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Short Communication

The assay of benzalkonium chloride in pilocarpine, hypromellose and polyvinyl alcohol ophthalmic drops by second-order derivative ultraviolet spectrophotometry

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Introduction

Benzalkonium chloride (BAK) is an antibacterial preservative widely used in pharmaceutical products. It consists of a variable mixture of the C_8–C_{18} alkyl homologues of alkylbenzyldimethylammonium chlorides with the C_{12} and C_{14} homologues predominating [1, 2]. It is usually used as BAK Solution of the BP [1] or USP [2] which, in the case of the BP, consists of a 50% w/v solution of BAK (limits for BP preparation: 47.5–52.5% w/v; previously 49.0–51.0% w/v [3]) calculated as $C_{22}H_{40}ClN$ and determined as total quaternary bases by titration.

It is assayed in pharmaceutical products by a variety of methods including colorimetric [4–9] and titrimetric procedures [10, 11] which quantitate total BAK and a variety of homologue-specific chromatographic [12–17] and mass spectrometric procedures [18].

The disadvantages of these methods are that they are time-consuming and often require complex work-up procedures. This paper reports a simple method involving second-order derivative spectrophotometry which enables BAK to be quantitated in a variety of eye-drop formulations provided they do not display high absorbances due to other components. BAK has not previously been assayed by this method, however, it has been demonstrated that second-order derivative spectrophotometry can be used to enhance the limits of detection of the preservative over conventional zero-order spectrophotometry [19].

Products evaluated in this study are pilocarpine drops and artificial-tear products based on hypromellose and polyvinyl alcohol (PVA) (Fig. 1).

Materials and Methods

Equipment

A Hewlett–Packard HP 8450A spectro-

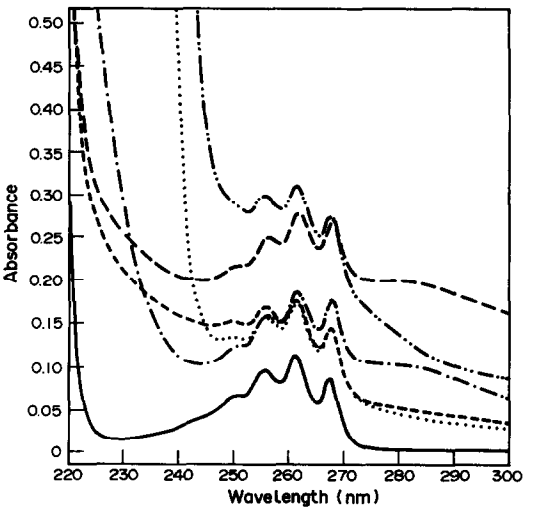

Figure 1
Ultraviolet spectra of formulations used in this study all containing BAK (1×10^{-2}% w/v). ——, BAK standard; ....., pilocarpine drops (0.5% w/v); — -- —, pilocarpine drops (2.0% w/v); ----, hypromellose drops; – · – · – · –, PVA drops A; and — — — , PVA drops B.

photometer equipped with a HP 7470A plotter was used in these studies.

Solvents and chemicals

All reagents and materials used in the preparation of the formulations were of BP quality. Two grades of PVA were used to prepare drops — (a) Mowiol® (Hoechst, Germany); and (b): general reagent grade P-1763 (Sigma, USA). The BAK solution used for the preparation of standards and formulations was Vantoc CL® (ICI, UK) and the comparative study was performed on two additional batches of Vantoc CL and one from TCI (Japan).

Water was purified by means of a Milli-Q system (Millipore, Australia) and the methanol was of AR grade.

Preparation of sample formulations and standards

The products used to assess the analytical method and standards were made with analytical precision from a common stock solution of BAK (0.5% w/v). Ophthalmic drops included in the study were pilocarpine drops (0.5, 1.0 and 2.0% w/v) and hypromellose drops (0.3% w/v) of the Australian Pharmaceutical Formulary and Handbook [20] and two PVA drops (1.4% w/v) in normal saline using the two different grades of PVA [21].

Analytical determination

To 5 ml of ophthalmic product or standard BAK solution in a glass vial was added methanol (2 ml) and the second-order derivative spectrum obtained against water as a blank using a 1 cm cell. To measure the amplitude with maximum precision, the spectrum was plotted using the default values of the plotter (maximum axes) over a wavelength range of 260–275 nm. The tangent was drawn as shown in Fig. 2 and the height measured in millimetres.

