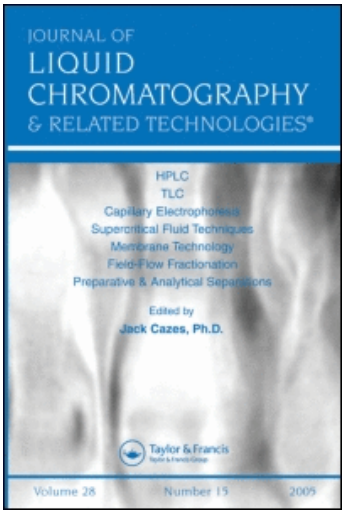

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LIQUID CHROMATOGRAPHIC SEPARATION OF CALIXARENES

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ABSTRACT

In this paper, the retention behavior of p-t-butyl calixarenes: p-t-butylcalix[4]arene, p-t-butylcalix[5]arene, p-t-butylcalix[6]-arene, p-t-butylcalix[7]arene, p-t-butylcalix[8]arene, p-t-butyl-calix[9]arene, p-t-butylcalix[10]arene and the bis-homo-oxacalix[4]arene was examined by reverse phase liquid chroma-tography, on a silica based octadecyl bonded phase with non-aqueous tertiary eluent in a linear gradient elution. The effect of various mobile phase compositions on the capacity ratio, peak asymmetry and resolution were studied. It was found that log k' did not vary linearly with the percentage of methanol, and that the symmetry of peaks was improved when shorter elution times and/or methanol percentage were decreased.

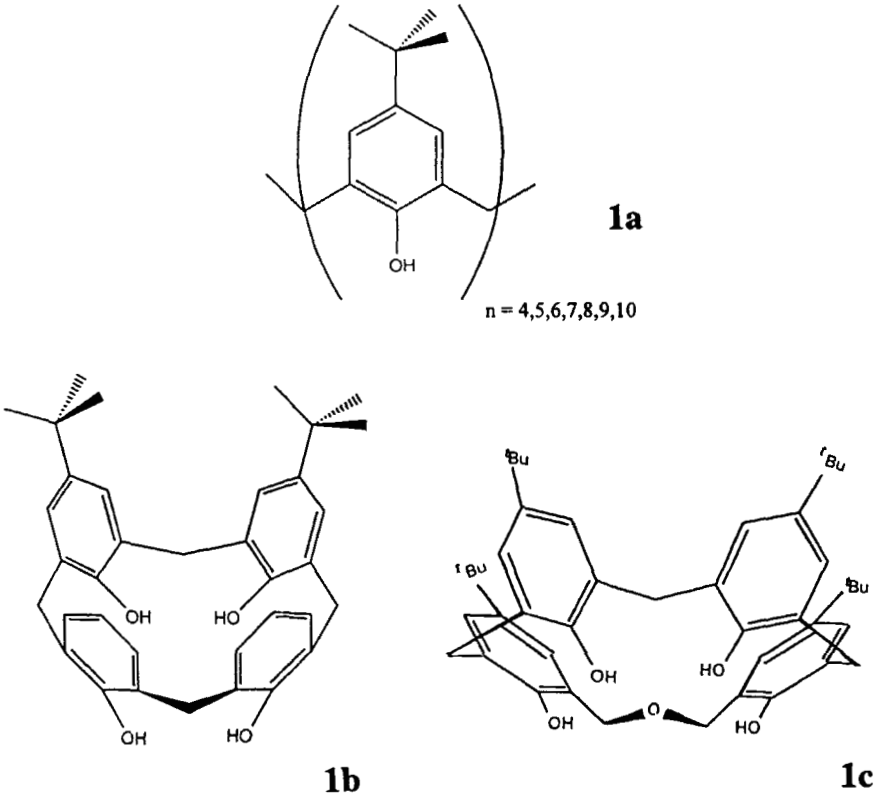

Figure 1. Structures of calixarenes: (1a), basic monomeric structure, (1b), *p*-t-butylcalix[4]arene (front t-butyl groups are omitted for clarity), (1c) bis-homo-oxacalix[4]arene.

INTRODUCTION

Calixarenes are macrocyclic oligomers which are obtained from the condensation of p-t-butylphenol and formaldehyde.[1] The name calixarene[2] is due to the cone conformation adopted by the smallest oligomers, p-t-butylcalix[4]arene, *p*-t-butylcalix[5]arene and bis-homo-oxacalix[4]arene. Their structures can be seen in Figure 1 In order to maximize their *intramolecular* hydrogen bond, when the numbers of phenol units increase the structures become flatter.

