

Chromatographic separation of 3- and 4-substituted anilines and correlation of their R_M values with chosen physicochemical parameters

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A procedure for chromatographic separation of 3- and 4-substituted anilines on thin layers of silica gel in various systems has been developed with purpose of their identification and control of their purity. The chromatographic values R_M both from adsorption and partition chromatographies were correlated with physicochemical parameters pK_a , σ , and $\bar{\pi}$. The relationship between R_M from partition chromatography and the substituent parameter $\bar{\pi}$ has proved to be linear.

Разработана процедура хроматографического разделения 3- и 4-замещенных анилинов в тонком слое силикагеля в различных системах с целью их идентификации и контроля чистоты. Хроматографические величины R_M , полученные по данным поглотительной и распределительной хроматографии, коррелировались с физико-химическими параметрами pK_a , σ и $\bar{\pi}$. Показано, что существует линейная зависимость между величиной R_M по данным распределительной хроматографии и параметром заместителя $\bar{\pi}$.

At our department we have been preparing new compounds, basic esters of substituted phenylcarbamic acid, with presumed local anaesthetic activity and activity influencing cardiovascular system (β -adrenolytics, antiarrhythmics). The starting compounds used in their synthesis have been 2-, 3-, and 4-substituted anilines, which may be contaminated with their positional isomers or decomposition products. Besides, these anilines may occur as degradation products of the final compounds, *i.e.* derivatives of phenylcarbamic acid, or as undesired unreacted products already in preparation of the intermediates, substituted phenyl isocyanates. With regard to these facts, attention has been paid to identification and checking of the purity of the starting anilines. For these studies thin-layer chromatography was utilized.

Separation of aromatic amines on silica gel or alumina is based on absorptivity of the respective amine on the sorbent, while significant role is ascribed to basicity of the amine [1]. Primary aromatic amines were separated on silica gel

by using the following mobile phases: benzene—methanol (ϕ_r (volume ratio) = 95:5) [2], benzene—ethanol ($\phi_r = 19:1$), benzene—tetrahydrofuran ($\phi_r = 4:1$), benzene—ethyl acetate ($\phi_r = 5:1$), benzene—diethyl ether ($\phi_r = 1:1$) [3], benzene with addition of acetone [4, 5] as well as phases containing benzene and acetic acid [5, 6]. Isomeric diamines were separated on silica gel in the system benzene—acetone ($\phi_r = 3:4$) [7] or hexane—acetone ($\phi_r = 3:1$) [8].

On loose layer of alumina alkyl-, alkoxy-, hydroxy-, halo-, and nitroanilines were separated by using benzene, however, the separation achieved with some positional isomers was not sufficient [9]. Good separation of aniline and its nitro derivatives was achieved on loose layer of alkali alumina in benzene, chloroform, and in the system of benzene—ethanol ($\phi_r = 98:2$ and $\phi_r = 95:5$) [10].

In order to improve the separation of amines, *Gasparič* [11] suggested to impregnate the layers with formamide and use the same mobile phase as in paper chromatography (heptane, benzene). Good separation of isomeric amines has been achieved on thin layer of silica gel, impregnated with cadmium sulfate in the system benzene—acetic acid ($\phi_r = 9:1$) or benzene—methanol—acetic acid ($\phi_r = 8:1:1$) [12]. In this as well as in the work [13] the author expressed the chromatographic values R_M in dependence on pK_a and found a good correlation. The behaviour of aromatic amines was studied on thin layer of silica gel impregnated with sodium, potassium or ammonium oxalate, sodium chloride, sodium acetate, and sodium sulfate. Separation of isomers was achieved by suitable combination of mobile phase and impregnation of the sorbent [14]. In some cases better separation was achieved by conversion of the aromatic amine to its derivatives, such as azo [15] or dinitro compounds [16].

Determination of the purity of aromatic amines has not often been the subject of study despite the fact that these compounds may be contaminated by their isomers, accompanying products or decomposition products, when considering the sensitivity of aromatic amines to light. Impurities from aromatic amines were separated on thin layers of silica gel in mobile phases propanol—methanol—water—acetic acid ($\phi_r = 65:15:15:20$) and butanol—water—acetic acid ($\phi_r = 66:17:17$) [17]. Quantitative evaluation of the separated spots of anilines was performed densitometrically on the basis of reflection in the UV region or in the visible region after diazotization. On thin layer of alumina in benzene aromatic amino compounds were analyzed in order to check the purity of the intermediates in the synthesis of procaine and benzocaine [18].

