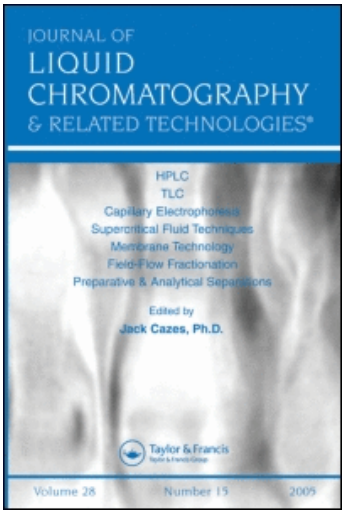

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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Bruce D. Johnson[a]; Nelu Grinberg[a]; Gary Bicker[a]; Dean Ellison[a]
[a] Analytical Research Merck Research Laboratories Merck & Co. Inc., Rahway, NJ

To cite this Article Johnson, Bruce D. , Grinberg, Nelu , Bicker, Gary and Ellison, Dean(1997) 'The Quantitation of a Residual Quaternary Amine in Bulk Drug and Process Streams Using Capillary Electrophoresis', Journal of Liquid Chromatography & Related Technologies, 20: 2, 257 — 272
To link to this Article: DOI: 10.1080/10826079708010651
URL: http://dx.doi.org/10.1080/10826079708010651

J. LIQ. CHROM. & REL. TECHNOL., 20(2), 257–272 (1997)

THE QUANTITATION OF A RESIDUAL QUATERNARY AMINE IN BULK DRUG AND PROCESS STREAMS USING CAPILLARY ELECTROPHORESIS

Bruce D. Johnson,* Nelu Grinberg, Gary Bicker, Dean Ellison
Analytical Research
Merck Research Laboratories
Merck & Co. Inc.
P. O. Box 2000
Rahway, NJ 07065

ABSTRACT

A capillary electrophoretic method for the determination of a residual alkyl quaternary amine, tetra-n-butylammonium ion (TBA^+), in bulk drug and process streams was developed. Since the analyte does not have a chromophore, detection was performed utilizing indirect photometric detection. The influence of the quinine, tetrahydrofuran and sodium acetate concentrations and pH_{app} in the background electrolyte solution upon the efficiency of the separation and effective electrophoretic mobility of both TBA^+ and electro-osmotic flow were studied. The ranges in which the various parameters were examined had considerable effects upon both the efficiency and the effective electrophoretic mobility of the electro-osmotic flow; however, the effective electrophoretic mobility of TBA^+ was not significantly affected. The optimized method was validated in terms of detector linearity, sensitivity, precision and accuracy.

INTRODUCTION

Capillary electrophoresis (CE) has been used to quantitate a broad range of organic and inorganic ions in a wide variety of matrices.[1-3] CE offers a uniquely different form of separation selectivity in comparison to conventional ion-analysis chromatographic methods. CE separates ions according to their mobility as they travel through a small diameter capillary filled with an electrolyte solution resulting in highly efficient separations and rapid analysis times. Additionally, CE offers the practical benefit of lower operating costs manifested by less solvent consumption and inexpensive column replacement.[1,2,4] Thus, CE is beginning to play an important role in pharmaceutical research towards the development and analysis of complex molecules.

Alkyl quaternary amines have proven to be difficult analytes to analyze for in drug substance matrices, due to the absence of a chromophore, which significantly limits detection by conventional UV detectors.[5] However, CE utilizing indirect photometric detection (IPD) is an appropriate option. In IPD, a UV-absorbing solute of the same charge as the separands (a co-ion) serves as an additive to the background electrolyte. This additive, known as a visualizing reagent (VR), elevates the baseline. When solute ions are present, they displace the VR and are measured as negative ions as they migrate past the detector window. IPD has proven to be a powerful technique due to its sensitivity without the need for derivatization.[1,2,6,7]

A literature survey revealed few reports of the separation of alkyl quaternary ammonium compounds including both CE[8-10] and IC.[11,12] This paper describes the quantitation of residual amounts of an alkyl quaternary amine, tetra-n-butylammonium ion (TBA^+), in both *indinavir* monohydrate intermediate and process streams. *Indinavir* monohydrate intermediate is the freebase form of the drug substance *Crixivan*, a potent inhibitor of the HIV protease.[13,14] The conversion step to form *indinavir* monohydrate intermediate from its precursor involves an alkylation reaction with 3-picolyl chloride in the presence of sodium iodide and tetra-n-butylammonium bromide in an aqueous/organic biphasic solution.[15,16] TBA^+, a commonly used phase transfer catalyst, facilitates rapid alkylation.

