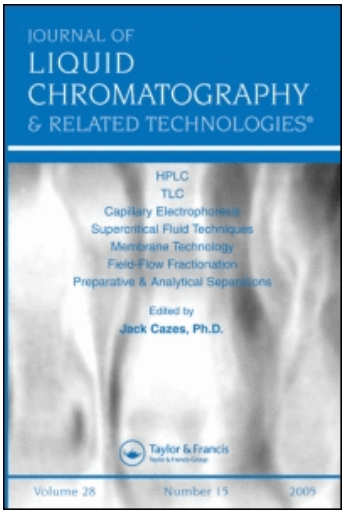

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

EFFECT OF SURFACTANT CONCENTRATION ON THE DETERMINATION OF *TRANS,TRANS*-MUCONIC ACID IN URINE BY CATIONIC MICELLAR ELECTROKINETIC CHROMATOGRAPHY WITH THERMO-OPTICAL ABSORBANCE DETECTION

Jianjun Li[a]; Karen C. Waldron[b]
[a] Institute for Biological Sciences, Ottawa, Canada [b] Department of Chemistry, University of Montréal, Montréal, Canada

Online publication date: 13 January 2005

To cite this Article Li, Jianjun and Waldron, Karen C.(1999) 'EFFECT OF SURFACTANT CONCENTRATION ON THE DETERMINATION OF *TRANS,TRANS*-MUCONIC ACID IN URINE BY CATIONIC MICELLAR ELECTROKINETIC CHROMATOGRAPHY WITH THERMO-OPTICAL ABSORBANCE DETECTION', Journal of Liquid Chromatography & Related Technologies, 22: 12, 1919 — 1933
To link to this Article: DOI: 10.1081/JLC-100101776
URL: http://dx.doi.org/10.1081/JLC-100101776

J. LIQ. CHROM. & REL. TECHNOL., 22(12), 1919–1933 (1999)

EFFECT OF SURFACTANT CONCENTRATION ON THE DETERMINATION OF *TRANS,TRANS*-MUCONIC ACID IN URINE BY CATIONIC MICELLAR ELECTROKINETIC CHROMATOGRAPHY WITH THERMO-OPTICAL ABSORBANCE DETECTION

Jianjun Li,[1] Karen C. Waldron[2,]*

[1] Institute for Biological Sciences
National Research Council
Ottawa, ON, K1A 0R6, Canada

[2] Department of Chemistry
University of Montréal
Montréal, Québec, H3C 3J7, Canada

ABSTRACT

A rapid method is described for the determination of *trans,trans*-muconic acid (2,4-hexadienedioic acid), a biomarker of benzene exposure in urine, by micellar electrokinetic chromatography (MEKC) with thermo-optical absorbance (TOA) detection. Addition of the cationic surfactant cetyltrimethylammonium bromide (CTAB) to a pH 7.0 background electrolyte induces a reversal in the direction of electroosmotic flow (EOF), from cathode to anode. The negatively charged *trans,trans*-muconic acid ion migrates rapidly (< 3 min) toward the anode owing to its electrophoretic mobility supplemented by the surfactant-induced anodic EOF.

A rigorous investigation into the effect of CTAB concentration on EOF showed that 0.05 to 5 mM surfactant permits *trans,trans*-muconic acid to elute before most endogenous urine components. For a urine sample doped with 0.8 mg/L *trans,trans*-muconic acid, the relative standard deviations of the analyte migration time and peak height were 1.5 % and 5.7 % respectively (n=6), for this home-built analysis system. The detection limit (3 SD) was 0.1 mg/L (7×10^{-7} M) *trans,trans*-muconic acid based on calibration standards and the linear dynamic range extended to 50 mg/L.

INTRODUCTION

Although benzene has been classified as a group I carcinogen by the International Agency for Cancer Research, it is still a widely used industrial solvent, a constituent of unleaded gasoline, and a component of tobacco smoke.[1,2] According to U.S. EPA studies from the 1980s, a typical smoker takes in roughly 2 mg benzene per day.[3] Benzene is metabolized in the liver into benzene epoxide which, via different pathways, results in the excretion of various phenolic compounds and several minor metabolites such as mercapturic acids and *trans,trans*-muconic acid.[4-6] Methods for the biological monitoring of benzene and its metabolites in exhaled air, blood, and urine have been reviewed[2,7] and related issues to benzene exposure continue to be raised in the literature.[8-10]

Trans,trans-muconic acid (ttMA) has been proposed as an indicator for monitoring benzene exposure by various analytical techniques that involve the separation and quantification of ttMA.[1,2,11-16] For example, gas chromatography (GC) was used to measure urinary ttMA after exposure to benzene contained in environmental tobacco smoke.[11] Ruppert *et al.*[12] developed a sensitive and specific method for the determination of ttMA in urine by GC–mass spectrometry (GC-MS). Bartczak *et al.*[13] modified an HPLC-based assay for urinary ttMA with the use of a diode array detector. The majority of analytical methods for determining ttMA in urine require lengthy sample preparation steps like solid-phase extraction on an anion exchanger,[4,14] or ether extraction and derivatization for GC separation.[11] In an effort to circumvent these steps, we have investigated free solution capillary electrophoresis (CE) and CE–related techniques for the determination of ttMA in urine.

