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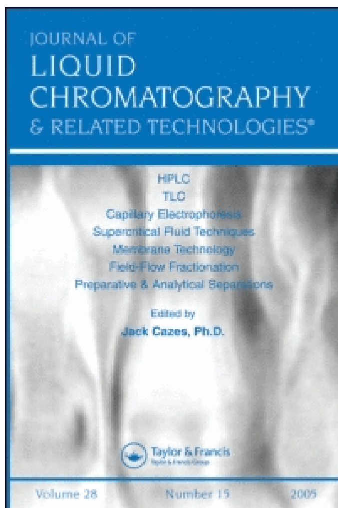

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SEPARATION OF XANTHINES AND URIC ACIDS BY CAPILLARY ZONE ELECTROPHORESIS AND MICELLAR ELECTROKINETIC CAPILLARY ELECTROPHORESIS

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ABSTRACT

A CZE method was developed for the separation of xanthines and uric acid derivatives which are normally present in human plasma and urine as metabolites of caffeine. The methyl-substituted uric acids were separated using a 0.05 M sodium phosphate buffer (pH 7.0) at an applied voltage of 10 kV. In contrast, the separation of the methyl-substituted xanthines was only possible in the MECE mode, where SDS (0.15 M) was added to the buffer system. When both types of compounds were present in the same sample, optimum resolution (12 peaks for 13 standard solutes) was realized in the MECE mode with 0.15 M SDS added to the 0.05 M, pH 7 phosphate buffer at an applied voltage of 15 kV.

INTRODUCTION

Capillary zone electrophoresis (CZE) is recognized as a highly efficient and sensitive microanalytical technique. It has rapidly developed since Jorgenson and co-workers (1,2) realized the advantages of using small diameter (<100 μm) fused silica columns. In this technique ionic substances of different molecular size and shape are separated according to their differential mobilities in semiconducting mobile phases under the influence of an

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electric potential gradient. As a consequence of the applied electric field a strong electroosmotic flow is created inside the capillary. With untreated fused silica under normal aqueous conditions with small binary electrolytes, the wall surface has a net negative charge resulting in a flat velocity profile flow which causes all kinds of solutes (cationic, anionic and neutral) to elute at the cathodic end of the tube.

As a separation technique CZE is limited to the separation of charged substances which exhibit differential electrophoretic mobility. Neutral molecules migrate with the same velocity as the solvent under the influence of electroosmotic flow.

In order to take advantage of the high separation efficiencies obtained by CZE (normally in the range of 1×10^5 to 1×10^6 theoretical plates) for the separation of neutral molecules modification of the buffer should be undertaken. Terabe, et al (3) introduced micellar electrokinetic capillary electrophoresis (MECE), an adaptation of CZE where the addition of an ionic micellar agent to the electrolyte allows the separation of neutral molecules based on chromatographic distribution principles. MECE also offer advantages over CZE for the separation of ionic substances in terms of improved selectivity and peak shape (4-6). In MECE, the migration time of an ionic substance is a function of three factors: (1) the electrophoretic mobility of the solute itself; (2) the distribution ratio of the solute between the micellar phase and the aqueous phase; and (3) ion-pair formation between the solute molecules and the micelles.

In this work the separation of a standard mixture of xanthines and uric acids using CZE and MECE is developed and the factors affecting the separation are evaluated. Xanthines and uric acids are present in human plasma and urine as metabolites of caffeine and many are pharmacologically active. Their separation and quantification is of great importance in the study of therapeutic and metabolic problems related to their presence.

EXPERIMENTAL

Materials:

The xanthines and uric acids used in this study were purchased from Fluka AG, Switzerland and used without further purification. Sodium phos-

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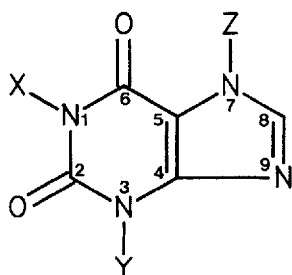
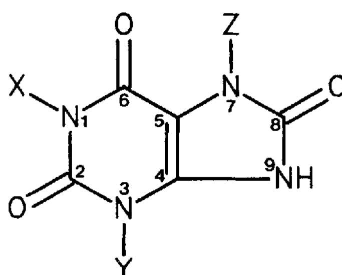
Table I. List of Solutes





