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Isocratic reversed-phase high-performance liquid chromatographic separation of simple perhalogenated compounds

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ABSTRACT

An isocratic method for the separation of perhalogenated compounds using reversed-phase high-performance liquid chromatography is described. Nine perhalogenated compounds (CCl_4, $CBrCl_3$, $CBrCl_2$, CBr_4, C_2Cl_4, C_2Br_4, C_2Cl_6, $C_2Br_2Cl_4$ and C_2Br_6) were divided into three groups as a function of carbon number and unsaturation and their separations were optimized. Methanol–water mobile phases in different proportions were used and the optimum conditions were determined for each of the groups and for a mixture of all nine compounds, based on resolution and separation factors. These conditions were then used to separate and identify perhalogenated compounds resulting from radiolysis, photolysis or thermolysis of different test solutions. Inversions of retention times were observed for some of the compounds as a function of the mobile phase polarity. This suggests that a gradient elution separation of these compounds would not result in a better separation.

INTRODUCTION

The determination of perhalogenated compounds, often associated with water pollutants, is frequently carried out by gas chromatography [1]. However, many of the possible $C_nBr_yCl_z$ compounds are thermosensitive and decompose or rearrange on injection or during temperature-programed analysis. A high-performance liquid chromatographic (HPLC) procedure would eliminate the thermosensitivity problem and present similar quantitative characteristics.

Little was found in the literature on the use of HPLC for the determination of mixtures of perhalogenated compounds. HPLC determination of C_2Cl_4 [2,3] and C_2Cl_6 [4] using reversed-phase columns with methanol–water eluents has been reported.

This paper describes the separation of several C_1 and C_2 perhalogenated compounds using isocratic elution.

EXPERIMENTAL

Reagents

Most of the perhalogenated compound standards were used as received. Both CBr_4 and C_2Br_6 were sublimed under vacuum in the absence of light immediately

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TABLE I

PERHALOGENATED COMPOUNDS USED AS STANDARDS

Compound	Form	Melting point (°C)[a]	Boiling point (°C)	Source
CCl_4	Liquid	−23	76.5	Merck
$CBrCl_3$	Liquid	5.65	104.7	Eastman-Kodak
CBr_2Cl_2	Semi-solid	22	150.2	Alfa
CBr_4	Solid	90–91	189–190	ICN
C_2Cl_4	Liquid	−19	121	Merck
C_2Br_4	Solid	56.5	226–227	ICN
C_2Cl_6	Solid	186–187 (s)	(186)	Carlo Erba
$C_2Br_2Cl_4$	Solid	220–222	Decomp.	Aldrich
C_2Br_6	Solid	200–210 (d)	Decomp.	K & K Labs.

[a] (s) Sublimes; (d) with decomposition.

before use. The physical properties of the nine perhalogenated standards used are summarized in Table I.

Other perhalogenated compounds were obtained by gamma radiolysis of CBr_4 (3%) in CCl_4 and by photolysis (250–700 nm) of a mixture of C_2Br_6 and Cl_2 in CCl_4.

Chromatographic system

The HPLC system consisted of an Altex Model 110A reciprocating pump, a Rheodyne Model 7010 injection valve with a 10-μl loop and a Schoeffel Spectroflow Model 770 spectrophotometric detector (Kratos Analytical) used with an 8-μl flow cell at 220 nm. The chromatograms were registered on a Model RB-102 recorder (Equipamentos Científicos do Brasil).

Stationary phases

Several columns were tested. Their characteristics are summarized in Table II.

Mobile phases

Methanol (analytical-reagent grade, Merck), acetonitrile (LiChrosolv, Merck) and distilled, deionized water were used in different proportions to prepare mobile phase binary mixtures, which were ultrasonically degassed before use.

TABLE II

CHARACTERISTICS OF THE REVERSED-PHASE COLUMNS USED

Mobile phase, methanol–water (70:30) at 1.0 ml/min; test compound, naphthalene; detection, 220 nm.

