

Capillary Electrophoresis with Indirect Ultraviolet Detection for Pharmaceutical Counterion Analysis



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ABSTRACT

Capillary electrophoresis (CE) with indirect UV detection is a proven technology for the analysis of counterions. CE offers short analysis and cycle times, broad tolerance for sample matrices, small sample- and buffer-volume requirements, and low waste production. These traits make it a desirable alternative or complementary analysis method to ion chromatography. We present here the characterization of a counterion analysis method based on CE with indirect UV detection. The method characterization includes determination of the linear range of quantitation and limit of detection for commonly employed organic and inorganic counterions. We also demonstrated the ability of the method to accurately analyze samples prepared in several organic solvents commonly used to address drug solubility.

MATERIALS AND METHODS

Data were collected and analyzed on a PA 800 Series capillary electrophoresis system with 32 Karat software using indirect UV detection. Anion analysis was performed using SCIEX Anion Analysis Kit (PN A53537) by following kit instructions. Cation analysis was performed using SCIEX Cation Analysis Kit (PN A53540) by following kit instructions. The run conditions are summarized in Figures 1 and 2. Purchased drugs were solubilized and diluted in distilled, deionized (DDI), and filtered (0.2 µm) water except for ergotamine tartrate and atropine sulfate which were solubilized in dimethylsulfoxide (DMSO) and diluted in water. Calibration curves for sodium and chloride were prepared by dissolving 0.5 g NaCl in 100 mL filtered DDI water (f.c. of 5000 ppm) and diluting from this stock. Calibration curves for TFA were prepared by dissolving trifluoroacetic acid to 9700 ppm and diluting from this stock. A curve of 10 calibration levels was run for each ion with five replicates per level. All calibration peaks were automatically integrated by the software using method integration parameters. For testing the effect of organic solvents on the anion analysis method, a sample of organic ions was prepared as follows: 4.6 g phosphoric acid 85%, 15.7 g sulfuric acid 25%, 120 g DDI water, 54 g dicyclohexylamine, 1.5 g tartaric acid, 1.18 g succinic acid, and 1.07 g malonic acid were combined and DDI water was added to 200 g. The resulting solution was filtered before diluting 200 µL into 20 mL of solvent/water mixture. The test mix was analyzed using standard and reduced injection pressure of 0.1 psi. To increase the signal an additional 20 µL of the test mix was spiked into the sample vials. The tests for the effect of organic solvents were run on a SCIEX P/ACE™ MDQ series capillary electrophoresis system.

Initial Conditions	Anion	Cation
Bare Fused Silica Capillary ID	75 µm	75 µm
Capillary Length to Detector	50 cm	50 cm
Capillary Temperature:	25° C	25° C
Sample Storage Temperature	25° C	25° C
Indirect UV Detection Wavelength	230 nm	200 nm
Polarity	Reverse	Normal

Figure 1. Initial Conditions for counterion analysis methods

RESULTS

Anion Timed Program			
Rinse with Anion Dynamic Capillary Coating Solution	20 psi	30 sec	
Rinse with Anion Separation Buffer	20 psi	30 sec	
Inject Sample	0.5 psi	8 sec	
Inject Water	0.1 psi	1 sec	
Separate Anions Reverse Polarity	30 kv	8 min	
Autozero		After 1.25 min	
Stop Data		After 8 min	
Rinse with Conditioner Na	20 psi	30 sec	
Rinse with Rinse Solution	20 psi	30 sec	
End			

Cation Timed Program			
Rinse with Cation Dynamic Capillary Coating Solution	20 psi	30 sec	
Rinse with Cation Dynamic Capillary Coating Solution	20 psi	30 cm	
Rinse with Cation Separation Buffer	20 psi	90 sec	
Water Dip		12 sec	
Inject Sample	0.5 psi	5 sec	
Inject Water	0.1 psi	10 sec	
Separate Cations Normal Polarity	30 kv	5 min	
Autozero		After 2 min	
Stop Data		After 5 min	
Rinse with Conditioner Na	20 psi	30 sec	
Rinse with Rinse Solution	20 psi	30 sec	
End			

Figure 2. UV detector settings and timed run program for Anion Analysis Method (A) and Cation Analysis Method (B)

