. Recoveries of spiked samples were 78–95% at 0.05 ppm level and 83–88% at 0.2 ppm level with a lower limit of detection of 0.005 ppm (tomato and cucumber) and 0.01 ppm (cabbage). This HPLC method was more specific for dazomet than the conventional colorimetric method.

INTRODUCTION

Dazomet (tetrahydro-3, 5-dimethyl-1, 3, 5-thiadiazine-2-thione) is a temporary soil sterilant which controls annual and perennial weeds, nematodes, soil fungi and soil insects.

Few method for the determination of dazomet residues have been reported. Dazomet cannot be analyzed directly by gas chromatography (GC) because it decomposes at high temperature. HPLC was used for the analysis of dithiocarbamates including *N*-methyl dithiocarbamate, decomposition product of dazomet,[1] but no practical application of the analysis of dazomet residues in crops was demonstrated. The only method available for residue analysis of this chemical is a colorimetric procedure which involves acid hydrolysis to carbon disulfide.[2] However, this method is unspecific, since it suffers from interference by other dithiocarbamate pesticides and endogenous compounds of environmental samples.

It is known that dazomet in water breaks down gradually forming methyl isothiocyanate (MITC) when heated to 100°C.[3] In our laboratory, with the modification of the method of MITC analysis,[4] a gas chromatographic technique in which dazomet was converted to MITC was examined (GC-MITC method). This GC-MITC method was not strictly specific for dazomet residue, so we also investigated the use of HPLC for determining dazomet residues in cucumber, tomato and cabbage, and compared it with the GC-MITC method.

MATERIALS AND METHODS

1. Apparatus

The high performance liquid chromatography system used was a Jasco TRI-ROTAR and a Jasco UVIDEC-100 III, UV detector which was connected to a Shimadzu model R-101 recorder with a 1.01 mV span. The chromatographic column (4.6 mm i.d. $\times$25 cm, stainless steel) was packed with Nucreosil 5 CN (5 μm) by a slurry technique, using slurry solvent A CONC (Macherey-Nagel, Germany),

(theoretical plate, N=5,200/25 cm). The mobile phase consisted of 40% ethyl acetate in 2,2,4-trimethylpentane. The flow rate was 1.0 ml/min for all analyses (column pressure=110 kg/cm^2). Samples were injected onto the column via a Jasco VL-611 variable loop injector and the injection volume was 10 μl. UV wavelength was 285 nm and sensitivity was 0.02 a.u.f.s. Chromatography was conducted at room temperature. Under these conditions, retention time of dazomet was 7.2 min (capacity factor, k'=0.8) and the minimum detectable amount of dazomet was 0.5 ng per injection.

2. Reagent

Distilled-in-glass grade dichloromethane was used for extraction and preparation of the standards, and other organic solvents were analytical grade. Stock solution of dazomet was prepared in dichloromethane at a concentration of 100 μg/ml. Spiking solution and standard for HPLC were prepared by dilution of the stock solution with dichloromethane.

Pure dazomet was provided by KANESHO Co., Ltd. (Japan). Methyl isothiocyanate (MITC) was purchased from Tokyo Chemical Industry Co., Ltd. (Japan). Florisil (60–100 mesh) for column chromatography was from Floridin Company. Silver diethyldithiocarbamate (DDTC-Ag) (Dojin Laboratories, Japan), ammonium pyrrolidinedithiocarbamate (APDC) (Wako Pure Chemical Industries, Ltd., Japan), diphenylthiocarbazone (Dithizone) (BDH Chemicals Ltd., England), 8-hydroxyquinoline (Oxine) (Wako), tetrasodium ethylenediaminetetraacetate (EDTA-4Na) (Dojin) and sodium diethyldithiocarbamate (DDTC-Na) (Wako) were analytical grade.

3. Extraction

The crops (about 500 g) were homogenized with DDTC-Ag (200 mg/500 g of sample). Fifty grams of the homogenate was weighed into a 500-ml flask (in case of tomato, an appropriate volume of 1 N sodium hydroxide was added to the homogenate so as to adjust the pH to 7.0), added with 150 ml of dichloromethane and shaken for 30 min at room temperature. The mixture was dried over about 50 g of anhydrous sodium sulfate and filtered through Whatman 1 PS filter paper under atmosphere into a 500-ml round bottom flask. The filtrate was evaporated to 1–2 ml below 30°C using a rotary evaporator.

Care must be taken not to allow the residue to evaporate to dryness or losses will result.

