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ISOCRATIC HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION AND MULTIPLE-WAVELENGTH ULTRAVIOLET DETECTION OF ALDICARB AND ITS SOIL DEGRADATION PRODUCTS

OPTIMIZATION OF STATIONARY PHASE SELECTIVITY

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SUMMARY

The unique selectivities which can be generated through optimization of stationary/mobile phase combinations have been applied to the development of an isocratic, reversed-phase high-performance liquid chromatographic method for the separation of aldicarb and its primary soil degradation products. The method utilizes a cyanopropyl bonded stationary phase and water–acetonitrile mobile phase and is capable of separating aldicarb and its various sulfoxide, sulfone and oxime derivatives in less than 10 min. When combined with multiple-wavelength UV detection and an appropriate preconcentration step, this method can in principle be applied to the routine monitoring of aldicarb and its soil derivatives in water at concentrations less than 1 μg/l.

INTRODUCTION

Carbamates are finding widespread use as commercial pesticides because of their broad spectrum of activity, high effectiveness and generally low mammalian toxicity. One carbamate pesticide that is causing growing concern, however, is aldicarb [2-methyl-2-(methylthio)-propionaldehyde-O-(methylcarbamoyl) oxime]. Aldicarb is sold by Union Carbide under the trade name Temik for use as a systemic soil insecticide, acaricide and nematicide.

Aldicarb is metabolized in soils primarily to aldicarb sulfoxide, aldicarb sulfone, aldicarb oxime, aldicarb sulfoxide oxime, aldicarb sulfone oxime (see Fig. 1 for these structures), the corresponding nitriles, and other compounds that have not yet been fully characterized. Aldicarb and its sulfoxide and sulfone derivatives have high anti-cholinesterase activity, while the corresponding oximes are approximately 1/1000 as active. Aldicarb sulfoxide, the most potent cholinesterase inhibitor of the group, is responsible for the high systemic activity and long term persistence of insecticidal activity after soil application[1,2]. There is currently evidence that indiscriminate use of aldicarb is causing ground water contamination in some parts of the United States,

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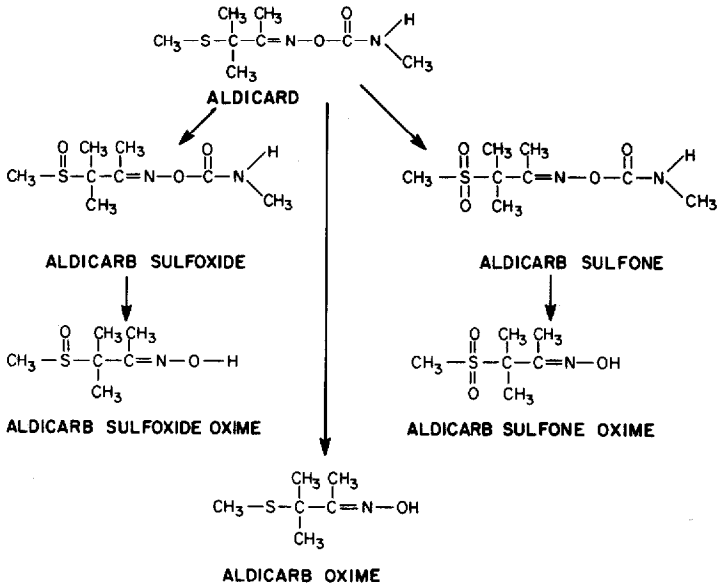

Fig. 1. Structures of aldicarb and its primary soil degradation products.

particularly in Florida and New York. There is thus a need for a rapid and sensitive method for screening water samples for the presence of aldicarb and its metabolites.

Numerous gas chromatographic (GC) methods for the determination of aldicarb and its sulfoxide and sulfone derivatives have been reported in the literature[2–4]. Packed-column GC, however, suffers the disadvantage of poor selectivity, since aldicarb and aldicarb sulfoxide have retention times too short to allow sufficient separation from the solvent peak. It is therefore necessary to convert all three to the sulfone with peracetic acid, thus providing no information regarding the relative concentrations of the three compounds.

Residue characterization of the parent pesticide and its individual metabolites is critical in order to properly evaluate the degree of contamination of potable waters, since the sulfone reportedly is 25–30 times less toxic than aldicarb and its sulfoxide[5]. If quantification of the individual metabolites is desired, it is necessary to first separate them by column chromatography. Aldicarb and aldicarb sulfoxide are then oxidized separately to aldicarb sulfone before GC analysis. These procedures are both time consuming and contribute to inaccurate determinations. Trehy *et al.*[6] developed a capillary GC–mass spectrometric (MS) method for aldicarb, aldicarb oxime and aldicarb nitrile, but they did not include sulfoxide or sulfone, or the corresponding oximes, in their scheme.

