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Normal- and Reversed-Phase of 16,17-Secoestra-1,3,5(10)triene Derivatives on Chemically Bonded Phases

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Abstract

The retention behaviour of a number of 16,17-secoestra-1,3,5(10)triene derivatives has been studied by HPLC on two chemically bonded polar stationary phases: aminopropyl and diol- and one non-polar C-18 phase, all of which are commercially available columns, using non-aqueous and aqueous-organic mobile phases. The retention behaviour has been discussed in terms of nature of the solute, eluent and stationary phase and compared with results of the same derivatives obtained in earlier investigations. The correlation between the retention constants of 16,17- secoestra-1,3,5(10)triene derivatives obtained from reversed-phases and commercially available ACD log *P* software (Advanced Chemistry, Toronto, Canada) has also been examined.

Keywords

Aminopropyl-, 1,2-dihydroxypropylether- (diol-) and octadecyl silica gel (C-18)
16,17-secoestra-1,3,5(10)triene derivatives
Normal-phase and reversed-phase
Correlation analysis

Introduction

Estradiol is the most important human estrogen produced by the ovaries. Other estrogens (estrone, estriol) may be weaker, but in principle, have the same effect. The importance of the functional groups in the activity of natural estrogens has been recognized. Some simple chemical modifications of the basic structure of estrogens can have a direct effect on their activity, primarily by modification of the binding activity of the steroid to receptor.[1] Thus, small conformational changes in estrogens have an important role in the design of new estrogens and antiestrogens.[2] The development of steroidal compounds is often followed by research on structure-activity relationship studies.[3-6] Knowledge of the stuctural characteristics

of a compound is one of the most significant factors for understanding the structure-acitivity relationships underlying its biological activity.

In our previous papers[7-10] we have described the retention behaviour and retention mechanism of some estradiol and estrone derivatives chromatographed on: silica gel, alumina, cyanopropyl silica gel, C-8 and C-18 bonded silica gel in normal- and reversed-phase, using several non-aqueous and aqueous eluents. It was observed that the type of stationary and mobile phases, and the nature, number, and position of substituents in the compound molecule steroids have significant and distinct effects on retention.

Aminopropyl- and diol- stationary phases bonded on a silica gel comprising three carbon atoms are less polar than non-modified silica gel or alumina adsorbents. The bonded chains are not long enough to provide efficient shielding of the residual silanol groups, which could not be modified in the silanization procedure because of steric reasons. Because of that chemically bonded polar stationary phases provide for more specific interaction at the surface and have operating advantages over traditional silica gel.[11-13] The specific properties of a polar chemically bonded phases in the interaction with steroids have been shown in normal- and reversed- phase partition mode.[8,9,14]

On the other hand the most popular are the reversed- phase materials in which the ligand is most commonly a saturated hydrocarbon chain of 18 or 8 carbon in length. The specific properties of the octyl- and octadecyl- silica gel chemically bonded phases in the interaction with steroids have been shown in reversed- phase partition mode.[10,14]

For initial chemical screening of activity of newly synthesized compounds, it is first recommended to determine their lipophilicity. The most widely accepted measure of lipophilicity is the octanol-water partition coefficient, which is expressed in its logarithmic form as log *P*.[6,11] Literature is rich in research articles investigating similarities/dissimilarities between log *P* and chromatographic retention.

This paper investigates retention behaviour and retention mechanism of 16,17-secoestra-1,3,5(10)triene derivatives on diol-, aminopropyl- and C-18 columns using heptane-propan-1-ol, acetonitrile-water and isopropanol-water eluents in order to determine the retention constants of 16,17-secoestra-1,3,5(10)triene derivatives, and to correlate these constants with log *P* calculated by the fragmental based method using the ACD log *P* software (Advanced Chemistry, Toronto, Canada).

The compounds and their molecular structures are listed in Table 1.

Table 1.

Experimental

HPLC separations were performed on an Agilent 1100 Series HPLC (Agilent Technologies, USA) including a degasser G1379 A, binary G1312 pump, ALS G1313A, COLCOM G1316A and DAD G1315B. The columns used were commercially available particle size 5 μm: LiChrosorb NH_2, 250 × 4 mm i.d. and LiChrosorb DIOL, 250 × 4 mm i.d. (both E. Merck, Darmstadt, Germany) and Spherisorb ODS-2.5 μm, 124 × 4 mm i.d. (Agilent).

