PHARMACEUTICAL Application Brief

Brief Number 1004

System Suitability Evaluation of a Small Molecule Pharmaceutical Capillary Electrophoresis Separation

930780

Introduction

To be broadly accepted, a new separation technique must be accurate and reproducible. For capillary electrophoresis reproducibility includes both migration time and peak area or peak height measurements. Migration time provides information concerning a compound identity and peak area or peak height is used for quantitation. These parameters are monitored over an extended period to evaluate a method's reproducibility. Separation parameters which change impact sample throughput. For example, if a fifteen minute separation must be checked with standard(s) on an hourly basis, then 20 - 25 % of the day can be consumed running standards and recalibrating the instrument. A robust and reproducible CE method only needs a complete daily calibration. Samples are bracketed with standards to make minor adjustments to migration time or response, increasing laboratory throughput without sacrificing confidence in the results.

Capillary Electrophoresis Reproducibility

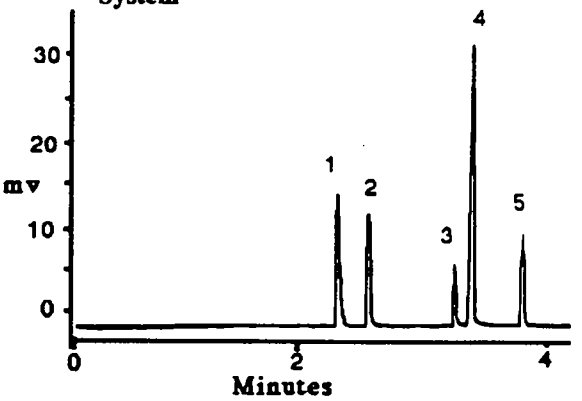
Capillary electrophoresis with its inherent high efficiency (typically over 100,000 plates) is only advantageous if the method is reproducible. Migration time reproducibility is extremely important to ensure proper identification of the compounds since many of these peaks are separated by only a few seconds. If high plate counts were achieved while sacrificing the method's reproducibility, then sample throughput would be compromised.

To evaluate the reproducibility of the Quanta 4000™ Capillary Electrophoresis system, a five component analgesic separation was evaluated. This separation utilized micellar conditions as listed in the caption of Figure 1. Data was collected on Waters 860 VAX Based Data System and evaluated by Waters Expertease™ System Suitability Software.

Evaluating Capillary Electrophoresis System Suitability

Waters System suitability software statistically evaluates GC, HPLC or CE reproducibility and adheres to the USP XXII (method 621) physical test procedure. To completely understand a CE method's suitability the short term and long term effects on migration time and area are investigated. From multiple electrophoresis runs a trend plot reveals variations occurring from run to run. The overall precision and accuracy of the method are also measured from multiple runs. These results are generally expressed as the % RSD (per cent relative standard deviation). Together this data determines how often a method must be validated with standards. With a low % RSD for area, height and migration time, the confidence in the derived answer is high. With a high % RSD, the number of standards and samples which must be duplicated increases while decreasing overall laboratory throughput.

Figure 1 Four Minute Analgesic Separation on Waters Quanta™ 4000 Capillary Electrophoresis System



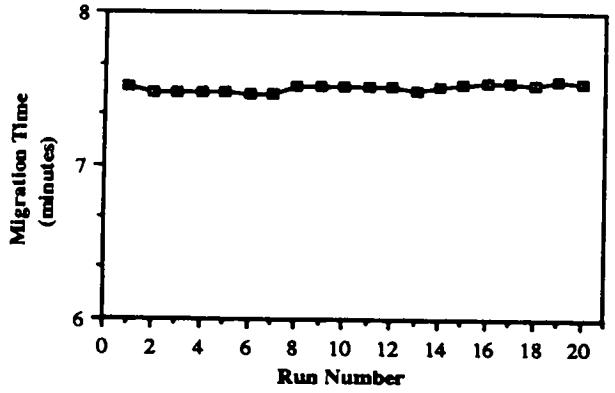
Analgesics separated on a 75µm x 60 cm capillary with 20 mM sodium phosphate buffer adjusted to pH = 11 with NaOH and 50 mM SDS. Peak Identity 1. Caffeine, 2. Acetaminophen, 3. Acetylsalicylic Acid, 4. Salicylamide and 5. Salicylic Acid

Factors Affecting Reproducibility

Parameters affecting capillary electrophoresis reproducibility include capillary wall contamination, changes in buffer strength, changes in electrolyte pH, and variation in capillary temperature (internal and external). The separation described in figure 1 was repeated twenty times to evaluate these variables. Each peak was evaluated by the system suitability software package, but to simplify the discussion only the salicylamide results are illustrated. Similar results were obtained with all peaks.

