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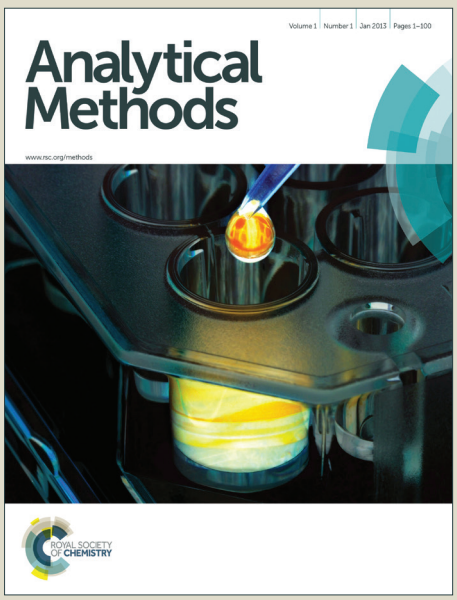

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A flow injection low-pressure chromatographic system exploiting fused-core columns

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Abstract

Chromatography with ultra-short monolithic columns, although attractive in view of its operation at low pressure without the need for expensive pumps, presents limited selectivity due to the low availability of stationary phases. In this work, fused-core columns are pioneered exploited in flow injection systems aiming at low-pressure chromatography. The proposed approach expanded selectivity of flow injection low-pressure chromatography by considering the range of different stationary phases available for fused-core columns. Separation of methyl, ethyl and propylparabens was selected as an application. A critical comparison of chromatographic efficiency of four columns (C18, RP-amide, F5 and Phenyl-hexyl) is presented. Acetonitrile/phosphoric acid pH 2.5 solution was selected as mobile phase, with specific ratios for each column. RP-amide provided best chromatographic efficiency, performing quantitative separation of the three analytes in 8.0 min, with resolutions > 1.72, peak symmetry < 1.66, LODs between 0.12 and 0.39 mg L^{-1}, linear response ranges up to 5.0 mg L^{-1} ($r > 0.996$) and coefficients of variation of peak heights < 3.5% (n=10). The procedure was applied to parabens determination in personal care products and the results agreed with the HPLC reference procedure at the 95% confidence level.

Keywords: flow analysis, fused-core columns, low-pressure chromatography, parabens

1. Introduction

Monolithic stationary phases were introduced on liquid chromatography as an alternative to particle-packed columns in highly efficient separations. Due to their high porosity, they can be operated at relatively high flow-rates with low backpressure.[1] A logical evolution was therefore to implement monolithic columns to sequential injection analysis (SIA) to perform chromatographic separations, increasing the potential of flow analysis to multicomponent determination.[2] This combination was originally named sequential injection chromatography (SIC).

Different monolithic stationary phases (silica, RP-C8, RP-C18, CN, NH_2 and HILIC) are currently available. However, the availability of only silica and RP-C18 phases for the pioneering works restricted the SIC applications and introduction of fused-core particle columns overcame this drawback.[3] These columns are composed by a fused-silica particle (*e.g.* 1.7 μm diameter) covered by a shell (*e.g.* 0.5-μm thickness), which acts as stationary phase. This physical constitution improves chromatographic performance by operation at the backpressure of 3-μm particle columns, but achieving the performance of the sub-2-μm particle ones.[4] Because the analytes cannot penetrate the solid inner core, the fused-core particle provides shorter diffusion path compared with traditional silica particles, thus lessening the resistance to mass transfer[5] and reducing the axial dispersion, especially at high flow rates. Moreover, compared to porous particles, these columns present narrower particle size distribution and higher packing density, promoting a lower eddy diffusion,[6] thus resulting in low peak broadening and high number of theoretical plates. Fused-core columns were pioneered used in a SIC system for separation of four estrogens

with similar structure, taken ethylparaben as internal standard. The chromatographic performances of fused-core and monolithic columns, both with C18 stationary phases, were evaluated highlighting the benefits of the former on SIC system.[3] Further work pointed out the superior performance of pentafluorophenylpropyl fused-silica columns in comparison to the monolithic ones to the separation of eight sulfonamides.[7]

