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Development of a solid-phase microextraction gas chromatography with microelectron-capture detection method for the determination of 5-bromo-5-nitro-1,3-dioxane in rinse-off cosmetics

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ABSTRACT

5-Bromo-5-nitro-1,3-dioxane (bronidox) is a bromine-containing preservative often used in rinse-off cosmetics but also subjected to several restrictions according to the European Cosmetic Products Regulation. Thus, as a part of a quality control procedure, analytical methods for the determination of this compound in different types of cosmetics are required. In the present work, a solvent-free and simple methodology based on solid-phase microextraction (SPME) followed by gas chromatography with microelectron capture detection (GC-μECD) has been developed and validated for the determination of bronidox in cosmetic samples such as shampoos, body cleansers or facial exfoliants. As far as we know, this is the first application of SPME to this preservative. Negative matrix effects due to the complexity of the studied samples were reduced by dilution with ultrapure water. The influence of several factors on the SPME procedure such as fiber coating, extraction temperature, salt addition (NaCl) and sampling mode has been assessed by performing a 2^4-factorial design. After optimization, the recommended procedure was established as follows: direct solid-phase microextraction (DSPME), using a PDMS/DVB coating, of 10 mL of diluted cosmetic with 20% NaCl, at room temperature, under stirring for 30 min. Using these suggested extraction conditions, linear calibration could be achieved, with limits of detection (LOD) and quantification (LOQ) well below the maximum authorized concentration (0.1%) established by the European legislation. Relative standard deviations (RSD) lower than 10% were obtained for both within a day and among days precision. The method was applied to diverse types of formulations spiked with bronidox at different concentration levels (0.008–0.10%); these samples were quantified by external calibration and satisfactory recoveries (≥70%) were obtained in all cases. Finally, the SPME–GC-μECD methodology was applied to the analysis of several cosmetics labeled or not as containing bronidox. The presence of this preservative in some of these samples was confirmed by GC–MS.

1. Introduction

Cosmetics are important consumer products with an essential role in everyone's life: apart from *traditional* cosmetic products, such as make-up and perfumes, it also includes products for personal hygiene, for example tooth-care products, shampoos and soaps [1]. The addition of preservatives is absolutely essential to ensure that cosmetics are safe to use for a long time, since these compounds protect formulations from contamination by microorganisms such as bacteria and yeasts [2]. Nevertheless, preservatives together with certain ingredients used in cosmetics such as fragrances can cause allergic reactions, namely contact allergy and dermatitis [3]. Furthermore, products containing amines or amino derivatives may form nitrosamines (which are known potent carcinogens) if they also contain a component which acts as a nitrosating agent [4], as in the case of some preservatives. In order to ensure the free circulation of cosmetic products in the internal market and to guarantee the safety of cosmetics placed on it, Council Directive 76/768/EEC (known as Cosmetics Directive) was adopted by the European Union (EU) in 1976 [5]. Since its implementation, this Directive was amended by the European legislators seven times in order to reflect new trends and challenges concerning cosmetic products [1]. Recently, on 30 November 2009, a new Cosmetic Products Regulation (which is, to a great extent, a recast of the previous Directive and its successive amendments and adaptations) has been adopted in order to strengthen certain elements of the regulatory framework for cosmetics, such as in-market control, with a view to ensuring a high level of protection of human health [6].

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5-Bromo-5-nitro-1,3-dioxane (bronidox) is a bromine-containing preservative often used in cosmetics and pharmaceutical preparations including shampoos, body cleansers and facial exfoliants [7]. Bronidox has recently attracted attention as potential allergen and as nitrosating agent. According to the current European legislation, this preservative is restricted to rinse-off cosmetics with a maximum authorized concentration (MAC) of 0.1% and with the requirement of avoiding the formation of nitrosamines [6]. Bronidox is even not permitted in other more preventive legislations, as the Japanese [8].