The utility of CE has been demonstrated in many different areas of separation science and its significance continues to grow.[17-19] Surfactant–modified CE, known as micellar electrokinetic chromatography (MEKC), was introduced in 1984 by Terabe *et al.*[20] and a large number of applications based

on this technique have been reported.[21-23] Typically, higher peak efficiency is exhibited with MEKC than with HPLC separations although the two maintain similar selectivity for neutral analytes. In some instances, MEKC has replaced HPLC as the analytical method of choice,[21] e.g., for determining low molecular weight acids in foods. As far as we are aware, there has only been one report on the application of free solution CE or MEKC to the monitoring of benzene exposure by determination of ttMA[24] since we first presented this work.[25]

The reason for so few CE applications may be its inferior detection sensitivity with photometric detectors compared to HPLC. Large capillaries (i.e., ≥ 75 μm i.d.) or extended pathlength cells are required to obtain sub-micromolar detection limits for CE with UV/Visible absorbance.[24] Alternatively, thermo-optical absorbance (TOA) detection is well suited to detection in capillary tubes because the signal is independent of the optical pathlength (50 μm) of the excitation source. Although excitation is at a single wavelength, TOA has previously demonstrated excellent detection sensitivity for analytes that have absorbance maxima as far as 20 nm away from the excitation wavelength.[26,27]

In this paper we show a rapid MEKC separation method with TOA detection for the determination of ttMA in urine. Cationic surfactant cetyltrimethylammonium bromide (CTAB) is added to the background electrolyte (BGE) to reverse the direction of electroosmotic flow (EOF) from cathode to anode, a well known phenomenon in MEKC.[28-31] This flow reversal increases the analyte's apparent (net) velocity toward the detector (placed near the anode) owing to the strong anodic electrophoretic mobility of the divalent ttMA anion. By manipulating the concentration of CTAB, the selectivity of the separation is modified. We have studied this effect on the elution of ttMA relative to the interfering endogenous components of urine, typically present at high concentrations.

EXPERIMENTAL

Materials

Trans,trans-muconic acid, mesityl oxide and sodium tetraborate were purchased from Sigma (St. Louis, MO) and used without further purification. Sodium phosphate (dibasic) was from Anachemia (Montréal, Canada). Cetyltrimethylammonium bromide (CTAB) and HPLC-grade methanol were purchased from Aldrich (Milwaukee, WI). Sodium hydroxide was obtained from BDH (Toronto, Canada). In-house distilled water was purified using a multi-cartridge Millipore water filtration/deionization system before using.

Nylon syringe filters, 0.2-μm pore size, were purchased from Chromatographic Specialties (Brockville, Canada). Fused silica capillary tubing externally coated with polyimide was purchased from Polymicro Technologies (Phoenix, AZ). Platinum wire and 600-μL microcentrifuge tubes were obtained from Fisher Scientific (Montréal, Canada).

Instrumentation

The CE-TOA detection system was built in-house as described previously.[27] All components were assembled on an optical breadboard. The pump laser beam intensity was attenuated two-fold with a neutral density filter (Melles Griot, Nepean, Canada). Separations were performed at room temperature in a 45-cm-long 50-μm-I.D., 190-μm-O.D. fused silica capillary tube. On-column detection was performed 40 cm from the injection end of the capillary.

Preparation of Background Electrolyte and Standards

Purified water was used to make all background electrolyte (BGE) solutions and standards. A 1–mL volume of BGE was re-filtered through a syringe filter before use in the electrophoresis buffer reservoirs. BGE buffers for free solution CE consisted of 18 mM citric acid–sodium phosphate ranging in pH from 3.0 to 7.6. MEKC buffers consisted of 1.7 mM citric acid, 16.5 mM dibasic sodium phosphate, 0.5-30 mM CTAB, pH 7.0. A stock solution of 1 g/L ttMA was prepared in purified water and further dilutions were made in BGE.

