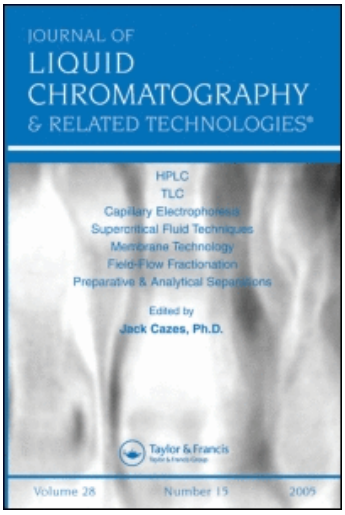

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

DETERMINATION OF DAZOMET IN BASAMID GRANULAT BY NORMAL PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Biljana Petanovska-Ilievska[a]
[a] Department of Chemistry, Faculty of Agriculture, Bul. Aleksandar Makedonski bb, Laboratory for Pesticide Formulation Control, Skopje, Republic of Macedonia

Online publication date: 31 August 2001

To cite this Article Petanovska-Ilievska, Biljana(2001) 'DETERMINATION OF DAZOMET IN BASAMID GRANULAT BY NORMAL PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY', Journal of Liquid Chromatography & Related Technologies, 24: 14, 2209 — 2216
To link to this Article: DOI: 10.1081/JLC-100104903
URL: http://dx.doi.org/10.1081/JLC-100104903

J. LIQ. CHROM. & REL. TECHNOL., 24(14), 2209–2216 (2001)

DETERMINATION OF DAZOMET IN BASAMID GRANULAT BY NORMAL PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Biljana Petanovska-Ilievska

Laboratory for Pesticide Formulation Control, Department of Chemistry, Faculty of Agriculture, Bul. Aleksandar Makedonski bb, 91000 Skopje, Republic of Macedonia
E-mail: bpetanovska@zsv.ukim.edu.mk

ABSTRACT

The normal phase determination of tetrahydro-3,5-dimethyl-1,3,5-thiadiazine-2-tione (IUPAC), with commercial name dazomet in pesticide formulation, Basamid Granulat (Dazomet Technical Granules), produced by BASF has been studied. The analysis was performed on a LiChrosorb CN analytical column and UV detection at 285 nm. The mobile phase of n-hexane/dichlormethane (50/50 v/v), and flow rate of 1,3 mL/min at constant column temperature (25°C) gave a retention time of 3.59 min. The evaluated repeatability value of results based on peak area was 1.7%.

INTRODUCTION

Dazomet (I), tetrahydro-3,5-dimethyl-1,3,5- thiadiazine-2-tione (IUPAC) is a soil fumigant, which acts by decomposition to methyl isothiocyanate.[1] The

application of this pesticide is based on a wide range of its activities. Therefore, dazomet is used as a soil sterilant, controller for soil fungi, nematodes, germinating weed seeds, and soil insects. It is also used as a slimicide in pulp and paper manufacture, and as a preservative in adhesives and glues.

The HPLC method is used for determination of residues of parent compound in soil[1] and urinary metabolites[2] in rats and mice. The GLC method is applied for determination of methyl isothiocianate in crops.[1] Details are available from BASF AG.[1]

Osselton and Snelling,[3] have used HPLC with Spherisorb S5W silica and ODS-Hypersil for separation of 51 common pesticides including dazomet. Identification of individual pesticides was confirmed with measured values of capacity factors and absorption maximum, from ultraviolet spectra of obtained peaks. The supplemental quantitative investigation was not performed.

Dazomet is an active ingredient in the pesticide formulation Basamid Granulat with declared content of 98% ± 4%. The possible technical impurities in this formulation are following the dehydro-dimer forms of dazomet:

Ia 5,5'-ethylenedi[tetrahydro-3-methyl-1,3,5-thiadiazine-2-thione].
Ib 3,5-ethylenedi[tetrahydro-3-methyl-1,3,5-thiadiazine-2-thione].
Ic 3,3'-ethylenedi[tetrahydro-3- methyl-1,3,5-thiadiazine-2-thione].

