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Short communication

Determination of bronopol and its degradation products by HPLC

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Abstract

Bronopol (2-bromo-2-nitropropane-1,3-diol) is widely used as an anti-microbial in cosmetics, external medicaments, shampoos and bath preparations. A reversed-phase high performance liquid chromatographic separation method with UV spectrophotometric detection was developed for the determination of bronopol and its degradation products. Degradation of bronopol was observed in aqueous medium, giving rise to non-reproducible data. To overcome this problem, HPLC grade methanol (water content $< 0.05\%$) was used for sample preparation. In addition, the parameters for the HPLC analysis of bronopol were optimised. It was found that bronopol standards and product samples were stable in methanol for at least 1 month at ambient temperature, thus allowing a quantitative and reproducible determination of bronopol.

Keywords: Bronopol; Bromonitroethanol; Bromonitromethane; Degradation; HPLC

1. Introduction

Bronopol (2-bromo-2-nitropropane-1,3-diol) is widely used as an antimicrobial in cosmetics, external medicaments, shampoos and bath preparations [1]. It is important to determine its content in these commercial products as a part of the quality control procedure. Various different analytical techniques, for example, enzymatic [2], ultraviolet spectrophotometric [3], gas chromatography [4], HPLC with UV [5,6] and electrochemical [7] have been reported. However, some of these analytical procedures are time-consuming, and as with our preliminary experiments, degradation of bronopol was observed in some instances, giving rise to non-reproducible data. Furthermore, there appears to be little comment on the determination of the degradation products of bronopol.

In this paper, the conditions for sample preparation and the HPLC analysis for the determination of bronopol and its degradation products were investigated. The factors influencing the stability of bronopol are discussed. The content of bronopol and its degradation products in some bronopol containing products is given.

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2. Experimental

2.1. Materials

Benzyl benzoate application, triple orange flower water, cornflower water and ginger tincture were manufactured by William Ransom & Son plc., Hitchin, UK. Four different shampoo products were purchased from a local supermarket.

2.2. Reagents and chemicals

Acetonitrile (HPLC grade) and methanol (HPLC grade) and orthophosphoric acid (analytical grade) were purchased from Fisher Scientific (Essex, UK). Bronopol was purchased from Fluka (Dorset, UK). Bromonitromethane was purchased from Aldrich (Dorset, UK), and 2-bromoethanol was purchased from Sigma (Dorset, UK). The water used in HPLC and for sample preparation was produced with a Super Purity Water System (Purite Ltd., UK) with a resistivity over 17.5 MΩ cm.

2.3. Preparation of standard solution

Stock standard solutions were prepared separately by accurately weighing 100 mg of bronopol, bromonitromethane and 2-bromoethanol reference standards into 100-ml volumetric flasks and dissolving in methanol (HPLC grade, water content $< 0.05\%$) with the aid of sonication. Working standard solutions, 0.02–0.8 mg ml^{-1}, were prepared from the stock standard solutions by diluting with methanol.

2.4. Sample preparation

Five hundred milligrams of each product, accurately weighed, were dissolved in methanol as for the standard solutions and made up to 50 ml with the same solvent. Approximately 1 ml of each final solution was filtered through a 0.45 μm membrane filter (Nylon Acrodisc 13, Gelman) prior to injection for HPLC analysis.

2.5. Instrumentation

An HP 1100 series liquid chromatograph system comprising vacuum degasser, quaternary pump, auto-sampler, thermostatted column compartment, and diode array detector was used. The column used was a Kingsorb 5 μm C18, (150×4.6 mm). The column was operated at 30 °C. The mobile phase eventually adopted for this study was methanol/water/orthophosphoric acid (5:95:0.1, v/v/v) and the flow rate was 1.5 ml min^{-1}. Detection wavelength was 210 nm. The sample injection volume was 10 μl. The chromatographic peaks of 2-bromoethanol, bromonitromethane and bronopol were conformed by comparing their retention times and UV spectra with those of the reference standards. The peak of bromonitroethanol was identified by referring its retention time with a reference [3]. Working standard solutions were injected into the HPLC, and peak area responses obtained. Standard graphs for bronopol and bromonitromethane were prepared by plotting concentration versus area. Quantification was carried out from integrated peak areas of the product samples using the corresponding standard graph for bronopol and bromonitromethane. The content of bromonitroethanol, due to the lack of a standard, was determined using the response factor for bromonitromethane.

