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Short communication

Chemometric analysis of retention data from salting-out thin-layer chromatography in relation to structural parameters and biological activity of chosen sulphonamides

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Abstract

Salting-out thin-layer chromatography of several chosen sulphonamides on silica gel has been examined with aqueous solutions of salts: sulphates, chlorides, nitrates, phosphates, acetates, thiocyanates. It was established that applied salts have different effects on retention of sulphonamides accordingly to Hofmeister's clasification (e.g. kosmotropes, chaotropes and neutral).

The parameters of the linear regression analysis of dependences between the R_M values and concentration of the salt in the eluent system were correlated with QSAR ones. It appeared that chromatographic parameters obtained by SOTLC method reflect not only physico-chemical properties of examined compounds but also they include information about their activity. 3D graph revealing pharmacological properties of analytes was constructed. Universal character of this method for predicting and classification of drug containing sulphonamide group was confirmed by localisation of additional compounds structurally similar but acting antagonistically towards sulphonamides.

Keywords: Salting-out thin-layer chromatography; Sulphonamides; Quantitative structure–retention relationships; Cluster analysis

1. Introduction

Salting-out thin-layer chromatography (SOTLC) is an example of chromatographic technique based on the use of concentrated aqueous solutions of different inorganic salts as mobile phase and sorbents with high polarity such as silica gel, cellulose, polyacrylonitrile.

So far, solutions of ammonium sulphate have been most extensively used as eluent in SOTLC, due to its high solubility in water and high salting out effect.

From the beginning when Rutter described "streaming potential in paper chromatography" [1] and Hagdahl and Tiselius [2] published their dissertations on salting out chromatography of amino acids, alkaloids, β-indolyl acids, quaternary amines not many further paper concerning this subject have appeared in scientific literature until the present time.

After series of Lederer and co-workers' publications [3–5] who separated many classes of organic compounds on cellulose controlled by salting-out effect, Janjić, Tešić, Vučković et al. published results of experiments devoted to separation of some mixed aminocarboxylatocobalt(III) and diamine Co(III) complexes in SOTLC systems consisting of different polar stationary phases [6–13]. The effect of chelate ring size and charge of Co(III) complexes was analysed under salting-out thin-layer chromatography conditions.

This work concerns application of salting out effect on thin-layer of silica gel for analysis of chosen sulphonamides.

Sulphonamides as congeneric group of substances with ampholytic character differing in activity exhibit similarity to amino-acids analyzed by the precursors of SOTLC technique. They are ideal choice to prove suitability of parameters achieved owing to salting-out process in QSAR analysis.

Anticipation of pharmacological activity of the analytes was proved by cluster analysis of chromatographically determined retention values and computer generated molecular descriptions.

According to our knowledge these are the first studies presenting the usage of chromatographic parameters achieved by SOLTC to foreseeing the activity and exploration of the retention mechanism in this chromatographic technique.

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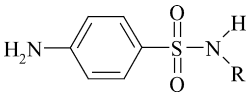
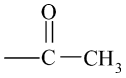
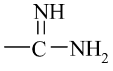
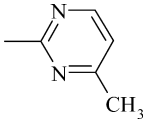
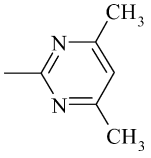
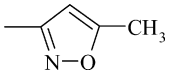
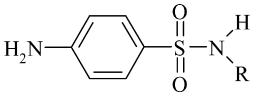
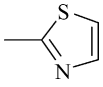
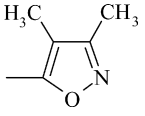
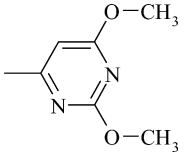
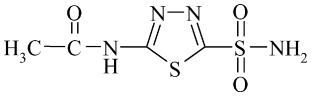
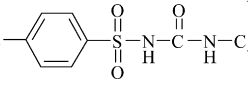
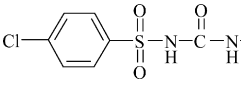
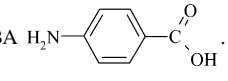
Table 1
Investigated compounds: general structure



