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Retention of some monoamine oxidase inhibitory drugs on a β-cyclodextrin polymer-coated silica column

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ABSTRACT

The retention of seventeen monoamine oxidase inhibitory drugs (mono- and disubstituted derivatives of propargylamine) was determined on a β-cyclodextrin polymer (β-CDP)-coated silica column using methanol–0.05 *M* K_2HPO_4 (6:4, v/v) as the eluent. The inclusion complex formation between the drugs and a water-soluble β-cyclodextrin polymer was studied by charge-transfer chromatography carried out on reversed-phase TLC layers. The capacity factors were correlated with the various physicochemical parameters and with the inclusion complex-forming capacity of the monoamine oxidase inhibitory drugs. Calculations indicated that the inclusion complex-forming capacity of the drugs does not influence their retention on a β-CDP column, that is, the water-soluble and water-insoluble β-CDPs show different retention characteristics. The specific hydrophilic adsorption surface of the solutes significantly influences the retention in HPLC. The results suggest that the selectivity of the β-CDP-coated silica support may be different from that of the traditional alkyl-bonded reversed-phase columns.

INTRODUCTION

Cyclodextrins (CDs) and various CD derivatives have found growing acceptance and application in many fields of chromatography [1]. They have been used in reversed-phase thin-layer chromatography (TLC) to study their interaction with various bioactive compounds such as barbiturates [2,3] and chlorophenol derivatives [4,5]. CDs modify the effective mobilities of various inorganic ions in isotachophoresis [6], improve the separation of peptides in capillary electrophoresis [7] and enhance the efficiency of enantiomeric separation in gas chromatography [8–11]. In high-performance liquid chromatography (HPLC), CDs are used in two different manners, either by adding CDs to the eluent [12–15] or by covalently bonding CDs to the silica surface [16–19]. CDs are used either to improve the separation of non-chiral compounds [20] or to separate enantiomers in both normal- [10] and reversed-phase systems [21]. So far as we are aware, CD polymers have not been applied frequently in HPLC [22].

Propargylamine derivatives are selective inhibitors of B-type monoamine oxidase [23,24] and the determination of their lipophilicity [25] and their TLC behaviour [26] have been recently reported.

The objectives of this work were to explore the separation characteristics of a β-cyclodextrin polymer (β-CDP)-coated HPLC column [27] using propargylamine derivatives as model compounds and to find the relationships between their retention behaviour and molecular structure.

EXPERIMENTAL

The structures of the steroid drugs studied are given in Table I.

Determination of the retention behaviour of drugs by high-performance liquid chromatography

The β-CDP-coated support (patent pending) was prepared at the Cyclolab Research and

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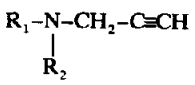
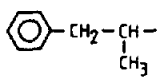
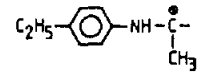
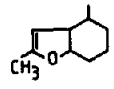
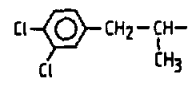
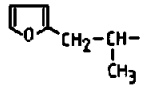
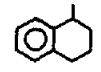
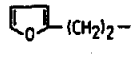
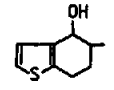
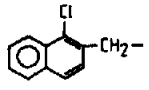
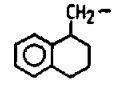
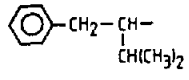
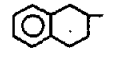
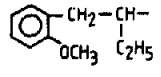
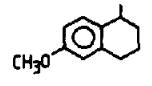
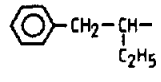
TABLE I

STRUCTURES OF MONOAMINE OXIDASE INHIBITORS



No.	R_1	R_2	No.	R_1	R_2
1 (+)		CH_3	10		CH_3
2 (−)		CH_3	11		H
3		CH_3	12		CH_3
4		H	13		CH_3
5		CH_3	14		CH_3
6		CH_3	15		CH_3
7		CH_3	16		CH_3
8		CH_3	17		CH_3
9		C_4H_7			

