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Characterization and comparison of the chromatographic performance of different types of reversed-phase stationary phases

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Abstract

The chromatographic performance of several base-deactivated stationary phases was evaluated with a specific chromatographic test. Seven basic test compounds, possessing different physico-chemical properties were injected on different supports with two mobile phases: one at pH 7.0 (acetonitrile–phosphate buffer, 40:60, v/v), and the other at pH 3.0 (acetonitrile–phosphate buffer, 15:85, v/v). Chromatographic parameters obtained under these conditions were treated by principal component analysis (PCA) to separate base deactivated supports according to their silanol activity (pH 7.0 mobile phase) and hydrophobic properties (pH 3.0 mobile phase). The information given by the specific test column evaluation was improved with complementary chemometric tools such as hierarchical cluster analysis. The same base deactivated supports were also tested following a general test procedure issued from the literature and obtained fundamental properties (in particular silanol activity and hydrophobicity) were compared with column evaluation obtained with the specific test: results were in good agreement, although the use of the specific test offered a better differentiation between numerous base-deactivated supports.

Keywords: High-performance liquid chromatography; Basic compounds; Principal component analysis; Chromatographic test

1. Introduction

Reversed phase liquid chromatography (RPLC) is considered as the method of choice for the analysis of pharmaceutical compounds for several reasons such as its wide applicability, its compatibility with aqueous and organic solutions as well as with different detection systems [1–3]. Sensitive and accurate RPLC analysis, whether in the pharmaceutical or bioanalytical field necessitates the use of stationary phases which give symmetrical and efficient peaks. Therefore, manufacturers are continuously improving and introducing new RPLC phases. In particular for the analysis of basic compounds which represent almost 80–90% of the pharmaceutical compounds, the so-called base-deactivated columns have been largely developed last 15 years [4–7].

Unfortunately the generic name of these supports does not sufficiently describe their chromatographic behaviour. Differences in similarly labeled commercial columns lie in both the nature of the silica support and the technique used to produce the bonded phase. Factors such as particle size, surface area, pore size, trace metal activity, bonded phase surface activity, bonding chemistry, silica deactivation process can all influence retention selectivity and peak shape properties of analytes. All these variables will result in significant differences in chromatographic performances among packings as well as batch differences for a given packing [8]. For these reasons, several procedures have been published to evaluate interactions between solutes and the packing material [9,10]. A general test for bonded silicas based on three criteria (i.e. the shape discrimination facility for isomers based on their configuration, the level of silanol activity and the hydrophobicity) has been reported elsewhere by one of

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us [11]. More than 130 commercial C_{18} materials, including embedded, hydrophilic end-capped and polar end-capped alkyl bonded silicas were tested. As a result, stationary phases were partially ranked according to the level of residual silanols. It is then possible to select those which possess similar and different behaviour.

For the analysis of basic compounds, chromatographic performances are strictly compounds dependent and for this reason approaches considering a set of basic test compounds possessing different physico-chemical properties supports [12–14] are often preferred to the currently used general test procedures [15–17]. In a previous work [18] different base deactivated columns with reduced silanophilic interactions were initially tested with a set of 14 basic test compounds, covering a wide range of physico-chemical properties. This procedure was successively simplified and applied to build a database of different base deactivated columns. Results demonstrated that it was possible to separate columns with closely related characteristics. Furthermore, a reduced methodology with mobile phases at pH 7.0 and 3.0 allowed the evaluation of silanol groups masking capacity and hydrophobic properties of the selected supports, respectively [19].

The aim of this work was to achieve the better characterization of a set of stationary phases especially dedicated to the analysis of basic compounds. For this reason, the previously developed specific test for base deactivated supports [19] was applied on 27 stationary phases and results compared to those obtained following other procedures issued from the literature such as Tanaka's, Engelhardt's and Cruz's test [15–17]. Among all the tested columns, some of them were conventional columns. They were included for improving the reliability of our specific test to select the dedicated column for basic compounds analysis. As already demonstrated elsewhere [8,20–22], chemometric tools can be used to better evaluate the huge amount of data obtained during the chromatographic test. In addition, the reliability of two-dimensional principal component analysis (PCA) plots obtained with the specific test was improved by applying another multivariate analysis, namely hierarchical cluster analysis (HCA).

2. Experimental

2.1. Materials and chemicals

Test solutes used for the characterization of chromatographic supports were of analytical reagent grade. Procainamide hydrochloride (PR), nicotine (NI), pyridine (PY), amylbenzene (AB), butylbenzene (BB), triphenylene (TP), *o*-terphenyl (TE), caffeine (CF), phenol (PH), *N*,*N*-dimethylaniline (NN) were provided by Fluka (Buchs, Switzerland). Uracil (UR) was obtained from Sigma (Buchs, Switzerland). Methadone hydrochloride (MT) and quinine hydrochloride (QN) were from Hänseler AG (Herisau, Switzerland). Fentanyl citrate (FN) was obtained from Macfarlan Smith Limited (Edinburgh, Scotland). Chloroprocaïne hydrochloride (CL) was provided by Orgamol (Evionnaz, Switzerland). Aniline (AN) was obtained from Acros