No.	Name	R	pK_{a1}	pK_{a2}
1	Sulphacetamide		5.01	1.87
2	Sulphaguanidine		9.68	3.31
3	Sulphamerazine		7.13	2.34
4	Sulphadimidine		7.23	2.36
5	Sulphanilamide	—H	10.52	2.30
7	Sulphamethoxazole		7.67	2.18
8	Sulphaproxyline		5.01	1.85
9	Sulphathiazole		6.74	1.56
10	Sulphafurazole		6.17	2.05
11	Sulphadimethoxine		6.17	2.41
6	Acetazolamide*		8.15	−2.77

Acetazolamide structure differs from other sulphonamides in Table. Full structural formula of this compound is included in section R.

Tolbutamide  . Chlorpropamide  . PABA  .

2. Experimental

2.1. Materials

The several sulphonamides used in the study were obtained from Sigma (St. Louis, MO, USA). The structures and pK_a values of the above compounds are shown in Table 1.

Inorganic salts: $(NH_4)_2SO_4$, $CaCl_2$, NaCl, $MgCl_2$, NH_4Cl, NH_4SCN, NaH_2PO_4, CH_3COONH_4, NH_4NO_3, $Mg(NO_3)_2$ were of analytical grade from P.O.Ch.Gliwice Poland. They were used in the preparation of aqueous solutions of various concentrations (Table 2). Water used for preparation of eluent systems was obtained from Barnstead deionising system (Dubuque, IA, USA).

Table 2
Concentration of inorganic salts additives in water used as mobile phases in each set of experiments

Kind of salt	Examined concentration range (M)*
$(NH_4)_2SO_4$	0.5–5.3
$CaCl_2$	2–8
NaCl	1–6
$MgCl_2$	0.36–2.86
NH_4Cl	1–6
NH_4SCN	0.5–6
NaH_2PO_4	3.75–7.13
CH_3COONH_4	0.6–4.8
NH_4NO_3	3–10
$Mg(NO_3)_2$	1–8

* Range of salt concentrations were chosen according to solubility of salts and linearity of R_M vs. c_m relationships.

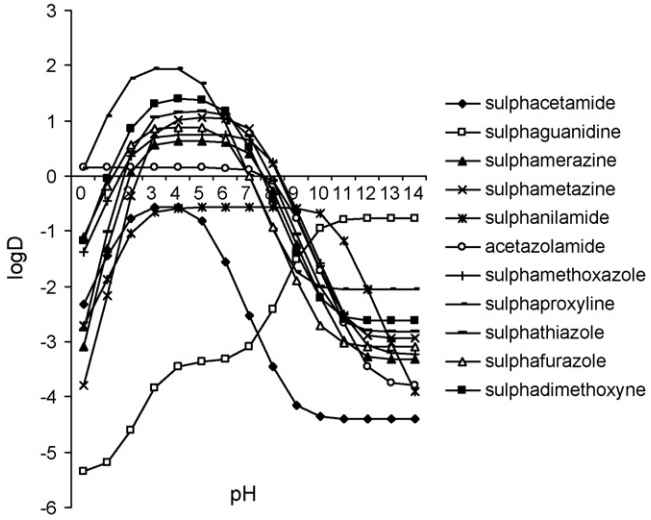


Fig. 1. The plots of log D against pH predicted by PrologD, which is a module of the Pallas system.

2.2. Chromatographic studies

Investigated compounds were dissolved in methanol at concentration of 3 mg mL^{-1} and samples (10 μm) of the solutions were spotted on the plates.

Chromatography was performed on 10 cm × 20 cm HPTLC plates precoated with 0.25 mm layers of silica gel 60 F_{254} (Merck, Darmstadt, Germany). The plates were developed in horizontal Teflon DS chambers (Chromdes, Lublin, Poland) and after drying visualized under λ = 254 nm UV light. Chromatograms were developed to a distance of 9 cm.

