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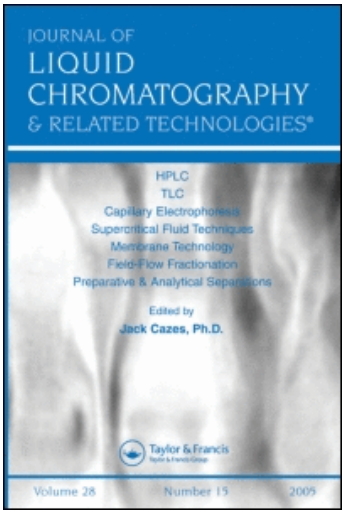

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A STABILITY-INDICATING HPLC METHOD FOR THE DETERMINATION OF BENZALKONIUM CHLORIDE IN PHENYLEPHRINE HCl 10% OPHTHALMIC SOLUTION

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ABSTRACT

A high-performance liquid chromatographic (HPLC) procedure employing ultraviolet (UV) detection for the analysis of benzalkonium chloride (BAK) in Phenylephrine HCl 10% ophthalmic solution is reported. The method requires no sample pretreatment and is sensitive, accurate, and reproducible. The peak area versus BAK concentration is linear over the range of 50-150% of its label claim of 0.05 mg/mL. The mean absolute recovery of BAK using the described method is 103.2 ± 0.6%, (mean ± SD, n = 10). A stress study with heat, acid, base and UV radiation indicates that the method is stability-indicating with no interference from drug, excipients or their degradation products.

INTRODUCTION

Phenylephrine HCl 10% is an alpha sympathetic receptor agonist producing mydriasis of short duration and vasoconstriction. Its indications of use include

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prevention of posterior synechiae associated with uveitis and as a mydriatic prior to intraocular surgery [1,2]. Phenylephrine HCl 10% is preserved with benzalkonium chloride, which is a mixture of alkylbenzyldimethylammonium chlorides of the general formula $[C_6H_5CH_2N(CH_3)_2R]Cl$. "R" represents a mixture of alkyls with the $n\text{-}C_{12}H_{25}$, $n\text{-}C_{14}H_{29}$, and $n\text{-}C_{16}H_{31}$ homologues comprising the major portion.

Several HPLC methods exist for the determination of BAK in ophthalmic solutions [3-9], however, these formulations do not contain phenylephrine. When using these methods, a separation problem exits because phenylephrine is 2000 times more concentrated than BAK. Recently, our laboratory investigated several developmental parameters in the separation of BAK from sulfacetamide in Vasocidin® ophthalmic solution [10]. This work has expanded to include quantitating BAK in the presence of high phenylephrine concentrations. This manuscript describes a sensitive, accurate, and reproducible, ion-pair reversed-phase HPLC method for the determination of BAK in an ophthalmic solution containing phenylephrine. Moreover, this method was determined to be stability-indicating.

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According to the USP XXII quidelines, analytical methods for the quantitation of major components of bulk drug substance or preservatives in finished pharmaceutical products fall under Assay Category I [11]. Data elements required for Assay Category I include precision, accuracy, selectivity, range, linearity, and ruggedness. The method for BAK in Phenylephrine HCl 10% ophthalmic solution satisfies all of these requirements.

EXPERIMENTAL

Chemicals and Reagents

Phenylephrine HCl 10% ophthalmic solution was formulated at IOLAB Corporation (Claremont, CA, USA).

Phenylephrine HCl and benzalkonium chloride were USP grade. HPLC grade acetonitrile, 1 **N** sodium hydroxide, hydrochloric acid, and ACS reagent grade potassium phosphate monobasic, monohydrate were purchased from J.T. Baker (Phillipsburg, NJ, USA). Hexane sulfonate, sodium salt, HPLC grade, was purchased from Eastman Kodak (Rochester, NY, USA). The water was deionized and distilled. All reagents were used without further purification.

Apparatus

The chromatographic system consisted of a Waters model 600E system controller and pump, a WISP 712D autosampler, and a Waters 486 variable-wavelength UV detector set at 215 nm (Waters Associates, Milford, MA, USA). A stainless-steel μBondapak™ phenyl column (30 cm x 3.9 mm, 10 μm, Waters Associates) was maintained at ambient temperature.

