Agilent
Trusted Answers

CE/MS Principles and Practices

A guidebook for novices and practitioners

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Content

1 Aim of this Booklet

Since its inception as an instrumental analysis method in the 1990s, capillary electrophoresis (CE) has evolved as a prime separation tool for charged compounds. CE is used for the analysis of low molecular weight (LMW) pharmaceuticals (especially for the separation of enantiomers) and many other LMW molecules of high biological relevance, and for ion analysis. CE has become an indispensable tool for the determination of structural homogeneity, size, charge heterogeneity, and especially glycosylation patterns of endogenous and recombinant proteins. This is because CE pairs high resolving power with high sensitivity, and requires minimal sample volumes.

Electrospray ionization mass spectrometry (MS) coupled with liquid chromatography (LC) became available by the end of the 1980s and has been a crucial tool in development of new pharmaceuticals. In life science research, LC with MS detection gathers invaluable structural information, resulting in unambiguous confirmation of peptide and protein identity, structure, and heterogeneity.

Not surprisingly, soon after LC/MS became established, successful efforts to couple CE with MS were reported in the late 1980s. The method became commercially available with the introduction of the Agilent Technologies (then Hewlett-Packard) CE/MS interface in 1999. It has since demonstrated wide versatility and practical applicability in bio-analytical measurements.

Recently, capillary electrophoresis coupled with mass spectrometry (CE/MS) evolved into an essential tool in development, characterization, and manufacturing of biopharmaceuticals and in biomarker discovery. Currently 35 to 50 % of new drugs that are under development in the pharmaceutical industry are proteins or protein-like molecules. These biopharmaceuticals require more extensive bio-analytical verifications than small molecule drugs for regulatory approval. Besides that, intellectual property rights (IPR) on first-generation biopharmaceuticals are running out, triggering the introduction of biosimilars. These biosimilars require proof of identity and of structural equivalence when introduced to the market.

More recently in metabolomics, CE/MS has gained the interest of many groups for the screening of cationic/anionic and polar metabolites. CE/MS has been developed into a standard method and has become available as a commercial product.

These trends have given strong drive growth of the CE/MS market.

Unlike HPLC though, CE has been regarded a method for experts and specialists, which has inhibited its broad application in bio-analysis and in life science research. However, the factors that render a CE separation a reproducible, repeatable, robust, and sensitive method are now well understood. Both CE and CE/MS have therefore become a valuable separation method providing structural and compositional information of biomolecules complementary to HPLC/MS.

In coupling CE with MS, another degree of “difficulty” is added through the interface that is required for their connection when compared to an LC/MS interface.

It is the intention of this guidebook to help novices to step into the practical application of CE/MS, and to provide current users with relevant information on method development and on diagnosis of practical problems. Towards this goal, the booklet is divided into two parts:

Part 1: Concepts of CE/MS

Description of the concept of CE/MS coupling with detailed emphasis to help understand the technique and to choose proper starting conditions.

Part 2: Practice of CE/MS

Detailed description of the practice according to recommendations of Agilent that help to set up the CE instrument, the interface, the MS, and to provide current users with relevant information on method development and on diagnosis of practical problems. Also detailed guidelines to do system performance verification will be given.

2 Foreword

Dr. Julie Schappler
Former Lecturer at School of Pharmaceutical Science, University of Geneva, Switzerland

Capillary electrophoresis is a powerful separation technique that features many assets such as high efficiency, rapid method development, simple instrumentation, low sample consumption, and reduced cost. UV-Vis spectrophotometry is probably the most widely used detection technique with CE because of the simplicity of the on-capillary configuration. However, its sensitivity, which is directly related to the internal diameter of capillaries, is rather low. Because analysts must deal with complex samples, particularly in the bio-analytical field, highly selective detectors are required. In this context, the online combination of high-efficiency CE separations with mass spectrometry is an attractive perspective and presents major benefits such as enhanced sensitivity and selectivity.

CE/MS is a relevant option for many applications such as pharmaceutical and biopharmaceutical analysis, bio-analytical assays, including metabolomics studies. The performance offered by this technique can sometimes be found to be inadequate though. This misconception is mainly due to a lack of training and expertise leading to improper operational conditions. Special attention needs to be paid to instrumental aspects, particularly to the interface conditions, to ensure stable and successful CE/MS operation. Over the last years, many technical improvements regarding the interface have enabled the production of high-quality data. The commercially available sheath-flow interface has proven for several years now to be a valuable choice to combine CE with ESI-MS detection. The clear advantage of this interface is to almost entirely decouple the CE and ESI processes. Therefore, enabling the ability to work over a wide range of BGEs with standard capillaries. This set-up is robust, considering that a constant flow rate of the sheath liquid is obtained, ensuring high spray stability. The many quantitative studies published by several research groups have shown the potential of this coupling. Therefore, circumventing the often held notion that CE/MS cannot produce quantitative results and demonstrating that CE/MS performs as well as any other analytical techniques.

This guidebook gives an excellent insight into the concepts and the practical aspects that have to be considered for successful CE/MS operation.
The readers will learn why and how to choose the correct experimental parameters, according to their own applications and objectives, to get sensitive, reliable, and repeatable results. They will also be convinced that CE/MS enables solutions to challenging analytical problems and has its place in modern separation science.

Julie Schappler

Prof Dr. Christian Neusüß
Professor for General and Analytical Chemistry at Aalen University of Applied Sciences, Germany

When starting CE/MS more than 15 years ago, it required not only knowledge in capillary electrophoresis and mass spectrometry on its own. Numerous intuitive experiments and trial and error were necessary to obtain meaningful methods and results. Thus, there were only a few people running routinely CE/MS. However, it turned out that the combination of the selectivity of CE and the sensitivity and amount of information of MS provides a powerful tool in many fields of application. Today CE/MS is applied in many areas including food control, metabolomics, and especially biopharmaceutical analysis, where CE/MS has demonstrated its power for intact protein and glycan analysis.

To a large extent, CE/MS has been made possible by the sheath liquid interface from Agilent. It is robust and easy to use so that CE/MS can be set up in minutes. Furthermore, it is flexible as ionization conditions can be chosen to a large extent independent of the separation. In this way, the dilution effect is partly compensated by choosing best ESI conditions. We have been using this interface for many different applications and it is the routine tool in our lab having become the standard against which other techniques are assessed.

This guidebook will help the increasing number of people applying CE/MS to find faster methods for their application. It closes the gap between practice and theory of CE/MS since it provides information on important practical parameters often not described in sufficient detail in scientific publications. In the first part, this guidebook is mostly independent of instrumentation, thus, providing useful information for all potential or experienced CE/MS user. It will be also a stable base for future developments such as microchip electrophoresis or two-dimensional capillary electrophoresis coupled to mass spectrometry.