The measurements were carried out at room temperature (20 ± 2 °C). Salt concentrations were adapted to the retention values of the solutes, solubility of salt in water, linearity of dependences: $R_M = f(c_m)$ as described in Table 2. The R_f determination were run in duplicate and the mean values were used for calculation of R_M according to the equation: $R_M = \log(1/R_f - 1)$. The R_M values were than extrapolated to 100% water giving value of intercept known as R_{M0} parameter. In all cases at least three different salt concentrations were used for extrapolation to R_{M0}.

2.3. Data analysis

Linear multivariate regression analysis and multidimensional cluster analysis were performed by the use of the K-mean clustering algorithm within the Statistica v. 6.0 package. (StatSoft Inc., Tulsa, OK, USA).

3. Results and discussion

3.1. Retention mechanism under salt effect

As the major aim of this investigation was to determine the salt-out effect of different inorganic salts, it was necessary to operate under conditions of high salt concentration above 1 M.

While in very diluted solutions of salt both ion-exchange and ion exlusion mechanism play a dominant role than in more concentrated eluent systems (above 1 M) nonspecific hydrophobic adsorption probably decides about retention.

It was assumed that the adsorption mechanism is mainly a kind of hydrophobic interactions between the hydrated silica gel and the aqueous solution of salt [14]. The enlargement of the hydrophobic interactions between solutes and polar adsorbent deactivated in aqueous eluent could be based on solvation mechanism [15].

It is well known that the influence of salt additions is very complex. The hydrophobic interaction between hydrophobic parts of sorbents and analytes originates from the repulsion of their dehydrated forms connected with increasing the surface tension. In turn, degree of hydration primarily depends on lipophilicity and ionization of sulphonamides. In aqueous salt solution the degree of ionization will be affected by pH, ionic strength and type of additive [16].

For the majority of sulphonamides, there is an anilinium group for which pK_{a1} is around 2–2.5. pK_{a2} applies to the ionization of the sulphonamide nitrogen and lies between 7 and 8. As it can be seen, in Fig. 1 representing relationships between log D (octanol–water distribution coefficient in its logarithmic form) values and pH, the investigated compounds exist in neutral or anionic forms in the examined conditions.

An ionic forms of sulphacetamide, sulphaguanidine, sulphaproxyline will be surrounded by a bigger hydration shell in solution than other nonionic compounds. The hydration shell associated with any species in aqueous solution prevents them from biding on the surface. This situation changes in the presence of salt, due to the hydration effect of the salt. Thus, the dehydrated fraction of sulphonamides increases gradually with the increasing concentration of salt. The visible result of these changes in solution will be an increase of analytes retention with increasing salt concentration, making the hydrophobic adsorption with adsorbent surface stronger.

3.2. Analysis of linear dependences between the R_M values and the concentration of salt in the solvent system

The almost parallel salting out curves presented relationship between the retention coefficient values vs. molar concentration of salt indicate the same adsorption mechanism for all the investigated sulphonamides differing in molecular size and polarity. The results showed that the marked improvements in selectivity can be achieved by changing the kind and concentration of inorganic additives. Data of linear regression analysis for chosen salts are presented in Table 3. Demonstrated results indicate that the salt additives can significantly affect the retention of sulphonamides. Taking into account only type of anions, the effective salting-out strength could be ordered in the following way: $SO_4^{2-} > Cl^- > H_2PO_4^- > NO_3^- > SCN^- > CH_3COO^-$. This order is closely consistent with the Hofmeister effect expressed the ability of anions to cause salting-out and salting-in [17,18] Kosmotropic salts at the beginning of above series promote adsorption due to enhancing different intermolecular interactions (hydrophobic, charge–charge attractions, dipole, π-electron coupling) connected with water structuring properties of these salts. The strongest salting-out factor for investigated sulphonamides appears to be ammonium sulphate (average value of $S = 0.26$) and chlorides (average value of $S = 0.16$).

