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Note

Direct gas chromatographic method for determining the homologue composition of benzalkonium chlorides

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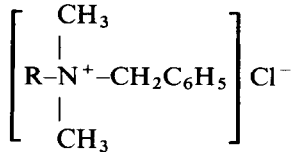
Benzalkonium chlorides (I) are surface active agents which exhibit antiseptic properties. They have found widespread use in many applications, which include pharmaceutical preparations and laundry and sanitation products.



(I)

In most benzalkonium chlorides (where R represents a saturated straight fatty chain of varying length), the C_{12}, C_{14} and C_{16} predominate, while C_{18} may also be present. We are interested in the determination of the homologue distribution of benzalkonium chlorides since it has a bearing on the tariff classification of these goods imported into Canada.

Various techniques have been applied to the quantitative analysis of the cationic surfactant. For example, reversed-phase high-performance liquid chromatography was developed to quantitate benzalkonium chlorides in an ophthalmic system[1]; mass spectrometry using unconventional soft ionization techniques such as fast atom bombardment[2] and laser[3] has also been attempted on benzalkonium chlorides.

We favor a gas chromatographic (GC) approach since the gas chromatograph is one of the principal analytical instruments in our laboratory. GC methods used for the determination of the homologue composition of benzalkonium chlorides usually involve the use of strongly alkaline columns[4–6] which are not favoured in GC because of their poor stability and short lifetime. Other GC approaches require sample pretreatment such as debenzylation[7], conversion to alkoxide[8] and Hofmann degradation[9] before GC analysis. A direct injector pyrolysis method using a non-alkaline column has been reported by Uno *et al.*[10] but the results reported were not quantitative.

In this paper, we describe the quantitative analysis of benzalkonium chlorides

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employing the injector port pyrolysis technique on a gas chromatograph with a flame ionization detector and a fused silica DB-5 column. In this method, benzalkonium chlorides were thermally degraded to alkyldimethylamines upon injection onto the gas chromatograph and the response of the tertiary amines was measured. The pyrolysis products were identified by electron impact mass spectrometry on a gas chromatograph–mass spectrometer.

EXPERIMENTAL

Reagents

Acetonitrile (HPLC grade) was obtained from Caledon Labs. (Ontario, Canada), C_{14} benzalkonium chloride (97%), C_{16} benzalkonium chloride (99%), C_{18} benzalkonium chloride (95%), Hyamine 3600, Maquat MC 1416 and Maquat MC 1412 were obtained from various commercial sources. Dodecyl alcohol was obtained from Analabs (CT, U.S.A.).

Gas chromatography

A Hewlett-Packard Model 5840A gas chromatograph equipped with a flame ionization detector, a 15 m × 0.242 mm I.D. J & W DB-5 capillary fused-silica column and a Hewlett-Packard recorder–integrator 5840A was employed. The chromatograph was operated in the split injection mode using a 100:1 split ratio. The injector temperature was 250°C, unless otherwise specified, and 1 μl of the acetonitrile solution of benzalkonium chloride was injected at an oven temperature of 100°C. After an initial hold of 2 min, the oven temperature was programmed at 10°C/min until 280°C. Flow-rates of the carrier gas (He) and make-up gas (He) were 1.0 ml/min and 60 ml/min respectively.

Gas chromatography–mass spectrometry (GC–MS)

The gas chromatograph–mass spectrometer (Finnigan Model 1020 OWA) was equipped with an electron impact source and a Nova 4 data system. The scanning rate was 1 s/scan in the range 40–650 a.m.u. The ion source temperature was held at 90°C. Electron impact spectra were obtained at 75 eV. The GC instrument (Perkin-Elmer Sigma 3B) was fitted with a 20 m × 0.242 mm I.D. DB-5 capillary fused-silica column used in a splitless mode. The oven temperature was kept at 80°C for 3 min, then programmed at 20°C/min until 280°C and held at this temperature for 10 min. The injector temperature was set at 250°C and the flow-rate of the carrier gas (He) was 1.5 ml/min.

Effect of injector temperature

An acetonitrile solution of C_{16} benzalkonium chloride at a concentration of 5 mg/ml was used for the study. The injector temperature of the gas chromatograph was incremented by 50°C from 200–350°C. Three GC runs were obtained for each temperature. For each GC run 1 μl of the solution was injected.

