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## Note

# Separation of methyl-substituted benz[*c*]acridines by reversed-phase high-performance thin-layer chromatography

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Several methods have been reported for the detection and determination of methyl-substituted benz[*c*]acridines (BAc) in air pollution sources and urban atmospheres[1–6], tobacco smoke[7] and coal-derived fuel samples[8–11]. Their analysis is important because of their carcinogenic and mutagenic properties[12–15].

Most work on the analysis of BAc has involved gas chromatography[4,16–18] and high-performance liquid chromatography (HPLC)[10,18,19]. Also, a few papers have been published on the thin-layer chromatography (TLC) of BAc[3,4,19–21]. TLC is especially used as a final step in the analysis of BAc for the purposes of both detection and clean-up for the elimination of interfering materials. However, no studies have been published on the analysis of BAc by reversed-phase high-performance TLC (RP-HPTLC). This technique is particularly suitable for the separation and analysis of polar compounds that do not migrate in conventional TLC. Also, the resolution achieved by RP-HPTLC is far superior to that obtained by conventional TLC.

In this work, we have developed a solvent system for the separation of BAc by RP-HPTLC.

## EXPERIMENTAL

### *Chemical and apparatus*

BAc were synthesized according to the literature[22–28] and purified as described in a previous paper[29]; benz[*c*]acridine, 7-methylbenz[*c*]acridine, 8-methylbenz[*c*]acridine, 9-methylbenz[*c*]acridine, 10-methylbenz[*c*]acridine, 11-methylbenz[*c*]acridine, 5,7-dimethylbenz[*c*]acridine, 7,9-dimethylbenz[*c*]acridine, 7,10-dimethylbenz[*c*]aridine, 7,11-dimethylbenz[*c*]acridine, 7,9,10-trimethylbenz[*c*]acridine and 7,9,11-trimethylbenz[*c*]acridine.

HPTLC pre-coated RP-18 plates from E. Merck (Darmstadt, F.R.G.) were used.

The reagent and solvents were of analytical-reagent grade (Wako, Osaka, Japan) and were used without further purificatin.

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TABLE I

$R_F$ VALUES OF BAc OBTAINED USING SINGLE SOLVENTS

| *Compound* | *Solvent* | | | | | |
|---|---|---|---|---|---|---|
| | *Methanol* | *Ethanol* | *Acetonitrile* | *Acetone* | *Ethyl acetate* | *Chloroform* |
| Benz[*c*]acridine | 0.31 | 0.53 | 0.33 | 0.88 | 0.93t* | 0.83t |
| 7-Methylbenz[*c*]acridine | 0.27 | 0.48 | 0.18 | 0.85 | 0.88t | 0.68t |
| 8-Methylbenz[*c*]acridine | 0.27 | 0.48 | 0.38 | 0.84 | 0.93t | 0.82t |
| 9-Methylbenz[*c*]acridine | 0.25 | 0.46 | 0.36 | 0.83 | 0.93t | 0.81t |
| 10-Methylbenz[*c*]acridine | 0.27 | 0.48 | 0.23 | 0.83 | 0.93t | 0.77t |
| 11-Methylbenz[*c*]acridine | 0.21 | 0.43 | 0.32 | 0.81 | 0.94 | 0.91 |
| 5,7-Dimethylbenz[*c*]acridine | 0.22 | 0.43 | 0.14 | 0.80 | 0.89t | 0.56t |
| 7,9-Dimethylbenz[*c*]acridine | 0.22 | 0.43 | 0.13 | 0.80 | 0.89t | 0.56t |
| 7,10-Dimethylbenz[*c*]acridine | 0.22 | 0.43 | 0.11 | 0.78 | 0.85t | 0.47t |
| 7,11-Dimethylbenz[*c*]acridine | 0.17 | 0.38 | 0.28 | 0.77 | 0.94 | 0.91 |
| 7,9,10-Trimethylbenz[*c*]acridine | 0.19 | 0.42 | 0.07 | 0.76 | 0.81t | 0.38t |
| 7,9,11-Trimethylbenz[*c*]acridine | 0.14 | 0.29 | 0.23 | 0.75 | 0.94 | 0.91 |

* t = Tailing.

Chromatography was performed in glass chromatography tanks (12 × 20 × 23 cm). The plates were spotted using a Hamilton mucrosyringe and were viewed under short-wave UV light (254 nm) using a chromato-UV instrument (Ultra-violet Products, San Gabriel, CA, U.S.A.).

*Thin-layer chromatography*

Standard solutions of 100 ppm (w/v) were prepared in ethanol and 1 μl was spotted 2.0 cm from the edge of the plate. The solvent was allowed to migrate 10 cm from the starting line. The chromatograms were developed at room temperature (20 ± 3°C) in a chamber pre-saturated with before use. Only single developments were tested in this work.

