PHARMACEUTICAL *Application Briefs*

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Maximizing Available Plates for Drug Purity Assays Using Capillary Electrophoresis

Introduction

Ensuring product purity of bulk drugs and formulations continues to be a high priority for pharmaceutical companies. To ensure impurities and degradation products are identified and quantitated, various complementary but different analytical (orthogonal) methods are employed

The need for purity determinations has lead pharmaceutical chemists to utilize new, faster, and more efficient separations. Today, these analytical methods include gas chromatographic or liquid chromatographic methods where retention time and peak area are used for identification and quantitation. Purity determinations for the more structurally complex drugs including antibiotics and prostaglandins require orthogonal methods to ensure co-elution has not occurred.

A New Method to Solve Old Problems

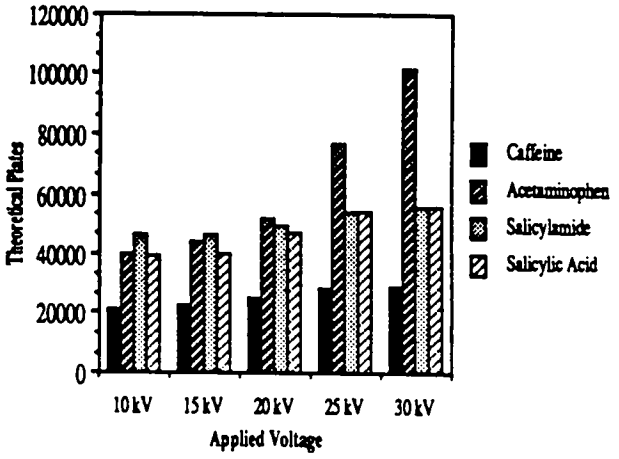
Capillary Electrophoresis (CE) is a new separations technology which complements both GC and HPLC. The chemist obtains similar qualitative and quantitative data from CE but in many instances, since the separation mode is different, the selectivity obtained is very different from GC or HPLC. Therefore CE is finding quick acceptance as an ancillary tool for confirmational work.

CE offers advantages over HPLC and GC including minimum sample preparation, shorter analysis times and highly efficient separations with minimal turn around time between sample runs. For HPLC separations the highest efficiency is achieved with very small particles and low flow rates which necessitate longer analysis times. For CE separations the relationship between analysis time and efficiency is complementary. Separations which generate the highest efficiency require the least time.

Higher Voltages Are Usually Preferred

Determination of operational voltage for optimum efficiency and resolution is a commonly asked question. Increasing the applied voltage in CE is not directly analogous to increasing the HPLC flow rate or GC temperature. Four pharmaceutical compounds, caffeine, acetaminophen, salicylamide and salicylic acid were separated on Waters Quanta™ 4000 Capillary Electrophoresis System using a 75 µm x 60 cm capillary. The efficiency of each peak was measured following the United States Pharmacopoeia method for HPLC peaks (tangent method with the peak width measured at 5.0% from baseline). For all compounds the efficiency increases with an increase in the applied voltage. (Figure 1) For example, the salicylamide plate count increased from 53,000 to over 100,000 plates when the applied voltage was increased from 10 kV to 30 kV.

Figure 1 Relationship of Applied Voltage and Efficiency



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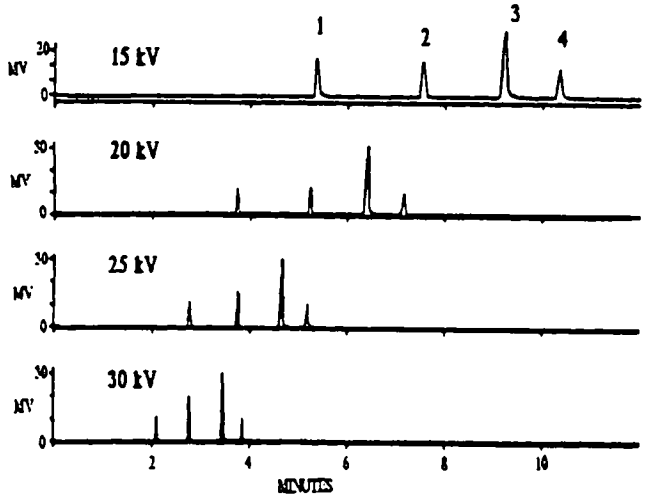
Disadvantages of Operating at Lower Applied Voltage

The two disadvantages of operating a CE system below its maximum voltage are a decrease in efficiency and an increase in analysis time. Figure 2 shows how increasing the voltage decreases total analysis time. Analysis time and applied voltage is analogous to flow rate in HPLC. Increasing the applied voltage decreases the analysis time. Capillary electrophoresis separations have the added benefit that when the applied voltage increases, the efficiency of the separation increases.

Figure 2 Lower Applied Voltage Increases Analysis Time



Peak Identity:

1. Caffeine
2. Acetaminophen
3. Salicylamide
4. Salicylic Acid

Limitations to Increasing the Voltage

Increasing the voltage however has limitations. The major limitation is that joule heat generated inside the capillary increases with voltage. If this heat is not effectively removed from the total capillary length, localized outgassing or bubble formation can occur. Once the electrolyte outgasses, the continuity of the electrical circuit is broken and electrophoresis stops. The Waters Quanta 4000 Capillary Electrophoresis System has been designed to effectively dissipate the joule heat generated. The maximum applied voltage for any given separation will depend on the electrolyte and the capillary dimensions (internal diameter and total length). To achieve the highest efficiencies therefore the instrument should be operated at the highest possible applied voltage continuously while preventing outgassing.

Summary

Capillary electrophoresis offers many new advantages to the analytical chemist. The Quanta 4000 Capillary Electrophoresis System combines new separation modes with achievable high efficiency, short analysis times and very fast cycle times to offer the analytical chemist another tool to meet the regulatory challenges of the 1990's.