A chromatography column (1.2 cm i.d.) was filled with a slurry prepared by shaking 10 g of Florisil with *n*-hexane and allowed to settle. After gradually replacing *n*-hexane in the column with dichloromethane, the residue after the evaporation was transferred to the column with two 5 ml portions of dichloromethane. The first portion was allowed to be almost completely adsorbed before adding the other. After adsorption of the sample, the column was washed with dichloromethane (15 ml) and the eluate was discarded. Dazomet was eluted with the next 30 ml of dichloromethane. After the eluate was concentrated to 2–3 ml on a rotary evaporator, the residue was transferred to a 5-ml or appropriate volume volumetric flask and diluted to the mark with dichloromethane. An aliquot (10 μl) of this solution was injected onto the liquid chromatographic column.

The amount of dazomet was determined from a standard plot of peak height vs. nanogram of dazomet. The plot was linear over the range of 0.5 to 5.0 ng of dazomet (r=0.9999).

4. GC-MITC Method

100 g of homogenate sample was transferred in a liquid extractor. The following procedures were the same as that for MITC analysis,[4] except for the use of *n*-hexane in place of diethyl ether as an extracting solvent. Two microliters of the final *n*-hexane extract (10 ml) was injected into the following GC.

The gas chromatograph used was a Hewlett-Packard 5710A equipped with a dual Nitrogen-Phosphorus Flame Ionization Detector (N-P FID). Glass column (6 ft×3 mm i.d.) packed with 5% Carbowax 20 M on Chromosorb W (HP), 100–120 mesh was used. The flow rate of carrier gas (N_2) was 30 ml/min. Gases for N-P FID detector were hydrogen (3 ml/min) and air (50 ml/min). The temperature at column oven, injection port and detector were 100, 150 and 300°C, respectively.

Standard solution of MITC for GC was prepared in *n*-hexane. Calibration curve was

used for the quantitation of MITC. The relationship between the amount of MITC and peak height was linear in the range from 0.2 to 1.6 ng (r=0.9996). The residues of dazomet were calculated using the formula: Dazomet (ppm)=MITC (ppm) × 2.22 (2.22=molecular weight ratio, dazomet/MITC).

RESULTS AND DISCUSSION

1. HPLC Operational Conditions

Dazomet in dichloromethane has two maximum absorptions (λ_{max}) at 245 and 285 nm, and molar extinction coefficients (ϵ) of 7.63 $\times 10^3$ and 1.38×10^4, respectively. Since the measurement at 285 nm was more sensitive and less interfered by extraneous peaks, this wavelength was selected for the detector of HPLC.

For residue analysis of dazomet, a variety of columns were tested. For initial experiment, several reversed-phase columns (octadecyl bonded, cyanopropyl bonded and porous polystyrene divinylbenzene co-polymer) were investigated using a methanol-water or an acetonitrile-water mobile phase. In general, however, poor reproducibility of peak height and a decrease in response were observed in repeated injections whenever any combination of the column packings and mobile phase mentioned above were used. The octadecyl bonded phase resulted in the largest decrease in response. The reason for the reduced response was not investigated and the use of reversed-phase HPLC was discontinued.

Normal-phase chromatography gave generally good results. Silica gel (SS-05, Jasco), cyanopropyl phase (Nucreosil 5 CN, Nargel) and amino propyl phase (SN-02, Jasco) were examined using two different solvent systems: *n*-hexane–dichloromethane and 2,2,4-trimethylpentane–ethyl acetate. Among these systems tested, cyanopropyl phase for packing in combination with 2,2,4-trimethylpentane–ethyl acetate (6 : 4, v/v) for solvent gave the best results with regard to sensitivity and separation of dazomet from coextractives of samples including cabbage, tomato and cucumber, and this combination was selected for all analyses.

2. Extraction and Clean Up Procedure

As extracting solvents, methanol, acetonitrile, acetone and dichloromethane were examined. Among these, dichloromethane was the most suitable because it gave good recoveries.

Florisil column chromatography was necessary to remove coextractives which showed large peaks of the liquid chromatograph and prevented quantitation of dazomet.

