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Review

Optimisation and characterisation of silica-based reversed-phase liquid chromatographic systems for the analysis of basic pharmaceuticals

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Abstract

Reversed-phase liquid chromatography using silica-based columns is successfully applied in many separations. However, also some drawbacks exist, i.e. the analysis of basic compounds is often hampered by ionic interaction of the basic analytes with residual silanols present on the silica surface, which results in asymmetrical peaks and irreproducible retention. In this review, options to optimise the LC analysis of basic pharmaceutical compounds are discussed, i.e. eluent optimisation (pH, silanol blockers) and stationary phase optimisation (development of new columns with minimised ionic interactions). The applicability of empirical based, thermodynamically based and test methods based on a retention model to characterise silica-based reversed phase stationary phases, as well as the influence of the eluent composition on the LC analysis of basic substances is described. Finally, the applicability of chemometrical techniques in column classification is shown.

Keywords: Reviews; Optimization; Mobile phase composition; Stationary phases, LC; Basic pharmaceuticals

Contents

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1. Introduction

World-wide approx. 75% from the total amount of liquid chromatographic (LC) analysis is performed using the reversed phase mode, i.e. using C_8 or C_{18} modified silica columns [1]. Especially in the industry the large majority of chromatographers are using LC systems of conventional size, i.e. columns with internal diameters of 4–5 mm and 10–25 cm length. The broad range of compounds that can be analysed, i.e. non-ionic, ionisable and ionic compounds has caused this widespread applicability. Other main contributions are the short equilibrium times, the possibility to perform gradient analysis and the possibility to use water rich eluents and samples [2]. Although applied in most chromatographic separations, reversed phase (RP) LC has also some drawbacks. Many pharmaceutical compounds contain basic nitrogen atoms, e.g. central nerve system drugs, cardiovascular drugs, amino acids, peptides etc. The ability of basic nitrogen atoms to interact with residual silanols on the packing material hampers the RPLC analysis of these compounds and asymmetrical peaks and irreproducible retention are the result. The use of different packing materials often results in different chromatographic performance. Since world-wide approx. 600 different brands of packing material are available, differences exist between type of ligand, end-capping, type of silica, residual silanols, bonding density, pore size etc. [3–5]. Analysts do not recognise that a large group of sometimes nominally identical materials often show very different chromatographic properties [6]. Moreover the nomenclature of RPLC stationary phases is not clear and therefore confusing. It is obvious that test procedures enabling a well considered selection of a column are necessary to select a column to solve a specific separation problem and to develop reliable and validated analysis protocols. However, also for test procedures, various approaches are available each with their advantages and disadvantages. As with the choice of the optimal column, the chromatographers get confused which test method to select to characterise columns. In the literature empirical, thermodynamically as well as test methods based on retention models have been described [7–11], whereas also test methods specially developed for specific classes of compounds have been reported [12,13].

In this review the RPLC analysis of basic pharmaceutical compounds is discussed. First, the optimisation of the mobile phase is discussed. Secondly, optimisation of stationary phase with respect to the preparation of silica and the manufacturing of modern packing materials optimised for the analysis of basic pharmaceutical compounds, is described. Thirdly, various column characterisation procedures, divided into empirical methods, thermodynamically based methods, and model based methods, are discussed for the applicability in classifying columns. Fourthly, the influence of the eluent composition on eluent pH, and on pK_a of the basic analytes is shown. Finally, the use of chemometrical techniques to analyse the data obtained when classifying columns, enabling categorisation of columns with equal characteristics, is discussed.

2. Applicability of liquid chromatography for the analysis of basic pharmaceuticals

2.1. Silica based packing materials

Compounds with a basic nitrogen atom in the chemical structure often cause problems when analysed with RPLC. Asymmetrical peaks, irreproducible retention and non-robust separating methods are frequently obtained. Basic nitrogen atoms can be protonated, depending on the pK_a of the analyte and the pH of the eluent. These protonated basic com-

pounds can interact with residual silanol groups of the stationary phase, as shown in the equation:

$$XH^+ + SiO^-Na^+ \Leftrightarrow Na^+ + SiO^-XH^+ \quad (1)$$

Thus, besides the reversed phase retention mechanism also an ion-exchange retention mechanism occurs often resulting in distorted peak shapes. Asymmetrical peaks can be explained in terms of kinetic phenomena, i.e. if the kinetics of mass transfer of one type of column site is slower than from the other. Fornstedt et al. showed that the most pronounced peak tailing occurs when the slow sites provide a smaller contribution to retention than the fast ones and if the rate constant of mass transfer for the slow sites is between 20 and 2000 times smaller than that of the fast sites [14]. For basic analytes, the kinetics of the ion-exchange interaction with silanol groups may be slower than those with the alkyl ligands, giving rise to peak tailing. Moreover, overloading of ionised silanols by the basic solute can occur which also will contribute to tailing [15].

To improve the peak shape, optimisation of the mobile phase can be considered. Eq. (1) suggests several approaches to reduce the ionic interaction between analyte and acidic sites on the column packing [16]:

- selection of a suitable mobile phase pH, i.e. $pH < pK_{a_{silanol\ groups}}$, or $pH > pK_{a_{basic\ analyte}}$, to suppress ionisation of either the silanol groups or the basic analytes,
- addition of a silanol blocker to the eluent, i.e. $pK_{a_{silanol\ blocker}} \gg pK_{a_{basic\ analyte}}$, the more basic compound will stronger interact with residual silanols allowing the less basic compound to interact solely with the alkyl ligand of the stationary phase, i.e. hydrophobic interaction,
- increasing the ionic strength of the mobile phase in order to drive the reaction in the equation to the left and/or replacement of Na^+ by more strongly retained ions such as K^+, Rb^+ etc., and,
- reduction of the sample concentration to alleviate saturation of the acidic sites.

Improvements of the stationary phase can also lead to improved peak shapes for basic analytes. As a result, a new generation of newly developed stationary phases is available and still increasing. Today analysts can choose a column from an enormous amount of stationary phases [3,4,17]. Stationary phases specially developed for the analyses of basic compounds are phases in which the ionic interaction between basic analyte and residual silanols, responsible for asymmetrical peaks, are minimised. In the literature various approaches of stationary phase manufacturing have been described:

- High purity silica [18–21]: conventional silica gels contain approx. 10 ppm of trace metals. However, the level of trace metals may differ from batch to batch. Residual metals can influence the acidity of adjacent silanols, and thus indirectly the interaction between silanols and basic analytes. Removal of metals can be achieved via extensive acid treatment of the silica before preparation of the alkyl modified stationary phase. Using the acid treatment, generally two thirds of the metals can be removed still leaving significant amounts of metals in the silica.
- End-capping procedures [22–25]: preparation of alkylsilylated silica's means that approx. 50% of the silanols reacted, meaning that still approx. 50% of the silanols are left. Removal of these silanols can be achieved by end capping, i.e. reaction of short chain alkylsilanes with these silanols. Trimethylchlorosilane (TMCS) and hexamethyldisilazane (HMDS) are known as suitable end-capping agents. HMDS was found to be suitable to end-cap stationary phases for the analyses of bases, whereas TMCS was found to be suitable to end-cap stationary phases for the analysis of acids. It was shown that end-capping of stationary phases could be performed efficiently and sharp peaks for basic drugs were obtained using these stationary phases [26].
- Polymer encapsulation [27–30]: coverage of the silica with a thin layer of organic silicone polymer and subsequent introduction of long alkyl chains. The polymer coating process solves problems generating from the original silica surface, i.e. an inert surface free from residual silanols and metal impurities. The inertness of such a polymer encapsulated stationary phase was demonstrated showing good peak profiles of pyridine (residual silanols) and hinokitiol (metals). However, from NMR studies it was concluded that after the coating still residual silanols were present [31]. The polymer encapsulated stationary phase was

found to be stable when using a mobile phase containing borate buffer pH 10.