Although SIC performs chromatographic separations at much lower backpressures than conventional liquid chromatography, it still needs a special propulsion unit (syringe pump) to assure the flow rate of the mobile phase, reaching pressures of up to 600 psi. Peristaltic pumps normally used in flow analysis do not allow the achievement of such high pressures. An alternative to reduce backpressure is the use of short columns (*e.g.* 0.5 cm length). Coupling of short monolithic columns to FIA manifolds was named as flow injection chromatography (FIC), which was successfully applied to the separation of theobromine, theophylline and caffeine in coffee brewed samples.[8] The flow system was composed by a peristaltic pump, an injection valve and a 0.5-cm long monolithic column, usually used as the guard column in chromatography. Resolution > 1.83 was achieved for all analytes in 6.5 min. A FIC system was also employed for aspartame, saccharin, metylparaben, ethylparaben, propylparaben, butylparaben, propylgallate and butylhydroxyanysole determinations in food and cosmetic samples.[9] Gradient elution was required for complete separation of the analytes with a 0.5-cm long C18 monolithic column. A similar strategy was further used for parabens determination in cosmetics with spectrophotometric[10] or chemiluminometric[11] detection. The main disadvantage of FIC is the limited column size, which may hinder the

separation efficiency due to the low number of theoretical plates. Because monolithic phases were the only available option for operation at low pressures, the selectivity was restricted as well.

The goal of this work was to evaluate the chromatographic performance of short-fused core columns with different stationary phases in a flow system with a peristaltic pump as propulsion unit. Separation of parabens was taken as a model, with their determinations in different personal care products.

2. Experimental

2.1. Apparatus

The flow injection low-pressure chromatographic system comprised a peristaltic pump (Ismatec IPC, Switzerland; model CP 78017-10) with Tygon® pumping tubes; three-way solenoid valves (NResearch, USA); Teflon™ confluence and flow lines of 0.25 mm i.d PEEK tubes. The system was controlled by a microcomputer through a parallel interface connected to a current drive based on an ULN2803 integrated circuit, as previously described[12]. The control software was developed in Visual Basic 6.0 (Microsoft, Redmond, USA). The detection unit was a USB2000 fiber-optic CCD UV–Vis spectrophotometer (Ocean Optics, Dunedin, FL, USA), a DH-2000 deuterium UV light source (Ocean Optics), SMA ended optical fibers with a 600-μm core diameter (CeramOptec®, East Longmeadow, MA, USA) and Z-shaped flow-cell with a 20-mm optical path and 9 μL inner volume (FIAlab Instruments®, Bellevue, WA, USA). Data acquisition was performed by the OOIBase32 software provided by the spectrophotometer manufacturer.

The reversed-phase fused-core columns (C18, F5, RP-Amide and Phenyl-hexyl; 5 mm x 4.6 mm, particle size 2.7 μm) were purchased from Supelco, USA.

2.2. Reagents and solutions

Methyl (MP), ethyl (EP) and propyl (PP) parabens were purchased from Sigma Aldrich. Stock 1.000 g L^{-1} solutions were prepared in methanol and stored at 5 ºC. Working solutions within 1.00 and 5.00 mg L^{-1} were daily prepared by dilutions on the mobile phase. Different ratios of acetonitrile and phosphoric acid solution (pH 2.5) were used as mobile phases. Personal care products were purchased from a local market.

2.3. Procedure

The low-pressure chromatographic flow manifold was designed with two convergent flow lines (Figure 1), with both solutions flowing at 0.6 mL min^{-1}. The mobile phase was continuously pumped through valve V_2 and sample solutions were inserted into the system by simultaneously switching V_1 and V_2. The sample volume (10 μL) was defined by the flow-rate and the valve switching time. Detection was carried out at 255 nm (maximum absorption for all parabens). Chromatograms were evaluated by using the graphical OriginLab® software and peak heights were used as the measurement basis, as in previous works with SIC.[2,13] Retention time, resolution, peak symmetry, number of theoretical plates and height equivalent to a theoretical plate were calculated from experimental data based on FDA recommendations.[14]

The reference procedure for accuracy assessment relied on reversed-phase HPLC.[15] The parabens extractions from the samples were performed by the procedures previously described for wet wipes[16] or gel and cream samples.[17]

3. Results and discussion

The requirements for low-pressure chromatography (*i.e.* a highly efficient separation with low backpressure) are fulfilled by fused-core columns, which also show a higher diversity of stationary phases. During the experiments, backpressure did not exceed 80 psi and suitable resolution was achieved even with short 5-mm columns. In addition, the propulsion unit needs to allow a reproducible flow rate to achieve reliable results, including reproducible peak heights and retention times. Flow-rate stability was evaluated during all experiments and no significant variation was observed, demonstrating the robustness of the peristaltic pumping. Another concern on this kind of system was the resistance of the pumping tubes to organic solvents that could harm its performance. The same tubes were employed during all experiments and no damage was observed by using acetonitrile.

