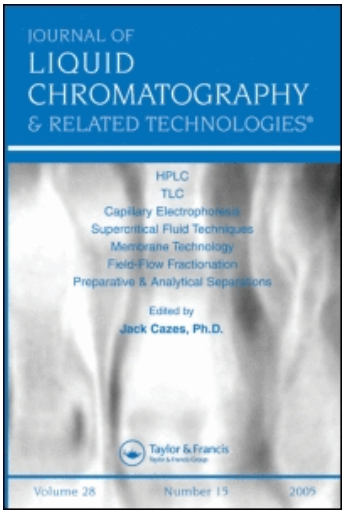


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# Lipophilicity of Selected Steroid Compounds. I. Investigations on RP18W Stationary Phase by RP-HPTLC

A. Pyka[a]; M. Babuska[a]
[a] Department of Analytical Chemistry, Faculty of Pharmacy, Silesian Academy of Medicine, Sosnowiec, Poland

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# Lipophilicity of Selected Steroid Compounds. I. Investigations on RP18W Stationary Phase by RP-HPTLC

**A. Pyka and M. Babuska**
Department of Analytical Chemistry, Faculty of Pharmacy,
Silesian Academy of Medicine, Sosnowiec Poland

**Abstract:** The selected steroid compounds (androsterone, epi-androsterone, dehydro-epi-androsterone, testosterone, stigmasterol, $\beta$-sitosterol, estradiol, hydrocortisone, and cholesterol) were investigated with the use reversed-phase high performance thin layer chromatography on RP18W plates (#1.14296, E. Merck), using methanol-water, acetonitrile-water in different volume compositions as a mobile phase. The chromatographic parameters of lipophilicity ($R_{MW}$ and $\varphi_0$) of the studied steroids were determined. Lipophilic parameters ($R_{MW}$ and $\varphi_0$) were compared both, with measured ($logP_{exp}$), and calculated partition coefficients ($AlogP_S$, IAlogP, $logP_{KOWIN,}$ xlogP, ClogP, miLogP). Comparing all calculation procedures, generally ClogP and IAlogP are more appropriate for chromatographic parameters of lipophilicity and experimental *n*-octanol-water partition coefficients of studied steroid compounds. The results indicate that chromatographic parameters of lipophilicity may be used as a measure of lipophilicity of the investigated steroid compounds.

**Keywords:** RP-HPTLC, Steroid compounds, Drugs, RP18W, Lipophilicity, QSAR *n*-Octanol-water partition coefficients

## INTRODUCTION

Lipophilicity is one of the parameters of chemical substances which influence their biological activities. Lipophilicity is a prime parameter in describing both pharmacodynamic and pharmacokinetic aspects of drug action.[1–5]

Address correspondence to A. Pyka, Department of Analytical Chemistry, Faculty of Pharmacy, Silesian Academy of Medicine, 4 Jagiellońska Street, PL-41-200 Sosnowiec, Poland. E-mail: alinapyka@wp.pl

Lipophilicity is defined by the partitioning of a compound between a non-aqueous and an aqueous phase. The *n*-octanol-water partition coefficient ($\log P_{ow}$) is generally accepted as a useful parameter in structure activity relationship studies (QSAR) for the prediction of biological or pharmacological activity of compounds. The different partition chromatographic techniques,[2–21] and theoretical methods[1,5,22–32] have been widely used as a reliable alternative to classical determination of logP.

Steroids are compounds having a four ringed carbon skeleton derived from 1,2-cyclopentanoperhydrophenanthrene with a cyclic structure. Many steroids are present in plants and animals. They can not be found only in bacterium and cyanosis.[33] Cholesterol is the most important sterol representative of animal origin. A series of cholesterol analogs have been isolated from many organisms. Steroids have definite physiological activity.[33–36] Some steroid compounds, i.e., testosterone, estradiol, estriol, progesterone, hydrocortisone, $\beta$-sitosterol, are used as drugs in modern therapy.

The aim of this work was to compare the lipophilicity of selected steroids determined by RP-HPTLC on RP18W plates using different mobile phases with lipophilicity values estimated by computational methods.



