

Correlation Between Retention and 1-Octanol-Water Partition Coefficients of Some Estrane Derivatives in Reversed-Phase Thin-Layer Chromatography

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Abstract

The retention constants (R_M) of a series of estrane and secoestrane derivatives are experimentally determined on C18-modified silica gel layers with methanol–water mobile phases of various concentrations. The slopes (m) and intercepts (R_{MW}) of the linear relationships between R_M and the volume fraction of methanol are calculated. Both constants increase when the retention of compounds increases, and there is a linear dependence between them indicating their additivity; they represent the sums of particular retention contributions of skeleton and substituents. The contributions, particularly the retention fragmental constants, are calculated by combining the linear relationships $R_M/\log P$, R_M/m , and $R_{MW}/\log P$. The $\log P$ values of the compounds and skeleton were calculated using Rekker's fragmental constants.

Introduction

It is widely accepted that in reversed-phase liquid chromatography (RPLC) a linear relationship between the retention constant (R_M and $\log k'$) and the volume fraction of organic modifier (O_{org}) in a binary aqueous mobile phase exists and can be expressed by the well-known Equation 1:

$$R_M = R_{MW} - m o_{org} \quad \text{Eq. 1}$$

where R_{MW} is the value of R_M extrapolated to $o_{org} = 0$, and m is a constant. The R_{MW} value is a widely used chromatographic hydrophobicity parameter. Because the constants R_{MW} and m increase with increasing retention, a linear relationship mainly between them existed (1–5), which indicated that both constants seem to be related to the same physicochemical factors and, therefore, they are intercorrelated. It is evident that the m value reflects the nature of the solute and is mainly determined by the interaction between the solute and the mobile

phase. Consequently, the m value can be treated as an alternative chromatographic parameter, which indicates the mechanism of retention.

A number of authors correlated retention data in RPLC and 1-octanol-water partition coefficients ($\log P$) mostly for the interpretation of the retention mechanism and, in some cases, example references for the retention prediction (6–9). In RPLC with methanol–water mobile phase retention, the constants ($\log k'$, R_M , $\log k'_W$, and R_{MW}) generally correlated well with the calculated $\log P$ values (6,9).

This study reports a method for the retention prediction of a series of variously substituted estrane and secoestrane derivatives by correlating retention data that was obtained in reversed-phase thin-layer chromatography (bonded C18 silica gel plates) with methanol–water mobile phase and calculated $\log P$ values.

Experimental

Thin-layer chromatography was performed on 10- × 20-cm or 10- × 10-cm glass high-performance thin-layer chromatographic plates precoated with C18 bonded silica gel with a fluorescence indicator (Merck, Darmstadt, Germany). The samples were dissolved in methanol (2 mg/mL) and 1-mL volumes of the solutions were applied to the chromatoplate with a micropipette. The methanol used to prepare mobile phases was of analytical grade and the water was distilled twice. The R_f values were the averages from at least three chromatograms developed for each solute–mobile phase combination; spots were observed under UV light (at $\lambda = 254$ nm). The R_M values were calculated using the formula $R_M = \log [(1/R_f) - 1]$.

The $\log P$ values for each compound and skeleton were calculated using Rekker's fragmental constants (10). Each calculated $\log P$ value was obtained by the addition of the corresponding fragmental constants. The retention fragmental

constants (RFC) values from Table I were calculated statistically. The structures of the compounds and skeletons are given in Figure 1.

Results and Discussion

The retention data of the examined compounds, obtained with methanol–water mobile phase of various compositions as well as calculated $\log P$ values, are collected in Table I. Correlation coefficients from linear regression of experimental R_M values varied from 0.9924 to 0.9999. Both of the constants (m and R_{MW}) increased with increasing hydrophobicity (i.e., retention of compounds). Therefore, there is a linear relationship with a high correlation coefficient (r) between these two constants, expressed by the following equation:

$$R_{MW} = 1.156m - 1.126$$

($r = 0.9998$, $SD = 0.0392$, and $n = 45$) Eq. 2

indicating their additivity which can be shown in the following ways:

$$m = m_{sk} + \sum \Delta m_{sb}$$
 Eq. 3

$$R_{MW} = \Delta R_{MWsk} + \sum \Delta R_{MWsb}$$
 Eq. 4

where the subscripts sk and sb stand for skeleton and sub-

stituents, respectively. Thus, the constants (m and R_{MW}) of particular steroids studied depended upon the type and number of substituents in the molecule and on the skeletal structure; they are the sums of the particular Δm or ΔR_{MW} values.

