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Characterization of several stationary phases prepared by thermal immobilization of poly(methyltetradecylsiloxane) onto silica surfaces

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ABSTRACT

Variations of a thermal immobilization procedure using poly(methyltetradecilsiloxane) and silica produced fourteen stationary phases with carbon contents of 4–18%. The stationary phases were chromatographically evaluated with the Engelhardt, SRM 870 and Tanaka tests. Classifications using USP and Euerby procedures indicate that the new immobilized phases are different from most commercial phases although there was some similarity with phases that have high ion-exchange interactions. The retention mechanism involved in the separation of basic solutes on several of the new stationary phases was studied by varying pH, type of Lewis base and the ionic strength of the eluent. The separations are strongly influenced by the chemistry of the accessible free silanols. The stationary phases present good selectivity at intermediate pH where the basic analytes were protonated, suggesting use of intermediate pH for these separations. Stability tests show that the stationary phases have poor stability at very high pH, even at 23 °C, but good stability in acidic mobile phases, even at 75 °C, as expected for an immobilized polymer stationary phase.

1. Introduction

Reversed-phase high performance liquid chromatography (RP-HPLC) is a well-known technique for the determination of many different types of compounds having different polarities, molar masses and functionalities, such as pharmaceuticals, pesticides and petrochemicals. RP-HPLC presents several advantages [1], including the use of less noxious and less expensive mobile phases, such as solutions of methanol or ethanol with water [2,3], fast equilibration of the column after changing the mobile phase, usefulness with gradient elution, high speed analyses and good repetitivity of retention times [4]. Stationary phases are the most important component of an HPLC system and, despite the large number of phases now available, the development of new stationary phases still occupies a prominent place in the literature [5]. Most commercially available stationary phases are prepared by chemical bonding C18 or C8 groups with the silanols of bare silica. However, novel phases can provide alternative and complementary separations for many analyses that are difficult to perform with C8 or C18 stationary phases. In many instances, the elution order of solutes differs on the novel phases, thus providing enhanced selectivity for difficult-to-separate compounds. This complementary approach can aid in identification, proof of purity, and quantitation [6]. Novel phases also offer chromatographers the flexibility to use simpler mobile phases, thereby avoiding ion pair reagents, exotic buffer systems, extreme pH conditions, and complex mobile phase preparations [7].

For a number of reasons, chromatographers also want to improve chemical and thermal stabilities, and thus the longevity of their stationary phases, and to adequately and quickly develop new analysis protocols by exploring all the experimentally available parameters, especially with respect to pH and temperature [8]. However, the range of these experimentally available parameters is very narrow since with silica-based stationary phases the support dissolves in alkaline mobile phases and the use of inorganic buffers (carbonate and phosphate) and temperatures equal to or higher than 60 °C increases the rate of dissolution [8].

Some novel phases present high chemical and thermal stabilities making possible the use of extreme pH mobile phases and higher temperatures [9]. Ethylene-bridged hybrid stationary phases from Waters [10] and polymer-coated zirconia stationary phases from ZirChrom [11–14] must be considered when highly alkaline mobile phases are required to change the selectivity of basic solutes or for solute stability reasons, especially when elevated temperatures are necessary to reduce analysis time [9]. These novel stationary phases show very different retention mechanisms. The former shows only hydrophobic interactions, while the latter has a mixed-mode separation mechanism. Although mixed-mode retention mechanisms in RP-HPLC are usually considered

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to be undesirable [15], good basic solute selectivities have been shown when polymer-coated zirconia columns are optimized for mixed-mode separations involving both hydrophobic and ionic interactions. It is possible to adjust selectivity by changing the type of buffer, the pH and the ionic strength of the eluent as well as the type and amount of organic modifier.

Similar to the approach taken by ZirChrom, another alternative for the preparation of stationary phases for HPLC consists of immobilizing linear polymer molecules into the silica pore system. Polysiloxanes are ideal for this application as the apparent distance between the monomeric units is nearly optimal for multipoint adsorption of the siloxane $(–Si–O–)_n$ chain onto the silica surface [16,17]. Stationary phases prepared by the immobilization of polysiloxanes onto silica have been shown to separate multiresidues of pesticides and their metabolic/degradation products (weak acids and bases), benzodiazepines and basic pharmaceuticals [16]. Similar phases have also been used for concentration and clean-up procedures using solid phase extraction [17].

The present paper describes the preparation and chromatographic evaluation of stationary phases by thermal immobilization of poly(methyltetradecylsiloxane) (PMTDS) onto silica (PMTDS–SiO_2). PMTDS was chosen as Szabó et al. [18] have suggested that the fourteen carbon chain, with a length intermediate between C8 and C18, should perform separations similar to those obtained with both these phases. The PMTDS–SiO_2 stationary phases were evaluated initially with some classical tests such as the Engelhardt [19], SRM 870 [20] and Tanaka tests [21]. These results were compared with literature data [22–25] for commercial phases to obtain an idea of their chromatographic behavior. One of the stationary phases with intermediate carbon content was evaluated with a in-house test that evaluated the effect of different pH, buffer type and buffer concentration on retention factors and asymmetry factors of several basic solutes (hydrophobic and hydrophilic), as proposed Carr and co-workers [11–14]. The importance of the ion exchange mechanism to the retention was evaluated using mobile phases with different phosphate buffer concentrations. The chemical and thermal stabilities were also evaluated at both high [8] and low [26] pH. Finally, the applicability of the PMTDS–SiO_2 stationary phases was compared with that of a stationary phase with the same carbon content prepared by the thermal immobilization of poly(methyloctylsiloxane) onto the same silica using both isocratic and gradient mode elutions.

2. Experimental

2.1. Chemicals and reagents

The mobile phases were prepared with ultrapure water from a Millipore Direct-Q™ system (Billerica, USA). Methanol and isopropanol were from Tedia (Fairfield, USA). Tetrahydrofuran was from J.T. Baker (Phillipsburg, USA). Pentane was purchased from Merck (Darmstadt, Germany).

The reagents used to prepare the mobile phases were: KH_2PO_4 (98%), K_2HPO_4 (99%) and $KHCO_3$ (99.7–100.5%) from Synth (Diadema, Brazil), sodium citrate tribasic dihydrate (99%) and N-(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)glycine (99%) (tricine) from Sigma (St. Louis, USA), sodium borate was from Fisher (Fairlawn, USA), triethylamine (99%) (TEA) and trifluoroacetic acid (99.5%) (TFA) from Vetec (Duque de Caxias, Brazil). 2-Amino-2-hydroxymethyl-propane-1,3-diol (Tris) from Mallinckrodt (Paris, France), ammonium hydroxide (28–30%) from LabSynth (Diadema, Brazil).

The silica was Kromasil, lot no AT 1959, from Akzo Nobel (Bohus, Sweden) with 5 μm particle size, 11.1 nm pore size and 313 m^2/g specific surface area. Kromasil is a type B silica that has only a small amount of contaminant metals. The polysiloxanes used were poly(methyltetradecylsiloxane), average molar mass (*M*) 9500, from Petrarch/Huls America (Piscataway, USA) and poly(methyloctylsiloxane), number-average molar mass, M_n, 6200, and weight-average molar mass, M_w, 16,000, from United Chemicals Technologies (Bristol, USA).

Table 1
Percentage of PMTDS per gram of silica (%PMTDS), time (*t*) and temperature (*T*) of immobilization used to prepare the stationary phases, %C from elemental analysis, and the final percentage of PMTDS per gram of silica after thermal immobilization (%$PMTDS_f$).

