J.Serb.Chem.Soc. 68(12)971–977(2003)
JSCS – 3119

UDC 543.544.5:547.233+547–326
Original scientific paper

Normal-phase high performance liquid chromatography of estradiol derivatives on amino- and diol- columns

MARIJANA M. AČANSKI

Department of General and Inorganic Chemistry, Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia and Montenegro (e-mail: marijana@tehnol.ns.ac.yu)

(Received 7 April, revised 13 August 2003)

Abstract: The retention behaviour of estradiol derivatives was studied by HPLC on chemically bonded polar stationary phases: commercially available amino- and diol- columns, as a function of the heptane-propan-1-ol as the mobile phase, when the volume fraction of propan-1-ol in the binary mobile phase was low, even less than 5 %. The relationship between the logarithm of the retention constant (log k) and the logarithm of the volume fraction of propan-1-ol (–log φ) in the eluent was linear for all solutes studied. The results are discussed in terms of the solute and stationary phase properties and compared with the results of the same derivatives obtained in earlier investigations.

Keywords: HPLC, amino- and diol- columns, non-aqueous eluent, estradiol derivatives, retention behaviour.

INTRODUCTION

Estrogens[1] are important physiologically active substances produced by the ovaries. Among the most important estrogens is estradiol. Some simple chemical modification of the basic structure of the steroid can have a direct effect on the activity, in particular on the binding activity, of estradiol.

In our previous papers[2–6] the retention behaviour and retention mechanism of some estradiol and estrone derivatives chromatographed on silica gel, alumina, chemically bonded polar phases and C-18 bonded silica gel in normal and reversed phase, using several non-aqueous and aqueous eluents, was described. The type of the stationary and mobile phases, as well as the nature, number, and position of substituents in the molecule of the steroids were observed to have significant and distinct effects on the retention.

Non-aqueous mobile phases are more often used with chemically bonded polar stationary phases[7–10] than are aqueous mobile phases. Amino- and diol- stationary phases bonded on a silica gel support, comprising three carbon atoms (aminopropyl and 1,2-dihydroxypropylether), 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. As a result of this, chemically bonded polar stationary phases provide for more specific interaction at the surface and have operating advantages over traditional silica gel columns.

Several studies have reported that the competitive model of adsorption can be used to describe retention on chemically bonded polar phases in normal-phase chromatography.[4,11–13] This model yields, with some simplification, Eq. (1) describing the dependence of the retention (capacity factor, k) on the mole fraction of the polar solvent, N_b, in a binary mobile phase comprised of a polar solvent and a none-polar one[14]:

$$\log k = \log k_0 - n \log N_b \quad (1)$$

In this exponential equation, n is a constant giving the ratio of the molecular area on the adsorbent surface occupied by one molecule of the sample solute to that occupied by one molecule of the polar solvent. The concentration can often be expressed as the volume fraction, φ, instead of the mole fraction:[15]

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

In addition, the competitive model does not take into account so-called secondary solvent effects. These effects, resulting from solute–solvent interactions in both the mobile and adsorbed phases, give rise to some of the most useful changes in retention and are often the source of chromatographic selectivity.

Although Eq. (2) is widely known in the chromatographic literature, Jandera and Churaček[15] have shown that for normal phase liquid–liquid chromatography the relationship between logarithm retention constant log k and volume fraction of the more polar component, φ, in a binary eluent is better represented by the quadratic expression:

$$\log k = a\varphi^2 + b\varphi + c \quad (3)$$

Assuming that the quadratic term can be ignored, to a first approximation, they obtained Eq. (4):

$$\log k = \log k_0 - m\varphi \quad (4)$$

This model[15] interprets that the retention and retention mechanism are governed by a partitioning process.

This paper investigates the retention behaviour and retention mechanism of estradiol derivatives on amino- (aminopropyl) and diol- (1,2-dihydroxypropylether) columns using heptane-propan-1-ol eluents. The volume fraction of propan-1-ol in the binary mobile phases was low, even less than 5 %.

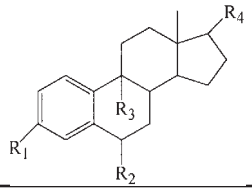
The compounds and their molecular structures are listed in Table I.