Table 1
Tested columns characteristics

Column	Alkyl chain	Bonding type	Particle size/pores	Column dimensions (mm)	Manufacturer	Batches tested	Abbreviation
Acclaim	C_{18}	–	5 μm/120 Å	4.6 × 150	Dionex®	1	ACL
Chromolith performance	C_{18}	Monolith	–	4.6 × 100	Merck®	3	PER
Chromolith speedrod	C_{18}	Monolith	–	4.6 × 50	Merck®	3	CHR
Discovery RP amide C_{16}	C_{16}	Polar group	5 μm	4.6 × 150	Supelco®	3	DIS
Hypersil GOLD	C_{18}	Ultra pure silica	5 μm/175 Å	4.6 × 150	Thermo electron corp.	1	THERMO
Luna	C_{18}	High density	5 mm/100 Å	4.6 × 150	Phenomenex®	1	LUN
Nucleodur	C_{18}	Endcapping	5 μm/100 Å	4.0 × 125	Macherey-Nagel®	3	NUC
Nucleodur 100-5 CN-RP	CN	Cyano	5 μm/100 Å	4.0 × 125	Macherey-Nagel®	1	NUCN
Nucleodur C18 gravity	C_{18}	High density	5 μm/100 Å	4.0 × 125	Macherey-Nagel®	3	GRA
Nucleosil C_{18} AB	C_{18}	Polymeric bonding	5 μm/100 Å	4.0 × 125	Macherey-Nagel®	3	AB
Nucleosil C_{18} nautilus	C_{18}	Polar group	5 μm/100 Å	4.0 × 125	Macherey-Nagel®	3	NAU
Nucleosil HD	C_{18}	High density	5 μm/100 Å	4.0 × 125	Macherey-Nagel®	3	HD
Nucleosil protect	C_8	Polar group	5 μm/100 Å	4.0 × 125	Macherey-Nagel®	3	PRO
Purospher STAR	C_{18}	Dense polymeric	3 μm	4.0 × 55	Merck®	2	PUR
Pyramid	C_{18}	Hydrophilic endcapping	5 μm/110 Å	4.0 × 125	Macherey-Nagel®	3	PYR
Stability BS C_{23}	C_{23}	Charged polar group	5 μm/100 Å	4.6 × 250	CIL-Cluzeau®	1	STA 100
Stability BS C_{23}	C_{23}	Charged polar group	5 μm/300 Å	4.6 × 250	CIL-Cluzeau®	1	STA 300
Supelcosil ABZ plus	C_{16}	Polar group	5 μm	4.6 × 150	Supelco®	3	ABZ
Symetry shield	C_{18}	Polar group	5 μm/100 Å	4.6 × 100	Waters®	3	SYM
Uptisphere 5 HDO	C_{18}	Endcapping	5 μm/120 Å	4.6 × 250	Interchim®	3	UPHDO
Uptisphere 5 HSC	C_{18}	Endcapping	5 μm	4.6 × 250	Interchim®	3	UPHSC
Uptisphere 5 NEC	C_{18}	–	5 μm/110 Å	4.6 × 250	Interchim®	3	UPNEC
Uptisphere 5 ODB	C_{18}	Endcapping	5 μm/110 Å	4.6 × 250	Interchim®	3	UPODB
Uptisphere 5 TF	C_{18}	Polymeric bonding	5 μm	4.6 × 250	Interchim®	3	UPTF
Xterra RP C_{18}	C_{18}	Hybrid support	5 μm/100 Å	4.6 × 150	Waters®	3	TER
Zorbax Eclipse XDB	C_{18}	High density	5 μm	4.6 × 150	Agilent®	1	ECL
Zorbax extend C_{18}	C_{18}	Bidentate bonding	5 μm	4.6 × 150	Agilent®	3	EXT

Organics (Geel, Belgium) and toluene (TN) from SDS (Peypin, France). Acetonitrile and methanol were of HPLC gradient grade from SDS (Peypin, France). Water was obtained with the Milli-Q Water Purification System from Millipore (Milford, MA, USA). Aqueous buffers were prepared with di-potassium hydrogen phosphate and potassium dihydrogen phosphate (Fluka-Buchs, Switzerland) by measuring pH with a Metrohm pH meter (Herisau, Switzerland).

Tested columns and their characteristics are listed in Table 1. The selected untreated special base bonded silicas (ACL, PER, CHR, UPTF, UPODB, UPHDO, UPNEC, UPHSC, NUC and AB) possess different molecular discrimination properties and silanol activities according to Ref. [11]. Furthermore, THERMO, GRA, HD, EXT, LUN, ECL displayed very low accessibility to residual silanols and consequently could be well adapted to the analysis of basic compounds, as well as polar embedded bonded silicas (DIS, NAU, PRO, STA 100, STA 300, ABZ and SYM). Some additional stationary phases displaying a low accessibility to residual silanols (PYR, PUR, and TER) or a particular selectivity (NUCN) were included.

2.2. Apparatus

Column testing was performed with a Merck-Hitachi LiChrograph constituted of a L-6200 pump, an AS-2000 automatic injector and a L-4250 UV–vis programmable detector operating at 215 nm. Data acquisition and evaluation were performed by the D-7000 HPLC System Manager Software. Connections were made with minimum lengths of 0.25 mm i.d. tubing.

2.3. Specific test

Two different mobile phases were used to test the selected chromatographic supports. The chromatographic performances of these columns both at neutral (pH 7.0) and acidic (pH 3.0) pH values were compared:

- Mobile phase 1: acetonitrile—pH 7.0, 0.0375 M phosphate buffer (40:60, v/v)
- Mobile phase 2: acetonitrile—pH 3.0, 0.0265 M phosphate buffer (15:85, v/v)

For the mobile phase 1, buffers were prepared by dissolving the appropriate amount of KH_2PO_4 and K_2HPO_4 in water and by mixing these two solutions to attain pH 7.0. The mobile phase 2 buffer was prepared by dissolving the appropriate quantity of KH_2PO_4 in water and adjusting the pH with concentrated phosphoric acid [18–20]. In all cases, the pH was measured before adding the organic modifier.