The actual CIPAC Handbook[4] referee method for determination of dazomet in Technical Granules is based on active ingredient decomposition with hydriodic acid. The produced carbon disulfide is trapped in methanolic KOH as xanthogenate and determined by iodometric titration. The results obtained from this analysis are the sum of contents of dazomet and its dehydro-dimer forms. However, the HPLC method for determination of dazomet in this technical formulation was not done. As it is known, the solubility of dazomet in nonpolar solvents, such as cyclohexane (400g/kg) is better than in polar solvent, such as water (3g/kg)[1]. Therefore, the aim of this study was to develop a normal phase HPLC method for determination of dazomet in the pesticide formulation Basamid Granulat.

EXPERIMENTAL

Equipment and Materials

The chromatographic system consisted of a Binary Pump (model 250, Perkin Elmer), and an UV Diode Array Detector (model 235, Perkin Elmer). Constant column temperature was maintained with Columnthermostat Spark Holland "Mistral" (type 880). Chromatographic separation was accomplished using HS Pecospher 3 × 3 Silica (3 μm, 3.3 × 0.46 cm, Perkin Elmer),

LiChrosorb Si 60 (5 μm, 25 × 0.4 cm, Merck), and LiChrosorb CN (5 μm, 25 × 0.4 cm, Merck) analytical columns. All eluents with HPLC-grade purity were obtained by Sigma-Aldrich. The pure analytical standard of dazomet and analytical standards of its isomer forms were gifts from BASF.

Preparation of Standard Solutions

Stock solution of dazomet was prepared by dissolving 0.0175 g of pure analytical standard with a mixture of n-hexane/dichlormethane (50/50, v/v) in a 25 mL volumetric flask. Working solutions were prepared from 0.1; 0.2; 0.4; 0.5; 0.875; 1.25; and 2.5 mL of stock solution in 10 mL volumetric flasks and dissolved with the mixture of the same solvents. From these solutions three injections were performed with 5 μL each.

Sample Solution

Samples were prepared in a 25 mL volumetric flask by dissolving the weighed amounts of 0.0350, 0.0175, and 0.0088 g in a solvent made by mixing equal volumes of n-hexane/dichlormethane. The samples were degassed for 10 min in an ultrasonic bath, and from each solution 1 mL was transferred in a 10 mL volumetric flask. The solutions injected (5 μL each) into the chromatograph, were filtered through 0.45 μm Spartan-T syringe filters.

RESULTS AND DISCUSSION

LiChrosorb Si 60 column, mobile phase of n-hexane/dichlormethane (40/60 v/v to 10/90 v/v) with flow-rate of 2mL/min at constant column temperature of 25°C, and UV detection at 285 nm was used in a 23 min run (Figure 1). At such conditions, the peak of dazomet was not obtained against the results of Osselton and Snelling,[3] which measured a capacity factor of 2.19 using different type of silica column (Spherisorb S5W silica, 25×5cm) and an eluent comprising isooctane/dichlormethane (40/60, v/v) at a flow-rate of 2 mL/min.

On the other hand, silica gel is acidic and organic amines are very strongly retained, often eluting as very asymmetrical peaks from silica columns.[6] Therefore, to confirm this assumption, the HS Pecosphere 3×3 Silica was used with a mobile phase of n-hexane/dichlormethane in a volume ratio of 20/80. The flow rate of 2 mL/min and column temperature of 25°C gave the retention time of dazomet at approximately 3.45 min. The column dead time for this chromatography condition was 0.2 min., hence, the capacity factor had a high value of 16,25.

Figure 1. Chromatographic condition on LiChrosorb Si 60 column; mobile phase n-hexane/dichlormethane (10/90 v/v); flow rate 2 mL/min; column temperature 25°C; UV detection at 285 nm, where the peak of dazomet was retained.