## RESULTS AND DISCUSSION

More than 40 solvent systems have been tried for the separation of BAc with the TLC pre-coated RP-18 plates. The $R_F$ values of the BAc obtained using single solvents for development are listed in Table I, and those using combined solvents in Table II. Each $R_F$ value represents the mean of three determinations. The detection limit was found to be *ca.* 2 ng for each compound. When developed with methanol or acetonitrile, the BAc were distributed at low $R_F$ values. The spots of all the BAc except 10-methylbenz[*c*]acridine, 7,10-dimethylbenz[*c*]acridine and 7,9,10-trimethylbenz[*c*]acridine showed tailing with the solvent systems chloroform–ethyl acetate–acetonitrile–ethyl acetate and acetonitrile–chloroform–acetic acid.

TABLE II

$R_F$ VALUES OF BAc OBTAINED USING COMBINED SOLVENTS

Solvent systems: I = acetonitrile–acetone (8:2); II = acetonitrile–chloroform (8:2); III = acetonitrile–ethyl acetate (8:2); IV = acetone–water (8:2); V = acetonitrile–acetone–water (7:2:1); VI = acetonitrile–acetone–0.1 *M* sodium 1-pentanesulphonate (8:2:1); VII = acetonitrile–chloroform–water (8:1.5:0.5); VIII = acetonitrile–chloroform–25% ammonia (8:1.5:0.5); IX = acetonitrile–chloroform–acetic acid (8:1.5:0.5); X = acetonitrile–chloroform–0.1 *M* sodium 1-pentanesulphonate (8:1.5:0.5).

| *Compound* | *Solvent system* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *I* | *II* | *III* | *IV* | *V* | *VI* | *VII* | *VIII* | *IX* | *X* |
| Benz[*c*]acridine | 0.49 | 0.50 | 0.49t* | 0.36 | 0.37 | 0.36 | 0.51 | 0.58 | 0.58t | 0.59 |
| 7-Methylbenz[*c*]acridine | 0.38 | 0.32 | 0.35t | 0.32 | 0.32 | 0.36 | 0.48 | 0.54 | 0.52t | 0.55 |
| 8-Methylbenz[*c*]acridine | 0.47 | 0.45 | 0.45t | 0.31 | 0.30 | 0.33 | 0.48 | 0.53 | 0.56t | 0.54 |
| 9-Methylbenz[*c*]acridine | 0.44 | 0.44 | 0.42t | 0.29 | 0.28 | 0.30 | 0.44 | 0.50 | 0.52t | 0.51 |
| 10-Methylbenz[*c*]acridine | 0.42 | 0.40 | 0.40t | 0.30 | 0.30 | 0.34 | 0.47 | 0.52 | 0.54t | 0.53 |
| 11-Methylbenz[*c*]acridine | 0.45 | 0.52 | 0.48 | 0.24 | 0.24 | 0.23 | 0.40 | 0.45 | 0.50 | 0.48 |
| 5,7-Dimethylbenz[*c*]acridine | 0.30 | 0.28 | 0.27t | 0.26 | 0.25 | 0.33 | 0.43 | 0.48 | 0.53t | 0.51 |
| 7,9-Dimethylbenz[*c*]acridine | 0.30 | 0.27 | 0.28t | 0.26 | 0.25 | 0.39 | 0.43 | 0.48 | 0.53t | 0.51 |
| 7,10-Dimethylbenz[*c*]acridine | 0.26 | 0.24 | 0.25t | 0.26 | 0.25 | 0.39 | 0.43 | 0.48 | 0.53t | 0.53 |
| 7,11-Dimethylbenz[*c*]acridine | 0.41 | 0.48 | 0.44 | 0.21 | 0.19 | 0.20 | 0.36 | 0.41 | 0.47 | 0.44 |
| 7,9,10-Trimethylbenz[*c*]acridine | 0.20 | 0.19 | 0.18t | 0.23 | 0.22 | 0.41 | 0.44 | 0.45 | 0.52t | 0.54 |
| 7,9,11-Trimethylbenz[*c*]acridine | 0.36 | 0.44 | 0.39 | 0.17 | 0.15 | 0.13 | 0.31 | 0.35 | 0.42 | 0.38 |

* t = Tailing.

The combination of acetonitrile with chloroform and acetone gave satisfactory separations. When developed with acetonitrile–chloroform (8:2), the BAc were distributed, with good separation, between $R_F$ 0.19 and 0.52. The $R_F$ values of BAc obtained using acetonitrile–chloroform (8:2) as the solvent system followed the order of decreasing number of carbon atoms. However, the $R_F$ values behaviour of compounds substituted with a methyl group at position 11 apparently differed from those of other derivatives. This effect, which can be related to the steric hindrance of substituens located near the nitrogen atom, was similar to that observed when using HPLC[18]. We also examined the effect of water, acids, bases and sodium 1-pentanesulphonate as ion-pair reagent on the resolution of BAc. In general, in reversed-phase chromatography, the $R_F$ values can be influenced by changes in the composition of the developing solvent. However, we observed that such changes had no pronounced effect on the resolution and the use of acetic acid caused tailing.

From the results, it can be concluded that the system described here is useful for the simple identification of BAc.

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