Stationary phase	Column dimensions (mm)	Theoretical plates per metre	Asymmetry (10% of peak height)
Vydac TP201, 10 μm (laboratory packed)	124 × 4.6	14 307	1.10
Spherisorb ODS-1, 5 μm (laboratory packed)	100 × 4.6	42 920	1.04
Ultrasphere ODS, 5 μm (Beckman)	250 × 4.6	53 400	0.93

RESULTS AND DISCUSSION

Separations were obtained with all of the columns listed in Table II. Typical results, as a function of mobile phase composition, are shown in Figs. 1 and 2. As can be seen, each group of perhalogenated compounds can be resolved, but with a different mobile phase composition. Of interest is the inversion of elution order observed as the methanol–water composition changes (compare Fig. 1a and d).

Similar results were obtained using acetonitrile–water mixtures. Despite the differences in polarity of methanol and acetonitrile, the separation showed similar retention times and resolutions with similar mobile phase compositions.

Table III summarizes the retention times and resolutions observed with column 3 as a function of mobile phase composition. Mixtures containing more than 50% water were not tested, owing to the very low flow-rates and the resulting long retention times. However, the superposition of the C_1 perhalogenated compounds at a methanol–water composition of 50:50 suggests that inversion of retention times would also occur at higher water contents with this column.

The similar behaviours of all three columns with respect to these polarity changes in interesting, as a recent paper [5] gives a different classification to each of the three stationary phases used. It therefore seems that for such small hydrophobic

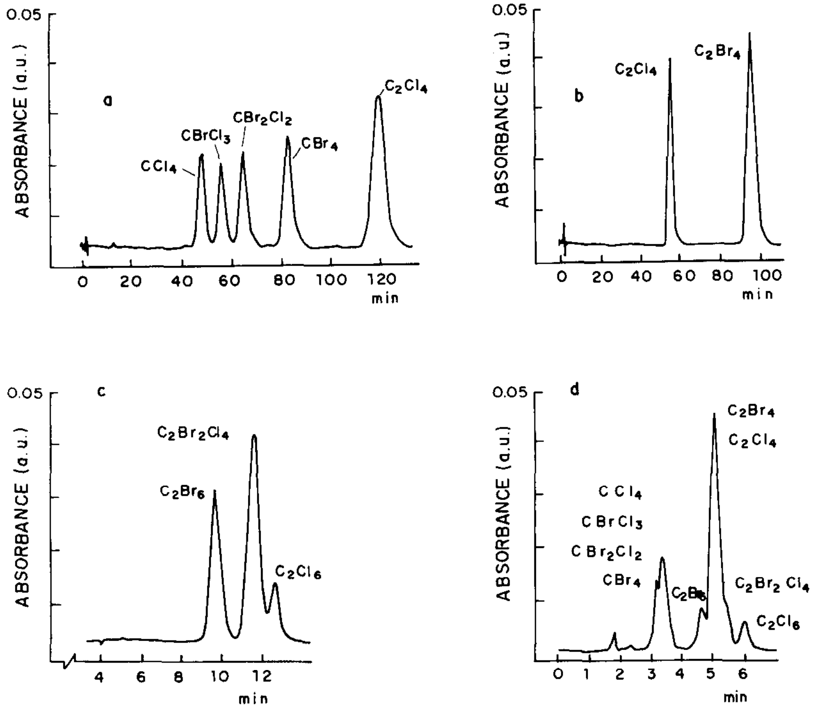

Fig. 1. Chromatograms of various mixtures of perhalogenated compounds obtained with column 1. Stationary phase, Vydac TP210 (124 × 4.6 mm I.D.); injection volume, 10 μl; detection, 220 nm. Methanol–water mobile phase: (a) 22:78 at 1.0 ml/min; (b) 35:65 at 1.0 ml/min; (c) 70:30 at 0.5 ml/min; (d) 70:30 at 0.4 ml/min.