Procedure

Samples and standards were injected hydrostatically by raising the inlet end of the capillary to a height of 6 cm for 30 s, timed by a stopwatch (6 nL injected). Mesityl oxide (MO) was co-injected as a neutral marker for EOF determination. The separation capillary was rinsed manually by syringe for 2 min with BGE (for free solution CE) or BGE plus surfactant (for MEKC) between each analysis. After a number of analyses had been carried out, the capillary was washed for 2 min with 1.0 M NaOH, 1.0 M HCl, water, and BGE respectively. Urine was collected on the same day of analysis from a normal adult non-smoker. Urine samples, neat or doped with ttMA, were diluted 2-fold in purified water then passed through a 0.22 μm–pore syringe filter before analysis. Recovery studies were performed by comparing peaks heights of ttMA in doped urine samples against a calibration curve constructed from ttMA standards.

RESULTS AND DISCUSSION

Free Solution CE Versus MEKC

A variety of BGEs (without surfactant) were initially investigated for ttMA determination by free solution CE, including phosphate and borate at various ionic strengths and pHs. Because the effective electrophoretic mobility (μ_{eff}) of the ttMA divalent anion toward the anode is large and opposite in direction to the electroosmotic mobility (μ_{eof}) of the BGE at pH > 6, the apparent or net mobility (μ_{app}) of ttMA is very small; $\mu_{app} = \mu_{eff} + \mu_{eof}$. Migration times were long (> 9 min) and the ttMA peak was broad under free solution CE conditions (data not shown). When pH < 6, the effective mobility of ttMA toward the anode decreases as its overall negative charge is reduced. However, the concomitant decrease in EOF at lower pHs lead to elution times of 16 min or more for ttMA. As Kaneta *et al.*[32] point out, control of the electroosmotic mobility and solute charge are very important for improving separations and minimizing analysis time. The fastest elution time we achieved for ttMA was 9.3 min for a pH 7.0 BGE. The neutral EOF marker, MO, eluted well ahead of this at 2.7 min. Given the complexity of urine and its numerous UV–absorbing components, free solution CE (no micelles) conditions would have led to elution of ttMA in a crowded region of the electropherogram.[33]

It is well documented that cationic surfactants adsorb on the inner capillary surface by dynamic electrostatic interactions with the negatively charged free silanol groups of fused silica.[28-30,32,34-36] At low concentrations, the surfactant monomers tend to shield the negative wall charge and reduce the overall EOF, as shown in Figure 1a for ≤ 0.03 mM CTAB and in other studies.[32,34] Reversing the polarity of high voltage (inlet = cathode) for analysis at CTAB concentrations less than 0.02 mM did not permit elution of ttMA, i.e., the normal EOF was too strong. However, at the critical micelle concentration (cmc), a bilayer of cationic surfactant molecules (hemimicelle) is formed at the capillary wall which essentially makes the inner surface positively charged. The electric double layer formed with BGE counterions leads to reversed (anodic) EOF when potential is applied across the capillary. This phenomenon distinguishes cationic-MEKC from free solution CE (no micelles) and is demonstrated in Figure 1a for CTAB concentrations ≥ 0.04 mM. Under these conditions (i.e., 0.05 to 0.5 mM CTAB), ttMA elutes at 2.4 min, well ahead of the neutral marker (t_{MO} = 6.9 min).

When adding charged surfactants to CE, the mechanism of separation involves contributions from several interactions owing to the multiple equilibria involved. Above the cmc, partitioning or complexation of analyte with micelles contributes significantly to overall selectivity. Below the cmc, analyte–

