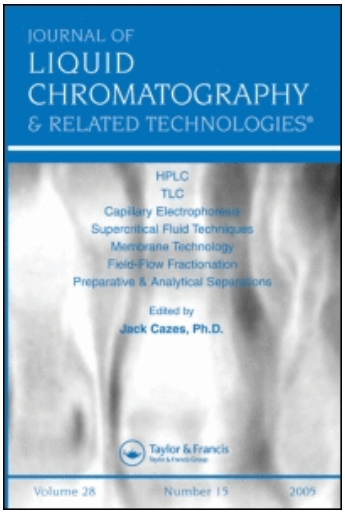

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Normal-Phase Liquid Chromatography on Amino-Bonded-Phase Column of Fluorescence Detected Pesticides

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NORMAL-PHASE LIQUID CHROMATOGRAPHY ON AMINO-BONDED-PHASE COLUMN OF FLUORESCENCE DETECTED PESTICIDES

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ABSTRACT

A liquid chromatographic method with fluorimetric detection is developed for the simultaneous determination of aminocarb, carbaryl, carbendazim, fuberidazole and 1-naphthylacetamide. An amino-bonded column was used and the optimun mobile phase was selected through fitting a mathematical model to experimental data. Separations were accomplished in less than 20 min. Recoveries from spiked grapes ranged from 90 % to 103 % and detection limits between 5.5 x 10^{-3} mg kg^{-1} for fuberidazol and 0.08 mg kg^{-1} for 1-naphthylacetamide and relative standard deviation lower than 6 %.

INTRODUCTION

In normal-phase liquid chromatography only a few quantitative rules exist for predicting how change in mobile phase composition will affect sample

retention [1-3] and locating the overall optimal set of chromatographic conditions is often an unsuccesful trial and error procedure. However, as multifactor methods have been developed for optimization of reverse-phase liquid chromatographic separations [4] they would provide a means to optimization of normal-phase liquid chromatographic separation of pesticides.

Aminocarb, carbaryl, carbendazim, fuberidazol and 1-naphthylacetamide (1-NA) are two insecticides, two fungicides and a plant growth regulator, respectively. They can be found in fruits, vegetables, cereals and other types of crops as a consequence of the pre- and post-harvest treatment. Methods for carbaryl determination include liquid chromatography (LC) [5-8] and gas chromatography (GC) [9,10], also aminocarb is determined by LC [5,11] and GC [12,13]. Determination of carbendazim is mainly by LC [14-16] and also by GC with nitrogen-phosphorous detection after derivatization [17]. Fuberidazol is determined by GC with flame ionization detector after derivatization [18] and no LC methods are available. Determination of 1-NA is by LC [19]. So, the simultaneous determination of the five pesticides is unavailable at present by GC or LC, mainly as a result of their thermal lability or their polar character.

In residue analysis, GC gives usually lower detection limits than LC. However, LC facilitates the direct determination of acidic and thermally labile pesticides without the need for derivatization. For the more polar samples an amino-bonded column is preferable to separations on silica or alumina and because of the nature of the five pesticides a normal-phase LC on amino-bonded column determination is developed. In this work, a multifactor chromatographic optimization is used to find the optimum chromatographic system. The deduced mobile phase is used to develop a rugged residue method of the five fluorescent pesticides in grapes.

EXPERIMENTAL

Instrumentation

The measurements were performed with a Merck-Hitachi liquid chromatograph (Darmstadt, Germany) which incorporates a L-6200 pump, AS-4000 autosampler, L-4250 UV-visible detector and D-6000 interface. Integration was made with a PC/AT computer and the instrumental parameters were controlled by Hitachi-Merck HM software. A Perkin-Elmer LS-50 fluorescence detector (Beaconsfield, UK) placed in series with and after the UV-visible spectrophotometer, is equipped with a xenon discharge lamp and two monochromators. Software Fluorescence Data Manager (FLDM) (LC program) and RS232C interface was used to send information to an external computer. For graphical recording, a NEC Silenwriter2 S60P laser printer was connected to the spectrofluorimeter.

The calculations were made with PC-MATLAB software (MatWorks Inc., Sherborn, MA). To resolve the linear system with more equations than unknowns was used orthogonal factorization. Of the many solution vectors, orthogonal factorization found the best solution in a least squares sense. Surface graphs were obtained by using Surfer software (Golden Software, Golden, CO).