EXPERIMENTAL

The separations were performed with a Milton Roy (Riviera Beach, FL, USA) Consta Metric 3000 pump and a Milton Roy Spectro Monitor 3160 variablewavelength UV-vis detector set at 254 nm. The samples were injected using a Rheodyne 7125 valve (Cotati, CA, USA) fitted with a 20 μL loop. The columns

used were commercially available 5 μm LiChrosorb NH_2 and LiChrosorb DIOL, 250×4 mm i.d. (E. Merck, Darmstadt, Germany).

TABLE I. IUPAC names and chemical structures of the studied compounds



No.	IUPAC Name	R_1	R_2	R_3	R_4
1	3,17β-Dihydroxyestra-1,3,5(10-triene (estradiol)	OH			OH
2	3-Methoxy-17β-hydroxyestra-1,3,5(10)-triene	[a)]OMe			OH
3	3-Acetoxy-17β-hydroxyestra-1,3,5(10)-triene	[b)]OAc			OH
4	3,17β-Diacetoxyestra-1,3,5(10)-triene	OAc			OAc
5	3-Propionoxy-17β-hydroxyestra-1,3,5(10)-triene	[c)]OPr			OH
5a	3-Hydroxy-17β-propionoxy estra-1,3,5(10)-triene	OH			OPr
6	3,17β-Dipropionoxyestra-1,3,5(10)-triene	OPr			OPr
7	3-Benzoyloxy-17β-propionoxy estra-1,3,5(10)-triene	[d)]OBz			OH
8	3,17β-Dibenzoyloxyestra-1,3,5(10)-triene	OBz			OBz
9	3-Acetoxy-17β-benzoyloxyestra-1,3,5(10)-treiene	OAc			OBz
10	3,17β-Dihydroxyestra-1,3,5(10)-triene-6-one	OH	=O		OH
11	3-Methoxy-17β-hydroxyestra-1,3,5(10)-triene-6-one	OMe	=O		OH
12	3-Hydroxy-17β-propionoxy estra-1,3,5(10)-triene-6-one	OH	=O		OPr
13	3,17β-Dipropionoxy-1,3,5(10)-triene-6-one	OPr	=O		OPr
14	3,9α-Dihydroxy-17β-propionoxyestra-1,3,5(10)-triene-6-one	OH	=O	α-OH	OPr
15	3,17β-Dipropionoxy-9α-hidroxyestra-1,3,5(10)-triene-6-one	OPr	=O	α-OH	OPr

[a)]OMe-OCH_3, [b)]OAc-$OCOCH_3$, [c)]OPr-$OCOCH_2CH_3$, [d)]OBz-$OCOC_6H_5$

The estradiol derivatives (Table I), synthesized by original reactions or according to literature methods,[16] were dissolved (0.005 mg mL^{-1}) in methanol and the solutions prefiltered through a 0.2 μm Chromafil filter (Macherey-Nagel, Düren, Germany).

One binary solvent system, heptane–propan-1-ol was used as the mobile phase with varying contents of the more polar component; propan-1-ol 1–20 %; increments 2 and 5 %. The solvents used to prepare the mobile phases were of analytical grade and were filtered through a 0.45 μm filter and deggased before use. The flow rate was 1 mL min^{-1} at room temperature.

The retention 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 obtained when trace amounts of methanol were injected into the column. Each t_r value was measured in triplicate and averaged.

RESULTS AND DISCUSSION

The logarithmic relationship between the retention factor, k, of the investigated compounds and the volume fraction, φ, of the propan-1-ol as the stronger solvent in the binary

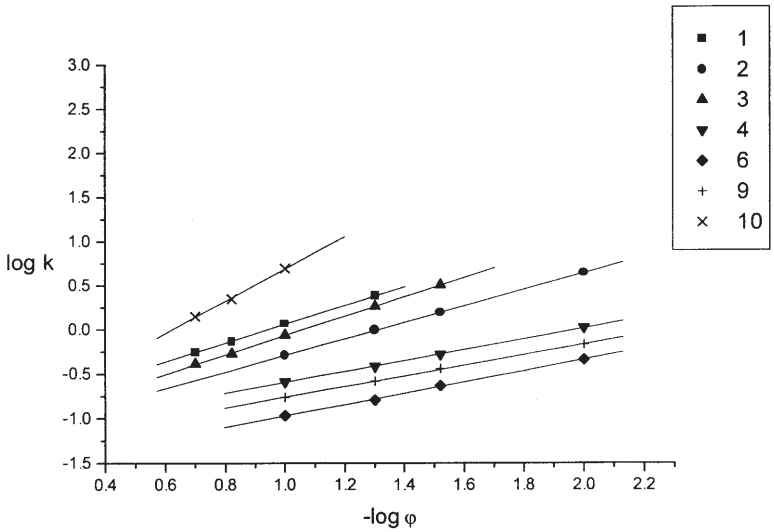


Fig. 1. Correlation lines of Eq. (2) for the amino column and eluent heptane–propan-1-ol; compound designation as in Table I.

