Reverse-Phase Liquid Chromatographic Behavior of Some Carbamate and Urea Pesticides

Sensitivities (absorbance at 254 nm) and retention volumes with carriers of various polarities are reported for a group of carbamates and substituted ureas on a nonpolar column. Sensitivities varied widely (0.01 to 1 absorbance unit/μg) depending on the compound and the retention volume. The ureas had the largest sensitivities. The retention volumes were near 3.5 mL for most of the samples with methanol as carrier. However, either increasing or decreasing the polarity of the carrier usually increased the retention volumes. The signals were all sharp in the most polar carriers but tailing was serious for five cases in the carriers less polar than methanol.

Recent reviews have described the use of high-pressure liquid chromatography for pesticides (Moye, 1975) and various methods of analysis for carbamates (Dorough and Thorstenson, 1975). Kirkland (1969) demonstrated the analysis of some substituted ureas on a column of silica particles coated with β,β′-oxydipropionitrile. Sparacino and Hines (1976) carried out a survey of a number of carbamates on a variety of column-carrier combinations. They noted that reversed-phase chromatography gave the best overall results. However, their work stressed separations of carbamates from each other. This is not a particularly important consideration in residue analysis of foodstuffs: generally, groups of carbamates are not used on one crop. Rather, in developing or using an analytical procedure for a residue it may be helpful to be able to manipulate the retention volume of a particular compound relative to solvents and plant materials which may interfere. We present here detailed results for several carbamate and urea compounds and for several solvents in a reverse-phase chromatography system; a Partisil-10 ODS column with carriers of various polarities. The column is packed with 10 μm diameter silica particles coated with a C_{18} saturated hydrocarbon layer chemically bonded to the particles. It is noted particularly how the retention volumes vary with solvent polarity on this column and approximately how the sensitivity varies (UV absorbance). The effect of carrier on peak widths and tailing is described. Two simple examples are presented to illustrate how these results might be applicable to residue analysis.

EXPERIMENTAL SECTION

The measurements were made with a Nester-Faust Model 1240 chromatograph and a Reeve-Angel Partisil-10 ODS column, 0.46 × 25 cm. Two detectors were used to measure absorbance at 254 nm; the original Nester-Faust detector and a new Glenco Model 5480. The Glenco detector was about five times more sensitive, and all sensitivities cited here are with respect to this device. All results were obtained at room temperature, about 27 °C.

The samples were donated by the various manufacturers as "analytical standards" and were used without further purification. No impurities were observed in the chromatograms. The trade names and chemical names are listed in Table I.

The solvents were laboratory distilled water and Fisher certified ACS grade. Methanol, 2-propanol, and mixed hexanes were used without further purification. Acetonitrile was redistilled.

RESULTS AND DISCUSSION

The results are listed in Table II. The compounds are separated into several groups; *N*-methylcarbamates, thiocarbamates, "other" carbamates, metabolites, and solvents. The second column lists the sensitivities in either

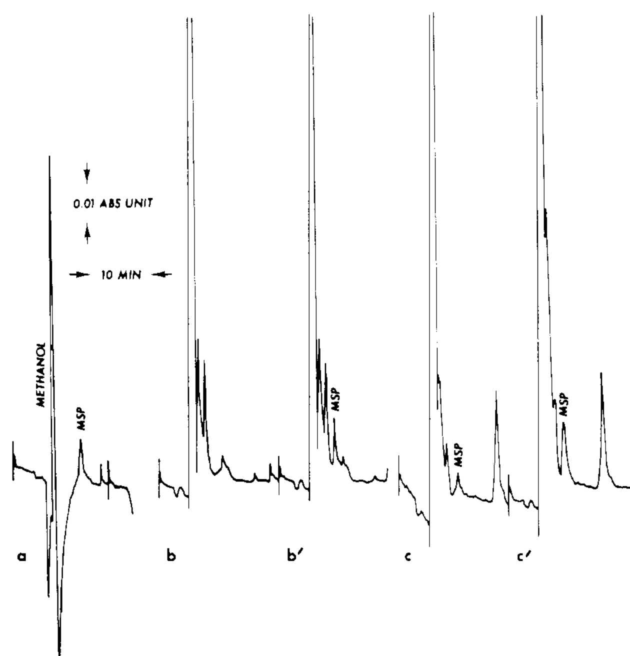

Figure 1. Chromatograms for Mesurol analysis (as the sulfone phenol, MSP) in Brussels sprouts. Chromatograph conditions: Partisil 10-ODS column, acetonitrile–water (1:2, v/v) carrier, 0.72 mL/min, 10-μL samples in methanol; (a) 0.04 μg of MSP, (b) blank Brussels sprouts preparation, (b′) blank with added 0.05 μg of MSP, (c) Brussels sprouts plus Mesurol preparation (Mesurol content equivalent to 0.05 μg of MSP), (c′) sample c with added 0.05 μg of MSP.