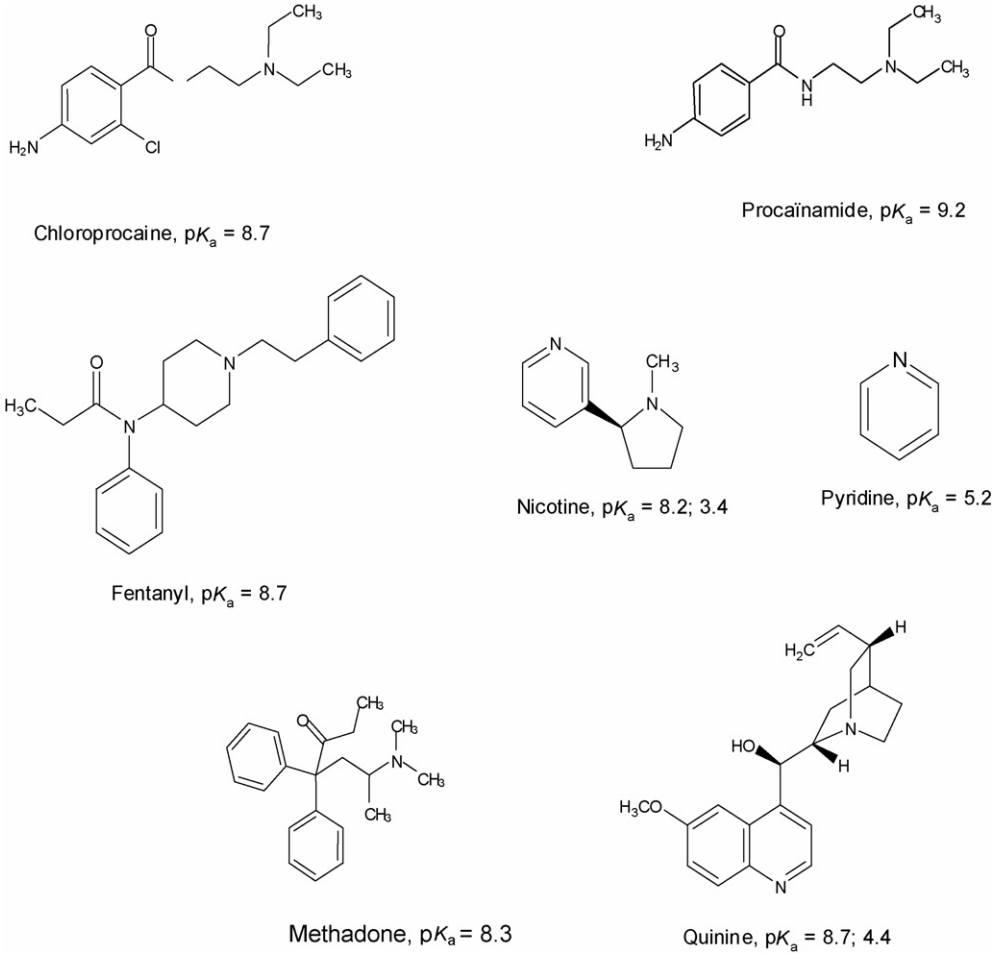

Fig. 1. Basic test compounds and pK_a values.

Seven previously selected basic test compounds (Fig. 1) were injected on each chromatographic support with both mobile phases in isocratic mode [19]. In order to assess the batch-to-batch variability, three columns of different batches were tested for each support when available and two chromatographic parameters, namely retention factor (k) and asymmetry (As) were measured:

$$\text{Retention factor}: k = \frac{t_r - t_0}{t_r} \quad (1)$$

where t_r was the retention time and t_0 the column void volume retention time (measured with $NaNO_3$)

$$\text{Asymmetry}: \text{As} = \frac{1 + B/A}{2} \quad (2)$$

where A and B were peak widths evaluated at 5% of the peak height.

2.4. General tests

Selected supports were also tested according to experimental conditions given in the literature [15–17] and the following properties were measured:

- *hydrophobicity* (k_{AB}): the measure of the hydrophobic retention capacity of the stationary phase determined from the amylbenzene retention factor (k_{AB}).
- *methylene selectivity* (α_{CH_2}): the ability of a phase to distinguish two compounds differing of a single methylene ($-CH_2-$) unit substitution determined by injecting simultaneously amylbenzene and butylbenzene.
- *steric selectivity* ($\alpha_{T/O}$): characterized by the separation of triphenylene and *o*-terphenyl presenting similar polarity but different shapes. This parameter allows the characterization of the alkyl chain density and the bonded phase surface.
- *silanol activity at pH* 2.5 ($\alpha_{B/P\ pH\ 2.5}$) *and at pH* 7.5 ($\alpha_{B/P\ pH\ 7.5}$): the amount of ion exchange interactions with basic compounds determined under conditions in which the majority of silanol groups was uncharged (pH 2.5) and charged (pH 7.5). This was characterized by the selectivity obtained between benzylamine and phenol in both mobile phases. When comparing caffeine/phenol or benzylamine/phenol selectivities with *trans*-β-carotene/zeaxanthin selectivity, identical conclusions on silanol activity were obtained [11].
- *chromatographic behaviour of a weak basic compound* ($\alpha_{An/Ph}$): selectivity between aniline and phenol is used to determine the silanol activity.
- *chromatographic behaviour of a strong basic compound* ($\alpha_{D/T}$): selectivity between *N*,*N*-dimethylaniline and toluene is measured to determine the silanol activity.
- *Asymmetry of aniline* (As_{An}) *and N,N-dimethylaniline* (As_D): the asymmetry factor of both compounds is measured to determine the chromatographic behaviour of a weak and a strong base, respectively, obtained with an unbuffered mobile phase.

2.5. Software

Data handling (principal component analysis and hierarchical cluster analysis) was performed with the XLStat 6.5 (AddinSoft, France) and Simca-P 11.0 (Umetrics AB, Sweden) software packages.