I wish this guidebook many critical readers using this important collection of essential CE/MS parameters. The analytical scientists should always scrutinize what they read to further improve and spread this still young technique.

Christian Neusüß

3 About the Authors

Gerard Rozing completed his undergraduate studies in chemical engineering and organic chemistry in 1971 at the University of Amsterdam, the Netherlands. He obtained a Ph.D. in organic chemistry early 1977 from the University of Amsterdam, followed by a postdoctoral stay at the State University of Ghent, Belgium as a NATO Science Fellow until early 1978. Further postdoctoral studies in chromatography and analytical chemistry followed at the University of Amsterdam in the group of Professor Hans Poppe. In 1979, Gerard joined Hewlett-Packard, Waldbronn, Germany as an R&D Chemist working on stationary phase development and HPLC system evaluation. Gerard held positions as an R&D project leader and later an R&D section manager. In 2000, Gerard became the University Relations and External Collaborations Manager and held that position until retirement on September 1, 2012. Gerard became the first Agilent Research Fellow in the Chemical Analysis Group of Agilent Technologies in 2006. Gerard has been the conference chairman of MicroScale Bioseparation Series of Symposia in 2006, Amsterdam and 2012, Geneva. He has been involved in the organization of recent issues of the HPLC symposium series and a member of the editorial advisory board of Electrophoresis and Journal of Separation Science. He now serves as a guest scientist at the Free University of Brussels and at the University of Amsterdam and works as an independent consultant for liquid phase separation science with ROZING.COM Consulting.

Hans Brunnert and **Sibylle Aldridge** are both Support Specialists at Agilent Technologies, Waldbronn, Germany. They have many years of experience in capillary electrophoresis (and other techniques such as microfluidic "Lab on a Chip," HPLC, and the coupling with mass spectrometry). From a technical point of view, both provide high-level support and consulting to the worldwide Agilent support organizations and their customers.

Christian Wenz is an R&D scientist at Agilent Technologies, Waldbronn, Germany. He studied Biochemistry at the University of Hannover, Germany. He did his Ph.D. in the group of Professor Alfred Pingoud, Department of Biochemistry, Justus-Liebig-University Giessen, Germany on the structure and function of proteins that interact with nucleic acids. After a postdoctorate at the Swiss Institute for Experimental Cancer Research (ISREC) in Epalinges, Switzerland, he joined Agilent Technologies in 2002. During the last 10 years, Christian was involved in instrument and application development for automated electrophoresis systems, for example, the Agilent 3100 OFFGEL fractionator and the Agilent 2100 Bioanalyzer system. Since 2009, he has worked with the Agilent 7100 Capillary Electrophoresis system. He is the author of several application notes and technical literature published by Agilent Technologies for this instrument.

4 Acknowledgements

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5 Symbols and Abbreviations

APCI	Atmospheric pressure chemical ionization
APPI	Atmospheric pressure photo ionization
BGE	Background electrolyte
CE	Capillary electrophoresis
CE/MS	Capillary electrophoresis coupled with mass spectrometry*
CEC	Capillary electrochromatography
CGE	Capillary gel electrophoresis
CIEF	Capillary iso-electric focusing
CITP	Capillary isotachophoresis
CZE	Capillary zone electrophoresis
DAD	Diode array detector
EOF	Electro-osmotic flow
ESI	Electrospray ionization
GC	Gas chromatography
GC/MS	Gas chromatography coupled with mass spectrometry*
HMW	High molecular weight
HPLC	High-performance liquid chromatography
id	Inside diameter
IF	Interface
IPR	Intellectual property rights
LC	Liquid chromatography
LC/MS	Liquid chromatography coupled with mass spectrometry*
LMW	Low molecular weight
MALDI	Matrix-assisted laser desorption ionization
MEKC	Micellar electrokinetic chromatography
MEEKC	Micro-emulsion electrokinetic chromatography
MS	Mass spectrometry
od	Outside diameter
SMIL	Successive multiple ionic-polymer layers
SPE	Solid phase extraction
SST	Stainless steel
UHPLC	Ultrahigh-performance liquid chromatography

**The use of a hyphen (-) or a slash (/) for coupling analytical techniques is not unambiguously regulated. Agilent Technologies has conformed to using a slash. For reference see K.K. Murray, J. Chrom. A, 1217 (2010) 3922–3928*

Part 1 Concepts of CE/MS

The first part of this guidebook provides details to the hyphenation of capillary electrophoresis separation with mass spectrometry detection. Starting with the basic concept of CE/MS, the chapter continuous with insights to the different types of ionization and concerning parameter.

6 Coupling Liquid-Phase Separations with Mass Spectrometry

Since HPLC technology matured in the early 1980s, its coupling with mass spectrometry remained a challenge for some time. Bringing the analytes out of liquid phase into the gas phase, ionizing them, and transferring the molecular ions into ultrahigh vacuum, is far more difficult for liquid-phase separations (LC) than for gas-phase separations (GC). The field has seen multiple iterations in LC/MS interfacing methodology since the 1970s. For example, by transport of the column eluate by a moving belt or moving wire into vacuum, by direct liquid inlet introduction[1] by thermospray ionization[2] by particle beam ionization technique[3] or by offline coupling with MALDI plates.

Fenn[4] and Alexandrov[5] first reported electrospray ionization (ESI) at the end of the 1980s. In this approach, the eluate from the HPLC column exits through a narrow orifice and forms a spray of fine solvent droplets by being exposed to a strong electrical field. From the droplets, eventually charged molecules (ions) evaporate and enter the mass spectrometer through an inlet capillary. Despite great initial promise, electrospray ionization was confined to flow rates of maximally 10 µL/min and to mobile phases with low aqueous content to establish a stable electrospray.

However, in the 1980s, most columns used in HPLC separations were of reversed-phase type using water-rich mobile phases and had inside diameters of 2.1 to 4.6 mm. Therefore, these columns were typically operated at flow rates between 0.2 and 2.0 mL/min. To establish a stable spray under these conditions, Henion and coworkers came up with an interface concept, which they called “ion spray” ionization[6]. In this approach, spray formation (nebulization) is assisted with a co-axially delivered flow of nitrogen gas.

With further improvements over time and its commercialization, the ion spray interfacing technology became broadly available allowing users to work at flow rates up to 1 to 2 mL/min. By definition, ion spray ionization is not the same as “electrospray” ionization. However, ion spray ionization has become the standard for LC/MS coupling for more than 20 years and is now called electrospray ionization.

Electrospray in its original form, operating at flow rates significantly below 10 µL/min, is nowadays called nano-electrospray ionization. This technique has become an exceptionally versatile and sensitive interfacing method in proteomics, biomarker discovery, and in metabolomics, where small sample volumes (low µL) with low analyte concentration are common. The low flow rate mandates the use of low inside diameter HPLC separation columns (less than 150 µm), which are typically operated at low flow rates (less than 1 µL/min). In addition, it has been found that at flow rates below 100 nL/min, the MS signal becomes significantly enhanced, while at the same time the matrix effect (ion suppression) becomes less prominent.

