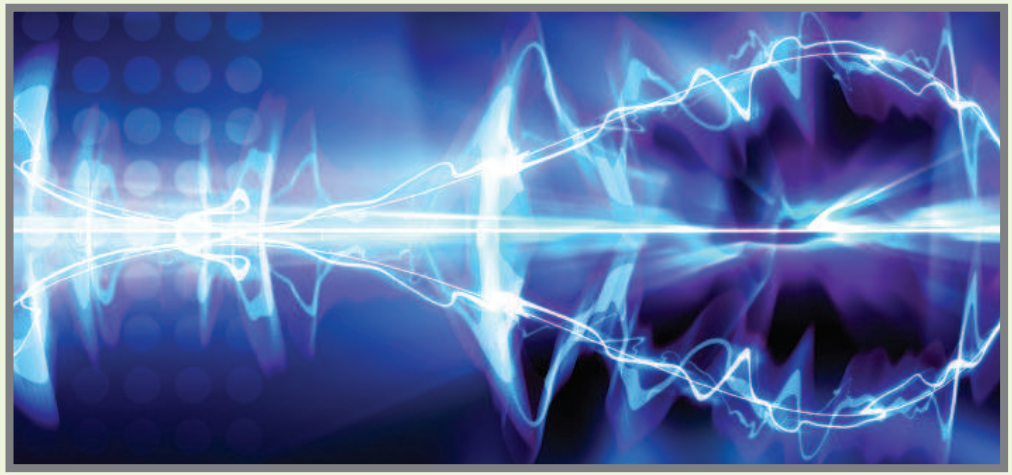
CE Solutions

separation | science
new horizons in chromatography and detection techniques

Cari Sänger is best known as one of the world's foremost CE troubleshooting authorities. Separation Science and Cari Sänger have collaborated to offer this digital learning platform providing valuable advice on everyday issues, problems and challenges faced by CE practitioners. Importantly, you will also have the opportunity to interact with Cari through our online questions submission system.

Tech Tip

Why CE?



This article is the first in a new series about capillary electrophoresis (CE), aims to be a low-threshold, practical and pragmatic aid for that implementation process by focusing on good CE working practices and troubleshooting.
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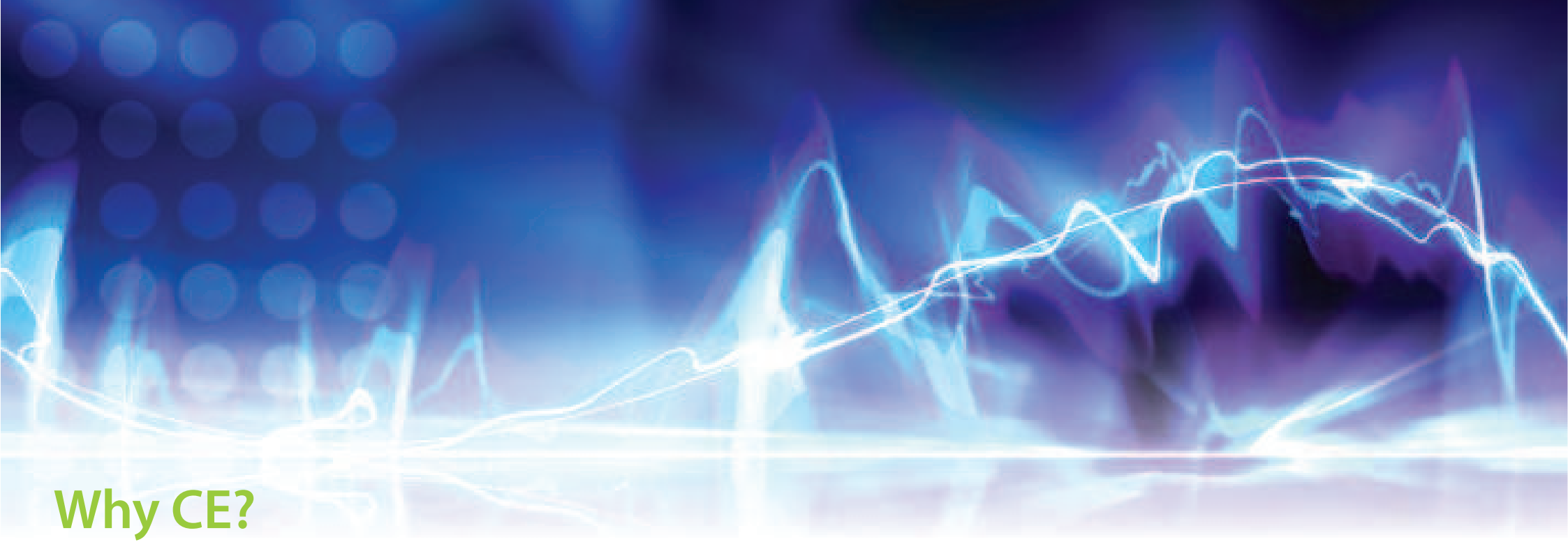
Workshops