Calibration curves

The calibration curves for C_{14}, C_{16} and C_{18} benzalkonium chlorides in the concentration range (1–8 μg/μl) were obtained as follows.

A stock solution of each benzalkonium chloride (C_{14}, C_{16}, and C_{18}) was prepared by dissolving 1.6 g of the salt in acetonitrile in a 100-ml volumetric flask. A stock solution (10 mg/ml) of internal standard (dodecyl alcohol) in acetonitrile was similarly prepared. Suitable volumes (1–7.5 ml) of each stock solution of benzalkonium chloride were introduced to 10-ml volumetric flasks and the solution made up to 10 ml by the addition of acetonitrile. Then 1 ml of each of these solutions was mixed with 1 ml of the internal standard stock solution to obtain a series of working standards. A 1-μl volume of each working standard was injected into the gas chromatograph. This procedure was repeated three times and the calibration curves of the area ratio between the analyte and internal standard, plotted against the concentration of the analyte in the working standard solution, were constructed from the averages obtained.

RESULTS AND DISCUSSION

All benzalkonium chlorides gave similar GC trace patterns as shown in Fig. 1 for the C_{16} homologue. The three major chromatographic peaks identified by mass spectrometry are benzyl chloride, alkyldimethylamine and benzylalkylmethylamine, where alkyl is C_{14}, C_{16} or C_{18} fatty chain corresponding to the alkyl chain of the benzalkonium chloride injected into the gas chromatograph. The mass spectra of both alkyldimethylamine (Fig. 2) and benzylalkylmethylamine (Fig. 3) are characterized by the base peaks at *m/e* 58 ($[CH_2\text{-}N(CH_3)_2]^+$) and *m/e* 134 ($[CH_2\text{-}N(CH_3)CH_2C_6H_5]^+$), respectively, which are fragments resulted from α-cleavage. The molecular ions, M^+, are also present in significant intensity and are used to determine the identity of the alkyl chain. Uno *et al.*[10] failed to report the presence of the much higher boiling benzylalkylmethylamine because of the low isothermal column temperature (150°C) used in their investigation. Minor peaks due to alkyl (fatty) halide and dimethylbenzylamine were also detected.

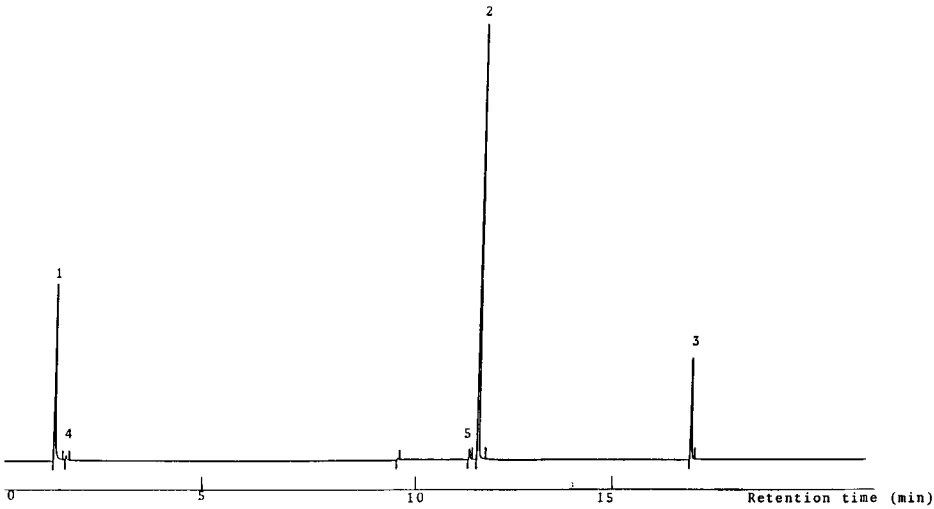
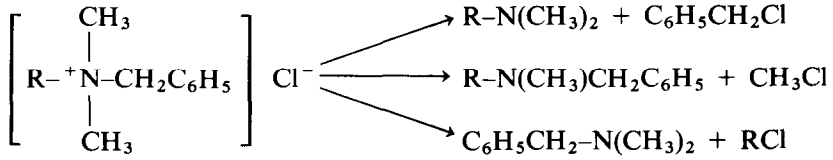


Fig. 1. Gas chromatogram of C_{16} benzalkonium chloride: benzyl chloride (peak 1), C_{16} alkyldimethylamine (peak 2), C_{16} benzylalkylmethylamine (peak 3), benzyldimethylamine (peak 4) and C_{16} alkyl chloride (peak 5).