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Mobile Phase

The mobile phase consisted of acetonitrile - buffer (65:35 V/V), where the buffer was comprised of 50 **mM** potassium phosphate monobasic, monohydrate and 57 **mM** hexane sulfonate, sodium salt, adjusted to pH 6.3 with 1**N** NaOH. The mobile phase was filtered through a 0.45 μm filter and degassed for 30 minutes. The flow rate was 1.8 mL/minute with a typical operating pressure of **ca.** 80 bar. Under these conditions, the retention times of the C_{12} and C_{14} homologues of BAK were 7.5 and 12.1 minutes, respectively.

Preparation of BAK Solutions

A BAK Stock Solution was prepared by accurately weighing BAK (W_s) into a tared volumetric flask (V_1) and diluting to volume with water.

A BAK Standard Solution was prepared by pipeting the BAK Stock Solution (V_2) into a volumetric flask (V_3) and diluting to volume with water.

System Suitability

The system suitability results were calculated according to Chromatography <621> of the USP XXII from typical chromatograms [11]. The instrument precision as determined by six successive injections of a BAK Standard Solution should provide a relative standard deviation (RSD) not greater than 1.0%. The column efficiency, when calculated using the C_{14} peak should be greater than 2000 theoretical plates. The tailing factor for any homologue should not exceed 2.0 at 5% peak height. Finally, the resolution between peaks must be greater than 1.5.

The Test Solution (Phenylephrine HCl 10% ophthalmic solution with no sample work-up) is used to verify that the method meets all of the suitability limits.

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Stress Study

The specificity of the method was studied through the analysis of stressed Test Solutions containing the label claim of BAK (0.05 mg/mL) and stressed Placebo Solutions (Test Solution without BAK). The stressed samples were subjected to heat, acidic, basic and UV light environments.

Five mL aliquots of the Test and Placebo Solutions were sealed in transparent glass containers and exposed to a UV radiation source (200-400 nm, 40 mWatt/cm^2) for 16 days. Other 5.0 mL aliquots were adjusted to either pH 2 with concentrated HCl or pH 12 with 50% NaOH and sealed in glass containers with equal head space and stored at 88°C for up to 16 days.

Data Acquisition

The peak areas of the C_{12} and C_{14} homologues of BAK were measured using a PE Nelson 900 series interface and down-loaded to PE Nelson Turbochrom 3 workstation (Perkin-Elmer Corporation, Cupertino, CA, USA). The chromatographic data was automatically processed for peak area followed by an unweighted linear regression analysis.

Calculations

The BAK content of the Test Solution was calculated according to the individual BAK homologue peaks. The total BAK content was obtained by combining the BAK homologue concentrations of the Test Solution. The response factor, RF_i, for each BAK homologue peak is:

$$RF_i = \frac{W_S \times F \times P_i \times V_2}{V_1 \times V_3 \times PA_{Si} \times 100}$$

where W_S is the quantity (mg) of the BAK standard used, F is the purity factor (mg/mg) of the BAK standard, P_i is the composition (%) of any homologue in the BAK standard, V_1 is the volume of the flask used to prepare the BAK Stock Solution, V_2 is the pipetted volume of the BAK Stock Solution, V_3 is the volume of the volumetric flask used to prepare the BAK Standard Solution, and PA_{Si} is the peak area of the corresponding homologue of the BAK Standard Solution.

The concentration of any BAK homologue, C_{Ti}, is:

$$C_{Ti}\ (mg/mL) = RF_i \times PA_{Ti}$$

where RF_i is the response factor of any homologue peak and PA_{Ti} is the peak area of the corresponding homologue of the Test Solution. The total BAK content, C, is:

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$$C\ (mg/mL) = \Sigma C_{Ti}$$

where ΣC_{Ti} is the sum of the BAK homologue concentrations calculated in the above section.

RESULTS AND DISCUSSION

Chromatography

Typical chromatograms obtained from a 50 μL injection of a Standard, Test Solution and Placebo are illustrated in Figure 1 (a-c), respectively. The retention times of the C_{12} and C_{14} homologues of BAK were 7.5 and 12.1 minutes, respectively. The overall chromatographic run time was 20 minutes.

