Journal of Pharmaceutical & Biomedical Analysis
Vol. 2, Nos 3/4, pp. 409–415, 1984
Printed in Great Britain

0731–7085/84 $3.00 + 0.00

Direct quantitation of antiseptic quaternary ammonium compounds by fast atom bombardment mass spectrometry

M. BAMBAGIOTTI ALBERTI[1]*, S. PINZAUTI[1], G. MONETI[2], G. AGATI[2], V. GIANNELLINI[1], S. A. CORAN[1] and F. F. VINCIERI[1]

[1] *Dipartimento di Scienze Farmaceutiche, Università di Firenze, Via G. Capponi 9, 50121 Firenze, Italy*
[2] *Centro di Spettrometria di Massa e Analisi Frammentografiche, Facoltà di Medicina e Chirurgia, Università di Firenze, Viale G.B. Morgagni 65, 50134 Firenze, Italy*

Abstract: The medicinally important quaternary ammonium salts benzyldimethyltetradecylammonium chloride (BDTA), cetylpyridinium chloride and benzethonium chloride, all afford, under fast atom bombardment (FAB) mass spectrometric conditions, abundant and persistent $[M-Cl]^+$ species usefully amenable to quantitative analysis with the aid of thioglycerol as a liquid FAB matrix. The use of BDTA as an internal standard allowed a direct, precise and accurate determination of cetylpyridinium and benzethonium chlorides, either as pure samples or in dosage forms, in the concentration range 0.05–2 mg/ml.

Keywords: *FAB mass spectrometry; quantitative application; quaternary ammonium salts determination; pharmaceutical preparations.*

Introduction

Various methods have been employed for the determination of quaternary ammonium compounds [1]. These non-specific methods consisted essentially of titrimetric or colorimetric procedures using antagonist dyes as reagents [2, 3].

In the last few years, a rapid evolution of the methods dedicated to this class of compounds has occurred, keeping pace with the development of particular chromatographic and potentiometric techniques. Thus, reversed-phase high-performance liquid chromatography was claimed to allow specific and direct quantitation of benzalkonium chloride in an ophthalmic system [4], even if the approach was criticized shortly thereafter [5], when it was proposed that an extraction step should be reintroduced in the procedure. On the other hand, potentiometry was recently employed successfully by one of the present authors [6, 7] for the rapid quantitation of cationic surfactants in pharmaceutical preparations without extraction procedures.

Attempts to apply mass spectrometry to quaternary ammonium compounds date back

* To whom correspondence should be addressed.

to 1965 [8], with results strictly depending on the new ionization methods becoming available from time to time. Thus, quaternary ammonium cations from quaternary ammonium salts have been observed by soft ionization techniques such as field desorption (FD) [9], ^{252}Cf plasma desorption (PD) [10], secondary-ion mass spectrometry (SIMS) [11], laser desorption (LD) [12] and very recently electron impact [EI]; apparently none of these approaches was undertaken primarily in order to develop a quantitative application. In the last few years, however, the fast atom bombardment (FAB) ionization technique has found increasing application in the analysis of polar and nonvolatile compounds, for which it possesses significant advantages compared with the other soft ionization methods in terms of simplicity and speed of execution.

In the work presented here, an investigation has been designed to establish the efficiency of FAB mass spectrometry as an analytical tool for the direct detection and quantitation, on a microsample scale, of quaternary ammonium compounds, both as pure samples and in pharmaceutical dosage forms.

Experimental

Materials

Analytical reagent grade benzyldimethyltetradecylammonium chloride (BDTA), benzethonium chloride U.S.P., cetylpyridinium chloride B.P. and benzalkonium chloride U.S.N.F., were obtained through normal commercial channels. Commercial disinfectant solutions and lozenges were purchased locally.

Apparatus

FAB mass spectra were obtained by means of a VG Analytical model 7070 EQ instrument fitted with its own standard FAB ion source employing argon atoms of 7-keV kinetic energy. Recordings in positive ion mode were taken at a resolution of 3000, with a run speed of 20 s/decade; data were processed by a Digital PDP8/A computer system.

