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# EVALUATION OF LIPOPHILIC PROPERTIES OF BETAMETHASONE AND RELATED COMPOUNDS

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**Abstract:** The lipophilicity ($R_{MW}$) of betamethasone and its four related compounds: betamethasone-17,21-dipropionate, betamethasone-17-valerate, betamethasone-21-valerate and also betamethasone disodium phosphate was determined by reversed phase HPTLC and various mobile phase systems (methanol-water, dioxane-water and acetonitrile-water). The chromatographic lipophilicity parameters obtained for all examined compounds using abovementioned mobile phases onto three chromatographic plates (RP-$2F_{254}$, RP-$8F_{254}$, RP-$18WF_{254}$) were compared with the theoretical partition coefficients which have been calculated by different computing programs: AlogPs, AClogP, AlogP, MlogP, KOWWIN, xlogP2, xlogP3, $logP_{ChemDraw}$ as well as with logP measured by shake-flask method. The results of this work demonstrate that regardless of applied method the greatest similarity in lipophilic properties show betamethasone-17-valerate, betamethasone-21-valerate and also betamethasone 17,21-dipropionate. The influence of solvent system as mobile phase on $R_{MW}$ values of examined compounds was observed. Among different mobile phases (organic modifier-water) proposed in this study, which allowed obtaining the reliable chromatographic lipophilicity parameters for all studied compounds is methanol-water mixture. The performance investigations showed that RP-HPTLC method has proved to be a rapid and cost effective analytical tool for describing the lipophilic properties of betamethasone and its related compounds.

**Keywords:** betamethasone, lipophilicity, cluster analysis, logP, $R_{MW}$, RP-HPTLC

Betamethasone (9α-fluoro-16β-methyl-11β, 17α,21-trihydroxy-1,4-pregnadiene-3,20-dione) is a synthetic glucocorticoid (GC). Some esters and salts of this compound are widely used as anti-inflammatory, immunosuppressive and antiproliferative agents, and also in dermatological therapy of different skin diseases (1, 2). Despite minor differences in chemical structures, betamethasone derivatives may have various pharmacological effects. From the data which are available in literature, it is obvious that among different physicochemical properties, lipophilicity of drugs including skin preparations has significant impact on their biological activity and dermal permeation (3, 4). The quantitative descriptor of lipophilicity is the partition coefficient (P) or its decimal logarithm (logP) which is usually determined by traditional, but time consuming shake-flask method. Theoretically, logP value can be calculated from molecular structure with the use of commercial computational programs (5, 6). Recently, a new possibility in prediction of the lipophilicity parameters was opened by use of the following chromatographic methods: RP-TLC, RP-HPTLC and also RP-HPLC. The main advantages of the chromatographic methods are: decreasing of development time and small amount of compounds needed for the determination of lipophilicity parameters. Numerous research papers involved the application of reversed thin-layer chromatography and high-performance chromatography to determine the lipophilicity of various organic compounds (7-13). Many of the original works which were focused on QSAR study (quantitative structure-activity relationship) indicated that the lipophilicity parameter determined using various methods (including liquid chromatography) correlates well with other physicochemical descriptors such as dissociation constant, polarity and also with topological indices and plays important role in predicting of drug behavior in biological system (e.g., in biological membranes and tissues). Significant correlations obtained between the chromatographic lipophilicity parameters of different organic compounds (determined by means of RP-

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Table 1. Lipophilicity parameters of examined compounds obtained using calculating procedures and also by shake-flask method (25).

| Compound | Lipophilicity parameters calculated by the use of theoretical methods | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $logP_{exp}$ | $AlogP_s$ | AClogP | AlogP | xlogP2 | xlogP3 | $logP_{KOWWIN}$ | MlogP | $logP_{ChemDraw}$ | $logP_{average}$ (± SD) |
| Betamethasone | 1.83 | 1.93 | - | - | 1.14 | - | 1.72 | - | 1.94 | 1.60 ± 0.41 |
| Betamethasone-17,21-dipropionate | - | 3.69 | 3.99 | 2.89 | 2.83 | 6.38 | 3.97 | 3.10 | 4.68 | 3.84 ± 1.22 |
| Betamethasone-17-valerate | 3.60 | 3.76 | 3.52 | 3.19 | 2.84 | 4.60 | 3.94 | 2.84 | 3.91 | 3.53 ± 0.64 |
| Betamethasone-21-valerate | 3.87 | 3.65 | 3.52 | 2.90 | 2.84 | 4.99 | 3.94 | 2.84 | 3.91 | 3.52 ± 0.78 |
| Betamethasone disodium phosphate | - | 3.62 | -0.99 | - | 0.56 | 2.23 | - | - | 1.07 | 1.36 ± 2.00 |

SD = standard deviation.

TLC or RP-HPLC techniques) and their biological activity, e.g., antitumoral, antibacterial and also cardiovascular, are widely presented in literature from the last decade (10-13).