16,17-secoestra-1,3,5(10)triene (Table 1), synthesized by original reaction or according methods in the literature[2] were dissolved (0.05 mg mL^{-1}) in methanol, and the solutions filtered through a 0.2 μm Chromafil filter (Macherey-Nagel, Duren, Germany). The three binary solvent systems, hexane-propan-1-ol, acetonitrile-water and isopropanol-water were used as a mobile phase with a varying content of organic modifier, propan-1-ol 5-20%, acetonitrile 70-90% and isopropanol 70-95 in 5% increments. The water used as a mobile phase component had been

distilled twice. The eluents used to prepare the mobile phases were of HPLC grade (E. Merck). The flow rate was at 1mL min^{-1} at room temperature.

The retention factor, *k*, was calculated from Eq. (1):

$$k = \frac{t_r - t_0}{t_0} \qquad (1)$$

where t_r is the retention time of the solute and t_0 the column void time. Each t_r value was measured in triplicate and averaged.

The behaviour and separation of the two mixtures (compounds **7**, **9**, **11** and **13** and compounds **8**, **10**, **12** and **14**) were also investigated. Both mixtures were applied to the columns as 0.001% solutions in methanol.

The correlation analysis and all calculations were performed by the use of the computer program Origin 6.1.

Results and Discussion

Two types of compounds were studied: 16,17-secoestra-1,3,5(10)triene derivatives hydroxylated at the 3-position (odd numbered compounds) and their 3-benzyloxy counterparts (even numbered compounds), Table 1.

Retention Behaviour of 16,17-Secoestra-1,3,5(10)triene Derivatives on Diol- and Aminopropyl- Columns Using Hexane-Propan-1-ol

The relationship between the logarithmic retention constant, log *k*, and the volume fraction, -log φ, of the propan-1-ol in binary eluent was in accordance with the well-known Eq. (2), generally accepted in adsorption chromatography, (Figs. 1 and 2):

$$\log k = \log k_0 - n \log \varphi \qquad (2)$$

Figs. 1 and 2

The numerical data constants *n* and log k_0 for each compound studied for aminopropyl- and diol- columns in eluent hexane-propan-1-ol are presented in Table 2.
Correlation coefficients from linear regression analysis of experimental log *k* values varied between $r^2 =$ 0.9030 to 0.9999, respectively.

Table 2.

The retention behaviour of 16,17-secoestrone derivatives on the both columns were very similar and in accordance with general retention behaviour in normal-phase liquid chromatography. The retention sequence obtained with elunent hexane-propan-1-ol is that predicted on the basis of the polarity of the compounds; the more polar solutes were more strongly retained and vice versa. Hydroxylated derivatives were generally more retained compared with their even numbered compounds, due to increased polarity of the former (Table 1). The most retained compounds were compounds **1**, **5** and **3**.

All 16,17-secoestra-1,3,5(10)triene derivatives have been retained more strongly on the aminopropyl- column than on the diol- column.

The slopes for the same 16,17-secoestra-1,3,5(10)triene derivatives determined on different columns were generally similar, Table 2.

The retention of compounds with a halogen atom at position 17 was low and decreased from fluorine to iodine. When the two mixtures were studied on both columns (compounds **7**, **9**,

11, **13**, and **8**, **10**, **12**, **14**) co-elution was observed. It is in accordance with the previous investigations on cyanopropyl- column.[9]

Generally, the same retention order was obtained on diol- column compared to aminopropyl- column, but band spacing was sometimes different (Figs. 1 and 2). This suggests that the choice of stationary phase may offer some opportunity to gain resolution but the dramatic changes in selectivity are not likely.

