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Effect of β-cyclodextrin derivatives on the retention of steroidal drugs

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Abstract

The retention of eighteen steroid drugs was determined on a β-cyclodextrin polymer (βCDP)-coated silica column using methanol–water mixtures as eluents. The relative strength of inclusion formation between the drugs and hydroxypropyl-βCD (HPβCD) and dimethyl-βCD was determined by charge transfer chromatography carried out on reversed-phase thin-layer chromatography plates. The retention characteristics of drugs were correlated with their physicochemical parameters and with their inclusion complex-forming capacity. Calculations indicated that the inclusion complex-forming capacity of the drugs has little impact on the retention that is due to the HPβCD and water-insoluble βCD polymers exposed to different retention characteristics. The hydrophilic molecular parameters of drugs significantly influenced their retention. This result suggests that the selectivity of the βCDP-coated column may be different from that of the traditional reversed-phase columns.

Keywords: Steroids; β-Cyclodextrin

1. Introduction

Over the last decades 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 (RP-TLC) to study their interaction with various bioactive compounds such as barbiturates [2,3], chlorophenol derivatives [4,5], etc. CDs modify the effective mobilities of various inorganic ions in isotachophoresis [6], improve 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 [16–20]. CDs are used either to improve separation of non-chiral compounds [21] or to separate enantiomers both in direct and reversed-phase systems [22]. According to our knowledge, CD polymers have not been frequently applied in HPLC [23].

Many HPLC methods have been developed for the separation of bioactive steroids [24] such as ion-pair HPLC [25], porous graphitic carbon column [26], CD bonded phase [27], etc.

The objectives of our investigations were to study the retention behaviour of a βCD polymer (βCDP)-coated silica support using biologically active ster-

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oids as model compounds, to find the physicochemical parameters of solutes governing the retention, to compare the inclusion forming capacity of various βCD derivatives and to determine the impact of inclusion complex formation on the retention.

2. Experimental

The chemical structures of steroid drugs are compiled in Table 1.

2.1. Determination of the retention behaviour of drugs by HPLC

The βCDP-coated silica support (patent pending) was prepared at the CYCLOLAB Research and Development Laboratory (Budapest, Hungary). The silica to be coated was the product of Merck (Darmstadt, Germany, particle size 5 μm, pore size 60 Å). As the CD was polymerized directly onto the surface of the silica support, we do not have information about the molecular mass distribution of this polymer coating. A 250 × 4 mm I.D. column was filled in our laboratory with a Shandon analytical HPLC packing pump (Pittsburgh, PA, USA) 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 CE-212 spectrophotometer (Cambridge, UK) used as the detector, a Valco 20-μl injector (Houston, TX, USA) and a Waters 740 integrator (Milford, MA, USA). The flow-rate was 0.6 ml/min and the detection wavelength used was 225 nm. Mixtures of methanol–water were used as the eluents. The methanol concentration ranged from 50 to 80% (v/v). The application of methanol as the organic modifier, both in HPLC and TLC experiments, was influenced by the fact that methanol forms only weak inclusion complexes with CDs [28,29] and, according to our preliminary investigations, methanol, together with acetonitrile, ethanol, tetrahydrofuran and dioxane, does not dissolve the polymer. The drugs were dissolved in the eluent at a concentration of 0.05 mg ml^{-1}. The retention time of each compound was determined by three consecutive determinations. As the correlation between the $\log k'$ and the organic phase concentration is generally linear in HPLC, we also applied linear equations to describe the dependence:

$$\log k' = \log k'_0 + b \cdot C \quad (1)$$

where: k'=capacity factor; k'_0=capacity factor extrapolated to zero methanol concentration in the eluent; b = change of $\log k'$ caused by unit change (1%, v/v) of methanol in the eluent and C = concentration of methanol (vol.%).

To test the validity of the hypothesis, that in the case of an homologous series of compounds the slope and intercept values of Eq. 1 are intercorrelated [30,31], the linear correlation was calculated between the two retention parameters.

To find the physicochemical parameters of steroids influencing their retention on the βCDP column, stepwise regression analysis was applied [32]. Parameters included in the calculation were: π = Hansch–Fujita's substituent constant characterizing hydrophobicity; *H-Ac* and *H-Do* = indicator variables for proton acceptor and proton donor properties, respectively; *M-RE* = molar refractivity; *F* and *R* = Swain-Lupton's electronic parameters characterizing inductive and resonance effect, respectively; σ = Hammett's constant, characterizing the electron-withdrawing power of the substituent; *Es* = Taft's constant, characterizing the steric effects of the substituent; *B*1 and *B*4 = Sterimol width parameters determined by the distance of the substituent at its maximum point perpendicular to the attachment bond axis.

