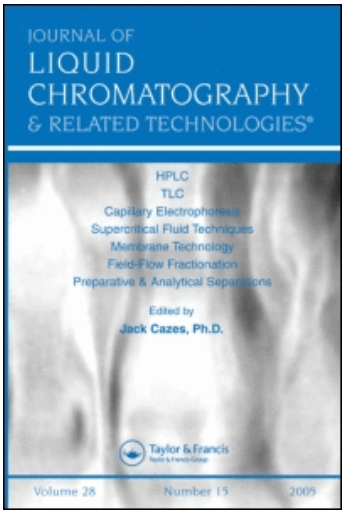

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Comparison of Selected Stationary Phases for Determination of Vancomycin and Ciprofloxacin Using Buffered Mobile Phases, With and Without Triethylamine

P. Forlay-Frick[a]; J. Fekete[a]
[a] Faculty of Chemical Engineering, Budapest University of Technology and Economics, Budapest, Hungary

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Comparison of Selected Stationary Phases for Determination of Vancomycin and Ciprofloxacin Using Buffered Mobile Phases, With and Without Triethylamine

P. Forlay-Frick and J. Fekete*

Faculty of Chemical Engineering, Budapest University of Technology and Economics, Budapest, Hungary

ABSTRACT

In our study we measured the retention factors, the theoretical plate numbers, and the symmetry factors in the case of phosphate buffer and triethylamine containing phosphate buffer, in order to get some information about the surface characteristic of tested columns. Three solutes (*N*,*N*-dimethyl-aniline, Ciprofloxacin, and Vancomycin) and 7 stationary phases (Nucleosil® 100-C-8, LiChrosorb® RP-select B, LiChrospher® RP-18, Purospher® RP-18e, Prontosil® 120-5-C18-AQ, Symmetry® C-18, and Chromolith® SpeedROD RP-18e) were used in this study. One of the solutes, *N*,*N*-dimethyl-aniline (121 g/mol) is a basic, low molecular weight compound. This is widely used for

*Correspondence: J. Fekete, Faculty of Chemical Engineering, Budapest University of Technology and Economics, Gellért tér 4, Budapest 1111, Hungary; E-mail: fekete@mail.bme.hu.

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characterization of silanol activity of chemically bonded phases. The other two, Ciprofloxacin (331 g/mol) and Vancomycin (1449 g/mol), contain both basic and acidic groups, and Vancomycin has a high molecular weight. These latter two are real life samples and we tried to find a correlation between results of test compounds and these widely used antibiotics. For this purpose, we selected widely different columns: end-capped phases, non end-capped phases with high and low metal content, and last but not least, a recently developed monolithic silica column. According to selection criteria ($N/m > 20{,}000$; $0.8 < A_S < 1.8$), we evaluated the effect of masking agent (triethylamine) and the role of organic solvents (methanol and acetonitrile). It was found that the Symmetry C-18 was the best in the case of Ciprofloxacin and *N*,*N*-dimethyl-aniline, and the Chromolith SpeedROD RP-18e monolithic silica rod in the case of high molecular weight Vancomycin in these experiments. In most of the cases the LiChrosorb RP-select B had to be excluded. Based on selection criteria, the eluent composition and the structure and size of tested solutes strongly influenced the applicability of other columns. Influence of TEA on theoretical plate number and symmetry factor was very high in the case of these columns.

Key Words: Ciprofloxacin; Vancomycin; RP stationary phases; Triethylamine; Buffer.

INTRODUCTION

There are several approaches to evaluate reversed phase columns for practical purposes.[1] Mostly, unbuffered mobile phases are used.[2–4] The effect of the polar surface must be evaluated based on retention, plate number, and symmetry factor. In the second approach, buffered mobile phases are applied for simulating real life conditions.[5,6]

We do not intend to write a review about column tests; only a few were cited above from a large number of publications. The common feature of these approaches is that the selected solutes represent all possible interactions with the reversed phase material. In most cases, the selected solutes have low molecular weights and one or two interactions might be responsible for the retention. This may not represent the real situation. In the practical applications, when multiple interactions occur it is hard to predict the influence of stationary phases on retention, plate number, and peak symmetry. The most efficient way is to find literature sources for the intended applications, but several methods can be found and the selection of appropriate stationary phase is always difficult.

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In our study, we compare seven different stationary phases (Table 1) for separation of three compounds. The structure and the calculated pKa value of polar groups of the solutes by Pallas 3.0 and Marvin Sketch software can be seen in Fig. 1. One of the solutes, *N*,*N*-dimethyl-aniline ($\log P = 2.03$; $\text{pKa} = 5.06$) is a basic compound with a single polar group. Its $\log D$ value as a function of the pH can be seen in Fig. 2. This model compound was chosen to monitor the polar surface of tested stationary phases. To avoid the detrimental ion-exchange interaction with silanols, the pH of the mobile phase was adjusted to 7.5.

The other two, Ciprofloxacin ($\log P = 1.76$) and Vancomycin ($\log P = -0.21$), are often used antibiotics in the pharmaceutical industry. The Ciprofloxacin contains two polar groups: one is acidic and the other is basic. The Vancomycin is a highly polar compound. It contains several acidic and basic groups. The pKa value of basic and acidic groups of Ciprofloxacin are 9.08 and 6.27, respectively. Similar pKa values are published by Maurer[7] and by Ross and Riley.[8] The pKa value of the most acidic, and the two most basic, groups of Vancomycin are 3.18, 9.36, and 6.32. The acidity of the phenolic and amide groups is too low. The $\log D$ value of these compounds as a function of the pH can be seen in Figs. 3 and 4. The molecular weight of Vancomycin, Ciprofloxacin, and *N*,*N*-dimethyl-aniline is 1449, 331, and 121 g/mol. The effect of pore diameter on retention, plate number, and symmetry factor is directly related to molecular mass of selected solutes.