Solute	X	Y	Z
Xanthine (X)	H	H	H
1-MeX	Me	H	H
3-MeX	H	Me	H
7-MeX	H	H	Me
1,3-Mex	Me	Me	H
1,7-MeX	Me	H	Me
3,7-MeX	H	Me	Me
1,3,7-MeX	Me	Me	Me

Solute	X	Y	Z
Uric Acid (U)	H	H	H
1-MeU	Me	H	H
3-MeU	H	Me	H
7-MeU	H	H	Me
1,3-MeU	Me	Me	H
1,7-MeU	Me	H	Me
3,7-MeU	H	Me	Me

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phate-dibasic, phosphoric acid and sodium dodecyl sulphate (SDS) were purchased from Fisher Scientific. The buffers were prepared by dissolving the appropriate amounts of sodium phosphate-dibasic and phosphoric acid in distilled and deionized water. The pH was adjusted to 7.0 using phosphoric acid.

Apparatus and Methods:

A Beckman CZE system (Model P/ACE) equipped with a UV detector, an automatic injector, column cartridge (50 cm x 75 μm i.d., surrounded by coolant), autosampler, and a printer was used in this study. A Fisher Accumet

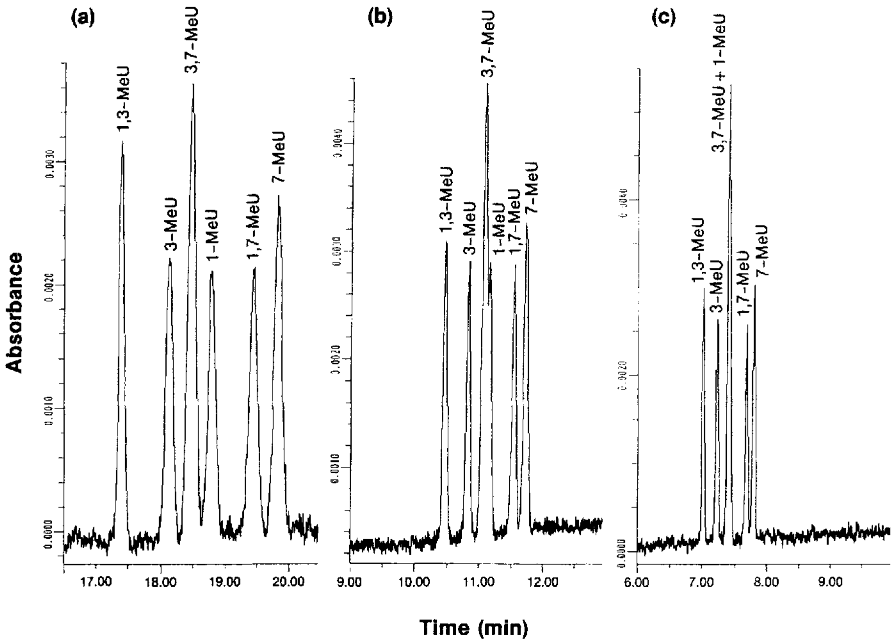

Figure 1 Electropherograms of six methyl-substituted uric acids. Capillary: 50 cm x 75 μm i.d.; buffer: 0.05 M Na_2HPO_4; pH 7.0; pressure injection mode: 2 sec at 0.5 psi; temperature: 25°C; detector: UV at 280 nm. Solute symbols as given in Table I. Applied voltage: (a) 10 kV, (b) 15 kV, (c) 20 kV.

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selective ion analyzer Model 750 was used for pH measurements. All experiments were carried out at 25°C and were run at least in triplicates to insure reproducibility. Injections were made using the pressure mode for 2 seconds. Buffer standard solutions were degassed and filtered through a 0.2 μm Nylon 66 filters. Solute standards were prepared to be 40-120 μg/mL, and were monitored at 280 nm.