Several analytical procedures for the determination of bronidox in cosmetic samples have been reported, most of them based on thin layer chromatography (TLC) [9,10] and liquid chromatography [11–15]. Gas chromatography-based methods have been also developed but all of them came out in low international repercussion and, consequently, hard-to-reach journals [16,17]. In the present work, a procedure for the determination of preservative bronidox in rinse-off cosmetics has been developed employing SPME combined with GC-μECD. Even though solid-phase microextraction has been previously applied to the analysis of other kind of preservatives (parabens) in cosmetic samples [18], it is important to highlight that this is the first time that bronidox has been extracted by SPME, which is considered as a valuable alternative analytical technique to more traditional procedures, reducing the laboratory-generated waste and time for sample preparation. SPME integrates sampling, extraction, concentration and sample introduction into a single uninterrupted process, resulting in high sample throughput [19]. A factorial design was selected to study and optimize main experimental factors affecting SPME. Finally, the optimized method was validated and applied to the determination of the target compound in different cosmetic samples.

2. Experimental

2.1. Reagents, materials and solutions

5-bromo-5-nitro-1,3-dioxane (Bronidox) was acquired from Fluka (Buchs, Switzerland). Methanol (gradient grade) was purchased from Merck (Mollet del Vallés, Barcelona, Spain) while ethyl acetate (HPLC grade) and sodium chloride (99.7%) were supplied by Prolabo (VWR, Fontenay-sous-Bois, France). Water was deionised and further purified in a Milli-Q water purification system from Millipore (Bedford, MA, USA).

A standard stock solution of 16,077 μg mL^{-1} of bronidox was prepared in methanol. Working solutions in the same solvent were prepared by convenient dilution of the stock solution to spike cosmetic samples (when needed). For daily evaluation of the GC equipment, a solution of 1 μg mL^{-1} of the target compound in ethyl acetate was also prepared to direct injection into the chromatograph. Stock and working solutions were stored in a freezer at −20 °C protected from light.

Commercially available 100 μm polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane–divinylbenzene (PDMS/DVB), 85 μm polyacrylate (PA), 75 μm carboxen–polydimethylsiloxane (CAR/PDMS), and 50/30 μm divinylbenzene–carboxen–polydimethylsiloxane (DVB/CAR/PDMS) fibers housed in manual SPME holders were obtained from Supelco (Bellefonte, PA, USA). The fibers were conditioned as recommended by the manufacturer.

2.2. Cosmetic samples

Shampoos, body cleansers and facial exfoliants were purchased from local supermarkets and drugstores. Four of these products (three shampoos and one gel) were labeled as containing bronidox, while the remaining ones did not have declared the presence of this preservative. Several *blank* samples were spiked with known concentrations of the target compound for method optimization and validation. Such samples were fortified at levels between 0.16% and 4.5×10^{-4}% (w/w) by addition of small volumes (<140 μL) of methanol solutions containing bronidox, in the concentration range of 16,077–81.4 μg mL^{-1}, over accurately weighed aliquots of approximately 1 mL. Subsequently, spiked samples were homogenized by agitation in a vortex and then kept at 4 °C for 1 h to allow analyte–matrix interactions.

Due to the complexity of the analyzed samples, all SPME experiments were performed with cosmetic samples diluted with ultrapure water in order to avoid damages and contaminations in the fiber coating and the GC instruments. With this aim, aliquots of approximately 1 mL of the cosmetic samples were diluted firstly with 9 mL of Milli-Q water. Then, a new dilution with ultrapure water was performed to achieve a final dilution ratio of 1:10,000 (for GC-μECD analyses) or 1:1000 (for GC–MS analyses). Such variations in the dilution ratios were due to the large differences between μECD and MS sensitivity for bronidox detection.

2.3. SPME extraction procedure

The diluted cosmetic samples (10 mL) were placed in 10 or 22 mL vials. Sodium chloride was added in proportion of 20% (w/v) in the required experiments. The vials were sealed with a headspace aluminum cap furnished with a PTFE-faced septum. Then, samples were let to equilibrate at the working temperature for 5 min and the SPME fiber was then immersed into the sample (direct solid-phase microextraction, DSPME) or exposed to the headspace over the sample (headspace solid-phase microextraction, HSSPME) for 10–60 min, depending on the experiment. In all extractions, the samples were magnetically stirred during the sampling. After extraction, the fiber was retracted and transferred to the GC injection port and chromatographic analysis was carried out. Desorption time was set at 5 min and desorption temperature was 250 °C. Next, the fiber was conditioned in another GC injector for 5 min at 260–290 °C (depending on the fiber coating) to avoid any carryover-effect. In order to extend the lifetime of the fibers and to protect the chromatographic system, fibers were immersed for a few seconds (three times) in ultrapure water after DSPME extractions with NaCl.