eluent, for both columns was linear. This behaviour suggests that the model based on Eq. (2) is suitable to describe the experimental behaviour of estrodiol derivatives on both columns. The numerical data for log k and the constants n and log k_0 for each studied compound for the amino- and diol- column with heptane–propan-1-ol as the eluent are presented in Tables II and III, respectively. The correlation coefficients from linear regression analysis of the experimental log k values varied from 0.9959 to 1. The linear relationship between the logarithm of the retention constant and the logarithm of the volume fraction of

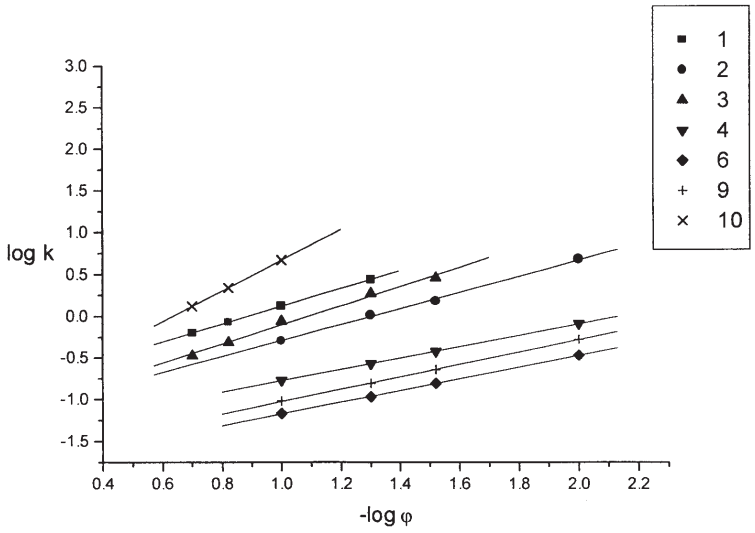


Fig. 2. Correlation lines of Eq. (2) for the diol column and eluent heptane–propan-1-ol; compound designation as in Table I.

propan-1-ol in the eluent for compounds 1, 2, 3, 4, 6, 9 and 10 on the amino- and diol- columns are presented in Figs. 1 and 2, respectively.

TABLE II. Retention data and constants n and log k_0 of Eq. (2) of the estradiol derivatives for the amino column; r-correlation coefficient; φ is the concentration of propan-1-ol % (v/v) in the binary mobile phase heptane–propan-1-ol

φ	0.2	0.15	0.1	0.05	0.03	0.01			
–log φ	0.7	0.82	1	1.3	1.52	2	n	log k_0	r
Comp.	log k								
1	– 0.25	– 0.134	0.065	0.383	–	–	1.06	– 0.998	0.9999
2	–	–	– 0.291	– 0.004	0.191	0.643	0.93	– 1.221	0.9999
3	– 0.386	– 0.271	– 0.06	0.265	0.506	–	1.1	– 1.159	0.9999
4	–	–	– 0.586	– 0.412	– 0.28	0.002	0.59	– 1.176	0.9999
5	– 0.526	– 0.381	– 0.18	0.158	0.41	–	1.14	– 1.317	0.9999
5a	– 0.468	– 0.313	– 0.098	0.313	0.529	–	1.24	– 1.328	0.9988
6	–	–	– 0.966	– 0.78	– 0.63	– 0.34	0.63	– 0.1599	0.9944
7	– 0.468	– 0.318	– 0.131	0.2	0.44	–	1.1	– 1.229	0.9998
8	–	–	– 0.852	– 0.676	– 0.53	– 0.24	0.61	– 1.469	0.9998
9	–	–	– 0.762	– 0.58	– 0.44	– 0.17	0.59	– 1.35	0.9996
10	0.153	0.35	0.698	–	–	–	1.83	– 1.133	0.9991
11	– 0.177	– 0.029	0.257	0.63	–	–	1.36	– 1.129	0.9991
12	– 0.294	– 0.163	0.1	0.454	–	–	1.26	– 1.182	0.9999
13	–	–	– 0.278	– 0.043	0.131	0.504	0.78	– 1.06	0.9999
14	0.112	0.301	0.646	–	–	–	1.79	– 1.151	0.9986
15	– 0.267	– 0.082	0.217	0.62	–	–	1.48	– 1.294	0.9988

