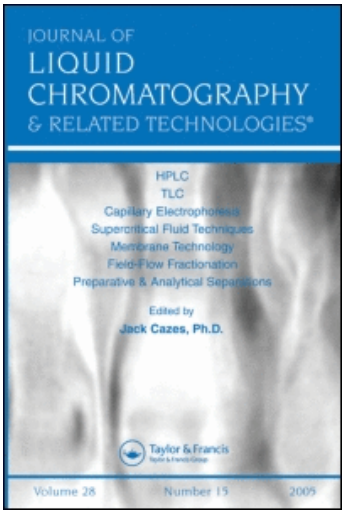

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Application of Alkylamide Phases to Separate Compounds of Different Polarity Under Reversed Phase Conditions

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APPLICATION OF ALKYLAMIDE PHASES TO SEPARATE COMPOUNDS OF DIFFERENT POLARITY UNDER REVERSED PHASE CONDITIONS

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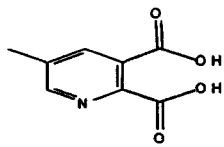
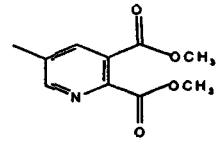
ABSTRACT

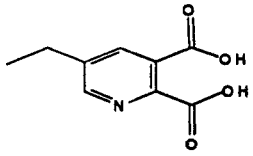
The effects of pH and ionic strength of the mobile phase, as well as the concentration of organic modifiers, on the retention and elution order of zwitterionic and neutral compounds related to imidazolinone herbicides was studied by using polymeric alkylamide stationary phases. It is shown that retention of these compounds on alkylamide phases is governed by a mixed ion-exchange and partition-displacement mechanism. After adjusting pH, ionic strength and composition of the eluent, these phases allow a simultaneous separation of nonpolar and ionic compounds.



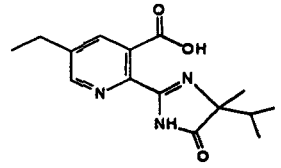
5-Methylpyridinedicarboxylic Acid (MPDC)



5-Methylpyridinedicarboxylic Acid Dimethyl Ester (MPDC.DME)



5-Ethylpyridinedicarboxylic Acid (EPDC)



5-Ethyl-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid (Imazetaphyr)

Figure 1. Structures of the solutes studied.

INTRODUCTION

Chemically bonded phases, containing a ligand with an internal polar group, appeared to be promising for separation of basic compounds under reversed phase conditions.[1,2] Among this type of packing materials the alkylamide phases, which contain an internal amide functional group, become very popular because they exhibit a combination of specific and non-specific interactions with respect to the solutes of various polarities.[3,4]

These phases are usually synthesized by a two-step process, in which initial aminopropyl phase is prepared and subsequently reacted with a suitable alkanoyl chloride.[2-10] This synthesis pathway gives phases which, in addition to alkylamide ligands and residual silanols, contain unreacted aminopropyl ligands. It is suspected that unreacted aminopropyl ligands increase the structural stability of the stationary phase and change significantly its sorption affinity to solvent molecules.[5,6]

O'Gara et al.[11] synthesized an octylcarbamate phase by using monofunctional bonding chemistry. The carbamate functional group was incorporated into an octyldimethylchlorosilane compound and than the entire ligand was directly attached to the silica surface. This synthesis pathway gives bonded phases without residual aminogroups. A comparative study of

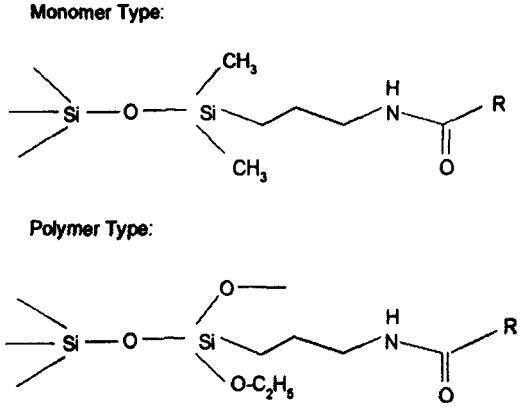


Figure 2. Schematic representation of alkylamide ligands in monomeric and polymeric bonded phases.

alkylamide phases prepared, according to both pathways, would allow the estimation of the effect of residual aminopropyl groups on the retention mechanism of basic solutes.