***Separation Science*, in conjunction with Cari Sänger, offer a "CE Method Development" & "CE Method Validation" Master Classes in Basel, Switzerland.**
Click here to learn more

Ask the Doctor

Through 'CE Solutions' you will be able to ask questions directly. So if you have problems with low signal, detection, precision or any other CE issues then *click here* to contact Cari.



Why CE?

This article is the first in a new series about capillary electrophoresis (CE). The CE techniques have been around since the 1980s, and commercial instruments available since the end of that decade. So why would we start on such a topic now? There are several indications for a growing interest in the CE techniques. Instrument sales are increasing. An important driving force is the biopharmaceutical industry, where the capillary format replaces the slab gel. This process has been driven by several biotech products coming off patent, with CE methods in their pharmacopoeial monographs. Also, more and more commercial application kits have become available. Many determinations are now available as ready-made solutions. For successful implementation, well-trained personnel both on theory and practice are a must. This series aims to be a low-threshold, practical and pragmatic aid for that implementation process by focusing on good CE working practices and troubleshooting.

OK, so the world is more mature now for the successful application of CE. Why would one choose CE to start with; can't we solve all our problems with (UHP)LC and GC?

CE can be seen as an automated, analytical version of the conventional electrophoresis techniques. The main advantages of doing electrophoresis in a capillary are magnificent efficiencies and automation. Because of the small diameters of the capillary, typically in the 20–100 µm inner diameter range, the Joule heat dissipation is very efficient. This means that high voltages, usually up to 30000 V, can be applied. That in its turn, results in fast separations with very little band broadening. Small bands means efficient peaks with high plate numbers.

Because the capillary is usually made from fused silica, on-column UV detection is easily achievable. This means that the time-consuming staining and destaining known from slab gel electrophoresis is no longer needed. The combination of a small inner diameter (ID) capillary and on-column UV detection means that automated equipment has been developed. And with peaks instead of bands, quantitation is no longer an issue.

The capillary format creates an electrophoresis technique that is applicable over a wide range of analytes. Anything from small anions and cations to chiral separations, large proteins, DNA, cell organelles and even complete cells and viruses have been analysed with CE.

The small scale of CE makes it a very green technique. A 50 cm long 50 µm ID capillary has a volume of 1 µL. Only a few nanoliters are injected.

