Universal procedure for the assessment of the reproducibility and the classification of silica-based reversed-phase packings
II. Classification of reversed-phase packings

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Abstract

The tools developed for the testing of the reproducibility of several commercial packings have been used to study the differences and similarities of over 50 different commercial reversed-phase packings. The tests employed allow a characterization of the hydrophobicity of packings, the silanol activity at neutral and acidic pH, a differentiation between classical packings and packings with an embedded polar functional group, and a differentiation between C_{18} and C_8 packings. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Today, a large number of different reversed-phase packings is available to the chromatographer. However, the selection of the most suitable material for a particular application is still a mystery. It is difficult to obtain suitable information to select packings that are similar to each other, for example for the purpose of selecting an alternative supplier for a column used in an important assay. Conversely, during method development it is often desirable to select packings with significantly different properties. In this paper, we will use the techniques employed for the quality control of a range of packings [1] to examine the diversity of a large number of reversed-phase packings. The ultimate purpose of this exercise is a classification of these packings. While a complete characterization of a packing is still not possible with the simple tools used here, a range of relevant properties such as hydrophobicity and silanol activity are accessible.

Numerous studies of packing selectivity fill the literature. Many of these studies focus only on a few analytes or examine only a very limited number of columns, while a few of them either use a large set of analytes or probe a representative number of columns to be able to generalize the findings. For example, a typical example of the column selection problems encountered in the pharmaceutical industry can be found in the publication by Verne-Mismer et al. [2]. The analytes were a cardioselective anti-oxidant and its impurities. The authors concluded that for their separation problem ''base-deactivated'' columns worked best.

An example of a broader scope is the study by Verhoort and coworkers [3–5]. The initial goal [3]

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was an understanding of the retention behavior of basic drugs. The investigation then expanded [4,5] into a characterization of different packings using principal component analysis of the retention of a selected group of basic pharmaceutical compounds under several mobile phase conditions. The authors established that the pH of the mobile phase affects the peak shape for some packings, but not for others. The authors also found similarities between different packings included in their comparison.

Sýkora et al. [6] studied the interaction of basic compounds with the surface of modern reversed-phase packings over a broad pH range. They reported that the "ability of a basic solute to reveal non-hydrophobic interactions depends mainly on its dissociation constant. The strongly basic compounds are the most sensitive probes." Different packings behaved quite differently, and the authors were capable of selecting the best packings for the analysis of basic compounds.

McCalley evaluated the performance of reversed-phase columns for the analysis of basic analytes in a series of papers spanning over more than a decade [7–13]. Some studies were dedicated to the understanding of the stereochemistry of the analytes [9], others to the choice of mobile phase conditions [10,11] or sample load [13]. In Ref. [12], several reversed-phase packings were compared to each other using a range of basic analytes.

It can be shown that basic analytes like tricyclic antidepressives are retained on underivatized silica at pH 7 via an ion-exchange mechanism [14,15]. In order to understand the influence of variable levels of silanols, Neue and Murphy prepared reversed-phase packings with different levels of derivatization and studied the peak shape of basic organic analytes as a function of the degree of derivatization [14]. They found that excellent peak shapes could be obtained for tricyclic antidepressants at a low level of derivatization, at a ligand density below 0.5 $\mu mol/m^2$. It was especially surprising to find that silica itself gave symmetrical peaks for these compounds, while high levels of derivatization with or without endcapping resulted without fail in strongly tailing peaks. Most details of this investigation can be explained best by the presence of two adsorption sites with different kinetics, as outlined for example in Ref. [16].

Many researchers made attempts to classify pack-ings based on their retention behavior for selected substances. Some of the tests developed for this purpose can be categorized as simple use tests, while other assays are designed to measure the properties of a reversed-phase packing in a more fundamental fashion.

The test developed by Sander and Wise [17,18] belongs to the first category. Their test mixture, which is available as a NIST standard reference material (SRM 869), comprises a set of aromatic hydrocarbons, whose elution order depends on the chain spacing of the bonded phase. With this test it is possible to categorize C_{18} packings into "monomeric" packings, i.e. with a surface coverage of <3.5 $\mu mol/m^2$ and "polymeric" phases, with a higher surface coverage. The test is primarily useful for the assessment of the value of reversed-phase packings for the separation of polynuclear aromatic hydrocarbons.

The chromatographic test developed by Engelhardt and coworkers [19–23] is representative of the methods designed to assess the fundamental properties of a reversed-phase packing. A group of compounds that include toluene, ethylbenzene, phenol, benzoic acid, aniline, *N,N*-dimethylaniline and later *p*-ethylaniline are injected onto C_{18} and C_8 columns in a methanol–water (49:51, w:w) eluent. The compounds were carefully selected to yield information about the hydrophobic, polar and silanophilic interactions of the packing. Generally, the retention of toluene and ethylbenzene is a measure of the hydrophobicity of a packing, while the retention and the tailing factor of the bases is a measure of the silanophilic interaction. Only limited information is available on the reproducibility of this test method. The test was also subjected to a chemometric analysis [23] that demonstrated that the retention of *N,N*-dimethylaniline correlated unexpectedly with the retention of the hydrophobic solutes and does not measure primarily the silanophilic properties of the packings.

Tanaka and coworkers [24,25] published a comprehensive chromatographic characterization procedure for reversed-phase packings. The characterization scheme includes compounds that are designed to measure the hydrophobic retentivity of the packing, the steric selectivity (in analogy to the Sander–Wise test [17,18]), the hydrogen bonding properties, and the ion-exchange properties of the packing at neutral

and acidic pH of the mobile phase. Three independent chromatographic tests are employed for this purpose. For subsets of the data, a good correlation between physical measurements and chromatographic test results were shown. Unfortunately, the study does not include any data on the reproducibility of the procedure nor any indication for the batch-to-batch reproducibility of the packings studied.

A fairly recent publication by Eymann [26] describes a battery of tests that include neutral analytes, amines, chelators, and acids. Once again, no data are presented that indicate the errors of the tests nor the reproducibility of packings. Another drawback of the procedure is a lack of internal reference compounds in each test, i.e. all analytes in each test are of the same category. The relative retention of analytes of different types is a better tool for the assessment of the properties of a packing.

Sándi et al. [27] have attempted to "develop a generally acceptable, unified testing procedure for all types of RP column packings with different pore sizes and containing different ligands". Test solutes comprise simple aromatic compounds with different solvatochromic properties (*p*-nitrophenol, phenol, benzyl alcohol, aniline, caffeine, *o*-toluidine, acetophenone, benzyl cyanide, anisole, methyl benzoate, ethyl benzoate, toluene and ethylbenzene), and retention times are obtained using an unbuffered water–acetonitrile gradient. The results are analyzed using principal component analysis. Some 50% of the total variance can be attributed to hydrophobic interaction, 32% due to the interaction of basic analytes (proton acceptors) and 13% to an interaction that the authors interpreted as hydrogen bonding. The results were compared to physical characteristics of the analytes, such as Van der Waals volume, polarizability, dipolar properties and hydrogen donor and acceptor characteristics. Ultimately, the columns could be characterized based on the principal components. Ref. [28] is a related study focusing on wide-pore reversed-phase packings. Refs. [29] and [30] expand the studies to other packings, including SymmetryShield RP$_8$ and RP$_{18}$ packings, and to a range of 34 simple analytes. Principal component analysis was used again to classify the different packings.

The characterization of a range of different stationary phases was attempted by Valko et al. [31]. The analysis was based on the chromatographic hydrophobicity index previously described in reference [32] and covered a broad range of packings, from C$_{18}$ packings to diol and NH$_2$ packings.

Recently, a comparison of different test methods developed for C$_8$ and C$_{18}$ packings was published by Claessens et al. [33]. They concluded that the hydrophobicity measurements of different tests are in good agreement, provided that one uses absolute retentions instead of relative retentions. However, significant differences between the tests were observed for the assessment of silanophilic activity. Several very good reviews summarize the development of the thinking about the activity of silanols and their influence on the retention of polar and basic analytes [34–37]. They are highly recommended to the interested reader.