Reagents

The solvents used were tetrahydrofuran (THF), methanol (MeOH), dichloromethane (CH_2Cl_2) and water gradient grade Lichrosolv (Merck); acetic acid, propan-2-ol, and acetonitrile pro analysis (Merck). Water was distilled and deionized or LC grade. The pesticide aminocarb (99%) was purchased from Dr. Ehrenstorfer (Augsburg, Germany); carbaryl (Pestanal, 99%), carbendazim

(Pestanal, 99%) and fuberidazol (Pestanal, 99%) were supplied by Riedel-deHäen (Hannover, Germany) and 1-naphthylacetamide. The solvents were previously sonicated for 30 min and filtered through 0.2 µm Nylon filters. Other chemicals used were celite (particle size 0.01-0.04 mm), sodium acetate and tetramethylammonium hydrogen sulphate (TMA) (Merck).

The stock standard solutions of aminocarb, carbaryl, 1-NA and fuberidazole of 1 g l^{-1} and carbendazim at 0.2 g l^{-1} were prepared by dissolving the pesticide in methanol and stored at 4 °C. Working solutions were prepared by dilution with tetrahydrofuran.

Buffer solution of pH 5.6 was prepared from 0.2 M acetic acid/sodium acetate, and that of pH 6.7 from 0.05 M acetic acid/sodium acetate. This solutions were filtered through 0.2 µm Nylon filters.

Extraction

50 g of grapes were chopped in a food chopper and transfered to a blender cup to be blended with 100 ml of acetonitrile, 25 ml water and 10 g of celite, for 15 min. The homogenate is filtered through fritted glass Buchner funnel (coarse porosity) under reduced pressure, the filtrate transfered to a 500 ml volumetric flask and diluted with acetonitrile: water (50 + 50).

An aliquot (10 ml) of the extract in acetonitrile:water was evaporated to dryness by a rotary evaporator. The residue was taken up in 3 ml of methanol, this solution filtered through 0.2 µm Nylon membranes filters and the filter cake washed with 2 ml of methanol. Finally, the residue was dissolved in 5 ml of THF. This solution was used for the analytical determination.

Chromatographic conditions

The extracts redissolved in THF are analyzed using an analytical column Spherisorb S5 amino, 250 x 4.6 mm id (Phase Separations, U. K.). Flow rate is

TABLE 1
Mobile Phase Composition and Wavelength Program

Time min	THF %	10 mM TMA %	Buffer pH 6.7 %	λ_{exc} nm	λ_{em} nm
0	99.8	0.2	0	255	367
3.6				280	333
7.0				282	340
10.0	99.8	0.2	0		
11.0	98.9	0.1	1	310	345
13.0				285	318
20.0	98.9	0.1	1	285	318

1 ml min^{-1} and injection volume 20 µl. The mobile phase composition and wavelength program for fluorimetric detection are detailed in Table 1. Measure peak area response at retention times of aminocarb (3.42 min), carbaryl (3.80 min), 1-NA (10.23 min), fuberidazol (11.90 min) and carbendazim (15.18 min). A linear regression curve of concentration versus peak area is calculated.

Recovery test

Grapes were spiked with a solution of pesticides in methanol at the levels indicated in Table 2 after checking for the absence of aminocarb, carbaryl, carbendazim, fuberidazole and 1-NA. They were subjected to the whole procedure and determined by the LC method.

RESULTS AND DISCUSSION

The structures of aminocarb, carbaryl, carbendazim, fuberidazole and 1-NA, are presented in Figure 1 in which is indicated the chemical name (IUPAC)

TABLE 2

Estimated Parameters

Compound	a	b	c	d	e
Aminocarb	-1.4580	324.7109	0.0145	-2.6779	-3.2481
Carbaryl	-1.3087	203.1251	0.0131	-1.5930	-2.0303
1-NA	-1.7409	205.0411	0.0175	-1.6388	-2.0452
Fuberidazole	-1.4823	232.4747	0.0149	1.6965	2.3269
Carbendazim	-1.1644	22.1096	0.0117	0.2391	-0.2229

