Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1030 (2004) 157–166
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Chromatographic and column stability at pH 7 of a C_{18} dimethylurea polar stationary phase

César Ricardo Silva, Carol H. Collins, Isabel Cristina Sales Fontes Jardim, Claudio Airoldi*

Instituto de Química, Universidade Estadual de Campinas, Caixa Postal 6154, 13083-970 Campinas, São Paulo, Brazil

Abstract

Chromatographic evaluations of a C_{18} dimethylurea phase in 150 mm × 3.9 mm HPLC columns were performed using the Tanaka and Engelhardt test mixtures. The applicability of the new C_{18} dimethylurea phase was also evaluated with a mixture of some herbicides and their metabolites. An artificial aging procedure was also performed by passing a potassium phosphate mobile phase buffered at pH 7.0 through C_{18} 50 mm × 3.9 mm dimethylurea columns. The column stability was evaluated by means of the chromatographic parameters obtained for the separation of some compounds from the Neue test mixture, using apolar, polar and highly basic analytes.

Keywords: Stationary phases, LC; Dimethylurea stationary phases; Silanols; Stability studies; Pesticides

1. Introduction

Several new stationary phases containing embedded polar groups have appeared on the scene, providing users with alternative selectivites for more difficult chromatographic separations involving polar, highly basic and ionizable compounds in reversed phase conditions. The successful performance of these reversed phases, having embedded polar groups, is attributed to lower tailing and retention for bases at neutral pH and different selectivities in comparison with classical C_{18} phases. These advantages over conventional C_{18} phases are provided by the presence of the polar groups, which minimize undesirable interactions with the residual silanols and are responsible for good peak shapes and high efficiencies for analytes with different physicochemical properties (nonpolar, polar and basic). Another important feature is that these phases can be used with mobile phases having a low percentage of organic solvent without stationary phase collapse, leading to stable and reproducible retention and faster gradient regeneration [1].

In a recent article, Przybyciel and Majors listed about 20 different polar embedded alkyl stationary phases from different suppliers that have become commercially available during the past several years [2]. In an article describing the new chromatography columns and accessories introduced at the 2003 Pittsburgh conference, Majors [3] reported that manufacturers were displaying a significant number of various types of embedded polar phases.

Embedded polar group bonded phases were first reported by Nomura et al. [4] in 1987. Their phases were prepared by a two step modification process. In the first step, the bare silica was grafted with aminopropyl moieties, which were acylated in a further step with acid chlorides to form an amide group [–NH–C(O)–R′]. In a systematic study, the reactivity of these amino groups were explored with different acylating reagents as a function of pore size of the silica support. However, the applicability of these amide phases remained unexplored.

Later, in 1990, Ascah and Feibush [5], from Supelco, reported the separation of basic compounds including pyridine and some substituted pyridines, under reversed phase conditions, on a bonded phase with an embedded polar group. In this work, the polar group was not revealed, but the good peak shapes observed for the pyridines in neutral pH without the addition of additives in the mobile phase was a clear indication for reduced silanophilic interactions. In this same year, this phase was patented by Supelco [6] and became commercially available on the market as Suplex.

In 1991, Buszewski et al. [7] reported the synthesis of new polar stationary phases prepared through the reaction of aminopropyl silica with an acid chloride following the two step procedure. The acylating reagent was prepared by the reaction of stearic acid with tionyl chloride. Later, Schmid

* Corresponding author. Fax: +55-19-3788-3023.
E-mail address: airoldi@iqm.unicamp.br (C. Airoldi).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2003.11.016

et al. [8] characterized these new polar phases by both ^{13}C and ^{29}Si nuclear magnetic resonance (NMR) spectroscopy. It was possible to obtain more information about the presence of the polar amide group embedded into the alkyl chain by the presence of a signal at 160 ppm in the ^{13}C NMR spectrum, related to the carbonyl of the amide function. The preparation of different amide phases, with terminal alkyl groups varying from C_3 to C_{18}, was reported by Buszewski et al. [9] allowing a better understanding of the selectivity of this new class of stationary phases.

In 1996, Ascah et al. [10] reported a new version of the amide phases called Supelcosil ABZplus. In this work, the phases were characterized by X-ray photoelectron spectroscopy. The liquid chromatographic performance of these phases was found to be superior to the phase prepared earlier and to the usual C_{18} phases. Nowadays, these phases are claimed to be prepared by one step process, using the type B silicas and commercialized by Supelco as Discovery Amide C_{16} phases [2].

Following a similar approach, a stationary phase, containing embedded amide groups with sterically protective diisopropyl groups and a C_{14} *N*-alkyl chain has been prepared by Kirkland et al. [11] and commercialized by Agilent as Zorbax Bonus RP.

However, the procedure involving a two step modification process suffers from the difficulty of achieving complete acylation of the amine groups in the second step. As a result, a heterogeneous surface is obtained having both acetylated and underivatized amine groups. The presence of these underivatized groups can, sometimes, be beneficial because they can also aid in shielding the residual surface silanols. However, a mixed mode separation mechanism can be observed especially in the separation of acidic ionizable compounds, resulting in high retention and tailed peaks due to an anion exchange mechanism with the protonated underivatized amino groups, at pH under 9 [12].

As an alternative approach, in 1995 O'Gara et al. [13] reported the preparation of a new C_8 phase containing embedded polar carbamate groups, using a single step modification process. The approach was based on the prior synthesis of the appropriate monofunctional chlorosilane containing polar functional carbamate groups [–O–C(O)–NH–R′], followed by chemical modification of the silica surface with this new chlorosilane reagent and then, endcapping. The synthesis of this new silane was patented by Waters in 1994 [14].

Following this synthetic route, a series of HPLC bonded phases containing an internal carbamate group were studied by changing the terminal *N*-alkyl group from C_8H_{17} to $C_{18}H_{37}$ in increments of two methylene units. These carbamate phases have had their chromatographic performance compared to their alkyl counterparts and to other commercially available embedded polar stationary phases [15,16]. Waters commercializes these phases as SymmetryShield columns and they are also available on hybrid silica (X-Terra RP).