Salts in the end of Hofmeister series such as thiocyanate and nitrate randomize the structure of liquid water and in consequence promote elution. This phenomenon is expressed by the negative values of slopes and ΔR_M obtained for acetates (−0.05) and thiocyanates (−0.01) in eluent systems. It is worth noticing that the highest values of S and ΔR_M belong to sulphaproxyline, which is similarly to sulphanilamide in strongly hydrated ionic form. Additionally pK_{a1} and pK_{a2} for above compounds are also almost identical. Thus it has been assumed that changes in retention will be similar under the same conditions. The values in Table 3 clearly indicate no identical behavior of these solutes in response to increasing salt concentration. Thus salting-out effect is discriminatory. The variation of results could be explained according to the structural differences. In the case of the smaller and more polar sulphacetamide, the salt-out effect is less visible than for larger and less polar molecule of sulphaproxiline. Similar behavior of these compounds was observed in capillary zone electrophoresis controlled by the high ionic strength of phosphate buffers [19–23]. Differences in size and polarity were also pointed out as a rational explanation of experimental results.

Summarizing, the main factor deciding about the effectiveness of salting-out process appears to be molar volume and lipophilicity of molecules.

Considering influence of the cation type on effectiveness of salting-out adsorption we can compare the slope values obtained for different chlorides: sodium, ammonium, calcium, magnesium. In all cases it is just the charge of cation that decides directly about the values of slope. For bivalent ions magnesium and calcium in comparison to monovalent ones, their favorable hydration causes more effective exclusion of analytes from eluent system and finally their stronger adsorption. Since adsorption is driven by the hydration of added ions by water molecules

Table 3
Coefficients of R_M vs. concentration of different salts in the mobile phase linear relationships

Investigated compounds	$(NH_4)_2SO_4$			NaCl			NH_4NO_3			CH_3COONH_4			NH_4SCN			$MgCl_2$		
	R_{M0}	S	R^2	R_{M0}	S	R^2	R_{M0}	S	R^2	R_{M0}	S	R^2	R_{M0}	S	R^2	R_{M0}	S	R^2
Sulphacetamide	−0.97	0.29	0.89	−0.84	0.17	0.99	−0.83	0.03	0.90	−0.79	−0.08	0.75	−1.93	−0.01	0.24	−0.87	0.25	0.99
Sulphaguanidine	−0.83	0.18	0.83	−0.94	0.12	0.96	−0.93	0.00	0.47	−0.73	−0.03	0.33	−1.92	0.01	0.10	−0.94	0.18	0.99
Sulphamerazine	−0.11	0.31	0.99	−0.06	0.14	0.99	−0.08	0.02	0.87	−0.01	−0.05	0.97	−0.32	−0.04	0.92	−0.02	0.20	0.99
Sulphadimidine	0.08	0.36	0.98	0.10	0.18	0.99	0.13	0.03	0.93	0.23	−0.07	0.92	0.08	−0.04	0.92	0.16	0.26	0.99
Sulphanilamide	−1.03	0.20	0.90	−1.01	0.11	0.99	−1.04	0.01	0.48	−0.70	−0.06	0.81	−2.03	0.02	0.24	−0.98	0.19	0.99
Acetazolamide	−0.92	0.21	0.87	−1.05	0.11	0.99	−1.06	0.01	0.62	−0.71	−0.04	0.99	−1.98	−0.02	0.55	−0.93	0.18	0.99
Sulphamethoxazole	−0.37	0.24	0.99	−0.40	0.17	0.99	0.38	0.02	0.94	−0.44	−0.06	0.99	−0.93	−0.01	0.60	−0.41	0.30	0.99
Sulphaproxyline	−0.12	0.39	0.99	−0.07	0.25	0.99	0.01	0.05	0.99	−0.30	−0.01	0.18	−0.21	0.01	0.73	−0.09	0.43	0.99
Sulphathiazole	−0.39	0.20	0.99	−0.35	0.13	0.99	−0.38	0.00	0.62	−0.33	−0.05	0.80	−1.01	−0.03	0.81	−0.32	0.21	0.99
Sulphafurazole	−0.41	0.28	0.99	−0.31	0.18	0.99	−0.24	0.04	0.98	−0.47	−0.07	0.99	−0.75	0.01	0.63	−0.33	0.29	0.99
Sulphadimetoxine	−0.13	0.28	0.99	−0.17	0.20	0.99	−0.10	0.02	0.91	−0.15	−0.08	0.96	−0.49	−0.04	0.92	−0.04	0.29	0.99
Average value	–	0.26	0.94	–	0.16	0.98	–	0.02	0.79	–	−0.05	0.79	–	−0.01	0.60		0.25	0.99
Standard deviation	–	0.06	0.06	–	0.04	0.01	–	0.01	0.20	–	0.02	0.28	–	0.02	0.29		0.07	0.00