6.1 Requirements for CE/MS coupling

Soon after CE became established in the mid-1980s, interest emerged to couple CE with MS. CE is by definition an ultralow flow rate separation technology, which would enjoy the advantages mentioned before when coupled with MS. As the problems related to bringing a dissolved analyte molecule from the liquid phase into the gas phase and ionizing it, there exist other factors specific for CE separations that complicate the coupling of CE with MS.

Electrical connections

In CE, an electrical field moves the solutes towards the outlet of the capillary electrophoretically. In addition, the field may drive the BGE in the same direction by electro-osmotic flow. Typically, voltages are applied on the inlet side (up to 30 kV) by a platinum or palladium electrode in a vial containing the BGE. In a CE system, the outlet vial with BGE contains the ground electrode of the same material, which closes the circuit. In coupling CE with MS, it is obvious that there cannot be an outlet vial. Nevertheless, an electrical contact such as a liquid vial must be present to provide a return path for the CE current.

Conversely, to generate an electrospray, a field is required between the end of the CE capillary from or to the MS inlet. A high voltage (up to 4 kV) is applied. Therefore, for electrospray two electrodes also are required; one at a positive or negative voltage, and one at ground.

It can easily be imagined that it is convenient when the ground electrodes for the two fields are shared. If so, the CE and electrospray power supplies have common current return paths with currents flowing to ground in equal direction. CE and electrospray currents do not interfere.

In practical implementation, where the CE and the MS share the ground electrical contact at the capillary outlet, the electrospray voltage is applied at the MS inlet capillary. In further electrical detail, the inlet capillary of these MS instruments is a glass tube and therefore insulates the electrospray voltage from the vacuum part of the MS.

Alternatively, if the electrospray voltage is provided from the CE capillary end through the spray needle and the mass spectrometer inlet is grounded, an awkward situation can occur where the electrospray voltage is of opposite charge. The same sign as the CE voltage thereby effectively reducing or increasing the electrical field in the CE capillary.

The current carried by the electrolyte in the CE separation capillary may be 2 to 3 orders of magnitude larger than the electrospray. If so, the CE current would be flowing towards the ground in the MS inlet, which may damage the MS. Therefore, an electrical resistance circuit needs to be added to lower the CE current by another path. These details will not be addressed here but should be available from other MS instrument suppliers.

Hydraulics

In contrast to HPLC, the flow rate or electro-osmotic flow (EOF) in CE is a parameter depending on BGE properties, charge of the fused silica capillary wall, and other factors. However, the EOF is not a parameter that can be set to control the volumetric flow as in HPLC. Under ideal conditions, the BGE is propagated through the capillary, at a velocity of 1 to 3 mm/s. This corresponds to 0.12 to 0.35 µL/min for a 50 µm id capillary. In practice however, a BGE composition that is optimal for CE separation may render low, none, or even reverse EOF. As will be explained later, sprayers used for CE/MS have been developed for higher flow rates. Therefore, assistance of spray formation by an auxiliary solvent flow is required.

Compromised CE separation methods

In CE separations, inorganic buffers are preferably used as the BGE. Besides, additives can be used in the BGE to add special selectivity (for example, cyclodextrin for separation of chiral molecules). In CE modes such as micellar electrokinetic chromatography (MEKC), capillary gel electrophoresis (CGE), and capillary isoelectric focusing (CIEF), nonvolatile surfactants, additives, hydrophilic polymers, or carrier ampholytes can be used in the BGE. Cationic or anionic polymers can be used to change the charge of the capillary inner surface. Since MS is a vacuum measurement technique, nonvolatile BGE components should be omitted from the BGE where possible. For example, a CE separation using phosphate buffer at acidic pH should be changed to formate buffer, which may compromise the efficiency of the CE separation. Neutral additives present can be avoided by using an orthogonal sprayer configuration.

CE/MS systems

MS instrumentation manufacturers that have the MS inlet at electrical ground and the spray needle at high voltage, have provided basic interfaces for CE/MS based on modified nano-electrospray LC/MS interfaces combined with some auxiliary parts (for example, a positioning device) to establish CE/MS coupling. Power, electrical, and hydraulic connections must be supplied by the user.

Over many years, Agilent Technologies has been the only analytical equipment manufacturer offering a complete system solution for CE/MS. The basis of the interfacing technology is the coaxial solvent sheath flow or triple tube CE/MS interface.

This guidebook focuses on the use of this interface for CE/MS coupling.

6.2 The coaxial sheath solvent flow concept

Smith and coworkers reported the use of an auxiliary solvent as a sheath around the CE separation capillary end to provide a wet, electrical contact on the outlet side[7]. They used a 1.6 mm od genuine PTFE tube to contain the CE capillary (0.2 mm od × 0.1 mm id). The genuine PTFE tube was connected to a syringe pump by a T-piece, which delivered the sheath solvent at flow rates of 5 to 10 µL/min. A metal needle of 0.46 mm od and 0.25 mm id was sealed into the genuine PTFE tube and was connected to the electrode providing the electrospray voltage. Towards its end, the CE capillary protruded through the metal needle and extended about 0.2 mm. In addition, nitrogen gas was delivered with gas flow rate of 0.1 to 1 L/min through a stainless-steel capillary of 0.5 mm id intended to shield and cool the spray.

The sheath liquid acts essentially as the outlet buffer for the CE and maintains a spray independent of the magnitude of the EOF. The sheath solvent should be chosen to provide optimal conditions for electrospray (low viscosity, low surface tension, high vapor pressure, and low aqueous, low pH solvent mixtures). In practice, most CE/MS separations are performed with BGE containing volatile buffers such as acetic acid for low pH, or ammonium acetate for high pH separation, and sheath solvent water with methanol or isopropanol containing 0.1 to 1.0 % acetic or formic acid.

In analogy to their development of pneumatically assisted ESI for LC/MS by Henion and colleagues (see section 6.1 “Requirements for CE/MS coupling”), this group applied their ion spray for CE/MS coupling. In their early work, a liquid junction approach was used to provide a contact for the electrospray voltage, which was applied to the spray needle. In later work, they described a coaxial sheath solvent interface with pneumatically assisted spray formation[8,9]. Banks of Analytica of Branford[10,11] realized that applying the electrospray potential to the end of the CE capillary caused the resulting electric field to be the difference between the applied CE voltage and the electrospray voltages. Consequently, he connected the exit of the CE capillary to ground and applied a (negative) voltage to the MS inlet for positive ion MS.