Experimental

For chromatographic separation ready-made plates of Silufol[®] and Silufol[®] UV 254 (150 mm × 150 mm) with a Silpearl[®] silica gel layer (Kavalier, Votice) were used. Freshly

Table 1

 pK_a , σ , and $\bar{\pi}$ Values of substituted anilines

Compound	Substituent	pK_a [19]	σ [20]	$\bar{\pi}$ [21]
<i>I</i>	3-OH	4.310	0.12	-0.66
<i>II</i>	4-OH	5.480	-0.37	-0.87
<i>III</i>	4-Br	3.888	0.23	1.13
<i>IV</i>	3-I	3.583	0.35	1.47
<i>V</i>	4-I	3.812	0.18	1.45
<i>VI</i>	3-F	3.570	0.34	0.47
<i>VII</i>	4-F	4.610	0.06	0.31
<i>VIII</i>	3-OCH ₃	4.200	0.12	0.12
<i>IX</i>	4-OCH ₃	5.310	-0.27	-0.12
<i>X</i>	3-Cl	3.521	0.37	1.04
<i>XI</i>	4-Cl	3.982	0.23	0.93
<i>XII</i>	3-CF ₃	3.200	0.43	1.49
<i>XIII</i>	3-C ₂ H ₅	4.700	-0.07	0.94
<i>XIV</i>	4-C ₂ H ₅	5.000	-0.15	0.98
<i>XV</i>	4-iso-C ₃ H ₇	4.850	-0.15	1.36
<i>XVI</i>	3-NO ₂	2.470	0.71	0.54
<i>XVII</i>	4-NO ₂	1.000	0.78	0.45
<i>XVIII</i>	3-CH ₃	4.690	-0.07	0.50
<i>XIX</i>	4-CH ₃	5.100	-0.17	0.48
<i>XX</i>	3-OC ₂ H ₅	4.180	0.10	0.62
<i>XXI</i>	4-OC ₂ H ₅	5.240	-0.24	0.35
<i>XXII</i>	H	4.596	0	0

prepared 0.1 % solutions of anilines in methanol were spotted (2 mm³) and the plates were developed by ascending technique. The length of the plates to be developed was 10 cm. The chromatograms after development were air-dried.

In chromatographic separation based on adsorption principle Silufol[®] UV 254 plates and the following elution systems were used: S_1 : toluene—ethyl acetate ($\varphi_r = 4 : 1$), S_2 : toluene—ethyl acetate—acetic acid ($\varphi_r = 4 : 1 : 1$), S_3 : toluene—ethyl acetate—acetone ($\varphi_r = 4 : 1 : 1$), and S_4 : toluene—ethyl acetate—chloroform ($\varphi_r = 4 : 1 : 1$). The plates were detected in UV light at 254 nm, produced by a mercury discharge lamp (UV-lamp, Camag, Muttenz, Switzerland).

For partition chromatography Silufol[®] plates, impregnated with 5 vol % octanol solution, were used. Impregnation was performed by allowing the plates to develop with the impregnating solution. After impregnation the solvent (ether) was removed from the chromatogram by 30 min air-drying at room temperature. The following mobile phases were used: S_5 : methanol—water ($\varphi_r = 1 : 1$) and S_6 : acetic acid—water ($\varphi_r = 1 : 9$). Prior to separation both systems were shaken with octanol and the phases were separated in a separating funnel. Developments were performed in vessels embedded with filtration paper and saturated with the developing system for 30 min prior to separation. The spots were visualized with the Dragendorff's reagent. The anilines studied are presented in Table 1.

