Comparison of Supercritical Fluid Chromatographic and High-Performance Liquid Chromatographic Separations of *p-tert*-Butylcalix[*n*]arenes

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Abstract

Supercritical fluid chromatography (SFC) using packed analytical columns is compared with high-performance liquid chromatography (HPLC) for the separation of the readily available calixarenes, *p-tert*-butylcalix[4]arene, *p-tert*-butylcalix[6]arene, and *p-tert*-butylcalix[8]arene. With methanol as a modifier in supercritical carbon dioxide, *p-tert*-butylcalix[8]arene fails to elute from any of the four different columns used (ODS, RP-18, Cyano, and Diol), although the smaller calixarenes elute; *p-tert*-butylcalix[4]arene always precedes *p-tert*-butylcalix[6]arene. By using chloroform modifier under gradient conditions, all three can be resolved, and a retention time as short as 1.4 min is obtained for *p-tert*-butylcalix[8]arene on an RP-18 column. The total run time including re-equilibration is slightly over 3 min. In contrast, the shortest retention time obtained for *p-tert*-butylcalix[8]arene by HPLC (150-mm ODS column, 3-µm particle size, isocratic conditions, acetonitrile–ethyl acetate mobile phase [80:20]) is approximately 5 min. Peaks are both narrower and more symmetrical under SFC rather than HPLC conditions.

Introduction

The calixarenes (1,2) are macrocylic oligomers formed by condensation of *p*-substituted phenols and formaldehyde (Figure 1). They are particularly readily synthesized when the number of phenolic residues in the macrocycle is 4, 6, or 8. The name given to the whole family is based on the known chalice or cuplike shape of the smallest member of the series, the one that contains four phenolic residues and is therefore designated *p-tert*-butylcalix[4]arene (1). The larger calixarenes usually adopt structures that are closer to planar, though all the parent (phenolic) calixarenes are conformationally flexible, and many different forms have been characterized through crystallographic studies (2,3). Evidence has been obtained (1,3) that calix[*n*]arenes with *n* values as great as 16 are present in reaction mixtures from which the species with *n* values of 4, 6, and 8 are isolated in good yields. Particular effort has been

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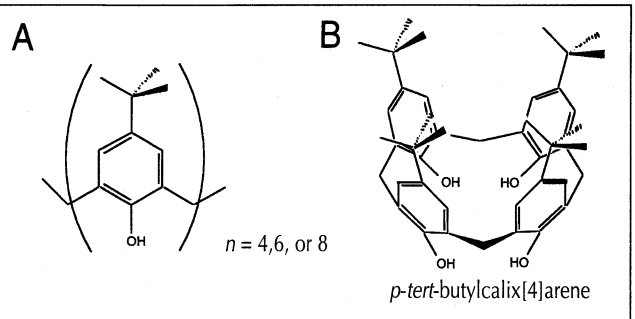

Figure 1. Generic structure of the *p-tert*-butylcalix[*n*]arenes (A) and the cone conformation of *p-tert*-butylcalix[4]arene (B).

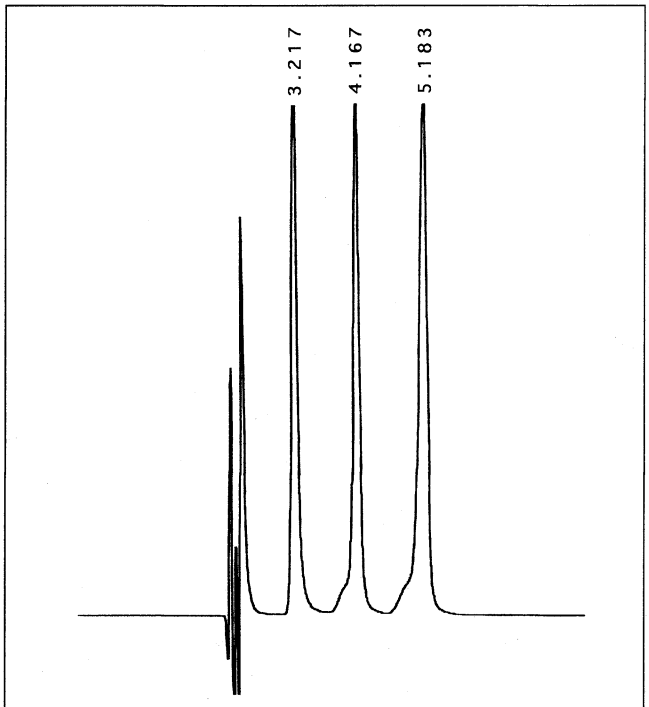

Figure 2. HPLC chromatogram with a mobile phase of MeCN–EtOAc–CH_3COOH (80:19.9:0.1). Flow rate, 1 mL/min; temperature, ambient. Elution order: solvent, *p-tert*-butylcalix[4]arene, *p-tert*-butylcalix[6]arene, *p-tert*-butylcalix[8]arene.