3. Addition of DDTC-Ag

No addition of DDTC-Ag resulted in the recoveries of only 28% (cabbage), 53% (cucumber) and 58% (tomato) of dazomet fortified at a level of 0.2 ppm when analyzed according to the method in "MATERIALS AND METHODS." These low recoveries were presumed to be caused by heavy metals in the crop homogenates, because methan-sodium (sodium *N*-methyldithiocarbamate, insecticide), chemically related compound, is broken down by heavy metals. Therefore, various reagents which can form a complex with heavy metal were added to the homogenate and examined for the effect of recovery of dazomet. As expected, the recovery was increased by addition of DDTC-Na, APDC and DDTC-Ag to the initial cucumber homogenate (Table 1). Among these reagents tested, DDTC-Ag was the most satisfactory. APDC gave a large peak just before the dazomet peak in the

Table 1 Effect of various reagents on recovery of dazomet from cucumber.[a]

Reagent[b]	% recovery
Non	53
EDTA-4Na	43
Oxine	60
DDTC-Na	86
APDC	80
DDTC-Ag	88
Dithizone	—[c]

[a] Each reagent was added to cucumber homogenate (20 mg/50 g of sample) and dazomet fortified at 0.2 ppm was analyzed.

[b] Abbreviation of reagent names is shown in "MATERIALS AND METHODS."

[c] Could not be determined due to interfering peak.

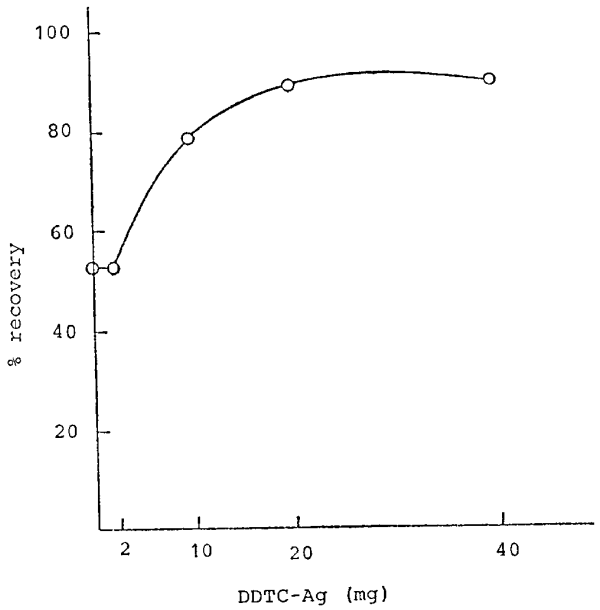

Fig. 1 Effect of amount of DDTC-Ag on recovery from cucumber.
DDTC-Ag was added to 50 g of cucumber homogenate and dazomet fortified at 0.2 ppm was analyzed.

chromatogram and DDTC-Na gave a low recovery from cabbage.

Effect of the amount of DDTC-Ag on recovery was investigated using cucumber homogenate fortified with dazomet at the 0.2 ppm level. As shown in Fig. 1, recovery of dazomet increased with an increasing amount of DDTC-Ag and reached a plateau above 20 mg/50 g of cucumber. Almost the same results were achieved for both cabbage and tomato. Since an excess of DDTC-Ag gave an interfering peak in the chromatogram, 20 mg of DDTC-Ag was added to 50 g of sample in subsequent work.

In the analysis of tomato, recovery of dazomet was still low despite the addition of DDTC-Ag or other reagents listed in Table 1 to the homogenate. Because the recovery of dazomet was low when pH was below 7.0 (Table 2), adjustment of pH of the tomato homogenate to 7.0 was necessary for good recovery.

The recovery was still low when pH was adjusted to 7.0 with no addition of DDTC-Ag and when only supernatant of the homogenate with DDTC-Ag was used without pH adjustment (Table 2). This fact suggested that dazomet may be decomposed by some compounds existing in supernatant fraction of tomato homogenate at low pH and this decomposition may be prevented by DDTC-Ag at pH 7.0.

Table 2 Effect of pH of tomato homogenate on recovery of dazomet.[a)]

pH	% recovery		
4[b)]	58	58[c)]	62[d)]
5	72		
6	76		
7	92	48[c)]	
8	84		

a) 50 g of tomato homogenate (with DDTC-Ag) was centrifuged at 3,000 rpm for 5 min and the supernatant was collected. After changing its pH to 4–8 with 1 N NaOH, this solution was recombined with the precipitate and analyzed. Fortified level of dazomet was 0.2 ppm.
b) Original pH of homogenate was 4.0.
c) Without DDTC-Ag.
d) Using only the supernatant as a sample.

Table 3 Recovery of dazomet by Florisil column chromatography with or without DDTC-Ag.