3.1. Chromatographic characteristics

Separation of parabens was exploited to evaluate the chromatographic performance achieved with different fused-core columns. Figure 2 shows the chromatograms obtained in the optimal compositions of the mobile phases, whereas Table 1 shows the corresponding chromatographic parameters. For F5, phenyl-hexyl and C18 columns, acceptable resolution (R_s > 1.5) was not achieved, even for mobile phases with high water content. Under these

conditions, high retention times were observed due to the increased polarity of mobile phase; moreover, broader peaks caused by higher analyte dispersion inside the column were observed.

The phenyl-hexyl phase shows π-π interactions with the analyte through its aromatic ring and delocalized electrons (Figure 3). The aromatic ring acts as donator of π electrons thus as a Lewis base that strongly interacts with π receptors. Consequently, good selectivity for aromatic molecules with electronegative groups, such as the parabens, is attained. Despite these favourable properties, exploitation of this phase leads to an acceptable resolution only at high retention times and with higher peak broadening, which hinders sensitivity and sample throughput. The F5 column is composed by electron-deficient phenyl rings due to the fluorine substituents (Figure 3), forming a less apolar reversed phase. Beyond π-π interactions, it retains analytes by polar and nonpolar interactions. Parabens presents high interaction with this phase, but the same drawbacks mentioned for the phenyl-hexyl columns were observed.

RP-amide, whose structure is similar to the C18 phase except for the amide linked to the silanyl group (Figure 3) is an alternative for reversed phase separations of polar compounds. Thus, this phase showed the best chromatographic performance for parabens separation. Satisfactory resolutions (Rs > 1.5) were achieved for MP and EP that present similar molecular structures, with lower retention times and less broadened peaks. Peak symmetries were in the 1.04 - 1.82 range and the higher values are due to the relatively high sample volume (10 µL) for the short column length. Another noteworthy behaviour observed in the separations with RP-amide was the high

dependence on the mobile phase composition. A 5% alteration in the ratio of the organic component of the mobile phase halved the retention time and the peak width of the more retained analyte (PP), without losses in resolution of the other peaks (MP and EP). This aspect is useful in the separation of polar compounds with similar properties and molecular structures, as slight changes on mobile phase composition result in significant alterations in the chromatographic performance. On the other hand, robustness of the procedure may become critical.

3.2. Optimization

The RP-amide column was used to evaluate other system parameters that might affect the chromatographic performance. Variation of mobile phase flow-rate demonstrated higher retention times and more broadened peaks for lower flow rates because of the higher sample dispersion. Peak resolution was not critically affected at the maximum evaluated flow-rate (0.6 mL min^{-1}), because it is still below the optimum flow-rate for fused-core columns according to van Deemter equation.[18] Higher flow rates were not evaluated due to the limitation of the peristaltic pumping and the backpressure promoted by the chromatographic column. In fact, 80 psi was necessary to maintain the flow rate at 0.6 mL min^{-1}.

Injection of higher sample volumes is an alternative to increase sensitivity in chromatography, but losses in peak resolution should be critically evaluated. These effects can be observed in Figure 4, as well as that even the highest evaluated sample volume did not hinder resolution of the two critical analytes (MP and EP). As sensitivity was not critical because of the relatively high

parabens concentrations in personal care products, the sample volume was selected as 10 μL.

3.3. Analytical features and application

The low-pressure chromatographic procedure presented a linear response within 1.0 and 5.0 mg L^{-1} for MP (A = 0.0479±0.0010 C + 0.008±0.002), EP (A = 0.0287±0.0009 C + 0.017±0.002) and PP (A = 0.0153±0.0015 C + 0.008±0.005) with linear correlation coefficients higher than 0.996. Limits of detection were estimated at 0.12, 0.21 and 0.39 mg L^{-1} for MP, EP and PP, respectively. Coefficients of variation for peak heights and retention times were lower than 3.5 and 4.1%, respectively, emphasising the repeatability of the separation process and the stability of mobile phase flow rate. Compared to previously described procedures for determination of parabens with chromatographic separation [10,11,19-22] (Table 2), the proposed system presents some advantages as the lowest consumption of organic solvent, shorter analysis time compared to HPLC[20,22] and electrophoresis[19] and lower coefficients of variation than those attained in FIC procedures.[10,11] The main advantage in comparison to FIC systems using ultra-short columns is the use of isocratic elution, thus simplifying the manifold architecture.

