Capillary Electrophoresis with Indirect Ultraviolet Detection for Pharmaceutical Counterion Analysis

C Boardman, J Dewald, JB Falmagne* and F de l’Escaillie*
Discovery Products Applications Group, Beckman Coulter, Inc.
*Analis R&D Diag. Namur, Belgium

Introduction

Capillary electrophoresis (CE) with indirect UV detection is a proven technology for the analysis of counterions. CE offers short analysis times 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 characteristics 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 cations. We additionally demonstrated the ability of the method to accurately analyze samples prepared in several organic solvents commonly used to address drug solubility.

Materials & Methods

Data were collected and analyzed on a Beckman Coulter PA 800c Advanced Protein Characterization System with 20 kV Version 8 software using indirect UV detection. Anion analysis was performed using Beckman Coulter Anion Analysis Kit (PN A53537) by following kit instructions. The cation analysis was performed using Beckman Coulter Cation Analysis Kit (PN A53546) by following kit instructions. The run conditions are summarized in Figures 1 and 2. Purchased drugs were solubilized and diluted in distilled, deionized water (ddH2O), and filtered (0.2 μm) water except for organotin tetraste and anionic sulfates which were solubilized in dimethylsulfoxide (DMSO) and diluted in water. Calibration curves for sodium and chloride were prepared by dissolving 0.1 g of NaCl and 0.1 g of NaHCO3 in 100 mL of distilled deionized water (ddH2O) and diluting from this stock. Calibration curves for TFA were prepared by dissolving 100 mg of TFA in 100 mL of 25% DMSO in water and diluting from this stock. A curve of 10 calibration levels was generated for each drug in each replicates per level. All calibration peaks were automatically integrated via the software using method integration parameters. For testing the effect of organic solvents on the analysis method, a sample of organic ions was prepared as follows: 4.0 g phosphoric acid 85%, 15.7 g sulfuric acid 25%, 13.0 g dioxane, 5.04 g dicyclohexylamine, 1.5 g hexadecylamine, and 17.0 mL methanol were combined and ddH2O was added to 200 mL. The resulting solution was diluted before obtaining 200 μL into 25 mL of 25% DMSO/water mixtures. 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 applied into the sample vials. The tests for the effect of organic solvents were run in an APCxP™ MDQ series capillary electrophoresis-system 20.

Results

By following the Anion Analysis Kit instructions, calibration curves for chloride and TFA were generated and found to be linear (R2 > 0.999) from 1.5 to 300 ppm and 4.8 to 970 ppm respectively (Fig. 1). LOD (S/N = 3.0) and LOQ (S/N = 10) for chloride were found to be 4.5 ppm and 15 ppm. For TFA the LOD and LOQ were found to be 1 ppm and 5 ppm. A sodium ion calibration was generated using the Anion Analysis Kit and shown to be linear from 1-100 ppm with LOQ and LOD (S/N = 4.0) being 1 ppm and 2 ppm respectively (Fig. 2). Purchased drug samples demonstrated the utility of the kit for stoichiometric analysis (Fig. 3). Using the standard method conditions the Anion Analysis Kit was able to resolve all six anion peaks from a drug sample up to 25% organic solvents. However by simply lowering the injection pressure and thereby reducing the size of the injection plug, the DMSO was well tolerated up to 50% (Fig. 4). Erucinates tetraste and Anionic sulfate, two drugs with low solubility in water, could be analyzed in solutions of up to 80% DMSO (data not shown) or DMSO using the modified injection conditions (Fig. 5).

Additionally it was shown that the method could address 40% organic solvent in the sample with only slight modifications to the standard method.

References

2. Little M. Quantifying Counterions in Drug Discovery (2008) ISPE ACE Sponsored by ISPE