CE Solutions

SeparationScience
PREMIER LEARNING FOR ANALYTICAL CHEMISTS

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

Troubleshooting – The Basics

You're whistling on your way to work because it is a beautiful morning and you are looking forward to tackle the enormous sequence you started yesterday. It is an important one that will give all the answers to an anxious project leader who needs the data for making decisions in how to move forward with the project. When you get into the lab and go through the results from last night, the first injections look fine, but then when the real samples come, you only see … nothing…. What happened?

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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.

Troubleshooting – The Basics

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Stay cool

First of all, don't panic. What is going to happen is that you'll need to troubleshoot and find out what went wrong and what to do to prevent this to happen next time. Troubleshooting is an investigative process for which you need all your knowledge and experience, and preferably that of others. You'll need to observe, think out of the box, analyse and make a plan. In this issue of CESolutions we will discuss the general approach for troubleshooting and in next issues we will look more specifically at certain cases.

Roughly, we divide the cause of problems as hardware related, software related or application related. Hardware related causes are for instance pressure failure, low lamp energies, a broken capillary. Software related issues are connected with the programming of the method and sequence in the software that comes with the instrument, so programming the method and sequence parameters.

Application related issues can relate to the sampling, the sample preparation, the CE part and the quantitative evaluation of the data.

The very first question we should ask ourselves concerning the application is whether the method we used was really developed and validated for the samples and sample matrix we want to analyse. This sounds like a silly remark. But it is not rare to encounter situations where an application has been in use for many years for a certain substance, while the matrix has undergone many small changes over time. If this for instance would result in an entirely different ionic strength of the sample injected, you might get problems with peak shape, resolution, stacking and destacking and so finally with sensitivity and reproducibility.

Even when the application passes muster and is being used for what it is intended to, we need to troubleshoot from time to time. Typical issues we might encounter when working with CE can be categorized as follows:

- No peaks
- Poorly resolved peaks
- Irreproducibility
- Baseline problems
- Current issues

Most of the problems we encounter can be sorted into one of these categories. John W Dolan calls this the "Divide and conquer rule" in his troubleshooting approach [1]. Classifying into these categories often

gives a good start as to where to look for the root cause.

Some examples of potential causes for the five categories are listed below, but the list is by no means complete.

No peaks
Peaks are missing from the electropherogram or there is no or a low signal. Missing peaks can be attributed to problems with the sample or the application, such as conditioning or the background electrolyte (BGE). No peaks detected during the run could come from issues with the sample or hardware or software failures. Hardware type of failures could be that injection failed or that the detector lamp was not turned on. In the software there might be a mistake in the method or sequence programming, for instance the detection wavelength or voltage polarity, or we are looking at the wrong scale.

Poorly resolved peaks
Poorly resolved peaks that were separated nicely before can result from loss of separation, loss of efficiency, or both. Loss of efficiency means broader peaks, tailing peaks or electromigration dispersed peaks. The capillary is one potential cause, for instance inappropriate conditioning or problems with the capillary inlet or outlet. Injection and stacking issues could be connected to the sample matrix or BGE. Loss of separation can be associated with changed migration times, e.g. by matrix effects or conditioning or BGE issues.

Irreproducible results
Poor migration time reproducibility can be divided in poor electrophoretic mobility reproducibility or poor electro-osmotic flow (EOF) reproducibility. Poor EOF reproducibility can be connected to conditioning or buffer depletion or other changes in the BGE composition, as well as temperature issues. Poor electrophoretic mobility reproducibility might for instance be caused by sample composition issues or adsorption.

Baseline problems
Baseline issues can come from hardware components such as the detector lamp or fibre optics or in appropriate detection slit/alignment interface. Software related issues can be the detection sampling rate. The BGE might need to be degassed or has particulate problems such as particles or microbial growth. Baseline drifts might be caused by temperature issues or capillary misalignment.