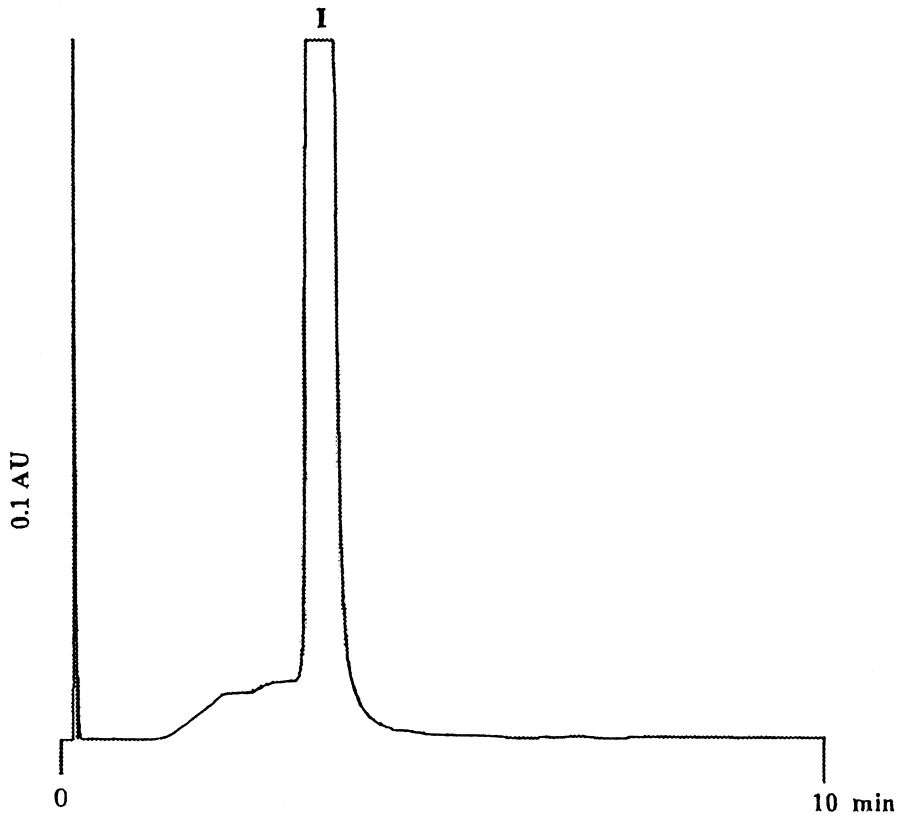

Figure 2. Chromatogram obtained from standard solution of dazomet on HS Pecosphere 3 × 3 Silica, mobile phase n-hexane/dichlormethane (20/80 v/v), flow rate 2 mL/min, column temperature 25°C; UV detection at 285 nm.

The obtained peak shape (Figure 2) was not fully symmetrical, because the base line on the front of the peak was raised and the purity index was not satisfactory.

The preliminary examination performed on CN column, mobile phase of n-hexane/dichlormethane in the ratio of 50/50 v/v flow rate of 1.3 mL/min, and column temperature of 25°C, achieved retention time with the mean value of 3.59 min (Figure 3). The obtained peak of interest was sharp, symmetrical, and with good index purity (1.0). In this mixture of solvents in region of 195 to 370 nm, the absorption spectra of dazomet gives rise to two bands with absorption maximum at around 246 and 287 nm (Figure 4). Because the long-wavelength band has high-intensity, the assay for determination of dazomet in pesticide formulation Basamid Granulat was performed at 285 nm.

Using the chromatographic conditions described, all constituents were well separated and their retention times were 4.08 min for isomer Ia, 7.57 min for isomer Ib, and 8.57 min for isomer Ic, respectively (Figure 3). The evaluated value

