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Correlation between retention and C-18 silica gel surface coverage in reversed-phase liquid chromatography

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The effect of C-18 silica gel surface coverage on the retention behaviour of some estrone derivatives in reversed phase high performance liquid chromatography has been studied. Two commercial columns with different C-18 coverage, Spherisorb ODS-1 (8 % carbon content) and Li Chrosorb RP-18 (22 % carbon content), using methanol-water as the eluent, were used.

Keywords: HPLC, reversed phase, estrone derivatives.

INTRODUCTION

The stationary phase plays an active role in the retention processes. Partitioning and selectivety are both highly dependent on the chain density of the bonded hydrocarbon phase.[1,2] The most popular are the reversed-phase materials in which the ligand is most commonly a saturated hydrocarbon chain of 18 or 8 carbons in length. These two bonded phases probably account for over 80 % of all liquid chromatography analysis. Because of steric hindrance, the maximum possible concentrations of alkyl groups in a bonded phase is ≈4.5 μmol/m^2 for C–18 ligands.

Steroids are of interest in chromatographic investitaions as they offer the opportunity to study the effect of substituents on the retention. A series of estrone derivatives were synthesized in order to functionalize ring B and D of the estrone skeleton and hence attempt to change the hormonal activity of estrone.[3,4]

In previous work,[5–7] we have described the retention behaviour in normal- and reversed-phase HPTLC and HPLC of several series of estrone derivatives. The type, the number and position of substituents in a compound molecule were observed to have significant and distinct effects on the retention in both normal- and reversed-phase chromatography.

This paper describes a study of the retention behaviour of a series of estrone derivatives on two commercial columns with different carbon contents. The compounds and their structures are listed in Table I.

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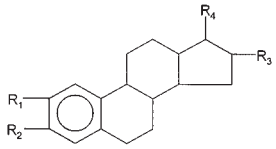
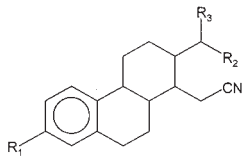
TABLE I. Chemical structures of the studied compounds



Compound	R_1	R_2	R_3	R_4
1.		OH		=O
2.		OCH_3		=O
3.		$OCH_2C_6H_5$		=O
4.		OCH_3	=NOH	=O
5.		$OCH_2C_6H_5$	=NOH	=O
6.		OCH_3	=NOH	OH
7.		$OCH_2C_6H_5$	=NOH	OH
8.	H_3COC	OCH_3		=O



Compound	R_1	R_2	R_3
9.	OCH_3	H	=O
10.	$OCH_2C_6H_5$	H	=O
11.	OCH_3	H,H	=O
12.	$OCH_2C_6H_5$	H,H	OH
13.	OCH_3	H,H	$OCOCH_3$
14.	OCH_3	H,H	$OSO_2C_6H_4CH_3$
15.	OCH_3	H,H	OSO_2CH_3
16.	OCH_3	C_6H_5	=O
17.	OCH_3	C_6H_5	OH

IUPAC names of steroids: Common name

1. 3-Hydroxyestra-1,3,5(10)-triene-17-one, Estrone
2. 3-Methoxyestra-1,3,5(10)-triene-17-one, Estrone-3-methyl ether
3. 3-Benzyloxyestra-1,3,5(10)-triene-17-one, Estrone-3-benzyl ether
4. 3-Methoxyestra-1,3,5(10)-triene-16,17-dione 16-oxime
5. 3-Benzyloxyestra-1,3,5(10)-triene-16,17-dione 16 -oxime
6. 17-β-Hydroxy-3-methoxyestra-1,3,5(10)-triene-16-one oxime
7. 17-β-Hydroxy-3-benzyloxyestra-1,3,5(10)-triene-16-one oxime
8. 2-Acetyl-3-methoxyestra-1,3,5(10)-triene-17-one
9. 3-Methoxy-17-oxo-16,17-secoestra-1,3,5(10)-triene-16-nitrile
10. 3-Benyloxo-17-oxo-16,17-secoestra-1,3,5(10)-triene-16-nitrile
11. 3-Methoxy-17-hydroxy-16,17-secoestra-1,3,5(10)-triene-16-nitrile
12. 3-Benzyloxy-17-hydroxy-16,17-secoestra-1,3,5(10)-triene-16-nitrile
13. 3-Methoxy-17-acetoxy-16,17-secoestra-1,3,5(10)-triene-16-nitrile
14. 3-Methoxy-17-p-toluenesulfonyloxi-16,17-secoestra-1,3,5(10)-triene-16-nitrile
15. . 3-Methoxy-17-methanesulfonyloxy-16,17-secoestra-1,3,5(10)-triene-16-nitrile
16. 3-Methoxy-17-oxo-17-phenyl-16,17-secoestra-1,3,5(10)-triene-16-nitrile
17. 3-Methoxy-17-hydroxy-17-phenyl-16,17-secoestra-1,3,5(10)-triene-16-nitrile