Code	%PMTDS	*t* (h)	*T* (°C)	%C	%$PMTDS_f$
SP1	30	4	100	4	6
SP2	60	4	100	5	7
SP3	30	8	100	6	9
SP4	60	8	100	15	21
SP5	30	4	130	9	13
SP6	60	4	130	10	14
SP7	30	8	130	18	26
SP8	60	8	130	19	27
SP9	20	6	115	8	11
SP10	70	6	115	9	13
SP15	45	6	115	8	11
SP16	45	6	115	9	13
SP17	45	6	115	11	16
SP18	45	6	115	10	14

The test solutes were: uracil (98%), butylbenzene (>99%) and amitriptyline hydrochloride (99%) from Aldrich (Milwakee, USA), benzylamine (>99%), pentylbenzene (>98%) and o-terphenyl (>99%) from Merck-Schuchardt (Hohenbrunn, Germany), caffeine from Medley (Campinas, Brazil), phenol (>99.5%), N,N-dimethylaniline (≥98%) and triphenylene (≥98.0%) from Fluka (Buchs, Switzerland), aniline (99.5%) from Merck, ethyl benzoate (99%) from Carlo Erba (Milan, Italy), HPLC grade toluene from Tedia (Rio de Janeiro, Brazil), and p-ethylaniline (98%), ethylbenzene (99%), quinizarin (96%), nortriptyline hydrochloride (98%), dextromethorphan hydrobromide, (−)-nicotine (98–100%) and (±)-chlorpheniramine maleate salt from Aldrich (Steinheim, Germany). Codeine sulfate, diphenhydramine hydrochloride, propanolol, salbutamol sulfate and methadone were kindly donated by Dr. Marcelo Ribani from TECPAR (Curitiba, Brazil) while the benzodiazepines and fluoxetine were kindly donated by Dr. Paulo César Pires Rosa of EMS (Hortolândia, Brazil).

2.2. Preparation of the stationary phases

The PMTDS–SiO_2 stationary phases were prepared using different amounts of PMTDS (g PMTDS/g silica), and different times (h) and temperatures (°C) of thermal treatment, as summarized in Table 1. The general procedure for the preparation of these stationary phases consists in dissolving PMTDS in 20 mL of pentane, then adding 1 g of Kromasil silica and 20 mL more of pentane. This mixture is stirred for 30 min at room temperature and then placed in a fume hood for the evaporation of the solvent at room temperature. The dried materials are then placed individually in an oven at the specified temperature for immobilization under an air atmosphere. The PMOS-SiO_2 stationary phase with carbon content of 18% was prepared as described elsewhere [27].

The stationary phases were slurry packed (0.8 g of stationary phase in 20 mL of 20:80 (v/v) isopropanol–tetrahydrofuran) into previously polished 50 mm × 4 mm columns [28] made from 316 stainless steel tubing at a constant packing pressure of 40 MPa, using a Haskel Packing Pump (Burbank, USA) with methanol as propulsion solvent. The pressure was maintained until the passage of 200 mL methanol to assure a good packing and removal of excess polysiloxane [29].

The PMTDS–SiO_2 stationary phases SP1–SP10, SP15 and SP16 were packed two months after the immobilization procedure, while SP17 and SP18 were packed three months after the immobilization procedure. All columns were conditioned for at least 2 h with mobile phase at 0.5 mL/min before the chromatographic evaluations.

2.3. Physical characterization of the stationary phases

2.3.1. Percent carbon

Elemental analyses were carried out on the material recovered after column packing with a Model CHN-2400 PerkinElmer Analyzer (Shelton, CT, USA). The final percentages of PMTDS per gram of silica after the thermal immobilization ($PMTDS_f$) were determined using the formula $\%PMTDS_f = (\%C/0.7)$ since 70% of the PMTDS refers to carbon.

2.3.2. Solid-state ^{29}Si CP-MAS NMR spectroscopy

Solid-state ^{29}Si NMR measurements were performed on an INOVA spectrometer (Varian, Palo Alto, USA) using cross polarization and magic angle spinning (CP-MAS). The contact time and pulse interval time were 5 ms and 1.5 s, respectively. A frequency of 59.6 MHz was used. Representative samples of 200–300 mg were spun at 4 kHz using 7 mm double ZrO rotors. Typically, 1.5 k free induction decays (FIDs) with an acquisition time of 35 ms were accumulated in 1 kb data points, zero-filling to 8 kb prior to Fourier transformation. The line broadening used was 30 Hz and the spectral width for all spectra was about 25 kHz.

2.3.3. Surface area and porosity—BET/BJH

Full adsorption–desorption isotherms of nitrogen at −195.8 °C on dried samples were measured at various partial pressures with a Micromeritics model ASAP-2010 apparatus (Norcross, USA). Specific surface areas (S_{BET}) and pore-size distributions (PSD) were determined with the Brunauer–Emmett–Teller (BET) and Barret–Joyner–Hallenda (BJH) methods, respectively. BET surface areas were obtained from the adsorption data points, whereas the PSD were derived from the desorption isotherm. Prior to the BET/BJH measurements, the samples were degassed for 3 h at 120 °C in the out-gassing station of the adsorption apparatus.

2.4. Mobile phase preparation

All mobile phases were prepared volumetrically. The pH was measured in the aqueous phase with a calibrated pH meter, Qualxtron model 8010 (Jundiaí, Brazil), before the addition of organic modifier. pH adjustments were made with hydrochloric acid solutions for organic buffers and phosphoric acid solutions for phosphate buffers, while potassium hydroxide solutions were used to adjust the pH with both kind of buffers.

2.5. Chromatographic evaluations

All the chromatographic evaluations were performed using a modular HPLC system with a Shimadzu LC 10AD pump (Kyoto, Japan), a Rheodyne model 8120i injection valve (Cotati, USA) with 5 μL loop, a Shimadzu CTO-10AC column oven and a Shimadzu Model SPD-10 AV UV–VIS detector. Data were processed using ChromPerfect software from Justice Innovations (Mountain View, USA). All tests were conducted at a flow rate of 0.5 mL/min. Van Deemter plots obtained using naphthalene as test solute in a 70:30 (v/v) mobile phase indicated an optimal flow rate of 0.3 mL/min but as the efficiency loss due to the use of a 0.5 mL/min flow rate was inferior to 10%, chromatographic evaluations and stability tests were carried out at this slightly faster flow rate. With flow rates of 0.5 mL/min for the 50 mm × 3.9 mm columns used in this work the retention time of uracil is near to 1 min, which indicates a flow rate of one column volume per minute. Some analyses were also carried out at flow rates of 1.0 and 1.5 mL/min without resolution loss.

Retention factors (k) were calculated using the relation $k = (t_R - t_M)/t_M$, where t_R is the retention time for the solute and t_M is the retention time of the unretained component, uracil. Asymmetry factors (As) were calculated using the relation $As = rw_{10\%}/lw_{10\%}$ and the USP tailing factor (Tf) was calculated as $w_{5\%}/2\,lw_{5\%}$, where $rw_{10\%}$, $lw_{10\%}$ and $lw_{5\%}$ are the right width and the left width, respectively, measured horizontally from the right or left edge of the peak to a vertical line from the peak apex, at the 5% or 10% level and $w_{5\%}$ is the total peak width at 5% of the peak height. Efficiency was calculated using $N = 5.54\,(t_R/w_h)^2$, where w_h is the peak width at 50% of the peak height.

2.5.1. Engelhardt test [19]

The two principal parameters, reflecting different chromatographic conditions, were: retention factor for ethylbenzene (k_E) that reflects the surface coverage or ligand density; and asymmetry factor of p-ethylaniline ($As_{p\text{-}E}$) that reflects the silanol activity.

Additionally, eleven other parameters were considered: the retention factors of phenol, aniline, p-ethylaniline, ethyl benzoate, N,N-dimethylaniline, toluene and ethylbenzene (k_P, k_A, $k_{p\text{-}E}$, k_N, k_T and k_E) were used as selectivity parameters, while the asymmetry factors of aniline and N,N-dimethylaniline (As_A and As_N), the asymmetry factor ratio between aniline and phenol (As_A/As_P) and the retention factor ratio between aniline and phenol (k_A/k_P) were used to measure silanol activity.

The test was carried out at a column temperature of 40 °C with a 55:45 (v/v) methanol–water mobile phase. Detection was done at 254 nm.

2.5.2. SRM 870 test [20,25]

The four parameters, reflecting different chromatographic conditions, were: retention factor for ethylbenzene (k_E) that reflects the surface coverage or ligand density; tailing factor of quinizarin (Tf_Q) that indicates activity toward metal chelators; retention factor for amitriptyline (k_{ami}) that reflects the ion-exchange properties of the stationary phase; and tailing factor of amitriptyline (Tf_{ami}) that indicates silanol activity.

Additionally, six other parameters were considered: retention factors of toluene and quinizarin (k_T and k_Q), efficiency in plates per meter of column of ethylbenzene and amitriptyline (N_E/m and N_{ami}/m) and the asymmetry factors of quinizarin and amitriptyline (As_Q and As_{ami}).