Retention Behaviour of 16,17-Secoestra-1,3,5(10)triene Derivatives on Diol- and Aminopropyl- Columns Using Acetonitrile-Water

The most popular are the reversed-phase in which the organic solvents are acetonitrile and methanol.[11,15] Since no advantage in selectivity was apparent with methanol as the strong solvent and greater peak tailing was observed, our work on polar chemically bonded phases was focused on acetonitrile-aqueous mobile phases.[8] The relationship between the logarithmic retention constant log *k* and the volume fraction φ of the organic component in binary eluent for both columns was in accordance with the well-known quadratic Eq. (3), Figs. 3 and 4.

$$\log k = A\varphi^2 + B\varphi + C \qquad (3)$$

Coefficients *A*, *B* and *C* are presented in Table 3. The correlation coefficients obtained from quadratic regression analysis of experimental log *k* values varied between r^2 = 0.9766 to 0.9988, respectively.

It should be noted that it was not possible to get retention data for all compounds when using the diol- and aminopropyl- columns and acetonitrile-water. Namely, with the eluent acetonitrile-water, retention data which fit Eq. (3) for both columns were obtained only for six compounds: **1**, **3**, **7**, **9**, **11** and **13**. These six 16,17-secoestra-1,3,5(10)triene derivatives have been retained more strongly on the aminopropyl- column than on the diol- column. Under reversed-phases, conditions analyte retention is determined by the hydrophobic properties of a molecule.[11] Thus, the change from hydroxylated group at position 3 to the less polar benzyloxy group led to increased retention of the 16,17-secoestra-1,3,5(10)triene derivatives. However, it was impossible to determine the values of retention constant log *k* for these compounds when using the diol- and aminopropyl- columns. This was due to excessive peak tailing and consistent with published observations.[8,9] The retention of halogen-bearing compounds (**7**, **9**, **11** and **13**) was low and increased from fluorine to iodine but the separation of these compounds in mixture I was not achieved.

Retention Behaviour of 16,17-Secoestra-1,3,5(10)triene Derivatives on C-18 Column Using Isopropanol-Water

Reversed-phase liquid chromatography (RP-LC) is currently the most popular method in the field of high-performance liquid chromatography (HPLC). Reversed-phase high-performance liquid chromatography (RP-HPLC) is widely used in pharmaceutical analysis because of its selectivity and sensitivity for a large range of compounds.

The retention data obtained on C-18 column are generally typical of reversed phase chromatographic behaviour: less polar solutes are more strongly retained and vice versa. The relationship between the logarithmic retention constant, log *k*, of the investigated compounds and volume fraction, φ, of the organic component in eluent isopropanol-water was linear and in accordance with the well-known Eq. (4), generally accepted in partition chromatography:

$$\log k = \log k_0 - S\varphi \qquad (4)$$

Correlation coefficients from linear regression analysis of the experimental log k values varied between $r^2 = 0.9034$ to 0.9999, respectively. The numerical data for the value of constant S and constant log k_0 for each compound studied are presented in Table 4.

Table 4

The retention order of 16,17-secoestra-1,3,5(10)triene derivatives is in accordance with their hydrophobicity. As expected, odd numbered compounds were generally less retained compared to their 3-benzyloxy counterparts; because benzyloxy group is more hydrophobic compared to hydroxy group. Retention order of 16,17- secoestra-1,3,5(10)triene derivatives increases in the order:

17-I > 17-Br > 17-Cl > 17-OTs > 17-F > 17=O > 17-OH

Compounds **5** and **6** possessing toluenesylfoniloxy function at position 17 were significantly more retained than compounds **7** and **8** bearing fluorine.

It is noteworthy that on C-18 column both mixtures of halogen derivatives were clearly resolved with eluent isopropanol-water into four peaks. The peaks always emerged in the order: fluoro-, chloro-, bromo- and iodo-derivatives. An example, the separation of mixture II (3-benzyloxy halogen derivatives) in eluent isopropanol-water (75:25 v/v) is shown in Fig. 5.

Fig. 5

Correlation Between Retention Constant log k_0 of 16,17-Secoestra-1,3,5(10)triene Derivatives Obtained on Diol-, Aminopropyl- and C-18 Columns and *ACD/*log *P*

The traditional experimental method for the detrmination of log $P_{o/w}$, is shake flask method. Nowadays liquid chromatography has a tendency to replace tedious and poor interlaboratory reproducible shake flask method for measuring partition coefficients. RP-HPLC and TLC provide a variety of descriptors that can be used as lipophilicity indices.[3-6]

The quadratic term in Eq. (3) can be ignored in the first approximation and Eq. (3) can be expressed as Eq. (4).