Table 2

 R_f and R_m Values of substituted anilines

Compound	Adsorption chromatography				Partition chromatography									
	R_f	S_1	R_m	R_f	S_2	S_3	S_4	R_f	S_5	R_m	R_f	S_6	R_m	
I	0.099		0.959			0.29				0.751		-0.479	0.695	-0.358
II	0.019		1.713			0.15				0.585		-0.149	0.790	-0.575
III	0.431		0.121							0.290		0.389	0.375	0.222
IV	0.547		-0.082		0.58					0.200		0.602	0.205	0.589
V	0.451		0.080		0.47					0.213		0.568	0.270	0.432
VI	0.482		0.031		0.56							0.398		0.181
VII	0.351		0.270		0.38					0.455		0.078	0.530	-0.052
VIII	0.340		0.288		0.33					0.583		-0.146	0.645	-0.259
IX	0.193		0.621		0.20					0.563		-0.110	0.640	-0.250
X	0.542		-0.073		0.59					0.265		0.443	0.185	0.644
XI	0.434		0.115		0.45					0.360		0.250	0.500	0.000
XII	0.581		-0.142							0.190		0.630	0.150	0.753
XIII	0.484		0.028				0.51			0.265		0.443	0.445	0.096
XIV	0.424		0.133				0.43			0.215		0.562	0.446	0.096
XV	0.424		0.133							0.165		0.704	0.490	0.017
XVI	0.406		0.165		0.43					0.410		0.158	0.420	0.140
XVII	0.289		0.391		0.33					0.448		0.091	0.265	0.443
XVIII	0.430		0.122		0.29		0.66			0.423		0.135	0.500	0.000
XIX	0.378		0.216		0.15		0.58			0.340		0.288	0.635	-0.240
XX	0.389		0.197		0.38					0.640		0.070	0.520	-0.035
XXI	0.255		0.467		0.24					0.480		0.035	0.560	-0.105
XXII	0.442		0.101							0.535		-0.061	0.660	-0.288

 R_f — mean value from five chromatograms.

The pK_a [19], σ [20], and $\bar{\pi}$ [21] values were taken from the literature. The correlation between the R_M values and the foregoing physicochemical parameters was calculated by the method of regression analysis, *i.e.* solution of the equation of linear relationship $y = a + bx$. The values taken from the literature were considered in all cases as independent variables (x) and the R_M values found herein from adsorption and partition chromatographies as dependent variables (y). The relationships were calculated with an RPP 16S computer by using the computation program for multiple regression.

Results and discussion

Complex analysis of a synthetic medicine requires not only assessment of the final product but includes also evaluation of raw materials and intermediates. The aim of the present work was to select sorbents and mobile phases where substituted anilines, starting compounds in the synthesis of basic esters of substituted phenylcarbamic acid, would appear as distinct spots with reproducible R_f values. This requirement was fulfilled by the system S_1 : toluene—acetic acid ($\varphi_r = 4 : 1$) (Table 2). In this system, however, the 3- and 4-substituted anilines, positional isomers, were not separated sufficiently. The differences in the R_f values were found to be greater with the isomeric pairs 3-I and 4-I, 3-F and 4-F, 3-OCH₃ and 4-OCH₃, 3-Cl and 4-Cl, 3-NO₂ and 4-NO₂, 3-OC₂H₅ and 4-OC₂H₅ in the system S_2 , *i.e.* in the one with addition of acetic acid. For separation of 3-OH and 4-OH, 3-CH₃ and 4-CH₃ addition of acetone (system S_3) was advantageous and separation of 3-C₂H₅ and 4-C₂H₅ was favourably influenced by addition of chloroform (system S_4) (Table 2). These systems were used for identification of the individual anilines and control of their purity or presence in the reaction mixture. In the aforementioned three-component mobile phases the compounds separated, when spotted on silica gel as a mixture of $\varphi_r = 1 : 1$, even in the case when one isomer was present beside the other only in 1 to 10 %. The smallest amount of anilines detectable in the UV light at $\lambda = 254$ nm was 0.02 μ g (2 mm³ of 0.001 % solution).

For partition chromatography on silica gel impregnated with octanol solution the systems methanol—water (S_5) and acetic acid—water (S_6) were chosen of a number of mobile phases examined. Separation on the impregnated layers was as to size and shape of the spots of less quality than on pure silica gel and, in some cases, separation of isomers was not achieved (Table 2).

