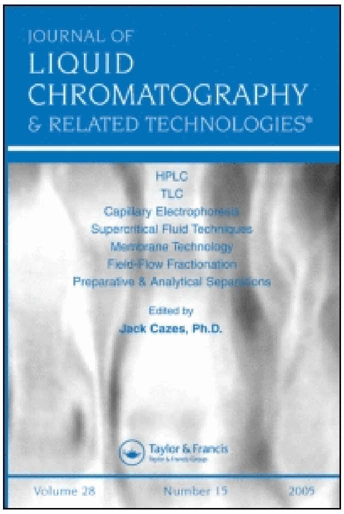

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Nakashima, Kenichiro , Fuchigami, Yusuke , Kuroda, Naotaka , Kinoshita, Toshio and Akiyama, Shuzo(2000) 'STUDY ON π-π INTERACTION IN HIGH PERFORMANCE LIQUID CHROMATOGRAPHY', Journal of Liquid Chromatography & Related Technologies, 23: 16, 2533 — 2540

To link to this Article: DOI: 10.1081/JLC-100100507

URL: http://dx.doi.org/10.1081/JLC-100100507

J. LIQ. CHROM. & REL. TECHNOL., 23(16), 2533–2540 (2000)

STUDY ON π-π INTERACTION IN HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

In high-performance liquid chromatography, the reversed-phase mode column is used most popularly. In the reversed-phase mode separation, several interactions between the stationary phase and analytes are concerned, i.e., partitioning, adsorption, hydrogen bonding, and π-π interactions. In this paper, a π-π interaction effect on high performance liquid chromatographic separation was studied using some *N*-substituted aminopropyl modified silyl silica gel stationary phases bearing aromatic moieties in their structures. A series of aromatic polycyclic hydrocarbons, i.e., benzene, naphthalene, phenanthrene, and naphthacene, were retained and separated on those columns in the order of their aromaticities, while no retention was observed by the non-substituted aminopropyl modified silyl silica gel column. These results strongly supported the existence of π-π interactions between *N*-substituted aminopropyl modified silyl silica gel and the hydrocarbons.

INTRODUCTION

High performance liquid chromatography (HPLC) is one of the excellent separation techniques in the pharmaceutical and biomedical sciences. Many kinds of stationary phases for HPLC, thus, have been developed. Among these, a reversed-phase HPLC using an octadecylsilyl modified silica gel (ODS) stationary phase is currently the most popular and widely applied to various research and practical fields. In parallel, recent trends have been directed toward the development of new phases having specialty and selectivity in the recognition of biologically important substances; mixed-mode stationary phases having a size-exclusion effect for small molecules in biological matrices by direct injection,[1] chiral stationary phases derived from L-proline for enantiomers of *N*-(3,5-dinitrobenzyl)amino acid esters and amides and the related analytes,[2] a chiral α_1-acid glycoprotein phase for enantiomers of nicotine and nicotine-like compounds,[3] a chiral crown ether stationary phase for *β*-*p*-chlorophenyl-*γ*-aminobutyric acid and its analogues,[4] polymer coated silica gels modified with alkyl groups for peptides,[5] a fluorocarbon-bonded silica gel for nucleic acids,[6] axially dissymmetric 2'-substituted-1,1'-binaphthyl-2-carboxylic acids bonded silica gel for enantiomeric acids, amines, and alcohols.[7]

Recently, we have developed new stationary phases by derivatizing aminopropyl-modified silyl silica gel (APS) with several heterocyclic compounds, and applied them to the separation of water-soluble vitamins in beverages,[8] nucleic acid related compounds in seasonings,[9] sugars in an infusion and soft drinks,[10] and components of an eye lotion.[11] Further, in order to develop new stationary phases having π-π interactions, APS derivatives carrying aromatic moieties have been prepared, and applied to the analysis of nucleic acid related compounds[12-15] and barbiturates.[16] In the course of these studies, the separation of nucleic acid related compounds with APS derivatives bearing aromatic moieties were suspected to be somewhat based on the reversed-phase mode possessing π-π interactions, but the details were not able to be clarified.

In this study, we prepared a series of APS-based stationary phases carrying aromatic moieties and tested the separation of aromatic polycyclic hydrocarbons to confirm the existence of π-π interactions.

EXPERIMENTAL

Preparation of Stationary Phases

The proposed structures of the stationary phases developed are illustrated in Figure 1. They have been prepared by the reaction of APS with acid anhydrides according to our previous method for the preparation of 3-(1,8-naphthalimido)propyl-modified silyl silica gel (NAIP).[14] They have π-electrons in their moieties in the density order of NAIP > 3-(1,2-phthalimido)propyl-modified silyl silica gel (PHIP) > 3-meleimidopropyl-modified silyl silica gel (MAIP), while APS has no π-electron. The prepared stationary phases were packed with a slurry method using a mixture of glycerol and methanol (50:50, v/v) in a stainless steel column (250 x 4.5 mm, i.d.).

