Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1055 (2004) 21–28
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

High-performance liquid chromatography of di- and trisubstituted aromatic positional isomers on *1,3-alternate* 25,27-dipropoxy-26,28-bis-[3-propyloxy]-calix[4]arene-bonded silica gel stationary phase

Magdalena Śliwka-Kaszyńska*, Katarzyna Jaszczołt, Dariusz Witt, Janusz Rachoń

Department of Organic Chemistry, Gdansk University of Technology, Narutowicza 11/12, 80-952 Gdańsk, Poland

Received 27 April 2004; received in revised form 4 August 2004; accepted 4 August 2004

Abstract

A new *1,3-alternate* 25,27-dipropoxy-26,28-bis-[3-propyloxy]-calix[4]arene-bonded silica gel stationary phase (*1,3-Alt* CalixPr) has been prepared and used for the separation of di- and trisubstituted aromatic positional isomers by HPLC. The effect of organic modifier content, pH and column temperature on retention and selectivity of the benzene derivatives were studied. The retention mechanism was also discussed. The results indicated that the stationary phase behaves like a reversed-phase packing. However inclusion, hydrophobic, hydrogen bonding and π–π interactions seem to be involved in separation process.

Keywords: High-performance liquid chromatography; Positional isomers separation; *1,3-alternate* 25,27-dipropoxy-26,28-bis-[3-propyloxy]-calix[4]arene; Stationary phase

1. Introduction

Calixarenes are macrocyclic molecules made up of phenolic units and *ortho* linked by methylene bridges. This interesting class of cavity-shaped cyclic compounds is known to have attractive structural properties and therefore are counted as the third generation of supramolecules, after cyclodextrin and crown ethers [1–3]. The cyclic tetramers known as calix[4]arene can exist in four distinctive conformations: first with all aryl groups *syn* to one another, second with three aryl groups *syn* and one *anti*, third with adjacent pairs of aryl groups *syn* and *anti*, and fourth with non-adjacent pairs of aryl groups *syn* and *anti*. These were later named by Gutsche as cone, partial cone, 1,2-alternate and 1,3-alternate respectively. Their properties are strongly influenced by conformation, which is fixed after introduction of four bulky substituents at the phenolic oxygen atoms. The interest in calixarenes in analytical and separation chemistry have been increased in recent years because of their ability to form reversible complexes with neutral as well as charged molecules [4]. Calixarenes have been utilized in gas chromatography [5–8], solid-phase extractions [9], and capillary electrophoresis [10–12]. Calixarenes as stationary phases in liquid chromatography have attracted many researchers' attention. Glennon and coworkers [13–15] prepared silica-bonded calix[4]arene tetraester and silica-bonded calix[4]arene tetradiethylamide stationary phases to separate metal ions and amino acid esters. Park et al. [16] synthesized calyx[6]arene-*p*-sulfonate-bonded silica stationary phase and carried out separation of aromatic positional isomers. Gebauer et al. [17–19] reported the chromatographic separation of disubstituted aromatics, nucleosides, uracil derivatives, estradiol epimers and *cis*/*trans* isomers of proline-cointaining peptides on calix[*n*]arene-bonded (*n* = 4, 5, 6, 8) silica gel. In the past few years the research group

* Corresponding author. Fax: +48 58 3472694.
E-mail address: pestka@chem.pg.gda.pl (M. Śliwka-Kaszyńska).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.08.006

from Wuhan University (Xiao and coworkers) prepared *p-tert*-butyl-calix[6]arene-bonded silica gel stationary phase with 3-glycidoxypropyltriethoxysilane [20] and *p-tert*-butyl-calix[*n*]arene bonded (n = 4, 8) silica gel stationary phases with γ-(ethylenediamino)-propyltriethoxysilane as coupling reagents [21–25] and carried out the chromatographic separation of some positional isomers, polycyclic aromatic hydrocarbons (PAHs), nucleosides, sulfonamides, quinolones and aromatic carboxylic acids. Sokoließ et al. compared selectivity of unsubstituted calixarenes of different ring size by separations of PAHs, barbituric acids and benzoxepin derivatives, xanthines and *cis*/*trans* isomers of thioxanthene and discussed the influences of binary eluents on the retention behavior [26–28]. Several previous works have shown that calixarene-bonded stationary phases possessing including capability are excellent in reversed-phase chromatography. Some research groups [16–18,24–26] described application of these phases in separation of analytes of very similar structure, e.g. aromatic positional isomers, by HPLC. As far as we know, except for the work of Casnati and coworkers [29–31] on synthesis of 1,3-alternate calix[4]arenecrowns for separation of Cs^+ and K^+ from alkali metal ions, there are no research papers concerning synthesis of 1,3-alternate calix[4]arene-bonded stationary phases and its application to resolution of organic compounds. In this paper we described the synthesis of new 1,3-alternate calix[4]arene-bonded silica gel stationary phase (*1,3-Alt* CalixPr) for separation of positional aromatic isomers. The efficiency of the column and the influence of various chromatographic conditions (pH of the mobile phase, column temperature and organic modifier addition) on retention and selectivity of chosen compounds were evaluated. The retention mechanism of the analytes was also discussed. The results show that *1,3-Alt* CalixPr column exhibits high selectivity for di- and trisubstituted aromatic positional isomers due to various retention mechanisms.