In recent literature much effort has been devoted to mathematical expression of the relation between the structure and physicochemical properties of structurally similar compounds. The relations between structure, thin-layer chromatographic behaviour, and other physicochemical properties of the basic esters of substituted phenylcarbamic acid prepared herein were studied earlier and correlation was confirmed between the chromatographic parameter R_M , logarithm

Table 3

Coefficients of linear relationship of equations $y = a + bx$

Mobile phase	n	a	b	r_k	s
$R_M = f(pK_a)$					
S_5	21	0.373	-0.040	0.135	0.3144
	18	1.214	-0.214	0.498	0.2591
S_6	22	0.969	-0.218	0.654	0.2665
	20	1.834	-0.408	0.778	0.2257
$R_M = f(\sigma)$					
S_1	22	0.334	-0.600	0.439	0.3731
	18	0.190	-0.616	0.738	0.1297
S_5	21	0.194	0.151	0.146	0.3210
S_6	22	-0.023	0.773	0.679	0.2586
	20	-0.015	1.114	0.744	0.2399
$R_M = f(\bar{\pi})$					
S_1	22	0.570	-0.515	0.821	0.2371
S_5	21	-0.050	0.443	0.934	0.1152
	20	-0.109	0.511	0.962	0.0870
S_6	22	-0.189	0.433	0.813	0.2048

n — number of dots in correlation equation, r_k — correlation coefficient, s — estimate of standard deviation.

of the distribution coefficient ($\log P$), and substituent constant π [22—24], respectively.

Therefore, we attempted further to correlate the lipophilic parameter R_M from various types of thin-layer chromatography (Table 2), respectively with other physicochemical properties, such as pK_a , the Hammett constant σ (steric parameter), and the substituent parameter $\bar{\pi}$. The coefficients of equations of linear relationships are presented in Table 3. In evaluation of relationships of the R_M values obtained and the pK_a values of substituted anilines the best correlation was achieved in the system acetic acid—water ($\varphi_r = 1:9$) on silica gel impregnated with octanol. The value of the correlation coefficient increased when the compounds with the substituents 3- NO_2 and 4- NO_2 were excluded ($r_k = 0.778$). The best correlation was obtained in the system acetic acid—water due to favourable influence of ionization of anilines by weak acid reaction of the developing system during chromatography. Correlation was not assumed between R_M from adsorption chromatography and pK_a with regard to the system used ($r_k = 0.338$). The relatively most advantageous correlation with the σ constants was achieved also in the system containing acetic acid ($r_k = 0.679$).

Better correlation was achieved when the compounds not falling into the given set of points, *i.e.* 3-NO₂ and 4-NO₂ were excluded ($r_k = 0.744$). Linear relationship was found between the substituent parameter π and R_M values from chromatography on silica gel, impregnated with octanol, in the system methanol—water ($\varphi_r = 1 : 1$). The value of the correlation coefficient for the whole set ($r_k = 0.934$) increased to 0.962 after exclusion of the compounds with the substituent 4-OH (Table 3, Fig. 1). On the basis of this favourable correlation we can assume that chromatography in the given system is governed predominantly by the partition principle. Subsequently, the R_M value from chromatography in polar system methanol—water on the sorbent impregnated with octanol can be used in assessment of lipophilic character of the substituted anilines.

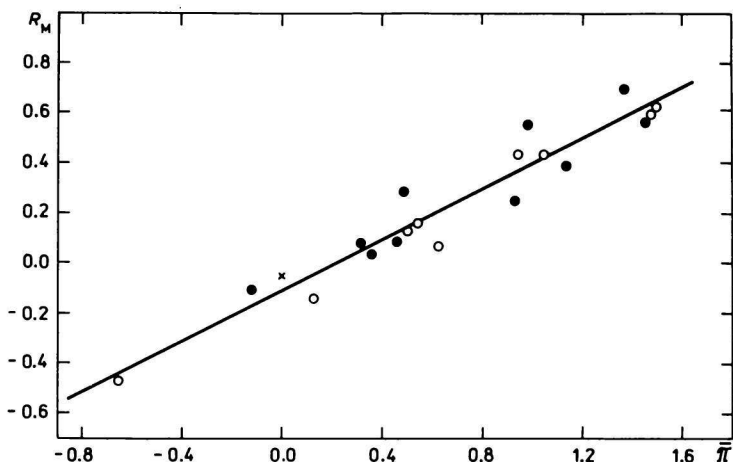


Fig. 1. $R_M = f(\bar{\pi})$.

Silica gel impregnated with octanol, mobile phase methanol—water ($\varphi_r = 1 : 1$).

○ 3-Substituted anilines; ● 4-substituted anilines; × aniline.

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