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Comparison of the performance of conventional microparticulates and monolithic reversed-phase columns for liquid chromatography separation of eleven pollutant phenols

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Abstract

The performance of isocratic separations of 11 pollutant phenols (PP) using monolithic (Chromolith RP-18e) and conventional reversed-phase 5 μm (Luna and Purospher C_{18}) and 4 μm (Synergi C_{12}) particulate size columns, selected from high purity silica materials, has been compared. The separations have been optimized based on a previously optimized separation in which a reversed-phase C_{18} Luna column and acetonitrile as organic modifier were used, allowing the separation of all phenols tested in 23 min. The optimization process was carried out for each column by studying the effect of the mobile phase (acetonitrile as organic modifier, pH, flow-rate) on phenols separation. Under the optimized separation conditions, all phenols were separated in less than 23 min for all columns tested. Asymmetry factors were further evaluated and used to estimate column efficiency using the Dorsey–Foley equation. The efficiency and asymmetry factors were lower for Chromolith than for Purospher and Luna columns respectively. The Chromolith column was finally selected, due to its lower flow resistance, analysis time and good efficiency and asymmetry factors. The PPs separation was achieved in 3 min. The asymmetry factors were in the range 0.9–1.5 using 50 mM acetate buffer (pH = 5.25)–ACN (64:36, v/v) as mobile phase, $T = 45\,^{\circ}C$ and 4.0 ml min^{-1} flow-rate.

Keywords: Reversed-phase columns; Monolithic column; Phenols

1. Introduction

Pollutant phenols (PPs) are important contaminants. They are well known for their toxicity and persistence in the environment. PPs are obtained as degradation products of humic substances, lignins and tannins or other pollutants, such as pesticides and herbicides, generated in different types of industries and used as preservatives for wood, textiles and leather [1–3]. Owing to their toxicity, both the United States Environmental Protection Agency and the European Union have included some phenols in their lists of priority pollutants. The structures and other characteristics of the phenols herein studied are summarized in Table 1.

Many high performance liquid chromatographic (HPLC) procedures for the analysis of mixtures of phenols with isocratic and gradient elution, using electrochemical or UV-diode array detection (DAD), have been reported [4–10]. In the last few years, many companies have been trying to increase the number of samples analyzed, whilst simultaneously being required to reduce the analysis time. This request has stressed the evolution of the columns from 10 μm particles introduced in the 1970s to the 2–5 μm packings used in most modern HPLC columns, to improve their efficiency. Column efficiency improves when the particulate size is reduced since the mass transfer process is directly proportional to the square of the particulate diameter. More-

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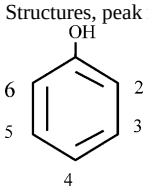
Table 1
Structures, peak number, dissociation constants (pK_a) and UV maxima absorbance wavelength for priority pollutant phenols



PPs	Peak number	λ (nm)	pK_a	C_2	C_3	C_4	C_5	C_6
Phenol (P)	1	195	9.99					
4-Nitrophenol (4NP)	2	360	7.23			NO_2		
2,4-Dinitrophenol (24DNP)	3	360	3.94	NO_2		NO_2		
2-Chlorophenol (2CP)	4	195	8.48	Cl				
2-Nitrophenol (2NP)	5	210	7.08	NO_2				
2,4-Dimethylphenol (24DMP)	6	195	10.58	CH_3		CH_3		
4-Chloro-3-methylphenol (4C3MP)	7	195	9.55		CH_3	Cl		
2,4-Dichlorophenol (24DCP)	8	200	7.85	Cl		Cl		
2-Methyl-4,6-dinitrophenol (46DNOC)	9	375	4.31	CH_3		NO_2		NO_2
2,4,6-Trichlorophenol (246TCP)	10	200	6.00	Cl		Cl		Cl
Pentachlorophenol (PCP)	11	220	5.25	Cl	Cl	Cl	Cl	Cl

over, the eddy diffusion term increases with increasing particulate diameter. However, when particulate size is reduced, the pressure increases noticeably in the instrumentation and, as a consequence, the life of pumps, seals and columns are also reduced. Due to the pressure, these columns can only be supplied in short format (typically 2–5 cm in length and 2–4.6 mm internal diameter) and poor separation can be obtained [11].

