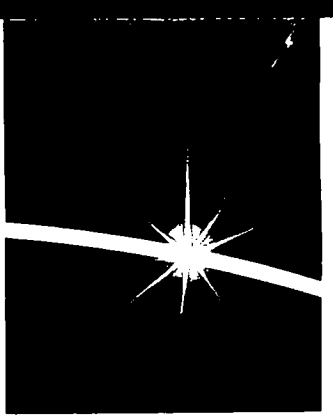
Capillary Electrophoresis

94-0423



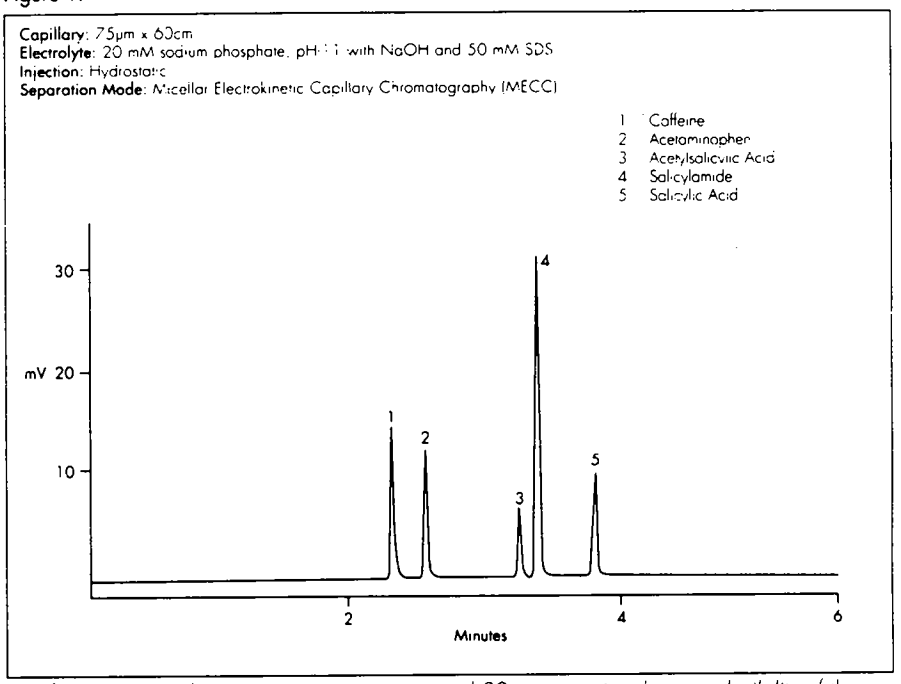
Capillary Electrophoresis
Application Brief Ph 1. 3/91

Proving the Reproducibility of Capillary Electrophoresis: A System Suitability Evaluation on the Quanta® 4000

CE Methods are Reproducible.
Capillary electrophoresis has proven to be an accurate analytical technique for many pharmaceutical and small molecule applications. This is due to the exceptional reproducibility of CE methods. Reproducibility is of the utmost importance - especially when identifying peaks where migration times differ by only a few seconds or for accurate quantitation.

The reproducibility of CE can be affected in a number of ways, including capillary wall contamination, changes in buffer strength and electrolyte pH, and variation in both internal and external capillary temperature. To evaluate the suitability of CE methods, the short- and long-term effects on migration time and area were investigated. Multiple runs were used to evaluate the overall precision and accuracy of the method.

Figure 1.



This four minute analgesic separation was repeated 20 times, testing the reproducibility of the Quanta 4000.

Reproducibilty Accurate Over 20 Injections.
To evaluate the reproducibility of the Quanta 4000 system, five component analgesics were run 20 times over 15 hours using micellar conditions. Data was collected on the Waters 860 Chromatography Networking Computer System and evaluated by Waters Expert™ Ease System Suitability Software. The salicylamide results will be discussed here, although each peak was evaluated and showed similar results.

Figure 1 shows the results of the four minute separation. Figure 2 demonstrates the excellent migration time reproducibility (0.5%) achieved over the 15 hour experiment. This migration time trend plot was measured electronically and was automatically generated by the data system, eliminating possible manual errors.

Time-Consuming Purges Not Needed.
The analgesic separation was further evaluated to test whether capillary purging after every run was necessary for removing capillary surface contaminates. Triplicate injections of each sample followed by 0.2 M KOH purges and were compared to injections without purging. After 20 separations of each method, there was little difference — in fact, statistically, the migration time did not vary.

