Journal of Chromatography, 478 (1989) 191–203
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 604

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF ALKYLAMIDOPROPYL-N,N-DIMETHYL-N-(2,3-DIHYDROXYPROPYL)AMMONIUM CHLORIDES IN AQUEOUS SOLUTIONS AND COSMETIC FORMULATIONS

ROLAND CAESAR[a], HENRY WEIGHTMAN and GILBERT R. MINTZ*[,b]
Inolex Chemical Company, Analytical Group, Philadelphia, PA (U.S.A.)
(First received December 12th, 1988; revised manuscript received April 26th, 1989)

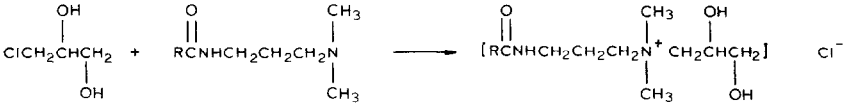
SUMMARY

A reversed-phase high-performance liquid chromatographic method is described to determine the quaternary ammonium compounds myristamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)ammonium chloride and oleamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)ammonium chloride in aqueous solutions and cosmetic formulations. Fractions containing quaternary chlorides are isolated from their reactants and by-products by semi-preparative liquid chromatography and are used as standards to quantify the quaternium compound in selected samples. Analytical liquid chromatography is performed by an ion-pairing reversed-phase technique using two alkyl/cyano columns. This method is applicable as a quality control assay procedure to quantify these cationics in finished cosmetic formulations.

INTRODUCTION

Alkylamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)ammonium chlorides were manufactured in 1952 as novel wetting and emulsifying agents[1]. Their use has extended into the personal care industry which results in a need to determine the amount of these quaternary ammonium compounds in aqueous solutions and cosmetic formulations[2–4]. These cationics offer excellent conditioning and emulsifying properties in a broad range of cosmetic formulations[4].

The quaternium chlorides are synthesized by alkylation of alkylamidopropyldimethylamines with α-monochlorohydrin in aqueous solution at 80–85°C and pH 8.0–8.5:



[a] Present address: Ethyl Corporation, Orangeburg, SC, U.S.A.
[b] Present address: Centerchem, Inc., 660 White Plains Road, Tarrytown, NY 10591, U.S.A.

0021-9673/89/$03.50

The possible impurities present in the product may be unreacted alkylamidopropyldimethylamine and α-monochlorohydrin. Other impurities that may be present are sodium chloride that results from the neutralization of sodium hydroxide with hydrochloric acid and glycerol that results from the nucleophilic substitution of chlorine in α-monochlorohydrin by hydroxide.

The determination of quaternary ammonium compounds is classically performed by potentiometric titration with anhydrous perchloric acid in the presence of mercury(II) acetate[5]. However, the presence of sodium chloride interferes with the determination because mercury(II) acetate complexes with chloride to form titratable acetate resulting in a falsely high result. Another classic determination involves titration of a quaternary ammonium chloride with standard sodium lauryl sulfate in a two phase water–chloroform system using methylene blue as the indicator[5]. Ideally the endpoint is observed when the intensity of the blue color is equally distributed between the two phases. However, with these particular quaternary compounds, the alkylamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)ammonium–methylene blue complex is not soluble in chloroform and chloroform–alcohol solutions making endpoint detection impossible.

Another determination involves the formation of a water insoluble complex between the quaternary ammonium compound and hexacyanoferrate(III) ion[5]. Using this method, we determined the quaternary ammonium chloride concentration in an aqueous solution of Lexquat® AMG-O[a]. We found excellent correlation between this method and the quantity of desired product calculated from the amount of residual reactants remaining in the product. However, a low value was obtained in a complex cosmetic formulation containing additional surfactants.

In recent years, several papers have been published on the determination of quaternary ammonium compounds by high-performance liquid chromatography (HPLC) with reversed-phase packing containing octadecyl and alkylcyano silane groups chemically bonded to silica gel[6–8].

Ion-pair reversed-phase chromatography was chosen because without ion-pairing the quaternary ammonium chlorides gave tailing peaks when used with an alkylcyano stationary phase. The ion-pairing technique was also chosen because the capacity factor (k') of the analyte did not change with concentration when trifluoroacetic acid was used as a counter-ion.

A reversed-phase liquid chromatography method has been developed for quantitation of the surfactant(s) in the Lexquat® AMG product line. Furthermore, this procedure separates the alkylamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)-ammonium chlorides from process impurities and was used to quantify the level of quaternary ammonium chloride in two cosmetic formulations.