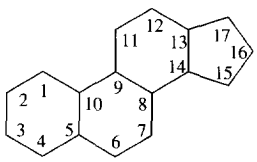
Stepwise regression analysis was carried out twice, with the intercept and slope values being the dependent variables separately, whereas the physicochemical parameters listed above were the independent variables in both cases. The acceptance level for the individual independent variables was set to a 95% significance level.

To check the inclusion complex forming capacity of the βCDP column, linear correlations were calculated between the parameters of Eq. 1 and the relative complex stability values determined by RP-TLC.

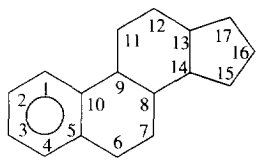
2.2. Determination of the interaction between drugs and βCD derivatives by RP-TLC

RP-TLC was performed on pre-coated silica plates of 0.25 mm thickness (Silcoplat UV_{254}, Labor MIM),

Table 1
Chemical structures of steroid drugs

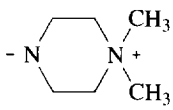
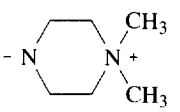
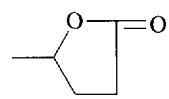
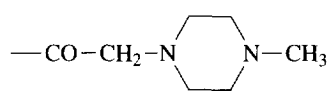


A



B

General structures

Number of compound	General structure	Substituent position 2	3	7	11	13	16	17
								
1	A		$-O-CO-CH_3$	–	–	$-C_2H_5$		$-O-CO-CH_3$
2	A[a]	–	=O	–	–	–	–	–OH $-CO-CH_3$
3	A[a]	–	=O	–	–OH	–	–	–OH $-CO-CH_2OH$
4	A[a]	–	=O	–	–	–	–	–OH –C≡CH
5	A[a]	–	=O	–	–	–	–	–OH $-CO-CH_2OH$
6	A[a]	–	–	–	–	–	–	–OH $-CH_2-CH=CH_2$
7	A[a]	–	=O	$-S-CO-CH_3$	–	–	–	
8	A[a]	–	=O	–	–	$-C_2H_5$	–	–OH –C≡CH
9	A[a]	–	=O	–	–	–	–	$-O-CO-CH_2-C_6H_5$
10	A[a]	–	$-O-CO-CH_3$	–	–	–	–C≡CH	$-O-CO-CH_3$
11	A[a]	–	=O	–	–	–	–	$-O-CO-CH_3$–C≡CH
12	A[b]	–	=O	–	–OH	–	–	$-CO-CH_3$ $C_{16}-O-C(CH_3)_2-O-C_{17}$
13	A[b]	–	=O	–	–OH	–	–	
14	A[c]		$C_2-CH=N-O-C_3$	–	–	–	–C≡CH	–OH
15	B	–	–OH	–	–	–	–	–OH
16	B	–	$-O-CO-C_6H_5$	–	–	–	–	–OH
17	B	–	$-O-CO-CH_3$	–	–	–	–	=O
18	B	–	–OH	–	–	–	–C≡CH	–OH

[a] Double bond between C_4 and C_5.
[b] Double bonds between C_1–C_2 and C_4–C_5.
[c] Double bonds between C_2–C_3 and C_4–C_5.

impregnated with *n*-hexane–paraffin oil (95:5, v/v). The impregnation was carried out by overnight pre-development. The drugs were separately dissolved in methanol to give a concentration of 5 mg/ml, and 2-μl samples of the solutions were separately spotted onto the plates. The developments were carried out in sandwich chambers (22×22×3 cm) at room temperature, and the running distance was ca. 15 cm.

The chambers were not pre-saturated. The eluent contained 0–55 vol.% methanol that was increased in steps of 5 vol.%. The concentration of HPβCD (with an average number of hydroxypropyl groups per molecule of βCD = 2.4) and dimethyl-βCD (DIMED) varied in the eluent between 2.5–20 and 1–50, respectively and both derivatives were the product of the CYCLOLAB Research and Development Laboratory. After development, the plates were dried at 105°C, and the spots were detected under UV light and with iodine vapour. Each determination was run in quadruplicate. The R_M values were calculated from Eq. 2:

$$R_M = \log (1/R_F - 1) \quad (2)$$

The dependence of the R_M value on the eluent composition was calculated from Eq. 3:

$$R_M = R_{M0} + b_1 \cdot C_1 + b_2 \cdot C_2 \quad (3)$$

where R_M = actual R_M value of a compound determined at a given methanol and HPβCD, or DIMEB, concentrations; $R_{M0} = R_M$ value of a compound extrapolated to zero methanol and HPβCD, or DIMEB, concentrations; b_1 = 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) [33]; b_2 = decrease in the R_M value caused by 1 mg/ml change in the concentration of HPβCD, or DIMEB, in the eluent (related to the relative strength of the interaction); C_1, C_2 = methanol and HPβCD, or DIMEB, concentrations, respectively.