In light of the technique and the large number of columns currently available, high-performance liquid chromatography (HPLC) would seem to be ideally suited for aldicarb analysis. HPLC methods presently available require either expensive MS detection[7,8] or post-column derivatization and fluorometric detection[9–12] due to a lack of chromatographic resolution. Cochrane and co-workers[13,14] developed reversed-phase methods for aldicarb, aldicarb oxime and aldicarb nitrile in water and

food that utilized UV detection, but separate mobile phases were necessary. Spalik *et al.*[15] determined aldicarb alone using a 254-nm detector. In spite of these studies, no single, comprehensive LC separation of aldicarb and all of its primary soil degradation products has yet been developed.

The present study has focused on the development of an isocratic, reversed-phase HPLC separation combined with multiple wavelength UV detection. Methyl- (C_1), octyl- (C_8), phenyl- and cyano-silica columns were evaluated for their ability to separate aldicarb and the primary metabolites illustrated in Fig. 1. The effects of mobile phase pH and ionic strength were also evaluated, since these variables can have significant impacts on the retention of ionizable solutes. These studies have led to the development of a separation of aldicarb and its oxidative and hydrolytic derivatives that can be accomplished isocratically in less than 10 min. The application of such a rapid, simple and inexpensive separation method should allow much more widespread studies on the behavior and fate of aldicarb in the environment.

EXPERIMENTAL

Apparatus

All retention measurements were obtained using an IBM 9533 ternary gradient liquid chromatograph equipped with a Rheodyne 7125 LC sampling valve (20 μl sample loop) and an LC 9552 fixed-wavelength UV detector operated at 254 nm. For detection at wavelengths other than 254, a Hitachi Model 100-10 spectrophotometer and Model 155-00 flow cell was used.

Columns

Methyl-, octyl- and cyanopropyl-silica columns were obtained from IBM Instruments (Wallingford, CT, U.S.A.) and were endcapped by the manufacturer. The Adsorbosphere phenyldimethyl-silica column was obtained from Alltech (Deerfield, IL, U.S.A.) and was endcapped with a commercial endcapping reagent (Alltech). All columns were packed with 5 μm particles and were 25 cm $\times$ 4.6 mm I.D.

Solvents and solutes

All organic solvents used as mobile phase modifiers were of LC grade obtained from Mallinkrodt (Paris, KY, U.S.A.). Water was distilled, deionized and then further purified by passage through granulated active carbon filters. Ionic strength and pH were controlled by using standard acetate buffer solutions prepared from sodium acetate and acetic acid mixed in proportions necessary to produce the desired values[16].

Aldicarb, aldicarb sulfoxide, aldicarb sulfone, aldicarb sulfoxide oxime and aldicarb oxime were obtained from the U.S. Environmental Protection Agency's Pesticide and Industrial Chemical Repository (Research Triangle Park, NC, U.S.A.). Stock solutions of aldicarb, aldicarb sulfoxide and aldicarb sulfone were prepared by adding accurately weighed quantities of the analyte to varying volumes of solvent that was made from 200 ml of acetonitrile and 1.3 ml of sulfuric acid added to 1.2 l of HPLC grade water. Stock solutions of the oximes were prepared in the same way except that no acid was added to the original solvent. Serial dilutions of these stock standards were then made with the same solvent that was used to prepare those stock solutions.

Procedures

Reported solute capacity factors are the result of at least two injections. Differences in retention times were never greater than 5%. Column void volumes were determined with methanol, with the first baseline disturbance taken as the void volume. Flow-rates were maintained at 1.2 ml/min. No less than ten column volumes were allowed for column equilibration upon a change of mobile phase.

RESULTS AND DISCUSSION

Optimization of stationary/mobile phase combinations

The results presented in a previous paper[17] demonstrated that although the overall differences in octyl, phenyl and cyano bonded stationary phases are largely related to differences in phase ratios and accessible residual silanols, unique chromatographic selectivities could be achieved with proper matching of mobile and stationary phases. For this reason these three bonded phases were evaluated for their ability to separate aldicarb and its derivatives in the same three solvent mixtures utilized in our previous evaluation of bonded phase characteristics. In addition, a methyl (C_1) phase was also evaluated. Methyl bonded phases behave much like deactivated silica and have been used in normal-phase separations, but they have not found widespread use in the reversed-phase mode.