The test was carried out at a column temperature of 23 °C with a 80:20 (v/v) methanol–phosphate buffer (pH 7; 20 mmol/L) mobile phase. Detection was at 254 nm.

2.5.3. Tanaka test [21]

The six parameters, using different chromatographic conditions, were: retention factor for pentylbenzene (k_{PeB}) that reflects the ligand density; hydrophobicity or hydrophobic selectivity (CH_2) that is the retention factor ratio between pentylbenzene and butylbenzene and is a measure of the surface coverage of the phase as the selectivity between the alkylbenzenes differentiated by one methylene group also depends on the ligand density; shape selectivity (T/O), the retention factor ratio between triphenylene and o-terphenyl, which is influenced by the spacing of the ligands; hydrogen bonding capacity (C/P), the retention factor ratio between caffeine and phenol, which is a measure of the number of available silanol groups and the degree of end capping; total ion-exchange capacity (B/P 7.6), the retention factor ratio between benzylamine and phenol at pH 7.6, which is an estimate of the total silanol activity; and total ion-exchange capacity (B/P 2.7), which is the retention factor ratio between benzylamine and

phenol at pH 2.7, an estimate of the acid activity of the silanol groups.

Additionally, five other parameters were considered: the efficiency per meter for pentylbenzene (N_{PeB}/m), the asymmetry factors of caffeine in an unbuffered mobile phase and of benzylamine at both pH 2.7 and 7.6 (As_C, $As_{B2.7}$ and $As_{B7.6}$), and aromatic selectivity (PeB/O) which is the retention factor ratio between n-pentylbenzene and o-terphenyl. This descriptor is believed to be a measure of the aromatic selectivity, which is influenced by the density of aromatic character on the stationary phase.

The tests were carried out at a column temperature of 40 °C. k_{PeB}, CH_2, T/O, N_{PeB} and PeB/O were evaluated using a 80:20 (v/v) methanol–water mobile phase. C/P and As_C were evaluated using a 70:30 (v/v) methanol–water mobile phase. B/P 7.6 and $As_{B7.6}$ were evaluated using a 70:30 (v/v) methanol–phosphate buffer (pH 7.6; 20 mmol/L) mobile phase. B/P 2.7 and $As_{B2.7}$ were evaluated using a 70:30 (v/v) methanol–phosphate buffer (pH 2.7; 20 mmol/L) mobile phase. Detection was at 254 nm.

2.6. Effect of mobile phase pH and buffer type on retentions and asymmetry factors

The retention factors and asymmetry factors of nine basic solutes were evaluated using 80:20 (v/v) methanol–buffer solutions. For pH 6, 7, 8, and 11 phosphate buffers were used, for pH 8.5 Tris buffer, for pH 9 and 10 borate buffer, for pH 10.5 ammonium buffer and for pH 11.5 triethylamine buffer. The buffer solutions were 20 mmol/L, except for the borate buffers that were 5 mmol/L. The test was carried out at a column temperature of 23 °C. Detection was at 220 nm.

2.7. Effect of buffer concentration

The retention factors and asymmetry factors of eight basic solutes were evaluated using 65:35 (v/v) methanol–buffer solutions at pH 7 at concentrations of 10 mmol/L, 50 mmol/L and 100 mmol/L. The analyses were performed at 23 °C. Detection was at 220 nm.

2.8. Stability evaluations

The column stability tests were performed using a modular HPLC system from Shimadzu equipped with a LC-10AD LC pump, a SPD-10A UV–Vis detector, a CTO-10AS column oven, a SIL-10AD automatic injector and a SCL-10A system controller. Data were acquired and processed using the CLASSVP program (Shimadzu). The columns under test were continuously purged with fresh mobile phase, not recycled, at 0.5 mL/min.

Throughout this work the mobile phase volume that passed through the column is expressed in column volumes (Vc), as is commonly done in the literature. The column volume was calculated from the retention volume of an unretained solute (uracil). For the columns used in this work Vc is 0.50 mL. The PMTDS–SiO_2 column used for the stability tests was SP7.

2.8.1. Acidic mobile phase

The acid test was done using 50:50 (v/v) methanol–0.2% trifluoroacetic acid mobile phase at 75 °C. The test solutes were toluene, ethylbenzene and propanolol.

2.8.2. Basic mobile phase

The basic test was done using 65:35 (v/v) methanol–phosphate buffer (pH 11; 20 mmol/L) mobile phase at 23 °C. The test solutes were toluene, ethylbenzene and amitriptyline.

2.9. The applicability of some PMTDS–SiO_2 stationary phases

The gradient separations were performed using a modular HPLC system from Shimadzu LC having a 10AT VP pump, SIL-10AF auto injector, a CTO-10AS VP column oven, a SPD-M10A VP diode array detector and a SCL-10A VP system controller. The solvents were degassed with a DGU-2A degasser using helium at 50 kPa. The amounts of each solvent were determined by a FCV-10AL VP programmer. Data were processed using Class VP software. All tests were conducted at a flow rate of 0.5 mL/min.

2.10. Data analysis

Hierarchical cluster analyses (HCAs) were performed using Pirouette Version 3.11 from Infometrix, Inc. (Woodinville, USA). The data from the present study were combined with the data from Euerby et al. [24] and from The United States Pharmacopeia [25]. To give all variables the same importance, they were "auto scaled", i.e., the average was subtracted from each variable and each variable was divided by its standard deviation, before applying the HCA. For the HCA, Euclidian distances and centroid linkages were used. All graphs were constructed using OriginPro 7.5 SRO v7.5714 (B714) (Northampton, USA). The correlations and basic statistics were calculated with STATISTICA 6.0 from STatSoft (Tulsa, USA).

3. Results and discussion

The overall purpose of this work was to understand the mechanisms involved in producing the retentions and asymmetry factors of several basic pharmaceuticals with different pH, buffer types and buffer concentrations using stationary phases prepared by immobilization of PMTDS onto silica (PMTDS–SiO_2) and to compare these phases with commercial stationary phases using the well known chromatographic tests of Engelhardt, SRM 870 and Tanaka, making comparisons with the literature data for these tests. The stabilities of some of these stationary phases were evaluated in both highly basic and highly acidic mobile phases.

3.1. Physical characterizations of the stationary phase

As shown in Table 1 the use of a larger amount of PMTDS, longer times and/or higher temperatures of immobilization leads to larger amounts of PMTDS being immobilized onto the silica. The carbon contents (%C) were higher than those obtained for zirconized [30] and titanized Kromasil silica [31] and similar to that obtained with unmodified Kromasil silica [32] for similar PMTDS loadings.

The ^{29}Si NMR spectra of SP7 and SP8 (data not shown) indicate the existence of $D^1{}_H$, $D^{2'}$ and $D^{2''}$ groups from PMTDS on PMTDS–SiO_2 stationary phase and Q^2, Q^3 and Q^4 from silica. Q^2 indicates geminal silanols, the Q^3 signals are isolated or vicinal silanols and Q^4 are tetrasiloxane in the silica backbone. A $D^1{}_H$ signal represents the breaking of some Si–O–Si bonds of the siloxane chain during the thermal immobilization processes, $D^{2''}$ indicates that the PMTDS chains are loosely attached to or physically adsorbed onto the silica, while $D^{2'}$ represents chemical bonding between the PMTDS and the silica surface [33,34]. The $D^1{}_H$, $D^{2'}$, $D^{2''}$, Q^2, Q^3 and Q^4 groups mentioned here are shown in Fig. S1 of the Supplementary material. The NMR spectra confirm the presence of some bonds linking the polysiloxane to the silica surface [34].

Comparison of some textural and physical–chemical properties of Kromasil silica with those of SP8, which is the PMTDS–SiO_2 stationary phase with the highest %C, indicate significantly reduced surface area (301 vs. 87 m^2/g) and pore volume (0.85 vs. 0.24 cm^3/g) while the mean pore diameter was almost unchanged (11.2 vs. 10.8 nm).

Table 2
Data obtained with the Engelhardt test for some PMTDS–SiO_2 stationary phases. The identification of the symbols and the chromatographic conditions are in Section 2.5.1.