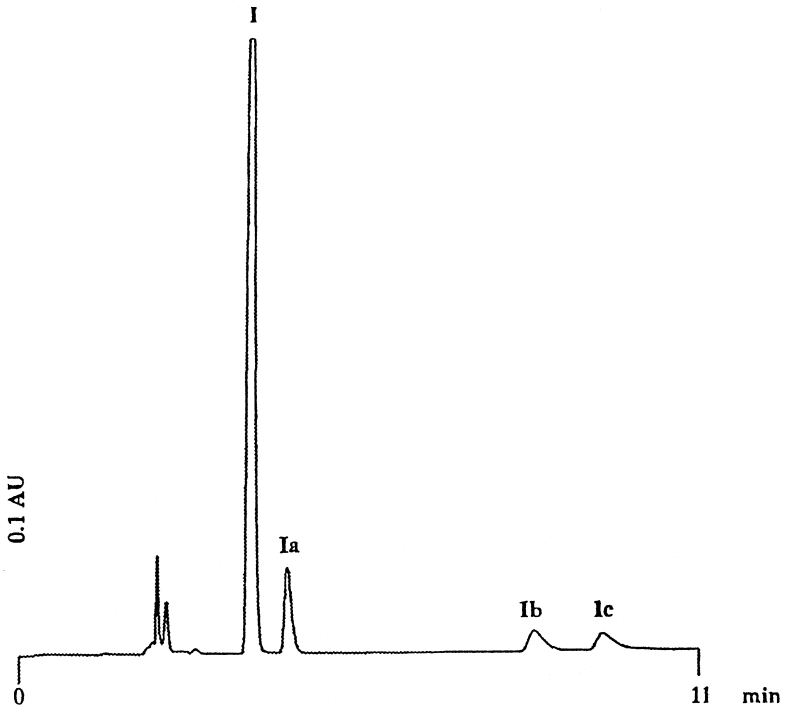

Figure 3. Chromatogram of dazomet (I) and its dehidro-dimer forms (Ia; Ib; Ic) separated on LiCrosorb CN, mobile phase n-hexane/dichlormethane (50/50,v/v); flow rate 1.3 mL/min; column temperature 25°C; UV detection at 285 nm.

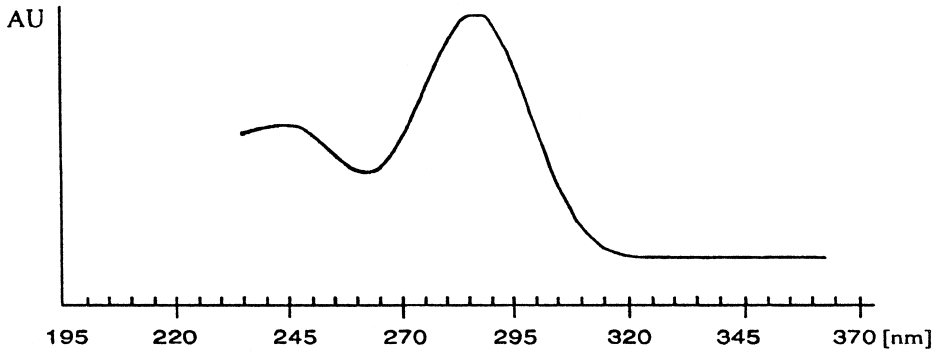

Figure 4. Overlay spectra of dazomet from analytical standard and dazomet from pesticide formulation (Index purity = 1.0).

for the separation factor measured from the adjacent peaks was 1.21, 2.21, and 1.16. In addition, to confirm the specificity of the developed method, UV diode array detection was used to check the peak purity and analyte peak identity.[5] Figure 4 shows the overlay spectra with a purity index of 1.0, obtained by comparing the absorption spectra of a pure analytical standard and absorption spectra of an active ingredient in formulation.

Repeatability[6,7,8] of results was evaluated from obtained values for retention times and peak areas of dazomet after eight successive injections of analytical standard of dazomet, with concentration of 43.75 μg/mL (Table 1). The percent of relative standard deviation for retention time was 0.6 and 1.7 for peak area.

