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SUPERCRITICAL FLUID CHROMATOGRAPHY OF BARBITURATES

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SUMMARY

A number of barbiturates have been separated by supercritical fluid chromatography on columns packed with polystyrene–divinylbenzene or ODS-silica using carbon dioxide containing methanol as a modifier. The effect of the proportion of modifier on the capacity factors, relative capacity factors, and retention indices based on the alkyl aryl ketone scale has been studied.

INTRODUCTION

Over the last few years there has been considerably interest in the application of supercritical fluids as the mobile phase in chromatographic separations using either capillary or packed columns[1–3]. These methods offer an alternative to conventional gas or liquid chromatography but the influence of the separation conditions on the resolution and retention particularly for polar compounds has not yet been fully examined. Much work is still in progress to determine the scope and potential applications of supercritical fluid chromatography (SFC). Although the technique has been applied to the separation of a number of individual drugs and pharmaceutical compounds[1,4,5] and the separation of the opium alkaloids using different stationary phases and modifiers has been studied in detail[6], few other systematic studies of groups of related drugs have been reported.

The separation of the barbiturates by gas–liquid chromatography (GLC)[7,8] and high-performance liquid chromatography (HPLC)[8–10] has been widely examined in therapeutical and toxicological studies because of their widespread application for the treatment of epilepsy. It was therefore of interest to examine their separation by SFC to determine if this method could provide an alternative selectivity that might be advantageous for the confirmation of identification or to improve the resolution in complex mixtures. By using a different mobile phase the relative separation of the barbiturates compared to each other or to other drug groups might be enhanced.

Phenobarbitone had been chromatographed previously by Later *et al.*[4] using capillary SFC with a polymethylsiloxane stationary phase and in an initial study in

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this laboratory[11] a number of barbiturates were examined using a polystyrene–divinylbenzene (PS–DVB) packed column with carbon dioxide as the mobile phase. However, in that case the peak shapes were poor compared to less polar aromatic test compounds and on a ODS-silica column all the barbiturates were totally retained.

In the present paper the use of a modifier in the mobile phase to improve the separation of the barbiturates using these two stationary phases has been examined in detail and the influence of the proportion of modifier on the retention and selectivity has been determined.

EXPERIMENTAL

Chemicals

The alkyl aryl ketones from acetophenone to octadecanophenone were laboratory grade from a range of suppliers. Barbiturates were from the reference collection of the Central Research Establishment, Home Office Forensic Science Service, U.K. Carbon dioxide was industrial grade (99.98%) from British Oxygen Company, U.K.

Equipment

The separations were carried out using a packed column chromatograph[1,12] consisting of a Jasco BIP-1 pump, with cooled check valves and pumping head, operating under constant pressure conditions, a Pye Unicam LC-XPS pump for the addition of modifier, a Rheodyne 7125 injection valve with a 20-μl loop and a Pye 104 gas chromatographic oven (Pye Unicam, Cambridge, U.K.). The analytes were detected at 254 nm using an ACS 750/12 variable-wavelength ultraviolet spectrophotometric detector (Applied Chromatography Systems, Macclesfield, U.K.) fitted with a high-pressure flow cell.

The samples were separated on either a 150 × 4.6 mm column packed with PLRP-S (PS–DVB) 5-μm particles (Polymer Labs., Church Stretton, U.K.) or a 250 × 4.6 mm column packed with Ultrasphere-ODS 5-μm particles (Beckman) and peaks were recorded using a Hewlett-Packard 3390 integrator.

Method

Samples of the barbiturates and alkyl aryl ketones in methanol were injected onto the column and eluted with supercritical carbon dioxide. Selected percentages of methanol by weight were added to the eluent as required. A sample of acetone was used as the column void volume marker.

Capacity factors k' were calculated as $k' = (t_R - t_0)/t_0$ (where t_R = retention time and t_0 = column void volume and the mean of triplicate injections was used in calculations). Retention indices based on the alkyl aryl ketones were calculated as described for HPLC[13] by fitting log k' against carbon number × 100 for the standards to a linear correlation using a least squares routine and then interpolating the log k' values for the test compounds.

RESULTS AND DISCUSSION

In an initial study, it was found that there were problems with the separation of barbiturates on either PS–DVB or ODS bonded-silica columns using unmodified carbon dioxide as the mobile phase[11]. On the ODS-bonded silica column, the barbiturates were totally retained and on the PS–DVB column the peak shapes were poor and considerable tailing was observed. It has been suggested that residual silanol groups on the surface of the silica may be a cause of tailing[4] but the PS–DVB column contains no silanol groups. Other workers have suggested that a more important factor is the solubility of the analyte in the mobile phase and that phenolic, acidic and amide groupings cause particular problems. In both cases it is reported that the effects can be reduced by the addition of a modifier to the mobile phase[14–16].