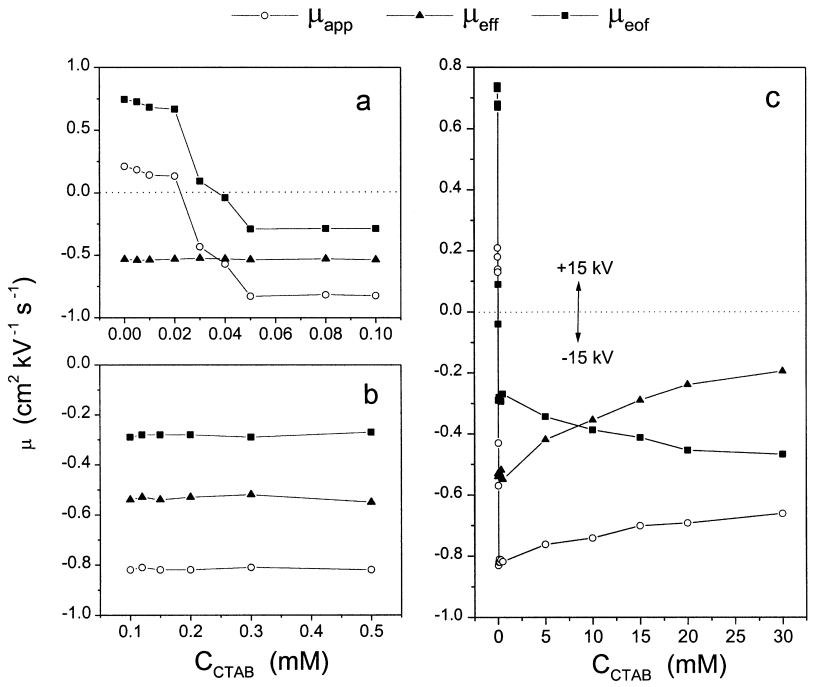

Figure 1. Plots of the relationship between apparent (μ_{app}) and effective (μ_{eff}) mobility of ttMA, and electroosmotic mobility (μ_{eof}) as a function of CTAB concentration showing regions of a) flow reversal; b) constant mobility; c) increasing analyte–micelle interaction. Separations were run at +15 kV or –15 kV in buffer consisting of 1.8 mM citric acid, 16.5 mM dibasic sodium phosphate, pH 7.0, with varying concentrations of CTAB.

surfactant interactions in CE are more poorly defined.[35] From Fig. 1a for $C_{CTAB} < 0.04$ mM, there appears to be no ion-pair formation between ttMA and CTAB monomers since a neutral ttMA–surfactant complex would have been expected to migrate at the same rate as MO, the neutral EOF marker. This result was not observed. When the concentration of cationic surfactant is greater than the cmc, the reversed EOF has been shown to be largely independent of pH.[30] In our experience, changing the pH from 3.0 to 7.6 had little effect on the migration times of ttMA in the MEKC separations (results not shown). However, increased peak tailing seen at pH 9.0 suggests strong interaction between the ttMA divalent anion and cationic hemimicellar layer.[32] Therefore, a pH 7.0 citrate-phosphate buffer was accepted as a favorable BGE with which to further explore the effects of CTAB concentration.

Effect of CTAB Concentration in MEKC

Figure 1a-c shows the effect of CTAB concentration on the electroosmotic mobility, μ_{eof}, and the apparent, μ_{app}, and effective mobilities, μ_{eff}, of ttMA under constant pH for the same ionic strength BGE. The μ_{eff} is a function of the electrophoretic mobilities of both free ttMA anion and ttMA-CTAB complex. It is important to point out the conventions used in plotting our data. Mobilities have been defined as positive for cathodic EOF (flow of BGE from anode to cathode as seen typically in bare fused silica capillaries) and negative for anodic EOF (flow from cathode to anode). Thus in Figure 1c, the downward sloping μ_{eof} curve as CTAB is increased from 5–30 mM actually represents an increasing mobility toward the anode, where detection takes place.

The dependence of EOF on CTAB concentration (Fig. 1) shows four domains, similar to that observed previously for cetyltrimethylammonium chloride (CTAC):[32] i) a gradual decrease in EOF as the wall charge is neutralized by adsorption of CTAB monomers; ii) flow reversal between concentrations of 0.02 and 0.05 mM CTAB (Fig. 1a) as the hemimicelle layer is established at the capillary wall; iii) a concentration region where the mobilities remain virtually unchanged (Fig. 1b); iv) an increasing EOF at high CTAB concentration (Fig. 1c). No mobility corrections were made for increasing viscosity or ionic strength with CTAB concentration. However, we expected to see the EOF decrease slightly at high CTAB concentrations (domain iv) due to increased viscosity and ionic strength; this was not observed. These factors may be "self-correcting" in that Joule heating from the higher ionic strength medium cancels out any viscosity increase arising from addition of CTAB. Lucy and Underhill[30] also saw an increase in the magnitude of EOF with increasing CTAB (domain iv) up to a total ionic strength of 50 mM, attributed to an increased surface concentration of surfactant as the electrostatic repulsion between surfactant head groups decreased.

By definition, the concentration of surfactant monomers in solution is constant at the cmc and above. However, micelle formation occurs within a transition zone and defining a unique cmc is inexact but convenient.[37] From the data in Figure 1a, the cmc is estimated to be 0.05 mM CTAB, corresponding to the point where reversed EOF becomes constant.[30] This value is three times lower than we expected for our 18.3 mM citrate-phosphate buffer system compared to results from CTAC and CTAB studies performed in higher ionic strength BGEs.[30,32] On the other hand, the cmc is slightly higher than that seen for CTAB in a reported 5 mM BGE solution.[31] The discrepancies may arise from the use of different electrolytes in each study. For this work, the reversed EOF was constant (domain iii) from the cmc up to 3 mM CTAB (estimated graphically), which Kaneta *et al.* attribute to the onset of multi-layer adsorption of surfactant at the capillary wall.[32]