SP	k_P	k_{EB}	k_T	k_E	k_A	$k_{p\text{-}E}$	k_N	As_A	$As_{p\text{-}E}$	As_N	As_A/As_P	k_A/k_P	$(N_E/m) \times 10^{-3}$
1	0.1	1.2	2.1	3.7	0.3	1.0	1.9	2.6	5.9	3.2	1.5	2.5	55
2	0.1	1.3	2.3	4.0	0.3	0.8	1.7	1.9	3.1	1.7	0.9	2.0	47
3	0.1	1.4	3.0	5.3	0.3	1.1	2.1	2.0	2.2	3.0	1.2	2.9	13
4	0.7	4.9	9.8	17.3	0.8	2.3	5.7	2.7	4.3	1.4	1.7	1.2	36
5	0.5	3.6	6.9	12	0.6	1.5	3.5	1.8	1.3	0.9	1.3	1.1	22
6	0.5	3.5	6.6	11.6	0.6	1.6	3.6	2.3	2.0	1.2	1.2	1.1	30
7	0.5	4.0	8.6	15.2	0.6	1.9	4.8	2.0	3.0	1.2	1.3	1.3	37
8	0.6	4.8	9.6	16.8	0.8	2.7	5.9	3.1	4.5	1.1	2.3	1.3	74
9	0.2	1.8	3.5	6.2	0.3	0.9	2.1	1.5	1.6	1.2	0.7	1.4	49
10	0.3	2.4	4.5	8.0	0.4	1.2	2.6	1.9	2.2	1.5	1.3	1.2	27
15	0.5	2.8	5.1	9.0	0.6	1.5	3.2	1.6	2.4	1.4	1.4	1.3	20
16	0.3	2.4	4.5	8.0	0.4	1.2	2.7	1.7	1.8	1.1	1.3	1.4	19
17	0.5	3.1	5.8	10.2	0.8	1.9	3.8	1.6	1.8	1.1	1.2	1.6	20
18	0.3	2.4	4.6	8.1	0.6	1.5	3.2	2.2	2.2	1.4	1.5	1.9	19

3.2. Chromatographic evaluations

3.2.1. Engelhardt test

The results of the Engelhardt test are shown in Table 2. The retention factors of the poorly retained test solutes aniline and phenol and the asymmetry factors of the basic test solutes (aniline, p-ethylaniline and N,N-dimethylaniline) had no correlation with the other test parameters, while the retention factors of the retained test solutes (ethyl benzoate, toluene and ethylbenzene, N,N-dimethylaniline and p-ethylaniline) were highly correlated, results that are in concordance with the work of Schmitz et al. [35]. On the PMTDS–SiO_2 stationary phase ethyl benzoate elutes after N,N-dimethylaniline, while toluene elutes before N,N-dimethylaniline, as usually observed for immobilized polymer phases.

When aniline is more retained than phenol, it indicates that the stationary phase strongly interacts with basic solutes. Also, the asymmetry of the aniline peak divided by that of phenol should be less than 1.3. Engelhardt and Jungheim [36] explain that calculating the ratio of asymmetries allows being independent of the extra-column effects that can alter peak width and that aniline should elute after phenol.

With all the PMTDS–SiO_2 stationary phases aniline elutes after phenol and the ratio As_A/As_P was near to 1.3, except for SP8. The asymmetry factors of p-ethylaniline were smaller than 2.5, showing intermediate silanol activity, except for SP1 and SP8, which had the lowest and the highest %C. N,N-dimethylaniline presents good peak shapes for PMTDS–SiO_2 stationary phases, except for SP1 and SP3, while aniline presents poor peak shapes with all the phases, possibly due to an extra-column effect, as it elutes very close to the dead time.

3.2.2. SRM 870 test

The results of the SRM 870 test are shown in Table 3. The PMTDS–SiO_2 stationary phases prepared with smaller amounts of PMTDS and lower times and temperatures of immobilization had hydrophobicities (k_E) lower than the lower quartile of the commercial phases ($k_E < 1.3$), while phases prepared with higher amounts of PMTDS and longer times and higher temperatures of immobilization had hydrophobicities higher than the upper quartile of commercial phases ($k_E > 2.2$).

With PMTDS–SiO_2 stationary phases the retention factors of all the test solutes were highly related but presented large variations, measured as relative standard deviation (RSD). As expected, those stationary phases with high %C were highly retentive for amitriptyline while even the PMTDS–SiO_2 stationary phases with lower %C had retention factors for amitriptyline higher than the upper quartile of commercial phases ($k_{ami} > 7.5$). SP4, SP7 and SP8, with high %C, were more retentive for amitriptyline than the most retentive commercial phases, Alltima HP C18 AQ and Prevail C10, both from Grace Davison, and Aquasil C18 from Thermo Science, with the USP test [25]. The high retention for amitriptyline and the different %C with PMTDS–SiO_2 stationary phases were not reflected in the asymmetry factors of quinizarin and amitriptyline, which are smaller than the lower quartile of commercial stationary phases ($As_Q < 1.5$ and $As_{ami} < 2.5$, respectively).

3.2.3. Tanaka test

The results obtained with the Tanaka test are shown in Table 4. With most of the PMTDS–SiO_2 stationary phases, caffeine elutes after phenol with C/P values between 1.4 and 2, independent of the %C, and benzylamine elutes after phenol at pH 7.6, with B/P 7.6 values between 8 and 11, also independent of the %C. On SP8 caffeine

Table 3
Data obtained with the SRM 870 test for some PMTDS–SiO_2 stationary phases. The identification of the symbols and the chromatographic conditions are in Section 2.5.2.

SP	k_T	k_E	As_Q	k_Q	As_{ami}	k_{ami}	$(N_E/m) \times 10^3$	$(N_{ami}/m) \times 10^3$	Tf_{ami}	Tf_Q
1	0.3	0.5	1.4	0.9	1.5	7.8	41	39	1.2	1.3
2	0.4	0.6	1.6	1.1	1.5	7.4	28	30	1.3	1.3
3	0.5	0.7	1.3	1.0	1.5	8.2	12	7	1.3	1.1
4	1.4	2.0	1.5	4.1	1.3	22.6	27	9	1.1	1.3
5	0.8	1.2	1.2	2.0	1.1	10.5	42	32	0.9	1.1
6	0.9	1.3	1.4	2.2	1.3	13.0	31	22	1.2	1.3
7	1.7	2.4	1.2	4.3	1.0	26.9	49	22	1.0	1.1
8	1.9	2.6	1.3	4.7	1.2	28.0	43	13	1.1	1.2
9	0.7	1.1	1.5	1.8	1.2	11.3	37	32	1.0	1.3
10	0.8	1.2	1.6	2.2	1.2	16.6	28	23	1.1	1.4
15	0.8	1.1	1.5	2.1	1.3	15.4	35	32	1.1	1.3
16	0.9	1.2	1.2	2.1	1.2	15.7	43	35	1.1	1.2
17	1.0	1.4	1.2	2.4	1.2	18.7	48	31	1.0	1.2
18	0.9	1.3	1.3	2.3	1.2	17.3	39	27	1.2	1.3

Table 4
Data obtained with the Tanaka test for some PMTDS–SiO_2 stationary phases. The identification of the symbols and the chromatographic conditions are in Section 2.5.3.

SP	k_{PeB}	CH_2	T/O	C/P	B/P 7.6	B/P 2.7	$(N_{PeB}/m) \times 10^3$	PeB/O	As_C	$As_{B7.6}$	$As_{B2.7}$
7	6.3	1.6	1.6	1.4	10.9	0.0	59	1.0	0.6	2.2	3.6
8	8.7	1.8	1.8	0.5	24.8	0.0	59	1.0	1.6	4.6	2
9	2.7	1.4	1.5	2.0	7.4	0.0	48	1.0	1.4	1.8	3.4
10	3.1	1.4	1.6	1.8	9.9	0.0	29	0.9	1.5	1.5	2.7
15	3.0	1.4	1.5	1.6	9.1	0.0	51	1.0	1.4	2.8	1.5
16	3.9	1.5	1.4	1.6	7.7	0.0	52	1.0	1.2	1.7	3.7
17	3.4	1.4	1.5	1.6	8.9	0.0	54	1.0	1.2	2.3	1.5
18	3.0	1.4	1.5	1.6	9.8	0.0	42	0.9	1.3	2.7	2.6

elutes before phenol and this phase also presents the highest B/P 7.6 value (24.8). The PMTDS–SiO_2 stationary phases with higher %C present higher k_{PeB}, while T/O, CH_2 and B/P 2.7 were similar for all the stationary phases.

