SEPARATION OF METHYL-SUBSTITUTED BENZ[c]ACRIDINES BY CATION-EXCHANGE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

High-performance liquid chromatography on an ion-exchange column for the separation of methyl-substituted benz[c]acridines was investigated. A cation-exchange column (Partisil 10 SCX) was used with acetonitrile–0.001 M $(NH_4)_2HPO_4$ as the mobile phase. The retention times depended on the composition of the mobile phase. An elution order depending on the pK_a values was established.

INTRODUCTION

Methyl-substituted benz[c]acridines (BAcs) are widespread environmental pollutants[1–6] and are well known for their carcinogenic and mutagenic characteristics[7–10]. Therefore, there is considerable interest in their qualitative and quantitative analysis in complex mixtures.

Methods for the determination of BAcs using paper chromatography[11,12], thin-layer chromatography[1–4,13–16] and gas–liquid chromatography[15,17–23] have been reported. Recently, in order to improve the sensitivity and specificity, high-performance liquid chromatography (HPLC) methods have been developed[5,6,15,22,24–29]. In many of these studies, both reversed-phase (RP)[5,6,15,22,24–26,28] and normal-phase (NP) HPLC were used[22,24,25,29]. Very little work, however, has been done on the determination of BAcs by ion-exchange HPLC[27]. This technique is particularly suitable for the separation and determination of polar compounds that are not separated by RP- or NP-HPLC. In this study, the separation of BAcs by cation-exchange HPLC (CE-HPLC) was investigated.

EXPERIMENTAL

Materials

Twelve BAcs were synthesized according to the literature[30–36] and purified as

described in a previous paper[15]: benz[c]acridine (1), 7-methylbenz[c]acridine (2), 8-methylbenz[c]acridine (3), 9-methylbenz[c]acridine (4), 10-methylbenz[c]acridine (5), 11-methylbenz[c]acridine (6), 5,7-dimethylbenz[c]acridine (7), 7,9-dimethylbenz[c] acridine (8), 7,10-dimethylbenz[c]acridine (9), 7,11-dimethylbenz[c]acridine (10), 7,9,10-trimethylbenz[c]acridine (11) and 7,9,11-trimethylbenz[c]acridine (12).

A 100 μg/ml stock solution of BAcs in methanol was prepared and diluted as required.

The reagents (analytical-reagent grade) and solvents (HPLC-grade) were obtained from Wako (Osaka, Japan).

Chromatographic apparatus and conditions

The HPLC apparatus consisted of a JASCO Model BIP-I pump (Japan Spectroscopic, Tokyo, Japan), a Rheodyne (Berkeley, CA, U.S.A.) Model 7125 injector equipped with a 20-μl loop, a JASCO Model 860-CO column oven and a JASCO UVIDEC-100 III spectrophotometer. The HPLC columns used were strong cation exchangers: (1) Partisil 10 SCX (10 μm, 250 × 4.6 mm I.D.) (Whatman, Clifton, NJ, U.S.A.) and (2) TSKgel SP-2SW (5 μm, 250 × 4.6 mm I.D.) (TOSOH, Tokyo, Japan). Various proportions of acetonitrile–$(NH_4)_2HPO_4$ buffer and methanol–$(NH_4)_2HPO_4$ buffer were used as mobile phases and were filtered through Millipore membrane filters (0.45 μm) and degassed under vacuum prior to use. The sample injected was 2 μl of a methanol solution containing 10 ng of each BAc. Separations were carried out at a flow-rate of 1.0 ml/min and a column oven temperature of 40°C.

Elution patterns were monitored at 280 nm. The results were evaluated with a Shimadzu Chromatopac CR-3A digital integrator.

RESULTS AND DISCUSSION

The composition of the mobile phase (percentage of water, salt concentration, nature of the buffer and pH) had a strong influence on the quality of the CE-HPLC separation. The optimum mobile phase composition was chosen for each column to give the best separation of the BAcs and to detect as many as possible of the components in one injection. The column efficiency was checked by making injections of a mixture of BAcs dissolved in methanol. Isocratic elution was used.

