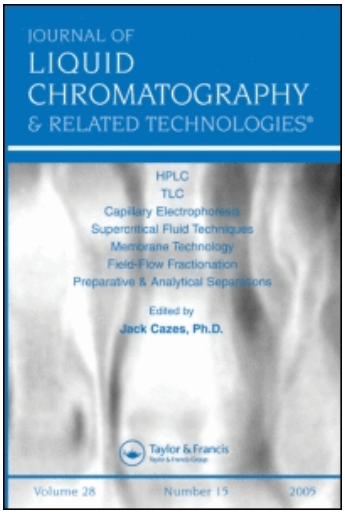

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Ion Exchange High Performance Liquid Crhomatography Separations Using a High Content Organic Modifier Mobile Phase

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ION EXCHANGE HIGH PERFORMANCE LIQUID CRHOMATOGRAPHY SEPARATIONS USING A HIGH CONTENT ORGANIC MODIFIER MOBILE PHASE

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ABSTRACT

The separation of a mixture of structurally similar compounds was achieved using a cation exchange column and a buffered mobile phase rich in an organic modifier 50-75%. The addition of different modifiers to the mobile phase affected not only the selectivity but the resolution.

INTRODUCTION

Separations in liquid chromatography (LC) are the result of the interactions between the solute, the mobile phase and the stationary phase. When the interactions between them are optimum, the separations will be optimum, too. In ion exchange chromatography (IEC) the properties of the mobile phase, pH, buffer used and its concentration (ionic strength) and

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the addition of organic modifiers, type and concentration, will enhance resolution.

Although the mechanism of separation in adsorption and partition LC is better understood, sample retention in IEC is less understood than in the other LC methods (1).

In this study the effect of the addition of high percentage of an organic modifier to the mobile phase on the separation of structurally similar compounds (Figure 1) was probed, using a strong cation exchange

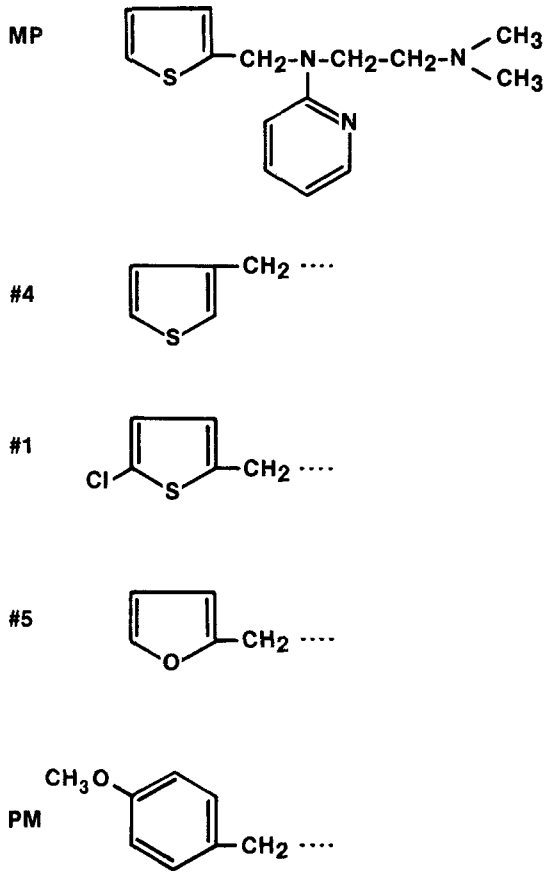

Figure 1. Structural formula of the 5 compounds used in this study.

column. Also, the effect of the addition of different organic modifiers, such as acetonitrile, methanol, and tetrahydrofuran, was studied.

EXPERIMENTAL

Materials: Methapyrilene (MP) and pyrylamine maleate (PM) were from Sigma Chemicals. The other three compounds were synthesized according to published procedures, N,N-dimethyl-N'-(2-pyridyl)-N'-(3-thenyl)ethylenediamine (2), N,N-dimethyl-N'-(2-pyridyl)-N'-(5-chloro-2-thenyl)ethylenediamine (3), and N,N-dimethyl-N'-(2-pyridyl)-N'-(2-furfuryl)ethylenediamine (4). Acetonitrile, tetrahydrofuran (THF) and methanol (MeOH) were glass distilled (Burdick and Jackson).

Apparatus: A modular HPLC system consisting of Laboratory Data Control (LDC) Constametric I and II pumps attached to a LDC Gradient Master, a Chromatronix dual channel UV detector, a Rheodyne injector and a strip chart recorder operated at 0.2 in/min. was used for this study. The column was 250 mm long x 4.6 mm i.d. prepacked with a 10μ partisil-10 SCX material (Whatman, Inc.). Experiments were run at room temperature using a mobile phase flow of 3 ml/min. The isocratic mobile phase was 50-75% organic modifier in a .0005M phosphate buffer having a pH of 7.3. The mobile phase was passed through a Millipore 0.45μ filter and degassed daily in an ultrasonic bath under vacuum before use. Detection was carried out at 254 nm.