invested in optimizing yields for and isolating the odd species with n values of 5 and 7 (and higher) (C.D. Gutsche and D. Stewart, Texas Christian University, personal communication, 1995). High-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), and flash chromatography have been extremely useful in these efforts (1,4–6). Nonetheless, there remains a significant need for an efficient chromatographic method for the separation of complex mixtures commonly obtained from both direct calixarene syntheses and the formation of calixarene derivatives (7,8). The present work describes our initial results in attempting a thorough assessment of the potential of supercritical fluid chromatography (SFC) to meet this need. A particular stimulus in this work was our interest in the coordination chemistry of the calixarenes (3) and the possibility of the use of calixarenes in the solvent extraction of metals (9,10). Because this work was largely based on the easily isolated and purified *p-tert*-butylcalix[n]arenes (n = 4, 6, and 8), we chose to commence our SFC studies with the same compounds. Since the first HPLC studies of calixarene separations (1,4–6), both HPLC and SFC technologies have improved considerably, and the efficiency of SFC has been demonstrated in many particularly difficult systems (11,12), therefore, we made a careful comparison of the two techniques.

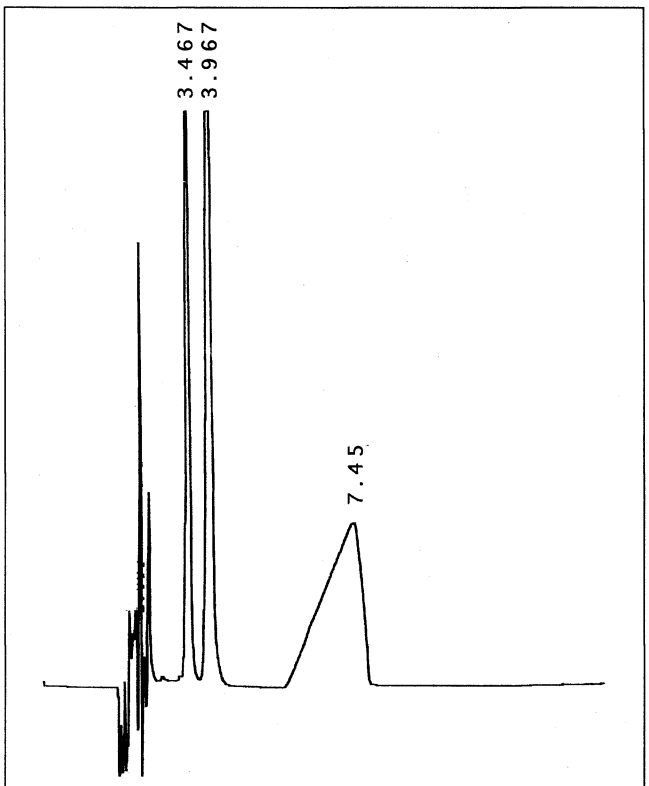

Figure 3. HPLC chromatogram with the earlier mobile phase of MeOH–EtOAc–CH_3COOH (80:19.9:0.1). Flow rate, 1 mL/min; temperature, ambient. Elution order: solvent, *p-tert*-butylcalix[4]arene, *p-tert*-butylcalix[6]arene, *p-tert*-butylcalix[8]arene (tailing badly at 7.5 min).