EXPERIMENTAL

Reagents for synthesis of quaternaries

Myristic acid is commercially available at greater than 95% purity from Emery

[a] Lexquat AMG-O is a registered trade name for an aqueous solution of oleamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)ammonium chloride.

and oleic acid was obtained from Witco with a greater than 70% $C_{18:1}$ content (Industrene 205). α-Monochlorohydrin at greater than 99% purity was obtained from Dixie (Houston, TX, U.S.A.).

Reagents and chemicals

HPLC grade acetonitrile, tetrahydrofuran (THF) and methanol (Burdick and Jackson Labs., Muskegon, MI, U.S.A.) and trifluoroacetic acid (Aldrich, Milwaukee, WI, U.S.A.) were used as components in various mobile phases.

Apparatus

The instrument employed was a Waters Assoc. high-performance liquid chromatography Model ALC-201 equipped with a Model 6000A pump and Model 401 differential refractometer. Chromatograms were obtained using a Waters Assoc. Model 730 data module. Infrared spectra were obtained using an IBM Model 32 IR spectrometer. NMR spectra were obtained using an Bruker WP 270 SY spectrometer at Betz Labs., Trevose, PA, U.S.A.

Semi-preparative chromatography

Separation of the quaternary ammonium chloride from impurities was accomplished using a 30 cm × 7.8 mm I.D. μBondapak C_{18} column. The mobile phase used was water–methanol (25:75, v/v) for Lexquat AMG-O and Lexquat AMG-M[a]. The flow-rate was 1.5 ml/min. The fraction containing the quaternary ammonium chlorides and mobile phase was allowed to dry by evaporation in a hood overnight to remove the bulk of the remaining solvent, and further dried at 80°C at atmospheric pressure for 8 h. The samples were then placed in a vacuum oven (25 mmHg) for three hours until a constant weight (agreed to between 0.04% of each other) was obtained[9,10].

Analytical chromatography

Oleamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)ammonium chloride and myristamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)ammonium chloride in aqueous systems were determined by reversed-phase ion-pairing liquid chromatography. Two alkyl/cyano columns (μBondapak CN, Waters Assoc.) 15 cm × 4 mm I.D. were used in series. The mobile phase used for the analytical HPLC in assaying the quaternary ammonium chloride in Lexquat samples was prepared by mixing 1100 ml of water, 900 ml of acetonitrile and 2 ml of trifluoroacetic acid. The same mobile phase was used to quantify oleamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)-ammonium chloride in the skin moisturizer formulation containing Lexquat AMG-O. The mobile phase used for the determination of myristamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)ammonium chloride in the clear conditioning shampoo containing Lexquat AMG-M was prepared by mixing 1140 ml of water and 840 ml of acetonitrile, 20 ml THF and 2 ml of trifluoroacetic acid.

[a] Lexquat AMG-M is a registered trade name for an aqueous solution of myristamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)ammonium chloride.

Standard and sample preparation

Approximately 50 mg of standard obtained from the semi-preparative liquid chromatography, approximately 200 mg of aqueous Lexquat AMG sample, and approximately 650 mg of the prototype formulation were accurately weighed into separate 10-ml volumetric flasks and diluted to volume with the mobile phase.

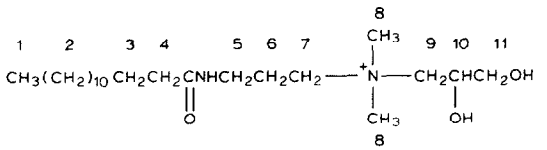
Characterization of isolated quaternary chloride homologues

Elemental and infrared analyses. The evaporated fractions from the semi-preparative liquid chromatography containing the quaternary chlorides were dried at 80°C at atmospheric pressure for 8 h then placed in a vacuum oven (25 mmHg) for 3 h and until a constant weight (agreed to between 0.04% of each other) was obtained prior to submission to Galbraith Laboratories (Knoxville, TN, U.S.A.) for elemental analyses. The elemental analysis obtained for the myristyl and oleyl homologues were, respectively: calculated for $C_{22}H_{47}N_2O_3Cl$: C, 62.48%; H, 11.12%; N, 6.63%; O, 11.36%; Cl, 8.40%. Found: C, 62.00%; H, 11.30%; N, 6.50%; O, 11.75%; Cl, 8.45%. Calculated for $C_{26}H_{55}N_2O_4Cl$: C, 63.09%; H, 11.12%; N, 5.66%; O, 12.94%; Cl, 7.18%. Found: C, 63.11%; H, 10.71%; N, 5.58%; O, 13.23%; Cl, 7.75%.