3. Results and discussion

A new generation of "base deactivated" stationary phases is now commercially available for the analysis of basic compounds. Unfortunately, strong ionic interactions of cationic analytes with residual silanol groups on the chromatographic support could occur, leading in asymmetrical peaks and irreproducible retention. Therefore, a great variety of especially designed packings, which reduce the accessibility and activity of free silanols (high density, sterically hindered, dense polylayer and embedded polar group, bonded ultra pure silicas as well as polymer coated bonded ultra pure silicas or bonded ultra pure hybrid silica) have been developed. To characterize and evaluate their relative performances, a chromatographic test was previously developed [18] and optimized [19]. Briefly, the different chromatographic supports presented in Table 1 were tested at two pH values (pH 3 and 7) with isocratic mobile phases. The test compounds were individually injected to avoid any intermolecular interaction. When available, inter and intra-batch variabilities were evaluated. Preliminary data analysis achieved for individual columns demonstrated a stable batch clustering. Therefore and for sake of clarity, only the inter-batch average chromatographic parameters were used in this work to perform column evaluation studies.

For a simplified data representation, principal component analysis (PCA) was applied as a reduction technique to summarize many different variables (i.e. chromatographic observations) in a simple graphical display with minimal loss of information and assess relationships between variables. The PCA application demonstrated a relatively good separation of the chromatographic supports and allowed the performance evaluation in the analysis of basic compounds [18,19].

To better extract the information obtained with the 14 observed variables (retention factor and asymmetry values for the 7 tested compounds), autoscaled PCA and hierarchical cluster analysis (HCA) based on the application of Ward linkage rules and Euclidian distances calculation were sequentially used in the present work. In a first step, PCA was used to reduce the data dimensionality and only principal components (PCs) explaining 95% of the total variance were selected, because the supplementary axes mainly expressed the random "noise" in the original data set. The latter can therefore be discarded without reducing the amount of relevant information. In a second step, hierarchical cluster analysis (HCA) was performed on the principal coordinates of the tested supports to obtain tree diagrams. The latter were reported on the PCA representation to both ensure identification of groups of chromatographic supports and combine the bi-dimensional graphical visualisation with the multi-dimensional clustering afforded by HCA.

3.1. Column evaluation with specific test

3.1.1. Hydrophobic properties

Base deactivated supports were firstly tested in a mobile phase at pH 3.0. Due to the reduction of secondary interactions in this acidic mobile phase (silanols are mostly uncharged), chromatographic supports were mainly clusterised in relation to their hydrophobic properties. Nevertheless, asymmetry factors of basic test compounds were measured and treated by PCA together with retention factors with the aim of determining supports with a silanol activity at low pH. Preliminary data treatment showed that one support (UPHSC) exhibited important an unexpected asymmetry values (data not shown) and therefore, the latter was discarded for the PCA data analysis. As this support exhibited a strong hydrophobicity while a very low residual silanol activity was observed by Lesellier and Tchapla [11], one could emit the hypothesis that interactions occurred between basic solutes and end-capping groups bonded of the stationary phase surface, leading to an important peak shape alteration.

All retention variables were well represented in the first two PC axes and obtained score plots are presented in Fig. 2A. PC axes, as a multilinear combination of all variables, were constituted of about 95% of k and 5% of As for PC1, 95% of As and 5% of k for PC2, respectively. Hence, positions of chromatographic supports along PC1 are due to differences in retention behaviour and this axis can be used as a ranking criterion of the tested supports in relation to their hydrophobicity. Due to the strong contribution of asymmetry factor on PC2, chromatographic supports were orthogonally ranked in relation to their silanol activity and help finding out base deactivated supports possessing a silanol activity even in an acidic mobile phase. HCA was performed on the first six principal components to take into account the information of about 95% of the variance (see Fig. 2B). The obtained groups were reported on the PCA graphical output, allowing a complete evalua-