2. Experimental

2.1. Chemicals and reagents

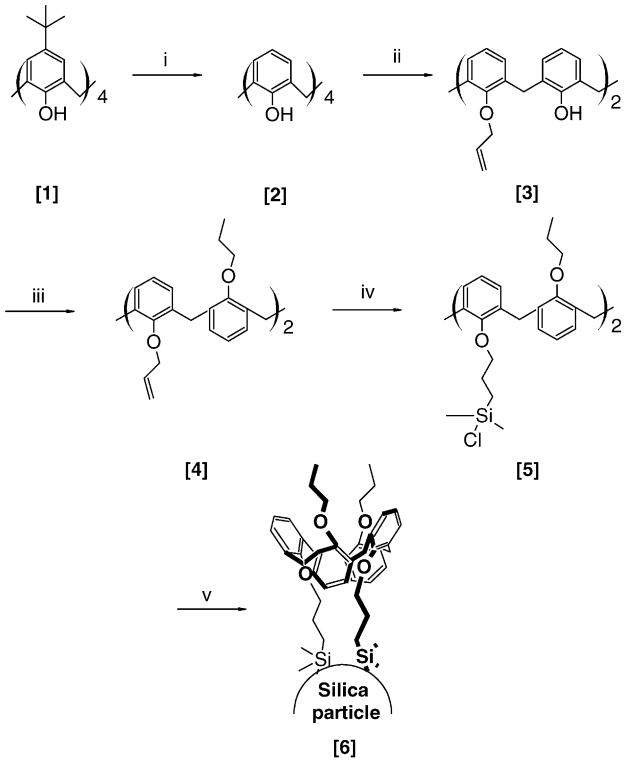
Phenol, allyl bromide and iodide, dimethylchlorosilane, propyl iodide, caesium carbonate, sodium carbonate and silica gel LiChrosorb Si 100 (particle size 5 μm, pore size 100 Å and specific surface area 300–400 m^2/g) were obtained from Merck (Darmstadt, Germany). Positional isomers (*ortho*, *meta* and *para*) of: nitrobenzoic acid, chlorobenzoic acid, 2-amino-chlorobenzoic acid, hydroxybenzoic acid, aminobenzhydrazide, hydroxybenzyl alcohol, aminophenol, hydroxypyridine, dinitrobenzene, nitrophenol and nitroaniline were obtained form Lancaster (Eastgate, England). Reversed Phase Test Mix was obtained from Supelco (Deisenhofen, Germany). Silica gel was refluxed with 2 M hydrochloric acid for 4 h to reduce metals content, washed with water and acetone and dried in an oven at 160 °C for 15 h prior to use [32]. All solvents used as reaction media were of analytical grade and were obtained from POCh (Gliwice, Poland). Acetonitrile and methanol used as mobile phases were of HPLC grade and were purchased from Merck (Darmstadt, Germany). Water was obtained by bidistillation.

2.2. Equipment

Chromatographic analyses were performed using a Hewlett-Packard liquid chromatograph type 1090 equipped with autosampler, thermostated column compartment and diode-array detector. Elemental analyses were obtained using Perkin-Elmer elemental analyzer PE 240. ^{1}H NMR spectra were recorded on Varian Gemini 500 MHz spectrometer. Reactions were monitored by TLC on precoated silica gel plates (SiO_2, Merck, $60F_{254}$). Flash column chromatography was performed on silica gel 60 (SiO_2, Merck, 230–400 mesh).

2.3. Preparation of 1,3 alternate 25,27-dipropoxy-26,28-bis-[3-propyloxy]-calix[4]arene-bonded silica gel stationary phase 1,3-Alt CalixPr

The multistep synthesis of new 1,3-alternate 25,27-dipropoxy-26,28-bis-[3-propyloxy]-calix[4]arene-bonded silica gel stationary phase is shown in Scheme 1.



Scheme 1. Synthesis of the 1,3-alternate 25,27-dipropoxy-26,28-bis-[3-propyloxy]-calix[4]arene-bonded silica gel stationary phase [6]; (i) C_6H_5OH, $AlCl_3$, dry toluene, stirring for 1 h at room temperature; (ii) allyl bromide, ACN, K_2CO_3, reflux for 24 h; (iii) propyl iodide, Cs_2CO_3, ACN, reflux for 72 h; (iv) $(CH_3)_2SiHCl$, H_2PtCl_6, $CHCl_3$, reflux for 4 h; (v) activated silica gel, pyridine, shaking for 4 days at room temperature.

25,27-Diallyloxy-calix[4]arene **3** was prepared according to the reported procedure [33]. 25,27-Diallyoxy-26,28-dipropoxycalix[4]arene **4** was obtained by refluxing compound **3** with excessive amount of propyl iodide in dry acetonitrile for 3 days under an atmosphere of nitrogen, in the presence of cesium carbonate as base and a catalyst. The solvent was then removed under reduced pressure. The residue was dissolved in CH_2Cl_2 and washed with 1N NH_4Cl and water. The organic phase was separated, dried with $MgSO_4$ and evaporated. The flash chromatography (SiO_2, $CHCl_3$) of crude product yielded white powder. Yield 52%, mp 195–198 °C.

^{1}H NMR (*CDCl$_3$*): δ 7.2–7.0 (m, 8H, Ar**H**-*m*); 6.72 (t, 2H, Ar**H**-*p*, *J* = 7.3 Hz); 6.63 (t, 2H, Ar**H**-*p*, *J* = 7.3 Hz); 5.83–5.76 (m, 2H, $OCH_2C\mathbf{H}{=}CH_2$); 5.16–5.14 (d, 4H, $OCH_2CH{=}C\mathbf{H}_2$, *J* = 4.4Hz); 3.15 (d, 4H, $OC\mathbf{H}_2CH{=}CH_2$, *J* = 4.4 Hz); 3.62 (s, 8H, $ArC\mathbf{H}_2Ar$); 3.51 (t, 4H, $OC\mathbf{H}_2CH_2CH_3$, *J* = 7.3 Hz); 1.68–1.62 (m, 4H, $OCH_2C\mathbf{H}_2CH_3$); 0.91 (t, 6H, $OCH_2CH_2C\mathbf{H}_3$, *J* = 7.3 Hz).