RESULTS AND DISCUSSION

Caffeine (1,3,7-trimethylxanthine) undergoes oxidative demethylation and hydroxylation reactions in man and various other species to yield a number of

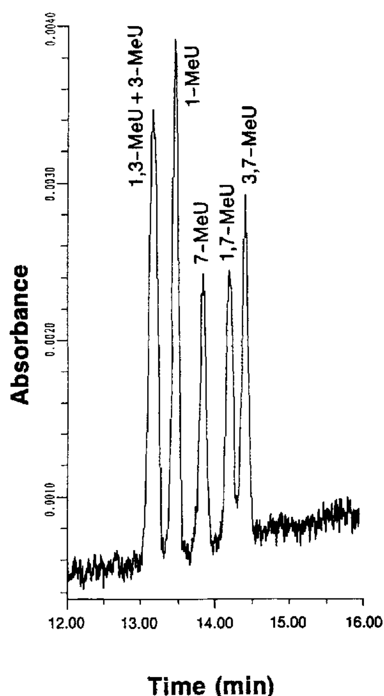

Figure 2 Electropherogram of six methyl-substitubed uric acids. Capillary: 50 cm x 75 μm i.d.; buffer: 0.05 M Na_2HPO_4 + 0.15 M SDS; pH 7.0; pressure injection mode: 2 sec at 0.5 psi; temperature: 25°C; detector: UV at 280 nm; voltage: 15 kV. Solute symbols as given in Table I.

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urinary excretion products (7). Several HPLC methods were developed (8,9) and optimized for resolution and analysis time (7,10).

In this work, we investigated the possibility of using CZE for this type of analysis. Although CZE is at least an order of magnitude lower in sensitivity compared to HPLC, the method is favored for situations where high peak capacity is required such as in the analysis of drug metabolites and biotechnological products.

In a previous study we showed that changing the buffer type results, among other things, in changes in selectivity for small charged molecules (11-

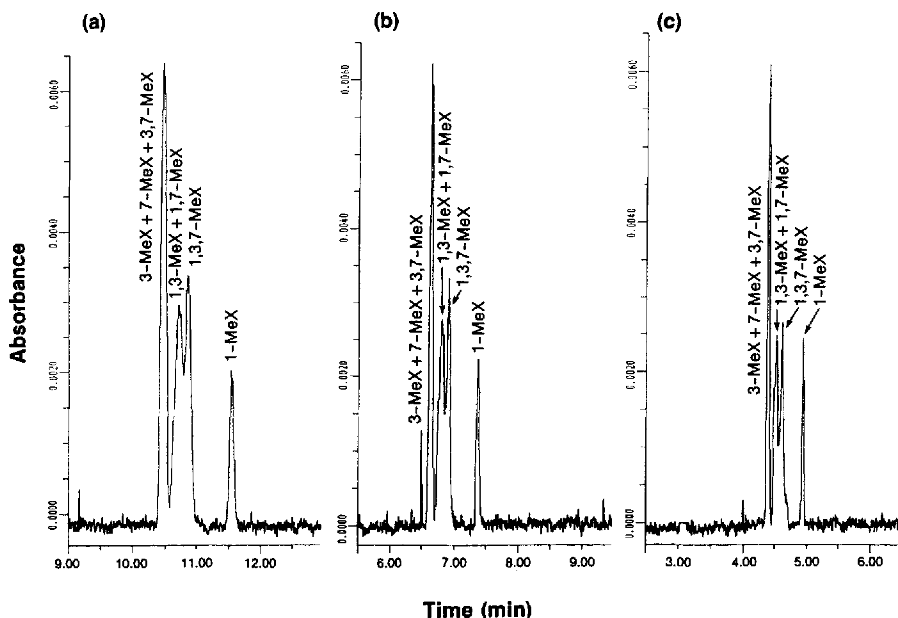

Figure 3 Electropherograms of seven methyl-substituted xanthines. Experimental conditions: same as Figure 1.

13). In trying to arrive at the optimum conditions for the separation of xanthines and uric acids we studied the effect of buffer type and concentration, pH, SDS concentration and applied voltage. Within the series of buffers tested (sodium citrate at pH = 3.0; sodium acetate at pH = 5.0; sodium phosphate at pH = 7.0; and sodium tetraborate at pH = 8.5), the sodium phosphate buffer at concentration of 0.05 M gave the best results. The operating conditions were optimized to provide good resolution in a reasonable analysis time for low current. The solutes studied are listed in Table I, together with their symbols.