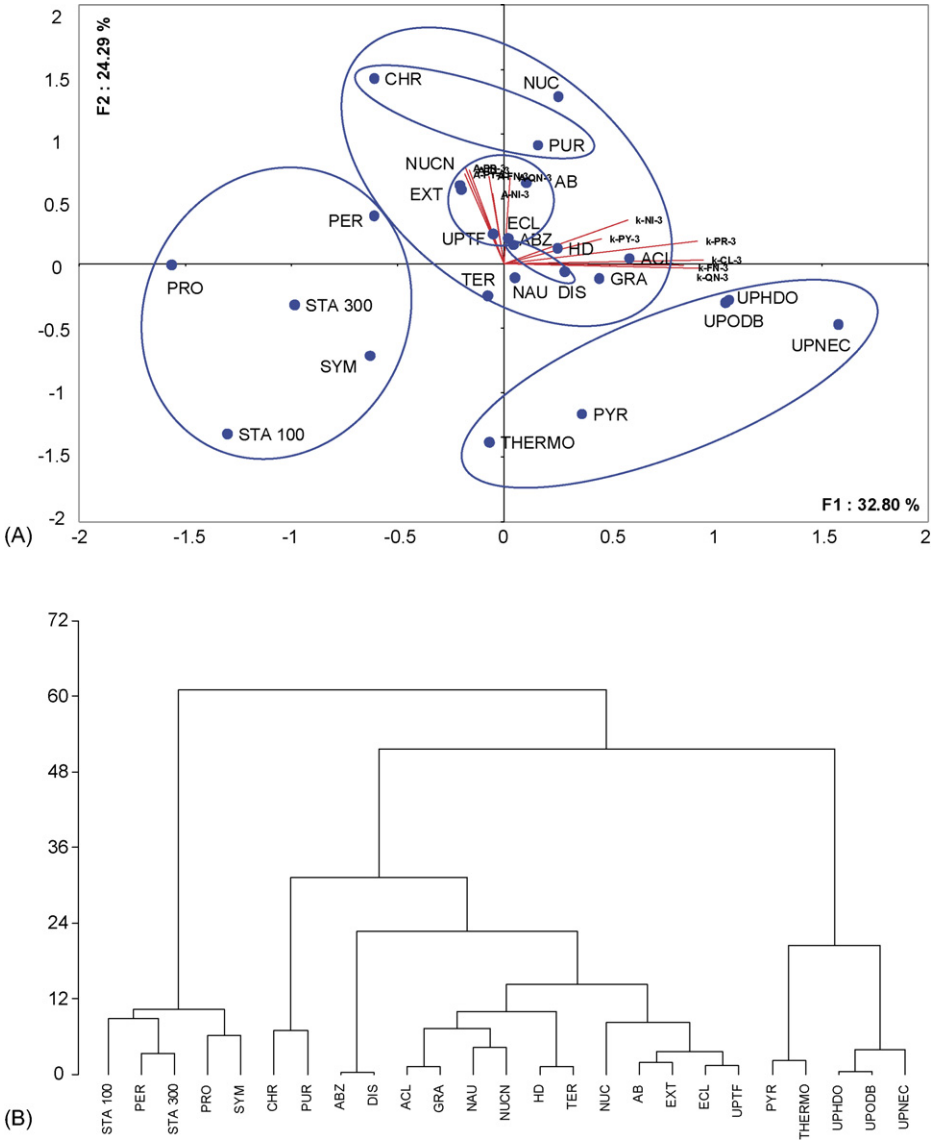

Fig. 2. (A) PCA representation at pH 3. (B) HCA representation at pH 3.

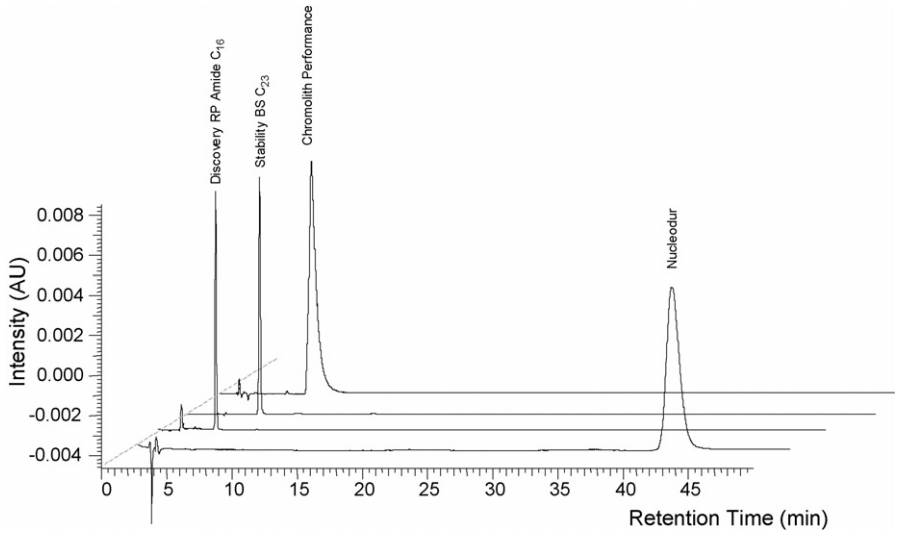

Fig. 3. Retention of diphenhydramine on different selected chromatographic supports (mobile phase 1).