The coefficients included in the table were obtained on the basis of the linear regression analysis of the relationships between retention parameter values R_M ($R_M = \log(1 - R_f/R_f)$) and molar concentration of appropriate salt in eluent system. R_{M0} values reflect the intercepts, S—the slope values and R^2—the correlation coefficient squared of these dependences.

Table 4
The structural parameters of the sulphonamides tested

Name	MiLogP[a]	Volume[a]	Surface area (Å^2)[b]	Molecular volume (Å^3)[b]	Area properties[b]	Volume (properties)[b]	Molar ref.[c]
Sulphacetamide	−0.557	174.71	225.01	182.89	238.96	221.65	52.21
Sulphaguanidine	−0.842	172.752	224.87	182.06	240.59	221.12	50.8
Sulphamerazine	0.396	218.823	274.73	229.39	291.05	276.98	67.76
Sulphadimidine	0.835	235.384	296.55	246.68	313.48	297.55	72.39
Sulphanilamide	−0.293	138.052	181.13	144.71	195.72	176.24	42.8
Acetazolamide	−1.146	157.109	209.45	166.02	227.13	203.84	45.95
Sulphamethoxazole	0.609	204.547	260.07	214.75	277.96	260.22	62.45
Sulphaproxyline	1.911	288.492	356.14	300.51	366.98	360.29	87.89
Sulphathiazole	0.833	197.13	250.31	206.09	267.96	250.93	63.48
Sulphafurazole	0.986	221.108	280.47	231.99	295.08	280.04	67.07
Sulphadimethoxine	0.753	253.353	318.79	265.41	336.70	320.30	75.87
PABA	0.924	122.333	160.86	128.05	174.74	156.27	37.41
Tolbutamide	2.543	242.791	310.89	252.43	325.35	304.02	70.76
Chlorpropamide	2.213	222.964	283.54	231.99	299.21	281.05	66.33

[a] http://www.molinspiration.com/cgi-bin/properties?textMode=1.
[b] Program Titan 1.0.7 (Jun 19, 2004) equilibrium geometry with semi-empirical AM1, with NOPSEUDO QSAR option.
[c] ChemSketch.

which are in turn released from hydration shells of the analytes. Thus bigger charge and smaller size of ions cause their stronger hydration. Number of water molecules released from hydration shell could be expressed by the slope values of correlation between RM and molar concentration of salt. This parameter reflects ability of salt to salting-out properties and depends on kind of salt, whereas R_{Mo} values obtained by extrapolation to pure water reflects differences in degree of hydration of individual solutes that is why they could be used for describing their properties.

For the better characterization of the compounds studied and applied SOTLC data for classification of drugs according to their pharmacological and pharmacokinetic properties, the numerical values of the structural parameters of the sulphonamides examined were established and collected in Table 4.

3.3. QSRR analysis

The QSRR analysis was derived by means of multiple regression using retention data as the dependent variable and structural parameters as the independent ones [24,25]. We found a few structural descriptors: molecular volume, surface area, molar refractivity, miLogP, volume properties and area properties to be highly correlated with retention data obtained in chromatographic systems containing kosmotropic salts extrapolated linearly. Among these salts with positive values of S it is sodium chloride that has been shown to give the best correlations.