Figure 1 shows electropherograms of six methyl-substituted uric acids at different voltages. Although the separation improved as the voltage was decreased from 15 kV to 10 kV the analysis time was almost doubled. Figure 2

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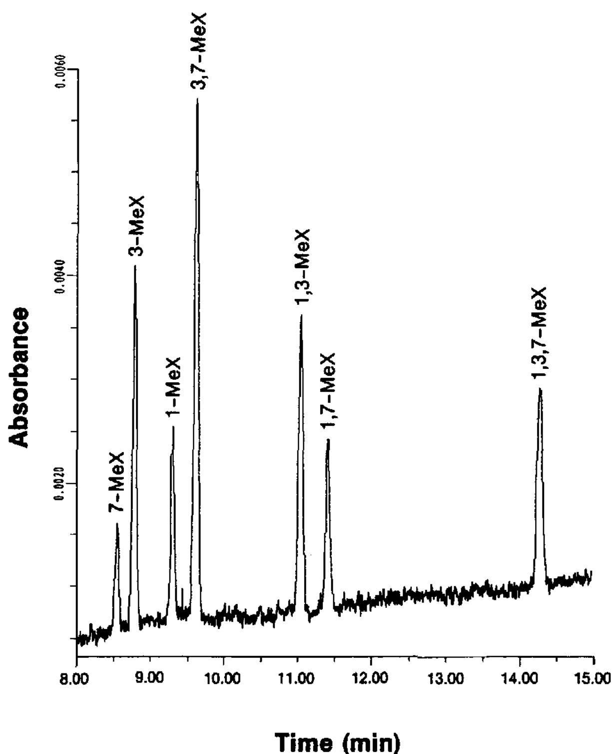

Figure 4 Electrpherogram of seven methyl-substituted xanthines. Experimental conditions: same as Figure 2.

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shows the effect of the addition of SDS on the separation of the same set of solutes. Even at the optimum SDS concentration (0.15 M), the resolution was not complete as five peaks were observed for a set of six solutes. Furthermore, the separation did not improve with a change in voltage.

Figure 3 shows the separation of seven methyl-substituted xanthines (see Table I for solute symbols). Only four peaks were observed and the separation did not improve with a change in voltage. However, a dramatic improvement was observed as shown in figure 4, when SDS was added to the buffer at a concentration of 0.15 M. All seven xanthine solutes were completely resolved with

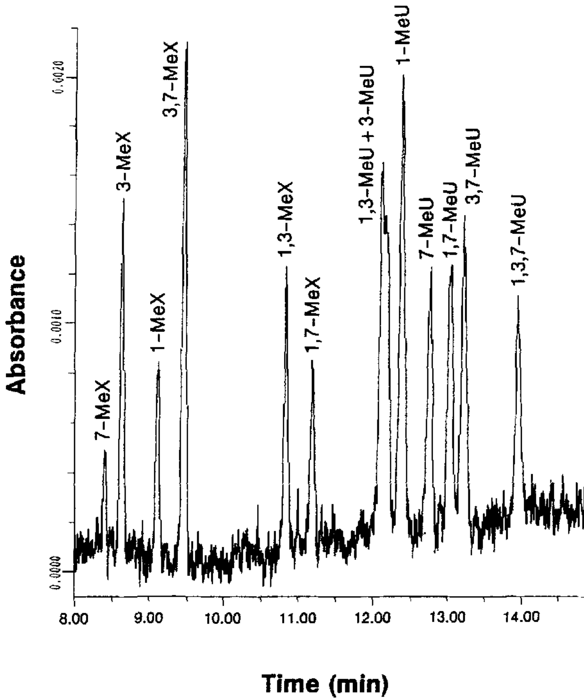

Figure 5 Electropherogram of a mixture of seven methyl-substituted xanthines and six methyl-substituted uric acids. Experimental conditions: same as in Figure 2.

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the mono-methyl xanthines eluting first, followed by the di-substituted xanthines and finally 1,3,7-trimethyl xanthine. It is curious to note that this is the same order of retention as reported with HPLC (7-10). In contrast the order of elution of the xanthines with no SDS added to the operating buffer was random, as shown in figure 3.

Finally, figure 5 shows the separation of a standard mixture of xanthines and uric acids at the optimum experimental conditions stated in the figure caption. The analysis time is only half that obtained by reversed-phase HPLC (7).

It is clear from the above results that MECE is a useful analytical technique for both charged and neutral molecules. This study shows that the separation of most caffeine metabolites by MECE is possible. However, at the present time it is not possible to quantitate these metabolites in biological fluids due to sensitivity restraints in current UV detection methods.

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