Calixarenes can undergo conformational conversion in solution, as evidenced by NMR studies,[3] by free rotation about the σ bonds of the Ar-CH_2-Ar groups. The energy barrier for the ring conversion is decreased by more polar solvents and particularly by solvents which can break hydrogen bonds.[3]

The growing interest in calixarenes derives from their ability to reversibly include smaller molecules and ions,[4] and to act as hosts of neutral organic molecules[5] as well as their selective complexation capability for metal cations such as cesium,[6] and organic cations such as amines.[7,8] These properties are more pronounced in the case of special derivatives than in the parent calixarenes.[9] The high interest in these compounds is also due to the large accessibility of these compounds from inexpensive and readily available starting materials. Accordingly, the synthetic chemistry of calixarenes is widely investigated to explore new aspects in their chemistry.

In order to analyze the reaction mixture and isolate specific compounds, the first chromatographic methods were developed more than 10 years ago, including thin layer chromatography (TLC),[10] flash column chromatography on silica gel,[11] and high performance liquid chromatography (HPLC).[12] In their work on HPLC, Ludwig and Bailie[12] separated reaction mixtures containing up to the heptamer linear oligomer and the octamer cyclic oligomer using a RP-18 reverse phase column and gradient elution with methanol, ethyl acetate and acetic acid. Under these conditions the separation took 35 min, baseline resolution between the trimer and tetramer linear oligomers was not achieved, and calix[8]arene had a very asymmetric peak shape.

In this work we report an alternative HPLC method for the analysis of calixarenes up to the 10^{th} cyclic oligomer. Furthermore, attempts are made to improve the symmetry of peaks and to shorten analysis time.

MATERIALS

Reagents and Materials

P-t-butylcalix[n]arenes standards (Calix[n], n = 4,6,8) and bis-homo-oxacalix[4]arene were synthesized in one of our laboratories (University of Western Australia) by literature methods, whereas calix[5]arene, calix[7]arene, calix[9]arene and calix[10]arene were supplied by Prof. C. D. Gutsche and Dr. D. Stewart. All solvents were of HPLC grade.

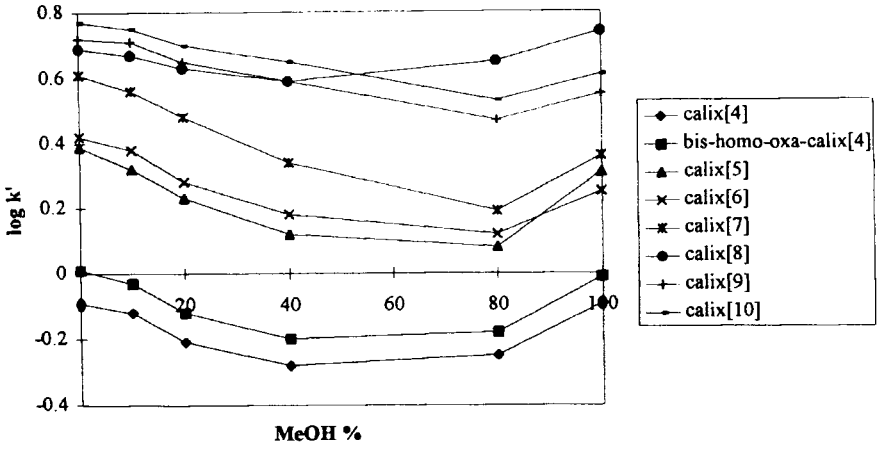

Figure 2. Variation of log k' with the percentage of methanol added in the mobile phase.

Toluene, acetonitrile (ACN), methanol (MeOH) and ethylacetate (EtAc) were purchased from J.T. Baker (Phillipsburg, USA). Chloroform was purchased from Lab Scan (Dublin, Ireland). Dichloromethane was purchased from Fisher Chemical (New Jersey, USA).

HPLC Instrumentation and Conditions

The column used was a Spherisorb ODS (3 μm; 4.6x150 mm) from Phase Separations (Deeside, UK). The flow rate was maintained at 1mL/min. HPLC experiments were conducted at ambient temperature in a Waters 600 E solvent delivery system. Absorbance was measured at 288 nm with a 0.001 absorbance range in a Waters 486 UV detector. Usually injection was of 20μl, performed by a Waters 700 Satellite WISP injector. Data was processed by a Waters Maxima 820 chromatography workstation equipped with version 3.3 software (Waters, Massachusets, USA).