2.4. Chromatographic conditions

GC-μECD analyses were carried out in a Hewlett-Packard 6890 GC system fitted with a ^{63}Ni microelectron capture detection and equipped with a split/splitless injector. Data were acquired and processed by GC Chemstation software. Injector operated in the splitless mode (2 min) at 250 °C. A HP-5MS (30 m × 0.32 mm × 0.25 μm) column was employed in combination with the following GC oven temperature program: 60 °C held for 1 min, rate 10 °C min^{-1} to 150 °C, rate 20 °C min^{-1} to 210 °C, with a total run time of 13 min. Helium was used as carrier gas at a constant flow rate of 1 mL min^{-1}, while nitrogen was employed as make-up gas at the flow of 30 mL min^{-1}. Detector temperature was maintained at 300 °C.

In addition, some samples labeled as containing bronidox were also analyzed using a Varian 450 gas chromatograph (Varian Chromatography Systems, Walnut Creek, CA, USA) equipped with a 1079 split/splitless injector and an ion-trap mass spectrometer Varian 240 (Varian Chromatography Systems). For these GC–MS analyses, a 30 m × 0.25 mm i.d. HP-5MS column coated with a 0.25 μm film was employed. Oven temperature program was the same than for GC-μECD analyses. Helium was employed as carrier and damping gas, with a constant flow rate of 1 and 2.5 mL min^{-1}, respectively. Injector, trap, manifold, transfer line and ion source temperatures were 250, 150, 40, 290 and 200 °C, respectively. The ion-trap

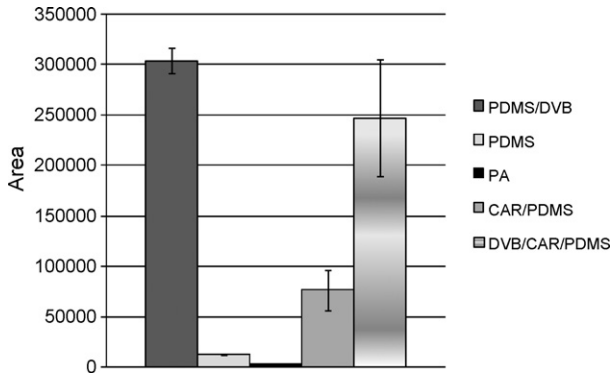

Fig. 1. Influence of the fiber coating in the SPME extraction of bronidox.

mass spectrometer was operated in the electron-ionization mode at 70 eV. A range of *m/z* 40–300 was scanned at a scan rate of 0.53 s/scan. The multiplier was set at a nominal value of 1750 V.

3. Results and discussion

3.1. Optimization of the SPME process

An experimental design was set up to evaluate the simultaneous effect of the main parameters affecting the SPME extraction of bronidox from rinse-off cosmetics. The studied variables were: type of fiber coating (A), extraction temperature (B), sampling mode (C) and addition of sodium chloride (D). First of all, preliminary experiments using PDMS, PDMS/DVB, PA, CAR/PDMS and DVB/CAR/PDMS coatings were performed in order to select the levels of this factor to be included in the above-mentioned experimental design. In such trials, 1 mL of shampoo samples fortified with 0.10% (w/w) of bronidox and diluted with ultrapure water (1:10,000 dilution ratio) were extracted in 10 mL vials, at room temperature for 30 min with constant stirring. As it is deduced in Fig. 1, PDMS/DVB and DVB/CAR/PDMS led to greater bronidox responses. Based on these results, both fibers were included in the corresponding experimental domain in which each investigated factor was evaluated at two levels (Table 1).