## EXPERIMENTAL

### Chemicals

The following components of the mobile phase: methanol (Merck, Germany; for liquid chromatography), acetonitrile (Merck, Germany; for liquid chromatography), and redistilated water were used for RP-HPTLC analysis. The commercial samples of androsterone (A), epi-androsterone (EP), dehydro-epi-androsterone (DHEA), testosterone (T), stigmasterol (ST), $\beta$-sitosterol (S), estradiol (E), hydrocortisone (H), and cholesterol (CH) (E. Merck, Germany) were used as test solutes. Methanol (POCh, Gliwice, Poland; pure p. a.), ethanol (ZPS Polmos, Kutno, Poland), chloroform (POCh, Gliwice, Poland), and acetone (Chempur, Piekary Śląskie, Poland) were used to prepare the solutions of steroid compounds. Sulfuric acid, 95% (Chempur, Piekary Śląskie, Poland) and methanol (POCh, Gliwice, Poland) were used to prepare the visualizing reagent.

### Sample Preparation

Standard solutions of steroid compounds (5 mg/1 mL) were prepared in methanol (for androsterone, epi-androsterone, and estradiol, cholesterol), chloroform (for dehydro-epi-androsterone, stigmasterol, and $\beta$-sitosterol), ethanol (for testosterone), or a mixture of chloroform and acetone ($7 + 3$, $v/v$; for hydrocortisone).

## Reversed-Phase Thin-Layer Chromatography

Thin–layer chromatography was done on RP-HPTLC RP18W (E. Merck, #1.14296) glass plates. Solutions of examined bile acids were spotted on chromatographic plates in quantities of 10 μg of each steroid in 2 μL of solution. The particular compounds were spotted separately on the plates. The chromatograms were developed by using the mixture of organic modifier-water in the following volume compositions:

a. methanol-water, the content of methanol in mobile phase was gradually varied by 5% (%, v/v) from 50–100 (%, v/v);
b. acetonitrile-water, the content of acetonitrile in mobile phase was gradually varied by 5% (%, v/v) from 30–80 (%, v/v);

Fifty mL of mobile phase was placed into a classical chromatographic chamber (Camag, Switzerland). The chamber was saturated with solvent for 20 min. The chromatograms were developed at room temperature, e.g., 20°C. The development distance was 8.5 cm. The plates were dried at room temperature, e.g., 20°C. The mixture of sulfuric acid and methanol (1:9, v/v) was used as the visualizing agent, and a 10 cm × 20 cm plate was sprayed with 5 mL of this visualizing agent. The plate was then heated at 120°C for 15 min. A Camag densitometer was used to obtain $R_F$ values. The chromatograms were done in triplicate and mean $R_F$ values were calculated.



## Determination of Lipophilicity Parameters

### Theoretical Partition Coefficients

The values of theoretical partition coefficients such as: AlogPs, IAlogP, ClogP, $logP_{Kowwin}$, xlogP, and miLogP[23–28,30–32] were calculated with the use of the Internet databases.[30]

## Application of Reversed–Phase High Performance Thin–Layer Chromatography for Determination of Lipophilicity of Examined Steroids

### Parameter of Lipophilicity $R_{MW}$

The parameter of lipophilicity determined by RP-HPTLC can be expressed by $R_M$ value and can be calculated using the formula (1). The $R_M$ values obtained for studied steroids on RP18W plates, using the following mobile phases: methanol-water and acetonitrile-water, were extrapolated to zero

concentration of organic modifier in eluent ($R_{MW}$), in accordance with Soczewiński-Wachtmeister equation:[5]

$$R_M = R_{MW} - S \cdot \varphi \quad (1)$$

where: $R_M$ is the $R_M$ value of the examined substance by content $\varphi$ of volume fraction of the organic modifier in mobile phase; $R_{MW}$ is the theoretical value of $R_M$ of analyte extrapolated to zero concentration of organic modifier in mobile phase; S is the slope of the regression curve; $\varphi$ is the volume fraction of organic modifier in the mobile phase.

