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Determination of atropine sulphate and benzalkonium chloride in eye drops by HPLC

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Summary

A reversed-phase high-performance liquid chromatographic method has been developed for the simultaneous determination of atropine sulphate and C_{12}-C_{16} benzalkonium chloride homologues in eye drops. The elution was isocratic using a C-8 column and acetonitrile-diluted acetic acid (80:20, v/v) as mobile phase with 6 mM tetramethylammonium bromide. Detection was carried out at 260 nm with a diode array. Tropic acid can also be detected and quantified under the same chromatographic conditions.

In ophthalmic solutions, atropine sulphate (a midriatic) at the concentration of 10 mg ml^{-1} is usually available, while benzalkonium chloride (BAK), a mixture of alkylbenzyldimethylammonium chlorides with alkyl groups which may extend from n-C_8H_{17} to n-$C_{18}H_{37}$, is used in the bacteriostatic concentration range of 0.04–0.2 mg ml^{-1}.

The USP requires that the amounts of homologue components having chain lengths of C_{12} and C_{14} comprise no less than 70% of the total BAK content. Actually, the homologues do not possess identical antimicrobial activities and BAK, being a mixture of homologues of undefined relative proportions, presents more analytical problems than the other monocomponent quaternary ammonium salts. Of the methods available for determination of BAK homologues, high-performance liquid chromatography (HPLC) with UV detection has been recently described for aqueous ophthalmic systems in the presence of slightly water-soluble products or common components (Ambrus et al., 1987; Gomez-Gomar et al., 1990). HPLC methods have also been described for atropine (Brown and Sleeman, 1978; Arvidsson et al., 1990).

Methods designed to analyse active ingredients and preservatives with a single assay are in great demand in pharmaceutical quality control. The

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present work proposes a reversed-phase HPLC method with UV diode-array detection that allows the simultaneous determination of atropine and BAK homologues with the use of tetramethylammonium bromide (TMA) as mobile phase modifier to enhance the chromatographic performance of BAK. The effects of mobile phase, pH and modifying agents on the retention time of atropine and BAK homologues were also studied.

Reagents and chemicals: Atropine sulphate (Sigma), diphenydramine hydrochloride (internal standard, Merck), BAK homologues (Sigma), tetramethylammonium bromide (Janssen Chimica) and acetic acid (Carlo Erba) were used as received. Acetonitrile (Carlo Erba) and water were HPLC analytical-reagent grade. Samples of two batches of eye drops manufactured for the Italian army were obtained from the Stabilimento Chimico Farmaceutico Militare, Florence. The labeled composition was: atropine sulphate 1 g, benzalkonium chloride 0.02 g, sodium chloride 0.70 g, distilled water sufficient to produce 100 ml. Stock solutions of atropine sulphate (10 mg ml^{-1}), BAK homologues (C_{12}, C_{14}, C_{16}; 0.2 mg ml^{-1}) and diphenhydramine hydrochloride (10 mg ml^{-1}) were prepared with water. Working standard solutions were prepared by diluting the stock solutions with mobile phase and the concentrations ranged from 1 to 4 mg ml^{-1} for atropine and from 0.02 to 0.08 mg ml^{-1} for BAK homologues. The concentration of diphenydramine hydrochloride was fixed at 1 mg ml^{-1}.

Apparatus: Analyses were performed using a Perkin-Elmer Series 3B liquid chromatograph equipped with a Reodyne injector 7125 (20 μl loop), a UV diode-array detector LC 235 and an LC 100 integrator. A 10 cm × 4.6 mm i.d. reversed-phase column (HPLC Technology, Technosphere RP C-8, 5 μm particle size) was used.

Chromatographic conditions: The elution was isocratic. The mobile phase consisted of acetonitrile-acetic acid (0.25 M, pH 2.5) (80:20, v/v) and contained 6 mM TMA; it was degassed prior to use. A flow rate of 1.4 ml min^{-1} was employed; all determinations were performed at room temperature in triplicate. The column effluent was monitored at 260 nm with a bandwidth of 5 nm (0.2–0.005 absorbance units full scale). Because of the low levels of BAK, at 3.0 min a change of absorbance units full scale from 0.2 to 0.005 was effected.