A full factorial design (2^4), which involves 16 experiments, was proposed to assess the influence of the studied variables (Statgraphics Plus®). This design has resolution V, meaning that the evaluation of all main effects and all two-factor interactions is allowed. In each experiment, 10 mL of diluted shampoo samples fortified with 0.10% (w/w) of bronidox were extracted in 22 mL vials (due to the impossibility of carrying out HSSPME experiments over 10 mL of diluted cosmetic samples in 10 mL vials), for 30 min with constant stirring.

The graphic tools provided by Statgraphics are very useful to easily interpret the results of the factorial design. In the Pareto chart (Fig. 2a), the bar length is proportional to the influence that each factor or interaction exerts on bronidox response. This chart also includes a vertical line corresponding to the 95% confidence interval; the main effects or interactions that exceed this reference line may be considered statistically significant. In the main effects plot (Fig. 2b), the length of the line drawn between the low and the high level of each factor is proportional to the effect of such variable in the extraction process, while the slope sign indicates the level of the factor producing the highest response.

Table 1
Levels and factors selected for the proposed experimental design.

Code	Factor	Low level (−)	High level (+)
A	Fiber	PDMS–DVB	DVB–CAR–PDMS
B	Temperature (°C)	25	100
C	Sampling mode	DSPME	HSSPME
D	NaCl (%, w/v)	0	20

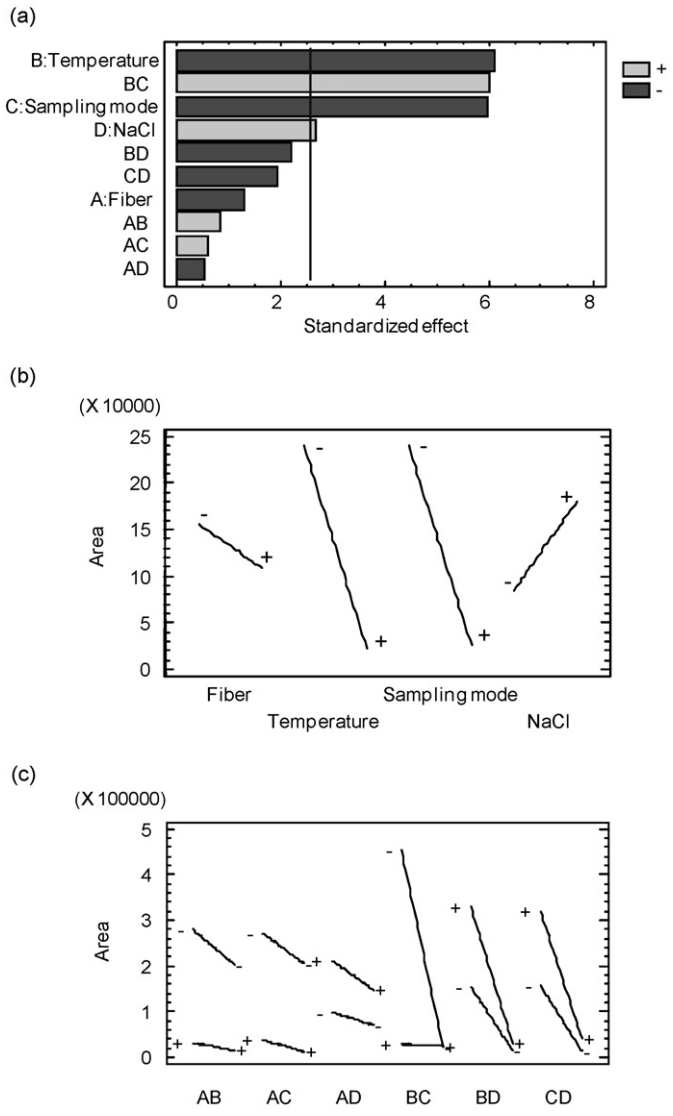

Fig. 2. Graphic tools to interpret the results of the factorial design: (a) Pareto chart; (b) main effects plot; and (c) interactions plot.