methanol or 2-propanol carrier. The sensitivity values vary with retention volume such that the product (signal height × retention volume) is approximately constant for a particular compound. This is a rough approximation; values for some compounds vary by ±50%. Large deviations from this relation were accompanied by tailing of the peak and were found only in the least polar carriers. The solvents listed (except for nitrobenzene) are transparent at 254 nm but do give optical signals, apparently from a refractive index gradient. These signals depend strongly on the carrier, of course. The remainder of the table contains retention volumes for the different compounds in six carriers. The carriers are arranged in order of decreasing polarity.

A further result pertaining to sensitivities may be noted. It might be expected that the absorption spectra of these compounds shift substantially from one solvent to another. Absorption spectra were obtained from the compounds Mesurol, Sevin, benomyl, diuron, fluometuron, and monuron in the solvents water–methanol (1:2, v/v), methanol, 2-propanol, and ethyl acetate. The spectra were essentially unaffected by solvent changes. The only change

Table I. Trade and Chemical Names of the Sample Compounds[a]

Formetanate	*N,N*-Dimethyl-*N'*-(3-((methylamino)carbonyl)oxy)phenylmethanimidamide
Matacil	4-(Dimethylaminophenyl)-3-methylphenyl *N*-methylcarbamate
Mesurol	4-(Methylthio)-3,5-xylyl *N*-methylcarbamate
Methomyl	*S*-Methyl *N*-[(methylcarbamoyl)oxy]thioacetimidate
Oxamyl	Methyl *N',N'*-dimethyl-*N*-[(methylcarbamoyl)oxy]-1-thiooxamimidate
Sevin (carbaryl)	1-Naphthyl *N*-methylcarbamate
Temik	2-Methyl-2-(methylthio)propionaldehyde *O*-(*N*-methylcarbamoyl)oxime
Zectran	4-(Dimethylamino)-3,5-xylyl *N*-methylcarbamate
Eptam	*S*-Ethyl *N,N*-dipropylthiocarbamate
Ro-neet	*S*-Ethyl *N*-cyclohexyl-*N*-ethylthiocarbamate
Benomyl	Methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate
CIPC	2-Propyl *N*-(3-chlorophenyl)carbamate
Chlorbromuron	3-(4-Bromo-3-chlorophenyl)-1-methoxy-1-methylurea
Diuron	3-(3,4-Dichlorophenyl)-1,1-dimethylurea
Fluometuron	1,1-Dimethyl-3-(2,α,α-trifluoro-*m*-tolyl)urea
Linuron	3-(3,4-Dichlorophenyl)-1-methoxy-1-methylurea
Monuron	3-(*p*-Chlorophenyl)-1,1-dimethylurea

[a] Thomson (1975).

Table II. Sensitivities at 254 nm and Retention Volumes for Carbamate and Urea Pesticides and for Several Solvents