In the previous paper [1] we have shown that the techniques described there are useful for the establishment of the batch-to-batch reproducibility of a single packing material. Since the evidence showed that at least the presence and activity of surface silanols can be measured unequivocally using a single type of packing, it became interesting to apply the technique to a range of different packings. While the major focus was to establish a broad measure of silanol activity at neutral pH, the range of compounds included in the test procedure at neutral pH made it possible to assess other parameters as well. This allows for an additional classification of packings; for example, the hydrophobicity of a packing can be measured via the retention of neutral analytes. In addition, the newer generation packings with an embedded polar functional group can be identified chromatographically with little difficulty. The errors of the method were an important aspect of its use in the quality control of a packing and are covered in detail in the previous paper [1]. The results of the method obtained with over 50 different reversed-phase packings are discussed in the current paper. Furthermore, a second test was developed for the measurement of silanol activity at acidic pH, and the results are compared to those obtained at neutral pH.

2. Experimental

The analytes used in this study (Fig. 1) were uracil (16 mg/l) as marker for the column dead volume, naphthalene (60 mg/l) and acenaphthene (200 mg/l)
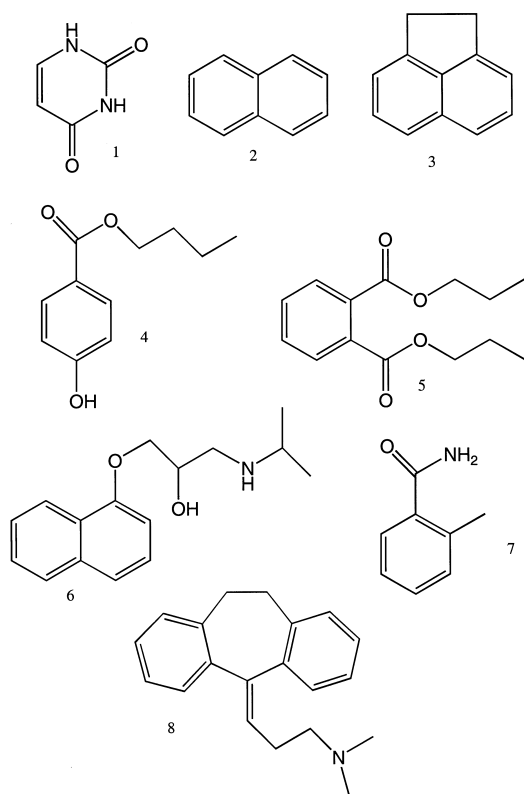
Fig. 1. Structures of test compounds: 1=uracil, 2=naphthalene, 3=acenaphthene, 4=butylparaben, 5=propranolol, 6=toluamide, 7=amitriptyline.

as hydrophobic markers, butylparaben (20 mg/l) and dipropylphthalate (340 mg/l) as polar probes, and propranol (400 mg/l) and amitriptyline (100 mg/l) as basic probes at pH 7.0. At pH 3.5, the samples were chlorpheniramine maleate (500 mg/l), propranolol (500 mg/l) and o-toluamide (700 mg/l). Uracil, propranolol, dipropylphthalate, amitriptyline, o-toluamide, potassium phosphate monobasic and potassium phosphate dibasic, trihydrate were obtained from Aldrich, butylparaben, naphthalene, acenaphthene and chlorpheniramine maleate were obtained from Sigma.

The mobile phase for the test at neutral pH consisted of 35% of a 20 mM K$_2$HPO$_4$–KH$_2$PO$_4$ buffer, pH 7.00 and 65% methanol. For the test under acidic conditions we used 20% of a 50 mM H$_3$PO$_4$–KH$_2$PO$_4$ buffer, pH 3.00 and 80% acetonitrile.

Data analysis was performed on a Microsoft EXCEL spreadsheet. The cluster analysis was performed using Data Desk Professional from Odesta, USA.

Table 1 contains a list of the columns used in this study. The number in the second column is used to identify columns in those charts, where a direct identification was necessary. The third column contains a comment on a special property of the packing. All columns were tested as received from the manufacturer and no other analysis was performed on these columns prior to the tests described. It is important to carry out these tests on new columns to avoid unusual results caused by column aging.

3. Classification of reversed-phase packings

In the following, we will examine several different parameters that we found to be useful in the classification of the different packings. In some cases, related test parameters provided us with similar information.

The test at neutral pH contains the compound uracil as a marker of the column breakthrough volume, naphthalene and acenaphthene as neutral hydrophobic compounds, propranolol and amitriptyline as basic analytes, and dipropylphthalate and butylparaben as probes for hydrophilic interactions. At acidic pH, the basic probes were propranolol and chlorpheniramine and the neutral reference compound was toluamide. Originally, the unretained peak marker at acidic pH was maleate, the counterion in the chlorpheniramine sample. Maleate, however, was retained on packings that contained a basic functional group. Therefore, we used the retention of uracil at neutral pH to determine the retention factors of the test compounds used at acidic pH.

For a comparison of the different packings, a range of parameters can be considered: the retention times, the retention factors or the relative retention values between different analytes. In the following discussion, those parameters have been selected that provide that largest amount of information. Let us first explore a very simple relationship, the retention factor of the neutral analytes naphthalene and acenaphthene measured at neutral pH (Fig. 2). For these purely hydrophobic and structurally related compounds, a straight line is obtained for all packings