- Horizontal polymerisation [32–34]: with this method of stationary phase synthesis a high density is achieved at the silica surface by mixing long chain and short chain trifunctional silanes. Horizontal polymerisation refers to the notion that there is significant Si–O–Si bridging parallel to the silica substrate. The reaction of trifunctional silanes is performed in an anhydrous environment, except for a monolayer of water on the silica. A humidity of approx. 50% was found to be optimal for stationary phase synthesis. Phases prepared by horizontal polymerisation were found to be stable at acidic (at least 2400 column volumes at pH 1.8) and basic (at least 900 column volumes at pH 10.0) eluent conditions.
- Embedded polar groups [5,35]: a stationary phase is obtained showing different selectivity compared to ordinary alkyl based stationary phases. The polar function shields the silica surface electrostatically, preventing the interaction of analytes with residual silanols on the silica surface. An additional advantage is that stationary phases with embedded polar groups can be used with eluents containing high amounts of water, without the problem of folding of the stationary phase chains.
- Bidentate stationary phases [21,36]: main advantage of bidentate stationary phases, compared to ordinary alkyl phases, is column stability. It is shown that C_{18}/C_{18} bidentate synthesized columns with a propylene bridge exhibit excellent stability at low and intermediate pH. However, the main advantage is the excellent stability of bidentate phases especially at high pH. This means that basic analytes can be analysed using a mobile phase $pH > pK_{a_{basic\ analyte}}$. Analysing basic analytes as uncharged compounds will reduce the interaction with residual silanols. Other advantages of analysing bases at high pH are situations in which the analyte is unstable at low pH, the desired selectivity cannot be obtained at low pH, and at low pH protonated hydrophilic compounds are poorly retained.
- Stationary phases prepared using surface modified silica [37,38]: with this type of stationary phases the silica backbone is modified, resulting in a reduction of residual silanols. In preparing the silica, organic functional groups have become a constituent of the silica backbone as well as of the surface. Compared to ordinary silicas, OH groups have been replaced by CH_3 groups. After introduction of the alkyl chains, for the ordinary phases the ratio between OH and alkyl chains is 1:1. Using the surface modified silica, the ratio is 1/3 OH, 1/3 alkyl chain and 1/3 CH_3, leading to a reduction of residual silanols. Another advantage is the improved pH stability, i.e. this phase has been used up to mobile phase pH 12 showing good stability.
- Monolithic silica phases [39,40]: these phases are prepared according to a new sol–gel process, based on hydrolysis and polycondensation of alkoxysilanes in the presence of water soluble polymers. The method results in silica rods made of a single piece of porous silica with a defined pore structure. Compared to conventional silica based columns, the porosity of silica rod columns is approx. 15% higher. Moreover, silica rod columns maintain high performance at high flow rates. Silica rod columns will be available in the near future, allowing LC analysis at high flow rates and thus short analysis times. The applicability of silica rod columns in preparative analysis was also shown [41]. Due to the high efficiencies obtained at high flow rates, high throughput and productivity was demonstrated. The applicability of silica rod columns in the analysis of basic pharmaceuticals to our knowledge so far has not been reported in the literature.

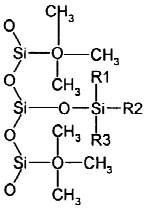
In Table 1 some of the structures of the stationary phases described are shown.

2.2. Preparation of silica based reversed phase packing materials

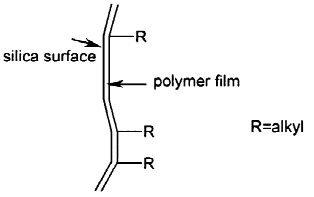
The manufacturing and quality of the silica used to synthesise reversed phase packing materials determine amongst other parameters the quality of the ultimate stationary phase. The silica used for column packings is porous and non-crystalline with the general formula $SiO_2 \cdot xH_2O$, and water is chemically bound forming Si–OH bonds which is most important for use in LC packing materials [42–45]. These silanol groups and siloxane bridges (Si–O–Si)

Table 1
Structures of silica based stationary phases specially developed for minimised interactions with residual silanols

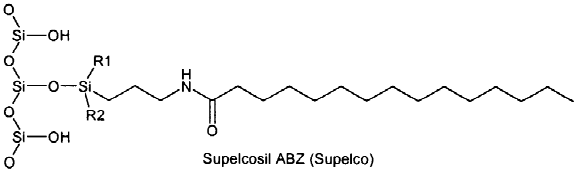
endcapping

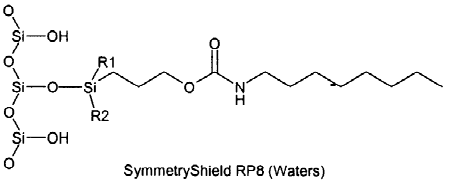


polymerisation

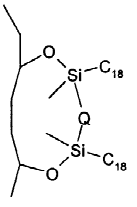


embedded polar groups

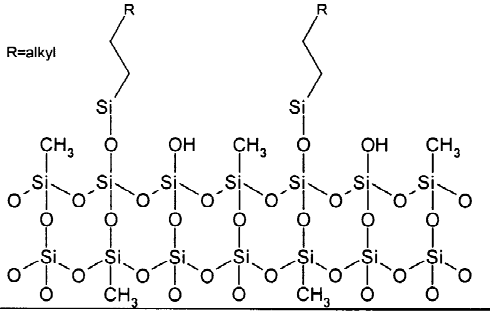




bidentate



surface modified silica



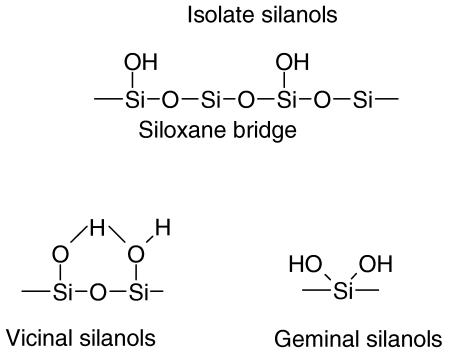


Fig. 1. Types of surface silanols.

compose the surface of silicas. In Fig. 1 the possible types of surface silanols are shown, i.e. isolated, geminal and vicinal silanols, together with siloxane bridges. The silanol groups are hydrophilic, i.e. they can interact with polar groups, whereas the siloxane bridges possess hydrophobic properties [42]. In chromatography the silica particles today in most cases are spherical and the main characteristics of silica as chromatographic material are particle size and specific area (typically in the range of 200–400 m^2/g) [46].

The concentration of surface silanols of a fully hydroxylated surface is approx. 8 $\mu mol/m^2$. Modification of the silica with C_{18} or C_8 results, due to steric hindrance effects, in the reaction of approx. 50% of the silanols, leaving approx. 4 $\mu mol/m^2$ of residual silanols. These residual silanols can interact with basic compounds. For the silanols present, isolated silanols are responsible for strong, undesired interactions with basic compounds in RPLC [47,48]. Furthermore, the existence of concentrated groups of residual silanols on the silica surface results in a small population of silanols, which are able to strongly interact with bases. This population is less than 1% of the total amount of residual silanols [49].

Depending on the manufacturing route of the silica, two types of silica can be distinguished which can be defined by their different physical and chromatographic properties. Particles made by gelling soluble silicates are characterised by higher surface areas, higher porosities and irregular pore shapes with variable wall thickness, and are named SilGel silicas. Particles made by aggregating silica–sol particles have lower surface areas, lower porosities, and more regular pores with thicker walls defined by surrounding silica sol micro particles, and are named SolGel silicas [50]. In LC, the majority of separations are performed in the reversed phase mode, i.e. an apolar stationary phase with a polar mobile phase. Manufacturing of the reversed phase packing material is generally performed by reaction of an organosilane with a silanol group at the silica surface yielding octyl (C_8) or octadecyl (C_{18}) modified packings. Both SolGel and SilGel type silicas are used to manufacture these reversed phase packing materials. Comparance of stationary phases prepared from both silicas showed that phases prepared from SolGel silicas are more durable, compared to phases prepared from SilGel silicas [51].

2.3. Alternative solutions

Besides silica based reversed phase packing materials, also alternative solutions have been proposed. The use of bare silica in combination with a reversed phase like mobile phase showed acceptable performance [52,53]. The main disadvantage of this option is the disability to use gradient elution. Other alternative solutions are the use of columns based on alumina, titanium oxide or zirconium oxide. The main advantage of these materials is, compared to silica, the better pH stability, i.e. approx. from pH 0 to 13, enabling the chromatography of strong bases as unprotonated compounds [54,55]. However, with these packings also secondary retention mechanisms occur. Polymer based column packings are also pH stable, i.e. for a polystyrene–divinylbenzene packing from pH 0 to 14. The main disadvantages, however, are the limited pressure resistance, hindered mass transfer and swelling/shrinking properties [5]. Using polymer based columns, symmetrical peaks were obtained for basic analytes, however, due to the hindered mass transfer properties a reduction in plate number must be taken into account [13]. Taking the drawbacks of the alternative solutions into account, for many applications silica based packing materials are still preferred.

3. Characterisation of silica based packing materials

A disadvantage of the large amount of columns available is how to select the optimal column for the chromatographers application. Classification of columns into groups with equal characteristics would make the choice of a column easier. Most characterisation studies have been performed using either spectroscopic or chromatographic methods. Spectroscopic characterisation of stationary phases is performed using IR and NMR spectroscopy. Bonded phase, silanols and silanes on the solid support can be identified and information about the type of bonding can be qualitatively obtained using diffuse reflectance infrared fourier transform IR [56]. The various types of silanols (isolated, geminal and vicinal) can be determined using ^{29}Si solid state NMR, whereas the type of bonding (mono, di or trifunctional) and type of endcapping can be determined using ^{13}C solid state NMR [57]. Hetem showed the applicability of various ^{29}Si NMR techniques to study the chemical modifications of nonporous and porous silica supports, as well as the stability of various modified silicas under conditions normal for daily chromatographic practice [31]. Scholten presented evidence of the decreased hydrogen bonding of residual silanols of di-isobutyl-*n*-octadecylsilane derivatized silica (Zorbax Stable Bond phases), compared to dimethyl-*n*-octyl derivatized silica, using NMR. This illustrated the increased steric protection of the ligand siloxane bonds, which is assumed to be the reason for improved hydrolytic stability in aqueous mobile phases with low pH [58].