The need for fast, high-resolution separations has made the columns to evolve from a bed packed with porous particles to a straight rod of highly porous silica with a bimodal pore structure (monolithic columns). These columns possess a unique combination of very large internal surface area, over which chemical adsorption can take place due to mesopores (13 nm), together with significantly higher total porosity (2 μm macropores) to transport mobile phase and analytes, reducing the diffusion path and provides high permeability (and thus low pressure). This behavior allows to use monolithic columns at flow-rates close to 9 ml min^{-1} without problems and enables faster separations than with a standard column [12,13]. In addition, efficiency for monolithic columns does not decrease significantly when the flow-rate is increased due to their flow-trough pores, thus the diffusion path is reduced, resulting in a reduction in mass transfer effects. However, for traditional particulate columns, using high flow-rates, the efficiency decreases [14,15].

In the present study, the performance of the optimized isocratic separations of 11 pollutant phenols using monolithic (Chromolith RP-18e) and conventional reversed-phase 5 μm (Luna and Purospher C_{18}) and 4 μm (Synergi C_{12}) particulate size columns, have been compared. In the optimization process, the effect of mobile phase (acetonitrile as organic modifier, pH, flow-rate) on phenols separation were studied. Asymmetry factors were assessed and further used to estimate column efficiency using the Dorsey–Foley equation [16].

2. Experimental

2.1. Chemicals

Phenol (P), 4-nitrophenol (4NP), 2,4-dinitrophenol (24DNP), 2-chlorophenol (2CP), 2-nitrophenol (2NP), 2,4-dimethylphenol (24DMP), 2-methyl-4,6-dinitrophenol (46DNOC), 4-chloro-3-methylphenol (4C3MP), 2,4-dichlorophenol (24DCP), 2,4,6-trichlorophenol (246TCP) and pentachlorophenol (PCP) were obtained from Aldrich Chemie (Beerse, Belgium). A stock solution of these analytes (1000 μg ml^{-1}) was prepared in methanol. A single or a mixture of the phenolic compounds was prepared daily by diluting the stock solution with methanol (MeOH) and used for different studies.

HPLC-grade methanol (MeOH), acetonitrile (ACN) (Scharlau, Barcelona, Spain) and Milli-Q (Millipore, Molsheim, France) water were used. Millipore 0.45 μm nylon filters (Bedford, MA, USA). Acetic acid, sodium acetate, sodium dihydrogen phosphate and other reagents were of the maximum purity available and obtained form (Merck, Darmstadt, Germany).

2.2. Apparatus

The chromatographic system consisted of the following components all of them from (Jasco Analítica, Madrid, Spain): a 3-line degasser DG-980-50, a ternary gradient unit LG-980-02S, an HPLC pump PU-980 and a multiwavelength (190–650 nm) diode array detector MD-910. A 6-port Rheodyne valve with a 20 μL sample loop injector (Cotati, CA, USA), and a Jones-Chromatography block heated series 7971 for thermostating columns in the range 30–70 °C (Seagate Technology, Scotts Valley, CA, USA) were used. The following reversed-phase columns were used: Luna ODS (250 mm × 4.6 mm i.d., 5 μm) column from

Phenomenex (Torrance, CA, USA), Synergi RP-MAX C_{12} (250 mm × 4.6 mm i.d., 4 μm), Purospher START RP-18 (250 mm × 4.6 mm i.d., 5 μm) column and Chromolith RP-18e (100 mm × 4.6 mm i.d.) column from (Merck, Darmstadt, Germany).

2.3. Mobile phase and chromatographic analysis

Isocratic binary mobile phases were prepared by mixing 50 mM phosphate buffer (pH 3.0) with ACN. Binary mobile phases consisted of 50 mM phosphate buffer (pH 5.0) and ACN (34–55%). All solvents and mobile phases were firstly filtered under vacuum through 0.45 μm nylon filters and degassed using a vacuum degasser.

Once the column had been conditioned with the mobile phase, chromatograms were obtained at the programmed temperature (45–50 °C). The injection volume used for the phenols analysis was 20 μl. For optimization purposes based on the use of different mobile phases, a methanolic solution containing an appropriate mixture of phenols (10 μg ml^{-1}) was injected. The flow-rates were in the range 0.5–1.5 ml min^{-1} for microparticulate columns and 1–4 ml min^{-1} for the monolithic one and UV absorbance-DAD detection in the range 190–360 nm was also used. Peaks identification and peak purity were performed by comparing the retention time and UV spectra of the chromatographic peaks with those of reference compounds previously registered. Phenols analysis was carried out at different wavelength values shown in Table 1.