TABLE 1

Average least-square regression equations of atropine and benzalkonium chloride homologues

Drug	Slope	Intercept	r
Atropine	3.151	−0.452	0.9994
C_{12}	2.551	−0.002	0.9996
C_{14}	3.729	−0.001	0.9987
C_{16}	6.306	0.000	0.9993
$C_{12} + C_{14}$	4.586	−0.029	0.9992

Assay: The sample solution (2 ml) and the stock diphenydramine hydrochloride solution (1 ml) were transferred into a 10 ml volumetric flask and diluted to volume with the mobile phase. The diluted samples were filtered (0.45 μm) and injected. The peak-height ratios between the compound and the internal standard were plotted against the corresponding analyte concentrations (mg ml^{-1}) on the abscissa.

The response linearity of atropine sulphate was verified in the range 1–4 mg ml^{-1} and that of BAK homologues in the range 0.02–0.08 mg ml^{-1}. The average least-square regression equations (n = 4) are reported in Table 1.

In the present work, only C_{12}, C_{14} and C_{16} homologues were monitored since they are the major components of benzalkonium chloride. In particular, in the ophthalmic preparation only C_{12} and C_{14} BAK homologues were found and the C_{12} was the most prevalent component. This situation was previously observed during the analysis, by fast atom bombardment mass spectrometry, of several hard contact lens solutions (Pinzauti et al., 1989).

Under the described chromatographic conditions atropine and C_{12}–C_{16} BAK homologues were separated and quantitated quite accurately (Fig. 1). The analytical results from the assay of two batches of eye drops and two admixtures containing known quantities of atropine sulphate and BAK in ratios equivalent to those declared in the dosage form are reported in Table 2. The proposed method can also be applied to stability

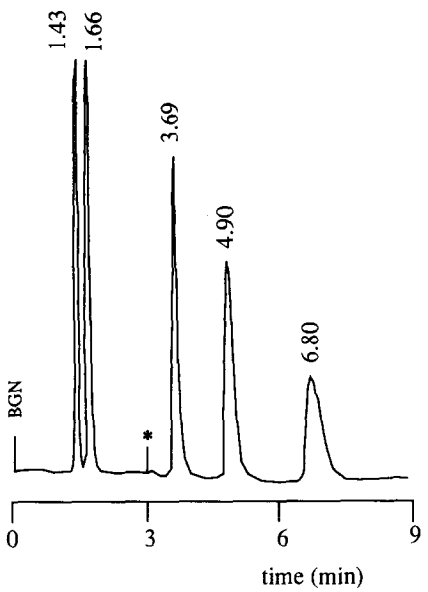

Fig. 1. Typical chromatogram of atropine sulphate (R_t 1.43 min), diphenydramine hydrochloride internal standard (R_t 1.66 min), C_{12} (R_t 3.69 min), C_{14} (R_t 4.90 min), C_{16} (R_t 6.80 min) BAK homologues; (*) from 0.2 to 0.005 absorbance units full scale.

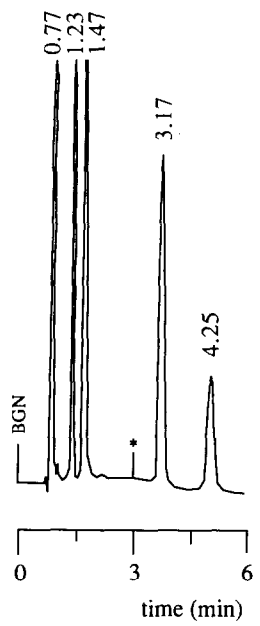

Fig. 2. Chromatogram of eye drops spiked with tropic acid (R_t = 0.77 min), corresponding to 5% w/w of atropine. Other sample components are in sequence: atropine sulphate, diphenydramine hydrochloride internal standard, C_{12} and C_{14} BAK homologues; (*) from 0.2 to 0.005 absorbance units full scale.

studies: in fact tropic acid, a major hydrolytic product of atropine, can be detected and assayed in the same chromatographic conditions as shown in Fig. 2.

