



Brief Number 1003

930779

Detector Linearity A Prerequisite for Capillary Electrophoresis

Introduction

Analytical chemists require new separation techniques to be reproducible and exhibit linear detector response. Without linear detector response, obtaining any meaningful analytical data requires multiple level calibration curves. This problem has been apparent in HPLC and Ion Chromatography utilizing older style electrochemical or conductivity detectors. A non-linear detector response requires routine recalibration of the system across the expected concentration range. The number of standards and the frequency of recalibration is dramatically decreased with linear detector response.

Capillary Electrophoresis Benefits

Capillary electrophoresis (CE) is a relatively new analytical tool with high resolving power generated by very high efficiencies (theoretical plates in excess of 100,000 plates are common). CE also has different selectivity compared to normal or reverse phase HPLC, or GC. CE selectivity is affected by pH, ionic strength, micelle structure and concentration and other electrolyte additives including organic solvents. A CE separation's high efficiency and different selectivity is only advantageous however if the method exhibits adequate sensitivity and has a linear detector response. Detector linearity ensures the method's suitability for analyzing trace components in the presence of major components. Examples where detector sensitivity and linearity are important include bulk drug purity, chiral purity or assaying compounds at low concentrations.

Experimental Conditions

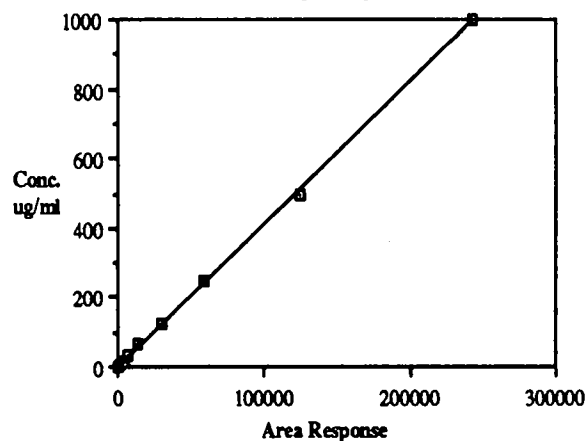
The Waters Quanta 4000™ Capillary Electrophoresis System with its selectable UV/VIS detector was evaluated for both linearity and sensitivity. A positive polarity power supply, zinc lamp with 214 nm filter and the hydrostatic injection mode were used in all experiments. Electrolyte solutions were prepared from stock solutions of HPLC grade reagents and degassed daily. Data was collected and reprocessed using Waters 845 Chromatography Workstation. Fifty and seventy-five micron internal diameter capillaries were used during this study. Both capillaries were 60 cm long.

Salicylamide standard solutions were prepared from a concentrated solution. In all experiments a 10 second x 10 cm hydrostatic injection was utilized to eliminate sampling discrimination with other injection techniques. For this study the salicylamide concentration and the internal diameter of the capillary were the controlled variables.

Both the 50 μ m and 75 μ m Capillary Show Excellent Linearity

The Quanta 4000 Capillary Electrophoresis System detector response for salicylamide was linear for sample concentrations from less than 1 μ g/ml up to 1 mg/ml. Figures 1 and 2 are the calibration curves generated with the 50 μ m and 75 μ m capillary respectively. At concentrations above 0.5 mg/ml the detector response for the 75 micron capillary is linear with a coefficient of variation of 0.998. Excellent linearity over a concentration range of three orders of magnitude is shown. If the 1 mg/ml concentration is eliminated from the calibration curve the correlation coefficient is 1.00. This is important for drug purity or chiral purity determinations where the impurities may be at levels less than 0.1% of the major component.

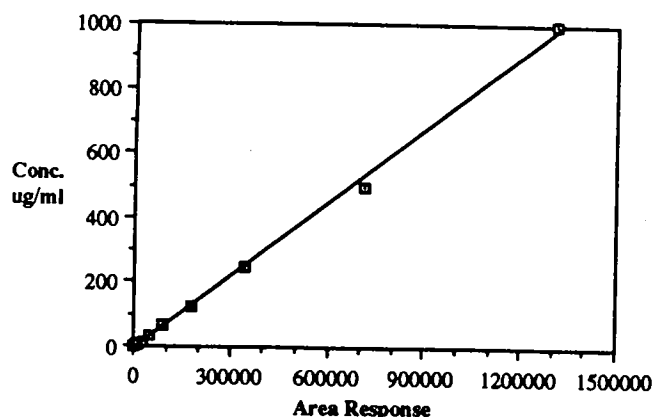
Figure 1 CE Response Curve
(50 Micron i.d. Capillary)



Minimum Detection Limit

The minimum detectable quantity of material was evaluated by performing serial dilution of standards. The concentration of material which provided a signal to noise ratio of three to one was determined to be the minimum detection limit. For this experiment all hydrostatic injection times were held constant at 10 seconds x 10 cm. For the 75 micron capillary, the minimum detectable quantity was 73 ng/ml and for the 50 micron capillary, the minimum detectable quantity was 489 ng/ml. The detection limit differences between the different internal diameter capillaries result from the narrower detector cell path (50 μ m) and less total sample applied during the 10 second injection (see the next section on injection volume effects). Similar minimum detection limits for the other compounds were achieved. (Table 1).