EXPERIMENTAL

HPLC separations were performed with a Milton Roy (Riviera Beach, FL, USA) consta Metric 3000 pump and a Milton Roy spectro Monitor 3100 variable-wavelenght UV-Vis detector set at 280 nm. The samples were injected using a Rheodyne 7125 valve (Cotati, CAL, USA) fitted with a 20 μL loop. The columns used were the commercially available Li Chrosorb RP-18, 5 μm, 22% carbon content (E. Marck, Darmstadt, Germany) and Spherisorb ODS-1, 5 μm, 8 % carbon content (Phase Separations, Queensferry, UK).

The synthesized[3,4] estrone derivatives (Table I) were dissolved (0.05 mg ml^{-1}) in methanol and the solutions prefiltered through a 0.2 μm Chromafil filter (Macherey-Nagel, Duren, Germany).

All solvents used to prepare the mobile phases were of analytical grade and were filtered through a 0.45 μm filter and degassed before use. The flow-rate was 1 ml min^{-1} at room temperature.

The capacity factor, k', was calculated from $k' = (t_r - t_0)/t_0$, where t_r is the retention time of the solute and t_0 the column void time measured using the solvent disturbance peak. Each t_r value was measured in triplicate and averaged.

RESULTS AND DISCUSSION

The retention data for the estrone derivatives on a Spherisorb ODS-1 column and on Li Chrosorb RP-18 column, using methanol-water (8 : 2) as the elutant, are given in Table II.

TABLE II. Retention data for the estrone derivatives on a Spherisorb ODS-1 and Li Chrosorb RP-18 columns, using methanol-water (8 : 2) as the elutant

Compound	Spherisorb ODS 1	Li Chrosorb
	log k	
1.	0.067	0.175
2.	0.158	0.261
3.	0.699	0.803
4.	–0.122	–0.039
5.	0.396	0.485
6.	–0.162	0.003
7.	0.381	0.501
8.	–0.051	0.052
9.	–0.350	–0.244
10.	0.128	0.242
11.	–0.451	–0.299
12.	0.063	0.158
13.	–0.204	–0.141
14.	–0.083	0.240
15.	–0.550	–0.200
16.	–0.034	0.026
17.	0.079	0.168

The compounds were retained longer on the Li Chrosorb RP-18 column than on the Spherisorb ODS-1 column. The elution order of the compounds was very similar on

both columns and consequently the analysis time is shorter on the Spherisorb ODS-1 column.

The sequence of separation on the ODS-1 column is:

$$3>5\geq 7>2>10>17\geq 1 = 12>16\geq 8\geq 14>4>6>13>9>11>15$$

The sequence of separation on the RP-18 column is:

$$3>7>5>2>10\geq 14>1\geq 17>12>8>16>6>4>13>15>9>11$$

On the basis of the separation sequence, it is shown that the retention behaviour of the estrone derivatives is in accordance with their polarity. Namely, less polar solutes are more strongly retained and *vice versa*.

On both columns estrone-3-benzyl ether was retained the longest. Compounds 5 and 7 were not or only poorly resolved, because in reversed-phase HPLC the retention is determined by the benzyloxy functions only, *i.e.,* the polar keto and hydroxy groups at position 17 did not affect the retention.

The compounds possessing the benzyloxy function were retained significantly longer than the compounds bearing a methoxy group, because the phenyl ring is more hydrophobic compared to hydrogen atom. Hence the retention of compounds 9, 11, 13 and 15 resulted in lower retention.

Values of $\Delta\log k$ for the substitution of the BzO- with the MeO- function for various solute pairs (solute pairs 3–2, 5–4, 7–6, 10–9, 12–11) are reasonably constant for the particular columns (Table III). On the other hand, compound 14 possessing tosyloxy function was retained significantly longer than compound 15 bearing a mesyloxy group, because the tosyloxy function is more hydrophobic than the mesyloxy group.[5,8,9]

TABLE III. $\Delta\log k$ values for conversion of the substituent -OBz in -OMe in estrone derivatives; Compound numbering as in Table I

Converted functional group	Compounds compared	ODS-1, $\Delta\log k$	RP-18, $\Delta\log k$
–OBz[a)] →–OMe	3 and 2	0.541	0.542
	5 and 4	0.518	0,524
	7 and 6	0.543	0.498
	10 and 9	0.478	0.486
	12 and 11	0.514	0.457