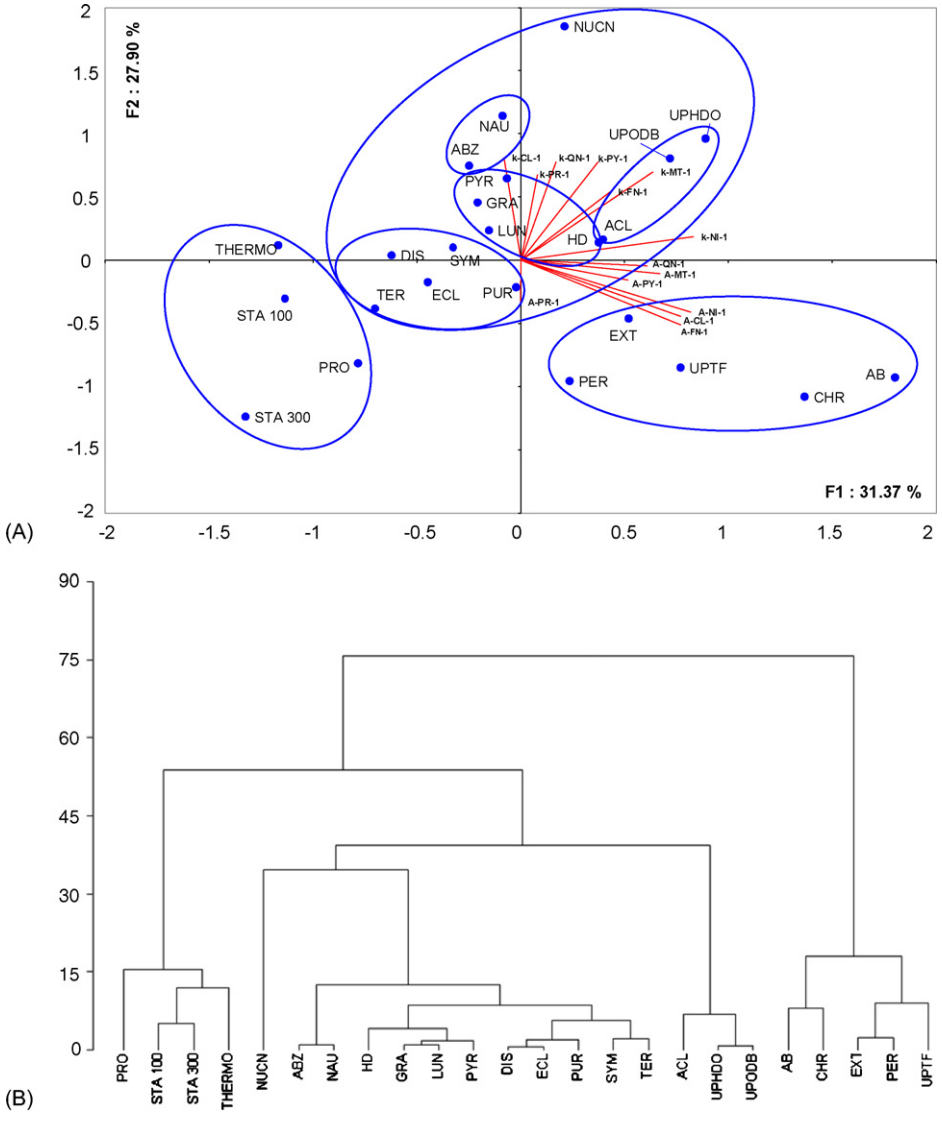

Fig. 4. (A) PCA representation at pH 7. (B) HCA representation at pH 7.

tion on the tested supports and an unambiguous clusterisation (Fig. 2A).

At this acidic pH, embedded polar group supports showed a quite similar behaviour in terms of silanols activity, but were clearly distinguished in relation to their hydrophobicity, which can be partially related to their carbon chain length (e.g. C_8, C_{16} and C_{18}). Supports possessing a C_{16} and C_{18} carbon chain were clusterised (DIS, ABZ, NAU, TER). Embedded polar group support possessing a C_8 carbon chain (PRO) was situated in the region of the plot characterized by the lowest hydrophobic character, with SYM, PER, STA 100 and STA 300. The latter with a total of C_{23} carbon chain (three methylene groups as spacer and a $C_{18}H_{37}$ group) embedded with a positively quaternary ammonium group allowed to conclude that the electrostatic repulsion effect of the cationic embedded moiety appeared predominant for the analysis of basic compounds. Monolithic supports (PER and CHR) appeared relatively less retentive than other conventional particle based supports with significant asymmetry. THERMO, PYR, UPHDO, UPODB and UPNEC, which corresponded to UPODB non-endcapped [11] were characterized by very low asymmetry values at this pH and clustered together; the last three supports exhibiting relevant retention. The high-density supports (ECL, HD, GRA) were clustered with C18 crosslinked polylayer bonded stationary phases (PUR, AB), hybrid particles (TER), bidendate bonding (EXT) and other end-capped support (NUC).

3.1.2. Silanol masking capacity

Selected supports were further tested with a pH 7.0 mobile phase to observe silanophilic interaction. All chromatographic parameters measured were treated by PCA to therefore characterize chromatographic supports mainly in relation to their silanol activity. Among the tested supports reported in Table 1, UPHSC, UPNEC and NUC presented an important retention of the tested analytes. As shown in Fig. 3, retention values measured in the same conditions for different chromatographic supports were significantly higher on ultra pure silica support (NUC). Results measured with UPHSC, UPNEC and NUC were discarded to obtain a better discrimination of the set of stationary phases. Obtained score plots are presented in Fig. 4A. Most of the variables were well represented in the first two axes and closed to each other (data not shown), indicating a high degree of correlation between asymmetry and retention variables. PCs axes were composed of about 66% of As and 33% of *k*, 20% of As and 80% of *k*, for the first and second PC axes, respectively. It is interesting to note that, as expected, at pH 7 most of the total variability was related to As on PC1, while at pH 3 the first PC axis was mainly constituted with retention data. Positions along the first PC axis was taken as supports ranking criteria in relation to their silanol masking capacity, which is the most important information obtained at pH 7. As asymmetry vectors were highly correlated, an "average" asymmetry vector was drawn on the corresponding score plot, indicating supports possessing the best silanol masking capacity in a pH 7.0 mobile phase. HCA clusterisation was achieved on the first six principal components to explain 95% of the total data variability (Fig. 4B). Obtained groups were reported on the PCA (Fig. 4A) as described for the acidic mobile phase.

Among all tested supports, best results in terms of silanol masking capacity were obtained with embedded group supports. There were embedded supports (STA 100 and 300) presenting a permanent charged group (quaternary ammonium) and supports (PRO, DIS, SYM, ABZ, NAU) presenting a polar group (amide or carbamate). Some other embedded polar groups could be used such as an ester, an urea or a sulfamide group. Excellent results obtained on STA supports were due to the repulsion effect between basic compounds and ammonium groups, both positively charged. The presence of an embedded polar group also greatly reduced ion exchange interactions thanks to the formation of an electrostatic shield on the surface of the packing. The