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System Suitability

The column efficiency for the C_{14} homologue of BAK was 2500 theoretical plates. The tailing factors of the C_{12} and C_{14} homologues were 1.4 and 1.3, respectively. The resolution was 5.9 between the C_{12} and C_{14} peaks. The instrument precision, determined by 6 replicate injections of the BAK Standard Solution, exhibited a RSD of 0.4%.

Precision and Accuracy

The precision (RSD) and accuracy (relative error, RE) were determined by analyzing Placebo Solutions spiked with BAK, in replicates of six, ranging from ca. 50-150% (23.5-80 μg/mL) of its label claim in Phenylephrine HCl 10% (Table 1).

Recovery

The recovery of BAK was determined by comparing the concentration found in Phenylephrine HCl 10% to that of the BAK Standard Solution. The mean found recovery of BAK over the range of 50-150% its label claim was 103.2 ± 0.6% (mean ± SD, n=10, Table 2).

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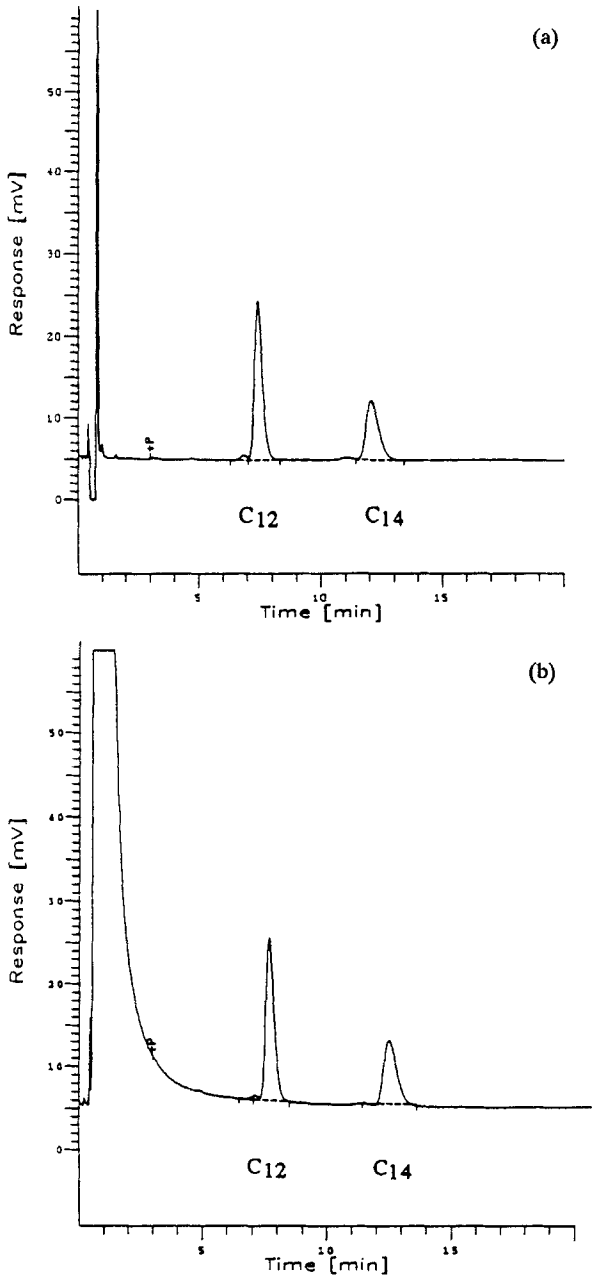

FIGURE 1. Typical chromatograms of (a) a Standard Solution of BAK, (b) Phenylephrine HCl 10% ophthalmic solution containing BAK and (c) Phenylephrine HCl 10% ophthalmic solution not containing BAK.

(continued)

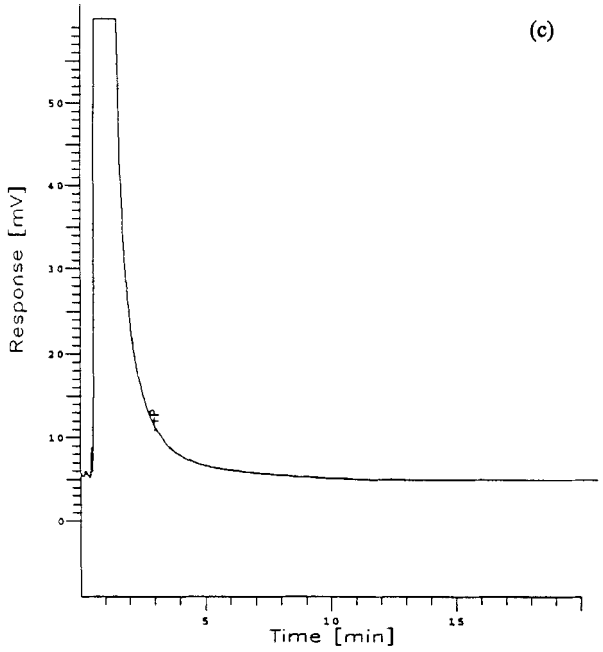

FIGURE 1 (Continued).

