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Determination of bronopol in cosmetic products by HPLC with electrochemical detection

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A procedure is described for the assay of bronopol in cosmetics by HPLC coupled with constant-potential amperometric detection. Samples were analysed on an Alltima C_{18} column with methanol-phosphate buffer as the eluent and detected at a porous graphite electrode set at a reduction potential of −0.9 V. The recovery of bronopol from different cosmetic matrices was between 96.4 and 98.8% and the precision of the method was better than 4.5% relative standard deviation.

1. Introduction

Bronopol (2-bromo-2-nitropropane-1,3-diol) is a broad spectrum antibacterial agent [1] which is widely used as preservative in cosmetic detergents and creams [2, 3]. It is included in the list of antimicrobial agents authorized by the European Economic Community (EEC) directive on cosmetics [4], the maximum allowed level being 0.1% (w/w). Hence, the assay of bronopol in final products is important for quality control purposes and for checking compliance with the EEC legislation.

Published chromatographic methods for the determination of bronopol in cosmetic products are based on TLC [5, 6], GC [7, 8] and HPLC [3, 9–11]. These techniques, however, suffer from several drawbacks such as inadequate resolving power and accuracy (TLC) and laborious sample pretreatment and derivatization (GC). Moreover, since bronopol lacks a strong chromophore, HPLC with conventional UV detection requires the selection of short UV wavelengths [9–11] which results in increased interference with the complex cosmetic matrices.

HPLC in conjunction with electrochemical detection (ED) provides enhanced selectivity as a result of the limited number of solutes which can undergo redox reactions under certain conditions [12, 13]. Weyland et al. [14] have reported a method for the determination of bronopol and other preservatives (i.e., bronidox and methyldibromo glutaronitrile) in cosmetic preparations using HPLC coupled with pulsed amperometric detection at a gold electrode. However, the complexity of the equipment and the procedure hamper the applicability of this system to routine quality control assays. Moreover, the operating conditions [14] were not optimized specifically for the analysis of bronopol.

This study describes a method for the determination of bronopol in cosmetics by HPLC combined with a constant-potential amperometric detector. This system was selected because it represents the most commonly employed and the easiest type of electrochemical detection to implement for direct coupling with HPLC [12]. The application of this procedure to the assay of commercial cosmetics and its comparison with classical UV detection in terms of sensitivity and specificity are also presented.

2. Investigations, results and discussion

The objectives of this study were to evaluate the use of conventional controlled-potential amperometry for the HPLC-ED determination of bronopol in complex cosmetic matrices.

Several parameters were investigated to optimize the electrochemical detection of bronopol. Initial experiments were carried out on an octadecyl-silica stationary phase using methanol-0.01 M potassium phosphate (pH 6.0) as the eluent. Under these conditions, bronopol (retention time 5.5 min) produced a detectable signal at reduction potentials higher than −0.4 V. Enhanced signals were observed as the working electrode voltage was varied from −0.4 to −1.2 V. However, the operating reduction potential was set at −0.9 V, since at higher values the rise in the background current diminishes the signal-to-noise ratio. The ED performance was markedly affected by the molarity of the mobile phase buffer. Increasing the concentration of potassium phosphate in the eluent from 0.01 to 0.05 M enhanced the bronopol electrochemical response by a factor of 7, although a rise in the noise was observed with increasing ionic strength in the mobile phase. Consequently, the potassium phosphate molarity was fixed at 0.04 M. The detector response was not significantly influenced, in the range 4.0–6.0, by the phosphate buffer pH which was set at 6.0.

The limit of quantitation for bronopol, using the optimized HPLC-ED conditions, was 6.1 ng on-column weight, which corresponds to a concentration in the final product of 0.003% (w/w). There was a linear correlation between peak area and amount injected up to 230 ng ($r = 0.998$, slope $= 0.18$, intercept $= -0.01$).

A cream and a bath foam placebo were spiked with bronopol at 0.01% (w/w) and subjected to analysis by HPLC-ED. No interference was observed from the formulation excipients. The average recoveries ($n = 6$) from the cream and the bath foam matrices were 96.4% with a relative standard deviation (RSD) of 4.0% and 98.8% with a RSD of 2.3%, respectively.

The precision of the method, determined by six replicate measurements, was shown by RSD of 2.1% for the intra-assay reproducibility and 4.5% for the inter-assay reproducibility.