Table 1
List of packings and their code numbers on the charts

| Manufacturer/supplier	Packing	Code	Comments
Waters	Symmetry C_8	1	High-purity silica
	Symmetry C_{18}	2	High-purity silica
	Symmetry300 C_{18}	52	High-purity silica
	SymmetryShield RP8	3	Embedded polar group
	SymmetryShield RP18	4	Embedded polar group
	Nova-Pak C_8	5	
	Nova-Pak C_{18}	6	
	μBondapak C_{18}	7	
	Waters Spherisorb ODS-2	8	
YMC	YMC Basic	9	
	YMC J'Sphere L80	10	
	YMC J'Sphere M80	11	
	YMC J'Sphere H80	12	
	YMC-Pack Pro C_{18}	44	High-purity silica
Hewlett-Packard	Zorbax ODS	53	
	Zorbax Rx C_8	13	High-purity silica
	Zorbax Rx C_{18}	15	High-purity silica
	Zorbax SB C_8	14	High-purity silica
	Zorbax SB C_{18}	16	High-purity silica
	Zorbax Eclipse XDB C_8	17	High-purity silica
	Zorbax Eclipse XDB C_{18}	49	High-purity silica
Alltech	Alltima C_8	19	
	Alltima C_{18}	18	
	Platinum C_{18}	50	
	Platinum EPS	54	
Keystone	Spectrum	20	Embedded polar group
	Prism	47	Embedded polar group
Supelco	Supelcosil LC DB-C_8	21	
	Supelcosil LC DB-C_{18}	22	
	Supelcosil LC-ABZ	55	Embedded polar group
	Supelcosil ABZ+Plus	41	Embedded polar group
	Discovery RP Amide 16	42	Embedded polar group
Macherey–Nagel	Nucleosil C_{18}	23	
Merck	LiChrospher Select B	24	
	LiChrosorb Select B	25	
	Purospher RP18	27	Amine endcapping
	Purospher RP18e	48	High-purity silica
Hypersil	Hypersil ODS	29	
	Hypersil BDS C_8	30	
	Hypersil BDS C_{18}	31	
	Hypersil Elite C_{18}	32	High-purity silica
	Hypurity Elite C_{18}	51	High-purity silica
Akzo Nobel	Kromasil C_8	33	High-purity silica
	Kromasil C_{18}	34	High-purity silica
GL Sciences	Inertsil C_8	35	High-purity silica
	Inertsil ODS-2	36	High-purity silica
	Inertsil ODS-3	37	High-purity silica
Chemical Inst. of Japan	L-Column ODS	38	High-purity silica
Nomura Chemical	Develosil ODS-UG-5	39	High-purity silica
Phenomenex	Prodigy C_8	45	High-purity silica
	Prodigy C_{18}	40	High-purity silica
	Luna C_{18}	46	High-purity silica
Toyo Soda	TSK-Gel ODS 80TS	43	High-purity silica
Shiseido	Capcell Pak C_{18}	56	Polymer coated
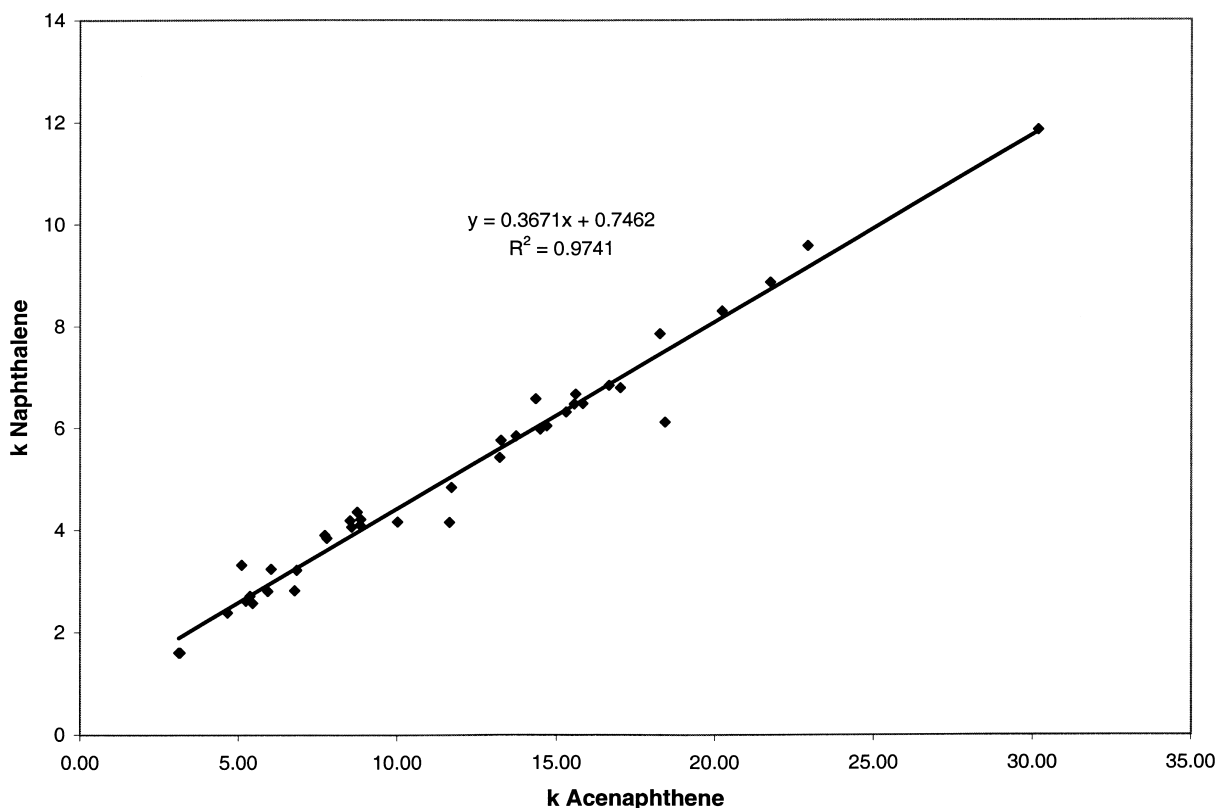
Fig. 2. Plot of the retention factor of naphthalene vs. the retention factor of acenaphthene. The correlation coefficient is 0.9741.

tested with a correlation coefficient of 0.9741. Selectivity differences are small, and no general patterns can be found that would distinguish different groups of packings from each other. One can conclude that the choice of the hydrophobic reference compound needed for further analysis is rather immaterial. On the other hand, the retention factor of acenaphthene varies from about 3 to over 30. Since the retention factor of a hydrophobic compound can be used as a measure for the hydrophobicity of the packing, we selected acenaphthene due to its higher retention factor for this purpose. It is also used as a convenient reference factor for the calculation and the comparison of relative retention values. However, a word of caution needs to be included here. We need to recognize that the correlation between the retention factors of different compounds is not perfect. This is not due to measurement errors, but is caused by subtle selectivity differences of the different packings. This means that the choice of the reference

compound(s) can influence the position of a packing on a scale used for the assessment of the hydrophobicity of packings. On the other hand, due to the fact that the purely hydrophobic selectivity differences are rather subtle, scales based on different analytes will to a large extent agree with each other [33].

Next we examine a plot of the relative retention of dipropylphthalate and acenaphthene vs. the relative retention of naphthalene and acenaphthene (Fig. 3) obtained at 65% methanol. The x-axis is primarily a measure of hydrophobic selectivity, in a similar way to the tools used by Sander and Wise [17,18]. A line is drawn through the three YMC J'Sphere packings, which are based on the same silica but contain different C_{18} coating levels. The YMC J'Sphere packing with the highest coating level is found on the left-hand side of the line. C_8 type packings, marked with a circle, are on the right-hand side, while most C_{18} packings are found on the left-hand
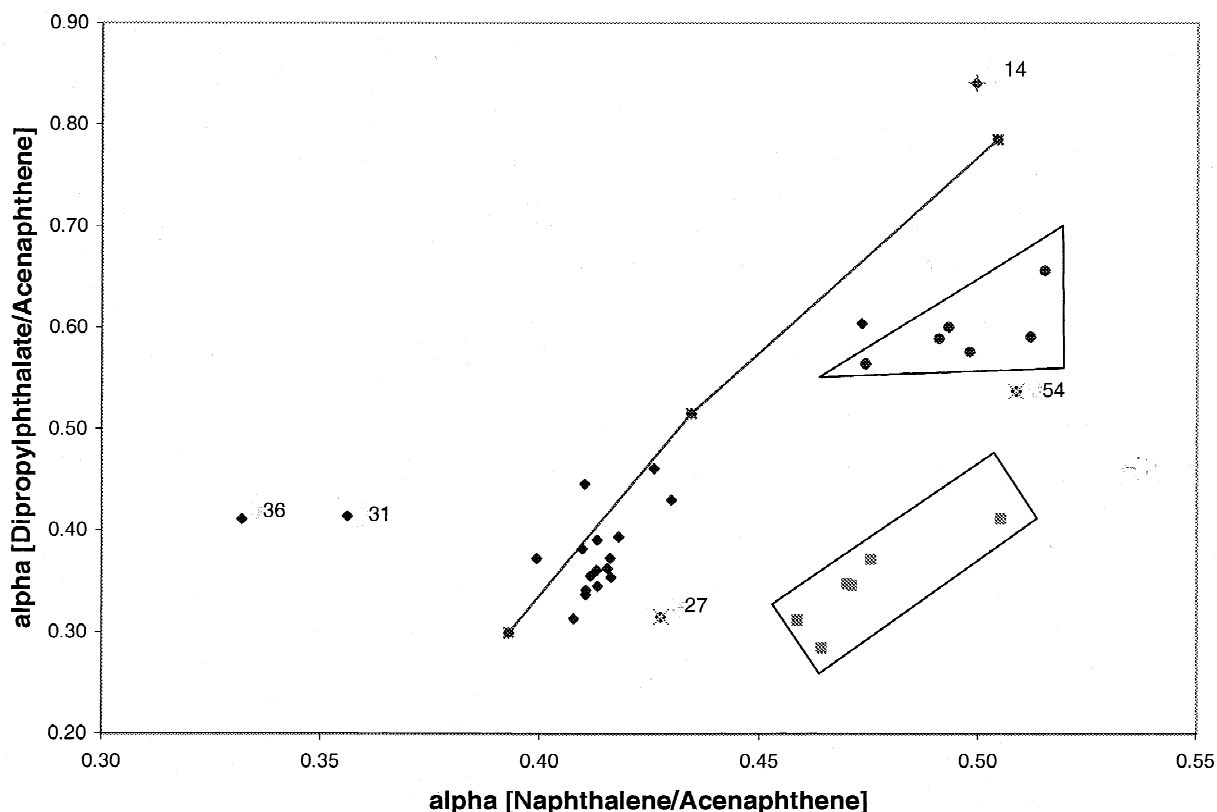
Fig. 3. Plot of the relative retentions of dipropylphthalate/acenaphthene vs. naphthalene/acenaphthene. (squares)=packings with incorporated polar functional groups, (circles)=C_8 packings, +=packing based on a sterically hindered silane, x=packings containing a basic functional group, (triangle)=Capcell Pak C_{18}. The line connects the three YMC J'Sphere packings.

side. Inertsil ODS-2, no. 36, and Hypersil BDS C_{18}, no. 31, are found on the far left of the graph. Nevertheless, a unique differentiation between a C_8 and a low coating of a C_{18} packing is not possible with these two parameters. At the right top of the graph, the Zorbax SB C_8 packing, no. 14, is found, which is based on a unique sterically protected silane [38]. Away from the general pattern of C_8 and C_{18} packings, two stationary phases are marked with an X: one of them, Purospher RP_{18}, no. 27, is known to be endcapped with an amino-functional silane [29]. The other one is Alltech Platinum EPS C_{18}, no. 54, whose endcapping procedure is not known to us. The group of packings marked by a square are modern packings that use the incorporation of a polar functional group into the chain as a tool for further suppression of silanol groups [39–42]. Capcell Pak C_{18} is marked with a triangle. It is based on a surface

polymerization of a silicon polymer and therefore different from all other packings in this study. The grouping of the different packings observed in this graph encourages a further interpretation of the selectivity patterns found.