The aim of our study is to compare results of different columns and analytes, and to get information of amine (triethylamine) effects on chromatographic parameters. We monitored, on the one hand, the change of the retention of compounds in cases of buffered mobile phases with and without triethylamine (TEA). On the other hand, we also tested the change of theoretical plate number and symmetry factor. To reduce the huge amount of data, we evaluated only those stationary phases, which fulfilled the following criteria: $N/m > 20{,}000$ and $0.8 < A_S < 1.8$. Evaluation of the measured data was conducted at two different retention factors: $\log k = 1$ and 0.1. At lower retention factors, the overloading effect of polar groups is more pronounced.

EXPERIMENTAL

Equipment

The separation was performed using isocratic elution at a flow rate of 0.5 mL/min for Vancomycin and 0.6 mL/min for Ciprofloxacin and *N*,*N*-dimethyl-aniline. We used a Spectra Focus HPLC-system (Thermoseparation

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Table 1. The columns used in our study.

	Nucleosil® 100-C-8 250 × 4 mm[a]	LiChrosorb® RP-select B 250 × 4 mm[a]	LiChrospher® RP-18 100 × 4 mm[a]	Purospher® RP-18e 125 × 3 mm[b]	Prontosil® 120-5-C18-AQ 120 × 5 mm[b]	Symmetry® C-18 150 × 4.6 mm[b]	Chromolith® SpeedROD RP-18e 50 × 4.6 mm[b]
Particle Size [μm]	5 ± 1.5	5	5	5	5	5	One rod of continuous monolithic porous silica
Pore diameter [Å]	100	60	100	120	120	100	120
Macropore/mezopore size	–	–	–	–	–	–	2 μm/13 nm
Pore volume [mL/g]	1	0.75	1.25	1	1	0.90	1
Specific surface area [m^2/g]	350	300	350	350	300	335	300
Surface coverage [μmol/m^2]	Not known	4.21	3.61	Not known	Not known	3.13	Not known
Plate number [1/m]	Not known	55.000	55.000	>80.000	Not known	Not known	Not known
C-content [%]	9.0	11.4	21.0	18.0	13.0	19.1	17.0
pH range	1–9	2–7.5	2–7.5	2–7.5	2–7	2–8	2–7
Metal content [ppm]	<350	>350	<350	<5	<1	<10	<10
End-capped	No	No	No	Yes	Yes	Yes	Yes

[a]Prepared from Silica A (high metal content silica gel).
[b]Prepared from Silica B (high purity silica gel).

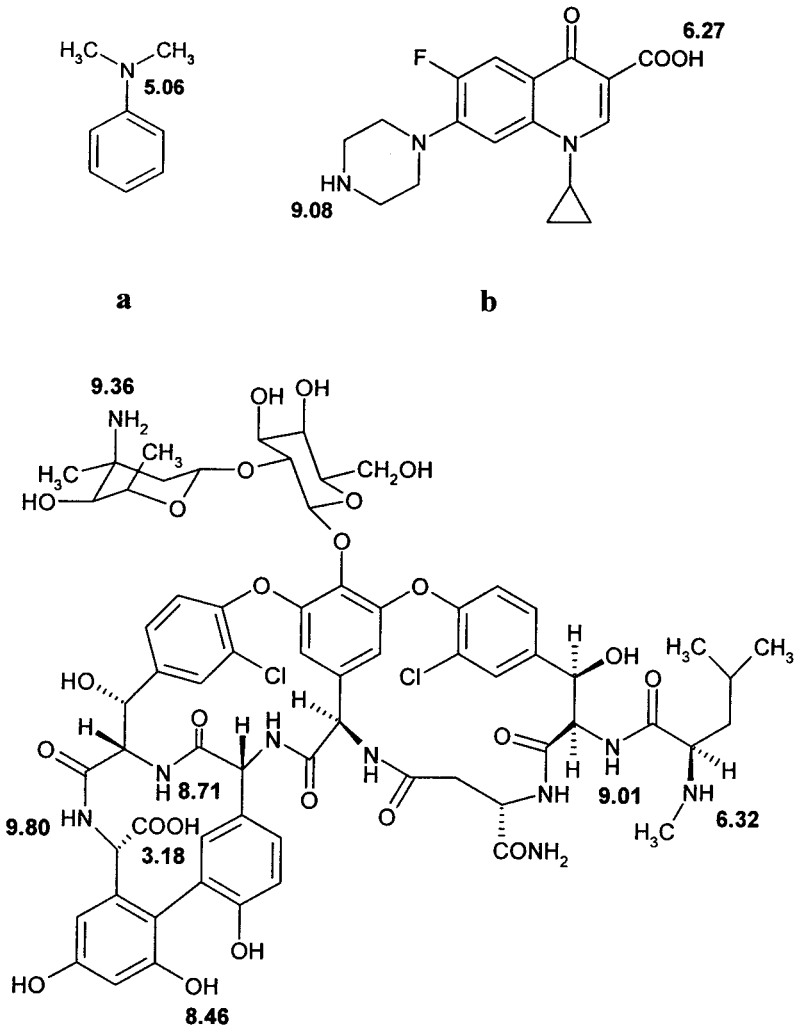

Figure 1. The structure and the calculated pKa value of polar groups of *N*,*N*-dimethyl-aniline (a), Ciprofloxacin (b), and Vancomycin (c).

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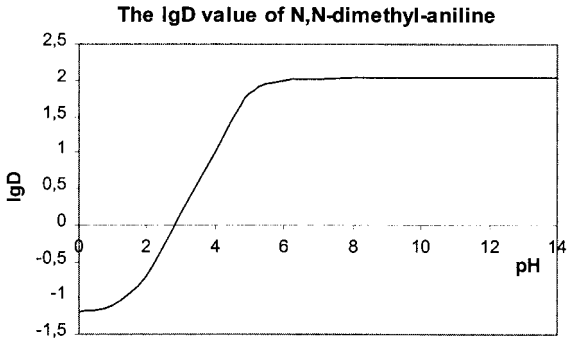

Figure 2. The log *D* value of *N*,*N*-dimethyl-aniline as a function of the pH.

