ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
15 (1997) 667–671

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Short communication

A study on the stability of bronopol in bronopol lotion by ion-paired reversed-phase high performance liquid chromatography[1]

H.Z. Lian[a,*], W.B. Zhang[a], J. Miao[a], Q. Jiang[a], L. Mao[a], L. Zong[b], L. Li[b], X.X. Wu[b], R.M. Cheng[b]

[a]*Center of Materials Analysis, Nanjing University, 22 Hankou Road, Nanjing 210093, People's Republic of China*
[b]*The Institute of Pharmaceutical Industries Jiangsu, 86 Yingtuo Cun, Nanjing 210042, People's Republic of China*

Received 15 September 1995; accepted 3 July 1996

Keywords: Bronopol; *Pseudomonas aeruginosa*; Polargraphy; Gas chromotography; HPLC

1. Introduction

Bronopol (2-bromo-2-nitro propane-1,3-diol), shows activity against a broad spectrum of bacteria, including Gram-negative bacteria such as *Pseudomonas aeruginosa* which is resistant to many antibacterial agents [1,2]. This compound has been officially accepted as a preservative in cosmetic and pharmaceutical products [3–6] for its high activity and low toxicity. The effective concentration of bronopol in pharmaceutical preparations ranges from 0.01 to 0.1%, with the common concentration being 0.02% [6]. Generally, 5% bronopol solution was prefabricated by mixing bronopol and some auxiliary materials in water. The stock solution is diluted to 0.02% with water before use, with the pH of diluted solution being 6.8–7.2 [7].

It was known that bronopol in aqueous base could hydrolyze. The decomposition mechanism was preliminarily studied by Bryce et al., and subsequently by Schmeltz and Wenger [8,9], but both of these previous works were inconclusive. Satisfactory results were obtained by Challis and Yousaf in 1991 [10]. Bronopol hydrolyzes in aqueous medium to give tris(hydroxymethyl)-nitromethane, glycolic acid, formic acid, methanol and 2,2-dinitroethanol. It also releases NO_2^- and Br^-. These products were shown to form via four concurrent decomposition pathways, three of which involved 2-bromo-2-nitroethanol as a reactive intermediate which increased following pseudo first-order kinetics. So it is necessary to establish the shelf-life of 5% bronopol stock lotion for pharmaceutical use.

* Corresponding author.

[1] Presented at the Fifth International Symposium on Drug Analysis, September 1995, Leuven, Belgium.

0731-7085/97/$17.00 Copyright © 1997 Elsevier Science B.V. All rights reserved
PII S0731-7085(96)01895-X

The main problem is that the concentration of bronopol in decomposed lotions must be determined accurately. The methods developed for bronopol analysis included polargraphy [8], gas chromatography [8], thin-layer chromatography [8,11] and high performance liquid chromatography (HPLC) [12], most of which regarded the determination of bronopol in cosmetic products, aiming at quality control checking of these products. The official analytical method presented for the raw material is also not suitable for the determination in complex liquid formulations [5]. In recent years, a few methods have been reported about the determination of bronopol in pharmaceutical ingredients, such as ion-paired reversed-phase HPLC by Lian et al. [13] and bioassay by Sanyal et al. [14]. In this paper, an ion-paired reversed-phase HPLC method for study of the stability of bronopol lotion by accelerated-chemical kinetic test was described.

2. Experimental

2.1. Reagents and chemicals

Methanol (analytical grade) was purchased from Shanghai Zhenxing First Chemical Plant (Shanghai, PRC) and was redistilled before use. Pentanesulfonic acid (HPLC grade) was purchased from Tianjing Second Reagent Factory (Tianjing, PRC). Water was distilled twice. The aqueous phase was prepared by dissolving 5.0 g pentanesulfonic acid in 1000 ml of water. Methanol and water containing 0.5% pentanesulfonic acid were purified with the Millipore Milli-Q system (Millipore Corporation, Bedford, MA, USA). Other reagents and chemicals were analytical or biochemical reagent grade.

Bronopol reference substance was provided by The Institute of Pharmaceutical Industries Jiangsu (Nanjing, PRC) and 5% bronopol stock solution was prepared by dissolving 50 g bronopol and adding other auxiliary materials just before the heating test.