Upon completion of the alkylation reaction, the organic reaction mixture is extracted several times with water to remove TBA^+ along with other polar impurities. Alkyl quaternary amines are known to form micelles; thus, both the organic and aqueous cuts are analyzed for TBA^+.[17] Additionally, the isolated intermediate is analyzed for residual TBA^+ remaining from the extraction process.

The factors that contribute to the electrophoretic separation and quantitation of TBA^+ have been investigated. The effects of varying the concentration of THF, sodium acetate, quinine and the pH_{app} of the background electrolyte (BGE) upon the efficiency of the separation and the effective electrophoretic mobility of both TBA^+ and electro-osmotic flow are discussed. The method has been validated in terms of precision, accuracy, detector linearity and sensitivity. Additionally, the method was applied to the analysis of process streams and *inidinavir* monohydrate intermediate used in the manufacture of drug substance used for both clinical studies and commercial sale.

MATERIALS

Instrumentation

The CE experiments were carried out on a HP^{3D} CE (Hewlett-Packard, Germany) system. The HP^{3D} CE instrument was used with a 56 cm effective length fused-silica capillary (65 cm total length, 75μm I.D., Hewlett-Packard, Germany) and a diode-array detector (Hewlett-Packard, Germany). The wavelengths for the sample and reference signals were reversed to provide positive peaks for analysis. The capillary temperature was maintained at 30±0.1°C, a hydrodynamic sampling injection model was applied for 3 s at 50 mbar, and the applied voltage was 30 kV (current typically ~ 20 mA). The electrode polarity was in the 'standard' mode i.e. anode (+) at the inlet/injector and cathode (-) at the outlet/detector. Data collection, integration and efficiency calculations were accomplished by using the PE Nelson ACCESS CHROM system (Cupertino, CA). The spectra of the BGE at various pH_{app}'s was collected by making a 300-fold dilution of the BGE into 70:30 water:THF and measuring the spectra on a Shimadzu UV-2101PC UV-VIS spectrometer (Columbia, MD). The concentration of quinine was confirmed by potentiometric titrations using a Metrohm 716 DMS Titrino Dosimat and a Titrino Workcell titroprocessor (Brinkman, Westbury, NY).

Reagents

Indinavir monohydrate intermediate and reaction mixtures were obtained from the Chemical & Engineering department in Merck Research Laboratories, Rahway, NJ. The tetra-n-butylammonium bromide was purchased from Aldrich (Milwaukee, WI, USA), quinine, anhydrous obtained from either

Janssen Chimica (Belgium) or Aldrich. sodium acetate was obtained from Sigma (St. Louis, MO, USA), methanol and tetrahydrofuran (THF) were obtained from Fisher Scientific (Spring Field, NJ, USA) and concentrated acetic acid was obtained from EM Sciences (Gibbstown, NJ) . All commercial chemicals were used directly without any further purification. Water used in the study was purified with a HYDRO Picosystem (Hydro Service and Supplies, Inc., Research Triangle Park, NC, USA).

METHODS

Background Electrolyte (BGE) Solution

The BGE was prepared by dissolving sodium acetate in water. Separately, the quinine was dissolved with the THF and diluted to volume with the sodium acetate solution. The pH was adjusted with concentrated acetic acid and the solution was filtered through a 0.45 μm nylon membrane before use (the concentration of THF reported reflects the volume percent of the original amount of THF and water that were mixed).

Sample Solutions

Samples and standards were dissolved in methanol to provide sufficient solubility of the *indinavir* monohydrate intermediate and the reaction mixtures. The stock solution of tetra-n-butylammonium ion (TBA^+) was prepared by dissolving 360 mg of tetra-n-butylammonium bromide in 25 mL of water (approx. 4.5 mM TBA^+). Standard solutions were made from the stock solution by dilutions into methanol. Dry powder sample solutions were made by dissolving 700 mg of *indinavir* monohydrate intermediate into 10 mL of methanol. The organic layer reaction mixtures were prepared by dissolving approximately 40 g of material into 250 mL of methanol. The aqueous layer reaction mixtures were diluted with methanol. The methanol diluent was used as the neutral marker (NM).

