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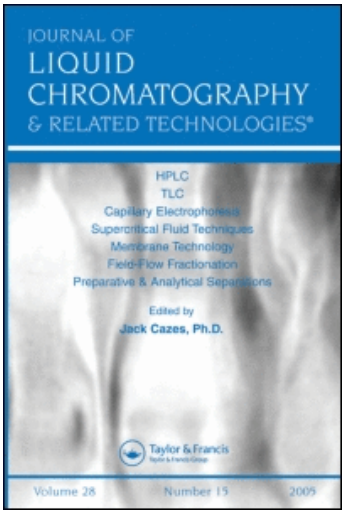

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Kenneth D. McMurtrey[a]; Paul J. Deslauriers[a]; Thomas J. Knight[a]
[a] Department of Chemistry, University of Southern Mississippi, Hattiesburg, MS

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LIQUID CHROMATOGRAPHIC BEHAVIOR OF SELECTED AZA-ARENES AND POTENTIAL METABOLITES

Kenneth D. McMurtrey, Paul J. DesLauriers, and Thomas J. Knight
Department of Chemistry
University of Southern Mississippi
Hattiesburg, MS 39406

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ABSTRACT

Reversed phase HPLC was applied to analyses of selected aza-arenes and potential metabolites (5,6-benzoquinoline, 5,6-benzoquinoline-N-oxide, and the N-methyliodide salt of 5,6-benzoquinoline). Naphthalene and anthracene were employed as reference materials. Water-methanol and ammonium phosphate-methanol mobile phases were used with several commercial octadecyl reversed phase columns and a column using laboratory synthesized ODS stationary phase. Chromatographic behavior of the hydrocarbon reference compounds were excellent on all packings with either mobile phase. Benzoquinoline and derived materials were more difficult analytical subjects; water-methanol mobile phases proved unsuitable giving very long retention times with unacceptable efficiencies. Ammonium phosphate containing mobile phases were more appropriate for the nitrogen containing materials especially when used with end capped or polymeric end capped stationary phases.

INTRODUCTION

Liquid chromatography has emerged as the method of choice for analysis of polynuclear aromatic hydrocarbon (PAH) carcinogens and their metabolites(1). Normally reversed phase columns are used with mobile phases consisting of water-methanol, water-acetonitrile, or water-tetrahydrofurane mixtures. Under these

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conditions chromatographic performance is usually excellent with theoretical plate heights near two particle diameters for well packed columns. There appears to be little difference between analytical efficiency for PAH and their metabolites. Elution of metabolites between the column void volume and the substrate retention volume is an added advantage during metabolism studies (metabolism is usually accompanied by gains in polarity).

Aza-arenes (NPAH) and their metabolites appear to be more difficult analytical subjects than PAH since the parent compounds and their metabolites are subject to protonation. In addition, metabolism of NPAH might produce metabolites with a partial or full positive charge localized on the nitrogen atom (amine oxide or N - methyl salt). As a necessary prelude to an investigation of the metabolism and biological actions of NPAH the liquid chromatographic behavior of a mixture of 5,6-benzoquinoline (BQ), 5,6-benzoquinoline-N-oxide (BQNO), and the N-methyl iodide derivative of 5,6-benzoquinoline (BQMeI) on several reversed phase stationary materials was studied.

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METHODS AND MATERIALS

A Perkin-Elmer 3B liquid chromatograph with column temperature control was used in these studies. Chromatography columns (5 μ ODS, 4.6 X 250 mm) were purchased from commercial vendors (Alltech, Houston, TX; Laboratory Data Control, Riviera Beach, FL; Perkin-Elmer, Norwalk, CT; Supelco, Bellefonte, PA), or packed using techniques previously described(2). End capped

ODS packing material was synthesized by reaction of octadecyl bonded silica with trimethylsilyl chloride in distilled carbon tetrachloride for two hours at room temperature, followed by filtering, washing (carbon tetrachloride, methanol, methylene chloride) and drying overnight at room temperature. Polymeric ODS packings were prepared by reaction of silica gel (Analtech, Newark, DE, 10 μ silica gel without binder) with octadecyltrichlorosilane and end capping according to a published procedure (3).

Model metabolites were prepared from 5,6-benzoquinoline (Aldrich Chemical, Milwaukee, WI). The amine oxides was synthesized by oxidation of the NPAH with m-chloroperbenzoic acid at room temperature in methylene chloride for 2 hours. Methyl iodide and BQ were heated in benzene to 100°C in a sealed tube for 4 hours and precipitated BQ methiodide recrystallized from methanol-benzene.

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RESULTS AND DISCUSSION

Although reversed phase columns with water-organic solvent mobile phases are well suited for analysis of PAH and their metabolites it was immediately obvious that these conditions were not appropriate for NPAH and model metabolites. While naphthalene and anthracene gave excellent peaks under these conditions BQ showed considerable evidence of non-ideal behavior (Fig. 1). In general the number of theoretical plates for BQ was no more than 100 for 25 cm columns. At the same time plate height counts