The solvent systems finally chosen were acetonitrile–0.001 M $(NH_4)_2HPO_4$ (adjusted to pH 3.0 with phosphoric acid) (60:40, v/v) and methanol–0.001 M $(NH_4)_2HPO_4$ (pH 3.0) (70:30, v/v) for the Partisil 10 SCX column and acetonitrile–0.01 M $(NH_4)_2HPO_4$ (pH 3.0) (50:50, v/v) and methanol–0.01 M $(NH_4)_2HPO_4$ (pH 3.0) (65:35, v/v) for the TSKgel SP-2SW column. The retention times of BAcs using either acetonitrile–$(NH_4)_2HPO_4$ or methanol–$(NH_4)_2HPO_4$ as eluent are given in Table I and the chromatograms are shown in Figs. 1 and 2. The HPLC patterns of BAcs on the Partisil 10 SCX and TSKgel SP-2SW columns were very similar to each other and also to that obtained previously by NP-HPLC[22]. The resolution of BAcs was considerably better using the Partisil 10 SCX than the TSKgel SP-2SW column. The former column was particularly useful for separating compounds 3 and 4, which could not be sufficiently separated on the TSKgel SP-2SW column. With respect to the retention time of BAcs on the Partisil 10 SCX column, acetonitrile–$(NH_4)_2HPO_4$ was better than methanol–$(NH_4)_2HPO_4$ (Fig. 1).

TABLE I

HPLC RETENTION TIMES OF BAcs

Mobile phase: (A) acetonitrile–0.001 M $(NH_4)_2HPO_4$ (pH 3.0) (60:40); (B) methanol–0.001 M $(NH_4)_2HPO_4$ (pH 3.0) (70:30); (C) acetonitrile–0.01 M $(NH_4)_2HPO_4$ (pH 3.0) (50:50); (D) methanol–0.01 M $(NH_4)_2HPO_4$ (pH 3.0) (65:35).

Compound	$pK_a{}^a$	Retention time (min)			
		Partisil 10 SCX column		TSKgel SP-2SW column	
		A	B	C	D
1	2.80	9.8	10.7	13.0	14.8
2	3.55	24.4	29.8	28.1	34.9
3	3.12	12.4	14.0	16.5	19.7
4	3.11	12.0	13.6	16.4	19.6
5	3.37	17.9	20.6	22.4	26.9
6		3.1	3.5	5.3	9.8
7	3.68	26.5	34.3	32.0	44.2
8	3.70	28.3	36.9	33.0	47.0
9	3.91	37.9	52.2	40.4	62.6
10		3.8	4.7	6.0	11.3
11	4.25	41.2	60.6	46.2	84.0
12		4.2	5.5	6.4	12.1

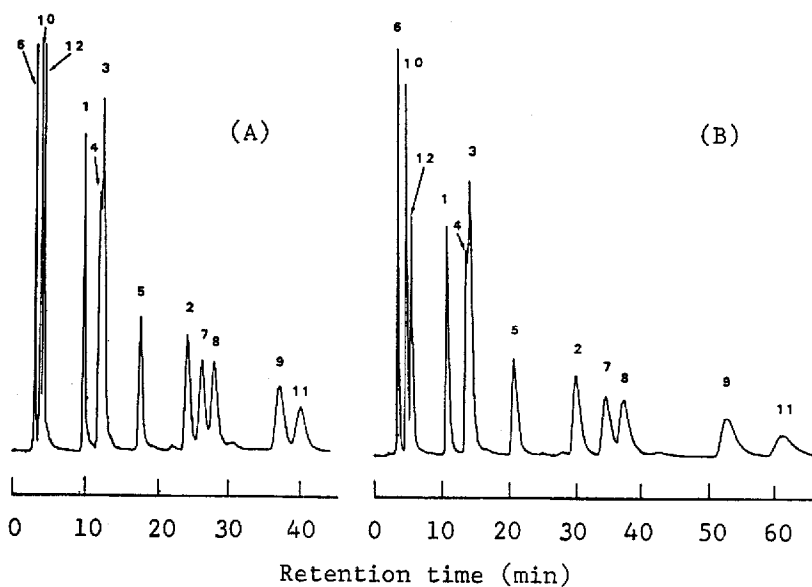^{a} pK_a values taken from ref. 37.