The present work is continuation of earlier investigations concerning TLC lipophilicity study of selected steroid compounds which have different pharmacological effect such as free and conjugated (with taurine or glycine) bile acids present in bile, other bile acids like ursodeoxycholic acid and dehydrocholic acid, which are widely applied in hepatic diseases in form of respective pharmaceutical formulations and also some anabolic steroids: stanozolol, 19-nortestosterone and mesterolone (14-23). Previous papers allowed to confirm the compatibility of the lipophilicity parameter $R_{MW}$ (determined by RP-TLC and RP-HPTLC) of abovementioned compounds with their theoretical partition coefficients (logP) calculated by use of various computational programs: AlogPs, $logP_{KOWWIN}$, xlogP2, xlogP3, milogP, AlogP, MlogP and also with measured (by shake-flask method) *n*-octanol-water partition coefficient ($logP_{exp}$).

Continuing research in this field, the objective of the current study was to evaluate and compare the lipophilicity ($R_{MW}$) of betamethasone (B) and its related compounds such as: betamethasone-17,21-dipropionate (BP), betamethasone-17-valerate (BV17), betamethasone-21-valerate (BV21) and also betamethasone disodium phosphate (BPh) by means of reversed phase high performance thin-layer chromatography (RP-HPTLC) under different chromatographic conditions. Moreover, the theoretical determinations of logP values obtained using the internet module (AlogPS 2.1-vcclab): AlogPs, AClogP, AlogP, MlogP, KOWWIN, xlogP2, and xlogP3 and by software Cambridge ChemDraw were performed. According to our knowledge, until today the lipophilicity parameters obtained by chromatographic methods of betamethasone derivatives such as BP, BV17, BV21 and also BPh have not yet been published in the literature and in DrugBank (24). Therefore, the main objective of this work was to determine and compare the lipophilicity parameters of betamethasone and its four related compounds obtained by means of RP-HPTLC under various chromatographic conditions and also using the computational methods.

Moreover, the influence of the chromatographic conditions such as the kind of mobile phase system and the type of chromatographic plates on lipophilicity measurements of investigated compounds by RP-HPTLC method was discussed.

## EXPERIMENTAL

### *Chemicals and reference standards*

Mobile phase components: methanol, dioxane and acetonitrile for liquid chromatography were purchased from POCh (Gliwice, Poland). Distillated water was obtained from Department of Analytical Chemistry (Medical University of Silesia, Sosnowiec, Poland). The reference standards of betamethasone (CAS No. 378-44-9), betamethasone-17,21-dipropionate (CAS No. 5593-20-4), betamethasone-17-valerate (CAS No. 2152-44-5), betamethasone-21-valerate (CAS No. 2240-28-0) and also betamethasone disodium phosphate (CAS No. 151-73-5) were from commercial source (Sigma-Aldrich, St. Louis, MO, USA). Standard solutions of five examined compounds at concentration of 5 mg/mL each were prepared in ethanol (96%, pure for analysis) from POCh (Gliwice, Poland).

### Determination of chromatographic parameter of lipophilicity ($R_{MW}$) by use of RP-HPTLC

Lipophilicity of betamethasone and its related compounds was determined by thin-layer chromatography on 10 × 10 cm RP-HPTLC plates: RP-$8F_{254}$ (E. Merck, Darmstadt, Germany, Art. 1.15424), RP-$2F_{254}$, (E. Merck, Darmstadt, Germany, Art 1.13726) and also on RP-$18WF_{254}$ (E. Merck, Darmstadt, Germany, Art. 13124). The solutions of examined compounds were spotted separately onto chromatographic plates in quantity of 3 μL (15 μg per spot). The chromatograms were developed using the mixtures of organic modifier-water in different volume compositions. The content of organic modifier (e.g., methanol, dioxane, acetonitrile) in mobile phase was gradually varied by 5% (v/v) from 20 to 100 (%, v/v). Fifty mL of respective mobile phase was placed into a classical chromatographic chamber (Camag, Muttenz, Switzerland). The chamber was saturated with solvent vapor for 30 min. The chromatograms were developed to distance of 80 mm at temperature of 20 ± 2°C. After development, the plates were dried at room temperature. Each chromatogram was run in triplicate. The spots were localized in UV at λ = 254 nm (Camag, Muttenz, Switzerland). For subsequent calculations, mean $R_F$ values obtained for B, BP, BV17 BV21 and also BPh on three chromatographic plates: RP-$2F_{254,}$ RP-$8F_{254,}$ RP-$18WF_{254}$ and using mobile phases: methanol-water, dioxane-water, acetonitrile-water in various compositions were converted to $R_M$ values according to the expression:

$$R_M = \log\left(\frac{1}{R_F} - 1\right) \quad [1]$$

Linear correlation between $R_M$ and volume fraction of organic modifier in mobile phase (φ) permits an extrapolation of calculated $R_M$ values to the zero concentration of organic modifier accordance with Soczewiński-Wachtmeister's equation [2] and it allowed to determine the relative chromatographic parameter $R_{MW}$ (3):

$$R_M = R_{MW} - S \times \varphi \quad [2]$$

where: $R_M$ is the $R_M$ value of examined compound, $R_{MW}$ is the $R_M$ value extrapolated to zero concentration of organic modifier in mobile phase, S is the slope of the regression plot, φ is the volume fraction of organic modifier (e.g., methanol, dioxane, acetonitrile) in mobile phase.

