

A Comparative Evaluation of Continuous Flow Fast Atom Bombardment and Ion Spray Ionization Techniques for the Simultaneous Determination of Alkyltrimethylammonium Surfactants by Mass Spectrometry

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This study compares the performances of two mass spectrometric ionization techniques, i.e. fast atom bombardment (FAB) and ion spray ionization (ISI), in the simultaneous quantitation of dodecyl-, tetradecyl- and hexadecyltrimethylammonium halides in aqueous media. Continuous flow FAB and flow injection analysis (FIA)-ISI, both in selected ion monitoring and selected reaction monitoring modes, were evaluated. Quantitation was performed by dilution with deuterium labelled homologues synthesized by a simple procedure. A comparison of the data indicated FIA-ISI as the more sensitive (limit of detection = 10 ppb). Good linearity, precision and accuracy were obtained by the tested techniques in the concentration range 0.125–4.0 ng μL^{-1} . Hair softeners, commercial surfactant mixtures and hematology lysing reagents were used as test samples. © 1998 John Wiley & Sons, Ltd.

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Alkyltrimethylammonium compounds are widely employed in many industrial applications and pharmaceutical and cosmetic preparations as softeners, antistatics, bactericides and also for the formulation of reagent solutions to be employed in instrumental blood analysis.

We report here a study aiming to define a direct, rapid and sensitive mass spectrometric procedure that can simultaneously quantify dodecyl-, tetradecyl- and hexadecyl trimethylammonium halides (DTA, TTA and HTA, respectively) in aqueous media. Both continuous flow fast atom bombardment (CF-FAB) and ion spray ionization (ISI) were considered as the mass spectrometric ionization techniques of choice for compounds existing as pre-formed ions. FAB-MS and ISI-based analytical procedures appear well suited to analysis of quaternary ammonium salts^{1–3} and competitive with other procedures^{4–6} especially those involving UV detection.⁷ Moreover ISI, a softer ionization technique, does not usually yield any fragmentation so that the analysis of quaternary ammonium ions can allow better sensitivity.⁸

We were interested in developing a method based on flow injection analysis (FIA) in order to obtain rapid simultaneous determination of the three analytes, without the use of any separation technique. Consequently, possible interferences from the matrices or the background must be minimized. For this reason when using ISI we tested tandem mass spectrometry (MS/MS), making a comparison

between selected ion monitoring (SIM) in single MS mode and selected reaction monitoring (SRM) in MS/MS mode.

EXPERIMENTAL

Materials

Unlabelled DTA-Br, TTA-Br and HTA-Br were purchased from Sigma-Aldrich (Milan, Italy); N,N,N-trimethyl-d9 labelled analogues were synthesized as previously described.⁹ Arquad MC-50, obtained from Akzo Chemicals (Amersfoort, The Netherlands), is a 50% w/w mixture of DTA-Cl, TTA-Cl and HTA-Cl (68:29:3) in water:isopropanol 1:1. The hematology lysing reagent solutions A–E were commercial samples. The hair conditioners F and G, selected as representative of complex matrix products, were purchased locally.

Equipment and operating conditions

CF-FAB spectrometric measurements in positive ion mode were carried out using a 7070-E mass spectrometer (VG Analytical, Manchester, UK) equipped with a standard FAB ion source and a computer system with a Teknivent Vector Two interface. The CF-FAB system consisted of a home-made probe, Rheodyne 7250 injector with a 0.5 μL internal loop (Rheodyne, CA, USA) and a fused-silica capillary column (1 m; 50 μm i.d.).

Acetonitrile:water:2,2'-dithiodiethanol¹⁰: oxalic acid saturated solution(75:23:1:1) was used as the mobile phase and delivered by a Kontron Micropump (Kontron Instru-

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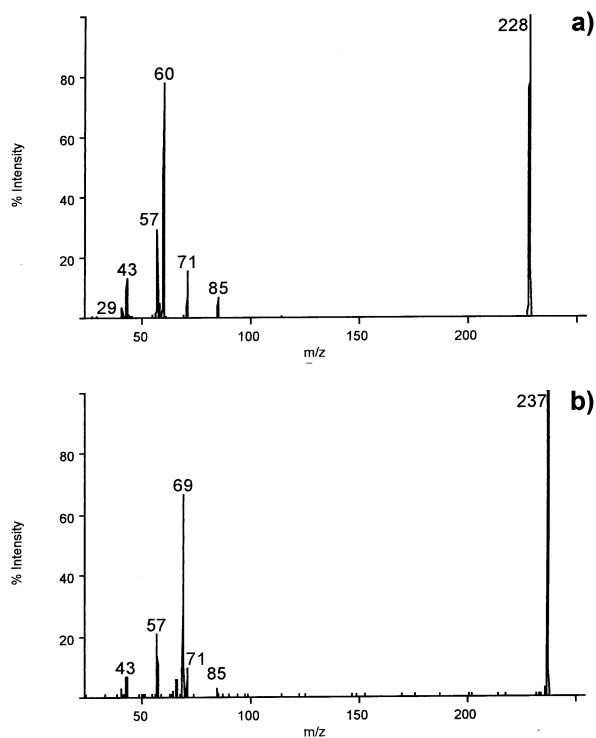