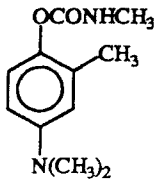
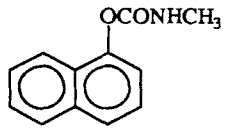
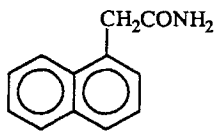
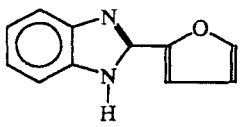
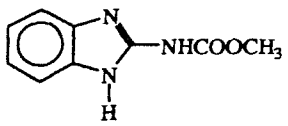
STRUCTURE	RETENTION TIME (min.)	IUPAC NAME
	3.42	4-Dimethylamino-m-tolyl methylcarbamate (Aminocarb)
	3.80	1-Naphthyl methylcarbamate (Carbaryl)
	10.23	1-naphthylacetamide
	11.90	2-(2-furyl) Benzimidazole (Fuberidazole)
	15.18	Methyl benzimidazol -2-yl carbamate (Carbendazim)

FIGURE 1. Structure of the pesticides

and common name [20]. These compounds posses enough natural fluorescence to enable their detection without pre- or post-column derivatization steps. The use of selective fluorescence detector minimizes interferences of coextractives and permits very sensitive determinations, so fluorimetric detection was selected. To maximize sensitivity and selectivity the maximum emission wavelength of each pesticide must be used. From this a wavelengths program for the best mixture resolution is deduced. In Table 1 are the wavelengths selected.

Generally is observed that more polar samples present additional problems in separations on silica or alumina and because of the polar character and hydrogen bonding properties of the studied pesticides an amino-bonded column was selected for the separation. Optimization of mobile phase composition was initially performed by trial and error procedures. Thus, retention data were obtained for a single solvent, methanol (MeOH), dichloromethane (CH_2Cl_2) and tetrahydrofuran (THF) (Solvent strength parameter on aminopropyl column: 0.24, 0.13 and 0.11, respectively); with MeOH the sample is not retained, with CH_2Cl_2 carbendazim, fuberidazole and 1-naphthalenacetamide are strongly retained and by using THF as mobile phase only carbendazim is retained. Binary mixtures of CH_2Cl_2-MeOH, CH_2Cl_2-propan-2-ol, THF-propan-2-ol, THF-water, -acetic acid, -acetate buffer pH 5.6 and acetate buffer of pH 6.7 and ternary mixtures of THF-water-acetate buffer pH 5.6, or THF-propan-2-ol-acetic acid, at different proportions were tested but no adequate separation selectivity was achieved.

Thus, the trial and error procedures were not satisfactory for optimization of the chromatographic system and a multifactor optimization strategy based in the "windows diagrams" [21] was used to predict the mobile phase giving optimal separation. From the previously tested systems, a combination of THF with controlled amounts of aqueous solutions gave the best results. An increase in the water concentration of the mobile phase decreases retention of the

pesticides, so the amino-bonded phase functioned in the normal mode. Because of the basic nature of solutes the addition of surfactants such as TMA (tetramethylammonium hydrogen sulphate) is successful in supressing anomalous retention behaviour and peak shape degradation [22]. So, a binary system THF-TMA aqueous was tuned in composition following a factorial design [16] to select the overall optimum. Experiments were carried out to determine the retention times of each pesticide. The results for each pesticide were fitted to a full second-order polynomial model, including a mathematical interaction effect:

$$\log K = aX_1 + bX_2 + cX_{12} + dX_{22} + eX_1X_2 \qquad (1)$$

where K is the capacity factor for each pesticide *a, b, c, d* and *e* are parameters of the model and X_1 and X_2 represent the percentage in mobile phase of THF and TMA, respectively. Table 2 contains the best least-squares estimates for the parameters of eq.1 for each of the five pesticides. Table 3 gives predicted and observed values of K for the separation under optimal conditions; the close agreement between predicted and observed values demonstrated the ability of the fitted equation to accurately predict capacity factors. Fig. 2 is a representation of resolution in vertical axis against a variable chromatographic factor for the worst separated pair of compounds (1-NA and fuberidazole). The value of the chromatographic factor that corresponds to the top of the tallest peak gives the best possible separation of the worst separated pairs of compounds; the conditions located by the tallest point in the Figure 1 corresponds to a mobile phase composed of 99.8 % THF and 0.2 % TMA 10 mM. This mobile phase separates the five pesticides but the peak of carbendazim has a low efficiency (effective plate number N = 37), to increase efficiency for carbendazim a mobile phase of composition 98.9 % THF, 0.1 % TMA 10 mM and 1 % buffer acetate pH 6.7 is needed. So, to achieve the best separation a gradient elution as the indicated in Table I was used. Chromatographic peaks were examined by asymmetry factor measurements and an asymmetry factor of 1.25 and effective