RESULTS AND DISCUSSION

The separation of methapyrilene hydrochloride from feed and sleep aid tablets was reported using a reversed phase C_{18} column using a mobile phase

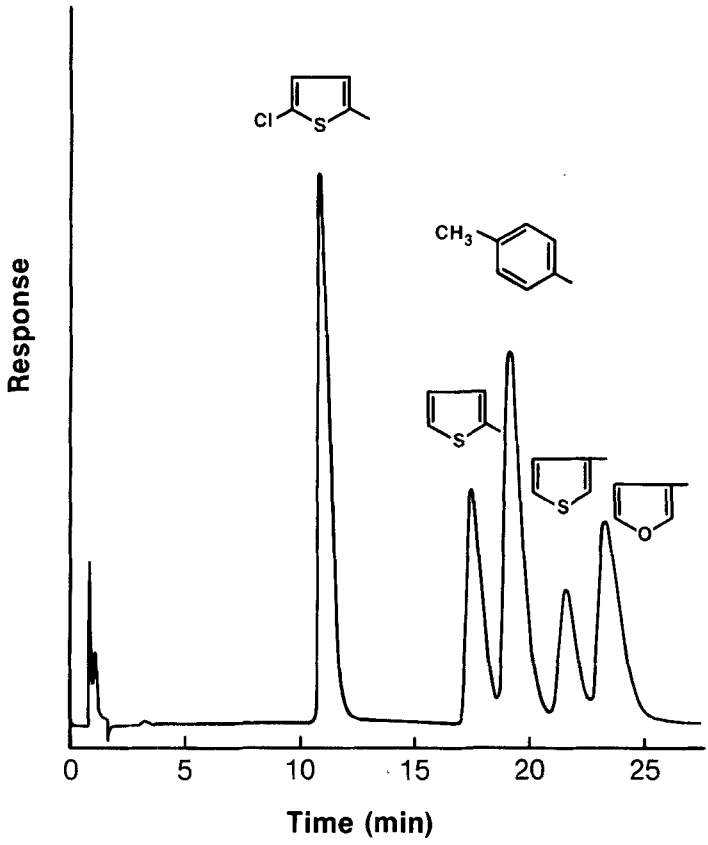

Figure 2. An HPLC chromatogram of the five compounds separated on a cation exchange volumn using 75% acetonitrile: 25% 0.0005M phosphate buffer having a pH of 7.3.

of a (1:1) mixture of acetonitrile and 1.1% ammonium carbonate (5). However, tailing of methapyrilene was observed.

Due to the basic nature of methapyrilene and its structural analogs, it was decided to use a cation exchange column rather than a reversed phase one. Preliminary results indicated that the addition of an organic modifier such as methanol, acetonitrile or tetrahydrofuran to the aqueous buffer solution would prevent tailing of the methapyrilene peak. Acetonitrile

was selected as the organic modifier since it is the least viscous of the other two organic modifiers, resulting in reduced back pressure and better separation.

The separation of the mixture of the five components listed in Figure 1 was achieved using a 1:3 mixture of 0.0005M phosphate buffer (pH 7.3) and acetonitrile (Figure 2). It is generally known that sample retention in IEC is reduced with an increase in the amount of organic modifier added to the mobile phase (6). However, in this case, increasing the volume of acetonitrile from 50% to 75% increased the retention time from 13 to 23 minutes.

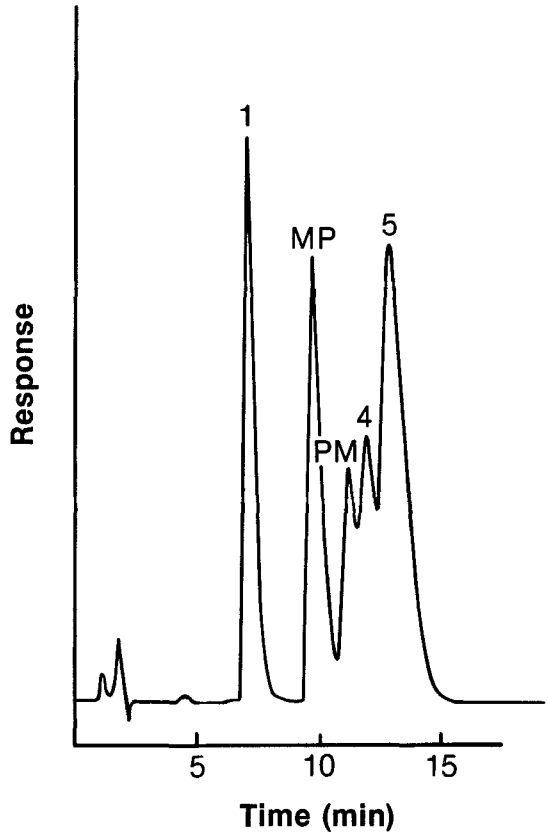

Figure 3. Same as Figure 2 except that a 1:1 acetonitrile: buffer mobile phase was used.

