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High-performance liquid chromatography of benzodiazepines using sorbents with thermally immobilized Carbowax 20M

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ABSTRACT

The use of siliceous supports (controlled-porosity glass or silica gel) with thermally immobilized Carbowax 20M as sorbents for the separation of benzodiazepines by high-performance liquid chromatography (HPLC) was studied. The physico-chemical and chromatographic properties of these sorbents were compared with those of LiChrosorb DIOL and LiChrosorb RP-18. The results indicate that normal-phase HPLC chromatography of benzodiazepines using sorbents with thermally immobilized Carbowax 20M is a simple and effective method characterized by a simple binary mobile phase, very short column equilibration, high selectivity and a short time of analysis.

INTRODUCTION

Benzodiazepines belong to a group of anti-insomnia and anti-anxiety drugs [1]. In some instances the level of these drugs and their metabolites in body fluids such as blood or urine need to be monitored and controlled [2]. The methods most frequently used for this purpose are spectrophotometry [3,4], electrochemical methods [5,6], radioimmunoassay [7–9], supercritical fluid chromatography [10], gas chromatography (GC) [4,11,12], thin-layer chromatography [13–15] and high-performance liquid chromatography (HPLC) [8,14,16–21]. Chromatographic methods are superior to the others owing to their selectivity. They allow the separation and determination of individual drugs and their metabolites. However, GC analysis is complicated by the need for derivatization and the thermal instability of some drugs, particularly chlordiazepoxide and oxazepam. For this reason, HPLC analysis is prefered to GC.

According to the literature, the separation of benzodiazepines by HPLC is performed mainly using reversed-phase (RP) systems on sorbents composed of a silica support material with chemically bonded alkyl radicals (octyl or octadecyl) [9,14,22]. In these instances multi-component and buffered mobile phases are used [14,19,23,24]. However, RP columns working with buffered phases have a short lifetime. In addition, the analysis requires time-consuming pH adjustment depending on the composition of the sample mixture. Ion-pair chromatography [20,25] involves prolonged column equilibration before analysis.

In order to avoid the above disadvantages, in normal-phase chromatography can be considered [21,26,27]. Our preliminary investigations with silica gel as a column packing showed that unmodified silica materials are unsuitable. However, it was found that the use of less polar sorbents should resolve the problem. Such sorbents can be prepared by chemical bonding or immobilization of medium-polarity stationary phases to a silica support. This paper deals with the determination of benzodiazepines using a normal-phase HPLC system on siliceous supports with a bonded diol phase and a Carbowax 20M chain. The results are compared with those obtained in RP systems.

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EXPERIMENTAL

Preparation of sorbents with immobilized Carbowax 20M

The modified Aue–Daniewski procedure was employed [28,29]: 4-g portions of LiChrosorb Si 100 (Merck, Darmstadt, Germany) or controlled-porosity glass (CPG) (prepared in our laboratory) were placed in a round-bottomed flask that contained 100 ml of hexadecane (International Enzymes, Windsor, UK). A stream of nitrogen with an oxygen content below 2 ppm (Róży-Luxemburg Factory, Warsaw, Poland), additionally dried on molecular sieves 4A and 5A (Fluka, Buchs, Switzerland), was turned on 30 min before the heating began. A 4-g amount of Carbowax 20M (Aldrich, Milwaukee, WI, USA) was placed in a small funnel located between the flask and a reflux condenser. Heating was then started. First, most of the hexadecane liquefied on the wall of the condenser without touching the polymer. Subsequently, the cold finger of the condenser was cooled with air and the walls of the flask and the condenser were insulated, so vapour of hexadecane melted the polymer so that it dropped into the flask. After about 15 min all the polymer was in the flask and the process was continued for 5 h. The temperature of immobilization was controlled by a thermocouple set in the flask and maintained in the range 270–275°C. After allowing it to cool under nitrogen to 25–30°C, the hexadecane was decanted and the sorbent washed a few times with hexane (Reachim, Moscow, USSR) and chloroform (POCH, Gliwice, Poland). The material was then placed in a stainless-steel column (300 × 8 mm I.D.) thermostated at 60°C and washed with 250 ml each of hexane, chloroform and methanol (POCH) at a flow-rate of 1 ml/min. The solvents were pumped by means of a syringe-type pump. The sorbent wat then removed from the column and dried on a Schott funnel. Controlled-porosity glass and silica gel with immobilized Carbowax 20M are referred to as CPG-C and Si100-C, respectively.