Figure 1

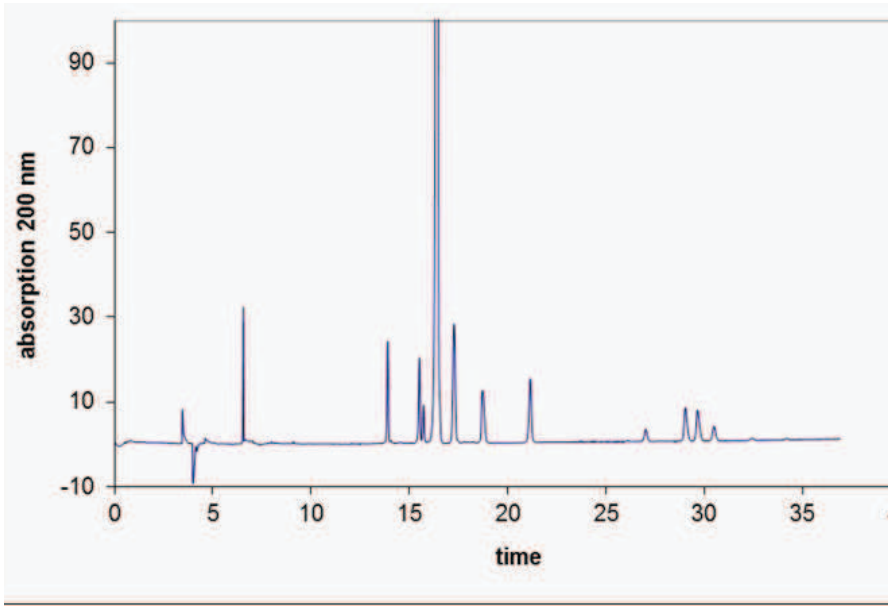


Figure 1: Impurity analysis of a potential new drug molecule by MEKC. Potential impurities include positional isomers (migration times 15 – 18 min), doubly charged synthesis impurity (peak at 6-7 min) and dimers (migration times around 30 min). Conditions: Electrophoresis solution, 20 mM TRIS pH 8.0, 50 mM SDS, 24% (v/v) acetonitril; capillary, 48.5 (40.0) cm x 50 µm fused silica; V = 20 kV (I = 33 µA); T = 30 °C.

On top of that, most of the time the separation medium is aqueous meaning that the consumption of chemicals is very low.

Electrophoresis is a fundamentally different separation technique than chromatography. Chromatographic separations are based on partition differences of the analytes between the stationary and mobile phases. Electrophoresis is based on differences in migration of charged particles in an electric field. This means that chromatography and electrophoresis are complementary tools in the analytical chemist's toolbox. Of course there are separation problems that can be solved with both techniques. But even so there are problems where one technique proves superior. The art of good analytical science is to use the correct tool for analytical challenge at hand.

When use CE?

As listed in the sidebar adjacent, CE can be used for a diversity of analytes. Looking from another angle we can see what CE has been used for e.g., in the pharmaceutical industry, the list is long and covers from early discovery research through to pharmacopoeial monographs.

So when is CE a good choice? First, of course, when an analyte is charged. It is also useful for biomolecules, and not only for replacing slab gel methods. There are many situations when the extensive resolution from a capillary gel electrophoresis (CGE) method such as CE-SDS is not needed and the fast CZE is preferred.

Chiral CE is also a strong methodology. Small differences in affinity for the chiral selector can

CE in the Pharmaceutical and Biotech Industry

In the pharmaceutical and biotech industries, CE has seen its ups and downs in use. Strong areas are biotech, chiral separations and small cations/anions. The latter, especially, can be found in widespread applications, such as counter ion stoichiometry determination, counter ion impurities, analysis of broths, stability tests, etc. The list below is extensive, but probably far from complete.

Identity/Assay
- Active components
- Counter ion
- Important impurities
- Excipients

Impurities
- Related substances
- Enantiomeric purity

Chemical characterisation
- pKa / pI determinations
- logP, logD
- Drug-protein binding

Formulation development
Confirmation & troubleshooting
DNA, RNA
Biomarker research
Metabolomics
CE-MS

Biotech
- Identity
- Isoform distribution by CZE
- cIEF
- Post-translation modifications
- CGE (CE-SDS)
- Peptide mapping
- Amino acid analysis
- Heterogeneity
- Carobydrates
- Assay of small anions and cations, such as TFA, Ca^{2+}, Mg^{2+}

Electrophoresis

Migration of charged particles in an electric field (left figure below). The driving force for migration is the charge q of the analyte and the strength of the electric field E. Upon movement, friction occurs. This friction is influenced by size/shape (6π r) of the analyte, its velocity v_e and the viscosity η of the medium. In equilibrium, driving force and friction are in balance. The velocity of an analyte is then dependant on its charge, size/shape, the electric field strength and the viscosity. As charge and size are analyte-dependent attributes, we here have our mechanism of separation.