The salicylamide migration time trend plot is shown in figure 2. The migration time was measured electronically by the data system and the trend plot was generated automatically, eliminating possible manual transcription errors. Over the 15 hour experiment, excellent migration time reproducibility (0.5 %) was achieved for these electrophoresis separations.

Figure 2 Migration Time Trend Plot for Salicylamide

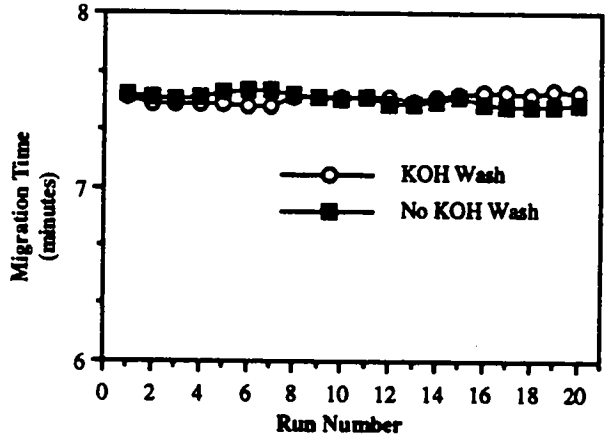


Frequency of Cleaning the Capillary with KOH

A common practice in capillary electrophoresis specifies washing the capillary with KOH to remove any surface contaminates and regenerate the silica surface. Capillary surface contamination causes electroosmotic flow changes with subsequent changes in migration times. To evaluate whether a KOH wash was required between all capillary electrophoresis runs, the analgesic separation was further evaluated.

Triplicate injections of each sample were followed by a 0.2 M KOH purge. After this regeneration step, the capillaries electrolyte was automatically replenished and the analysis repeated. This experiment was repeated but the KOH wash step was eliminated. Figure 3 illustrates the system suitability migration time trend plot for twenty consecutive analgesics electrophoresis separations with and without the KOH column regeneration. Statistically the migration time variation is the same whether or not the system was purged with KOH. To further verify these results the peak area and peak height for each injection were evaluated as shown in figure 4.

Figure 3 Comparing Migration Time Trends With and Without KOH Washes



The area or height did not vary significantly whether the capillary was washed with KOH. Therefore in all subsequent small molecule pharmaceuticals CE separations, we have adopted the routine of washing the capillary with KOH at the beginning of an analysis day. Eliminating the KOH wash had no detrimental effects on reproducibility or quantitation but sample throughput increased by eliminating the need to re-equilibrate the capillary after each KOH wash.

Figure 4 Quantitation Trend Plot for Salicylamide

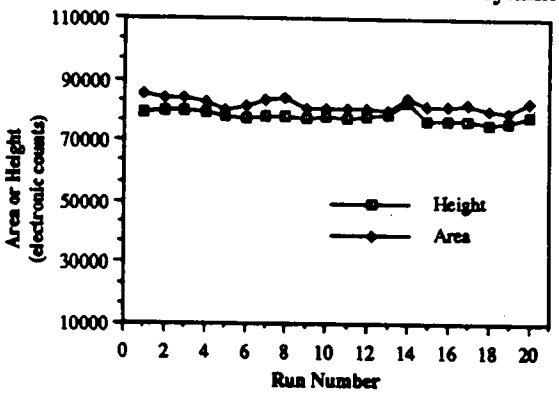
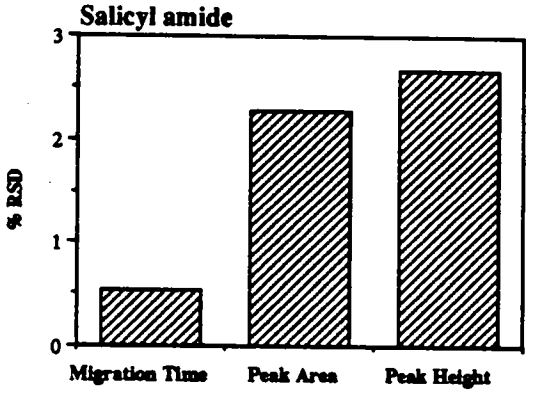


Figure 5 Relative Standard Deviation Plot for Salicyl amide



Overall system suitability evaluation for twenty CE separations which occurred during a fifteen hour experiment

Excellent Overall Method Reproducibility

Capillary Electrophoresis reproducibility includes short term variations as displayed in trend plots and long term trends expressed as overall % RSD. Figure 5 shows the overall % RSD for each experimental parameter. The RSD for peak area was 2.2%, peak height varied by less than 3% RSD and migration time varied less than 0.5 %. Therefore capillary electrophoresis separations on Waters Quanta 4000 Capillary Electrophoresis System provides reproducible separations with a high degree of confidence in the quantitation. The excellent reproducibility minimizes the number of standards required between samples and increases the laboratory throughput without sacrificing confidence in the results.