3. Results and discussion

In a previous work, a systematic optimization of the HPLC separation of a mixture containing eleven phenols using an Hypersil ODS (250 mm × 4.6 mm i.d., 5 μm) column and UV detection was described [17]. Isocratic mobile phases ranged from binary to quaternary consisted of a 50 mM phosphate buffer (pH 3.0) and organic modifiers such as MeOH, ACN and THF were studied. After selecting ACN as organic modifier, the effect of pH (range 3.0–5.6) and temperature (30–55 °C) on phenols separation were studied. A strong decrease in the retention factor for 24DNP, 46DNOC, PCP, and a slight one for 246TCP as pH increase in the range studied were observed (see pK_a values in Table 1). In this way, all phenols were separated for pH 5.0 and 5.6. However, a mobile phase 50 mM acetate buffer (pH 5.0)–acetonitrile (60:40, v/v) at 50 °C was selected which allowed the separation of all phenols in 22 min. To improve this separation, especially for a broad peak observed for PCP, several columns were tested and the optimum separation was achieved using a Luna ODS (250 mm × 4.6 mm i.d., 5 μm) column (50 °C), obtaining a separation of all phenols in 23 min with better performance [17].

The long analysis time found was mainly due to the strong retention of PCP and 246TCP. To improve the performance of the above separation, this initial mobile phase (IMP) was selected as a starting point. For this purpose, several columns at 50 °C were also selected in the optimization process by studying the effect of the mobile phase (acetonitrile as organic modifier, pH, flow-rate) on phenols separation.

Asymmetry factors (A_s) and column efficiency were assessed using the Dorsey–Foley equation, $N_{df} = 41.7 [t_r/w_{0.1}]^2/[A_s + 1.25]$, where N_{df} is the Dorsey–Foley efficiency, in terms of A_s, the asymmetry factor (calculated at 10% of the peak height); t_r, the retention time for a given compound and $w_{0.1,}$ the width peak at 10% of the peak height [16]. It has been shown that is a reasonable way to estimate the true efficiency for asymmetric peaks [18]. Berthod [19] have also used the Dorsey–Foley equation to calculate N_{df} to give meaningful Van Deemter plots. In addition, plate height (H_{df}) values were also calculated from Dorsey–Foley efficiency, N_{df}.

The retention factors, k, $A_{s,}$ N_{df} and H_{df} values found for the Luna column are in Table 2.

Table 2
Performance obtained for phenols separation using several HPLC microparticulate and monolithic columns

PPs	Luna				Synergi				Purospher				Chromolith			
	k	A_s	N_{df}	H_{df}[a]	k	A_s	N_{df}	H_{df}[a]	k	A_s	N_{df}	H_{df}[a]	k	A_s	N_{df}	H_{df}[a]
P	1.62	0.97	13337	0.002	2.56	2.16	12737	0.002	2.42	1.22	25021	0.001	0.96	1.12	3790	0.003
4NP	1.85	0.98	12105	0.002	2.96	2.96	5195	0.005	2.68	1.21	23652	0.001	1.20	0.83	5751	0.002
24DNP	0.40	0.51	9519	0.003	1.06	2.65	6515	0.004	1.16	1.43	14494	0.002	0.40	0.93	3115	0.003
2CP	2.93	1.03	9603	0.003	4.28	2.07	12173	0.002	3.66	1.06	27070	0.001	1.76	1.17	4621	0.002
2NP	3.76	1.00	9959	0.002	5.31	1.88	13821	0.002	4.73	1.09	22846	0.001	2.24	1.21	6027	0.002
24DMP	4.46	1.03	9165	0.003	6.16	1.98	13319	0.002	4.99	0.94	28561	0.001	2.72	1.25	7741	0.001
4C3MP	5.05	1.15	9775	0.003	6.99	1.85	10702	0.002	5.47	0.97	27791	0.001	3.32	1.27	4940	0.002
24DCP	6.25	1.14	9573	0.003	8.49	2.28	8298	0.003	6.52	1.14	22908	0.001	4.20	1.29	5892	0.002
46DNOC	0.57	1.03	11844	0.002	1.50	2.57	9909	0.002	1.56	1.44	19224	0.001	0.60	1.31	3293	0.002
246TCP	10.19	1.20	7448	0.003	13.45	3.60	4596	0.005	10.45	1.59	17584	0.001	7.41	1.17	6488	0.001
PCP	5.56	2.04	2517	0.010	9.86	2.86	749	0.033	7.18	2.98	5622	0.004	5.65	1.41	5096	0.002

