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Determination of Benzalkonium Chloride by Gas Chromatography

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Abstract □ A new, simple, and useful approach for the analysis of benzalkonium chloride is presented. A gas chromatograph (GC) has been used to pyrolyze benzalkonium chloride in a specific and reproducible manner to yield two tertiary amines for each homologue of benzalkonium chloride present. These are separated by GC and are used to determine the homologue composition of the benzalkonium chloride. These determinations can be made with an analysis time of 25 min/sample.

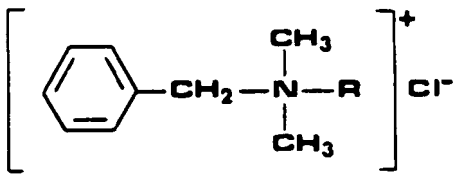
Keyphrases □ Benzalkonium chloride—determination by GC □ GC—determination of benzalkonium chloride

Benzalkonium chloride is used in pharmaceuticals as an antimicrobial preservative and is a mixture of homologous compounds (I), where the R group represents an *n*-alkyl chain which can vary from 10–18 carbon atoms. The relative amounts of the different *n*-alkyl species in benzalkonium chloride solutions greatly affect the antimicrobial spectrum and activity on the mixture (1). The current methods for analysis of benzalkonium chloride solutions according to the USP (2) rely upon titrimetric analysis of the total alkylbenzyldimethylammonium chloride based upon potassium iodate equivalents. Additionally, the ratio of *n*-alkyl components of benzalkonium chloride can be determined by microhydrogenation followed by solvent extraction and gas chromatography (GC). The ratio of alkyl components is then calculated and must meet specific USP requirements. These requirements state that the C_{12} homologue must comprise at least 40% of the total benzalkonium chloride content and that the C_{14} homologue must be at least 20%. Furthermore, these two homologues together must comprise not less than 70% of the total content.

Other methods have been reported for the determination of benzalkonium chloride, for example, ion-pairing techniques (3–5), direct titration involving tetraphenylboron sodium (6) and iodate (7). Later methods of analysis have included MS using laser ionization (8), chemical ionization (9), and HPLC (10).

GC methods include one based on a modified Hofmann degradation of benzalkonium chloride with subsequent analysis of the formed benzyldimethylamine and the corresponding alkene (11). Another method involves chemical derivatization of the benzalkonium chloride to introduce specific functional groups into the derivatives which are amenable to GC with enhanced detectability using electron capture and nitrogen-specific thermionic detecting systems (12).

The procedure presented here determines directly the alkyl chain-length ratios of benzalkonium chloride species in samples of benzalkonium chloride in an easy, sensitive, and reliable operation.



(I)

EXPERIMENTAL SECTION

Apparatus—Measurements were carried out using a GC[1] fitted with a flame ionization detector. The column used was a 0.9 m (2 mm i.d.) glass column packed with OV17 (3%) on Chromosorb W HP (80/100 mesh). A 10-μL syringe[2] was used to inject samples. The chromatograms were recorded on a potentiometric recorder[3] and peak area measurements were obtained using an integrator[4].

Mass spectrometric measurements for identification of the GC pyrolysis products were conducted using a spectrometer[5] operating in the electron impact ionization mode. The mass spectrometric data was handled by a data system[6].

Reagents, Solvents, and Standards—The carrier gas for the GC work was nitrogen; for MS work, the carrier gas was helium. The commercial samples of benzalkonium chloride were obtained from various manufacturers. Samples of benzalkonium chloride for analysis were prepared as solutions in methanol (0.5%). For confirmation, a benzalkonium chloride standard was obtained[7]. Individual pure benzalkonium chloride standards were examined with R = C_{14}, C_{16}, and C_{18}. The standards were also examined as solutions in methanol (0.5%).

[1] Model 204; Pye-Unicam.
[2] S.G.E.
[3] Model 2S; Servoscribe.
[4] Model 3390A; Hewlett Packard.
[5] Models MS30 and MS50; Kratos.
[6] Model DS55; Kratos.
[7] USP Convention Sample.

