Phosphorylated calix[4,8]arenes improve the RP HPLC separation of benzene derivatives

Aim. To study the effect of 5,11,17,23-tetrakis(diisopropoxyphosphonyl)-25,26,27,28-tetrapropoxy-calix[4]arene and octakis(diethoxyphosphoryloxy)-tert-butylcalix[8]arene additives to the MeCN–H₂O mobile phase (86:14) on the selectivity of the separation of aromatic compounds by the reversed-phase high performance liquid chromatography (RP HPLC) using a Separon SGX C18 support.

Results and discussion. The process of complexation of phosphorylated calix[4,8]arenes with benzene derivatives in the mobile phase plays a key role in the RP HPLC separation of analytes. The stability constants of the inclusion complexes and the chromatographic separation coefficients of the analytes depend on the nature of the aromatic compounds and the cavity size of the calixarene macrocycle.

Experimental part. The HPLC analysis was performed in acetonitrile–water (86:14) solution using a Separon SGX C18 column. The stability constants of the calixarene complexes were determined using the dependence of 1/k' on the calixarene concentration in the mobile phase. Molecular modelling of the calixarene complexes was carried out using a Hyper Chem 8.0 program.

Conclusions. The phosphorus-containing calixarenes due to their ability to form supramolecular complexes with aromatic molecules can be used as additives to the RP HPLC mobile phase and improve separation of benzene derivatives.

Key words: calixarenes; benzene derivatives; inclusion complexes; stability constants; separation selectivity; RP HPLC; molecular modelling
the host–guest inclusion complexes of benzene derivatives were found. The complexation is influenced by log \( P \), the volume and other parameters of analytes (Fig. 1).

Results and discussion

Complexation of calix[4]arene 1 and calix[8]arene 2 with benzene derivatives was studied by the RP HPLC method described in papers [18, 19]. Calix[4]arene 1 and calix[8]arene 2 were characterized by linear adsorption isotherms (Fig. 2, 3) that indicated their reversible adsorption by the column surface. Due to these characteristics, calix[4,8]arenes 1, 2 are potential selectors for the RP HPLC separation of analytes.

The calixarene additives to the mobile phase decrease the capacity coefficient \( k' \) of benzene derivatives due to the formation of the host–guest inclusion complexes. The linear plots of \( 1/k' \) vs the calix[4]arene and calix[8]arene concentration (Fig. 4, 5) indicate the formation of the host–guest inclusion complexes with stoichiometry in the ratio of 1:1. It allows using the equation (1) for calculation of their stability constants \( K_A \):

\[
\frac{1}{k'} = \frac{1}{k'_0} + K_A \times [\text{CA}] / k'_0,
\]

where: \( k'_0, k' \) are capacity factors of the benzene derivatives determined in the absence and in the presence of calix[4,8]arenes in the mobile phase.

It has been shown that the addition of calixarenes to the mobile phase improves the separation selectivity of benzene analytes (Tables 1, 2). The separation coefficient \( (\alpha) \) calculated as the ratio of the retention times of the pair of analytes depends on the size of the macrocyclic skeleton, as well as the nature, quantity and position of the substituent in the molecules of aromatic analytes.

According to the data presented in Table 1, the addition of phosphorylated calix[4]arene 1 to the mobile phase increases the analyte separation \( \alpha_{10}/\alpha_0 = 1.10–2.05 \).

The best separation has been observed for the pairs of analytes, such as \( p \)-aminophenol vs iodobenzene.
(α₁/α₂ = 1.78), phenol vs p-aminophenol (α₁/α₂ = 2.05),
veratrole vs guaiacol (α₁/α₂ = 1.42), m-toluidine vs
N,N-dimethyl-p-toluidine (α₁/α₂ = 1.42), and m-xylene
vs chlorobenzene (α₁/α₂ = 1.36). Other pairs of ana-
lytes were separated by calixarene 1 less effectively.

Calix[8]arene 2 additive to the mobile phase also
improves the separation selectivity of benzene deri-
vatives (α₁/α₂ = 1.06–1.74). The best separation was
observed for the following analytes: resorcinol vs
trichloromethylbenzene (α₁/α₂ = 1.74), toluene vs
resorcinol (α₁/α₂ = 1.71), toluene vs p-methylphenol
(α₁/α₂ = 1.58), trichloromethylbenzene vs trifluoro-
methylbenzene (α₁/α₂ = 1.49) and phenol vs veratrol
(α₁/α₂ = 1.32) (Table 2).