The current work is focused on the effects of pH and ionic strength of the mobile phase, as well as the role of organic modifiers, on the retention of zwitterionic and neutral compounds related to imidazolinone herbicides. This work is a continuation of the studies reported previously.[3,4]

EXPERIMENTAL

The retention measurements at different pH, ionic strength (concentration of phosphate buffer) and various compositions of the hydro-organic eluent were carried out at 35°C by using flow rate of 1 mL/min. Acetonitrile and methanol were used as organic modifiers. Chromatographic measurements were performed by using the 15 cm, 4.6 mm I.D. column packed with polymeric dodecylamide phase bonded to 5 μ Eka Nobel Kromasil silica. This column denoted as AA-PC-12 was described previously.[4] For the comparative purposes, the following 15 cm x 4.6 mm columns packed with the conventional alkyl phase were also used: Kromasil C-8 from Eka Nobel and Inertsil C-8 prepared at Kent State University.[3,4] Organic solvents were obtained from Baxter.

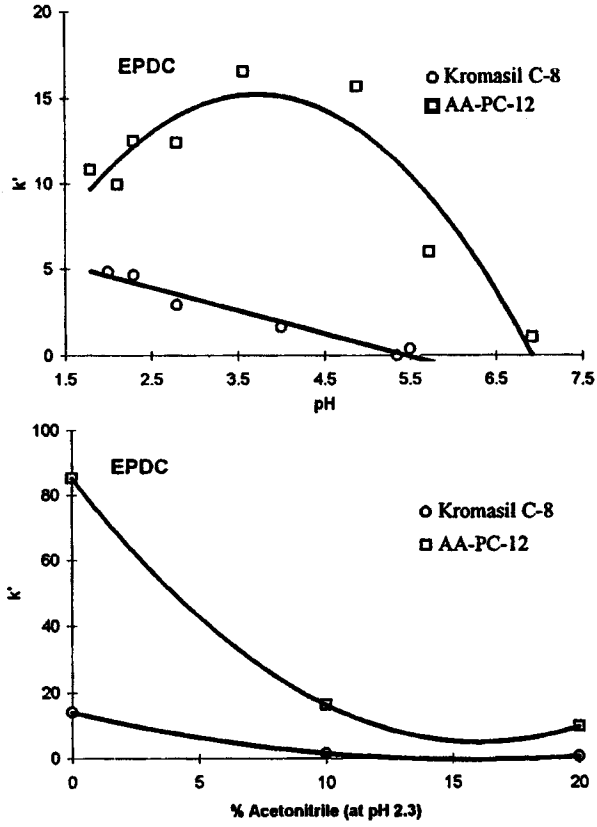


Figure 3. A comparison of the capacity factor k' vs pH and % acetonitrile for 5-ethylpyridinedicarboxylic acid on conventional alkyl and alkylamide columns.

Deionized water was purified using a Millipore Milli-Q system. Aldrich and American Cyanamid were sources of the chromatographic solutes (see Figure 1).

The Hewlett-Packard HP 1050 and a modular liquid chromatograph with a Spectra Physics SP8800 pump, a LKB Model 2125 column oven, a variable wavelength UV detector (ABI 785A) and a Hewlett-Packard 1050 autosampler were used in the current study. Data were acquired and processed using a Hewlett Packard 3350 Laboratory Data System.