At pH 2.7 benzylamine was not retained with the PMTDS–SiO_2 stationary phases, as expected for new generation stationary phases based on type B silica [37]. On the other hand, at pH 7.6 the PMTDS–SiO_2 stationary phases show significant ion exchange properties and present B/P 7.6 values higher than those observed for almost all of the new generation stationary phases [37]. SP8 presents higher ion exchange values at pH 7.6 than any of the 229 commercial stationary phases.

Another characteristic of new generation phases is good peak shapes for benzylamine at both pH values [37]. Most of the PMTDS–SiO_2 stationary phases present poor peak shapes for benzylamine at both pH values. Poor peak shapes at pH 2.7 can be attributed to extra-column effects that affect the peak width, since benzylamine is not retained at this mobile phase pH. However, the poor peak shapes for benzylamine at pH 7.6 is a surprisingly result since the PMTDS–SiO_2 stationary phases present good peak shapes for amitriptyline with the SRM 870 test. This poor peak shape for benzylamine may be attributed to the mobile phase used in the Tanaka test [1].

3.3. Classifications using HCA

3.3.1. Classifications using HCA and literature data for the SRM 870 test

Extensive study by the United States Pharmacopeia on the characterization of stationary phases using the SRM 870 test [25] has provided a database with the characterization of 111 stationary phases, which was used for comparisons with the PMTDS–SiO_2 stationary phases. The data from the PMTDS–SiO_2 stationary phases obtained with the SRM 870 test were submitted to HCA together with the literature data presented by USP [25] for these commercial phases, excluding Resolve C8 and Spherisorb ODS1, based on type A silica and unable to elute quinizarim due to their high metal contents. The variables were k_E, Tf_Q, k_{ami} and Tf_{ami}. The HCA is shown in Fig. S2 of the Supplementary material and the commercial stationary phases mentioned are described in Table S1 of the Supplementary material. In this HCA PMTDS–SiO_2 stationary phases with high %C (SP4, SP7 and SP8) are placed near to Prevail C18 and Alltima HP C18 AQ, both from Grace Davison, and to Aquasil C18 from Thermo Scientific, which are commercial "aqua" phases with high %C and that possess significant ion-exchange properties due to their polar end-capping. The PMTDS–SiO_2 stationary phases with a intermediate %C (SP5, SP6, SP9, SP10, SP15, SP16, SP17 and SP18) are located near commercial stationary phases Cosmosil 5C18-AR-II from Nacalai Tesque, YMC ODS-AL from YMC, ProntoSil 120-5-C18-AQ Plus and ProntoSil 120-5-C18-SH, both from Bischoff, Alltima HP C18 EPS from Grace Davison, Allure from Restek, and Hypersil PAH from Thermo Scientific, while PMTDS–SiO_2 stationary phases with low %C (SP1, SP2 and SP3) are located near the commercial stationary phase Alltima HP C18 EPS (from Grace Davison), a phase with extended polar selectivity and low carbon content (~4%). All the commercial phases that are near the PMTDS–SiO_2 stationary phases present similar retention factors for ethylbenzene and amitriptyline as well as similar tailing factors for quinizarim, but higher tailing factors for amitriptyline than do the relevant PMTDS–SiO_2 stationary phases.

3.3.2. Classifications using HCA and literature data for the Tanaka test

Euerby et al. [22,24] have done extensive studies on the characterization of stationary phases using the Tanaka test. They have provided a freely available database with the characterization parameters of 229 stationary phases [24] for making comparisons with new stationary phases. The PMTDS–SiO_2 stationary phases were also submitted to HCA together with the literature data [24] for commercial stationary phases evaluated with the Tanaka test. The variables used were k_{PeB}, CH_2, T/O, C/P, B/P 7.6 and B/P 2.7. CH_2 and T/O have small relative standard deviations (RSD), while C/P, B/P 7.6 and B/P 2.7 have high RSD. However since Euerby et al. [22,24] considered all the variables determined by the Tanaka test [21], all the variables were included in this work. The HCA was performed without the type A silica phases Resolve C18, Spherisorb ODS1 and Spherisorb phenyl from Waters and Hypersil phenyl from Hypersil as well as the polar phases that do not retain pentylbenzene ($k_{PeB} \sim 0$), Luna NH2 from Phenomenex and MonoChrome Diol from Varian.

The HCA constructed using the Tanaka test (see Fig. S3 and Table S2 in the Supplementary material) places SP8, which is the phase with the highest B/P 7.6, higher than those of Zirchrom MS and Zirchrom PDB, zirconium oxide stationary phases from ZirChrom, and Primesep A, Primesep 100 and Primesep 200, which have embedded carboxylic groups, from SIELC Primesep™, a B/P 2.7 lower than the lower quartile (~0.1) and C/P near the upper quartile (~0.8), far from all other commercial phases and even from the other PMTDS–SiO_2 stationary phases (SP7, SP9, SP10, SP15, SP16, SP17 and SP18) that are placed near to Altima HP C18 EPS and Platinum C18 EPS, both from Grace Davison, and Acquity HSS C18 SB from Waters, which are all stationary phases with extended polar selectivity, as well as Primesep 200 (carboxylic groups with pK_a 2). These commercial stationary phases have C/P and B/P 7.6 values similar to the indicated PMTDS–SiO_2 stationary phases, but present lower %C and higher B/P 2.7 values than these PMTDS–SiO_2 stationary phases.

The commercial phases ZirChrom MS and ZirChrom PDB, the aluminum oxide-based phase Spherisorb A5Y from Waters, which has the highest B/P 2.7 value, the phases with embedded carboxylic groups, Primesep 100 (carboxylic groups with pK_a 1) and Primesep A (carboxylic groups with pK_a 0), and the mixed-mode phase containing discrete C18 and cation-exchange particles, Hypersil Duet from Thermo Scientific, have all been described as highly retentive for basic pharmaceutical solutes due to ion exchange interactions but give high efficiencies and good peak shapes for basic solutes [11,13–15]. These commercial phases have high C/P, B/P 7.6 and B/P 2.7 values (see Table S2).

Table 5
Influence of mobile phase pH and buffer type used on retention factors (k) and asymmetry factors (As) of basic solutes on PMTDS–SiO_2 stationary phase SP9. The tests done at pH 6–11 were carried out in a 80:20 methanol:buffer mobile phase. Other chromatographic conditions are in Section 2.6. The letter after the mobile phase pH indicates the buffer used; P, phosphate buffer; B, borate buffer; A, ammonia buffer; T, triethylamine buffer. Solute identifications: sa, salbutamol; D, diphenidramine; me, methadone; pr, propanolol; ami, amitriptyline; nor, nortriptyline; ni, nicotine; co, codeine; Ch, chlorpheniramine; n.d., not determined.

Solutes	sa	D	me	pr	ami	nor	ni	co	Ch
k									
pH									
6 P	1.2	4.4	6.3	2.0	7.2	6.7	1.1	4.2	10.0
7 P	1.7	3.0	8.8	2.8	6.2	12.9	0.6	2.3	6.1
8 P	1.2	2.4	6.9	2.2	5.3	10.4	0.4	1.9	4.8
8 tricine	2.0	5.2	5.6	10.8	5.6	11.2	1.3	2.6	7.3
8.5 tris	3.6	9.3	16.5	4.8	15.6	17.0	3.2	8.4	14.8
9 B	5.0	4.8	14.6	5.2	10.4	24.1	0.7	2.8	9.8
10 B	0.7	1.4	4.1	1.5	3.5	8.6	0.2	1.2	2.5
10.5 A	8.9	10.7	36.1	8.4	18.7	49.0	1.6	5.6	22.4
11 P	0.5	1.1	2.9	1.1	2.7	5.9	0.2	1.1	1.9
11.5 T	1.2	2.1	5.4	2.2	5.0	11.4	0.3	1.2	3.6
As									
pH									
6 P	1.1	1.3	1.4	1.2	2.1	1.6	2.3	1.9	2.3
7 P	1.2	1.1	1.4	1.2	1.3	1.2	1.9	1.6	2.0
8 P	1.6	1.1	1.4	1.0	1.2	1.0	1.7	1.5	2.0
8 trine	1.7	1.8	2.1	1.1	0.9	1.4	1.3	1.3	1.1
8.5 tris	0.9	1.6	1.5	1.2	1.2	2.8	0.9	1.6	1.6
9 B	n.d.	0.5	1.6	1.2	0.8	1.0	1.8	1.5	1.9
10 B	n.d.	0.7	0.8	1.3	1.1	0.7	0.8	1.6	1.7
10.5 A	1.4	1.0	2.0	1.5	1.6	1.2	2.4	2.2	3.0
11 P	1.9	1.0	0.9	1.3	1.1	1.0	1.0	1.3	1.5
11.5 T	1.5	1.0	0.8	1.1	0.9	0.9	1.2	1.1	0.9