2.6. Recovery of bronopol

The recovery was determined by spiking a selected sample of benzyl benzoate application with bronopol standard solution.

3. Results and discussion

3.1. Effect of solutions and mobile phases

Bronopol standard solution, 0.02 mg ml^{-1}, in different solvents (acetonitrile, methanol, acetonitrile/water (50:50, v/v) and methanol/water (50:50, v/v)) was analysed by HPLC, and the effects of the solvents compared. As shown in Fig. 1, bronopol degradation was observable after 1 h

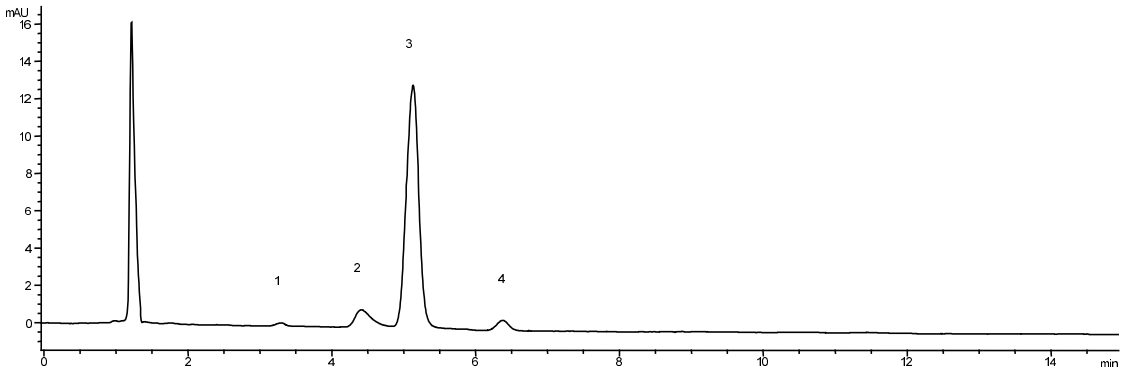


Fig. 1. Chromatogram of bronopol standard (0.02 mg ml^{-1}) prepared in methanol/water (50:50, v/v) after being kept at ambient temperature for 1 h. The chromatographic conditions are described in Section 2.5; (1) 2-bromoethanol, (2) bromonitromethane, (3) bronopol, (4) bromonitroethanol.

when the sample was prepared with methanol/water (50:50, v/v), and the decrease in bronopol continued with time (Fig. 2). After storage at ambient temperature for 24 h, the content of bronopol had decreased by about 20%. As a result, bromonitroethanol, a degradation product of bronopol, at first increased and then with time slightly reduced due to degradation to produce bromonitromethane. The same situation was observed when bronopol solutions were prepared with acetonitrile/water (50:50, v/v). No decomposition of bronopol could be detected when methanol was used, and the bronopol content remained unchanged over 1 month. Furthermore, no degradation was found in a bronopol standard solution prepared with methanol after it had been stored in a refrigerator at 4 °C for 1 year. However, in a methanol/water mixture, as reported by Ferioli et al. [5], the bronopol standard solution had to be prepared daily. Acetonitrile gave similar results to methanol but with a very small amount of decomposition product detectable.

Benzyl benzoate application was dissolved in the same four solvents as for the bronopol standards, and the stability of bronopol in this product in each solvent was compared. The results showed that a sample dissolved in methanol gave a good chromatographic profile (Fig. 3) and the data for bronopol and its decomposition products remained stable for a period of 1 month. No interference was observed from the other components of the formulation. However, the corresponding sample prepared in aqueous medium showed not only bronopol decomposition with time but also the chromatography deteriorated. These results suggest that, for sample preparation, mixtures containing water are unsuitable, whereas organic solvents of high purity, preferably methanol, should be used for preparing standard and sample solutions.

Two mobile phases, methanol/water/orthophosphoric acid (5:95:0.1, v/v/v) and acetonitrile/water/orthophosphoric acid (5:95:0.1, v/v/v) were compared by analysing a standard bronopol solution prepared with methanol. There was no significant difference between the two mobile phases, although the results using the methanol system showed less variation (CV% = 0.13 for methanol and $CV\% = 0.81$ for acetonitrile). From both a chromatographic and economic point of view, methanol was chosen as the preferred mobile phase for the analysis.