If the relative retention of butylparaben/acenaphthene is plotted vs. the relative retention of dipropylphthalate/acenaphthene (Fig. 4), most packings exhibit a very similar trend. A straight line can be drawn through the majority of packings with a correlation coefficient of 0.8975. This means that for the majority of packings, both parameters measure the same property. A discrimination between C_8 and C_{18} packings is not possible. However, this is not true for the packings with an incorporated polar functional group (squares) and Capcell Pak C_{18} (triangle). They exhibit a different selectivity pattern for these polar compounds, away from the trend of
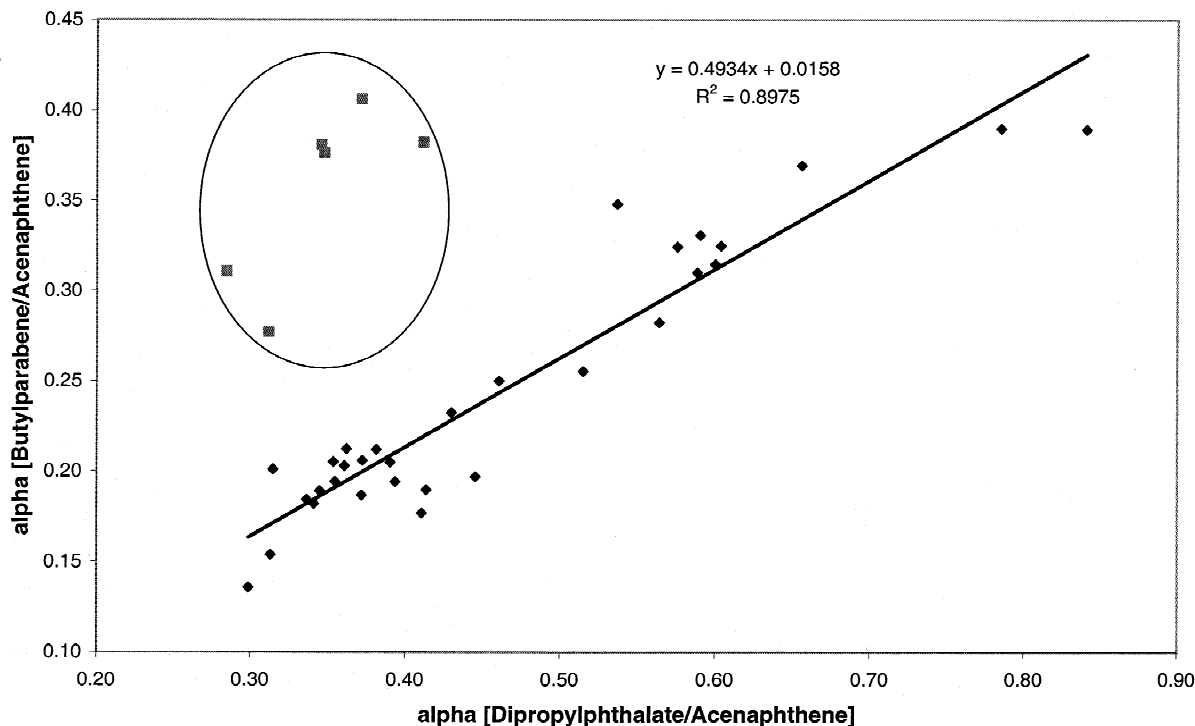
alpha Values of Polar Compounds at Neutral pH

Fig. 4. Plot of the relative retentions of butylparabene/acenaphthene vs dipropylphthalate/acenaphthene. The correlation coefficient for standard C_8 and C_{18} packings is 0.8975. The packings enclosed with a circle and marked with a square on the upper left of the graph are packings with an incorporated polar functional group. The triangle is Capcell Pak C_{18}.

the classical C_{18} and C_8 packings. The underlying factor is the relative retention between butylparaben and dipropylphthalate, shown in Table 2. The typical value for all regular reversed-phase packings is less than 0.59; the value for packings with an incorporated polar group is larger than 0.85. The value for Purospher RP_{18}, endcapped with an amino silane, is 0.639. Therefore, the relative retention between butylparaben and dipropylphthalate can be used without difficulty to distinguish between classical C_8 and C_{18} packings and packings with an incorporated polar functional group, including packings with unusual endcapping procedures.

There is little question that the retention of acenaphthene can be used as a measure of the hydrophobicity of a packing. However, it is not a priori evident, if the relative retention values between different basic analytes and a neutral reference

compound are telling a consistent story. Claessens et al. [33] found significant differences between the results obtained with different tests, which generally use the relative retention between a basic analyte and a neutral analyte as a measure of silanol activity. Since our test procedure uses several structurally quite different basic analytes at neutral and acidic pH, it is possible to test whether different basic analytes give similar results under a given set of test conditions and if different test conditions give similar results. At acidic pH, the test compounds were propranolol and chlorpheniramine, and at neutral pH propranolol and amitriptyline had been selected. The selection of propranolol under both conditions also allows for a comparison of the information obtained at both pH values.

The relative retention of basic analytes relative to the neutral reference compound toluamide under

Table 2
Relative retention of butylparaben and dipropylphthalate

Name	Butylparaben/dipropylphthalate
Inertsil ODS-2	0.431
Zorbax SB-C_{18}	0.442
YMC J'Sphere H80	0.455
Hypersil BDS C_{18}	0.459
Zorbax SB C_8	0.463
Purospher RPe 18	0.492
Inertsil ODS-3	0.493
YMC J'Sphere M80	0.496
YMC J'Sphere L80	0.497
Zorbax Eclipse XDB C_8	0.501
Zorbax Eclipse XDB C_{18}	0.502
Inertsil C_8	0.524
Supelcosil LC DB-C_{18}	0.525
Symmetry C_8	0.527
Symmetry C_{18}	0.534
Alltech Platinum C_{18}	0.538
Phenomenex Luna C_{18}	0.540
TSK-Gel 80Ts	0.542
Hypersil Elite C_{18}	0.548
Kromasil C_{18}	0.548
Phenomenex Prodigy C_{18}	0.553
YMC Pack Pro C_{18}	0.556
Phenomenex Prodigy C_8	0.560
YMC Basic	0.563
Develosil ODS UG 5	0.563
Kromasil C_8	0.564
Symmetry300 C_{18}	0.580
Hypersil HyPurity Elite C_{18}	0.586
Purospher RP 18	0.639
Alltech Platinm EPS C_{18}	0.649
Capcell Pak C_{18}	0.857
SymmetryShield RP18	0.889
SymmetryShield RP8	0.929
Spectrum	1.084
Supelcosil LC-ABZ-Plus	1.093
Prism	1.093
Discovery RP Amide 16	1.102

acidic test conditions is shown in Fig. 5. All 49 tested packings exhibit a very high correlation, $r^2 = 0.9595$, and no specific selectivity patterns are observed. Such a high correlation – and consequently lack of packing selectivity – is rather unexpected. At least for the basic analytes used here, propranolol and chlorpheniramine, despite their structural differences shifts in selectivity from packing to packing are small. Since the study included the entire range of commercially available packings, endcapped and unendcapped packings, packings with an incorporated polar functional group, based on

monofunctional, multifunctional and sterically hindered silanes, such a finding is remarkable. A consequence of this finding is the fact that an assessment of the underlying parameter is possible with either compound.

