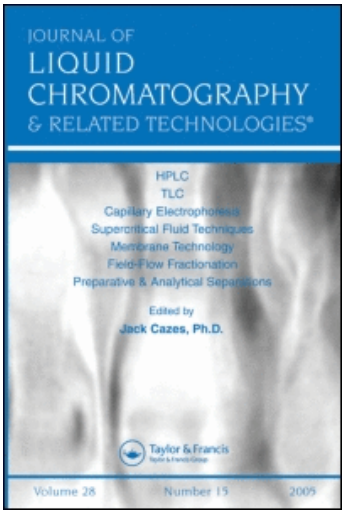

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Determination Of Mexacarbate And Five Of Its Derivatives By High-Performance Liquid Chromatography

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DETERMINATION OF MEXACARBATE AND FIVE OF ITS DERIVATIVES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A simple, sensitive and reliable analytical method has been developed and reported for mexacarbate (4-dimethylamino-3,5-xylyl N-methylcarbamate) and five of its possible degradation products likely to be found in environmental samples using reversed-phase high-performance liquid chromatography with isocratic and gradient solvent systems. All chromatogram peaks were identified through comparison to standards. The method has been used to identify and separate the six compounds from a mixture of the standards. It has been evaluated under different column conditions and with different mobile phases. Best resolution of the analytes was obtained by using a gradient solvent system consisting of CH_3CN and H_2O detecting at 200 nm and 30°C using a HP-RP8, 10 μm, 20 cm x 4.6 mm column.

INTRODUCTION

Mexacarbate (4-dimethylamino-3,5-xylyl N-methylcarbamate), a broad spectrum insecticide, was introduced by the Dow Chemical

 0148-3919/85/0814-2579$3.50/0

Company under the trade name Zectran® in 1961 (1). The chemical was field tested for control of spruce budworm (*Choristoneura fumiferana*, Clemens) larvae in different eastern provinces of Canada during the 1972-73 spray seasons (2). The chemical is currently being re-examined for large scale forestry use in Canada because of its desirable properties such as pest selectivity (3), relatively low mammalian toxicity (4) and low persistence in the environment (3,5). One of the primary requirements, with such extensive operational use patterns, is to have sensitive and reliable residue methods to study the distribution, persistence and metabolic fate of the sprayed material found in various forestry substrates. Union Carbide claims (3) that mexacarbate may be highly labile in the environment but that some of its degradation products, especially the 4-methylamino and the 4-amino-3,5-xylyl N-methylcarbamates, were found to be more toxic to some of the nontarget species than the parent material (6). It has therefore become necessary to monitor simultaneously the parent material and its likely breakdown products from environmental samples. To date, relatively few residue methods have been reported for the derivatives of mexacarbate.

Colorimetric methods (7,8), gas-liquid chromatographic (GLC) methods using an electron capture detector (9), electrolytic conductivity detector (10,11), flame ionization detector (12-16) and N-P specific thermionic detector (17,18) have been reported in literature for the parent material. These methods are either tedious and time consuming due to derivatization, or insensitive because of on-column decomposition of carbamate insecticides (19). Indeed, Zweig and Sherma (11) reported that the percent breakdown of mexacarbate, depending on GLC column packing and temperature, varied from 8.6 to 55.8.

High-performance liquid chromatography (HPLC) with its high speed and sensitivity offers a promising alternative system in analyzing thermally labile compounds such as carbamates. Earlier HPLC studies (20-22) were mostly concerned with the separation and quantification of different types of carbamates by normal and re-

verse phase modes including post-column derivatization techniques. Since we are interested in the identification and quantification of the active material and its breakdown products, the HPLC methods reported in literature are not sufficient for our purpose. In this paper, we report not only the analysis of mexacarbate by HPLC using UV absorbance but also the simultaneous analysis of five of its possible degradation products that are most likely to be found in forestry substrates. It has been reported (23) that aminocarb (4-dimethylamino-m-tolyl N-methylcarbamate), a structurally similar insecticide, subsequent to its release in the environment, gave similar degradation products due to hydrolysis and oxidative demethylation. Emphasis is placed here on the optimization of HPLC parameters, judicious selection of solvents necessary to separate all six compounds by both isocratic and gradient systems, the response coefficient of the instrument to the analytes at the chosen wavelength and their minimum detectable limits (MDL).