[a)] –OBz, benzyloxy; –OMe, methoxy

A linear relationship between the retention constant of a compound (log k) and the methanol concentration in the mobile phase (φ_{org}) exists according to the well-known equation:

$$\log k = \log k_w - m\varphi_{org} \quad (1)$$

where m and k_w are constants. The linear relationship between the retention con-

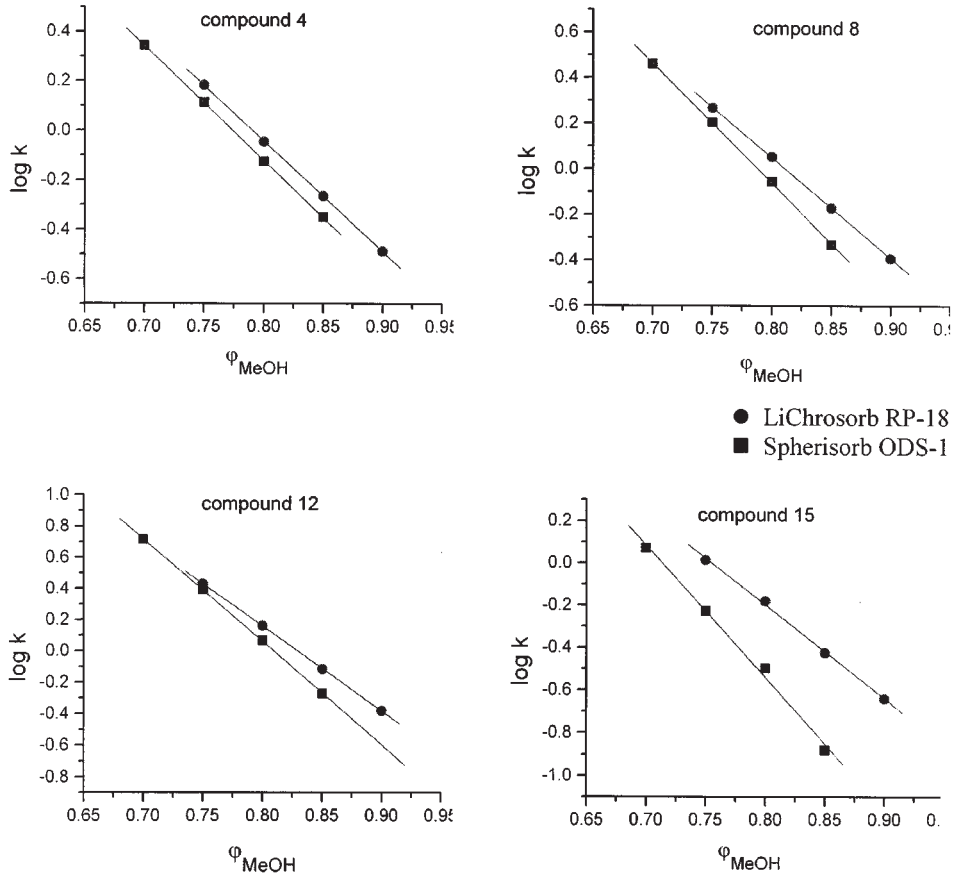


Fig. 1. Linear relationship between the retention constant (log k) and the methanol concentration in the mobile phase (φ_{MeOH}) for compounds 4,8,12 and 15.

stant and the methanol concentration for compounds 4, 8, 12 and 15 are presented in Fig. 1. The numerical values of the constants m and k_W for each compound are presented in Table IV. Correlation coefficients from linear regression analysis of the experimental log k values varied from 0.9799 to 0.9999. It is apparent from the data in Table IV that both constants m and log k_W are higher on the Spherisorb ODS-1 than on the Li Chrosorb RP-18 column. The lower log k_W values on the Li Chrosorb RP-18 column are the consequence of lower m values. The higher m values on the Spherisorb ODS-1 column could be explained in terms of solute Raoult law activity coefficients in the mobile (γ_m) and the stationary (γ_s) phase:

$$\log k = \log \gamma_m - \log \gamma_s + \log \phi \qquad (2)$$

where ϕ is the phase ratio of a column. Combining Eqs. (1) and (2), Eq. (3) is obtained:

$$\log \gamma_m = \log \gamma_{mw} - \log \gamma_{sw} + \log \gamma_s - m\varphi_{org} = const. - m\varphi_{org} \quad (3)$$

According to Raoult law, the activity of a compound in the mobile phase a_m is equal to $c_m \gamma_m$, *i.e.*, $\gamma_m = a_m / c_m \approx 1/c_m$ where c_m is the concentration of a compound in the mobile phase. Hence, Eq. (3) can be written in the following form:

$$\log c_m = m\varphi_{org} - const. \quad (4)$$

According to Eq. (4), constant m is given by:

$$m = \frac{d(\log c_m)}{d\varphi_{org}} \quad (5)$$

As c_m in the column with the lower carbon content (Spherisorb ODS-1) is higher than in the Li Chrosorb RP-18 column, constant m is also higher.