Stepwise regression analysis was used, in the same manner as described above, in order to find the physicochemical parameters that had significant influence on the relative strength of interaction, .

3. Results and discussion

The drugs were eluted as symmetrical peaks in each eluent (Fig. 1), without the eluent being buffered. This finding suggests that βCDP evenly covers the silica surface and that the polar silanol groups have a negligible effect on the retention. The parameters of Eq. 1 are compiled in Table 2 (compounds 1, 6, 11, 13 and 16 were strongly retained on the column). The drugs showed no anomalous re-

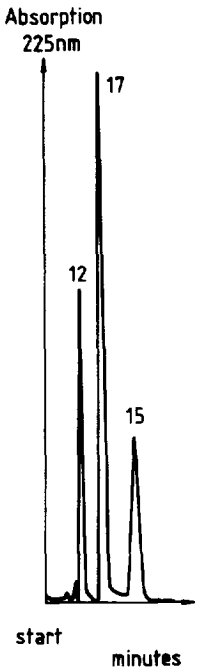


Fig. 1. Separation of some steroid drugs on a β-cyclodextrin polymer-coated column. Eluent used was methanol–water (75:25, v/v); flow-rate was 0.6 ml min^{-1}; detection was carried out at 225 nm. Numbers refer to the drugs named in Table 1.

Table 2
Linear correlations between between log k' and methanol concentration (C) in the eluent

Number of compound[a]	$\log k' = \log k'_0 + b \cdot C$			
	$\log k'_0$	$-b \cdot 10^2$	$S_b \cdot 10^3$	r
2	2.195	3.40	1.60	0.9976
3	1.228	2.27	2.60	0.9872
4	2.136	3.42	2.54	0.9943
5	1.931	2.99	2.04	0.9954
7	2.106	3.33	1.92	0.9967
8	2.232	3.54	1.50	0.9982
9	3.130	4.10	2.29	0.9969
10	3.049	4.14	2.06	0.9975
12	1.598	3.03	2.41	0.9937
14	2.040	3.26	1.66	0.9974
15	2.766	3.34	1.00	0.9991
17	2.559	3.55	2.30	0.9958
18	2.958	3.58	1.25	0.9988

[a] Numbers refer to steroid drugs in Table 1.
[b] S_b = standard deviation of slope.

Table 3
Correlations between R_M values of steroid drugs and the concentration of methanol (C_1 and hydroxypropyl-β-cyclodextrin (C_2) in the eluent

	$R_M = R_{M0} + b_1 \cdot C_1 + b_2 \cdot C_2$														
Parameter	No. of compound[a]														
	2	3	4	5	7	8	9	11	12	13	14	15	16	17	18
n	16	16	16	16	17	17	7	13	16	10	15	17	10	8	17
R_{M0}	2.40	1.58	2.67	2.18	2.95	2.79	6.39	2.81	1.94	1.66	2.98	2.32	2.29	1.29	2.15
$-b_1$	3.81	3.13	3.77	3.51	4.70	4.37	8.58	4.22	3.40	n.s.	4.02	3.57	2.47	n.s.	3.33
S_{b1}[b]	0.78	0.59	0.61	0.57	0.61	0.56	0.89	0.83	0.65	–	0.75	0.49	1.06	–	0.54
b_1' %	n.s.[c]	n.s.	74.77	63.39	70.19	78.50	67.46	–	–	–	69.79	51.40	–	–	54.09
$-b_2$	–	–	3.46	4.53	5.31	3.19	5.68	n.s.	n.s.	2.80	4.54	8.98	n.s.	6.73	7.52
S_{b2}	–	–	1.49	1.27	1.62	1.48	1.22	–	–	1.02	1.95	1.31	–	1.45	1.43
b_2' %	–	–	25.23	36.61	29.81	21.50	32.54	–	–	–	30.21	48.60	–	–	45.91
r^2	0.6311	0.6690	0.7953	0.7966	0.8244	0.8202	0.9625	0.7012	0.6616	0.4820	0.7196	0.8668	0.4041	0.7832	0.8107
$F_{calc.}$	–	–	27.61	25.45	32.87	31.93	51.30	–	–	–	15.39	45.56	–	–	29.97

[a] Numbers refer to steroid drugs in Table 1.
[b] S_b = standard deviation of slope.
[c] n.s.= not significant.

tention behaviour, the retention time decreasing uniformly with increasing concentrations of methanol in the eluent. The correlation coefficient in most cases was greater than 0.99, thus confirming the applicability of Eq. 1. The slopes and intercepts differed considerably from each other. This means that the steroid derivatives can be easily separated on the βCDP column.

