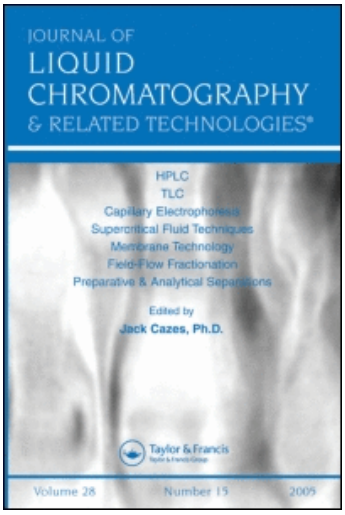

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LIPOPHILICITY DETERMINATION OF SOME MONOAMINE OXIDASE INHIBITORS BY REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY. THE EFFECT OF pH

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ABSTRACT

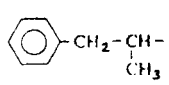
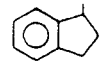
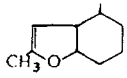
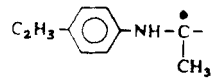
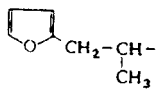
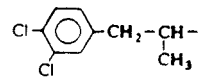
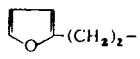
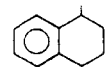
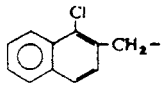
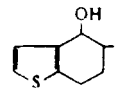
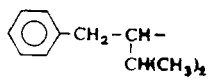
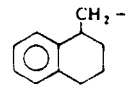
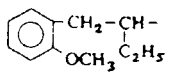
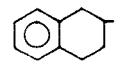
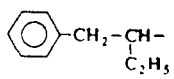
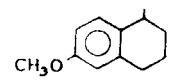
The lipophilicity (R_M value) of 17 monoamine oxidase inhibitory drugs was determined by reversed-phase thin-layer chromatography and the effect of various eluent additives such as acetic acid, sodiumacetate and sodium chloride on the retention was studied. Each drug exhibited regular retention behavior, their R_M value linearly decreased with the increasing concentration of methanol in the eluent. Acetic acid decreased the retention, the effect was higher at lower acid concentrations which indicates that the phenomena is of saturation character. The effect of sodiumchloride and sodiumacetate was negligible. The lipophilicity and specific hydrophobic surface area values of the drugs were intercorrelated, however, their information content is different, therefore their simultaneous application in quantitative structure-activity relationship studies is recommended.

INTRODUCTION

In recent decades quantitative structure-activity relationship (QSAR) methods have found growing acceptance

TABLE 1.
Chemical Structures of Monoamine Oxidase Inhibitory Drugs.

R_1-N-CH_2-C CH
R_2
General structure

No of compound	R_1	R_2	No of compound	R_1	R_2
1 (+) 2 (−)		CH_3	10		CH_3
3		CH_3	11		H
4		H	12		CH_3
5		CH_3	13		CH_3
6		CH_3	14		CH_3
7		CH_3	15		CH_3
8		CH_3	16		CH_3
9		C_4H_7	17		CH_3

and application not only in the design of new drugs, pesticides, etc (1,2) but also in the study of various biochemical (3) or biophysical processes (4). In the search for the best correlation between chemical structure and biological activity, a wide range of molecular parameters have been applied in QSAR studies (5). Many of these parameters can readily be determined by various chromatographic techniques (6). Chromatographic methods have some advantages: they are rapid and relatively simple; very small quantities of the substances are required and; the compounds need not be very pure. Lipohilicity is the molecular parameter used most frequently in QSAR studies (7,8). Lipophilicity can be determined via reversed-phase high performance liquid chromatography (RP-HPLC) (9) and by reversed-phase thin-layer chromatography (RP-TLC) (10). Recent research indicates that both methods are equally suitable for the determination of lipophilicity (11,12). The R_M value (related to the molecular lipophilicity), determined through the use of RP-TLC, generally linearly depends on the concentration of the organic component in the eluent (13). In order to increase the accuracy of the lipophilicity determination, the R_M value extrapolated to zero organic component concentration (R_{M0}) has been calculated from the linear correlation between the actual R_M value and the concentration of the organic component in the eluent (14). It has been additionally stated that not only the RM value extrapolated to zero organic component concentration but also the slope (b) value of the linear correlation, is characteristic of the lipophilicity (15). In the case of homologous series of compounds the slope and R_{M0} values exhibited significant linear correlation (16), but for a non-homologous series both parameters were needed to describe the lipophilicity accurately (17). The slope has been regarded as a charac-

teristic of the specific hydrophobic surface area of the compounds (18).