In our laboratory, we have prepared new stationary phases containing embedded urea groups [–NH–C(O)–NH–R′] by a single-step modification process, based on the prior synthesis of trifunctional or monofunctional urea-alkoxysilanes by a proprietary chemical process [17], followed by modification of the bare silica and further endcapping. A series of embedded polar phases based on urea were prepared through modification of LiChrosorb silica with trifunctional urea-alkoxysilanes with *N*-alkyl terminal groups varying from C_5 to C_{12} [18]. A trifunctional C_{18} urea phase, prepared by the modification of ProntoSil silica, showed promise for the separation of the compounds of the Neue test mixture, and of polycyclic aromatic hydrocarbons with good peak shapes and column efficiencies [19]. In another paper, we described the successful separation of some triazine herbicides at neutral pH with isocratic elution, which is very difficult to achieve in conventional C_{18} phases [20]. The column aging test, performed at pH 7 using a buffered phosphate mobile phase at room temperature, showed a considerable decrease in efficiency after the passage of 16 000 column volumes of mobile phase [20].

More recently, a new generation of monofunctional C_{18} urea phases was prepared by the modification of ProntoSil silica with monofunctional dimethyl and diisopropyl urea-alkoxysilanes [21]. By the separation of Neue test mixture, we have confirmed our prediction that the dimethyl C_{18} urea phase showed better peak shapes for amitriptyline and propranolol in comparison to both the C_{18} diisopropyl phase and the polymeric C_{18} urea phase synthesized earlier, based on the same silica support [21].

The present paper reports further chromatographic evaluations with the Tanaka and Engelhardt test mixtures, as well as the separation of some herbicides and their metabolites, to better illustrate the advantages of the C_{18} dimethylurea phase. A column stability test in a buffered phosphate mobile phase at pH 7 was also performed in order to compare the stability of the dimethyl embedded urea phase with the polymeric C_{18} urea phase.

2. Experimental

2.1. Chemicals

Uracil, caffeine, 3,4-dichloroaniline, phenol and phosphoric acid were obtained from Aldrich (Milwaukee, WI, USA) and were used as received. Aniline and *N*,*N*-dimethylaniline were from Fluka (Buchs, Switzerland). Cyanazine, simazine and atrazine were supplied from Norvatis. Diuron and linuron were from DuPont and Hoechst, respectively. The atrazine metabolites, 2-chloro-4,6-diamino-1,3,5-triazine and 2-hydroxyatrazine were purchased from Chem Service (West Chester, USA). Potassium salts (KH_2PO_4 and K_2HPO_4), benzylamine, toluene, ethylbenzene, butylbenzene, pentylbenzene and *o*-terphenyl were obtained from Merck (Darmstadt, Germany). Methanol and acetonitrile

were HPLC grade and were also purchased from Merck. Deionized water was purified using a Milli-Q water system (Millipore, Bedford, MA, USA). A 150 mm × 3.9 mm Nova-Pak C_{18} HPLC column (4 μm dimethyloctadecylsilyl bonded silica particles with 6 nm pore size, lot No. W 11421) was purchased from Waters (Milford, MA, USA).

2.2. C_{18} Dimethylurea phase

ProntoSil spherical silica (3 μm, 20 nm pore size) from Bischoff Chromatography (Leonberg, Germany) was chemically modified with the monofunctional alkoxysilane [(3-octadecyl-urea)propyl]dimethylethoxysilane to obtain the C_{18} dimethylurea phase with a surface coverage of 3.3 μmol m^{-2}. The C_{18} dimethylurea phase was slurry packed in 150 or 50 mm × 3.9 mm i.d. HPLC columns. Additional details about the preparation, column packing, physicochemical properties of this phase have been published elsewhere [21].

2.3. HPLC separations

All chromatographic evaluations were performed using a modular HPLC system from Shimadzu (Kyoto, Japan) equipped with a LC-10AD liquid chromatography pump, a SPD-10A UV-Vis detector, a CTO-10A column oven and a Rheodyne 8125 injector (Cotati, USA) with a 5 μl loop. Data were acquired and processed using ChromPerfect software (Justice Innovations, Mountain View, USA). All solvents were filtered and degassed before use. The mobile phases were prepared volumetrically from individually measured amounts of each component.

2.3.1. Tanaka test procedure

A 150 mm × 3.9 mm i.d. HPLC column packed with the C_{18} dimethylurea phase was used in these tests at the optimal flow rate of 0.8 ml min^{-1} and detection at 254 nm. The first chromatographic evaluations were performed using a mixture of uracil (10 mg l^{-1}), used as the marker for the column dead time, butylbenzene (1000 mg l^{-1}), *o*-terphenyl (80 mg l^{-1}), pentylbenzene (1100 mg l^{-1}) and triphenylene (50 mg l^{-1}), using methanol–water (80:20, v/v) as mobile phase at 298 K. Retention factors, plates per meter, separation factors for butyl and pentylbenzene (methylene selectivity, α_{CH2}) and for *o*-terphenyl and triphenylene (steric selectivity, $\alpha_{T/O}$) were calculated as recommended [22]. A second mixture was composed of uracil (10 mg l^{-1}), caffeine (135 mg l^{-1}) and phenol (270 mg l^{-1}) and was chromatographed using methanol–water (30:70, v/v) as mobile phase at 303 K. The quotient of the retention factor of caffeine to that of phenol (hydrogen bonding capacity, $\alpha_{C/P}$) was calculated. A third test was performed by separating uracil (10 mg l^{-1}), benzylamine (100 mg l^{-1}), and phenol (120 mg l^{-1}), dissolved in a mobile phase composed of methanol–20 mmol l^{-1} KH_2PO_4/K_2HPO_4 (30:70, v/v) at pH 7.6. The buffer was prepared by dissolving 1.25 g of K_2HPO_4 and 0.39 g KH_2PO_4 in a 500 ml volumetric flask. The pH was adjusted to 7.60 using a calibrated pH meter before addition of methanol. The ion-exchange capacity ($\alpha_{A/P}$) was calculated by the relation between the retention factors for benzylamine and phenol. The last test was performed using these same compounds dissolved in a methanol–20 mmol l^{-1} phosphate buffered mobile phase at pH 2.7 (30:70, v/v). The buffer was prepared by dissolving 0.985 g of KH_2PO_4 and 0.28 g of H_3PO_4 in a 500 ml volumetric flask, and the pH adjusted to 2.70. The ion exchange capacity ($\alpha_{A/P}$) at lower pH was again calculated [22,23].