The following statistical significant one-parameter regression equations Eqs. (1)–(6) containing the molecular size related descriptors and R_{M0} (NaCl) were obtained:

$$R_{M0} = 0.035(\pm0.003)\mathrm{RM} - 2.624(\pm0.236),$$
$$n = 10,\quad r = 0.9549,\quad s = 0.13,\quad F = 82,91 \qquad (1)$$

$$R_{M0} = 0.469(\pm0.089)\mathrm{miLogP} - 0.576(\pm0.068),$$
$$n = 10,\quad r = 0.8793,\quad s = 0.21,\quad F = 27,28 \qquad (2)$$

$$R_{M0} = 0.009(\pm0.001)A - 2.774(\pm0.384),$$
$$n = 10,\quad r = 0.9040,\quad s = 0.19,\quad F = 35,78 \qquad (3)$$

$$R_{M0} = 0.010(\pm0.001)V - 2.612(\pm0.332),$$
$$n = 10,\quad r = 0.9153,\quad s = 0.18,\quad F = 41,33 \qquad (4)$$

$$R_{M0} = 0.008(\pm0.001)A^* - 2.883(\pm0.404),$$
$$n = 10,\quad r = 0.9033,\quad s = 0.19,\quad F = 35,49 \qquad (5)$$

$$R_{M0} = 0.008(\pm0.001)V^* - 2.658(\pm0.343),$$
$$n = 10,\quad r = 0.9139\ ,s = 0.18,\quad F = 40,59 \qquad (6)$$

R_{M0} is the retention parameter for pure water as the eluent recorded as the intercept of the relationship between retention parameter ($R_M = \log\ 1 - R_f/R_f$) and molar concentration of sodium chloride. RM (ChemSketch), A, V, A^*, V^* (Titan 1.0.7), miLogP (http://www.molinspiration.com/cgi-bin/properties?textMode=1) are the molar refractivity, surface area, molecular volume, area properties, volume properties, respectively. Outlier is sulphaproxiline as identified by the standardized residual value.

The above equations are established after exlusion of sulphaproxiline differing in degree of ionization.

Each of the derived equations contains a descriptor related to the size and lipophilicity of solutes. This is consistent with the theory that non-specific hydrophobic interactions are decisive in this chromatographic technique. Thus, the QSRR analysis could describe very well salting-out process in thin layer chromatography where the primary driving force is hydrophobic interactions.

3.4. QSAR analysis applying chromatographic data obtained by SOTLC technique

To find relation between the structural parameters, chromatographic behavior and pharmacological activity of investigated

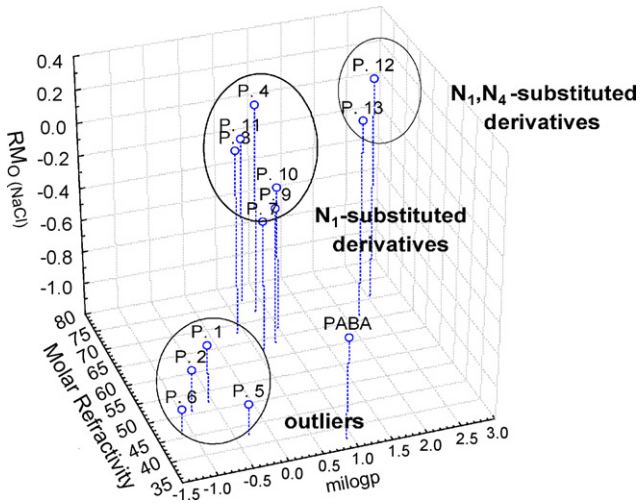


Fig. 2. 3D graph illustrating relationship between R_{M0} (NaCl) values obtained chromatographically and QSAR descriptors exhibiting size (RM) and lipophilicity (miLogP) of the investigated compounds.

compounds multidimensional cluster analysis has been conducted [26,27]. On the basis of obtained result 3D scatterplot of the chromatographic- R_{M0} (NaCl) and molecular parameters-molar refractivity (RM), miLogP was constructed (Fig. 2).