Figure 1. ISI-MS/MS (product ion spectra) of dodecyltrimethyl ammonium ion (a) and its nonadeuterated analog (b).

ments, Milan, Italy) at a flow rate of 2–3 $\mu\text{L min}^{-1}$. A 7–8 keV beam of xenon atoms was used as the primary beam.

Ion spray measurements were performed using a PE Sciex API III Plus triple quadrupole mass spectrometer (Sciex, Thornhill, Ontario, Canada) equipped with a Sciex API source and articulated ion spray interface. The experimental conditions used included: ion spray voltage (ISV) 5500 V, orifice voltage (OR) 60 V; selected ion monitoring, m/z 228.3, 237.4, 256.3, 265.4, 284.3, 293.4; 200 ms dwell time, total cycle time ~ 1.3 s. SRM: 228.3 \rightarrow 60.1, 237.4 \rightarrow 69.1, 256.3 \rightarrow 60.1, 265.4 \rightarrow 69.1, 284.4 \rightarrow 60.1 and 293.4 \rightarrow 69.1; dwell time 200 ms, total cycle time ~ 1.3 s; collision energy 30 eV, collision gas thickness (CGT) 230×10^{13} molecules/cm² (CGT is defined as the collision gas density times the length of the collision cell, and gives better information on the collision conditions with respect to the gas pressure).

The samples were injected by FIA using a Perkin-Elmer LC200 dual solvent delivery system equipped with a 7125 Rheodyne injector with a 4 μL loop. The mobile phase was a water: methanol (1:1) mixture flowing at 50 $\mu\text{L min}^{-1}$.

Standard and sample preparations

Three stock solutions, each containing 100 $\text{ng } \mu\text{L}^{-1}$ of the unlabelled target trimethylalkylammonium cation, were made by dissolving accurately weighed amounts of each trimethylalkylammonium bromide (DTA-Br 13.509 mg, TTA-Br 13.125 mg and HTA-Br 12.817 mg) in water: methanol (1:1), in 100 mL volumetric flasks. Similarly, three stock internal standard solutions, each containing 100 $\text{ng } \mu\text{L}^{-1}$ of the corresponding labelled cation, were prepared by weighing DTAd₉-Br 13.376 mg, TTAd₉-Br 13.019 mg and HTAd₉-Br 12.730 mg. Stock sample solutions were obtained by diluting the commercial products in