2.3.2. Engelhardt test procedure

Some of the compounds of the Engelhardt test mixture were chosen for chromatographic evaluation of the C_{18} dimethylurea phase. A solution, containing uracil (12 mg l^{-1}), aniline (100 mg l^{-1}), phenol (220 mg l^{-1}), *N*,*N*-dimethylaniline (40 mg l^{-1}), toluene and ethylbenzene (1500 mg l^{-1}) was separated using a methanol–water (55:45, v/v) mobile phase at 313 K [24,25]. Plate number, *N*, retention factor, *k*, and tailing factor at 5%, T_F, were used for column performance evaluation.

2.3.3. Separation of some herbicides

The separation of the mixture containing uracil (5 mg l^{-1}), 2-chloro-4,6-diamino-1,3,5-triazine (1.5 mg l^{-1}), 2-hydroxyatrazine (2 mg l^{-1}), cyanazine (2 mg l^{-1}), simazine (2 mg l^{-1}), atrazine (2 mg l^{-1}), 3,4-dichloroaniline (1 mg l^{-1}), linuron (2 mg l^{-1}) and diuron (2 mg l^{-1}) was performed at 303 K using methanol–water (50:50, v/v) as mobile phase with detection at 230 nm. The mixture was also separated on the Nova-Pak C_{18} column using similar conditions.

2.4. Column aging study

The column aging procedure was performed using a modular HPLC system with a Waters 486 tuneable wavelength absorbance detector, a Waters 510 pump and a Rheodyne 7725i injector (Cotati, USA). For the test, a 50 mm × 3.9 mm i.d. HPLC column, packed with the C_{18} dimethylurea phase was employed. To simulate the usual chromatographic practice, the column was continuously purged at 1.0 ml min^{-1} with methanol–20 mmol l^{-1} KH_2PO_4/K_2HPO_4 buffer (65:35, v/v) at pH 7.0 (not recycled) as mobile phase at room temperature. The buffer was prepared by dissolving 1.68 g of K_2HPO_4 and 1.33 g KH_2PO_4 in a 1 l volumetric flask. The pH was adjusted to 7.0 using a calibrated pH meter before addition of methanol. The column was periodically tested by the separation of uracil (6 mg l^{-1}), naphthalene (80 mg l^{-1}), dipropyl and dibutyl phthalate (500 mg l^{-1}), with propranolol (350 mg l^{-1}), and amitriptyline (100 mg l^{-1}) as basic probes, at the optimal flow rate of 0.8 ml min^{-1}. Not all components of the Neue test mixture [26] were used for this test, because they were not well separated due the short length of the column employed for this study.

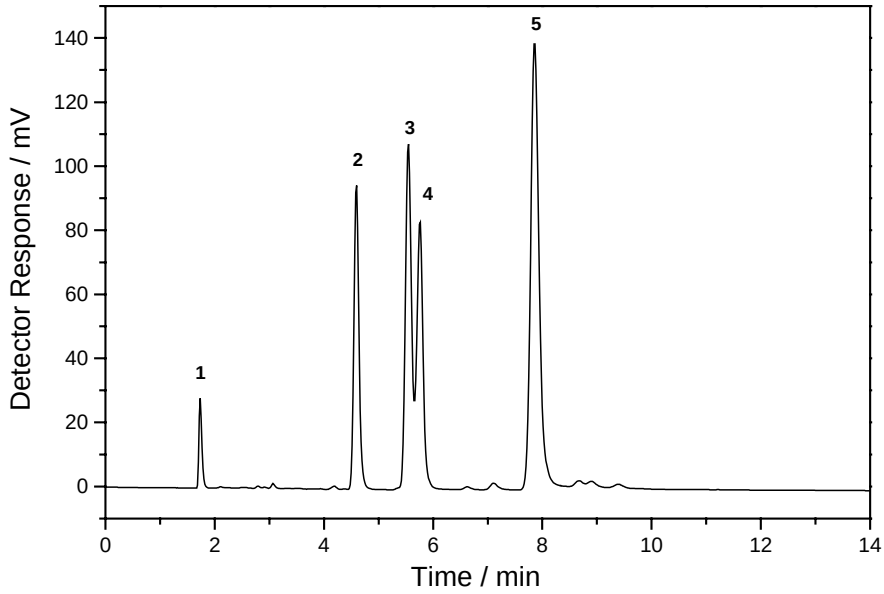

Fig. 1. Chromatogram of the separation of the Tanaka test mixture composed of uracil (1), butylbenzene (2), pentylbenzene (3), *o*-terphenyl (4) and triphenylene (5). Conditions: 150 mm × 3.9 mm i.d. column packed with the C_{18} dimethylurea phase, mobile phase: methanol–water (80:20, v/v); flow rate: 0.8 ml min^{-1}; injection volume: 5 μl; temperature: 298 K; detection: UV at 254 nm.

3. Results and discussion

3.1. Column characterization

Following the evaluation of the C_{18} dimethylurea phase using the test procedure of Neue et al. [26], the characterization was extended by studying the evaluation parameters of two other popular test procedures, those of Tanaka and Engelhardt.

The Tanaka test [22] is based on the separation of seven compounds, using four different mobile phase compositions. The chromatogram of Fig. 1 shows the separation of the alkyl benzenes and the polyaromatic hydrocarbons, where it was possible to observe that pentylbenzene was not well separated from *o*-terphenyl. A steric selectivity ($\alpha_{T/O}$) of 1.44 and a methylene selectivity (α_{CH2}) of 1.45 were obtained. The hydrogen bonding capacity ($\alpha_{C/P}$) was calculated by the relation of retention factors of caffeine and phenol from the chromatogram of Fig. 2, obtaining a value of 0.45. For the chromatograms of Fig. 3, the ion exchange capacities, $\alpha_{A/P}$, in acidic and neutral medium were determined by the separation of benzylamine and