At the same time, engineers at Hewlett-Packard had conceived an ion spray type interface for LC/MS in which the sprayer was oriented orthogonally to the inlet capillary of the MS. This enabled large droplets and neutral mobile phase additives to pass by the MS inlet capillary minimizing contamination of the inlet.

6.3
The triple tube sprayer

By taking the best of the approaches described in section 6.2 "The coaxial sheath solvent flow concept," and taking advantage of the development of LC/MS sprayers, engineers at Hewlett-Packard came up with the triple tube sprayer for CE/MS in 1995. This triple tube sprayer consisted of a spray needle through which the sheath solvent was delivered coaxially with the CE capillary inside the spray needle and a nebulizing gas to assist the spray formation delivered by an outer tube (Figure 1 and Figure 2).

The triple tube sprayer is a high precision engineered part. It accepts the standard CE capillary with 365 µm od, co-aligns it concentrically in the surrounding sprayer needle, which has 0.4 mm id and enables adjustment of the CE capillary and sprayer needle in axial direction in a reproducible manner.

An isocratic pump delivers the sheath liquid, which operates in a split flow mode at 1 to 20 µL/min. The nebulizing gas is nitrogen at gas flow rate of 3 to 10 L/min.

The sprayer tube is grounded. This is the common ground for the CE current and the electrospray current. The field for electrospray is delivered by a voltage on the MS (not shown here).

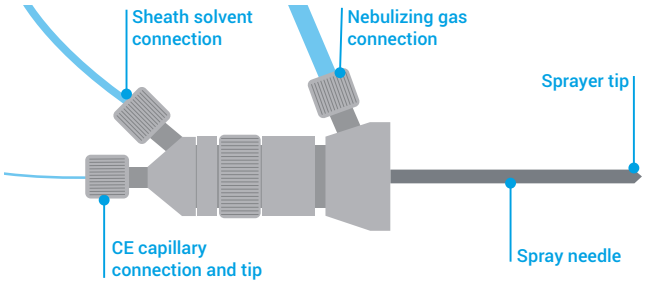


Figure 1. Schematic view of the triple tube sprayer.

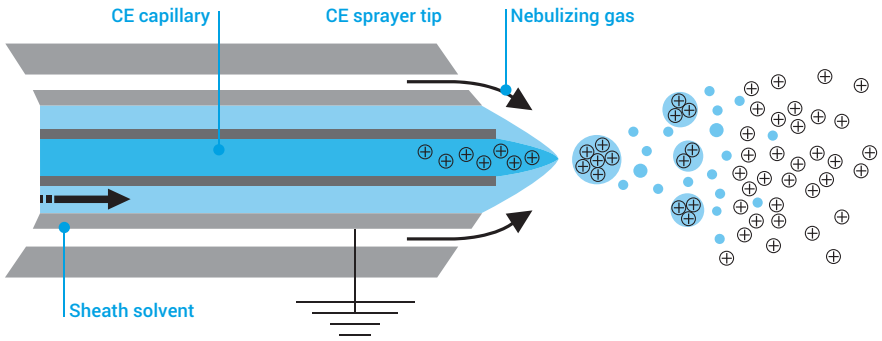


Figure 2. Simplified view of sprayer tip area.

As shown in Figure 3 and Figure 4, in the Agilent CE/MS system the sprayer is placed orthogonally to the mass spectrometer inlet capillary as with Agilent LC/MS interfaces. This has the inherent advantage that neutral additives in the separation buffer and/or large droplets that are formed will not enter the mass spectrometer.

This interface introduces several experimental parameters, which require careful adjustment and optimization to obtain a stable spray and ionization process. Especially regarding sheath solvent flow, composition of the sheath solvent, and nebulizing gas pressure. Recommended setting for these parameters will be described in following paragraphs.

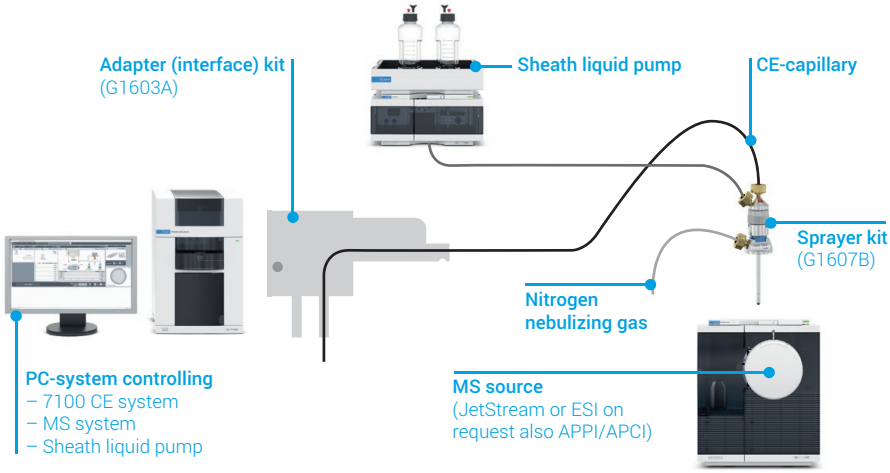


Figure 3. Agilent CE/MS system with control through Agilent OpenLab CDS (ChemStation Edition) software.

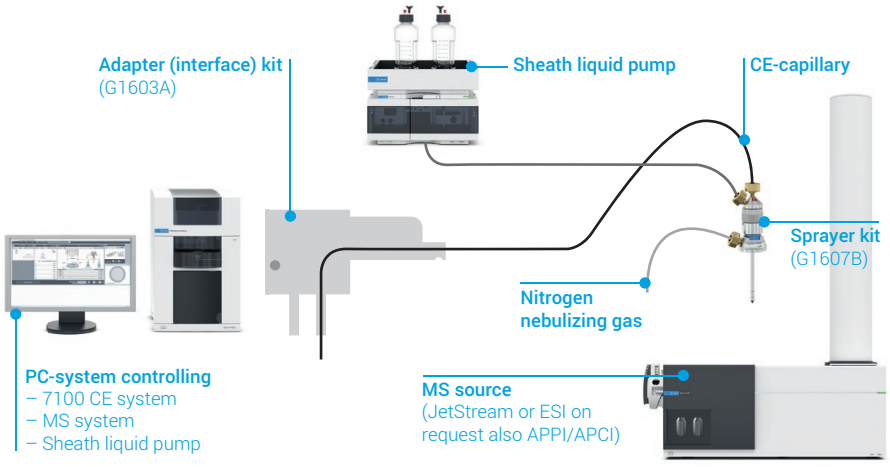


Figure 4. Agilent CE/MS system with control through Agilent MassHunter workstation software.