		Retention volume, mL					
		Carrier[c]					
Compound	Sensitivity[a]	I	II	III	IV	V	VI
Formetante	0.017	4.15	3.51	3.98	3.35	4.07	4.50
Matacil	0.35	13.91	6.08	4.14[d]	4.38	3.77[d]	>19[d]
Mesurol	0.046	13.65	6.62	3.51	3.45	3.50	3.69
Methomyl	0.069	4.41	3.78	4.84	3.40	8.93[d]	9.00[d]
Oxamyl	0.25	4.06	3.69	4.31	3.49	6.40	15.3[d]
Sevin	0.15	7.69	4.64	3.53	3.36	3.50	3.78
Temik	0.10	5.62	4.28	3.98	3.38	3.48	4.32
Zectran	0.16	19.52	6.65	4.29	3.42	6.31	8.40[d]
Eptam	0.011	17.04	6.81	5.83	3.39	3.51	3.25
Ro-neet	0.0075	11.81	6.72	7.18	3.46	3.40	3.27
Benomyl	0.046	8.36	4.71	6.18	4.52	3.85	4.29[d]
CIPC	0.086	12.87	6.21	3.51	3.26	3.08	3.15
Chlorbromuron	0.76	15.44	6.39	3.47	3.51	3.84	3.87
Diuron	1.5	13.13	6.17	4.45	3.45	4.21	4.86
Fluometuron	0.36	9.43	5.09	4.00	3.24	3.51	4.23
Linuron	0.53	14.52	6.57	3.54	3.46	3.72	4.05
Monuron	1.1	7.96	4.93	4.91	3.49	4.14	5.31
1-Naphthol (Sevin)	0.095[b]	7.83	4.67	4.67	3.44	3.26	3.33
Mesurol sulfone phenol	0.60[b]	4.62	3.80	3.65	3.31	3.24	3.40
Water	0.0030	3.71	3.44		3.84	3.80	5.44
Methanol	0.0085[b]	3.28	3.13	3.73		3.71	3.91
2-Propanol	0.0020	4.07	3.87		3.60		3.19
Ethyl acetate	0.0080	4.57	3.89		3.50	3.29	3.30
Dimethylformamide	0.095	4.13	3.81		3.94	6.15	7.17
Nitrobenzene	0.85[b]	6.50	4.62	4.73	3.40	3.30	3.23

[a] Sensitivity is in absorbance units per microgram or microliter of compound; see text. The values vary with the carriers and retention volume such that approximately the product signal height × retention volume is constant for a particular compound. Sensitivities cited here are for methanol carrier unless noted otherwise. [b] 2-Propanol carrier. [c] Carriers were the following: (I) methanol–water 1:1 (v/v), (II) methanol–water 1:2 (v/v), (III) acetonitrile, (IV) methanol, (V) 2-propanol, (VI) 2-propanol-mixed hexanes 1:1 (v/v). [d] Signal was broad and tailed.

observed was for Mesurol: λ_{max} values were 255, 263, 260, and 240 nm, respectively, in these solvents. Ethyl acetate distorted the spectra of the three ureas because their absorption maxima were near 242 nm and the solvent absorption became strong below 250 nm.

The compounds listed in Table II all gave sharp signals which would be suitable for an analytical procedure with the more polar carriers, I–IV (except Matacil in CH_3CN). The dimensionless ratio of peak width at half-height to retention volume V_w/V_r was typically in the range 0.06 ± 0.02. No tailing was observed in these carriers. In the least polar carriers, V and VI, the potential resolution decreased markedly: V_w/V_r rose to 0.13 ± 0.07. Peaks for the compounds Matacil, methomyl, Oxamyl, Zectran, and benomyl were so broad and tailed in carriers V and VI as to be unsuitable for analysis (see Table II).

The retention volumes represent the partitioning of the samples between the column coating and the carrier. For the compounds studied the retention volumes were generally minimal and similar for the carriers of intermediate polarity, methanol and acetonitrile. Thus, for effecting separations of these compounds from others it is usually necessary to use carriers which are more or less polar than methanol and acetonitrile. Two examples are described below.

Sometimes it may be desired to determine a pesticide and its metabolite simultaneously as in the case of sevin and 1-naphthol. As shown in Table II, the retention volumes of these compounds are nearly identical over the entire range of carriers. A separation was obtained in the least polar carrier, VI, because the difference in retention volumes became as large as 0.4 mL. Since both compounds gave reasonably sharp peaks in this carrier, V_w/V_r = 0.1, the analysis is potentially feasible in this system or with a slightly less polar carrier. The analysis is not possible with the polar carriers. This implies that a normal phase

system might be suitable for this separation. The results of Sparacino and Hines (1976) support this suggestion in their Figure 1 and Table II where with a silica column and heptane carrier these compounds are well separated.

