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Rapid ultraviolet spectrophotometric determination of bronopol: application to raw material analysis and kinetic studies of bronopol degradation

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Abstract

A simple and rapid spectrophotometric assay is described for bronopol determination in raw material. The method is based on the measurement of absorbance (at 244 nm) of bromonitroethanol (sodium salt of aci-form), the chromophoric derivative formed from bronopol instantaneously by a retroaldol reaction in the presence of 0.1 M NaOH. This derivative has a molar absorption coefficient of 8330 (at absorption maximum, 244 nm) and its absorbance follows a linear relationship with the concentration of bronopol within the range 5–25 μg ml^{-1}. It has been validated that the spectrophotometric method can be applied successfully to the analysis of bronopol in raw material. The sensitivity of the method towards bronopol degradation in aqueous solution is comparable to that obtained by gas–liquid chromatography and enzymatic assays described elsewhere. Thus the proposed method is suitable for use in the study of the kinetics of bronopol degradation. A simple and sensitive detection of bronopol and bromonitroethanol (down to 2 μg) on a silica gel 60 F254 precoated thin layer chromatography plate based on the formation of the chromophoric derivative in the presence of ammonia vapour has been described.

Keywords: Bromonitroethanol formation; Bronopol degradation; Bronopol determination; Nitrite liberation; Raw material analysis; Ultraviolet spectrophotometry

1. Introduction

Bronopol (2-bromo-2-nitropropane-1,3-diol) is a broad spectrum antibacterial with high activity against *Pseudomonas aeruginosa*. The compound was recommended as suitable for the preservation of oral medicaments [1] and officially accepted as a preservative for pharmaceutical use [2]. Because of its high activity over a wide pH range (5–8) bronopol is increasingly used nowadays in liquid oral formulation. A convenient method of analysis for this preservative is therefore highly desirable. An enzymatic method for microdetermin-

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ation of bronopol in complex pharmaceuticals was described earlier [3]. However, such a method cannot provide the high degree of accuracy and precision necessary for raw material analysis.

The only officially accepted method available [2] for the analysis of bronopol in raw material is very time-consuming and involves titrimetric analysis of bromide liberated from bronopol by a tedious process.

During the authors' investigation of the light absorption characteristics of bronopol it was observed that at low concentration (15 μg ml^{-1}) the compound has practically no absorbance in aqueous solution in the UV region 220–350 nm. However, a shift in pH of the solution to alkalinity resulted in the development of a strong UV absorption with a peak at 244 nm due to the formation of a chromophoric derivative (sodium salt of the aci-form of bromonitroethanol). This UV absorption appeared to enable the determination of bronopol by direct spectrophotmetry. Being unaware of any assay method based on this observation an investigation was carried out into the development of a suitable spectrophotometric method for the analysis of bronopol in raw material. A preliminary investigation of the possible application of the method in stability analysis and in studies of the kinetics of bronopol degradation are also reported.

2. Experimental

A Hitachi model U-3210 spectrophotometer was used. An authentic sample of bronopol was obtained from Aldrich (Milwaukee, WI). A bronopol sample used as a reference standard during assay of raw material samples was obtained from TTK Pharma Ltd., (Madras, India). The characteristics of the material (e.g. IR absorption spectrum, melting point, colour, odour, texture and solubility) were identical with those of the authentic bronopol sample. The substance also conformed to the British Pharmacopoeia [2] standard with respect to all the tests indicated in the monograph. The contents of bronopol and water in the sample estimated according to the British Pharmacopoeia [2] were 99.9% and 0.15% respectively. All the chemicals used in this investigation were of analytical-reagent grade.

2.1. Standard and sample preparation

About 60 mg bronopol reference standard or sample (raw material) was accurately weighed and disolved in 200 ml water. This served as the stock solution. For the preparation of an acidic solution 50 ml of 0.1 M HCl was added to 25 ml of the stock (standard or sample) in a 500 ml volumetric flask, the volume was made up to the mark with water and the contents mixed well. An alkaline solution of the standard or sample was prepared by diluting a mixture of 25 ml of the respective stock solution, 50 ml of 0.1 M HCl and 50 ml of 1 M NaOH to 500 ml with water.