TABLE 1

Accuracy and Precision of BAK in Phenylephrine HCl 10%

Nominal Conc. (μg/mL)	n	Mean Found Conc. (μg/mL)	%RSD	%RE
23.50	6	24.29	0.61	3.4
37.50	6	39.01	0.47	4.0
47.00	6	48.55	0.45	3.3
56.40	6	58.03	0.46	2.9
80.00	6	82.73	0.20	3.4

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TABLE 2

% Recovery of BAK in Phenylephrine HCl 10%

% Label Claim of BAK in Phenylephrine HCl 10%	% Recovery
50	102.6
50	103.3
75	104.1
75	104.2
100	103.1
100	103.1
125	102.8
125	102.2
150	103.4
150	103.2

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Linearity

A linear response in peak area for BAK over the range of 50-150% of its label claim in Phenylephrine HCl 10% was observed. The correlation coefficients were 1.000 (n=6).

Stress Study

Phenylephrine HCl 10% ophthalmic solution was stressed with heat, acid, base, and UV radiation for up to 16 days or until approximately 10% degradation of phenylephrine was achieved. The acid stressed samples were adjusted to pH 2 with concentrated HCl and heated at 88°C for 16 days. No degradation was observed for the acid stressed samples under the described conditions. The base stressed samples were adjusted to pH 12 with 50% NaOH and heated at 88°C for 65 hours. Phenylephrine degradation of 9.2% was observed for the base stressed samples under the described conditions. Ultraviolet light stressed samples were placed in the path of a UV lamp at 40 mWatt/cm^2 for 16 days. No degradation was observed for the UV stressed samples under the described

conditions. Furthermore, no interfering peaks at the retention times for the BAK homologues were observed in any of the stressed sample.

Conclusion

The described assay for the analysis of BAK in an ophthalmic solution containing phenylephrine HCl is sensitive, accurate, and reproducible. Furthermore, the method is stability-indicating with no interference from phenylephrine HCl or excipients or their degradation products under the described stress conditions.

REFERENCES

1. Physicians' Desk Reference For Ophthalmology®, pp. 275, Medical Economics Data, Montvale, NJ, USA (1994).

2. N. Weiner, in The Pharmacological Basis of Therapeutics, 7th edition, (A.G. Gilman, L.S. Goodman, T.W. Rall, and F. Murad, eds.), pp. 170-180, Macmillian, New York, NY, USA (1985).

3. L. Elrod, Jr., T.G. Golich and J.A. Morley, J. Chromatogr., 625: 362-367 (1992).

4. R.C. Meyer, J. Pharm. Sci., 69: 1148-1150 (1980).

5. M.R. Euerby, J. Clin. Hosp. Pharm., 10: 73-77 (1985).

6. G. Ambrus, L.T. Takahashi and P.A. Marty, J. Pharm. Sci., 76: 174-176 (1987).

7. A. Gomez-Gomar, M.M. Gonzalez-Aubert, J. Garces-Torrents and J. Costa-Segarra, J. Pharm. Biomed. Anal., 8: 871-876 (1990).

8. D.F. Marsh and L.T. Takahaski, J. Pharm. Sci., 72: 521-525 (1983).

9. J.J. Halvax, G. Wiese, J.A. Arp, J.M.P. Vermeer, W.P. Van Bennekom and A. Bault, J. Pharm. Biomed. Anal., 8: 243-252 (1990).

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10. R.B. Miller, C. Chen and C.H. Sherwood, J. Liq. Chromatogr., 16: 3801-3811 (1993).

11. The United States Pharmacopeia XXII, pp. 1558-1567, U.S. Pharmacopeial Convention, Rockville, MD (1990).

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