3.3.3. Comparison between the classifications obtained using the SRM 870 test and Tanaka test

The HCAs with the SRM 870 test and with the Tanaka test reveal how the choice of the test can affect the final classification. For example, Platinum C18 EPS was not placed near the PMTDS–SiO_2 stationary phases in the HCA using SRM 870 test, as it presents poor peak shape for amitriptyline (3.5) and poor retention for ethylbenzene (0.4) while, using the Tanaka test, this phase is placed quite near to some PMTDS–SiO_2 stationary phases. The phases Aquasil C18 and Altima HP C18 AQ, which were placed near to SP8 and SP7 using the HCA with SRM 870, are placed far from the PMTDS–SiO_2 stationary phases in the HCA based using the Tanaka test, as they present much smaller B/P 7.6 values than SP8 and SP7. Thus the choice of the classification test can be very important, depending on the goals of the classification process.

3.4. Effect of mobile phase pH and buffer type on retention and asymmetry factor

As the pH is changed, all the chromatographic parameters for the basic solutes also change. Normally a mobile phase buffered at a pH value higher than the pK_a of the solutes is used with chemically bonded phases [1,38] to ensure that the solute is unprotonated, avoiding ion exchange interactions and enriching the hydrophobic interactions. This results in better peak shapes and increased retentions for the solutes than when the separation is conducted in mobile phases buffered at pH equal to or less than the pK_a of the solutes.

To understand the nature of the ion exchange interactions that take place with the PMTDS–SiO_2 stationary phases, SP9 was evaluated with nine basic solutes in 80:20 (v/v) methanol–buffer mobile phases with the pH ranging from 6 to 11.5, using both organic and inorganic buffers, to verify the influence of pH and the nature of the buffer on both the retention and asymmetry factors. The results are shown in Table 5.

The PMTDS–SiO_2 stationary phases present exponential increases in the retention factors for basic solutes with the increase in %C. Thus SP9, with an intermediate %C, was chosen for these tests. Mobile phase pH before the addition of organic modifier is a function of buffer type and concentration as well as the type of organic modifier used in the mobile phase [39]. Different kinds of buffer result in different selectivities. Thus the same buffer type must be used to evaluate the effect of mobile phase pH on chromatographic performance [40]. Table 5 shows that, for mobile phases buffered with phosphate at pH 6, 7, 8 and 11, the retention factors of nortriptyline, methadone, salbutamol and propanolol increase, while the retention factors of methadone, nicotine, codeine, amitriptyline, chlorpheniramine and diphenhydramine decrease as the mobile phase pH increases from 6 to 7, probably due to the different ion-exchange interactions of these solutes with the stationary phase surface at a mobile phase pH of 6. However the retention factors of all test solutes decrease as the pH increases from 7 to 11 (with phosphate buffer). The peak shapes also are better as the pH goes from 6 to 7, remaining similar as the pH goes from 7 to 11 except for salbutamol whose peak shapes worsen as the mobile phase pH increases. At pH 6–8 with phosphate buffer the test solutes were almost 100% protonated and at pH 11 almost 100% unprotonated [41], showing that a predominant ion exchange mechanism occurs with this PMTDS–SiO_2 stationary phase at the intermediate pH while the use of a highly alkaline mobile phase with phosphate buffer (pH 11) suppresses these effects, resulting in decreased retention, although slightly better peak shape. In all mobile phases above pH 6 nortriptyline eluted after amitriptyline, which is "anti-reversed phase" retention behavior [14]. In contrast, at pH below 7, nortriptyline eluted before amitriptyline, indicating that the ion exchange interactions are not the dominant retention mechanism in acidic mobile phases where both the free silanols and the test solutes are protonated.

Different elution orders were observed with each mobile phase used. Some solutes have better peak shapes at one pH and other solutes at another pH, as previously observed by McCalley [40], who has suggested that more than one solute and mobile phase pH must be evaluated in any stationary phase evaluation. Also, higher retention factors were observed with tricine buffer at pH 8, tris buffer at pH 8.5, borate buffer at pH 9 and with ammonium buffer at pH 10.5 than with phosphate buffer at pH 7. Phosphate is a harder

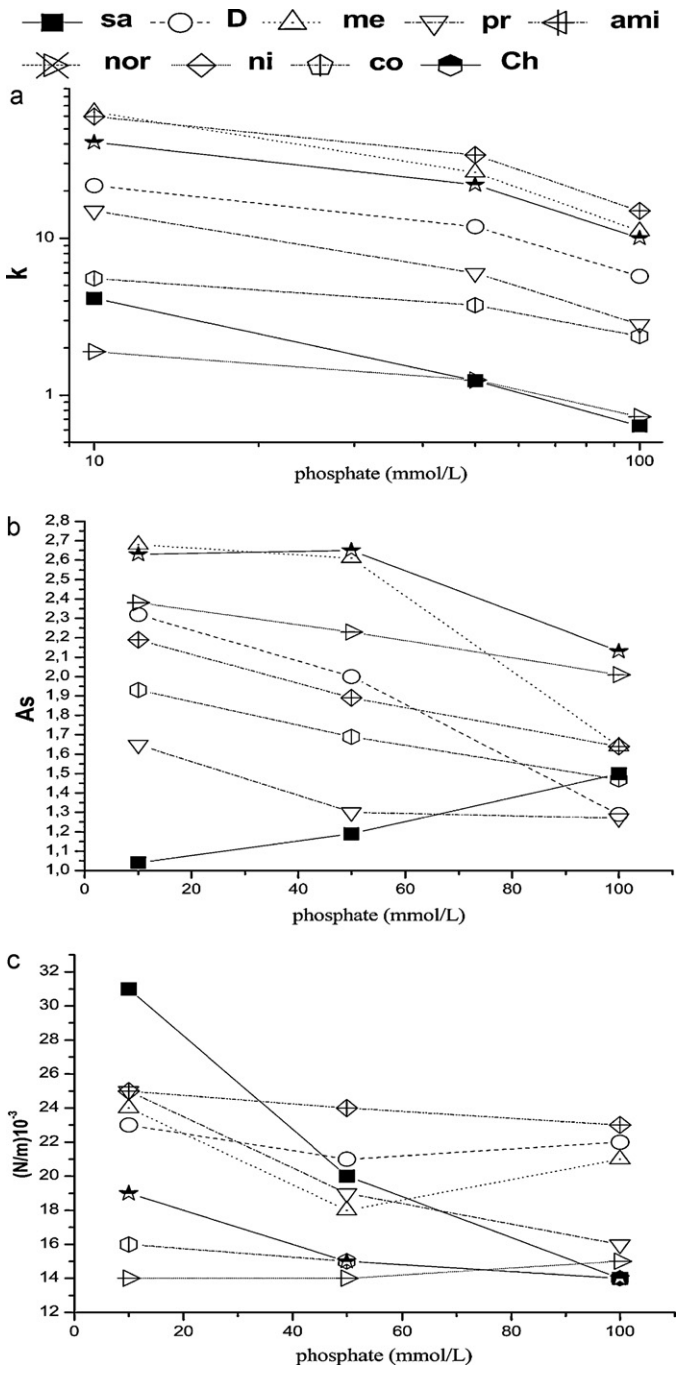

Fig. 1. Effect of buffer concentration on (a) retention factors (k); (b) asymmetry factors (As) and (c) efficiencies (N/m) for basic solutes. Solute identifications in Table 5. Chromatographic conditions in Section 2.7.