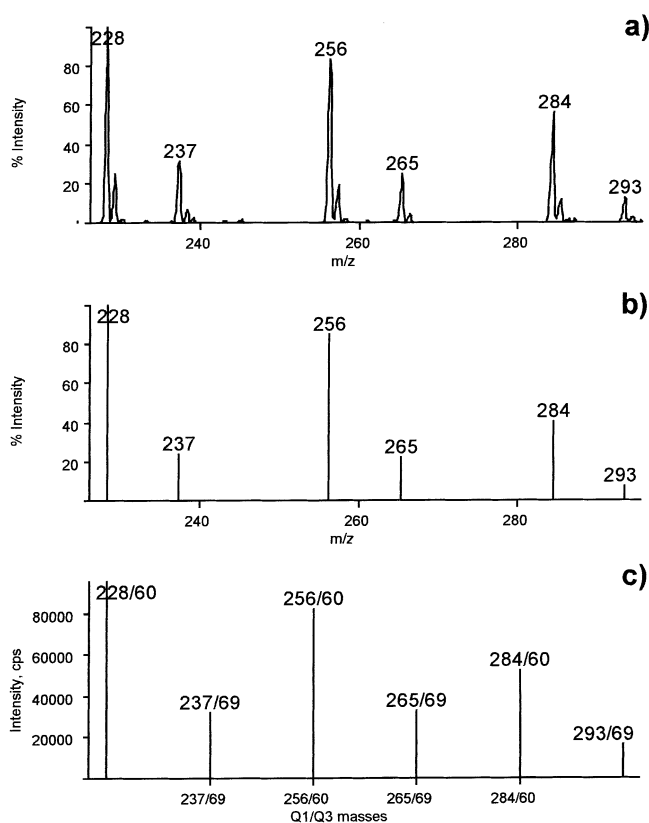


Figure 2. MS and reconstructed MS spectra of a standard mixture containing dodecyltrimethyl ammonium, tetradecyltrimethylammonium, hexadecyltrimethyl ammonium bromides and their nonadeuterated analogs. (a) Single MS, full scan mode, (b) single MS, SIM mode; (c) tandem MS, SRM mode.

water:methanol (1:1) as follows: Arquad MC-50, 504.0 mg to 50 mL; lysing reagent solutions A–E, 5.0 mL to 100 mL; hair conditioners FG, 250.0 mg to 50 mL.

Calibration and assay

500 μL of each labelled surfactant stock solution were mixed in a 25 mL volumetric flask and diluted with water:methanol (1:1) to obtain an internal standard working solution containing 2 $\text{ng } \mu\text{L}^{-1}$ of each TMAc₉ cation. A similarly prepared working solution with each analyte concentration of 8 $\text{ng } \mu\text{L}^{-1}$ was used to obtain, by serial dilution, six solutions (8.0, 4.0, 2.0, 1.0, 0.5, 0.25 $\text{ng } \mu\text{L}^{-1}$).

To a series of vials, each containing 100 μL of internal standard working solution, equal amounts (100 μL) of the six solutions were added, thus obtaining six calibration solutions each containing 1 $\text{ng } \mu\text{L}^{-1}$ of internal standard and 4.0, 2.0, 1.0, 0.5, 0.25 and 0.125 $\text{ng } \mu\text{L}^{-1}$ of chemical standard, respectively. Commercial and simulated samples were assayed by mixing 100 μL of opportunely diluted stock sample solutions with 100 μL of internal standard working solution. Three injections were made for each point of the calibration curves, as well as for analysis of each sample.

RESULTS AND DISCUSSION

CF-FAB spectra exhibit, together with a prominent cation peak, minor fragment ions not exceeding 3–5% relative abundance. The most important of these fragment ions

Table 1. Calibration parameters (linear regression)

TMAAC Cation	Technique	Correlation coefficient	Slope	Intercept
DTA	CF-FAB	0.9967	1.242	0.133
	ISI-SIM	0.9970	0.912	0.127
	ISI-SRM	0.9957	0.550	0.024
TTA	CF-FAB	0.9937	1.488	0.141
	ISI-SIM	0.9971	0.945	0.129
	ISI-SRM	0.9968	0.668	0.022
HTA	CF-FAB	0.9961	1.406	0.111
	ISI-SIM	0.9987	1.000	0.073
	ISI-SRM	0.9976	0.759	0.013

corresponds to the neutral loss of methane and therefore does not interfere with the simultaneous determination of the lower homologues. It is worth noting that, under the CF-FAB conditions used, neither cluster ions nor extensive fragmentation are observed.^{8,9} Moreover discrimination effects previously observed in analysing mixtures of homologues by static FAB,^{1,2} were not found.

ISI spectra of the title compounds are very simple, consisting essentially of only the molecular cation peak without any fragmentation. The spectra of the title mixtures are consequently free of mutual interferences. However, the relative response factors for equimolar concentrations are quite different (DTA : TTA : HTA = 1 : 0.65 : 0.45, for both unlabelled and labelled compounds).

On the other hand, a background ion at m/z 237 interfering with the DTAd₉ measurement motivated us to the use of MS/MS, even if the other cations can be reliably determined. As an example, the MS/MS product ion spectra of DTA and DTAd₉ are reported in Fig. 1, showing a simple fragmentation pattern consisting mainly of trimethylammonium ions at m/z 60 and 69, respectively. Alkyl fragments at m/z 29, 43, 57 and 85 are also present in lower abundances.