### Determining the theoretical partition coefficients (logP)

The computationally calculated logP values expressed as AlogPs, AClogP, AlogP, MlogP, KOWWIN, xlogP2, xlogP3, their mean value (average logP) and also experimental $logP_{exp}$ for all examined compounds were determined *via* on line at VCCLAB.org website (25). Additionally, in order to determine the logP value of examined compounds the software Cambridge ChemDraw 13.0 Ultra was applied.

### Regression and cluster analysis

Regression and cluster analysis of obtained results were performed with the use of computer software STATISTICA 10.0.

## RESULTS AND DISCUSSION

In order to estimate the lipophilic properties of five examined compounds: B, BP, BV17, BV21 and also BPh, the partition coefficient (logP) determined on the basis of their chemical structures by use of various computational programs from internet database (*via* on-line at VCCLAB.org website) and by software Cambridge ChemDraw 13.0 Ultra were compared. The values of logP: AlogPs, AClogP, AlogP, MlogP, KOWWIN, xlogP2, xlogP3, their mean value (average logP), measured logP value (determined by shake flask method) and also logP calculated by software Cambridge ChemDraw 13.0 Ultra ($logP_{ChemDraw}$) are presented in Table 1. Analysis of obtained logP results: AlogPs, xlogP2, $logP_{average}$ and also $logP_{ChemDraw}$, which are available for all (five) tested compounds (Table 1), indicates that generally the theoretical log P value obtained for each investigated compound, especially for BPh,

Table 2. Parameters of the linear correlations (± SD) between $R_M$ and φ values of examined compounds obtained on different chromatographic plates and by use of various content of organic modifier in mobile phase: methanol-water, Eq. $R_M = R_{MW} - S \times \varphi^*$.

| Parameters of linear correlations (± SD) $R_M = R_{MW} - S \times \varphi^*$ | | | | | | |
|---|---|---|---|---|---|---|
| Compound | $R_{MW}$ | S | r | s | F | n |
| RP-2F$_{254}$ | | | | | | |
| Betamethasone | 2.255 (± 0.158) | 3.627 (± 0.203) | 0.995 | 0.060 | 320.4 | 8 |
| Betamethasone-17,21-dipropionate | 3.418 (± 0.218) | 4.478 (± 0.275) | 0.992 | 0.084 | 265.9 | 9 |
| Betamethasone-17-valerate | 3.258 (± 0.179) | 4.345 (± 0.226) | 0.995 | 0.069 | 370.9 | 7 |
| Betamethasone-21-valerate | 3.583 (± 0.138) | 4.748 (± 0.174) | 0.997 | 0.053 | 745.9 | 8 |
| Betamethasone disodium phosphate | 2.448 (± 0.351) | 3.773 (± 0.406) | 0.983 | 0.078 | 86.3 | 6 |
| RP-8F$_{254}$ | | | | | | |
| Betamethasone | 3.093 (± 0.325) | 4.407 (± 0.445) | 0.989 | 0.080 | 98.1 | 9 |
| Betamethasone-17,21-dipropionate | 3.876 (± 0.421) | 4.666 (± 0.505) | 0.983 | 0.116 | 85.3 | 8 |
| Betamethasone-17-valerate | 4.093 (± 0.325) | 4.896 (± 0.389) | 0.991 | 0.089 | 158.1 | 8 |
| Betamethasone-21-valerate | 4.457 (± 0.398) | 5.338 (± 0.477) | 0.988 | 0.109 | 125.1 | 9 |
| Betamethasone disodium phosphate | 0.776 (± 0.091) | 1.699 (± 0.114) | 0.996 | 0.034 | 224.0 | 6 |
| RP-18WF$_{254}$ | | | | | | |
| Betamethasone | 2.090 (± 0.135) | 3.066 (± 0.171) | 0.994 | 0.052 | 322.7 | 9 |
| Betamethasone-17,21-dipropionate | 3.027 (± 0.178) | 3.714 (± 0.222) | 0.993 | 0.071 | 278.9 | 8 |
| Betamethasone-17-valerate | 3.027 (± 0.195) | 3.714 (± 0.243) | 0.992 | 0.078 | 232.8 | 9 |
| Betamethasone-21-valerate | 2.958 (± 0.089) | 3.699 (± 0.011) | 0.998 | 0.034 | 1056.2 | 8 |
| Betamethasone disodium phosphate | 1.980 (± 0.383) | 2.781 (± 0.458) | 0.962 | 0.110 | 36.9 | 5 |

n - number of points used to derive the particular regressions; r - correlation coefficient; s - standard error; F - value of Fisher test, SD - standard deviation, * - for all equations the significance level $p < 0.001$.