Monoamine oxidase inhibitory drugs are promising therapeutic compounds (19,20), however, their exact mode of action has not been elucidated in detail (21).

The objectives of our investigation were to determine for future QSAR calculations the lipophilicity and specific hydrophobic surface area of 17 monoamine oxidase inhibitory drugs and to find correlations between the various molecular parameters.

EXPERIMENTAL

Silkoplat plates (Labor MIM, Budapest, Hungary) were impregnated with paraffin oil as described earlier (22). The chemical structures of drugs are listed in Table 1. The compounds were dissolved separately in methanol to give a concentration of 5 mg/ml, and 2μl of solution were spotted on the plates. The developments were carried out in sandwich chambers, the running distance being about 16 cm. Water:methanol (methanol concentration varied between 0 - 60 vol.% in steps of 10 vol.%) mixtures containing various quantities of acetic acid (0-1M end concentration), sodium acetate (0-0.7M) and sodium chloride (0-0.7M) were applied as eluents. The application of acidic, basic and neutral eluent additives were motivated by the fact, that the pH (23) and salt concentration (24) may considerably influence the reversed-phase retention behavior of compounds with one or more polar substructures. After development the plates were dried at 105°C, and the spots were detected with iodine vapors. Each determination was run in quadruplicate. When the relative standard

deviation of parallel determinations was higher than 8%, the data were omitted from the calculations.

It was assumed that the concentration of methanol (x_1 vol.%)), acetic acid (x_2 M), sodium acetate (x_3 M) and sodium chloride (x_4 M) may simultaneously influence the lipophilicity of the drugs:

$$R_M = R_{M0} + b_1.x_1 + b_2.x_2 + b_3.x_3 + b_4.x_4 + b_5.\ \log x_2 \qquad 1.$$

where R_{M0} = lipophilicity value extrapolated to zero methanol, acetic acid, sodium acetate and sodium chloride concentrations; b_1 = specific hydrophobic surface area; $b_{2\text{-}5}$ = sensitivity of the retention to acetic acid, sodium acetate and sodium chloride concentrations.

The inclusion of the logarithm of the acetic acid concentration in Eq.1 was motivated by the fact that the dependence of R_M values showed a marked nonlinear (saturation-like) dependence on the acetic acid concentration.

To select the independent variables significantly influencing the lipophilicity of the drugs stepwise regression analysis was used (25). The calculation was carried out separately for each compound according to Eq.1. The number of the accepted variables was not limited and the acceptance limit was set to 95% significance level. To find the similarities or dissimilarities between the effect of various eluent additives, linear correlations were calculated between the R_M values of the compounds determined at 30% methanol and 0.7 M eluent additive concentrations.

To test the validity of the hypothesis discussed in refs 15-17, linear correlations were calculated between the R_{M0} and b_1 values of eq.1.