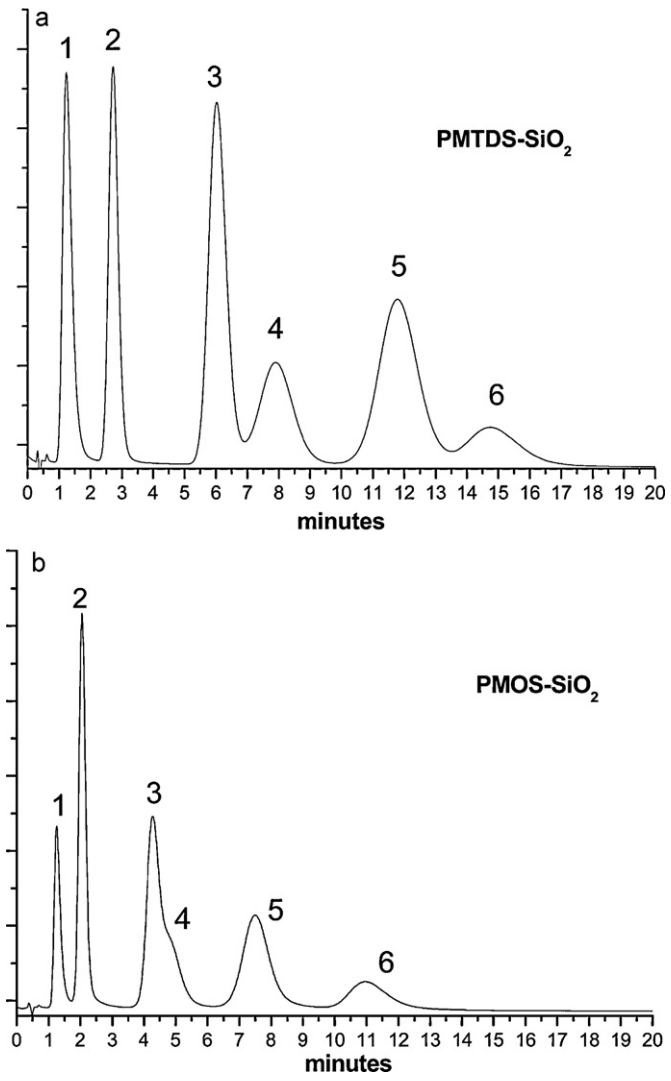

Fig. 2. Chromatograms for the separations of some basic pharmaceuticals on (a) PMTDS–SiO_2 stationary phase SP7 and (b) PMOS-SiO_2 stationary phase. Mobile phase: 80:20 (v/v) ethanol–phosphate buffer (pH 7.5; 20 mmol/L); flow rate: 1.5 mL/min; detection: UV at 220 nm; injection volume: 5 μL; temperature: 23 °C. Solutes: 1 = codeine; 2 = diphenhydramine; 3 = fluoxetine; 4 = amitriptyline; 5 = nortriptyline; 6 = dextromethorphan. (a) Asymmetry factors 1 = 1.8; 2 = 1.3; 3 = 1.4; 4 = 1.2; 5 = 1.2; 6 = 1.6. (b) Asymmetry factors 1 = 1.6; 2 = 1.2; 5 = 1.1; 6 = 1.4.

Lewis base than tricine, tris, and borate, and these soft buffers interact better with the soft ion-exchange sites (SiOH) on the PMTDS–SiO_2 stationary phase surfaces, making the PMTDS–SiO_2 stationary phases stronger cation exchangers. This results in longer retention times for the basic solutes since ion exchange interactions are stronger than hydrophobic interactions. At high pH both the solutes and the free silanols are unprotonated (SiO^-) and ion exchange interactions are suppressed. The retention factors with borate at pH 9 are greater than with phosphate at pH 7, while the retention factors with borate at pH 10 are lower than with phosphate at pH 7 and near to those obtained with phosphate at pH 11, due the suppression of ion exchange interactions at high pH. Some of these differences may result from the fact that the actual pH of the mobile phase is not the pH of the aqueous phase, since the pH changes after the addition of the organic constituent [39].

3.5. Effect of buffer concentration

The mobile phase buffer concentration has a big impact on solute retention in ion-exchange chromatography but only a minor effect in RP-HPLC [14]. Thus, to examine how changes in buffer concentration can affect retention on PMTDS–SiO_2 stationary phases, 10, 50 and 100 mmol/L phosphate buffers were chosen to ensure that any effect would be large enough to be detected. The mobile phases used were 65:35 (v/v) methanol–phosphate buffer because it is not possible to prepare 100 mmol of phosphate in a mobile phase with 80% methanol. Nortriptyline was taken out of the test solutes due to its extremely long retention time in this more aqueous mobile phase.

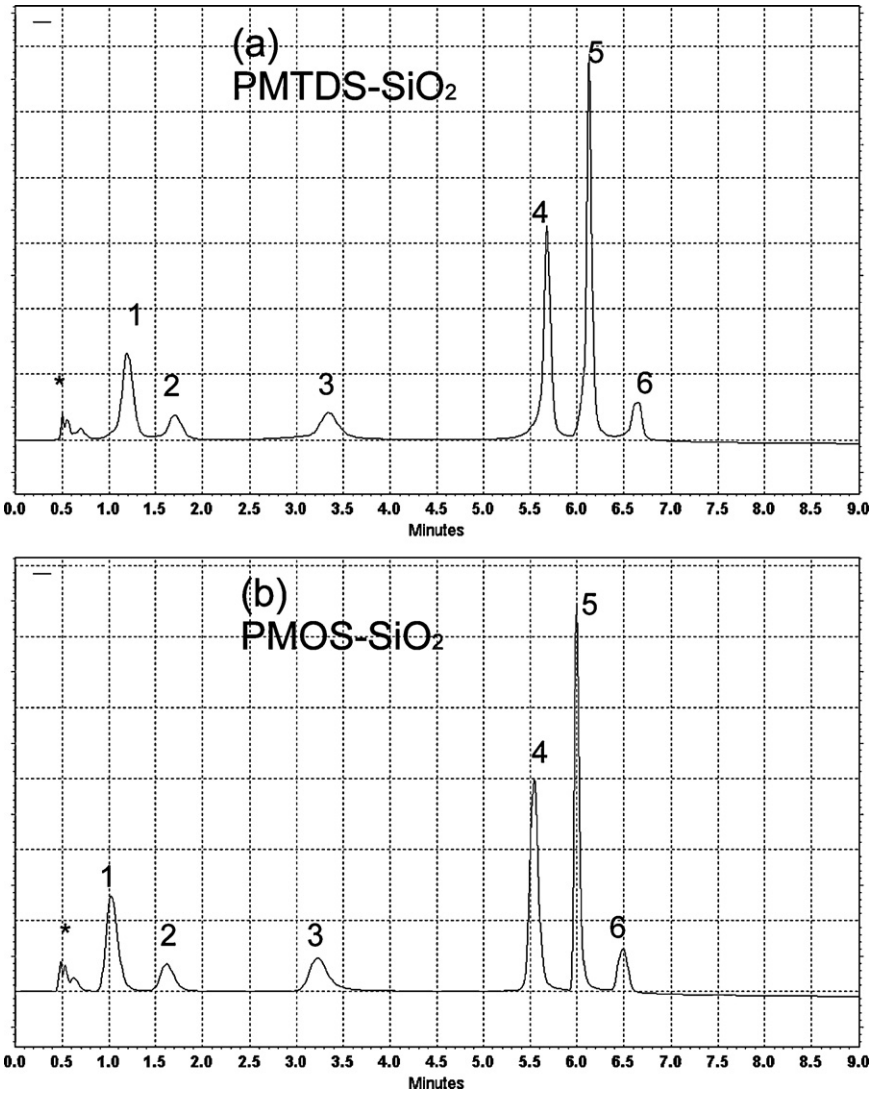

Fig. 3. Chromatogram for a green separation of some benzodiazepines on (a) PMTDS–SiO_2 stationary phase SP7 and (b) a PMOS-SiO_2 stationary phase. Mobile phase: A = ethanol; B = phosphate buffer (pH 7; 20 mmol/L). Step gradient: 30% A for 7 min, 55% A for 10 min, return to 30% A in 3 min; flow rate: 0.5 mL/min; detection: UV at 226 nm; injection volume: 10 μL; temperature: 23 °C. Solutes: 1 = bromazepam; 2 = clonazepam; 3 = lorazepam; 4 = alprazolam; 5 = diazepam; 6 = midazolam.