Preparation of porous glass

Controlled-porosity glass (CPG) was obtained from Vycor-type alkali–boron–silica glass. This material was converted into the porous form by appropriate thermal and chemical treatment as decribed elsewhere [30]. In order to obtain the packing for the HPLC columns, the porous glass was ground and the *ca.* 8-μm fraction was separated.

Other materials

LiChrosorb DIOL (referred to as SiDiol) and LiChrosorb RP-18, particle size 10 μm (Merck), were used as additional columns packings.

Chromatographic measurements

Chromatographic measurement were made using a Liquochrom 2010 liquid chromatograph (MIM, Budapest, Hungary) equipped with a UV detector. The sorbents were packed into stainless-steel columns (250 × 4 mm I.D.) using the balanced-density slurry method. As the mobile phase in the normal-phase system isopropanol–hexane (30:70) was employed and in the reversed-phase system methanol–acetonitrile–0.005 *M* KH_2PO_4 buffer (pH 6.0) (26.5:16.5:57) was used (isopropanol from POCH; acetonitrile from Fluka). In all instances the flow-rate was 1 ml/min. Analyte substances (see Table I) were obtained from Polfa (Tarchomin, Poland).

Physico-chemical measurements

Specific surface area, pore diameter and pore volume were determined using a Sorptomat 1800 (Carlo Erba, Milan, Italy) on the basis of nitrogen adsorption–desorption hysteresis. The Carbowax content was calculated from CHN analysis data (Type 185 CHN Analyzer; Hewlett-Packard, Avondale, PA, USA).

Particle size fractionation

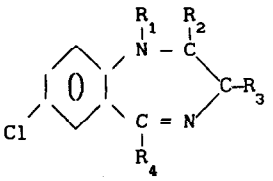
Particle size fractionation was carried out using Multi-Plex Laboratory Classifier 100 MZR (Alpina, Germany).

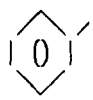
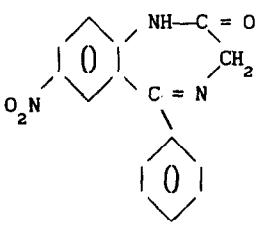
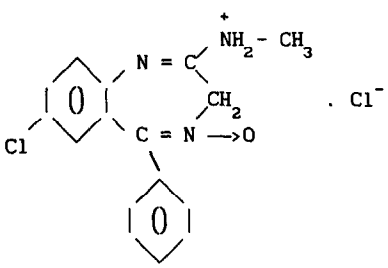
RESULTS AND DISCUSSION

The physico-chemical properties of the sorbents and siliceous supports of immobilized Carbowax 20M employed are given in Table II. The values of the specific surface area (S), mean pore diameter (D) and pore volume (V_p) of the porous glass (CPG) and silica gel (Si100) are similar. Immobilization of Carbowax 20M leads to some changes in the physico-chemical properties of sorbents obtained in this way (CPG-C and Si100-C), mainly a decrease in S

TABLE I

STRUCTURAL FORMULAE OF BENZODIAZEPINES



Name	R_1	R_2	R_3	R_4
Diazepam (Relanium)	$-CH_3$	=O		
Oxazepam (Serax)	-H	=O		
Medazepam (Norbrium)	$-CH_3$			
Nitrazepam (Mogadon)				
Chlorodiazepoxide (Elenium)				

and V_p. As can be seen from Table II, the amounts of bonded polymer are almost the same.

Retention data obtained for a benzodiazepine mixture on columns packed with the discussed materials are given in Table III. The capacity factors indicate that unmodified siliceous materials are not useful for analyse for benzodiazepines, at least with the applied chromatographic system. The differences among the capacity factors of the injected compounds are very small and some of the compounds, *e.g.*, chlordiazepoxide and oxazepam, do not appear in the chromatogram. This is connected with

TABLE II

PHYSICO-CHEMICAL PROPERTIES OF THE SORBENTS

S = specific surface area; V_p = pore volume; D_{max} = pore diameter corresponding to the maximum of the pore size distribution function; w/S = amount of Carbowax 20M immobilized on 1 m^2 of sorbent surface.