Product **4** was dissolved in dry CH_2Cl_2, in the presence of excess of dimethylchlorosilane, and catalytic amount of hexachloroplatinum acid (H_2PtCl_6) in isopropanol was added. The mixture was heated under nitrogen atmosphere for 4 h. Then the excess of silane was evaporated under vacuum and dry residue was used for immobilization of compound **5** on silica gel without purification. ^{1}H NMR spectra of the crude product **5** showed no signals of vinyl protons. The 1,3-alternate calix[4]arene-stationary phase **6** was prepared by shaking of activated silica gel with product **5** and catalytic amount of pyridine in dry toluene for 4 days under nitrogen atmosphere at room temperature according to the reported procedure [34,35]. The bonded phase was filtered and washed with $CHCl_3$, MeOH and acetone. Elemental analysis gave %C 14.54, %H 1.92 (coverage density of the gel 0.303 mmol/g). Finally, unreacted silanol groups were end-capped by reaction with 1,1,1,3,3,3-hexamethyldisilazane.

2.4. Column packing and column evaluation

Stainless steel column (250 mm × 4.6 mm i.d.) was packed with modified calix[4]arene-silica gel according to a slurry packing procedure [36]. The gel (3.5 g) was ultrasonically dispersed in acetone-isopropanol mixture (1:1, v/v) and the column was packed using methanol as displacing agent (750 bar, 30 min). The column efficiency was determined with a commercially available test mixture containing uracil, acetophenone, benzene and toluene using methanol–water (60:40, v/v) as the mobile phase at flow rate 1.0 ml/min. The efficiency of prepared column was about 40,000 plates/m.

2.5. Chromatography

All separations were performed isocratically. The mobile phases contained different proportions of A: ACN/MeOH (1;1, v/v) mixture in B: water or 5 mM water solution of phosphate buffer. The pH value of this buffer was adjusted with H_3PO_4 to 2.0 or with NaOH to 6.0. The flow-rate was 1.0 ml/min and injection volume was 20 μl. The UV detector was operated at 254 nm (DAD in single wavelength mode). Analytes were dissolved in ACN/MeOH/H_2O (1:1:1, v/v/v) mixture at the concentration between 0.25 and 0.5 mg/ml. The retention time of the aqueous solution of potassium nitrate was used as void time marker for the calculation of capacity factor.

3. Results and discussion

In order to exploit the great potential of calix[4]arenes in molecular recognition for liquid chromatography, *1,3-alternate* 25,27-dipropoxy-26,28-bis-[3-propyloxydimethylsililoxy]-calix[4]arene was chemically immobilized on silica gel matrix with a short hydrophobic spacer. The stability of the resulting support was evaluated over a 6-month period of use. Various analyte mixtures and different chromatographic conditions were used. No loss of retention power of the prepared column was observed during that time. The prepared stationary phase showed high chemical stability with respect to water (buffer pH from 2.0 to 6.0), methanol and acetonitrile mixture used as a mobile phase in isocratic as well as gradient mode. Separation of di- and trisubstituted benzene derivatives on *1,3-Alt* CalixPr column was investigated. Positional isomers representing compound with acidic, basic and neutral character were selected as the analytes. Retention capacity factors (*k*) and separation factors (α) of these isomers at the best chromatographic conditions are given in Tables 1 and 2. Chromatographic condition was individually optimized for each set of analytes. Most of the positional isomers were well resolved except xylene, dichlorobenzene and chlorotoluene isomers which were only partially resolved. An example of resolution of a number of aromatic positional isomers in a single run is shown in Fig. 1. Based on the chromatographic

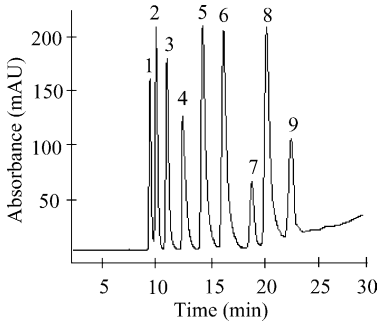


Fig. 1. Chromatogram of a mixture of benzene positional isomers. Chromatographic conditions: mobile phase A: 5 mM KH_2PO_4 buffer solution at pH 5.0; B: ACN/MeOH (1:1, v/v); gradient from 5 to 30% B within 10 min and then 30% B isocratic for 30 min; flow rate 0.8 ml/min, detection UV at 254 nm, temperature 30 °C. Analytes: (1) *p*-aminobenzhydrazide, (2) *m*-aminobenzhydrazide, (3) *o*-aminobenzhydrazide, (4) *p*-hydroxypyridine, (5) *o*-hydroxypyridine, (6) *m*-hydroxypyridine, (7) *p*-nitroaniline, (8) *m*-nitroaniline, (9) *o*-nitroaniline.