It is obvious in RPLC that steroid skeletal structure significantly affects retention. However, the effect of skeletal structure on retention can not be experimentally determined because estrane and secoestrane skeletons are not commercially or synthetically available; they are not of any biological or chemical interest. Therefore, the effect of estrane and secoestrane skeletons on the retention of their derivatives should be calculated. The calculations were performed using $\log P$ values of examined steroids from Table II. Namely, there is a good linear dependence between experimental R_M and calculated $\log P$ values. For example, the linear relationship of experimental R_M values at $O_{org} = 0.85$ and the $\log P$ values from Table II is presented in Figure 2 and given by the equation:

$$R_M^{85} = 0.295 \log P - 1.122$$

($r = 0.8625$, $SD = 0.2265$, and $n = 40$) Eq. 5

There is also a good linear dependence between the R_M values at O_{org} and the values of constant m (Table II), therefore:

$$R_M^{85} = 0.308m - 1.128$$

($r = 0.9970$, $SD = 0.0427$, and $n = 45$) Eq. 6

Combining Equations 5 and 6 and introducing the calculated $\log P$ value for estrane skeleton ($\log P_{sk} = 6.955$) or secoestrane skeleton (ssk) ($\log P_{ssk} = 6.597$) we obtained Δm_{sk} values for estrane and secoestrane skeletons (Figure 1) that were $\Delta m_{sk} = 6.68 \sim 6.7$ and $\Delta m_{ssk} = 6.34 \sim 6.3$, respectively. ΔR_{MWsk} values from Equation 4 were calculated by the equation:

$$R_{MW} = 1.130 \log P - 1.372$$

($r = 0.8684$, $SD = 0.2211$, and $n = 40$) Eq. 7

because there is a linear relationship between the extrapolated R_{MW} and calculated $\log P$ values (Figure 3); the calculated ΔR_{MWsk} values for estrane and secoestrane skeletons were $6.487 \sim 6.49$ and $6.083 \sim 6.08$, respectively.

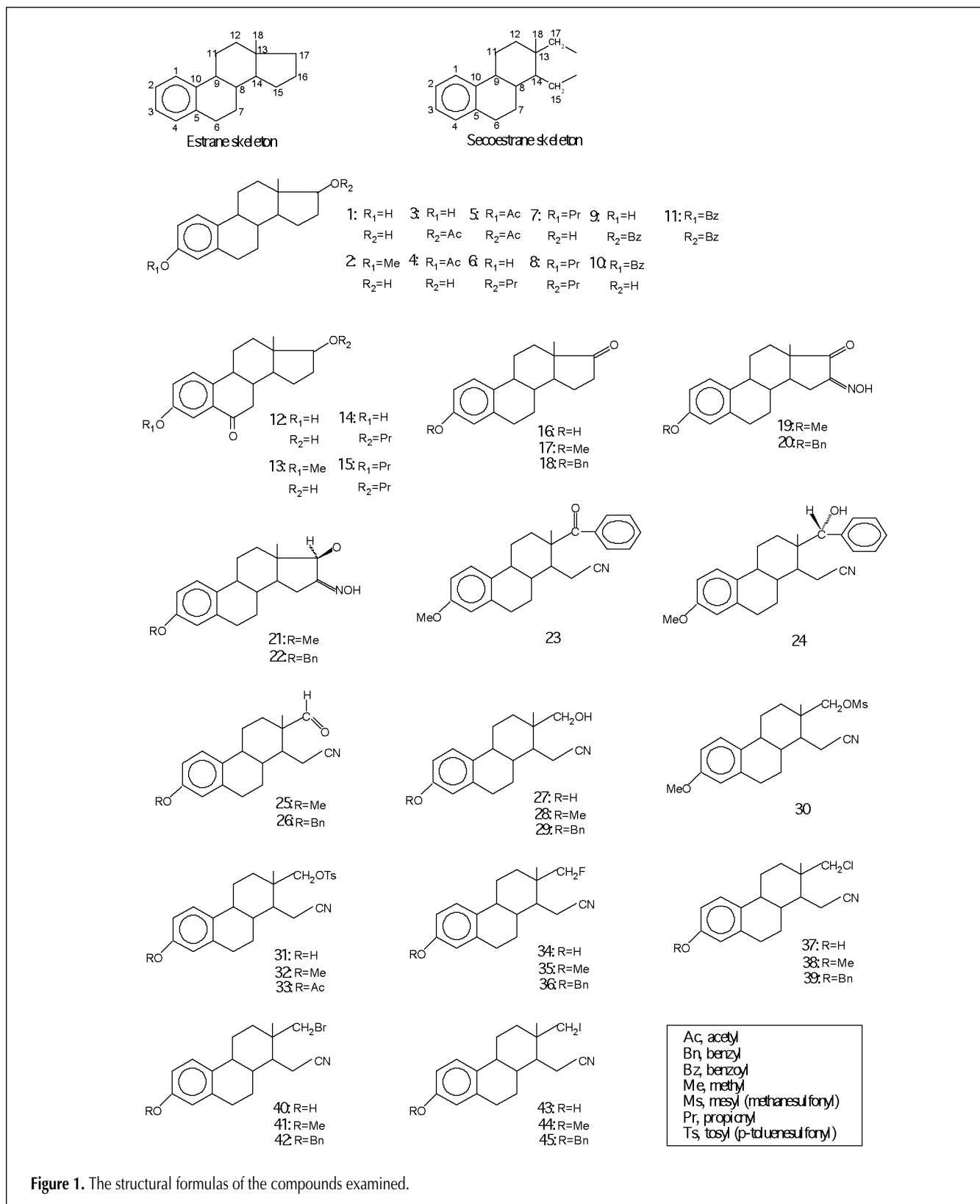
The RFC for steroids substituents (i.e., Δm_{sb} and ΔR_{MWsb} values determined by means of Equations 3 and 4) are presented in Table I. The RFC for polar substituents were negative. The phenolic 3-hydroxy function was acidic in comparison with the alcoholic 17-hydroxy group and therefore more negative. The RFC values for the oxo function at positions 6 and 17 were equal. Previously it was argued that the hydroxy group was slightly more polar than the mesyloxy function (4,11). Our experimental data (Table II) confirmed it; compound 30 was slightly more retained than compound 28. For such a reason, the RFC values for the mesyloxy function are highly negative. The RFC values for the lipophilic substituents (such as methoxy, propionoxy, benzoiloxo, or benzyloxy) are positive and increase with increasing hydrophobicity. Calculated constants m_{calc} and R_{MWcalc} utilizing RFC values from Table I and Equation 3 and 4 are presented in Table III.