A disadvantage of spectroscopic techniques, however, is that they can only be used to determine bulky characteristics of the stationary phase. Since especially in relation to the analyses of basic compounds subtle differences between the phases can have a large impact on the shape of the chromatographic peak, a chromatographic characterisation is preferred. Another advantage is that the stationary phases can be tested in the same condition as they are used in daily practice, i.e. as packed columns. The majority of chromatographic methods used to characterise silica based reversed phase stationary phases determine roughly two stationary phase properties, i.e. hydrophobic properties and ionic properties. The chromatographic characterisation of stationary phases can be divided into three classes:

- Empirical based evaluation methods: this section can be subdivided into two groups: (i) evaluation based on chromatographic data of test compounds chosen according to a certain line of thought, and (ii) evaluation based on chromatographic data of dedicated test compounds. In the case of testing columns for the applicability in analysing basic compounds, basic compounds are used as test probes;
- Thermodynamically based methods: the obtained information is based on studying enthalpies and entropies of transfer of solutes from the mobile to the stationary phase;
- Evaluation methods based on a retention model: the obtained information is based on a specific retention model such as Quantitative Structure Retention Relationship (QSRR) studies.

In this review various methods for characterising columns with respect to the analysis of basic pharmaceutical compounds are discussed.

3.1. Empirical based evaluation methods

3.1.1. Hydrophobic properties of stationary phases

With respect to the determination of hydrophobic properties, most tests described in the literature are based on retention of benzene derivatives. To determine hydrophobic stationary phase characteristics Tanaka [7] used amylbenzene and butylbenzene, Walters [8] used anthracene and benzene, Engelhardt [9] used toluene and ethylbenzene, Gonnet [59] used benzene and toluene, Eymann [60] used nitrobenzene and toluene, and Neue [61] used toluene, acenaphtene and naphtalene. Although the experimental part of these test methods is comparable, the calculations are confusing. Determination of amount of alkyl chains, carbon content, hydrophobicity, hydrophobic selectivity and surface coverage are mentioned. However, in several cases different stationary phase characteristics were determined. Hydrophobicity, which can be considered as the retentivity for compounds, based on interactions between the compound and the ligand on the silica, is calculated as

the relative retention of two benzene derivatives by some researchers [7–9]. However, relative retention values of many reversed phase columns were found to be comparable, whereas difference in % carbon load, ligand length and applied bonding chemistry must lead to different hydrophobicity [6]. Other researchers use the retention values of aromatic hydrocarbons as a measure of hydrophobicity [62,63]. Recently a good correlation between the absolute retention values of the aromatic hydrocarbons of the Engelhardt and Tanaka test was obtained, resulting in column classification independent of the applied test. Furthermore, it was concluded that the relative retention values should be considered as methylene selectivity, i.e. the ability of a given stationary phase to separate structurally closely related compounds [63].

3.1.2. Silanol activity of stationary phases

Determination of the silanol activity of stationary phases is important for the analysis of basic compounds. Determination of the silanol activity of reversed phase stationary phases is, compared to hydrophobic properties, a more difficult topic. As reviewed by Nawrocki, silanol activity comprises a number of stationary phase–solute interactions such as ion–ion (ion exchange), ion–dipole, dipole–dipole (e.g. hydrogen bonding), dipole-induced dipole and induced dipole-induced dipole (London forces) of which ion–ion and hydrogen bonding are probably the most important [49]. Since large differences between packing materials exist due to different manufacturing processes used, large differences between peak shapes obtained with various stationary phases can be obtained. Since symmetrical peaks are favourable for a high selectivity and sensitivity, determination of silanol activity of a given stationary phase is important in order to select a suitable stationary phase for a specific separation.

The empirical tests described in the literature are mainly based on relative retention values between compounds from which the retention is assumed to be caused by hydrophobic and silanol interactions, and compounds from which the retention is assumed to be based on hydrophobic interaction only. Furthermore, the nomenclature used in these tests is not identical. Tanaka differentiates between hydrogen bonding and ion exchange using the relative retention of caffeine and phenol, and benzylamine and phenol, respectively [7], whereas for silanol activity without further specification Walters [8], Engelhardt [9], Gonnet [59] and Verzele [64] used the relative retention values of *N*,*N*-diethyl-*m*-toluamide and anthracene, aniline and phenol, theophyline and caffeine, and naphtalene and nitronaphtalene, respectively. Additional experiments are included in the Engelhardt test, e.g. the separation of three isomeric toluidines, and recently the peak symmetry of 4-ethylaniline was introduced as a measure of ionic interactions [65]. Daldrup [66] used the relative retentions of diphenhydramine, 5-(*p*-methylphenyl)-5-phenylhydantion and diazepam to determine silanol interactions. Another test was developed by Neue for silanol interactions based on the relative retention of a basic compound and a hydrophobic compound, at neutral and acidic eluent pH. The test was used to assess the reproducibility of column material [61] and to classify 50 different commercial available stationary phases [67]. It was shown that modern packing materials could be manufactured reproducibly. Kele and Guiochon [68–70] used a mix of the tests based on the tests of Tanaka [7] and Engelhardt [9] to evaluate the column-to-column and batch-to-batch reproducibility of modern packing materials. They found that packing materials were reproducible with respect to analysing neutral compounds. However, contradictory to the results of Neue et al. [61], differences between batches of identical packing material were observed with respect to analysing basic substances. These data confirmed our own findings, i.e. batch-to-batch differences can be obtained in the analysis of basic pharmaceutical compounds [71]. In Table 2 a selection of the previously discussed tests is summarised. The large variety of the tests described in the literature make it difficult to the user to judge the quality of these tests. Claessens recently showed the low correlation between the tests of Walters, Tanaka and Engelhardt [63], meaning that these tests describe different ionic and polar properties. Moreover, as discussed in Ref. [72], the correlation of these tests with peak shape data obtained when analysing basic pharmaceutical compounds using seven modern stationary phases is low: the tests of Engelhardt and Tanaka were found to be of limited use to differentiate between columns of the latest gene-

Table 2
Selection of empirical tests to determine ionic properties of stationary phases

Calculation	Nomenclature	Eluent %(V/V)	Ref.
$k_{caffeine}/k_{phenol}$	Hydrogen bonding capacity	MeOH + H_2O/30 + 70	Tanaka [7]
$k_{benzylamine}/k_{phenol}$	Ion exchange capacity pH > 7	MeOH + 0.02 M phos. pH 7.6/30 + 70	
$k_{benzylamine}/k_{phenol}$	Ion exchange capacity pH < 3	MeOH + 0.02 M phos. pH 2.7/30–70	
$k_{DETA}/k_{anthracene}$	Free SiOH test	Acetonitrile	Walters [8]
$k_{nitrobenzene}$	Free SiOH test	Dry *n*-heptane	
$k_{aniline}/k_{phenol}$	Silanol activity	MeOH + H_2O/55 + 45	Engelhardt [9]
$\alpha_{ortho\text{-, meta- and para-toluidine}}$ $k_{N,N\text{-dimethylaniline}}/k_{phenol}$			
$k_{theophyline}/k_{caffeine}$	Test on residual silanols	MeOH + H_2O/40 + 60	Gonnet [59]
k and α of: 1 and 3-methyl uric acid, 1 and 3-methyl xanthine, 1,3-dimethyl uric acid		MeOH + NaH_2PO_4(10 g/l) /9 + 91	
k and α of: 1 and 3-methyl uric acid, 1 and 3-methyl xanthine, 1,3-dimethyl uric acid, theobromine, para xanthine, theophyline		*Solvent 1:* MeOH + 0.01 M acetate/ 50 + 50 TBA $5 \cdot 10^{-3}$ M *Solvent 2:* 0.01 M acetate, TBA $5 \cdot 10^{-3}$ M; 10 to 30% of solvent 1 in 15 min	
k of dopamine, tyramine, theophyline, caffeine, bamifylline and M119		MeOH/0.1 M NaH_2PO_4 pH $2_{45/56}$; $5 \cdot 10^{-2}$ M NaCl, $8 \cdot 10^{-3}$ M heptane sulph.	
k and peak shape of: benzylamine, 2-(4-methoxy-phenyl)-ethylamine, *N*-naphtyl-ethylene-1,2-diamine	Elution of amines	A: water; B: acetoni./ water 65/35; C acetoni. /water 40/60 + 10 ml 1 N H_2SO_4 1 L^{-1}; D acetoni. /water 40/60 + 10 ml	Eymann [60]
k and peak shape of: Bayer research compound, 4,4′-bipyridine, 2,2′-bipyridine, 2,3-dihydroxynaphtalene	Elution of chelating compounds	1 M pH 7 buffer 1 L^{-1} t_0: %A: 90, %B 0, %C or D 10; t_{20}: %A: 0, %B 90, %C or D 10	
k and peak shape of: 4-hydroxy benzoic acid, 2-nitrobenzoic acid, 2-hydroxy benzoic acid	Elution of acids		
k and α diphenydramine, 5-(*p*-methylphenyl)-5-phenylhydantoin (MPPH), diazepam	''DMD'' test	156 g acetonitrile + 340 g buffer; buffer: 6.66 g KH_2PO_4 + 4.8 g of 85% H_3PO_4 in 1 l water, pH 2.3	Daldrup [66]
$\alpha_{naphtalene/nitronaphtalene}$	Degree of activity or deactivation	MeOH + 5% sodium acetate/60 + 40	Verzele [64]