7 Agilent CE/MS Systems

7.1 System overview

The development of CE/MS systems by Agilent was paralleled by development of data acquisition and data handling software for the Agilent chromatography systems and mass spectrometric analyzers. At introduction of CE/MS in 1999, control of interface parameters, MS data acquisition, and data processing was embedded in the graphical user interface of the Agilent ChemStation software for CE. The mass spectrometer in the system was a single quadrupole mass analyzer, which was also controlled through the ChemStation software. Meanwhile the Agilent CE/MS triple tube interface can be coupled with all Agilent MS analyzers and is available on two software platforms as shown in section 7.2 "System details."

7.2 System details

The Agilent CE/MS system with Agilent OpenLab CDS comprises the following components:

- Agilent 7100 Capillary Electrophoresis system
- Agilent CE/MS adapter kit (CE/MS cassette, PEEK alignment interface for detector, and accessories) (G1603A)
- Agilent CE sprayer kit (Agilent Jet Stream compatible ESI-MS sprayer, flow splitter for sheath liquid, and accessories) (G1607B)
- Agilent InfinityLab LC/MSD Series system with API electrospray source (G1948B) or Agilent Jet Stream source (G1958B)
- Agilent 1260 Infinity II Isocratic Pump (G7110B)
- Agilent 1260 Infinity II Degasser (G7122A)
- Agilent OpenLab CDS (ChemStation Edition) software (M8301AA)
- CE driver for OpenLab CDS software (M8501AA)
- MS driver for OpenLab CDS (only required for use with single quadrupole MS) (M8361AA)

With the introduction of new mass analyzers, MS data acquisition and data processing became more demanding and control of CE setpoints and automation became embedded in the Agilent MassHunter workstation software. The Agilent CE/MS system with an Agilent Time of Flight, Triple Quadrupole, or Quadrupole Time of Flight LC/MS comprises the following components:

- Agilent 7100 Capillary Electrophoresis system
- Agilent CE/MS adapter kit (CE/MS cassette, detector alignment interface, and accessories) (G1603A)
- Agilent CE sprayer kit (Agilent Jet Stream compatible ESI-MS sprayer, flow splitter for sheath liquid, and accessories) (G1607B)
- Agilent Quadrupole Time of Flight LC/MS system with Agilent dual-spray ESI source (G3251B) or Agilent Jet Stream dual-spray ESI source (G1959A)
- Agilent 1260 Infinity II Isocratic Pump (G7110B)
- Agilent 1260 Infinity II Degasser (G7122A)
- Agilent MassHunter workstation software for Agilent Time of Flight LC/MS, Triple Quadrupole LC/MS, or Quadrupole Time of Flight Series LC/MS systems.

8 Optimum Parameters for a CE/MS Analysis System

CE is a versatile analytical separation technique, enabling differing modes of operation as well as a method that can be tuned optimally for the analytes involved*. Each CE operational mode works with a set of parameters of the CE separation that are highly specific for the particular mode. These parameters need to be chosen and optimized carefully to obtain highest resolution and sensitivity. However, the parameters that are optimal for the CE operational mode may have adverse effect on sensitivity of MS detection. The selection of CE mode of separation, the selection of BGE, and the optimization of CE parameters need to be well considered. The Agilent coaxial sheath solvent flow interface enables multiple ionization modes. Each of these modes will dictate parameters that need to be chosen carefully to obtain highest signal-to-noise ratio and stability of operation. Finally, parameters specific to the mass spectrometer will need to be adjusted for best signal-to-noise ratio and mass resolution.

In summary:

1. **Choosing the CE separation method and parameters:**
    a. Mode of operation such as CZE, ***MEKC, CGE, CIEF, CITP***, or ***CEC****.
    b. Composition of the background electrolyte (BGE), pH, ion strength, and type of buffer, organic solvents, additives
    c. The separation capillary, dimensions, wall coating, temperature
    d. The electric field, polarity
    e. The injection method (in particular regarding preconcentration techniques)
2. **Choosing the CE/MS interface:**
    a. Type of ionization; ESI, APCI, APPI
    b. Sheath solvent flow rate, composition and delivery
    c. Other operational parameters; nebulizing gas pressure, drying gas temperature, and flow rate
    d. Electric field and polarity for electrospray formation
3. **Optimization of MS data acquisition parameters:**
    a. (Auto) Tune signal-to-noise ratio, mass resolution, fragmentor voltage
    b. Acquisition rate, dwell times

This large set of parameters will confront a user in practice with a multifactor optimization challenge. It is the intention of this chapter to deliver good starting conditions at the beginning of CE/MS method development.

The optimization of MS data acquisition parameters is beyond the scope of this guideline. The reader is referred to the appropriate MS publications. In addition, they will be specified as used in the methods described and application notes referred to in Part 2 “Practice of CE/MS.”

** For a concise description of all CE modes of operation, refer to the Agilent Technologies Primer “High Performance Capillary Electrophoresis” (publication number 5990-3777EN). MEKC, CGE, CIEF, or CEC modes are typically incompatible with CE/MS hyphenation and therefore mostly avoided.*

9 Choosing the CE Separation Method and Parameters

9.1 The CE mode of operation

CZE is currently the most widely used operation mode of CE. It is optimally suited for the separation of charged, ionizable, polar, and HMW and LMW compounds.

In MEKC, surfactants such as sodium dodecyl sulfate are present in the BGE. These reduce MS signal response significantly while increasing noise, which has strong adverse effects on the limit of detection of ESI. As an alternative to ESI, APPI has been successfully applied in MEKC and MEEKC with MS with the triple tube interface. The ionization mechanism in APPI is less affected by the presence of surfactants in the BGE (see section 9.7 "Types of ionization – ESI, APCI, and APPI").

With CGE and CIEF, additives are also present in the BGE, which affect the ionization process leading to low ionization efficiency and high background. Special precautions in the CE method such as partial filling of the capillary are needed to avoid the undesired entrance of polymer gels or ampholytes into the MS.

In contrast, CITP separation is well suited for coupling with MS since the leading electrolyte can be the same as the sheath solvent. In practice CITP is used with CZE, serving as a preconcentration method (see section 9.5 "Sample introduction methods").

CEC is well suited to be coupled with MS detection since the BGEs used are similar to the solvents used in LC/MS. However, CEC did not find wide application in CE separations. One obstacle here is that CEC is usually run by applying pressure on both sides of the capillary to avoid the formation of gas bubbles within the capillary. Further, robustness and reliability of CEC is lagging behind CZE.

Reference information about the modes of operation of CE is available in the Agilent Technologies Primer "High Performance Capillary Electrophoresis," publication number 5990-3777EN.

9.2 Composition of the BGE

In general, inorganic buffers such as phosphate and borate are used preferably in the BGE of a CE separation. They have high buffering capacity and ion strength, and can be used in low concentrations, resulting in narrow, high efficiency peaks. However, use of inorganic nonvolatile buffer constituents in the BGE should be avoided in CE/MS. Nonvolatile buffer constituents cause salt buildup in the electrospray chamber and in the MS inlet, which can block the inlet capillary. This blocking will eventually result in loss of sensitivity, reliability of operation, and even failure of the MS.