Table I. HPLC Retention Times (R_t) and Capacity Factors (k') of Calixarenes with Varying Percentages of Chloroform in the Mobile Phase

	p-tert-Butylcalix[4]arene		*p-tert*-Butylcalix[6]arene		*p-tert*-Butylcalix[8]arene	
% Chloroform	R_t	k'	R_t	k'	R_t	k'
0	3.21	0.33	4.16	0.64	5.18	1.02
4	2.91	0.29	3.48	0.53	4.11	0.83
8	2.79	0.25	3.23	0.45	3.65	0.63
12	2.67	0.21	3.02	0.37	3.30	0.50

Experimental

Synthesis

p-tert-Butylcalix[4]arene (13), *p-tert*-butylcalix[6]arene (14), and *p-tert*-butylcalix[8]arene (15) were prepared and purified by methods in the literature.

Chromatography

HPLC experiments were conducted with a system comprising a Shimadzu (Kyoto, Japan) LC-9A pump and a Shimadzu SPD-6A ultraviolet (UV) detector with a Rheodyne (Berkeley, CA) 20-µL injection valve connected to a Phase Separations Spherisorb (Norwalk, CT) ODS column (150 × 4.6-mm i.d., 3-µm particle size). All separations were conducted at ambient temperature and a flow rate of 1 mL/min unless otherwise stated. The absorption due to eluted calixarenes was monitored at a wavelength of 288 nm, and a Shimadzu C-R6A Chromatopac integrator was used for data analysis.

All SFC experiments were conducted using a Hewlett-Packard (Wilmington, DE) G1205A SFC with two pumps and diode array detection. The chromatograph was used in its "downstream" pressure-regulation configuration; it was equipped with an HP 7673 autosampler connected to a Rheodyne model 7410-077 valve with a 0.5-µL internal loop. HP MS-DOS Chemstation software was used to collect and process data. A monitoring wavelength of 288 nm was used in all chromatograms shown herein. Column temperatures of 40, 60, and 80°C were used with a column flow rate of 2 mL/min unless otherwise stated. The columns for the SFC experiments were a Hypersil (Runcorn, UK) ODS (200 × 4.6-mm i.d., 5-µm particle size), a LiChrosorb (Kilsyth Vic, Australia) RP-18 (200 × 4.6-mm i.d., 5-µm particle size), a Deltabond (Bellefonte, PA) Cyano (250 × 4.0-mm i.d., 5-µm particle size), and a Phenomenex (Torrance, CA) Diol (250 × 4.6-mm i.d., 5-µm particle size).

The carbon dioxide used for SFC was Air Liquide (Perth, Australia) anaerobic grade, with a stated purity of 99.99%. Methanol (Fisher Chemicals, Fair Lawn, NJ) modifier in SFC was dried by reaction with magnesium turnings and subsequently distilled, then filtered through a Whatman (Singapore) 0.2-µm membrane before use. Chloroform (LabScan, Dublin, Ireland) modifier was fractionally distilled from molecular sieves and shielded from light during storage. HPLC-grade ethyl acetate and acetonitrile were purchased from J.T. Baker (Phillipsburg, NJ) and used without further treatment.

Results and Discussion

The extensive inclusion chemistry of the calixarenes (1,3) indicates that an appropriate choice of stationary phase could be the basis of highly discriminatory separations. For example, it is known (1) that H-bond acceptor solvents can disrupt the phenolic H-bonding array that stabilizes the cone conformation of *p-tert*-butylcalix[4]arene, and hence a polar surface may alter the effective size and shape of this molecule in a very different way than a nonpolar surface. It is also well-known that methyl groups adjacent to polar centers readily insert into the cone of *p-tert*-butylcalix[4]arene (3), but there is only evidence of very weak (if any) interactions with methyl groups at the termini of long aliphatic chains (16,17). Hence, a reversed-phase ODS or RP-18 chromatographic support might be expected to interact with calixarenes through nonspecific dispersion forces and thus discriminate on the basis of size only. Indeed, retention times dependent on ring size were found in early HPLC studies of calixarene separation on reversed-phase columns (as well as on normal silica columns, where it is possible that the number of surface oxide–phenolic hydroxyl interactions determines retention times) (4–6), and our present results confirmed these observations for separations of somewhat improved quality. Thus, a chromatogram for the separation of *p-tert*-butylcalix[4]arene, *p-tert*-butylcalix[6]arene, and *p-tert*-butylcalix[8]arene by HPLC on an ODS column using acetonitrile–ethyl acetate–acetic acid 80:19.9:0.1 as the mobile phase is shown in Figure 2. The calixarenes eluted with baseline separation and in order of ring size; the largest one eluted in slightly over 5 min. Considerable reduction of retention times could be achieved by the addition of chloroform to create a quaternary mobile phase (Table I). Good peak symmetry was observed with the addition of up to 12% of chloroform, but baseline resolution could not be achieved. Use of the methanol–ethyl acetate–acetic acid mobile phase developed for earlier work (4,5) provided acceptable separation (Figure 3) approximately three times faster than in the original work, but the *p-tert*-butylcalix[8]arene peak was still very broad.