Conditions: Luna (250 mm × 4.6 mm i.d., 5 μm) column (column temperature, 50 °C; injection volume, 20 μl; concentration level of each phenol, 10 μg ml^{-1}; mobile phase, 50 mM phosphate buffer (pH 5.0)–ACN (60:40, v/v); and flow-rate, 1 ml min^{-1}); Synergi column (conditions as in Fig. 1); Purospher column (conditions as in Fig. 2); Chromolith column (conditions as in Fig. 3).

[a] H_{df} is in cm.

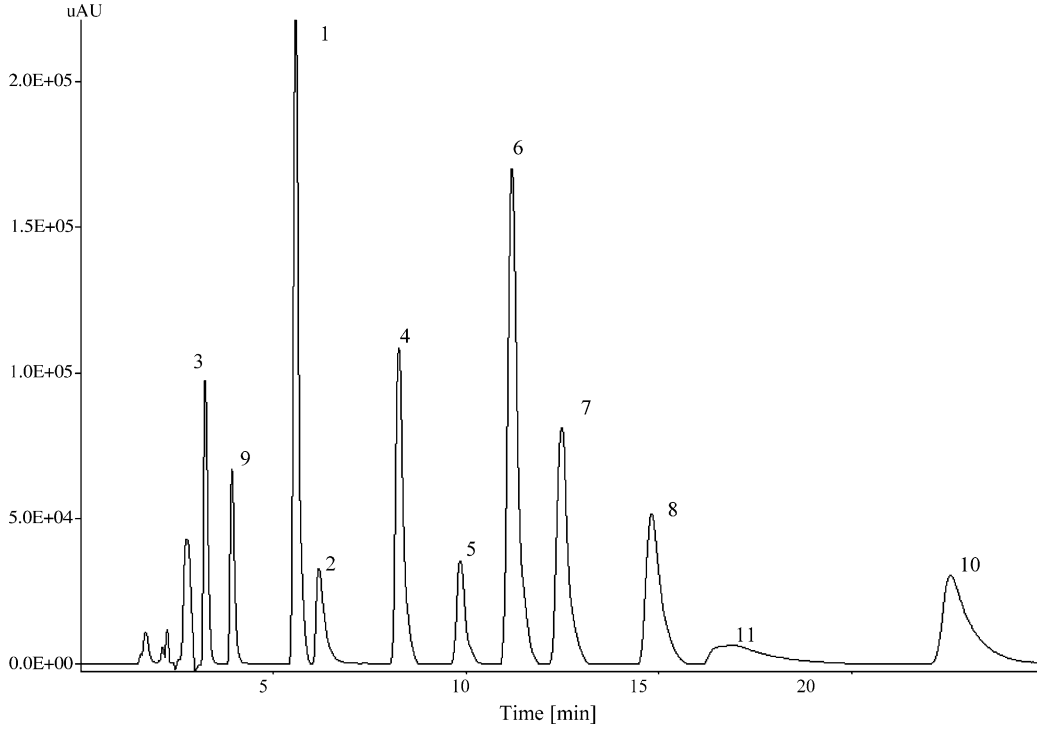


Fig. 1. Chromatogram for PPs obtained under optimal conditions using a Synergi MAX-RP C_{12} (250 mm × 4.6 mm i.d., 4 μm) column (50 °C). Conditions: injection volume 20 μl, concentration level of each phenol 10 μg ml^{-1}, mobile phase 50 mM phosphate buffer (pH 5.0)–ACN (60:40, v/v) and flow-rate 1 ml min^{-1}. For peaks identification and UV detection see Table 1.

3.1. Optimization using a Synergi column

Compounds were tested using the IMP conditions described above, a Synergi RP-MAX C_{12} (250 mm × 4.6 mm i.d., 4 μm) column and 1 ml min^{-1} flow-rate. All phenols were separated to base line in about 23 min. To reduce the time, ACN was varied in the range 40–50%. Improvements in the separation were not significant using 45 or 50% ACN since P and 4NP overlapped and poor resolution were obtained for them. In addition, a similar behavior with regard to pH was observed when compared this column with the Hypersil one. For this reason pH was slightly modified in the range 5.0–5.5 to improve the above separation, especially for the most retained phenols 246TCP and PCP (pK_a values are in Table 1). Using 40% ACN, the separation was not improved because at pH 5.25 and 5.50 the pairs PCP/24DCP and PCP/4C3MP overlapped, respectively. In summary, IMP conditions were selected. The separation and the elution order obtained are shown in Fig. 1. The k, A_s, N_{df} and H_{df} values are also summarized in Table 2. This elution order differs from the one obtained for Luna column (PCP and 24DCP are interchangeable).