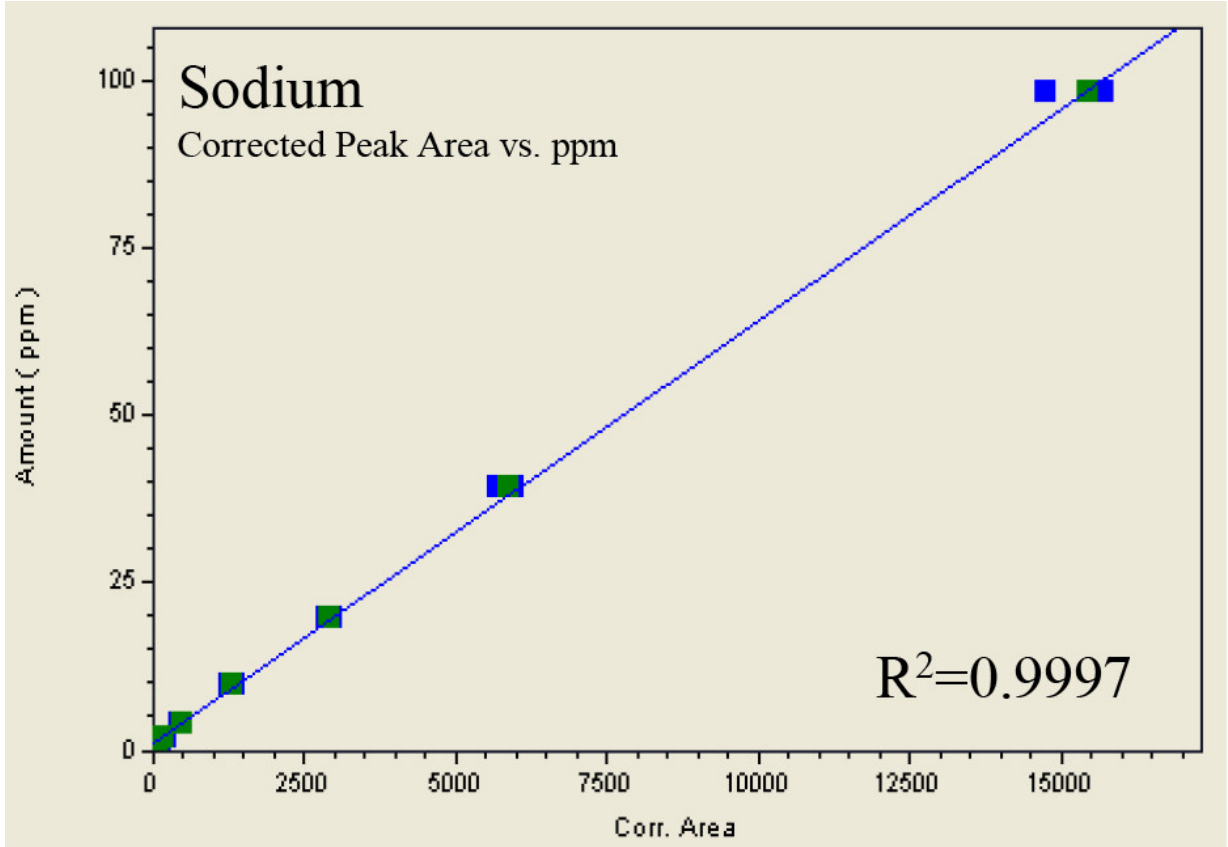


Figure 3. Calibration curve generated with Cation Analysis Kit for sodium ion showing linear quantitation from 1-100 ppm Na. Peaks were automatically integrated via batch analysis using method integration parameters.