Development Laboratory (Budapest, Hungary). A 25 cm × 4 mm I.D. column was filled in our laboratory with a Shandon (Pittsburgh, PA, USA) analytical HPLC packing pump by the procedure proposed for the filling of reversed-phase columns. The HPLC equipment consisted of a Liquopump Type 312 (Labor MIM, Budapest, Hungary), a Cecil (Cambridge, UK) CE-212 spectrophotometer used as the detector, a Valco (Houston, TX, USA) 20-μl injector and a Waters (Milford, MA, USA) Model 740 integrator. The flow-rate was 0.6 ml/min and the detection wavelength was 225 nm. The eluent was methanol–50 m*M* K_2HPO_4 (6:4, v/v). Methanol was used in both the HPLC and TLC experiments because it forms only weak inclusion complexes with CDs [28,29]. The drugs were dissolved in the eluent at a concentration of 0.05 mg/ml. The retention time of each compound was determined by three consecutive determinations. The capacity factor and the relative standard deviation (R.S.D.) of the capacity factor were calculated for each compound.

Determination of the interaction between drugs and water-soluble β-cyclodextrin polymer by reversed-phase thin-layer chromatography

Silcoplat UV_{254} plates (Kavalier, Brno, Czech Republic) were impregnated with *n*-hexane–paraffin oil (95:5, v/v) by overnight predevelopment. The eluents were methanol–water mixtures, the methanol concentration varying between 45 and 60% (v/v) in steps of 5% (v/v). To determine the strength of the interaction between the drugs and β-CD, a water-soluble β-CDP (SCDP) was added to the eluent. Its concentration in the eluent was varied between 0 and 20 mg/ml. SCDP was prepared by cross-linking β-CD with butylene glycol bis(epoxypropyl ether) in aqueous alkaline solution. The product contained 66.04% β-CD. It should be emphasized that β-CDP and SCDP were prepared with different processes of polymerization, and therefore their inclusion forming capacities may also differ. After development the plates were dried at 105°C and the spots were detected under UV radiation and with iodine vapour. Each determination was run in quadruplicate.

The R_M values were calculated from the equation

$$R_M = \log(1/R_F - 1) \quad (1)$$

The dependence of the R_M value on the eluent composition was calculated from

$$R_M = R_{M0} + b_1 C_1 + b_2 C_2 \quad (2)$$

where R_M is the actual R_M value of a compound determined at given methanol and SCDP concentrations, R_{M0} is the R_M value of a compound extrapolated to zero methanol and SCDP concentrations, b_1 is the decrease in the R_M value caused by a 1% increase in the methanol concentration in the eluent (related to the specific hydrophobic surface area [30], b_2 is the decrease in the R_M value caused by 1 mg/ml change in the concentration of SCDP in the eluent (related to the relative strength of interaction) [31,32] and C_1 and C_2 are the methanol and SCDP concentration, respectively.

Calculation of relationships between retention behaviour and physico-chemical parameters of propargylamine derivatives

To elucidate the role of the physico-chemical parameters of drugs on their retention behaviour on a β-CDP column, stepwise regression analysis was applied [33]. The capacity factors of drugs determined on the β-CDP column were the dependent variables. The independent variables were the various hydrophobic and hydrophilic physico-chemical parameters of drugs taken from ref. 26 and the relative strength of inclusion formation with SCDP (b_2 value in eqn. 2). When the b_2 value was not significant, $b_2 = 0$ was included in the data matrix. The number of accepted independent variables was not limited and the acceptance limit was set to a 95% significance level.

RESULTS AND DISCUSSION

The retention order of propargylamine derivatives does not follow the lipophilicity order (Fig. 1); the less lipophilic compound **17** elutes after the more lipophilic compound **15**. This suggests that the retention characteristics of the β-CDP column differ from those of alkyl-bonded silica columns, where the retention order is determined by the lipophilicity order of the solutes.