Capillary Conditioning

New capillaries were conditioned with a 1*N* NaOH solution for 30 min., deionized H_2O for 10 min., 0.2 *N* NaOH solution for 30 min., deionized H_2O for 10 min., 75:25 (v/v) H_2O: THF for 10 min. and BGE for 15 minutes. Between each injection, the capillary was flushed with the BGE for 10 min.

The capillaries were flushed with 75:25 (v/v) H_2O: THF for 20 min subsequent to usage. Care was taken not to expose the capillary with BGE to aqueous solutions (i.e. water or NaOH solutions) due to the insolubility and precipitation of quinine.

Calculations

The electrophoretic mobility of the electro-osmotic flow (μ_{EOF}) and the net effective electrophoretic mobility of TBA^+ (μ_{TBA+}) were calculated using Equations 1 and 2, respectively,

$$\mu_{EOF} = \frac{l\ L}{V\ t_{EOF}} \quad (1)$$

$$\mu_{TBA+} = \left(\frac{1}{t} - \frac{1}{t_{EOF}}\right)\frac{l\ L}{V} \quad (2)$$

where l is the effective capillary length, L is the total capillary length, V is the applied voltage, t_{EOF} is the migration time of the NM (methanol), and t is the migration time of TBA^+.[18]

For calculation of the efficiency of the separation, the Foley-Dorsey equation was used.[19]

RESULTS AND DISCUSSION

Selection of Wavelength for Indirect Detection

The optimal wavelength for detection is determined by two factors. First, a wavelength where the maximum difference in the extinction coefficient between the analyte and the visualizing reagent (VR) is determined. Secondly, the absorbance of the VR at this wavelength should not be too high so as to saturate the detector.[2,6,7,20] Because TBA^+ does not possess a chromophore in the UV-visible wavelength ranges, the only wavelength consideration is the absorbance of the VR, quinine. To select the proper detection wavelength, spectra were obtained of the BGE as the pH was varied as follows: 3.5, 4.0,

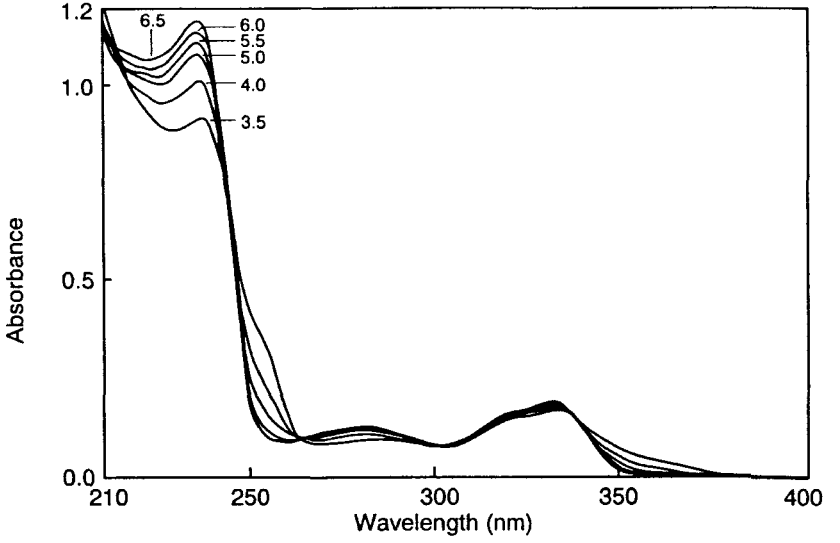

Figure 1. Effect of the pH_{app} on the absorbance spectra of quinine (absorbance decreases with decreasing pH_{app} at 235 nm). BGE: 7 mM sodium acetate, 12 mM quinine and 30 v/v % THF.

4.5, 5.0, 5.5 and 6.5 (Figure 1). Quinine exhibits absorbance maxima's at approximately 235, 285 and 335 nm's. The detector was saturated at the 235 nm maxima, however, an acceptable absorbance of approximately 0.5 AU was found for the 335 nm maxima. Additionally, the absorbance spectra of quinine is pH sensitive as it exhibits pKa's of approximately 5.1 and 9.7.[21] Therefore, the 335 nm maxima was selected as the optimal wavelength of detection because it does not saturate the detector and this wavelength is minimally sensitive to pH changes.