Significant linear correlation was found between the slope and intercept value of Eq. 1. (r = 0.8965; n = 13) indicating that the drugs can be considered as an homologous series of solutes. Stepwise regression analysis found significant correlation between the retention parameters (slope and intercept of Eq. 1) and the physicochemical characteristics of the drugs:

$$\log k_0' = 3.04 - (0.47 \pm 0.07) \cdot H - Ac - (4.68 \pm 1.42) \cdot Es \quad (4)$$
$$n = 13, \quad f = 27.52, \quad r^2 = 0.8462$$

$$b = 3.95 - (0.63 \pm 0.07) \cdot H - Do \quad (5)$$
$$n = 13, \quad r = 0.9022$$

The fact that polarity parameters primarily influence the retention of drugs on βCDP columns makes it probable that the hydrophilic surface of CD and/or the crosslinking agent turns towards the eluent and the CD's cavities are therefore not available (or barely available) for the solutes in the eluent. The role of structural parameters is of secondary importance (the path coefficients in Eq. 4 are 68.74 and 31.26% for *H-Ac* and *Es*, respectively).

The parameters of Eq. 3 are compiled in Table 3 and Table 4. Compounds one and six were very near to the start when HPβCD was used as the eluent additive, therefore their interaction with HPβCD cannot be determined under the experimental conditions applied. In contrast to HPβCD, the majority of drugs were always on the DIMEB front, indicating that that they form very strong complexes with DIMEB. The few cases where the calculation could be carried out (Table 4) suggested that DIMEB forms more stable inclusion complexes with steroids than HPβCD does and that there is no correlation between the complexing characteristics of these two βCD derivatives. We have to stress that, due to the low number of data, the conclusions mentioned above are only tentative ones and that much more data are needed to exactly compare the complexing capacity of HPβCD and DIMEB.

The complex forming capacity of HPβCD significantly depended on the bulkiness of the steroid drugs

$$b_2(\text{HP}\beta\text{CD}) = 7.55 - (6.37 \pm 2.78) \cdot M - RE \quad (6)$$
$$n = 10, \quad r = 0.6291$$

Table 4
Correlations between R_M values of steroid drugs and the concentration of methanol (C_1 and dimethyl-β-cyclodextrin (C_2) in the eluent

$$R_M = R_{M0} + b_1 \cdot C_1 + b_2 \cdot C_2$$

Parameter	No. of compound[a]					
	6	9	10	11	13	16
n	17	18	20	21	22	18
R_{M0}	1.09	3.39	3.10	3.51	1.85	3.15
$-b_1$	n.s.[c]	4.32	3.65	5.28	1.24	3.66
S_{b1}[b]	–	1.05	0.95	0.67	0.42	1.15
b_1%	–	42.66	37.74	46.30	36.20	38.32
$-b_2$	4.34	11.31	10.96	11.24	1.97	11.49
S_{b2}	0.49	2.05	1.73	1.23	0.37	2.24
b_2' %	–	57.72	62.26	53.70	63.80	61.68
r^2	0.8394	0.6891	0.7089	0.8228	0.6342	0.6960
$F_{calc.}$	16.62	20.69	41.81	16.47	17.17	

[a] Numbers refer to steroid drugs in Table 1.
[b] S_b = standard deviation of slope.
[c] n.s. = not significant.

This result indicates that the interaction is really due to inclusion complex formation. The inclusion forming capacity of drugs has a significant effect on their retention on a βCDP column, however, the variance explained is fairly low (46.1%):

$$\log k_0' = 1.56 + (0.16 \pm 0.06) \cdot b_2(\text{HP}\beta\text{CD}) \qquad (7)$$
$$n = 9, \quad r = 0.6790$$

This finding proves that not only the inclusion complex formation, but also other interactions, has a considerable impact on the retention times of steroid drugs on βCDP columns.

It can be concluded from the data that the βCDP-coated silica is a promising reversed-phase support exposing different selectivities than those found with ODS silica. However, the exploration of its capacity as a chiral stationary phase needs further investigation.

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