Instead, for low pH separations, we recommended using volatile acidic and basic buffers such as formic and acetic acid. For high pH separations, we recommended ammonium salt or trialkylammonium compound buffers.

To avoid high currents (and resulting Joule heating) during the CE separation, the concentration of these buffers should be kept as low as possible (for example, 50 mM) without compromising the separation (peak shape and resolution).

The pH of the separation buffer (BGE) will render the analyte molecule in a cationic (positive) or anionic (negative) charge state. This charge will determine whether to use a positive (normal polarity) or a negative (reversed polarity) CE voltage. The sheath solvent though may have a different pH from the separation buffer and will be present in excess during spray formation. Hence weak acids that are separated as anions in the CE separation will become neutralized by the sheath solvent and form cations in the electrospray.

When developing a CE separation coupled with MS, these considerations must be addressed and will constrain the CE separation parameters during method development. In practice, a CE method is developed with spectrophotometric detection but with the requirements for MS detection in mind.

Special cases for BGEs

BGEs may contain additives required to obtain a selective separation. For example, cyclodextrin, a chiral crown ether, macrocyclic antibiotics, or chiral surfactants are added to the separation buffer for separation of enantiomeric mixtures. Surfactants are used in MEKC to induce hydrophobic interactions to allow separation of, for example, neutral or weakly acidic/basic drugs.

If these additives bear no charge, they will not become drawn into the mass spectrometer. This is because of the orthogonal position of the triple tube sprayer assembly relative to the inlet of Agilent mass spectrometers.

However, many of these additives are charged to control the CE separation time and augment selectivity (for example, ampholytes in CIEF). They will be present during the ESI ion formation process and they may compete with the analytes for the available charges in the ionization process (which is called “ion suppression”).

When the additives carry the same charge as the analyte, they may become drawn in the mass spectrometer. Although the mass analyzer can be operated in a mass selective mode, additive ions contribute to a high background in the mass spectrometer. In addition, surfactants present in the separation buffer used in MEKC contaminate the interface, are difficult to remove, and significantly reduce sensitivity with electrospray ionization.

Consequently, techniques such as MEKC and CIEF have not been used frequently in ESI CE/MS. Special techniques such as partial filling of the CE capillary[12] are applied to avoid these problems. However, these special techniques make the separation method circumstantial and are not discussed here.

9.3 The separation capillary

Fused silica tubing is drawn from wide inside diameter silica, which is preformed at a temperature around 1300 °C in an inert atmosphere. Under these conditions, silanol groups present at the capillary surface condense completely to form siloxane bonds. During the drawing process, a polyimide skin is deposited on the outside of the capillary, which gives fused silica tubing its extreme robustness and pressure stability while remaining flexible. The inner surface of a pristine fused silica capillary thus is hydrophobic. Over time during storage, the inner surface becomes slowly rehydroxylated by humidity and eventually by the buffers used in CE.

Batch-to-batch variability of the EOF in fused silica tubing depends on the residual surface silanol concentration and on the amount of residual (metal) impurities in the silica. Rigorous pretreatment and preconditioning procedures should be applied to render the capillary in a well-defined, reproducible state of surface hydroxylation to begin with. Capillary pretreatment is commonly done with 1 M sodium hydroxide. Alternatively, overnight treatment with high concentration hydrochloric acid will do too. Afterwards the capillary should be flushed with water and the BGE.

It has become common practice in routine CE work, to maintain a rigorous protocol of preconditioning operations. Washing procedures between runs with brief cleaning and reconditioning with the BGE should be part of a routine method. Eventually, the magnitude and reproducibility of electro-osmotic flow that is generated in a fused silica separation capillary will depend strongly on pretreatment and its previous operation as well as the composition, pH, and ionic strength of the BGE.

For further details on procedures for capillary pretreatment, refer to section 4.2 in the Agilent Technologies Primer "High Performance Capillary Electrophoresis," publication number 5990-3777EN. In "Capillary Electrophoresis Methods for Pharmaceutical Analysis" (Eds. S. Ahuja and M.I. Jimidar, Academic Press, 2008, London) a wealth of practical information is given on capillary pretreatment and handling for reproducible CE analyses. It is beyond the scope of this guidebook to provide details on these aspects.

Fused silica capillaries for CE/MS

Capillaries for CE and for CE/MS have inside diameters from 20 to 100 µm with 50 and 75 µm being the most widely used. The standard outside diameter is 365 µm in Agilent CE/MS systems. Capillaries with 180 to 220 µm od and 20 to 30 µm id have found application in special cases. However, these capillaries are not recommended for use in the Agilent 7100 CE System. Since the separation capillary must bridge the distance between two instruments, typically capillaries of 1000 mm length are used (especially when the diode array detector of the CE system is used).

Analogous to normal CE operation, in CE/MS the ends of the capillary at inlet and outlet side must be flat and undamaged. On the capillary inlet side, an uneven or fractured end renders the electrical field distribution inhomogeneous, which results in broadening during sample injection. When the outlet end of the capillary for CE/MS is damaged, the spray will become distorted and unstable causing high noise and low sensitivity in the mass spectrometer. Bare fused silica capillaries can be cut from the reel. It is therefore important to use a precision cutting tool like the Agilent CE capillary column cutter (p/n 5183–4669).

Nevertheless, it is recommended to use Agilent precut fused silica capillaries for CE/MS, which have 1 to 2 mm of polyimide removed at the ends. From practical experience, a more stable spray is obtained with the polyimide removed at the CE/MS capillary end. In addition, these precut capillaries have a window for UV-Vis detection. For more information, see the "Agilent Guide to Capillaries, Reagents, and Supplies for CE," publication number 5991-5623EN.

If fused silica tubing from the reel is used, we recommended following the instructions for capillary cutting as given in section 14.1 "Preparing the capillary."

Capillaries with covalently bonded neutral or charged layers

For the separation of peptides and proteins, it may be advantageous or even mandatory, to have a permanent charge at the capillary wall to minimize adsorption and to sustain a strong EOF. The wall charge must have the same polarity as the analytes to avoid their adsorption. Therefore, a high EOF is needed to transport the solutes against their electrophoretic mobility towards the capillary exit. For example, with a positively charged wall coating, the EOF is carried by the negative ions in the BGE and the electrode at the exit must be the anode. Hence, positively charged solutes will move opposite to the EOF towards the cathode. It has been reported that applying a slight pressure on the BGE in the inlet vial assists the EOF and sweeps opposite charged analytes towards the capillary exit (here, the sprayer needle).