The effective mobility of ttMA remains essentially constant from 0 to 2 mM CTAB (estimated graphically from a log-linear plot of C_{CTAB} versus mobility) implying that there are no interactions between analyte and CTAB monomers or micelles up to this concentration. A previous study by Shirao *et al.* on the determination of endogenous organic acids in urine by CE with cationic surfactant–induced flow reversal also suggested that the analytes did not interact with the surfactant.[36] This is in contrast to experiments done by Kaneta *et al.* who demonstrate that a selection of monovalent organic acids interact with CTAC monomers based on ion-pairing equilibria and interact with the hemimicelles formed on the capillary wall.[32]

In this work, a threshold concentration of CTAB micelles seems to be required to effect a change in mobility of ttMA. From 5 to 30 mM CTAB, the magnitude of the effective mobility of ttMA decreases as complexation with CTAB micelles occurs, presumably by electrostatic interaction. We assume the decreasing mobility is not viscosity related, otherwise a viscosity effect would be seen at lower CTAB concentrations too (domain iii), and the mobility of mesityl oxide would decrease. It is interesting to point out here that the analyte migrates ahead of the EOF marker, a result that is not often encountered in MEKC experiments.

Determination of ttMA in Urine

In general, increasing the concentration of surfactant in MEKC is expected to improve separation selectivity compared to free solution CE for components of a complex sample like urine. For example, as CTAB concentration is increased, uncharged hydrophobic species should partition with the micelles and show decreasing apparent mobility. Organic anions are also expected to show decreased apparent mobility, whereas cationic species should show little change in mobility as the amount of cationic surfactant is increased. However, because the magnitude of the EOF increased with increasing CTAB concentration above 0.5 mM, the apparent mobility of ttMA and presumably other anions decreased only slightly (Fig. 1c). Given that we observed four domains of EOF behavior and two distinct regions of ttMA migration behavior, the choice of MEKC conditions for urine analysis was not intuitively obvious.

In the previous report employing 0.1 mM CTAB[24] for free solution CE, the surfactant was used simply to reverse EOF rather than capitalize on global effects that it might have for MEKC analysis of ttMA in urine. Therefore, we used a ttMA–doped urine sample to investigate the effect of CTAB concentration on the separation of analyte from the components in the urine matrix. These results are shown in Figure 2a–e.