Quaternary ammonium compounds are known to decompose thermally, thus the GC results can be explained by the pyrolysis reactions summarized in Scheme 1. The elimination of alkyl (fatty) halide is not as significant as debenzylation and demethylation.



Scheme 1.

Since alkyldimethylamine, benzylalkylmethylamine and alkyl chloride contain the fatty chain, they could be used to determine the homologue distribution in mixed benzalkonium chlorides. Because of the highest GC response of alkyldimethylamine, their GC peaks were used for quantitation purpose. These amines are well separated with retention times of 7.26, 9.66, 11.87 and 13.87 min for C_{12}, C_{14}, C_{16} and C_{18} homologues, respectively.

In searching for an optimal response for alkyldimethylamine, the effect of injector port temperature was investigated. The detector response of C_{16} alkyldimethylamine to a given amount of C_{16} benzalkonium salt was found to vary with temperature as shown in Table I. The optimal injector temperature was found to be 250°C. At lower temperature (200°C), the peak shape of benzyl chloride was broad

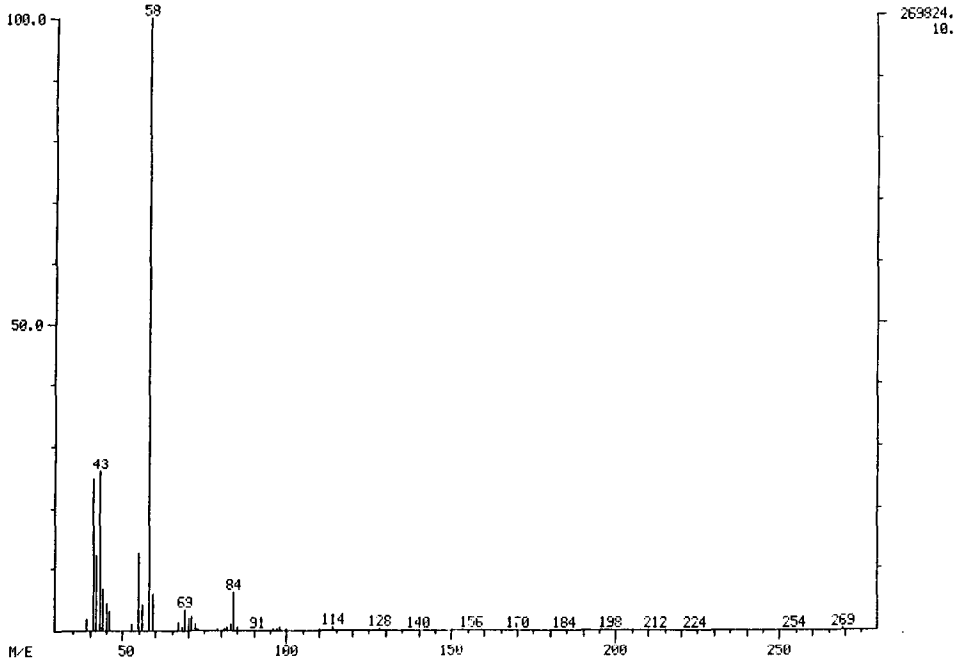


Fig. 2. Mass spectrum of C_{16} alkyldimethylamine.