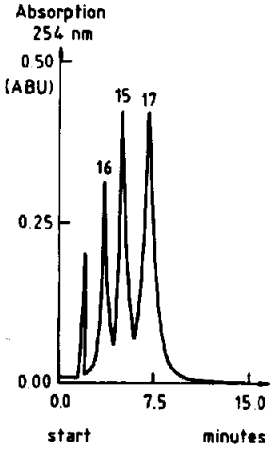


Fig. 1. Separation of monoamine oxidase inhibitory drugs on a β-cyclodextrin polymer-coated column. Numbers refer to drugs in Table I.

The log k' values and the R.S.D.s are given in Table II. The log k' values show wide variations, supporting our previous qualitative conclusions that the drugs can be separated on this column. The R.S.D.s are fairly low (in most instances less than 0.5%), indicating good reproducibility of retention times on the β-CD column.

The R_M values of some drugs decrease linearly with increasing concentration of water-soluble β-CDP in the eluent (Fig. 2). This can be explained by the assumption that the hydrophilic β-CDP interacts with the more lipophilic drugs and the complexes (probably inclusion complexes) exhibit lower lipophilicity.

The parameters of eqn. 2 are given in Table III. Eqn. 2 fits the experimental data well, the significance level always being over 95%. The ratio of variance explained by the independent variables varied between 45 and 95% (see r^2 values). Both the lipophilicity value extrapolated to zero methanol and SCDP concentration (R_{M0})

TABLE II

RETENTION OF MONOAMINE OXIDASE INHIBITORY DRUGS ON A β-CYCLODEXTRIN POLYMER-COATED COLUMN

Eluent; methanol–50 m*M* K_2HPO_4 (6:4, v/v).

Compound	Log k'	
	Mean[a]	R.S.D. (%)[a]
1	0.167	0.13
2	0.207	0.24
3	0.110	0.41
4	−0.178	0.19
5	0.166	0.25
6	0.748	0.61
7	0.463	>0.10
8	0.174	0.12
9	0.705	0.61
10	0.186	0.49
11	−0.132	0.18
12	0.524	0.14
13	0.526	0.36
14	−0.029	0.16
15	−0.033	0.16
16	0.289	0.11
17	0.525	0.29

[a] $n = 3$

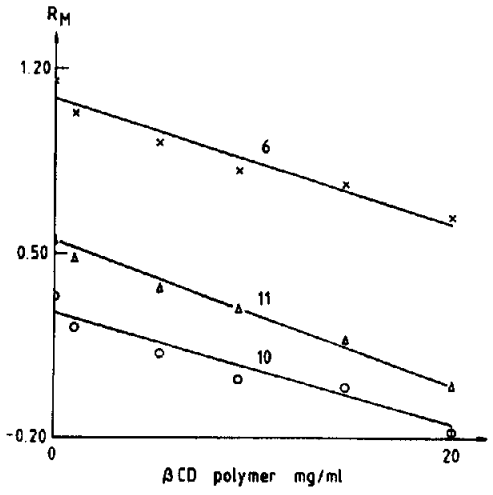


Fig. 2. Relationship between the R_M value of monoamine oxidase inhibitory drugs and the concentration of water-soluble β-cyclodextrin polymer in the eluent [water–methanol (45:55, v/v)]. Numbers refer to drugs in Table I.

and the specific hydrophobic surface area (b_1) differ considerably. This indicates that these parameters can be applied in quantitative structure–activity relationship (QSAR) calculations, and the drugs can easily be separated under reversed-phase chromatographic conditions. The interaction of SCDP with drugs **3–5**, **8**, **9** and **14** was not proved by the experiments. This result indicates that either the interaction does not take place or it is so weak that it is below the detection limit of the method.