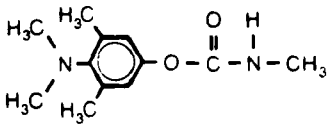
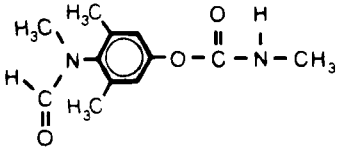
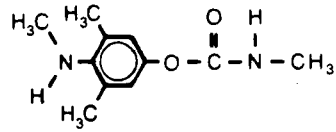
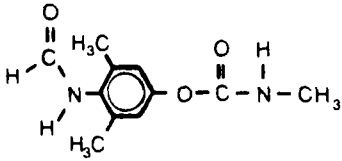
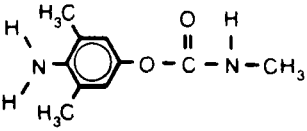
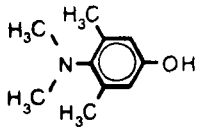
MATERIALS

Analytical standards of mexacarbate (Zectran®) and the five derivatives used in the present study were supplied by Union Carbide. The structural formulae, International Union of Pure and Applied Chemistry (IUPAC) usage and the abbreviations used for them in this paper are given in Table 1.

All solvents were HPLC grade received from Fisher and tested prior to use for their spectral purity. They were filtered through appropriate Millipore filters and degassed before use. All compounds were soluble in methanol and acetonitrile and showed good stability. Standard stock solutions were prepared in the solvents and subsequently diluted as required. All standards were thoroughly filtered prior to injection into the HPLC system. Prolonged storage (> 4 weeks) of stock solutions of compounds 2, 3, 4 and 6 (Table 1) in tightly sealed volumetric flasks at 1°C, even if they were completely covered with aluminum foil to shield them

TABLE 1

Some Common Metabolites of Mexacarbate Used in the Study.

No.	CHEMICAL STRUCTURE	NAME (IUPAC USAGE)	ABBR.
1		4-Dimethylamino-3,5-xylyl N-methylcarbamate	M
2		4-Methylformamido-3,5-xylyl N-methylcarbamate	MFM
3		4-Methylamino-3,5-xylyl N-methylcarbamate	MAM
4		4-Formamido-3,5-xylyl N-methylcarbamate	FAM
5		4-Amino-3,5-xylyl N-methylcarbamate	AM
6		4-Dimethylamino-3,5-xylenol	DMAX

from light, showed progressive formation of colourless degradation products with the following retention times [RT (min)] (under the experimental conditions discussed below using an RP-8 column).

Compound No.	RT (min)
2	3.51, 3.92
3	3.90
4	4.55
6	4.54

The other solutions were quite stable under these conditions for the entire period of study (> 8 weeks).

No structural correlations could be established with the HPLC peaks observed for them with the compounds studied in Table 1 except that the presence of N-formyl and N-methylamino functional groups generally added instability to the molecular species. The formation of such intermediates indicate that mexacarbate is relatively labile in solution and an in depth investigation of its metabolic fate is warranted.

METHODS

A Hewlett-Packard model 1084B high-performance liquid chromatograph equipped with a variable wavelength detector (190 - 600 nm), microprocessor and electronic integrator was used for this study. The instrument also employed an automatic degassing system, dual solvent system and dual pumpheads with common drive which gave stable and reproducible flows. A Hewlett-Packard LC terminal (79850B) provided the chromatogram, area, area %, retention time, etc., for each peak. The operating parameters were as follows:

Columns: (a) Hewlett-Packard (HP) RP-8, 10 μm, 20 cm x 4.6 mm ID
(b) Regis Spherisorb Hi-Chrom Rev. ODS2 Octadecyl II, 5 μm, 15 cm x 4.6 mm ID

Column Pressure: 16 - 116 bars (1 bar = 14.5 psig.)