Recoveries from 93% to 102% were estimated for parabens spiked to different samples of personal care products, thus demonstrating the absence of matrix effects. Samples were analysed by the proposed and a reference HPLC procedure[15] for accuracy assessment (Table 3). Spikes of ethyl and propylparabens were also exploited to demonstrate the separation capability.

Variances were not significantly different and all results agreed at the 95% confidence level.

4. Conclusions

Exploitation of ultra-short fused-core columns for low-pressure chromatographic separations in FIA was pioneering proposed and the developed analytical procedure was successfully applied to parabens determination in personal care products. The evaluation of different columns highlighted the importance of a suitable stationary phase to achieve efficient separations in FIC, as the chromatographic efficiency is limited by the short column length. The critical dependence of the mobile phase composition was demonstrated as well. Use of fused-core columns enhanced the potentialities of the system by increasing selectivity, besides presenting the potential to perform in-line sample treatment and derivatization steps by resorting from the multicommutation approach.

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Table 1. Chromatographic parameters estimated for the different fused core particle columns.

Stationary phase	Parameter	MP	EP	PP	MP	EP	PP
		Water (pH 2.5)/Acetonitrile (85:15)			Water (pH 2.5)/Acetonitrile (80:20)		
Phenyl-Hexyl	RT (min)	2.13	4.58	12.2	1.42	2.57	5.70
	PW (min)	0.78	1.25	3.27	0.47	0.63	1.35
	PS	1.48	1.28	1.10	2.05	1.56	1.27
	NTP	119	214	222	140	260	281
	PR	1.42[a]	1.98[b]	—	1.22[a]	1.85[b]	—
	HETP (µm)	4.22	2.34	2.26	3.56	1.92	1.78
		Water (pH 2.5)/Acetonitrile (85:15)			Water (pH 2.5)/Acetonitrile (80:20)		
C18	RT (min)	1.58	3.78	11.6	1.18	2.20	5.47
	PW (min)	0.92	1.41	3.47	0.47	0.59	0.91
	PS	1.36	1.28	1.01	2.22	1.59	1.34
	NTP	47.6	116	179	103	222	574
	PR	1.12[a]	1.89[b]	—	1.14[a]	2.57[b]	—
	HETP (µm)	105	43.1	27.9	48.5	22.5	8.71
		Water (pH 2.5)/Acetonitrile (85:15)			Water (pH 2.5)/Acetonitrile (80:20)		
F5	RT (min)	2.98	5.93	13.7	1.88	3.15	6.12
	PW (min)	1.28	1.89	3.92	0.75	0.96	1.72
	PS	1.20	1.18	1.16	1.51	1.33	1.28
	NTP	87.2	158	197	102	173	202
	PR	1.10[a]	1.59[b]	—	0.88[a]	1.31[b]	—
	HETP (µm)	5.73	3.16	2.54	4.91	2.89	2.47
		Water (pH 2.5)/Acetonitrile (75:25)			Water (pH 2.5)/Acetonitrile (80:20)		
RP-amide	RT (min)	1.68	3.17	7.18	2.23	4.87	12.7
	PW (min)	0.42	0.60	1.30	0.55	0.93	2.47
	PS	1.50	1.66	1.28	1.82	1.23	1.04
	NTP	261	446	489	264	435	426
	PR	1.72[a]	2.49[b]	—	2.09[a]	2.73[b]	—
	HETP (µm)	1.91	1.12	1.02	1.90	1.15	1.17

RT: retention time; PW: peak width; PS: peak symmetry; NTP: number of theoretical plates; PR: peak resolution; HETP: height equivalent to a theoretical plate; [a] MP/EP; [b] EP/PP

Table 2. Analytical figures of merit for some procedures for parabens determination by different separation techniques.