The experimental log k values of the compounds **1**, **3**, **7**, **9**, **11** and **13** obtained on diol- and aminopropyl- columns also fit Eq. (4). The numerical values of the constants log k_0 and S for each compound examined are presented in Table 4. The correlation coefficients of the linear regression of the experimental log k values varied beetwen $r^2 = 0.9158$ to 0.9886, respectively. The intercept log k_0 corresponds to the retention in water as mobile phase, and represents the commonly employed chromatographic hydrophobicity parameter.

Correlations between the retention constants log k_0 of investigated compounds determined in RPHPLC on all three columns and log P calculated by *ACD*/log P are presented on Fig 6.

Fig. 6

There was better correlation between the retention constants determined by RP-HPLC on C-18 silica gel column and calculated log P using the *ACD/*log$P$ method in comparison with same correlation on diol- and aminopropyl- columns.

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References

1. Palomino E.; in E. J. Parish, W. D. Nes (eds.), Biochemistry and Function of Sterols, CRC Press, Boca Raton, FL, Chapter 18, 1997.

2. Jovanović-Šanta S.; Andrić S.; Kovačević R.; Pejanović V. *Collect. Czech. Chem. Commun.* **2000.** *65,* 77-82.

3. Kaliszan R. *Chem. Rev.* **2007.** *107,* 3212-3246.

4. Heberger K. *J. Chromatogr. A.* **2007.** *1158,* 273-305.

5. Testa B.; Kramer S. D. Wunderli-Allenspach H, Folkers G In: Pharmacokinetics Profiling in Drug Research, Wiley VCH, 2006.

6. Todeschini R.; Consonni V. Handbook of Molecular Descriptors Wiley VCH, 2000.

7. Ačanski M. M.; *J. Serb. Chem. Soc.*, **2003.** *68*, 971- 977.

8. Ačanski M. M.; *Chromatographia*, **2005.** *62*, 475-482.

9. Ačanski M. M.; Dobrijević J. P.; Redžepović A. S.; Vujić Đ. N.; Jovanović Šanta, S: S. *Chromatographia*, **2009.** *70,* 1679-1683.

10. Ačanski M. M.; Dobrijević J. P.; Redžepović A. S.; Vujić Đ. N.; Sakač M. N.; Jovanović Šanta, S: S. *Chromatographia*, **2010.** *71,* 913-916.

11. Cazes J.; Encyclopedia of Chromatography, Marcel Dekker, 2004.

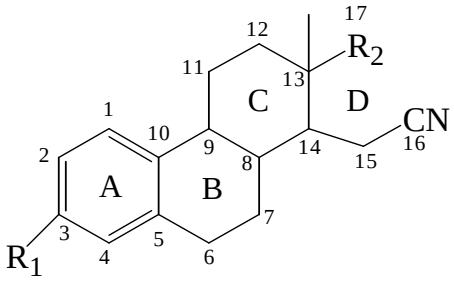
12. Tanaka H.; Zhou X. Ohira M. *J. Chromatogr. A* **2003.** *987,* 119-125.

13. Olsen B. *J. Chromatogr. A.* **2001.** *913,* 113-122.

14. Gaica S. B.; Opsenica D. M.; Šolaja B. A.; Tešić Ž. Lj.; Milojković-Opsenica D. M. *J. Planar Chromatogr.*, **2004.** *17,* 299-305.

15. ChromBook, 2nd edn. Merck KgaA, Darmstadt, Germany.

Table 1. The chemical structure of the compounds studied.



Compound	R_1	R_2
1	OH[a)]	CH_2OH
2	OBn[b)]	CH_2OH
3	OH	CHO
4	OBn	CHO
5	OH	OTs [c)]
6	OBn	OTs
7	OH	CH_2F
8	OBn	CH_2F
9	OH	CH_2Cl
10	OBn	CH_2Cl
11	OH	CH_2Br
12	OBn	CH_2Br
13	OH	CH_2I
14	OBn	CH_2I

[a)] –OH, hydroxy [b)] –OBn = $–OCH_2C_6H_5$, benzyloxy,
[c)] –OTs = $CH_2OSO_2C_6H_4CH_3$, toluenesulfonyloxy