Samples	DDTC-Ag[a)]	% recovery
Dazomet standard (10 μg)	—	88
〃	+	100
Cabbage extract[b)] + Dazomet (10 μg)	—	58
〃	+	95
Cabbage extract (with dazomet)[c)]	+	50
Cabbage extract (with DDTC-Ag)[d)] + Dazomet (10 μg)	—	92

a) Samples for column chromatography were dissolved in 2 ml of 1% DDTC-Ag/dichloromethane solution (+) or 2 ml of dichloromethane (−) and applied to Florisil column.
b) 50 g of cabbage homogenate (without DDTC-Ag) was extracted and dazomet was added just before Florisil column chromatography.
c) Dazomet was added to 50 g of cabbage homogenate (without DDTC-Ag) and extracted.
d) 50 g of cabbage homogenate (with DDTC-Ag) was extracted and dazomet was added just before Florisil column chromatography.

Loss of dazomet during the analytical process was determined. As shown in Table 3, 47% of dazomet added to cabbage homogenate was lost at the initial extraction step and 42% was lost during the Florisil column chromatography, and DDTC-Ag could prevent these losses.

4. Recovery and Detection Limit

By the proposed procedure, known amounts of dazomet were added to crop homogenates and the recoveries were determined. The mean values of recovery are summarized in Table 4 and are satisfactory for the concentration tested in all samples, the reproducibility was also satisfactory.

Figure 2 shows the chromatograms in the analysis of cabbage and cucumber fortified and unfortified with dazomet. The chromatogram for tomato has almost the same features as that for cucumber. All samples gave a large peak eluted before dazomet and a small peak after dazomet, but these peaks did not interfere the quantitation.

Table 4 Recoveries of dazomet from three crops by HPLC and GC-MITC procedure.

Crops	HPLC		GC-MITC	
	Fortification ppm	% recovery[a)]	Fortification ppm	% recovery[b)]
Cabbage	0.05	95.0±3.0	0.1	83.9
	0.2	88.0±1.0		
Cucumber	0.05	78.3±3.5	0.1	87.1
	0.2	83.3±4.7		
Tomato	0.05	86.3±1.9	0.1	92.8
	0.2	87.7±4.1		

a) Means of three determinations±standard error.
b) Means of duplicate determinations.

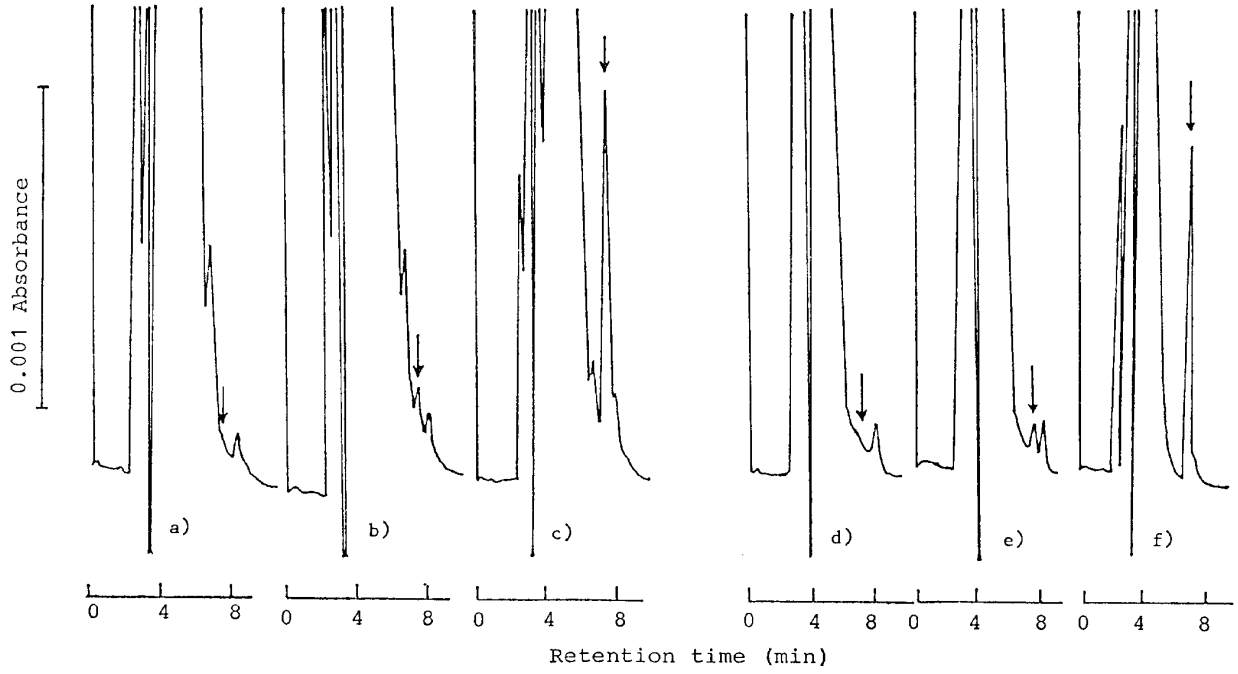

Fig. 2 High performance liquid chromatograms of extracts of cabbage and cucumber fortified and unfortified with dazomet.
Arrows indicate the retention time of dazomet. HPLC parameters are described in the text. (a) cabbage blank, (b) cabbage+0.01 ppm dazomet, (c) cabbage+0.2 ppm dazomet, (d) cucumber blank, (e) cucumber+0.005 ppm dazomet, (f) cucumber+0.2 ppm dazomet.