The addition of methanol to the carbon dioxide eluent in the present study caused a sharpening of the peaks for the barbiturates on the PS–DVB column (Fig. 1) and reductions in their retentions, which were directly dependent of the proportion of modifier (Table I). The capacity factors of all the barbiturates changed markedly but smaller changes were observed for the N-methyl substituted barbiturate, methohexitone. The order of elution differed from that found on HPLC which was generally in the order of increasing molecular size[9,10]. In the SFC separation, talbutal, which contains an unsaturated side chain, was retained much more than amylobarbitone and pentobarbitone, the corresponding isomeric saturated compounds. The cyclic derivatives phenobarbitone and heptabarbitone also were very highly retained.

Retention indices based on the alkyl aryl ketones have been shown to provide a robust method for recording the retentions of the barbiturates by HPLC, which were less dependent than capacity factors on small variations in the operating conditions, such as temperature and eluent composition and in the determination of the column void volume[9,10]. This approach was initially investigated in the earlier study of the SFC separation[11]. In the present work the retentions of the homologous alkyl aryl ketones, acetophenone to heptanophenone were recorded (Table I) and these were used to calculate the retention indices of the barbiturates (Table II). However, the indices were very dependent on the proportion of modifier. This was in agreement with a study of a series of aromatic test compounds on a PS–DVB column in which

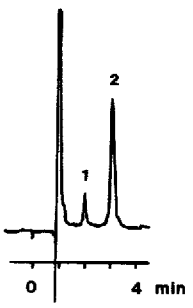

Fig. 1. Separation of quinalbarbitone (1) and heptabarbitone (2) on a PS–DVB column. Conditions: mean column pressure, 2200 p.s.i.; temperature, 60°C; eluent, carbon dioxide containing 9% methanol; detection, UV spectroscopy at 254 nm.

TABLE I

CAPACITY FACTORS OF BARBITURATES IN DIFFERENT METHANOL CONCENTRATIONS ON A PS–DVB COLUMN

Conditions; column, PLRP-S; eluent carbon dioxide plus methanol; mean column pressure, 2200 p.s.i.; temperature, 60°C; detection, UV spectroscopy at 254 nm.

Compound	*Capacity factor*			
	Methanol (%, w/w)			
	0	*4.0*	*9.1*	*14.6*
Barbiturates				
Barbitone	5.32	1.34	0.67	0.37
Butobarbitone	6.51	1.73	0.78	0.39
Amylobarbitone	6.55	1.74	0.78	0.40
Pentobarbitone	6.56	1.75	0.79	0.39
Talbutal	7.50	2.06	0.92	0.47
Quinalbarbitone	8.34	2.19	0.95	0.48
Methohexitone	6.16	2.27	1.11	0.69
Phenobarbitone	>24	4.87	1.91	0.91
Heptabarbitone	>24	5.07	1.98	0.98
Alkyl aryl ketones				
Acetophenone	2.27	1.33	0.89	0.67
Propiophenone	2.99	1.70	1.07	0.78
Butyrophenone	3.41	1.85	1.17	0.87
Valerophenone	4.15	2.11	1.36	0.99
Hexanophenone	5.21	2.56	1.59	1.14
Heptanophenone	6.57	3.06	1.85	1.31

TABLE II

RETENTION INDICES OF BARBITURATES IN DIFFERENT METHANOL CONCENTRATIONS ON A PS–DVB COLUMN

Based on alkyl aryl ketone standards, butyrophenone–heptanophenone[17]. Conditions as in Table I.

Compound	*Retention index*			
	Methanol (%, w/w)			
	0	*4.0*	*9.1*	*14.6*
Barbitone	1207	829	641	378
Butobarbitone	1299	974	740	424
Amylobarbitone	1302	976	734	442
Pentobarbitone	1303	978	742	433
Talbutal	1364	1072	848	567
Quinalbarbitone	1412	1106	867	570
Methohexitone	1274	1126	966	842
Phenobarbitone		1558	1321	1040
Heptabarbitone		1582	1342	1089

TABLE III

RELATIVE CAPACITY FACTORS OF BARBITURATES IN DIFFERENT METHANOL CONCENTRATIONS ON A PS–DVB COLUMN

Conditions as in Table I.