	Δm	ΔR_{MW}
Estrane skeleton	6.7	6.49
Secoestrane skeleton	6.3	6.08
3 – OH	$-1.59 \pm 0.13 \approx -1.6$	-1.76 ± 0.08
17 – OH	$-1.30 \pm 0.10 \approx -1.3$	-1.49 ± 0.15
6 = O	$-1.18 \pm 0.06 \approx -1.2$	-1.34 ± 0.09
17 = O	$-1.18 \pm 0.06 \approx -1.2$	-1.34 ± 0.09
16 = NOH	$-0.98 \pm 0.06 \approx -1.0$	-1.14 ± 0.06
16 – CN	$-1.40 \pm 0.10 \approx -1.4$	-1.64 ± 0.14
3 – OAc	-0.2	-0.11
17 – OAc	-0.4	-0.37
3 – OBn	$1.65 \pm 0.08 \approx 1.7$	2.01 ± 0.10
3 – OBz	1.6	1.99
17 – OBz	1.2	1.39
3 – OMe	$0.40 \pm 0.05 \approx 0.4$	0.50 ± 0.11
17 – OMs	$-1.56 \approx -1.6$	-1.73
3 – OPr	$0.37 \pm 0.07 \approx 0.4$	0.43 ± 0.09
17 – OPr	$0.22 \pm 0.04 \approx 0.2$	0.26 ± 0.07
17 – OTs	$-0.37 \pm 0.14 \approx -0.4$	-0.41 ± 0.16
17 – F	$-0.50 \pm 0.07 \approx -0.5$	-0.55 ± 0.05
17 – Cl	$-0.18 \pm 0.09 \approx -0.2$	-0.16 ± 0.08
17 – Br	$0.11 \pm 0.11 \approx 0.1$	0.11 ± 0.04
17 – I	$0.34 \pm 0.12 \approx 0.3$	0.37 ± 0.10
17 – C ₆ H ₅	1.08 \approx 1.1	1.27

* Δm and ΔR_{MW} values of Equations 3 and 4 for steroids skeletons and substituents.

The predictive ability of RFC and Equation 3 and 4 is illustrated in Table IV in which the slopes and intercepts of linear correlation between R_{Mexp} and R_{Mcalc} for particular O_{org} values are given. The slope values for unity and intercepts near zero indicate good linear correlation between the experimental and calculated R_M values.