Products, CA). The apparatus consisted of a Spectra P2000 pump attached to an AS 3000 autosampler, a Spectra UV2000 detector, a SN 4000 interface, and a SCM 1000 solvent degasser. The ultraviolet detection was carried out at 230 nm for Vancomycin, at 250 nm for *N*,*N*-dimethyl-aniline, and at 280 nm for Ciprofloxacin. Injection volume for all analytes was 20 μL. The software used was a PC 1000 System Software Version 3.03. The mobile phase was ultrasonicated in a Realsonic RS-95 SF ultrasonic bath (Budapest, Hungary), and its pH was adjusted using a Radiometer pH-meter 28 (Copenhagen, Denmark).

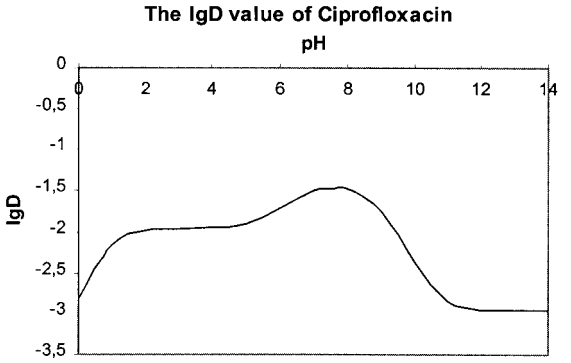

Figure 3. The log *D* value of Ciprofloxacin as a function of the pH.

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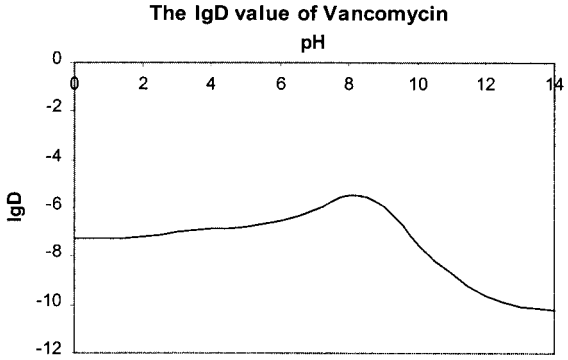

Figure 4. The log *D* value of Vancomycin as a function of the pH.

Chemicals

Acetonitrile (AcN) and methanol (MeOH), super gradient, were supplied by Labscan (Dublin, Ireland). For the preparation of the aqueous phases, potassium dihydrogen phosphate (Reanal, Budapest, Hungary), triethylamine (Aldrich, Milwaukee, WI), concentrated ortophosphoric-acid (Reanal, Budapest, Hungary), and potassium hydroxide (Reanal, Budapest, Hungary), analitical grade, were used. The high purity water was generated in a Millipore (Milli-Q water purification system, Bedford, TX).

The test samples were obtained from the companies identified below: Ciprofloxacin from Bayer AG (Leverkusen, Germany) as Ciprobay® infusion for intravenous use (containing 2 mg/mL of Ciprofloxacin hydrochloride), Vancomycin (Fig. 1) from Lilly Deutschland GMBH (Giessen, Germany) as Vancocin® injection (containing 50 mg/mL of Vancomycin hydrochloride), and *N*,*N*-dimethyl-aniline (99%) from Aldrich (Gillingham, UK).

Columns

The physical–chemical properties of columns used in our experiments are very different (Table 1).[9–11] Among these columns, beyond the non end-capped and end-capped phases there was a column, which presented the new generation of stationary phases. This was the Chromolith SpeedROD. This column is developed based on a new sol gel process for the preparation of monolithic porous silica rods, using highly pure metal free alkoxysilanes. The Chromolith columns consist of macropores and mezopores in the skeleton, providing a higher porosity. Consequently, these columns can be operated at

higher flow rates without loss of performance and limitations due to low column backpressure.

Mobile Phase Compositions

In our experiments we used four different eluents: AcN-KH_2PO_4 buffer, AcN-TEA buffer, MeOH-KH_2PO_4 buffer, or MeOH-TEA buffer. The KH_2PO_4 and the triethylamine buffers were 25 mM. The pH of these aqueous solutions was adjusted to 2.80 ± 0.05 for Vancomycin, 3.00 ± 0.05 for Ciprofloxacin, and 7.50 ± 0.05 for *N*,*N*-dimethyl-aniline. For adjustment of the pH we used concentrated ortophosphoric-acid and potassium hydroxide.

The measured range of retention factors was between $\log k = 0.1$ to $\log k = 1$. During the measurement, the concentration of buffer was constant and the organic content of the mobile phase was gradually changed. The theoretical plate number and symmetry factor of chromatographic peaks were calculated at every measuring point.

Calculations

All column characteristics, retention factor (k), number of theoretical plates (N), and symmetry factor (A_S), were calculated according to European Pharmacopoeia.[12] The log P, log D and pKa values of *N*,*N*-dimethyl-aniline and Ciprofloxacin were calculated by Pallas 3.0 intelligent software package. In the case of Vancomycin, the calculation was completed by Marvin Sketch calculation software. The calculated pKa value of polar groups in the case of all solutes can be seen in Fig. 1. The log D value of compounds as a function of pH can be found on Figs. 2–4.

From the measured data, the deviations of theoretical plate numbers (ΔN) and symmetry factors (ΔA) were calculated as follows: the difference between the value obtained with KH_2PO_4 buffer and that with TEA buffer is divided by the value with TEA.