The increase in separation selectivity of the analytes
after the calixarene addition is explained by the forma-
tion of the host–guest inclusion complexes with dif-
ferent adsorption on the stationary phase compar-
ted to free analytes. The stability constants K₁ of
the complexes depend on the calixarene structure,
as well as on the nature, quantity, position of the sub-
stituents in the benzene analytes. The ratio of the se-
paration selectivity (α₁/α₂) induced by calixarene ad-
ditives 1, 2 correlates with the ratio of the stability
constants of its complexes (S = K₁/K₂) (Fig. 6, 7).

The complexes can be stabilized by hydrogen bonds,
π–π, C–H···π, solvophobic interactions and van der Waals
forces between the calixarene and analytes. Fig. 8 il-
lustrates the energy minimized molecular structures
of calixarene 1 and its complex with p-aminophenol.

Calixarene 1 functionalized by phosphonyl groups
at the upper rim of the macrocycle exists in the cone-
conformation (Fig. 8a). Complexation with p-aminophenol
does not change the conformation. For calix-
arene 1 there is the possibility of forming two types
of the complexes with p-aminophenol. In the comp-
lex b (Fig. 8b), the phenolic group forms the hydro-
gen bond with an oxygen atom of the phosphoryl
group at the upper rim. In the complex c (Fig. 8c),
the phenolic group creates the hydrogen bond with
an ether oxygen atom at the lower rim.

Octaphosphorylated calix[8]arene 2 (Fig. 9a) that
is larger by size with free rotation of the aromatic
fragments around Ar–CH₂–Ar bonds changes its confor-
mation after complexation with phenol. A phenol
molecule is located in the center of the calixarene cavi-
ty and forms the hydrogen bond Ph–O–H···O(Ph=O)Ar
with an ether oxygen atom (Fig. 9b).

**Experimental part**

The calixarenes were synthesized by the methods
described previously: 1 [18] and 2 [19]. Acetonitrile
was obtained from the Acros Organics (Thermo
Fisher Scientific, New Jersey, USA).

**HPLC analysis**

The HPLC analysis was performed using the liquid
chromatography system (Hitachi, Ltd., Tokyo, Japan).
The column (250 × 4.6 mm id) was packed with Sepa-
ron SGX C18 (Merck, Darmstadt, Germany). Experi-
ments were performed in isocratic conditions. The acet-
onitrile–water (86:14, v/v) mixture was used as the mo-
bile phase. The calixarene concentrations in the mo-
bile phase were 0.05–0.6 mM. The UV detector was
operated at the wavelength of 254 nm, and the flow
rate was 0.8 mL/min. The samples of the analytes used
for injections were dissolved in the same acetonitrile–
water (86:14, v/v) mixture (c = 0.01 mM). All chro-
matograms were obtained at 26°C. The mobile phase
which contained the calixarene additive was equili-
brated for 3 h before the analysis. Under these con-
ditions the chromatographic column was saturated
with the calixarene additive.

![Fig. 4. Plots of 1/k’ for some benzene derivatives: veratrol, guaiacol, phenol, benzene, toluene and trichloromethylbenzene vs the calix[4]arene 1 concentration in the mobile phase (R² = 0.95–0.99)](image)

![Fig. 5. Plots of 1/k’ for some benzene derivatives: p-bromophenol, p-fluorophenol, chlorobenzene, p-chlorophenol, trichloromethylbenzene, m-xylene and p-aminophenol vs the calix[8]arene 2 concentration in the mobile phase (R² = 0.95–0.99)](image)
Table 1

| Analytes separated                   | Selectivity without calixarene 1 additive, \( \alpha_0 \) | Selectivity with calixarene 1 additive, \( \alpha_1 \) | Selectivity ratio, \( \alpha_1/\alpha_0 \) | Stability constant ratio, \( S = K_1/K_2 \) [18] |
|------------------------------------|----------------------------------------------------------|-----------------------------------------------------|---------------------------------------------|--------------------------------------------------|
| veratrol vs guaiacol               | 1.18                                                     | 1.67                                                | 1.42                                        | 2.02                                             |
| p-xylene vs isopropylbenzene       | 2.00                                                     | 2.20                                                | 1.10                                        | 1.11                                             |
| guaiacol vs trifluoromethylbenzene | 1.78                                                     | 1.88                                                | 1.10                                        | 1.09                                             |
| p-xylene vs benzyl alcohol         | 1.82                                                     | 1.98                                                | 1.10                                        | 1.39                                             |
| p-cyanophenol vs p-aminophenol     | 1.20                                                     | 1.44                                                | 1.20                                        | 1.06                                             |
| m-xylene vs chlorobenzene          | 1.00                                                     | 1.36                                                | 1.36                                        | 2.58                                             |
| p-fluorophenol vs trifluoromethylbenzene | 1.00                                         | 1.11                                                | 1.11                                        | 1.03                                             |
| m-toluidine vs N,N-dimethyl-p-toluidine | 3.65                      | 5.20                                                | 1.42                                        | 1.88                                             |
| toluene vs p-methoxytoluene        | 3.31                                                     | 4.50                                                | 1.36                                        | 1.81                                             |
| phenol vs guaiacol                 | 1.03                                                     | 1.77                                                | 1.71                                        | 4.06                                             |
| p-aminophenol vs iodobenzene       | 1.53                                                     | 2.72                                                | 1.78                                        | 4.70                                             |
| phenol vs m-nitropenol             | 1.68                                                     | 1.86                                                | 1.11                                        | 1.22                                             |
| phenol vs p-aminophenol            | 1.11                                                     | 2.28                                                | 2.05                                        | 10.28                                            |
| trifluoromethylbenzene vs trichloromethylbenzene | 2.28                  | 3.47                                                | 1.52                                        | 10.92                                            |

