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Application of packed column supercritical fluid chromatography to the analysis of barbiturates*

ROGER M. SMITH† and M. MARSIN SANAGI

Department of Chemistry, Loughborough University of Technology, Loughborough, Leicestershire, LE11 3TU, U.K.

Abstract: The analysis of barbiturates using packed column supercritical fluid chromatography (SFC) has been investigated. The separations were carried out on either an ODS bonded silica or polystyrene–divinylbenzene polymer column with carbon dioxide as the mobile phase and flame ionisation detection. All the barbiturates were strongly adsorbed on the ODS-silica column, but reasonable separations were obtained by using the polymer column.

Keywords: *Supercritical fluid chromatography; barbiturates; flame ionisation detection; polymer column material.*

Introduction

Recently there has been a growing interest in supercritical fluid chromatography (SFC) as an analytical tool for the separation of a wide range of compounds [1–4]. The solvating power of supercritical fluids allows the separation of low volatility and high molecular mass compounds, often without prior derivatisation [5]. Many supercritical fluids have low critical temperatures (less than 100°C), which gives SFC an advantage over GLC for the separation of thermally unstable substances. Furthermore, with many supercritical fluids, SFC allows the direct use of the flame ionisation detector (FID) for sensitive, universal detection of organic compounds. SFC has potential as an alternative separation method with a selectivity different from more conventional techniques, which may enable improved separations of complex mixtures or matrices to be obtained.

Sensitive and reliable methods for the determination of barbiturates are of great interest in the therapy of epilepsy and in barbiturate toxicology. Numerous methods have been reported in the literature, mainly based on GLC [6] and HPLC [7–9]. However, no previous studies have reported the separation of barbiturates using SFC.

The present paper describes results of an investigation on the application of a packed column SFC, with flame ionisation detection, to the separation of barbiturates.

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†To whom correspondence should be addressed.

Experimental

Materials

Standard grade liquid carbon dioxide was obtained from a cylinder with a dip tube (British Oxygen Co., Brentford, Middlesex, U.K.). Methanol and dichloromethane were HPLC grade (BDH, Poole, U.K.). Reference samples of alkylarylketones and *n*-alkanes were of laboratory reagent grade from a range of different suppliers. Barbiturates were from the Central Research Establishment, Home Office Forensic Science Service, U.K.

The test substances were dissolved in dicholoromethane or methanol. The injected volume was 5 μl containing about 10 μg of each test substance.

Chromatography

The supercritical fluid chromatograph used in the present study has been described elsewhere [10] but with a few additional changes. It consisted of a modified Jasco Familic 300S HPLC pump (Jasco International Co. Ltd., Japan) and a Pye-Unicam 104 gas chromatograph. The three pump heads, the check valves and the eluent mixers were enclosed in a cooling box. Cold ethylene glycol–water mixture (−20°−−5°C) was circulated through the cooling box during SFC operations to prevent gasification of the carbon dioxide mobile phase. Injection was via a Rheodyne 7125 injector (Berkeley, CA, U.S.A.) fitted with a 20 μl loop. A T-piece effluent splitter was used to allow a small fraction of the effluent (about 60 ml min^{-1} of gas) to flow to the FID. The majority of the effluent was passed through a short length of stainless steel tubing as a waste restrictor, into a heated expansion chamber. The expansion chamber was made from a copper tube (⅛ inch o.d.), which is placed in a close contact with a heating block at the base of the FID. This simple device eliminated problems with CO_2 condensation in the waste restrictor.

Separations were carried out on either a 150 × 4.6 mm i.d. column packed with 5 μm PLRP-S polystyrene divinylbenzene (PS-DVB) particles (Polymer Laboratories Ltd.), or a 200 × 3 mm i.d. column packed with 5 μm Spherisorb ODS-2 particles (Phase Separations). The oven temperature was 60°C and column pressures of up to 240 kg cm^{-2} were used. Retention times and peak areas were recorded using a HP 3390A integrator (Hewlett Packard Co. Avondale, CA, U.S.A.). Acetone was coinjected with the samples to serve as dead volume marker. The capacity factors were calculated using $k' = [t_r - t_o]/t_o$. The retention indices based on the alkylarylketone scale [11], and the *n*-alkane scale [12] were calculated (retention indices of reference compounds = 100 × carbon number of the standards, respectively).

Results and Discussion

The barbiturates are derivatives of malonyl urea (Fig. 1) and contain secondary amido groups, which contribute to their high polarity. It was of interest to determine whether

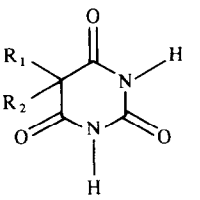

Figure 1
General molecular structure of barbiturates. R_1 and R_2 can be either alkyl, aryl or alicyclic groups.

Table 1
Capacity factors and retention indices based on *n*-alkanes and alkylarylketones of some barbiturates at two different column pressures. Other operating conditions as in Fig. 2

Compound	Column pressure: 155 kg cm^{-2}			Column pressure: 187 kg cm^{-2}		
	k'	RI–HC	RI–AAK	k'	RI–HC	RI–AAK
Barbitone	5.32	1771	1208	3.17	1746	1149
Methohexitone	6.16	1835	1282	3.43	1786	1194
Amylobarbitone	6.55	1862	1313	3.57	1805	1216
Butobarbitone	6.51	1860	1310	3.70	1822	1235
Pentobarbitone	6.56	1863	1314	3.78	1833	1247
Talbutal	7.50	1922	1380	4.30	1896	1318
Quinalbarbitone	8.34	1968	1434	4.54	1923	1349
Phenobarbitone	—	—	—	13.28	2448	1941
Heptabarbitone	—	—	—	13.69	2463	1958

Note: RI–HC and RI–AAK are retention indices based on *n*-alkanes and alkylarylketones, respectively.