IUPAC names of steroids:

1. 3,17-Dihidroxy-16,17-secoestra-1,3,5(10)triene-16-nitrile
2. 3-Benzyloxy-17-hidroxy-16,17-secoestra-1,3,5(10)triene-16-nitrile
3. 3-Hydroxy-17-oxo-16,17-secoestra-1,3,5(10)triene-16-nitrile
4. 3-Benzyloxy-17-oxo-16, 17-secoestra-1,3,5(10)triene-16-nitrile
5. 3-Hydroxy17p-toluenesulfonyloxy1617-secoestra-1,3,5(10)triene-16-nitrile
6. 3-Benzyloxy17p-toluenesulfonyloxy16,17-secoestra-1,3,5(10)triene-16-nitrile

7. 3-Hydroxy-17-fluoro-16,17-secoestra-1,3,5(10)triene-16-nitrile
8. 3-Benzyloxy-17-fluoro-16,17-secoestra-1,3,5(10)triene-16-nitrile
9. 3-Hydroxy-17-chloro-16,17-secoestra-1,3,5(10)triene-16-nitrile
10. 3-Benzyloxy-17-chloro-16,17-secoestra-1,3,5(10)triene-16-nitrile
11. 3-Hydroxy-17-bromo-16,17-secoestra-1,3,5(10)triene-16-nitrile
12. 3-Benzyloxy-17-bromo-16,17-secoestra-1,3,5(10)triene-16-nitrile
13. 3-Hydroxy-17-iodo-16,17-secoestra-1,3,5(10)triene-16-nitrile
14. 3-Benzyloxy-17-iodo-16,17-secoestra-1,3,5(10)triene-16-nitrile

Table 2. Coefficients for the linear relationship between log k and –log φ of propan-1-ol Eq. (2), for diol- and aminopropyl- columns; r^2-correlation coefficient. Compound numbering is as in Table 1.

Comp.	Diol- column			Aminopropyl- column		
	-log k_0	n	$r^2 \pm SD$	-log k_0	n	$r^2 \pm SD$
1	0.017	0.083	0.9995 ± 0.0010	0.010	0.079	0.9947 ± 0.0018
2	1.164	1.197	0.9999 ± 0.0024	1.168	1.187	0.9999 ± 0.0039
3	1.190	1.262	0.9998 ± 0.0045	0.854	0.784	0.9030 ± 0.0823
4	1.064	0.848	0.9998 ± 0.0033	1.146	0.849	0.9999 ± 0.0027
5	0.835	0.927	0.9999 ± 0.0012	0.890	0.894	0.9998 ± 0.0045
6	1.136	0.988	0.9999 ± 0.0021	1.226	1.006	0.9780 ± 0.0483
7	1.611	1.707	0.9999 ± 0.0041	1.636	1.797	0.9999 ± 0.0065
8	1.557	0.965	0.9998 ± 0.0035	1.625	0.088	0.9993 ± 0.0084
9	1.632	1.632	0.9999 ± 0.0010	1.689	1.693	0.9973 ± 0.0282
10	1.413	0.855	0.9997 ± 0.0051	1.496	0.922	0.9891 ± 0.0310
11	1.707	1.789	0.9999 ± 0.0037	1.838	1.846	0.9998 ± 0.0084
12	1.407	0.894	0.9985 ± 0.0112	1.635	1.108	0.9939 ± 0.0277
13	1.785	1.912	0.9958 ± 0.0396	1.874	2.057	0.9999 ± 0.0057
14	1.456	1.009	0.9999 ± 0.0016	1.605	1.073	0.9997 ± 0.0064

Table 3. Coefficients for the quadratic relationship between log k and φ of acetonitrile Eq. (3) for diol- and aminopropyl- columns; r^2-correlation coefficient. Compound numbering is as in Table 1.