An infrared spectrum was obtained on the dried residue of myristamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)ammonium chloride isolated by semi-preparative procedures and the following peak assignments were made: 3299 cm^{-1} due to the hydroxyl stretch, 1653 cm^{-1} due to carbonyl stretch vibration of amide (amide I band), and 1545 cm^{-1} due to N–H bending vibration of amide (amide II band). The IR spectrum of the isolated oleamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl) ammonium chloride fraction provided the following peak assignments; 3286 cm^{-1} due to the hydroxyl stretch vibration, 1650 cm^{-1} due to the carbonyl stretch vibration of the amide (amide I bond), 1547 cm^{-1} due to the N–H bending vibration of amide (amide II bond), 3007 cm^{-1} due to the olefinic C–H stretch vibration and 985 cm^{-1} due to the C–H out of plane bending vibration.

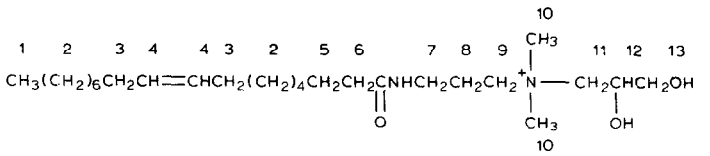
NMR analysis. The isolated fractions from the semi-preparative liquid chromatography containing the myristyl and predominately the oleyl quaternary chlorides were subjected to ^1H NMR analysis in ^2H_2O. The proton assignments are as follows:

myristyl:



Myristamidopropyl-N,N-dimethyl-(2,3-dihydroxypropyl)ammonium chloride: ^1H NMR (^2H_2O) δ 0.92–0.94 (t, 3 H, No. 1 protons), δ 1.25 (m, 20 H, No. 2 protons), δ 1.58 (m, 2 H, No. 3 protons), δ 1.90–2.15 (m, 2 H, No. 4 protons), δ 2.15–2.35 (m, 2 H, No. 6 protons), δ 3.11–3.21 (d, 6 H, No. 8 protons), δ 3.21–3.38 (m, 2 H, No. 7 protons), 3.38–3.75 (m, 6 H, No. 5, No. 9, and No. 11 protons), 4.18–4.42 (m, 1 H, No. 10 proton).

oleyl:



Oleamidopropyl-N,N-dimethyl-(2,3-dihydroxypropyl)ammonium chloride: ^1H NMR (^2H_2O) δ 0.82–0.94 (t, 3 H, No. 1 protons), δ 0.95–1.47 (m, 20 H, No. 2 protons), δ 1.48–1.70, (m, 2 H, No. 5 protons), δ 1.80–2.13 (m, 6 H, No. 3 and No. 6 protons), δ 2.20–2.38 (m, 2 H, No. 8 protons), δ 2.84–3.20 (d, 6 H, No. 10 protons), δ 3.20–3.35 (m, 2 H, No. 9 protons), δ 3.37–3.72 (m, 6 H, No. 7, No. 11 and No. 13 protons), δ 4.18–4.36 (m, 1 H, No. 12 proton), δ 5.18–5.41 (d, 2 H, No. 4 protons).

RESULTS AND DISCUSSION

The alkylamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)ammonium chloride for myristic and oleic acids were synthesized and separated from unreacted impurities by semi-preparative liquid chromatography. Fig. 1A and B shows the preparative HPLC chromatogram of Lexquat AMG-M and Lexquat AMG-O, respectively. In separate chromatograms, we established the retention volumes of the possible reactants and any by-products: 3-chloro-1,2-propanediol, glycerol, sodium chloride and myristamidopropyl dimethylamine and oleamidopropyl dimethylamine and related alkylamidopropyl dimethylamine homologues present in commercially available oleic acid to ensure that these components were well resolved from the quaternary chloride of interest (see Fig. 1). We observed that the unalkylated alkylamidopropyldimethylamine does not elute using this particular column and mobile phase.

Having identified the peak(s) due to the alkylamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)ammonium chlorides the chromatographic separation was repeated a number of times on a semi-preparative scale to obtain additional quantities of the myristyl quaternary present in Lexquat AMG-M and all homologues of alkylamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)ammonium chlorides contained in Lexquat AMG-O, greater than 70% of which is the $C_{18:1}$ oleyl. The appropriate fractions containing the quaternary chloride(s) of interest were collected and the mobile phase evaporated to concentrate the desired ingredient(s).