RESULTS

The measured theoretical plate number and symmetry factor values can be seen in Tables 2 and 3. We used the following abbreviations: Nu: Nucleosil, Lb: LiChrosorb, L: LiChrospher, Pu: Purospher, P: Prontosil, Sy: Symmetry, Sp: Chromolith SpeedROD, M: methanol, A: acetonitrile, T: TEA buffer, and K: KH_2PO_4 buffer. On the basis of this, we use NMK to signify a Nucleosil

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column in the case of using mobile phase containing MeOH and KH_2PO_4 buffer.

To reduce the huge amount of data for evaluation of a masking agent (TEA) and the role of organic solvents, we took only those stationary phases into consideration, which fulfilled the following criteria: $N/m > 20{,}000$ and $0.8 < A_S < 1.8$.

In the case of Ciprofloxacin (331 g/mol), at high retention ($\log k = 1$) the Symmetry column whether with methanol or with acetonitrile, both in the presence of TEA and in the absence of TEA, meets the selection criteria. The influence of TEA on retention, plate number, and peak symmetry was negligible. Thus, it may be concluded, that the surface coverage of this stationary phase is high due to good end-capping technology. The polar or silanophil interactions are highly excluded. The measured theoretical plate numbers approach the highest possible available theoretical plate numbers, given by the manufacturer. On Purospher and Chromolith SpeedROD the advantageous effect of TEA on both parameters was found. For Purospher, high theoretical plate number and good peak symmetry was found with methanol containing mobile phase, even if the mobile phase does not contain TEA. However, when KH_2PO_4 (pH = 3.0) and AcN containing eluent was used, the theoretical plate number was only 18,700. The Chromolith SpeedROD can be used with AcN containing eluent without TEA. Using MeOH in the absence of TEA, the symmetry factor was only 1.87, but when TEA was added to the mobile phase this value was improved (1.65). It can be seen from the Table 3, that LiChrospher and Prontosil gave more than 20,000/m for plate number and less than 1.8 for symmetry factor with TEA containing mobile phase at retention factor of 10. The Prontosil column is tailor-made for highly aqueous mobile phases. The specially developed surface structure of the stationary phase does not collapse at high water content according to the manufacturer. The LiChrospher column belongs to the higher metal content, silica A based stationary phases. Triethylamine containing mobile phases were necessary to get acceptable theoretical plate numbers and peak symmetry. At $\log k = 0.1$ the Purospher, the Chromolith SpeedROD, and the LiChrospher could not be used because of low plate number and non adequate symmetry factor.

Vancomycin has higher molecular weight analytes (1449 g/mol) and contains more polar groups than Ciprofloxacin. In consequence of its high molecular size, one part of the unreacted silanol groups was not available due to steric inhibition. On the other hand, the diffusion rate and mass transfer between the mobile and stationary phases decreased. The former caused improvement in the peak symmetry. The latter, inasmuch the size of compound and size of some pores were comparable, reduced the theoretical plate number. As for the size of Vancomycin, the exclusion from smaller pores

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Table 2. The measured theoretical plate numbers (N) at $\log k = 1$ and 0.1 in case of all columns and compounds.

N for Ciprofloxacin [1/m]		N for Vancomycin [1/m]		N for *N*,*N*-dimethyl-aniline [1/m]	
$\log k = 1$	$\log k = 0.1$	$\log k = 1$	$\log k = 0.1$	$\log k = 1$	$\log k = 0.1$
2,440 (LbMK)	3,058 (LbMK)	5,300 (LbMK)	4,088 (PuMK)	9,244 (LbMK)	5,648 (LbAK)
4,616 (LbAK)	5,748 (LbAK)	5,320 (LbMT)	4,504 (PuMT)	11,072 (LbAK)	5,704 (LbMK)
5,644 (NMK)	6,184 (LbMT)	7,032 (LbAK)	5,836 (LbMK)	12,512 (LbAT)	11,660 (LbAT)
5,780 (LbMT)	8,520 (LbAT)	7,032 (PuMK)	6,356 (LbMT)	15,268 (LbMT)	13,148 (LbMT)
7,444 (LbAT)	8,960 (NMK)	7,588 (LbAT)	6,744 (NMK)	36,096 (LMK)	24,460 (SpAK)
17,496 (LMK)	11,456 (LMK)	8,608 (PuMT)	7,632 (LbAK)	39,632 (PuAK)	26,120 (PuMK)
18,688 (PuAK)	11,744 (PuAK)	12,084 (NMK)	8,400 (LbAT)	39,920 (LMT)	26,220 (SpAT)
19,360 (NMT)	12,336 (PuMK)	15,104 (PuAK)	8,696 (PuAK)	40,184 (PuMK)	27,648 (LMK)
20,512 (LAK)	13,576 (PuAT)	17,568 (LMK)	9,440 (PuAT)	46,980 (SpAK)	28,512 (LMT)
22,324 (NAK)	14,816 (PuMT)	18,240 (LMT)	12,460 (SyMT)	48,384 (LAK)	28,528 (PuMT)
25,904 (PuMK)	15,192 (LAK)	20,232 (NMT)	12,824 (LMK)	49,120 (SpAT)	30,832 (PuAK)
29,792 (PuMT)	20,016 (LMT)	23,227 (SyMT)	12,992 (LMT)	50,020 (NMK)	30,920 (SpMK)
30,464 (PuAT)	25,826 (SyAT)	23,296 (PuAT)	17,247 (PMK)	50,956 (NMT)	31,960 (SpMT)
33,584 (LMT)	26,167 (PMK)	25,472 (LAK)	17,624 (LAK)	51,048 (PuMT)	38,408 (LAT)