Results and Discussion

Initial studies were undertaken to assess the procedure involving direct measurement without dilution of the drops with methanol. It was found in all cases studied that the presence of other electrolytes subtly modified the spectral characteristics of the BAK resulting in a small bathochomic shift in the spectrum and a reduction in amplitude. Table 1 demonstrates the effect of sodium chloride, a component of all six formulations, on the magnitude of the measured amplitude obtained for an 0.01% w/v solution of BAK.

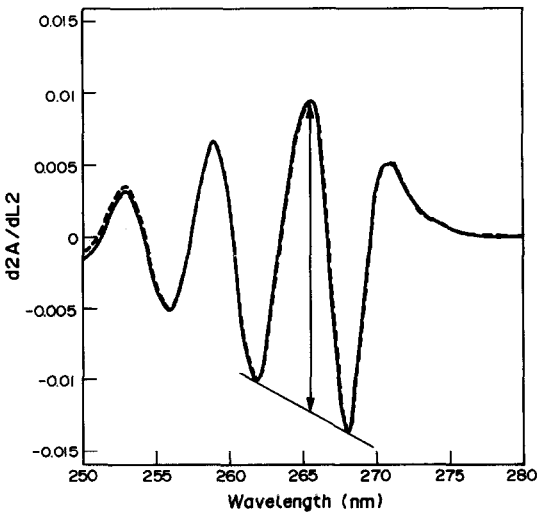

Figure 2
Second-order derivative ultraviolet spectra of samples following dilution with methanol. ——, BAK standard (1×10^{-2}% w/v); and ----, pilocarpine eye drops (0.5% w/v).

Table 1
Effect of sodium chloride on the assay of 0.01 w/v benzalkonium chloride ($n = 2$)

Concentration of sodium chloride (% w/v)	Assay without dilution	Assay following dilution with methanol
—	1.000	1.000
0.1	1.000	1.004
0.2	0.984	1.000
0.5	0.961	1.008
1.0	0.954	0.996

Expressed as a fraction of the BAK response found for water (standard).

The most likely explanation for this phenomenon resides in the fact that BAK, being a surface-active agent, self-associates and this is modified by the presence of electrolytes [22]. The addition of a small amount of methanol to the sample to be analysed overcomes this association by changing the polarity of the medium and a consistent second-order derivative spectrum is obtained (Fig. 2). It has been demonstrated previously that low molecular weight alcohols such as methanol, at the concentrations employed in this assay, totally inhibit micelle formation by cationic surface-active agents [23]. In all cases, the second-order derivative spectra, following the addition of methanol, were superimposable for identical

concentrations of BAK in the wavelength range 255–275 nm. This enables the concentration of the BAK to be determined without interference (Table 2).

Table 2
Analytical results obtained for a range of ophthalmic drops containing 0.01% w/v benzalkonium chloride (n = 2)

Product		Conc. ($\times 10^{-2}$% w/v)
Pilocarpine	0.5% w/v*	0.998
Pilocarpine	1.0% w/v*	0.996
Pilocarpine	2.0% w/v*	1.001
Hypromellose	0.3% w/v*	0.988
Polyvinyl alcohol	1.4% w/v (Source A)	1.004
Polyvinyl alcohol	1.4% w/v (Source B)	1.008

* Australian Pharmaceutical Formulary and Handbook.

The method affords a linear relationship between measured amplitude versus concentration for BAK for standards over the range of 0–1.5 $\times$ 10^{-2}% w/v: amplitude (mm) = 1.105 $\times$ 10^4 conc. (% w/v) − 0.899 (r = 0.9999; n = 6). A relative standard deviation of 0.8% was found for repetitive assay of a 1 $\times$ 10^{-2}% solution of BAK (n = 8).

To ensure that the method is applicable to all BAK solutions irrespective of homologue content, four samples of BAK solution (50% w/v) from different sources were diluted to 1 $\times$ 10^{-2}% w/v and submitted to analysis. All four samples afforded a concentration of BAK as determined by the method within ±1% of the mean value. Analysis of the solutions by a previously-reported chromatographic procedure [15] demonstrated variable homologue composition and gave results within ±1.8% of the mean.

Conclusion

The quantitation of BAK in ophthalmic products especially those containing polymeric products such as hypromellose and PVA is difficult. Second-order derivative spectrophotometry provides a simple method for the determination of total BAK in products such as artificial tears which is rapid and reliable.

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