Compound	*Relative capacity factor*			
	Methanol (%, w/w)			
	0	*4.0*	*9.1*	*14.6*
Barbitone	63.8	61.2	70.5	77.1
Butobarbitone	78.1	79.0	82.1	81.3
Amylobarbitone	78.5	79.5	82.1	83.3
Pentobarbitone	78.7	79.9	83.2	81.3
Talbutal	89.9	94.1	96.8	97.9
Quinalbarbitone	100	100	100	100
Methohexitone	73.9	104	117	144
Phenobarbitone		222	201	190
Heptabarbitone		232	208	204

the effect of the modifier varied markedly with the nature of the functional groups in the analyte[17]. Changing the proportion of modifier can cause large differences in relative retentions for unrelated compounds, thus because of the structural differences between the alkyl aryl ketone standards and the acidic barbiturates, retention indices are unlikely to be as useful as in HPLC as a method of recording robust retention values. However, the more detailed study of separation on the PS–DVB column found that the retention indices of the test compounds were robust to small changes in the temperature, pressure and density of a unmodified carbon dioxide mobile phase[17].

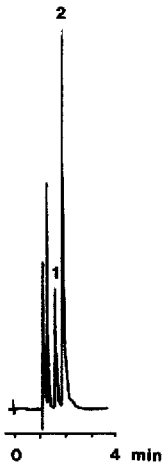

Fig. 2. Separation of amylobarbitone (1) and phenobarbitone (2) on a ODS-silica column. Conditions: mean column pressure, 1950 p.s.i.; temperature, 60°C; eluent, carbon dioxide containing 4.2% methanol; detection, UV spectroscopy at 254 nm.

An alternative method is to record retentions relative to an internal standard closely related to the analytes of interest and this approach was very succesful in the HPLC studies as it compensated for small pH changes in the eluent[18]. The relative retention times were therefore calculated compared to quinalbarbitone as an internal standard (Table III). Although these values showed a smaller variation than capacity factors or retention indices there were still considerable changes with the proportion of methanol. Thus neither relative method of recording retentions would be robust to small changes in the proportion of methanol, such as might be found in using a specified method on different equipment or in different laboratories.

The separation of the barbiturates on an ODS-silica column with methanol as a modifier was then examined. Again the capacity factors and retention indices were determined using different proportions of modifier (Table IV) and the retention times were reduced as the proportion of modifier was increased. Good peak shapes were obtained for most of the compounds (Fig. 2), although the more retained analytes

TABLE IV

CAPACITY FACTORS OF BARBITURATES IN DIFFERENT METHANOL CONCENTRATIONS ON AN ODS-SILICA COLUMN

Conditions: column, Ultrasphere ODS; eluent carbon dioxide plus methanol; mean column pressure, 1950 p.s.i.; temperature, 60°C; detection, UV spectroscopy at 254 nm.

Compound	*Capacity factor*		*Retention index*	
	Methanol (%, w/w)[a]		*Methanol (%, w/w)*[a]	
	4.2	*8.4*	*4.2*	*8.4*
Barbiturates				
Barbitone	0.30	0.16	619	398
Butobarbitone	0.37	0.17	751	437
Amylobarbitone	0.42	0.17	831	467
Pentobarbitone	0.37	0.17	747	437
Talbutal	0.41	0.22	825	631
Quinalbarbitone	0.46	0.24	893	681
Methohexitone	0.51	0.28	953	793
Phenobarbitone	0.65	0.28	1111	793
Heptabarbitone	0.78	0.35	1223	964
Alkyl aryl ketones				
Acetophenone	0.35	0.25	726	722
Propiophenone	0.47	0.32	902	888
Butyrophenone	0.53	0.35	984	970
Valerophenone	0.63	0.42	1090	1088
Hexanophenone	0.75	0.49	1199	1202
Heptanophenone	0.89	0.57	1307	1311
Octanophenone	1.06	0.66	1413	1417
Decanophenone	1.48	0.88	1622	1623
Dodecanophenone	1.93	1.13	1790	1806
Tetradecanophenone	2.76	1.51	2013	2012
Hexadecanophenone	3.73	1.95	2202	2196
Octadecanophenone	4.97	2.50	2382	2374

[a] At 0% methanol all the barbiturates were fully retained[11].

showed some tailing. Generally using a similar pressure of carbon dioxide, the retentions were shorter than on the PS–DVB column probably because of a lower overall retention capacity, which is also found in HPLC[19]. The elution orders of the barbiturates on the two columns were the same except for the closely eluted compounds, amylobarbitone and pentobarbitone. Again the retention indices (Table IV) changed markedly with the proportion of organic modifier and this can be attributed to differences in the selectivity of the separation observed for compounds with different functional groups[20].

CONCLUSION

The barbiturates can be successfully separated by SFC on both PS–DVB and ODS-silica columns by incorporating methanol as a modifier into the mobile phase with a different selectivity to HPLC separations. The proportion of the modifier has a marked effect on the retentions and particular care will be needed to reproduce these separations in different laboratories. Because of the changes in relative retentions with the proportion of modifier, retention indices and relative retention times cannot successfully compensate for small changes in the composition of the eluent.

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