34,663 (PMK)	28,920 (LAT)	27,500 (PMK)	18,420 (SyMK)	52,184 (LAT)	39,008 (LAK)
36,060 (SpAK)	29,076 (NMT)	28,192 (LAT)	18,840 (NMT)	53,487 (PMT)	39,032 (PuAT)
36,956 (NAT)	32,013 (PMT)	30,340 (NAK)	19,744 (LAT)	54,633 (PAT)	40,153 (PMT)
41,913 (PAK)	32,313 (PAK)	30,873 (SyMK)	21,307 (PMT)	56,947 (PMK)	40,540 (NMK)
43,376 (LAT)	33,028 (NAK)	31,147 (PMT)	23,353 (SyAT)	58,440 (SpMK)	44,833 (PMK)
48,520 (SpAT)	35,060 (SpAK)	35,564 (NAT)	26,156 (NAK)	60,080 (PAK)	44,992 (NMT)
49,793 (PMT)	37,307 (PAT)	35,727 (PAK)	27,253 (PAK)	61,584 (PuAT)	45,627 (SyMT)
51,940 (SpMK)	38,084 (NAT)	38,607 (SyAT)	29,400 (NAT)	62,320 (SpMT)	48,433 (SyMK)
53,486 (SyAT)	38,740 (SpAT)	39,407 (SyAK)	31,353 (SyAK)	62,724 (NAK)	49,272 (NAK)
55,873 (PAT)	39,293 (SyMT)	44,860 (PAT)	36,807 (PAT)	63,432 (NAT)	56,056 (NAT)
61,640 (SyMT)	41,200 (SpMK)	67,800 (SpMK)	52,240 (SpMK)	67,573 (SyMT)	57,693 (SyAT)
67,140 (SpMT)	44,893 (SyMK)	75,780 (SpAK)	58,120 (SpMT)	77,120 (SyMK)	59,547 (SyAK)
76,980 (SyMK)	51,340 (SpMT)	76,520 (SpAT)	61,160 (SpAK)	78,060 (SyAT)	63,120 (PAT)
86,360 (SyAK)	56,246 (SyAK)	83,900 (SpMT)	69,700 (SpAT)	82,927 (SyAK)	68,020 (PAK)

Abbreviations: Nu: Nucleosil, Lb: LiChrosorb, L: LiChrospher, Pu: Purospher, P: Prontosil, Sy: Symmetry, Sp: Chromolith SpeedROD, M: methanol, A: acetonitrile, T: TEA buffer and K: KH_2PO_4 buffer. On the basis of this NMK: Nucleosil column in the case of using MeOH and KH_2PO_4 buffer containing mobile phase.

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Table 3. The measured symmetry factors (A_S) at log $k = 1$ and 0.1 in case of all columns and compounds.

A_S for Ciprofloxacin		A_S for Vancomycin		A_S for *N*,*N*-dimethyl-aniline	
log $k = 1$	log $k = 0.1$	log $k = 1$	log $k = 0.1$	log $k = 1$	log $k = 0.1$
0.959 (SyAT)	0.994 (SyAT)	0.821 (NAT)	0.959 (NAT)	0.947 (LbMT)	0.881 (NAT)
1.014 (SyMT)	1.211 (PuMT)	0.859 (NMT)	1.035 (NAK)	0.958 (NAK)	0.887 (NAK)
1.094 (SyMK)	1.228 (PuAT)	0.961 (NAK)	1.058 (NMT)	0.971 (NAT)	0.891 (NMK)
1.173 (PuMT)	1.267 (SyMT)	1.002 (PuAT)	1.070 (PuMT)	0.980 (SpAK)	0.953 (NMT)
1.197 (PuAT)	1.347 (SyAK)	1.021 (SyMT)	1.081 (PuAT)	0.993 (LMT)	1.008 (LbMT)
1.206 (PuMK)	1.386 (PuMK)	1.028 (PAT)	1.092 (PuMK)	0.994 (SpAT)	1.123 (SyMT)
1.215 (SyAK)	1.427 (SyMK)	1.028 (PuMT)	1.103 (PuAK)	1.003 (LbMK)	1.128 (LMT)
1.220 (PuAK)	1.532 (PuAK)	1.037 (LMT)	1.107 (SyMT)	1.012 (SyMT)	1.138 (SyAT)
1.371 (SpAT)	1.572 (PAT)	1.043 (PuMK)	1.142 (SyAT)	1.020 (NMT)	1.152 (LbMK)
1.503 (PMT)	1.645 (LAT)	1.045 (PuAK)	1.168 (LMT)	1.026 (LAT)	1.171 (LAT)
1.510 (PAT)	1.715 (PMT)	1.052 (SyAT)	1.172 (NMK)	1.044 (SyAT)	1.187 (SyAK)
1.513 (LAT)	1.749 (NAT)	1.068 (PMT)	1.177 (LMK)	1.046 (SpMT)	1.192 (LMK)
1.547 (SpAK)	1.814 (LMT)	1.071 (SpMT)	1.205 (SyAK)	1.063 (SpMK)	1.224 (SyMK)
1.551 (LMT)	1.904 (LbMT)	1.073 (SpAT)	1.216 (LAT)	1.087 (SyMK)	1.241 (PuMT)