Diol- column				
Comp.	***A***	***-B***	***C***	$r^2 \pm SD$
1	1.484	7.730	12.544	0.9884 ± 0.0342
3	1.069	4.953	7.943	0.9988 ± 0.0070
7	1.087	3.859	4.143	0.9915 ± 0.0229
9	1.430	6.900	10.509	0.9931 ± 0.0168
11	1.637	7.810	11.905	0.9872 ± 0.0284
13	1.917	9.446	15.401	0.9869 ± 0.0315
Aminopropyl- column				
Comp.	***A***	***-B***	***C***	$r^2 \pm SD$
1	2.190	12.825	22.303	0.9766 ± 0.0463
3	1.530	8.552	15.204	0.9937 ± 0.0120
7	1.449	6.855	10.501	0.9974 ± 0.0089
9	1.475	5.996	7.608	0.9860 ± 0.0356
11	1.686	7.795	12.514	0.9911 ± 0.0195
13	2.011	10.030	17.001	0.9554 ± 0.0613

Table 4. Constants -log k_0 and S of Eq. (4). Values of log P of compounds 1 – 14 are presented in the last column of Table 3. The correlation coefficients of the linear regression between log P and log k_0 are presented in the last row.

Comp.	Diol- column		Aminopropyl-column		C-18		log *P*
	-log k_0	*S*	-log k_0	*S*	-log k_0	*S*	
1	1.105	3.22	1.336	3.91	***0.941***	1.55	***2.72***
2	**-**	-	**-**	-	***2.555***	2.78	***5.03***
3	0.789	1.90	0.938	2.42	***1.680***	2.18	***2.99***
4	**-**	-	**-**	-	***2.792***	2.93	***5.30***
5	**-**	-	**-**	-	***2.051***	2.53	***4.08***
6	**-**	-	**-**	-	***3.664***	3.88	***6.39***
7	***0.959***	2.45	***1.073***	2.80	***1.530***	1.98	***3.90***
8	**-**	-	**-**	-	***3.211***	3.39	***6.21***
9	***1.051***	2.83	***1.172***	2.89	***1.876***	2.28	***4.36***
10	**-**	-	**-**	-	***3.511***	3.63	***6.67***
11	***1.203***	3.24	***1.198***	2.75	***1.972***	2.36	***4.54***
12	**-**	-	**-**	-	***3.521***	3.64	***6.84***
13	***1.350***	3.44	***1.312***	3.00	***2.133***	2.48	***4.89***
14	**-**	-	**-**	-	***3.745***	3.81	***7.19***
$r^2 \pm SD$	***0.9363 ± 0.0532***		***0.9803 ± 0.017***		***0,9421 ± 0.2264***		

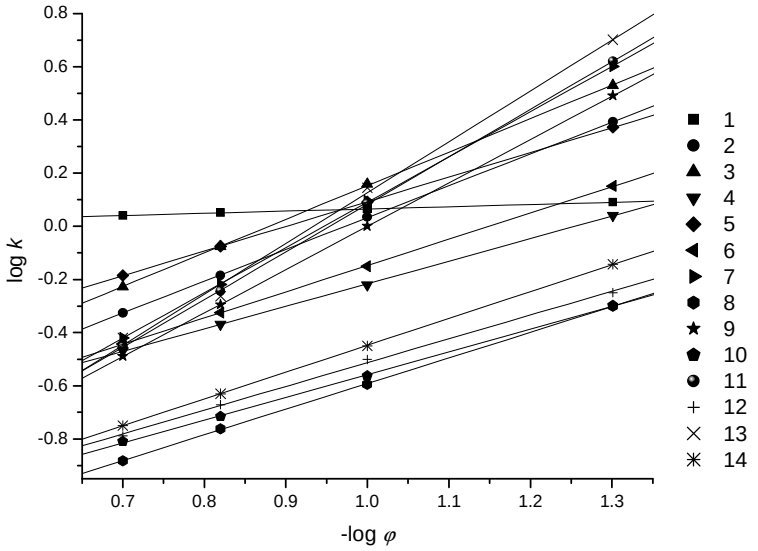
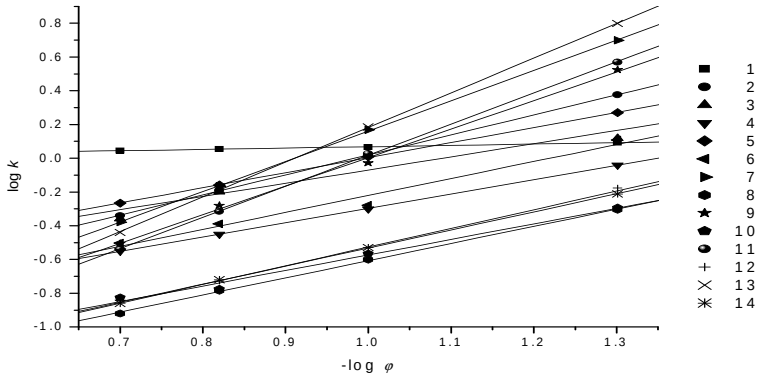