$$\text{Driving Force: } F_e = q \cdot E$$

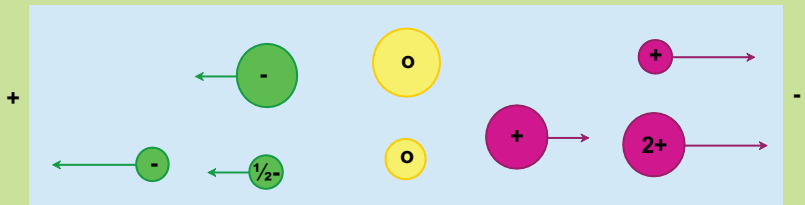
$$\text{Friction Force: } F_d = 6\pi \cdot \eta \cdot r \cdot v_e$$

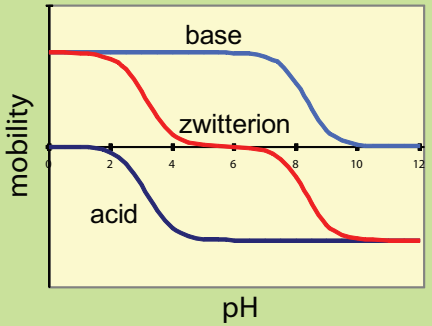
$$\text{Velocity: } v_e = \frac{q \cdot E}{6\pi \cdot \eta \cdot r}$$

$$\text{Mobility: } \mu_e = \frac{v_e}{E} = \frac{q}{6\pi \cdot \eta \cdot r}$$

In capillary electrophoresis we often talk about mobility instead of velocity. The electrophoretic mobility μe is the velocity independent of the field strength, and is an intrinsic property of the analyte in a given medium.

Because analytes can carry either a positive or a negative charge, electrophoretic separations are bi-directional. Our analyte of interest has to carry charge. This we play upon by selecting a pH that favours protonation or deprotonation of our analyte (right figure below). If our analyte has no charge, there are several modes of capillary electrophoresis we can use instead to induce charge, for example MEKC (micellar electrokinetic chromatography). The precise mechanisms of MEKC and other modes will be discussed later.





already result in resolution. Chiral selector screening is fast, because the selector is dissolved in the electrophoresis buffer. The possibility to vary the selector concentration is a strong parameter for optimizing the selectivity.

When dealing with analytes with a wide range of lipophilicity, MEKC or MEEKC methods might be beneficial over gradient LC. Figure 5 shows an example of a real life sample where potential impurities varied from doubly charged compounds, positional isomers from the main component and lipophilic dimers.

As the CE separation mechanism is different from chromatography, CE is a powerful technique for complementary separations and special investigations.

What are reasons then not to use CE? Frankly, when another method proves superior. This may sound silly, but is seriously meant. As I said before, it is important to use each technique in its strength for the most sensitive and robust, or even sensible, applications. When you push a technique to the limits of its possibilities, you might achieve *separation*, but it takes more to develop a robust, reproducible *method*.

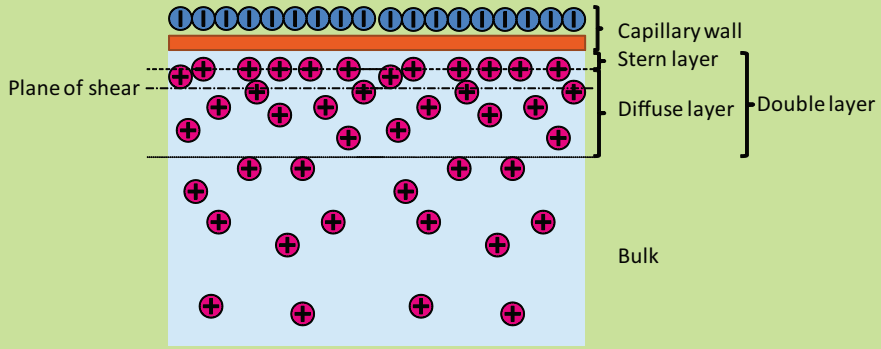
Drawbacks?