1.653 (SpMT)	1.944 (PAK)	1.077 (SyAK)	1.242 (LAK)	1.091 (PuMT)	1.260 (PuMK)
1.873 (SpMK)	1.950 (LbAT)	1.084 (NMK)	1.300 (PAT)	1.092 (NMK)	1.282 (PuAT)
1.956 (LbMT)	1.956 (PMK)	1.096 (LMK)	1.307 (LbAT)	1.108 (SyAK)	1.292 (PuAK)
1.987 (LbAT)	2.073 (SpAT)	1.112 (LAT)	1.328 (PMT)	1.119 (PuMK)	1.339 (PMT)
2.168 (PMK)	2.078 (SpMT)	1.120 (LbAT)	1.333 (LbMT)	1.125 (PuAT)	1.384 (LAK)
2.243 (NAT)	2.164 (SpAK)	1.124 (SyMK)	1.343 (LbAK)	1.129 (LAK)	1.387 (SpAT)
2.363 (PAK)	2.428 (SpMK)	1.125 (LAK)	1.354 (PMK)	1.148 (LMK)	1.402 (SpMT)
2.784 (NMT)	2.547 (LAK)	1.155 (PMK)	1.391 (SyMK)	1.215 (PuAK)	1.407 (PAT)
2.930 (LMK)	2.695 (LMK)	1.172 (LbAK)	1.413 (LbMK)	1.243 (PMT)	1.419 (SpMK)
3.154 (LAK)	3.112 (NAK)	1.213 (LbMT)	1.508 (PAK)	1.255 (PMK)	1.420 (PAK)
3.501 (NMK)	3.697 (LbAK)	1.236 (SpMK)	1.597 (SpMT)	1.287 (PAT)	1.445 (SpAK)
3.909 (LbMK)	3.707 (LbMK)	1.254 (SpAK)	1.674 (SpAT)	1.325 (PAK)	1.462 (PMK)
3.947 (LbAK)	3.768 (NMT)	1.272 (PAK)	1.730 (SpMK)	1.651 (LbAT)	1.789 (LbAT)
5.120 (NAK)	5.640 (NMK)	1.287 (LbMK)	1.789 (SpAK)	1.714 (LbAK)	1.903 (LbAK)

Abbreviations: Nu: Nucleosil, Lb: LiChrosorb, L: LiChrospher, Pu: Purospher, P: Prontosil, Sy: Symmetry, Sp: Chromolith SpeedROD, M: Methanol, A: Acetonitrile, T: TEA buffer and K: KH_2PO_4 buffer. On the basis of this NMK: Nucleosil column in the case of using MeOH and KH_2PO_4 buffer containing mobile phase.

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reduced the possibility of the polaric interactions with silanols. For this reason, the most critical factor was the symmetry factor for Vancomycin in the case of the tested stationary phases. In consequence of its significant decrease, more columns were suitable for testing of Vancomycin compared to Ciprofloxacin. The Chromolith SpeedROD met the prescribed requirements in the case of using both TEA and KH_2PO_4 also at $\log k = 0.1$, where the zone broadening effects are well known to be more significant. This can be explained with the smaller depth of diffusion pores. For Symmetry at $\log k = 1$ ($k = 10$), similarly to Chromolith SpeedROD, when MeOH or AcN was used we got good N and A_S in cases of both TEA and its absence. However, MeOH containing mobile phases were not used at $\log k = 0.1$, because of the low theoretical plate number. In the case of Symmetry, the measured N values were roughly half of the measured N values for Ciprofloxacin and *N*,*N*-dimethyl-aniline. For Nucleosil, the TEA improved the peak symmetry in such a way, that this high metal content stationary phase was also acceptable for the measurement. In small diameter pores the Vancomycin molecule was not able to penetrate because of its high molecular size, so the zone broadening effects significantly decreased. Only the AcN containing eluents were useful for both high and low retentions as far as Nucleosil was concerned. The LiChrospher seemed to be suitable to examine Vancomycin at $\log k = 1$, but only in AcN. The Prontosil at both examined $\log k$ values seemed to be well suited for analysis of Vancomycin in cases of all four eluents, although at $\log k = 0.1$ we obtained a theoretical plate number of only 17,200, when MeOH-KH_2PO_4 buffer eluent was used. This value does not meet the requirement. For Vancomycin the highest theoretical plate numbers were measured using Chromolith SpeedROD columns in the case of all four eluents, which can be explained by the smaller diffusion path. These measured values approach the highest possible theoretical plate number given by manufacturer.

All stationary phases except LiChrosorb were well suited for measurement of *N*,*N*-dimethyl-aniline (121 g/mol). The highest theoretical plate number was obtained, when Symmetry was used, which can be explained by excellent coverage of the column and the few available micropores.

From the measured data we calculated deviations of theoretical plates numbers and symmetry factors (Tables 4 and 5). For example: PuM is the abbreviation of the deviation of measured N or A_S values, which were measured using TEA buffer or KH_2PO_4 buffer containing eluents in the case of Purospher, using MeOH. The effects of TEA are reflected well in these calculated values. It can be seen, that high N and A_S deviations (ΔN and ΔA) were measured in the case of silica A based stationary phases, which contain a lot of available and unreacted silanol groups. In silica B based stationary phases (for example, Symmetry and Purospher), which contain few, but

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Table 4. Deviations of measured theoretical plate numbers (ΔN) in case of all columns and solutes at $\log k = 1$ and 0.1.

ΔN for Ciprofloxacin [%]		ΔN for Vancomycin [%]		ΔN for *N*, *N*-dimethyl-aniline [%]	
$\log k = 1$	$\log k = 0.1$	$\log k = 1$	$\log k = 0.1$	$\log k = 1$	$\log k = 0.1$
15.0 (PuM)	10.5 (SpA)	0.4 (LbM)	1.3 (LM)	1.1 (NA)	−1.5 (LA)
19.9 (SyM)	12.5 (SyM)	1.0 (SpA)	8.6 (PuA)	1.9 (NM)	3.1 (SyA)
29.3 (SpM)	15.3 (NA)	2.0 (SyA)	8.9 (LbM)	4.6 (SpA)	3.1 (LM)
33.3 (PA)	15.5 (PA)	3.8 (LM)	10.1 (LbA)	5.9 (SyA)	3.4 (SpM)
34.6 (SpA)	15.6 (PuA)	7.9 (LbA)	10.2 (PuM)	6.1 (PM)	5.8 (SyM)
38.1 (SyA)	20.1 (PuM)	10.7 (LA)	11.3 (SpM)	6.6 (SpM)	7.2 (PA)
43.6 (PM)	22.3 (PM)	13.3 (PM)	12.0 (LA)	7.9 (LA)	7.2 (SpA)
61.3 (LbA)	24.6 (SpM)	17.2 (NA)	12.4 (NA)	9.1 (PA)	9.2 (PuM)
63.0 (PuA)	48.2 (LbA)	22.4 (PuM)	14.0 (SpA)	10.6 (LM)	10.4 (PM)
65.5 (NA)	54.1 (SyA)	23.7 (SpM)	23.5 (PM)	12.4 (SyM)	11.0 (NM)
92.0 (LM)	74.7 (LM)	24.8 (SyM)	25.5 (SyA)	13.0 (LbA)	13.8 (NA)
111.5 (LA)	90.4 (LA)	25.6 (PA)	32.4 (SyM)	27.0 (PuM)	26.6 (PuA)
136.9 (LbM)	102.2 (LbM)	54.2 (PuA)	35.1 (PA)	55.4 (PuA)	106.4 (LbA)
243.0 (NM)	224.5 (NM)	67.4 (NM)	179.4 (NM)	65.2 (LbM)	130.5 (LbM)