Analytical procedures

To record full spectra required about 2–4 μl of a 0.005–0.1% m/v aqueous solution of each cationic surfactant, which was added by microsyringe to a layer of thioglycerol on the FAB target. For quantitative measurements BDTA was adopted as an internal standard. The calibration curves for benzethonium and cetylpyridinium chlorides in the appropriate concentration range were obtained simultaneously as follows: to a series of vials each containing 50 μl BDTA aqueous solution (2 μg/μl), suitable volumes (2–40 μl) of cetylpyridinium chloride (5 μg/μl) and 20–80 μl benzethonium chloride (5 μg/μl) stock solutions were added and the mixtures made up to 200 μl to obtain a series of working standards. A 2–4 μl quantity of each working standard was added to the FAB target as indicated above and measurements performed as usual in the multiple ion detection (MID) mode, using the intact cation peaks $[M-Cl]^+$ (where $M = R_4N^+Cl^-$) appearing at *m/z* 332, 304 and 412 for BDTA, cetylpyridinium chloride and benzethonium chloride, respectively. The procedure was repeated four times and the calibration curves of the area ratios between the analyte and internal standard cations, plotted against the concentration of the analyte surfactant in the working standard solution, were constructed from the averages obtained.

Cetylpyridinium chloride lozenges (1.42 mg each) were assayed by weighing and finely powdering five lozenges. A sample equivalent to 5 mg of cetylpyridinium chloride was

weighed and stirred magnetically for 15 min with 30 ml water. The slightly turbid solution was made up to volume in a 50-ml volumetric flask. To 100 μl of this solution, 50 μl BDTA standard solution and 50 μl water were added. An aliquot of 2–4 μl of this solution was placed on the FAB target and analysed as described above.

Cetylpyridinium chloride commercial disinfectant solution (0.2% m/v) was assayed simply by mixing a 50-μl aliquot with 50 μl BDTA standard solution and 100 μl water and placing 2–4 μl of this mixture on the FAB target.

Analysis of benzethonium chloride disinfectant solution (0.1% m/v) was performed in a similar manner, starting from a mixture of 150 μl commercial sample and 50 μl BDTA standard solution.

Results and Discussion

Calibration curves were rectilinear in the working standard solution concentration range 0.05–1 μg/μl for cetylpyridinium chloride (slope = 4.77, intercept = 0.023, r = 0.9986) and 0.5–2 μg/μl for benzethonium chloride (slope = 0.180, intercept = 0.013, r = 0.9906). A typical average recovery (n = 4) at the 0.5-μg/μl level was 100.8 ± 1.2% for benzethonium chloride and 100.6 ± 0.8% for cetylpyridinium chloride.

Table 1 lists the results obtained from three commercial dosage forms. FAB mass spectrometric analyses provided rapid, accurate and precise quaternary ammonium compound determinations in a wide range of amounts (about 5–150 μg) without interference from other labelled ingredients.

FAB mass spectrometry has thus been shown to be very suitable for determining quaternary ammonium compounds. In fact these compounds have a charged site, a pre-formed ion which does not have to be generated by bombardment, but only requires to be desorbed from the condensed phase. A large portion of the energy required to remove the quaternary ammonium cation from its counter-ion has already been provided as energy of solvation by the liquid matrix in which the sample is dissolved. Consequently, only a relatively small amount of energy is required to desolvate these pre-formed ions, which then give persistent, intense peaks and negligible or no fragmentation.

Full spectra of cetylpyridinium chloride, benzethonium chloride and BDTA aqueous solutions are reported in Fig. 1, together with the spectra of the two commercial disinfectant solutions, analysed by directly placing them undiluted into the ion source. The peaks of intact cations are largely predominant in all spectra. The small degree of fragmentation taking place with BDTA and benzethonium chloride is consistent with the structure, the fragment ions at m/z 240 and m/z 320 corresponding in both cases to the loss of toluene from $[M-Cl]^+$. Similar behaviour has been recently described in the analysis of benzalkonium chloride by LD mass spectrometry [15]. The spectrum of cetylpyridinium chloride also contains significant fragments, i.e. m/z 80, 93, 109, which are consistent with the loss of the lipophilic chain or its portions. It is noteworthy, from an analytical point of view, that the excipients present in the commercial solutions (Fig. 1, c and e) do not interfere at all with the detection of quaternary ammonium compounds under consideration.

A crucial requirement in the quantitative application of every MS ionization technique is to obtain a stable ion current during measurement in the MID mode. In the present case glycerol, the most widely employed FAB matrix, failed to afford the required stability. The use of thioglycerol, however, overcame the difficulty. A comparison

Table 1
Analyses on samples of the same batch of commercial dosage forms

Formulation*	Quantity of active principle taken† (μg)	Average recovery (%) (RSD, n = 4)	Average recovery by alternative method‡ (%) (RSD, n = 4)	Other declared ingredients
(A) Cetylpyridinium chloride disinfectant solution, 0.2% m/v	100	100.3 (0.4)	99.6 (0.3)	2-Phenoxyethanol, lignocaine, patent blue V
(B) Cetylpyridinium chloride lozenges, 1.42 mg	10	98.7 (1.3)	98.0 (1.6)	Benzyl alcohol, sucrose, essential oils, tartrazine
(C) Benzethonium chloride disinfectant solution, 0.1% m/v	150	100.4 (0.2)	100.7 (0.3)	Acetone, isopropyl acetate, ponceau 4R

* Manufactured by: (A) Farmacosmici (Italy); (B) Merrel (Italy); (C) Formenti (Italy).
† About 1/40 was transferred on to the FAB target.
‡ Cetylpyridinium and benzethonium disinfectant solutions: potentiometric titration with a silver–silver sulphide electrode [7]; cetylpyridinium lozenges: potentiometric titration with a mercury-coated platinum electrode [6].