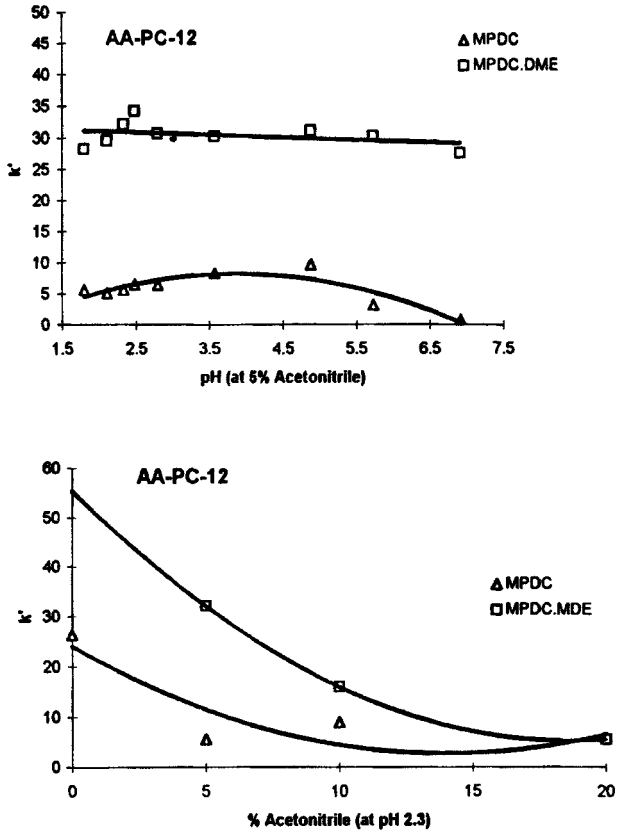


Figure 4. A comparison of the capacity factor k' vs pH and % acetonitrile for 5-methylpyridinedicarboxylic acid and its dimethyl ester on the AA-PC-12 column.

RESULTS AND DISCUSSION

Separation of the imidazolinones and their very polar impurities, such as pyridine based acids under reversed phase conditions, is difficult since these zwitterionic compounds tend to interact strongly with the uncovered surface of silica. Organic acids, especially those with the carboxylic group in the α position to the ring nitrogen, are substances difficult to analyze due to their poor peak shape, irreversible adsorption or complete lack of retention. Even when an ion pairing reagent is added, in order to improve their peak shape and to modify the retention mechanism, the pyridine diacids are practically unretained under conditions suitable for separation of imidazolinones (~20%

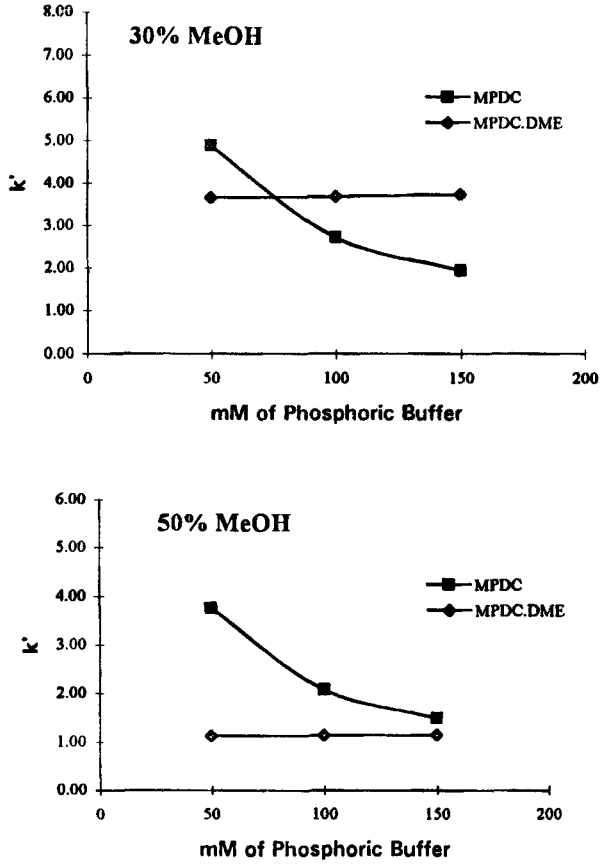


Figure 5. Dependence of k' *versus* phosphate buffer concentration (pH = 2.8) for MPDC and MPDC.DME at two different concentrations of methanol on the AA-PC-12 column.

of organic modifier). The monomeric and polymeric alkylamide packings are chemically bonded phases in which alkyl chains contain an internal amide group (see Figure 2). The retention of polar, ionic and zwitterionic compounds on alkylamide phases occurs according to a mixed ion-exchange and partition-displacement mechanism.[3,4] The dependencies of the capacity ratio on pH and the acetonitrile concentration for a very polar compound EPDC on the conventional alkyl bonded columns and a polymeric dodecylamide column are compared in Figure 3. As can be seen in this figure, the ionic type of interactions plays a substantial role in the retention mechanism.