3.2. Optimization using a Purospher column

Using IMP conditions, a Purospher START RP-18 (250 mm × 4.6 mm i.d., 5 μm) column and 1 ml min^{-1} flow-rate, 10 phenols were separated in about 23 min (the pair PCP/24DCP coeluted). To improve this separation ACN was varied in the range 40–55%. Using 45 and 50% ACN, PCP and 24DCP were completely separated, and all phenols were resolved in 19 and 17 min, respectively. However, using 55% ACN, 9 compounds were separated. Finally, 50% ACN was selected for further experiments. As above mentioned, pH was slightly modified in the range 5.0–5.25 without improving the separation (at pH 5.25 the pair PCP/4C3MP overlapped). In summary, a mobile phase phosphate buffer 50 mM (pH 5.00)–ACN (50:50, v/v) was selected and all phenols were separated in 17 min. Fig. 2 shows the separation obtained and Table 2 summarizes the k, A_s, N_{df} and H_{df} values obtained for the optimal conditions.

3.3. Optimization using a Chromolith column

Owing to its physical characteristics, column temperature for Chromolith RP-18e (100 mm × 4.6 mm) columns can not exceed 45 °C. For this reason, column temperature was set at 45 °C when using this column. Moreover, as mentioned above, the Chromolith column can use higher flow-rates than microparticulate ones, reducing analysis time. For this reason, 4 ml min−1 flow-rate was selected for further experiments. Phenols were eluted under IMP conditions at 45 °C and 4 ml min^{-1} flow-rate. In these conditions 10 phenols were separated in 3 min. To improve this separation, ACN was varied in the range 34–40%. In this way, 10 phenols were separated in 3.5 min (38% ACN) and 11 phenols in 3.5 min and