The effect of buffer concentration on the efficiencies, asymmetries and retention factors of the test solutes are shown in Fig. 1. For all the solutes, non linear retention decreases are seen as the buffer concentration is increased. The variations in retention factors with the increase in buffer concentration for the different solutes are different. This means that when the concentration of the mobile phase additive changes, the retentions of the different solutes differ. Thus, the selectivity (band spacing) will vary as the buffer concentration is changed. The elution order of salbutamol and nicotine was changed as the buffer concentration increased. The increase in buffer concentration results in slightly better peak shapes and slightly worse efficiencies. This confirms that ion exchange interactions contribute substantially to retention on the PMTDS–SiO_2 stationary phases.

3.6. Stability evaluations

3.6.1. Acidic mobile phase

A test with an acidic mobile phase, Fig. S4 of the Supplementary material, was carried out until the passage of 6500 column volumes (Vc) at 75 °C. The retention of toluene and ethylbenzene showed only a slight decrease by the end of the test, while the retention of propanolol fell by 25%. Also, only minor changes were observed in efficiencies and peak shapes at the end of the test.

The good stability presented for some PMTDS–SiO_2 stationary phases at low pH, even at high temperature, are probably due to the fact that the cleavage of Si–C bond in PMTDS is harder than in chemically bonded phases due to steric hindrance and that silica is highly stable in most acid media.

3.6.2. Basic mobile phase

With the PMTDS–SiO_2 stationary phases the basic stability test showed that amitriptyline presents lower retentions since the ion exchange interactions are suppressed. The alkaline stability test evaluated the viability of the phases in conditions that suppress the ion exchange interactions, as shown in Fig. S5 of the Supplementary material. This test was carried out at pH 11 and at 23 °C until the passage of 2500 Vc. The retention factors of toluene and ethylbenzene had smaller decreases (10%) than did amitriptyline (30%). The efficiencies for the test solutes were unchanged up to the passage of 1200 Vc. After this volume the stationary phase loses 60% of its efficiency, although the asymmetry factors were essentially unchanged. These results are similar to a previous investigation [42], which showed that a stationary phase prepared by

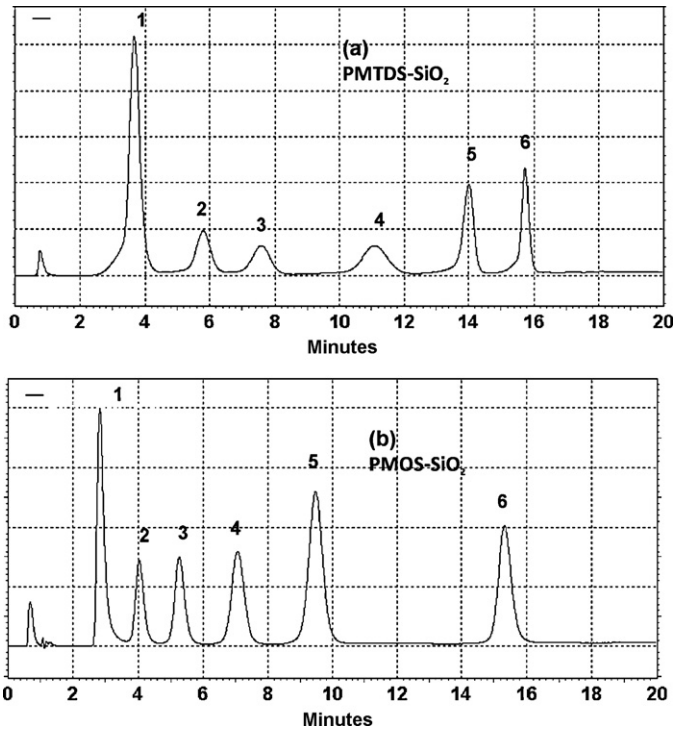

Fig. 4. Chromatogram for the separation of some agrochemicals on (a) on the PMTDS–SiO_2 stationary phase SP7 and (b) a PMOS-SiO_2 stationary phase. Mobile phase: A = 90:10 (v/v) acetonitrile:water; B = 10:90 (v/v) acetonitrile:water. Step gradient: 15% A for 2 min, 100% A for 6 min, return to 15% A in 3 min; flow rate: 1 mL/min; detection: UV at 249 nm; injection volume: 10 μL; temperature: 40 °C. Solutes: * = degradation product of imidacloprid; 1 = aldicarb; 2 = carbendazim; 3 = 3,5-dichloroaniline, 4 = diuron; 5 = diflubenzuron; 6 = diphenoconazol.

thermal immobilization of poly(methyloctylsiloxane) with high %C (23%) did not present any variation in retention factor, asymmetry factor and efficiency (naphthalene as test solute) until the passage of 1600 Vc of 70:30 (v/v) methanol–carbonate buffer (pH 10; 50 mmol/L) at 60 °C. After this volume the stationary phase lost 60% of its efficiency, while the asymmetry factors were again essentially unchanged.

The lower stability presented for PMTDS–SiO_2 stationary phases at high pH, even at room temperature, is due to the dissolution of the silica back bone, confirming that the PMTDS is immobilized onto silica surface as plugs [43]. This gives these stationary phases high ion-exchange properties but also instability in highly alkaline mobile phases. However these phases present a unique selectivity using neutral mobile phases with amino buffers and relative high amounts of organic modifiers could be used to obtain rational retention factors. Under these conditions acceptable stationary phase lifetime can be achieved.

3.7. *The applicability of some PMTDS–SiO_2 stationary phases*

The Tanaka test indicated that the major difference between the PMTDS–SiO_2 stationary phase SP7 and a PMOS-SiO_2 stationary phase with the same %C are higher ion exchange properties at neutral mobile phases and higher shape selectivity for SP7 (Table 4). To understand what this implies in terms of selectivity and peak shape for basic solutes both phases were used to separate a mixture of basic solutes as shown in Fig. 2. The higher ion-exchange properties of SP7 allow the separation of amitriptyline and fluoxetine, while this separation fails on the PMOS-SiO_2 stationary phase. Dextromethorphan was better resolved and has a slightly better asymmetry factor on the PMOS-SiO_2 stationary phase than on SP7. Fig. 3 shows the separation of benzodiazepines on the same stationary phases, using ethanol as organic modifier. The benzodiazepines are weaker bases than the pharmaceuticals used in the example of Fig. 2 but the differences in selectivity for the separations between these two stationary phases are significant. The asymmetry factors for the benzodiazepines with SP7 are close to 0.9, while the PMOS-SiO_2 stationary phase affords asymmetry factors nearer to 1.0. Also, the use of ethanol as organic modifier afforded a good separation without the generation of hazardous waste [2,3]. However, since both phases have the same %C they provide almost the same selectivity for the separation of a mixture of neutral agrochemicals, as shown in Fig. 4.

4. Conclusion

The small RSD presented for some test parameters (such as asymmetry factor) and the high correlation of the retention factors obtained with the SRM 870 and Engelhardt tests leads to the conclusion that the Tanaka test is the best one to evaluate these PMTDS–SiO_2 stationary phases, since the variables were highly uncorrelated. Comparison of several PMTDS–SiO_2 stationary phases with commercial phases using the Tanaka test and literature data suggests that the PMTDS–SiO_2 stationary phases do not have ion exchange properties in acidic mobile phases but that ion exchange interactions are significant at neutral pH and that these stationary phases have different chromatographic properties than most commercial phases. Our in-house test adds evidences that ion-exchange interactions are increased in the presence of the soft buffers, which results in large variations in the retention factors of basic solutes with pH, buffer type and buffer concentration variations. The PMTDS–SiO_2 stationary phases present reasonable stability with acidic mobile phases, as expected for immobilized polymer phases, although these phases were not stable in highly alkaline mobile phases. The use of highly basic mobile phases suppresses ion-exchange interactions, resulting in lower selectivities and slightly better peak shapes than those obtained with neutral mobile phases. However, better selectivities and adequate asymmetries are found in neutral mobile phases using amino buffers, reducing the need for alkaline mobile phases, avoiding possible negative consequences of using highly alkaline mobile phases on the instrument and permitting the use of mass spectrometric detection. The use of green mobile phases with the PMTDS–SiO_2 stationary phases merits further investigation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.05.007.

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