Fig. 6 shows a comparison of the base–neutral α values measured at neutral pH. The relative retention between propranolol and acenaphthene is plotted vs. the relative retention between amitriptyline and acenaphthene for 34 different commercial packings. The correlation coefficient of 0.83 indicates that for the most part the relative retention between the
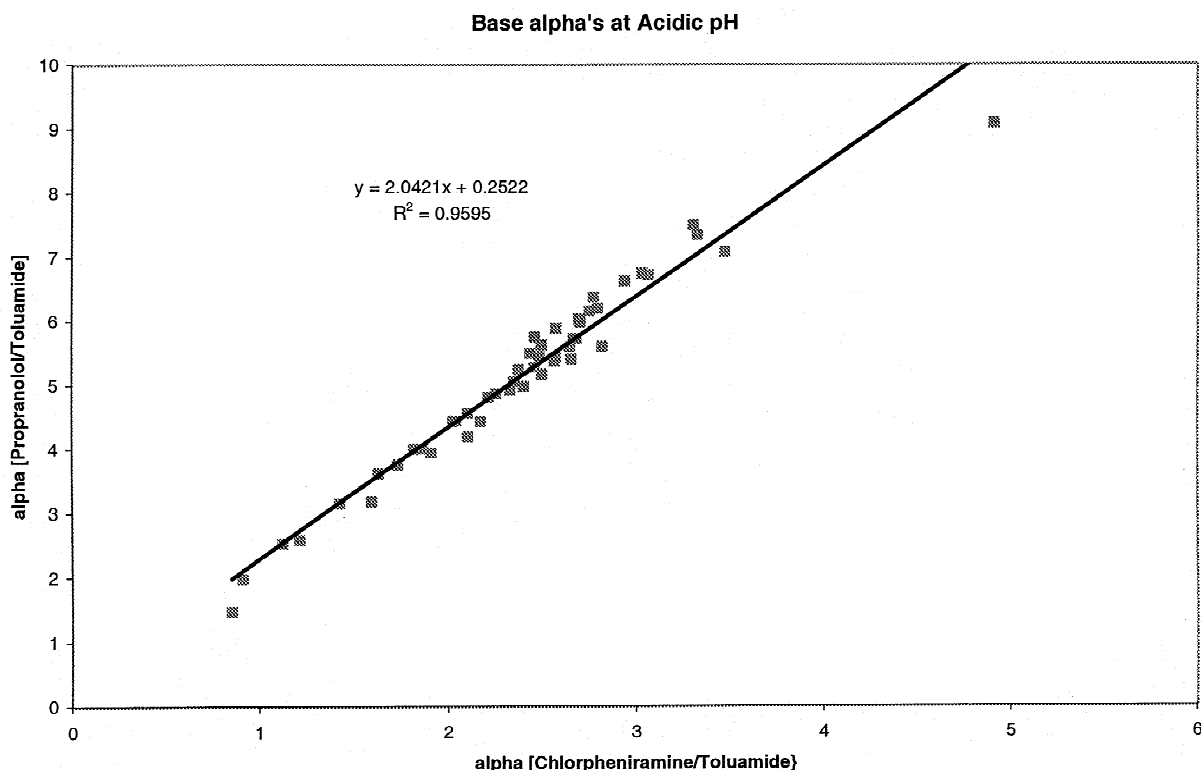
Base alpha's at Acidic pH

Fig. 5. Plot of the relative retention of propranolol/toluamide vs. the relative retention of chlorpheniramine/toluamide; a consistent pattern is obtained with a correlation coefficient of 0.9595.

different bases and the neutral reference compound tell a similar story about the silanol activity of a packing. Considering the breadth of the packings, with different surface chemistries ranging from multifunctional silanes to sterically protected mono-functional silanes, different surface coverage, different pore structures and different silica purity, this correlation is quite good. This corroborates the findings of the batch-to-batch reproducibility studies: one generally finds a good correlation between the relative retention of both basic analytes.

Nevertheless, a further examination of the fine structure of this graph is warranted. On the left side of the graph, a group of packings can be found that contain an amide or carbamate functional group. Clearly, the incorporation of the polar functional group changes the selectivity pattern of the packings. In addition, a line can be drawn through the group of classical C_8-type packings, which includes LiChros-

pher Select B and LiChrosorb Select B. The correlation coefficient between both base–neutral α values is 0.9569. The only C_8 packings that do not fit this general pattern are Zorbax Rx C_8 and Zorbax SB C_8. Both are prepared using a unique silane with sterically hindered side chains. Similarly, a line with a correlation coefficient of 0.9649 can be drawn through the C_{18} packings used in this study. Consequently, the basic analytes selected for the test at neutral pH discriminate between C_{18} packings, C_8 packings and packings with a polar functional group with a high degree of certainty.

Since an excellent correlation between different bases has been obtained at acidic pH, and good correlations were still found at neutral pH, the next interesting question is, whether there is a correlation between the results obtained at both pH values. Since propranolol was used as a test compound under both conditions, the relative retentions between proprano-
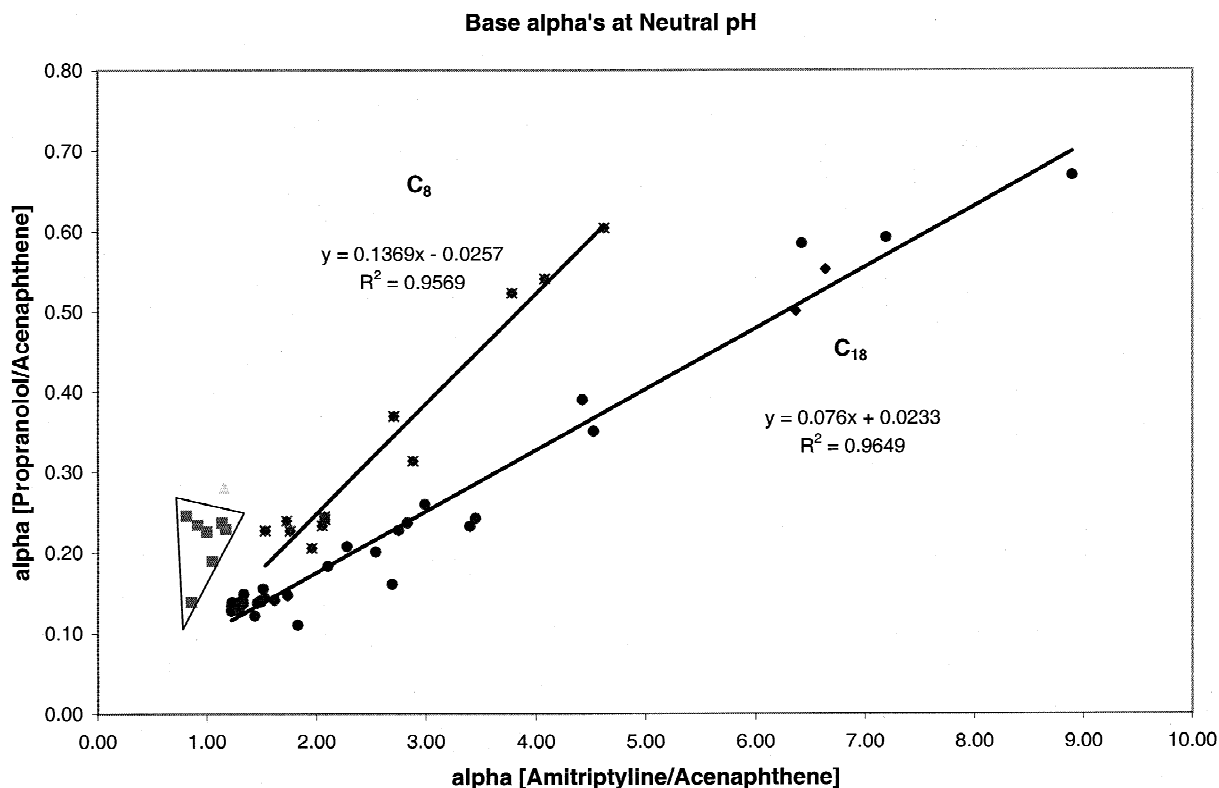
Base alpha's at Neutral pH

Fig. 6. Plot of the relative retention between propranolol and acenaphthene vs. the relative retention between amitriptyline and acenaphthene at neutral pH. Trend lines can be drawn through the classical C_8 packings (cross) and the classical C_{18} packings (circles). The packings in the left corner (squares) are packings with an incorporated polar functional group. The triangle is Capcell Pak C_{18}.

lol and the neutral reference compounds were plotted against each other. Yet no correlation is found ($r^2 = 0.003$). The relative influence of silanols on retention at acidic and neutral pH is different for the different packings, which means that the activity of silanols is a function of multiple parameters, a fact that has been discussed extensively in the literature (e.g. [37,43–46]). A good correlation would have indicated that pH alone is the determining parameter for silanol activity, and that other influences such as metal content of the silica and surface deactivation procedures play a secondary role only. This is clearly not the case. This limits our ability to assign simple universal quality criteria to any single packing.