Table II. Dependence of SFC Retention Times and Capacity Factors on Pressure and Temperature*

	Temperature (°C)	150 bar R_t	150 bar k'	200 bar R_t	200 bar k'	250 bar R_t	250 bar k'
p-tert-Butylcalix[4]arene	40	2.782	1.53	2.500	1.27	2.340	1.13
	60	2.709	1.46	2.228	1.03	1.992	0.81
	80	3.223	1.93	2.191	0.99	1.832	0.67
p-tert-Butylcalix[6]arene	40	3.768	2.43	3.264	1.97	2.986	1.71
	60	3.631	2.30	2.792	1.54	2.405	1.19
	80	4.776	3.34	2.800	1.55	2.185	0.99

* Column: Hypersil ODS (200 x 4.6 mm; 5-µm particle size); modifier: 20% MeOH (v/v).

Table III. SFC Retention Times for Varying Pressures and Modifier Percentages*

	Modifier % (v/v)	150 bar R_t	150 bar k'	200 bar R_t	200 bar k'	250 bar R_t	250 bar k'
p-tert-Butylcalix[4]arene	5	4.660	3.24	3.607	2.28	3.089	1.81
	10	3.629	2.30	3.002	1.73	2.760	1.51
	20	2.782	1.53	2.500	1.27	2.340	1.13
	30	1.487	0.04	2.344	1.13	2.245	1.04
p-tert-Butylcalix[6]arene	5	9.899	8.00	6.836	5.21	5.482	3.98
	10	6.667	5.06	4.647	3.22	3.958	2.60
	20	3.768	2.43	3.264	1.97	2.986	1.71
	30	3.103	1.82	2.878	1.62	2.742	1.49

* Column: Hypersil ODS (200 x 4.6 mm; 5-µm particle size); column temperature: 40°C.

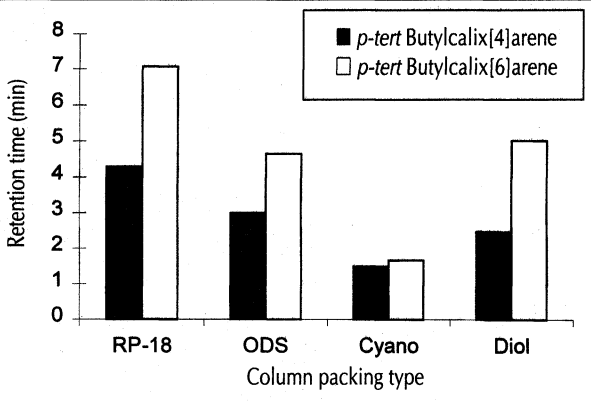

Figure 4. Relative retention times for *p-tert*-butylcalix[4]arene and *p-tert*-butylcalix[6]arene on the different stationary phases. Pressure, 200 bar; temperature, 40°C; flow rate, 2 mL/min.

The four different columns used in SFC were chosen in part because of their range across non-H-bonding, H-bond accepting, and H-bond donating properties. It was not possible on any of the columns, however, to elute the calixarenes with pure supercritical carbon dioxide as the mobile phase; therefore, various modifiers were introduced, and their choice was influenced by consideration of their known inclusion chemistry with calixarenes. Addition of methanol led to appreciable rates of elution for *p-tert*-butylcalix[4]arene and *p-tert*-butylcalix[6]arene. The detailed results of this study into the influence of various physical factors on the separations are given in Tables II and III. *p-tert*-Butylcalix[8]arene did not elute under any conditions with the use of methanol modifier. Retention times decreased as the pressure (and density) of the mobile phase was increased at a constant temperature and as the modifier concentration was increased at a constant temperature and pressure. Globally, these effects are presumably attributable to enhanced calixarene solvation. For a fixed methanol concentration of 20% by volume (14.5