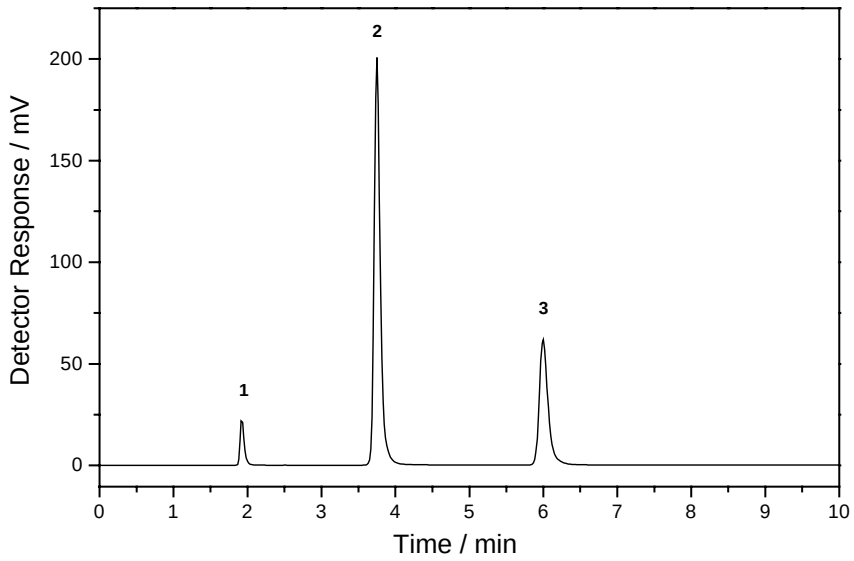

Fig. 2. Chromatograms of the separation of uracil (1), caffeine (2) and phenol (3) using an unbuffered mobile phase. Conditions: 150 mm × 3.9 mm i.d. column packed with the C_{18} dimethylurea phase, mobile phase: methanol–water (30:70, v/v); flow rate: 0.8 ml min^{-1}; injection volume: 5 μl; temperature: 303 K; detection: UV at 254 nm.

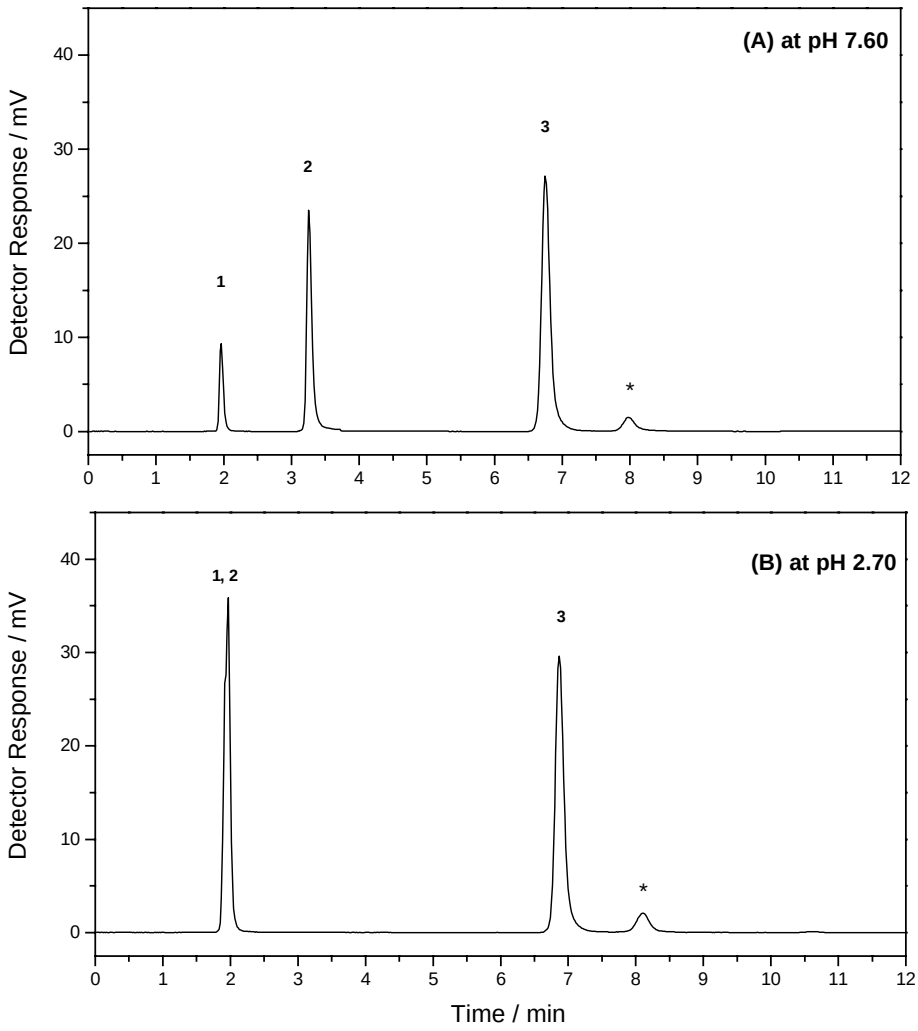

Fig. 3. Chromatograms of the separation of uracil (1), benzylamine (2) and phenol (3) at pH 7.60 (A) and pH 2.70 (B). Conditions: 150 mm × 3.9 mm i.d. column packed with the C_{18} dimethylurea phase, mobile phase: methanol-buffer phosphate (30:70, v/v); flow rate: 0.8 ml min^{-1}; injection volume: 5 μl; temperature: 303 K; detection: UV at 254 nm. The small peak is an impurity of benzylamine.

phenol using buffered mobile phases at pH 2.70 and 7.60, respectively.

At low pH, benzylamine is not retained and coelutes with uracil, indicating a null ion exchange capacity, $\alpha_{A/P} = 0$, a behavior which is expected due to the high purity silica support used in the preparation of the C_{18} dimethylurea phase. The ion exchange capacity at low pH is more pronounced on older type A silica supports with higher metal contents, especially those of iron and aluminum, which increase the acidity of the residual surface silanols, so that they are still ionized at low pH values. On the other hand, the separation in pH 7.6 maximizes the ion exchange activity of the residual silanols where the great majority of the silanols are in their ionized form and benzylamine ($pK_a = 9.3$) is partially protonated. In a recent publication, Neue et al. [27] described this as similar to his test procedure, also using an amine, amitriptyline. Chromatogram A of Fig. 3 shows good peak shape for benzylamine with lower tailing. The ion exchange capacity, $\alpha_{A/P}$, was calculated by the relation between the retention factors for benzylamine and phenol, obtaining a value of 0.28. Retention factor, *k*; plate number per meter, *N*/*m*, and tailing factor at 5%, T_F, were calculated for all components and the results are summarized in Table 1. The overall results are quite similar and comparable to those found in the recent publications of Euerby and Petersson [28,29] on the characterization of more than eighty commercially available RPLC columns, especially for six polar embedded phases from different suppliers.