Table 1
Retention (k) and separation factor (α) for benzene positional isomers on *1,3-Alt* CalixPr stationary phase

Compounds	Isomer	k	α
Dinitrobenzene[a] DNB	1,4-Dinitrobenzene	34.90	1.07
	1,3-Dinitrobenzene	37.46	1.18
	1,2-Dinitrobenzene	44.06	
Nitroaniline[a] NAN	4-Nitroaniline	17.77	1.06
	3-Nitroaniline	18.86	1.22
	2-Nitroaniline	23.09	
Hydroxypyridine[b] HPyr	4-Hydroxypyridine	4.96	1.14
	2-Hydroxypyridine	5.64	1.40
	3-Hydroxypyripine	7.89	
Aminobenzhydrazide[c] ABHz	4-Aminobenzhydrazide	8.90	1.06
	3-Aminobenzhydrazide	9.39	1.62
	2-Aminobenzhydrazide	15.21	
Nitrophenol[a] NPhol	4-Nitrophenol	16.39	1.04
	3-Nitrophenol	17.03	1.08
	2-Nitrophenol	18.37	
Aminophenol[d] APhol	4-Aminophenol	7.85	1.12
	3-Aminophenol	8.77	1.13
	2-Aminophenol	9.96	
Hydroxybenzyl alcohol[e] HBnol	4-Hydroxybenzyl alcohol	9.45	1.24
	3-Hydroxybenzyl alcohol	11.70	1.17
	2-Hydroxybenzyl alcohol	13.68	
Xylene[c] Xyl	4-Xylene	4.52	1.00
	3-Xylene	4.52	1.00
	2-Xylene	4.54	
Dichlorobenzene[c] DClB	4-Dichlorobenzene	3.88	1.00
	3-Dichlorobenzene	3.90	1.00
	2-Dichlorobenzene	3.90	
Chlorotoluene[c] ClT	4-Chlorotoluene	7.11	1.00
	3-Chlorotoluene	7.11	1.00
	2-Chlorotoluene	7.11	

Chromatographic conditions: flow rate 1 ml/min, detection UV at 254 nm, temperature 30° C; mobile phases—A: ACN/MeOH (1:1, v/v) in B: water.
[a] 30% A.
[b] 35% A.
[c] 10% A.
[d] 20% A.
[e] 7% A.

data, the *1,3-Alt* CalixPr has reversed-phase property and exhibits strong retention power, which is similar in many cases to the calixarene stationary phases synthesized by the other research groups [16–18,25,26]. It can be shown that separation of positional isomers, for example *ortho-*, *meta-* and *para*-nitroaniline, which only partly succeeded with ODS stationary phase, can be executed very well with the novel phase used in the present study. Aromatic carboxylic acids also exhibit better selectivity on calixarene phase than on ODS one [24]. The retention times of the compounds containing nitro substituents at the phenyl ring were very high in comparison to the rest of investigated analytes (e.g. isomers of dinitrobenzene eluted after 30 min). This behavior may be due to π-electron transfer interaction resulting from the electron-withdrawing effect of the nitro group of analyte and electron-releasing effect of the propyloxy groups of calixarene.

The elution order of disubstituted benzene isomers possessing neutral and basic character as well as for the phenols was always $p > m > o$. Different observations were found for nitrophenol, aminophenol and nitroaniline isomers separated on *p-tert*-butylcalix[*n*]arene (n = 4, 6, 8) as stationary phase in HPLC [17,18,24] and capillary GC [37], *p*-sulfoniccalix[4]arene in capillary electrophoresis [38] and on Caltrex® phases [26], but our data correspond to that obtained on ocatadecyl silica gel columns in HPLC [17]. The elution order of hydroxypyridine isomers was in contrast $p > o > m$. *ortho*-Hydroxypyridine may form intermolecular hydrogen bonding, which can predominate over the weaker orientation and induction interactions between the polar groups of the pyridine and the stationary phase. On the other hand we did not observe such behavior for isomers of aminophenol also possessing two polar substituents capable of hydrogen bond formation. One should notice that both *para* and *ortho* isomers of hydroxypyridine exist likewise in tautomeric form of pyridin-(1H)-one that may interact with the stationary phase in different way. We cannot at present rationalize our observations. The elution order of the substituted benzoic acid isomers was $o > m > p$, which is opposite to the previously discussed and follows pK_a values order of these acids.

3.1. Influence of organic modifier content

Methanol/water and acetonitrile/water have generally been used as a mobile phase in reversed-phase chromatography. A number of studies have been made on the influence of the mobile phase conditions on the retention behavior in RP-LC in connection with the specification of the separation mechanism. The linear expression of the relationship between the retention parameter (i.e. $\ln k$) of a solute in RP-LC and the composition of the binary eluent was proposed by Snyder et al. [39]:

$$\ln k = \ln k_w - S\Phi$$

where k denotes the capacity factor of solute, k_w the capacity factor extrapolated for pure water as mobile phase, S a constant characteristic for a given stationary phase and analyte, and Φ the volume fraction of organic modifier in the mobile phase.

It is understandable that modeling of complex retention phenomenon by means of a simple relationship, primarily devised for reversed-phase systems, can only be a moderate success. Schoenmakers et al. [40] proposed one of the most widely recognized curvilinear equations:

$$\ln k = A\Phi^2 + B\Phi + C$$

where A, B, and C are the equation constants.