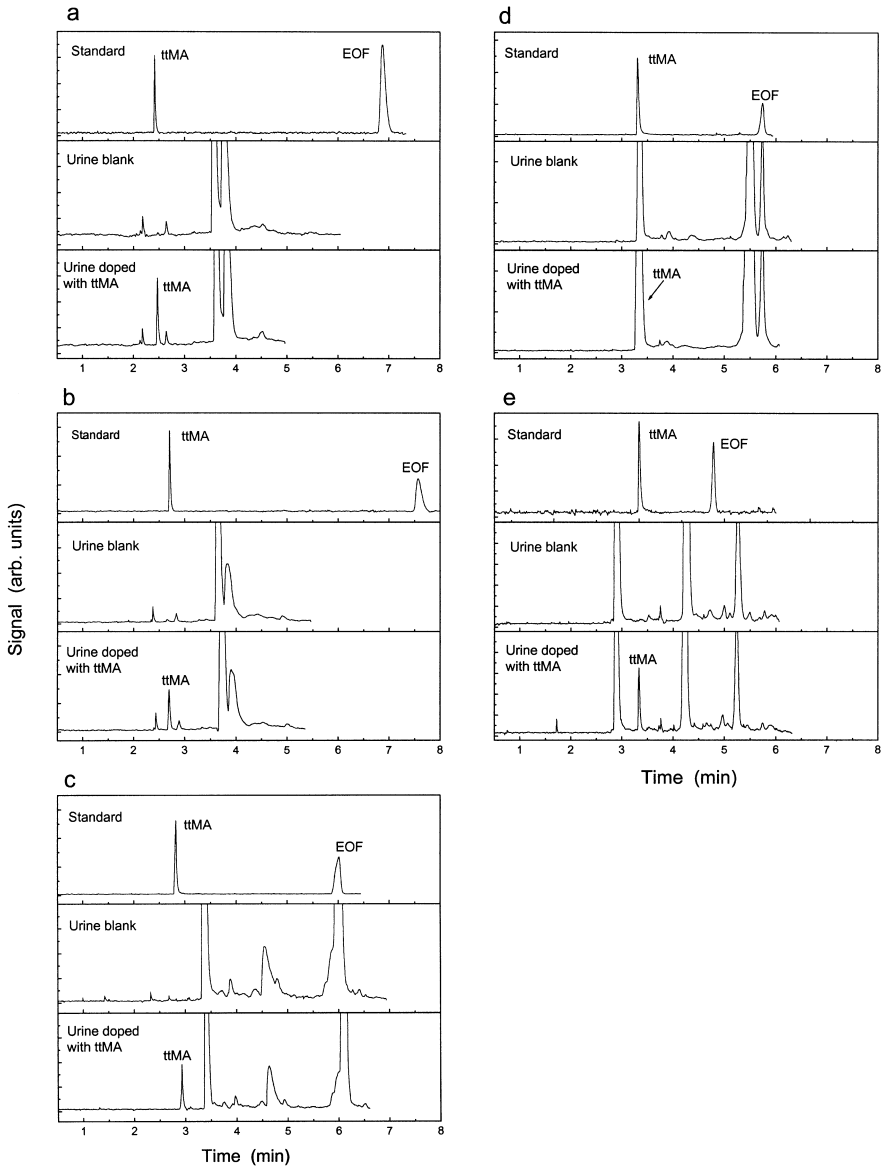

Figure 2. Electropherograms of a ttMA standard (top panel), blank urine sample (middle panel), and urine doped with 6.5 mg/L ttMA (bottom panel) showing the effect of different CTAB concentrations on separation selectivity at: a) 0.05 mM CTAB; b) 0.5 mM CTAB; c) 5 mM CTAB; d) 15 mM CTAB; e) 30 mM CTAB. All other conditions were the same as in Fig. 1.

Figure 2a shows that ttMA migrated much faster than the major UV–absorbing matrix components (unidentified) in urine when the CTAB concentration (0.05 mM) was near the cmc. A similar urine profile was observed when the CTAB concentration was increased to 0.5 mM (Fig. 2b). In both cases a few urine–based peaks eluted between 2 and 3 min indicating the potential for interference with ttMA determination. For example, in the report by Tavares *et al.*[24] using 0.1 mM CTAB, ttMA eluted in a region crowded by peaks from a normal urine sample. These interferents could be either minor urine constituents or major constituents poorly detected by, in our case, TOA at λ=248 nm. At 5 mM CTAB, well above the cmc, the migration time of ttMA was again faster than the major urine matrix peaks but with little additional interference from early eluting urine components (Fig. 2c). On the other hand, the separation of ttMA from endogenous components completely deteriorated when the CTAB concentration was increased to 15 mM (Fig. 2d). Further increasing the CTAB concentration to 30 mM (Fig. 2e) caused the ttMA peak to elute in a region crowded by major urine component peaks. Although ttMA was resolved from the components of normal urine at 30 mM CTAB, other biological metabolites might exist in this elution region contributing to interference with ttMA determination. Clearly, conditions that forced ttMA to migrate well away from a crowded chromatographic region were considered the most desirable for unambiguous determination of the analyte.

On the basis of these results, the best buffer for ttMA determination was chosen to be 5 mM CTAB, 1.8 mM citrate, 16.5 mM phosphate, pH 7.0. Since electrophoretic and electroosmotic mobility occur in the same direction, analysis times for ttMA were short (< 3 min) for a 40-cm-long effective capillary length. Quantification and recovery studies of ttMA in urine were carried out using the conditions of analysis above. A calibration curve based on peak heights of five ttMA standards injected in triplicate was constructed ($y = 33.9 \pm 2.6\ \mu V/ppm\ x - 5.9 \pm 2.2\ \mu V$; $R^2 = 0.982$) to estimate the limit of detection of the MEKC-TOA analysis technique. The standard deviation of the regression fit was 1.65. For a signal-to-noise ratio of 3 and sensitivity of 0.0339 mV/ppm, the concentration detection limit was 0.1 mg/L (7×10^{-7} M) ttMA and the absolute detection limit was 0.6 pg (4 fmol). The linear dynamic range of the measurement technique extended to 50 mg/L ttMA.

Recovery studies of added analyte were performed for six assays of three different concentrations of ttMA doped in urine, as described in the experimental section. These results are summarized in Table 1. We assume that the low recoveries (83–85%) originate from comparing the urine matrix to low salt standards (i.e., in BGE) in which some stacking during injection has biased the peak heights and areas. Urine samples were diluted only 2-fold in pure water to cut the high salt content without sacrificing detectability.

Table 1

Recovery of Added Analyte and Repeatability for the Determination of ttMA in Urine

Added ttMA to Urine Blank	Mean C_{ttMA} Found (μg/mL)*	Mean Recovery %	RSD(%) C_{ttMA}	RSD(%) t_m
0.80 mg/L	0.66	83	5.7	1.5
1.3 mg/L	1.1	85	6.3	2.8
6.5 mg/L	5.5	85	7.8	4.0

* n = 6.

Using 80% as a conservative estimate of ttMA recovery from urine, a more reliable estimate of the limit of detection (3 SD) of ttMA in urine is 0.1_3 mg/L (9×10^{-7} M) based on external calibration. These results are five times worse than those reported by Tavares *et al.*[24] who determined a lower limit of 0.025 mg/L ttMA from calibration standards using a 3-mm-pathlength detection cell and 90 nL injection volume. However, our absolute detection limit was about 4 times better than theirs. We found relative standard deviations in migration time and peak height as high as 4% and 7.8%, respectively (Table 1). With automated pressure injection and a thermostated capillary (available in commercial CE instruments), reproducibility in migration time and quantification on the order of 1% are expected.