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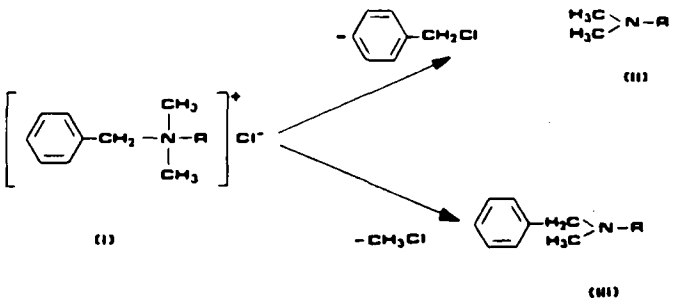

Scheme I—Gas chromatography pyrolysis products of benzalkonium chloride (I).

Assay—All benzalkonium chloride samples were dissolved in methanol to give 0.5% solutions which were used for the GC determinations. The optimum conditions for programmed GC were as follows: the oven start temperature was 100°C and this was programmed at 8.0°C/min up to 280°C; the flame ionization detector temperature was set at 350°C; the injection port temperature was 250°C; the carrier gas flow-rate was 40 mL/min. The injection size was 2 μL. The flame ionization detector sensitivity was 6.4×10^{-9} *A* full scale deflection.

Table I—MS Data on the GC Pyrolysis Products of Pure C_{14} Benzalkonium Chloride

GC Peak Retention time, min	Molecular Ion, *m/z*	Base Peak, *m/z*	Identity of Compound
0.8	126/128	91	Benzyl Chloride
5.2	241	58	Dimethylalkylamine (II) R = C_{14}
13.0	317	134	Benzylmethylalkylamine (III) R = C_{14}

Table II—Analysis of USP Benzalkonium Chloride Sample

Homologue	Label Claim, %	Found, %
C_{10}	0.8	0.0
C_{12}	41.5	41.3
C_{14}	49.1	50.5
C_{16}	8.7	8.2
C_{18}	0.0	0.0

RESULTS AND DISCUSSION

It has been shown previously (14) that when certain quaternary ammonium halides are pyrolyzed they yield tertiary amines. Benzalkonium chloride samples, when analyzed using the conditions described, yield two tertiary amines for each homologue of benzalkonium chloride present (Scheme I). The MS data supporting the structure of the pyrolysis products of the pure C_{14} benzalkonium chloride homologue are shown in Table I.

The method was applied to mixtures of benzalkonium chlorides. Using the retention times of the pyrolysis products of pure benzalkonium chloride homologues, the distribution of benzalkonium chloride was determined in a USP convention sample. The result is shown in Table II.

As there are two compounds formed for each homologue present, there are three possible methods available for calculating the percentage distribution of benzalkonium chloride homologues:

Method 1—Determination of the ratio of the responses for pyrolysis products (dimethylalkylamines) (II).

Method 2—Determination of the ratio of the responses for pyrolysis products (benzylmethylalkylamines) (III).

Method 3—Ratio of the sum of the responses determined for the corresponding pyrolysis products II and III.

Table III shows the reproducibility of the assay using the USP convention sample. The results of all three methods for calculating the homologue distribution are shown.

Since there is considerable variation in homologue composition between samples of benzalkonium chloride from different commercial sources, one commercial sample was analyzed by the new procedure. The chromatogram of the sample is shown in Fig. 1 and the homologue distribution and reproducibility data are presented in Table IV.

This assay was also conducted using nonpolar silicone phases such as SE-30, OV-1, and OV-101 (all 0.9 m columns with 3% loadings). The results obtained were reproducible for benzalkonium chloride samples which were composed predominantly of the C_{12}, C_{14}, and C_{16} homologues, although they proved to be unsuitable in cases where the C_{18} homologue was present as the C_{18} dimethylalkylamine could not be resolved from the C_{12} benzylmethylalkylamine. To demonstrate the ability of the described system to separate the pyrolysis products, a commercial sample was spiked with pure C_{18} benzalkonium chloride homologue. The resulting chromatogram is shown in Fig. 2.