Retention model devised by Schoenmakers is not meant for description of the adsorption liquid chromatography systems or for solutes whose mechanism of retention was

Table 2
Retention (k) and separation factor (α) and dissociation constants (pK_a) of benzoic acid positional isomers on *1,3-Alt* CalixPr stationary phase

Compounds	Isomer	pK_a	k	α
Hydroxybenzoic acid[a] HBA	2-Hydroxybenzoic acid	2.97	5.71	1.06
	3-Hydroxybenzoic acid	4.06	6.07	1.02
	4-Hydroxybenzoic acid	4.48	6.20	
Nitrobenzoic acid[b] NBA	2-Nitrobenzoic acid	2.17	4.51	1.35
	3-Nitrobenzoic acid	3.45	6.10	1.05
	4-Nitrobenzoic acid	3.54	6.44	
Chlorobenzoic acid[b] ClBA	2-Chlorobenzoic acid	2.94	4.31	1.29
	3-Chlorobenzoic acid	3.82	5.57	1.08
	4-Chlorobenzoic acid	3.98	6.04	
Dihydroxybenzoic acid[b] DHBA	2,3-Dihydroxybenzoic acid	2.94	7.85	1.09
	2,5-Dihydroxybenzoic acid	2.97	8.60	
2-Aminochlorobenzoic acid[c] AClBA	2-Amino-6-chlorobenzoic acid	1.21	4.39	1.74
	2-Amino-5-chlorobenzoic acid	1.69	7.62	1.19
	2-Amino-4-chlorobenzoic acid	1.86	9.08	

Chromatographic conditions: flow rate 1 ml/min, detection UV at 254 nm, temperature 30 °C; mobile phases—A: ACN/MeOH (1:1, v/v) in B: 5 mM KH_2PO_4 buffer solution at pH 5.0.
[a] 20% A.
[b] 30% A.
[c] 35% A.

based either on partition or on adsorption, depending on quantitative proportions of organic modifier in the eluent. However, it was successfully applied for RP-LC as well as for NP-LC systems. Its indisputably high performance results from the algebraic qualities of the equation used (which is a flexible parabolic dependence) rather than from the strength of its physical background. Fig. 2 illustrates the plots of logarithmic capacity factor of *para* isomers of dinitrobenzene, hydroxybenzyl alcohol, aminobenzhydrazide, nitroaniline, hydroxypyridine and aminophenol against the fraction of ACN/MeOH (1:1, v/v) in mobile phase. As can be seen, increase in the organic modifier in mobile phases led to a decrease in the retention of the analytes on *1,3-Alt* CalixPr. This result indicates that the new stationary phase behaves as a reversed-phase packing and the hydrophobic interaction is one of the factors playing a role in the retention

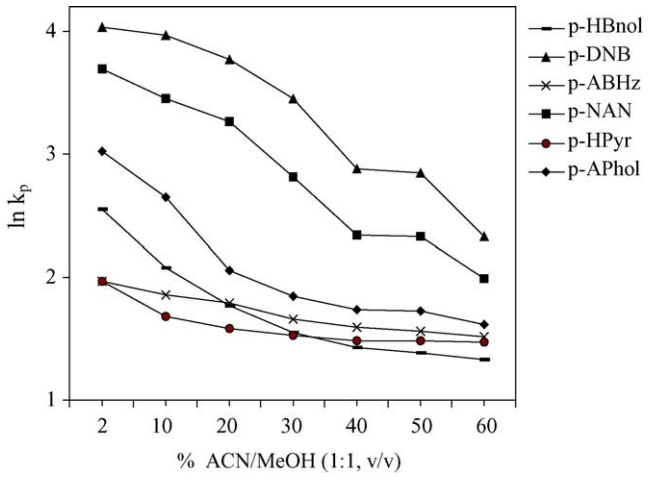


Fig. 2. Influence of the acetonitrile/methanol (1:1, v/v) content of the mobile phase on logarithmic capacity factor of hydroxybenzyl alcohol (HBnol), dinitrobenzene (DNB), nitroaniline (NAN), hydroxypyridine (HPyr) and aminophenol (APhol) *para*-isomers. Chromatographic conditions: ACN/MeOH (1:1, v/v) in water; flow 1 ml/min, UV at 254 nm, temperature 30 °C.

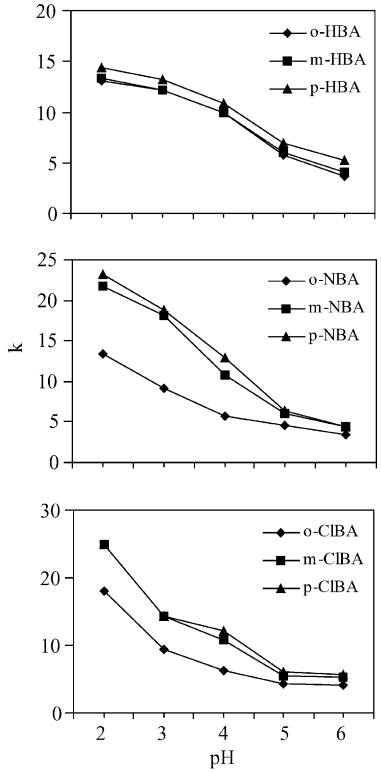


Fig. 3. Influence of the pH of mobile phase on the capacity factors of hydroxybenzoic acid (HBA), nitrobenzoic acid (NBA) and chlorobenzoic acid (ClBA) positional isomers. Chromatographic conditions: 30% ACN/MeOH (1:1, v/v) in 5 mM KH_2PO_4 buffer solution, flow 1 ml/min, UV at 254 nm, temperature 30 °C.

of positional isomers of benzene. However, it is obvious that the relationship between $\ln k$ and φ (organic modifier content of mobile phase) is not linear. Hence it can imply that hydrophobic interaction was not only factor in the separation of these compounds and the hydrogen bonding interaction between the solutes and residual phenolic hydroxyl group or ether-oxygen atom of *1,3-Alt* CalixPr may also be responsible for the retention behavior.

The peak symmetry was improved after addition of organic modifier, but the resolution of isomers was gradually lost as the capacity factors were decreased.