METHODS

To achieve greatest solubilities, calix[4] was dissolved in toluene, calix[6] was dissolved in dichloromethane, and calix[8], calix[9], calix[10] were dissolved in chloroform. Bis-homo-oxacalix[4], calix[5], calix[7] were

dissolved in ethylacetate. Before injection the standard solution was diluted in the mobile phase to the appropriate concentration. Separation was performed using linear gradient elution of 15 min and a tertiary mobile phase of acetonitrile: methanol: ethylacetate. 0.1% of trifluoroacetic acid (TFA) was added to deactivate the free silanols of the partially end-capped column.

RESULTS AND DISCUSSION

The effect of various mobile phase compositions on the retention behavior was studied. The variation of the values of log k' with the percentages of methanol can be seen in Figure 2. It is evident that the retention curves of the calix[5]arene cross over that of calix[6] and the retention curves of calix[8] cross over those of calix[9] and calix[10], i.e. selectivity reversals occur. On the other hand, in contrast to the regular reverse phase behavior, as the percentage of ACN is reduced by increasing volumes of MeOH, the retention factors decrease until between 50% to 80% of MeOH is reached, then retention factors increase again. The retention of the calixarenes in principle is expected to be governed by their hydrophobic properties, π-electron interactions, and attractive interactions of the t-butyl substituents of the solute, and the C_{18} chain of the stationary phase. Methyl groups at the termini of the long aliphatic chains are expected to have very little interaction in terms of inclusion into the hydrophilic cone cavity.[8,13] When we used increased percentages of methanol, additional polar effects were expected to come into play that decrease k' values. We can expect that after the addition of methanol, polar silanols become solvated and therefore silanophilic interactions are lowered. This leads to a decrease in retention times until saturation is reached, then retention times start to increase.

Therefore, we may say that in addition to solvophobic interactions, silanophilic interactions between the solute and accessible silanols are important mechanisms for the separation. There is no evidence of a specific inclusion process that might influence the order of retention of the calixarenes.

Generally, surface silanol interactions can be significant particularly when the eluent has a high organic solvent concentration. We found that the use of a surface modifier is absolutely necessary, and that trifluoroacetic acid is appropriate. Without the addition of TFA, the peaks of calixarenes tailed and finally split indicating a strong interaction with the stationary phase. We have tested the effect of triethylamine (TEA) as a silanol masking agent. We obtained only one peak which was not retained by the column indicating that calixarenes did not interact with the reverse phase stationary phase.

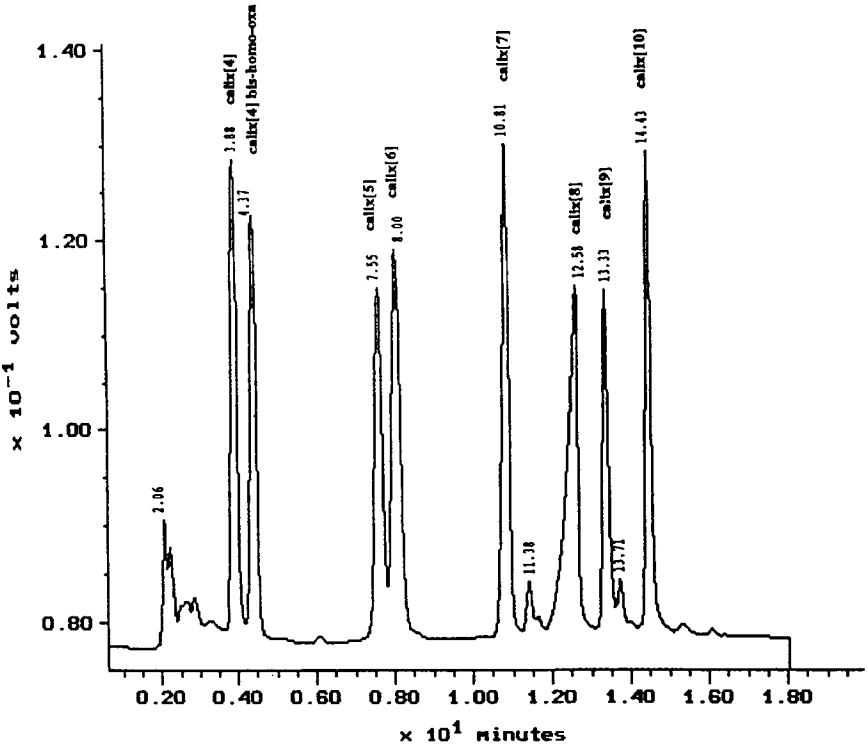

Figure 3. Liquid chromatogram of calixarenes. Conditions: Column, Sperisorb ODS (3μm; 4.6 x 150 mm); flow rate, 1 mL/min; linear gradient elution time, 15min; initial solvent composition 100% ACN, 0% EtAc; final solvent composition 40% ACN, 60% EtAc.