differed depending on the powers of calculated programs. On the basis of logP values determined by means of the algorithm xlogP2 and also by software ChemDraw ($logP_{ChemDraw}$), it could be observed that the lipophilicity of the examined compounds decreases in the following order: BP ≅ BV17 ≅ BV21 > B > BPh and BP > BV17 ≅ BV21 > B > BPh, respectively. Thus, it shows the lowest lipophilicity of betamethasone disodium phosphate (BPh) among all tested compounds. In the case of logP predicted using the procedure $AlogP_S$, the theoretical logP value for this compound (BPh) indicates relatively a higher value in comparison with those predicted as xlogP2 and $logP_{ChemDraw}$ and is evidently comparable with logP obtained for BV17, BV21 and BP (Table 1). Further interpretation of the theoretical logP values obtained from databases as $logP_{avarage}$ (± SD) confirms the fact that the theoretical lipophilicity factors such as $logP_{avarage}$ of examined compounds should be critically discussed on the basis of statistical parameters like, for example, standard deviation (SD). Taking into account the standard deviation of estimate of $logP_{average}$, particularly that obtained for previously discussed betamethasone disodium phosphate (BPh), it may be noted that including the SD value to arithmetic mean

value of log P ($logP_{average}$) of BPh and other studied compounds (B, BV17, BV21, BP) has significant impact on $logP_{average}$ value and changes the relation between lipophilicity properties of five examined substances in comparison with those, which were obtained by means of arithmetic mean value of log $P_{average}$ only. The values of $logP_{average}$ obtained after adding to them the SD value allow to perform the studied compounds in the following order of decreased lipophilicity: BP > BV21 ≅ BV17 > BPh > B. Subtracting the SD value from arithmetic mean value of log P ($logP_{average}$) of each compound resulted in obtaining the following relation between lipophilicity properties: BV17 ≅ BV21 > BP > B > BPh. The results which were presented above have shown that computational methods are still in development phase and they are imperfect (26) in comparison with experimental methods e.g., chromatography. From this fact the following conclusion can be made that the theoretical determination of logP allows for preliminary estimation of lipophilicity range of betamethasone and its related compounds. In order to obtain more reliable and accurate lipophilicity values of betamethasone derivatives, correlation of the theoretical lipophilicity parameter (logP) with experimental values should be executed. Finally, to describe the relationship between lipophilic properties of all examined compounds on

Table 3. Parameters of the linear correlations (± SD) between $R_M$ and φ values of examined compounds obtained on different chromatographic plates and by use of various content of organic modifier in mobile phase: dioxane-water, Eq. $R_M = R_{MW} - S \times \varphi^*$.

| Parameters of linear correlations (± SD) $R_M = R_{MW} - S \times \varphi^*$ | | | | | | |
|---|---|---|---|---|---|---|
| Compound | $R_{MW}$ | S | r | s | F | n |
| RP-$2F_{254}$ | | | | | | |
| Betamethasone | 2.325 (± 0.313) | 3.932 (± 0.414) | 0.989 | 0.135 | 90.3 | 9 |
| Betamethasone-17,21-dipropionate | 3.068 (± 0.826) | 4.597 (± 1.060) | 0.908 | 0.464 | 18.8 | 8 |
| Betamethasone-17-valerate | 2.803 (± 0.789) | 4.333 (± 1.013) | 0.906 | 0.444 | 18.3 | 9 |
| Betamethasone-21-valerate | 4.178 (± 0.237) | 5.909 (± 0.314) | 0.997 | 0.102 | 355.2 | 7 |
| Betamethasone disodium phosphate | 0.426 (± 0.262) | 1.743 (± 0.301) | 0.971 | 0.072 | 33.5 | 5 |
| RP-$8F_{254}$ | | | | | | |
| Betamethasone | 1.762 (± 0.356) | 3.042 (± 0.457) | 0.958 | 0.200 | 40.2 | 9 |
| Betamethasone-17,21-dipropionate | 2.998 (± 0.567) | 4.222 (± 0.688) | 0.962 | 0.230 | 37.6 | 9 |
| Betamethasone-17-valerate | 2.803 (± 0.581) | 4.034 (± 0.705) | 0.957 | 0.236 | 32.7 | 9 |
| Betamethasone-21-valerate | 3.197 (± 0.605) | 4.438 (± 0.735) | 0.961 | 0.246 | 30.8 | 8 |
| Betamethasone disodium phosphate | 0.266 (± 0.253) | 1.709 (± 0.349) | 0.943 | 0.122 | 23.9 | 6 |
| RP-$18WF_{254}$ | | | | | | |
| Betamethasone | 2.451 (± 0.606) | 4.154 (± 0.736) | 0.956 | 0.246 | 70.7 | 9 |
| Betamethasone-17,21-dipropionate | 3.907 (± 0.489) | 5.688 (± 0.593) | 0.984 | 0.198 | 91.9 | 8 |
| Betamethasone-17-valerate | 3.694 (± 0.511) | 5.467 (± 0.621) | 0.981 | 0.208 | 77.6 | 9 |
| Betamethasone-21-valerate | 3.932 (± 0.507) | 5.709 (± 0.616) | 0.983 | 0.206 | 85.9 | 8 |
| Betamethasone disodium phosphate | 0.248 (± 0.298) | 1.984 (± 0.353) | 0.956 | 0.134 | 31.6 | 5 |

n - number of points used to derive the particular regressions; r - correlation coefficient; s - standard error; F - value of Fisher test, SD - standard deviation, * - for all equations the significance level $p < 0.001$.

