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Determination of benzalkonium chloride in contact lens solutions by positive-ion fast atom bombardment mass spectrometry*

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Abstract: Under positive-ion fast atom bombardment (FAB) mass spectrometric conditions, benzalkonium chloride (BAK) afforded intense peaks at *m/z* 304 and 332, corresponding to the intact cations $[M—Cl]^+$ of C_{12} and C_{14} homologues, respectively. The use of benzethonium chloride as an internal standard and thioglycerol as a FAB matrix allowed the direct and specific determination of the BAK content (0.004–0.020%) in commercial hard contact lens solutions through the individual assay of the two alkyl homologues. A linear relationship between the homologue concentration and the peak–area ratio was observed over the concentration range 3–180 μg ml^{-1}.

Keywords: *FAB mass spectrometry; benzalkonium chloride determination; contact lens solutions.*

Introduction

Benzalkonium chloride (BAK) is a mixture of alkylbenzyldimethylammonium chlorides with alkyl groups which may extend from *n*-C_8H_{17} to *n*-$C_{16}H_{33}$. The amounts of homologue components having chain lengths of C_{12} and C_{14} comprise no less than 70% of total BAK content [1]. BAK is widely used as an antimicrobial in hard contact lens solutions [2] and ophthalmic products [3], in the bacteriostatic concentration range of 0.004–0.020% m V^{-1}.

Actually 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 its determination in pharmaceutical preparations, some have been based on titrimetric, potentiometric, or colorimetric procedures with antagonist reagents [4–8], and others on high-performance liquid chromatography (HPLC) [9–13]. The former, even if quite simple and suitable for routine use, are non-specific; the latter proved unsatisfactory for ophthalmic systems

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containing a polymeric matrix [9], or required complicated sample manipulation and/or derivatization [10, 11]; moreover, the sensitivity was generally low, due to the poorly-absorptive chromophore involved.

To overcome these difficulties a new direct method, which would combine simplicity and speed of execution with specificity and sensitivity, was clearly desirable. Positive-ion FAB mass spectrometry (FAB–MS) of the cationic moiety of BAK, $[M—Cl]^+$ (where $M = R_4N^+Cl^-$), appeared to be particularly suitable in view of the fact that the authors had recently succeeded in applying this technique for the first time, for quantitative purposes, namely, for the assay of the cationic surfactants cetylpyridinium chloride and benzethonium chloride, in antiseptic formulations with benzyldimethyltetradecyl-ammonium chloride as internal standard [14]. In the present work BAK was similarly quantitated in hard contact lens solutions using benzethonium chloride as internal standard. In general all the homologues constituting the BAK test sample could be individually assayed by the proposed method. However, on survey runs, the nine commercial preparations examined (with one exception) and the two BAK standard samples employed appeared to contain the C_{12} and C_{14} homologues alone (Fig. 1). Consequently, the assay was limited to the individual determination of these two intact cations, allowing direct, specific and reliable analyses without any extraction procedure or interference from the other constituents of the commercial formulations including various polymeric cleaning, "cushioning" and wetting agents.

Experimental

Materials

The commercial hard contact lens solutions (Table 1) were selected as being representative of single- and multiple-task products currently marketed in Italy, and were purchased locally. Product labels indicated the presence of various polymers (non-ionic surfactants, cellulose gum derivatives and wetting agents, such as polyvinyl alcohol) as well as preservative (0.004–0.020%), buffering, chelating and isotonicity agents.