mole %), retention times at a constant pressure increased with increasing temperature at 150 bar but decreased at 200 and 250 bar. This is believed to be due to two competing effects caused by the increase in temperature at isobaric conditions. The first effect was the decrease in density and hence solvating power of the mobile phase, which led to longer analyte residency times in the stationary phase and therefore longer elution times. The other effect of isobaric temperature increase was the desorption of the mobile phase and analyte from the stationary phase, which decreased the capacity factor and hence retention time (18). At 150 bar, the decrease in solvation of the calixarenes was the dominant effect, and the retention times increased. At the higher pressures, the drop in density with increasing temperature was less pronounced, so the decrease in column capacity was dominant. This resulted in the calixarenes preferentially residing in the mobile phase and therefore eluting faster.

A comparison of the chromatographic behavior of *p-tert*-butylcalix[4]arene and *p-tert*-butylcalix[6]arene on the four columns at 200 bar, 40°C, and 20% methanol concentration is given in Figure 4. The smaller calixarene always eluted first, and the Cyano column gave both the poorest resolution and the shortest retention times; this suggested that the surface might have competed only weakly and unselectively with methanol for the solutes.

The use of chloroform as a modifier resulted in the elution of all three calixarenes; this result was consistent with the fact that their solubility in chloroform is far greater than in methanol. Retention times increased with the macrocycle size as before, and the effects of variations in modifier concentration, temperature, and pressure are summarized in Tables IV and V. Differences in the phase behavior of chloroform–CO_2 and methanol–CO_2 were reflected in the different pressures at which the effect of an increase in temperature changed from an increase to a decrease in retention time. This indicated that the mobile phase desorption and subsequent reduction in the capacity factor were not as great for the chloroform–CO_2 system as for the corresponding methanol-based mobile phase. The effect of a change of chloroform modifier concentration at a constant temperature (40°C) and pressure for *p-tert*-butylcalix[4]arene is shown in Figure 5. Variation as a function of modifier concentration was greatest at the lowest concentrations, where the solubility of the calixarene was limited. An optimal separation of all three calixarenes is shown in Figure 6. The peak symmetry was excellent for *p-tert*-butylcalix[4]arene and *p-tert*-butylcalix[6]arene but slightly fronted for *p-tert*-butylcalix[8]arene, which eluted in less than 1.5 min.

Table IV. Dependence of Retention Times and Capacity Factors on Temperature and Pressure*

	Temperature	150 bar		200 bar		250 bar	
	°(C)	R_t	k'	R_t	k'	R_t	k'
	40	1.488	0.35	1.495	0.36	1.447	0.32
p-tert-Butylcalix[4]arene	60	1.517	0.38	1.405	0.28	1.365	0.24
	80	1.816	0.65	1.447	0.32	1.334	0.21
	40	1.675	0.52	1.597	0.45	1.552	0.41
p-tert-Butylcalix[6]arene	60	1.784	0.62	1.558	0.42	1.469	0.34
	80	2.452	1.23	1.689	0.54	1.463	0.33
	40	2.219	1.02	2.004	0.82	1.890	0.72
p-tert-Butylcalix[8]arene	60	2.529	1.30	1.981	0.80	1.759	0.60
	80	4.149	2.77	2.286	1.08	1.797	0.63

* Column: LiChrosorb RP-18 (200 × 4.6 mm; 5-µm particle size); modifier: 20% $CHCl_3$ (v/v).

Table V. Dependence of SFC Retention Times and Capacity Factors with Differing Percentages of Chloroform Modifier*

	Modifier	150 bar		200 bar		250 bar	
	% (v/v)	R_t	k'	R_t	k'	R_t	k'
	15	1.820	0.65	1.718	0.56	1.673	0.52
p-tert-Butylcalix[4]arene	20	1.488	0.35	1.495	0.36	1.447	0.32
	25	1.302	0.18	1.296	0.18	1.306	0.19
	30	1.195	0.09	1.203	0.09	1.218	0.11
	15	2.335	1.12	2.095	0.90	1.977	0.80
p-tert-Butylcalix[6]arene	20	1.675	0.52	1.597	0.45	1.522	0.38
	25	1.368	0.24	1.342	0.22	1.342	0.22
	30	1.198	0.09	1.203	0.09	1.218	0.11
	15	4.137	2.76	3.379	2.07	2.980	1.71
p-tert-Butylcalix[8]arene	20	2.219	1.02	2.004	0.82	1.890	0.72
	25	1.522	0.38	1.482	0.35	1.459	0.33
	30	1.267	0.15	1.257	0.14	1.262	0.15

* Column: LiChrosorb RP-18 (200 × 4.6 mm; 5-µm particle size).