ration for the analysis of basic pharmaceutical compounds. Other empirical tests are based on measuring, besides retention, also other chromatographic parameters such as peak symmetry and efficiency. These tests are dedicated to the analysis of a specific class of compounds, and therefore basic compounds

are used as test probes to classify columns for the analysis of basic compounds. The test of Eymann [60] used the retention and peak shapes of basic amines to determine silanol interactions. In a series of papers McCalley described the development of a test using generally available basic analytes as pyridine, benzylamine, codeine, diphenhydramine, amphetamine, nicotine, quinine and nortryptyline [12,73–77]. It was found that at mobile phase pH 3, deterioration of the peak shape often is caused by overloading. It is suggested to use low amounts of analyte (0.1 μg) for testing of stationary phases, and stationary phases are compared using efficiency and peak shape data. Although these test compounds were claimed to be generally available, both amphetamine and codeine are controlled drugs and therefore are not generally available to many analysts worldwide. A convenient way of developing a test method is to use compounds structurally closely related to the compounds for which the stationary phases have to be used. In Ref. [13] the selection of test compounds out of a large number of basic compounds is described. The disadvantage of this approach is that the test compounds usually are not generally available and therefore cannot be used by other chromatographers.

From a historical point of view, clear differences in the development of column test methods can be observed. The tests of Tanaka [7] and Engelhardt [9] are a decade old, meaning that these tests were developed using stationary phases of the same period. The test methods developed by Gonnet, Daldrup, Verzele, Neue and Walters [8,59,60,64,66] were developed from 1982 to 1987. Since that period many new stationary phases have been developed showing improved performance in the analysis of basic solutes. These phases were developed with respect to minimising ionic interactions and as a result the differences between these phases with respect to ionic interactions will be small compared to the ''older phases''. We therefore believe that the ''older'' tests are suitable to differentiate between generations of columns, as is demonstrated in Fig. 2 were clearly is shown the differences between a Symmetry C_8 and a Hypersil MOS column using the test of Engelhardt. For the modern phases of today only subtle differences are present, which cannot be visualised using tests like the Engelhardt and Tanaka test, and can best be detected using basic compounds as test substances. The comparison of test methods for the suitability to characterise stationary phase for the analysis of basic pharmaceutical compounds was performed recently. Column classification using the test developed by McCalley was in fair agreement with column classification based on the data obtained with a set of seven basic pharmaceuticals from daily practice [72]. Therefore, it can be concluded that classification of stationary phases for the analysis of basic pharmaceuticals is best performed using basic compounds as test probes.

3.1.3. Metal activity of stationary phases

As discussed before, metal impurities can influence the chromatographic properties of stationary phases and thus also the performance of a chromatographic analysis. Metals can increase the acidity of adjacent silanol groups as well as being strong

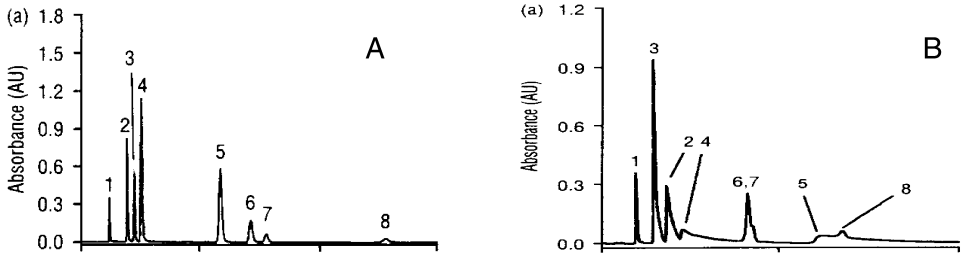


Fig. 2. Separation of the Engelhardt test mixture using a Symmetry C_8 (A) and a Hypersil MOS (B) column. Eluent methanol–water/49–51%w/w. Analytes: 1: thiourea, 2: aniline, 3: phenol, 4: *m*- and *p*-toluidine, 5: dimethylaniline, 6: benzoic ester ethylester, 7: toluene and 8: ethylbenzene. Reprinted from Ref. [65].

adsorption sites for complexing solutes. To determine the metal content of stationary phases chromatographically, various suggestions have been proposed. The first test solute was proposed by Verzele and Deweale [64]. They suggested that the peak shape and retention of acetyl acetone was a measure of metal contamination. Recently it was demonstrated that the peak of acetyl acetone was also affected by keto enol tautomerism effects [78] and therefore the effects observed cannot be described to metal activity solely. Other tests use aromatic di- or trihydroxy compounds [79–81], however, as demonstrated by Engelhardt and Lobert [82] these compounds were found to be insensitive in detecting metal impurities. In the same paper, Engelhardt and Lobert described the use of 2,2′-bipyridyl and 4,4′-bipyridyl to determine metal impurities. 2,2′-bipyridyl can form complexes with metals, whereas 4,4′-bipyridyl cannot.

Since both compounds are bases, also interaction with residual silanols can occur. However, for both compounds the pK_a and basic nitrogen atom are comparable. Therefore, the relative asymmetry of both compounds was found to be a good measure of metal content. In Fig. 3A examples of various columns are shown. The test on metal activity was performed using an unbuffered eluent. To demonstrate the influence of buffering the eluent, in Fig. 3B the difference between an unbuffered eluent and the use of 1 mM phosphate buffer pH 7 in the eluent is shown. As can be seen, using buffered eluents metal activity is suppressed. Therefore, Engelhardt and Lobert concluded that metal activity should be determined using unbuffered eluents. However, basic

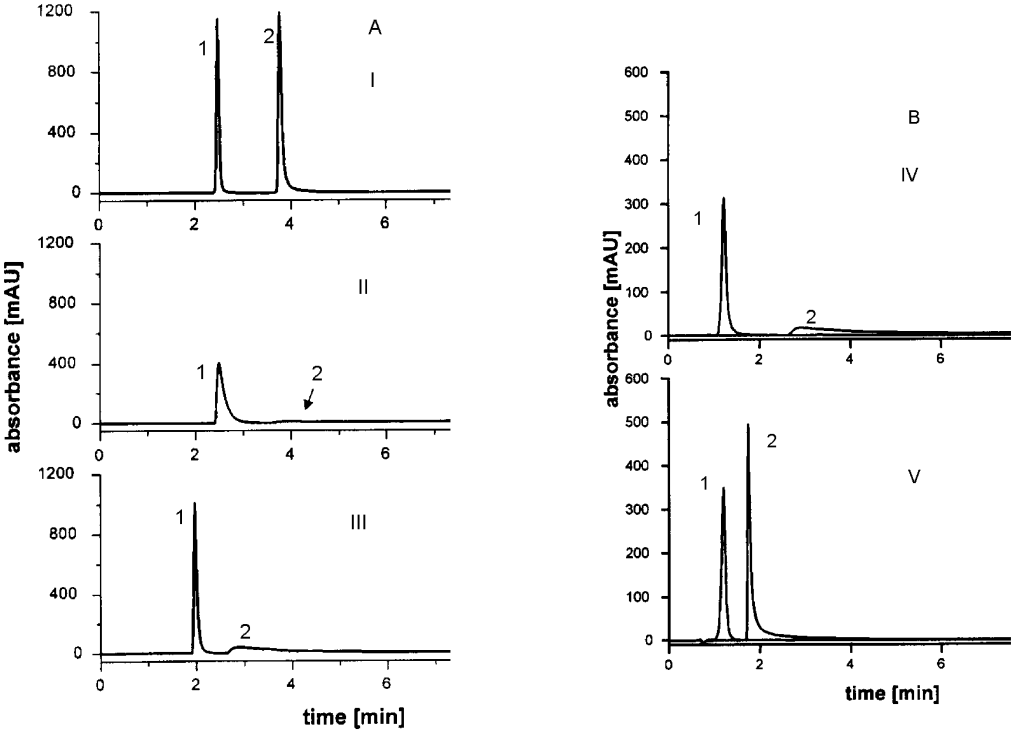


Fig. 3. Determination of metal activity on RP 18 columns. A: differentiation between silanophilic and metallophilic activity; I: low silanophilic and low metallophilic activity, II: high silanophilic activity, high metal content, III: low silanophilic activity and high metal content. B: Influence of buffer: IV: unbuffered eluent and V: buffered with 1 mM phosphate pH 7. Compound 1: 4,4′-bipyridyl, compound 2: 2,2′-bipyridyl. The eluent used was methanol + water/49 + 51%(w/w), the flow-rate was 1.0 ml/min, the column temperature was set to 40°C and detection was performed with UV at 254 nm. Reprinted from Ref. [82].

analytes are always analysed using buffered eluents. Therefore, metal activity data obtained with buffered eluents also show valuable information with respect to the analyses of basic pharmaceutical compounds.