Procedure	Stationary phase	Organic solvent consumption (mL)	Analysis time (min)	CV (%)	Reference
FIC	Monolithic C18 (5 x 4.6 mm)	1.9	3.3	0.65 – 1.80	[10]
FIC	Monolithic C18 (5 x 4.6 mm)	1.6	2.8	3.5 – 6.2	[11]
Capillary electrophoresis	Fused silica capillary	—	16.0	0.86 – 2.48	[19]
HPLC	C18 (125 x 4 mm i.d., 5 µm)	21	40.0	1.53 – 3.23	[20]
HPLC	C8 (150 x 4.6 mm i.d., 5 µm)	6.0	10.0	2.0 – 3.1	[22]
FIC	Fused-core RP-amide (5 x 4.6 mm, 2.7 µm)	1.4	8.0	2.9 – 3.5	This work

Table 3. Mean values (% w/w) and standard deviations for determination of parabens in personal care products samples (n=3) by the proposed procedure and HPLC.

Sample	Proposed procedure			Reference procedure[15]		
	MP	**EP**	**PP**	**MP**	**EP**	**PP**
Intimate lubricant 1	0.34 ± 0.01	0.41 ± 0.03*	0.39 ± 0.02*	0.32 ± 0.01	0.40 ± 0.02*	0.40 ± 0.01*
Intimate lubricant 2	0.32 ± 0.01	0.41 ± 0.03*	0.40 ± 0.01*	0.30 ± 0.01	0.40 ± 0.01*	0.39 ± 0.02*
Intimate lubricant 3	0.43 ± 0.02	0.40 ± 0.01*	0.39 ± 0.03*	0.44 ± 0.01	0.41 ± 0.01*	0.40 ± 0.02*
Insect repellent	0.45 ± 0.02	—	—	0.44 ± 0.01	—	—
Ointment	0.52 ± 0.02	0.38 ± 0.02*	0.37 ± 0.01*	0.51 ±0.01	0.39 ± 0.01*	0.38 ± 0.01*
Wet wipe 1	0.70 ± 0.04	—	—	0.70 ± 0.01	—	—
Wet wipe 2	0.45 ± 0.02	—	—	0.44 ± 0.01	—	—
Wet wipe 3	0.58 ± 0.01	—	—	0.57 ± 0.01	—	—
Wet wipe 4	0.39 ± 0.02*	0.40 ± 0.01*	0.39 ± 0.02*	0.40 ± 0.01*	0.41 ± 0.02*	0.40 ± 0.02*

*Samples spiked with 0.40 % w/w of each paraben

FIGURES CAPTIONS

Figure 1. Flow diagram of the system for parabens determination. V_1 and V_2: three-way solenoid valves; CC: fused-core chromatographic column; D: detection unit; S: sample; MP: mobile phase; X: confluence; W: waste vessel. Dashed and continuous lines in the valves indicate the flow pathways when the valves are switched on and off, respectively.

Figure 2. Chromatographic separation of methyl (MP), ethyl (EP) and propyl (PP) parabens with different stationary phases and ratios of mobile phase. (1) C18 column, (a) Water (pH 2.5)/Acetonitrile (80:20), (b) Water (pH 2.5)/Acetonitrile (85:15); (2) Phenyl-hexyl column, (a) Water (pH 2.5)/Acetonitrile (80:20), (b) Water (pH 2.5)/Acetonitrile (85:15); (3) F5 column, (a) Water (pH 2.5)/Acetonitrile (80:20), (b) Water (pH 2.5)/Acetonitrile (85:15) and (4) RP-amide column, (a) Water (pH 2.5)/Acetonitrile (75:25), (b) Water (pH 2.5)/Acetonitrile (80:20).

Figure 3. Chemical structures of the stationary phases of the evaluated fused-core columns.

Figure 4. Influence of sample volume in the chromatographic separation of parabens (5.0 mg L^{-1}).