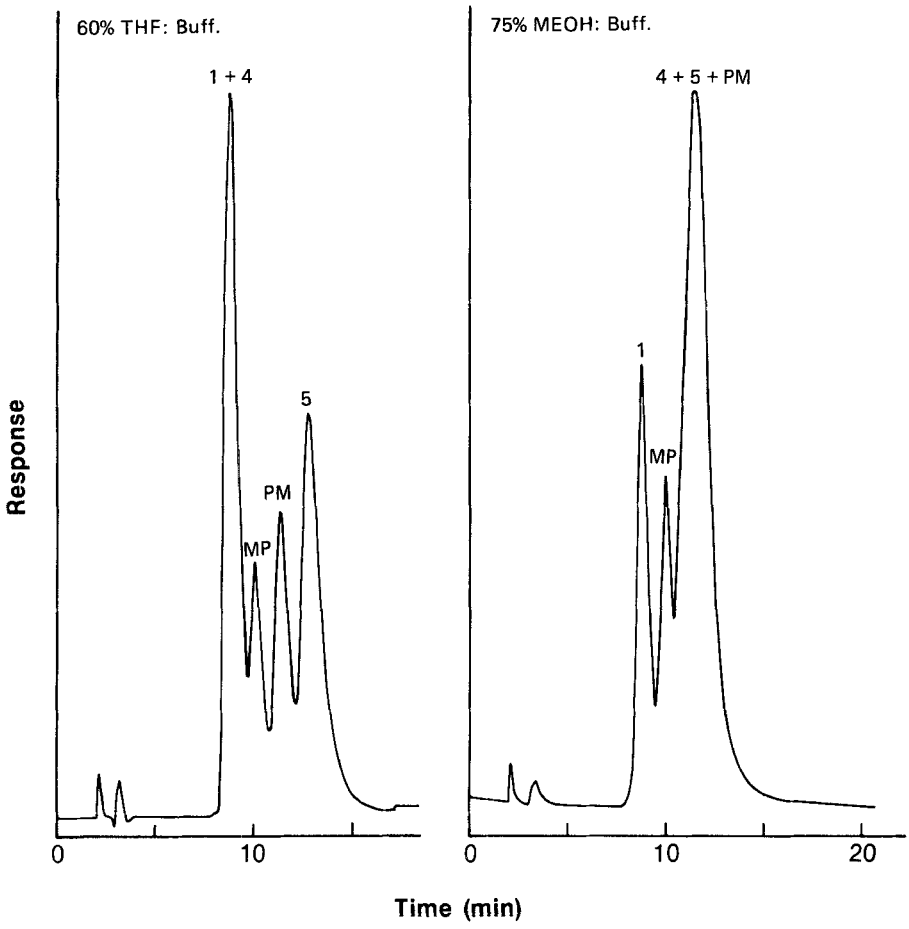

Figure 4. Chromatograms of the 5 component mixture using a cation exchange column and 60% THF: buffer (left) and 75% methanol: buffer (right).

Also, when the volume of the organic modifier was increased from 50% acetonitrile to 75% acetonitrile, better resolutions were observed, compare Figures 2 and 3. However, when methanol: buffer (75:25) and tetrahydrofuran: buffer (60:40) were used as mobile phases, Figure 4, the separations were not as good as when acetonitrile was used in the mobile phase (Figure 3).

Also, the selectivities, i.e., elution order of the 5 components were different using different organic modifiers.

Although slight changes in the structure differentiates between the components of the mixture studied, especially between MP and #4 with only a positional change of the thiophene substitution, and between #4 and #5 where the sulfur atom was replaced by an oxygen atom, separation was achieved in 75% acetonitrile: buffer. When a chlorine atom replaced a hydrogen atom on the thiophene ring (#1) the retention time difference between MP and #1 was approximately 10 minutes in the same mobile phase.

The results (Figure 2), therefore, indicate that IEC HPLC can be used to separate positional isomers as well as other analogs with slight structural differences. The results also indicate that the use of high percentages of an organic modifier in IEC would lead to otherwise unachievable separations.

REFERENCES

1. Snyder, L.R. and Kirkland, J.J., Introduction to Modern Liquid Chromatography, 2nd Edition, J. Wiley and Sons, Inc., New York, (1979), p. 419.

2. Campaigne, E. and LeSuer, W.M., J. Am. Chem. Soc., 71, 333, (1949).

3. Clapp, R.C., Clark, J.H., Vaughan, J.R., English, J.P., and Anderson, J.W., J. Am. Chem. Soc., 69, 1549 (1947) and Tyler, L.J. Sommer, L.H., and Whitmore, F.C., J. Am. Chem. Soc., 69, 980 (1947).

4. Kyrides, L.T. and Zientry, M.S., J. Am. Chem. Soc., 71, 1121 (1949).

5. Shaikh, B. and Hallmark, M., J. Association Off. Anal. Chem., 64, 889, (1981).

6. Snyder, L.R. and Kirkland, J.J., Introduction to Modern Liquid Chromatography, 2nd Edition, J. Wiley and Sons, Inc., New York, (1979), p. 426.