Fig. 1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-log φ
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0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
0.7
0.8
0.9
1.0
1.1
1.2
1.3
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2
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4
5
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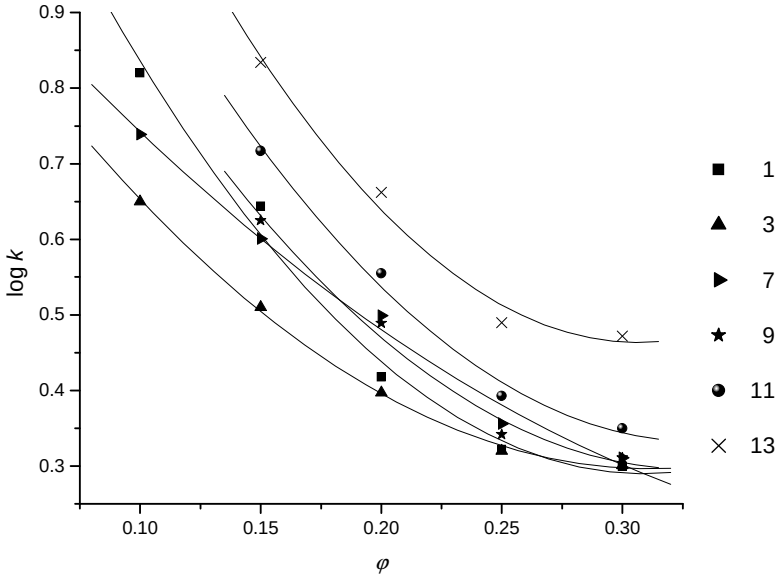
13
14

Fig. 2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φ
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0.7
0.6
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0.25
0.30
1
3
7
9
11
13

Fig. 3

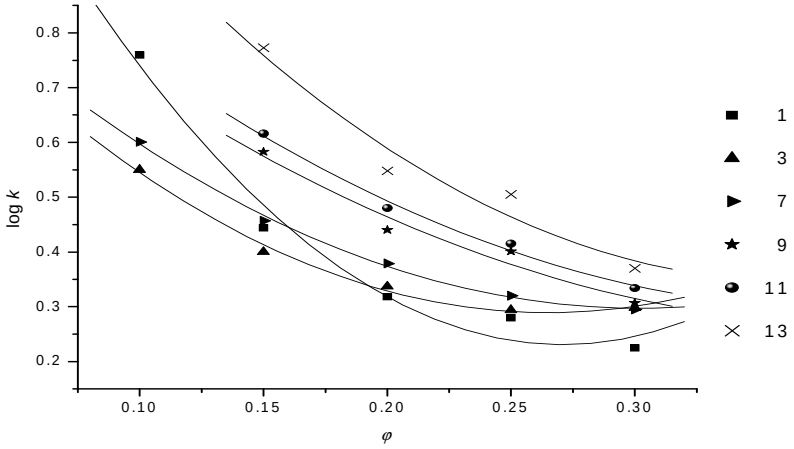
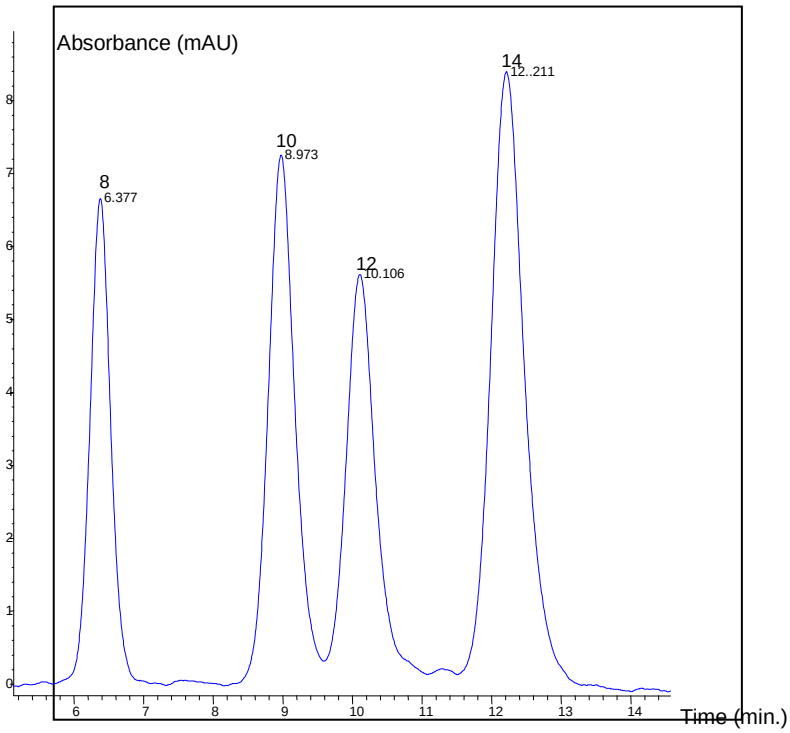