Mobile Systems (v/v): (a) CH_3CN/H_2O
(b) CH_3OH/H_2O

Flow Rate: 1 mL/min

Oven Temperature: 30°C and 50°C

Wavelengths: Sample (S): Reference (R) = 200:430 nm
220:430 nm
254:430 nm

Injection System: Rheodyne model 7120 syringe loading injector with 20 µL loop size

Sample Size: 20 µL of 10 µg/mL standard stock solution

Chart Speed: 0.5 cm/min

Attenuations: 2^2 and 2^5

Slope Sensitivity: 0.2

Various isocratic and gradient elution systems were developed and used to obtain the basic chromatograms of Zectran® and 5 of its metabolites. Solutions of the parent material, the individual metabolites and the mixture were injected separately several times ($n > 4$) to obtain reproducible results. The chromatograms obtained were well defined, having sharp peaks showing a deviation in RT of less that 5% for each injection. The linearity of the UV detector was measured by injecting, several times during the study, aliquot quantities of different concentrations (range 0.10 µg/mL to 10 µg/mL) of the chemicals analysed and the response was found to be adequate. Under the experimental conditions stipulated above, the relative sensitivity of the UV detector was good and MDL for the metabolites ranged from 5 ng (compounds 3, 5 and 6) to 10 ng (compounds 2 and 4), and for the mexacarbate it was found to be 3 ng.

RESULTS AND DISCUSSION

The solubility (wt %) of mexacarbate at 25°C varied widely between water (0.01%) and polar organic solvents such as acetonitrile and methanol (>100%). Consequently, these solvents served as the best choice for optimizing the separation of mexacarbate and its metabolites by HPLC.

The average RTs of mexacarbate and its metabolites studied using the isocratic solvent system at different % (v/v) compositions of CH_3CN/H_2O are given in Table 2. From the values recorded, it is evident that the solvent system containing 40:60% of

TABLE 2

RT (min) of Mexacarbate and Some of Its Metabolites* by Using a RP-8, 10 μm Column with Different Isocratic Solvent Systems: Flow Rate 1 mL/min, Temp. 30°C, λ = 200 nm

Cmpd. No. with Abbreviated Name	$CH_3CN:H_2O$				
	80:20	60:40	55:45	50:50	40:60
1 M	3.34	5.43	6.70	8.28	17.20
2 MFM	2.81	3.11	3.17	3.27	4.27
3 MAM	2.81	3.45	3.75	3.91	5.23
4 FAM	2.81	3.11	2.90	3.27	3.48
5 AM	2.81	3.11	3.31	3.27	3.85
6 DMAX	3.34	5.00	6.00	7.19	13.61

* Compounds with similar RTs were confirmed by injecting individual samples

CH_3CN and H_2O is the best mobile phase suitable for separating all of the six compounds using an RP-8, 10 μm, 20 cm x 4.6 mm column under the optimum operational conditions listed above. The chromatogram obtained (Fig. 1) depicts the excellent separation obtained. All other solvent compositions gave either poor resolution or overlapping peaks. In fact, in the 80:20 and 60:40 solvent ratios, compounds 2, 4 and 5 (Table 1) are completely overlapped. Lower CH_3CN ratios ($< 40\%$) yielded higher RTs and peak broadening, probably due to solute sorption on the stationary phase. Detection above 200 nm lowered the detector sensitivity. The compound 4 with its formamido group seems to be highly polar because it was readily soluble in the mobile phase and was eluted quickly with low RT (3.48 min) compared to the others. Mexacarbate, being lipophilic, is the least polar (RT 17.20 min) of the compounds. Considering the intact carbamate moieties listed in Table 1 and using the observed RTs (Table 2), one could predict the decrease in the order of polarity as 1 <3 <2 <5 <4. The high RT of compound 6 is probably due to its phenolic group causing it to interact selectively through H-bonding with the stationary phase.