Are there drawbacks in using CE? Of course, every technology has its pros and cons. CE is a group of several techniques with many

Electrophoresis in a Capillary: The Electroosmotic Flow

The electrophoresis capillary is often made of fused silica. If a silica capillary is filled with a (aqueous) medium, the silanol groups in the capillary wall deprotonate. This gives the wall a negative charge. How much or little deprotonation occurs depends on the pH of the medium.

The positively charged cations in the medium, mostly the buffer cations, are attracted to the negatively charged wall (Figure 1). They form a double layer at the wall that partly compensates the negative charge. Part of the double layer, the so called Stern layer, is adsorbed to the wall. The other part, the diffuse layer, forms the plane of shear when the voltage is applied. The excess of positively charged cations in this layer make it move to the negatively charged cathode when the voltage is applied dragging along the bulk liquid. The flow that thus occurs is called the electro(end)osmotic flow, the EOF. The EOF has a flat profile, so does not result in band broadening, and sharp peaks with good resolution are maintained (Figure 2).



The size of the EOF depends on many factors. First, the pH of the medium, which dictates how little or much the silanol groups in the capillary wall deprotonate. At a low pH, the EOF is slow and at a high pH, the EOF is very fast (Figure 3). Between pH 4 and 7, a small change in pH results in a large jump in EOF. Other parameters influencing the EOF are the ionic strength of the medium, temperature, viscosity, dielectric constant of the medium and the applied field strength.

You can manipulate the EOF; e.g., by coating the capillary wall covalently or dynamically. More of that later.

Figure 2

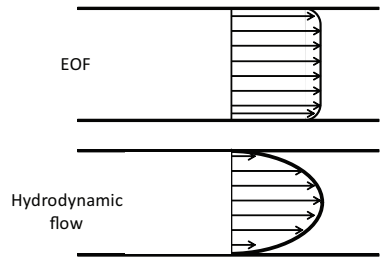


Figure 2: The EOF has a flat profile and does not contribute to band broadening. Hydrodynamic flows, such as pumped flows, are parabolically shaped. This means that particles in the centre move faster than particles at the wall, and band broadening occurs.

Figure 3

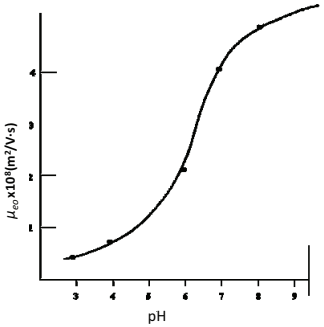
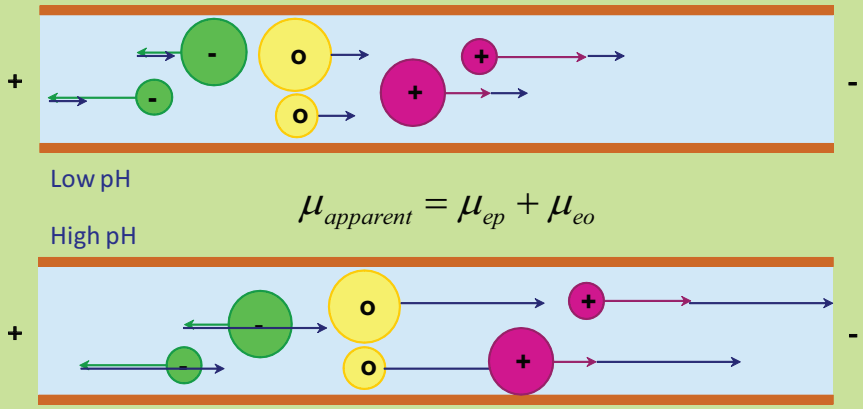


Figure 3: The pH dependency of the EOF (from KD Lukacs, JW Jorgenson, JHRC 8 (1985) 407 – 411)