Freshly packed OV-17 columns required considerable conditioning before they were suitable for use. New columns appeared to adsorb the dimethylalkylamine degradation products strongly resulting in poor peak shape (*i.e.*, considerable peak tailing). This was overcome by conditioning the columns by silylation and repeated injection with a benzalkonium chloride sample, such

Table III—Assay Reproducibility of the USP Sample

Sample	C_{12}	C_{14}	C_{16}	Total[a]
		Method 1		
1	40.8	51.0	8.3	100.0
2	41.4	50.4	8.2	105.1
3	41.2	50.6	8.2	99.5
4	41.3	50.4	8.2	101.2
5	41.3	50.4	8.1	102.4
Mean	41.2	50.6	8.2	101.6
CV	± 0.21	± 0.23	± 0.06	± 2.00
		Method 2		
1	42.1	50.0	7.8	100.0
2	42.0	49.8	8.2	106.3
3	42.1	50.0	7.9	100.0
4	42.0	49.8	8.2	103.9
5	42.0	50.2	7.8	103.6
Mean	42.0	50.0	8.0	102.8
CV	± 0.05	± 0.15	± 0.18	± 2.44
		Method 3		
1	41.0	50.8	8.2	100.0
2	41.5	50.8	8.2	105.3
3	41.4	50.5	8.2	99.6
4	41.4	50.3	8.2	101.4
5	41.4	50.5	8.1	102.6
Mean	41.3	50.5	8.2	101.8
CV	± 0.17	± 0.19	± 0.04	± 2.06

[a] Total response relative to sample 1 (100%).

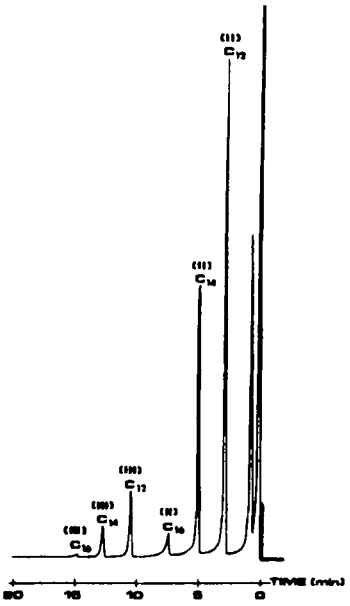

Figure 1—*Chromatogram of a commercial benzalkonium chloride sample.*

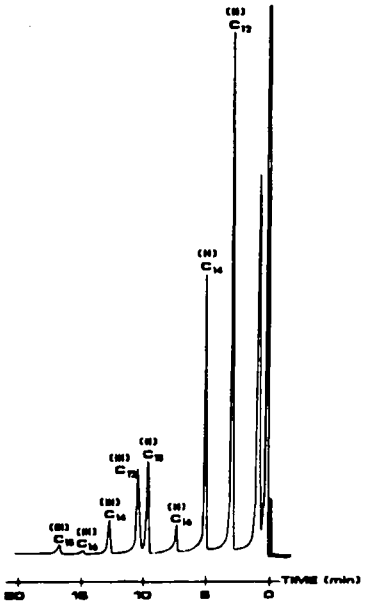

Figure 2—*Chromatogram of a commercial benzalkonium chloride sample spiked with C_{18} benzalkonium chloride homologue.*

Table IV—Assay Reproducibility of Commercial Sample

Sample	C_{12}	C_{14}	C_{16}	Total
		Method 1		
1	58.4	34.9	6.7	100.0
2	58.7	34.7	6.6	103.7
3	59.8	33.8	6.4	105.1
4	58.7	34.7	6.5	104.7
5	58.9	34.5	6.6	101.9
Mean	58.9	34.5	6.6	103.1
CV	± 0.48	± 0.38	± 0.10	± 1.89
		Method 2		
1	58.8	34.7	6.5	100.0
2	58.9	34.4	6.6	103.0
3	58.9	34.5	6.6	100.9
4	59.1	34.3	6.6	105.0
5	59.0	34.4	6.6	99.9
Mean	58.9	34.5	6.6	101.8
CV	± 0.10	± 0.14	± 0.04	± 1.97
		Method 3		
1	58.5	34.9	6.6	100.0
2	58.7	34.7	6.6	103.6
3	59.6	33.9	6.5	104.5
4	58.8	34.7	6.5	104.7
5	58.9	34.5	6.6	101.6
Mean	58.9	34.5	6.6	102.8
CV	± 0.37	± 0.34	± 0.05	± 1.81

as the USP convention standard, until an adequate peak shape was achieved from all thermal degradation products.

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