Since the polarity of the compounds varied considerably, an octadecylsilyl (ODS) bonded phase column (Regis Spherisorb S5 ODS II, 5 μm, 15 cm x 4.6 mm) was tested isocratically using varying ratios of CH_3OH/H_2O in the hope of obtaining a better performance in the separation of analytes. The higher UV cutoff (205 nm) and higher viscosity (0.6 cP at 20°C) of methanol compared to acetonitrile (190 nm and 0.37 cP at 20°C) (24) made it necessary for detections to be done at 220 nm and 50°C. Retention times obtained for mexacarbate and its metabolites are given in Table 3. Because of smaller particle size and higher viscosity, back pressure was appreciable and the anticipated column efficiency was not observed. Also, poor and unpredictable peak separations, peak overlaps and irregular peak shapes, peak broadening, variation in RTs and at times lack of response, especially for compounds 2 and 4 (Table 3), were observed. Because of these difficulties, the analyses of these compounds isocratically using the ODS column was not continued.

Although separation and detection of the compounds listed in Table 1 are possible by using the isocratic solvent system $CH_3CN:H_2O$, 40:60 (Table 2), the selection and use of some of the

TABLE 3

RT (min) of Mexacarbate and Some of Its Metabolites by Using a Regis 5 μm Column with Different Isocratic Solvent Systems: Flow Rate 1 mL/min, Temp. 50°C, λ = 220 nm

Cmpd. No. with Abbreviated name	$CH_3OH:H_2O$ 75:25	65:35	60:40	55:45
1 M	3.29	5.49	8.14	11.99
2 MFM	1.83	2.04	2.23	NR*
3 MAM	1.98	2.30	2.57	2.93
4 FAM	1.73	NR	NR	NR
5 AM	1.81	1.97	2.06	2.21
6 DMAX	3.06	4.94	6.94	9.65

*NR - No response

TABLE 4

Solvent Systems Used in Gradient Elution of Mexacarbate and Its Metabolites: Flow Rate 1 mL/min, Temp. 30°C, λ = 200 nm.

Solvent System	Elution Time (min)	Time (min)	Acetonitrile (%)	Water (%)
A	15	0.0	28	72
		5.0	28	72
		5.5	55	45
B	20	0.0	40	60
		10.0	40	60
		10.5	50	50
C	20	0.0	28	72
		7.0	28	72
		8.0	50	50
D	20	0.0	30	70
		4.0	30	70
		5.0	25	75
		6.0	25	75
		7.0	55	45
Solvent System*	Elution Time (min)	Time (min)	Methanol (%)	Water (%)
E	20	0.0	45	55
		8.0	45	55
		9.0	65	35

* Flow rate and temp. as above except detection at λ = 254 nm.

TABLE 5

RT (min) for the Gradient Elution of Mexacarbate and Its Metabolites in CH_3CN/H_2O and CH_3OH/H_2O Systems

Cmpd. No. and Abbreviated Name	SOLVENT SYSTEM*				
	A	B	C	D	E
1 M	12.95	14.60	16.67	14.12	16.49
2 MFM	7.40	3.79	7.14	6.52	6.87
3 MAM	8.86	4.68	9.15	8.20	8.00
4 FAM	4.67	3.02	4.41	4.33	3.34
5 AM	6.31	3.37	6.07	5.76	5.07
6 DMAX	12.03	12.16	15.22	13.17	15.24

* A to D: CH_3CN/H_2O, detection at λ = 200 nm; E: CH_3OH/H_2O, detection at λ = 254 nm. Refer Table 4 for the mobile phase compositions.