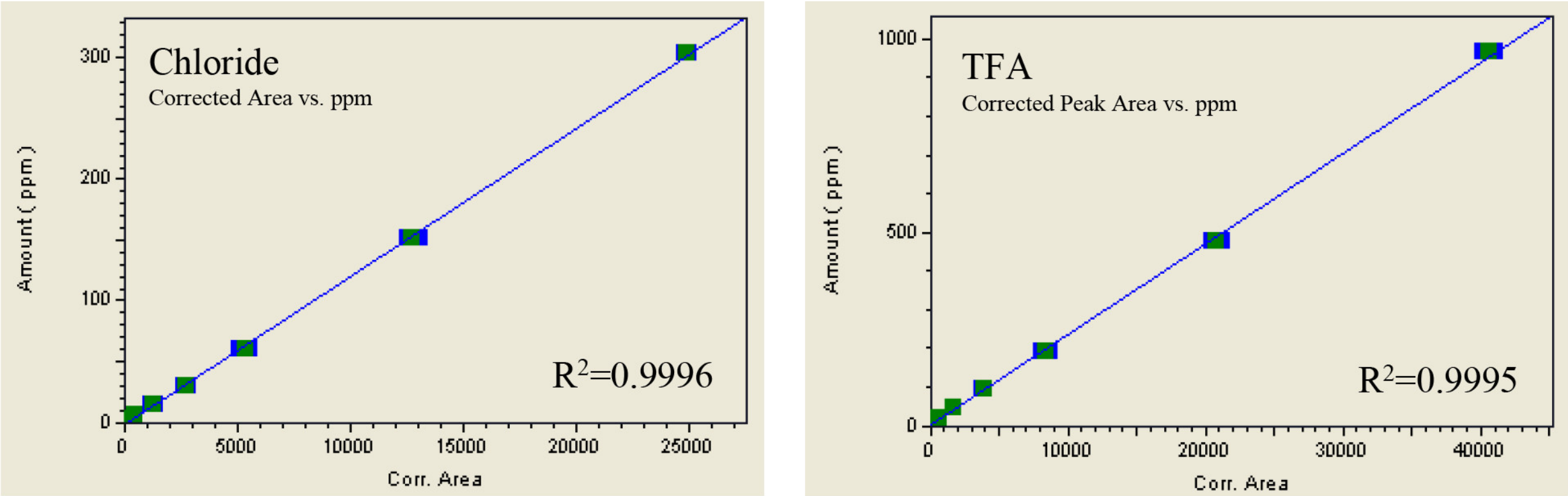


Figure 4. Calibration curves generated using Anion Analysis Kit for chloride (A) and TFA (B) showing linear range from 1.5 to 300 ppm Cl and 4.85 to 970 ppm TFA. Peaks were automatically integrated via batch analysis using method integration parameters.