Interaction of TEA with calixarenes has been reported before[8] and it was concluded that interaction between p-t-butylcalix[4] and TEA occurred predominantly through hydrogen bonding or ion pairing formation. However, due to the increased acidity of the hexamer and octamer their interaction resulted in the formation of ions and ion-pairs.

The symmetry of calix[9], calix[10] and especially calix[8] peaks is the most affected when methanol is introduced in the mobile phase. The chromatograms obtained for a linear gradient of 15 min and different percentages of MeOH in the mobile phase are shown in Figures 3 to 7. Calix[8]arene peak does not show a very good symmetry in Figure 3 where the mobile phase did not contain MeOH.

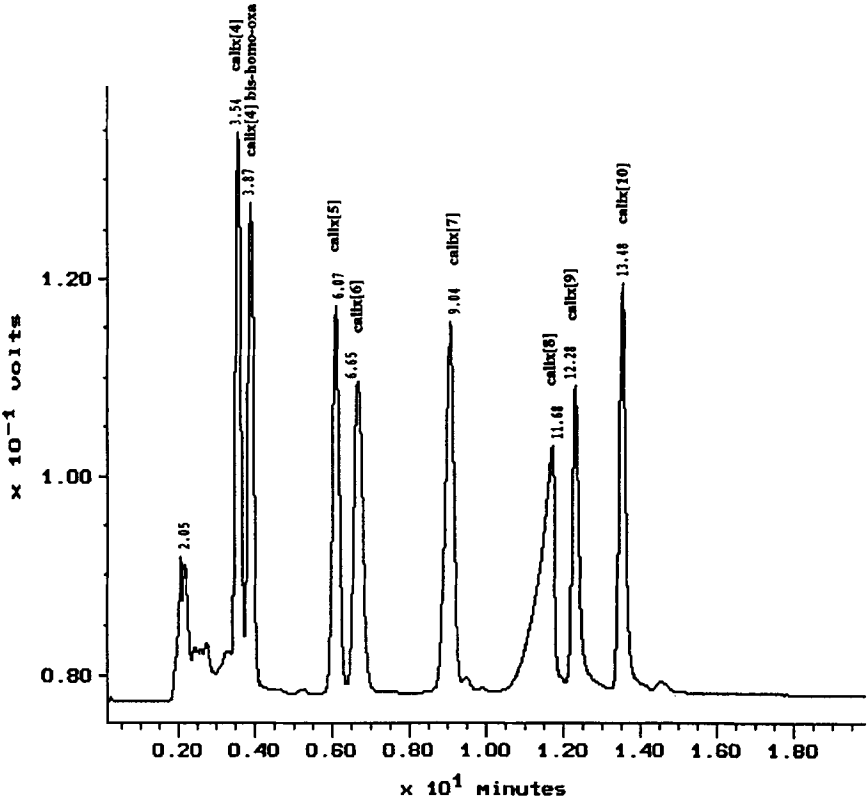

Figure 4. Liquid chromatogram of calixarenes. Conditions: Column, Sperisorb ODS (3μm; 4.6 x 150 mm); flow rate, 1 mL/min; linear gradient elution time, 15min; initial solvent composition 80% ACN, 20% MeOH, 0% EtAc; final solvent composition 40% ACN, 60% EtAc.

The symmetry was strongly affected when increased amounts of methanol were added in the mobile phase as can be seen from Figures 4 to 7. This fact may be due to a special interaction with the stationary phase.[11] Peak symmetry was also influenced by the gradient elution time. Asymmetry values for three different gradient elution times, 15 min, 30 min and 60 min and for two mobile phases; without the addition of methanol and with 10% of methanol, can be seen in Table 1 (only the four calixarenes which have variable asymmetry values are listed). From the asymmetry values we can see that calix[7] and calix[8] exhibit fronting whereas calix[9] and calix[10] exhibit tailing.