Fig. 4 shows the chromatograms and Table 2 summarizes the chromatographic parameters obtained for the separation of some compounds from the Engelhardt test mixture, also used to evaluate the C_{18} dimethylurea phase. Lower tailing factors for the basic probes, aniline and *N*,*N*-dimethylaniline, and higher *N*/*m* values of 100 400 and 103 600 for the hydrophobic probes, toluene and ethylbenzene, were observed. The relative retention between phenol and toluene ($\alpha_{Phenol/Toluene}$) was calculated, obtaining a value of 0.22. This value is similar to those obtained by Engelhardt et al.

Table 1
Chromatographic parameters obtained for the separation of some compounds of the Tanaka test mixture composed of nonpolar, polar and basic analytes on the C_{18} dimethylurea phase

Compound	k	N/m	T_F
Butylbenzene[a]	3.02	95 200	1.10
Pentylbenzene[a]	4.38	96 400[e]	1.09[e]
o-Terphenyl[a]	4.54	95 900[e]	1.12[e]
Triphenylene[a]	6.55	93 400	1.10
Caffeine[b]	0.95	72 500	1.28
Benzylamine	0[c]	38 500[c,e]	1.20[c,e]
	0.67[d]	58 100[d]	1.35[d]
Phenol	2.13[b]	89 900[b]	1.30[b]
	3.14[c]	92 900[c]	1.29[c]
	2.44[d]	91 600[d]	1.32[d]

Chromatographic conditions: 150 mm × 3.9 mm column; flow rate: 0.8 ml min^{-1}; detection: UV at 254 nm; injection volume: 5 μl at different mobile phase compositions.

[a] Methanol–water (80:20, v/v).

[b] Methanol–water (30:70, v/v).

[c] Methanol–20 mmol l^{-1} H_3PO_4/KH_2PO_4 buffer (30:70, v/v) at pH 2.70.

[d] Methanol–20 mmol l^{-1} KH_2PO_4/K_2HPO_4 buffer (30:70, v/v) at pH 7.60.

[e] This value was obtained from separated injection.

[25] for amide and carbamate polar reversed phases. This behavior is attributed to the hydrogen-bonding contribution of the polar urea group close to the silica surface.

3.2. Separation of herbicides

The experiment was based on the separation of some important triazine herbicides, including two metabolites and two urea herbicides, diuron and linuron, and their metabolite, 3,4-dichloroaniline. The structure of each compound is shown in Fig. 5. The chromatographic determination of these substances is of great importance in environment control due to their potential for groundwater contamination and they are quite difficult to separate on conventional C_{18} phases without pH adjustments or additives in the mobile phase to suppress the tailing for the basic molecules. Fig. 6 shows the separations obtained on the C_{18} dimethylurea phase and also on a commercial C_{18} phase (Nova-Pak) under the same separation conditions, using isocratic elution with a methanol–water (50:50, v/v) mobile phase.

The advantage of using the C_{18} dimethylurea phase over the commercial C_{18} Nova-Pak phase is shown by the different elution order obtained. With the commercial phase, atrazine and 2-hydroxyatrazine are more retained, while a different selectivity is observed in the phase containing the polar urea groups. According to the structure of 2-hydroxyatrazine shown in Fig. 5, the presence of the –OH group makes this molecule more basic than the other triazines [30].

Table 2
Chromatographic parameters obtained for the separation of some compounds of the Engelhardt test mixture on the C_{18} dimethylurea phase

Compound	k	N/m	T_F
Aniline	0.38	55 300	1.34
Phenol	0.64	67 000	1.32
N,N-DMA	2.13	93 600	1.16
Toluene	2.93	100 500	1.18
Ethylbenzene	4.92	103 600	1.10

Chromatographic conditions: 150 mm × 3.9 mm column; mobile phase: methanol–water (55:45, v/v); flow rate: 0.8 ml min^{-1}; temperature: 313 K; detection: UV at 254 nm; injection volume: 5 μl.

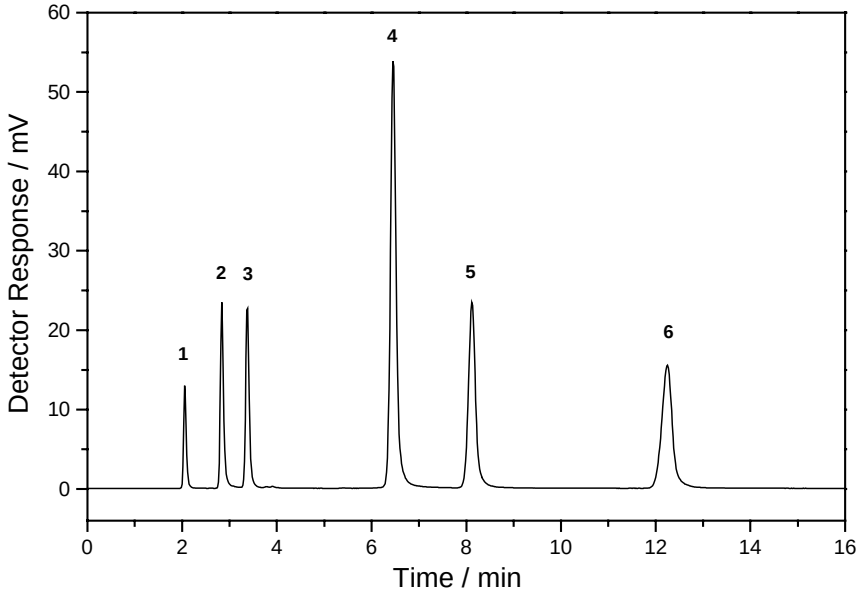

Fig. 4. Engelhardt test mixture separation on the phase C_{18} dimethylurea. Column: 150 mm × 3.9 mm i.d., mobile phase: methanol–water (55:45, v/v); flow rate: 0.8 ml min^{-1}; temperature: 313 K, detection: UV at 254 nm. Peaks: uracil (1), aniline (2), phenol (3), *N,N*-dimethylaniline (4), toluene (5) and ethylbenzene (6).