Table 2. Precision and recovery of TMAAC theoretical spike

TMAAC	Added (ng/μL)	Found (ng/μL)	Recovery (%)	CV (%)	Technique
DTA	0.50	0.54	109.2	3.42	CF-FAB
		0.56	111.3	1.04	ISI-SIM
		0.38	76.0	2.60	ISI-SRM
	1.00	0.98	97.9	2.54	CF-FAB
		1.27	127.3	0.91	ISI-SIM
		0.87	87.0	1.15	ISI-SRM
TTA	0.50	0.42	83.8	1.62	CF-FAB
		0.52	104.0	0.00	ISI-SIM
		0.44	88.0	2.27	ISI-SRM
	1.00	1.01	101.3	5.99	CF-FAB
		1.21	120.7	0.48	ISI-SIM
		0.98	98.0	0.00	ISI-SRM
HTA	0.50	0.49	97.4	4.96	CF-FAB
		0.50	100.7	2.29	ISI-SIM
		0.42	84.0	0.00	ISI-SRM
	1.00	1.02	102.5	1.99	CF-FAB
		1.15	115.3	2.18	ISI-SIM
		1.01	101.0	0.02	ISI-SRM

The higher homologues TTA, HTA, TTA₉ and HTA₉ behave similarly.

The quantitation was performed by SRM of the trimethylammonium ion formation (m/z 228 →60 for DTA; 237 →69 for DTAd₉; 256 →60 for TTA; 265 →69 for TTA₉; 284 →60 for HTA, and 293 →69 for HTA₉). We did not observe any cross-talk effect, potentially a problem when monitoring a common fragment ion from different precursors, as illustrated in Fig. 2.

Both CF-FAB and ISI (both SIM and SRM) gave linear calibration curves of FIA peak area ratios (analyte:deuterated standard) vs the corresponding concentration ratios in the 0.125–4.0 ng μL⁻¹ range. The calibration parameters are reported in Table 1.

During the analysis of the samples we found that, even

Table 3. Comparison of the results afforded by the analytical techniques tested

Sample	Technique	DTA (ng/μL)	CV (%)	TTA (ng/μL)	CV (%)	HTA (ng/μL)	CV (%)
Lyse A	CF-FAB	1.78	1.13				
	ISI-SIM	2.33	0.66				
	ISI-SRM	1.70	0.58				
Lyse B	CF-FAB	1.84	1.32	0.32	3.24		
	ISI-SIM	1.93	0.30	0.31	0.00		
	ISI-SRM	1.44	0.58	0.32	3.12		
Lyse C	CF-FAB	2.86	1.50			0.10	1.78
	ISI-SIM	3.66	0.42			0.07	7.87
	ISI-SRM	2.79	0.35			0.10	5.0
Lyse D	CF-FAB			1.51	3.7		
	ISI-SIM			3.11	1.03		
	ISI-SRM			1.60	0.62		
Lyse E	CF-FAB	2.68	0.79	0.23	1.87		
	ISI-SIM	3.29	1.39	0.19	0.00		
	ISI-SRM	2.57	0.39	0.22	4.54		
Arquad MC-50	CF-FAB	3.28	2.09	1.04	6.08	0.14	3.19
	ISI-SIM	3.94	0.82	1.69	0.34	0.15	3.77
	ISI-SRM	3.42	1.08	1.52	0.66	0.18	3.15
Hairconditioner F	CF-FAB					1.21	1.90
	ISI-SIM					1.02	2.48
	ISI-SRM					0.94	1.08
Hairconditioner G	CF-FAB					0.12	2.56
	ISI-SIM					0.10	5.59
	ISI-SRM					0.14	7.14

though the calibration curves were measured down to the $0.125 \text{ ng } \mu\text{L}^{-1}$ level, the real detection and quantitation limits appeared to be lower, as TMMAC concentrations of the order of $0.1 \text{ ng } \mu\text{L}^{-1}$ could still be determined.

To assess the accuracy we decided to proceed by recovery of theoretical spike from one of the most complex matrices examined, i.e. hair conditioner F. While no problems were encountered with ISI, CF-FAB suffered from interferences, presumably from some matrix component. To overcome this difficulty, the commercial sample Arquad MC-50 was employed to test the CF-FAB technique. The accuracy and precision data are summarized in Table 2.

Two hair conditioners, five hematology lysing reagents and the Arquad MC-50 (a commercial surfactant mixture), were employed as test samples after appropriate dilutions. The results, reported in Table 3, show a good agreement for the three instrumental approaches. However, ISI-SRM appears to be the technique of choice due to the higher sensitivity ($\text{LOD} = 10 \text{ pg } \mu\text{L}^{-1}$) and specificity. Alternatively, when a triple quadrupole or other MS/MS instrument is not available, satisfactory results can also be pursued by the other two techniques provided that specific interferences are not present.

In conclusion, the present procedure allows the simulta-

neous determination of the title surfactants without any complex sample pre-manipulation, in a very short time averaging two minutes per sample.

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