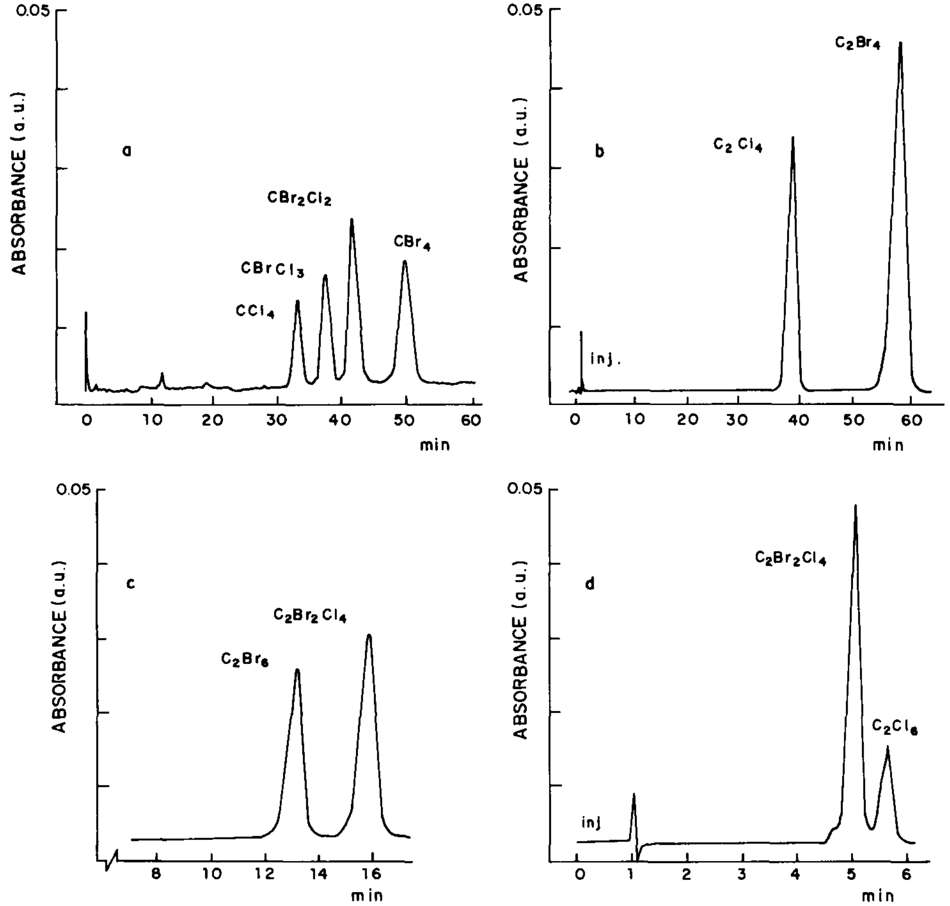

Fig. 2. Chromatograms of various mixtures of perhalogenated compounds obtained with column 2. Stationary phase, Spherisorb ODS-1 (100 × 4.6 mm I.D.); injection volume, 10 μl; detection, 220 nm. Methanol–water mobile phase: (a) 30:70 at 1.0 ml/min; (b) 40:60 at 1.0 ml/min; (c) 60:40 at 1.0 ml/min; (d) 70:30 at 1.0 ml/min.

molecules there is no discernible difference between the monomeric (Ultrasphere), dimeric (Spherisorb) and polymeric (Vydac) phases.

Considering as an objective the separation of as many perhalogenated compounds of the type $C_nBr_yCl_2$ as possible, including those not available as standards, the most appropriate mobile phase composition was fixed as methanol–water (63:37) for use with the Ultrasphere ODS column (Fig. 3). At this composition, the C_1 and C_2 saturated compounds are satisfactorily separated. The high resolution between CBr_2Cl_2 and CBr_4 and between the C_2 perhalogenated standards suggests that other $C_nBr_yCl_2$ compounds could be determined. That this is so is shown in Fig. 4, where two different reaction mixtures reveal the presence of CBr_3Cl, $C_2Br_3Cl_3$ and C_2BrCl_5. These compounds were identified by extrapolation [6] and their identities confirmed by gas chromatography–mass spectrometry.