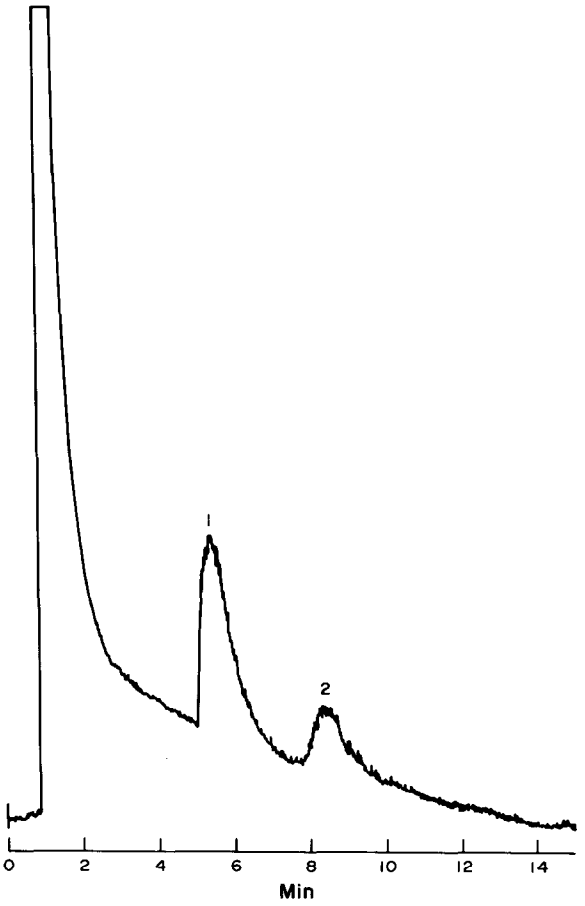

Figure 2
SFC separation of two barbiturates, on a PS–DVB polymer column with carbon dioxide as mobile phase. Column, 150 × 4.6 mm i.d. packed with 5 μm PLRP-S particles. Temperature 60°C, column inlet and outlet pressure 147 and 187 kg cm^{-2}, respectively. Attenuation 200. Key: 1, barbitone; 2, quinalbarbitone.

the high polarity of a weakly acidic analyte would permit separations to be carried out on either a non-polar PS-DVB or ODS-silica, with its residual silanols, using non-polar supercritical carbon dioxide as the mobile phase. However, when samples were injected onto the ODS-bonded silica column, none of the barbiturates under investigation were eluted with carbon dioxide as mobile phase over a range of operating pressures. This can probably be explained by the strong specific interactions between the polar functional groups and residual free silanol groups on the stationary phase. This assumption is consistent with previous suggestions [13, 14] in SFC that amido and other polar substituents, carboxy and amino groups, contributed strongly to retention on silica adsorption systems.

The separation was therefore studied using a PS-DVB polymer column, which should not show polar–polar interactions. On this column the barbiturates were eluted at a moderate pressure with reasonable retention times. The capacity factors, retention indices compared to the alkylarylketones [11], and Kovats retention indices [12] of the barbiturates were calculated (Table 1). The separation of barbitone and quinalbarbitone is illustrated in Fig. 2. The capacity factors of the barbiturates generally decreased with pressure. Unlike HPLC, when elution is in order of increasing molecular size [8], the capacity factors of the compounds did not always correspond with the molecular weight

Figure 3
SFC separation of alkylarylketones on a PS–DVB polymer column with carbon dioxide as mobile phase. Attenuation 1000. Other operating conditions as in Fig. 2. Key: 1, acetophenone; 2, propiophenone; 3, butyrophenone; 4, valerophenone; 5, hexanophenone; 6, heptanophenone.

order. Phenobarbitone and heptabarbitone appeared to be particularly strongly retained while the capacity factors of the other barbiturates were very close to each other.

As with all chromatographic methods some form of standardisation is needed. The retention indices compared to *n*-alkanes and alkylarylketones as standards were measured. The values for the latter can be compared to the values obtained earlier in the laboratory for HPLC on ODS-silica (e.g. 40:60 methanol–aqueous buffer, barbitone $RI = 579.0$, quinalbarbitone $RI = 930.4$) [8]. Although in the SFC separation the retention indices of the barbiturates on both scales decreased with increased pressure, the changes in retention indices based on the alkylarylketones appeared to be greater than the corresponding values based on *n*-alkanes, suggesting selectivity differences of the groups with pressure and solvating power of the fluid.

Unfortunately, even when using a polymer column, peak tailing and adsorption remained a significant problem with all the barbiturates (Fig. 2). In contrast, peak tailing was not observed for the *n*-alkanes or alkylarylketones retention index standards (Fig. 3). Since silanol groups are absent from the polymer column, this means that the tailing effects observed for the barbiturates cannot be because of solute–silanol interactions, and it appears that a more complex mechanism may be involved in the retention of these polar compounds.

Previous reports [14, 15] have suggested that the addition of small amounts of organic modifier, such as methanol, as modifier to the carbon dioxide mobile phase can give better separation of polar compounds. However, on trial runs, this caused problems with the background signal from the flame ionisation detector.

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