**Figure 2 CE Response Curve
(75 Micron i.d. Capillary)**



Injection Time (Volume) Effects Efficiency

To increase sensitivity in HPLC separations it is common to increase the sample volume injected. Initially it was thought capillary electrophoresis sensitivity and detection limits could be further enhanced by increasing the quantity of material injected into the capillary. For a hydrostatic injection increasing the sample volume is accomplished by increasing the time the sample carousel is raised above the electrophoresis operating position. Figure 3 illustrates increasing the injection time (volume) dramatically reduces efficiency. As injection times (volume) increase, the peak height does not increase as rapidly as the peak width causing a loss in efficiency. Sample volume is critical and detector sensitivity is extremely important for separations requiring high efficiency such as chiral analysis, bulk drug purity, or inorganic analysis. Without excellent detector sensitivity CE requires pre-concentration of compounds from very dilute solutions prior to injection to prevent efficiency losses.

Figure 3 Injection Time Effects on Efficiency

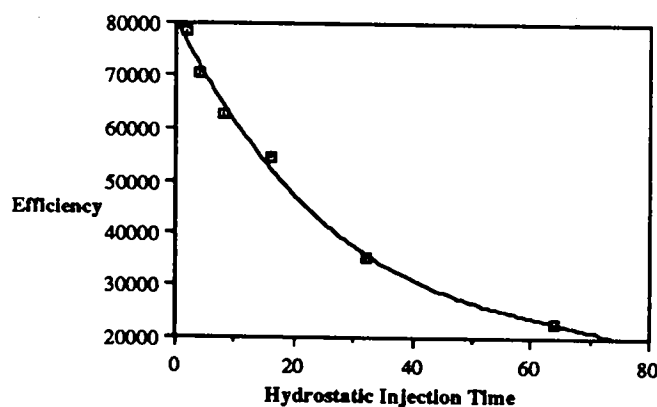


Table I

QUANTA™ 4000 Capillary Electrophoresis System Limits of Detection*

Component	Capillary	Conc.	Hydrostatic Inj. Time
Caffeine**	75 µm x 60 cm	1 µg/ml	10 sec
Salicylamide	50 µm x 60 cm	489 ng/ml	15 sec
Salicylamide	75 µm x 60 cm	73 ng/ml	15 sec
Neuromedin	75 µm x 60 cm	900 ng/ml	20 sec
ACTH (1 - 4)	75 µm x 60 cm	1 µg/ml	20 sec
Angiotensin II	75 µm x 60 cm	1 µg/ml	20 sec

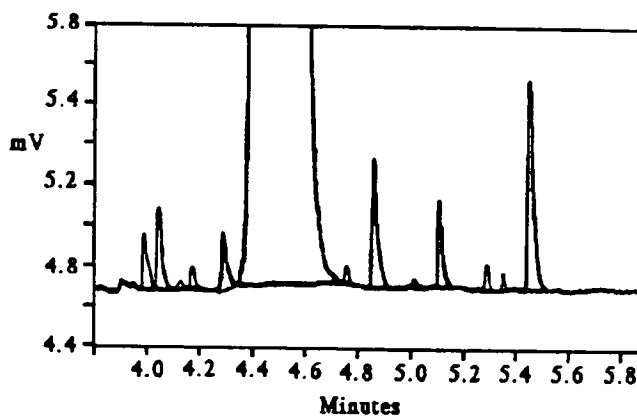
* L.O.D. S / N = 3

** Data courtesy of Dr. D. Edward Burton,
The Proctor and Gamble Company

Determination of Minor Components

Figure 4 shows a CE separation of a sample resulting from organic synthesis. By utilizing the Waters 845 data work station, the major component has been electronically expanded off scale to show the minor impurities. The high efficiency separation resolves thirteen components in less than two minutes. The linearity and sensitivity of the Quanta 4000 detector provides quantitation of impurities below 0.1%.

**Figure 4 Determination of Impurities with
Capillary Electrophoresis**



Summary

The excellent linearity and sensitivity of the Quanta 4000 UV/VIS discretely variable detector combined with the high efficiency, unique selectivity and small volume injections provide capabilities unavailable with other analytical methods. The high efficiency is further enhanced by the sensitivity and linearity of the Quanta 4000 discretely variable UV/Vis detector when using either 50 µm or 75 µm capillary.