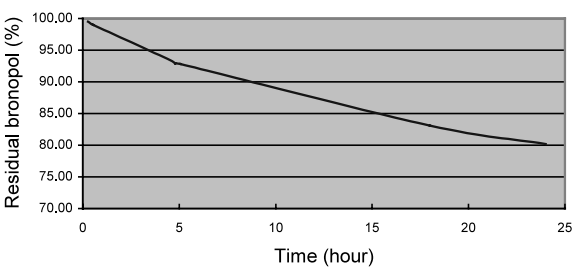


Fig. 2. Stability of bronopol in aqueous medium (methanol/water 50:50 v/v) at ambient temperature.

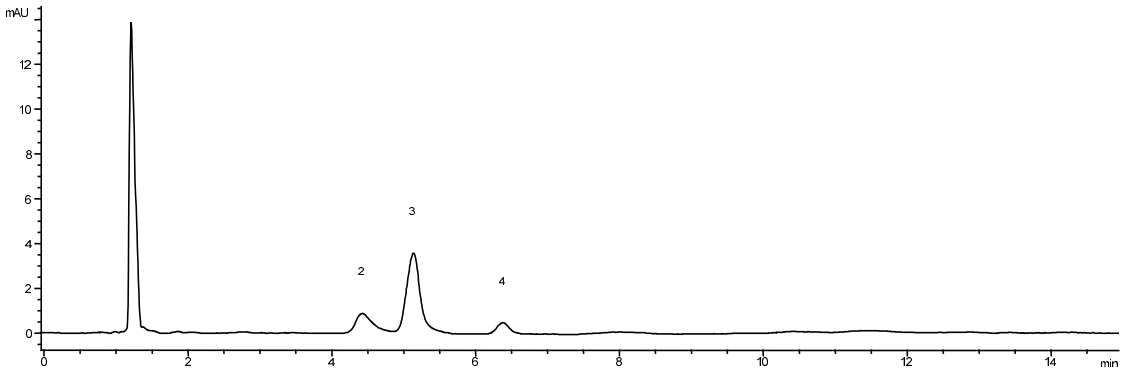
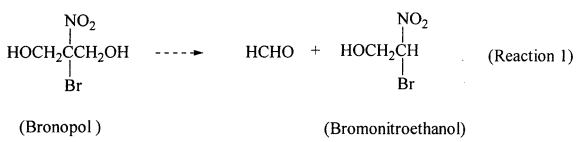


Fig. 3. Chromatogram of benzyl benzoate application in methanol (HPLC grade). The chromatographic conditions are described in Section 2.5; (2) bromonitromethane, (3) bronopol, (4) bromonitroethanol.

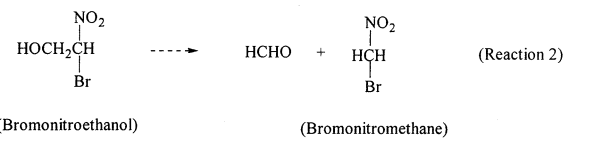
3.2. Effect of temperature on the stability of bronopol

The effect of temperature on the stability of bronopol was investigated over 30 min at 40 and 100 °C. This demonstrated that at 40 °C bronopol in methanol was stable with no detectable bromonitroethanol. However, as shown in Fig. 4, in aqueous solution at 40 °C bronopol degraded significantly to produce bromonitroethanol and bromonitromethane and a small amount of 2-bromoethanol. Similar results to those given by bronopol in aqueous solution at 40 °C were observed for bronopol standard solution in methanol after being heated at 100 °C for 30 min, but, in this case, no 2-bromoethanol was detectable.

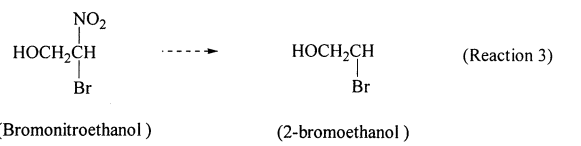
It had been reported by Bryce et al. [4], that the formation of bromonitroethanol from bronopol could take place by a retroaldol reaction with the liberation of an equimolar amount of formaldehyde according to reaction (1) as below.



As indicated above, it was found that the bromonitroethanol formed by bronopol decomposition also decreased with time. Therefore, it was thought that the reaction mechanism would be similar with the liberation of another equimolar amount of formaldehyde as shown in reaction (2) to produce bromonitromethane.



The occurrence of 2-bromoethanol was assumed to be formed from bromonitroethanol by the release of a nitrite ion as a reactive intermediate via reaction (3). Sanyal et al. [3] found that the absorbance of bromonitroethanol at 244 nm decreased rapidly with the concomitant liberation of nitrite ion when its alkaline solution was heated in a boiling water bath (100 °C). However, the production of 2-bromoethanol was found to be very limited in aqueous solution, especially at ambient temperature.



