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## Note

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# Determination of fungistatic quaternary ammonium compounds in beverages and water samples by high-performance liquid chromatography

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Fungistatic and fungicidal quaternary alkylammonium compounds are widely used in the prevention of staining of timber. They may also find application in the steeping of barley for restricting the growth of fungi. Although they show only low toxicity, sensitive methods are required for their trace determinations as contaminants in food products and water.

Problems arise with their analysis because they are of low volatility, they do not carry any chromophore and they are difficult to derivatize. The traditional method for their determination has been based on ion-pair extraction followed by photometric detection. Naturally such a system has low selectivity and is therefore most suitable for quality control purposes. Ion-pair extraction has also been applied as a detection system after high-performance liquid chromatographic (HPLC) separation<sup>1</sup>, and the other method applied is so-called vacancy chromatography<sup>2</sup>. The former method requires specific equipment and the latter is susceptible to disturbances from the matrix. The use of a refractive index detector, although possible for various formulations<sup>3</sup>, is normally affected by other substances in more complicated matrices.

The aim of this study was to develop a simple procedure for the determination of quaternary alkylamines at the  $\mu\text{g/ml}$  level.

## EXPERIMENTAL

The instrument consisted of an M-6000A pump, a U6K manual injector, a Novapak CN column (100 × 8 mm I.D. packed with 4- $\mu\text{m}$  particles) in an RCM-100 column chamber, an M-411 refractive index detector and Model 820 Maxima chromatographic workstation (all from Millipore/Waters).

Sodium benzenesulphonate (Fluka, Switzerland), sodium *p*-toluenesulphonate (Aldrich, U.S.A.) and sodium *dl*-10-camphorsulphonate (Eastman Kodak, U.S.A.) were dissolved in water to give 0.5 *M* solutions, filtered through a 0.45- $\mu\text{m}$  filter and used as stock solutions from which various dilutions were made.

As reference compounds didecyldimethylammonium chloride (Lonza, U.S.A.), and dodecyltrimethylammonium bromide (Aldrich) were used as solutions in water for the spiking of samples, and for standard solutions these compounds were diluted with the eluent.

### Sample preparation

To 100 ml of decarbonated beer or water, 100 ml of methanol and 10 ml of 50 mM benzenesulphonic acid solution were added. The solution was filtered through a medium-fast filter-paper, then through a Sep-Pak C<sub>18</sub> cartridge (preconditioned with 5 ml of methanol and 10 ml of water) by suction. The quaternary alkylamine-benzenesulphonic acid ion pair was then eluted from the column with 2 × 2 ml of 95% methanol in water and adjusted to 5 ml with water. For wort samples the sample size was restricted to 50 ml because of plugging of the Sep-Pak cartridge. Injections of 50 µl were made into the HPLC instrument.

## RESULTS AND DISCUSSION

In Table I the effect of the concentration of benzenesulphonic acid on retention is shown. An increase in the concentration of an ion-pairing agent normally increases the retention, but in this instance for both compounds the opposite trend was observed. The same phenomenon was also reported by Abidi<sup>4</sup> for similar compounds using perchlorate ion pairs and a CN column. This must be the reason for the high selectivity of these quaternary alkylamines for such a complex matrix as beer and wort (Fig. 1). The selectivity between didecyldimethylammonium and dodecyltrimethylammonium is poor, but as these compounds show the expected increase in retention with decreasing methanol content in the eluent, the selectivity may be improved simply by decreasing the methanol concentration (Table I). Experiments with a Novapak C<sub>18</sub> column showed that the same phenomenon occurs, but is less pronounced. As the

TABLE I

EFFECT OF CONCENTRATION OF BENZENESULPHONIC ACID AND METHANOL ON THE RETENTION OF DIDECYLDIMETHYLAMMONIUM (DE) AND DODECYLTRIMETHYLAMMONIUM (DO)

Column: Novapak CN.