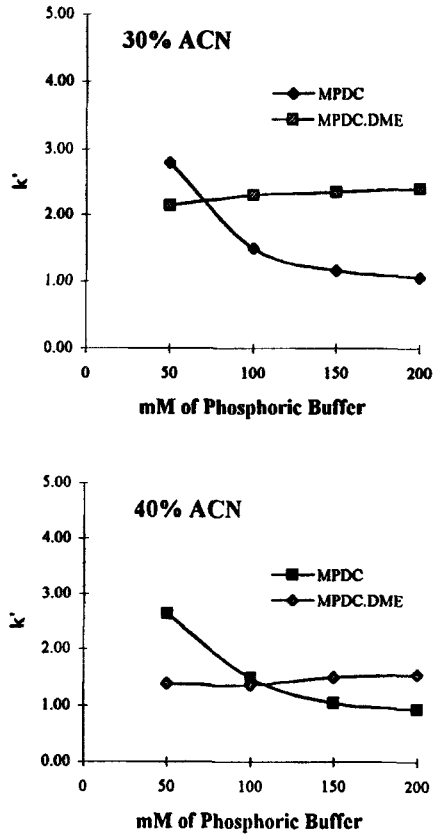


Figure 6. Dependence of k' *versus* phosphate buffer concentration (pH = 2.8) for MPDC and MPDC.DME at two different concentrations of acetonitrile on the AA-PC-12 column.

In the case of the alkyl column, the retention of EPDC decreases with the degree of acid ionization, while on the polymeric alkylamide column EPDC is retained longer at higher pH values. Also, the effect of the acetonitrile concentration on the retention of EPDC on alkyl and alkylamide columns is different. Over the entrie region of acetonitrile concentrations, EPDC is retained longer on the alkylamide column and after an initial significant decrease, the retention volume remains practically unchanged up to 20% acetonitrile.

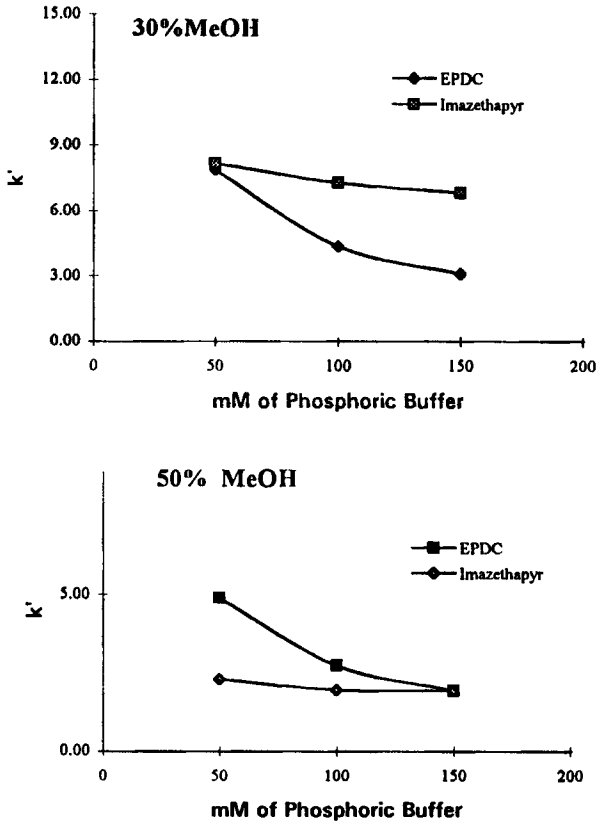


Figure 7. Dependence of k' *versus* phosphate buffer concentration (pH = 2.8) for EPDC and Imazethapyr at two different concentrations of methanol on the AA-PC-12 column.

Retention behavior of two compounds of different polarity on the polymeric dodecylamide phase AA-PC-12 is compared in Figure 4. The capacity factors of ionic 5-methylpyridine dicarboxylic acid (MPDC) and its analogue, i.e., neutral dimethyl ester (MPDC.DME), are plotted versus pH of the mobile phase and acetonitrile content. As can be seen, the retention of a neutral compound does not depend on pH and decreases faster with increasing acetonitrile concentration than the retention of the ionic component.

In Figures 5-8 the capacity factors of MPDC-MPDC.DME and EPDC-Imazethapyr pairs on the AA-PC-12 column are plotted against the concentration of phosphate buffer for different kinds and levels of organic