Different commercially available cosmetic preparations were assayed using the HPLC-ED procedure developed in this study and the results are listed in the Table. Since the

Table: Assay results for bronopol in cosmetic products determined by HPLC-ED

Formulation	Concentration* (%, w/w)	RSD
Liquid soap	0.023	2.9
Shampoo	0.010	3.2
Cream	0.015	4.5

* Each value is the mean of five determinations

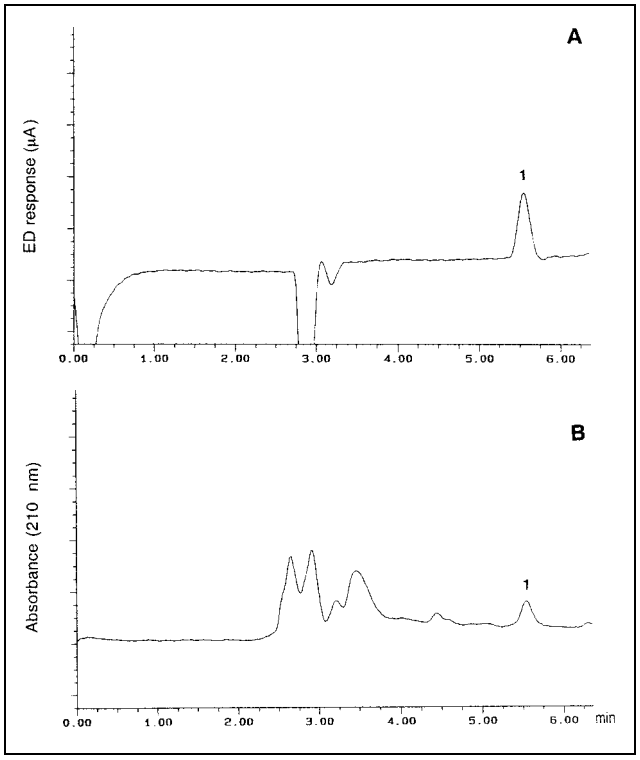

Fig. 1: Chromatographic recordings of a liquid soap preparation (containing 0.023% bronopol) obtained by: (A) ED or (B) UV detection. Operating conditions as described under Experimental. Peak: 1 = bronopol

electrochemical detector was coupled on-line with the UV detector, the trace from the ED was monitored in series with the UV trace. Representative chromatograms of a liquid soap extract recorded simultaneously with the two detection systems are reported in Fig. 1. The HPLC traces indicate that higher sensitivity is achieved by ED (Fig. 1A). Moreover, compared to UV monitoring (Fig. 1B), ED produces a chromatogram (Fig. 1A) with a lower background from matrix components, thus affording enhanced selectivity. This is further demonstrated by the comparison of the recordings obtained for a shampoo product with the two detectors (Fig. 2A and B). The ED response was not affected by cosmetic excipients (identified as sodium benzoate and nicotinamide) which generated peaks interfering with the UV determination of bronopol (Fig. 2B). Moreover, strongly retained substances that interfered with successive analyses were observed in the UV chromatogram of the cream preparation. This was not found to be the case with HPLC-ED, confirming the improved selectivity achieved by this technique. The data presented in the Table illustrate the precision of the method and indicate compliance with the EEC legislation [4].

In conclusion, HPLC coupled with constant-potential amperometry offers a means of enhancing the sensitivity and selectivity of conventional HPLC-UV analysis of the poorly absorbing bronopol. In addition, the proposed HPLC-ED method represents a valuable alternative to HPLC with pulsed amperometric detection [14] in terms of sensitivity, simplicity of the technique, signal stability, electrode maintenance and lifetime. Because of the high selectivity, good accuracy and precision the HPLC-ED procedure developed in this study is suitable for routine assays of bronopol in cosmetic products.