2.2. Measurement of absorbance

The absorbances of the acidic and alkaline solutions of the standard and sample were measured at 244 nm against water. Absorbance of the NaOH blank prepared by diluting 50 ml of 1 M NaOH to 500 ml with water was also measured against water.

2.3. Calculations

The content of bronopol in raw material was calculated by using the following equation.

$$\text{Content of bronopol } (\%) = (U-b)/(S-b) \times c \times (100/m) \times (100/100-x) \times D$$

where S and U are the differences between the absorbances of the alkaline and acidic solutions of bronopol at 244 nm for the standard and sample respectively, b is the absorbance of the NaOH blank, c is the concentration of the standard preparation (mg ml^{-1}), m is the weight of the sample taken for analysis (mg), x is the moisture content of the sample (%) and D is the sample dilution. The raw material is determined on an anhydrous basis.

2.4. Identification of bronopol and bromonitroethanol by thin layer chromatography

Bronopol solution (12 μl) equivalent to 9.82 μg bronopol with or without alkali treatment (Fig. 2) was applied as a compact spot on a silica gel 60 F254 precoated plate (10 × 5 cm^2; layer thickness, 0.2 mm; Merck, Darmstadt, Germany) 1 cm from the bottom. The spots were dried under a hot air stream and the plate was developed (in a tank lined with filter paper and saturated with the developing solvent) with a $CHCl_3$–methanol (4:1, v/v) solvent system described by Bryce et al., [4] for the identification of bronopol degradation products. When the solvent front had travelled 7 cm, the plate was removed from the developing tank, dried under a hot air stream and inserted in a tank saturated with ammonia vapour. After 5 min ammonia exposure the plate was immediately placed under shortwave UV radiation. Bronopol and bromonitroethanol show intense spots with R_f values of 0.62 and 0.74 respectively.

2.5. Determination of formaldehyde produced during the generation of the chromophoric derivative

Approximately 50 mg of bronopol was accurately weighed and dissolved in 50 ml vater. Volumes of the solution equivalent to 5, 7.5 and 10 mg bronopol were then transferred to three 100 ml volumetric flasks and diluted to 10 ml with water. 1.11 ml 1 M NaOH was then added and mixed well. The solutions were then acidified with 1.11 ml 1.5 M HCl and diluted to volume with water. Formaldehyde generated from bronopol in the dilute reaction mixtures (100 ml) was estimated colorimetrically by the reaction with DNPH (2,4-dinitrophenylhydrazine). The absorbance at 450 nm of the DNPH derivative formed followed a linear relationship with the concentration of formaldehyde within the range 3–20 μg ml^{-1}. The colour which developed completely within 5 min was found to be stable for at least 30 min. 2 ml of standard formaldehyde solution (10 μg ml^{-1}) or sample solution (reaction mixture) was treated with 1 ml of DNPH reagent (0.1% DNPH in 2 M HCl).

The reagent blank was prepared using 2 ml of water instead of standard or sample solutions. The absorbances of the solutions at 450 nm were measured after 15 min and the formaldehyde concentration in the sample was calculated from the sample absorbance value by comparison with that of the standard.

2.6. Detection of nitrite generation by the chromophoric derivative

The chromophoric derivative formed from bronopol (15 μg ml^{-1} in 0.1 M NaOH) was heated in a boiling water bath and at different time intervals the amount of nitrite in the solution was evaluated colorimetrically [5] by the Bratton–Marshall reaction. The colour produced (absorbance at 538 nm) by the reaction of nitrite with sulphanilamide and *N*-1-(naphthyl)-ethylenediamine dihydrochloride under acidic conditions represents the level of nitrite ion.

2.7. Spectrophotometric evaluation of bronopol degradation

Degradation of bronopol in aqueous solution was carried out at two different bronopol concentrations. For studying degradation at low concentration as described by Sanyal et al., [3] a solution containing 12 μg ml^{-1} bronopol prepared in Tris–HCl buffer (0.02 M, pH 8) was heated at 60°C for different time intervals and preserved at −20°C until analysis. For spectrophotometric assay a 9 ml portion of the sample was treated separately with 1 ml of either 0.1 M HCl or 1 M NaOH to obtain the acidic or alkaline solution respectively, and the bronopol content was calculated from the absorbance values (at 244 nm) of the sample and standard after proper corrections for the HCl and NaOH blanks as described above.