Influence of the pH_{app}

The pH_{app} of the BGE was varied between 3.5 and 6.5 in the same increments as described in the previous section. TBA^+ is a quaternary amine and, as expected, the μ_{TBA^+} changed minimally throughout the pH range studied. However, the μ_{EOF} increased over 180% when the pH_{app} was increased (Figure 2). The electro-osmotic flow (EOF) is generated by the silanol groups (SiO^-) present on the capillary wall and their ionization is significantly affected by pH_{app} changes.[1,18,22]

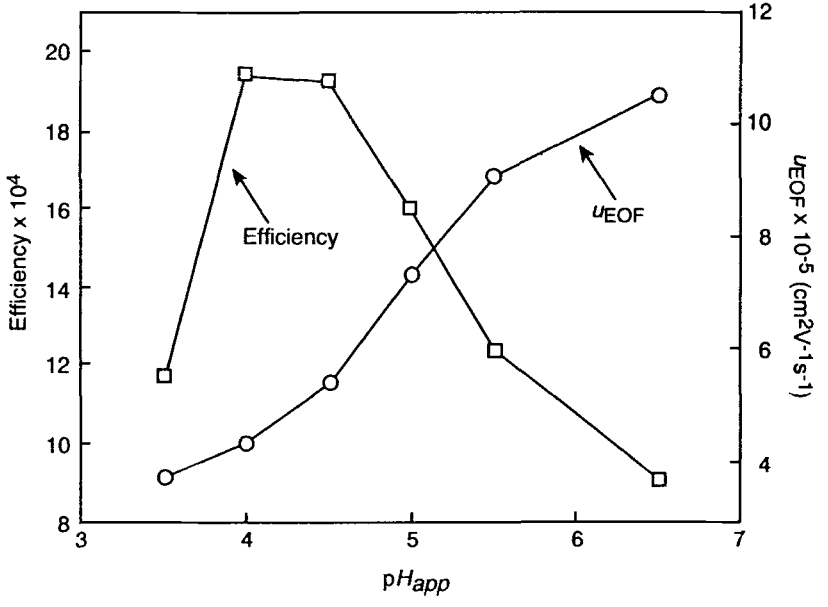

Figure 2. Effects of the pH_{app} on the effective electrophoretic mobility of the electroosmotic flow and efficiency of the separation. BGE: 7 mM sodium acetate, 12 mM quinine and 30 v/v % THF.

As the present study deals with the analysis of trace amounts of TBA^+, the method should be optimized such that the efficiency of the TBA^+ peak should be maximized to insure lower detection limits. From the data plotted in Figure 2, the maximum efficiency was obtained between the pH range of 4.0 to 4.5. The pH_{app} of 4.5 was selected because it is nearest to the pKa's of acetate (4.8) and quinine (5.1) thus, insuring the maximum buffering capacity. Therefore, in the following experiments the pH_{app} was maintained at this value.

Influence of the Concentration of Quinine

Quinine is an appropriate selection as a VR as it satisfies the general conditions required for successful capillary ion analysis of TBA^+.[1,2,23] Additionally, quinine has previously been utilized as a VR in CE.[10,24-26] The concentration of quinine in the BGE was varied between 5.3 and 26 mM as follows: 5.3, 11, 13, 21, and 26 mM. The μ_{TBA+} varied minimally in the concentration range of quinine studied. However, the μ_{EOF} decreased more