Alternatively, it may be undesired for a particular CE separation that an EOF exists. If so, the capillary must have a permanent coating rendering the surface neutral and hydrophilic, which suppresses EOF and, minimizes solute adsorption. Capillaries with neutral hydrophilic coatings are commercially available from several manufacturers. For more information, see the "Agilent Guide to Capillaries, Reagents, and Supplies for CE," publication number 5991-5623EN.

Successive multiple ionic layers

CE capillaries with covalently bonded permanent positive or negative layers are expensive and have not found widespread use. Dynamically generated wall coatings have been proven to serve equally well in controlling or neutralizing the EOF in many CE analyses. In addition, this can be done with standard fused silica CE capillaries and is therefore much more affordable. In an extensive and detailed recent review, Lucy et al.[13] and Huhn et al.[14] discussed approaches to control the EOF by coatings and BGE additives.

The method of dynamic coating of the fused silica capillary surface by successive multiple ionic-polymer layers (SMIL), has become well established. SMIL leads to coatings that are easily generated, inexpensive, applicable over a wide range of buffer conditions, and do not interfere with MS detection.

SMIL coating procedure was proposed by Katayama[15]. A pictorial representation is given in Figure 5.

Polybrene (hexadimethrine bromide) is cationic polymer that is usually used for the first layer coating of the anionic silanol groups. Dextran sulfate or polyethylene sulfonate are examples of anionic polymers for the second layer. Triple layers, which have a cationic surface, have also been demonstrated.

The exceptional stability of SMIL coatings has been proven in BGE covering a wide pH range, buffer type, and additives without the need to have the ionic polymer present in the BGE. In practice, it suffices to recondition the capillary coating with the top layer ionic polymer intermittently. Therefore, CE with SMIL coating is well compatible and can be coupled with mass spectrometry[16]. Analis SA offers a wide choice of kits for SMIL[17].

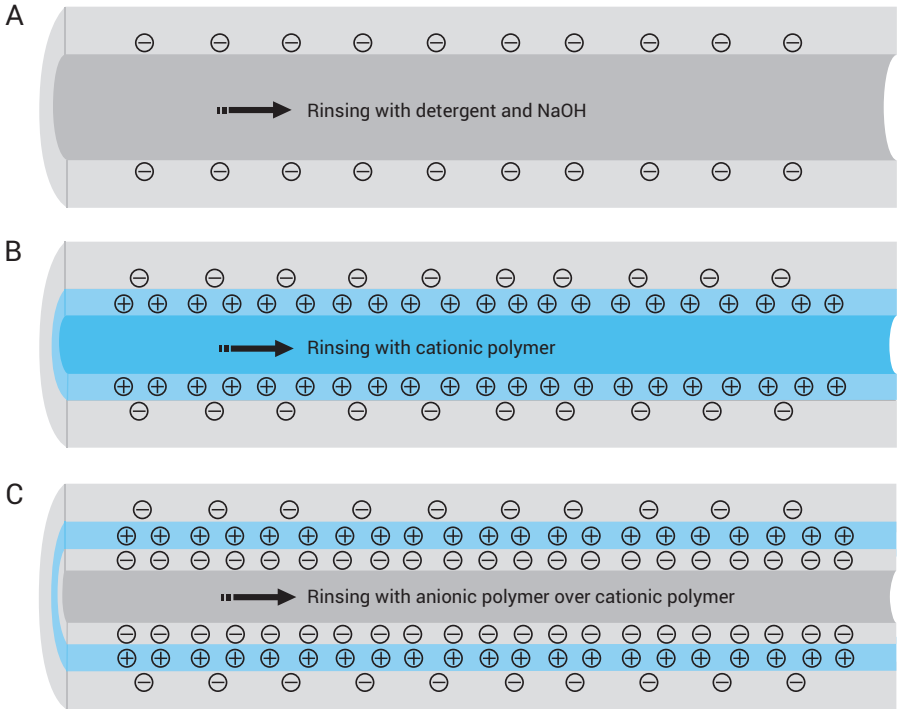


Figure 5. SMIL coating procedure. (a) Activation of the silanol groups; (b) first layer coating; (c) second layer coating.

Capillary installation and thermostatting

Details about handling capillaries and installation in the CE/MS cassette are given in section 14.2 "Installing the capillary in the CE/MS cassette."

In the Agilent 7100 CE System, the capillary is thermostatted apart from the parts of the capillary that protrude through the electrode in the inlet and outlet vial. Given that in connecting the CE capillary with the sprayer on the mass spectrometer the distance between the two instruments needs to be bridged, a section of the separation capillary will not be in a controlled temperature environment. It has been reported that fluctuations of the temperature of the environment affect migration time precision[18].

Therefore, the temperature in the capillary cassette will be set at the average temperature in the lab. A lab with temperature control is recommended, in addition the capillary should be protected against drafts in the room.

As described in section 14.2 "Installing the capillary in the CE/MS cassette," a PTFE tube is provided for electrical insulation. This tube will also serve to insulate the separation capillary to some extent.

9.4 Electric field and polarity

In CE, a dual-polarity DC power supply is used to apply up to about 30 kV. The Agilent 7100 CE System's power supply can deliver current levels of up to 300 µA (9 W). The voltage is applied to the electrode in the inlet vial. When a positive voltage is applied, the electrode in the inlet vial will be the anode. With bare fused silica capillaries, the EOF will be into the direction of the outlet. The spray needle in the triple tube interface will become the cathode and is connected to ground.

If the capillary surface charge is positive, it is mandatory to apply a negative voltage to the inlet side to direct the EOF towards the capillary outlet. Then, the electrode in the inlet vial becomes the cathode and sprayer needle becomes the anode and is also connected to ground potential.

Regarding the application of the electrical field when using the triple tube interface for CE/MS, uncompromised operation of the CE system is possible, and recommendations are given in section 3.1 of the Agilent Technologies Primer "High Performance Capillary Electrophoresis," publication number 5990-3777EN.

But there are a few important consequences that the user should be aware of, which are discussed in the following sections.

Electrode reactions

It is imperative to understand that during CE separation a DC current is flowing. This current is carried by the electrolyte ions in the BGE. At the electrodes, charge is transferred from the solution to the electrode by redox half reactions. In CE, electrodes typically are made of noble metals such as platinum or palladium.

In the normal polarity mode, when the inlet electrode is the anode, water is oxidized. Whereas at the outlet, which is the cathode, water is reduced. The corresponding half reactions are:

$$2H_2O - 4e^- \rightarrow O_2 + 4H^+$$

$$2H_2O + 2e^- \rightarrow 2H_2 + 2OH^-$$

In reversed polarity mode, the inlet side electrode becomes the cathode and the reverse for the electrode in the outlet vial. The half reactions also reversed.

The Agilent 7100 CE System's electrodes are made of noble metal tubes with an id of 0.5 mm and od of 1.0 mm. The material is a platinum/palladium alloy. This alloy is more noble than water so that there are no galvanic processes that change or erode the electrodes.