gradient mobile phase systems listed in Table 4 gave a faster and better separation of all the six compounds because of the wide range in their polarities. This is evident from the RTs recorded in Table 5. Also, Figs. 2a to 2e illustrate the advantages of using a gradient system over the isocratic phase (Fig. 1). Among the 5 eluent combinations, system A containing three gradient steps, was the best to separate, identify and quantify the six compounds. The separations were good and the analysis was rapid, yielding sharp symmetrical peaks (Fig. 2a). The RTs ranged from 4.67 min for the relatively polar compound 4 to 12.95 min for the nonpolar lipophilic mexacarbate (Table 5). The eluent combination B, yielded a poor differentiation of compounds 2, 4 and 5 (Fig. 2b) and also gave a longer run time (20 min). Similar longer elution time was also observed for the system C along with peak broadening because of solute diffusion (Fig. 2c) and low detector sensitivity to the analytes. Peak height reduction, peak overlapping (Fig. 2d) and longer elution time made the eluent combination D unsuitable. The final gradient system E, is different from the others because it contained CH_3OH and H_2O rather than CH_3CN and H_2O. Since the detection was done at 254 nm due to the higher UV cutoff (205 nm) of methanol, sensitivity for the analytes decreased (reduction in peak height) with increased run times. Furthermore, because of the increased elution strength of the solvent system E and poor detector response, differentiation between compounds of nearly equal polarity such as methylformamido and methylamino carbamates (compounds 2 and 3) was not possible (Fig. 2e).

This study has shown that HPLC with UV detection at 200 nm is a sensitive and reliable analytical technique to separate, identify and quantify mexacarbate and five of its metabolites either using isocratic or gradient solvent systems containing CH_3CN and H_2O. With sufficient modification and expansion, the method reported will be a viable tool to screen the active material and its degradation products from various forestry substrates. Further work along this line is in progress at our laboratory.

