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Determination of Dazomet in Basamid Granulat Using Reversed Phase HPLC

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The reverse phase HPLC determination of dazomet (tetrahydro-3,5-dimethyl-1,3,5-thiadiazine-2-tione) in a pesticide formulation has been studied. HS Pecosphere 3 × 3 C8 (3 μm, 3.3 × 0.46 cm) and LiChrosorb C8 (5 μm, 25 × 0.4 cm) analytical columns were tested at a flow rate of 1.0 cm^3 min^{-1}, column temperature of 25 °C and UV detection at 280 nm. The best separation of dazomet from its dehydro-dimer forms was achieved with a mobile phase containing acetonitrile-water in the volume ratio 15 : 85 on a HS Pecosphere column, and acetonitrile–water in the volume ratio 30 : 70 on a LiChrosorb C8 column. The HS Pecosphere column showed a better peak symmetry, separation factor, and multiple correlation coefficient. The retention time and peak area for the HS Pecosphere column were precise within a day and between days as indicated by the ANOVA test, in contrast to the LiChrosorb column, which showed lost of efficiency.

Key words: reversed-phase HPLC, tetrahydro-3,5-dimethyl-1,3,5-thiadiazine-2-tione.

INTRODUCTION

Dazomet, tetrahydro-3,5-dimethyl-1,3,5-thiadiazine-2-tione (**I**), is widely used as a nematicide, fungicide, herbicide and insecticide.[1] In the soil, in the presence of moisture, it undergoes degradation to methyl (methylaminomethyl) dithiocarbamic acid, which then undergoes further degradation into methyl isothiocyanate, formaldehyde, hydrogen sulfide and methylamine.[1]

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Residues of the parent compound were determinated in soil by HPLC, that of methyl isothiocyanate in crops by GLC.[1] Urinary metabolites[2] in rats and mice were determined using the reverse phase HPLC.

In the study of Osselton and Snelling,[3] the HPLC method with Spherisorb S5W and ODS-Hypersil columns were used for separation of 51 common pesticides. In order to confirm peak identification, the capacity factors and the absorption maximum from UV spectra were listed. No further quantitative investigation was performed.

The declared content of this active ingredient in the pesticide formulation Basamid Granulat is 98% (±4). Major impurities of dazomet in this formulation are **Ia**, **Ib** and **Ic**, dehydro-dimer forms with IUPAC names:

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 analysis for determination of dazomet in technical granules was performed using acid hydrolysis – absorption of the produced carbon disulfide in methanolic KOH and quantified by iodometric titration according to the CIPAC Handbook method.[4]

It is evident that the results will increase if the dehydro-dimer forms of dazomet are present in the formulation. However, no HPLC method for determination of dazomet in the pesticide formulation Basamid Granulat has been found.

It is known that the separation of basic compounds on 'acidic' silica (such as an ODS column) often gives marked peak tailing.[5] For this reason, the HPLC analysis of bases has been the subject of much discussion with many recommendations as to how to find the best way to tackle the problem.[5] It was suggested that a shorter alkyl chain, such as C8, gives better results than ODS.[5] Therefore, the aim of this paper is to investigate the possibility of developing a reversed phase HPLC method instead of the actual CIPAC reference method using two different types of C8 columns.

EXPERIMENTAL

Chemicals

Methanol, acetonitrile and water (HPLC-grade) were from Sigma-Aldrich (Deisenhofen, Germany). The pesticide formulation Basamid Granulat, the pure analytical standard of dazomet and its dehydro-dimers were gifts from BASF (Germany). The retention time of potassium nitrate, purchased from Alkaloid (Macedonia), was used as the column dead-time.

HPLC Analysis

A Perkin Elmer HPLC, with Binary LC Pump (model 250), and UV Diode Array Detector (model LC 235) were used. Constant column temperature was maintained with a column-thermostated Spark Holland »Mistral« (type 880). The investigations were carried out with a High-Speed Pecosphere 3 × 3 C8 analytical column, particle size 3 μm and column dimensions 3.3 × 0.46 cm, purchased from Perkin Elmer (Norwalk, Connecticut). LiChrosorb C8 analytical column, particle size 5 μm and column dimensions 25 × 0.4 cm, was purchased from Merck (Darmstadt, Germany).

