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Note

Use of UV absorbing species to detect and quantitate aliphatic alcohols and esters by high-performance liquid chromatography

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UV detection of non-UV-absorbing species without the necessity of pre- or post-column derivatisation has recently been shown to be feasible in reversed-phase liquid chromatography systems by the addition of UV-absorbing "ion-pairing" reagents to the eluate[1–5].

A method for the detection of aliphatic alcohols[6,7], ketones[6] and monosaccharides[8] has recently been reported using methylene blue in the eluate with UV–visible detection. The authors suggest that complex formation between the alcohol and methylene blue in the stationary phase is responsible[7] and that some form of "ion-pair" interaction is involved.

This paper describes the detection and quantitation of a range of aliphatic alcohols using a neutral UV-absorbing species in the mobile phase.

EXPERIMENTAL

Reagents and materials

The alcohols and esters used in the study were either from BDH (Poole, U.K.) or Ajax Chemicals (Sydney, Australia) and re-distilled prior to use. The *n*-propyl-*p*-aminobenzoate ester was prepared from *n*-propanol and *p*-aminobenzoic acid by the method of Kadaba *et al.*[9] and recrystallised repeatedly from ethanol–water as was the benzamide (Hopkin and Williams, U.K.). Both compounds were shown to be chromatographically pure by high-performance liquid chromatography (HPLC) using the solvent system in which they were used. The acetonitrile was HPLC grade (Ajax Chemicals).

Chromatographic equipment

The liquid chromatograph consisted of a pump and variable-wavelength detector (LC-3, Pye-Unicam, Cambridge, U.K.), 20-μl loop injector (Rheodyne 7125, Cotati CA, U.S.A.), integrating recorder (Hewlett-Packard 3380 A, PaloAlto, CA, U.S.A.) and a μBondapak C_{18} column (30 cm × 6.4 mm I.D., 10 μm particle size) (Waters Assoc., Sydney, Australia).

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RESULTS AND DISCUSSION

Two solvent systems were used in this study (Tables I and II): 4% acetonitrile containing 10^{-4} *M* benzamide for ethyl acetate and low-molecular-weight alcohols (C_3–C_5) and 25% acetonitrile containing $5 \cdot 10^{-5}$ *M* *n*-propyl-*p*-aminobenzoate for higher-molecular-weight esters (C_4–C_6) and alcohols (C_5–C_8). Injection of 1% (w/v) solutions of the alcohols, esters or ethers in the mobile phase showed that, for compounds with a shorter retention time than the UV-absorbing compound in the eluate

TABLE I

CHROMATOGRAPHY OF LOW-MOLECULAR-WEIGHT ALCOHOLS, ETHERS AND ESTERS

Solvent system: 4% acetonitrile containing 10^{-4} *M* benzamide.

Compound	*Capacity factor (k')*	*Area of 1% (w/v) solution (× 10^3)*
Benzamide	9.8	–
2-Propanol	2.2	16
1-Propanol	2.5	38
2-Methyl-2-propanol*	4.0	52
2-Butanol	5.0	83
2-Methyl-1-propanol	5.9	116
1-Butanol*	6.4	150
2-Pentanol	15.6	–
1-Pentanol	18.6	–
1,4-Dioxan	2.2	–
Tetrahydrofuran	4.8	–
Ethyl acetate	8.0	–

* These compounds were used to demonstrate linearity of response *versus* concentration.

TABLE II

CHROMATOGRAPHY OF HIGHER-MOLECULAR-WEIGHT ESTERS AND ALCOHOLS

Solvent system: 25% acetonitrile containing $5 \cdot 10^{-5}$ *M* *n*-propyl-*p*-aminobenzoate.

Compound	*Capacity factor (k')*	*Area of 1% (w/v) solution (× 10^3)*
n-Propyl-*p*-aminobenzoate	19.0	–
1-Pentanol	4.0	58
1-Hexanol*	8.5	404
1-Heptanol	20.0	–
2-Ethylhexanol	33.6	–
1-Octanol	49.2	–
Ethyl acetate	2.9	25
Methyl butyrate	5.7	47
Ethyl butyrate*	10.0	144
Butyl acetate	11.3	156

* The compounds were used to demonstrate linearity of response *versus* concentration.

a positive peak was obtained with a negative system peak corresponding to the retention time of the UV-ansorbing species (Figs. 1–3). For compounds with a longer retention time a negative peak was obtained with a corresponding positive system peak (Figs. 1 and 2). The peak corresponding to the compound and that due to the UV-absorbing compound in the eluate were approximately equivalent in area. Similar phenomena have been observed with chromatography using UV-absorbing ion-pair reagents[3]. For this reason UV-absorbing compounds were added to the eluate which had longer retention times than the compounds being studied, thereby giving these positive peaks. Greatest response was obtained for compounds which had retention times approaching that of the UV-absorbing species, however, compounds which eluted at the system peak were highly asymmetric. Similar phenomena have been observed with the detection of solvents using a refractive index detector[10].