As shown in Fig. 1B, the minor components (members of homologous series) present in Lexquat AMG-O were identified in the semi-preparative chromatogram of the figure legend.

As shown in Fig. 2, the absence of the reactants and by-products in the myristyl and oleyl amidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)ammonium chlorides was confirmed by reversed-phase ion-pairing liquid chromatography. Furthermore, Fig. 2A indicates that the myristamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)ammonium chloride fraction contains only one peak. It should be noted that the oleyl fractions of fatty acids used to prepare these surfactants contain additional alkyl chain lengths and varying degrees of unsaturation. Therefore, the oleyl fraction (see Fig. 2B) contains many quaternary chlorides of different alkyl chain lengths which,

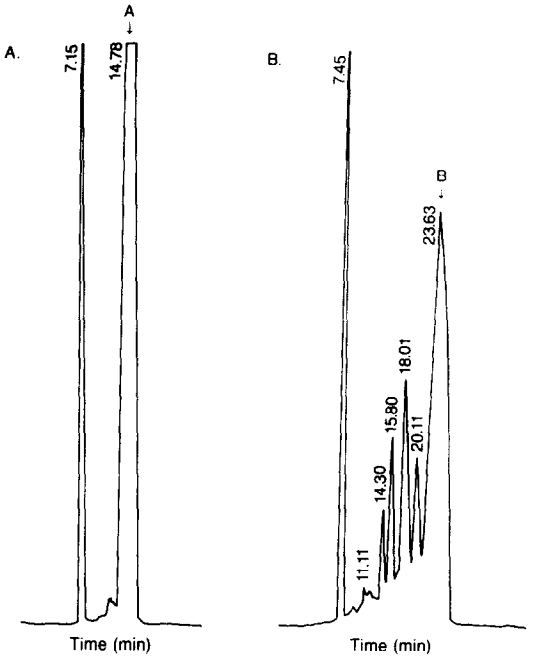


Fig. 1. Semi-preparative isolation of the surfactant components in Lexquat AMG-M and AMG-O on a μBondapak C_{18} reversed-phase column. (A) represents myristamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)ammonium chloride and (B) is a chromatogram of oleamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)ammonium chloride. The various quaternary homologues present in oleyl fraction were identified as follows: 14.30 min, myristyl; 15.80 min, palmitoleic; 18.01 min, linoleic; 20.11 min palmitic; 23.63 min oleic. The amount of each cationic injected onto the column was 15 mg.

within our limits of detection, is consistent with the known fatty acid composition of the starting material. As a manufacturer of large quantities of these quaternary chlorides for the cosmetics industry the economical considerations are such that we cannot use highly purified forms of the oleic acid, nor would it be necessary for specific applications in personal care formulations. We routinely use commercially available "oleic acid" which typically contains 2.4% myristic acid, 1.4% myristoleic acid, 0.2%

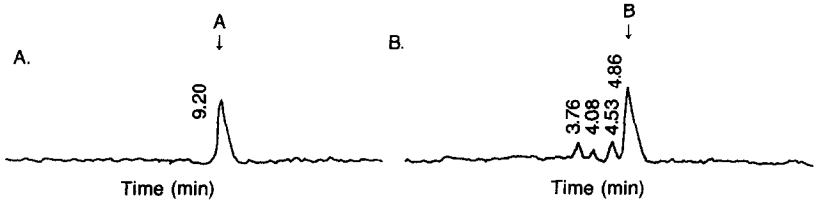


Fig. 2. Analytical chromatogram of (a) myristamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)ammonium chloride and (B) oleamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)ammonium chloride obtained by reversed-phase ion-pairing liquid chromatography. The amount of myristyl homologue injected onto the column was 52 μg and the amount of oleyl homologue was 48 μg. The various homologues present in the oleyl fraction have been identified as follows: 3.76 min, palmitoleic; 4.08 min, linoleic; 4.53 min, palmitic and 4.86 min, oleic.

myristolinoleic, 5.2% palmitic acid, 6.4% palmitoleic acid, 1.2% palmitolinoleic acid, 2.1% stearic acid, 72.7% oleic acid, 7.6% linoleic acid and 0.7% linolenic acid as determined by gas chromatography. The use of fatty acids containing other homologs to prepare the alkyl amidopropyldimethylamines prior to alkylation with α-monochlorohydrin will yield quaternary chlorides that contain various alkyl chain lengths. We have not attempted to identify and/or resolve in our chromatographic system the quaternary chlorides of the fatty acids that comprise less than 5% of the over all cationic species. We manufacture these products using the best available fatty acids with the highest concentration of alkyl groups of interest (*i.e.* myristyl and oleic) to prepare products with the desired functional properties. The presence of other alkyl homologues in the final product does not detract from the functional properties of these molecules.