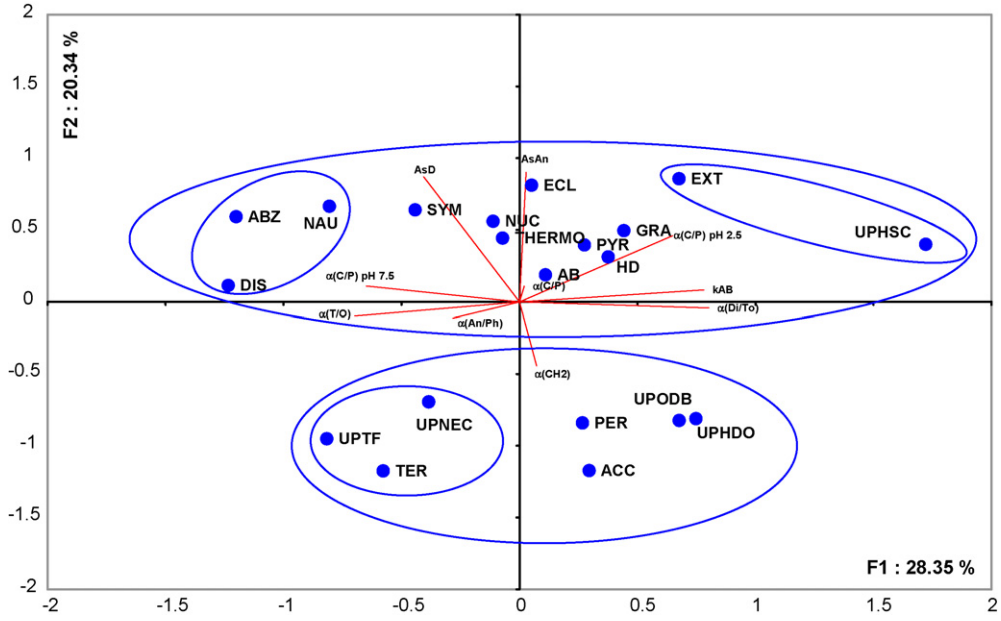

Fig. 5. PCA–HCA representation obtained with general tests.

performances obtained with high-density silica covering support (HD, GRA, ECL) were found to be comparable to embedded packings as well as ultrapure silica based material (THERMO). Endcapped materials such as UPODB, UPHDO exhibited higher asymmetry values. Monolitic supports (CHR, PER) as regular C_{18} grafted stationary phase were clusterised with polylayer (AB, UPTF) or bidentate (EXT) bonding and presented similar asymmetry values.

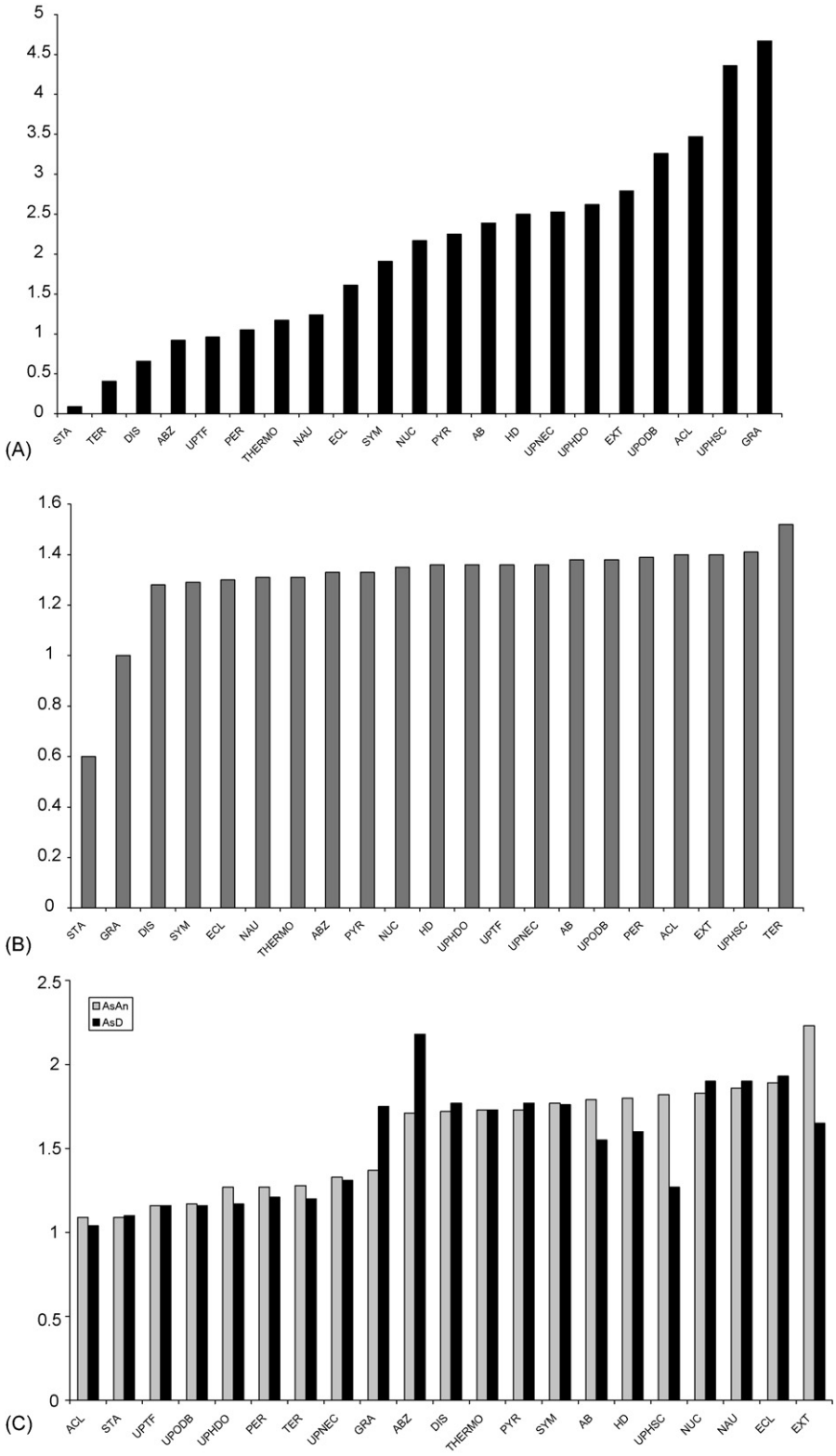

Fig. 6. Fundamental properties obtained with general tests (A) hydrophobicity (k_{AB}); (B) methylene selectivity (α_{CH_2}); (C) asymmetry factors for a weak base: aniline (AsAn) and *N*,*N*-dimethylaniline: strong base (AsD).