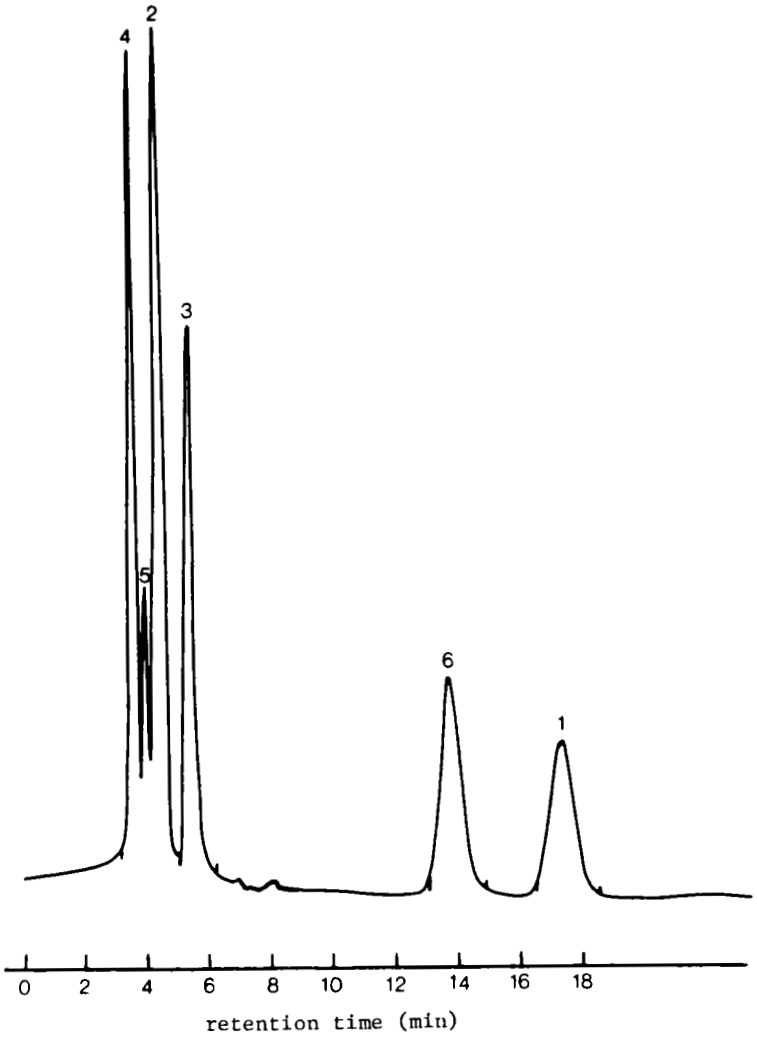
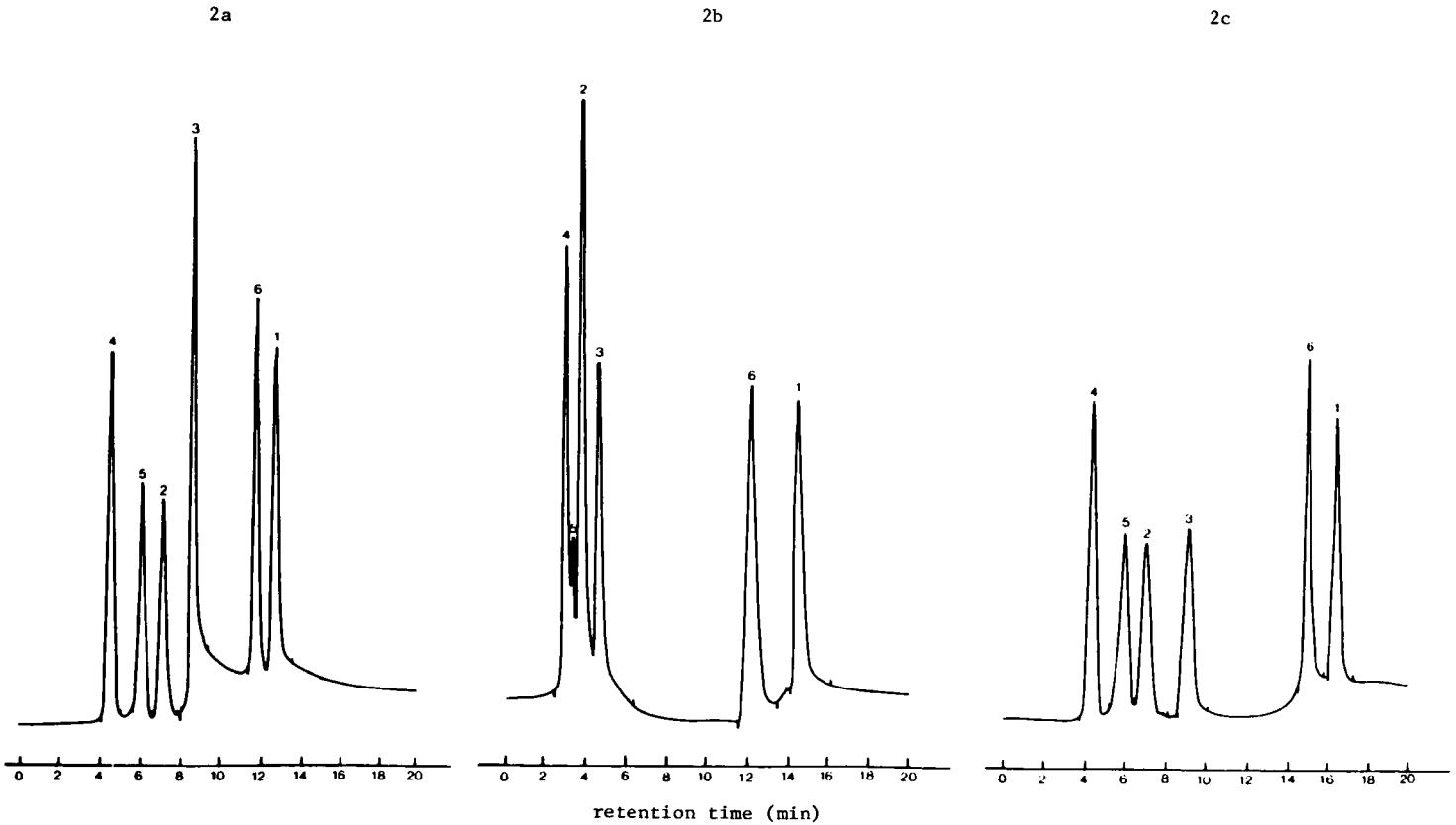