To achieve the best separation of dazomet from its dehydro-dimer forms on the High-Speed Pecosphere 3 × 3 C8 column, the mobile phase consisting of acetonitrile-water, volume ratio 15 : 85, was used. The flow rate of the eluent was 1 $cm^3\ min^{-1}$, and the column temperature was maintained at 25 °C. The diode array detector was set to monitor the signals of the analyte at a wavelength of 280 nm (0.5 AUFS). The chromatograms were integrated at a chart speed of 10 mm/min (Method A).

The mobile phase of the same composition at a volume ratio of 30 : 70 on the LiChrosorb C8 analytical column was applied. The other working conditions were identical with the previous method, except for the detector sensitivity level, which was 0.2 AUFS (Method B).

Preparation of Standard Solutions

The dazomet stock solution was prepared by dissolving 0.0155 g of pure analytical standard with methanol in a 25 cm^3 volumetric flask. Working solutions were prepared from 0.028 cm^3, 0.05 cm^3, 0.1 cm^3, 0.35 cm^3, 0.7 cm^3, 1 cm^3 and 1.5 cm^3 of stock solution in 10 cm^3 volumetric flasks and dissolved with a mixture of acetonitrile-water (volume ratio 50 : 50).

Calibration Curve

The calibration curve of dazomet was obtained with a triplicate injection (5 mm^3 each) of working solutions. The area and height counts of individual peaks and the corresponding amount of dazomet were used to construct the standard curve, using the least-squares method by the OMEGA[6] statistical program with external standard multilevel calibration by linear, quadratic and cubic fit. The curve followed Beer's law in the range of 8.68–465 ng (or 1.736–93 $\mu g\ cm^{-3}$).

Repeatability

The day to day and within day repeatability of the retention time and peak area were performed on three days by 8 successive injections of the analytical standard of dazomet (232 ng). The obtained results were tested with the ANOVA test.

Accuracy

The accuracy of the methods was evaluated by comparing the results obtained using peak areas of the amounts of analyte (200 ng and 400 ng) spiked into a mixture of metabolites with the true value.

Sample Solution

In order to determine the content of dazomet in Basamid Granulat, the samples were weighed in the amounts of 0.0300 and 0.0150 g in a 25 cm^3 volumetric flask, dissolved in an acetonitrile-water mixture (vol. ratio 50 : 50), degassed for 20 min in an ultrasonic bath, and then 0.5 cm^3 from each solution was transferred in a 10 cm^3 volumetric flask. These solutions were filtered through 0.45 μm Spartan-T syringe filters and three injections were performed with 5 mm^3 each for all cases.

RESULTS AND DISCUSSION

The UV spectrum of dazomet in an acetonitrile-water mixture (vol. ratios 30 : 70 and 15 : 85) had bands at approximately 210, 245 and 280 nm (Figure 1). The band that lies at the longest-wavelength had a high intensity, therefore measurements were performed at 280 nm. In addition, to confirm the specificity of the developed methods, the UV diode array detection was used to check the peak purity and analyte peak identity.[7] Figure 1 shows the overlay spectra obtained by comparing the absorption spectra of the pure analytical standard and absorption spectra of the active ingredient in the formulation. For both methods, the purity index was 1.0.

The chromatograms (Figures 2 and 3) show the resolution of dazomet (232 ng) from its dehydro-dimer forms. Good separation of dazomet from its dehydro-dimer forms was obtained in both tested methods. The value estimated for the retention time on the High-Speed column for dazomet was about 0.65 min. The column dead-time was approximately t_0 = 0.25 min, so the retention factor k was 1.6. In the case of dehydro-dimer forms from these chromatographic conditions, the retention factors were about 3.96 (**Ia**),

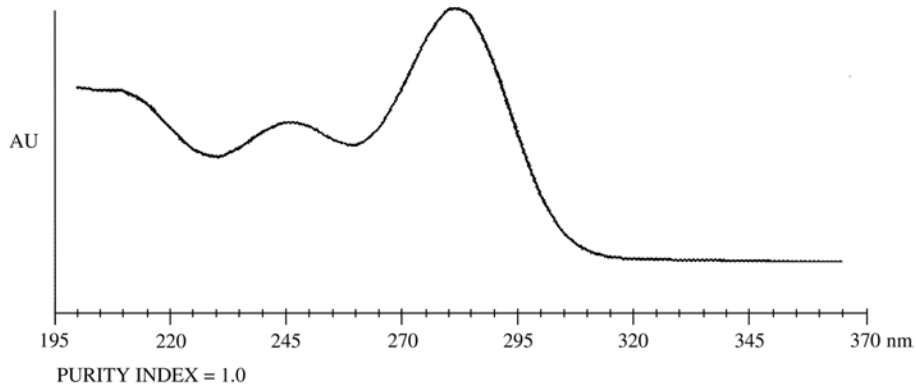

Figure 1. Overlay spectra of dazomet from analytical standard and dazomet from pesticide formulation.

