Quantitative Aspects of Capillary Electrophoresis

Capillary electrophoresis reproducibility includes both migration time and peak area or peak height measurements. Migration time reproducibility is an important factor for compound identity. Peak area or peak height is used for quantitation. The following experiments were designed to provide quantitative data on the performance of the Waters Quanta™ 4000 capillary electrophoresis system.

The parameters investigated in this experiment include:

A. Linearity of Hydrostatic Injection
B. Linearity of Detector Response
C. Detector Sensitivity and Detection Limits
D. Migration Time Reproducibility
E. Peak Area Reproducibility
F. Peak Height Reproducibility

These parameters were evaluated over an extended period using test solutes provided a Fine Chemical House. The results presented in this report relate specifically to the analysis of these test solutes supplied. As such, these results can be used to indicate the capability of the Waters Quanta 4000.

For all tests, anthraquinone - 1 - sulphonic acid (compound 1) concentrations were made from 8 µg/ml to 274 µg/ml with 105 µg/ml anthraquinone - 1, 5 - disulphonic acid (compound 2) added as internal standard (Figure 1). The hydrostatic injection mode introduces the sample into the capillary by using gravity as the pressure source and raising the inlet of the capillary to a specified height.

Quanta 4000 Conditions:
Injection Mode: Hydrostatic (10 cm height)
Injection Time: 25 seconds
Capillary: 75 µm i.d. x 60 cm length
Temperature: Ambient
Buffer: 10 mM Na₂B₄O₇, pH = 9.4
Sample Matrix: Water
Detector: 254 nm
Running Voltage: + 20 KV

These results were calculated and the linearity for compound 1 was R² = 0.997959 and the linearity for compound 2 was R² = 0.998772. The hydrostatic injection displays a high degree of linearity for the tested range - 10 to 50 seconds (Figure 2).

Figure 1 Quanta 4000 Separation of Test Molecules
Capillary Electrophoresis separation of substituted anthraquinones. Instrumental conditions and peak identification are outlined in the text.

Linearity of the Hydrostatic Injection System
The hydrostatic injection system was tested using triplicate injections of 10, 15, 20, 25 and 50 seconds for the 274 µg/ml sample. The linearity of response was calculated and the results expressed as coefficients. The linearity for compound 1 was R² = 0.997959 and the linearity for compound 2 was R² = 0.998772. The hydrostatic injection displays a high degree of linearity for the tested range - 10 to 50 seconds (Figure 2).

Figure 2 Quanta 4000 Hydrostatic Injection Linearity
The Quanta 4000 Hydrostatic Injection system is very linear over the tested range from 10 to 50 seconds.

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Linearity of Detector Response
The sample concentration series were hydrostatically injected five times each for 25 seconds (32 nl). A calibration curve of detector response versus concentration was generated. The linearity of compound 1 was \( R^2 = 0.999976 \). The Quanta 4000 detector response displays a high degree of linearity for compound 1 in the range of 8 - 274 \( \mu g / ml \) (Figure 3).

Detector Sensitivity and Detector Limits
The 274 \( \mu g/ml \) compound sample was diluted 1:1000 times and injected five times. The detection limit calculation was based on the Waters Expert Ease system suitability software. The Root Mean Square (RMS) Baseline Noise is automatically calculated and compared to the peak height of compound 1. The results are expressed as concentration detection limits and mass detection limits where the injection volume was 32 nl. The RMS baseline noise equaled 0.0112 mv (1% noise at a sensitivity setting of 0.001 AUFS). The concentration detection limit was 137 \( ng / ml \) and the mass detection limit was 4.38 picograms (137 \( ng / 32 \) nl).

Peak Area and Peak Height Reproducibility
The migration time experiment was evaluated using the twenty five hydrostatic injections from the migration time reproducibility study. The peak area reproducibility was 0.88% RSD. Area reproducibility is very good, to the extent that external standards quantitation is acceptable. Peak height reproducibility for the same samples was 1.41% RSD (Figure 4).

![Figure 4 Quanta 4000 System Suitability Study (N = 25)](image)

Using various concentrations of compound 1 test solutions with internal standard (compound 2), five hydrostatic injections of each vial were made (6 samples x 5 injections, \( N = 30 \)). The peak area % RSD for external standards varied from 0.23 - 0.72% and averaged 0.33 %. Reanalysis of this data using the internal standard method shows that the area % RSD varied from 0.14 - 0.66. The internal standard quantitation results are more precise, as expected, and the internal standard method would be preferred for some quantitative analysis. Peak height % RSD varied from 0.46 to 4.04% for the same samples. Peak area reproducibility is better in all experiments (Figure 4).

Conclusions
The Quanta 4000 capillary electrophoresis system with the hydrostatic injection system is reproducible. Both migration time and area results are acceptable for either external and internal standard quantitative methods. In this study, peak area was more reproducible than peak height. The UV detector is linear and has good sensitivity with minimal noise.