Standard solutions at concentration of 10–70 mg ml^{-1} of bronopol were prepared by accurately weighing out 0.1, 0.3, 0.5 and 0.7 g of bronopol reference substance into 10-ml volumetric flasks and adding water to mark, respectively. These solutions were used for calibration purposes.

2.2. Apparatus and chromatographic conditions

Instrumentation included a Varian 5060 HPLC coupled to a high-pressure loop injector with a standard 10-μl sample loop and a UV-100 variable wavelength detector (Varian Instrument Division, Walnut Creek, CA, USA) set at 254 nm with a sensitivity of 0.025 aufs. Chromatograms were recorded on a Yokogawa Hokushin Electric Type 3066 pen recorder (Sino-Japanese Sichuan No. 4 Meter Factory, Chongqing, PRC) and a VISTA-401 Chromatographic data system (Varian). Separation was accomplished using MicroPak MCH-5 column (15 cm × 4 mm i.d., Varian). The mobile phase was methanol—0.5% pentanesulfonic acid (11:89 v/v) at a flow-rate of 1.0 ml min^{-1}. The column temperature was held at 30°C.

2.3. Test of stability of bronopol lotion

Bronopol lotion (2 ml) prepared freshly was sealed up in ampoules and these ampoules were stored in several thermostated water baths of 85, 90, 95, 100°C, respectively. The hydrolysis of bronopol occurred and the concentration of bronopol decreased in varying degrees. At appropriate time intervals (0.5–15 h), the ampoules were successively removed from baths and then cooled suddenly in an ice-bath to stop the reaction. The aliquots were examined by HPLC described above. Three independent tests were performed in parallel in the same manner under the same temperature.

2.4. Determination of lotion and test of recovery

Bronopol lotion (1 ml), as well as intact and decomposed lotion was taken and filtered through a 0.45-μm micropore filter (Millipore) by means of a syringe. Aliquots (10 μl) of the filtered lotion were injected to the HPLC column. In order to estimate the efficiency of the recovery, the differ-

ent quantities of bronopol reference substance were mixed with auxiliary materials according to the ingredient. The authentic bronopol lotion was analysed the same as the sample. Recovery experiment was also performed on decomposed lotion. The concentration of bronopol in decomposed lotion was previously determined using a calibration curve. Then the recovery of bronopol from decomposed lotion was obtained by spiking bronopol reference substance to the lotion.

3. Results and discussion

3.1. Results for analysis and recovery

Typical HPLC chromatograms of bronopol standard solution, intact and decomposed lotions were shown in Fig. 1. Bronopol was clearly separated and recognized from other materials. In our experiment, ion-paired reagent pentanesulfonic acid was added into the mobile phase and the retention time of bronopol and its decomposition products which were ionized in water were increased. Therefore, the separation was satisfactory. Without pentanesulfonic acid, although changing the ratio of methanol and water to 10:90 (v/v), bronopol and other components could not be separated completely, but the peak width of bronopol was seriously increased (Fig. 2). The result seemed to indicate that these dissociative components were conversed to hydrophobic ion-paired compounds in presence of anionic counter ion.

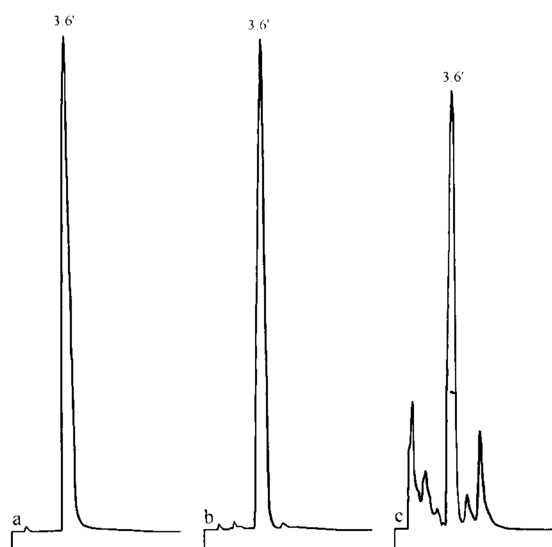

Fig. 1. Chromatograms of bronopol standard solution (a), intact lotion (b) and decomposed lotions—DL_2^* (c). *The condition of decomposition for DL_2 is described in the legend to Table 2.