### Parameter of Lipophilicity $\varphi_0$

In the case of curves which were applied to the equation of Soczewiński and Wachtmeister (1), parameter $\varphi_0$ is calculated with the use of the expression:[5]

$$\varphi_0 = \frac{R_{MW}}{S} \quad (2)$$



## RESULTS AND DISCUSSION

The selected steroid compounds (androsterone, epi-androsterone, dehydro-epi-androsterone, testosterone, stigmasterol, $\beta$-sitosterol, estradiol, hydrocortisone, and cholesterol) were studied. The theoretical partition coefficients calculated by use of different methods and for experimental *n*-octanol-water partition coefficients for these compounds are presented in Table 1. The scientific literature does not publish the experimental *n*-octanol-water partition coefficients for stigmasterol, $\beta$-sitosterol, and cholesterol.[30] It results from the fact, that practical problems of determination of experimental *n*-octanol-water partition coefficients arise for highly lipophilic compounds ($\log P > 4$).[1,5,22]

The above mentioned nine steroid compounds were investigated with the use reversed-phase high performance thin layer chromatography on RP18W plates (#1.14296, E. Merck), using methanol-water, acetonitrile-water in different volume compositions as a mobile phase. The $R_M$ values obtained for studied steroids were extrapolated to zero concentration of organic modifier in mobile phase in accordance with Soczewiński-Wachtmeister equation (1). The parameters of regression Equations (3–20), which describe dependencies between the $R_M$ values of steroids and the methanol content ($\varphi$) in methanol-water mobile phase ($R_M = R_{MW(m)} - S\varphi$) as well as between the $R_M$ values of steroids and the acetonitrile content ($\varphi$) in acetonitrile-water mobile phase ($R_M = R_{MW(a)} - S\varphi$) are presented in Tables 2 and 3, respectively.

***Table 1.*** The numerical values of *n*-octanol-water experimental partition coefficients and partition coefficients calculated by using different theoretical methods[23–28,30–32]

| | *n*-Octanol-water partition coefficient | | | | | | |
|---|---|---|---|---|---|---|---|
| Steroid | $\log P_{exp}$ | AlogPs | IAlogP | ClogP | $\log P_{Kowwin}$ | xlogP | miLogP |
| Androsterone | 3.69 | 3.71 | 3.46 | 3.55 | 3.07 | 4.30 | 3.742 |
| Epi-androsterone | 3.69 | 3.71 | 3.46 | 3.55 | 3.07 | 4.30 | 3.742 |
| Dehydro-epi-androsterone | 3.23 | 3.53 | 3.04 | 3.07 | 2.98 | 3.04 | 3.765 |
| Testosterone | 3.32 | 2.99 | 3.24 | 3.22 | 3.27 | 3.60 | 3.765 |
| Estradiol | 4.01 | 3.57 | 3.50 | 3.78 | 3.94 | 4.23 | 4.482 |
| Stigmasterol | — | 6.51 | 9.52 | 9.96 | 9.40 | 8.44 | 7.818 |
| $\beta$-Sitosterol | — | 7.24 | 9.64 | 10.45 | 9.65 | 9.06 | 8.058 |
| Hydrocortisone | 1.61 | 1.71 | 1.71 | 1.70 | 1.62 | 0.52 | 1.445 |
| Cholesterol | — | 7.00 | 8.89 | 9.52 | 8.74 | 8.20 | 7.469 |

***Table 2.*** Parameters of linear regression (±S.D.) between $R_M$ values of steroids and methanol content in methanol-water mobile phase (according to Eq. (1): $R_M = R_{MW(a)} - S \cdot \varphi^a$)

| Steroid | $R_{MW(m)}$ (±S.D.) | S (±S.D.) | n | r | s | F | Range of the volume fraction of methanol ($\varphi$) | Eq. no. |
|---|---|---|---|---|---|---|---|---|
| Androsterone | 2.693 (±0.114) | 3.20 (±0.14) | 11 | 0.990 | 0.078 | 462 | 1.00 ÷ 0.50 | (3) |
| Epiandrosterone | 2.507 (±0.104) | 2.951 (±0.14) | 11 | 0.990 | 0.072 | 467 | 1.00 ÷ 0.50 | (4) |
| Dehydroepiandrosterone | 2.392 (±0.104) | 2.86 (±0.14) | 11 | 0.989 | 0.071 | 442 | 1.00 ÷ 0.50 | (5) |
| Testosterone | 2.240 (±0.158) | 2.69 (±0.20) | 9 | 0.982 | 0.076 | 189 | 1.00 ÷ 0.60 | (6) |
| Estradiol | 2.388 (±0.099) | 2.90 (±0.12) | 10 | 0.992 | 0.057 | 524 | 1.00 ÷ 0.55 | (7) |
| Stigmasterol | 13.496 (±1.805) | 13.48 (±2.00) | 5 | 0.968 | 0.316 | 45 | 1.00 ÷ 0.80 | (8) |
| $\beta$-Sitosterol | 12.695 (±2.069) | 12.50 (±2.29) | 5 | 0.953 | 0.362 | 29 | 1.00 ÷ 0.80 | (9) |
| Hydrocortisone | 1.277 (±0.077) | 1.85 (±0.10) | 11 | 0.986 | 0.053 | 336 | 1.00 ÷ 0.50 | (10) |
| Cholesterol | 7.659 (±0.667) | 7.47 (±0.74) | 5 | 0.985 | 0.117 | 102 | 1.00 ÷ 0.80 | (11) |

*Note:* n, number of points used to derive the particular regression Eq. (1); r, correlation coefficients; s, standard error of the estimate; F, value of Fisher test.