Compound	Concentration of benzene sulphonic acid (mM)	<i>k'</i>		
		Concentration of methanol in aqueous eluent (%)		
		60	70	80
DE	10	7.7	6.6	1.2
	30	3.3	1.0	0.6
	50	2.8	0.8	0.5 <sup>a</sup>
DO	10	3.4	1.8	1.2
	30	1.8	1.0	0.6
	50	1.5	0.8	0.5 <sup>a</sup>

<sup>a</sup> 75% methanol.

capacity factor ( $k'$ ) on a  $C_{18}$  column with 80% methanol was five times larger and the solubility of benzenesulphonic acid begins to be a limiting factor with higher methanol concentrations, only a few experiments could be carried out [at benzenesulphonic acid concentrations of 5, 10 and 25 mM, the  $k'$  values of didecyldimethylammonium were 1.0, 0.86 and 0.66, respectively, using a Novapak  $C_{18}$  column and methanol-water (80:20) as the eluent].

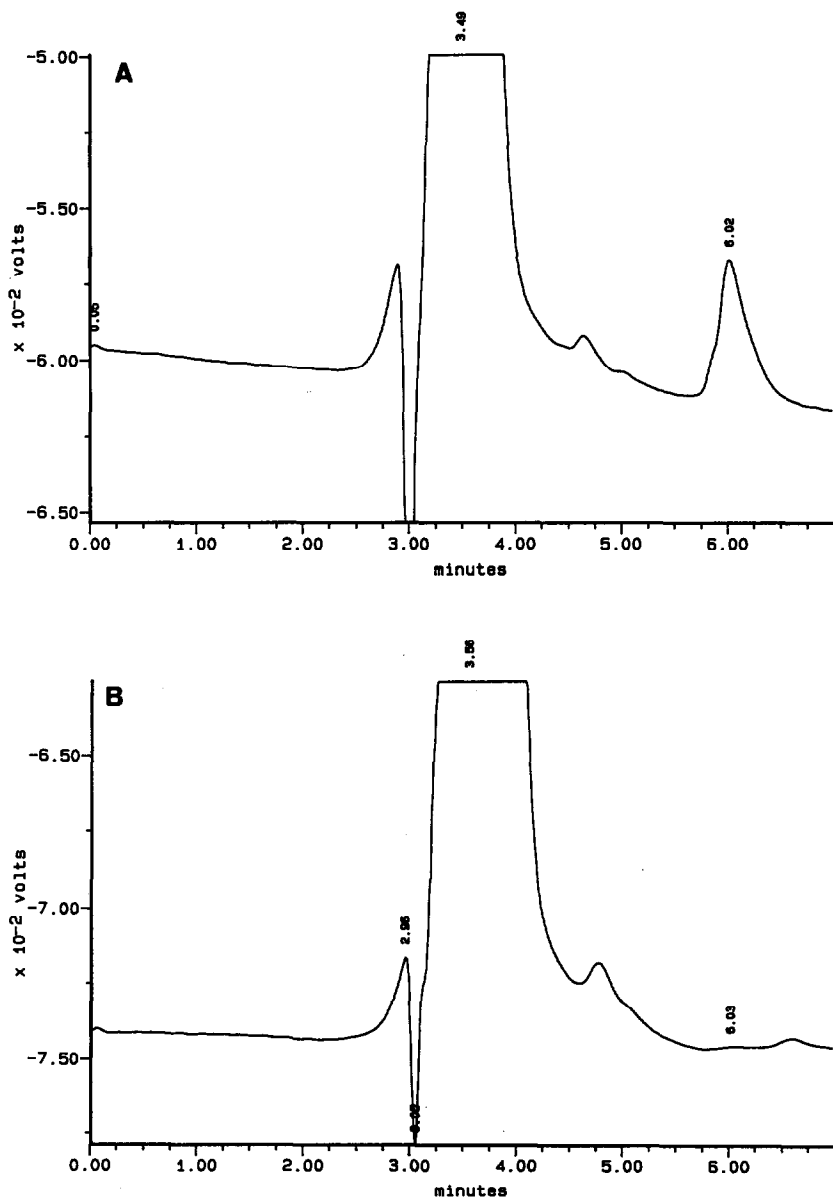


Fig. 1.