Table 4. Parameters of the linear correlations (± SD) between $R_M$ and φ values of examined compounds obtained on different chromatographic plates and by use of various content of organic modifier in mobile phase: acetonitrile-water, Eq. $R_M = R_{MW} - S \times \varphi^*$.

| Parameters of linear correlations (± SD) $R_M = R_{MW} - S \times \varphi^*$ | | | | | | |
|---|---|---|---|---|---|---|
| Compound | $R_{MW}$ | S | r | s | F | n |
| RP-2F$_{254}$ | | | | | | |
| Betamethasone | 3.410 (± 1.189) | 5.820 (± 1.578) | 0.934 | 0.249 | 13.6 | 9 |
| Betamethasone-17,21-dipropionate | 1.787 (± 0.287) | 2.768 (± 0.348) | 0.970 | 0.100 | 63.1 | 9 |
| Betamethasone-17-valerate | 1.990 (± 0.212) | 3.188 (± 0.270) | 0.989 | 0.056 | 139.6 | 9 |
| Betamethasone-21-valerate | 2.362 (± 0.133) | 3.605 (± 0.169) | 0.997 | 0.035 | 455.6 | 9 |
| Betamethasone disodium phosphate | 0.814 (± 0.119) | 1.256 (± 0.152) | 0.986 | 0.014 | 68.3 | 5 |
| RP-8F$_{254}$ | | | | | | |
| Betamethasone | 1.332 (± 0.444) | 2.620 (± 0.518) | 0.963 | 0.116 | 25.5 | 9 |
| Betamethasone-17,21-dipropionate | 1.956 (± 0.345) | 2.769 (± 0.407) | 0.969 | 0.105 | 46.4 | 9 |
| Betamethasone-17-valerate | 1.141 (± 0.188) | 1.897 (± 0.223) | 0.980 | 0.058 | 72.6 | 9 |
| Betamethasone-21-valerate | 1.566 (± 0.295) | 2.388 (± 0.348) | 0.970 | 0.090 | 47.2 | 9 |
| Betamethasone disodium phosphate | 0.974 (± 0.370) | 2.649 (± 0.543) | 0.974 | 0.058 | 23.8 | 5 |
| RP-18WF$_{254}$ | | | | | | |
| Betamethasone | 0.698 (± 0.097) | 1.502 (± 0.124) | 0.993 | 0.018 | 146.2 | 9 |
| Betamethasone-17,21-dipropionate | 0.782 (± 0.037) | 1.143 (± 0.037) | 0.998 | 0.017 | 972.7 | 9 |
| Betamethasone-17-valerate | 0.502 (± 0.047) | 0.796 (± 0.059) | 0.994 | 0.016 | 182.7 | 9 |
| Betamethasone-21-valerate | 0.674 (± 0.010) | 1.064 (± 0.012) | 0.999 | 0.003 | 7400.3 | 9 |
| Betamethasone disodium phosphate | 0.490 (± 0.056) | 0.384 (± 0.060) | 0.976 | 0.023 | 40.6 | 5 |

n - number of points used to derive the particular regressions; r - correlation coefficient; s - standard error; F - value of Fisher test, SD - standard deviation, * - for all equations the significance level $p < 0.001$.

the basis of obtained theoretical parameters of lipophilicity (logP), the computationally predicted logP values were analyzed by means of cluster analysis (CA). Dendrogram of the similarity analysis (single-bond method, *Euclidean-distance*) of theoretically determined lipophilicity parameters (AlogPs, xlogP2, $logP_{ChemDraw}$, $logP_{average}$ (± SD)) in Figure 1 demonstrates that the biggest similarity in lipophilicity parameters (logP) is between BV17, BV21 and BP which form one characteristic cluster. Similar dependence could be observed between B and BPh which form the second cluster on this dendrogram. It could be suggested that the applied cluster analysis permits grouping the studied compounds into two subgroups (Fig. 1). The first subgroup form BV17, BV21 and BP. To the second subgroup belong B and BPh.

In order to confirm the lipophilic properties of four steroid compounds of betamethasone as well as betamethasone which have been predicted by theoretical procedures, an experimental method such as high performance thin layer chromatography in reversed phase system (RP-HPTLC) was applied. The $R_F$ values measured for all examined compounds under different chromatographic conditions: on the chromatographic plates RP-2F$_{254}$, RP-8F$_{254}$,

RP-18WF$_{254}$ and mobile phases: methanol-water, dioxane-water, acetonitrile-water in various volume compositions were used to calculate the $R_M$ values. The relative chromatographic parameter of lipophilicity ($R_{MW}$) was determined for each compound by extrapolation of $R_M$ values to zero content of organic modifier (methanol, dioxane or acetonitrile, respectively) in mobile phase. Important parameters of obtained linear correlations between $R_M$ and φ (the content of organic modifier in mobile phase used) such as *r* - correlation coefficient, *s* - standard error, *p* - significance level and *F*-value of

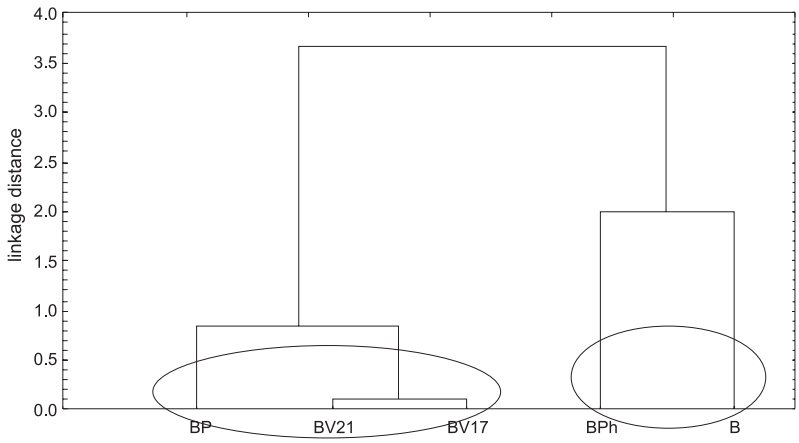


Figure 1.Cluster analysis of logP values of examined compounds determined by the use of selected computational procedures: AlogPS, xlogP2, logP$_{ChemDraw}$ and logP$_{average}$. B = Betamethasone, BV17 = Betamethasone-17-valerate, BV21 = Betamethasone-21-valerate, BP = Betamethasone-17,21-dipropionate, BPh = Betamethasone disodium phosphate