Fig. 1. Separation of twelve BAcs on a Partisil 10 SCX column. Mobile phase: (A) acetonitrile–0.001 M $(NH_4)_2HPO_4$ (pH 3.0) (60:40); (B) methanol–0.001 M $(NH_4)_2HPO_4$ (pH 3.0) (70:30).
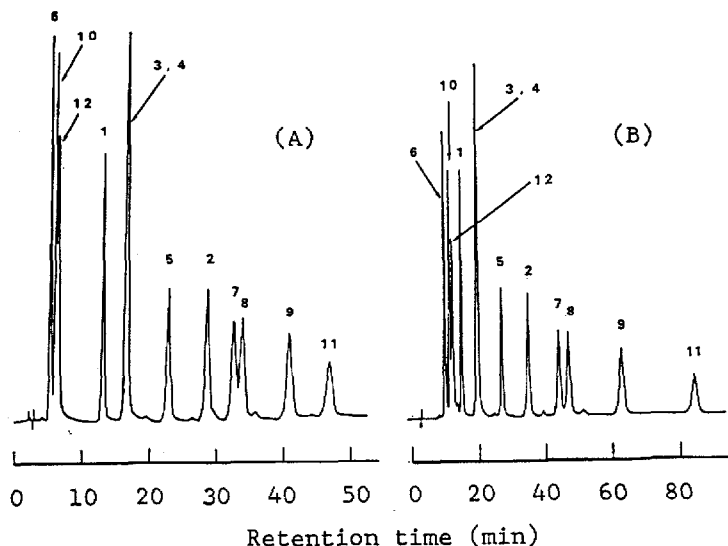
Fig. 2. Separation of twelve BAcs on a TSKgel SP-2SW column. Mobile phase: (A) acetonitrile–0.01 M $(NH_4)_2HPO_4$ (pH 3.0) (50:50); (B) methanol–0.01 M $(NH_4)_2HPO_4$ (pH 3.0) (65:35).

The influence of organic solvent concentration, buffer concentration and mobile phase pH on the chromatographic characteristics of BAcs was systematically examined and the results are shown in Figs. 3–5. The retention time increased with
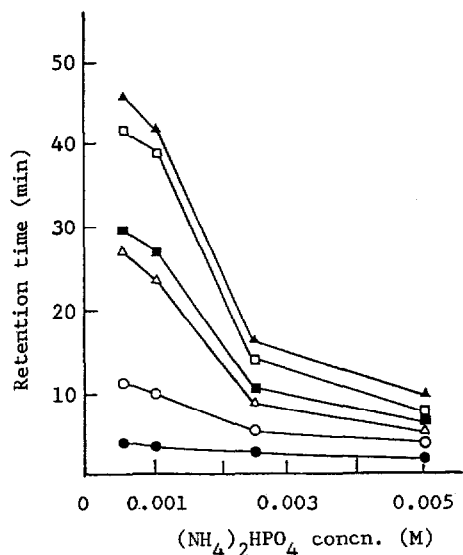

Fig. 3. Relationship between retention time and $(NH_4)_2HPO_4$ concentration in the mobile phase. Column Partisil 10 SCX; eluent, acetonitrile–$(NH_4)_2HPO_4$ (60:40), adjusted to pH 3.0 with phosphoric acid; flow-rate, 1.0 ml/min. Compounds: (○) = 1; (●) = 2; (□) = 6; (■) = 7; (△) = 9; (▲) = 11.
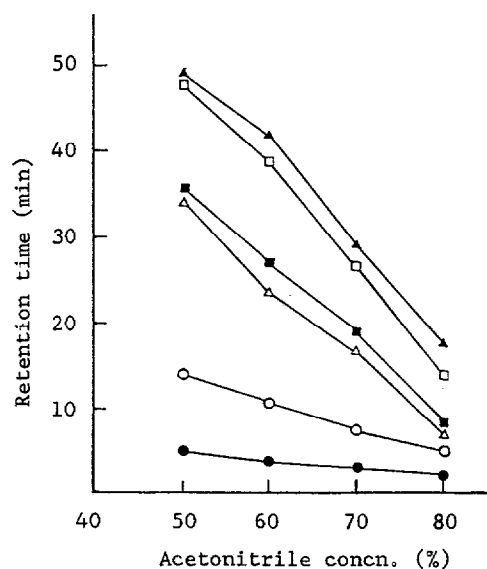
Fig. 4. Relationship between retention time and acetonitrile concentration in the mobile phase. Column, Partisil 10 SCX; eluent, acetonitrile–0.001 M (NH$_4$)$_2$HPO$_4$, adjusted to pH 3.0 with phosphoric acid; flow-rate, 1.0 ml/min. Symbols as in Fig. 3.