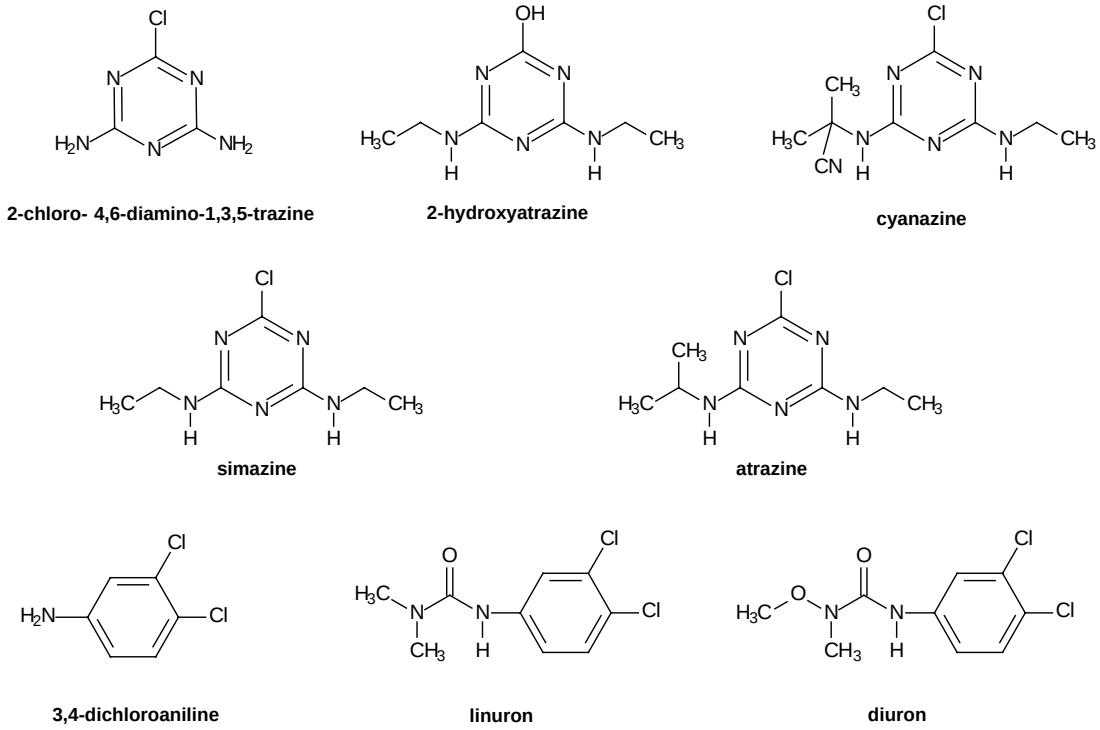

Fig. 5. Chemical structures of some herbicides and their metabolites.

On the C_{18} dimethylurea phase, all compounds are less retained, except for 3,4 dichloroaniline, when compared to the conventional C_{18} reversed phase. The chromatographic parameters were calculated and are listed in Table 3. *N*/*m* values ranged from 27 200 for the least retained compound to 81 900 for the most retained compound, linuron. These values are higher, when compared to those obtained with the commercial C_{18} phase. However, it should be noticed that tailing factor values are somewhat higher for all compounds, except for 2-hydroxyatrazine, on the C_{18} dimethylurea phase. The higher retention and the less than desirable tailing factor for 2-hydroxyatrazine on the conventional phase may be attributed to interactions with residual surface silanols. The results obtained show the potential application of the C_{18} dimethylurea stationary phase for the determination of these herbicides, without requiring pH adjustments in the mobile phase.

3.3. Column aging at pH 7

A shorter HPLC column packed with the C_{18} dimethylurea phase was chosen for this test. There are some advantages in using a shorter column, such as the lower quantity of stationary phase required, shorter analysis time and lower consumption of solvents during the artificial aging test. The column was purged with known volumes of the mobile phase at room temperature, periodically testing with some components of the Neue test mixture [26]. The mobile phase chosen was a mixture of methanol–20 mm mol l^{-1} phosphate buffer at pH 7.0 (65:35, v/v). This system was

Table 3
Chromatographic parameters obtained for the separation of the mixture of herbicides

	C_{18} dimethylurea			Nova-Pak C_{18}		
	k	*N*/*m*	T_F	*k*	*N*/*m*	T_F
2-Chloro-4,6-diamino-1,3,5-triazine	0.17	27 200	1.27	0.10	23 400	1.20
2-Hydroxyatrazine	1.07	39 000	1.47	3.68	1400	2.10
Cyanazine	1.69	54 900	1.29	1.78	42 900	1.03
Simazine	2.11	60 300	1.28	2.60	51 800	1.07
Atrazine	3.50	67 600	1.24	5.14	59 000	1.11
3,4-Dichloroaniline	4.35	75 900	1.44	4.64	75 750	1.05
Diuron	6.80	79 400	1.26	6.34	69 800	1.05
Linuron	9.10	81 900	1.25	10.3	75 200	1.06

Chromatographic conditions: 150 mm × 3.9 mm columns packed with C_{18} dimethylurea phase and Nova-Pak C_{18}; mobile phase: methanol–water (50:50, v/v); flow rate: 0.8 ml min^{-1}; temperature: 303 K; detection: UV at 230 nm; injection volume: 5 μl.