Sorbent	S (m^2/g)	V_p (cm^3/g)	D_{max} (Å)	$(w/S) \cdot 100$ (g/m^2)
CPG	347	1.16	100	–
Si100	318	1.32	140	–
SiDiol	230	0.90	100	–
CPG-C	92	0.39	100	0.154
Si100-C	119	0.62	130	0.147

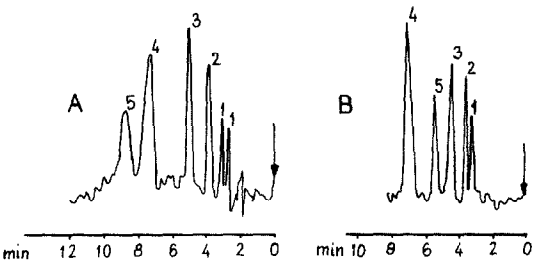


Fig. 1. Chromatograms of benzodiazepines in normal-phase system. Chromatographic conditions as described under Experimental. (A) CPG-C; (B) SiDiol. Peaks: 1 = medazepam; 2 = diazepam; 3 = chlordiazepoxide; 4 = oxazepam; 5 = nitrazepam.

the zone spreading effect caused by adsorption. When sorbents with an immobilized Carbowax 20M layer are used as column packings, the separations are considerably improved, especially with the CPG-C sorbent. This sorbent effects the complete separation of the benzodiazepines.

As was mentioned, SiDiol shows a polarity similar to those of packings with immobilized Carbowax 20M. The capacity factors of benzodiazepines obtained on SiDiol are given in Table III. The main difference between these data and those obtained on CPG-C is the elution sequence of oxazepam and nitrazepam. In addition, the capacity factors of benzodiazepines obtained on the SiDiol column are slightly lower than those obtained on the CPG-C column. The respective chromatograms are presented in Fig. 1. As can be seen from both Fig. 1 and Table III, medazepam is eluted from the CPG-C column as two peaks. Medazepam was obtained from a pharmaceutical manufacturer as a pure clinical preparation. It can be assumed that the two peaks correspond to geometric isomers and are separated owing to the high selectivity of the Carbowax 20M phase (the same is observed with Si100-C). It is also probable that they are products of the decomposition of medazepam (the drug was not capsuled), but Carbowax 20M still seems to be a more selective phase than SiDiol.

Fig. 2. shows a chromatogram of nitrazepam, oxazepam, chlordiazepoxide and diazepam obtained on LiChrosorb RP-18 in an RP system (the corresponding capacity factors are given in the last column of Table III). Medazepam was excluded from the mixture because of its extremely long elution time. Despite this, the analysis of the four drugs

TABLE III

CAPACITY FACTORS (k') OF BENZODIAZEPINES

Mobile phase (flow-rate 1 ml/min): (A) isopropanol–hexane (30:70); (B) methanol–acetonitrile–0.005 M KH_2PO_4 buffer (pH 6.0) (26.5:16.5:57).

Substance	k'					
	CPG (A)	CPG-C (A)	Si100 (A)	Si100-C (A)	SiDiol (A)	RP-18 (B)
Medazepam	0.59	0.51 0.77	0.56	0.44 0.61	0.43	–
Diazepam	0.83	1.22	0.86	1.13	0.65	19.3
Chlorodiazepoxide	–	1.90	–	1.64	1.05	11.0
Oxazepam	1.02	3.26	–	4.49	2.25	7.1
Nitrazepam	0.91	4.09	1.00	4.49	1.48	5.2

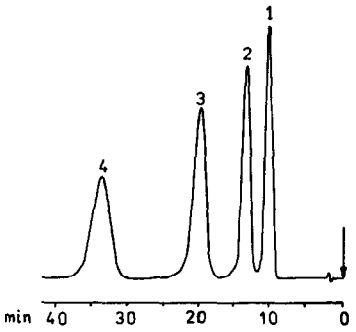


Fig. 2. Chromatograms of benzodiazepines in reversed-phase system. Chromatographic conditions as described under Experimental. Peaks: 1 = nitrazepam; 2 = oxazepam; 3 = chlordiazepoxide; 4 = diazepam.

present requires much more time than that for all five drugs on Carbowax 20M or Diol phase. Probably by using gradient elution and increasing the flow-rate it would be possible to separate all the described drugs (including non-polar medazepam) in an RP system. However, even then, comparing the mobile phase preparation and equipment, the advantages lie with isocratic chromatography in a normal-phase system.

CONCLUSIONS

Normal-phase chromatography of benzodiazepines using sorbents with thermally immobilized Carbowax 20M is a simple and effective method. Its main advantages are a simple binary mobile phase, very short column equilibration before analysis, high selectivity and a short time of analysis.

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