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Journal of Chromatography A, 692 (1995) 53–57

JOURNAL OF CHROMATOGRAPHY A

Comparison of high-performance liquid chromatographic methods for the determination of 1,2-dibromo-2,4-dicyanobutane in cosmetic products

Suresh C. Rastogi*, Sys S. Johansen

Department of Environmental Chemistry, National Environmental Research Institute, Ministry of Environment, Frederiksborgvej 399, P.O.Box 358, DK-4000 Roskilde, Denmark

Abstract

Three known HPLC methods for the analysis of 1,2-dibromo-2,4-dicyanobutane (BCB) were compared to evaluate the strength of the methods for the routine analysis of BCB in cosmetic products. Reversed- and normal-phase HPLC with UV detection were not suitable for routine analysis of BCB in cosmetics. Reversed-phase HPLC with electrochemical detection fulfilled the requirements of a method to be used on a routine basis.

1. Introduction

1,2-Dibromo-2,4-dicyanobutane (BCB), also known as methyl-dibromo glutaronitrile, is a biocide which is used as a preservative in cosmetic products, food packaging materials, paints, glues, etc. BCB is commercially marketed under the trade names Euxyl K400 and Mergaurd 1200, both being a mixture of 20% BCB and 80% phenoxy ethanol. The use of BCB as a preservative in cosmetics is regulated by the European Union's (EU) Cosmetic Directive 76/768/EEC [1]. The maximum allowed concentration of BCB in cosmetic products is 0.1%. Sun-care products, however, should not contain >0.025% BCB. Since the introduction of BCB in the European Market, about 10 years ago, a number of cases of contact dermatitis due to the use of BCB-containing cosmetics have been described (see Ref. [2]).

To monitor that the cosmetic products comply with the EU's Cosmetic Directive, a method for routine analysis of BCB in cosmetics was required. A literature survey revealed that BCB in cosmetics could be analysed by gas chromatography (GC) using nitrogen–phosphorus detection [3], reversed-phase high-performance liquid chromatography (HPLC) with UV detection [4], normal-phase HPLC with UV detection [5] and reversed-phase HPLC with electrochemical detection [6]. Our efforts to adapt the GC method [3] revealed that the method was not reproducible (data not shown) because of uncontrolled decomposition of BCB in the GC injector at temperatures >150°C. Analysis of BCB by GC–mass spectrometry employing cold on-column injection also revealed that the (decomposed) BCB was eluted when the GC column temperature was >150°C. Therefore, HPLC was preferred for BCB analysis. However, the λ_{max} of BCB (220 nm) is a problem for HPLC analysis of BCB with UV detection, because many cosmetic

* Corresponding author.

0021-9673/95/$09.50
SSDI 0021-9673(94)00609-1

ingredients as well as HPLC mobile phase(s) may also show significant absorbance at this wavelength. On the other hand, analysis of BCB by HPLC–electrochemical detection, which involves reductive electrochemical detection of bromine [6], may not be suitable for routine analysis because such systems are very sensitive to the presence of oxygen (air) in the HPLC system, and they are not very stable. Therefore, the known HPLC methods were compared for their applicability for the analysis of BCB in cosmetic products.

2. Experimental

2.1. Chemicals

BCB reference substance was obtained from Bureau Community References (BCR, Brussels, Belgium). HPLC-grade acetone was from Fluka (Buchs, Switzerland) and HPLC-grade methanol, acetonitrile, isopropanol and hexane were from Rathburn (Walkerburn, UK). All other chemicals of analytical grade were from E. Merck (Darmstadt, Germany).

2.2. HPLC equipment

HPLC pump Model 510, autosampler Wisp 712 and UV detector 490 were from Waters Chromatography, Millipore (Milford, MA, USA). The electrochemical detector (LC-4B amperometric detector) was from Bioanalytical Systems (West Lafayette, IN, USA). Chromatographic data were collected using a D-2000 Chromato-integrator from Hitachi (Tokyo, Japan).

2.3. Samples

Three cosmetic samples, a shampoo, a cream and a lotion were used for the comparison of HPLC methods. Thirty cosmetic products (17 hair and body shampoos and 13 creams and lotions including one sun-care product) were analysed for BCB by the selected HPLC method.