3.2. Effect of pH of the mobile phase

The influence of pH of the mobile phase on retention parameters of nitrobenzoic, chlorobenzoic and hydroxybenzoic acid isomers was studied. These acids have pK_a values in the range of 2.17–4.48. As can be seen in Fig. 3, the retention of the substituted benzoic acid isomers was strongly dependent on the pH of the mobile phases. The retention time of these acids decreased gradually in the pH range of 2.0–6.0. It is consistent with reversed phase retention mechanism in which ionic species are slightly retained. Surprisingly, the best resolution factor of the separated substituted benzoic acid isomers was observed at pH 5.0 of the mobile phase.

3.3. Effect of the temperature

In order to investigate the nature of the interactions involved in the discrimination process, the temperature effect on both the resolution factor and selectivity factor of selected aromatic positional isomers was studied. Several chromatograms of the hydroxybenzyl alcohol positional isomers, carried out at different temperatures (20–60 °C) on *1,3-Alt* CaixPr column are shown in Fig. 4. The results obtained clearly show that at the lower end of the temperature range investigated, the retention factors of all isomers increase while a certain amount of peak broadening occurs, which may due to a slow mass-transfer process occurring in the above temperature range. The retention of a solute in a chromatographic system is determined by the magnitude of the distribution coefficient of the solute between the two phases and by the amount of stationary phase available to the solute for interaction. The distribution coefficient in chromatography is equilibrium constant and it can be treated rationally by conventional thermodynamics. It can be expressed in terms of the standard energy of solute exchange between the phases:

$$RT \ln K = -\Delta G^{\circ}$$

where R is the gas constant, T the absolute temperature, and ΔG° the standard energy. Classical thermodynamics gives an expression for the standard energy, which separates it into

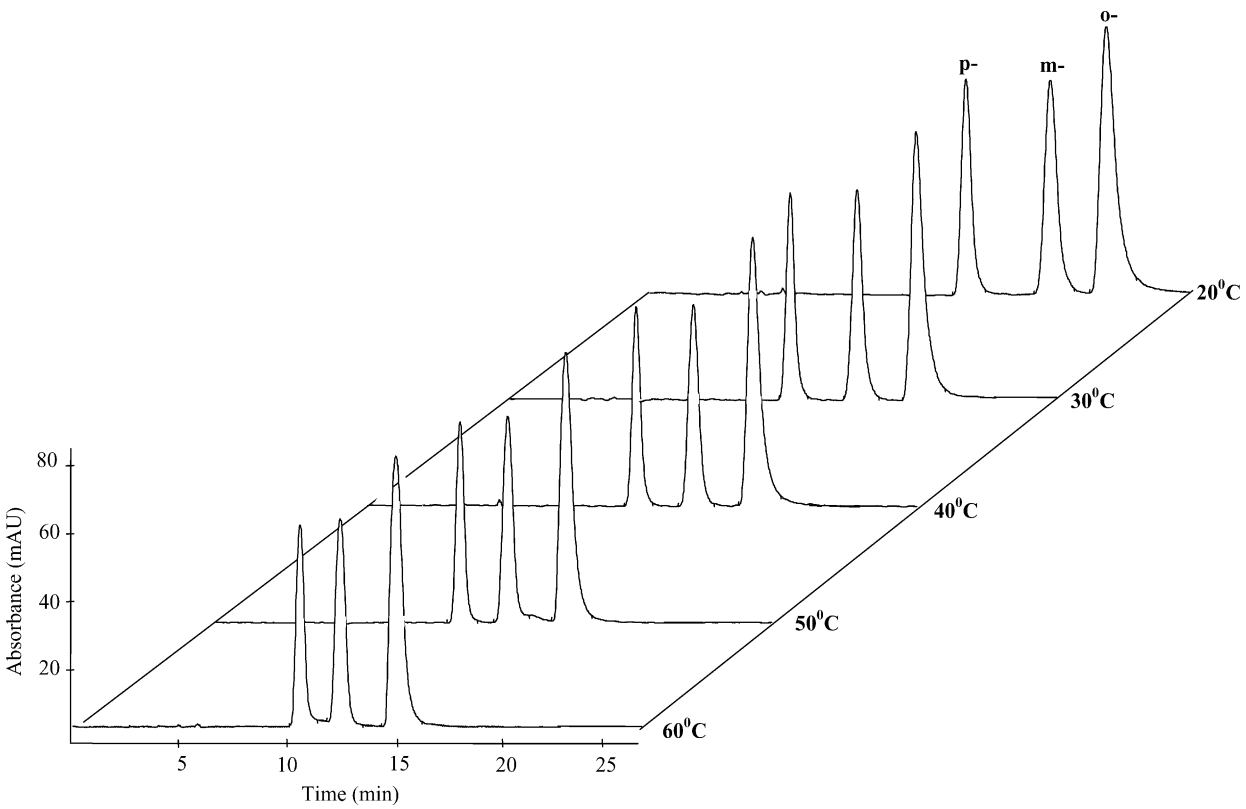


Fig. 4. Effect of temperature on the retention factor and peak symmetry for hydroxybenzyl alcohol positional isomers (*o*-, *m*-, *p*-). Chromatographic conditions: 7% ACN/MeOH (1:1, v/v) in water, flow 1 ml/min, UV at 254 nm.