The surface of a silica before bonding is populated by single and geminal silanols. Some of the silanols are close enough to each other to form –O–H· · ·O bridges. The population of the various silanols depends on the thermal history of the silica and/or

rehydroxylation processes [46]. Another important influence on the activity of silanols is the local chemical environment: metal impurities in the silica matrix affect the acidity of silanols on the surface of the silica [43–45]. The bonding process removes a large portion of the surface silanols, but the effectiveness of the bonding process varies significantly from packing to packing. All of these factors contribute to the activity of surface silanols and certainly to the activity pattern as a function of the pH.

These findings corroborate the results of the comparison of different silanol activity tests obtained by Claessens et al. [33]. One needs to recognize that the details of the test conditions determine what the test measures. Ohtsu et al. [25] discriminate between the measurement of ion-exchange capacity at neutral and acidic pH and the hydrogen bonding capacity. Our results support such a differentiation, at least as a function of the pH of the mobile phase. It should

be noted that the silanol activity tests used by Claessens et al. [33] were carried out using an unbuffered mobile phase, while our tests are performed at a carefully controlled pH. Considering the general practice to accomplish chromatographic separations of ionizable compounds using a buffered mobile phase, it appears to be more sensible to characterize different packings using a buffered mobile phase as well.

For a characterization of the different properties of the packings, we plotted the natural logarithm of the relative retention between propranolol and toluamide at pH 3 vs. the natural logarithm of the retention factor of acenaphthene in Fig. 7. The graph shows no relationship between both parameters, and no relationship is expected. One may consider the y-axis as a measure of silanol activity at acidic pH, while the x-axis is a measure of the hydrophobicity of the packing. Such a chart emphasizes the difference between different packings and allows for a judg-

ment of the similarities and dissimilarities of different packings. Consequently, the left-hand part of the chart is occupied by C_8 type packings, and the right-hand side by C_{18} type packings. Exceptions are μBondapak C_{18}, no. 7, and Platinum C_{18}, no. 50. Both are packings with a low C_{18} coating level. Also, Capcell Pak C_{18}, no. 54, is found among the group of C_8 packings. Among C_{18} packings, those with a lower specific surface area and a larger pore size, such as Hypurity Elite C_{18}, no. 51, are found on the left side of the cluster of other C_{18} packings. YMC Basic, which is prepared from an undisclosed mixture of short-chain silanes, is located on the far left of the chart. A straight line is drawn between the data points for packings 10, 11, and 12. These three packings, YMC J'Sphere L80, M80 and H80, are based on the same silica, but are prepared with different C_{18} coating levels. Below this line, several packings are found with an incorporated polar functional group: SymmetryShield RP_8, no. 3, Symme-
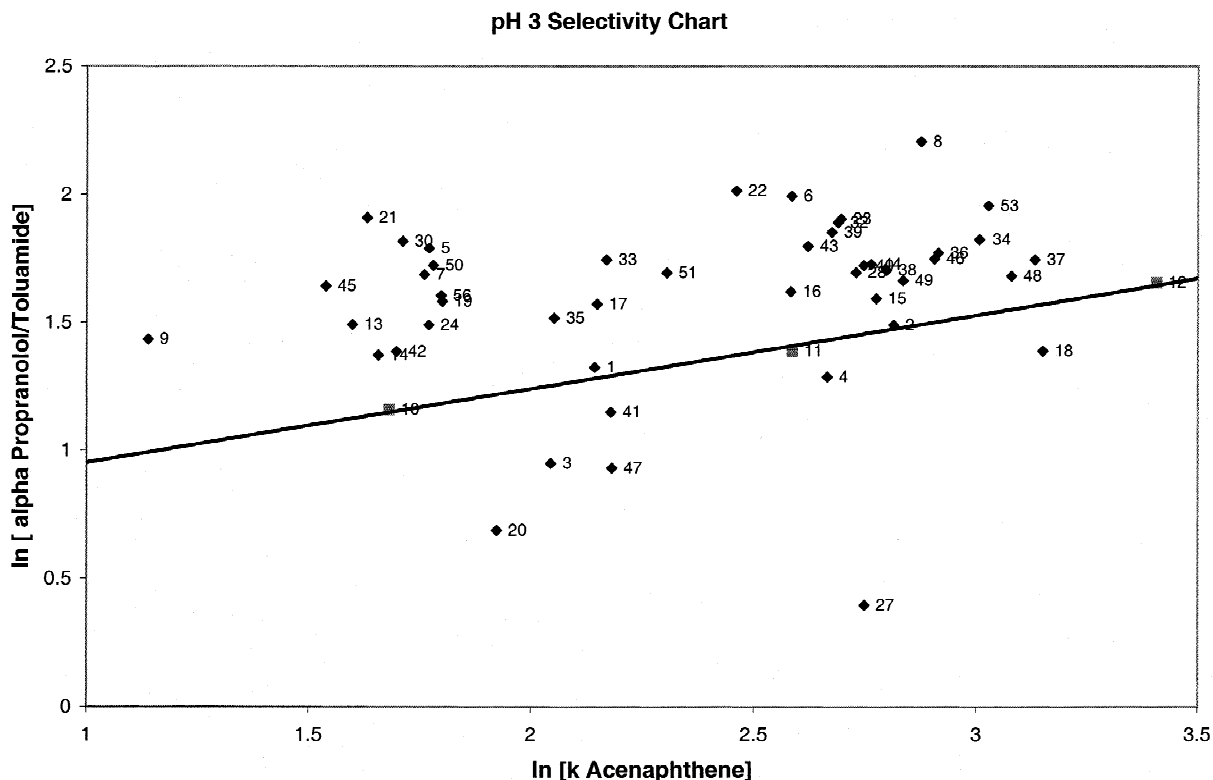

Fig. 7. Plot of the natural logarithm of the relative retention between propranolol and toluamide at pH 3 vs. the natural logarithm of the retention factor of acenaphthene.

tryShield RP$_{18}$, no. 4, Supelcosil ABZ+, no. 41, Prism, no. 47, and Spectrum, no. 20. The endcapping with an amino functional silane is the reason for the unusual position of Purospher RP$_{18}$, no. 27, on this chart. On the upper side of the chart, mostly older packings based on lower purity silicas can be found: Waters Spherisorb ODS-2, no. 8, Supelcosil LC DB-C$_{18}$, no. 22, Nova-Pak C$_{18}$, no. 6, and their equivalents on the C$_8$ side. Nevertheless, the range is narrower than at neutral pH (see below). At acidic pH, silanol activity is more suppressed than at neutral pH, and the difference between high-purity silicas and their older counterparts is less visible.

A similar plot has been created for the test results obtained at neutral pH (Fig. 8). On the *x*-axis, the retention factor of acenaphthene is plotted on a logarithmic scale. The retention factor of acenaphthene is used as measure for the hydrophobicity of a packing. On the *y*-axis, the relative retention between

amitriptyline and acenaphthene is also plotted on a logarithmic scale. We take this value as a measure of the silanol activity of a packing at pH 7. The *x*-axis is the same as on the previous chart, therefore the interpretation of the position of different packings on the *x*-axis has been discussed above.