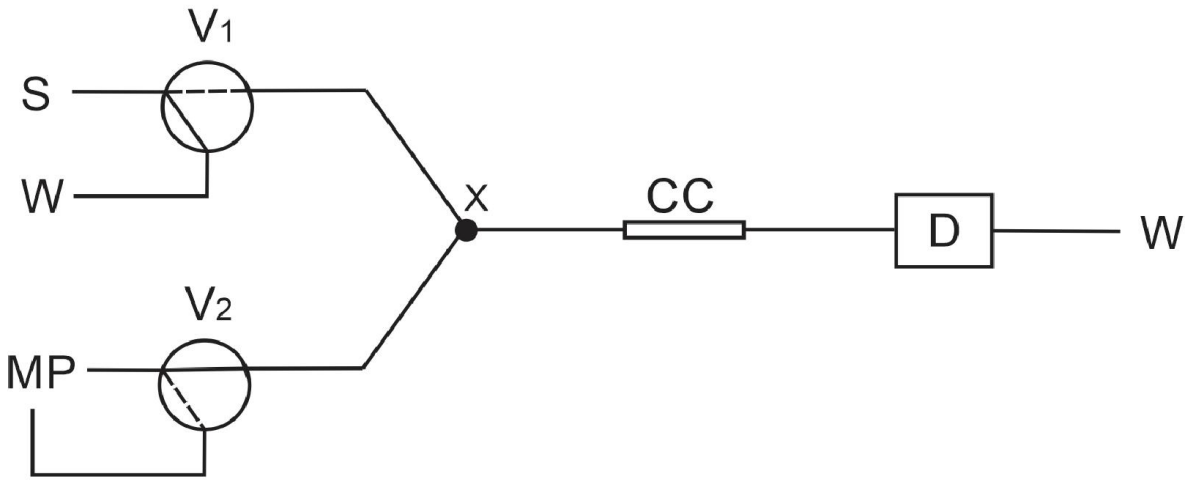


Figure 1

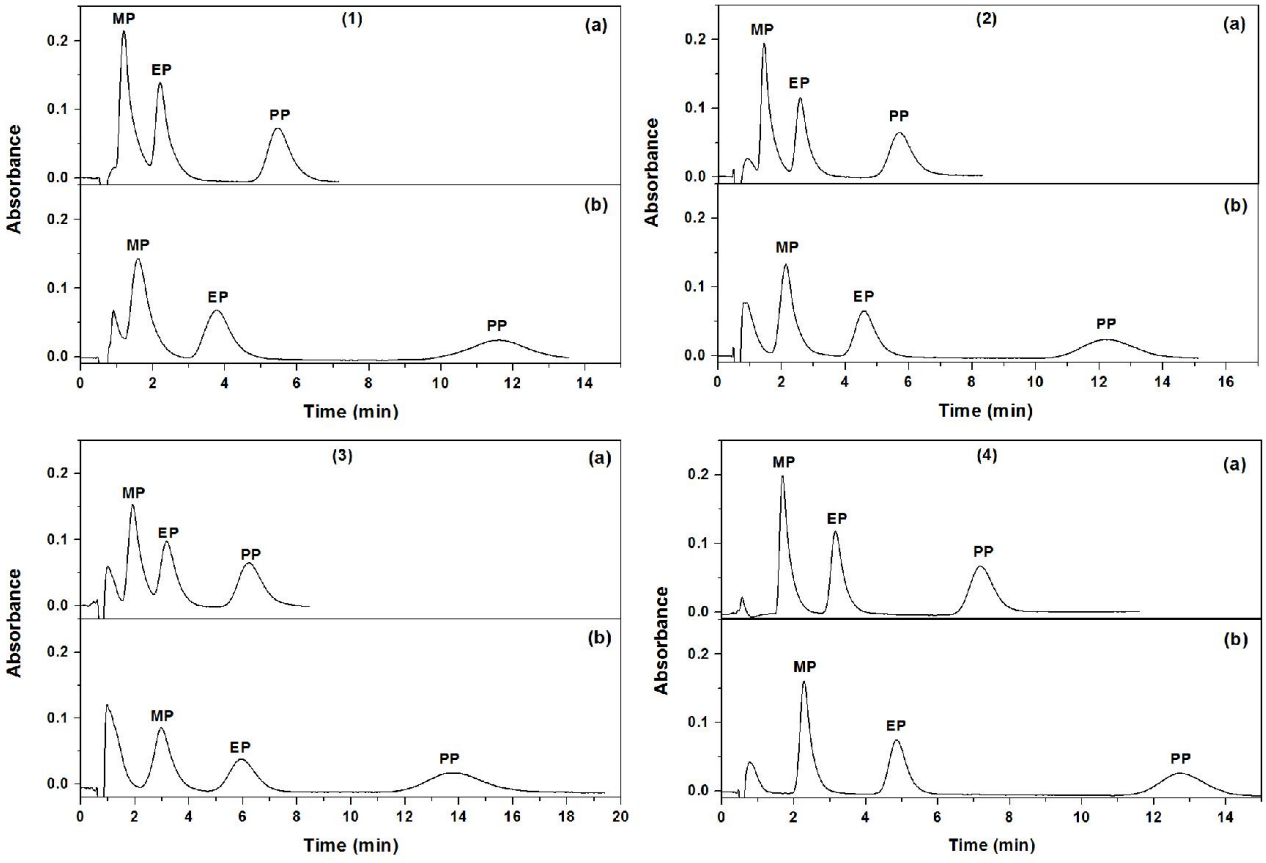
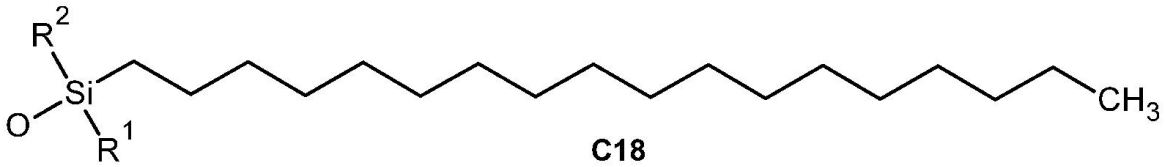
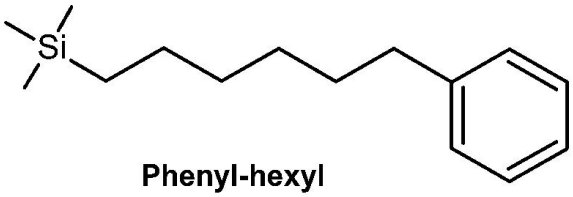