Figure 1. Separation of Mexacarbate and Its Five Metabolites by Using HPLC.
Column: RP-8, 10 μm Flow Rate: 1 mL/min
Solvent System: $CH_3CN:H_2O$ = 40:60
Numbers on the chromatograms correspond to the compounds given in Tables 1 and 2.



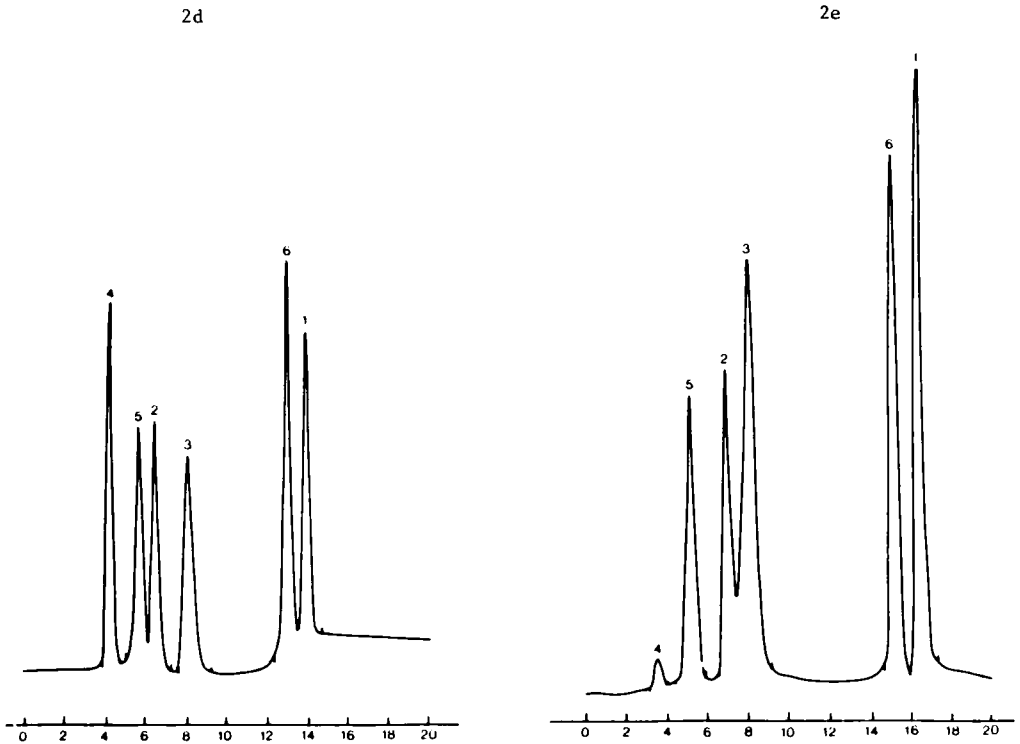


Figure 2. Separation of Mexacarbate and Its Five Metabolites by HPLC Using Different Gradient Elution Systems as Shown in Table 4.
Column: RP-8, 10 μm Flow Rate: 1 mL/min
Attn. for Figures 2a to 2d is 2^5, for Figure 2e is 2^2.
Figures 2a to 2e correspond to the gradient systems A to E respectively in Table 4.

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