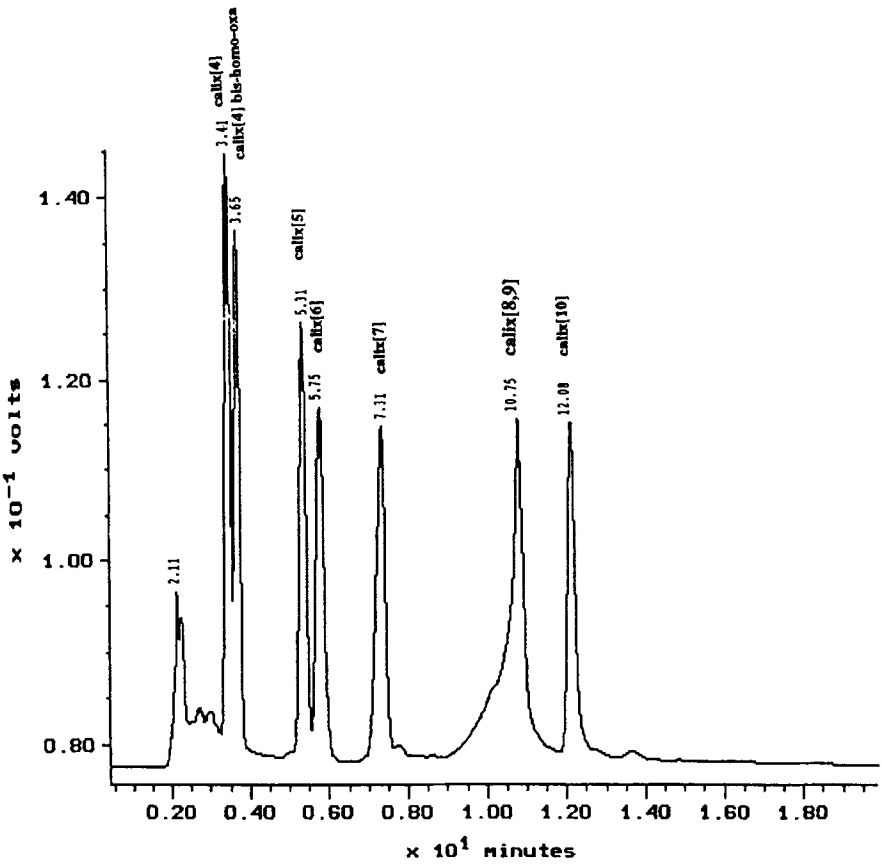

Figure 5. Liquid chromatogram of calixarenes. Conditions: Column, Sperisorb ODS (3μm; 4.6 x 150 mm); flow rate, 1 mL/min; linear gradient elution time, 15min; initial solvent composition, 60% ACN, 40% MeOH, 0% EtAc; final solvent composition 40% ACN, 60% EtAc.

Calix[8] shows very low asymmetry values as the gradient elution time is increased to 30 min and 60 min. The symmetry of calix[7], calix[9] and calix[10] decreases as well, with the increase in the gradient elution time, without improvement in resolution.

Separation of the calixarenes can be achieved without the use of methanol although 10% of methanol gives better baseline separation between calix[5] and calix[6] as can be seen by comparing Figure 3 and Figure 8, and from the log