For investigation of degradation at higher bronopol concentration (2 mg ml^{-1}) according to Bryce et al., [4] the solution prepared in sodium phosphate buffer (0.1 M, pH 8) was heated to 40°C for different time intervals and preserved at −20°C until analysis. For the spectrophotometric determiantion of residual bronopol, 0.37 ml of the

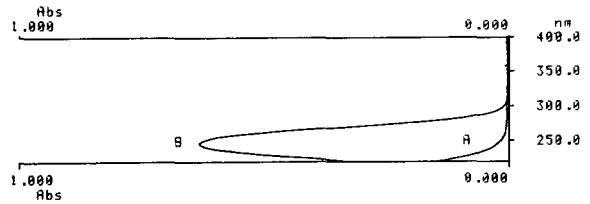
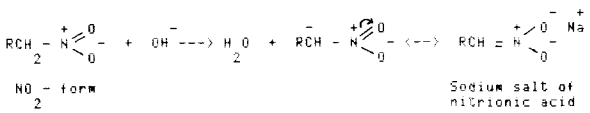


Fig. 1. Ultaraviolet absorption spectra of bronopol (14.5 μg ml^{-1}) in acidic (A: 0.01 M HCl, final concentration) and alkaline (B: 0.1 M NaOH, final concentration) solutions.

sample was treated separately with either 6 ml 0.1 M HCl or 5 ml 1 M NaOH in a 50 ml volumetric flask to obtain the acidic or alkaline solution respectively. The solutions were then diluted to volume with water and mixed well. The bronopol content of the sample was calculated from the absorbance values (at 244 nm) as described above.

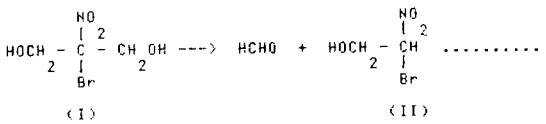
3. Results and discussion

An intense UV absorption band at 244 nm (Fig. 1) was observed when an aqueous solution of spectrophotometrically inactive bronopol was made alkaline with NaOH (0.1 M). The absorbance band disappeared under acidic conditions. Such spectral characteristics are expected only in the cases of primary and secondary aliphatic nitro compounds, as these contain an α-hydrogen atom which allows the formation of the sodium salt of the nitrionic acid:



These types of tautomeric changes are not feasible in the case of a *t*-nitro compound such as bronopol (**I**) which lacks an α-hydrogen atom. Thus the possibility of some chemical conversion(s) leading to the generation of primary or secondary nitro compounds from bronopol under alkaline conditions was investigated.

It was previously reported by Bryce et al., [4] that the formation of bromonitroethanol (**II**), a secondary nitro compound, from bronopol could take place by a retroaldol reaction with the liberation of an equimolar amount of formaldehyde according to the following reaction:



It was observed that when bronopol was treated with 0.1 M NaOH it lost its identity and was converted into a different compound. This has been confirmed by TLC (Fig. 2). TLC of degraded bronopol solution was earlier described by Bryce et al., [4]. The only spot with an R_f value (0.74) higher than that of bronopol (0.62) was identified as bromonitroethanol (Fig. 2D). Under the conditions of degradation followed by these workers (at pH 6) bromonitroethanol represents only a minor fraction of the total amount of bronopol spotted. Interestingly, it was found that when bronopol (0.9 mg) was treated with 0.1 M NaOH, complete conversion of bronopol to bromonitroethanol took place at room temperature (30°C) as indicited by the disappearance of the bronopol spot (Fig. 2B). This TLC identification of the chromophoric derivative (as bromoni-