The visual examination of a 3D graph suggested that investigated sulphonamides could be subdivided into separate clusters. It appears that such classification of the drug was generally in agreement with their known activity [28]. We can distinguish two main clusters. One of them named N_1 substituted derivatives is created by six sulphonamides commonly used as bacteriostatic drugs, second contains outliers: sulphanilamide, sulphacetamide, sulphaguanidine and acetazolamide. These sulphonamides located in second cluster differ from others mainly with their poor solubility or ionization at physiologic pH that is why they are used as a locally administrated drugs.

Acetazolamide which is one of the outliers from the main series was originally introduced as sulphonamide diuretic, but it is now used mainly as antiglaucoma drug for short term treatment preceding surgery. Its action is connected with inhibition of the enzyme carbonic anhydrase.

The activity of sulphonamides as a function of their structural parameters has been already reported in literature, especially in connection to their partition characteristics [29–33]. Kaliszan et al. found a significant correlation between in vitro activity of sulphonamides and their molar refractivity [34]. Nasal and coworkers performed extensive QSRR study for a series of sulphonamides on different HPLC stationary phases: octadecylsilica, trimethylsilane-bonded silica and an anion-exchange resin [35]. Chemometric analysis of retention parameters, structural ones with biological activity showed similar separation of investigated group according to their pharmacological activity.

To confirm the proposed scheme of sulphonamide derivatives investigation we decided to enclose additionally data of three substances: *p*-aminobenzoic acid, N1–N4 substituted derivatives: tolbutamide, chlorpropamide. First substance known as PABA is the naturally occurring substrate and that it is an essential metabolite bacteria. It is well demonstrated that PABA-containing extracts are able to reverse the sulphonamide-induced inhibition of bacterial growth in a competitive manner. Two next compounds in spite of the strong similarity to sulphonamide no longer exhibit antibacterial properties and were introduced as the orally active hypoglycemic drugs. Their location in graph space was pointed out as additional clusters. N1–N4 substituted analogues lying at a great distance from the main bacteriostatic sulphonamides cluster. Quite different location is possessed by PABA, which does not belong to any previous groups.

Three-dimensional, elaborated graph reflected activity of investigated compounds is statistically significant and could be expressed by the following Eq. (7):

$$R_{M0}\,(\mathrm{NaCl}) = -1.61(\pm 0.25) + 0.224(\pm 0.045)\mathrm{miLogP} + 0.0180(\pm 0.0040)\mathrm{RM},$$
$$n = 13,\quad r^2 = 0.916,\quad F(2, 10) = 24, 33 \qquad (7)$$

Cross-validated R^2 values determined by the leave-one-out method and error of estimation for this equation were 0.899 and 0.137, respectively.

4. Conclusion

The mechanism of hydrophobic interactions in SO-TLC was confirmed by QSRR analysis. It is molar refractivity, surface area and molecular volume that have appeared to be superior parameters for the description of the retention.

Influence of salts on decrease of retention was consistent with order of salts in Holfmeister series. The effectiveness of salt for salting-out could be expressed by the slope of the relationship between retention parameter and molar concentration of salt in mobile phase, whereas the R_{M0} values obtained by extrapolation of R_M vs. c curve to pure water relate to differences in solvation of analytes in aqueous system.

These parameters: molar refractivity, R_{M0}(NaCl) and additionally miLogP were chosen to derive three dimensional space model separating whole investigated group additionally including antagonistiacally acting compounds and those similar in structure into clusters characterized with different activity.

On the basis of the results obtained, it could be concluded that SO-TLC technique on silica gel of ampholytic substances is useful not only for improvement of separation selectivity in "ecological" conditions but also can generate parameters for further QSAR studies. We believe our studies offer very simple procedure for testing of also newly synthesized sulphonamide derivatives according to their potential application in pharmacotherapy.

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