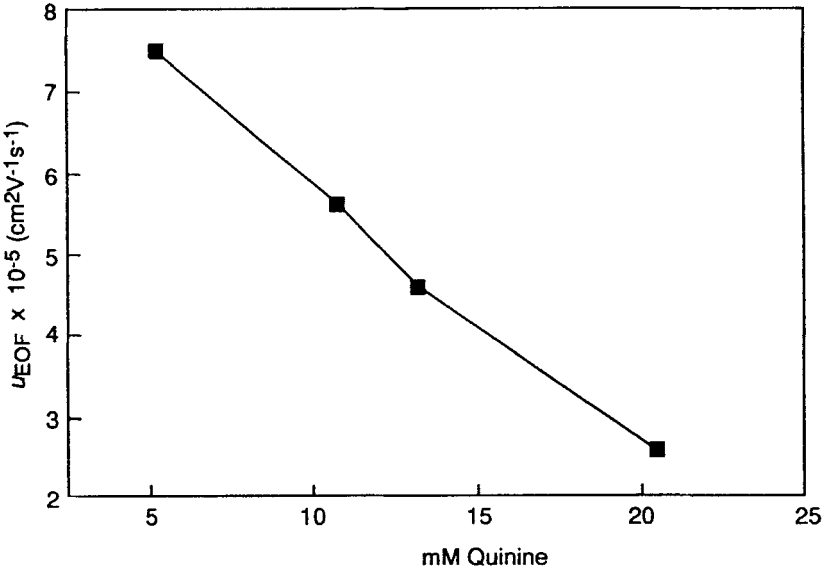

Figure 3. Effects of the concentration of quinine on the effective electrophoretic mobility of the electro-osmotic flow. BGE: 7 mM sodium acetate, pH_{app} 4.5 and 30 v/v % THF.

than 60% when the concentration of quinine was increased (Figure 3). This behavior is presumably due to the interaction of the positively charged quinine with the SiO^- groups on the capillary wall. As the concentration of quinine is increased, the zeta potential on the capillary wall is reduced thereby decreasing μ_{EOF}.

The increase of the concentration of quinine in the BGE has a positive effect on the efficiency of the TBA^+ peak (Figure 4). This efficiency increase is likely due to the minimization of the interactions of TBA^+ with the capillary wall. However, increasing the concentration of quinine in the BGE produced a decrease in area counts of TBA^+ (Figure 4). Therefore, an intermediate concentration of 12 mM quinine was maintained in the following experiments.

Influence of the Concentration of THF

THF was selected as an organic modifier as it provided solubility of quinine and is also known to inhibit the formation of micelles, a potential

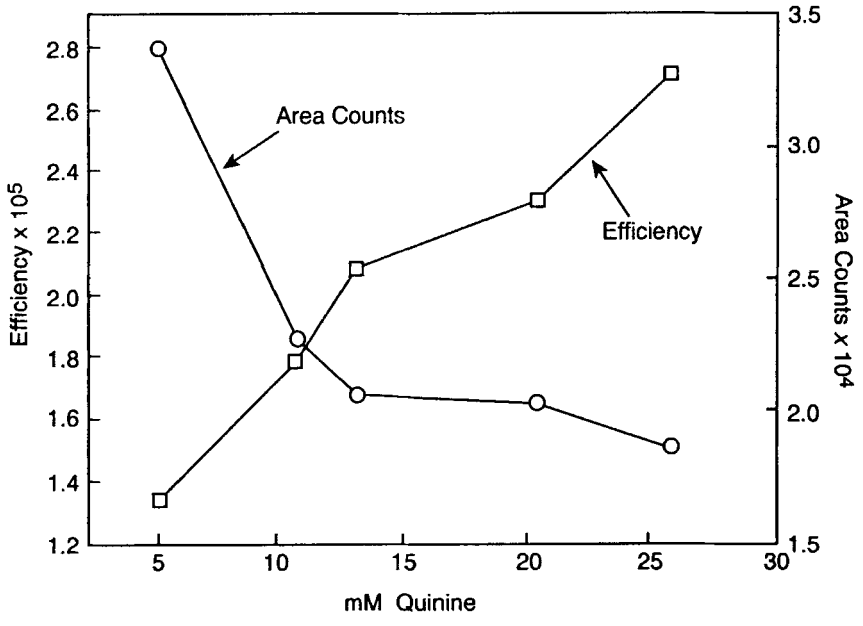

Figure 4. Effects of the concentration of quinine on the efficiency of the separation and the detector area counts. BGE: 7 mM sodium acetate, pH_{app} 4.5 and 30 v/v % THF.

problem towards the analysis of quaternary amines.[8,17,27] The volume percent of THF in the BGE was varied as follows: 20, 25, 30, 35, 40 and 50%. The μ_{TBA+} and the μ_{EOF} decreased by approximately 20 and 10%, respectively, as the concentration of THF was increased (Figure 5).