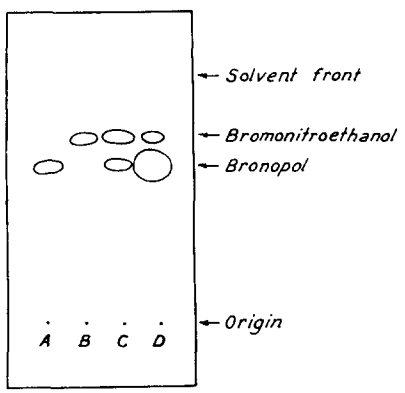


Fig. 2. Thin layer chromatographic detection of the chromophoric derivative: (A) 0.9 ml bronopol solution (1 mg ml^{-1}) + acidic mixture of 0.1 ml 1 M NaOH and 0.1 ml 1.5 M HCl; (B) 0.1 ml 1.5 M HCl + alkaline mixture of 0.9 ml bronopol solution (1 mg ml^{-1}) and 0.1 ml 1 M NaOH; (C) mixture of A and B (1:1, v/v); (D) bronopol solution containing 10% w/v bronopol (pH 6), degraded in a boiling water bath for 2 min according to Bryce et al., [4]. 12 μL each of (A) and (B), 24 μl of (C) and 2 μl of (D) were applied as spots on the TLC plate 1 cm apart and the chromatography was performed as described in the text.

Table 1
Evaluation of formaldehyde generation by bronopol

Bronopol taken for NaOH treatment (mg)[a]	Formaldehyde generation (mg)	
	Calculated (according to reaction(1))	Found[b]
5	0.750	0.749 ± 0.018
7.5	1.125	1.115 ± 0.012
10	1.500	1.512 ± 0.026

[a] Bronopol solution (10 ml) equivalent to stated amount of bronopol was made in 0.1 M NaOH and processed for formaldehyde determination as described in the text.
[b] Each result is the average of three independent determinations ± standard deviation.

troethanol) was further confirmed by the fact that the stoichiometry of formaldehyde generation (Table 1) was also in excellent agreement with the mechanism of bromonitroethanol formation by the retroaldol reaction (1) described by Bryce et al., [4].

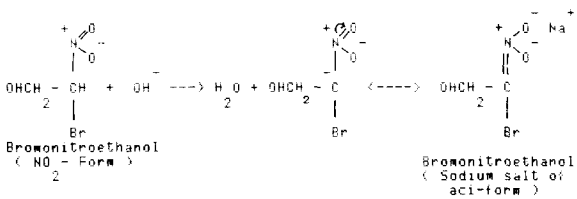
Table 2 shows the results of spectrophotometric studies on the formation and stability of the chromophoric derivative formed from bronopol in 0.1 M NaOH. When a solution of bronopol (2 ml, 15 μg ml^{-1}) taken in a spectrophotometric cuvette was made alkaline (0.1 M NaOH, final concentration) a strong absorbance was generated

Table 2
Spectrophotometric evaluation of the formation and stability of the chromophoric derivative

Reaction system[a]	Absorbance at 244 nm[b]	
	Instantaneous (5 s)	After 1 h
(A) Reaction (1)	0.031	0.030
(B) (A) made in 0.1 M NaOH	0.651	0.652
(C) (B) made in 0.03 M HCl	0.030	–
(D) (C) made in 0.1 M NaOH	0.654	–

[a] Reaction system (1) (taken in a spectrophotometric cuvette) contains (in a total volume of 2 ml in water) 30 μg bronopol.
[b] Absorbance values are corrected for NaOH and HCl blanks as well as for the volume changes of the reaction system (1) caused by NaOH and HCl additions.

instantaneously at 244 nm. The absorbance value remained unchanged, at least up to 1 h, indicating good stability of the chromophoric derivative at room temperature (30°C). On acidification of the solution the absorbance dropped instantaneously to the original low value again due to the transformation of the sodium salt of bromonitroethanol to its corresponding nitro form. It is thus the reversible conversion of bromonitroethanol to either the nitro or salt form in the presence of acid or alkali respectively that is responsible for the loss or generation of UV absorption at 244 nm shown by bronopol:



The chromophoric derivative (sodium salt of aci-form of bromonitroethanol) in 0.1 M NaOH exhibits only one absorption maximum (at 244 nm) between 220 and 350 nm (Fig. 1) and its molar absorption coefficient at this maximum is 8330. Although the derivative is highly stable at room temperature it is very unstable at elevated temperature. When the alkaline solution of the chromophoric derivative was heated in a boiling water bath (100°C) the absorbance at 244 nm decreased rapidly with the concomitant liberation of nitrite ion (Fig. 3).