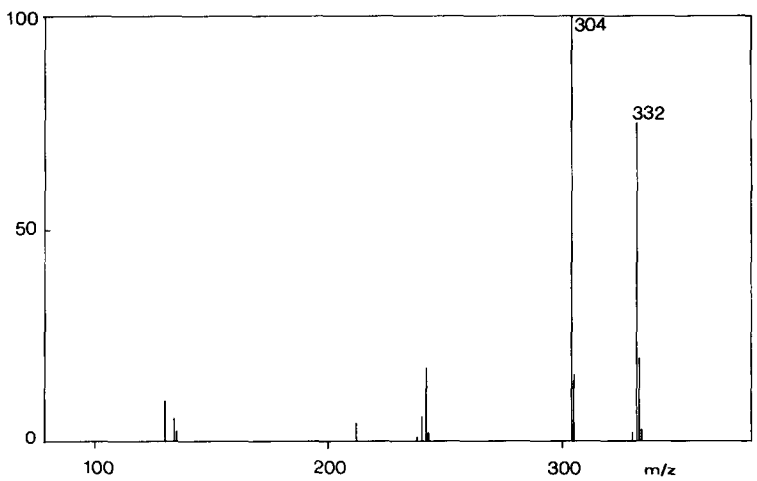

Figure 1
Positive-ion FAB mass spectrum of a commercial sample of BAK (Merck-Schuchardt) consisting of a C_{12} (*m/z* 304) and C_{14} (*m/z* 332) homologue mixture. Ordinate: relative abundance.

Table 1
FAB–MS BAK determination in commercial hard contact lens solutions

Brand*	BAK stated $\mu g\ ml^{-1}$	C_{12} found $\mu g\ ml^{-1}$ (SD, $n = 4$)	C_{14} found $\mu g\ ml^{-1}$ (SD, $n = 4$)	BAK found % average recovery	BAK % recovery by alternative method (RSD, $n = 4$)
A	40	26.9 (0.65)	13.3 (0.24)	100.5	100.9 (1.6)†
B	44	26.1 (0.23)	13.5 (0.23)	90.2	140.3 (1.8)†
C	100	65.3 (1.37)	29.5 (0.71)	94.8	96.3 (1.4)‡
D	40	29.9 (0.42)	20.5 (0.41)	125.0	142.6 (2.0)†
E	40	22.5 (0.34)	16.0 (0.13)	96.3	94.6 (0.7)†
F	40	27.4 (0.30)	12.3 (0.74)	99.3	—
G	100	66.1 (1.92)	25.1 (0.40)	91.2	94.0 (1.6)‡
H	200	—	168.2 (3.20)	84.1	—
I	40	24.0 (0.41)	13.5 (0.16)	93.7	—
Simulated Solution S_1	80	30.6 (0.36)	48.1 (0.38)	98.4	98.6 (0.6)†
Simulated Solution S_2	100	47.7 (0.61)	51.2 (0.79)	98.9	99.4 (1.3)†

*The identification key for the solutions can be obtained by individual request to the authors.
†Potentiometric method [6].
‡Colorimetric method [11].

Analytical reagent grade benzyldimethyldodecylammonium chloride (C_{12} homologue) and benzyldimethyltetradecylammonium chloride (C_{14} homologue), benzalkonium chloride (Merck–Schuchardt, average mol. wt 365, or Fluka, USNF) and benzethonium chloride USP, were obtained through normal commercial channels and were used without further purification. Their water content was determined with an automatic Karl Fisher titrator (Metrohm, E 547).

An isotonic pH 7.4 buffered medium, to be employed for the preparation of standard solutions, was made up by dissolving 1.95 g of sodium phosphate, 0.21 g of potassium acid phosphate, 0.1 g of disodium edetate and 0.37 g of sodium chloride in doubly-distilled water.

Two simulated contact lens solutions S_1 and S_2, containing 0.008 and 0.01% BAK, respectively, differed only in the BAK commercial source (S_1, Merck-Schuchardt and S_2, Fluka) and were prepared in the isotonic-buffered medium to which 0.5% hydroxyethylcellulose, 0.7% poloxamer 407 and 0.1% octoxynol 9 were added.

Apparatus

Mass spectral measurements were performed with a VG Analytical model 7070 EQ double-focusing instrument operating at 6 kV accelerating voltage and linked to a Digital PDP8/A computer system for data processing. The FAB ion source employed xenon atoms of 7 keV kinetic energy.