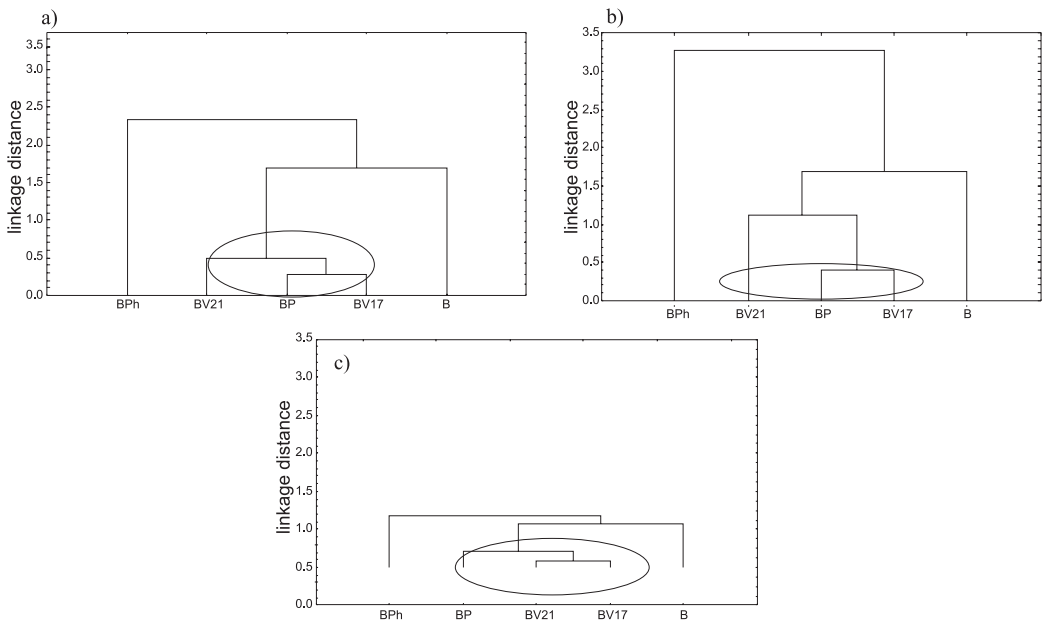


Figure 2. Cluster analysis of $R_{MW}$ values for examined compounds obtained on applied chromatographic plates developed using mobile phases: methanol-water (a), dioxane-water ( b) and acetonitrile-water (c). B = Betamethasone, BV17 = Betamethasone-17-valerate, BV21 = Betamethasone-21-valerate, BP = Betamethasone-17,21-dipropionate, BPh = Betamethasone disodium phosphate

Fisher test are presented in Tables 2-4. The best linear correlations were found by use of methanol-water as mobile phase. The high correlation coefficients (r) and also small values of the standard errors of the estimates (s) indicated that all obtained equations [2] are highly significant and can be used to calculate the $R_{MW}$ values of examined steroids (Table 2). Additionally, the data in Table 2 demonstrate that similarly like in the case of previously presented logP values, the lowest $R_{MW}$ value obtained by methanol-water system on three applied chromatographic plates is shown by BPh. Dendrogram of similarity of $R_{MW}$ values performed for studied compounds indicates that regardless of applied chromatographic plates (RP-$2F_{254}$, RP-$8F_{254,}$ RP-$18WF_{254}$) and using mixture of methanol-water mixture as mobile phase, the biggest similarity in lipophilic properties have: BV17, BP and BV21. Betamethasone (B) and betamethasone disodium phosphate (BPh) indicate poorer similarity in $R_{MW}$ values in comparison with them (Fig. 2a).

The linear dependencies between $R_M$ and φ obtained by means of other mobile phase systems: dioxane-water and also acetonitrile-water are presented in Tables 3 and 4. It can be noted that the change of the kind of organic modifier in mobile phase used from methanol to dioxane and also to acetonitrile has impact on $R_{MW}$ values of tested steroids, especially for disodium phosphate (BPh). For both mobile phases: dioxane-water and acetonitrile-water a significant decrease in $R_{MW}$ values for BPh in comparison with methanol-water mixture is shown. The data confirm some difficulties observed in determination of lipophilicity parameter for BhP by experimental methods, similarly like in the case of computed logP values which were determined for this compound by selected algorithms only: AlogPs, AClogP, xlogP2, xlogP3, $logP_{ChemDraw}$, $logP_{average}$.