The reduction in μ_{TBA+} is attributed to the simultaneous decrease of current (approximately 30%) as the concentration of THF was increased. The decrease in μ_{EOF} is due to the interaction of THF with the silanol groups at the capillary wall resulting in a decreased zeta potential.[22,28-30]

The increase of the concentration of THF in the BGE generally decreased the efficiency of the TBA^+ peak (Figure 6). A maximum in efficiency was obtained at 25 v/v % THF, therefore, the v/v % THF was maintained at this value.

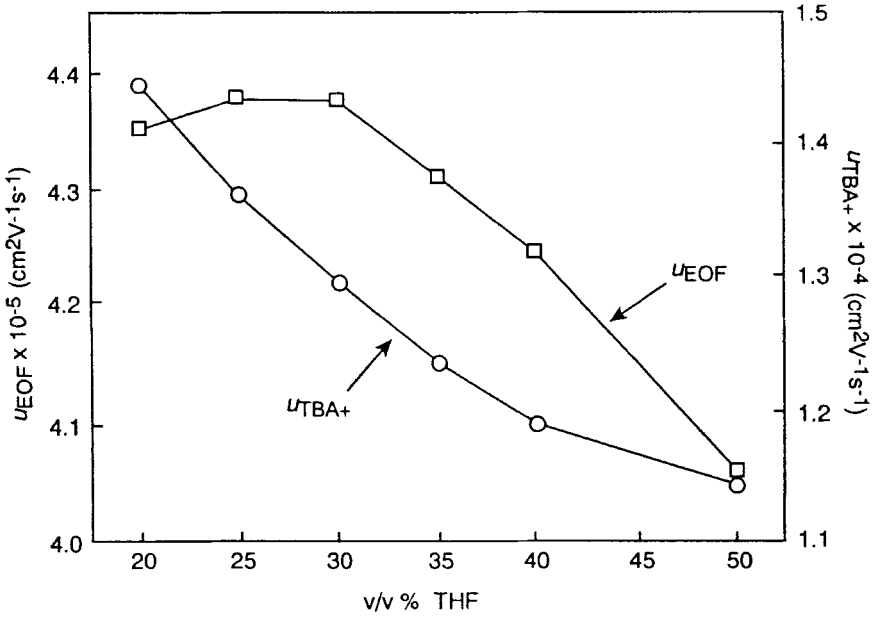

Figure 5. Effects of the v/v % THF on the effective electrophoretic mobility of the electro-osmotic flow and TBA^+. BGE: 7 mM sodium acetate, pH_{app} 4.5 and 12 mM quinine.

Influence of the Concentration of Sodium Acetate

Sodium acetate was utilized in the BGE to provide increased buffering capacity. The concentration of sodium acetate was varied between 3.0 and 30 mM as follows: 3.0, 7.0, 12, 15, 20, and 30 mM. The μ_{TBA+} varied minimally for the range of sodium acetate concentration studied. However, the μ_{EOF} decreased 25% when the concentration of sodium acetate was increased (Figure 7). This behavior was due to shielding of the SiO^- groups on the capillary wall as the ionic strength of the BGE was increased resulting in a decreased zeta potential.[1,18,22]

The increase of the concentration of sodium acetate in the BGE has a negative effect on the efficiency of the TBA^+ peak (Figure 7). Therefore, in the following experiments the concentration of sodium acetate was maintained at 3.0 mM.

