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Note

High-performance liquid chromatography of low-molecular-weight aliphatic alcohols using indirect photometric detection

J. E. PARKIN* and H. T. LAU
School of Pharmacy, Western Australian Institute of Technology, Kent Street, Bentley, Western Australia 6102 (Australia)
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Studies have shown that it is possible to detect and quantitate non-UV-absorbing ionic compounds by high-performance liquid chromatography (HPLC) by the addition of a UV-absorbing ion to the mobile phase with indirect photometric detection. The method is applicable to both organic and inorganic ions using ion-exchange[1,2] and reversed-phase chromatography in the ion-pair mode[3–9]. Recent reports have shown that this method of detection can be extended to neutral species such as alcohols[10], ketones[10,11] and monosaccharides[12] using methylene blue in the solvent. The authors suggest in the latter cases that some form of "ion-pair" complex is involved in the perturbation of the eluate concentration of the methylene blue[11]. More recently, it has been observed in this laboratory[13] that neutral molecules which absorb in UV light may be added to the chromatographic solvent to enable detection and quantitation of non-UV-absorbing neutral species such as aliphatic alcohols, ethers and esters by indirect photometric detection.

This paper reports a more detailed investigation of this phenomena using reversed-phase HPLC of C_3- and C_4-alcohols as a model system.

EXPERIMENTAL

Reagents and materials

The alcohols used in this study were obtained from Ajax Chemicals (Sydney, Australia) and re-distilled prior to use. The benzamide (Hopkin & Williams, U.K.) and the uracil and theobromine (Sigma, U.S.A.) were all shown to be chromatographically pure by HPLC using the solvent system employed in the present study. 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, U.S.A.), integrating recorder (Hewlett-Packard 3380 A, Palo Alto, 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

Chromatographic studies were undertaken on a mixture of isopropanol, *tert.*-butanol, *sec.*-butanol and *n*-butanol using acetonitrile–water mixtures containing a UV-absorbing neutral species[13] in the mobile phase. The UV-absorbing detection compounds (UV-ADCs) used in this study were selected on the basis that they provide a peak of reasonable shape and have retention times such that they are eluted

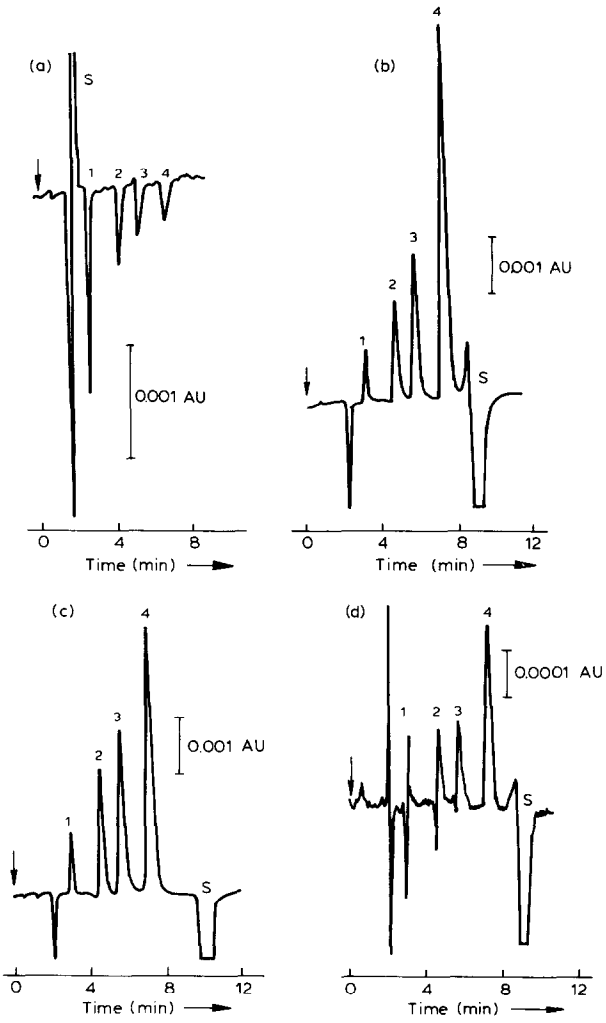

Fig. 1. Chromatograms of a 1% (w/v) solution of alcohols (20 μl). 1 = Isopropanol; 2 = *tert.*-butanol; 3 = *sec.*-butanol; 4 = *n*-butanol; S = system peak. (a) Solvent 4.5% acetonitrile containing $2 \cdot 10^{-4}$ *M* uracil; flow-rate 1.7 ml min^{-1}; monitoring wavelength 270 nm. (b) Solvent 4.5% acetonitrile containing $2 \cdot 10^{-4}$ *M* theobromine; flow-rate 1.5 ml min^{-1}; monitoring wavelength 300 nm. (c) Solvent 4.5% acetonitrile containing $2 \cdot 10^{-4}$ *M* benzamide; flow-rate 1.5 ml min^{-1}; monitoring wavelength 270 nm. (d) Solvent 4.5% acetonitrile containing $1 \cdot 10^{-5}$ *M* theobromine; flow-rate 1.5 ml min^{-1}; monitoring wavelength 300 nm.