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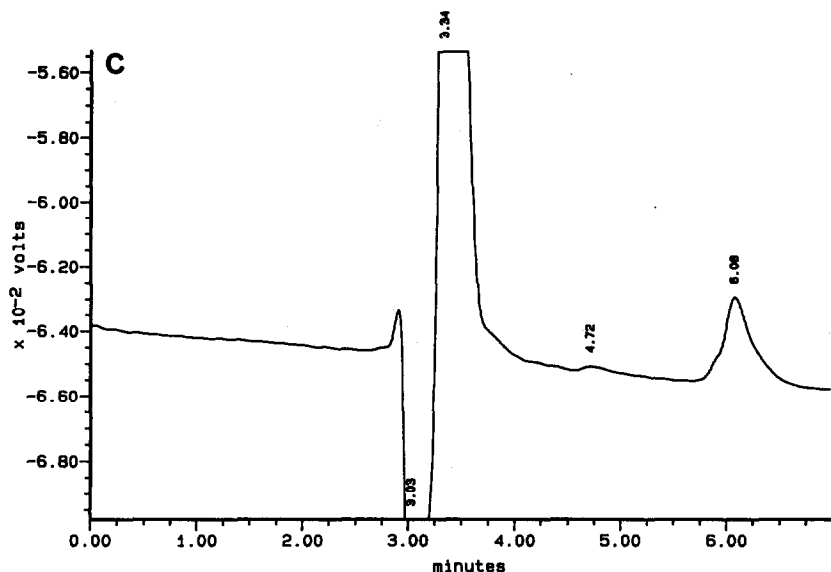


Fig. 1. Chromatograms of (A) a beer sample spiked at the 25 mg/l level with didecyltrimethylammonium (B) an unspiked beer sample and (C) a 200 mg/l standard. Eluent, methanol-water (80:20) with 10 mM sodium benzenesulphonate.

To clarify this phenomenon further, chromatography with toluenesulphonic acid and camphorsulphonic acids as ion-pairing agents was performed. With an 80% methanol concentration the retention of reference compounds was about 50% shorter using camphorsulphonic acid and about 20% longer using toluenesulphonic acid compared with chromatography using benzenesulphonic acid, so the normal ion-pairing seems to be the effective mechanism in spite of this unusual phenomenon of decreasing retention with increasing concentration of ion-pairing agent.

The calibration graph was linear ( $r^2 = 0.97$ ) from 500 to 50 mg/l. The injection of higher concentrations of standards gave tailing peaks with a sharp front, indicating overloading of the chromatographic system. Spiked samples containing about 10 g/l of quaternary amine showed symmetrical peaks with the same retention as the 500 mg/l standard.

The recovery from three beer samples spiked at the 25 mg/l level was 70% with a relative standard deviation (R.S.D.) of 7%. For three beer samples spiked at the 2.5 mg/l level the recovery was 62% with an R.S.D. of 9%. For three wort samples the corresponding values were 64% with an R.S.D. of 20% at the 25 mg/l level and 45% with an R.S.D. of 23% at the 2.5 mg/l level. The three samples spiked at the 2.5 mg/l level gave peaks that were about 20 times higher than the fluctuation of the baseline, so the detection limit would be about 0.3 mg/l and the determination limit in the 1 mg/l range. For water samples dodecyltrimethylammonium was used for spiking to imitate those fungistatic preparations which are based on cocoa fatty acids. The recovery for a sample spiked at the 50 mg/l level was 68% with an R.S.D. of 23%.

In conclusion, the use of the anomalous effect of the concentration of an

ion-pairing agent on C<sub>18</sub> and CN columns allows the determination of quaternary alkylammonium compounds in complex matrices with good sensitivity and selectivity.

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