The same reference line as in the previous chart is drawn between the data points for the YMC J'Sphere packings. As the coating level increases, the hydrophobicity of the packing increases. Also, the silanol activity is expected to decrease with an increase in the coating level. The fact that a straight line results for these three different packings based on the same silica confirms the validity of the chart.

In general, older packings exhibit a significant amount of silanol group activity and are found in the upper section of the chart. Examples are Waters Spherisorb ODS-2, no. 8, Nucleosil C$_{18}$, no. 23, or LiChrosorb Select B, no. 25. In general, unendcap-
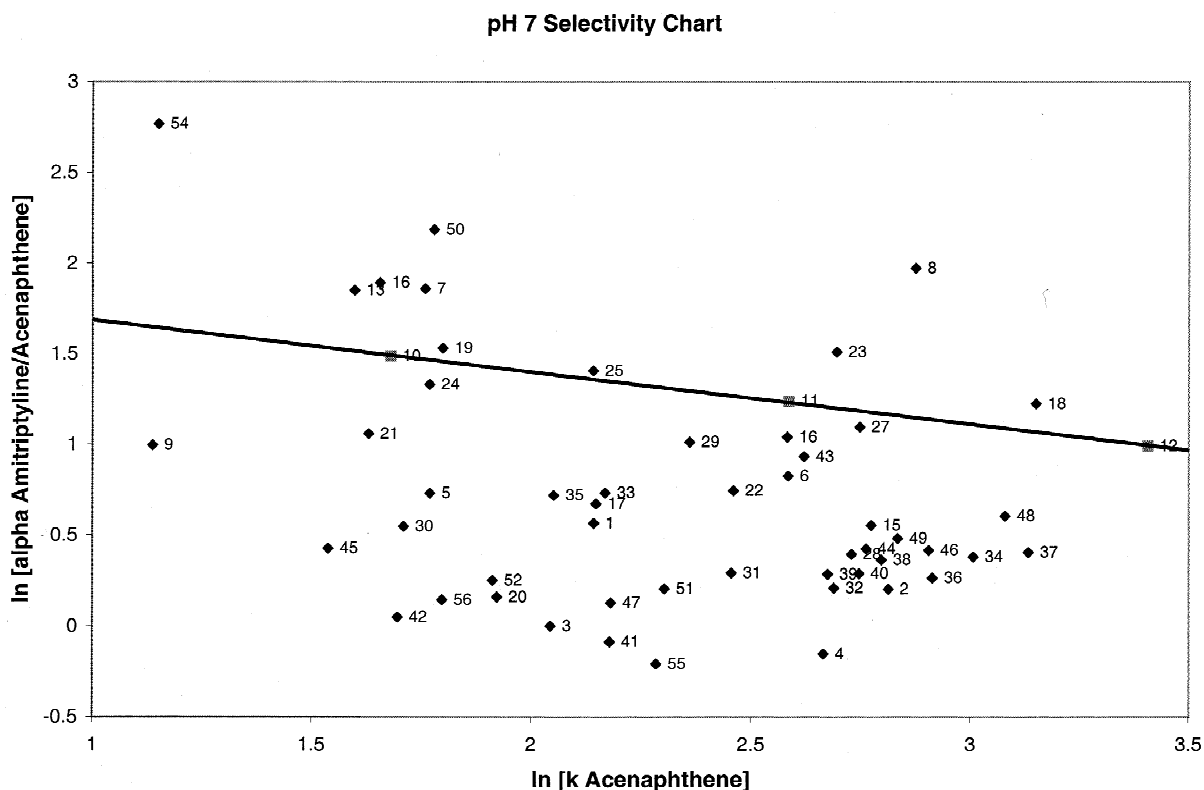

Fig. 8. Plot of the natural logarithm of the relative retention between amitriptyline and acenaphthene at pH 7 vs. the natural logarithm of the retention factor of acenaphthene.

ped packings based on classical silicas such as Resolve C_{18} are not found on this chart. The silanol activity of these packings is too large to fit reasonably on the same scale as modern packings. However, unendcapped packings based on high-purity silicas have a low enough silanol activity to still fit on the chart. Examples are the Zorbax Rx C_8, Zorbax SB C_8, Zorbax Rx C_{18}, and Zorbax SB C_{18} packings (nos. 13, 14, 15 and 16).

Fully endcapped C_{18} packings based on high-purity silicas are clustered on the lower right-hand side of the chart. The cluster contains a multitude of newer generation, fully endcapped packings: Symmetry C_{18}, no. 2, Hypersil Elite C_{18}, no. 32, Kromasil C_{18}, no. 34, Inertsil ODS-2, no. 36, and Inertsil ODS-3, no. 37, the L-Column ODS, no. 38, Develosil ODS-UG-5, no. 39, Prodigy C_{18}, no. 40, YMC-Pack Pro C_{18}, no. 44, Luna C_{18}, no. 46, and Zorbax Eclipse XDB C_{18}, no. 49. A similar cluster can be observed on the left side of the graph for fully endcapped C_8 packings based on high-purity silicas: Symmetry C_8, no. 1, Zorbax XDB C_8, no. 17, Kromasil C_8, no. 33 and Inertsil C_8, no. 35, with Hypersil BDS C_8, no. 30 and Prodigy C_8, no. 45, on the left side of the main cluster. Hypersil BDS bonded phases are based on a silica with a larger pore size and a lower surface area than the other packings in the same category. Symmetry300 C_{18}, no. 52, is found fairly low on the chart; this indicates that the silanol groups of a larger pore size silica can be inactivated more readily than the silanols of a classical 100 Å silica.

On the lower part of the graph a few packings can be found with exceptionally low silanol activity: Discovery RP Amide 16, no. 42, Spectrum, no. 20, Prism, no. 47, Supelcosil ABZ+, no. 41, and SymmetryShield RP_8, no. 3, and SymmetryShield RP_{18}, no. 4. As mentioned above, these bonded phases use an incorporated polar functional group to shield the silanols on the surface of the silica from an interaction with analytes [39–42]. The Supelcosil ABZ+ contains residues of an amine function stemming from a multi-step surface reaction. These amine functions cause tailing for acidic analytes. The composition of the Spectrum packing has not been published. According to our analysis, it contains an urea group. The Discovery RP Amide 16 packing, no. 42, contains an amide linkage, while the Symme-

tryShield RP packings, Nos. 3 and 4, use carbamate linkages to protect the analytes from the interaction with surface silanols. This reduced interaction with surface silanols results in different selectivities compared to classical packings and significantly reduced tailing of basic analytes.

In order to assess the overall similarity and dissimilarity of the columns studied, we subjected a selected group of data to cluster analysis. We selected the retention factor of acenaphthene, the relative retention between butylparabene and dipropylphthalate, the relative retention between propranolol and toluamide at pH 3, and the relative retention between amitriptyline and acenaphthene at pH 7 as the relevant data for the cluster analysis. All of these parameters have been discussed in detail in the previous paragraphs.

The results of the cluster analysis are shown in Fig. 9. The differences between packings and groups of packings in the cluster analysis is indicated by the length of the branches in a cluster, while the
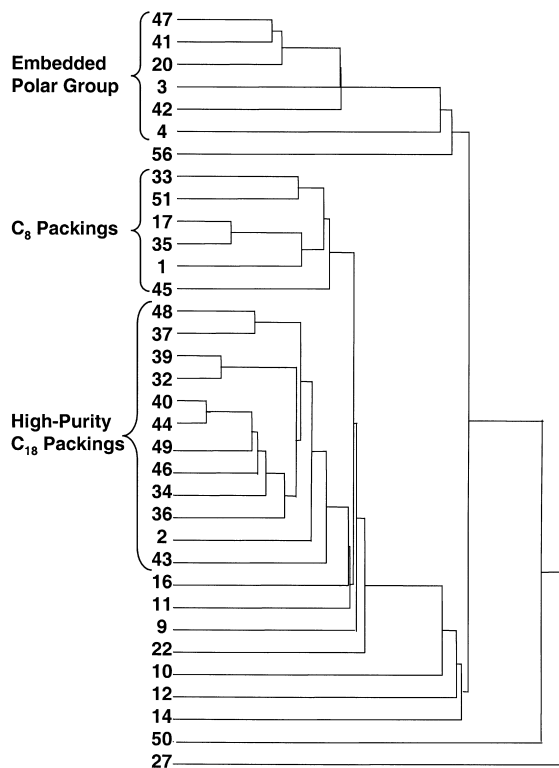