Notes: * – the uncertainties of the parameters were 5–7%.

Table 2

| Analytes separated                  | Selectivity without calixarene 2 additive, \( \alpha_0 \) | Selectivity with calixarene 2 additive, \( \alpha_1 \) | Selectivity ratio, \( \alpha_1/\alpha_0 \) | Stability constant ratio, \( S = K_1/K_2 \) [19] |
|------------------------------------|----------------------------------------------------------|-----------------------------------------------------|---------------------------------------------|--------------------------------------------------|
| benzene vs phenol                  | 1.11                                                     | 1.47                                                | 1.22                                        | 1.40                                             |
| phenol vs p-chlorophenol           | 1.04                                                     | 1.17                                                | 1.13                                        | 1.12                                             |
| phenol vs p-methylphenol           | 1.04                                                     | 1.06                                                | 1.02                                        | 1.18                                             |
| phenol vs guaiacol                 | 1.03                                                     | 1.09                                                | 1.06                                        | 1.18                                             |
| phenol vs resorcinol               | 1.08                                                     | 1.15                                                | 1.06                                        | 1.32                                             |
| phenol vs veratrol                 | 1.00                                                     | 1.32                                                | 1.32                                        | 1.25                                             |
| toluene vs resorcinol              | 1.12                                                     | 1.91                                                | 1.71                                        | 1.22                                             |
| benzene vs trichloromethylbenzene  | 2.55                                                     | 3.41                                                | 1.34                                        | 2.43                                             |
| benzene vs toluene                 | 1.04                                                     | 1.36                                                | 1.31                                        | 1.55                                             |
| benzene vs resorcinol              | 1.08                                                     | 1.40                                                | 1.30                                        | 1.89                                             |
| benzene vs trifluoromethylbenzene  | 1.77                                                     | 1.97                                                | 1.11                                        | 1.22                                             |
| trichloromethylbenzene vs trifluoromethylbenzene | 1.29                      | 1.92                                                | 1.49                                        | 2.96                                             |
| benzene vs guaiacol                | 1.02                                                     | 1.12                                                | 1.10                                        | 1.18                                             |
| benzene vs veratrole               | 1.00                                                     | 1.06                                                | 1.06                                        | 1.12                                             |
| toluene vs p-methylphenol          | 1.00                                                     | 1.58                                                | 1.58                                        | 1.07                                             |
| resorcinol vs p-fluorophenol       | 1.03                                                     | 1.43                                                | 1.38                                        | 2.02                                             |
| resorcinol vs p-chlorophenol       | 1.12                                                     | 1.34                                                | 1.20                                        | 1.21                                             |
| resorcinol vs trifluoromethylbenzene | 2.13                      | 2.49                                                | 1.17                                        | 1.55                                             |
| resorcinol vs trichloromethylbenzene | 2.75                  | 4.79                                                | 1.74                                        | 4.59                                             |

Notes: * – the uncertainties of the parameters were 5–7%.
The molecular modelling of calixarenes 1, 2 and their complexes with the analytes was carried out by the molecular mechanics MM+ method, the force field (PM3) (Hyper Chem software package, version 8) [20]. The structures were calculated by the semi-empirical method. The RMS (standard deviation of the word root mean square) gradient was equal to 0.01 kcal/A·mol.

Conclusions

The addition of phosphorylated derivatives of calix[4]arene and calix[8]arene to the acetonitrile–water mobile phase improves the selectivity of the chromatographic separation of benzene analytes on the Separ on SGX C18 stationary phase under HPLC. The improvement of the separation selectivity is explained by forming calixarene–analyte inclusion complexes with different sorption on the stationary phase compared to those of free analytes. The efficacy of such separation depends on the size of the calixarene backbone, as well as the nature, number and position of the substituents in the benzene analyte determining the structure and stability of the inclusion complexes.

Conflict of interests: authors have no conflict of interests to declare.
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