In comparison with previously reported chromatographic techniques for ttMA determination, our concentration limit of detection ranges from equivalent or 50 times poorer for some GC-MS and modified HPLC methods.[4,11-16] On the other hand, absolute detection limits were more than 2 orders of magnitude better than all HPLC methods reported. It is important to note that non-CE-based methods required extensive sample preparation, such as ttMA extraction and up to 20–fold preconcentration on solid phase ion-exchange media and/or time-consuming derivatization for GC analysis. In addition, interfering peaks in the chromatograms of these studies were problematic.[11,13-15] The proposed MEKC method ensures that ttMA elutes in a region essentially free of endogenous urinary components. The addition of a simple preconcentration step[38] with an added internal standard for precise recovery quantification[39] should give a 10-fold increase in detection sensitivity. This would bring the technique in line with the detection limits reported for GC-MS (0.01 mg/L) and within the background levels of ttMA reported for non-smokers who are not occupationally exposed to benzene (0.023–0.124 mg/L).[12,24]

Reports on biological monitoring of exposure to benzene typically measure the mass of urinary metabolites per gram of creatinine, a major endogenous constituent of urine. This method provides an absolute or "corrected" measure of ttMA. For example, people with occupational exposure to less than 1 ppm benzene have urinary ttMA concentrations ranging from 0.03 to 1.7 mg/g creatinine.[2,6,11,15] In the present study, this technique was not used, as justified in a previous report.[24] Nonetheless, there are several methods described for creatinine determination in urine by CE[40-42] and the present MEKC method could be adapted for simultaneous ttMA and creatinine quantification.

CONCLUSION

A rapid method for the separation and determination of ttMA by MEKC–TOA detection under cationic surfactant (CTAB)–induced flow reversal conditions has been described. In this study, the effects of cationic surfactant on EOF, ttMA mobility, and the resolution of ttMA from the major endogenous components in urine were investigated. The proposed MEKC procedure is faster than current chromatographic methods in terms of total analysis time including sample preparation. The reduction in sample pretreatment with CE-based analyses makes it an attractive alternative to traditional chromatographic techniques, particularly for rapid screening of large numbers of samples. In addition, the ability to rinse a fused silica capillary with a few microlitres of NaOH to regenerate the capillary surface makes it ideal for biofluid analyses where sample matrix components tend to foul the separation column. About 4–fold lower mass detectability of ttMA was achieved with TOA detection compared to the only other photometric technique reported for CE.

We believe that MEKC–TOA detection with preconcentration would be suitable for the determination of ttMA in urine of those chronically exposed to benzene and could be used to develop a protocol for monitoring such exposure.

ACKNOWLEDGMENTS

The authors would like to thank Dr. X. C. Le, Department of Public Health Sciences, University of Alberta, for his helpful comments and suggestions regarding this study. This work was first presented at the 43rd International Conference on Analytical Sciences and Spectroscopy (Abstract No. 16-4), August 10-13, 1997, Montréal, Canada. Funding for this research was provided by the Natural Sciences and Engineering Council of Canada and an FCAR Québec (Fonds pour la formation de chercheurs et l'aide à la recherche) grant for the establishment of new professors.

REFERENCES

1. C. N. Ong, B. L. Lee, C. Y. Shi, H. Y. Ong, H. P. Lee, Int. J. Cancer, **59**, 177-180 (1994).

2. C. N. Ong, B. L. Lee, J. Chromatogr. B, **660**, 1-22 (1994).

3. L. Wallace, Environ. Health Perspect., **106**, 1129-1136 (1996).

4. H. Schad, F. Schafer, L. Weber, H. J. Seidel, J. Chromatogr., **593**, 147-151 (1992).

5. R. Snyder, C. C. Hedli, Environ. Health Perspect., **104**, 1165-1171 (1996).

6. P. J. Boogaard, N. J. van Sittert, Environ. Health Perspect., **104**, 1151-1157 (1996).

7. A. M. Medeiros, M. G. Bird, G. Witz, J. Toxicol. Environ. Health, **51**, 519-539 (1997).

8. T. Ruppert, G. Scherer, A. R. Tricker, F. Adlkofer, Int. Arch. Occup. Environ. Health, **69**, 247-251 (1997).

9. P. W. Kok, H. Y. Ong, M. K. Wong, W. K. Au, K. T. Tan, W. H. Phoon, C. N. Ong, Environ. Mon. & Assess., **44**, 425-431 (1997).