TABLE 3
Predicted and Observed Capacity Factors at the Optimum Conditions

	Aminocarb	Carbaryl	1-NA	Fuberidazole	Carbendazim
Predicted K	0.15	0.23	1.95	2.51	3.80
Observed K	0.14	0.26	2.39	2.95	3.98
Δk	0.01	0.03	0.44	0.44	0.18

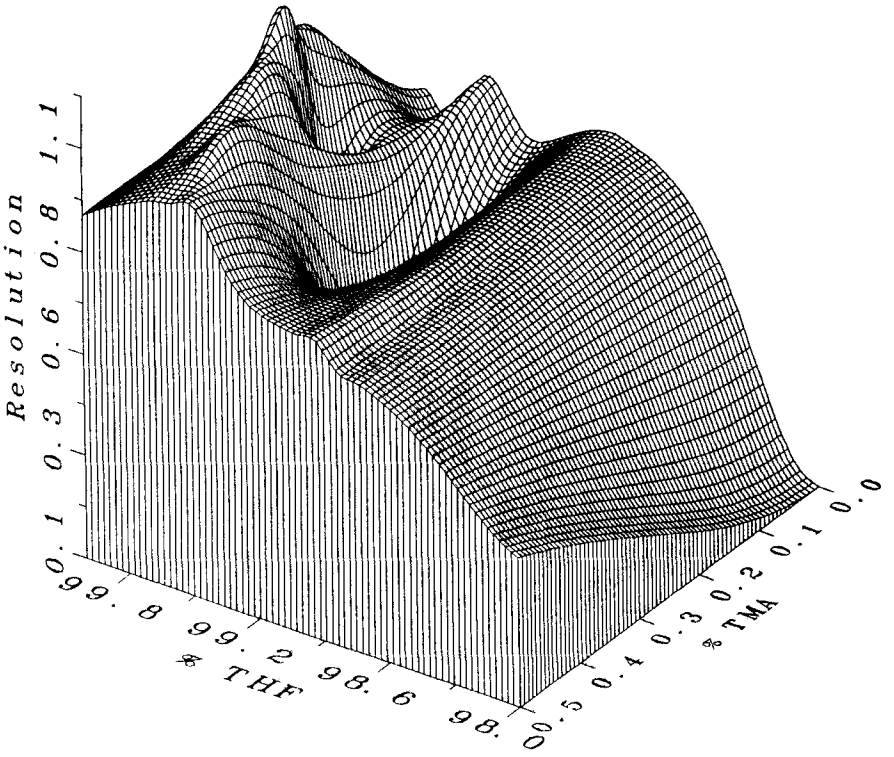


FIGURE 2. Predicted resolution of 1-naphthylacetamide and fuberidazole as a function of percent THF and TMA.

plate number N = 142 for carbendazim were obtained.

Calibration graphs were obtained by plotting the peak areas against the pesticides concentration. The correlation coefficients of the linear regression curves were 0.999, 0.996 and 0.999, over the concentration range 0.1-2 μg ml^{-1} for aminocarb, carbendazim and 1-NA respectively, 0.995 over the concentration range between 0.1-1.5 μg ml^{-1} for carbaryl and 0.998 over the concentration range between 5-100 ng ml^{-1} for fuberidazol.