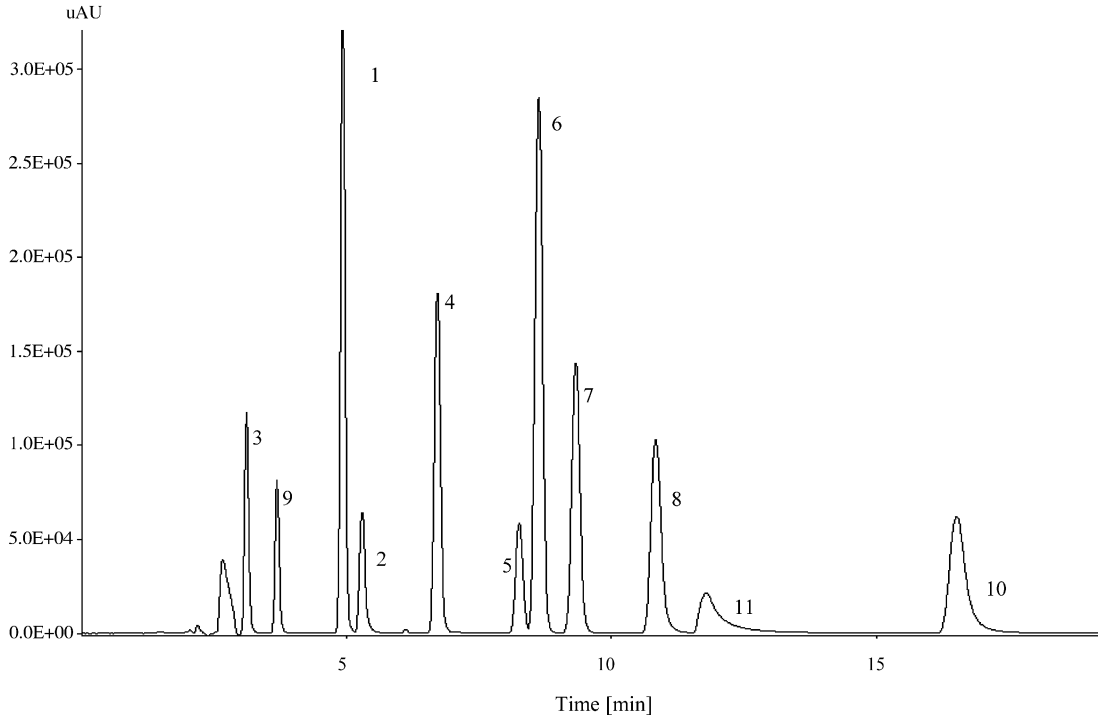


Fig. 2. Chromatogram for PPs obtained under optimal conditions using a Purospher STAR RP-18 (250 mm × 4.6 mm i.d., 5 μm) column (50 °C). Conditions: injection volume 20 μl, concentration level of each phenol 10 μg ml^{-1}, mobile phase 50 mM phosphate buffer (pH 5.0)–ACN (50:50, v/v) and flow-rate 1 ml min^{-1}. For peaks identification and UV detection see Table 1.

4.5 min when using 36 and 34% ACN, respectively. Finally, 36% ACN was selected. In these conditions, an inversion in the elution order for the pair PCP/246TCP was observed. pH was modified in the range 5.0–5.5. PCP retention was reduced, because its dissociation is favoured as pH increases. Thus, at pH 5.5 PCP and 24DCP coeluted. However, at pH 5.25 the separation improved since all phenols were separated to base line in an analysis time close to 3 min. In summary, a mobile phase phosphate buffer (pH 5.25)-ACN (64:36, v/v) (45 °C) was selected. Fig. 3 shows the separation obtained and Table 2 summarizes the k, $A_{s,}$ N_{df} and H_{df} values obtained for optimal conditions.

3.4. Effect of flow-rate on column efficiency

The influence of flow-rate of the mobile phase on the performance of the above-optimized separations using the described columns was carried out. The flow-rate for the Chromolith and microparticulate columns was varied in the range 1–4 ml min^{-1} and 0.5–1.5 ml min^{-1}, respectively. The Van Deemter plots (H_{df} versus flow-rate) for Synergi, Purospher and Chromolith columns are shown in Fig. 4 (A–C), respectively. As can be seen, there is no significant loss in efficiency for the Chromolith column in the range of flow-rates tested (H_{df} values are in the range 0.001–0.002 for all PPs, except for 24DNP range which is 0.003–0.004). Using microparticulate columns, a significant loss in efficiency was

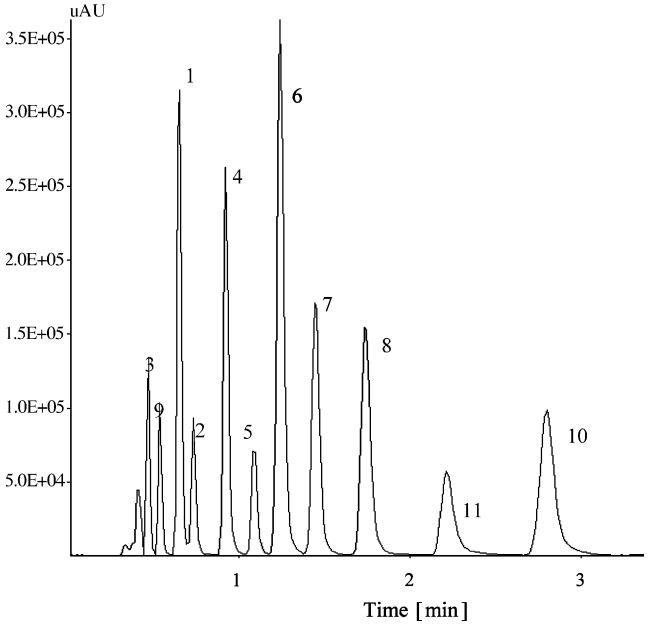


Fig. 3. Chromatogram for PPs obtained under optimal conditions using a Chromolith RP-18e (100 mm × 4.6 mm i.d.) column (45 °C). Conditions: injection volume 20 μl, concentration level of each phenol 10 μg ml^{-1}, mobile phase 50 mM phosphate buffer (pH 5.25)–ACN (64:36, v/v) and flow-rate 4 ml min^{-1}. For peaks identification and UV detection see Table 1.