Extraction temperature (B) was the most important variable (see Fig. 2a) with a negative effect (Fig. 2b), meaning that higher responses were obtained at room temperature. The next most influential factor was the sampling mode (C) and more efficient extractions were obtained by direct immersion of the SPME fiber (Fig. 2b). Influence of salt addition (D) was also statistically significant even though its influence in bronidox response was not so important; the salting-out effect was shown to enhance the extraction of the target preservative (Fig. 2b). Finally, fiber coating (A) was not a significant variable but higher chromatographic areas were observed when working with the PDMS/DVB fiber (Fig. 2b). Regarding second-order effects, only BC interaction showed a significant effect (Fig. 2a): using DSPME a significant response improvement was observed when working at 25 °C, while in the HSSPME mode the temperature effect was negligible (Fig. 2c).

After the optimization of the investigated factors, the recommended procedure for the SPME extraction of bronidox in rinse-off cosmetic samples was established as follows: PDMS/DVB fiber coating, room temperature, DSPME sampling mode, and addition

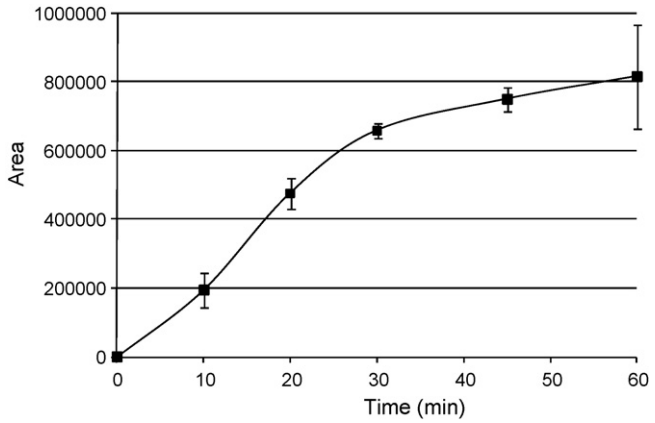

Fig. 3. SPME kinetic profile of bronidox under the recommended extraction conditions.

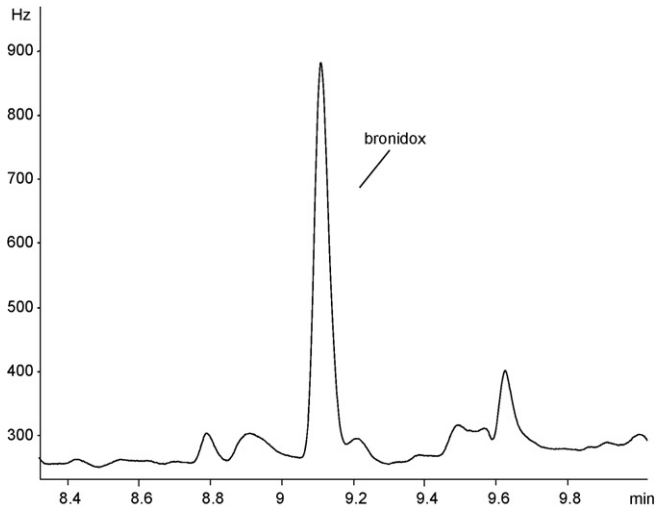

Fig. 4. DSPME–GC-μECD chromatogram obtained for a shampoo sample spiked with bronidox at 4.5×10^{-4}% used for the calculation of the detection and the quantification limits of the preservative.

of NaCl (20%, w/v). Since DSPME was observed as the optimal sampling mode and no significant differences between responses obtained using 10 and 22 mL vials were observed (data not shown), the subsequent experiments were carried out employing 10 mL vials.

Finally, the kinetic profile for the SPME extraction of bronidox was evaluated by performing extractions during different periods of time (10–60 min) under the recommended conditions mentioned above. As it is shown in Fig. 3, an important enhancement of the response was observed when extraction time increased up to 30 min. The equilibrium was not reached in the investigated range of time, but sampling during 60 min led to responses only a 20% higher than the corresponding ones at 30 min. Therefore, a compromise between duration of the analyses and sensitivity of the method was adopted by selecting 30 min as the optimum extraction time.

3.2. Method performance: precision, linearity and limits of detection and quantification

In order to assess the performance of the SPME procedure, analytical quality parameters were measured using spiked shampoo samples with known concentrations of bronidox. The validation parameters are presented in Table 2.