$$b_1 = a + b.R_{M0} \qquad 2.$$

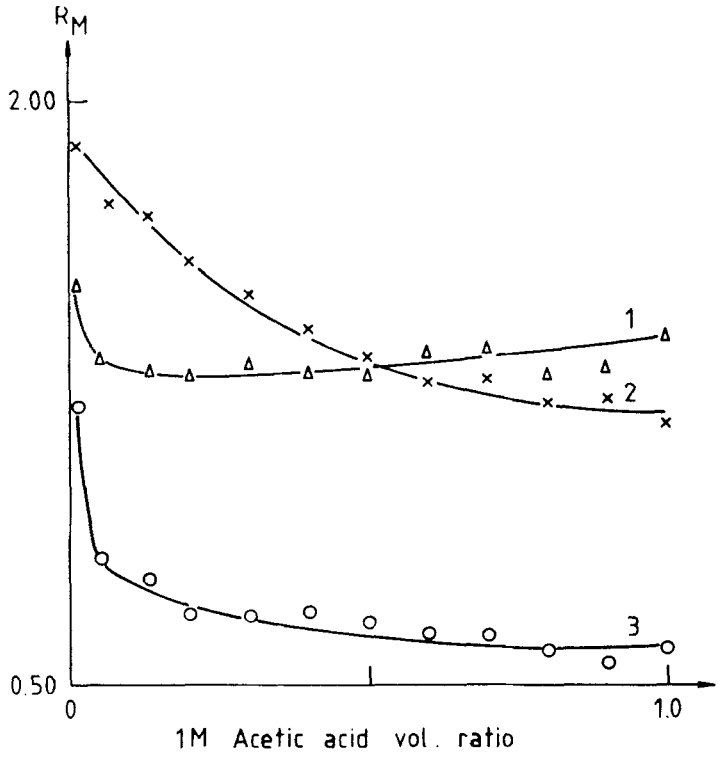


Figure 1. Effect of 1M acetic acid-water mixtures on the R_M value of compounds 3 (1), 5 (2) and 9 (3).

RESULTS AND DISCUSSION

The retention of compounds decreases in the present of acetic acid (Fig.1). This observation can be explained either by the salting-in effect (the dissociated drugs are better soluble in the aqueous phase) or by the competition between acetic acid and solutes for the free silanol groups on the silica surface resulting in decreased retention capacity. The effect of acetic acid depends on the chemical structure of the solute, the retention order

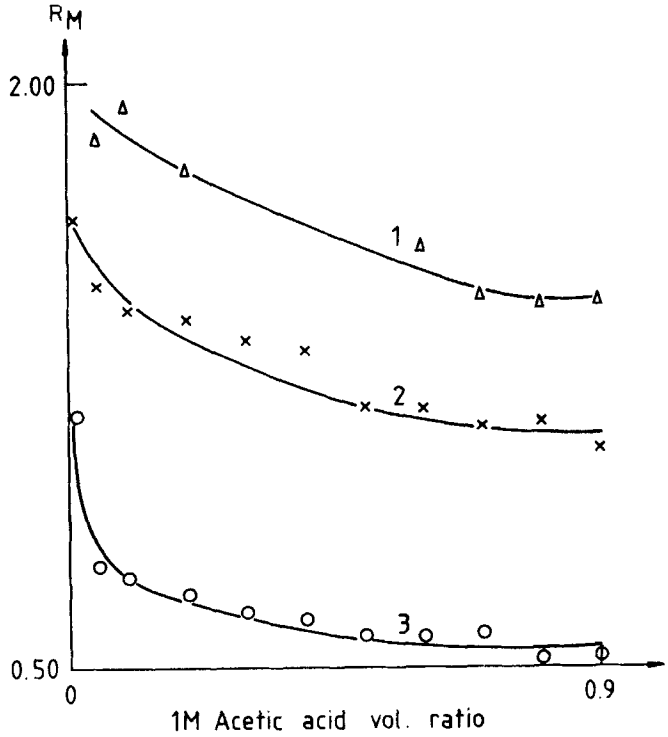


Figure 2. Effect of 1M acetic acid-1 M sodiumchloride mixtures on the R_M value of compounds 3 (1), 5 (2) and 9 (3).

changes at higher acid concentrations. Very low concentrations (0.025 M) of acid drastically decrease the retention of MAO inhibitors, however, higher concentrations have a lower effect on the retention. This observation can be explained by the supposition that the dugs are in dissociated form at low acetic acid concentrations and the further increase of acidity has no effect on the dissociation. Sodium chloride has a negligible effect on the retention behavior of solutes (Fig.2) that is the grade of dissociation of MAO inhibitory drugs rather de-

TABLE 2.

Dependence of the R_M Value of Monoamine Oxidase Inhibitory Drugs on the Methanol Concentration (x_1) and on the Logarithm of Acetic Acid Concentration (x_2). Results of Stepwise Regression Analysis. Numbers Refer to Drugs in TABLE 1. (p.c.% = path coefficient in percent)

$$R_M = R_{M0} + b_1.x_1 + b_5.\log x_2$$

Parameter	Number of drugs						
	1	2	3	4	5	6	7
n	32	32	32	32	32	31	32
R_{M0}	54.8	71.76	120.3	37.3	53.1	131.6	110.3
b_1	-2.11	-2.84	-3.86	-1.78	-1.94	-2.97	-2.82
s_{b1}	0.12	0.14	0.15	0.15	0.11	0.22	0.11
b_5	-23.0	-25.1	-20.8	-22.0	-28.1	-34.1	-22.1
s_{b5}	2.58	3.10	3.20	3.13	2.30	5.14	2.46
p.c.$_1$%	66.31	70.82	79.92	63.38	59.64	67.18	73.19
p.c.$_5$%	33.69	29.18	20.08	36.62	40.36	32.82	26.81
r^2	0.9353	0.9334	0.9587	0.8558	0.9338	0.8746	0.9556
$F_{calc.}$	134.9	170.8	336.5	86.1	204.4	97.6	311.9
$F_{99.9\%}$	8.85	8.85	8.85	8.85	8.85	8.93	8.85