For example: PuM the deviation of measured theoretical plate number values, which was measured using TEA buffer and KH_2PO_4 buffer containing eluents in case of Purospher, using MeOH.

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Table 5. Deviations of measured symmetry factors (ΔA) in case of all columns and solutes at $\log k = 1$ and 0.1.

ΔA for Ciprofloxacin [%]		ΔA for Vancomycin [%]		ΔA for *N*,*N*-dimethyl-aniline [%]	
$\log k = 1$	$\log k = 0.1$	$\log k = 1$	$\log k = 0.1$	$\log k = 1$	$\log k = 0.1$
−1.9 (PuA)	−1.9 (PuA)	−1.2 (LA)	−0.8 (LM)	1.4 (NA)	7.0 (NM)
−2.7 (PuM)	−2.7 (PuM)	−1.5 (PuM)	−1.9 (PM)	1.4 (SpA)	−0.7 (NA)
−7.3 (SyM)	−7.3 (SyM)	−2.3 (SyA)	−2.0 (PuA)	−1.0 (PM)	−0.8 (PuA)
−11.4 (SpA)	−11.4 (SpA)	−4.2 (PuA)	−2.0 (PuM)	−1.6 (SpM)	−0.9 (PA)
−11.7 (SpM)	−11.7 (SpM)	−4.4 (LbA)	−2.1 (LA)	−2.5 (PuM)	−1.2 (SpM)
−20.5 (NM)	−20.5 (NM)	−5.4 (LM)	−2.7 (LbA)	−2.9 (PA)	−1.5 (PuM)
−21.1 (SyA)	−21.1 (SyA)	−5.7 (LbM)	−5.2 (SyA)	−3.7 (LbA)	−4.0 (SpA)
−30.7 (PM)	−30.7 (PM)	−7.5 (PM)	−5.7 (LbM)	−5.6 (LbM)	−4.1 (SyA)
−36.1 (PA)	−36.1 (PA)	−9.1 (SyM)	−6.4 (SpA)	−5.8 (SyA)	−5.4 (LM)
−47.1 (LM)	−47.1 (LM)	−13.3 (SpM)	−7.3 (NA)	−6.6 (NM)	−6.0 (LbA)
−49.7 (LbA)	−49.7 (LbA)	−14.4 (SpA)	−7.7 (SpM)	−6.9 (SyM)	−8.3 (SyM)
−50.0 (LbM)	−50.0 (LbM)	−14.6 (NA)	−9.7 (NM)	−7.4 (PuA)	−8.4 (PM)
−52.0 (LA)	−52.0 (LA)	−19.2 (PA)	−13.8 (PA)	−9.1 (LA)	−12.5 (LbM)
−56.2 (NA)	−56.2 (NA)	−20.7 (NM)	−20.4 (SyM)	−13.5 (LM)	−15.4 (LA)

For example: PuM the deviation of measured symmetry factor values, which was measured using TEA buffer and KH_2PO_4 buffer containing eluents in case of Purospher, using MeOH.

Table 6. The measured retention factors in absence and presence of TEA in case of all tested stationary phases and solutes.

Column	Organic solvent used	Organic content of the mobile phase (v/v%)	Retention factor (k)		Deviation (%)
			Absence of TEA	Presence of TEA	
N,*N*-dimethyl-aniline					
LiChrospher	AcN	40	8.832	8.473	4.2
RP-18	MeOH	50	10.427	9.673	7.8
Nucleosil	AcN	27	10.448	9.906	5.5
100-C-8	MeOH	35	10.054	9.875	1.8
Prontosil	AcN	40	9.146	8.072	13.3
C18-AQ	MeOH	48	10.032	9.884	1.5
Chromolith	AcN	28	8.611	8.078	6.6
SpeedROD RP-18e	MeOH	35	10.102	9.843	2.6
Purospher	AcN	40	10.044	9.818	2.3
RP-18e	MeOH	52	10.173	9.923	2.5
Symmetry	AcN	40	10.177	10.148	0.3
C-18	MeOH	55	7.948	7.519	5.7
LiChrosorb	AcN	31	10.047	10.027	0.2
RP-select B	MeOH	49	6.905	6.369	8.4
Ciprofloxacin					
LiChrospher	AcN	13	10.061	6.233	61.4
RP-18	MeOH	24	10.19	8.736	16.6
Nucleosil	AcN	13	7.828	5.015	56.1
100-C-8	MeOH	24	6.424	4.948	29.8
Prontosil	AcN	14	6.362	3.975	60.1
C18-AQ	MeOH	27	9.967	8.013	24.4
Chromolith	AcN	9	7.911	5.95	33.0
SpeedROD RP-18e	MeOH	16	10.044	9.361	7.3
Purospher	AcN	13	5.083	4.687	8.4
RP-18e	MeOH	21	12.715	10.004	27.1
Symmetry	AcN	11	8.027	7.885	1.8
C-18	MeOH	19	10.796	6.358	69.8
LiChrosorb	AcN	9	12.826	9.795	30.9
RP-select B	MeOH	17	10.169	8.05	26.3
Vancomycin					
LiChrospher	AcN	8	9.938	5.219	90.4
RP-18	MeOH	16	10.018	7.332	36.6
Nucleosil	AcN	8	6.257	3.95	58.4

(*continued*)

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Table 6. Continued.