3.3. Validation of the method

The calibration graphs for bronopol and its degradation products were constructed from seven consecutive injections and were linear over the range of 0.02–0.8 mg ml^{-1} with a regression

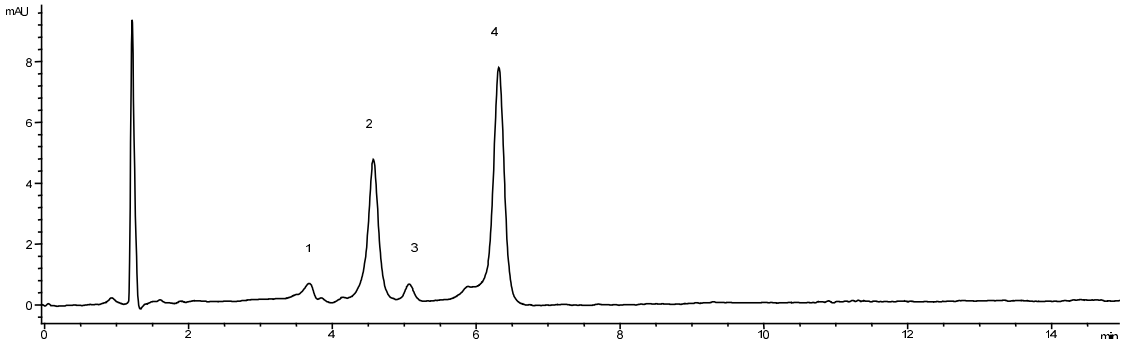


Fig. 4. Chromatogram of bronopol standard (0.02 mg ml^{-1}) prepared in methanol/water (50:50, v/v) after being kept at 40 °C for 30 min. The chromatographic conditions are described in Section 2.5; (1) 2-bromoethanol, (2) bromonitromethane, (3) bronopol, (4) bromonitroethanol.

Table 1
Percentage of bronopol and its degradation products in some commercial samples (%, w/w)

Compound	BBA[a]	Cornflower water	Orange flower water	Ginger tincture	Shampoo 1	Shampoo 2	Shampoo 3	Shampoo 4
Bromonitro-methane	0.015	0.016	0.016	0.014	0.023	0.019	0.013	0.015
Bronopol	0.075	0.080	0.013	0.051	0.028	0.067	0.061	0.011
Bromonitro-ethanol	0.006	n.d.	n.d.	0.012	n.d.	n.d.	n.d.	n.d.

n.d., Not detected.
[a] Benzyl benzoate application.

coefficient (R^2) of 0.999 for both bronopol and bromonitromethane. The limit of quantitation for bronopol was determined as 5 ng on-column weight by analysing various diluted standard solutions.

To test the precision of the assay method, a standard solution of bronopol and one of the product samples were injected ten times under the chromatographic conditions described above. The coefficient of variation for the standard solution was less than 0.13% and for the product solution, less than 0.47%. The recovery of bronopol added to the benzyl benzoate application was 98.6%. By running control samples which were absent of bronopol in our products, it was found that the ingredients did not generate peaks interfering with the determination of bronopol and its degradation products.

3.4. Determination of samples

Eight commercial products containing bronopol, including four shampoos were analysed. Triple orange flower water and cornflower water are ingredients in cosmetic products; ginger tincture is an ingredient for pharmaceutical use and benzyl benzoate application is used for the treatment of scabies. As indicated in Table 1, the content of bronopol was between 0.011 and 0.08% (w/w), which is in the range of effective antimicrobial concentration for bronopol (0.01–0.1%, w/w) [8]. Bromonitromethane and bromonitroethanol were also detected in some products in various amounts, indicating that the degradation of bronopol took place to a different extend in these products. No 2-bromoethanol was detected in all the samples.

4. Conclusions

It has been demonstrated that bronopol hydrolyses in aqueous media and under these conditions results in non-reproducible analytical data. The same effect has been reported by other researchers [6,9]. This problem can be overcome by using methanol for sample preparation. Bronopol standards and product samples were stable in methanol for in excess of 1 month at ambient temperature, thus allowing a quantitative and reproducible determination of bronopol. Bromonitroethanol and bromonitromethane, the degradation products of bronopol could be detected simultaneously with bronopol.

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