HPLC System

The HPLC system consisted of a Shimadzu LC 9A HPLC pump, Rheodyne 7125 injector with a 20 μL loop, an analytical column packed with APS derivative, a Shimadzu SPD 6A UV detector, and a Shimadzu CR 6A data processor. Aqueous acetonitrile solution was used as eluent at a flow rate of 1 mL/min, and the eluates were monitored at 260 nm.

RESULTS AND DISCUSSION

Separation of Aromatic Hydrocarbons

To study the separation mode of the developed stationary phases, the separation behaviors of a series of aromatic polycyclic hydrocarbons were exam-

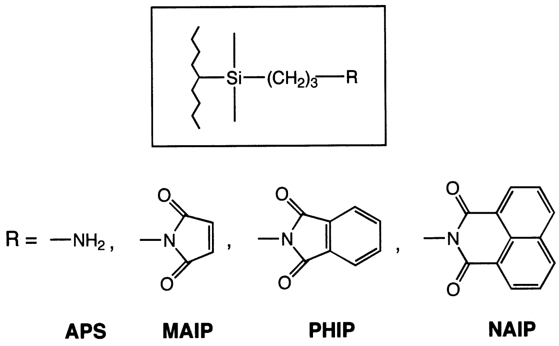

Figure 1. Structures of the stationary phases investigated.

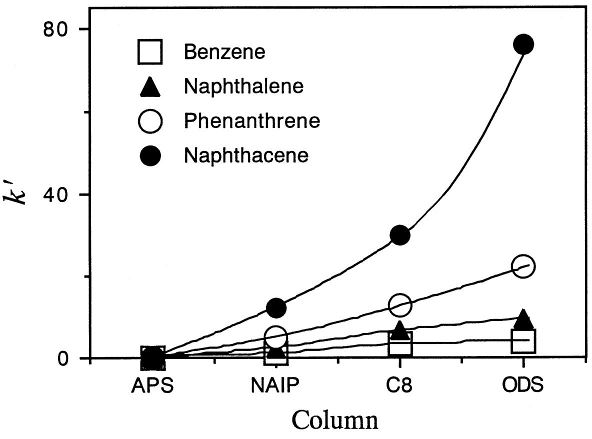

Figure 2. Capacity factors of the aromatic hydrocarbons with APS, NAIP, C8 and C18 column. Mobile phase: 50% aqueous acetonitrile solution. Other HPLC conditions are as described in the text.

ined. As aromatic hydrocarbons, benzene, naphthalene, phenathrene, and naphthacene were selected according to the increase of their π-electron numbers. When APS, NAIP, C8, and C18 (ODS) packed columns were used, the aromatic hydrocarbons were separated as shown in Figure 2. The mobile phase used was 70% aqueous acetonitrile solution. From the result, NAIP seemed to separate the hydrocarbons by a reversed-phase like mode as C8 and ODS, while APS scarcely retained them. The differences in retaining the hydrocarbons between NAIP and APS hint the existence of π-π interactions in the separation with NAIP.

To confirm the reversed-phase mode in NAIP, the effect of water contents in the mobile phase was examined. As shown in Figure 3, the capacity factors (*k'*) of all the hydrocarbons increased with the increase of water contents: these behaviors are similar to those with ODS, and, thus, might mean that the separation in NAIP was carried out by a reversed-phase like mode.

π-π Interaction on NAIP

As mentioned above, π-π interactions are assumed to exist in the NAIP separation mode. Thus, NAIP, PHIP, MAIP, and APS were tested for the separation of aromatic hydrocarbons. The mobile phase used was 50% aqueous ace-

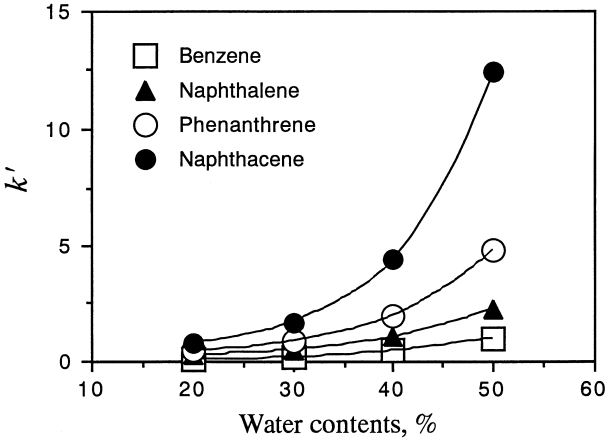

Figure 3. Effect of water contents in the mobile phase on the separation of aromatic hydrocarbons with NAIP. Mobile phase: aqueous acetonitrile. Other HPLC conditions are as described in the text.