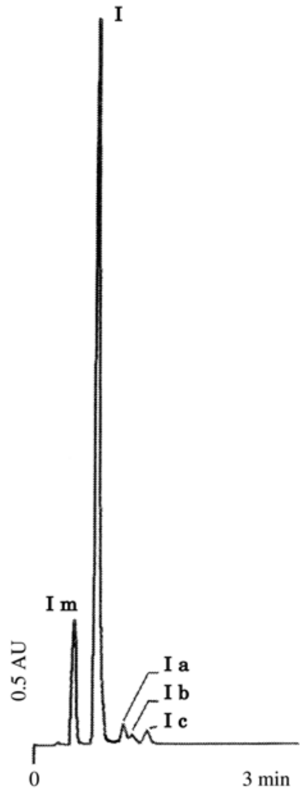

Figure 2. Chromatogram of dazomet (**I**) (232 ng), its dehydro-dimer forms **Ia**, **Ib**, **Ic** and methanol (**Im**), separated on HS Pecosphere 3 × 3 C8 column, mobile phase acetonitrile-water (15 : 85); flow rate 1 cm^3 min^{-1}, column temperature 25 °C; UV detection 285 nm.

4.56 (**Ib**), and 5.32 (**Ic**). The retention time for the LiChrosorb column was approximately 3.85 min (t_0 = 1.57), and the value assessed for the retention factor k was 1.45. Retention factors for dehydro-dimers were around 1.80 (**Ia**), 1.94 (**Ib**), and 2.11 (**Ic**). The values calculated for the separation factors between adjacent peaks were $\alpha_{\mathbf{I,Ia}}$ = 2.47, $\alpha_{\mathbf{Ia,Ib}}$ = 1.15 and $\alpha_{\mathbf{Ib,Ic}}$ = 1.17 (High-Speed column), in contrast to the LiChrosorb column where the obtained values were $\alpha_{\mathbf{I,Ia}}$ = 1.24, $\alpha_{\mathbf{Ia,Ib}}$ = 1.08 and $\alpha_{\mathbf{Ib,Ic}}$ = 1.09. Thus, the high efficiency of separation, reduced time of analysis and reduced mobile phase consumption,[8,9] made the High-Speed column suitable for HPLC analysis.

The detection limit (signal/noise = 3) for dazomet was determined as 881.6 pg (n = 5) for Method A and 2.88 ng (n = 5) for Method B.

Calibration graphs were constructed by plotting the injected amount of the active ingredient as a function of the peak area and height. Statistical

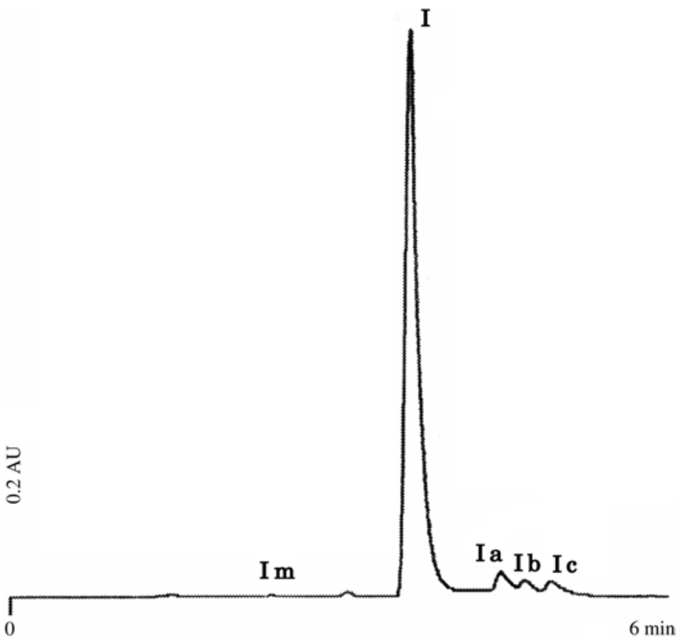

Figure 3. Chromatogram of dazomet (**I**) (232 ng), its dehydro-dimer forms **Ia**, **Ib**, **Ic** and metanol (**Im**) separated on LiChrosorb C8 column, mobile phase acetonitrile-water (30 : 70); flow rate 1 cm^3 min^{-1}, column temperature 25 °C; UV detection 285 nm.