Fig. 2. Chromatogram without pentanesulfonic acid in mobile phase for decomposed lotion— DL_2^*. *The condition of decomposition for DL_2 is described in the legend to Table 2.

The quantitation was based on a calibration curve. A linear regression of the relationship between peak height vs. amounts of standard was carried out within range of 100–700 μg of bronopol in 10-μl injection volume. The standard curve was linear with equation $y = 0.2455x + 0.0001$ ($r = 0.9994$), where y equaled peak height (cm) and x equaled final standard concentration (mg ml^{-1}).

Recoveries of bronopol from authentic lotion and spiked decomposed lotion demonstrated satisfactory accuracy and precision for the proposed methods (Tables 1 and 2).

3.2. Calculation of stability

The plots of logarithm of concentration ratio vs. heating time in different temperature were shown in Fig. 3, which showed linearity and significance. It was found that the decomposition of bronopol was a pseudo first-order reaction. That is:

Table 1
Recovery of bronopol from authentic lotion

Experiment	Bronopol added (mg ml^{-1})[a]	Recovery (%)[b]
1	10	98.9
2	30	99.1
3	50	98.7
4	70	98.8
Average		98.9

[a] Solutions containing the stated amount of pure bronopol represent authentic samples for recovery studies.
[b] Each recovery is the average of three determinations.

$$\log \frac{C_t}{C_0} = -\frac{k}{2.303} \cdot t \qquad (1)$$

where C_t was the concentration (mg ml^{-1}) of bronopol when the reaction was stopped and C_0 was intact concentration (mg ml^{-1}) of bronopol, k was the reaction rate constant (h^{-1}), t was heating time (h). The k in different temperature was obtained from the slope of the line in Fig. 3.

The plot of logarithm of reaction rate constants vs. reciprocal of temperature was a straight line. The variation of k with temperature could be expressed as Arrhenius equation:

$$\log k = \log A - \frac{E_a}{2.303R} \cdot \frac{1}{T} \qquad (2)$$

where A was frequency factor, E_a was activation energy (kcal mol^{-1}), T was abslute temperature (K), R was gas constant, 1.987×10^{-3} kcal (K·mol)$^{-1}$.

Table 2
Recovery of bronopol from spiked decomposed lotion

Decomposed lotion[a]	Bronopol (mg ml^{-1})		Recovery[b] (%)
	Previously determined	Added	
DL_1	47.5	10	101.6
DL_2	44.7	10	102.0
DL_3	42.6	10	101.9
Average			101.8

[a] Temperature and heating time—DL_1: 85°C, 1 h; DL_2: 90°C, 6 h; DL_3: 100°C, 6 h.
[b] Each recovery is the average of three determinations.

From linear regression ($r = 0.992$), log A was 12.07, $-E_a/2.303R$ was -5.17×10^3, E_a was 23.7 kcal mol^{-1}. Then the decomposition rate constant of bronopol in the lotion at room temperature (25°C) was obtained from Eq. (2):

$$\log k_{rt} = 12.07 - \frac{5.17 \times 10^3}{298} = -5.27,$$

$$k_{rt} = 5.36 \times 10^{-6} (\text{h}^{-1})$$

The half-life of decomposition of bronopol in bronopol lotion at room temperature was obtained from Eq. (1):

$$t_{0.5} = -\frac{2.303}{5.36 \times 10^{-6}} \cdot \log 0.5 = 1.29 \times 10^5 \text{ (h)} \approx 15 \text{ (years)}.$$

The shelf-life of bronopol lotion was also obtained from Eq. (1):

$$t_{0.9} = -\frac{2.303}{5.36 \times 10^{-6}} \cdot \log 0.9 = 1.97 \times 10^4 \text{ (h)} \approx 2 \text{ (years)}.$$