Parameter	Number of drugs						
	8	9	10	11	12	13	14
n	32	30	32	25	31	32	32
R_{M0}	82.0	130.8	57.9	132.2	141.3	64.2	55.4
b_1	-2.32	-2.60	-1.80	-4.32	-2.65	-1.52	-1.87
s_{b1}	0.18	0.12	0.11	0.32	0.16	0.12	0.11
b_5	-19.6	-55.1	-20.5	-26.1	-20.71	-20.73	-21.9
s_{b5}	3.88	3.17	2.35	6.94	3.86	2.62	2.30
p.c.$_1$%	71.77	55.78	65.24	78.27	75.03	61.10	64.60
p.c.$_5$%	28.23	44.22	34.76	21.73	24.97	38.90	35.40
r^2	0.8553	0.9562	0.9124	0.8947	0.9034	0.8666	0.9222
$F_{calc.}$	85.7	294.6	151.0	93.5	130.9	94.2	172.0
$F_{99.9\%}$	8.85	9.02	8.85	9.47	8.93	8.85	8.85

Parameter	Number of drugs		
	15	16	17
n	32	32	32
R_{M0}	107.9	90.3	111.4
b_1	-2.89	-2.38	-2.73
s_{b1}	0.16	0.12	0.13
b_5	-41.0	-23.0	-21.6
s_{b5}	3.49	2.66	2.71
p.c.$_1$%	60.13	68.90	73.01
p.c.$_5$%	39.87	31.10	26.99
r^2	0.9309	0.9314	0.9436
$F_{calc.}$	195.3	196.8	242.7
$F_{99.9\%}$	8.85	8.85	8.85