Table 3
Thermodynamic data

Parameter	Compounds in elution order			Remarks
	4-HPyr	**2-HPyr**	**3-HPyr**	
k (30 °C) =	4.96	5.64	7.90	Apparent entropy effect
ΔH° (kJ/mol) =	−2.24 ± 1.32	−2.64±1.86	−2.64±1.36	
$\Delta\Delta S^\circ$ (J/(mol K)) =	−7.15±1.59	−0.26±0.12		
	4-HBnol	**3-HBnol**	**2-HBnol**	Entropy effect visible but retention driven mainly by enthalpy
k (30 °C) =	9.45	11.70	13.68	
ΔH° (kJ/mol) =	−6.32 ± 0.29	−7.87 ± 0.38	−6.74 ± 0.28	
$\Delta\Delta S^\circ$ (J/(mol K)) =	5.00±0.32	−3.33±0.53		
	1,4-DNB	**1,3-DNB**	**1,2-DNB**	
k (30 °C) =	34.90	37.46	44.06	Separation of isomers entropy driven
ΔH° (kJ/mol) =	−14.49 ± 0.53	−15.02 ± 0.47	−4.40 ± 0.10	
$\Delta\Delta S^\circ$ (J/(mol K)) =	−1.18±0.21	−7.11±1.53		
	4-NAN	**3-NAN**	**2-NAN**	
k (30 °C) =	17.77	18.86	23.09	Apparent entropy effect
ΔH° (kJ/mol) =	−12.17 ± 0.57	−11.28 ± 0.53	−12.33 ± 0.54	
$\Delta\Delta S^\circ$ (J/(mol K)) =	3.45±0.93	−1.77±0.11		
	2-NBA	**3-NBA**	**4-NBA**	
k (30 °C) =	10.02	18.87	21.02	Retention entirely enthalpy driven
ΔH° (kJ/mol) =	−5.22 ± 0.79	−12.74 ± 0.72	−12.99 ± 0.32	
$\Delta\Delta S^\circ$ (J/(mol K)) =	0.0216±0.0003	0.10±0.24		

two parts, the standard enthalpy and the standard entropy:

$$\Delta G^\circ = \Delta H^\circ - T\,\Delta S^\circ$$

where ΔH° is the standard enthalpy, and ΔS° the standard entropy.

The enthalpy term represents the energy involved when the solute molecules break their interactions with the mobile phase and interact with the stationary phase. When the solute interacts with the stationary phase, the solute molecules are held more tightly and, consequently, are more restricted. This motion restriction, reduced freedom of movement or loss of randomness is measured as the entropy change. The thermodynamic properties of a distribution system can help explain the characteristics of the distribution (i.e. the standard enthalpy and standard entropy involved) and predict, quite accurately, the effect of temperature on the separation. Retention capacity factor is related to the thermodynamic equilibrium constant by equation:

$$k = \frac{KV_\mathrm{s}}{V_\mathrm{m}} = \frac{K}{a}$$

where V_s and V_m are volume of stationary and mobile phase available to the solute in the column, and $a = V_\mathrm{s}/V_\mathrm{m}$.

Combining the above equations one may obtain relationship of logarithmic capacity constant and the temperature:

$$\ln k = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} - \ln a$$

This equation can easily be used for evaluation of the enthalpy effect. Assessment of relative entropy effect of pair of the solutes is also possible on the basis of relationship of relative retention factor α on the temperature:

$$\ln \alpha = \ln\left(\frac{k_2}{k_1}\right) = -\frac{\Delta\Delta H^\circ}{RT} + \frac{\Delta\Delta S^\circ}{R}$$

where $\Delta\Delta H^\circ = \Delta H^\circ{}_1 - \Delta H^\circ{}_2$ and $\Delta\Delta S^\circ = \Delta S^\circ{}_1 - \Delta S^\circ{}_2$

The results of investigation of thermodynamics of the retention process of selected compounds are summarized in Table 3. Various contributions of enthalpy and entropy effects depending on the solute constitution were observed. Compounds bearing nitro group in phenyl ring have in general higher enthalpy of phase transfer process than the rest of examined solutes. This suggests that charge transfer mechanism may contribute to the retention of such solutes on *1,3-Alt* CalixPr stationary phase. The entropy effect was moderate and much more difficult to rationalize but in some cases it determined the elution order of positional isomers of the solutes. For example, enthalpy of retention process of 2-hyroxypyridine and 3-hydroxypyridine was the same under employed chromatographic conditions but the relative retention factor was quite substantial (α = 1.4) due to the difference in entropy. Furthermore, entire elution order of dinitrobenzene isomers was rather entropy than enthalpy dependent.

4. Conclusions

The positional isomers investigated were well resolved on new *1,3-alternate* 25,27-dipropoxy-26,28-bis-[3-

propyloxy]-calix[4]arene-bonded silica gel stationary phase. The elution order of the substituted benzoic acids positional isomers was *o* > *m* > *p* and follow the value of pK_a of these acids. The elution order of the remaining tested compounds was opposite expect for isomers of hydroxypyridine where *ortho* isomer eluted before *meta* isomer. According to the chromatographic data, it can be concluded that various chromatographic retention mechanisms occur in the separation of benzene positional isomers on *1,3-Alt* CalixPr, such as hydrophobic interaction, hydrogen-bonding interaction, π–π interaction with aromatic rings and perhaps inclusion complex formation. The influence of various factors such as pH of the mobile phase, column temperature and organic modifier content on retention and selectivity factor has been investigated.