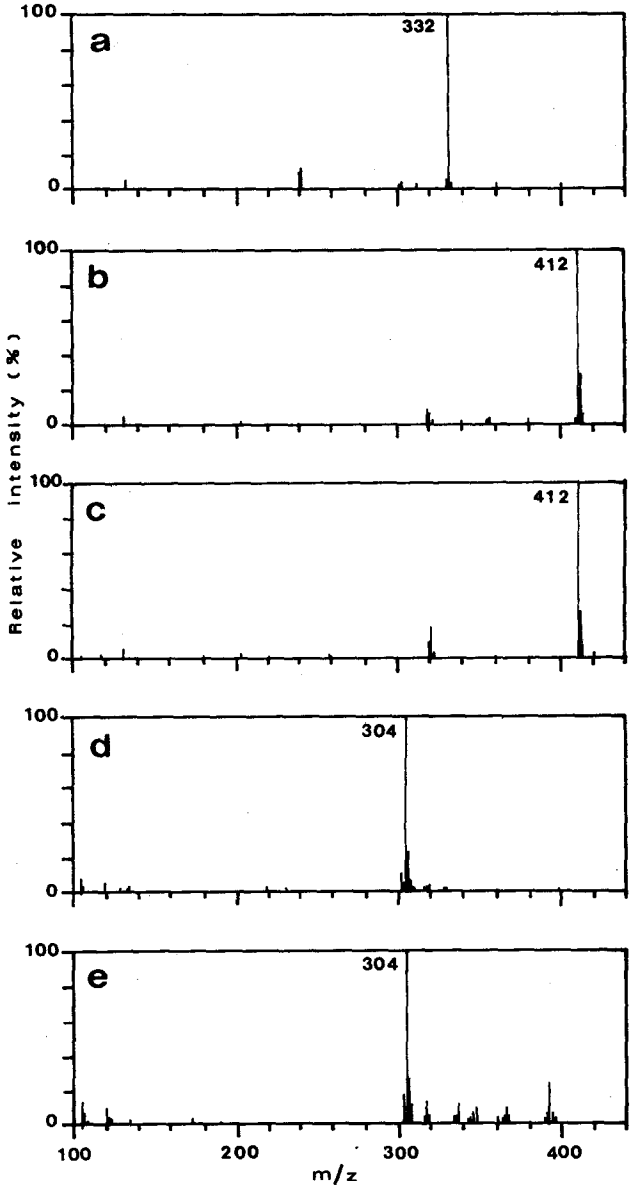

Figure 1
FAB mass spectra of BDTA (a), benzethonium chloride (b), 0.1% m/v benzethonium chloride disinfectant solution (c), cetylpyridinium chloride (d) and 0.2% m/v cetylpyridinium chloride disinfectant solution (e). All spectra were run in thioglycerol as a FAB matrix.

between the graphs reporting the rate of change of the ion current of each compound in glycerol (Fig. 2a) and thioglycerol (Fig. 2b) is enlightening in this respect. In glycerol, random and large variation in sputtering rate takes place independently for each compound; in thioglycerol this unwanted feature is suppressed and the individual ion current is consistent with the total ion current for several minutes, rendering the experiment amenable to quantitative application.

In preliminary experiments the FAB spectrum of the widely employed benzalkonium chloride has also been examined. As shown in Fig. 3 the spectrum is consistent with the