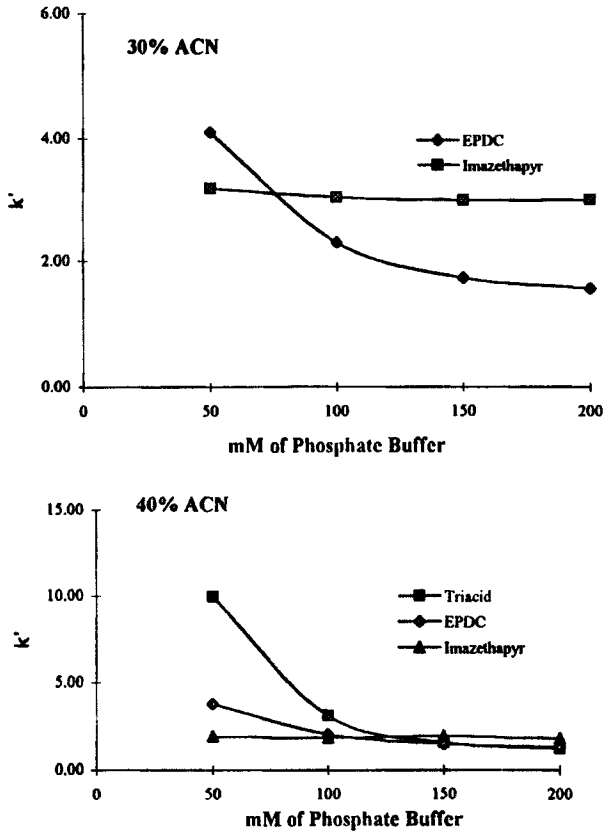


Figure 8. Dependence of k' *versus* phosphate buffer concentration (pH = 2.8) for EPDC, Triacid and Imazethapyr at two different concentrations of acetonitrile on the AA-PC-12 column.

modifiers in the mobile phase. These plots demonstrate that the retention of diacids is controlled by the ionic strength of the mobile phase, whereas the retention of neutral (MPDC.DME) or a less polar (Imazethapyr) compound is controlled by the nature and level of the organic solvent. For both solvents (acetonitrile and methanol) used, each combination of solvent and phosphoric acid concentrations may lead to an inverted elution order of polar and non-polar compounds. In addition, shown in Figure 8 is the dependence of the capacity factor k' on the concentration of phosphoric acid for 2,3,5-pyridine-tricarboxylic acid.

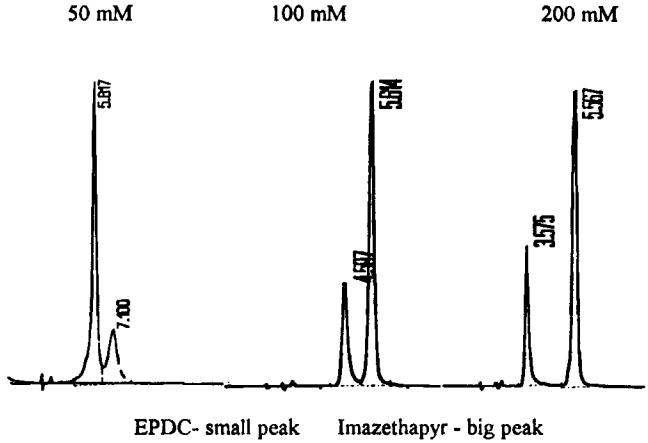


Figure 9. A comparison of the elution order and peak shape for 5-ethyl-pyridinedicarboxylic acid (EPDC) and Imazethapyr at different concentrations of phosphate buffer (pH = 2.8) and 30% acetonitrile on the AA-PC-12 column. Conditions: 35 °C, 1 mL/min, 254 nm. Small peak in each panel refers to EPDC.

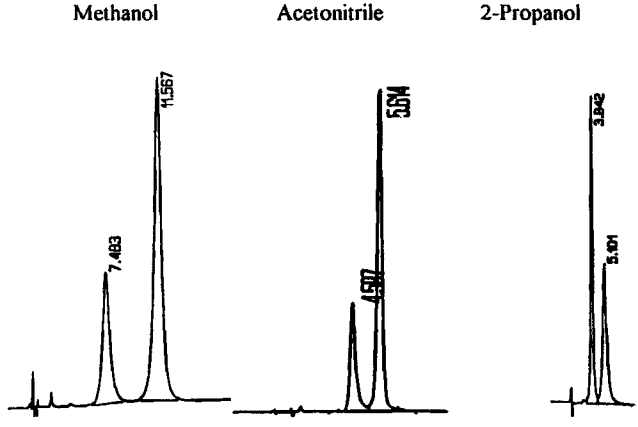
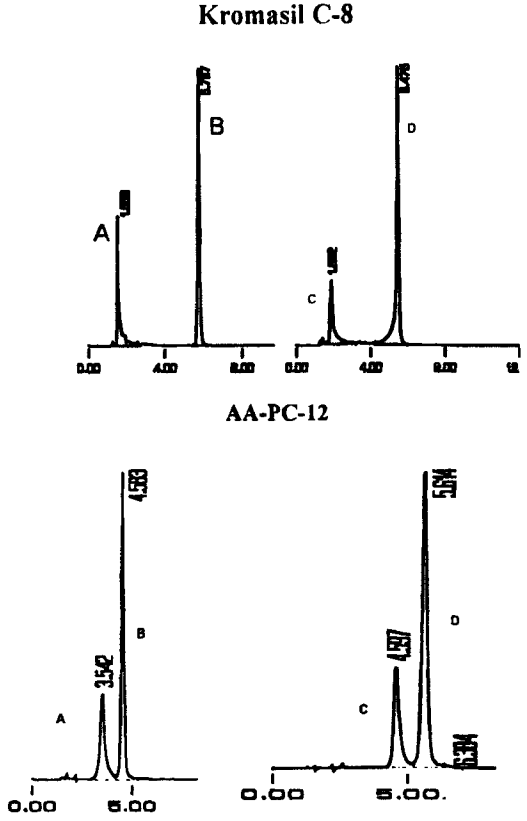