10. B. Javelaud, L. Vian, R. Molle, B. Allain, B. Allemand, B. Andre, F. Barbier, A. M. Churet, J. Dupuis, M. Galand, F. Millet, J. Talmon, C. Touron, M. Vaissiere, D. Vechambre, M. Vieules, D. Viver, Int. Arch. Occup. Environ. Health, **71**, 277-283 (1998).

11. R. Yu, C. P. Weisel, J. Toxicol. Environ. Health, **48**, 453-477 (1996).

12. T. Ruppert, G. Scherer, A. R. Tricker, D. Rauscher, F. Adlkofer, J. Chromatogr. B, **666**, 71-76 (1995).

13. A. Bartczak, S. A. Kline, R. Yu, C. P. Weisel, B. D. Goldstein, G. Witz, W. E. Bechtold, J. Toxicol. Environ. Health, **42**, 245-258 (1994).

14. M. Buratti, S. Fustinoni, A. Colombi, J. Chromatogr. B, **677**, 257-263 (1996).

15. B. L. Lee, A. L. New, P. W. Kok, H. Y. Ong, C. Y. Shi, C. N. Ong, Clin. Chem., **39**, 1788-1792 (1993).

16. D. Rauscher, G. Lehnert, J. Angerer, Clin. Chem., **40**, 1468-1470 (1994).

17. J. P. Landers, Clin. Chem., **41**, 495-509 (1995).

18. R. L. St. Claire, Anal. Chem., **68**, R569-R586 (1996).

19. S. C. Beale, Anal. Chem., **70**, 279R-300R (1998).

20. S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, T. Ando, Anal. Chem., **56**, 111-113 (1984).

21. C. Thompson, V. C. Trenerry, B. Kemmery, J. Chromatogr. A, **694**, 507-514 (1995).

22. I. Bechet, M. Fillet, P. Hubert, J. Crommen, Electrophoresis, **15**, 1316-1321 (1994).

23. M. G. Khaledi, S. C. Smith, J. K. Strasters, Anal. Chem., **63**, 1820-1830 (1991).

24. M. X. Coutrim, A. V. Jager, L. R. F. de Carvalho, M. F. M. Tavares, J. Cap. Elec., **4**, 39-45 (1997).

25. K. C. Waldron, J. Li, The 43rd International Conference on Analytical Sciences and Spectroscopy; Abstract No. 16-4, (Montréal, Québec, August 10-13, 1997).

26. K. C. Waldron, N. J. Dovichi, Anal. Chem., **64**, 1396-1399 (1992).

27. K. C. Waldron, J. Li, J. Chromatogr. B., **683**, 47-54 (1996).

28. T. Tsuda, J. High Resolut. Chromatogr. Chromatogr. Commun., **10**, 622-624 (1987).

29. G. M. Janini, K. C. Chan, J. A. Barnes, G. M. Muschik, H. J. Issaq, J. Chromatogr. A, **653**, 321-327 (1993).

30. C. A. Lucy, R. S. Underhill, Anal. Chem., **68**, 300-305 (1996).

31. M. F. M. Tavares, R. Colombara, S. Massaro, J. Chromatogr. A, **772**, 171-178 (1997).

32. T. Kaneta, S. Tanaka, M. Taga, J. Chromatogr. A, **653**, 313-319 (1993).

33. E. Jellum, H. Dollekamp, C. Blessum, J. Chromatogr. B, **683**, 55-65 (1996).

34. X. Huang, J. A. Luckey, M. J. Gordon, R. N. Zare, Anal. Chem., **61**, 766-770 (1989).

35. J. Varghese, R. B. Cole, J. Chromatogr. A, **652**, 369-376 (1993).

36. M. Shirao, R. Furuta, S. Suzuki, H. Nakazawa, S. Fujita, T. Maruyama, J. Chromatogr. A, **680**, 247-251 (1994).

37. C. Tanford, **The Hydrophobic Effect: Formation of Micelles and Biological Membranes**, John Wiley & Sons, New York, 1980.

38. D. K. Lloyd, J. Chromatogr., **735**, 29-42 (1996).

39. G. Renom, N. Bruneau, J. Mizon, Clin. Chem. Lab. Med., **36**, 111-113 (1998).

40. N. A. Guzman, C. M. Berck, L. Hernandez, J. P. Advis, J. Liq. Chromatogr., **13**, 3833-3848 (1990).

41. M. Miyake, A. Shibukawa, T. Nakagawa, J. High Res. Chromatogr., **14**, 181-185 (1991).

42. H. Shi, Y. Ma, Y. Ma, Anal. Chim. Acta, **312**, 79-83 (1995).

Received July 30, 1998
Accepted November 16, 1998
Manuscript 4853

Request Permission or Order Reprints Instantly!