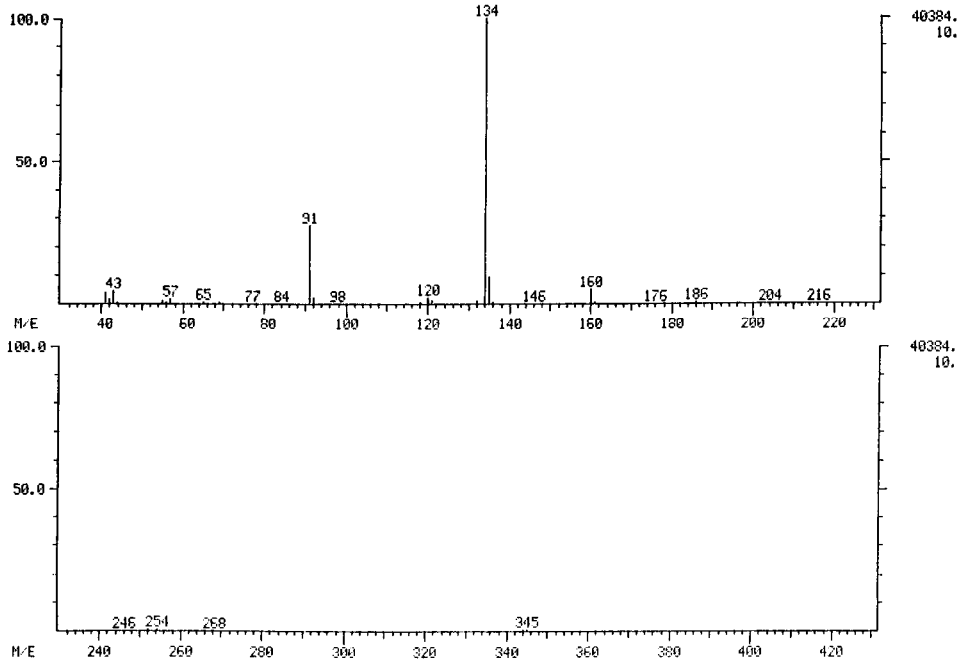


Fig. 3. Mass spectrum of C_{16} benzylalkylmethylamine.

and distorted, and the response count for alkyldimethylamine varied considerably (coefficient of variation 7%) from injection to injection, probably due to inefficient vaporization of the pyrolysis products which have their boiling points close to 200°C. At temperatures higher than 250°C, the drop in response count of alkyldimethylamine was accompanied by an increase in the response of benzylalkylmethylamine, an indication that higher temperature favors the second pyrolysis pathway.

Calibration curves were obtained by plotting the relative peak ratio between the alkyldimethylamine and the internal standard against the concentration ($\mu g/\mu l$) of the quaternary ammonium chloride injected into the gas chromatograph. Each calibration curve was linear in the working concentration range 1–8 $\mu g/\mu l$ for C_{14} (slope = 0.134, intercept = 0.0257, correlation coefficient = 0.9999), C_{16} (slope

TABLE I

EFFECT OF INJECTOR PORT TEMPERATURE ON THE GC RESPONSE OF C_{16} ALKYLDIMETHYLAMINE

Injector port temperature (°C)	*Response (peak area counts)*	*Coefficient of variation (%)*
200	17753	7
250	18340	2
300	15230	1
350	10387	2

TABLE II

CARBON CHAIN DISTRIBUTION IN COMMERCIAL BENZALKONIUM CHLORIDE SALTS

*Samples**	*Found (%)***				*Declared (%)****			
	C_{12}	C_{14}	C_{16}	C_{18}	C_{12}	C_{14}	C_{16}	C_{18}
Maquat MC 1416, 80%	6	59	31	4	5	60	30	5
Hyamine 3500, 80%	41	48	11	0	40	50	10	0
Maquat MC 1412, 80%	40	51	9	0	40	50	10	0

* These aqueous samples were diluted with acetonitrile to appropriate concentration (6 $\mu g/\mu l$) before injection.

** These are results of one GC run for each sample.

*** These values are adopted from ref. 11 for Maquat MC 1416 and Maquat MC 1412, from ref. 12 for the other sample.

= 0.131, intercept = 0.0061, correlation coefficient = 0.9995), and C_{18} homologues (slope = 0.140, intercept = 0.0021, correlation coefficient = 0.9996).

These results show that the response factors are almost the same for the three homologues of benzalkonium chloride. Although the response factor of the C_{12} homologues was not determined, it is safe to assume that it is the same as that of the higher homologues. Thus we can determine the homologue composition of benzalkonium chloride from the normalized peak areas of the alkydimethylamine homologues assuming the response is independent of the alkyl chain length.

Table II lists the results of three commercial aqueous benzalkonium chloride solutions. The homologue distribution determined by our method is in good agreement with the literature values.

In conclusion, GC analysis of benzalkonium chlorides based on injector port pyrolysis provides simple and rapid quantitative determination of homologue composition. This method does not require any sample pretreatment or the use of specially treated columns as described in the previously reported GC approaches.

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