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Original Paper

Preparation and HPLC evaluation of a new *1,3-alternate* 25,27-bis-[*p*-chlorobenzyloxy]-26,28-bis-[3-propyloxy]-calix[4]arene silica bonded stationary phase

The *1,3-alternate* 25,27-bis-[*p*-chlorobenzyloxy]-26,28-bis-[3-propyloxy]-calix[4]arene-bonded silica gel stationary phase was synthesized, structurally characterized, and used as a selector in high performance liquid chromatography. Selectivity studies on that phase used aromatic positional isomers, alkylbenzenes, polynuclear aromatic hydrocarbons, sulfonamides, and non-steroidal anti-inflammatory drugs as analytes. The effects of organic modifier content and pH of the mobile phase on retention and selectivity of selected aromatic positional isomers were studied. Selectivity comparisons of the novel phase *vs. 1,3-alternate* 25,27-di-[benzyloxy]-26,28-bis-[3-propyloxy]-calix[4]arene phase and commercially available RP-Phenyl phases were performed. The retention mechanism was also discussed. The results indicated that the calixarene stationary phase behaves like a reversed-phase packing; however, other retention mechanisms seem to be involved in the separation process.

Keywords: *1,3-alternate* calix[4]arene / Aromatic positional isomers / Non-steroidal anti-inflammatory drugs / PAHs / Sulfonamides

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1 Introduction

The search for more efficient stationary phases with the widest possible spectrum of application has become a significant direction in the study of compound separation by HPLC techniques. Presently, there are many commercially available stationary phases, *e.g.*, RP-C_{18}, RP-C_8, RP-Phenyl, and others. However, their application is limited, mainly due to specific features such as hydrophobicity and ionic or polar properties. In recent years, macrocyclic compounds (cyclodextrins, crown ethers, and calixarenes) capable of forming inclusion complexes with guest molecules have also been more commonly used in modern chromatography.

Interest in calixarenes as stationary phases in chromatography resulted from unique opportunities to influence the specificity and selectivity of these macrocycles [1, 2]. The host-guest interactions of calixarenes with solutes are not determined solely by their hydrophobic cavities, but are also influenced by additional functional groups attached at their rims, which can contribute to potential variations in these interactions. Several functionalized calixarenes were utilized as selectors in liquid chromatography [3, 4]. Modifications of these macrocycles comprised: conformations in which calixarene molecules are blocked; different types of functional groups and substituents present at their upper and lower rim; changes in the calixarene ring-size; and, finally, different types of spacer fixing macrocycles to the stationary phases. Calix[*n*]arenes (n = 4, 6, 8) in cone conformation functionalized at the upper rim by *tert*-butyl substituents and permanently attached to solid support attracted great attention. However, some examples of calixarenes functionalized with other substituents, including chiral residues, were also successfully used as selectors in HPLC separations [5, 6]. Recently in our laboratory we tested and reported the synthesis of calix[4]arene-bonded silica gel stationary phases blocked in *1,3-alternate* conformation which contained additional aliphatic [7]

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Abbreviations: ACN, acetonitrile; **CalixBz**, benzyloxy-calix[4]arene stationary phase; **CalixBz-Cl**, *p*-chlorobenzyloxy-calix[4]arene stationary phase; **ClPhol**, chlorophenol; **DAD**, diode array detector; **NSAID**, non-steroidal anti-inflammatory drugs; **PAH**, polynuclear aromatic hydrocarbons