[a]For all equations the significance levels $p < 0.05$.

***Table 3.*** Parameters of linear regression (±S.D.) between $R_M$ values of steroids and acetonitrile content in acetonitrile-water mobile phase (according to Eq. (1): $R_M = R_{MW(a)} - S \cdot \varphi^a$)

| Steroid | $R_{Mw(a)}$ (±S.D.) | S (±S.D.) | n | r | s | F | Range of the volume fraction of acetonitrile ($\varphi$) | Eq. no. |
|---|---|---|---|---|---|---|---|---|
| Androsterone | 2.107 (±0.081) | 3.03 (±0.13) | 13 | 0.990 | 0.087 | 551 | 0.90 ÷ 0.30 | (12) |
| Epi-androsterone | 2.365 (±0.078) | 3.47 (±0.14) | 11 | 0.993 | 0.072 | 663 | 0.75 ÷ 0.30 | (13) |
| Dehydroepiandrosterone | 2.447 (±0.080) | 3.91 (±0.14) | 11 | 0.994 | 0.072 | 799 | 0.75 ÷ 0.30 | (14) |
| Testosterone | 2.277 (±0.070) | 3.59 (±0.12) | 12 | 0.994 | 0.070 | 928 | 0.90 ÷ 0.30 | (15) |
| Estradiol | 2.071 (±0.039) | 3.37 (±0.06) | 13 | 0.998 | 0.042 | 2908 | 0.90 ÷ 0.30 | (16) |
| Stigmasterol | 11.806 (±1.087) | 11.24 (±1.20) | 5 | 0.983 | 0.190 | 87 | 1.00 ÷ 0.80 | (17) |
| $\beta$-Sitosterol | 11.710 (±1.308) | 11.17 (±1.45) | 5 | 0.976 | 0.229 | 59 | 1.00 ÷ 0.80 | (18) |
| Hydrocortisone | 1.424 (±0.046) | 2.73 (±0.08) | 11 | 0.996 | 0.042 | 1163 | 0.75 ÷ 0.30 | (19) |
| Cholesterol | 12.451 (±1.117) | 12.01 (±1.24) | 5 | 0.984 | 0.196 | 94 | 1.00 ÷ 0.80 | (20) |

*Note:* n, number of points used to derive the particular regression Eq. (1); r, correlation coefficients; s, standard error of the estimate; F, value of Fisher test.

[a]For all equations the significance levels $p < 0.005$.

The high correlation coefficients (r), the values of the Fisher test (F), the significance levels (p), and small values of the standard errors of the estimates (s) were indicated that all the equations obtained were highly significant.

It was found that the values of $R_{MW(m)}$ and $R_{MW(a)}$ lipophilicity parameters obtained by using RP-HPTLC depend linearly on the slope of regression curve S (from Eq. (1). The regression equations (21) and (22) describe these linear relationships with high correlation coefficients ($r = 0.999$):

$$R_{MW(m)} = -0.6299(\pm 0.0873) + 1.0624(\pm 0.0125)S$$
$$n = 9; r = 0.999; s = 0.160; F = 7235; p < 0.0001 \quad (21)$$
$$R_{MW(a)} = -1.9264(\pm 0.1359) + 1.2110(\pm 0.0189)S$$
$$n = 9; r = 0.999; s = 0.218; F = 4089; p < 0.0001 \quad (22)$$

Equations (21) and (22) confirm the fact that studied steroid compounds comply with Soczewiński-Wachtmeister equation (1). Therefore, the values of $\varphi_{o(m)}$ and $\varphi_{o(a)}$ lipophilicity parameters with the use of the expression (2) were calculated for studied steroid compounds. The calculated values of $\varphi_{o(m)}$ and $\varphi_{o(a)}$ lipophilicity parameters are presented in Table 4.