Based on the conversion of bronopol (poor UV absorber) to bromonitroethanol (strong UV absorber) in alkali, a simple and sensitive detection of bronopol (down to 2 μg) on a silica gel 60 F254 precoated TLC plate has been described. When the plate is exposed to ammonia vapour, bronopol is converted into bromonitroethanol within 5 min and shows an intense spot under shortwave UV in the alkaline atmosphere provided by ammonia. Thus both bromonitroethanol and bronopol can be detected on a TLC plate by this technique.

Under the conditions standardized in the proposed method the difference between the absorbances of alkaline and acidic solutions of

bronopol followed a linear relationship with concentration in the range 5–25 μg ml^{-1} (correlation coefficient (r) = 0.9999). This forms the basis of the proposed spectrophotometric assay of bronopol described in this paper. The recoveries obtained from authentic samples demonstrate the high degree of accuracy and precision of the proposed method (Table 3). The results of analyses of five different samples of raw material correspond excellently with that obtained by the official method (Table 4). The precision of the proposed spectrophotometric assay is also as good as the official method.

The response of the spectrophotometric assay towards degradation of bronopol is shown in Fig. 4. Degradation was carried out under two different conditions; (i) at low bronopol concentration (12 μg ml^{-1}) in Tris-HCl buffer (0.02 M, pH 8) at 60°C according to Sanyal et al., [3]; and (ii) at high bronopol concentration (2 mg ml^{-1}) in sodium phosphate buffer (0.1 M, pH 8) at 40°C as described by Bryce et al., [4]. The proposed assay has a high degree of sensitivity towards bronopol

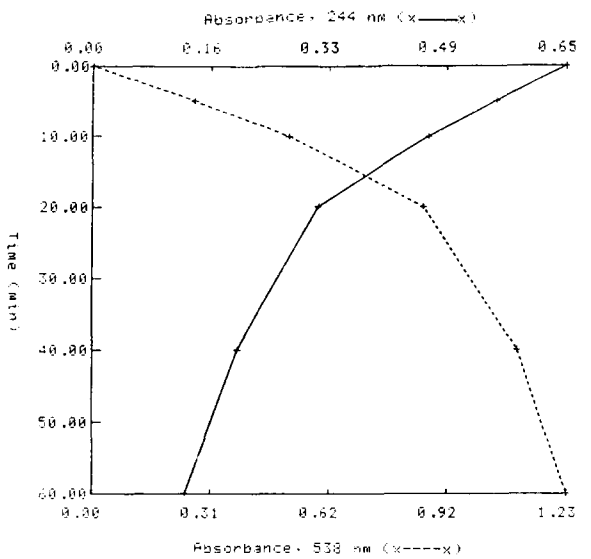


Fig. 3. Stability of the chromophoric derivative at elevated temperature (100°C). The solution of the chromophoric derivative in 0.1 M NaOH was heated in a boiling water bath (100°C). The absorbance at 244 nm and the nitrite formation were measured at different time intervals. Nitrite level (absorbance at 538 nm) was determined by the Bratton–Marshall reaction as described in the text.

Table 3
Recovery of bronopol from authentic samples

Experiment	Added (mg per 200 ml)[a]	Recovery (%)[b]
1	40	99.8
2	50	99.6
3	60	100.1
4	70	99.9
5	80	99.8
Average ± standard deviation		99.8 ± 0.18

[a] Solutions containing the stated amount of bronopol (pure bronopol used as the reference standard) represent the authentic samples for recovery studies. Processing of the samples for the determination of bronopol is described in Section 2.
[b] Each recovery is the average of three determinations.

degradation as indictaed by the drop in residual bronopol concentration with time. The results are comparable with those obtained previously by the GLC and enzymatic methods (Bryce et al., [4] and Sanyal et al., [3] respectively).