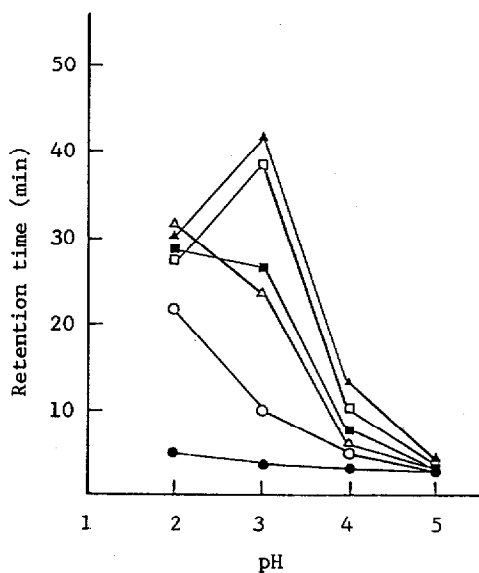
Fig. 5. Relationship between retention time and pH of mobile phase. Column, Partisil 10 SCX; eluent, acetonitrile–0.001 M (NH$_4$)$_2$HPO$_4$ (60:30), adjusted to various pH values with phosphoric acid; flow-rate, 1.0 ml/min. Symbols as in Fig. 3.
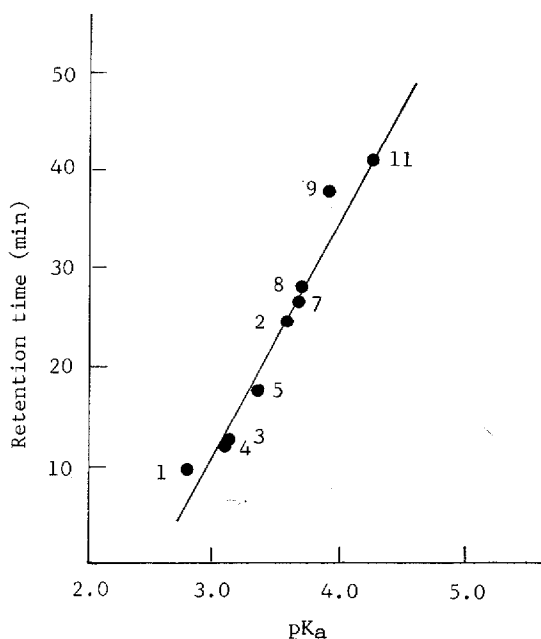
Fig. 6. Relationship between pK_a and retention times of BAcs on a Partisil 10 SCX column. Mobile phase, acetonitrile–0.001 M $(NH_4)_2HPO_4$ (pH 3.0) (60:40); flow-rate, 1.0 ml/min.

decreasing concentration of organic solvent and buffer and was sensitive to pH. The optimum pH for separating BAcs was 3.0.

The relationship between pK_a and retention time on the Partisil 10 SCX column using acetonitrile–0.001 M $(NH_4)_2HPO_4$ (pH 3.0) (60:40) as the mobile phase was linear (correlation coefficient 0.975), as shown in Fig. 6.

Previous investigations showed that the HPLC of BAcs provides satisfactory results[22]. Excellent separations of BAcs were obtained by a combination of RP-HPLC and NP-HPLC. Using the individual techniques, however, BAcs were not well separated. RP-HPLC did not give a good separation of compounds 7, 8 and 9, and in NP-HPLC compounds 6, 10 and 12 were eluted close to the solvent peak. These compounds were completely separated using CE-HPLC. In conclusion BAcs were separated in a relatively short time by CE-HPLC.