The retention behaviour of the estradiol derivatives on both columns were very similar and in accordance with the general retention behaviour in normal phase liquid chromatography. The retention sequence obtained with the eluent heptane–propan-1-ol is that predicted on the basis of the polarity of the compounds; the more polar solutes, compounds 1, 10 and 14 were more strongly retained and *vice versa*.

All the studied estradiol derivatives are more strongly retained on the amino column than on the diol column. Namely, both functions can form hydrogen bonds using both their own hydrogen atoms and hydrogen originating from the solute or solvent molecules, but only the amino function can be protonated.

The adsorption characteristics of the stationary phase of both columns are the consequence of proton acceptor-donor behaviour.

The slopes for the same compounds on the different columns (Tables II and III) were very similar. Because of this it was possible to average the values. Slope n in Eq. (2) is the change of the retention constant log k with the change of the volume fraction, φ, in the eluent and can be expressed as:

$$n = \frac{\mathrm{d}(\log k)}{\mathrm{d}(\log \varphi)} \tag{5}$$

TABLE III. Retention data and constants n and $\log k_0$ of Eq. (2) of the estradiol derivatives for the diol column; r-correlation coefficient; φ is the concentration of propan-1-ol % (v/v) in the binary mobile phase heptane–propan-1-ol

φ	0.2	0.15	0.1	0.05	0.03	0.01			
$-\log \varphi$	0.7	0.82	1	1.3	1.52	2	n	$\log k_0$	r
Comp.	$\log k$								
1	– 0.199	– 0.069	0.13	0.44	–	–	1.07	– 0.942	0.9999
2	–	–	– 0.289	0.018	0.185	0.676	0.96	– 0.243	0.9994
3	– 0.474	– 0.313	– 0.058	0.272	0.458	–	1.14	– 1.245	0.9959
4	–	–	– 0.769	– 0.568	– 0.42	– 0.088	0.68	– 1.453	0.9999
5	– 0.535	– 0.435	– 0.169	0.156	0.354	–	1.16	– 1.38	0.9960
5a	– 0.568	– 0.364	– 0.082	0.277	0.51	–	1.31	– 1.439	0.9961
6	–	–	– 1.165	– 0.966	– 0.805	– 0.468	0.7	– 1.869	0.9999
7	– 0.56	– 0.41	– 0.145	0.176	0.383	–	1.16	– 1.346	0.9969
8	–	–	– 1.12	– 0.896	– 0.736	– 0.388	0.73	– 1.849	0.9999
9	–	–	– 1.018	– 0.805	– 0.639	– 0.276	0.74	– 1.767	0.9999
10	0.123	0.343	0.67	–	–	–	1.82	– 1.152	1
11	– 0.201	– 0.051	0.17	0.535	–	–	1.32	– 1.173	0.9999
12	– 0.363	– 0.195	0.063	0.411	–	–	1.29	– 1.253	0.9985
13	–	–	– 0.482	– 0.24	0.078	0.274	0.75	– 1.227	0.9997
14	0.081	0.296	0.641	–	–	–	1.87	– 1.232	0.9998
15	– 0.375	– 0.195	0.091	0.479	–	–	1.43	– 1.362	0.9988

From Eq. (5) it is clear that the retention of a compound which has a slope decreases faster with increasing φ in comparison with a compound which has a lower values of the constant n. With increasing φ, the interations solute-stationary phase are reduced but the interactions solute–mobile phase are increased. On silica gel,[2,17–19] the retention and slope are strictly a function of the polarity of compound. More polar compounds have langer retentions and a bigger slope. However, in this and in earlier investigations[4] on chemically bonded phases, this rule was not confirmed. The retention order of he estradiol derivatives on both columns for $\varphi = 0.1$ and order of the average values of constant n are as follows:

Amino 10>14>11>.15>12>1>3>5a>7≥5>13≥2>4>9>8>6

Diol 10>14>11>1>15>12>3>5a≥7>5>2>13>4>9>8>6

n– *10 = 14> 15> 7*≥3>11>12=5a>5>1>2>13>9=9=6>4

As the slopes of the solutes were not in accordance with their retention values there was no correlation between n and $\log k_0$ of the compounds.