The obtained values of $R_{MW(m)}$, $R_{MW(a)}$, $\varphi_{o(m)}$, and $\varphi_{o(a)}$ lipophilicity parameters indicate that hydrocortisone shows the lowest lipophilic properties. Androsterone, epi-androsterone, dehydro-epi-androsterone, testosterone, and estradiol have intermediate lipophilic properties. However, stigmasterol, $\beta$-sitosterol, and cholesterol have the highest lipophilicities.

We compared the values of $R_{MW(m)}$ and $R_{MW(a)}$ lipophilicity parameters with the experimental and theoretical *n*-octanol-water partition coefficients for studied steroids. The $R_{MW}$ values obtained for androsterone, epi-androsterone, dehydro-epi-androsterone, testosterone, and estradiol are lower in relation to their values of experimental and theoretical partition coefficients. The best agreement of values of $R_{MW(m)}$ and $R_{MW(a)}$ lipophilicity parameters with

***Table 4.*** The values of lipophilicity parameters $\varphi_{o(m)}$ and $\varphi_{o(a)}$ for studied steroids investigated by using methanol-water and acetonitrile-water mobile phases according to Eq. (2)

| Steroid | $\varphi_{o(m)}$ | $\varphi_{o(a)}$ |
|---|---|---|
| Androsterone | 0.842 | 0.695 |
| Epi-androsterone | 0.850 | 0.682 |
| Dehydro-epi-androsterone | 0.836 | 0.634 |
| Testosterone | 0.833 | 0.634 |
| Estradiol | 0.823 | 0.615 |
| Stigmasterol | 1.001 | 1.050 |
| $\beta$-Sitosterol | 1.016 | 1.048 |
| Hydrocortisone | 0.690 | 0.522 |
| Cholesterol | 1.025 | 1.037 |

the experimental, as well as the theoretical *n*-octanol-water partition coefficients (except xlogP) for hydrocortisone was observed. For the remaining compounds, i.e., stigmasterol, $\beta$-sitosterol, and cholesterol, the $R_{MW}$ values in relation to their theoretical partition coefficients are greater.

The values of correlation coefficients of linear relationships between the chromatographic parameters of lipophilicity and experimental, as well as theoretical partition coefficients for all studied compounds are presented in Table 5.

It was stated that the highest intercorrelation exists between the $R_{MW(a)}$ and $\varphi_{o(a)}$ lipophilicity parameters, which were obtained using acetonitrile-water as a mobile phase:

$$R_{MW(a)} = -12.1050(\pm 1.3530) + 22.7893(\pm 1.7032)\varphi_{o(a)}$$
$$n = 9;\ r = 0.9810;\ s = 1.026;\ F = 179;\ p < 0.0001 \quad (23)$$

However, the intercorrelation between the $R_{MW(m)}$ and $\varphi_{o(m)}$ lipophilicity parameters, which were obtained using methanol-water as a mobile phase is considerably lower ($r = 0.8671$).

The intercorrelation between the $R_{MW(a)}$, $\varphi_{o(a)}$ lipophilicity parameters and the experimental n-octanol-water partition coefficients are comparatively low ($r < 0.828$). However, the $R_{MW(m)}$ and $\varphi_{o(m)}$ lipophilicity parameters correlate well with the experimental partition coefficients ($\log P_{exp}$). For example, the linear regression between the $R_{MW(m)}$ lipophilic parameter and $\log P_{exp}$ is given below:

$$R_{MW(m)} = 0.4641(\pm 0.3388) + 0.5479(\pm 0.1011)\log P_{exp}$$
$$n = 6;\ r = 0.9381;\ s = 0.193;\ F = 29;\ p < 0.01 \quad (24)$$