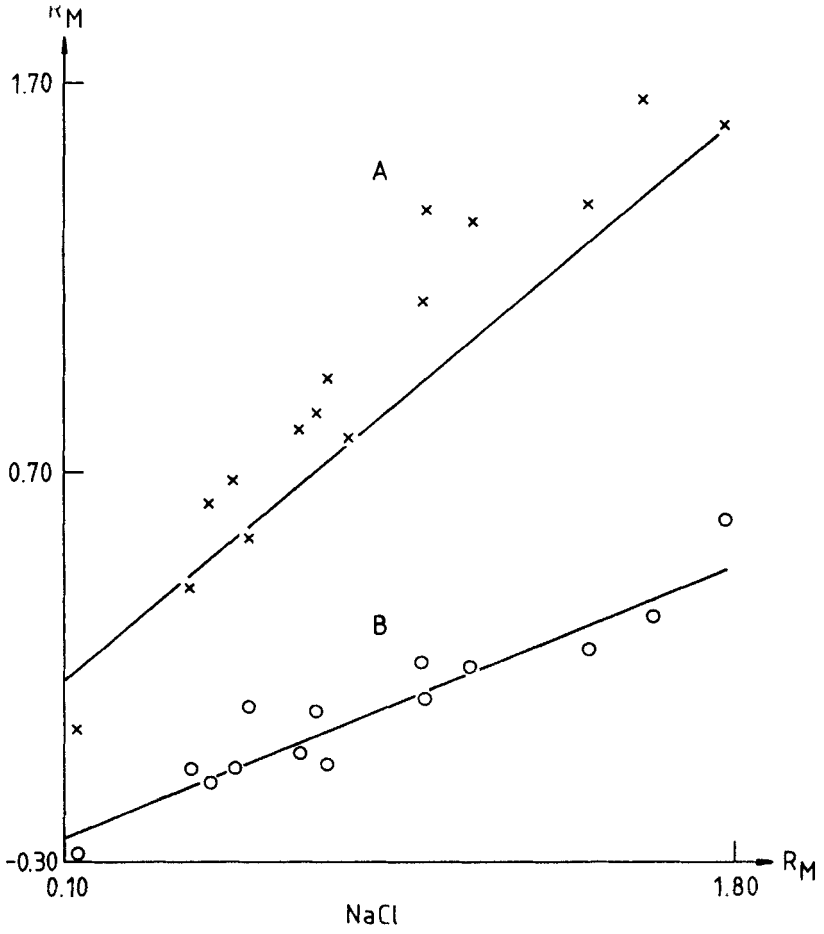


Figure 3. Relationships between the R_M values of MAO inhibitors determined in 1 M sodiumchloride:methanol 7:3 v/v, in 1 M sodiumacetate:methanol 7:3 v/v (curve A) and in 1 M acetic acid:methanol 7:3 v/v (curve B).

TABLE 3.
Parameters of Linear Correlations between the R_M Values Determined in the Presence of Acetic Acid, Sodiumacetate and Sodiumchloride. n = 15

I. R_M(acetic acid) = a + b.R_M(sodiumchloride)
II. R_M(sodiumacetate) = a + b.R_M(sodiumchloride)
III. R_M(acetic acid) = a + b.R_M(sodiumacetate)

Parameters	No of equation		
	I	II	III
a	-28.58	9.13	-28.50
b	0.42	0.98	0.39
r	0.8923	0.9570	0.8459

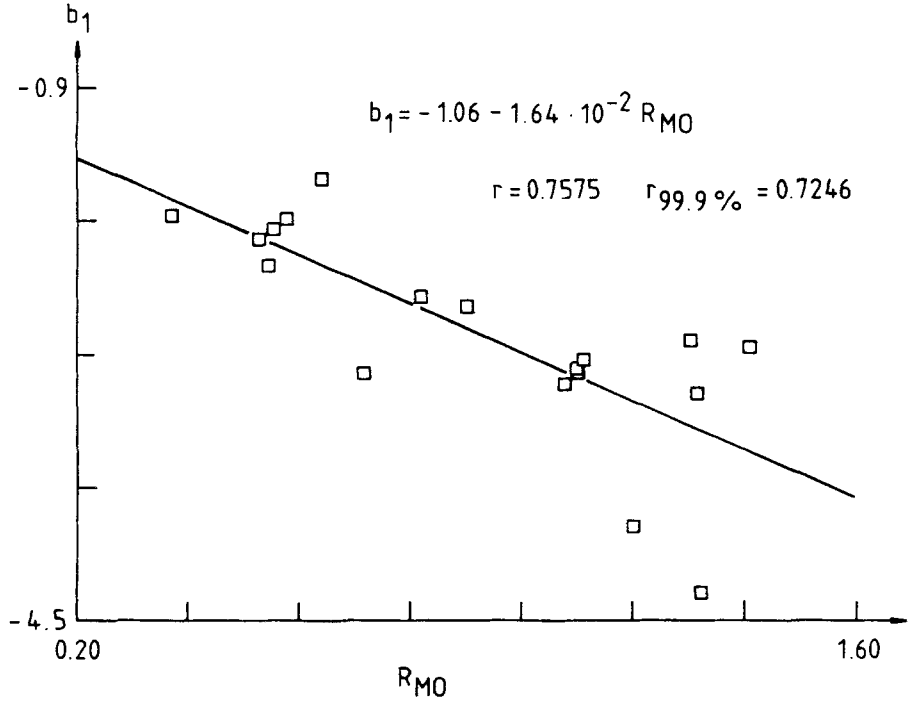


Figure 4. Correlation between the lipophilicity (R_{M0}) and specific hydrophobic surface area (b_1) of MAO inhibitors.

pends on the pH than on the ionic concentration of the environment.

The parameters of equations describing the dependence of R_M values on the methanol, acetic acid, sodium acetate and sodium chloride concentrations are compiled in Table 2. Sodium acetate and sodium chloride have no significant influence on the retention, the R_M values depended on the methanol concentration and on the logarithm of the acetic acid concentration. The equation selected by the stepwise regression analysis fits well to the experimental data, the significance level being over 99.9% in each case (compare calculated F values with the corresponding tabulated ones). The equations explain about 85-95% of the total variance (see r^2 values). The impact of both independent variables is commensurable, but the methanol concentration exerts a higher effect on the retention than the acetic acid concentration does (compare path coefficient values).