REFERENCES

1 E. Sawicki, T. W. Stanley and W. C. Elbert, *Occup. Health Rev.*, 16 (1964) 8.
2 E. Sawicki, S. P. McPherson, J. W. Stanley, J. E. Meeker and W. C. Elbert, *Int. J. Air Water Pollut.*, 9 (1965) 515.
3 C. R. Engel and E. Sawicki, *J. Chromatogr.*, 31(1967) 109.
4 T. W. Stanley, M. J. Morgan and E. M. Grisby, *Environ. Sci. Technol.*, 2 (1968) 699.
5 P. Maschlet, M. A. Bresson, S. Beyne and G. Mouvier, *Analusis*, 13 (1985) 401.
6 T. Yamauchi and T. Handa, *Environ. Sci. Technol.*, 21 (1987) 1177.
7 A. Lacassagne, N. P. Buu-Hoi, R. Dauel and F. Zajdela, *Adv. Cancer Res.*, 4 (1956) 315.
8 H. R. Glatt, H. Schwind, F. Zajela, A. Croisy, P. C. Jecquignon and F. Oesch, *Mutat. Res.*, 6 (1976) 307.
9 D. Niculescu-Duvas, T. Craescu, M. Tugulea, A. Croisy and P. C. Jacquignon, *Carcinogenesis*, 2 (1981) 269.

10 R. P. Deutsch-Wenzel, H. Brune and G. Grimmer, *Cancer Lett.*, 20 (1983) 97.
11 A. M. Luly and K. Sakodynsky, *J. Chromatogr.*, 19 (1965) 624.
12 S. Caroli and M. Lederer, *J. Chromatogr.*, 21 (1968) 333.
13 E. Sawicki, T. W. Stanley, J. D. Pfaff and W. C. Elbert, *Anal. Chim. Acta*, 31 (1964) 359.
14 E. Sawicki, W. C. Elbert and T. W. Stanley, *J. Chromatogr.*, 17 (1965) 120.
15 N. Motohashi and K. Kamata, *Yakugaku Zasshi*, 103 (1983) 795.
16 K. Kamata and N. Motohashi, *J. Chromatogr.*, 396 (1987) 437.
17 D. Brocco, A. Cimmino and M. Possanzini, *J. Chromatogr.*, 84 (1973) 371.
18 M. Pailer and V. Hlozek, *J. Chromatogr.*, 128 (1976) 163.
19 I. Ignatiadis, J. M. Schmitter and G. Guiochon, *J. Chromatogr.*, 246 (1982) 23.
20 J. M. Schmitter, I. Ignatiadis and G. Guiochon, *J. Chromatogr.*, 248 (1982) 203.
21 P. Burchill, A. A. Herod, J. P. Mahon and E. Pritchard, *J. Chromatogr.*, 265 (1983) 223.
22 K. Kamata and N. Motohashi, *J. Chromatogr.*, 319 (1985) 331.
23 G. Grimmer, K.-W. Naujack and G. Dettharn, *Toxicol. Lett.*, 35 (1987) 117.
24 M. Dong and D. C. Locke, *J. Chromatogr. Sci.*, 15 (1977) 32.
25 H. Colin, J.-M. Schmitter and G. Guiochon, *Anal. Chem.*, 53 (1981) 625.
26 L. J. Boux, C. M. Ireland, D. J. Wright, G. M. Holder and A. J. Ryan, *J. Chromatogr.*, 227 (1982) 149.
27 D. A. Hougen, M. J. Peak, K. M. Suhrbier and V. C. Stamoudls, *Anal. Chem.*, 54 (1982) 32.
28 L. A. D'Avla, M. Colin and G. Guiochon, *Anal. Chem.*, 55 (1983) 1019.
29 A. H. Siouffi, M. Righezza and G. Guiochon, *J. Chromatogr.*, 368 (1986) 189.
30 N. P. Buu-Hoi, R. Roger and M. Hubert-Habart, *J. Chem. Soc.*, (1965) 1082.
31 F. Ullmann and A. La Torie, *Chem. Ber.*, 37 (1904) 2922.
32 I. Y. Postovskii and B. N. Lundion, *J. Gen. Chem. USSR*, 10 (1940) 71.
33 N. P. Buu-Hoi, *J. Chem. Soc.*, (1949) 670.
34 N. P. Buu-Hoi and L. Lecocq, *C.R. Acad. Sci.*, 218 (1944) 794.
35 N. P. Buu-Hoi, *J. Chem. Soc.*, (1946) 792.
36 J. V. Braum and P. Wolff, *Chem. Ber.*, 55 (1922) 3675.
37 T. Okano, T. Horie, N. Motohashi, Y. Watanabe and Y. Nishimiya, *Gann*, 66 (1975) 529.