Calibration curves and contact lens solution assay

For quantitative measurements, benzethonium chloride was adopted as an internal standard. The individual calibration curves for C_{12} and C_{14} BAK homologues were obtained simultaneously by means of a series of seven working standards of each homologue (8–480 $\mu g\ ml^{-1}$) prepared in the isotonic-buffered medium described above. To a series of seven vials, each containing 50 μl of 400 $\mu g\ ml^{-1}$ benzethonium chloride

isotonic-buffered solution, equal volumes (75 μl) of the appropriate C_{12} and C_{14} working standard were added, thus obtaining a total of seven 200 μl assay samples with a final homologue concentration ranging from 3 to 180 μg ml^{-1}. A 2–4 μl quantity from each vial was added by microsyringe to a layer of thioglycerol on the FAB target, and measurements were performed by repetitive scanning in the mass range 210–500 *m/z*, at 1500 resolution, with a scan speed of 2 s/decade. The areas of the intact cation peaks $[M—Cl]^+$ which appeared at *m/z* 304, 332 and 412 for the C_{12} homologue, C_{14} homologue and benzethonium chloride, respectively, were taken from the 5th to the 20th scan and individually stored as relative intensity versus scan number plots. The procedure was repeated four times and the calibration curves of the peak–area ratios between the homologue and the internal standard cations, plotted against the analyte concentration, were constructed from the averages obtained.

Commercial and simulated contact lens solutions were assayed simply by mixing a 150-μl aliquot with 50 μl benzethonium chloride standard solution and placing 2–4 μl of this mixture on the FAB target as described above. The BAK concentration was calculated as the sum of the individual homologue concentrations obtained from calibration graphs.

Results and Discussion

Individual calibration curves for the C_{12} and C_{14} BAK homologues were rectilinear in the range 3–180 μg ml^{-1} (slope = 0.0183 and 0.0146; intercept = 0.0336 and −0.0201; r = 0.9995 and 0.9986, respectively). A typical average recovery (n = 4) at the 25 μg ml^{-1} level was 98.4 ± 0.9% for the C_{12} homologue and 101.2 ± 1.3% for the C_{14} homologue.

The results for the analysis of a selection of commercial hard contact lens solutions are presented in Table 1. For comparative purposes, a potentiometric procedure [6] was profitably employed for samples A, B, D, E, S_1 and S_2; while for samples C and G a colorimetric acid-dye method, described for determining chlorhexidine gluconate in contact lens solutions [15], worked better. However, neither of the assays proved suitable for the remaining commercial samples. In particular, the co-presence of significant amounts of chlorhexidine gluconate with BAK in the samples F and I ruled out both the methods which are not able to distinguish between the two antimicrobials in the mixture. Sample H gave an undefined potentiometric end-point and a permanent emulsion in the solvent extraction step of the colorimetric procedure.

Analyses of simulated solutions S_1 and S_2, which had been prepared to study the influence of buffering, isotonicity and viscosity-increasing agents on recovery, gave precise and accurate results in good agreement with the reference method. Taking the range 90–110% of the stated concentration as an acceptable limit, the majority of the brands examined, with the exception of samples D and H, were found to pass the analytical control and good agreement was generally noted with the alternative referee method (Table 1). On the other hand, the low or excessive BAK percentages recorded in samples H and D, respectively, might be ascribed as previously reported [4, 5, 16] to surface adsorption taking place on the plastic container, or to the manufacturer's incorporating an excess of BAK to counteract this process. It could be seen that the BAK used in brand H was actually an unusual C_{14} monocomponent product.

The C_{16} homologue (*m/z* 360) was observed in brand D only as a minor component of the BAK. It was therefore omitted in the authors' computation which, as mentioned above, considers the C_{12} and C_{14} homologues only. Accordingly, the percentage

recovery by the non-specific potentiometric alternative method was somewhat higher in the case of sample D.

Finally, the large discrepancy between the recoveries for brand B, obtained by the proposed and by the alternative method, still lacks a clear explanation, though it might be ascribed to interference by non-declared ingredients with the potentiometric referee procedure, which employed tetraphenylborate as titrant.