TABLE I

CAPACITY FACTORS AND REGRESSION LINES FOR PEAK-AREA RESPONSE *versus* CONCENTRATION OF ALCOHOL (% w/v)

Concentration of UV-ADC (M)	*Alcohol*	*Theobromine*				*Benzamide*			
		Capacity factor	*Slope ($\times 10^3$)*	*Intercept ($\times 10^3$)*	*Correlation coefficient*	*Capacity factor*	*Slope ($\times 10^3$)*	*Intercept ($\times 10^3$)*	*Correlation coefficient*
$4 \cdot 10^{-4}$	Isopropanol	1.96	50.2	+0.6	0.9980	1.96	62.1	+0.6	0.9996
	tert.-Butanol	3.56	166.3	−1.0	0.9978	3.56	222.4	+0.1	0.9999
	sec.-Butanol	4.59	330.6	+2.4	0.9968	4.61	322.7	−0.2	0.9999
	n-Butanol	6.08	941.2	−9.5	0.9995	6.10	600.9	0	0.9999
$3 \cdot 10^{-4}$	Isopropanol	1.98	45.6	+1.5	0.9996	1.98	45.2	+0.4	0.9990
	tert.-Butanol	3.56	131.5	+1.5	0.9999	3.55	159.9	+2.3	0.9994
	sec.-Butanol	4.60	251.3	0	0.9994	4.61	241.8	+1.4	0.9994
	n-Butanol	6.10	740.0	−4.0	0.9990	6.10	451.1	+3.1	0.9999
$2 \cdot 10^{-4}$	Isopropanol	1.98	28.0	+1.0	0.9996	1.96	32.3	−0.1	0.9999
	tert.-Butanol	3.56	80.0	+0.6	0.9998	3.54	108.8	+0.9	0.9997
	sec.-Butanol	4.61	150.5	−0.5	0.9994	4.58	165.9	+1.9	0.9995
	n-Butanol	6.10	461.0	−2.3	0.9994	6.08	315.3	+1.8	0.9997
$1 \cdot 10^{-4}$	Isopropanol	1.96	10.8	+0.1	0.9992	1.98	17.0	−0.4	0.9994
	tert.-Butanol	3.54	39.9	+0.1	0.9999	3.56	54.3	−0.2	0.9998
	sec.-Butanol	4.58	72.8	−1.1	0.9998	4.60	83.4	−0.6	0.9999
	n-Butanol	6.10	245.7	+0.9	0.9995	6.10	144.9	−0.6	0.9998
$0.5 \cdot 10^{-4}$	Isopropanol	1.96	13.4	−0.5	0.9860	1.97	16.1	−2.3	0.8576
	tert.-Butanol	3.56	26.0	+1.0	0.9991	3.57	29.4	+0.2	0.9960
	sec.-Butanol	4.60	46.1	−1.7	0.9991	4.60	45.9	−0.1	0.9996
	n-Butanol	6.06	129.1	−4.6	0.9998	6.08	77.6	−1.2	0.9997

just prior to or just after the series of alcohols used in the present study (Fig. 1a–c). The concentration of UV-ADCs employed and wavelengths monitored were selected so that the absorbance of a 1-cm layer of the resulting mobile phase was approximately 0.1–0.2. This provided the best compromise between response and noise level[9]. Uracil, which elutes prior to the alcohols afforded negative peaks corresponding to the alcohols and a positive system peak (Fig. 1a), whereas both benzamide (Fig. 1c) and theobromine (Fig. 1b), which elute after the alcohols, afford positive peaks for the alcohols and negative system peaks. This is consistent with earlier observations[13].