Increasing of standard error of linear estimates between $R_{MW}$ and φ obtained for acetonitrile-water as mobile phase indicates that this mobile phase is not suitable for describing the lipophilicity of disodium phosphate. This fact can be explained by different solubility of BPh in applied solvent systems and the differences in their power of elution. According to research paper prepared by Komsta et al. (26), the chromatographic parameters should be determined by means of optimum chromatographic conditions including suitable mobile phase and a proper kind of chromatographic plates. As was reported (26), among numerous mobile phases which are widely used in lipophilicity study the best are methanol-water and dioxane-water. Comparison of $R_{MW}$ results obtained in our experiment (Tables 2, 3 and 4) confirms this suggestion. Dendrogram of the similarity analysis of $R_{MW}$ values estimated using dioxane-water (Fig. 2b) shows that this mobile phase system gives similar results in $R_{MW}$ values of five examined steroids like previously described methanol-water. It could be noted that similarly like by methanol-water, the solvent system: dioxane-water gives the greatest similarity in $R_{MW}$ value between: BV17, BP and also B21 which form one cluster (one subgroup) (Fig. 2b). Dendrogram of the similarity analysis of $R_{MW}$ values of examined compounds obtained by the use of acetonitrile-water mixture as mobile phase is similar, but indicates the small differences in Euclidean distance between BV17, B21 and BP in relation to those obtained by methanol-water and also by dioxane-water. In this case, the smallest distance indicates not BP and BV 17, but rather BV17 and BV21 (Fig. 2c). Generally, it could be said that the three described steroids: BP, BV17 and BV21 form one cluster (one subgroup) on the dendrogram, which confirms their comparable lipophilic properties. Despite of this fact, about the usage of acetonitrile-water as the potential mobile phase, suitable for lipophilicity study decides comparison of $R_{MW}$ values obtained by this mobile phase with theoretical lipophilicity parameters (logP) and with experimental logP for examined compounds which are summarized in Table 1. Big differences observed between $R_{MW}$ results obtained by acetonitrile-water and logP values as well as with other $R_{MW}$ values (determined by methanol-water and dioxane-water) demonstrate that this mobile phase is not suitable to obtain the reliable value of $R_{MW}$ for examined compounds.

Next dendrogram of similarity analysis of all chromatographic parameters ($R_{MW}$) determined for five examined compounds under all variants of chromatographic systems used (different mobile phases and various chromatographic plates), which is presented in Figure 3, could be helpful to estimate the impact of the two components of chromatographic systems on lipophilicity study of betamethasone derivatives. Dendrogram of similarity analysis of $R_{MW}$ results obtained on the following chromatographic plates: RP-$2F_{254}$, RP-$8F_{254}$ and also RP-$18WF_{254}$ by respective mobile phases: methanol-water, dioxane-water and acetonitrile-water describes the greatest similarity in $R_{MW}$ results for all examined compounds which were predicted by methanol-water and dioxane-water solvent systems, because they form one cluster on dendrogram (first subgroup). To the second subgroup belong the $R_{MW}$ values obtained on all types of chromatographic plates used in this experiment and by acetonitrile-

Table 5. Correlation matrix between all $R_{MW}$ values obtained for examined compounds under various chromatographic conditions.

| $R_{MW}$ | $RP2_{(m)}$ | $RP2_{(d)}$ | $RP2_{(a)}$ | $RP8_{(m)}$ | $RP8_{(d)}$ | $RP8_{(a)}$ | $RP18W_{(m)}$ | $RP18W_{(d)}$ | $RP18W_{(a)}$ |
|---|---|---|---|---|---|---|---|---|---|
| $RP2_{(m)}$ | 1.000 | 0.780 | 0.121 | 0.753 | 0.843 | 0.594 | 0.954 | 0.799 | 0.269 |
| $RP2_{(d)}$ | | 1.000 | 0.517 | 0.962 | 0.956 | 0.670 | 0.800 | 0.940 | 0.568 |
| $RP2_{(a)}$ | | | 1.000 | 0.541 | 0.406 | 0.246 | 0.022 | 0.452 | 0.512 |
| $RP8_{(m)}$ | | | | 1.000 | 0.984 | 0.616 | 0.850 | 0.987 | 0.498 |
| $RP8_{(d)}$ | | | | | 1.000 | 0.699 | 0.916 | 0.996 | 0.527 |
| $RP8_{(a)}$ | | | | | | 1.000 | 0.593 | 0.696 | 0.915 |
| $RP18W_{(m)}$ | | | | | | | 1.000 | 0.898 | 0.286 |
| $RP18W_{(d)}$ | | | | | | | | 1.000 | 0.542 |
| $RP18W_{(a)}$ | | | | | | | | | 1.000 |

RP2, RP8 and RP18W are the $R_{MW}$ values obtained on the following chromatographic plates: RP-$2F_{254}$, RP-$8F_{254}$ and RP-$18WF_{254}$ which were developed by use of mobile phases: (m) - methanol-water, (d) - dioxane-water, (a) - acetonitrile-water.