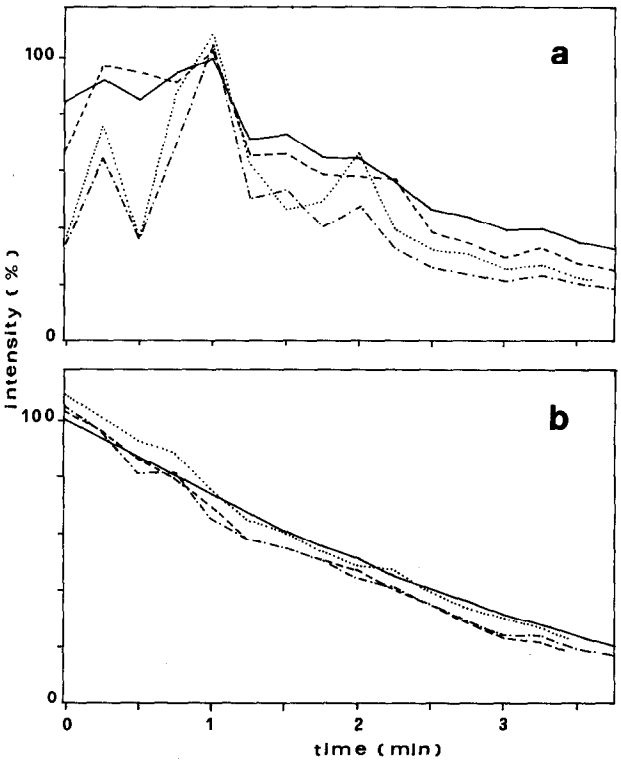

Figure 2
Data-system graphs of the rate of change of total ion current including matrix (———) and ion currents of *m/z* 304 cation (– – –) from cetylpyridinium chloride, of the *m/z* 332 cation (–·–) from BDTA, and of the *m/z* 412 cation (···) from benzethonium chloride, in the multiple ion detection mode: (a) in glycerol and (b) in thioglycerol FAB matrix.

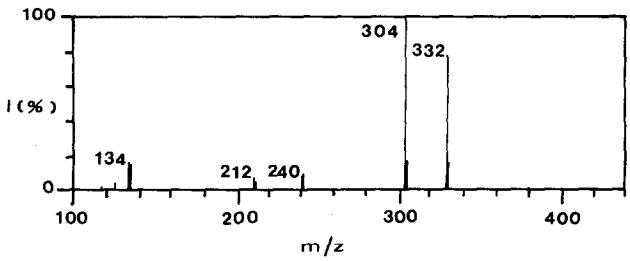

Figure 3
FAB mass spectrum of benzalkonium chloride in thioglycerol as a FAB matrix.

expected structure, ions at *m/z* 304 and *m/z* 332 corresponding to the intact cations with the R-alkyl $C_{12}H_{25}$ and $C_{14}H_{29}$, respectively. The spectrum is similar to that reported using LD mass spectrometry [15].

Direct inspection of the spectrum enables an approximate assessment to be made of the batch-to-batch variation in homologue composition. From the intensities of the intact cation peaks at *m/z* 304 and 332 appearing in Fig. 3, one can evaluate the percentages of the C_{12} and C_{14} homologues as 54 and 46% respectively, within the U.S.N.F. specifications. For a quantitative benzalkonium homologue assay, a suitable internal standard, different from the C_{14} homolog BDTA, should be found.

It is therefore apparent that the FAB technique is fully capable of quantitative performance, as illustrated in this first report of its application to quaternary ammonium compounds.

References

[1] M. J. Rosen and H. A. Goldsmith, *Systematic Analysis of Surface-Active Agents*, 2nd edn, pp. 444–464. Wiley–Interscience, New York (1972).
[2] J. T. Cross, *Analyst* **90**, 315–324 (1965).
[3] L. G. Chatten and K. O. Okamura, *J. Pharm. Sci.* **62**, 1328–1331 (1973).
[4] R. C. Meyer, *J. Pharm. Sci.* **69**, 1148–1150 (1980).
[5] D. F. Marsh and L. T. Takahashi, *J. Pharm. Sci.* **72**, 521–525 (1983).
[6] S. Pinzauti, E. La Porta, G. Papeschi and R. Biffoli, *J. Pharm. Belg.* **35**, 281–284 (1980).
[7] S. Pinzauti, G. Papeschi and E. La Porta, *J. Pharm. Biomed. Anal.* **1**, 47–53 (1983).
[8] M. Hesse, W. Vetter and H. Schmid, *Helv. Chim. Acta* **48**, 674–689 (1965).
[9] H. J. Veith, *Org. Mass Spectrom.* **11**, 629–633 (1976).
[10] B. Schueler and F. R. Krueger, *Org. Mass Spectrom.* **14**, 439–442 (1979).
[11] R. J. Day, S. E. Unger and R. G. Cooks, *J. Am. Chem. Soc.* **101**, 501–502 (1979).
[12] R. Stoll and F. W. Röllgen, *Org. Mass Spectrom.* **14**, 642–645 (1979).
[13] K. Isa and Y. Yamada, *Org. Mass Spectrom.* **18**, 229–233 (1983).
[14] C. Javanaud and J. Eagles, *Org. Mass Spectrom.* **18**, 93–97 (1983).
[15] K. Balasanmugam and D. M. Hercules, *Anal. Chem.* **55**, 145–146 (1983).

[*First received for review 27 January 1984; revised manuscript received 15 March 1984*]