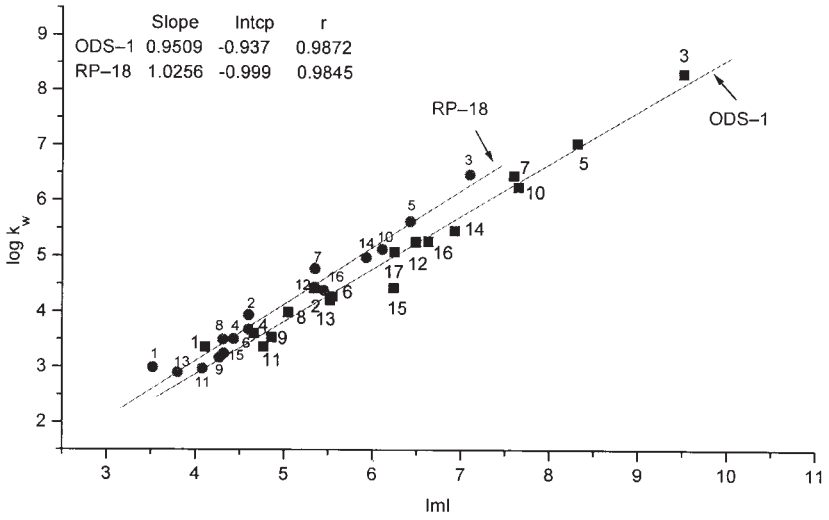


Fig. 2. Plots of log k_w against $|m|$ [Eq. (1)] on both columns using methanol-water as the mobile phase. Compound numbering as in Table I.

It is apparent from the data in Table IV that on both columns the constant log k_w and the absolute value of m increase with increasing hydrophobicity of the estrone derivatives. There is, therefore, a linear relationship between these two constants, on both columns, with high correlation coefficients (Fig. 2).

As can be seen from the values of the slopes in Fig. 2, the Li Chrosorb RP-18 column, with the higher carbon content, also has a higher separation efficiency.

TABLE IV. Constants in Eq. (1) for the estrone derivatives on both columns; *r*-correlation coefficient

Compound	Spherisorb ODS-1			Li Chrosorb RP-18		
	log k_w	$\|m\|$	*r*	log k_w	$\|m\|$	*r*
1.	3.36	4.11	0.9957	2.99	3.52	0.9967
2.	4.43	5.34	0.9985	3.94	4.60	0.9977
3.	8.32	9.52	0.9976	6.48	7.10	0.9988
4.	3.61	4.66	0.9999	3.51	4.43	0.9999
5.	7.05	8.32	0.9985	5.63	6.43	0.9992
6.	4.28	5.55	0.9987	3.68	4.60	0.9991
7.	6.46	7.60	0.9988	4.78	5.35	0.9992
8.	3.19	5.05	0.9998	3.50	4.31	0.9999
9.	3.54	4.86	0.9980	3.17	4.27	0.9997
10.	6.25	7.65	0.9989	5.13	6.11	0.9992
11.	3.37	4.77	0.9989	2.97	4.08	0.9994
12.	5.26	6.49	0.9999	4.43	5.34	0.9999
13.	4.21	5.52	0.9991	2.90	3.80	0.9899
14.	5.46	6.93	0.9960	4.98	5.93	0.9980
15.	4.44	6.24	0.9972	3.26	4.32	0.9991
16.	5.27	6.63	0.9990	4.39	5.45	0.9991
17.	5.08	6.25	0.9986	4.45	5.35	0.9988

ИЗВОД

КОРЕЛАЦИЈА ИЗМЕЂУ РЕТЕНЦИЈЕ И ПОКРИВЕНОСТИ НЕПОКРЕТНЕ ФАЗЕ ОКТАДЕЦИЛ ЛИГАНДИМА У ТЕЧНОЈ ХРОМАТОГРАФИЈИ НА ОБРНУТИМ ФАЗАМА

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Испитан је утицај покривености непокретне фазе октадецил – лигандима на ретенционо понашање деривата естрона у високоефикасној течној хроматографији, на обрнутим фазама. При томе су коришћене две комерцијалне колоне са различитим садржајем октадецил – лиганада: Spherisorb ODS-1 (8 % везаног угљеника) и Li Chrosorb RP-18 (22 % угљеника). Као покретна фаза коришћен је елуент метанол–вода.

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