3.2. Thermodynamically based evaluation method

Enthalpies and entropies of transfer of solutes from the mobile to the stationary phase can be calculated from retention data by evaluation of the van het Hoff plots [83]. The retention factor can be expressed in terms of standard enthalpies and entropies of transfer from mobile to stationary phase:

$$\ln k = \frac{-\Delta H^0}{RT} + \frac{\Delta S^0}{R} + \ln \phi \qquad (2)$$

The enthalpy (ΔH^0) represents the measure of energy exchange and a system strives to lowering ΔH^0. Entropy (ΔS^0) is the chaos and a system strives to ordering the chaos. ϕ is the phase ratio of the column. A plot of ln k vs. $1/T$ shows the van het Hoff plot and is linear if $\Delta H^0$and $\Delta S^0$are independent of the temperature. The slope of the van het Hoff plot gives the standard enthalpies of transfer, the standard entropies of transfer are calculated from the intercept and depend on the phase ratio. Comparison of stationary phases has been performed by studying the changes in mechanism of retention of test analytes with changing column temperature. Examples are the studies of Cole and Dorsey [84,85] who examined the influence of bonding density of stationary phases on the retention of non-polar solutes. They found that partition, rather than adsorption, was found to be the relevant model of retention for non-polar compounds. The columns which were compared differed in bonding densities ranging from 1.60 to 4.07 μmol/m^2. The entropic contribution to retention becomes more significant with respect to the enthalpy contribution as the stationary bonding density is increased. Using benzene as a test compound for the columns with a bonding density ≥3.06 μmol/m^2, as shown in Fig. 4, a non-linear van het Hoff plots was obtained. Other examples are the studies of Purcell et al. [86] using van het Hoff plots to study changes in the secondary structure of peptides and the mechanism of interaction with hydrophobic surfaces, and Philipsen et al. [87] using van het Hoff plots to study the retention of polystyrene and polyester oligomers. For basic solutes, retention is believed to be a combination of hydrophobic and ionic interaction [16]. Nonlinear van het Hoff plots might be indicative of a change of retention mechanism [87,88]. For basic compounds this means that the ratio of the hydrophobic and ionic interaction could change. However, studying the retention of basic compounds linear van het Hoff plots were obtained. As described in Ref. [72], no clear deviation from linearity was observed studying the retention from 10 to 80°C for both the McCalley test compounds and a set of basic compounds from daily practice. In Fig. 5 the van het Hoff plots for two basic compounds are shown using MeOH+25 mM NH_4Ac/50+50% v/v at buffer pH's 3 and 7 as eluent. It was therefore concluded that detecting changes in mechanisms of retention

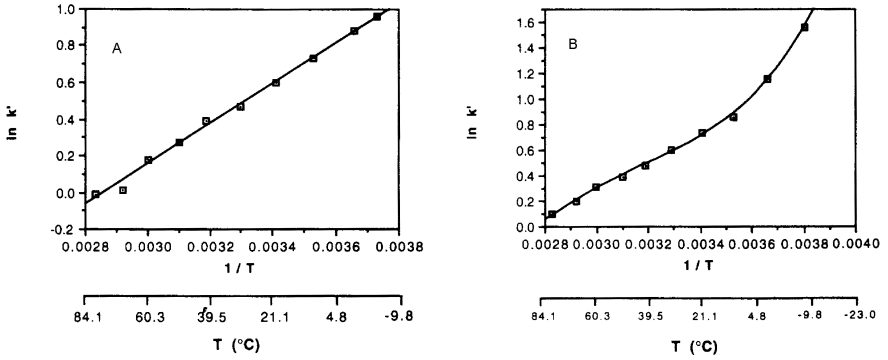


Fig. 4. van het Hoff plots for benzene on columns with bonding density 2.84 μmol/m^2 (A) and 3.06 μmol/m^2 (B). The eluent used was acetonitrile+water/60+40% v/v. Reprinted from Ref. [84].

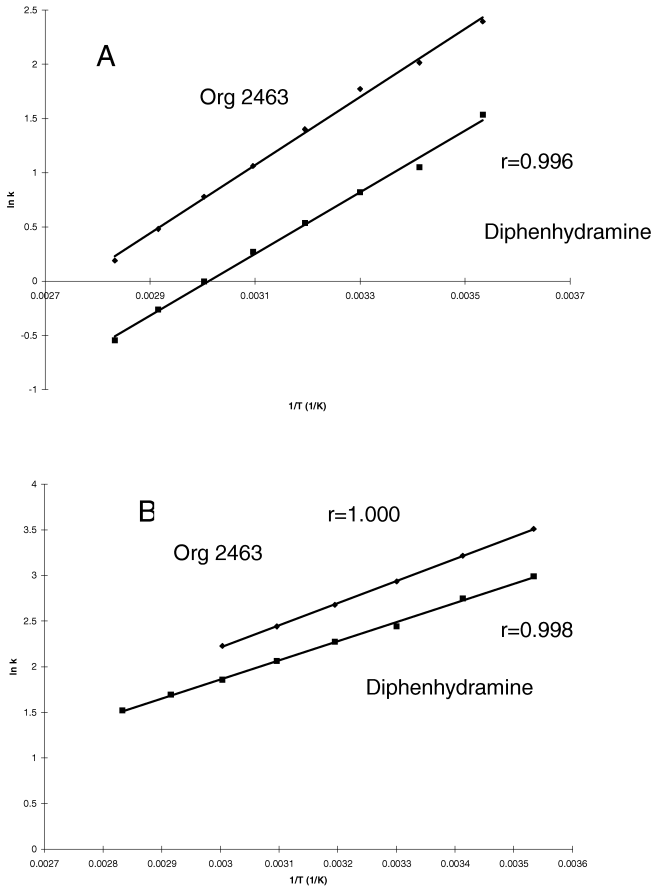


Fig. 5. van het Hoff plots for two basic compounds using MeOH+25 mM NH_4Ac/50+50% v/v at buffer pH's 3 (A) and 7 (B) as eluent. Reprinted from Ref. [72].

involved in the analysis of basic solutes was not straightforward studying the linearity of van het Hoff plots.

3.3. Evaluation methods based on retention models

As in most situations for the empirical and thermodynamically based tests, the model based evaluation methods are based on studying the retention of the test compounds, and differences between stationary phases are determined based on differences in retention behaviour of test compounds.

Galushko calculated retention in LC based on the molecular structure of the analyte and characteristics of the stationary and mobile phase. To calculate the retention, a two layer model of a chromatographic system is proposed [89,90]. The surface of a sorbent in reversed phase LC has a surface layer (SL) that involves octadecyl radicals and some of the components of a mobile phase, and SL is assumed to be a quasi-liquid that has its own characteristics, i.e. surface tension (γ_s) and dielectric constant (ε_s), and the SL characteristics vary with varying mobile phase and sorbent properties. The molecules of a retained analyte penetrate into the SL and the retention is determined by the difference in molecule solvation energies in the mobile phase and SL. The equation obtained for retention is:

$$\ln k = a\left(\sum_i V_i\right)^{2/3} + b\left(\sum_j \Delta G_{e.s.j,H_2O}\right) + c \qquad (3)$$

in which V_i are the increments of partial molar

volumes of the fragments of the test analytes in water, and $G_{e.s.j,H_2O}$ are the increments of energy of interaction of bond dipoles in water, respectively. Each column can be characterised quantitatively by using three parameters *a*, *b* and *c* that determine the retention. Using several reference compounds one can calculate the values of the parameters for each chromatographic system (mobile phase–stationary phase). Such set of parameters can thus be used to calculate retention of other compounds and for quantitative evaluation of differences between columns [91]. To determine column parameters accurately it is necessary to use test compounds that have substantial differences in V_i and $G_{e.s.j,H_2O}$, i.e. compounds differing in surface and polarity. The test mixture of Galushko to initially test stationary phases consists of aniline, phenol, benzene and toluene [91]. Column classification based on Galushko's approach was recently compared with column classification using the empirical Engelhardt and Tanaka tests [63]. For the hydrophobic column properties, good correlations were observed. However, for the ionic properties, low correlation between the tests was found. Judgement of which test yields the best information is difficult. In Ref. [72] various empirical and model based tests are compared and correlated with the peak shapes of a set of basic solutes. It was obvious that the Galushko test revealed only limited information about ionic properties of stationary phases as shown by the low agreement of the Galushko data with the peak shapes of the set basic compounds. However, the retention data of aniline, phenol, benzene and toluene are only used for an initial characterisation. A final characterisation of column and stationary phase is obtained using the retention data of the compounds of interest.

