CE & CE/MS Troubleshooting Guide

Your guide to solving common problems and staying productive

Agilent
Trusted Answers

Places to Start

Solvents

- Use CE-suitable vials that are pressure resistant.
- Prepare solvent volume to be used up within one day.
- Use only HPLC-grade solvents filtered through 0.2 to 0.45 µm filters.

Preparing and powering up the CE

- Inspect vials and electrode for damage or precipitation.
- Conditioning of uncoated capillary with MeOH, 1 M NaOH, water (5 to 10 min each), run buffer (20 min) or flush with 1 M NaOH (5 min), wait 5 min, water (5 min), run buffer (20 to 30 min).

Daily tasks CE-UV

- Replace background electrolyte (BGE) every day.
- Flush the capillary with 0.1 M NaOH or 10% (v/v) phosphoric acid (10 min each) depending on the pH of the BGE, followed by water (10 min) and conditioning with BGE (10 min).
- Flush the system with cleaning and storage solvent after application.

Storage of capillary

- A bare fused silica capillary should be cleaned properly with NaOH followed by an extensive water flush and then blown dry (with air from an empty vial).
- Coated capillaries should be stored as described in the respective product description.

Daily tasks CE/MS

- If the CE and MS systems are in idle state, keep the sheath solvent flow, nebulizing gas, and drying gas running.
- If you stop measuring for the day or a longer period:
  - Clean the sheath flow splitter and the CE/MS sprayer needle.
  - Clean and store the CE/MS capillary.
  - Inspect and store the sprayer assembly.

BGE for CE/MS

- Nonvolatile buffers such as phosphate or borate should be avoided in CE/MS as they cause salt buildup in the electrospray chamber and in the MS inlet, which can block the inlet capillary.
- Instead, for low-pH separations, we recommend using volatile acidic and basic buffers, such as formic and acetic acid. For high-pH separations, we recommend ammonium salt or trialkylammonium compound buffer.

Maintenance

Agilent Lab Advisor software helps you manage your Agilent CE instrument to achieve high-quality electrophoretic results in the most efficient way by ensuring high instrument performance, productivity, and reliability. It is available free of charge.

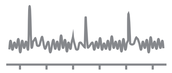
- Diagnostic tests to evaluate performance
- Easier maintenance of Agilent 7100 CE System
- Comprehensive reports generated to ease communication with Agilent

CE

Unstable/no current

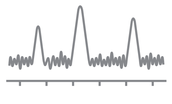
Possible Cause	Solution
Empty capillary/wrong solutions in buffer vials	Fill/change buffer vials
Clogged capillary	Flush capillary with absorbing solution and a baseline jump should be observed; if still plugged, flush with high pressure; if not successful, replace capillary
Large injection volume with different sample matrix (stacking)	Normal condition; current should stabilize during analysis
Different cathode/anode buffers	Check buffer identity
Broken or cracked capillary	If the current breaks down repeatedly after flushing with buffer, the capillary is probably cracked
System shortcut (buffer on vial cap)	Clean and dry cap or replace

Spikes on baseline



Possible Cause	Solution
Precipitates/contaminates in buffer or sample	Filter through 0.2–0.45 µm filter and verify solubility
Microscopic bubbles in buffer	Degas buffer

Unstable baseline



Possible Cause	Solution
Optical slit on capillary interface occluded	Clean with methanol or water; check with magnifying glass
Ageing deuterium lamp	Use diode array detector (DAD) test feature to measure lamp output and time on; replace

Tailing peaks

Possible Cause	Solution
Adsorption to capillary wall	Use pH extremes, buffer additives, polymer additives, or coated capillary
Capillary end(s) damaged or resting on vial surface	Check capillary ends are properly cut and check capillary distance from vial surface

Shifting migration times

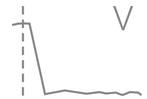
Possible Cause	Solution
Changes to capillary surface (due to pH changes or adsorption)	Conditioning of capillary to achieve surface equilibrium and/or avoid batch-to-batch capillary differences; do not cycle pH (surface charge hysteresis)
Change in buffer composition (due to electrolysis, buffer evaporation, conditioning waste flushed into outlet vial)	Increase buffer concentration (capacity) or replenish regularly to limit buffer depletion; to avoid evaporation check seal on vial caps, check destination of conditioning waste
Siphoning due to unleveled buffer vials	Use replenishment for automated leveling
Sample overload	Decrease sample injection or concentration; particularly evident with indirect UV detection of small ions

CE/MS

Current unstable/ spiky/too low

Possible Cause	Solution
Poor electrical contact at sprayer tip	Adjust axial position of the CE capillary in the CE-ESI-MS sprayer
Bubble formation at the sprayer needle	Increase the sheath liquid flow rate to flush out electrolysis gas

CE current breaks down after injection



Possible Cause	Solution
A liquid gap is formed in the BGE by the suction effect formed by the nebulizing gas	Time program the nebulizing gas pressure during the injection time to a low value
A liquid gap is formed by siphoning towards the capillary outlet	Check the height level of the inlet and the height of the sprayer

The baseline MS signal (TIC) varies

Possible Cause	Solution
The sheath solvent flow rate is unstable, is fluctuating, and/or is poorly degassed	Use recommended isocratic pump with online degassing
Polyimide of the CE capillary has swollen or has become detached	Avoid the use of high concentration of acetonitril in BGE and/or sheath solvent

Decreased migration times and wider peaks

Possible Cause	Solution
Hydraulic flow exists towards the capillary outlet	Check level of inlet vial and height of sprayer to avoid siphoning; reduce nebulizing gas pressure to avoid suction effect

ESI current is unstable or too low

Possible Cause	Solution
Drying gas flow rate too high	Reduce drying gas flow rate as much as possible

Poor reproducibility of peak heights (TIC)

Possible Cause	Solution
Variability of ionization efficiency	Use deuterated analogs of your solutes as internal standard
Low injection precision of the CE instrument: field variation on the inlet side of the CE capillary as cut or damaged	Use internal standard

Background MS current too high

Possible Cause	Solution
Dirty spray needle, CE capillary or sheath liquid pump	Flush capillary and clean spray needle or the the sheath liquid pump
Dirty BGE or sheath liquid	Use utmost cleanliness in all parts of your system

More troubleshooting information can be found in the following Agilent publications:

- High Performance Capillary Electrophoresis (primer, 5990-3777EN)
- CE/MS Principles and Practices (guidebook, 5994-0112EN)

Discover more about the Agilent 7100 CE System:
www.agilent.com/chem/ce

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