2.4. Sample preparation and analysis of BCB

Previously described sample preparation methods [4–6] prior to HPLC analysis of BCB were adapted with the exception that the filtration of sample extract in one of the methods [6] was substituted by centrifugation. The known HPLC methods [4,5] were optimized, using different HPLC columns and mobile phases, as described elsewhere [7]. For the optimization of reversed-phase HPLC [4], efficiencies of Spherisorb S 5 ODS and Hypersil 5 ODS (both 5 μm particle size) columns were compared; and the four-solvent (methanol, acetonitrile, tetrahydrofuran and water) optimization method [7] was used for the mobile phase. Efficiencies of Hibar LiChrosorb, Hypersil CN and Nucleosil CN columns were compared for the optimization of normal-phase HPLC [5]; and the mobile phase composition was optimized by varying the ratio of hexane–isopropanol. For the electrochemical detection of BCB separated by HPLC [6], the detector parameters were optimized for the analysis of 20 ppm (w/w) BCB solution. In brief, the optimized methods for the analysis of BCB in cosmetics were as follows.

(1) Approximately 1 g cosmetic product was suspended in 10 ml methanol, followed by ultrasonic treatment and filtration through a 0.45-μm membrane filter. HPLC of BCB in the filtrate was performed employing a 250 mm × 4.6 mm I.D. Spherisorb 5 ODS column and acetonitrile–tetrahydrofuran–water (20:10:70) as mobile phase. The flow-rate of mobile phase was 2 ml/min and UV detection was performed at 220 nm.

(2) Approximately 1 g sample suspended in isopropanol was heated at 60°C followed by filtration through a 0.2-μm membrane filter. HPLC of BCB was performed employing a 250 mm × 4.6 mm I.D. Hibar LiChrosorb, particle size 7 μm, column and hexane–isopropanol (87:13) as mobile phase. The flow-rate of the mobile phase was 1 ml/min and UV detection was performed at 230 nm [5].

(3) Approximately 1 g sample suspended in 80% aqueous methanol was heated at 60°C followed by centrifugation at 600 *g* for 5 min.

BCB analysis in the filtrate was performed by HPLC employing a 250 mm × 4.6 mm I.D. Zorbax C_8 column and 40% aqueous acetone containing 0.02 *M* sodium sulphate and 0.002 *M* sodium chloride as mobile phase. Reductive electrochemical detection was performed using a gold electrode. An Ag/AgCl electrode was used as the reference electrode [6]. The flow-rate of the mobile phase was 1 ml/min and the detector settings were: range 500 nA and measuring potential −0.6 V.

3. Results and discussion

The three available HPLC methods for the analysis of BCB in cosmetics were compared to evaluate their applicability for routine analysis of BCB in cosmetic products. The HPLC methods were optimized and then applied for the analysis of BCB in three samples (a shampoo, a lotion and a cream). Various aspects of the methods, technical, economical and physical resources, and work environment were compared (Table 1). Chromatograms of BCB in a shampoo, spiked with 0.1% BCB, by the optimized HPLC methods are shown in Fig. 1.

The analytical method employing reversed-phase HPLC and UV detection at 220 nm is the simplest and very stable. However, the recovery of BCB from the samples was very low (< 30%). This could probably be increased by modifying the sample preparation method, but the method has other problems as well: not a

Table 1
Comparison of the three HPLC methods for BCB analysis in cosmetics

Parameters	Reversed-phase HPLC–UV detection	Normal-phase HPLC–UV detection	Reversed-phase HPLC–electrochemical detection	Method of choice
Detection limit (ppm) ($S/N > 3$)	50	50	0.5	3
Linearity of calibration curve (ppm)	25–250	10–300	0.5–40	3
Selectivity of BCB detection	*	*/**	SD	3
Column efficiency (number of plates)	4900	9800	25 000	3
Capacity factor, k' BCB	5.6	2.8	3.9	2/3
Stability of eluent	**/***	*	*/**	1
HPLC analysis time per sample (min)	25–90	15	20	2
Recovery of BCB (%)	15–30	75–115	96–102	3
Repeatability of:				3
standard BCB (%)	3	11	1	
sample extract (%)	–	6	1	
Simplicity of sample preparation	*	*	*	–
Physical and mental strain	*/**	*	**	3
Resources	**/***	**	*/**	1
Work environment	**	*	**/***	3

Qualitative evaluation: *** = good; ** = acceptable; * = less good. SD = Selective detection. – = could not be evaluated. Repeatability was determined for the 10-replicate analysis of 10 ppm solution of standard BCB by electrochemical detection (150 ppm of standard BCB by UV detection) and for the 10-replicate analysis of a shampoo spiked to contain 0.1% BCB. Physical and mental strain refer to the efforts needed for the optimal functioning of a method on routine basis.