Stationary/mobile phase pairs were initially evaluated according to their ability to separate aldicarb and its most toxic metabolites, aldicarb sulfoxide and aldicarb sulfone. The content of each organic modifier added to the mobile phase was varied from 10 to 50%. The results of these initial evaluations are summarized in Table I. Acceptable separations were achieved with the phenyl column using a methanol–water mobile phase, and with the cyano column using methanol–water and acetonitrile–water mobile phases. Acceptable separation was defined as a range of capacity factors, k', of 0.5–10 and a minimum separation factor ($\alpha = k'_2/k'_1$) of 1.5 for sulfone and sulfoxide. Although analytical separations can normally be accomplished with α values as low as 1.1, 1.5 was chosen here because of the need to include the oxime derivatives in the analysis. It will be seen that the oximes generally elute between the sulfone and sulfoxide, and thus a somewhat larger than necessary separation of these two was desired in order to eventually incorporate the oximes into a single isocratic separation. Both the methyl and octyl columns gave results with methanol–water that indicated they were not appropriate for this application, and no further evaluations of these columns were carried out.

Previous attempts to develop an isocratic separation of aldicarb and its sulfone and sulfoxide derivatives using purely alkyl bonded phases were unsuccessful because the high organic modifier contents necessary to elute the relatively non-polar parent compound aldicarb within a reasonable time (*i.e.*, $k' < 10$) resulted in an inadequate separation of the sulfone and sulfoxide[13,14,18]. A number of workers have shown that phenyl and cyano phases are "weaker" than alkyl phases[17,19,20], and indeed we observed that aldicarb can be eluted from these columns with acceptable capacity factors in water-rich mobile phases. However, the mobile phases used with these weaker columns must exhibit some selectivity toward the sulfone–sulfoxide pair in order that they can be adequately separated. This selectivity is demonstrated by the data of Table II, which contains capacity factors of aldicarb sulfoxide, aldicarb sul-

fone and aldicarb tabulated as a function of organic modifier content. Table II includes retention data for the three systems listed in Table I which gave acceptable separations: phenyl/water–methanol, cyano/water–methanol and cyano/water–acetonitrile. For purposes of comparison, Table II also contains data for octyl/water–methanol.

The retention data of Table II has been fit to eqn. 1 by a linear least squares method

$$\ln k' = \ln k'_w - B\Phi \quad (1)$$

In eqn. 1, Φ is the volume fraction of organic modifier, B a constant related to the solubility of the solute in the pure organic solvent, and k'_w the capacity factor of the solute in a purely aqueous mobile phase. Although it is generally accepted that ln k' values are more accurately described as a quadratic function of Φ[21–23], several studies have demonstrated that, over a range 20–50% organic modifier in a binary mobile phase, retention is adequately described by eqn. 1[17,23–27]. The correlation coefficients included in Table II indicate that, over the range of modifier contents used in this study, the retention of aldicarb sulfoxide, aldicarb sulfone and aldicarb in these stationary/mobile phase pairs does indeed behave according to eqn. 1.

The constant B of eqn. 1 is directly related to the solvent strength of the pure organic modifier[17,24–27]. Comparison of these B values for modifiers when they are used in binary aqueous mixtures to elute aldicarb, aldicarb sulfoxide and aldicarb sulfone can give insight into the retention mechanisms responsible for the separation of these compounds in the stationary/mobile phase pairs listed in Table II. For example, the values included in Table II indicate that the solvent strengths of methanol toward these solutes in an octyl column follow the order: sulfoxide < sulfone < aldicarb. Since this trend in methanol solvent strength is opposite to the trend in solute polarity for this group (as inferred from the order of retention), we conclude that solvophobic interactions are primarily responsible for the retention of these solutes in an octyl/water–methanol system. When solvophobic retention dominates, organic modifiers are more effective at eluting less polar solutes.