It was stated, that the values of $R_{MW(a)}$ and $R_{MW(m)}$lipophilic parameters correlate best with the following theoretical *n*-octanol-water partition coefficients: IAlogP, ClogP, and $\log P_{Kowwin}$. Moreover, it was also stated, that $R_{MW(a)}$ correlates well with AlogPs. However $\varphi_{o(m)}$ and $\varphi_{o(a)}$ lipophilicity parameters correlate well with all theoretical partition coefficients. The selected regression equations presented intercorrelations between chromatographic lipophilic parameters and theoretical partition coefficients are listed below:

$$R_{MW(a)} = -2.1984(\pm 0.5768) + 1.4032(\pm 0.09105) \cdot C\log P$$
$$n = 9;\ r = 0.9856;\ s = 0.894;\ F = 237;\ p < 0.0001 \quad (25)$$
$$R_{MW(m)} = -2.1776(\pm 0.9698) + 1.4400(\pm 0.1622) \cdot IA\log P$$
$$n = 9;\ r = 0.9584;\ s = 1.460;\ F = 78;\ p < 0.0000 \quad (26)$$
$$\varphi_{o(a)} = -0.4394(\pm 0.0201) + 0.0607(\pm 0.0032) \cdot C\log P$$
$$n = 9;\ r = 0.9906;\ s = 0.031;\ F = 365;\ p < 0.0001 \quad (27)$$
$$\varphi_{o(m)} = -0.6310(\pm 0.0186) + 0.0560(\pm 0.0039) \cdot A\log Ps$$
$$n = 9;\ r = 0.9836;\ s = 0.022;\ F = 208;\ p < 0.0001 \quad (28)$$

***Table 5.*** The values of correlation coefficients of linear relationships between experimental and theoretical partition coefficients as well as chromatographic parameters of lipophilicity (n = 9)

| | $R_{MW(a)}$ | $R_{MW(m)}$ | $\varphi_{o(a)}$ | $\varphi_{o(m)}$ | AlogPs | IAlogP | ClogP | $logP_{Kowwin}$ | xlogP | miLogP | $logP_{exp}$[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $R_{MW(a)}$ | 1 | | | | | | | | | | |
| $R_{MW(m)}$ | 0.9292 | 1 | | | | | | | | | |
| $\varphi_{o(a)}$ | 0.9810 | 0.9389 | 1 | | | | | | | | |
| $\varphi_{o(m)}$ | 0.9249 | 0.8671 | 0.9671 | 1 | | | | | | | |
| AlogPs | 0.9585 | 0.9042 | 0.9838 | 0.9836 | 1 | | | | | | |
| IAlogP | 0.9864 | 0.9584 | 0.9926 | 0.9546 | 0.9795 | 1 | | | | | |
| ClogP | 0.9865 | 0.9549 | 0.9906 | 0.9530 | 0.9816 | 0.9994 | 1 | | | | |
| $logP_{Kowwin}$ | 0.9817 | 0.9560 | 0.9812 | 0.9470 | 0.9741 | 0.9970 | 0.9976 | 1 | | | |
| xlogP | 0.9250 | 0.9050 | 0.9708 | 0.9842 | 0.9842 | 0.9689 | 0.9696 | 0.9652 | 1 | | |
| miLogP | 0.9438 | 0.9207 | 0.9683 | 0.9792 | 0.9838 | 0.9784 | 0.9790 | 0.9825 | 0.9884 | 1 | |
| $logP_{exp}$[a] | 0.7566 | *0.9381* | 0.8277 | *0.9232* | 0.9466 | 0.9895 | 0.9991 | 0.9438 | 0.9841 | 0.9731 | 1 |

[a] n = 6 (no experimental data of partition coefficients for: stigmasterol, $\beta$-sitosterol, and cholesterol).

However, the highest values of correlation coefficients were obtained for the relationships between the experimental *n*-octanol-water partition coefficient and theoretical partition coefficients IAlogP, as well as ClogP ($r = 0.9895$ and $r = 0.9991$, respectively).

Comparing all calculation procedures, generally ClogP and IAlogP are more appropriate for chromatographic parameters of lipophilicity and the experimental *n*-octanol-water partition coefficient of studied steroid compounds.

Further investigations are in progress and concern the use of RP-TLC and RP-HPLC to evaluate the lipophilicity of different steroid compounds.

## CONCLUSIONS

The results from these investigations indicate that theoretical partition coefficients can be used for the study of QSAR analysis of selected steroid compounds. Theoretical determination of logP values on the basis of different procedures has special significance if standards of organic compounds are not available. These methods of determining lipophilicity on the basis of theoretical calculation of logP and chromatographic methods complement well established methods and applications, i.e., methods of normal measurement in the *n*-octanol–water system. Because of experimental difficulties including solubility limits, chemical instability, formation of emulsions or impurities of the compounds, the evaluation of logP values by the analytical methods in this paper are well founded.

The methodology worked out in these investigations can be used for study and comparison of lipophilic properties of different organic compounds of biological significance.



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