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Short communication

Quantitative determination of several simple perhalogenated compounds by high-performance liquid chromatography

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Abstract

A method for quantifying perhalogenated compounds with the general formula $C_xBr_yCl_z$ (where $x=1$ or 2 and $y+z=4$ or 6) by high-performance liquid chromatography (HPLC) was developed. The interest in such a determination by HPLC is related to the use of mild conditions, avoiding possible compound decomposition from the higher temperatures used in gas chromatography. Analytical curves were obtained using both the external calibration and the internal standard ($CHCl_3$) methods. The detection limits for CBr_4, CCl_4, C_2Br_4, C_2Cl_4, $CBrCl_3$, C_2Cl_6 and $C_2Br_2Cl_4$ were, respectively, 2, 4, 1, 1, 1, 5 and 2 mg l^{-1}. For determination of unknown samples, the internal standard method is preferred as several standard solutions proved to be unstable in methanol–water solution.

Keywords: Halogenated compounds

1. Introduction

Halogenated organic compounds, those with one or more hydrogen atoms and those without (perhalogenated compounds) belong to the category of organic substances used as solvents which, due to their inherent volatility, are frequently encountered at trace levels in the environment. In addition to being readily absorbed by all animal species, including humans, their presence in the environment leads to a significant number of photochemically and thermally induced reactions with other substances present in the air, water or soil to produce undesired and reactive intermediates or products, the best known example being the interaction of free halogen atoms with ozone in the upper atmosphere.

Many different methods have been proposed for identifying and quantifying these halogenated compounds. Many are based on selective or specific gas chromatographic detection, after headspace collection of the volatile halogenated organic [1–13] with electron-capture detection among the most cited [3–5,13].

Perhalogenated compounds ($C_xBr_yCl_z$, $x=1$ or 2, $y+z=4$ or 6) present special problems of quantification by gas chromatography (GC) as they may undergo on-column thermal decomposition during the GC analysis, impeding quantification [14–17], results confirmed by parallel quantitative analysis of the solutions of several two-carbon perhalogenated compounds by GC, GC–mass spectrometry (MS), and reversed-phase high-performance liquid chromatography (RP-HPLC) [18,19]. The milder conditions

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encountered in RP-HPLC permit detection of the saturated $C_2Br_yCl_z$ ($y+z=6$) compounds while GC indicates significant amounts of unsaturated decomposition products.

The present work is a continuation of these studies, comparing the external calibration and internal standard methods of quantification of several of these perhalogenated compounds.

2. Experimental

2.1. Instrumentation

The modular liquid chromatographic system used a Waters Model 510 pump, a Rheodyne Model 7125 Rheodyne injector (10 μl loop), and a Schoeffel Spectroflow 770 UV–Vis detector (at 220 nm) with an 8-μl flow cell, coupled to a RB 102 recorder from Equipamentos Científicos do Brasil (São Paulo, Brazil). The column (250×4.6 mm) containing 5 μm Ultrasphere-ODS was fabricated by Altex-Beckman. The mobile phase for quantification was methanol–water (80:20, v/v) at 0.6 ml min^{-1}.

2.2. Reagents

Tetrachloromethane (Merck, Rio de Janeiro, Brazil) was purified by treatment with potassium hydroxide, followed by washing with slightly acidified (H_2SO_4) deionized water until no coloration was observed, drying with $CaCl_2$ and distillation [20]. Tetrabromomethane (ICN), tetrabromoethene (ICN) and hexabromoethane (K & K) were purified by vacuum sublimation in the absence of light. Bromotrichloromethane (Eastman, Rochester) was distilled. Dibromodichloromethane (Alfa Products), 1,2-dibromotetrachloroethane (Aldrich), tetrachloroethene (Merck), hexachloroethane (Carlo Erba) and trichloromethane (Lichrosolv, Merck) were used without purification. Methanol (LiChrosolv, Merck) and deionized water (Nanopure, Barnstead) were used to prepare the mobile phases.

2.3. Quantification

The most concentrated of the standard solutions for external calibration was prepared by weighing appropriate quantities of the perhalogenated compound directly into a previously calibrated 10.03-ml volumetric flask, dissolving the compound in 2 ml of degassed methanol and completing the volume with methanol–water (80:20, v/v). More dilute solutions were prepared by successive dilutions to 10.03 ml with MeOH–water (80:20, v/v), taking 4.00 ml of the previous solution. A total of 10 solutions were prepared for each compound for which a complete study was made (Table 1) while the lower detection limits for the other compounds were determined using a smaller number of similarly prepared solutions.