Samples of the chromatographically isolated quaternary fractions and dried forms of the myristyl, oleyl and related homologues were subjected to elemental analysis (see Experimental). The results indicate that the desired compounds were obtained. Despite rigorous drying the oleyl homologue was dried consistently to the monohydrate as calculated from the elemental analysis.

Additional characterization of the isolated quaternary fractions was done by Fourier transform (FT)-IR and NMR (see Experimental). The FT-IR and NMR data indicate that the absorbances and signals observed from the respective instruments are consistent with the structures of the predominant cationic species in both standards. Furthermore, the absence of extraneous absorbances coupled with the expected integration values are consistent with the proposed structures of both standards.

The calibration plot for myristamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)ammonium chloride is shown in Fig. 3. This calibration curve was obtained using acetonitrile–water (45:55) containing 0.1% trifluoroacetic acid as mobile phase and was used to assay Lexquat AMG-M. A separate calibration curve was generated using acetonitrile–water–THF (42:57:1) containing 0.1% trifluoroacetic acid as mobile phase for quantifying the myristyl homologue in the clear conditioning shampoo formulation (standard curve not shown). The linearity of response for the myristyl standards was tested between 50–420 μg equivalent to 5–40% for the Lexquat AMG-M in aqueous solutions and includes a concentration equivalent to 1–10% in cosmetic formulations. The correlation coefficient (r) by peak area was 0.999.

The calibration plot for the oleamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)ammonium chloride containing fraction is shown in Fig. 4. This calibration curve was obtained using acetonitrile-water (45:55) containing 0.1% trifluoroacetic acid as mobile phase and was used to assay both the Lexquat AMG-O and the skin moisturizer formulation. The linearity of response of the oleyl standards was tested between 48–600 μg equivalent to 6–54% for Lexquat AMG-O in aqueous solutions and a concentration range equivalent to 1–16% in the cosmetic formulations. The correlation coefficient (r) by peak area measurements was 0.992.

As a routine procedure in the laboratory we were interested in establishing the accuracy and precision for determining alkylamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl) ammonium chloride levels in production batches of the aqueous solutions of Lexquat AMG-M and AMG-O. Table I summarizes the determination of each alkylamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)ammonium chloride using the analytical HPLC methods described in this paper. The Lexquat

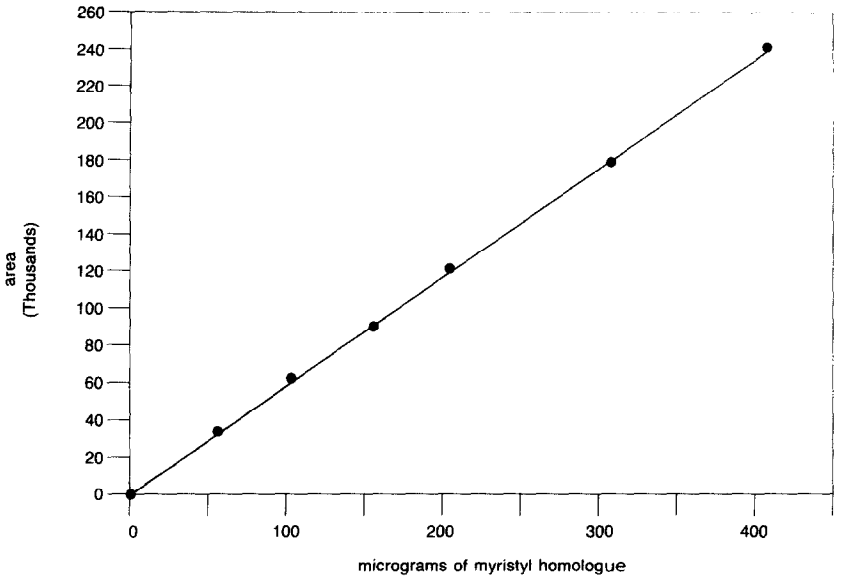


Fig. 3. Standard curve for the purified myristyl quaternary chloride. Chromatographic conditions were the same as Fig. 2A. Quantitative determination was based on peak area of the ion-pair.