evaluations for both tested columns are listed in Tables I and II. Regression equations and the value of the multiple correlation coefficients (R^2) showed better linearity of the peak area *vs*. the corresponding amount of analytical standard in the investigated area. The values estimated for the High-Speed

TABLE I

Statistical evaluation of dazomet calibration curves, Method A[a]

	Regression equation	Multiple correlation coefficient
Area	$y = -1.08900e^{0} + 5.52606e^{-5}x$	0.99981
Height	$y = 2.87091e^{0} + 2.20290e^{-1}x$	0.99611
Area	$y = -7.44914e^{-1} + 5.48373e^{-5}x + 5.24518e^{-14}x^2$	0.99981
Height	$y = 1.81113e^{0} + 2.25788e^{-1}x - 2.75499e^{-6}x^2$	0.99615
Area	$y = -1.89283e^{0} + 5.75838e^{-5}x - 8.23347e^{-13}x^2 + 6.85950e^{-20}x^3$	0.99984
Height	$y = -6.21978e^{0} + 3.07077e^{-1}x - 1.11252e^{-4}x^2 + 3.52019e^{-8}x^3$	0.99765

[a] The mean of 3 determinations at each level.

TABLE II

Statistical evaluation of dazomet calibration curves, Method B[a]

	Regression equation	Multiple correlation coefficient
Area	$y = 1.91460e^{0} + 6.43440e^{-5}x$	0.99977
Height	$y = 1.07736e^{0} + 5.50887e^{-1}x$	0.99934
Area	$y = 2.01396e^{0} + 6.41902e^{-5}x + 2.15921e^{-14}x^2$	0.99977
Height	$y = 1.37360e^{0} + 5.47011e^{-1}x + 4.64911e^{-6}x^2$	0.99935
Area	$y = 1.19284e^{0} + 6.69866e^{-5}x - 1.15891e^{-12}x^2 + 1.12766e^{-19}x^3$	0.99978
Height	$y = 2.15520e^{0} + 5.24676e^{-1}x + 8.47396e^{-5}x^2 - 6.52051e^{-8}x^3$	0.99936

[a] The mean of 3 determinations at each level.

column showed that R^2 were similar for the quadratic and linear fit. The value of the cubic coefficient is slightly higher (0.00003) than the quadratic and linear ones. Hence, the linear equation was used for simplicity.

The within day precision[5,7,10] is expressed as percentage of the relative standard deviation and the results are listed in Tables III, IV and V. A good repeatability of all tested parameters was achieved for both methods.

The day to day analysis showed a loss of efficiency for the LiChrosorb column. After several injections, the peak asymmetry for dazomet was higher

TABLE III

Analysis of variance for intra and inter day precision of retention time (Method A)

Day/Assay	1	2	3	4	5	6	7	8
1	0.65	0.64	0.65	0.64	0.64	0.64	0.64	0.65
2	0.64	0.66	0.66	0.65	0.64	0.64	0.64	0.66
3	0.66	0.65	0.65	0.64	0.66	0.65	0.66	0.65
		Mean = 0.65		SD = 0.008		RSD = 1.23%		

ANOVA test

Source of variation	df	Sum of squares	Mean of squares	F	P	F crit.
Within day	7	0.000333	4.76 e^{-5}	0.747664	0.05	2.764196
Between days	2	0.000308	1.54 e^{-4}	2.420561	0.05	3.73889
Error	14	0.000892	6.37 e^{-5}			
Total	23	0.001533				

TABLE IV

Analysis of variance for intra and inter day precision of peak area (232 ng) (Method A)

Assay/Day	1	2	3
1	4186052	4213521	4180963
2	4180895	4104822	4261584
3	4251059	4253480	4252452
4	4223514	4249861	4228379
5	4252889	4218152	4138645
6	4257415	4203869	4143720
7	4221879	4111937	4153619
8	4251815	4243720	4299730
Mean = 4211832 SD = 50396.88 RSD = 1.20%			