Capillary Electrophoresis = Electrophoresis + Electroosmosis

In capillary electrophoresis, our analytes experience both the electrophoresis and the EOF (see schematic below). That means that the total mobility, also called apparent or effective mobility, is the vectorial sum of the electrophoretic mobility and the electroosmotic mobility. These mobilities can be either positive or negative. At a high pH, the EOF can be so high as to drag even negatively charged analytes to the negative cathode. The advantage of this is that all analytes move in the same direction and can all be detected. At a low pH the separation remains bidirectional and selective detection is possible by putting the detector at the appropriate side.



modes available. This means many possibilities for method development and optimization for the opportunistic people among us. Others see the many parameters to optimize rather as a disadvantage of CE. It is a challenge to understand where to start with the many possibilities there are, and I hope that this series will help.

Another drawback often mentioned in connection with CE is its lower detection sensitivity. This comes from the shorter path length for detection compared with LC, because the usual path length is the capillary diameter. CE shows high efficiencies with small peaks and high plate numbers. This means less dilution, and, therefore higher concentration in the analyte bands, which means higher peaks. Also, there are many opportunities for using concentration techniques in, e.g., the injection procedures. I hope to demonstrate in this series that although the detection sensitivity is less than for other techniques, method sensitivity does not necessarily need to follow.

So is everything ideal for implementation of CE techniques? No, far from it! My main reason for contributing to this series is that, although the CE community has learned a lot about good practice for implementation, this knowledge is not sufficiently widespread. Proper training of personnel is continuously required not only in theory, but also in good working practices of CE. The high time pressure in many industries does not make it easy to implement any new technique. The aim of this series is to present you with practical and pragmatic solutions to help you implement CE techniques in your laboratory by developing robust and sensitive methods.

Here we have touched upon the core of what this new series on CE will be about: the art of turning a separation into a method. That is, what does it take to develop and validate methods in such a way that they are good enough to be applied in routine analysis? And what should one do when problems arise? In other words: good CE working practice and troubleshooting.

FEATURED WORKSHOP

CE Method Development
25 April, 2012 - Basel, Switzerland
Price: US$795

Who should take this course?
Analytical scientists and technicians who want a better understanding of the capillary electrophoresis techniques and practical tips for method development. This course is designed for analysts who use CE as a part of their regular jobs, but technicians with some CE experience will also find the course valuable. No previous CE training is assumed, however, much of the course will appeal to the experienced method developer who wants to stand on firm ground in the basics of CE. Also lab managers who need to supervise CE method development and review CE methods will benefit.

Click here to learn more>>

CE Method Validation
26 April, 2012 - Basel, Switzerland
Price: US$795

Who should take this course?
Analytical scientists and technicians who are responsible for validating capillary electrophoresis methods. The course is also useful for managers and QA staff involved in the method validation process. For workers who develop, but do not validate methods, this course will give insight into how to develop methods that will be easier to validate. No prior CE experience is needed, although those with practical CE experience and those who attended the CE method development course will certainly benefit more than those with no experience at all.

Click here to learn more>>

Ask the Doctor

Cari Sänger is available to answer your specific method development and troubleshooting CE questions. Submitted Q & As will also form the basis of future CE Solutions.

NOTE! *"Help! I need a method to separate ____" Unfortunately, this is a question that Cari can't help you with. However, here are a few hints: (1) do a literature search using 'Pub Med' or one of the free search engines; (2) a good source of methods are Electrophoresis Journal of Chromatography A and B issues; (3) consult the applications literature of various manufacturers (4) visit Chrom Forum at www.chromforum.org*

Click here to submit your question

Next Issue's
Tech Tip

Next time we start with discussing the core of the method: how do you select your background electrolyte and what makes it a good one?

Don't miss the next instalment of *CE Solutions*.

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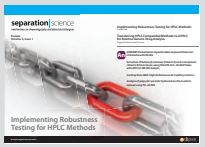

Implementing Robustness Testing for HPLC Methods

Transferring HPLC Compendial Methods to UHPLC for Routine Generic Drug Analysis