References

[1] C.P. Gutsche, Calixarenes, in: J.F. Stoddart (Ed.), Monographs in Supramolecular Chemistry, vol. 1, The Royal Society of Chemistry, Cambridge, 1989.
[2] J. Vicens, V. Böhmer (Eds.), Calixarenes—A Versatile Class of Macrocyclic Compounds, Kluwer Academic Publishers, Dordrecht, 1991.
[3] S. Shinkai, Pure Appl. Chem. 58 (1986) 1523.
[4] Z. Asfari, V. Böhmer, J. Harrowfield, J. Vicens (Eds.), Calixarenes 2001, Kluwer Academic Publishers, Dordrecht, 2001.
[5] P. Mnuk, L. Feltl, V. Schurig, J. Chromatogr. A 732 (1996) 63.
[6] P. Mnuk, L. Feltl, J. Chromatogr. A 696 (1995) 101.
[7] L. Lin, C.Y. Wu, Z.Q. Yan, X.Q. Yan, X.L. Su, H.M. Han, Chromatographia 47 (1998) 689.
[8] A. Mangia, A. Pochini, R. Ungaro, G.D. Andreetti, Anal. Lett. 16 (1983) 1027.
[9] S. Hutchinson, G.A. Kearney, E. Horne, B. Lynch, J.D. Glennon, M.A. McKervey, S.J. Harris, Anal. Chim. Acta 291 (1994) 269.
[10] D. Shohat, E. Grushka, Anal. Chem. 66 (1994) 747.
[11] K. Bächmann, A. Bazzanella, I. Haag, K.Y. Han, R. Arnecke, V. Böhmer, W. Vogt, Anal. Chem. 67 (1995) 1722.
[12] S. Sun, M.J. Sepaniak, J.S. Wang, C.D. Gutsche, Anal. Chem. 69 (1997) 344.
[13] J.D. Glennon, E. Horne, K. O'Connor, G. Kearney, S.J. Harris, M.A. McKervey, Anal. Proc. 31 (1994) 33.
[14] R. Bridle, K. Albert, S.J. Harris, C. Tröltzsch, E. Horne, J.D. Glennon, J. Chromatogr. A 731 (1996) 47.
[15] J.D. Glennon, E. Horne, K. Hall, D. Cocker, A. Kuhn, S.J. Harris, M.A. McKervey, J. Chromatogr. A 731 (1996) 41.
[16] Y.K. Lee, Y.K. Ryu, J.W. Ryu, B.E. Kim, J.H. Park, Chromatographia 46 (1997) 507.
[17] S. Friebe, S. Gebauer, G.-J. Krauss, G. Goermar, J. Krueger, J. Chromatogr. Sci. 33 (1995) 281.
[18] S. Gebauer, S. Friebe, G. Gübitz, G.-J. Krauss, J. Chromatogr. Sci. 36 (1998) 383.
[19] S. Gebauer, S. Friebe, G. Gübitz, G. Scherer, G.J. Krauss, J. Chromatogr. Sci. 36 (1998) 388.
[20] W. Xu, J.S. Li, Y.Q. Feng, S.L. Da, Y.Y. Chen, X.Z. Xiao, Chromatographia 48 (1998) 245.
[21] X.Z. Xiao, Y.Q. Feng, S.L. Da, Y.Y. Chen, Y. Zhang, Chromatographia 49 (1999) 643.
[22] X.Z. Xiao, Y.Q. Feng, S.L. Da, Y. Zhang, Anal. Lett. 33 (15) (2000) 3355.
[23] Y.X. Xiao, X.Z. Xiao, Y.Q. Feng, Z.H. Wang, S.L. Da, Talanta 56 (2002) 1141.
[24] L.S. Li, M. Liu, S.L. Da, Y.Q. Feng, Talanta 62 (2004) 643.
[25] L.S. Li, S.L. Da, Y.Q. Feng, M. Liu, J. Chromatogr. A 1040 (2004) 53.
[26] T. Sokoließ, D. Keßler, U. Menyes, U. Roth, T. Jira, Laborpraxis 7–8 (2000) 70.
[27] T. Sokoließ, U. Menyes, U. Roth, T. Jira, J. Chromatogr. A 948 (2002) 309.
[28] T. Sokoließ, U. Menyes, U. Roth, T. Jira, J. Chromatogr. A 898 (2000) 35.
[29] G. Arena, A. Casnati, A. Contino, L. Mirone, D. Sciotto, R. Ungaro, Chem. Commun. (1996) 2277.
[30] A. Casnati, A. Pochini, R. Ungaro, F. Ugozzoli, F. Arnaud, S. Fanni, M.J. Schwing, R.J. Egberink, J. Am. Chem. Soc. 117 (1995) 2767.
[31] A. Casnati, A. Pochini, R. Ungaro, C. Bocchi, F. Ugozzoli, R.J. Egberink, H. Struijk, R. Lugtemberg, F. Jong, D.N. Reinhoudt, Chem. Eur. J. 2 (1996) 436.
[32] K. Sugden, C. Hunter, G. Lloyd-Jones, J. Chromatogr. 192 (1980) 228.
[33] J.D. van Loon, A. Arduini, L. Coppi, W. Verboom, A. Pochini, R. Ungaro, S. Harkema, D. Reinhoudt, J. Org. Chem. 55 (1990) 5639.
[34] T. Ihara, Y. Sugimoto, M. Asada, T. Nakagama, T. Hobo, J. Chromatogr. A 694 (1995) 49.
[35] B. Feibush, M.J. Cohen, B.L. Karger, J. Chromatogr. 282 (1983) 3.
[36] D. Gasparrini, D. Misiti, C. Vallani, F. La Torre, M. Sinibaldi, J. Chromatogr. 457 (1999) 235.
[37] J.K. Suh, I.W. Kim, S.H. Chang, Bull. Korean Chem. Soc. 22 (2001) 409.
[38] T. Zhao, X. Hu, J. Cheng, X. Lu, Anal. Chim. Acta 358 (1998) 263.
[39] L.R. Snyder, J.W. Dolan, J.R. Gant, J. Chromatogr. 165 (1979) 3.
[40] P.J. Schoenmakers, H.A.H. Billiet, R. Tijssen, L. De Galan, J. Chromatogr. 149 (1978) 519.