TABLE I

SUMMARY OF RESULTS OF STATIONARY/MOBILE PHASE OPTIMIZATION FOR THE SEPARATION OF ALDICARB SULFONE (1), ALDICARB SULFOXIDE (2) AND ALDICARB (3)

Column	*Organic modifier*	*Comments*
Methyl	Methanol	Unacceptable separation of 1 and 2; very large k' (3)
Octyl	Methanol	k'(3) too large at Φ which separates 1 and 2.
Phenyl	Methanol	Acceptable separation of 1 and 2 at Φ = 20–30%; large k' values for all solutes in this range, however.
	Acetonitrile	No separation of 1 and 2.
	Tetrahydrofuran	Acceptable separation of 1 and 2, but k' for both too small.
Cyano	Methanol	Acceptable separation of 1 and 2 at Φ = 20–40%; k' range from 0.94–3.46 at Φ = 20%.
	Acetonitrile	Acceptable separation of 1 and 2 at Φ = 15–25%; k' range from 0.82–3.00 at Φ = 20%.
	Tetrahydrofuran	k' (1) too low.

TABLE II

RETENTION DATA FOR ALDICARB SULFOXIDE, ALDICARB SULFONE AND ALDICARB IN VARIOUS STATIONARY/MOBILE PHASE COMBINATIONS

	ln k' for indicated percent of organic modifier					*Slope [d(ln k')/dΦ]*	*Correlation coefficient*
Octyl/methanol	*10*	*20*	*30*	*40*	*50*		
Sulfoxide	–	0.44	0.26	−0.14	−0.44	3.04	0.991
Sulfone	–	0.69	0.41	−0.03	−0.44	3.83	0.996
Aldicarb	–	–	–	1.35	0.87	4.80	1.00
Phenyl/methanol	*10*	*20*	*30*	*40*	*50*		
Sulfoxide	2.5*	1.65	1.25	0.90	0.60	3.50	0.998
Sulfone	1.95*	1.30	0.95	0.65	0.45	2.85	0.993
Aldicarb	–	2.45	1.8	1.35	0.95	4.95	0.993
Cyano/methanol	*10*	*20*	*30*	*40*	*50*		
Sulfoxide	0.40	−0.05	−0.35	−0.40*	–	3.75	0.993
Sulfone	0.75	0.45	0.05	−0.15	−0.20*	3.10	0.993
Aldicarb	1.70	1.25	0.80	0.45	0.30	4.20	0.998
Cyano/acetonitrile	*15*	*20*	*25*				
Sulfoxide	−0.05	−0.15	−0.25			2.00	1.000
Sulfone	0.40	0.25	0.05			3.50	0.997
Aldicarb	1.35	1.10	0.95			4.00	0.990

* Non-linear behavior, not used in slope calculation.

Methanol solvent strengths in the phenyl and cyano columns, however, follow the order: sulfone < sulfoxide < aldicarb. We have shown previously that methanol is a particularly effective modifier in eluting basic solutes from these columns[17]. We ascribe this characteristic to a "mobile phase secondary solvent effect" that results from methanol's ability to hydrogen bond with certain basic solutes (*i.e.*, those with oxygen atoms). In phenyl and cyano columns, such hydrogen bonding in the mobile phase is apparently not balanced by hydrogen bonding in the stationary phase. This effect is due, we believe, to residual silanols on the silica support which are more accessible in phenyl and cyano packings than in octyl packings[17,20]. Methanol molecules sorbed onto the bonded stationary phase localize on these silanols, reducing their ability to hydrogen bond with solutes also sorbed into the stationary phase.

Differences in the B constants for sulfoxide and sulfone listed in Table II demonstrate that methanol and acetonitrile retain the selectivity exhibited in the octyl column, even in these weaker phenyl and cyano columns. We have shown that differences in these B values are related to selectivity by[17]

$$\Delta B = \frac{\mathrm{d} \ln \alpha}{\mathrm{d} \Phi} \tag{2}$$

That is, differences in B values indicate the extent to which the separation factor will change with a change in organic modifier content and these differences can be used as indicators of mobile phase selectivity.

The chromatographic systems which give the best separation of aldicarb and its sulfoxide and sulfone derivatives are a cyanopropyl bonded phase with 80:20 vol.% mixtures of water–methanol or water–acetonitrile as the mobile phase. These mobile phases produce almost identical selectivities, the acetonitrile–water mobile phase having a slight advantage in that aldicarb elutes with a slightly lower capacity factor (3.46 *vs.* 3.0). The cyano/acetonitrile–water system was therefore chosen for further study.

The retention of ionizable solutes such as aldicarb and its metabolites can be greatly affected by changes in pH and ionic strength. The resolution of the aldicarb sulfoxide and aldicarb sulfone peaks in the cyano/acetonitrile–water (20:80) system was thus monitored over ranges of pH and ionic strength typically employed in reversed-phase LC; pH = 3–6 and ionic strength = 0.04–0.20 Debye units.