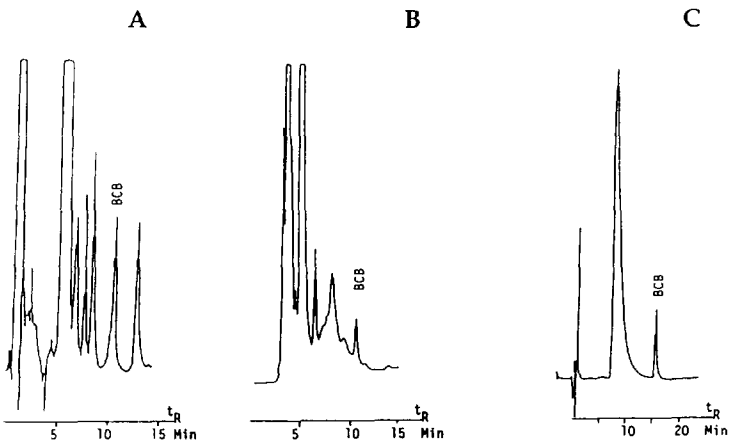

Fig. 1. Analysis of BCB (0.1%) in a shampoo by reversed-phase HPLC with UV detection (A), normal-phase HPLC with UV detection (B) and reversed-phase HPLC with electrochemical detection (C). See Experimental for details.

proper separation of BCB from other components in cosmetic products, non-specific detection of BCB at 220 nm where many cosmetic ingredients as well as HPLC mobile phase also show significant absorbance, and long analysis times because some of the cosmetic ingredients (especially in creams and lotions) elute very late (elution time >60 min). Although the method worked well for the analysis of a shampoo (Fig. 1A), analysis of BCB in a cream and a lotion by this method was not satisfactory, because the BCB peak was not properly resolved from the neighbouring peaks (results not shown). Changing the HPLC column to a Hypersil ODS column or changing the mobile phase composition [7] to optimize the method did not affect the resolution factor (<0.9) of BCB peak significantly. Thus, this method may only be suitable for the analysis of BCB in simple mixtures, for example, raw materials for cosmetics.

Analysis of BCB by normal-phase HPLC [5] showed a reasonable recovery ($>75\%$) of BCB from the cosmetic products, a reasonably good separation of BCB from other cosmetic ingredients (Fig. 1B) and short analysis times as well. The method, however, is not practical for routine analysis because a very long time (6–12 h) is required for the HPLC system to be stabilized and the UV detection at 230 nm, though better than that at 220 nm, is unspecific. Furthermore, the use of hexane is not considered to be safe.

The third method of BCB analysis by reversed-phase HPLC with reductive electrochemical detection of bromine, fulfilled the technical requirement for a routine method except that the baseline of the chromatogram was rather unstable and the retention time (t_R) of BCB was unstable (because of relatively quick evaporation of acetone from the constantly degassed mobile phase). Under the following conditions —replacement of PTFE tubings with steel tubings to stop the diffusion of air/oxygen into the HPLC system [6], column temperature at 40 ± 0.2°C [6] and room temperature at 20 ± 0.2°C— the baseline was stable (Fig. 1C). The major peak in Fig. 1C is a ghost peak of the HPLC system and that has nothing to do with the sample or standard BCB. The ghost peak does not interfere with the determination of BCB. Attempts to identify the cause of the ghost peak indicated that the ghost peak may have an association with the injection process, because a peak with the same t_R also appeared when the helium degassed mobile phase was injected. One of the possible reasons may thus be that a small portion of pressurized air, which is used for the injection process by autosampler, is introduced together

with the sample in the analytical column followed by the reductive electrochemical detection of the air/oxygen.

The HPLC system takes about 2 h to stabilize. The HPLC mobile phase which is recirculated, may be changed every 24 h. An internal standard, for example, bronidox or bronopol which are also used as preservatives in cosmetics, can be used in this method to ascertain the identification of BCB on the basis of its relative retention time (Fig. 2). This method is being used for the routine analysis of BCB in cosmetics in our laboratory without any problem.

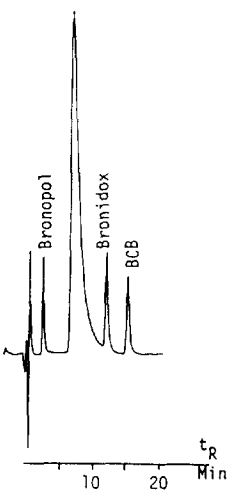

Fig. 2. Analysis of a solution of brominated preservative standards by reversed-phase HPLC with electrochemical detection. Concentrations of preservatives in the solution were Bronopol 220 ppm, Bronidox 57 ppm and BCB 10 ppm.

Acknowledgements

We thank Professor Steen Honoré Hansen for the fruitful discussions during the progress of the present work. Skillful technical assistance was provided by Mrs. Gitte H. Jensen.

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