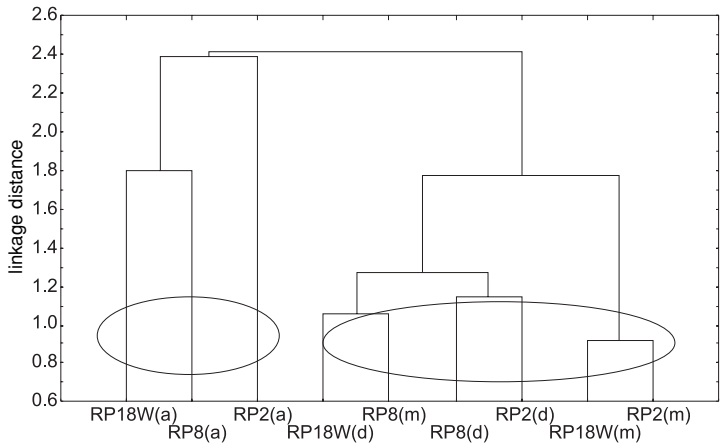


Figure 3. Cluster analysis of $R_{MW}$ values of examined compounds obtained on chromatographic plates: RP-$2F_{254}$, RP-$8F_{254}$ and RP-$18WF_{254}$ developed using all mobile phases used in the experiment. RP2, RP8 and RP18W are the $R_{MW}$ values obtained on the following chromatographic plates: RP-$2F_{254}$, RP-$8F_{254}$ and RP-$18WF_{254}$ which were developed by use of mobile phases: (m) - methanol-water, (d) - dioxane-water, (a) - acetonitrile-water

water as mobile phase. Of all $R_{MW}$ values presented in Figure 3 the smallest Euclidean distance (greatest similarity) indicate those $R_{MW}$ values which were determined on RP-$2F_{254}$ and RP-$18WF_{254}$ plates and by methanol-water. It could be suggested that RP-$2F_{254}$ plates may be alternatively applied to RP-$18WF_{254}$ when mobile phase methanol-water is used. To sum the results of experimental lipophilicity parameters of five examined compounds determined by use of different RP-HPTLC systems: on various chromatographic plates and mobile phases (designated as $R_{MW}$ values), the correlation matrix obtained between all measured $R_{MW}$ values was constructed (Table 5). The data presented in Table 5 confirm previous conclusions that the most similar $R_{MW}$ results to the best mobile phase system (methanol-water) gives dioxane-water because the highest correlation coefficients were obtained for the relationships between $R_{MW}$ values predicted by methanol-water and also by dioxane-water systems. Therefore, it may be suggested that dioxane-water could be applied as alternative mobile phase system in determining of chromatographic parameter of betamethasone derivatives by means of RP-HPTLC method. Additionally, the results of correlation matrix obtained for $R_{MW}$ values in Table 5 show that the best agreement in $R_{MW}$ values were obtained by mobile phase: dioxane-water and chromatographic plates: RP-$18WF_{254}$ and RP-$8F_{254}$ ($r > 0.99$). Therefore, it could be said that it is possible to use

of RP-8$F_{254}$ plates as alternative to the RP-18$WF_{254}$ in the case of mobile phase system: dioxane-water. For methanol-water, the best correlations between $R_{MW}$ results are observed in the case of RP-2$F_{254}$ and RP-18$WF_{254}$ plates. It could be concluded that RP-2$F_{254}$ and RP-8$F_{254}$ plates are a good alternative to RP-18$WF_{254}$ in lipophilicity study of betamethasone compounds depending on mobile phase system used. The results of cluster analysis indicate that chemometric methods such as CA are excellent tools in describing the lipophilicity of steroid compounds e.g., betamethasone derivatives. Moreover, they are helpful in optimization of chromatographic conditions needed to determine the reliable $R_{MW}$ values for examined compounds.

Further investigations are in progress and concern the use of described lipophilicity parameters in QSAR study of examined steroids.

## CONCLUSIONS

From the analysis of obtained data it can be concluded that:

- liquid chromatography in reversed-phase system such as RP-HPTLC is rapid, cost-effective method of describing the lipophilic properties of betamethasone and its related compounds;
- computational methods can be helpful in determining of partition coefficient (logP) for betamethasone derivatives as the measure of their lipophilicity;
- theoretically predicted lipophilicity parameter ($logP_{average}$) of examined betamethasone derivatives should be critically discussed on the basis of statistical parameters (e.g., standard error) in term of its application in lipophilicity study of these compounds;
- chromatographically determined $R_{MW}$ values (lipophilicity parameters) for five examined betamethasone compounds depend strongly on chromatographic conditions (e.g., the kind of mobile phase system);
- methanol-water in different volume compositions is the best mobile phase system for determining of the chromatographic lipophilicity parameters ($R_{MW}$) of betamethasone and its related compounds;
- dioxane-water gives the similar $R_{MW}$ values for examined compounds in comparison with those predicted by methanol-water, therefore it can be used as alternative mobile phase system in lipophilicity study of betamethasone derivatives;
- chemometric methods such as cluster analysis are excellent tool in describing the lipophilicity of betamethasone derivatives;
- CA is helpful in optimization of chromatographic conditions needed to determine the reliable $R_{MW}$ values for examined compounds.
- the results of lipophilicity parameters ($R_{MW}$) presented in this work may be applied in QSAR (quantitative structure-activity relationship) of betamethasone and its related compounds: 17,21-dipropionate (BP), betamethasone-17-valerate (BV17), betamethasone-21-valerate (BV21) and also betamethasone disodium phosphate (BPh).

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