Conclusion

SFC was significantly superior to HPLC for the separation of simple *p-tert*-butylcalix[*n*]arenes (n = 4, 6, and 8), though supercritical carbon dioxide alone was not a useful eluant and required high modifier levels to become effective. In the systems studied, there was no evidence for dramatic effects that might be attributed to specific inclusion characteristics of the calixarenes; retention times followed a simple order of size. It remains possible, however, that none of the chosen stationary phases were particularly appropriate for this purpose and that other surface coatings could prove more effective. We are proceeding to investigate the use of SFC on a wider range of simple calixarenes and on calixarene derivatives.

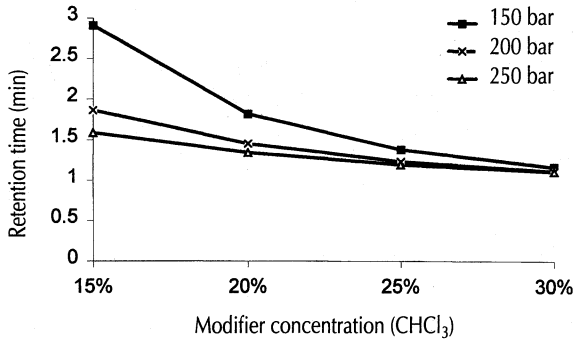

Figure 5. Retention times for *p-tert*-butylcalix[4]arene with varying chloroform modifier concentration at pressures of 150, 200, and 250 bar. Column, Hypersil ODS (200 x 4.6 mm, 5-µm particle size); temperature, 40°C; flow rate, 2 mL/min.

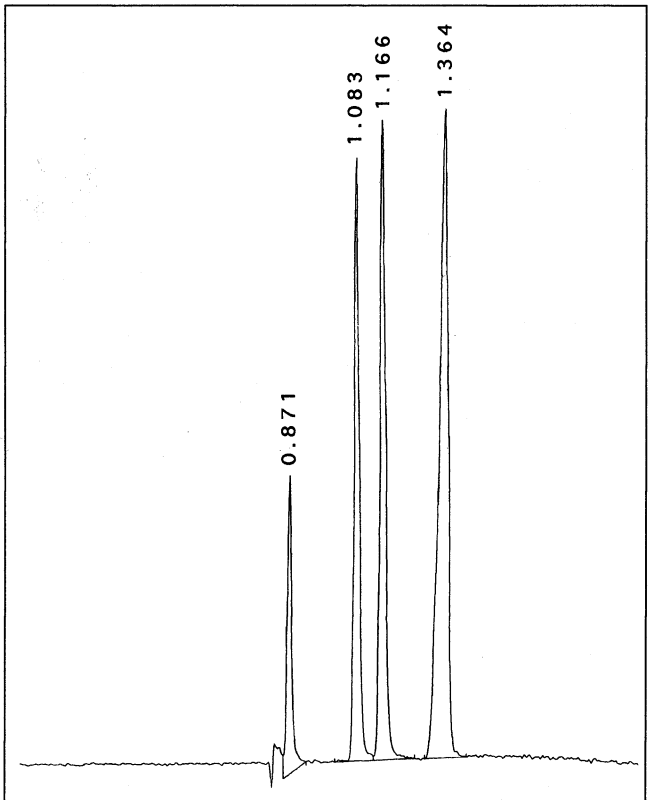

Figure 6. Optimal SFC chromatogram of calixarenes produced using a LiChrosorb RP-18 packed column (200 x 4.6 mm, 5-µm particle size). Eluent, chloroform-modified carbon dioxide; 20% initial modifier percentage, modifier increased 10% per minute to 30% (v/v); initial flow rate, 2 mL/min; gradient at 1 mL/min to 3.5 mL/min; pressure, 250 bar; oven temperature, 80°C; 0.5-µL injection. Elution order: toluene, *p-tert*-butylcalix[4]arene, *p-tert*-butylcalix[6]arene, *p-tert*-butylcalix[8]arene.

Acknowledgments

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