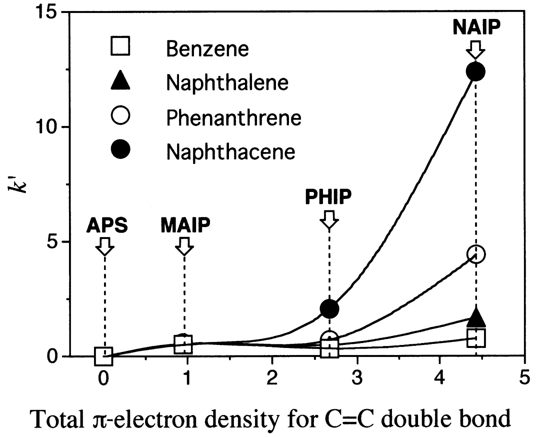

Figure 4. Capacity factors of aromatic hydrocarbons separated with NAIP, PHIP, MAIP and APS. Mobile phase: 50% aqueous acetonitrile. Other HPLC conditions are as described in the text.

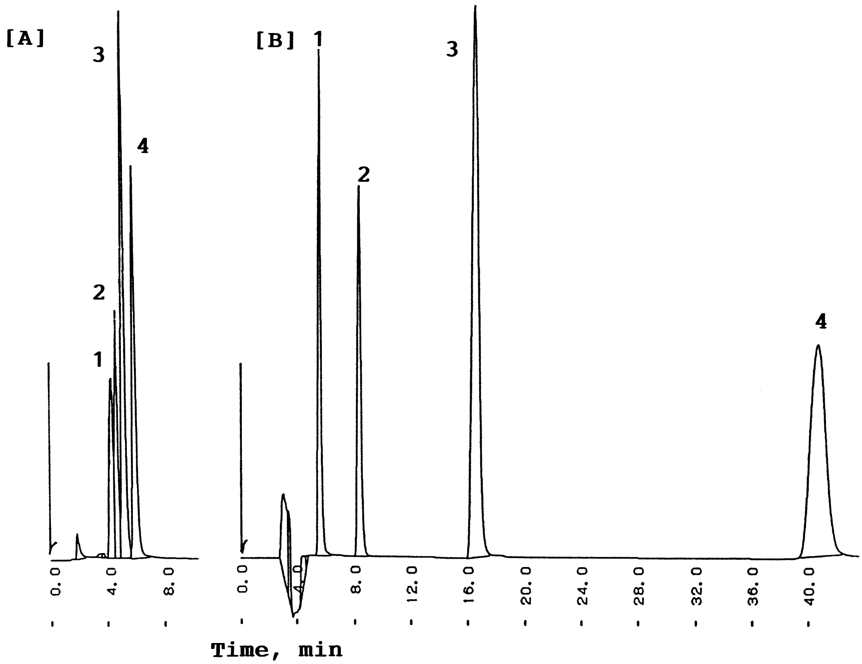

Figure 5. Chromatograms of aromatic hydrocarbons obtained with (A) PHIP and (B) NAIP. Sample (10^{-4} M in methanol): 1=benzene; 2=naphthalene; 3=phenanthrene; 4=naphthacence. Other conditions are as described in the text.

tonitrile solution. π-Electron density of each modifier of APS in Figure 1 was calculated using a PM3 parameter with MOPAC (version 6.01)/Anchor II (Fujitsu) software. We used total π-electron density for C=C double bond as an indication of π-electron density of each column, which was found as follows: 0.944563 for MAIP, 2.674254 for PHIP, and 4.432883 for NAIP. The other π-electron density, such as that derived from C=O double bond, did not show significant difference among the modifiers of APS in Figure 1 (ranging from 1.762215 to 1.645632). As shown in Figure 4, the capacity factor of each hydrocarbon increased with an increase of the total π-electron density with the order of NAIP>PHIP>MAIP>APS. Among those, NAIP possessing the highest π-electron density, powerfully retained the aromatic hydrocarbons, while APS having no π-electron of C=C double bond in the structure did not retain aromatic hydrocarbons. This result shows that the aromatic hydrocarbons apparently have been separated by the π-π interactions between the stationary phases and the analytes under the conditions studied. The representative chromatograms obtained with NAIP and PHIP are shown in Figure 5.

CONCLUSION

Generally, it is very difficult to prove the evidence of an interaction between a stationary phase and analytes. In this study, we have prepared some APS-based stationary phases having aromatic moieties in their structures. By using a series of aromatic polycyclic hydrocarbons, the π-π interactions of these stationary phases could be elucidated. Therefore, the separation of some nucleic acid related compounds, which were previously reported, should be based on the reversed-phase like mode with π-π interactions. The results should be informative to design and to prepare new stationary phases with π-π interactions for the separation and solid phase extraction of aromatic analytes.

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Received March 20, 2000
Accepted April 22, 2000
Author's Revisions May 9, 2000
Manuscript 5278