ИЗВОД

ВИСОКО ПРИТИСНА ТЕЧНА ХРОМАТОГРАФИЈА ДЕРИВАТА ЕСТРАДИОЛА НА АМИНО- И ДИОЛ- КОЛОНИ СА НЕВОДЕНОМ ПОКРЕТНОМ ФАЗОМ

МАРИЈАНА М. АЧАНСКИ

Одељење за општу и неорганску хемију, Технолошки факултет, Универзитет у Новом Саду, Булевар цара Лазара 1, 21000 Нови Сад

У раду је применом високо притисне течне хроматографије испитано ретенционо понашање деривата естрадиола на амино- и диол- хемијски везаној фази. Као покретна фаза коришћена је смеша хептан–1-пропанола, у различитим односима. Удео 1-пропанола био је низак, у неким покретним фазама мањи од 5 %. Ретенционо понашање испитиваних деривата је дискутовано са аспекта природе растворка, непокретне и покретне фазе. За све испитане деривате добијена је линеарна зависност између логаритма ретенционе константе и логаритма запреминског удела поларније компоненте покретне фазе тј., 1-пропанола. Иако су ретенциона времена деривата естрадиола дужа на амино колони, ефикасност раздвајања на различиим колонама је веома слична. Добијени резултати су упоређени и са ретенционим подацима истих једињења добијених у ранијим испитивањима на силика гелу.

(Примљено 7. априла, ревидирано 13. августа 2003)

REFERENCES

1. E. Palomino, in E. J. Parish, W. D. Nes, Eds., *Biochemistry and Function of Sterols*, CRC Press, Boca Raton, PL, 1997, p. 245, Chapter 18
2. S. M. Petrović, M. Ačanski, V. Pejanović, J. Petrović, *J. Planar Chromatogr.* **6** (1993) 29
3. S. M. Petrović, M. Ačanski, Lj. Kolarov, E. Lončar, *J. Planar Chromatogr.* **8** (1995) 200
4. S. M. Petrović, M. Ačanski, V. Pejanović, J. Petrović, *Chromatographia* **43** (1996) 551
5. M. M. Ačanski, S. M. Petrović, V. M. Pejanović, J. A. Petrović, *J. Serb. Chem. Soc.* **65** (2000) 811
6. M. M. Ačanski, *J. Serb. Chem. Soc.* **68** (2003) 163
7. M. Prošek, M. Pukl, *Kvantitativna planarna kromatografija*, Kemijski inštitut Boris Kidrič, Zavod Republike Slovenije za šolstvo in šport, 1991, p. 30 (in Serbian)
8. ChromBook Merck 2nd Edition, Darmstadt, Germany, p. 45
9. D. Milojković-Opsenica, *Chem. Review* **41** (2000) 38
10. J. G. Dorsey, W. T. Copper, *Anal. Chem.* **66** (1994) 857
11. J. Fischer, P. Jandera, *J. Chromatogr. A* **684** (1994) 77
12. S. Hara, S. Ohnishi, *J. Liq. Chromatogr.* **7** (1984) 59
13. S. Hara, S. Ohnishi, *J. Liq. Chromatogr.* **2** (1984) 69
14. L. R. Snyder, *Anal. Chem.* **46** (1981) 1384
15. P. Jandera, J. Churaček, *J. Chromatogr.* **91** (1974) 207
16. V. Pejanović, *Ph. D. Thesis*, Faculty of Science, Novi Sad, 1991
17. S. M. Petrović, Lj. A. Kolarov, E. S. Traljić, J. A. Petrović, *Anal. Chem.* **54** (1982) 934
18. S. M. Petrović, Lj. A. Kolarov, J. A. Petrović, *Chromatographia* **18** (1984) 145
19. S. M. Petrović, M. Ačanski, *J. Planar. Chromatogr.* **2** (1989) 214.