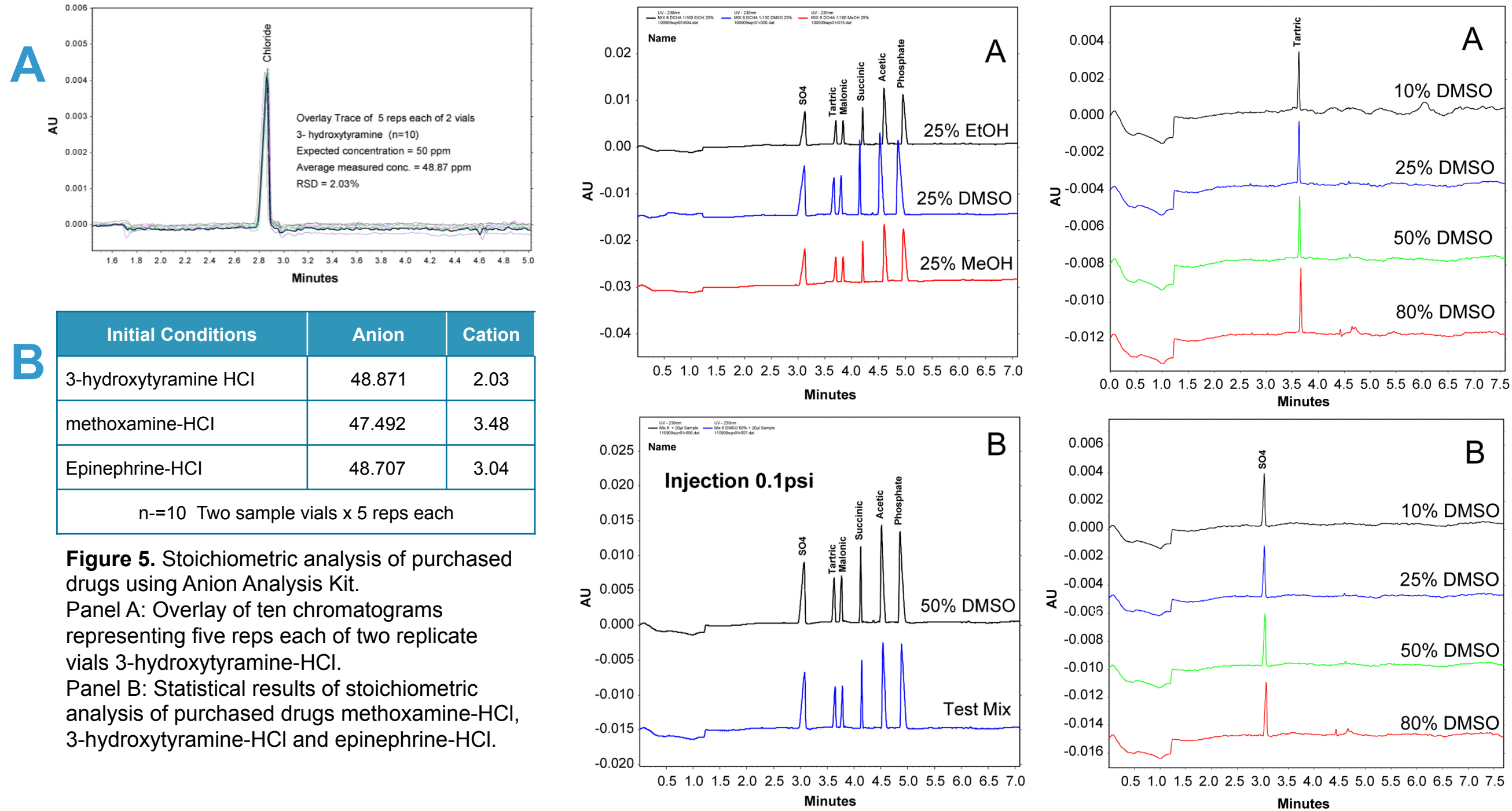


Figure 5. Stoichiometric analysis of purchased drugs using Anion Analysis Kit. Panel A: Overlay of ten chromatograms representing five reps each of two replicate vials 3-hydroxytyramine-HCl. Panel B: Statistical results of stoichiometric analysis of purchased drugs methoxamine-HCl, 3-hydroxytyramine-HCl and epinephrine-HCl.

Figure 6. Analysis of organic anion test mix in 25% organic solvents EtOH, MeOH, and DMSO (A), injection samples prepared as described in the methods section. Test mix diluted in water or in 50% DMSO analysed using modified injection condition of 0.1 psi 8 sec (B); injection samples prepared as described in the methods section with an additional 20 µL of anion stock solution spiked into the vials.

Figure 7. Counterion analysis of two drugs with low solubility in water ergotamine tartrate (A) and atropine sulphate (B) prepared to 2 mM in solutions of up to 80% DMSO.

RESULTS AND DISCUSSION

By following the Anion Analysis Kit instructions, calibration curves for chloride and TFA were generated and found to be linear ($r^2 > 0.999$) from 1.5 to 300 ppm and 4.85 to 970 ppm respectively (Figure 3). LOD (S/N > 3.0) and LOQ (S/N > 10) for chloride were found to be 0.6 ppm and 5 ppm. For TFA the LOD and LOQ were found to be 1 ppm and 5 ppm. A sodium ion calibration was generated using the Cation Analysis Kit and shown to be linear from 1-100 ppm with LOD and LOQ of 1 ppm and 4 ppm, respectively (Figure 4). Purchased drug samples demonstrated the utility of the kit for stoichiometric analysis (Figure 5). Using the standard method conditions the Anion Analysis Kit was able to resolve all six anion peaks of a test mix prepared in up to 25% organic solvents ethanol, methanol, or dimethylsulfoxide (DMSO) (Figure 6A). However by simply lowering the injection pressure and thereby reducing the size of the injection plug, the DMSO was well tolerated up to 50% (Figure 6B). Ergotamine tartrate and atropine sulfate, two drugs with low solubility in water, could be analyzed in solutions of up to 80% DMF (data not shown) or DMSO using the modified injection conditions (Figure 7).

CONCLUSION

Methods for capillary electrophoresis with indirect UV detection were found to be suitable for the analysis of pharmaceutical counterions. Calibration curves for chloride, TFA and sodium were linear between 1.5 to 300 ppm, 4.85 to 970 ppm, and 1-100 ppm respectively. Stoichiometric determinations on purchased drug samples showed good correlation with predicted outcomes. Additionally it was shown that the method could tolerate up to 80% organic solvent in the sample with only slight modifications to the standard method.

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Document number: RUO-MKT-10-6964-B