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34 Maple Street
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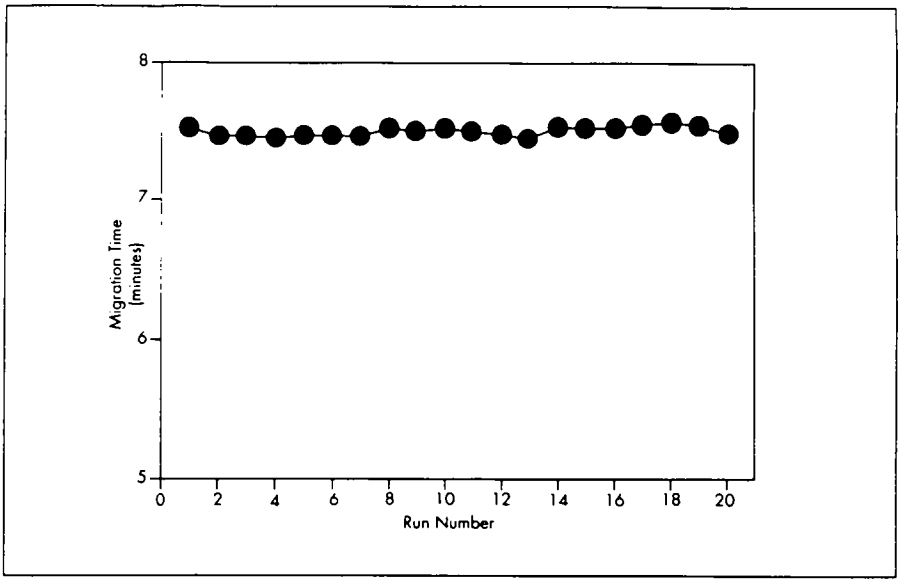
To further verify these results, the peak area and peak height for each injection were evaluated. Neither varied significantly, suggesting that only one purge at the beginning of an analysis day is necessary. Eliminating the KOH purge had no detrimental effects on reproducibility or quantitation. In fact, sample throughput increased because the need to purge and re-equilibrate after every sample had been eliminated.

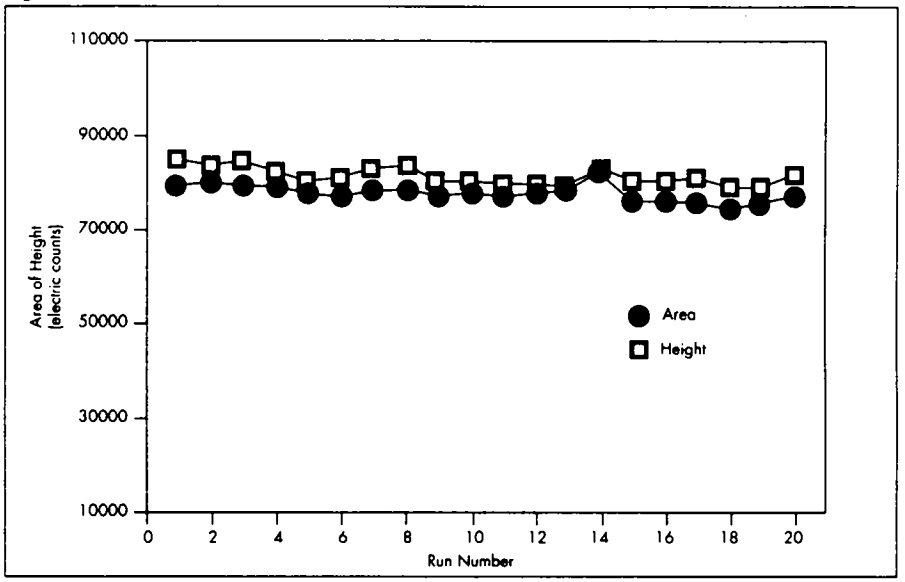
Quantitative Results in Less Time.
The excellent reproducibility of CE minimizes the number of standards required between samples and increases throughput without sacrificing quantitative results. The RSD for peak area over 15 hours was 2.2% and peak height varied by less than 3% RSD. Migration time varied less than 0.5%. Therefore, even over an extended analysis time, analyses on the Quanta 4000 system are reproducible and quantitative.

Figure 2



This migration time trend plot for salicylamide shows a 0.5% reproducibility for 20 runs over 15 hours.

Figure 3



Evaluation of both peak area and height showed sample quantitation did not vary significantly with or without purging. In fact, sample throughput was increased by eliminating the need to purge and re-equilibrate after every sample.

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