The calculation of the log P values can be performed also by a commercially available computer program. For example, ACD/Log P (Advanced Chemistry Development, Toronto, Canada) program calculated the log P value for the estrane skeleton to be 6.91 ± 0.24 , which is very close to that calculated by Rekker's fragmental constants. The correlation equation



between the experimental R_M^{85} values and calculated $\log P_{ACD}$ values for the particular steroid studied is:

$$R_{M85} = 0.300 \log P_{ACD} - 1.009$$

($r = 0.8384$, $SD = 0.2432$, and $n = 45$) Eq. 8

which, in combination with Equation 6, results in $\Delta m_{sk} = 7.1$. The RFC values obtained with this value are more negative for polar substituents and less positive for hydrophobic substituents. Thus, the RFC values depended on the method used for the calculation of the $\log P$ values.

Table II. R_M Values and the Constants m and R_{MW} of Equation 1 for Various ϕ_{org} (org = Methanol) and Calculated $\log P$ Values

Compounds	R_M ϕ_{org}						m	R_{MW}	$\log P$
	0.70	0.75	0.80	0.85	0.90	0.95			
1	0.589	0.410	0.213	0.052	-0.170	-0.337	3.73	3.205	4.77
2			0.935	0.659	0.339	0.078	5.78	5.562	5.38
3		0.770	0.513	0.308	0.052	-0.158	4.63	4.236	5.67
4		0.935	0.659	0.477	0.149	-0.070	5.04	4.714	5.28
5			1.158	0.778	0.501	0.213	6.24	6.129	6.29
6			0.807	0.575	0.269	0.026	5.30	5.055	6.20
7			0.931	0.659	0.337	0.087	5.71	5.498	5.92
8				1.061	0.630	0.327	7.34	7.279	7.35
9			1.172	0.826	0.513	0.224	6.31	6.209	6.86
10				1.094	0.689	0.378	7.16	7.164	6.57
11				1.751*	1.279	0.807	9.44	9.775	8.65
12	0.078	-0.052	-0.185	-0.327	-0.454		2.68	1.954	2.72
13	0.881	0.652	0.423	0.194	-0.035	-0.264	4.58	4.087	3.33
14	0.760	0.554	0.347	0.140	-0.066	-0.273	4.13	3.651	4.15
15			0.931	0.630	0.288	0.035	6.06	5.774	5.29
16	0.704	0.525	0.298	0.087	-0.105	-0.278	4.01	3.510	4.38
17			0.979	0.704	0.378	0.113	5.85	5.661	5.00
18				1.123	0.736	0.399	7.24	7.269	6.71
19		0.845	0.589	0.389	0.096	-0.122	4.85	4.485	
20			1.094	0.807	0.443	0.167	6.29	6.132	
21	1.072	0.831	0.589	0.347	0.105	-0.136	4.83	4.453	
22			1.049	0.770	0.421	0.149	6.10	5.993	
23		0.954	0.673	0.432	0.158	-0.070	5.13	4.787	6.14
24		0.954	0.673	0.432	0.158	-0.070	5.13	4.787	5.67
25	0.788	0.589	0.357	0.158	-0.070	-0.250	4.21	3.735	3.57
26			0.818	0.537	0.259	0.017	5.36	5.100	5.29
27	-0.222	-0.308	-0.421	-0.513			1.97	1.162	3.35
28	0.720	0.537	0.317	0.087	-0.096	-0.278	4.07	3.571	3.96
29		0.954	0.673	0.443	0.158	-0.070	5.13	4.789	5.67
30	0.589	0.410	0.213	0.035	-0.170	-0.337	3.74	3.210	
31	0.298	0.149	-0.017	-0.158	-0.327	-0.465	3.08	2.452	3.78
32		0.826	0.562	0.389	0.078	-0.131	4.80	4.421	4.39
33	0.845	0.644	0.410	0.213	-0.026	-0.217	4.30	3.855	4.39
34	0.167	0.035	-0.113	-0.250	-0.410		2.88	2.188	4.38
35		0.807	0.550	0.308	0.061	-0.140	4.77	4.368	4.99
36			1.032	0.753	0.410	0.140	6.04	5.867	6.70
37	0.347	0.203	0.026	-0.131	-0.308	-0.443	3.22	2.608	4.90
38		0.931	0.659	0.477	0.149	-0.078	5.06	4.725	5.51
39			1.158	0.865	0.489	0.222	6.37	6.256	7.23
40	0.477	0.317	0.131	-0.070	-0.231	-0.389	3.53	2.950	5.11
41		1.061	0.770	0.550	0.222	-0.008	5.37	5.085	5.72
42				0.931	0.562	0.269	6.62	6.545	7.43
43	0.575	0.399	0.194	-0.008	-0.176	-0.347	3.74	3.188	5.43
44			0.886	0.602	0.298	0.043	5.67	5.415	6.04
45				0.979	0.602	0.298	6.81	6.755	7.75

* Extrapolated value.