Method precision was studied within a day ($n = 5$) at three fortification levels (0.10, 0.040 and 0.008%) and among days ($n = 5$ days) at 0.10%. Relative standard deviations (RSD) for the intra-day precision ranged from 4 to 9%, while the RSD for the inter-day precision was 9% (Table 2). The limits of detection (LOD) and quantification (LOQ) were calculated as the concentration giving a signal-to-noise ratio of three (S/N = 3) and ten (S/N = 10), respectively. These limits were estimated using the GC-μECD chromatogram obtained for a shampoo sample fortified at 4.5×10^{-4}% with bronidox and analyzed using the proposed methodology (see Fig. 4). LOQ and LOD were 2.2×10^{-5} and 6.6×10^{-6}% (estimated taking into account the dilution factors), respectively, both values well below the maximum authorized concentration (0.1%) established by the EU in rinse-off cosmetics.

Table 3
Recoveries estimated in different cosmetic samples.

Cosmetic sample	Gel	Facial exfoliant	Bath oil	Shampoo
Spiked concentration (%, w/w)	0.10	0.060	0.028	0.008
Recovery (%)	92 ± 2	82 ± 10	71 ± 13	70 ± 4

The linearity of the method was tested over a wide range of concentrations between 0.16 and 0.004% (including seven concentration levels). At each concentration level triplicate analyses were performed and the response function was found to be linear with a determination coefficient (R^2) of 0.9951. To validate the regression data, an analysis of variance (ANOVA) was performed. The lack-of-fit (LOF) test is used for testing the adequacy of a linear regression model when replicates are available. The test compares the variability of the proposed model residuals to the variability between observations at replicate values of the independent variable. Results of the LOF test for the calibration range considered (at a confidence level of 95%) are also shown in Table 2. Since p-value is greater than 0.05, the linear regression model is adequate for the experimental data.

3.3. Accuracy evaluation

Recovery studies were carried out by applying the optimized SPME method to the extraction of four different types of cosmetic samples: shampoo, bath oil, facial exfoliant and gel. These samples were fortified at different concentration levels in the range 0.008–0.10% and quantified by external calibration. Recoveries were calculated as the ratio of the estimated concentration to the spiked concentration, and expressed as percentage. Satisfactory results were obtained in all cases with recoveries equal or higher than 70% (Table 3).

Table 2
Performance of the DSPME–GC-μECD method.

Precision (RSD, %)				LOD (%, w/w)	LOQ (%, w/w)	Linearity		
Intra-day ($n = 5$ days)			Inter-day ($n = 5$ days)			R^2	LOF test	
0.10% (w/w)	0.040% (w/w)	0.008% (w/w)	0.10% (w/w)				F-ratio	p-value
9	4	8	9	6.6×10^{-6}	2.2×10^{-5}	0.9951	2.19	0.1139

Table 4
Quantification of bronidox in cosmetics labeled as containing such preservative.

Cosmetic	Bronidox content (%, w/w)
Dermatologic shampoo	0.0077 ± 0.0004
Pediatric shampoo	0.0130 ± 0.0002
Anti-fall shampoo	0.0136 ± 0.0001
Aloe Vera gel	0.0028 ± 0.0003