log k
φ
1
3
7
9
11
13

Fig. 4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8
6.377
10
8.973
12
10.106
14
12..211
Time (min.)

Fig. 5

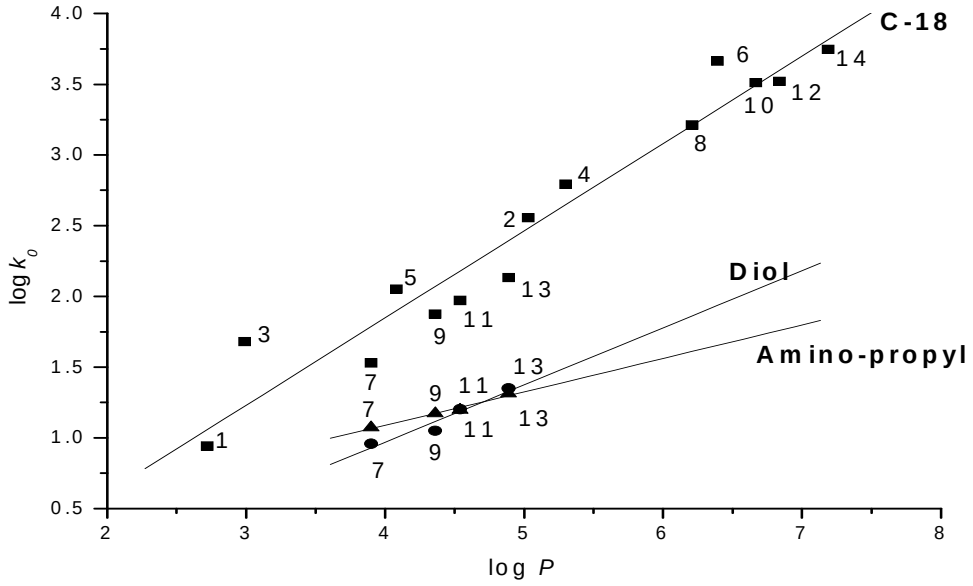

Fig. 6

Fig. 1. Correlation lines of Eq. (1) for diol- column with the eluent hexane-propan-1-ol. Compound numbering is as in Table 1.

Fig. 2. Correlation lines of Eq. (1) for aminopropyl- column with the eluent hexane-propan-1-ol. Compound numbering is as in Table 1.

Fig. 3. Correlation lines of Eq. (2) for diol- column with the eluent acetonitrile-water. Compound numbering is as in Table 1.

Fig. 4. Correlation lines of Eq. (2) for aminopropyl- column with the eluent acetonitrile-water. Compound numbering is as in Table 1.

Fig. 5. Separation of 3-benzyloxy halogen derivatives (compounds **8**, **10**, **12** and **14**) in eluent isopropanol-water, (75:25 v/v) in C-18 column. Compound numbering is as in Table 1.

Fig. 6. Plots of log k_0 against log P on C-18, diol- and aminopropyl- columns. Compound numbering is as in Table 1.