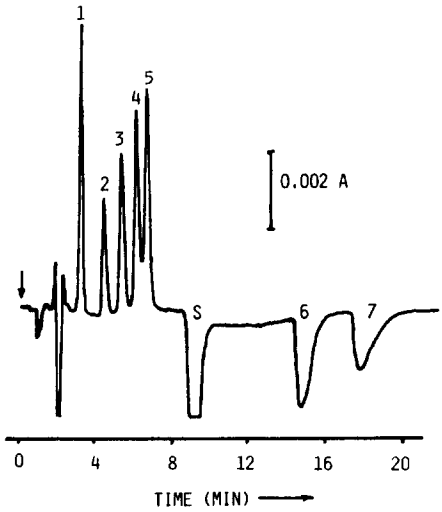

Fig. 1. Chromatogram of a solution of alcohols in 10% methanol. 1 = 1-Propanol (2%, w/v); 2 = 2-methyl-2-propanol (0.5%, w/v); 3 = 2-butanol (0.5%, w/v); 4 = 2-methyl-1-propanol (0.5%, w/v); 5 = 1-butanol (0.5%, w/v); 6 = 2-pentanol (1%, w/v); 7 = 1-pentanol (1%, w/v); S = system peak. Solvent 4% acetonitrile containing 10^{-4} *M* benzamide; flow-rate 1.5 ml/min; monitoring wavelength 270 nm.

Compounds marked with an asterisk (Tables I and II) were evaluated for linearity of response. In all cases excellent straight line correlations were obtained for concentration versus peak area over the range 0–1% (w/v) ($n = 4$, $r = 0.999$) with all calibration lines passing through the origin. The method would therefore appear to offer considerable potential as an analytical method. Greater selectivity could be achieved by the use of additives to the eluate which absorb in the visible region of the spectrum. It is necessary to purify scrupulously the UV-absorbing species as trace impurities can give to spurious peaks. The columns are rapidly equilibrated with the UV-absorbing compound, the time required being approximately that of the retention time of the species used. The column can be rapidly washed free of the material using blank solvent. This is an advantage over the method of Gnanasambandan and

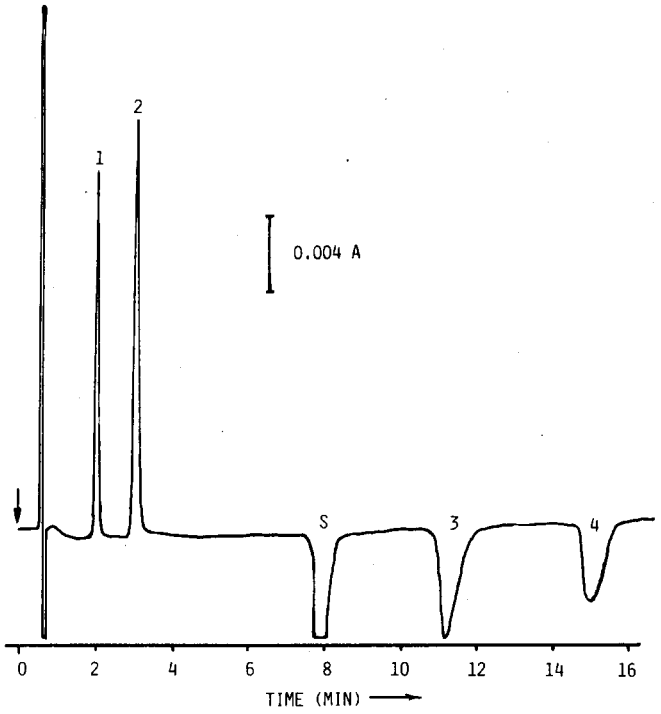

Fig. 2. Chromatogram of a solution of alcohols in 20% methanol. 1 = 1-Pentanol (2%, w/v); 2 = 1-hexanol (1%, w/v); 3 = 2-ethylhexanol (1%, w/v); 4 = 1-octanol (1%, w/v); S = system peak. Solvent 25% acetonitrile containing $5 \cdot 10^{-5}$ *M* *n*-propyl-*p*-aminobenzoate; Flow-rate 1.5 ml/min; monitoring wavelength 310 nm. Under these conditions 1-heptanol was eluted at the system peak.

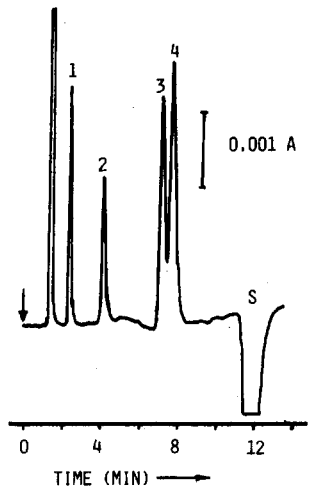

Fig. 3. Chromatogram of a solution of esters in 50% acetonitrile. 1 = Ethyl acetate (2%, w/v); 2 = methyl butyrate (1%, w/v); 3 = butyl acetate (1%, w/v); 4 = ethyl butyrate (1%, w/v); S = system peak. Solvent 25% acetonitrile containing $5 \cdot 10^{-5}$ *M* *n*-propyl-*p*-aminobenzoate; Flow-rate = 2.0 ml/min; monitoring wavelength 310 nm.

Freiser[7] using methylene blue where washing with 50% chloroform or 10% dimethyl sulphoxide in methanol is required.

For all compounds the eluting peak contains a vast excess of compound over UV-absorbing eluate species. A possible explanation for the observed effects is that the presence of the compounds perturbs the partitioning characteristics of the UV-absorbing species during its passage down the column. Gnanasambandan and Freiser[7] have speculated, for the case of methylene blue, that a specific complex is formed and this is substantiated by the observation that the capacity factors of alcohols were different in the presence and absence of the dye. Such studies were not performed with this system due to the unavailability of a refractive index detector.

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