A more detailed investigation was undertaken using benzamide and theobromine as the UV-ADCs in the mobile phase. Calibration curves were prepared by in-

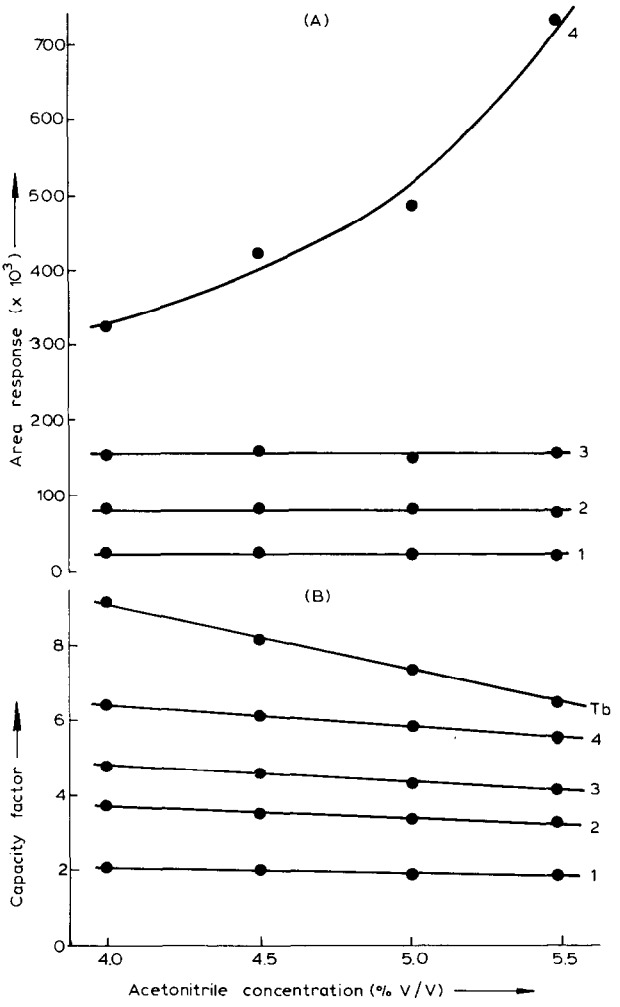

Fig. 2. Plot of (A) area responses and (B) capacity factors for alcohols *versus* concentration of acetonitrile (%, v/v) in mobile phase. UV-ADC concentration is $2 \cdot 10^{-4}$ *M* theobromine. 1 = isopropanol; 2 = *tert.*-butanol; 3 = *sec.*-butanol; 4 = *n*-butanol; Tb = theobromine.

jection of solutions containing 0.25, 0.5, 0.75 and 1.0% w/v of all four alcohols in water using a range of concentrations ($0.5 \cdot 10^{-4}$–$4 \cdot 10^{-4}$ *M*) of UV-ADC in the solvent. In all cases except for the lowest concentration of UV-ADC a linear relationship which passed through the origin was obtained for peak-area *versus* concentration of alcohol (Table I). The sensitivity of the method (as reflected in the slope of the calibration line) is highly dependent upon the capacity factor of the alcohol relative to that of the UV-ADC. It is greatest for *n*-butanol and least for isopropanol (Fig. 1b and c; Table I). Similar relationships were noted in the earlier study[13]. The sensitivity is also directly proportional to the concentration of UV-ADC in the mobile phase. When the slope of the calibration line for each alcohol (Table I) is plotted against concentration of UV-ADC in the mobile phase, a linear relationship is obtained which passes through the origin (correlation coefficient >0.99 in all cases). However, increasing the sensitivity of the method by increasing mobile phase concentration of UV-ADC is limited by the ability of the detector/recorder to correct for background absorbance[9].

The area response for the alcohols using the lowest concentration of UV-ADC ($0.5 \cdot 10^{-4}$ *M*) was abnormal in both linearity and slope due to the presence of a small negative peak eluting just prior to the alcohol peaks. This results in integration