AMG-M containing sample showed an error of 0.29% and relative standard deviation (R.S.D.) of 1.33% in six different runs. For the Lexquat AMG-O six different runs yielded an error of less than 1.41% and R.S.D. of 0.69%. The concentration of the analyte was measured by peak area. It is apparent that this analytical procedure

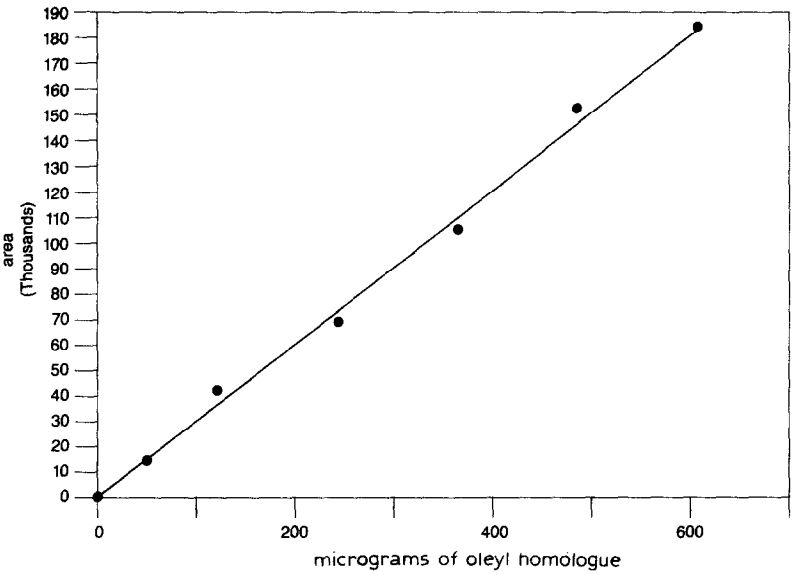


Fig. 4. Standard curve for the purified oleyl quaternary chloride. Chromatographic conditions were the same as Fig. 2B. Quantitative determination was based on peak area of the ion-pair.

TABLE I

ACCURACY AND PRECISION DATA FOR THE DETERMINATION OF QUATERNARY AMMONIUM CHLORIDES IN AQUEOUS SOLUTIONS

Weight percentages were determined by HPLC; the chromatographic conditions were the same as in Fig. 2. The analyses based on peak area of ion-pair.

Run no.	*Weight(%)*	
	Lexquat AMG-M	*Lexquat AMG-O*
1	34.5	28.7
2	33.5	28.8
3	34.1	28.8
4	34.2	28.6
5	34.8	29.0
6	33.9	28.6
Actual quaternary ammonium chloride level	34.1	28.4
Mean (%)	34.2	28.8
R.S.D. (%)	1.33	0.69
Error (%)	0.29	1.41

provides a reproducible method for quantifying the quaternary chlorides of interest in production batches.

The applicability of our analytical procedure for quantifying the quaternium ammonium compound in a complex cosmetic formula was demonstrated by determining the surfactant levels of Lexquat AMG-M and AMG-O in two different personal care prototypes. Two formulations were prepared according to methods described previously[4,11]; a clear conditioning shampoo containing Lexquat AMG-M (see Table II) and a skin moisturizer containing Lexquat AMG-O (see Table III). For purposes of this paper, we did not include fragrances usually found at low levels ($< 1.0\%$) in preparing these formulations. With regard to the chromatographic separations, it should be noted that fragrances are usually composed of aldehydes, ketones or hydrocarbons. Non-polar compounds of this type would tend to be strongly retained on the column and thus not interfere with the cationics being quantified.

Determination of myristamidopropyl-N,N-dimethyl-N-(2,3;dihydroxypropyl) ammonium chloride in the clear conditioning shampoo required a change in solvent strength of the mobile phase. As shown in Fig. 5, a multiplicity of peaks due to various components was obtained in the analytical chromatogram for the clear conditioning shampoo. It is apparent that the myristyl quaternary component (A) was sufficiently resolved from the other components in the formulation for quantification. The resolution (R_s) equals 0.9. A list of the components in the shampoo is shown in Table II.

A chromatogram of the skin moisturizer containing Lexquat AMG-O is shown in Fig. 6. It is apparent that the major cationic species (B) present in this fraction (oleyl quaternary) was sufficiently resolved from the other components in the formulation for quantification. R_s was equal to 0.7. The components in this formulation are listed in Table III.