Quantitative structure–retention relationships (QSRR) are relationships between physical parameters determined for test solutes and molecular descriptors which describe the structures of the test analytes. In fact, Eq. (3) can also be considered as a QSRR. In the last two decades QSRRs have often been applied to: (i) predict retention for a new solute, (ii) identify the most informative structural descriptors, (iii) gain insight into the molecular mechanisms of separation, (iv) evaluate complex physicochemical properties of analytes, and (v) predict relative biological activities within a set of drugs [92]. Different approaches have been described in the literature with respect to characterising chromatographic systems, i.e. regression of log *k* values against 1-octanol–water partition coefficients (log *P*) [93,94], linear solvent energy relationships (LSER) using experimentally determined molecular descriptors [95,96], and describing log *k* values in terms of calculated molecular descriptors [97,98].

Regression of log *k* against log *P* was performed by Kaliszan et al. [11] and revealed information about the hydrophobic properties of the columns. Log k_w data were obtained for 18 different columns and were linearly regressed against log *P*, i.e. log $k_w = k_1 + k_2$ log *P*. The value of k_2 reflects the degree to which the analyte is surrounded by the stationary phase. The more the solvated stationary phase is alike to octanol, the closer k_2 should be to one. In Fig. 6 the stationary phases are ordered by their ability to mimic octanol, i.e. hydrophobic properties. The Aluspher column was found to be the most hydrophobic, whereas the Nucleosil C_8 column was found to be least hydrophobic.

The general equation for LSER is:

$$\log SP = c + rR^2 + s\pi_2^H + a\sum \alpha_2^H + b\sum \beta_2^H + vV_xS \quad (4)$$

where *SP* is a solute property, e.g. solubility, partition coefficient or retention, V_x is a parameter characteristic of the solute size (e.g. molar volume), π_2^H a measure of the solutes capacity for dipole/polarizability interactions and α_2^H and β_2^H characterize the solutes hydrogen donor acidity and basicity, respectively. The terms *c*, *r*, *s*, *b*, and *v* are related to the chemical nature of the mobile and stationary phase and are unique for the combination of mobile and stationary phase. Therefore, these parameters can be suitable for stationary phase characterisation [99]. Chan et al. [100] used the retention data of 87 aliphatic and aromatic solutes obtained using five bonded phases and acetonitrile + water/50 + 50% v/v as eluent in LSER studies. The most important retention governing solute parameters were found to be solute volume and hydrogen bond acceptor basicity. The relative importance of the solute parameters in explaining the data did not differ for the five bonded phases studied. Buszewski et al. [101] used LSER to show differences between a conventional

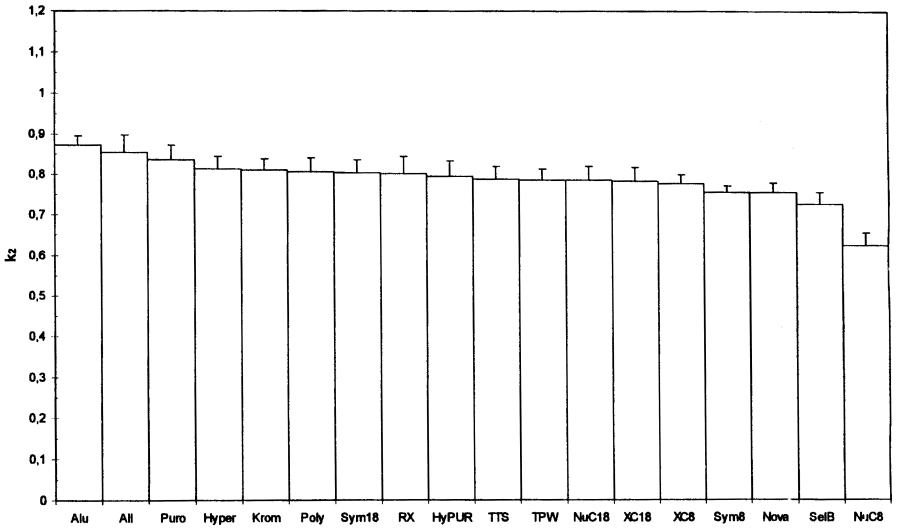


Fig. 6. k_2 values for 18 columns of log k_w vs. log P relationship. The log k_w data were obtained in methanol + water eluents. Reprinted from Ref. [11].

C_{18} bonded silica and *N*-acylaminopropyl silica. It was demonstrated that using the C_{18} bonded phase retention was dominated by the reversed phase mechanism. For the *N*-acylaminopropyl bonded phase, however, a significant effect on retention was shown for the structurally specific dipole–dipole and charge transfer interactions with the solutes. Sandi et al. [62,102,103] used the retention factors of 34 solutes obtained under isocratic conditions using 15 different LC columns. Principal components analysis (PCA) was performed to differentiate between the various columns. They found that the first principal component (PC1) described the hydrophobic character, the second PC described the hydrogen bonding acceptor basicity, whereas the third PC described the hydrogen bonding donor acidity of the various columns.

Column classification using calculated molecular descriptors was performed by Kaliszan [11]. The applicability was demonstrated by the ordering of 18 stationary phases according to their calculated total dipolarity properties. The ordering was found to be comparable to the ordering of the columns by the hydrogen-bond donor properties determined via LSER (see Fig. 7). Both total dipolarity and hydrogen-bond donor are related with the accessibility of the analyte with free silanols of the stationary phase.

4. Eluent composition

In Table 2 a selection of the discussed empirical based evaluation methods are shown. As can be seen,

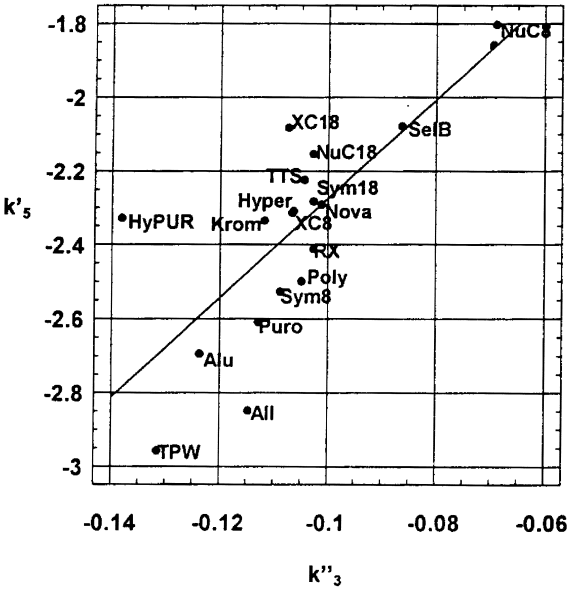


Fig. 7. Ordering of stationary phases according to their hydrogen-bond donor activity (k'_5) and total dipolarity (k''_3). Reprinted from Ref. [11].

some use buffered eluents, whereas others use non-buffered eluents to test columns for ionic interactions. Whether the eluent should be buffered or not, is a subject of discussion [9,12,63]. Since buffers can mask silanol interactions, testing in unbuffered systems can be advantageous for silanol determination [65]. In daily practice basic compounds are analysed using buffered eluents to control the ionisation of the analyte to obtain robust separations. Moreover, a test that is generally accepted for column characterisation should be rugged and reproducible in many different labs world-wide. This can only be achieved by using a rugged eluent, which can only be obtained using buffered eluents. To obtain rugged eluents, preparation and control of the pH of the eluent must be performed carefully. Therefore, the influence of eluent composition on the analysis of ionisable analytes is discussed.