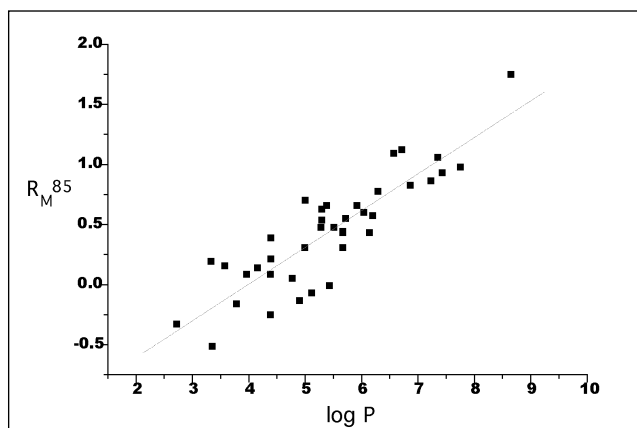


Figure 2. Plot of R_M at $O_{org} = 0.85$ against $\log P$ (Table I).

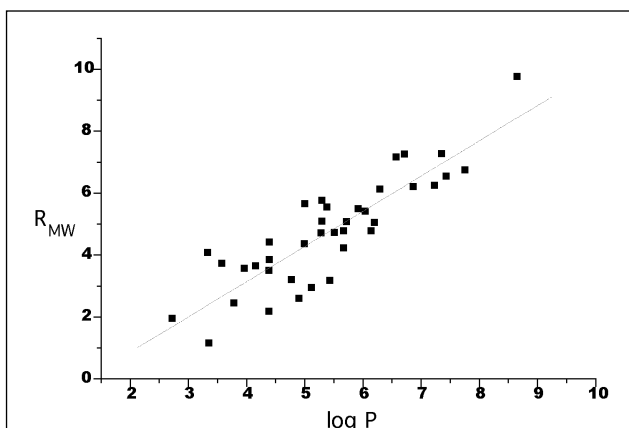


Figure 3. Plot of the constant R_{MW} against $\log P$.

Table III. Calculated Constants m_{calc} and R_{MWcalc} by Δm and ΔR_{MW} Values from Table III and Equations 3 and 4

Compound	m_{calc}	R_{MWcalc}	Compound	m_{calc}	R_{MWcalc}
1	3.8	3.24	24	5.1	4.72
2	5.8	5.50	25	4.1	3.60
3	4.7	4.36	26	5.4	5.11
4	5.2	4.89	27	2.0	1.19
5	6.1	6.01	28	4.0	3.45
6	5.3	4.99	29	5.3	4.96
7	5.8	5.43	30	3.7	3.21
8	7.3	7.18	31	2.9	2.27
9	6.3	6.12	32	4.9	4.53
10	7.0	6.99	33	4.3	3.92
11	9.5	9.87	34	2.8	2.13
12	2.6	1.90	35	4.8	4.39
13	4.6	4.16	36	6.1	5.90
14	4.1	3.65	37	3.1	2.52
15	6.1	5.84	38	5.1	4.78
16	3.9	3.39	39	6.4	6.29
17	5.9	5.50	40	3.4	2.79
18	7.2	7.16	41	5.4	5.05
19	4.9	4.51	42	6.7	6.56
20	6.2	6.02	43	3.6	3.02
21	4.8	4.36	44	5.6	5.31
22	6.1	5.87	45	6.9	6.82
23	5.2	4.87			

Table IV. Slopes, Intercepts, and Correlation Coefficients (r) for Linear Relationships Between the Experimental and Calculated R_M Values for Various ϕ_{org} Values

ϕ_{org}	0.70	0.75	0.80	0.85	0.90	0.95
Slope	0.993	0.963	1.016	1.014	1.001	1.008
Intercept	0.020	0.036	0.003	0.027	0.001	0.028
r	0.9900	0.9914	0.9921	0.9930	0.9918	0.9844

Conclusion

There is no fundamental law that can be used as a starting point to relate a compound molecular structure with its retention because the confident prediction of how it might interact with a support or eluent is still not possible. Retention models based on the solute descriptors (LFER, QSRR) are inconvenient for steroidal compounds due to tedious and unreliable calculations of these descriptors. The eminence of the retention prediction method for steroidal compounds proposed in this paper is due to its simplicity.

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