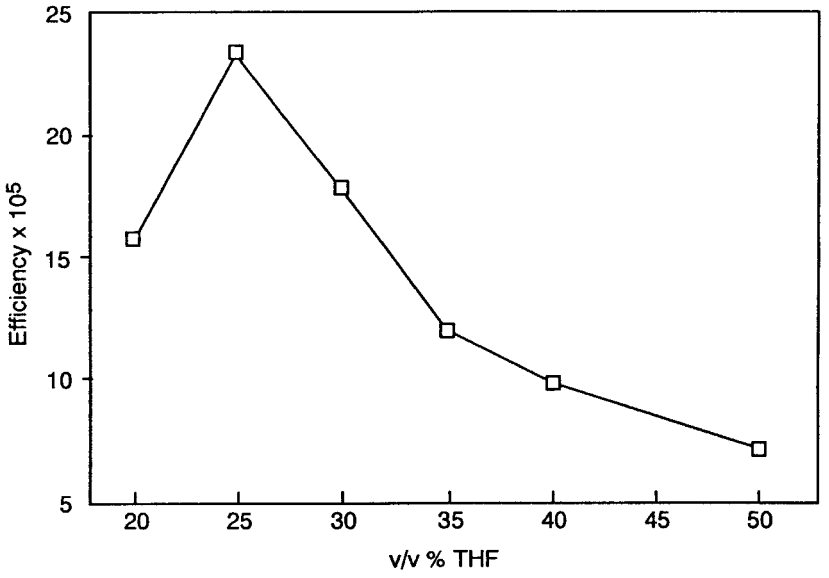

Figure 6. Effects of the v/v % THF on the efficiency of the separation. BGE: 7 mM sodium acetate, pH_{app} 4.5 and 12 mM quinine.

Influence of Methanol versus THF as the Organic Modifier

A direct comparison was made between methanol and THF as the organic modifier under the final BGE conditions. The μ_{TBA+} varied minimally, however, the μ_{EOF} was 20% slower with methanol. Methanol has a hydroxy group that can act both as an electron donor and acceptor while THF can only act as an electron donor. Thus, the decrease in μ_{EOF} is presumably due to the increased interactions of methanol with the capillary wall in comparison to THF. Additionally, the symmetry of the TBA^+ peak was reversed with methanol (fronting) in comparison to the THF (tailing). The asymmetry of a solute peak is a result of the difference between the electric field in the sample zone and the BGE.[1,2,18,31] The reversal of peak symmetry suggests that the conductivity of the BGE with methanol is lower (higher electric field) than the BGE with THF with respect to TBA^+. Because the efficiency and selectivity of the separation did not differ appreciably in the comparison between methanol and THF and because methanol does not inhibit the formation of micelles, THF was maintained as the organic modifer.[8]

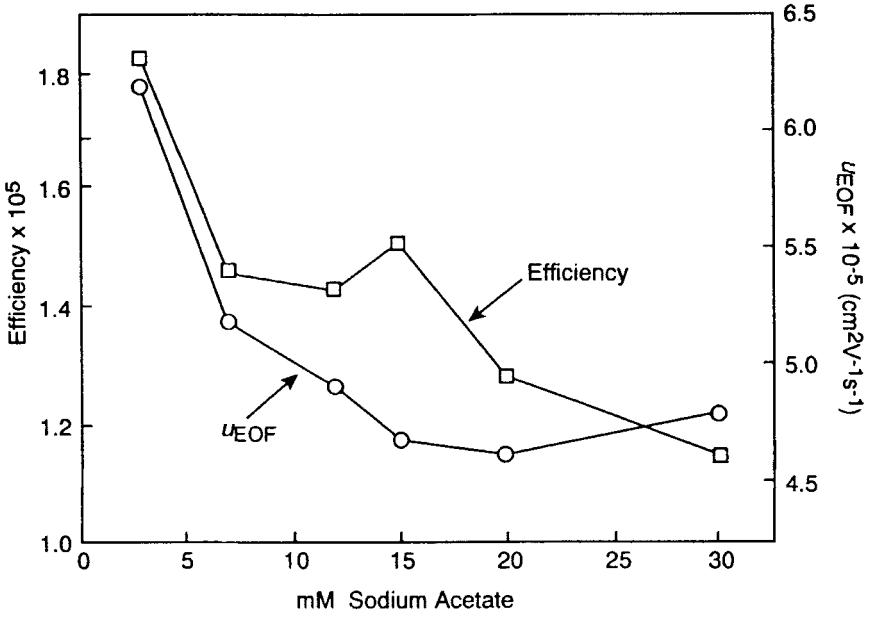

Figure 7. Effects of the concentration of sodium acetate on the effective electrophoretic mobility of the electro-osmotic flow and efficiency of the separation. BGE: 30 v/v % THF, pH_{app} 4.5 and 12 mM quinine.

The optimized composition of the BGE was 12 m*M* quinine and 3 m*M* sodium acetate in 75:25 (v/v) H_2O:THF at pH_{app} 4.5 and detection was performed at 335 nm. The ability to analyze real samples was the objective of this work and a typical electropherogram of an initial organic extraction layer (~0.1 mg/mL TBA^+) is shown in Figure 8. The method resolves several components in the process streams including potassium and sodium ions.