TABLE III

RETENTION TIMES (t_R, min) AND RESOLUTIONS (R_s) OF PERHALOGENATED COMPOUNDS AS A FUNCTION OF MOBILE PHASE COMPOSITION

Stationary phase, Ultrasphere ODS, 5 μm (250 × 4.6 mm I.D.); mobile phase, methanol–water of various compositions. Flow-rates selected to maintain the pressure at <600 bar.

Compound	Mobile phase composition and flow-rate (ml/min)													
	50:50, 0.7		60:40, 0.8		62.5:37.5, 0.9		63:37, 0.9		65:35, 1.0		70:30, 1.0		80:20, 1.0	
	t_R	R_s	t_R	R_s	t_R	R_s	t_R	R_s	t_R	R_s	t_R	R_s	t_R	R_s
CBr_4	66.3	–	23.1	1.9	21.1	2.0	17.2	2.5	12.3	2.6	9.4	1.9	5.6	2.6
CBr_2Cl_2	69.3	–	24.9	0.9	22.9	1.0	18.8	1.1	13.5	1.3	10.4	1.0	6.1	1.5
$CBrCl_3$	69.3	–	25.8	0.9	23.9	0.9	19.6	1.2	14.1	1.3	10.9	1.2	6.4	1.6
CCl_4	69.3		26.7		24.9		20.5		14.7		11.4		6.8	
C_2Br_4	179.9	5.3	54.1	–	50.3	–	40.1	–	27.3	–	19.2	1.4	9.6	n.c.[a]
C_2Cl_4	150.6		52.2		50.3		40.1		27.3		20.4		10.7	
C_2Br_6	247.0	4.8	60.4	6.2	54.1	6.3	41.8	6.8	26.8	7.0	18.3	5.2	8.3	n.c.
$C_2Br_2Cl_4$	298.0	2.3	75.2	3.6	68.9	3.5	53.4	3.6	34.1	4.1	23.3	4.1	10.3	n.c.
C_2Cl_6	329.5		85.3		80.0		62.0		39.2		27.1		11.9	

[a] n.c. = Not calculated.