Lower limits of detection were 0.005 ppm for tomato and cucumber and 0.01 ppm for cabbage. The dazomet peak is apparently shown on the chromatograms (Fig. 2-b, e).

5. *Comparison of HPLC with GC-MITC Method*

The proposed HPLC method was evaluated in comparison with the results obtained by the GC-MITC method. Samples of tomato, cucumber and cabbage were treated or untreated with dazomet and weathered in the field.

By the GC-MITC method, the recoveries were almost same as those by the proposed HPLC method (Table 4) and the lower limit of detection was 0.004 ppm using 100 g of sample. The results obtained by GC-MITC, however, were generally higher than those by HPLC, especially on treated samples (Table 5). It was considered that the results by GC may contain MITC generated from dazomet treated and/or from some constituents of the sample. Furthermore, results of the colorimetric procedure[2] were about forty times higher than that by GC-MITC when cabbage sample was analyzed. This higher result was probably caused by interferences from any other compounds which were converted to carbon disulfide by acid reflux.

From the results obtained above, HPLC offers a good alternative method of analysis for dazomet because the analytical procedure based on either GC-MITC or the colorimetric method is not strictly specific for this chemical. By HPLC method four samples can be analyzed in less than one day. It is said that dazomet in the soil under moisture conditions is converted to MITC, which has proposed fungitoxic action.[3,5] Therefore this method has potential applicability not only to routine analysis of dazomet residue but to determining the persistence of dazomet in the environment which could not be done by other methods.

Table 5 Results of determination of field samples treated and untreated with dazomet by HPLC and GC-MITC method (ppm).[a]

Samples		HPLC	GC-MITC[b]
Cabbage	Untreated	<0.01	0.018
	Treated	<0.01	0.020
Cucumber	Untreated	<0.005	0.004
	Treated	<0.005	0.018
Tomato	Untreated	<0.005	<0.004
	Treated	<0.005	0.022

[a] Means of duplicate determinations.
[b] Lower limit of detection was 0.004 ppm in three crops tested.

ACKNOWLEDGEMENTS

The authors thank Miss Keiko Ohtani for technical assistance.

REFERENCES

1) R. M. Smith, R. L. Morarji, W. G. Salt & R. J. Stretton: *Analyst* (*London*) **105**, 184 (1980)
2) H. A. Stansbury, Jr.: "Analytical Methods for Pesticides, Plant Growth Regulators, and Food Additives," ed. by G. Zweig, Vol. III, Academic Press, New York and London, p. 119, 1964
3) N. N. Melnikov: "Residue Reviews," ed. by F. A. Gunther, Vol. 36, Springer-Verlag, New York, Heidelberg and Berlin, p. 430, 1971
4) M. Ottnad, N. A. Jenny & C. H. Roder: "Analytical Methods for Pesticides and Plant Growth Regulators," ed. by G. Zweig & J. Sherma, Vol. X, Academic Press, New York, San Francisco and London, p. 563, 1978
5) R. L. Metcalf: "Pesticides in the Environment," ed. by R. White-Stevens, Vol. 1, Part 1, Marcel Dekker, Inc., New York, p. 127, 1971

要 約

高速液体クロマトグラフィーによる作物中のダゾメットの残留分析

志賀直史，俣野修身，後藤真康

トマト，きゅうり，きゃべつ中のダゾメットの残留分析を高速液体クロマトグラフィーを用いて検討した．試料の均質化等に伴うダゾメットの消失を防止するためジエチルジチオカルバミン酸銀塩を作物に加えて均質化し，ジクロルメタンを用いて抽出する．フロリジルカラムクロマトグラフィーで精製した後高速液体クロマトグラフィーで定量する．カラムは Nucreosil 5CN，移動相はイソオクタン-酢酸エチル (3:2) の混合溶媒を用い，検出は 285 nm の紫外部吸収で行なった．検出限界は 0.005～0.01 ppm，回収率は 78～95% である．本法はダゾメットをそのままの形で検出するので，二硫化炭素に分解する従来の方法に比べ選択性にすぐれ，感度および精度も良好である．