TABLE II

CLEAR CONDITIONING SHAMPOO CONTAINING LEXQUAT AMG-M

Procedure: add ingredients of part B to the water and heat to 60°C. When materials are completely dissolved, add part A to part B. Maintain temperature and mix. When uniform add Lexquat AMG-M to the mixture. Cool to room temperature and adjust the pH to 6 with citric acid. Fragrance was not included.

Component	*Weight (%)*
Part A	
Sodium C_{14-16} olefin sulfonate	4.63
Triethanolamine lauryl sulfate	9.85
Laura/myristamidopropyl betaine	9.82
Diethanolamide of coconut fatty acid	2.94
Potassium coco-hydrolyzed protein	2.94
Part B	
Water	64.37
Propylene glycol	2.90
Methyl paraben	0.29
Propyl paraben	0.10
Benzophenone-4	0.10
Tetrasodium EDTA	0.10
Myristamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)ammonium chloride	1.76
Citric acid	0.20

TABLE III

SKIN MOISTURIZER CONTAINING LEXQUAT AMG-O

Procedure: add hydroxyethyl cellulose to water while mixing and heating to 78°C. When the cellulose is completely hydrated, add remaining material of part A. Combine part B in a separate vessel and heat to 78°C. When uniform slowly add part B to part A maintaining mixing and temperature. Allow to mix at 78°C for 15 min then cool to room temperature. Fragrance was not included.

Component	*Weight (%)*
Part A	
Water	69.76
Hydroxyethyl cellulose	0.88
Glycerol	2.93
Propylene glycol	1.95
Methylparaben	1.95
Oleamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)ammonium chloride	1.91
Propylene glycol dinonanoate	14.66
Part B	
Glyceryl mono-, di-, and tristearates	1.95
Myristyl myristate	0.98
Stearyl alcohol	1.95
Cetyl alcohol	0.98
Propyl paraben	0.10

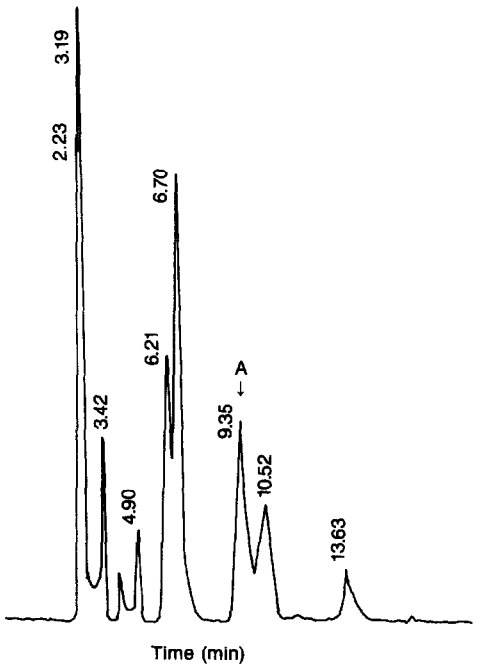


Fig. 5. Analytical separation and quantitation by HPLC of myristamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)ammonium chloride (peak A) in a clear conditioning shampoo. Other components in the formulation were not identified. Chromatographic conditions were as described under Experimental, *Analytical chromatography*.

The resolution obtained by the chromatographic systems described was suited for both cosmetic formulas as demonstrated by the accuracy and precision of quantifying the myristyl and oleyl quaternaries in six separate runs, as indicated in Table IV.

For the myristyl quaternary present in the clear conditioning shampoo we observed an error of 1.14% and R.S.D. of 2.81% in six different runs. For the Lexquat AMG-O containing skin moisturizer six different runs yielded an error of 2.62% and R.S.D. of 3.78%. The concentration of each cationic, identified in separate chromatograms using the appropriate mobile phases was determined by peak area. This HPLC procedure provides a reliable and reproducible method for quantitating each of these quaternary chlorides in a complex cosmetic formulation. In contrast, classical wet analysis methods are currently not available for quantifying the different types of quaternary surfactants commonly used in formulations.

Given the complex nature of individual cosmetic formulations, different prototypes may require changes in mobile phase and/or columns to obtain the desired resolution of the quaternary ammonium chloride and thus accurately quantify the level of the alkylamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)ammonium chloride in a personal care cosmetic formulation.