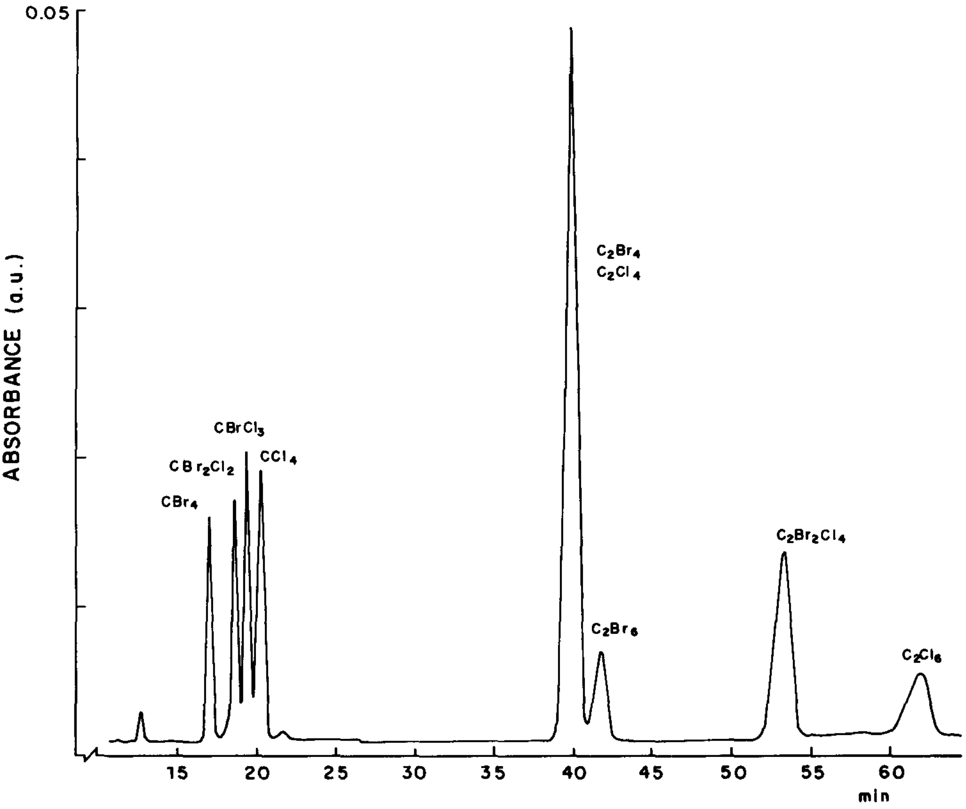

Fig. 3. Chromatograms of a mixture of perhalogenated compounds obtained with column 3. Stationary phase, Ultrasphere ODS (230 × 4.6 mm I.D.); mobile phase, methanol–water (63:37) at 0.9 ml/min; injection volume, 10 μl; detection, 220 nm.

If C_2 unsaturated compounds are present in the reaction mixtures a second chromatogram is run, at a lower water content, to permit the identification of these compounds, as with the chosen isocratic phase [methanol–water (63:37)] all C_2 unsaturated compounds are predicted to be superimposed.

The determinations reported here were applied to a large number of reaction mixtures of the types exemplified by Fig. 4. The retention times are remarkably reproducible, even after a chlorodimethyloctadecylsilane treatment of column 3.

With the methanol–water (63:37) system, the mass distribution ratios, *k*, for the C_1 compounds lie between 6 and 8 and those for the C_2 saturated perhalogenated compounds between 16 and 26. The latter higher values could be reduced by altering

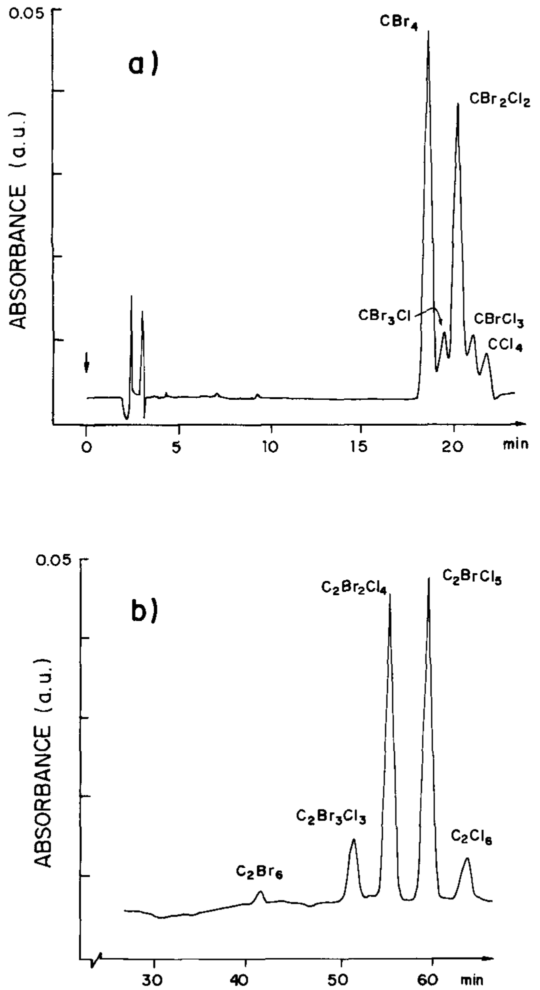

Fig. 4. Chromatograms of several reaction mixtures obtained with column 3. Stationary phase, Ultrasphere ODS (250 × 4.6 mm I.D.); mobile phase, methanol–water (63:37) at 0.9 ml/min; injection volume, 10 μl; detection, 220 nm. (a) Identification of gamma radiolysis (50 kGy) products from a solution of 3% CBr_4 in CCl_4; (b) identification of the UV photolysis products of a solution of C_2Br_6 in Cl_2-saturated CCl_4.

the mobile phase polarity, but this would sacrifice the separation of these compounds, and also the resolution of the C_1 compounds, if isocratic elution is used. On the other hand, the sensitivity of the retention times to the mobile phase composition, with possible superposition or even inversion of the elution order, suggests that gradient elution is not the solution to the problem presented by the long retention times of the C_2 compounds.

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