Validation Studies

The linearity of the detector response was evaluated using standard solutions of TBA^+ over the concentration range from 1.08 μg/mL to 1.08 mg/mL. This corresponds to approximately 15 ppm to 1.5 wt% TBA^+ in the *indinavir* monohydrate intermediate sample. Ten standard solutions were injected in triplicate and the detector response at 335 nm was found to be linear over the entire concentration range (R^2 = 0.9998). A signal to noise ratio (S/N) of three was measured for the lowest concentration tested during the linearity

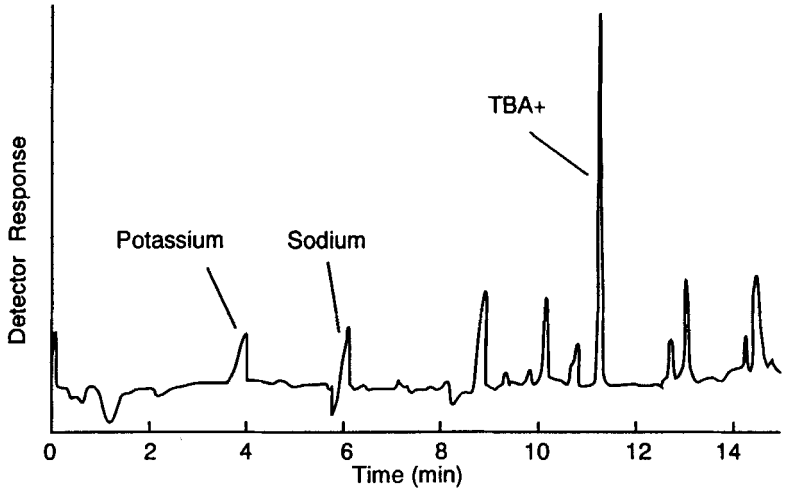

Figure 8. Typical electropherogram of an organic extraction layer. BGE: 25 v/v % THF, 3 mM sodium acetate, pH_{app} 4.5 and 12 mM quinine.

study, 1.08 μg/mL TBA^+. Therefore, the limit of detection and limit of quantitation (S/N=3xLOD) of the method was determined as 1.08 and 2.17 μg/mL, respectively. The linear range of the detector and sensitivity agrees well with the work of other laboratories quantitating structurally related alkyl quaternary ammonium compounds.[8-10]

The injection precision was evaluated by making three consecutive injections each of the standard solutions during the course of the linearity study. The %RSD for the area counts of TBA^+ ranged from 1.6% to 17.6% for the 1.08 mg/mL and 1.08 μg/mL standards, respectively. The average %RSD for the area counts and migration time of TBA^+ for each triplicate injection set of standards was 5.3 and 0.45%, respectively.

The accuracy of the assay was evaluated by the method of standard additions. Aliquots of TBA^+ stock solution (10.8 mg/mL) were spiked into a solution of *indinavir* monohydrate intermediate (71.7 mg/mL). The concentration of TBA^+ in the spiked samples ranged from 216 to 10.8 μg /mL. The amount of TBA^+ in the spiked samples was plotted against the amount of TBA^+ determined by linear regression of standards. A linear plot was obtained (R^2 = 0.998) with a slope close to 1.0 (0.98) and an intercept of zero.

Additionally, spiking analysis was performed at the 140 μg/mL level for both organic and aqueous reaction mixtures and recoveries between 96 and 102% were obtained.

CONCLUSIONS

The quantitation of TBA^+ in both drug substance and process streams was achieved by a CE method utilizing indirect detection. The studies described above demonstrated the effects of varying the pH_{app}, volume percent organic modifier, sodium acetate and quinine concentration in the BGE upon m_{TBA+}, μ_{EOF} and efficiency. The ranges in which the various parameters were examined had considerable effects upon both efficiency and m_{EOF}, however, μ_{TBA+} was not significantly affected. The method validation results demonstrated that the CE method is reproducible, accurate, precise and sensitive.

ACKNOWLEDGMENTS

The authors extend appreciation to Dr. Angelos Dovletoglou and Ms. Lili Zhou for the generous use of their CE system.

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Received August 5, 1996
Accepted August 27, 1996
Manuscript 4229