The R_M values determined in the presence of various eluent additives are correlated (Fig.3), that is, each data set can be used for QSAR studies. The parameters of linear correlations are compiled in Table 3. Each equation fits well to the experimental data the significance level was over 99.9% (see r values). The slope (b) values clearly show that the highest differences between the R_M values of MAO inhibitors can be expected in acidic environment. This finding suggests that the most effective reversed-phase separation of the drugs can be achieved in acidic eluents. A highly significant (significance level over 99.9%) linear correlation was found between the $_RM_0$ and b_1 values of equation 2 (Fig.4). This finding indicates that the lipophilicity and specific hydrophobic surface area of these MAO inhibitors are intercorrelated and they form a homologous series of compounds. However, we have to emphasize that the correlation is not strong

enough to substitute the parameters with each other in QSAR calculations. Their information content is somewhat different, therefore their separate application in the further calculations is proposed.

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REFERENCES

1. C.Hansch, in C.J.Cavallito (Editor), Structure-Activity Relationships, Pergamon Press, Oxford, 1973, p.73.

2. J.Andrew, W.E.Stuper, P.Brugger and S.Jurs, Computer Assisted Studies of Chemical Structure and Biological Function, J.Wiley and Sons, New York, 1979.

3. A.Lopata, F.Darvas, K.Valkó, Gy.Mikite, É.Jakucs and A.Kiss-Tamás, Pestic.Sci., 14 (1983) 513.

4. T.Cserháti, J.Bojarski, É.Fenyvesi and J.Szejtli, J.Chromatogr., 351 (1986) 356.

5. R.Franke, in J.K.Seydel (Editor), QSAR and Strategies in the Design of Bioactive Compounds. VCH Verlagsgesellschaft, Weinheim, 1985, p. 59.

6. R.Kaliszan, Quantitative Structure-Chromatographic Retention Relationships, J.Wiley and Sons, New York, 1987.

7. C.A.Lipinski, E.F.Fiese and R.J.Korst, Quant.Struct.-Act.Relat., 10 (1991) 109.

8. U.Norinder and T.Högberg, Quant.Struct.-Act.Relat., 10 (1991) 1.

9. J.M.McCall, J.Med.Chem., 18 (1975) 549.

10. C.B.C.Boyce and B.V.Milborrow, Nature, 208 (1965) 537.

11. G.L.Biagi, M.C.Guerra, A.M.Barbaro, S.Barbieri, M.Recanatini and P.A.Borea, J.Liqu.Chromatogr., 13 (1990) 913.

12. G.L.Biagi, M.C.Guerra, A.M.Barbaro, S.Barbieri, M.Recanatini, P.A.Borea and M.C.Pietrogrande, J.Chromatogr., 498 (1990) 179.

13. L.Ekiert, Z.Grodzinska-Zachwieja and J.Bojarski, Chromatographia, 13 (1980) 471.

14. T.Cserháti, B.Bordás and Gy.Ösapay, Chromatographia, 18 (1987) 184.

15. T.Cserháti, Chromatographia, 18 (1984) 18.

16. T.Cserháti, Chromatographia, 18 (1984) 318.

17. K.Valkó, J.Liqu.Chromatogr., 7 (1984) 1405.

18. C.Horváth, W.Melander and I.Molnár, J.Chromatogr., 125 (1976) 129.

19. J.Knoll, E.S.Vizi and G.Somogyi, Arzneimittel-Forsch., 18 (1968) 109.

20. J.Knoll and K.Magyar, Adv.biochem.Psychopharm. 5 (1972) 393.

21. J.Knoll, Z.Ecsery, K.Magyar and É.Sátory, Biochem. Pharmacol., 27 (1978) 1739.

22. T.Cserháti, B.Bordás, É.Fenyvesi and J.Szejtli, J.Chromatogr., 259 (1983) 107.

23. Gy.Vigh, Z.Varga-Puhony, J.Hlavay and E.Pap, J.Chromatogr., 236 (1982) 51.

24. E.Pap and Gy.Vigh, J.Chromatogr., 259 (1983) 49.

25. H.Mager, Moderne Regressionsanalyse, Salle, Sauerlander, Frankfurt am Main, 1982, p.135.