Figure 10. A comparison of the elution order and peak shape for 5-ethyl-pyridinedicarboxylic acid (EPDC) and Imazethapyr chromatographed in the mobile phase containing 100 mM of phosphate buffer (pH = 2.8) and 30% organic modifier on the AA-PC-12 column. Conditions: 35 °C, 1 mL/min, 254 nm. Small peak in each panel refers to EPDC.



A - MPDC, B - MPDC.DME, C - EPDC, D - Imazethapyr

Figure 11. A comparison of separation of the MPDC (A) - MPDC.DME (B) and EPDC (C) - Imazethapyr (D) pairs on the 15 cm Kromasil C-8 and AA-PC-12 columns with 30% acetonitrile and 70% 100 mM phosphate buffer at pH = 2.8. Conditions: 35 °C, 1 mL/min, 254 nm.

As can be seen, this very polar compound shows the highest retention on the AA-PC-12 column. However, it was eluted at the dead time on the conventional alkyl column with 40% acetonitrile. Exemplary chromatograms, shown in Figures 9 and 10, illustrate the reversed elution order where pyridine diacids elute after their nonpolar derivatives (methyl esters and/or pyridine imidazolinones). A comparison of selected chromatograms obtained on the AA-PC-12 column with those on the conventional RP Kromasil C-8 column is presented in Figure 11.

For 30% of acetonitrile in the mobile phase both diacids are virtually unretained, whereas their retention on the AA-PC-12 column is comparable to that of neutral components.

CONCLUSIONS

Comparative studies of retention of ionic and neutral compounds on the polymeric dodecylamide phase have shown that the specific interactions sites in this phase have a significant influence on the solute retention and selectivity. Since the retention of multifunctional organic acids is governed by a mixed ion-exchange and partition-displacement mechanism, while the retention of neutral compounds is governed by the latter mechanism only, the effective optimization of chromatographic conditions for simultaneous separation of ionic and non-polar compounds is possible.

REFERENCES

1. T. L. Ascah, B. Feibush, J. Chromatogr., **506**, 357-369 (1990).

2. B. Buszewski, J. Schmid, K. Albert, E. Bayer, J. Chromatogr., **552**, 415-427 (1991).

3. T. Czajkowska, I. Hrabowsky, B. Buszewski, R. K. Gilpin, M. Jaroniec, J. Chromatogr. A, **691**, 217-224 (1995).

4. T. Czajkowska, M. Jaroniec, B. Buszewski, J. Chromatogr. A, in press.

5. B. Buszewski, R. K. Gilpin, M. Jaroniec, Chem. Anal. (Warsaw), **39**, 673-679 (1994).

6. B. Buszewski, M. Jaroniec, R. K. Gilpin, J. Chromatogr. A, **668**, 293-299 (1994).

7. B. Buszewski, M. Jaroniec, R. K. Gilpin, J. Chromatogr. A, **673**, 11-19 (1994).

8. P. Kasturi, B. Buszewski, M. Jaroniec, R. K. Gilpin, J. Chromatogr. A, **659**, 261-265 (1994).

9. B. Buszewski, R. K. Gilpin, M. Jaroniec, Chem. Anal. (Warsaw), **39**, 653-661 (1994).

10. B. Buszewski, P. Kasturi, R. K. Gilpin, M. E. Gangoda , M. Jaroniec, Chromatographia, **39**, 155-161 (1994).

11. J. E. O'Gara, B. A. Alden, T. H. Walter, J. S. Petersen, C. L. Niederlander, U. D. Neue, Anal. Chem., **67**, 3809-3813 (1995).

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