Table 1. Repeatability of Retention Times and Peak Area of Dazomet

n	Rt	Area
1	3.52	3782328
2	3.53	3780908
3	3.49	3699758
4	3.55	3688373
5	3.56	3686188
6	3.54	3690347
7	3.52	3809471
8	3.54	3624092
$\bar{x}$	3.53	3720183.1
SD	0.022	63505.07
% RSD	0.623	1.707

Table 2. Statistical Evaluation for Calibration Curves of Dazomet

	Regression Equation	Correlation Coefficient
Area	$y = 3.43058e^{o} + 5.78664e^{-5}x$	0.99970
Height	$y = -4.01673e^{-1} + 2.64268e^{-1}x$	0.99277
Area	$y = 4.88519e^{o} + 5.70856e^{-5}x + 4.98100e^{-14}x^2$	0.99971
Height	$y = -1.2466e^{o} + 2.66287e^{-1}x - 5.85379e^{-7}x^2$	0.99277
Area	$y = 1.09874e^{1} + 5.1267e^{-5}x + 1.18026e^{-12}x^2 - 5.14108e^{-20}x^3$	0.99980
Height	$y = 2.37436e^{1} + 1.65947e^{-1}x + 8.25349e^{-5}x^2 - 1.6632e^{-8}x^3$	0.99451

The mean value for retention times of dazomet calculated from all injections in the assays was determined as 3.59, and percent of relative standard deviation calculated for this data was 1.85%.

The calibration curve of dazomet was obtained with triplicate injections (5 μL each) of varying concentrations of the analytical standard in a range from 35 to 875 ng (7-175 μg/mL). The peak areas and heights were used as dependent variables and their values were treated with the OMEGA[9] statistical program using external standard multilevel calibration by linear, quadratic, and cubic fit. The results for statistical estimation are presented in Table 2. It is evident that the obtained results for correlation coefficient indicated, preferably dependent of peak area as variable in all cases, to be of tested fit. The method showed good linearity, because the obtained value for correlation coefficient was 0.99970.

Minimum detection limit (signal to noise ratio 3:1) was determined as an absolute amount of 4.375 ng or concentration of 0.875 μg/mL at 0.05 AUFS sensitivity level.

Accuracy of the method was expressed as the deviation between the calculated mean value obtained by examination and the true value of the spiked amounts of analyte (20 μg/mL, 40 μg/mL, 80 μg/mL) in a mixture of isomer standards. The percentage of recovered analyte was 96.97, 99.16, and 103.02%. The obtained mean concentrations of active ingredient in tested amounts of pesticide formulations were 98.5, 99.2, and 99.4%, respectively, for high contents of weighted amounts of sample.

CONCLUSION

The HPLC method developed in this study allows the separation of dazomet and its dehydro-dimer forms. The proposed procedure is simple and relatively rapid for a routine analysis and precise enough for the quantification of active ingredient in pesticide formulation Basamid Granulat.

REFERENCES

1. Tomlin, C. *The Pesticide Manual Incorporating the Agrochemicals Handbook*, 11th Ed.; Crop Protection Publications: 1997; 279-280.
2. Lam, W.W.; Kim, J.H.; Sparks, S. E.; Quisted, G. B.; Casida. J.E. Metabolism in Rats and Mice of the Soil Fumigants Metham, Methyl Isothiocyanate, and Dazomet. J. Agric. Food Chem. **1993**, *41*, 1497-1502.
3. Osselton, M. D.; Snelling, R. D. Chromatographic Identification of Pesticides. J. Chromatogr. **1986**, *368*, 265 - 271.
4. CIPAC method, 1984; 2074 - 2077.
5. Jenke, D. R. Chromatographic Method Validation: A Review of Current Practices and Procedures. I. General Concepts and Guidelines. J. Liq. Chrom. & Rel. Technol. **1996**, *19* **(5)**, 737 - 757.
6. Lough, W. J.; Wainer, I. W. *High Performance Liquid Chromatography Fundamental Principles and Practice, Blackie Academic & Professional*, Chapman & Hall: Glasgow, UK, 1996; 50, 156-163.
7. Hearn, G.M. *A Guide to Validation in HPLC*, Perkin - Elmer Corp.: England, 1992; 1-20.
8. Meyer, V.R. *Practical High- Performance Liquid Chromatography*, 2nd Ed.; John Wiley & Sons, Ltd.: Chichester, 1994; 259-260.
9. OMEGA**,** Perkin - Elmer Corporation, 1989.

Received July 5, 2000 Manuscript 5324
Accepted August 27, 2000