Fig. 9. Cluster analysis of the relevant data.

similarities are indicated by the linkage pattern of the clusters. The first grouping of packings comprises those with an incorporated polar functional group and Capcell Pak C_{18}, no. 56. Packings with an incorporated polar group are Prism, no. 47, Supelcosil ABZ+ Plus, no. 41, Spectrum, no. 20, SymmetryShield RP8, no. 3, Discovery RP Amide 16, no. 42, and SymmetryShield RP_{18}, no. 4. Next is the group of packings based on an octyl silane: Kromasil C_8, no. 33, Zorbax Eclipse XDB, no. 17, Inertsil C_8, no. 35, Symmetry C_8, no. 1, and Prodigy C_8, no. 45. Closely associated with this group is Hypersil Hypurity Elite C_{18}, no. 51, which is based on a silica with a larger pore-size. What follows is a group of C_{18} packings based on high-purity silicas, ranging from Purospher RP_{18}e, no. 48, to Symmetry C_{18}, no. 2. Two of these packings exhibit a strong similarity: Prodigy C_{18}, no. 40 and YMC-Pack Pro C_{18}, no. 44. However, the origin of this similitude is not known to us. Next follow packings that exhibit different behavior from the groups discussed before. Zorbax SB C_{18}, no. 16 and Zorbax SB C_8, no. 14, are prepared using unique sterically hindered silanes. The YMC Basic packing, no. 9, is based on mixed short-chain silanes. The group of YMC J'Sphere packings, Nos. 10, 11 and 12, has been discussed before. Platinum C_{18}, no. 50, is a packing based on a low coating level. The Purospher RP_{18}, no. 27, is the C_{18} packing to which a unique endcapping with an amino-functional silane has been applied. Consequently, these results show that the cluster analysis is clearly capable of differentiating the different groups of packings and providing guidance as to the similarity and dissimilarity of packings.

4. Conclusion

The methods described here are effective tools for the grouping of a wide range of reversed-phase packings. For example, the relative retention between butylparaben and dipropylphthalate provides a unique measure to differentiate between classical packings and packings with an incorporated polar group. The retention factor of a simple aromatic compound can be used as a measure of hydrophobicity, while the relative retention between basic and neutral analytes can be used as a measure of the silanol activity of a packing. The tools provided here can be used to differentiate also between classical C_{18} and classical C_8 packings. At least for the parameters under study here, similarities and dissimilarities of different packings can be demonstrated. The relevant measurements have been subjected to a cluster analysis, which allowed a sensible ordering of the packings in the study.

References

[1] U.D. Neue, E. Serowik, P. Iraneta, B.A. Alden, T.H. Walter, J. Chromatogr. A 849 (1999) 87.
[2] J. Verne-Mismer, M. Lamard, J. Wagner, J. Chromatogr. 645 (1993) 251–258.
[3] R.J.M. Verhoort, F.A. Maris, H. Hindriks, J. Chromatogr. 623 (1992) 207–220.
[4] R.J.M. Verhoort, M.W.J. Derksen, F.A. Maris, J. Chromatogr. A 678 (1994) 1.
[5] R.J.M. Verhoort, M.W.J. Derksen, A.J.J. Debets, J. Chromatogr. A 765 (1994) 157–168.
[6] D. Sýkora, E. Tesarová, M. Popl, J. Chromatogr. A 758 (1997) 37–51.
[7] D.V. McCalley, J. Chromatogr. 357 (1986) 221–226.
[8] D.V. McCalley, J. Chromatogr. 636 (1993) 213–220.
[9] D.V. McCalley, J. Chromatogr. A 664 (1994) 139–147.
[10] D.V. McCalley, J. Chromatogr. A 708 (1995) 185–194.
[11] D.V. McCalley, J. Chromatogr. A 738 (1996) 169–179.
[12] D.V. McCalley, J. Chromatogr. A 769 (1997) 169–178.
[13] D.V. McCalley, J. Chromatogr. A 793 (1998) 31–46.
[14] U.D. Neue, B.P. Murphy, unpublished results, 1979.
[15] B.A. Bidlingmeyer, J.K. DelRios, J. Korpi, Anal. Chem. 54 (1982) 442.
[16] T. Fornstedt, G. Zhong, G. Guiochon, J. Chromatogr. A 741 (1996) 1–12.
[17] L.C. Sander, S.A. Wise, J. Chromatogr. A 656 (1993) 335–351.
[18] L.C. Sander, S.A. Wise, Anal. Chem. 67 (1995) 3284–3292.
[19] B. Dreyer, Dissertation, Universität des Saarlandes, Saarbrücken, 1984.
[20] H. Engelhardt, M. Jungheim, Chromatographia 29 (1985) 59–68.
[21] H. Engelhardt, H. Löw, W. Götzinger, J. Chromatogr. 544 (1991) 371–379.
[22] H. Engelhardt, M. Arangio, T. Lobert, LC·GC 15 (1997) 856–866.
[23] S.J. Schmitz, H. Zwanziger, H. Engelhardt, J. Chromatogr. 544 (1991) 381–391.
[24] K. Kimata, K. Iwaguchi, S. Onishi, K. Jinno, R. Eksteen, K. Hosoya, M. Araki, N. Tanaka, J. Chromatogr. Sci. 27 (1989) 721.
[25] Y. Ohtsu, Y. Shioshima, T. Okumura, J.-I. Koyama, K. Nakamura, O. Nakata, K. Kimata, N. Tanaka, J. Chromatogr. 481 (1989) 147–157.

[26] W. Eymann, Chromatographia 45 (1997) 235–242.

[27] Á. Sándi, Á. Bede, L. Szepesy, G. Rippel, Chromatographia 45 (1997) 206–214.

[28] G. Rippel, Gy. Bacsur, Á. Bede, Á. Sándi, L. Szepesy, J. Liq. Chromatogr. Rel. Technol. 20 (1997) 1667–1687.

[29] A. Sandi, L. Szepesy, J. Chromatogr. A 818 (1998) 1–18.

[30] A. Sandi, L. Szepesy, J. Chromatogr. A 818 (1998) 19–30.

[31] K. Valko, M. Plass, C. Bevan, D. Reynolds, M.H. Abraham, J. Chromatogr. A 797 (1998) 41–55.

[32] K. Valko, C. Bevan, D. Reynolds, Anal. Chem. 69 (1997) 2022.

[33] H.A. Claessens, M.A. vanStraten, C.A. Cramers, M. Jezierska, B. Buszewski, J. Chromatogr. A 826 (1998) 135–156.

[34] G.B. Cox, J. Chromatogr. A 656 (1993) 353–367.

[35] J. Nawrocki, Chromatographia 31 (1991) 177–192.

[36] J. Nawrocki, Chromatographia 31 (1991) 193–205.

[37] J. Nawrocki, J. Chromatogr. A 779 (1997) 29–71.

[38] J.J. Kirkland, J.L. Glajch, R.D. Farlee, Anal. Chem. 61 (1989) 2–11.

[39] J.E. O'Gara, B.A. Alden, T.H. Walter, J.S. Petersen, C.L. Niederländer, U.D. Neue, Anal. Chem. 67 (1995) 3809–3813.

[40] T.L. Askah, B. Feibush, J. Chromatogr. 506 (1990) 357–369.

[41] T.L. Askah, K.M.R. Kallury, C.A. Szafranski, S.D. Corman, F. Liu, J. Liq. Chromatogr. Rel. Technol. 19 (1995) 3049–3073.

[42] K.J. Duff, Am. Lab. (1998) 32AA–32JJ.

[43] K.K. Unger, Packings and Stationary Phases in Chromatographic Techniques, Marcel Dekker, New York, 1990.

[44] U.D. Neue, HPLC Columns, Wiley–VCH, New York, 1997.

[45] L.R. Snyder, J.J. Kirkland, J.L. Glajch, Practical HPLC Method Development, 2nd ed, Wiley–Interscience, New York, 1997.

[46] J. Köhler, D.B. Chase, R.D. Farlee, A.J. Vega, J.J. Kirkland, J. Chromatogr. 384 (1986) 275.