RETENTION BEHAVIOR OF BASIC AZAARENES IN REVERSE-PHASE LIQUID CHROMATOGRAPHY: THE EFFECT OF MOBILE PHASE pH

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Introduction

Azaarenes are of particular concern due to their carcinogenic and mutagenic properties and numerous sources in the environment. Separation of azaarenes by high performance liquid chromatography is usually done in reversed phase mode using octadecylsilica as the proper stationary phase. Neutral azaarenes can be successfully analysed with high efficiency and reproducibility, but separation of basic ones may be problematic because of specific interactions with column packing. These may be responsible for irreversible adsorption, peak tailing, low column efficiency or irreproducible retention. These effects are mainly supposed to be caused by the interactions of the basic compounds with acidic residual silanols of the silica support. Despite of numerous studies and theories, understanding of azaarene’s retention processes in RPLC is still quite incomplete. In the present study we analysed the effect of varying mobile phase pH on the retention behaviour of selected basic azaarenes. The character and complexity of the retention has been investigated in the system with two octadecylsilica stationary phases made by different manufacturers.
Method
All experiments were recorded under isocratic elution conditions with an eluent flow rate of 1 ml/min and UV-spectrophotometric detection at 254 nm and 231 nm. At first the evaluation of surface properties of two octadecylsilica stationary phases was carried out. The silanol activity was probed according to the Galushko test, the column hydrophobicity was checked as Engelhardt proposed. The separation experiments were carried out with eight members of basic azaarenes (quinoline and its mono-methyl derivatives, isoquinoline, benzo[h]quinoline, acridine and phenanthridine), forming three groups of similar hydrophobicities but with different pK_a constants. Isoeluotropic mixtures of methanol and acetonitrile in combination with phosphate buffer in the pH range 2,5-8,5 and concentrations of 2.5, 5 and 20 mM were used as mobile phases. All retention values were reported in terms of capacity factor (k’) and correlated with the varying mobile phase pH.

Results and conclusions
Differences in chromatographic properties towards to separation of basic azaarenes on two octadecylsilica sorbents of the same character and dimensions, but of different origin have been proved. The Purospher Star RP 18e has shown higher silanol activity and column hydrophobicity than the Zorbax Eclipse XDB-C18. The effect of mobile phase pH on the retention behaviour of studied azaarenes has been proved. In mobile phases of pH > 3,5 reproducible retention of all tested compounds with the elution order reflecting the azaarenes hydrophobicities has been found. On the contrary, unusual elution order of selected azaarenes in mobile phases of pH < 3,5 at all tested phosphate buffer concentrations have been observed. Retention of azaarenes with similar hydrophobicities has been found to be related to their pK_a values. Further experiments focused on retention behaviour of the studied compounds in a given system are being processed.

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