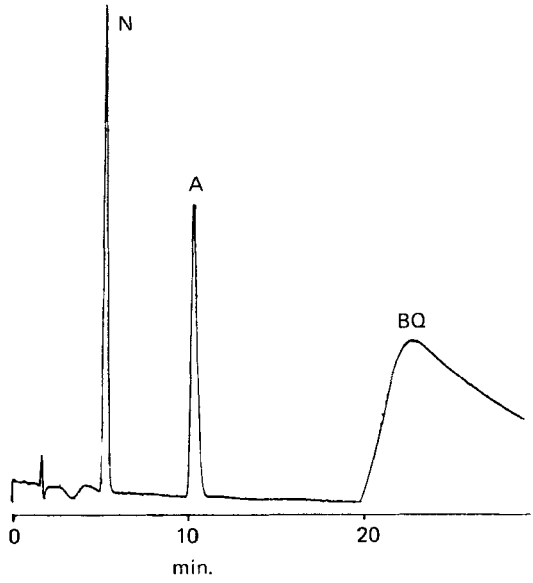

Figure 1. HPLC of anthracene (A), BQ, BQNO, BQMeI, and naphthalene (N) on Spherisorb ODS (4.6 X 150 mm), 7:3 methanol:water mobile phase at 1 ml/min.

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for naphthalene and anthracene were excellent. The model amine-oxide and methiodide metabolites appeared to be completely retained and did not elute from the column.

It is well known that bonded phase HPLC packings have many unreacted silanols that can act as ion exchange groups(4). The first modification of conditions was to change the mobile phases to mixtures of 0.1 M ammonium phosphate (pH 5.0) and methanol. As evidenced in Fig. 2, the chromatographic behavior of BQ was greatly improved. Furthermore BQ amine oxide (ret. time = 3.9 min) appears in the chromatogram. The quaternary methyl iodide salt was still completely retained under these conditions.

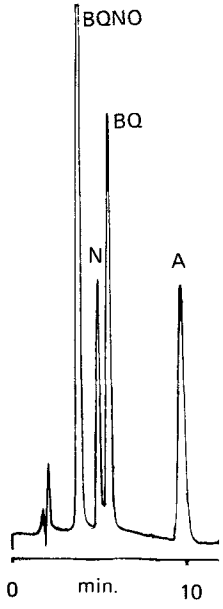

Figure 2. HPLC of mixture in Fig. 1 on Spherisorb ODS (4.6 X 150 mm), 7:3 methanol:0.1 M ammonium phosphate (pH 5.0) mobile phase at 1 ml/min.

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The next change in experimental conditions was to end cap a sample of Spherisorb ODS with trimethylsilyl chloride to decrease the number of available silanol groups. The chromatogram resulting from analysis of a mixture of anthracene, BQ, BQNO, BQMeI, and naphthalene is displayed in Fig. 3. For the first time BQMeI appears in the chromatogram (ret. time = ca. 8.6 min), although with unsatisfactory efficiency. In addition, BQNO elutes earlier on the end-capped column (2.8 min) than on the previous one (3.9 min), and the relative retention of BQ and naphthalene are reversed. These data suggest that there are fewer non-ideal

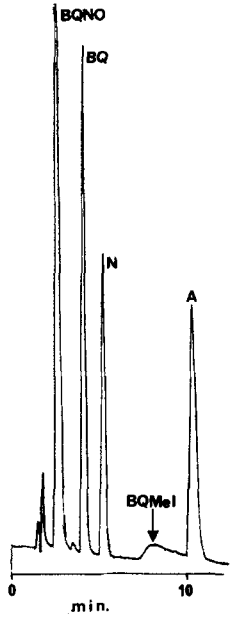

Figure 3. HPLC of mixture in Fig. 1 on Sperisorb ODS end-capped with trimethylsilyl chloride (4.6 X 150 mm) mobile phase in Fig. 2.

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solute-stationary phase interactions with the end-capped column. It also is clear that the fully charged methyliodide is the most difficult analytical subject in this group of compounds. The order of elution of the NPAH materials by a purely hydrophobic mechanism should be BQMeI<BQNO<BQ because of their relative polarities.

A number of commercial reversed phase columns and a polymeric ODS column prepared in this laboratory were examined. While there were considerable differences in chromatographic behavior of BQ, its amine oxide and methyliodide salt no column

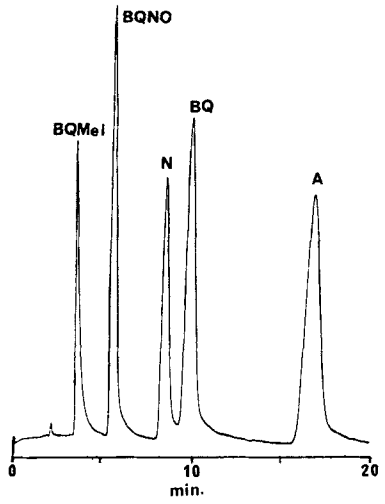

Figure 4. HPLC of mixture in Fig. 1 on semi-preparative column (10 X 200 mm), 1:1 methanol:0.1 M ammonium phosphate (pH 5.0) mobile phase at 5 ml/min.

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tested allowed chromatography of the fully charged methyl derivative with complete success. Columns with polymeric coatings appeared to perform best and gave the expected order of elution for BQMeI, BQNO, and BQ although peaks derived from BQMeI characteristically tailed to some degree. Under ideal conditions tailing of the methiodide salt is sufficiently suppressed to allow successful analytical or preparative analyses to be made. Fig.4 illustrates the chromatography of the mixture of PAH, BQ, BQNO, and BQMeI on a semi-preparative column prepared in the laboratory.

The mixture of BQ, BQNO, BQMeI and the two PAH appears to be an excellent probe for column evaluation.

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