According to the results of stepwise regression analysis, only the hydrophilic parameters of drugs influence their retention on the β-CDP column:

$$\log k' = 0.36 - (0.50 \pm 0.12)X_1 - (0.59 \pm 0.11)X_2 \quad (3)$$

$$n = 17;\ F_{calc.} = 43.41;\ r^2 = 0.8611$$

where X_1 = adsorption capacity of the drugs on a silica surface determined with acetonitrile–acetone eluents and X_2 = adsorption capacity of the drugs on an alumina surface determined with *n*-hexane–2-propanol eluents.

Eqn. 3 fits the experimental data well, the significance level being over 99.9% (compare $F_{calc.}$ values with the corresponding tabulated F

TABLE III

RELATIONSHIP BETWEEN THE LIPOPHILICITY (R_{M0}) OF MONOAMINE OXIDASE INHIBITORY DRUGS AND THE CONCENTRATIONS OF METHANOL (C_1) AND OF WATER-SOLUBLE β-CYCLODEXTRIN POLYMER (C_2) IN THE ELUENT

$R_M = R_{M0} + b_1C_1 + b_2C_2$. Compounds **1** and **2** were omitted from the calculations as they showed irregular spot shapes.

Parameter	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
n	13	13	13	13	13	13	11	13	13	13	13	12	13	13	13
R_{M0}	3.41	2.36	2.58	4.29	3.45	3.45	3.09	3.49	4.11	4.42	3.29	2.57	3.61	3.70	3.25
$-b_1$	5.58	4.70	4.79	5.92	4.59	5.29	2.92	5.98	6.71	6.27	4.64	5.43	5.77	5.18	4.75
s_{b1}	1.11	1.31	1.57	0.77	0.56	1.23	0.97	0.79	1.06	0.75	0.50	1.06	1.83	0.54	0.32
b_1' (%)	–	–	–	65.85	65.72	–	–	71.85	71.08	60.91	71.78	–	66.17	72.79	66.61
$-b_2$	n.s.[a]	n.s.	n.s.	1.90	1.48	n.s.	n.s.	1.45	1.69	2.50	1.13	n.s.	1.83	1.20	1.48
s_{b2}	–	–	–	0.48	0.35	–	–	0.49	0.66	0.46	0.31	–	0.45	0.34	0.20
b_2' (%)	–	–	–	34.15	34.28	–	–	28.15	28.92	39.09	28.22	–	33.83	27.21	33.39
r^2	0.6958	0.5408	0.4595	0.8582	0.8725	0.6277	0.5025	0.8523	0.8007	0.8852	0.8975	0.7235	0.8658	0.9011	0.9585
$F_{calc.}$	–	–	–	30.25	34.22	–	–	28.85	20.08	38.54	43.79	26.17	32.25	45.53	115.40

[a] n.s. = Not significant.

values). Two physico-chemical parameters explain about 86% of the retention behaviour of drugs on the β-CDP column (see r^2 value). Neither the lipophilicity parameters nor the strength of inclusion complex formation between the drugs and SCDP have a significant impact on the retention behaviour of drugs on the β-CDP column. This finding suggests that the retention characteristics of the β-CDP column differ from those of traditional reversed-phase columns, the impact of lipophilicity being insignificant, and the inclusion complex-forming capacities of water-soluble and water-insoluble β-CD polymers differ considerably, the complex stability values determined with the water-soluble β-CD polymer having no predictive value for the retention behaviour of the water-insoluble β-CD polymer.

The fact that only adsorption parameters influence the retention of drugs on the β-CDP column makes it probable that the hydrophilic surface of cyclodextrin and/or the cross-linking agent turns towards the eluent and the cyclodextrin cavities are not available or hardly available for the solutes in the eluent. The path coefficient values are comparable to each other (X_1 = 45.42% and X_2 = 54.58%), that is, the acidity (silica) or alkalinity (alumina) of the support surface has a commensurable effect on the retention. This suggests that silica is fairly well covered by the β-CD polymer.

It can be concluded that the β-CDP-coated silica is a promising reversed-phase support with a different selectivity to ODS silica. However, its capacity as a chiral stationary phase needs further investigation.

ACKNOWLEDGEMENT

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