Ionic strength had little effect on the resolution of these compounds over the range studied. In contrast, pH did have an effect, with optimum resolution of these compounds occurring at a pH of *ca.* 6. Retention was not studied in neutral or slightly alkaline mobile phases since these compounds are known to hydrolyze to their oxime derivatives in basic solutions.

The ability to separate aldicarb and its toxic derivatives (sulfone and sulfoxide) via a single isocratic HPLC analysis in less than 10 min represents a significant advance in the analytical chemistry of these compounds. To fully understand the environmental behavior of aldicarb, however, it is also necessary to monitor the oxime derivatives as well. Toward this end, the retention in a cyanopropyl column of aldicarb and the degradation products depicted in Fig. 1 was studied as a function of acetonitrile content. No standard was available for aldicarb sulfone oxime, however, and it was thus not included in the method development. Capacity factors and as-

TABLE III

CAPACITY AND SEPARATION FACTORS FOR ALDICARB AND METABOLITES IN CYANOPROPYL COLUMN AS A FUNCTION OF INDICATED VOLUME PERCENT OF ACETONITRILE IN WATER AS MOBILE PHASE

	15%		*20%*		*25%*	
	k'	α	*k'*	α	*k'*	α
Aldicarb sulfoxide oxime	0.73		0.68		0.65	
		1.27		1.19		1.09
Aldicarb sulfoxide	0.93		0.81		0.71	
		1.54		1.47		1.32
Aldicarb sulfone	1.43		1.19		0.94	
		1.52		1.61		1.65
Aldicarb oxime	2.18		1.91		1.55	
		1.51		1.42		1.25
Aldicarb	3.29		2.71		1.95	

sociated separation factors of the five compounds tested are summarized in Table III. These data demonstrate that acceptable separations of these compounds can be achieved with acetonitrile contents of 15–25%.

Fig. 2 is a representative chromatogram. The slight tailing of peaks evident in this chromatogram is most likely due to the presence of residual silanols on the silica support which have been shown to contribute significantly to retention in cyanopropyl columns[7]. These silanols could in principle be "masked" through the addition of small amounts of base to the mobile phase, but the tendency of aldicarb and its metabolites to hydrolyze in basic solutions brings into question their stability in the presence of such masking agents.

Miles and Delfino[18] have shown that aldicarb sulfone oxime elutes between aldicarb sulfoxide and aldicarb sulfone on an octyl column when using water–acetonitrile mobile phases. This same elution order would be expected on a cyanopropyl column, since retention mechanisms on these two columns have been shown to be

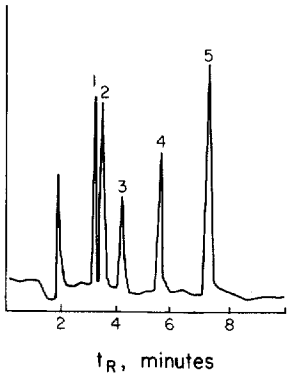

Fig. 2. Representative chromatogram of aldicarb metabolites: 1 = aldicarb sulfoxide oxime; 2 = aldicarb sulfoxide; 3 = aldicarb sulfone; 4 = aldicarb oxime; 5 = aldicarb. Cyanopropyl column with acetonitrile–water (20:80) mobile phase buffered at pH 6. Flow-rate, 1.2 ml/min.

"homeoenergetic"[17,28]. Thus, Fig. 2 and Table III indicate that aldicarb sulfone oxime could be incorporated into this separation method with little difficulty.

Multiple-wavelength UV detection

The separation of aldicarb and its environmental metabolites which can be accomplished with a cyanopropyl/acetonitrile–water RPLC system means that these compounds can be analyzed in less than 10 min provided that sensitive and selective detection can be achieved. The most sensitive LC detection method for aldicarb appears to that developed by Moye *et al.*[9] involving post-column derivatization with *o*-phthaldehyde and subsequent fluorescence detection. This method is also reasonably selective, but of necessity results in some peak broadening. Such broadening would neutralize the high-resolution separations which can be obtained with the cyanopropyl column. A study was thus undertaken to determine if multiple-wavelength UV detection would be suitable for this purpose. The use of multiple-wavelengths provides additional qualitative information. For example, calculation of absorbance ratios at two wavelengths will indicate if unwanted peaks are coeluting with the analyte, provided that the interferent does not have identical extinction coefficients.