In LC it is common practice to measure the pH of the mobile phase buffer before mixing with the organic modifier. However, it is also recognised that the pH changes after addition of organic solvent since the pK_a values of the acids used to prepare the buffers change with the solvent composition [104–106]. In Fig. 8 the influence of percentage methanol on the pK_a values of acids often used for preparing LC buffers are shown. The dissociation of electrolytes in binary solvents depends strongly on their preferential solvation and this is different for each solute [107]. Moreover, one should be aware that,

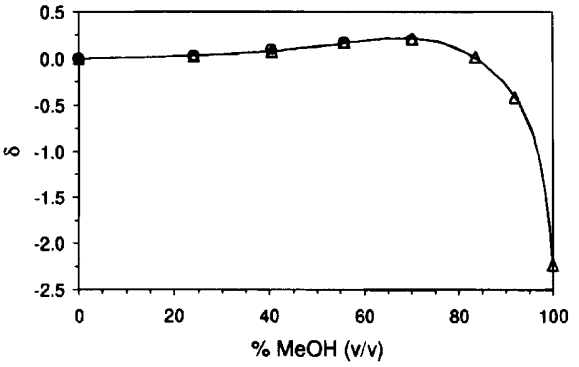


Fig. 9. Variation of δ with solvent composition. Reprinted from Ref. [111].

dependent on the type of buffer ions, addition of methanol can result in an eluent pH outside the pH range commonly used with silica based LC columns [108]. For organic modifier–buffer mixtures the pH can be calculated from measurements using conventional pH electrodes:

$$pH_x^* = pH_x^{app} - \delta \quad (5)$$

in which pH_x^* is the pH value in an aqueous–organic system, pH_x^{app} is the measured value in an aqueous–organic system and δ is a correction factor for the liquid junction between the electrode and the eluent [105]. Values of δ have been published for several methanol–aqueous systems, and as shown in Fig. 9 it

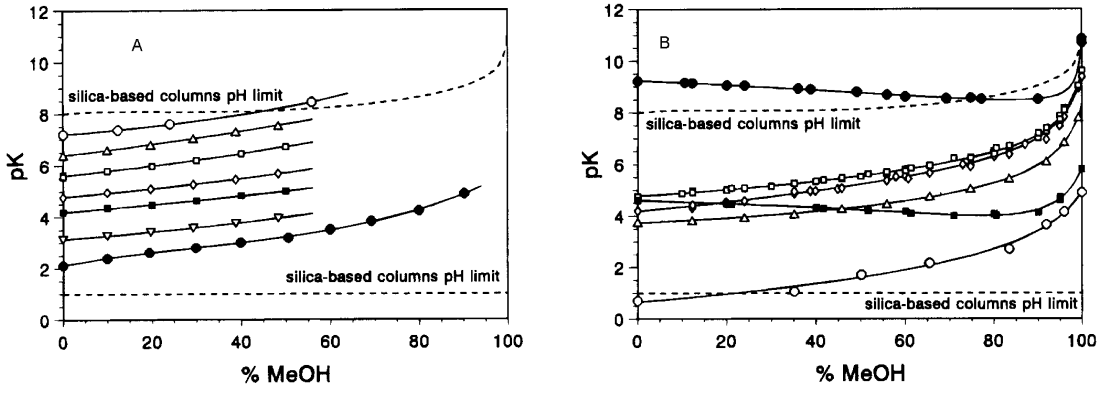


Fig. 8. Variation of pK_a values of acids with methanol–water composition. A: ● pK_{a1} of phosphoric acid, ∇ pK_{a1} of citric acid, ■ pK_{a1} of succinic acid, ◊ pK_{a2} of citric acid, □ pK_{a2} of succinic acid, △ pK_{a3} of citric acid and ○ pK_{a2} of phosphoric acid. B: ○ trichloroacetic acid, △ formic acid, ◊ benzoic acid, □ acetic acid, ■ anilinium and ● ammonium. Reprinted from Ref. [108].

Table 3
pH values in methanol+buffer mixtures at 25°C, reprinted from Ref. [104]

Buffer	% Methanol				
	0	10	30	40	50
KH_2–citrate	3.77	3.94	4.28	4.47	4.65
KH–phtalate	4.01	4.24	4.63	4.87	5.13
Acetate	4.65	4.78	5.13	5.35	5.49
$H_3PO_4/H_2PO_4^-$	2.12	2.38	2.68	2.86	3.12

can be concluded that for amounts of organic modifier up to 80% the correction factor is small, i.e. approx. 0.1 [109–111].

Recently Barbosa et al. [104] determined pH values for eluents using various organic modifiers as well as various buffers. In Table 3 the results obtained with methanol are shown. As can be seen, addition of 50% methanol to the buffers resulted in an increase of approx. 1 pH for the eluent. When using different modifiers and buffer ions, different pH values are obtained. For sodium phosphate the relation between buffer pH and measured pH of the eluent after preparing a 1:1 mixture with methanol with 25 mM buffers from pH 3 up to pH 7 was found to be approx. linear as shown by the correlation coefficient of >0.99. Comparable data were found for ammonium acetate, ammonium citrate and ammonium phosphate buffers [72]. However, small differences between the slopes of the regression lines were obtained.

Besides the pH of the eluent, the organic modifier will also influence the pK_a of the basic analyte. Comparable to the pH of the eluent, the pK_a values of the analytes are often measured in pure water. In Fig. 10 the influence of the methanol, acetonitrile and tetrahydrofuran concentration on the pK_a of the basic pharmaceutical mirtazipine is shown. As can be seen increasing the modifier concentration lowers the pK_a value of the basic analyte, which can influence the chromatographic performance of the basic analyte [72]. The effect was comparable for methanol and acetonitrile, but more significant for tetrahydrofuran.

An example of the influence of a changed pK_a and eluent pH as a result of addition of modifier to the aqueous solution was recently described by Neue et al. [61,67]. A shift in retention observed for propranolol at a given buffer pH did not correspond with the pK_a value, which were both measured without modifier. However, the pK_a of the analyte will be lower compared to the value measured in pure water, whereas the pH of the eluent will be higher as the pH value of the pure buffer. Therefore, the analyte might be analysed as a neutral compound, explaining the shift in retention. Other possible effects that should also be considered are the influence of the eluent pH on the stationary phase inside the column as well as

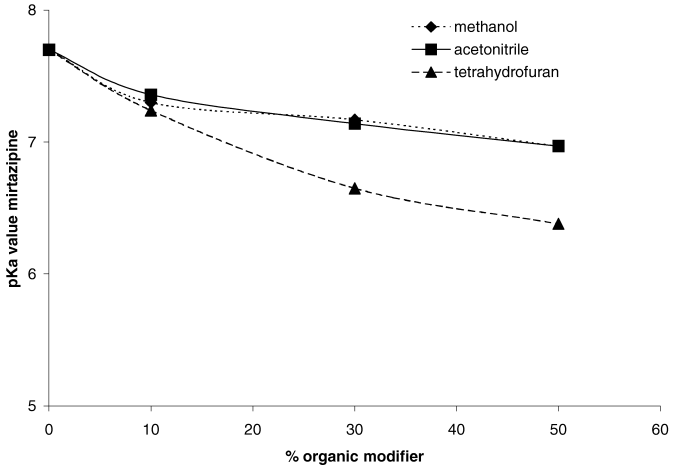


Fig. 10. Relation pK_a vs. % modifier for the basic pharmaceutical compound mirtazipine at 25°C. Reprinted from Ref. [72].

the effect of the organic modifier on the pK_a of the residual silanol groups [70].

5. Chemometric evaluation of characterisation data

Tests of stationary phases usually results in large amounts of data such as retention factors, peak asymmetries and plate numbers of the test compounds used with the various stationary phases tested. Extraction of valuable information from these large amounts of data is difficult. Chemometric techniques can be helpful tools to extract information from large datasets, enabling the classification of objects (stationary phases) with comparable characteristics [112]. The most frequently used tool is principal component analysis (PCA). With PCA, a set of new variables (principal components, PC) is defined instead of the original variables. To find the PC of a data matrix, X, the first step is to look for a vector t_1 (score)$=Xp_1$ that is a linear function of X with maximum variance, where p_1 (loading) is a vector of m constants $p_{11}, p_{12}, \ldots, p_{1m}$ and length 1. The second step is to look for a linear function Xp_2, orthogonal to Xp_1, which has maximum variance, etc. A datamatrix can thus be written as:

$$X = t_1 p_1^T + t_2 p_2^T + \cdots. \quad (6)$$

Since most of the variability of the data is present in the first PC's, it is possible to present the datamatrix X graphically [113] and the advantage of PCA is the ability to handle a more expansive dataset.

Delaney et al. [114] used PCA to classify columns tested using the method developed by Walters [8]. The use of PCA resulted in the same classification scheme as determined by a LC specialist. Schmitz et al. [115] and Walckzak et al. [116] presented differences between stationary phases using correspondence factor analysis (CFA) by which similarities in the structure of the objects (stationary phases) and the variables were presented simultaneously. Hamoir et al. [117] used spectral mapping analyses (SMA) to classify 16 stationary phases. Both CFA and SMA are chemometrical tools closely related to PCA.

In Ref. [118] the classification using PCA of 14 commercially available stationary phases characterised for the applicability to analyse basic analytes using phosphate buffers pH 3 and 7 is described. Brereton and McCalley [119], who classified eight commercially available stationary phases also with respect to the analysis of basic analytes used the same approach. In Fig. 11 the classification of the columns using buffers pH 3 and 7, and methanol, acetonitrile and tetrahydrofuran as modifiers, is shown. The influence of the nature of modifier on column classification is clear. Sandi et al. [62] compared PCA data of the test compounds with data from LSER studies. Solvatochromic parameters were regressed against the principal components (PC) and it was found that the first PC was a measure of hydrophobic strength whereas the second PC was positively correlated with hydrogen bond acceptor and polarizability/dipolarity. A negative correlation of the second PC was observed for the molecular size and hydrogen bond donor. The third PC was only positively correlated with hydrogen bond donor.