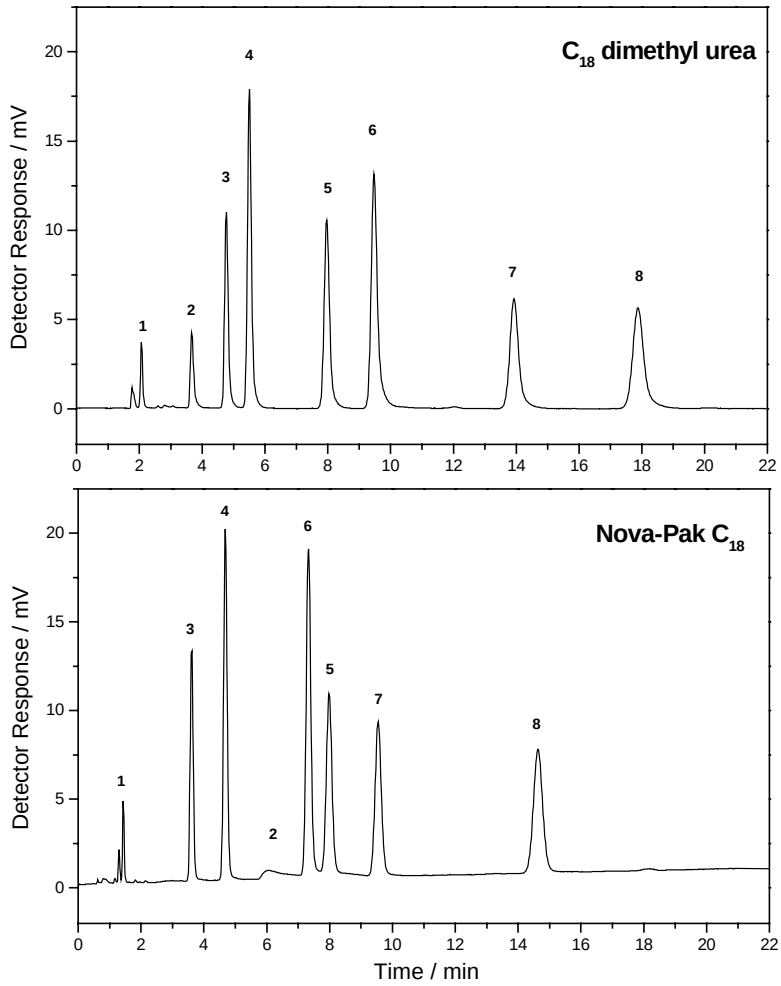

Fig. 6. Chromatograms of the separation of 2-chloro-4,6-diamino-1,3,5-triazine (1), 2-hydroxyatrazine (2), cyanazine (3), simazine (4), atrazine (5), 3,4-dichloroaniline (6), diuron (7) and linuron (8) performed on the C_{18} dimethylurea phase and on the Nova-Pak C_{18} phase. Conditions: 150 mm × 3.9 mm columns; mobile phase: methanol–water (50:50, v/v); flow rate: 0.8 ml min^{-1}; injection volume: 5 μl; temperature: 303 K; detection: UV at 230 nm.

selected as a critical test, since phosphate buffers are much more aggressive in the dissolution of the silica support, when compared to other buffers, for example citrate, Tris and others [31,32]. The chromatographic parameters, plate numbers expressed by *N*/*m*, tailing factor at 5% and retention factor for naphthalene (nonpolar probe), dibutyl phthalate (polar), amitriptyline and propranolol (basic analytes) were used to monitor column stability.

The retention of naphthalene during the aging test is a good measure of stationary phase hydrophobicity. The retention factors for amitriptyline and propranolol are known to be very influenced by the presence of residual silanols at pH 7.0 and thus measure any modification of the silanol population on the silica surface during the aging test.

The retention factors for amitriptyline and propranolol increased by 7 and 12% from their initial values, respectively, after the passage of 21 000 column volumes of the buffered phosphate mobile phase. The N/m values for amitriptyline also decreased by 13%. The slight increase in the retention factors for both basic probes can be interpreted as an increase in the number of the residual surface silanols. Consequently, a reduction in 7% in the retention factor of naphthalene was also observed. Thus, there is some evidence for the partial loss of the bonded phase ligands attached to the silica surface due to the silica dissolution, while the hydrolysis of the urea groups at pH 7 is not likely as the retention factor for naphthalene did not change significantly. To better illustrate the higher retention for the basic compounds, Fig. 7 shows the chromatograms before and after the passage of 21 000 column volumes of the buffered mobile phase, when the test was arbitrarily stopped.

By comparing the column aging study for the monofunctional C_{18} dimethylurea and those obtained on the polymeric C_{18} urea phase, under the same conditions and using the same silica support [20], it can be concluded that better stability was obtained from the monomeric C_{18} urea phase. In our early report on column stability of the polymeric C_{18} urea phase having similar surface coverage of 3.2 μmol m^{-2}, a considerable decrease in the *N*/*m* values and frontal tailing for all compounds were observed, as well as the higher

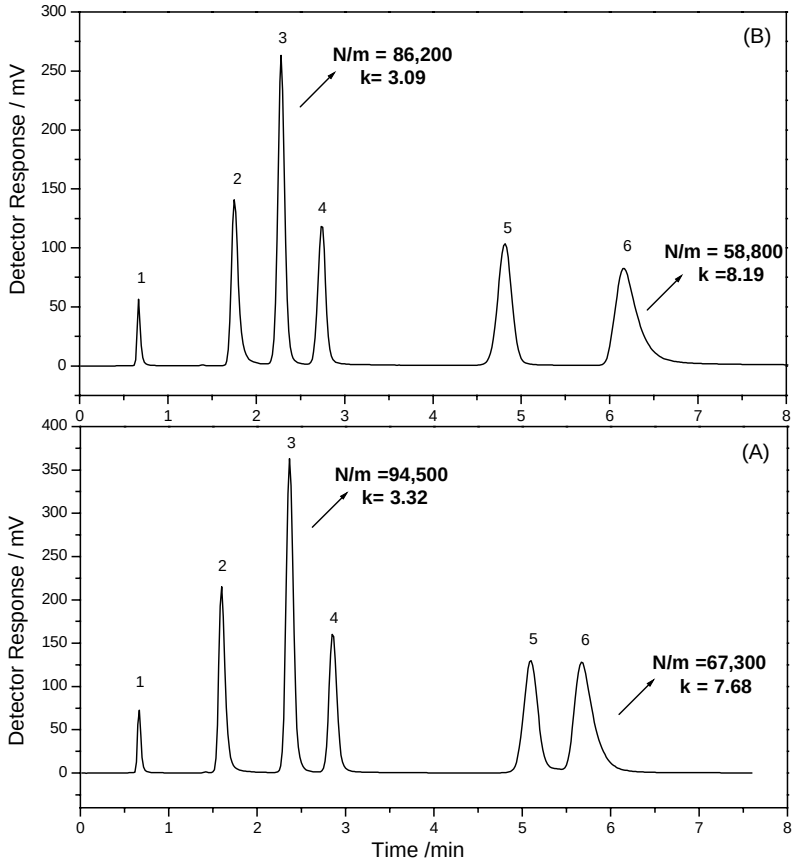

Fig. 7. Chromatograms obtained for the separation of uracil (1), propranolol (2), dipropyl phthalate (3), naphthalene (4), dibutyl phthalate (5) and amitriptyline (6) before (A) and after (B) the passage of 12 000 ml of buffered mobile phase at pH 7. Chromatographic conditions: 50 mm × 3.9 mm column; mobile phase: methanol–20 mmol l^{-1} KH_2PO_4/K_2HPO_4, pH 7.0 (65:35, v/v); flow rate: 0.8 ml min^{-1}; injection volume: 5 μl; temperature: 298 K; detection: UV at 254 nm.

retention for the bases after the passage of 16 000 column volumes (9500 ml) of the pH 7.0 buffered phosphate mobile phase, an effect attributed exclusively to the dissolution of the silica support [20].

