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Note

Separation of methoxyanilines by competing-ion reversed-phase high-performance liquid chromatography

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Reversed-phase high-performance liquid chromatography (HPLC) is well established as a powerful separation tool for both ionic and non-ionic compounds. A major reason for its widespread use for separation of ionic compounds has been the development of ion-pair chromatography[1–3]. This technique can be quite useful for both basic and acidic compounds, but the actual mechanism by which it functions is still widely debated[4]. One model explains retention by formation of ion pairs in the mobile phase. These non-polar ion pairs are then retained by the non-polar column[5,6]. Another theory proposes an ion-exchange mechanism after absorption of the ion-pair reagent onto the stationary phase[7–11]. Bidlingmeyer *et al.*[12] have proposed a third, an ion-interaction model, to explain retention characteristics by formation of a dynamic electrostatic double layer on the surface of the stationary phase. This charged layer then effectively shields charged solute molecules from the column bed.

Another technique commonly used for ionic compounds is ionic suppression[13,14]. With this technique, appropriate use of buffers in the mobile phase suppresses ionization of solute molecules and improves peak shape. Ionic suppression can be a powerful technique, since retention is governed principally by pH. Its use is limited, however, to relatively weak acids and bases, since ionization of stronger solutes cannot be suppressed without exceeding the pH limitation (pH = 2–8) of most silica-based columns[5,15].

This paper discusses competing-ion chromatography, another technique useful for ionic compounds. In this case, an ionic compound of similar charge to the solute molecule is added to the mobile phase and is adsorbed onto the stationary phase to form a charged layer. Hence, solute molecules are repelled from the column bed and show reduced retention and improved peak shape[16,17]. This technique is demonstrated in the separation of positional isomers of mono- and disubstituted methoxyanilines.

Björkqvist[18] has analyzed both aliphatic and aromatic amines by reversed-phase HPLC after derivatization with phenyl isocyanate, but water and some alcohols may interfere. Narang *et al.*[19] have discussed a normal-phase separation of aromatic amines while Lores *et al.*[20] have described a separation of halogenated aniline using a fairly harsh buffering system (pH 2.1) and gradient elution. Most other workers have used ionic suppression in their analysis of amines.

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EXPERIMENTAL

A Waters Assoc. microprocessor-based HPLC system, consisting of a Model 720 system controller, a Model 730 data module, a Model 710B sample processor, a Model 440 UV detector operating at 254 nm and 0.5 a.u.f.s., and two Waters pumps, was used. Separation was obtained on a 30 cm × 3.9 mm Waters μBondapak C_{18} column. Statistical calculations were performed by the Model 720 system controller with a BASIC program developed by the author.

HPLC-grade acetonitrile, glacial acetic acid and ammononium acetate were obtained from Baker. Distilled water was further purified using a Millipore Norganic™ cartridge. ACS grade dibutylamine and aniline purchased from Fisher Scientific and the methoxyaniline isomers purchased from Aldrich were used without further purification. PIC B-5™ and PIC B-7™ reagent cocktails were obtained from Waters Assoc. and prepared as recommended by the manufacturer.

RESULTS AND DISCUSSION

Repeated trials utilizing ion-pair chromatography proved unsuccessful. Waters PIC B-7 ion-pair reagent, as would be expected, gave more retention than PIC B-5, but neither gave adequate separation of the methoxyaniline isomers of interest. The use of ionic-suppression chromatography was also unsuccessful. While addition of ammonium acetate as a pH adjuster provided symmetrical peaks, several of the isomers were still not separated.

As seen in Fig. 1, competing-ion chromatography does provide excellent separation of all the methoxyaniline isomers of interest. The competing-ion reagent is 0.01 *M* in dibutylamine and contains 0.1% acetic acid as pH adjuster. If necessary, a final pH adjustment to 4.50 is made with sodium hydroxide. A similar reagent is available commercially from Waters Assoc., but it utilizes a phosphate buffer. Acetate is used here because of better solubility of acetate salts in organic modifiers[21] and less chance of bacteriological degradation[22]. The pH of the mobile phase at 4.5 is well within the limitations of silica-based columns and is, therefore, less likely to dissolve the column base. Formation of active silanol sites on the column is not of major concern, because the competing-ion reagent effectively shields these sites from

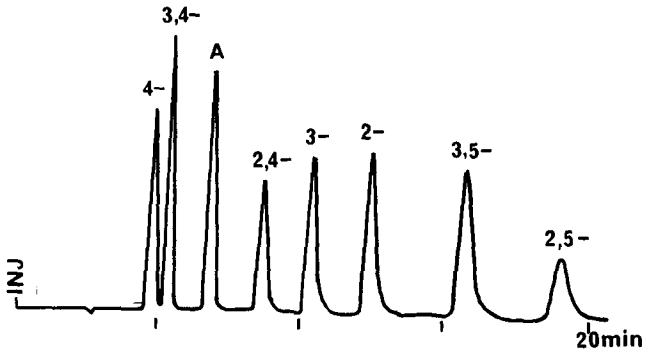

Fig. 1. Competing-ion separation of methoxyanilines. Mobile phase, acetonitrile–0.01 *M* dibutylamine (10:90) with 0.1% acetic acid; flow-rate, 1.0 ml/min. A = Aniline (internal standard).

TABLE I

PRECISION DATA FOR METHOXYANILINES BY COMPETING-ION CHROMATOGRAPHY (INTERNAL STANDARD)

HPLC conditions: 30 cm × 3.9 mm μBondapak C_{18} column; mobile phase, acetonitrile–0.01 *M* dibutylamine (10:90) with 0.1% acetic acid (pH 4.5); flow-rate, 1.0 ml/min.

Compound	*Retention*		*Quantitation*	
	Time	*% R.S.D.*	*Amount (mg/100 ml)*	*% R.S.D.*
4-Methoxyaniline	4.96	0.52	25.9	±1.00
3,4-Dimethoxyaniline	5.50	0.48	25.0	±1.00
Aniline internal standard	6.98	0.79	–	–
2,4-Dimethoxyaniline	8.78	0.21	27.1	±0.55
3-Methoxyaniline	10.42	0.10	25.6	±0.70
2-Methoxyaniline	12.50	0.05	35.0	±0.28
3,5-Dimethoxyaniline	15.82	0.06	25.0	±0.55
2,4-Dimethoxyaniline	19.10	0.08	25.0	±0.37

the solute molecules of interest. Columns may be used for extended periods without noticeable degradation of peak shape. As is the case with ion-pairing reagents, columns used with competing-ion reagents should be used exclusively for that purpose, since retention characteristics of the column may be changed even when used without these reagents.

Table I lists retention time and quantitation data for the methoxyaniline isomers. Aniline is added as an internal standard to improve precision. The precision data are based on nine duplicate injections and indicate that excellent agreement can be expected with this technique.

The competing-ion technique should be considered when developing separations of ionic compound by reversed-phase HPLC. Positional isomers are often better separated than with the more traditional ion-pairing techniques, and the resulting mobile phase is not usually as detrimental to silica-based columns. Peak broadening is better controlled, especially on silica-based columns which are not completely end-capped or partially hydrolyzed by harsh mobile phases.

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