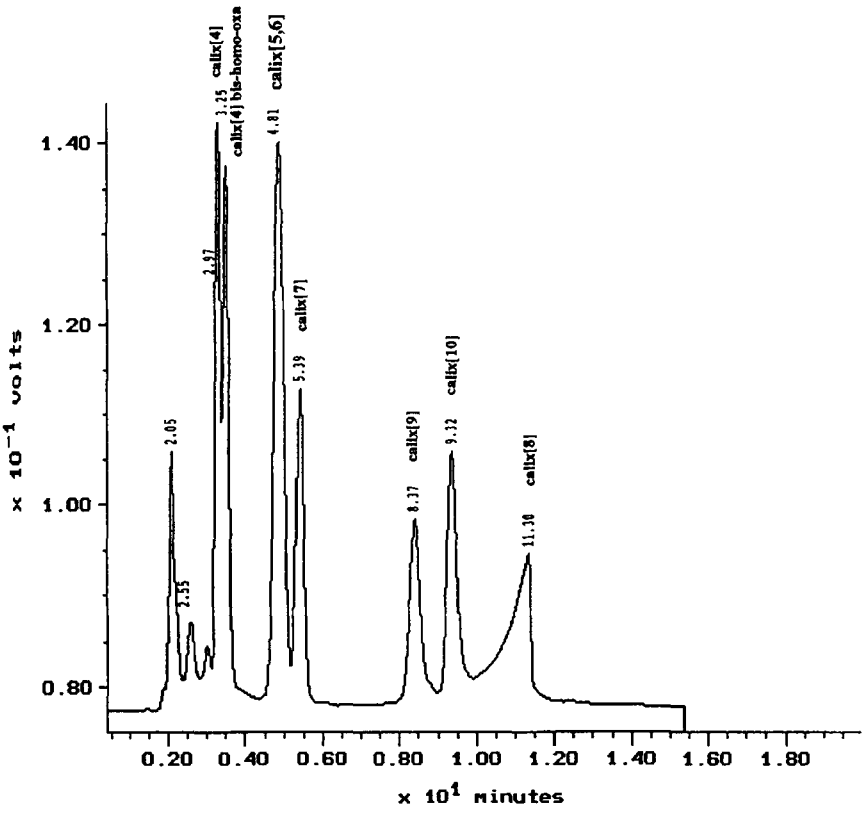

Figure 6. Liquid chromatogram of calixarenes. Conditions: Column, Sperisorb ODS (3μm; 4.6 x 150 mm); flow rate, 1 mL/min; linear gradient elution time, 15min; initial solvent composition 20% ACN, 80% MeOH, 0% EtAc; final solvent composition 40% ACN, 60% EtAc.

Table 1

Asymmetry Values for Different Gradient Elution Times and for Two Mobile Phase Compositions

	15 min		30 min		60 min	
	0% MeOH	10% MeOH	0% MeOH	10%MeOH	0% MeOH	10%MeOH
Calix[7]	1	1	0.79	0.96	0.76	0.76
Calix[8]	0.50	0.45	0.27	0.18	0.20	0.13
Calix[9]	1.16	1.19	1.57	1.79	1.77	2.50
Calix[10]	1.10	1.30	1.33	1.66	1.46	2.45

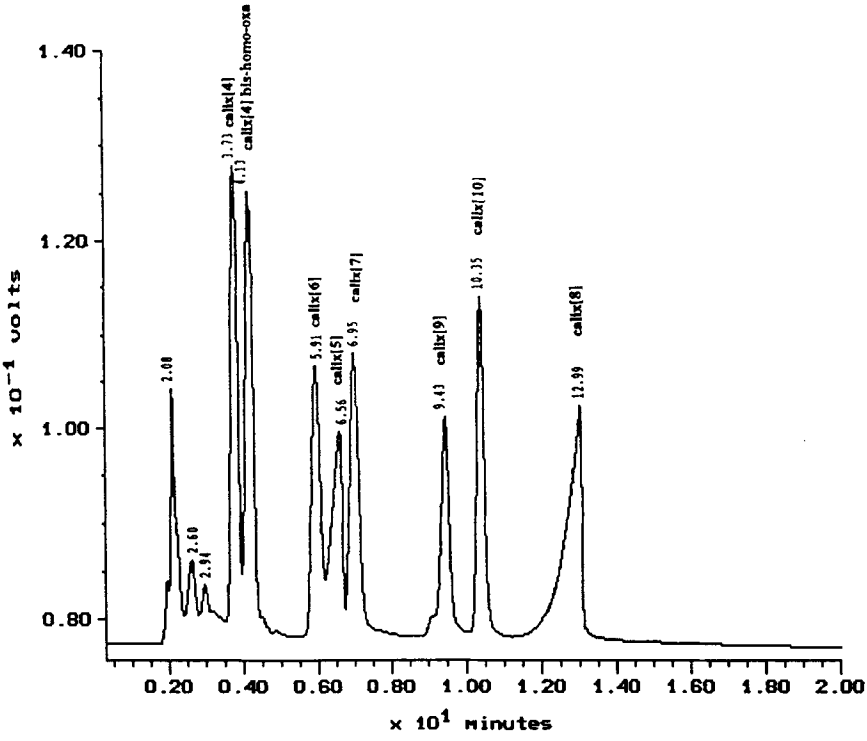

Figure 7. Liquid chromatogram of calixarenes. Conditions: Column, Sperisorb ODS (3μm; 4.6 x 150 mm); flow rate, 1 mL/min; linear gradient elution time, 15min; initial solvent composition 0% ACN,100% MeOH, 0% EtAc, final solvent composition 40% ACN, 60% EtAc.