The proposed spectrophotometric assay thus provides a simple and convenient means for studying kinetics of bronopol degradation. With regard to raw material analysis the method is unique not only in terms of its simplicity and rapidity but, more importantly, for its ability to detect the degradative change(s) in bronopol that might lead to loss of antimicrobial potency of the compound. The proposed spectrophotometric assay should therefore be a prospective candidate as

Table 4
Estimation of bronopol in raw materials

Sample[a]	Proposed spectrophotometric method[b]	Official method[b]
1	99.6 ± 0.24	99.4 ± 0.39
2	100.2 ± 0.38	100.6 ± 0.41
3	100.0 ± 0.28	99.9 ± 0.38
4	99.8 ± 0.30	99.3 ± 0.45
5	99.9 ± 0.35	99.5 ± 0.33

[a] Five samples of bronopol (raw material) were obtained from commercial sources
[b] Values in % w/w. Content of bronopol is calculated on an anhydrous basis. Each result is the mean of five independent determinations ± standard deviation

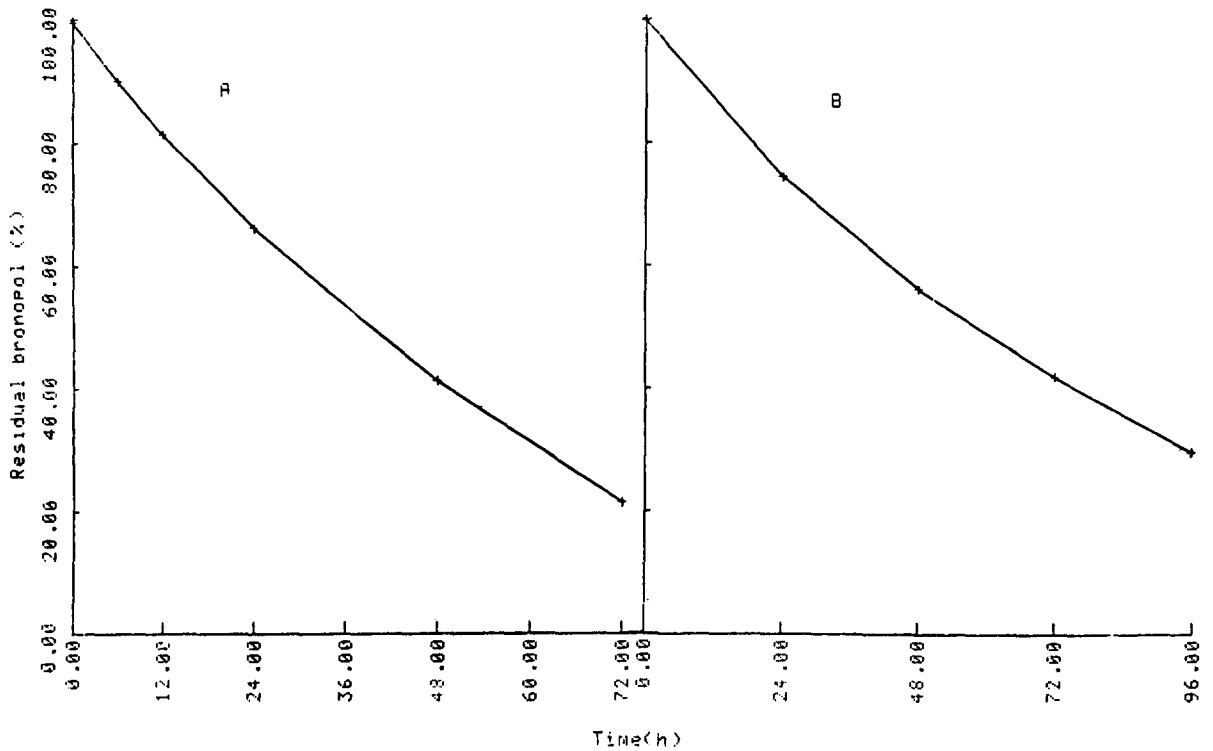


Fig. 4. Response of the proposed spectrophotometric method towards degradation of bronopol. Bronopol in solution at concentrations of 12 μg ml^{-1} (A) in Tris–HCl buffer (0.02 M, pH 8) and 2 mg ml^{-1} (B) in sodium phosphate buffer (0.1 M, pH 8) was subjected to degradation at 60 and 40°C respectively. The residual bronopol in the solutions was determined spectrophotometrically at different time intervals as described in the text.

a pharmacopoeial method for bronopol estimation in raw material.

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