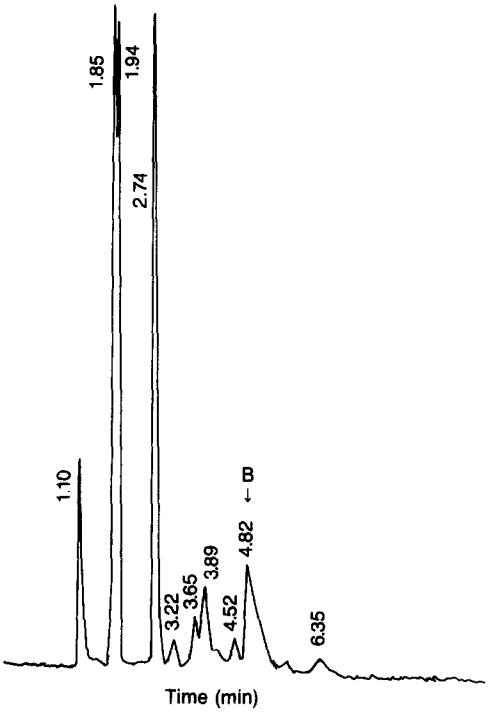


Fig. 6. Analytical separation and quantitation by HPLC of oleamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)ammonium chloride (peak B) in a skin moisturizer formulation. Other components in the formulation were not identified. Chromatographic conditions were as described under Experimental, *Analytical chromatography*.

TABLE IV

ACCURACY AND PRECISION FOR DETERMINATION OF ALKYLAMIDOPROPYL-N,N-DIMETHYL-N-(2,3-DIHYDROXYPROPYL)AMMONIUM CHLORIDE IN TWO COSMETIC FORMULATIONS

Weight percentages were determined by HPLC. Analyses based on peak area of the ion-pair.

Run No.	*Weight (%)*	
	Clear conditioning shampoo Lexquat AMG-M[a]	*Skin moisturizer Lexquat AMG-O*[b]
1	1.80	1.83
2	1.78	1.92
3	1.79	1.98
4	1.79	2.01
5	1.69	2.02
6	1.82	2.01
Amount (%) added to formulation	1.76	1.91
Mean (%)	1.78	1.96
R.S.D. (%)	2.81	3.78
Error (%)	1.14	2.62

[a] Chromatographic conditions were the same as in Fig. 5.
[b] Chromatographic conditions were the same as in Fig. 6.

CONCLUSIONS

The HPLC method described provides an accurate and reproducible procedure for quantifying the levels of two quaternary alkylamidopropyl-N,N-dimethyl-N-(2,3-dihydroxypropyl)ammonium chlorides in aqueous solutions and cosmetic formulations. Classical methods of potentiometric titrations and dye-binding methods were not suitable for quantitation of these quaternary compounds.

Accordingly, we used reversed-phase liquid chromatography to purify the myristyl and oleyl homologues from production batches of these cosmetic raw materials. Having isolated and identified the components of interest we used analytical ion-pairing chromatography to accurately quantify the levels of both quaternaries in production batches of the raw materials and two prototype personal care formulations.

ACKNOWLEDGEMENTS

We acknowledge the work of Tom Grebenar and Rocco Burgo in preparing Lexquat AMG-M and AMG-O. We thank B. Gesslein, L. Smith and T. Grebenar for useful discussions and for reviewing the manuscript.

REFERENCES

1 E. Cook and P. Moss, *U.S. Pat.*, 2,589,674 (1952).
2 A. Patel and H. Greenland, *U.S. Pat.*, 4,726,945 (1988)
3 D. E. Conner and A. W. Fogel, *U.S. Pat.*, 4,012,398 (1977).
4 J. J. Guth, G. M. Reinhart, L. R. Smith, B. W. Gesslein and G. R. Mintz, *U.S. Pat.*, pending (1988).
5 M. J. Rosen and H. A. Goldsmith, *Systemic Analysis of Surface-Active Agents*, Wiley, New York, 2nd ed., 1972, p. 445.
6 L. J. Cohn, V. J. Greely and D. L. Tibbetts, *J. Chromatogr.*, 321 (1985) 401.
7 N. Parris, *J. Liq. Chromatogr.*, 3(11) (1980) 1743.
8 G. Ambrus, L. T. Takahashi and P. A. Marty, *J. Pharm. Sci.*, 76(2) (1987) 174.
9 C. Paquot, *Standard Methods for the Analysis of Oils, Fats and Derivatives, Part 1*, Pergamon Press, New York, 6th ed., 1978, p. 8.
10 H. A. Boekenoogen, *Analysis and Characterization of Oils, Fats and Fat Products, Vol. 1*, Wiley, New York, 1964, p. 13.
11 B. Gesslein and L. Smith, *Inolex Technical Bulletin*, 1988, personal communication.