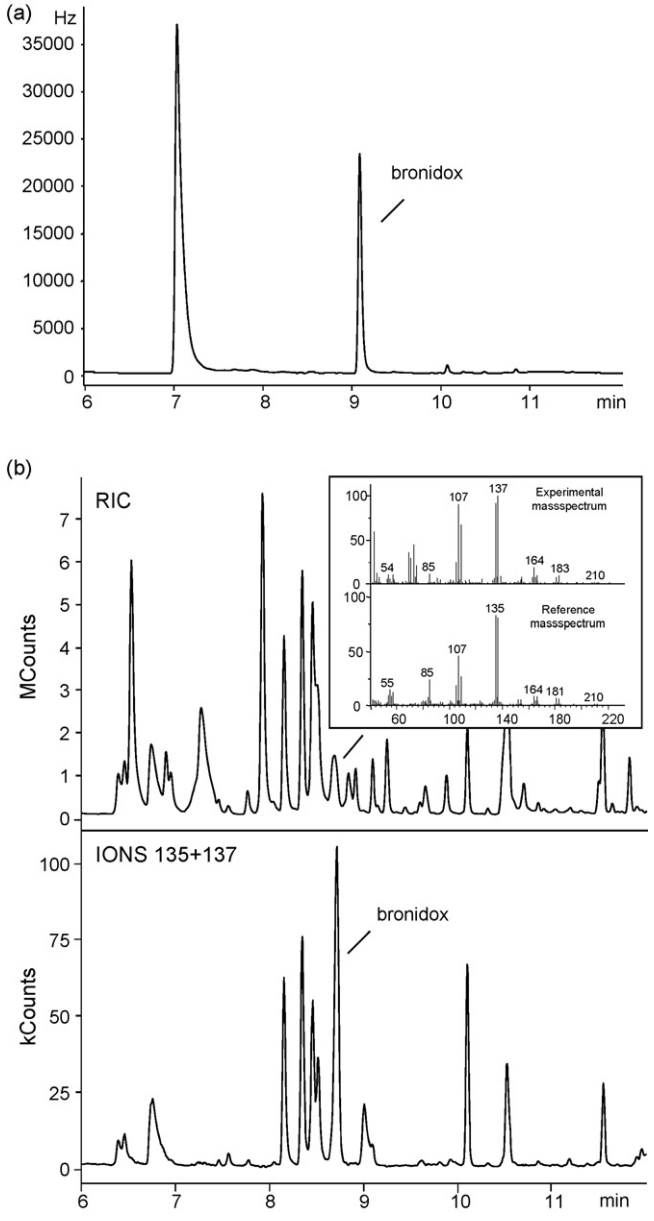

Fig. 5. DSPME–GC-μECD (a) and DSPME–GC–MS (b) chromatograms obtained for an anti-fall shampoo labeled as containing bronidox and mass spectra of such preservative.

3.4. Application to real samples

The SPME-based extraction procedure was applied to the determination of bronidox in 22 cosmetic products, four of which (three shampoos and one body cleanser) were labeled as containing the brominated preservative. External calibration was employed for accurate quantification of these four samples and the content of the target compound was within the MAC, that is, bellow 0.1% in all of them (Table 4). With the aim of confirming bronidox presence, GC–MS analyses were also performed. Fig. 5 shows the GC-μECD and GC–MS chromatograms obtained for the anti-fall shampoo, together with the experimental and reference mass spectra of bronidox. This figure clearly illustrates that, even when the ion GC–MS chromatogram is considered, the improvement in the selectivity is obvious when using μECD detection.

It is important to highlight that these four rinse-off cosmetic samples are also labeled as containing several fatty acid amides (FAA) such as cocamide diethanolamine (cocamide DEA), cocamide monoethanolamine (cocamide MEA) or lactamide monoethanolamine (lactamide MEA), which could react with nitrosating agents such as bronidox leading to the formation of carcinogenic nitrosamines [4]. Therefore, we can state that even though these four rinse-off cosmetic samples comply with the Cosmetic Products Regulation [6] regarding bronidox levels, they do not follow the recommendation of avoiding the formation of nitrosamines.

Finally, the remaining 18 products (12 gels, 5 shampoos, and 1 facial exfoliant) with no declared presence of bronidox were also analyzed. The investigated compound was not detected in any of these samples demonstrating the correct labeling of these formulations regarding bronidox occurrence.

4. Conclusions

This paper describes the optimization, validation and application of a novel SPME–GC-μECD method for the determination of bronidox in rinse-off cosmetics. An experimental design allowed estimating the effect of several SPME variables simultaneously. The proposed methodology has the advantages of short duration, simplicity and absence of organic solvents. Furthermore, the obtained results indicated that this method is capable of providing sensitive, reproducible and accurate data for the analyses of several types of formulations such as shampoos, gels or facial exfoliants.

Twenty-two cosmetic products were analyzed employing the recommended procedure and it was demonstrated that all of them were labeled correctly with respect to the presence of bronidox.

Acknowledgments

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