Current
The current itself can be an issue but it can also indicate in which direction to look for the root cause of the problems. For this reason, the current should always be recorded and a typical current trace should be part of the method documentation. Additionally, this gives you a good GXP tool to reject certain injections if needed. Typical current symptoms are no current, drifting current, irregular current or a sudden drop in current. If there is no current at all, for instance the capillary might be blocked or broken or is not filled properly or with the wrong solution. If the current drifts with time, excessive Joule heating might be an issue. Irregular current patters can happen when the sample composition is quite different from normal, if the capillary is cracked or if the liquid levels in inlet and outlet vial are too low or not level. Sudden current drop usually indicate a blocked or broken capillary or air bubbles.

Troubleshooting rules of thumb
Sometimes a problem fits in more than one category. For instance for a problem in any of the categories, the current trace is often a good troubleshooting tool to understand in which direction to look. To approach these multi-category issues, we have a strategy. As I cannot phrase this nicer than John Dolan did in his troubleshooting rules of thumb [1], I borrow that gladly with his consent. This strategy helps you making a troubleshooting plan.

1. The rule of one
Only change one thing at a time. Although I am a big advocate of multifactorial approaches for method development and robustness testing, when we need to troubleshoot we need to find the root cause. If you would change multiple things at once, you'll never identify the real issue. Your problem might be solved this time, but you don't know what to improve to prevent it happening again or help you short-cut the procedure next time it happens. We all ignore this rule every now and then. Especially, as John mentions, on Friday 4 pm.

2. The rule of two
Can you repeat the issue? When you inject the sample again, does the same thing happen? It is often easier to isolate a root cause when we detect a pattern in an issue. If the issue is not happening for all injections, maybe there is a pattern when distinguishing between samples and standards.

3. The divide and conquer rule
As discussed above it is good to be able to assign an issue to a certain category, so that you can concentrate the investigation and avoid spoiling time. Also here, pattern recognition helps a lot to eliminate those aspects that are not part of the issue at hand. If you cannot detect any patterns, design experiments that can exclude certain parts. For instance run an application you know very well to check whether the instrument is involved in the issue or can be absolved from the problems at hand.

4. The module substitution rule
Replace a suspect part with a good one. Typically, as capillaries are

relatively cheap, the first thing that gets replaced is the capillary. But also think of the BGE, the lamp, the sample prep. It could also mean that you check whether the problem remains when you reanalyse on another CE instrument or on an instrument from another brand.

5. The put-it-back rule

This rule follows logically to the previous rule, but is often forgotten. If replacing a part did not solve your problem, put the old part back into the system. For one thing, it could save a lot of money. But it is also in line with the Rule of One.

6. The documentation rule

When you solved the issue, you want to make sure that it does not happen again, but if it does, that you recall the root cause. Moreover, your colleagues want and need to learn from your findings. The only way is then to document and train others on the results of your troubleshooting. Documentation also gives you a tool to evaluate the long-term use of capillary electrophoresis. It shows you on what aspects training is needed, what to look for in a next equipment purchase or which methods need further development. Also from a QA perspective documentation is paramount. If you don't have a documentation system yet, make sure that the technicians and scientists are involved in creating one. Only that way you can come up with a system that is practical in use and that people are happy to comply to.

Troubleshooting examples

In this issue of CESolutions we discussed the general approach for troubleshooting. Problems can originate from the hardware, software or the application at hand. To prepare for a troubleshoot plan we categorize the problem. For CE we defined for instance No peaks, Poorly resolved peaks, Irreproducibility, Baseline problems and Current issues. With help of the troubleshooting rules of thumb we can systematically approach our task. In the coming issues of CE Solutions we will discuss several real-life examples. If you happen to have a real issue you wish to share or discuss with the CE community, feel free to contact us.

References

[1] John W Dolan, Troubleshooting Basics, Part I: Where to Start? in LC-GC, July 2011

Cari Sänger has more than 20 years of experience in pharmaceutical and chemical analysis. Her aim is to stimulate people to keep growing and learning, striving to get the best out of themselves. Cari is an independent, reliable, scientific people-manager and a globally recognized expert on separation science, especially within the capillary electrophoretic techniques. Cari's focus is primarily on implementation, knowledge transfer and good working practices.

Ask the Doctor

Cari Sänger is available to answer your specific method development and trouble-shooting 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*

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