For the internal standard procedure, calibrated solutions of a concentrated solution of each perhalogenated compound, prepared as previously described in a methanol–water solution, were placed, together with 1150 mg of chloroform, in a 10.03-ml

Table 1
Solutions for determination of the dynamic and linear range for external calibration quantification (concentrations in mg ml^{-1}±s)

Solution No.	Compounds			
	CBr_4	CCl_4	C_2Br_4	C_2Cl_4
1	2.841±0.004	51.08±0.07	1.057±0.002	0.698±0.001
2	1.446±0.002	40.43±0.06	0.857±0.001	0.399±0.001
3	1.133±0.004	20.37±0.08	0.422±0.002	0.278±0.001
4	0.578±0.002	16.12±0.06	0.342±0.001	0.159±0.001
5	0.452±0.002	8.12±0.03	0.168±0.001	0.1109±0.0004
6	0.231±0.001	6.43±0.02	0.136±0.001	0.0634±0.0002
7	0.180±0.001	3.24±0.01	0.0670±0.0003	0.0442±0.0002
8	0.0921±0.0004	2.56±0.01	0.0542±0.0002	0.0253±0.0001
9	0.0718±0.0003	1.29±0.01	0.0267±0.0001	0.0176±0.0001
10	0.0367±0.0001	1.021±0.004	0.0216±0.00004	0.01037±0.00004

Table 2
Solutions for determination of the linear regression coefficients by the internal standard method [concentrations (C) in mg ml^{-1}]

Solution No.	C_{CBr_4}	RM[a]	C_{CCl_4}	RM[b]	$C_{C_2Br_4}$	RM[c]	$C_{C_2Cl_4}$	RM[d]
1	2.02	0.0174	41.7	0.361	0.70	0.00607	0.437	0.00428
2	1.51	0.0131	31.50	0.272	0.52	0.00452	0.328	0.00321
3	1.01	0.0088	26.00	0.224	0.35	0.00302	0.219	0.00214
4	0.51	0.0043	13.78	0.1200	0.149	0.00129	0.109	0.00106
5	0.201	0.00173	9.08	0.0783	0.075	0.00065	0.088	0.00086
6	0.101	0.00087	1.900	0.01645	0.0373	0.00032	0.044	0.00043
7	0.050	0.00043	0.770	0.00663	–	–	0.022	0.00021

[a] RM=CBr_4:$CHCl_3$.
[b] RM=CCl_4:$CHCl_3$.
[c] RM=C_2Br_4:$CHCl_3$.
[d] RM=C_2Cl_4:$CHCl_3$.

volumetric flask, completing the volume with MeOH–water (80:20, v/v). Solutions with different concentration (mass) ratios were prepared (Table 2).

To construct the calibration curves, all solutions were injected a minimum of three times and the registered areas (paper speed 4 cm min^{-1}) were manually evaluated, by calculating the areas as the actual peak height multiplied by the width at half-height [21].

3. Results and discussion

Fig. 1 shows the separation of nine of the perhalogenated compounds, using a methanol–water (80:20, v/v) mobile phase. Modifications of the mobile phase polarity by increasing the water content change the peak resolutions, with concomittant increases in the total analysis time [19].

Triplicate injections of each of the prepared solutions using the methanol–water (80:20, v/v) mobile phase at 0.6 ml min^{-1} and UV detection at 220 nm, with absorbance ranges of 0.01, 0.02, 0.04 and 0.1, permitted determination the detection limits (3-times noise, Table 3), and linear range and linear regression coefficients (least-squares method, Table 4) of several one- and two-carbon perhalogenated compounds. Within these linear ranges, the peaks all show acceptable (<1.2) peak asymmetries which resulted in manual calculation errors (evaluated from more than 30 chromatograms done on mixtures of the perhalogenated compounds) of less than 1%. At concentrations higher than those of the linear range, peaks become increasingly distorted and the calcu-

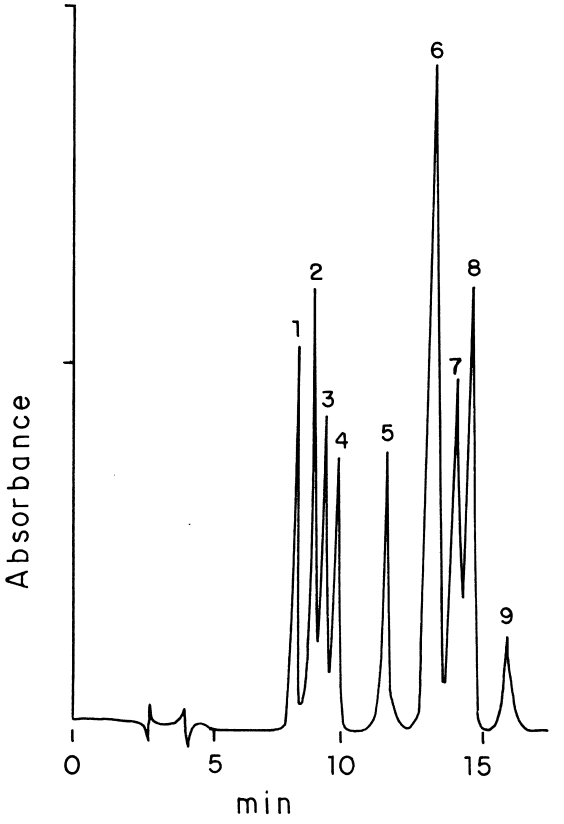

Fig. 1. Chromatogram showing the separation of nine perhalogenated compounds. Column: 250×4.6 mm Ultrasphere ODS, 5 μm, mobile phase: MeOH–water (80:20, v/v) at 0.6 ml min^{-1}. Injection volume: 10 μl. UV detection at 220 nm. Compound identification: 1=CBr_4; 2=CBr_2Cl_2; 3=$CBrCl_3$; 4=CCl_4; 5=C_2Br_6; 6=C_2Br_4; 7=$C_2Br_2Cl_4$; 8=C_2Cl_4; 9=C_2Cl_6.