Column	Organic solvent used	Organic content of the mobile phase (v/v%)	Retention factor (k)		Deviation (%)
			Absence of TEA	Presence of TEA	
100-C-8	MeOH	14	8.082	5.535	46.0
Prontosil	AcN	7	16.164	7.831	106.4
C18-AQ	MeOH	17	9.933	8.026	23.8
Chromolith	AcN	6	7.134	6.403	11.4
SpeedROD RP-18e	MeOH	13	8.69	6.404	35.7
Purospher	AcN	8	10.14	5.058	100.5
RP-18e	MeOH	16	6.935	6.364	9.0
Symmetry	AcN	8	6.322	3.656	72.9
C-18	MeOH	14	7.875	6.308	24.8
LiChrosorb	AcN	7	5.654	3.671	54.0
RP-select B	MeOH	13	8.375	4.399	90.4

probably strongly acid silanol groups we also measured high changes. Finally, the TEA causes significant advance in a manner similar to silica A based columns, because these strongly acid silanols are able to form strong interactions with solutes. These interactions cause zone broadening. Blocking these groups the deviation of the theoretical plate numbers and symmetry factors progresses. From the change of ΔN and ΔA, it can be also seen that the low molecular weight and small basic group containing solutes, such as *N*,*N*-dimethyl-aniline (pKa = 5.06) can be used as only primary screening for evaluation of columns. The compounds with higher basicity (higher pKa value), such as Ciprofloxacin (pKa = 9.08) could give better insight into the surface heterogeneity (polarity) of reversed phase columns.

DISCUSSION

According to our results, the silanol activity could be detected on all tested stationary phases. Using TEA containing mobile phase, the retention factors decreased in all cases (Table 6). The change of retention factors was different in MeOH and AcN. From the measured retention factors and their change, it can be seen that the columns cannot be grouped. We cannot declare in advance for which columns the change of retention factors will be the smaller or higher in the case of a solute. Namely, the pore diameter, the diffusion path, the surface of stationary phases, the size of solutes, the

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composition of eluent, the various effects and interactions jointly condition the retention of solutes.

The sign of the silanol activity was also the improvement of symmetry factor using TEA containing eluents. From measured data, it can be seen that the regular behavior, that is to say at high retention ($\log k = 1$), the value of symmetry factor and theoretical plate number advanced as compared with low retention, in consequence of zone broadening and overloading effects.

For even the high purity silica based stationary phases, the TEA decreased the effect of silanol groups, which caused the improvement of N and A_S as compared to mobile phases without TEA (Tables 2 and 3).

In way of theoretical plate number, we measured the highest values for Symmetry in cases of Ciprofloxacin and *N*,*N*-dimethyl-aniline, whether the eluent contained TEA or did not. This can be explained by the good coverage of the column. In the case of Vancomycin, the symmetry did not seem to be so good, because we got only half of the measured values compared to Ciprofloxacin and *N*,*N*-dimethyl-aniline. Its high molecular weight may be the cause of this. On account of high size, the molecule is not able to penetrate into the small pores (size exclusion effect), so the hindered pore diffusion and the significantly decreased mass transfer between the mobile and stationary phases cause decrease of the theoretical plate number. For determination of such high molecular weight compounds, the Chromolith SpeedROD seemed to be the best, whether the eluent contained TEA or did not. The pore structure of Chromolith SpeedROD (monolithic silica) is more favorable for separation of higher molecular weight compounds. The mean diameter of diffusion pores is 13 nm and the diffusion path is lower than in cases of particle based stationary phases.

For Ciprofloxacin and *N*,*N*-dimethyl-aniline in the case of Symmetry, and for Vancomycin in the case of Chromolith SpeedROD, the measured theoretical plate numbers approached the highest possible theoretical plate number values given by the manufacturer. The value of symmetry factor was better for *N*,*N*-dimethyl-aniline than for Ciprofloxacin and Vancomycin in all columns used. The worst symmetry factors were measured for Ciprofloxacin in all cases. The pKa of the basic group of Ciprofloxacin is about 9. At pH = 3 its protonated form is present, so the ion-exchange interaction with highly acidic dissociated silanol groups is probable. The deterioration in the peak symmetry may be explained by the effect of unreacted silanols in the small pores as well. The relatively small Ciprofloxacin can penetrate into these pores and desorption rate is lowered by strong polaric interaction and pore restricted diffusion. The peak symmetry for Vancomycin was better and acceptable ($1.0 < A_S < 1.8$). Its size (1449 g/mol) is much higher and, so, was excluded from small pores. The effect of the small pores on symmetry factor can be seen on Chromolith SpeedROD columns. The measured theoretical plate numbers

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approach the available highest possible value given by the vendor, but the peak symmetry was just acceptable at $\log k = 0.1$ ($1.6 < A_S < 1.8$). At $\log = 1$, the improvement in peak symmetry was significant ($1.1 < A_S < 1.3$), which can be explained by the overloading effect of the unreacted, but accessible silanol groups.

The retention, the theoretical plate number, and the peak symmetry have a complex function in pore structure and surface chemistry. Based on our results, a universal test might not be advised for selection of stationary phases for a given practical application. The multiple polar groups and size of solutes determine the possible interactions with unreacted silanols. Neither the unreacted, but accessible silanols, nor the penetration of solutes into the small pores can be predicted from given data by the manufacturer and from the results, which were obtained using small and simple solutes for column test.

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