Table 2

% RSD for the Peak Area and the Retention Time

Calixarene	[4]	oxa	[5]	[6]	[7]	[8]	[9]	[10]
RT	0.19	0.26	0.50	0.36	0.29	0.32	0.28	0.29
PA	2.16	2.37	2.61	2.51	3.44	1.89	4.63	2.52

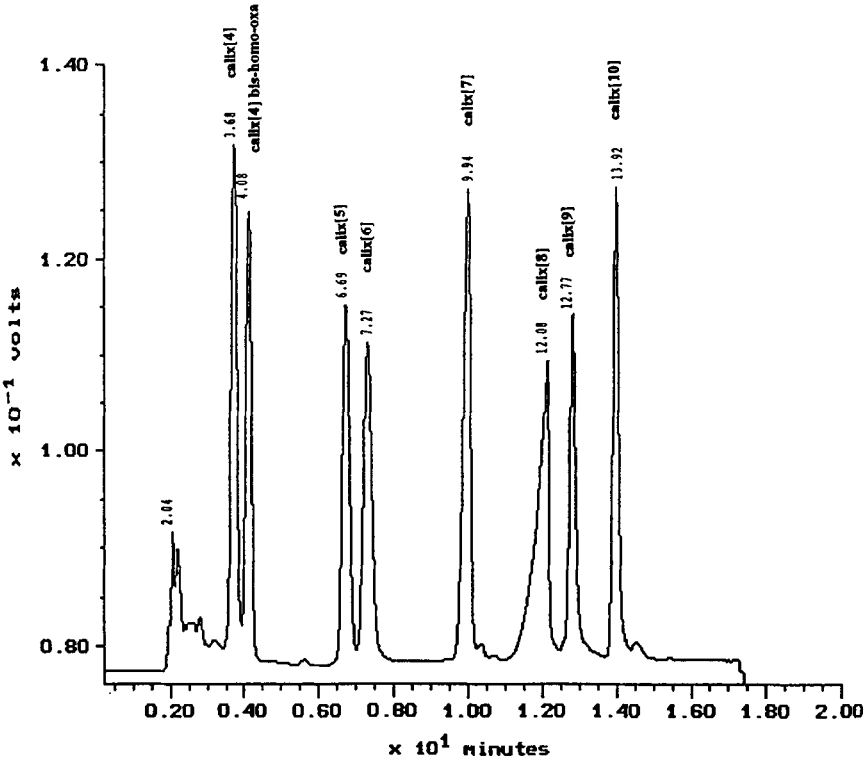

Figure 8. Liquid chromatogram separation of a mixture of calixarenes. Conditions: Column, Sperisorb ODS (3μm; 4.6 x 150 nm); flow rate 1mL/min; linear gradient elution time, 15 min; initial solvent composition 90% ACN, 10% MeOH, 0% EtAc; final solvent composition 40% ACN, 60% EtAC; UV detection at 288 nm; injection 20μL; temperature: abient. Concentration of each peak is ca. 5ppm.

k' values in Figure 2. Therefore, reductions of the gradient elution time and the percentage of methanol in the mobile phase were attempted. Optimum separation was obtained by using linear gradient elution time of 15 min and a tertiary mobile phase of ACN: MeOH: EtAc. The gradient was set as follows: Initial solvent composition was 90% ACN, 10% MeOH, 0% EtAc. Final solvent composition was 40% ACN, 0% MeoH, 60% EtAc. A chromatogram obtained using the optimum conditions is shown in Figure 8. The reproducibility of the separations was determined by repetitive analysis (n=5) of the calixarene standard sol'n. The values of the relative standard deviation, % RSD for peak area (PA), and retention time (RT) are in Table 2. The % RSD for peak area and retention time were less than 5% and 0.5%, respectively.

In summary, the decreases of the percentage of methanol in the mobile phase and the gradient elution time resulted in improvement in the separation of the calixarenes. Optimized separation was achieved by employing gradient elution time of 15 min with an initial solvent composition of 90% ACN, 10% MeOH, 0% EtAc and final solvent composition of 40% ACN, 0% MeoH, 60% EtAc. These conditions provided good resolution and satisfactory peak symmetry, and the analysis time was reduced to 14 min.

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