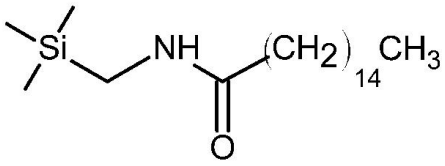
Figure 2



C18



Phenyl-hexyl



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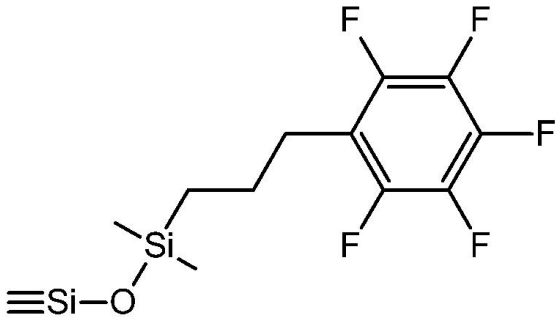

F5

Figure 3

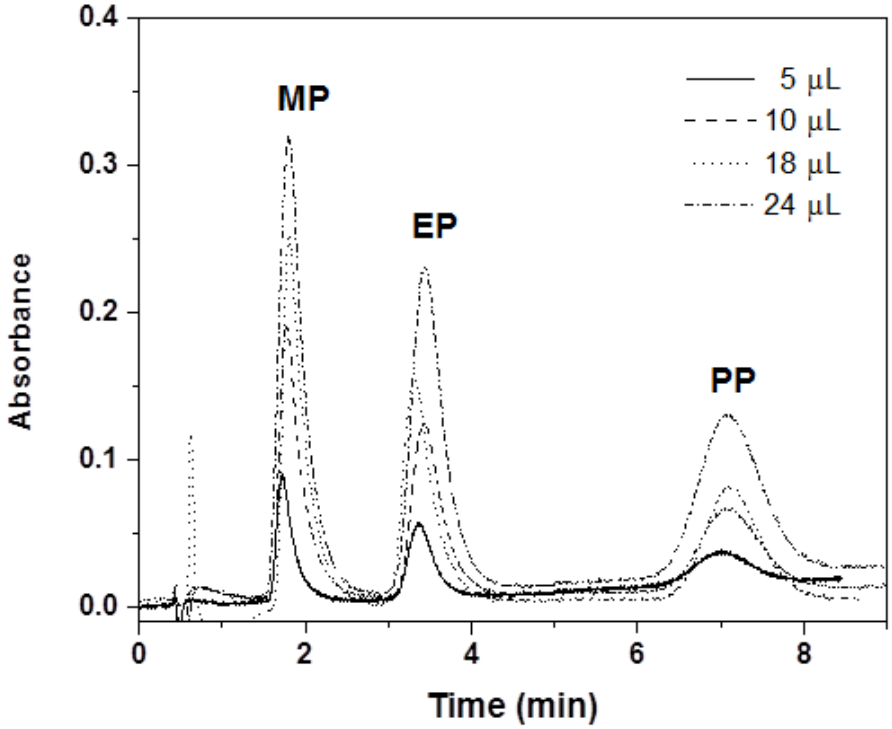

Figure 4