ANOVA test

Source of variation	df	Sum of squares	Mean of squares	F	P	F crit.
Within day	7	2.63 e^{10}	3.76 e^{9}	1.688394	0.05	2.764196
Between days	2	3.43 e^{9}	1.72 e^{9}	0.77063	0.05	3.73889
Error	14	3.12 e^{10}	2.23 e^{9}			
Total	23	6.1 e^{10}				

than 2.5 (*vs.* Method A, 1.0); compounds **Ia**, **Ib**, and **Ic** were overlapped. The chromatograms obtained for the tested columns were different (Figures 2 and 3), the methanol peak (**Im**) from the stock solution present in Method A, was noticeably higher than in the case of Method B. The peak area measured for the same content of analyte (Tables IV and V) was smaller for the LiChrosorb column, so we suppose that this behaviour may have arisen for several reasons: the extra column effect, unwanted sample interactions or other chromatographic problems.[12]

Similar behaviour of some substituted pyrimidines on RP C8 and RP C18 columns, when acetonitrile-water were used as mobile phase, were noticed by Cabras *et al.*[11]

The day to day repeatability of the retention times and peak areas for the High-Speed column are shown in Tables III and IV. The F values calculated for the retention times and peak areas, $F_{0.05}$ (2, 14) and $F_{0.05}$ (7, 14), were smaller than the table values $F_{0.05}$ (2, 14) = 3.739 and $F_{0.05}$ (7, 14) = 2.764, respectively. Thus, it was concluded, that there was no significant difference between the assays within and between days.

TABLE V

Intra day precision of retention times and peak area of dazomet (232 ng) (Method B)

n	1	2	3	4	5	6	7	8
t_R	3.81	3.80	3.84	3.86	3.83	3.80	3.83	3.82
	n = 8	Mean = 3.82	SD = 0.021	RSD = 0.55 %				
Area	3382436	3325239	3375545	3419833	3363627	3359388	3413694	3352732
	n = 8	Mean = 3374062	SD = 31398.073	RSD = 0.93 %				

The average percent of recovered analyte using the High-Speed column was 101.44% with RSD = 0.39% (n = 6) and 100.78% with RSD = 0.70% (n = 6) for high contents of spiked amounts of the standard.

The active substance quantity in pesticide formulation has the mean value equal to 98.63 with RSD = 1.01% (n = 18). The determined content corresponds to the values declared by the manufacturer.

CONCLUSION

This study shows the possibility of dazomet determination in the pesticide formulation Basamid Granulat by the reverse phase HPLC, using the HS Pecosphere 3 × 3 C8 and LiChrosorb C8 columns. The LiChrosorb column showed a loss of efficiency, contrary to the HS Pecosphere column which showed a better peak symmetry, separation factor, multiple correlation coefficient and reproducibility of retention time and peak area. Hence, the developed method on the HS Pecosphere 3 × 3 C8 column is simple, fast, economical and precise enough for the routine analysis of dazomet in the pesticide formulation Basamid Granulat.

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SAŽETAK

Određivanje dazometa u granulatu bazamida primjenom HPLC na reverznoj fazi

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Istraživana je mogućnost određivanja dazometa (tetrahidro-3,5-dimetil-1,3,5-tiadiazin-2-tiona) u pesticidnoj formulaciji primjenom HPLC na reverznoj fazi. Visokoprotočna analitička kolona Pecosphere 3 × 3 C8 (3 µm, 3,3 × 0,46 cm) i kolona LiChrosorb C8 (5 µm, 25 × 0,4 cm) testirane su pri brzini protoka eluensa 1,0 $cm^3\ min^{-1}$, temperaturi kolone 25 °C, te uz UV detekciju pri 280 nm. Na visokoprotočnoj koloni Pecosphere najbolje razlučivanje dazometa od njegova dihidro-dimernog oblika postignuto je s mobilnom fazom koja je sadržavala smjesu acetonitril-voda u volumnom omjeru 15 : 85, dok je za kolonu LiChrosorb najbolje razlučivanje dobiveno za volumni omjer acetonitril-voda 30 : 70. Visokoprotočna kolona Pecosphere dala je bolju simetriju vrha, bolji faktor razlučivanja, te bolju linearnost. Analiza varijacije upućuje na preciznost vremena zadržavanja i površine vrha visokoprotočne kolone Pecosphere u pokusima ponovljivosti provedenim tijekom jednog dana te kroz više dana. Nasuprot tome, kod kolone LiChrosorb utvrđen je gubitak djelotvornosti.