Thanks to this test, the most adapted stationary phase for the analysis of basic compounds could be easily selected not only on the basis of silanol activity, but also retention properties (positions along PC2) in the pH 7.0 mobile phase. As previously observed, supports with a charged polar group (STA) were found to be among the less retentive chromatographic columns, while some polar embedded material (NAU, ABZ) exhibited significant higher retention factors. It has to be noted that interesting results were obtained with both mobile phases for a cyano bonded support (NUCN), demonstrating the effectiveness of the presented methodology to integrate other reversed-phase chromatographic columns.

3.2. Comparison with general tests

3.2.1. Evaluation of fundamental properties

In order to validate column evaluation obtained with the specific test, the same chromatographic supports were also tested according to some general test procedures issued from the literature [15–17]. More in particular, the following fundamental properties: hydrophobicity, silanol activity, methylene selectivity and chromatographic behaviour towards strong and weak bases, were measured and compared to the column evaluation obtained with the specific *test*. The charged polar embedded support (STA) were clearly different from the others and discarded for the multivariate analysis.

PCA was able to explain less than 50% of the total variability on the first two axes. Data treatment, such as combined ACP–HCA, allowed to differentiate mainly two groups of columns (Fig. 5) where about 49% of the total variability was explained. The first axis was composed of k_{AB}, $\alpha(CH_2)$, α(T/O), α(Di/To), α(C/P) pH 2.5 and α(C/P) pH 7.5, while PC2 was essentially formed by AsAn and AsD, parameters explaining the two important clusters. Both groups were composed of various surface chemistry supports and did not distinguish particular stationary phases. Because a poor variability was observed with an overall data process, some fundamental properties were discussed one-by-one. Quite different values were obtained for the hydrophobic character of chromatographic supports with the tested columns (Fig. 6A). Embedded polar group supports showed a low hydrophobic character, in agreement with their bonding type and chain length. It is interesting to note that the great number of carbon atoms (C_{23}) of STA supports did not compensate their relatively low hydrophobic character essentially due to the presence of a quaternary ammonium group. Columns possessing a C_{18} carbon chain and a high density bonding showed, as expected, a strong hydrophobic character. These results corroborated column evaluation previously obtained in the acidic mobile phase, where supports were discriminated in relation to their bonding type and hydrophobic character. Methylene selectivity was also retained (Fig. 6B) since a high value indicated a high hydrophobic selectivity. This criterion is not very efficient for discriminating bonded supports with similar alkyl bonded chain length. As reported elsewhere, it permitted to characterize the true deepness of solute penetration inside the bonded chains [23]. Thus the low methylene selectivity measured for GRA was easily explained considering that it was a C_8 bonded silica. Furthermore, STA supports showed the smallest methylene selectivity, even if they possess 18 carbon atoms after the ammonium group. This charged group could lead to a particular conformation of bonded chains versus pure bonded C_{18}, avoiding the close contact between solutes and alkyl chains. By observing asymmetry factors measured with a strong base and a weak base (Fig. 6C), ACL, STA, UPTF UPODB, OPHDO, PER, TER and UPNEC exhibited lower values. On the other hand, shielded phases could not be distinguished from other bonding type supports. Some complementary informations were obtained from silanol activity measured with a weak basic compound. Embedded polar and charged group columns showed a low selectivity value indicating that this bonding type was particularly adapted for the analysis of basic compounds. All other supports presented a selectivity value close to 1.0, meaning that their silanol masking capacity is not as good as the one offered by shielded stationary phases. Ion exchange interactions were also evaluated in buffered mobile phases. More in particular, selectivity between a basic and a neutral compound was measured at two different pH values. At pH 7.5, tested supports showed a selectivity value lower than 1.0. At pH 2.5, a reduction of silanol activity (due to the reduction of secondary interactions at this pH), was observed for all stationary phases (data not shown).

All these results confirmed the column evaluation previously obtained with the specific test, but indicated that only chromatographic supports presenting very high (or very low) silanol activity could be clearly distinguished from all the others. When a more complete and precise evaluation of chromatographic performance of basic compounds is needed, a specific test as the one reported in Section 3.1 should thus be performed.

4. Concluding remarks

This paper described a column evaluation methodology, especially developed for base deactivated supports. Thanks to this specific test, different "special base" stationary phases have been characterized in terms of silanol masking capacity and bonding type. A set of seven basic test compounds, covering a wide range of physical–chemical properties, was injected on the selected supports with two different mobile phases. The first one, composed of a pH 7.0 phosphate buffer, allowed the evaluation of silanol activity, due to ion exchange interactions occurring in these chromatographic conditions between silanol groups and basic compounds. In the second mobile phase at pH 3.0, silanols were mostly uncharged and thus stationary phases could be evaluated in relation to their hydrophobicity and bonding type. All measured chromatographic parameters (k and As) were analysed to discriminate chromatographic supports. The results reported showed the effectiveness of PCA combined with HCA to obtain interesting information for the description of the tested chromatographic supports. This column evaluation procedure was compared with the evaluation obtained according to a general test protocol. It was a good correlation between the column evaluation obtained with the specific test and the fundamental properties measured with general tests. This comparison also highlighted that the specific test, especially developed for basic compounds, allowed to a better clusterisation between quite sim-

ilar supports. Moreover, base deactivated supports were subtly differentiated only with the specific test. In fact, with all fundamental properties measured following a general test procedure, only chromatographic supports presenting very different characteristics came into view.

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