As previously observed, FAB–MS was confirmed as being suitable for determining quaternary ammonium compounds, in that the intact cations $[M—Cl]^+$ arising from the analyte are pre-formed and have only to be desorbed from the liquid matrix. Here again thioglycerol was the liquid matrix of choice, which ensured that the individual ion-current intensity of the cations under examination kept pace with the ion-current intensity of the internal standard cation for at least 1 min, a feature which is essential for

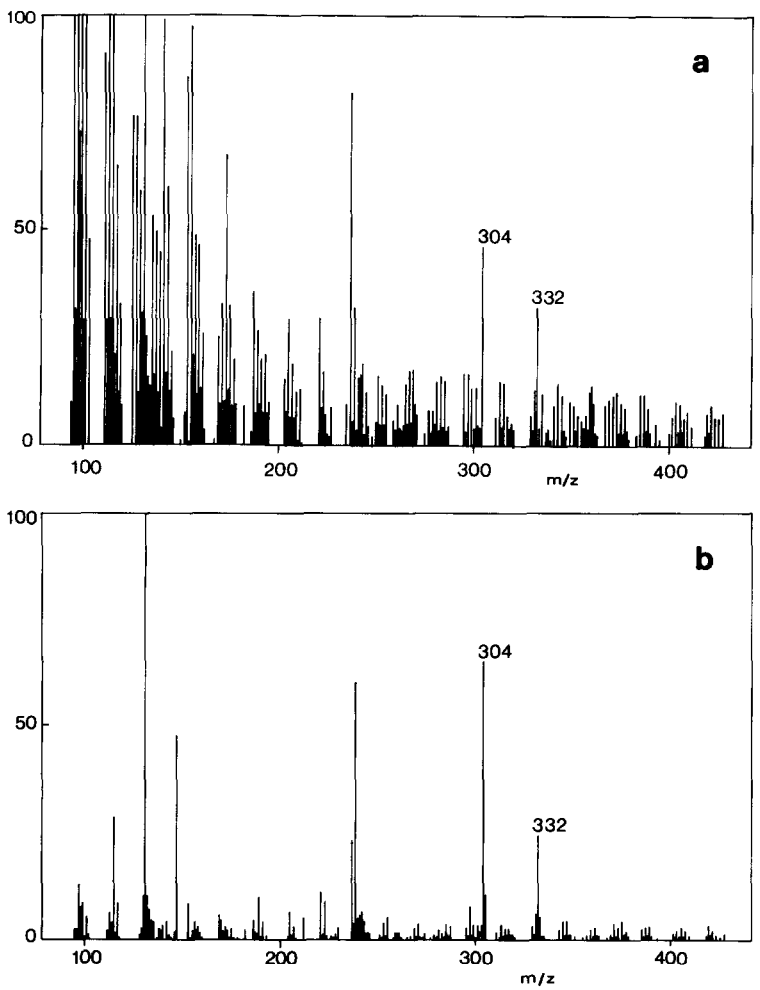

Figure 2
Positive-ion FAB mass spectra of commercial hard contact lens solution, showing the prominence of BAK ion pattern (*m/z* 304 and 332) against a more or less complex ion background (a: brand D, b: brand C; see Table 1). Ordinate: relative abundance.

quantitative analysis, as previously reported in an earlier study [14]. In the present case the cationic species $[M—Cl]^+$, relative to the C_{12} and C_{14} BAK homologues and the internal standard benzethonium chloride, gave rise to intense peaks at *m/z* 304, 332 and 412, respectively. Further fragmentation of these ions was negligible or absent, which contributes to the overall sensitivity of the method.

It is concluded that this direct method is particularly suitable for routine use, in that it is completely uninfluenced by the excipients commonly used in contact lens solutions (Fig. 2). These excipients may prove to be a source of problems with other methods, in particular by affecting column life in direct-injection HPLC procedures [9, 13].

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