It is a well known fact that the trifunctional alkoxysilane chemistry provides less reproducible surface coverage from batch to batch and, thus, monofunctional silanes are preferred [33]. Small amounts of water in the solvent or on the surface of the silica, which are difficult to control during the bonding procedure, promote the polymerization of the trialkoxysilane. However, extra silanols are turned after the hydrolysis of the trifunctional groups. Thus, even after endcapping, it is possible to have silanols which were not successfully blocked. It can be speculated that these extra silanols, which do not occur with modifications using monofunctional organosilanes, are responsible for the lower stability observed for the trifunctional C_{18} urea phase in comparison with the monofunctional phase reported here.

Knowing that stationary phases with embedded polar groups may have a higher water concentration near the underlying silica surface, due the hydrogen bonding ability of the polar group, it can be expected that the silica surface should be more exposed to the attack by the phosphate anions. As a consequence, such behavior can facilitate the hydrolysis of the siloxane bonds near the silica surface, increasing its solubility. For practical purposes, to improve the lifetime of the stationary phases containing embedded polar groups, the use of phosphate buffers in mobile phases should be avoided.

4. Conclusion

The ameliorating effects caused by the presence of the polar urea groups embedded in the C_{18} urea phase are clearly observed by the improved peak shapes obtained for the bases present in the Tanaka and Engelhardt test mixtures. The advantage of the monomeric C_{18} dimethylurea phase over the trifunctional analogous phase is supported by the improved performance on the aging test at pH 7.0 using a buffered phosphate mobile phase. The separation of some herbicides of two different classes and some of their metabolites, at neutral pH using isocratic elution, shows the potential application of this new kind of polar stationary phase in the determinations of these substances.

Acknowledgements

Financial support from FAPESP (Process 00/13698-6) is gratefully acknowledged. CRS also acknowledges fellowships from FAPESP. We especially thank FAEP-UNICAMP for a travel grant to present part of this work at HPLC 2003.

References

[1] M. Przybyciel, R.E. Majors, LC GC 20 (2002) 516.
[2] M. Przybyciel, R.E. Majors, LC GC 20 (2002) 584.
[3] R.E. Majors, LC GC 21 (2003) 240.
[4] A. Nomura, J. Yamada, K. Tsunoda, Anal. Sci. 3 (1987) 209.
[5] T.L. Ascah, B. Feibush, J. Chromatogr. 506 (1990) 357.
[6] B. Feibush, US Patent 5 137 627-A (1990).
[7] B. Buszewski, J. Schmid, K. Albert, E. Bayer, J. Chromatogr. 552 (1991) 415.
[8] J. Schmid, K. Albert, E. Bayer, J. Chromatogr. A 694 (1995) 333.
[9] B. Buszewski, M. Jaroniec, R.K. Gilpin, J. Chromatogr. A 668 (1994) 293.
[10] T.L. Ascah, K.M.L. Kallury, C.A. Szafranski, S.D. Corman, F. Lui, J. Liq. Chromatogr. Rel. Technol. 19 (1996) 3049.
[11] J.J. Kirkland, J.W. Henderson, J.D. Martosella, B.A. Bidlingmeyer, J. Vasta-Russell, J.B. Adams Jr., LC GC 17 (1999) 634.
[12] J.E. O'Gara, D.P. Walsh, C.H. Phoebe Jr., B.A. Alden, E.S.P. Bouvier, P.C. Iraneta, M. Capparella, T.H. Walter, LC GC 19 (2001) 632.
[13] J.E. O'Gara, B.A. Alden, T.H. Walter, J.S. Petersen, C.L. Niederländer, U.D. Neue, Anal. Chem. 67 (1995) 3809.
[14] U.D. Neue, C.L. Niederländer, J.S. Petersen, US Patent 5 374 755-A (1994).
[15] J.E. O'Gara, D.P. Walsh, B.A. Alden, P. Casellini, T.H. Walter, Anal. Chem. 71 (1999) 2992.
[16] U.D. Neue, Y.F. Cheng, B.A. Alden, P.C. Iraneta, C.H. Phoebe, K. van Tran, Chromatographia 54 (2001) 169.
[17] C.R. Silva, C. Airoldi, Brazilian Patent PI 9 903 110-8 (1999).
[18] C.R. Silva, I.C.S.F. Jardim, C. Airoldi, J. Chromatogr. A 913 (2001) 65.
[19] C.R. Silva, S. Bachmann, R.R. Schefer, K. Albert, I.C.S.F. Jardim, C. Airoldi, J. Chromatogr. A 948 (2002) 85.
[20] C.R. Silva, I.C.S.F. Jardim, C. Airoldi, J. Chromatogr. A 987 (2003) 139.
[21] C.R. Silva, I.C.S.F. Jardim, C. Airoldi, J. Chromatogr. A 987 (2003) 127.
[22] K. Kimata, K. Iwaguchi, S. Onishi, K. Jinno, R. Eksteen, K. Hosoya, M. Araki, N. Tanaka, J. Chromatogr. Sci. 27 (1989) 721.
[23] E. Cruz, M.R. Euerby, C.M. Johnson, C.A. Hackett, Chromatographia 44 (1997) 151.
[24] H. Engelhardt, M. Arangio, T. Lobert, LC GC 15 (1997) 856.
[25] H. Engelhardt, R. Grüner, M. Scherer, Chromatographia 5 (2001) S154.
[26] U.D. Neue, E. Serowik, P. Iraneta, B.A. Alden, T.H. Walter, J. Chromatogr. A 849 (1999) 87.
[27] U.D. Neue, K. van Tran, P.C. Iraneta, B.A. Alden, J. Sep. Sci. 26 (2003) 174.
[28] M.R. Euerby, P. Petersson, LC GC Eur. 13 (2000) 665.
[29] M.R. Euerby, P. Petersson, J. Chromatogr. A 994 (2003) 13.
[30] G. Sacchero, S. Apone, C. Sarzanini, E. Mentasti, J. Chromatogr. A 668 (1994) 365.
[31] J.J. Kirkland, J.W. Henderson, J.J. DeStefano, M.A. van Straten, H.A. Claessens, J. Chromatogr. A 762 (1997) 97.
[32] H.A. Claessens, M.A. van Straten, J.J. Kirkland, J. Chromatogr. A 797 (1998) 111.
[33] U.D. Neue, HPLC Columns, Theory, Technology and Practice, Wiley, New York, 1997.