4. Conclusions

The greatest advantages of HPLC lay in its high specificity and selectivity. The separation of some compounds, which had similar chemical structure, including their decomposition products was possible. In decomposed bronopol lotion there not only had bronopol and its hydrolysis products, but also had other auxiliary materials. An attempt was made to present a new method for determining the concentration of bronopol and calculating its stability in bronopol lotion. This experiment showed the activation energy (E_a) for decomposition of bronopol in aqueous base is 23.7 kcal mol^{-1}. The reciprocal of temperature was the negative exponent in the Arrhenius equation, the hydrolysis rate of bronopol varied remarkably with the temperature. This result verified the rationalization of the test for stability of bronopol lotion by accelerated-thermostatical method.

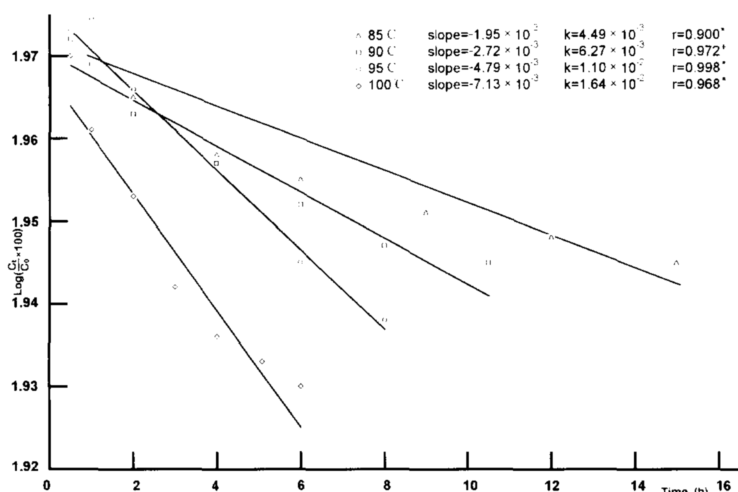

Fig. 3. Relationship between concentration of bronopol in the lotion and heating time $*n = 7$, $r > r_{1-0.01}(0.874)$, $\alpha = 0.01$. $^{+}n = 5$, $r > r_{1-0.01}(0.959)$, $\alpha = 0.01$.

References

[1] B. Croshaw, M.J. Groves and B. Lessel, J. Pharm. Pharmacol., 16 (1964) 127T–130T.

[2] D. M. Bryce and R. Smart, J. Soc. Cosmet. Chem., 16, 187–201 (1965).

[3] Commission Directive 82/368/EEC, Off. J. Eur. Community., 20 (1983) L275.

[4] Commission Directive 86/199/EEC, Off. J. Eur. Community., 38 (1986) L149.

[5] British Pharmacopoeia, HM Stationary Office, London, 1993, Vol. I, pp. 89–90.

[6] The Extra Pharmacopoeia Martindale, 30th edn, The Pharmaceutical Press, London, 1993, pp. 1134.

[7] Japan Pharmaceutical Information Center, Drugs in Japan, Ethical Drugs, Yakugyo Jiho, Tokyo, 1992, pp. 1076.

[8] D.M. Bryce, B. Croshaw, J.E. Hall, V.R. Holland and B. Lessel, J. Soc. Cosmet. Chem., 29 (1978) 3–24.

[9] I. Schmeltz and A. Wenger, Food Cosmet. Toxicol., 17 (1979) 105–108.

[10] B.C. Challis and T.I. Yousaf, J. Chem. Soc. Perkin Trans., 2 (1991) 283–286.

[11] N.D. Kruijf, M.A.H. Rijk, L.A. Pranoto-Soetardhi and A. Schouten, J. Chromatogr., 410 (1987) 395–411.

[12] N.D. Kruijf, A. Schouten, M.A.H. Rijk and L.A. Pranoto-Soetardhi, J. Chromatogr., 469 (1989) 317 328.

[13] H.Z. Lian, W.B. Zhang, L. Zong, L. Li, X.X. Wu, R.M. Cheng and L. Mao, Sepu (in Chinese), 7 (1989) 179–180.

[14] A.K. Sanyal, B. Chowdhury and A.B. Banerjee, Analyst, 118 (1993) 937–939.