Table 3
UV detection limits for several perhalogenated compounds (analytical conditions in text)

Analyte	Limit of detection (mg l^{-1})
CBr_4	2
CCl_4	4
C_2Br_4	1
C_2Cl_4	1
$CBrCl_3$	1
C_2Cl_6	5
$C_2Br_2Cl_4$	2

Table 4
Linear range and linear regression coefficients for several perhalogenated compounds (analytical conditions in text)

Analyte	Linear range (mg ml^{-1})	Linear regression coefficients			RSD (%)
		a	*b*	*r*	
CCl_4	1.021–20.370	4.438	0.213	0.9994	1.16
CBr_4	0.0367–1.133	95.938	−0.461	0.9999	0.44
C_2Cl_4	0.0104–0.399	338.890	2.387	0.9994	1.66
C_2Br_4	0.0216–0.857	312.387	4.189	0.9985	4.94

lated areas are not within the linear range. It should be noted, however, that all the compounds tested have significant linearity for quantification by HPLC, in contrast to GC where several perhalogenated compounds were found to lack quantitative linearity [16].

In quantitation using chloroform as an internal standard (Fig. 2), separate graphs were constructed of the area ratio (RA) versus the mass ratio (RM) and the RA versus analyte concentration (*C*, in mg ml^{-1}). As shown in Table 5, both methods lead to satisfactory correlation.

4. Conclusions

The results indicate that quantification of perhalogenated compounds using a non-destructive RP-HPLC method is feasible although the detection limits using UV detection indicate that preconcentration of environmental samples is necessary. Although both external calibration and the internal standard method give linear correlations, the in-

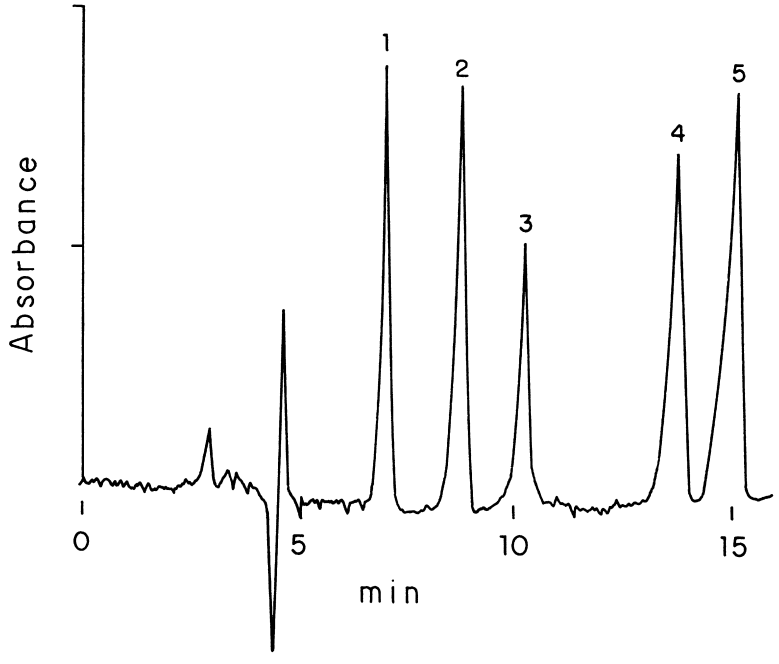

Fig. 2. Chromatogram showing the separation of several perhalogenated compounds and the internal standard (chloroform). Chromatographic parameters as in Fig. 1. Compound identification: 1=$CHCl_3$; 2=CBr_4; 3=CCl_4; 4=C_2Br_4; 5=C_2Cl_4.

Table 5
Linearity parameters for several perhalogenated compounds using $CHCl_3$ as internal standard for graphs of area ratio versus mass ratio and versus concentration

Analyte	Linear range (mg ml^{-1})	Linear regression coefficients vs. RM			Linear regression coefficients vs. *C* (mg ml^{-1})		
		a	*b*	*r*	*a*	*b*	*r*
CCl_4	0.770–41.70	0.0496	0.0566	0.9992	0.00655	0.0496	0.9992
CBr_4	0.050–2.020	0.0152	0.0991	0.9992	0.0147	0.0115	0.9990
C_2Cl_4	0.022–0.437	0.0210	4.340	0.9995	0.023	0.0443	0.9995
C_2Br_4	0.0373–0.70	0.0596	3.820	0.9990	0.0615	0.0440	0.9990

stability of some of the perhalogenated compounds in a methanol–water solution over time suggests that the internal standard method presents an advantage in that the calibration curve, with its series of standard solutions, does not have to be constructed each time the determinations are made.

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