Aldicarb and its derivatives are not particularly strong chromophores at 254 nm, the most common wavelength used in LC UV–VIS photometers. They do absorb more strongly at lower wavelengths, particularly at 190 nm[10]. This is below the UV cutoff of many organic modifiers used in mobile phases and is in a region where virtually all organic compounds absorb to some extent. Thus, we have chosen to evaluate the sensitivity of variable-wavelength detection over the range 210–254 nm. Even in this spectral region, however, many organic compounds absorb, and this points out the advantage of multiple-wavelength detection in assessing peak purity.

Table IV summarizes the detection limits which were obtained for aldicarb and its metabolites in the optimized RPLC system. Detection limit was taken to be the amount of compound that resulted in a peak height five times the average background noise. Detection limits for each compound over the range 210–254 nm were estimated by first determining the detection limit at 254 nm and then using eqn. 3

$$DL(\lambda) = DL(254\text{ nm})\,[S(254\text{ nm})/S(\lambda)]\,[N(\lambda)/N(254\text{ nm})] \quad (3)$$

TABLE IV

DETECTION LIMITS OF ALDICARB AND ITS METABOLITES AS A FUNCTION OF WAVELENGTH IN OPTIMIZED RPLC SYSTEM

	Detection limit (ng) at wavelength (nm)						
	*254**	*254*	*245*	*240*	*230*	*220*	*210*
Aldicarb	2.4	2.3	2.0	2.0	3.3	2.7	1.0
Aldicarb sulfoxide	2.2	2.8	1.4	1.1	1.0	0.9	0.6
Aldicarb sulfone	200.0	280.0	45.9	17.7	4.4	1.7	0.8
Aldicarb oxime	7.1	9.0	5.2	5.0	4.5	3.5	1.5
Aldicarb sulfoxide oxime	3.6	4.3	1.6	1.2	0.9	1.0	0.6

* Fixed-wavelength, UV photometer.

where $DL(\lambda)$ is the detection limit at the wavelength of interest, DL(254 nm) the detection limit at 254 nm, S(254 nm) and $S(\lambda)$ the sensitivity of the detector (in absorbance units/ng) for the compound at the two wavelengths, and $N(\lambda)$ and N(254 nm) the corresponding average background noise values.

The sensitivities used in the calculation of detection limits are also summarized in Table IV. These sensitivities can be used in the qualitative evaluation of peak purities by noting that, for a pure compound, the ratio of detector responses should be a constant

$$\frac{\text{Response}(\lambda_1)}{\text{Response}(\lambda_2)} = \frac{DL(\lambda_1) + S(\lambda_1)C_X}{DL(\lambda_2) + S(\lambda_2)C_X} = \text{constant} \quad (4)$$

Eqn. 4 is valid provided that the concentration of analyte X (C_X) is within the linear range of the detector at both wavelengths.

The mass detection limits reported in Table IV can be converted to concentration detection limits for this system by noting that these values were obtained with a 20-μl sample loop. Thus, the minimum concentration of aldicarb that can be analyzed at 220 nm, a common photometric line, is 2.7 ng/20 μl, or 0.14 μg/ml (0.14 mg/l). These limits of detection are comparable to those reported by Miles and Delfino[18] using a 200 nm analytical line. Detection at 220 nm has a number of advantages, however, primarily that associated with less interferents. The 220 nm line is also a common photometric line, and lower detection limits would be expected using a photometer instead of the spectrophotometers used in this study and that of Miles and Delfino (note the lower detection limits for the photometer *vs.* the spectrophotometer at 254 nm in Table IV).

The data of Table IV indicate that the detection limits for the metabolites will be within a factor of 0.3–1.5 of each other. Detection limits of 50–200 μg/l for aldicarb and its metabolites are not sufficient for either regulatory or research purposes. Spalik *et al.*[15], however, have developed a one-step extraction procedure using a C_{18} Sep-Pak cartridge that results in a concentration factor of 100:1 with over 90% efficiency. They also developed a two-step procedure in which the eluent from the Sep-Pak is further concentrated by solvent evaporation; this procedure gives concentration factors of 1000:1 with efficiencies approaching 100% over an aldicarb concentration range of 3–10 μg/l. Thus, when combined with the appropriate preconcentration and extraction technique, this simple, isocratic RPLC system with UV detection should be capable of determining aldicarb and its primary metabolites at concentrations at or below 1 μg/l.

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