With "real" samples two complications may appear. Although sample preparation schemes are intended to separate the residue from plant material, the separation is usually incomplete. In addition, the solvent the residue is in may be different from the chromatograph carrier. As an illustration, consider the determination of mesurol as mesurol sulfone phenol (Bowman and Beroza, 1969). Brussels sprouts were "spiked" with mesurol which was then converted to MSP and extracted, with the extract finally being dissolved in methanol. In carriers of low polarity the MSP was bracketed by methanol and some unidentified plant material. It was not possible to separate MSP from both simultaneously by varying the polarity of the carrier. Apparently, if a solvent other than methanol had been used for the sample, the nonpolar carrier would have provided a potentially useful separation. With polar carriers the MSP separated cleanly from both the methanol and the plant material. For example, in acetonitrile–water (1:2, v/v) the separation shown in Figure 1 was obtained.

In summary, reversed-phase liquid chromatography is a convenient analytical technique and appears to have useful potential for the analysis of residues of carbamates and substituted ureas in foodstuffs.

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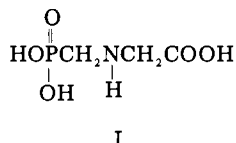
N-Nitrosamine Formation in Soil from the Herbicide Glyphosate

Formation of *N*-nitrosoglyphosate was observed when different soils were treated with sodium nitrite and the herbicide glyphosate at elevated levels. The highest yield was noted in soil low in organic matter and clay contents; however, nitrosation was not affected by soil pH. At low levels of glyphosate (5 ppm) and nitrite nitrogen (2 ppm) the formation of *N*-nitrosoglyphosate in soil was not observed.

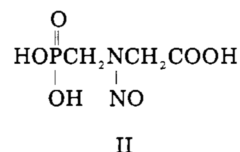
Since the discovery that some *N*-nitrosamines are carcinogenic (Barnes et al., 1954), there have been many studies on the formation, action, and analysis of this class of compounds (Mirvish, 1975; Scanlan, 1975; Montesano and Bartsch, 1976). Production of some *N*-nitrosamines in a soil environment may result from the interaction of nitrite with agricultural chemicals (Ayanaba et al., 1973; Tate and Alexander, 1974). The *N*-nitrosamines that form may be the *N*-nitroso derivative of the parent compound (Elespuru and Lijinsky, 1973; Eisenbrand et al., 1975; Uchiyama et al., 1975; Wolfe et al., 1976; Egert and Greim, 1976a) or a carcinogenic *N*-nitrosamine such as *N*-nitrosodimethylamine arising from chemical modification of the pesticide (Ayanaba et al., 1973; Ayanaba and Alexander, 1974; Egert and Greim, 1976b,c). *N*-Nitroso derivatives of some insecticides are both carcinogenic and mutagenic (Elespuru et al., 1974; Siebert and Eisenbrand, 1974; Eisenbrand et al., 1975; Uchiyama et al., 1975; Lijinsky and Taylor, 1976; Seiler, 1977). *N*-Nitrosodimethylamine is stable in soil (Tate and Alexander, 1975, 1976) and can be translocated from soil into vegetable crops (Dean-Raymond and Alexander, 1976).

Tate and Alexander (1974) were unable to detect any *N*-nitrosamines in soil treated with sodium nitrite and the herbicide glyphosate (I) at elevated levels. However, their



I

method would have detected only volatile *N*-nitrosamines. We have recently developed a method of analysis for glyphosate (Young et al., 1977) that involves formation of *N*-nitrosoglyphosate (II). We carried out a study similar



II

to that of Tate and Alexander (1974) using our method of detection (Young et al., 1977) and now report the formation of II from I in nitrite-treated soils.

EXPERIMENTAL SECTION

To 10-g portions of air-dried and ground soils (Table I) was added a solution of 1 mg of sodium nitrite (20 ppm nitrite nitrogen) and 10 mg of glyphosate isopropylamine salt (740 ppm acid equivalent) in sufficient distilled water to bring the soils to field capacity. The samples were thoroughly mixed and incubated in the dark at 25 °C. At regular intervals the soils were extracted with distilled water (2 × 50 mL), centrifuged, the combined supernatants concentrated under reduced pressure to ca. 10 mL, and centrifuged. The supernatant was washed with methylene chloride (5 × 10 mL), concentrated to 1.0 mL under reduced pressure, and diluted with 4.0 mL of acetonitrile. This solution was transferred to a column (1 × 5 cm) of Florisil (60–100 mesh, PR grade, moisture content 0.8%) and eluted with 20% water in acetonitrile (50 mL) and