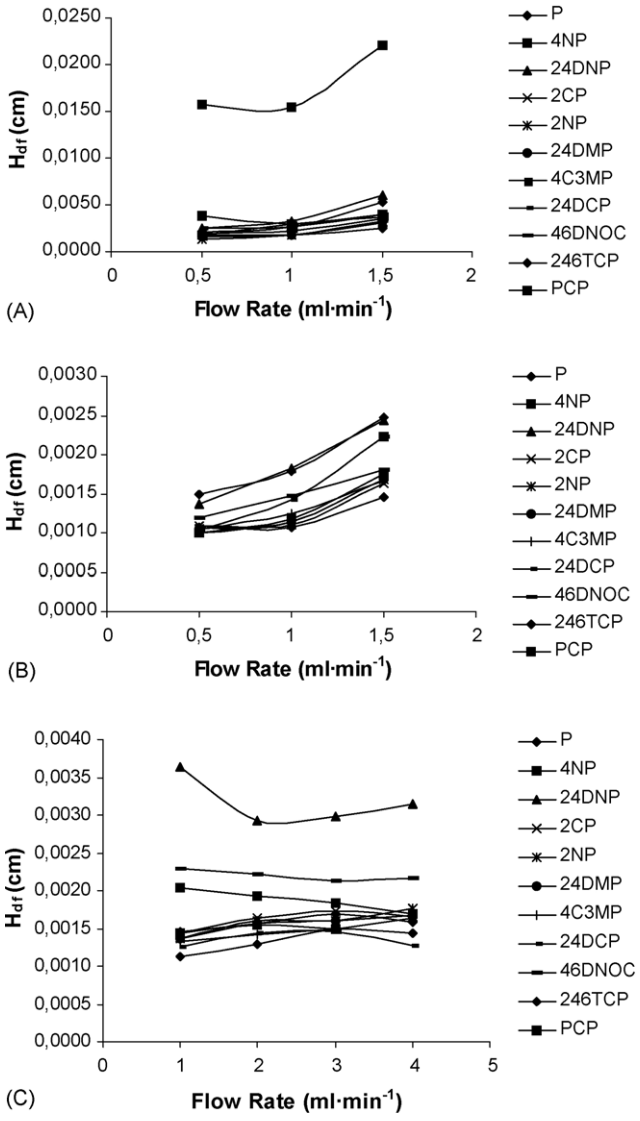


Fig. 4. Van Deemter plots. A: Synergi column; B: Purospher column; C: Chromolith column.

observed from flow-rates higher than 1 ml min^{-1} (Figs. 4(A and B)). However, the Purospher column has better efficiency than the other microparticulate ones in the range studied (see H_{df} values in Table 2). Considering efficiency and analysis time, 1 ml min^{-1} flow-rate for microparticulate columns, and 4 ml min^{-1} for the Chromolith column were selected as optimal.

4. Conclusions

The performances of different conventional microparticulate columns versus a monolithic one in the separation of phenols have been compared. For all columns the mobile phase (using ACN as organic modifier), pH and flow-rate were optimized. The optimal separation for each column was reached using different ACN concentration and pH of the mobile phase, flow-rate and temperature. In all cases, phenols were separated to baseline following the same elution order and analysis times were 22.5, 17 and 3 min, when using Synergi, Purospher and Chromolith columns, respectively. However, an inversion in the elution order of 24DCP and PCP was observed for the Luna column.

Van Deemter plots show that there is no loss in efficiency when using the Chromolith column if high flow-rates are employed. However, using the conventional columns the efficiency decreases when flow-rate increases.

The comparison of performance data was not obtained under identical conditions, even though some parameters may not be critical. However, in the optimization process we have checked that in spite of little changes over IMP conditions, the nature of columns can probably overcome the little changes made.

When the asymmetry factors were compared between columns, good results were obtained for Luna column (except for PCP) and poor for Synergi one. However, when the Chromolith and Purospher columns were compared, the monolithic column provided better results for P, 4NP, 24DNP, 46DNOC, 246TCP and PCP (the results for PCP were significant). The remainder phenols exhibited, however, better results using the Purospher column.

H_{df} parameters were used to evaluate column efficiency. The results obtained for H_{df} generally followed the sequence Purospher < Chromolith < Luna < Synergi. Exception is made for PCP. In this case, a better efficiency was achieved using the Chromolith column.

In summary, the optimal separation was obtained in about 3 min using the Chromolith column with acceptable A_s and H_{df} values as compared to the conventional columns. Additionally, PCP exhibited the best values (k, A_s, H_{df}) as compared with the others columns.

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