Besides the classification of stationary phases, PCA has also successfully been applied to reduce the number of test compounds. In Ref. [13] it is shown how PCA is used to reduce a set of 32 test compounds. The test compounds were presented in the score plots in which compounds describing comparable information are clustered. By selecting compounds situated apart from each other in the score plot, the number of compounds in the test set was reduced to five. In Fig. 12 the score plot is shown and the underlined compounds were selected to test columns.

In summary, the discussed examples clearly revealed that a graphical presentation of a large n-dimensional data set by means of chemometrical techniques can be very useful in classification of objects of interest, i.e. stationary phases and test compounds.

6. Conclusions

For the LC analysis of basic pharmaceuticals, optimisation of the mobile phase can be achieved by choosing the optimal eluent pH, addition of silanol blocking compounds etc., whereas optimisation of the stationary phase is achieved by minimising the

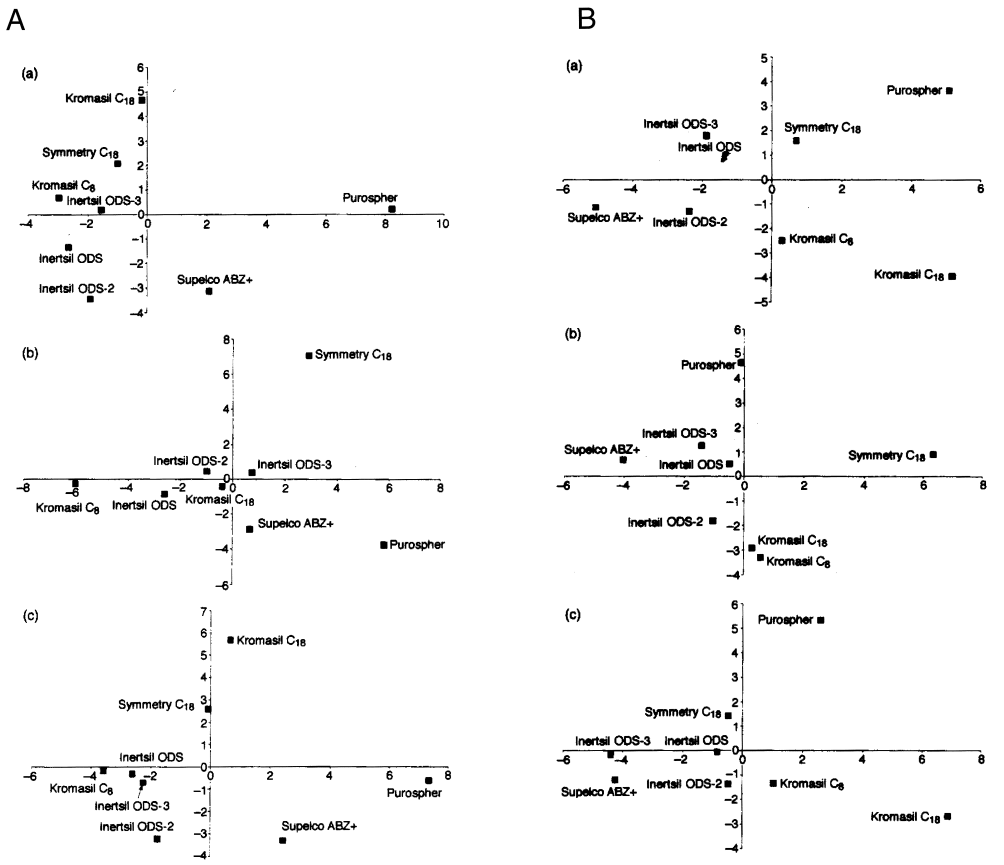


Fig. 11. Score plots of column classification at pH 3.0 (A) and pH 7.0 (B): (a) methanol, (b) tetrahydrofuran and (c) acetonitrile. Reprinted from Ref. [119].

interaction between analyte and residual silanols. Moreover, stationary phases have been developed which can be used at high eluent pH to enable the analysis of basic analytes as non-protonated compounds. Due to the large number of columns available, however, chromatographers get confused which column to use for their application. To allow the selection of suitable columns, the availability of a good and efficient column characterisation procedure would be of great help to classify columns into classes with comparable characteristics.

For the characterisation of stationary phases various attempts have been reported and it is confusing for the chromatographers which procedure should be used. The majority of column characterisation procedures can be divided into empirical methods, thermodynamically based and model based methods. The empirical methods are based on the chromatographic performance of compounds chosen according to a certain line of thought. Most of these methods were developed more than 10 years ago and use the retention of compounds like aniline, benzylamine and phenol to calculate characteristics like silanol activity and ion exchange capacity. The applicability of these methods to characterise the modern phases of today for the analysis of basic compounds is questionable. Other empirical methods use test compounds that are structurally related to the compounds of interest. For instance, the test developed by McCalley use amongst others compounds like diphenhydramine and nortriptyline to characterise columns for the applicability to analyse basic pharmaceutical compounds. Tests of this nature were found to be suitable to differentiate between columns with

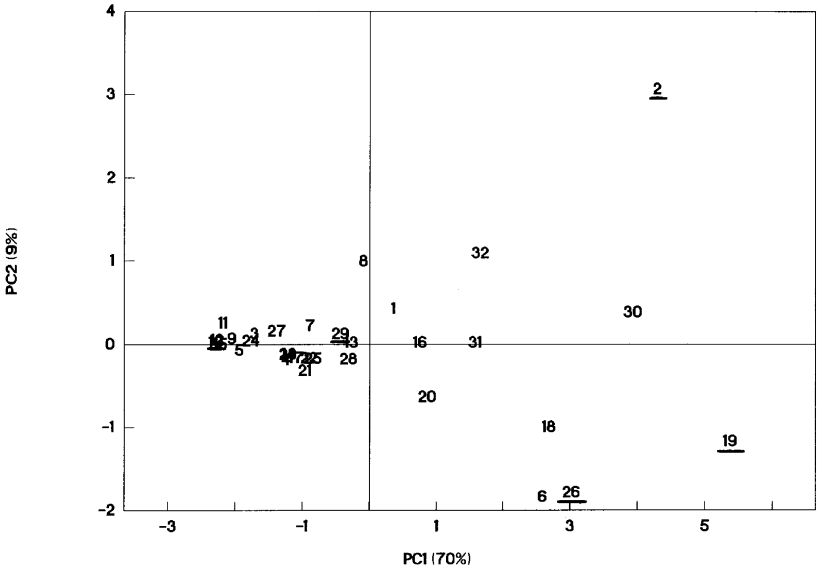


Fig. 12. PCA score plots for peak symmetry data of 32 basic compounds obtained with six different LC columns. Reprinted from Ref. [13].

respect to suitability for the analysis of basic pharmaceuticals. Therefore, in daily practice the McCalley test is a preferable tool to select suitable columns for the analysis of basic pharmaceuticals.

Van het Hoff plots can be used to study a change in retention mechanism caused by a variation of the column temperature. However, studies performed so far using basic pharmaceuticals showed that the change in retention mechanism cannot be detected investigating the linearity of van het Hoff plots.

Studies using quantitative structure–retention relationships (QSRR) require physical parameters determined for the test analytes. Meaningful classification was obtained using QSRR studies. Significant differences could be quantitated between a C_{18} and a *N*-acylaminopropyl bonded phase, whereas comparison of C_{18} phases resulted in a ordering of the phases. A comparable ranking of columns was obtained using data obtained from LSER and QSRR studies. A disadvantage of these methods is the circumstantial and laborious experiments needed to obtain the required retention parameters. Therefore, these methods are preferably not used in daily practice. Nevertheless, both LSER and QSRR reveal useful information about mechanisms of retention.

Special attention in the LC analysis of bases should be paid to the eluent composition. Some tests use buffered eluent, whereas others use non-buffered eluents to test the column for ionic interactions. Since buffers can mask silanol interactions, testing in unbuffered systems can be advantageous for silanol determination, however, in daily practice basic compounds are analysed using buffered eluents to control the ionisation of the analyte to obtain robust separations. A test that is generally accepted for column characterisation should be rugged and reproducible in many laboratories and in many countries. This can only be achieved by using a stable, i.e. a buffered eluent. However, one should be aware that the amount and nature of the modifier affects the pH of the eluent, as well as the pK_a of the basic analyte. Moreover, possible effects of the stationary phase to the pH of the eluent inside the column should also be considered as well as the effect of the organic modifier on the pK_a of the residual silanol groups.

Stationary phase testing usually results in large amounts of data such as retention factors, peak asymmetries and plate numbers of the test compounds used with the various stationary phases tested. Extraction of valuable information from these large amounts of data is difficult. Chemometric techniques have proved to be useful tools to extract information from such large datasets. Especially the use of principal components analysis or techniques

closely related, should be considered when columns and test compounds are characterised and classified into groups with similar characteristics.

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