The reversed-phase HPLC of quaternary ammonium ions has often been associated with irreversible binding of the solutes to the stationary phase or extreme peak tailing upon elution (Reynolds et al., 1983a). In the present case, preliminary experiments revealed that BAK homologues failed perceptibly to elute from a RP C-8 column using methanolic eluents or acetonitrile-water mixtures with a low percentage of the organic component. Similar effects have previously been attributed to the strong interactions between the quaternary ammonium ions and the residual silanols on the stationary phase (Reynolds et al., 1983b). The chemical derivatization of silica, by raising the presence of more than one type of adsorption site (hydrophobic and silanophilic), may result in a large separation selectivity but this, unfortunately, will also affect the peak symmetry values of solutes (Welsch et al., 1990).

In a previous work a methodology was developed for the analysis of a quaternary ammonium compound of pharmaceutical interest with the use of TMA as mobile phase modifier (Santoni et al., 1991). Mobile phase modifying agents having the same charge as the sample were used to reduce residual silanophilic interactions on reversed-phase surfaces and to improve the analysis of quaternary ammonium ions. The use of modifying agents in the mobile phase allowed the

TABLE 2

Analyses of eye drops and authentic admixtures of atropine sulphate and benzalkonium chloride (BAK) (percent average recovery; RSD, n = 5)

Sample	Atropine sulphate	C_{12}	C_{14}	C_{16}	Total BAK
Batch 1	104.3 (1.5)	70.8 (1.9)	27.4 (2.5)	–	98.2
Batch 2	103.5 (2.3)	74.4 (2.7)	28.1 (2.8)	–	102.5
Adm. 1	98.8 (2.4)	53.7 (2.9)	34.8 (2.9)	13.6 (2.8)	102.1
Adm. 2	97.9 (2.3)	51.2 (2.3)	30.4 (2.6)	16.5 (2.6)	98.1

reduction of the retention time and the improvement of peak symmetry. The retention time of positively charged samples is decreased by the formation of a positively charged layer of TMA adsorbed on the alkyl chains of the stationary phase, which might block charged solute molecules from hydrophobic interactions, while the peak symmetry is improved by ion-exchange interactions (Kiel et al., 1985). Thus, facilitation of elution by TMA may be explained in terms of a cation-exchange mechanism in which positive ions compete with residual silanol sites, thereby reducing solute retention. The effects of TMA concentration on the capacity and symmetry factors, calculated according to Lonardi and Mosconi, 1980, are shown in Figs 3 and 4, respectively.

The influence of mobile phase pH (Fig. 5) on the retention of atropine and BAK homologues was studied in 80% v/v acetonitrile-diluted acetic

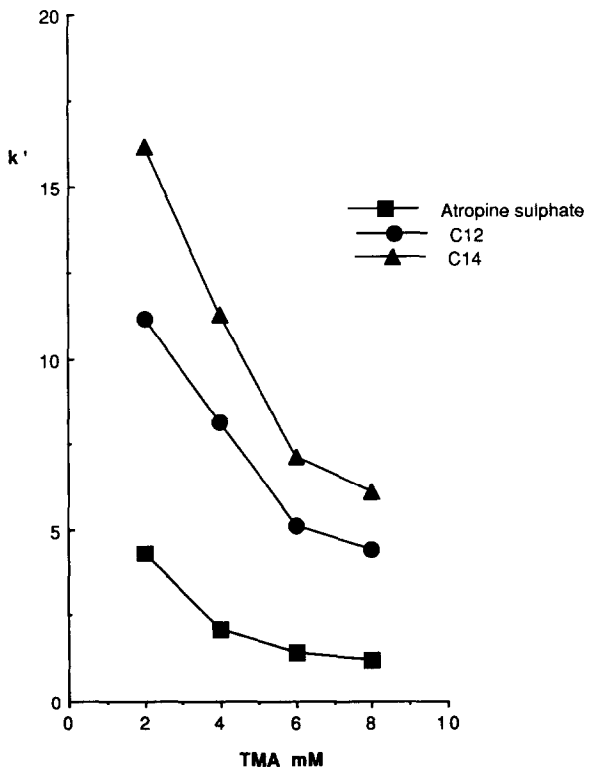

Fig. 3. Capacity factor (k') of atropine sulphate, C_{12} and C_{14} BAK homologues vs concentration of tetramethylammonium bromide.