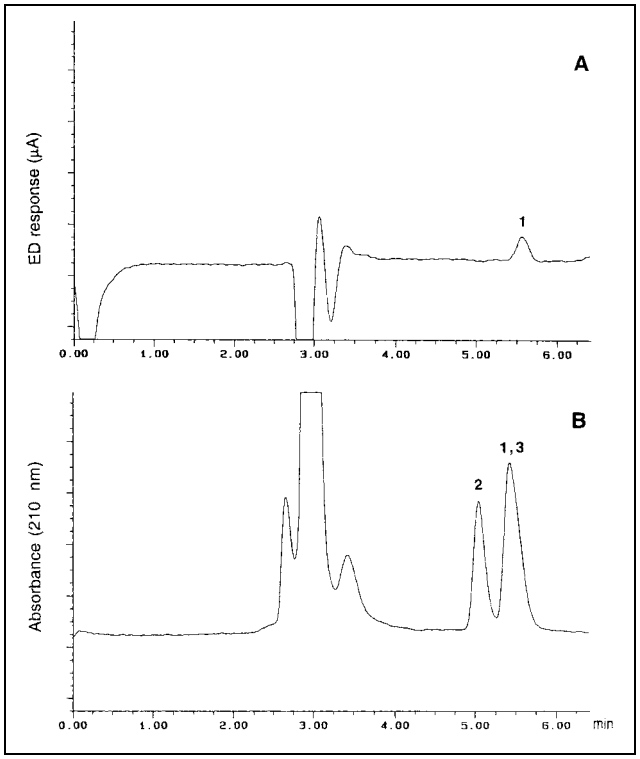

Fig. 2: Chromatographic recordings of a shampoo product (containing 0.010% bronopol) obtained by: (A) ED or (B) UV detection. Conditions and peak identification as in Fig. 1; 2 = nicotinamide; 3 = sodium benzoate

3. Experimental

3.1. Materials

Bronopol was obtained from Formenti (Milan, Italy). Methanol and water were HPLC grade from Baker (Phillipsburg, NJ, USA). All other chemicals were of analytical grade (Sigma, Milan, Italy). Commercial cosmetics from various manufacturers were from retail stores.

3.2. High performance liquid chromatography

The HPLC apparatus comprised two Model 510 pumps (Waters Corporation, Milford, MA, USA), a Model 712 WISP auto-injector (Waters) and a Model 5100A (Coulochem; ESA, Bedfod, USA) electrochemical detector which consisted of a control module and an analytical cell (Model 5010) containing two in-line porous graphite electrodes operating in the reductive mode, at voltages of −0.4 and −0.9 V, respectively. The ED sensitivity range and response time were set at 10 μA and 10 s, respectively. A single electrode guard cell (Model 5020), set at −1.0 V, was placed between the pump and the injector to suppress background current. The electrochemical detector was directly coupled to a Model 490E absorbance detector (Waters) set at 210 nm and 0.01 absorbance units full scale. Data acquisition and processing were accomplished with an APCIV computer system (NEC, Boxborough, MA) using Maxima 820 software (Waters).

Separations were carried out on a 5 μm Alltima C_{18} column (250 × 4.6 mm i.d.; Alltech Italia, Milan, Italy) fitted with a guard column (7.5 × 4.6 mm; Alltech) and eluted isocratically with 30% (v/v) methanol in potassium phosphate buffer (0.04 M, pH 6.0). The mobile phase was filtered through GV-type filters (0.22 μm, Millipore, Bedford, MA, USA) and on-line degassed with a Model ERC-3311 solvent degasser (Erma, Tokyo, Japan). Chromatography was performed at ambient temperature, at a flow rate of 1.0 ml/min. The identity of the bronopol peak was assigned by co-chromatography with the authentic standard. Quantification was carried out by integration of the peak areas using the external standardization method.

3.3. Sample preparation

The cosmetic (ca. 0.2 g) was accurately weighed into a 10-ml volumetric flask and dissolved in 30% (v/v) methanol in water (for aqueous products) or methanol (for emulsions) under mixing on a vortex-mixer. After dilution to volume, the sample was filtered (0.45 μm membrane filters, Millipore) and a portion (10 μl) of the resulting solution was directly analysed by HPLC.

3.4. Assay validation

A cream (oil-in-water emulsion) and a bath foam test samples were prepared in the laboratory by adding bronopol at a level of 0.01% (w/w) to

the formulation components (the cream excipients were: methyl silicone, chamomile oil, capryl glucoside, polyoxyethylene sorbitan monostearate, butylated hydroxyanisole, cetearyl alcohol, octyldodecanol, p-hydroxybenzoic acid ethyl ester, glycerin, citric acid, EDTA, water; the bath foam excipients were: cocamidopropyl betaine, magnesium laureth sulphate, arnica extract, citric acid, EDTA, glycerin, water). The percentage recovery was calculated by comparing the peak areas of bronopol extracted from the test samples with those obtained by direct injections of an equivalent concentration of the analyte dissolved in the mobile phase.

The intra-assay reproducibility was evaluated by repeated analyses ($n = 6$) of the same sample solution obtained from a cream containing 0.01% (w/w) bronopol. The inter-assay precision was calculated by extraction and HPLC assay of independent samples ($n = 6$) from the same cream formulation.

Calibration curves of peak areas versus concentration were generated with placebo extracts spiked with known amounts of bronopol.

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