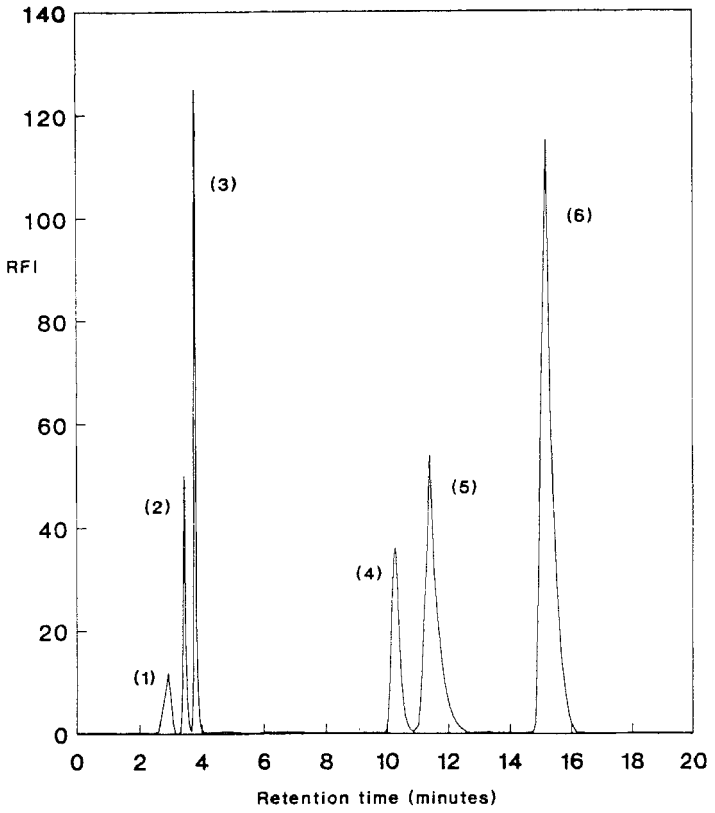


FIGURE 3. Chromatogram of grapes spiked. (1) peak from the matrix, (2) 0.8 μg ml^{-1} aminocarb, (3) 0.8 μg ml^{-1} carbaryl, (4) 0.8 μg ml^{-1} 1-NA, (5) 40 ng ml^{-1} fuberidazole, (6) 0.8 μg ml^{-1} carbendazim,

Application

Aminocarb, carbaryl, carbendazim, fuberidazol and 1-NA can be found in fruits, vegetables, cereals and other types of crops. Grapes were chosen for evaluation of the developed method.

TABLE 4
Recovery of Pesticides from Grapes[a]

Compound	Taken mg kg^{-1}	D_L[b] mg kg^{-1}	C_Q[c] mg kg^{-1}	Recovery %	RSD %
Aminocarb		0.07	0.25		
	1.00			95.0	5.3
	4.00			98.7	4.4
	8.00			100.0	3.1
Carbaryl		0.02	0.07		
	1.00			90.0	4.1
	4.00			94.0	3.1
	8.00			98.7	2.6
1-NA		0.08	0.26		
	1.00			90.0	3.5
	4.00			102.5	2.6
	8.00			99.4	0.8
Fuberidazole		5.5×10^{-3}	18.5×10^{-3}		
	0.05			95.4	2.6
	0.20			99.5	3.1
	0.40			100.8	0.8
Carbendazim		0.02	0.07		
	1.00			95.0	5.7
	4.00			96.2	4.3
	8.00			98.7	3.2

[a] n = 3, [b] detection limit for a signal-to-noise ratio = 3, [c] quantification limit for signal-to-noise ratio = 10.

Three different grape samples were spiked prior the extraction at three levels with a mixture of the pesticides in methanol. Extraction was performed with acetonitrile-water which is widely used for residue screening in non-fatty foods [23-27]. The extract in acetonitrile is evaporated to dryness, redissolved in methanol, concentrated to a 1 ml and diluted with THF. A previous redissolution in methanol is needed to impede residue precipitation. The chromatogram of an extract is shown in Fig. 3. In Table 4 the recoveries obtained are show. It can be observed that the recoveries are excellent giving values between 90 % and 102.5 %. The detection limit defined as the amount that gave a signal to noise ratio of 3 is between 5.5 x 10^{-3} mg kg^{-1} for fuberidazol and 0.08 mg kg^{-1} for 1-naphthylacetamide. The precision given by the relative standard deviation (RSD) is good and below of 6 %.

CONCLUSIONS

This study demonstrated that aminocarb, carbaryl, carbendazim, fuberidazole and 1-NA may be recovered simultaneously from food samples and then quantitatively determined by normal-phase LC on amino-bonded column. Because the unsuccessful separation based on systematic change in the mobile phase a graphical method is developed for optimization of normal-phase liquid chromatographic separation of pesticides.

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