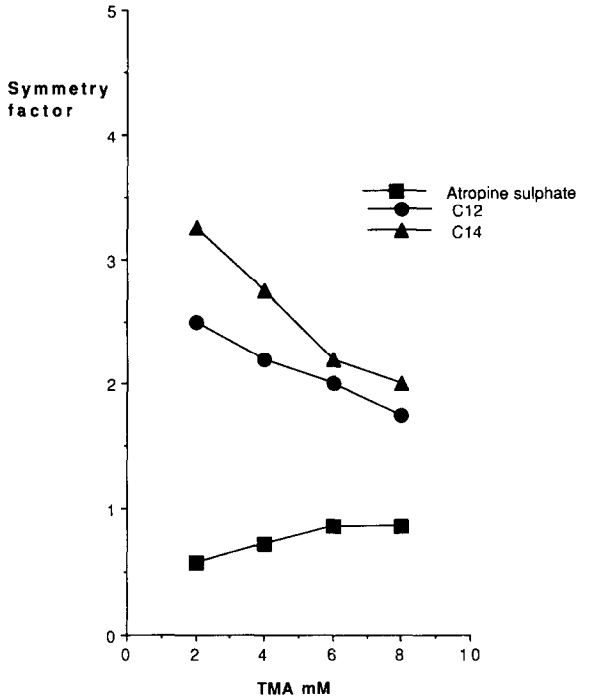

Fig. 4. Symmetry factor of atropine sulphate, C_{12} and C_{14} BAK homologues vs concentration of tetramethylammonium bromide.

acid mobile phases with TMA 2 mM. The function of acetic acid is the ionization control of atropine and the reduction of the interaction of the sample with accessible weak silanol groups on the stationary surface. The addition of the acid to the eluent results in greatly improved peak shapes, particularly for BAK homologues. The increased retention observed with pH increase may arise from the presence of strongly acidic sites on the silica surface which can increase their ion-exchange capacity (Flanagan and Jane, 1985).

The C_{14} homologue exhibited the greatest dependence of retention time and peak symmetry on variation in the mobile phase conditions (pH, TMA and acetonitrile concentration) while the symmetry factor of atropine was almost wholly unaffected by changes in pH or TMA concentration.

In conclusion, the use of TMA as a free silanol blocking agent adequately suppressed undesirable retardation effects and was able to improve peak symmetry. The result was a rapid method

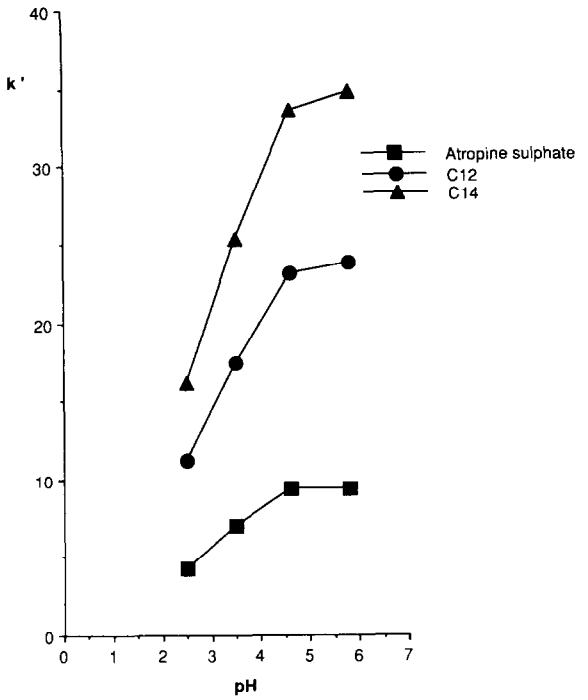

Fig. 5. Capacity factor (k') of atropine sulphate, C_{12} and C_{14} BAK homologues vs pH of aqueous component of the mobile phase.

allowing precise and accurate simultaneous determination of atropine sulphate (RSD < 2.5%) and BAK (RSD < 3%) in eye drops with good sensitivity and selectivity.

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