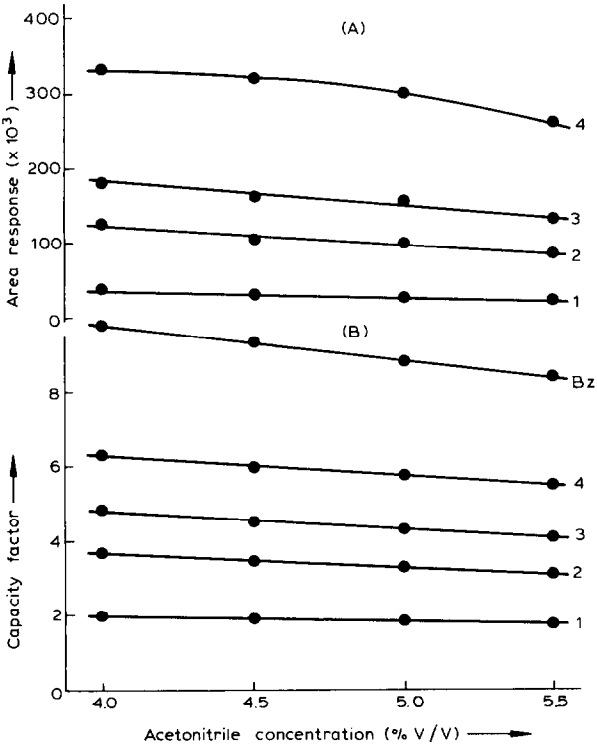

Fig. 3. Plot of (A) area responses and (B) capacity factors for alcohols *versus* concentration of acetonitrile (%, v/v) in mobile phase. UV-ADC concentration is $2 \cdot 10^{-4}$ *M* benzamaide. 1 = isopropanol; 2 = *tert.*-butanol; 3 = *sec.*-butanol; 4 = *n*-butanol; Bz = benzamide.

errors. To investigate this effect chromatography was performed using $1 \cdot 10^{-5}$ *M* theobromine as the UV-ADC and monitoring at both 300 and 270 nm. At 300 nm the absorbance of a 1-cm layer of the chromatographic solvent was approximately 0.02 whereas at 270 nm it was approximately 0.15. The chromatogram obtained by monitoring at 270 nm was normal whereas that obtained at 300 nm showed large negative peaks prior to elution of the alcohols (Fig. 1d). This appears to indicate that the effect is due to changes in the refractive index of the mobile phase due to the presence of the alcohols and is not due to changes in the concentration of the UV-ADC. If the latter were the case, the negative peaks would be present at both wavelengths monitored. Obviously care must be taken in the selection of concentration of UV-ADC and monitoring wavelength to avoid this effect.

Considerable intra-day variation in response occurred for the *n*-butanol when theobromine was used as the UV-ADC and this phenomenon was further investigated. The capacity factor for theobromine changes relative to the *n*-butanol with small changes in concentration of acetonitrile in the mobile phase (Fig. 2). As the area-response is influenced by the relative capacity factors of the alcohol and UV-ADC small changes in acetonitrile concentration result in large changes in response for the *n*-butanol. The capacity factors for benzamide and alcohols change little relative to each other with changes in acetonitrile concentration and peak-area response remains relatively constant (Fig. 3).

TABLE II

CAPACITY FACTORS FOR ALCOHOLS USING $2 \cdot 10^{-4}$ *M* THEOBROMINE AS THE UV-ADC

Alcohol	*Concentration (%, w/v)*	*Capacity factor*
Isopropanol	1	1.98
	2.5	1.93
	5.0	1.88
	7.5	1.83
	10.0	1.80
tert.-Butanol	1	3.56
	2.5	3.41
	5.0	3.27
	7.5	3.14
	10.0	3.02
sec.-Butanol	1	4.61
	2.5	4.42
	5.0	4.27
	7.5	4.12
	10.0	3.99
n-Butanol	1	6.10
	2.5	5.92
	5.0	5.75
	7.5	5.63
	10.0	5.48

The capacity factors for the alcohols remained constant with changes in both the nature and concentration of the UV-ADC in the mobile phase (Table I). This suggests that the presence of the UV-ADC in no way alters the chromatographic characteristics of the column. This is at variance with the observation of Gnanasambandan and Freiser[11] who found that the presence or absence of methylene blue on the column profoundly influenced the capacity factors of the alcohols, this being the principal evidence for their conclusion that some form of specific "ion-pair" complex occurred between the alcohol and methylene blue on the column. A possible explanation for their observation is that, in determining their capacity factors without dye they used 25% (v/v) injections of the alcohols to provide sufficient sensitivity to enable detection to be made using a refractive-index detector. At these loadings the column is overloaded and the capacity factor becomes smaller (Table II).

The evidence presented here supports the proposal that the presence of the alcohol perturbs the partitioning characteristics of the UV-ADC between the mobile and stationary phase. There is no evidence that the presence of the UV-ADC in any way influences the chromatographic characteristics of the column. This is at variance with ion-pair chromatography where the nature and concentration of the ion in the mobile phase can profoundly influence chromatographic behaviour[4,9,14].

The observations made in this and the preceding study[13] suggest that detection and quantitation of neutral compounds by the addition of a UV-ADC to the mobile phase could have general analytical applications and that the method may have advantages over refractive-index detection for non-UV-absorbing compounds being separated in a reversed-phase mode.

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