In CE, the user must be aware of these electrolytic processes and replace these vials or replenish their contents after several runs since eventually the pH of the BGE in the vials may change. In CE/MS, in normal polarity mode, water is reduced at the spray needle. Minute gas bubbles are formed in the annular space between CE capillary and the sprayer needle, which will become swept out by the sheath solvent through the open end of the sprayer.

Since the spray needle is made of stainless steel, in the reversed polarity mode (when the capillary surface is cationic and the EOF is carried by the anions in the BGE) the sprayer needle becomes the anode. Since iron and nickel are less noble than water, it has been observed that instead of water oxidation, iron, and nickel in the spray needle become oxidized and form their respective cations Fe^{2+} and Ni^{2+} [19]. These ions move against the EOF into the CE capillary and may form insoluble oxides, which obstruct the EOF in and eventually plug the capillary. Also, the stability of the electrospray and the sensitivity of MS detection are adversely affected.

As a remedy under these conditions, the use of a platinum spray needle is recommended (p/n G7100-60041). Instructions for replacing the stainless-steel spray needle are described in section 22.1 "Assembly of the CE/MS sprayer kit."

Alignment interface for the diode array detector

When using the Agilent CE/MS System, the user might opt to pass all solutes by the diode array detector (DAD) before entering the triple tube sprayer. Although the sensitivity of detection with the DAD is lower than with MS detection, the use of DAD has proven to be invaluable in CE/MS separation method development. When using the DAD in the CE system, the point of detection is at 21.5 cm with a further 80 cm to the triple tube sprayer.

The analysis time for a CE separation is inversely proportional to the square of the total capillary length. Therefore, when using the DAD in CE/MS, the analysis time is extended.

However, there is one important further consequence of using the DAD during CE/MS. At the point of detection, the voltage drop in the CE capillary has been about 20 %. Although the current is inside the capillary and fused silica is an excellent insulator, occasional arcing from the metal alignment interface to the grounded detector housing has been observed. Small imperfections or damage to the capillary wall or outside coating may lay at base of these occurrences. In normal CE operation, at the point of detection, 80 to 90 % of the voltage has dropped and reduced the importance of this problem.

To minimize these problems in CE/MS, a PEEK alignment interface is available and recommended for use. This interface insulates the CE capillary further from the detector housing. For the preceding reason, the use of extended light path (bubble cell) CE capillaries and the use of the high-sensitivity detector cell is discouraged in CE/MS.

9.5 Sample introduction methods

CE with spectrophotometric detection is a concentration-sensitive analysis method. Given the short path length of optical detection in CE, the lower limit detection for the solutes in the sample solution is in the 10 µM range. In principle, with mass spectrometric detection the LOD will be 10 to 100-times lower besides providing structural information.

In CE, the sample can be introduced with the EOF (field driven) or by hydraulic flow (pressure driven). In CE/MS, the preferred method of sample introduction in the CE separation capillary is by pressure injection**.** Typically, the sample volume is in the nanoliter range and can be calculated using the following formula:

$$V_{inj.} = \frac{\Delta P \cdot d_c^4 \cdot \pi \cdot t_{inj.}}{128 \eta \cdot L}$$

- ΔP: average pressure drop during injection
- d_c: capillary internal diameter
- t_{inj}: injection time
- η: viscosity of the sample solvent
- L: total length of CE capillary

The length of the injection zone amounts to a few millimeters. In contrast to HPLC injection methods, the user cannot increase the volume of the sample in CE without penalizing the separation efficiency. The injection of larger volumes in CE causes zones of larger width, not higher peaks, irrespective of the sensitivity of the detection method.

Widening the capillary inside diameter in CE may seem an attractive pathway to increase the injection volume. But in widening the capillary inside diameter, the current will increase with the square of the diameter and Joule heating will increase. Heat transport out of wide inside diameter fused silica capillaries is slow, which will lead to inhomogeneous radial temperature distribution and therefore zone distortion.

In CZE/MS, it is recommended to always use sample introduction by pressure. Electrical-field-driven sample introduction is recommended for CGE separation and for electrokinetic sample preconcentration mentioned in section "Electrokinetic sample preconcentration" of this chapter.

Sample introduction and carryover

As explained in section 9.4 "Electrode reactions," cylindrical tube electrodes (1.0 mm od and 0.5 mm id) are used. Although this is a robust design and protects the separation capillary when exposed outside a vial, the configuration bears a small risk for sample carryover. This may happen when the sample vial is exchanged with the BGE and sample solution remains behind in the annular space between capillary and electrode walls. Troubleshooting procedures are available that minimize this carryover, see Chapter 20 "Troubleshooting."

Electrokinetic sample preconcentration

To gain sensitivity in CE by injecting large sample volume, the injection zone needs to be reduced volumetrically as done by solid phase extraction (SPE) in HPLC. SPE has found limited application in CE since the integration of a short-packed particle zone in a CE capillary is cumbersome.

Compression of a long injection zone can be achieved by electrokinetic focusing. Several electrokinetic sampling procedures have found broad application in CE. An overview of such methods is given in section 3.2.3 of the Agilent Technologies Primer "High Performance Capillary Electrophoresis," publication number 5990-3777EN. Breadmore and Sänger-van de Grient have recently given an overview of these methods[20]. Not all the methods described in this overview can be applied for sample concentration in CE/MS. Some of these methods use additives in the sample or BGE, which should be avoided when using MS detection. The preconcentration methods listed in Table 1 are recommended for use in CE/MS.

Details of such methods are given in the references and can be found on pages 65–72 of the Agilent Technologies Primer "High Performance Capillary Electrophoresis," publication number 5990-3777EN.

Table 1. Recommended preconcentration methods for use in CE/MS

Electric-field-driven preconcentration methods	
Field-amplified sample stacking (FASS)	The sample is injected by pressure. The sample solvent has lower conductivity than the BGE. The field strength in the sample zone is high and the solutes move faster than in the BGE. They are slowed down and collect when reaching the BGE, which has higher conductivity[21].
Dynamic pH-junction method	The sample solvent has a different pH than the BGE. The analytes have higher mobility in the sample zone and collect at the boundary with the BGE[22].
pH-mediated FASS	The sample is sandwiched between a strong base and strong acid. When the voltage is applied, the OH^- and H^+ ions migrate through the sample zone and neutralize, thus reducing the conductivity. Collection can then take place[23].
Transient isotachophoresis (tITP)	The sample is sandwiched between a higher mobility co-ion (leading electrolyte) and a lower mobility terminating electrolyte. When the voltage is applied, the analytes are sorted in order of their mobility[24].
Liquid-phase microextraction (LPME)	Single-drop microextraction: analytes are extracted into a small organic drop suspended at the tip of the capillary[25]. This method can be performed in commercial instrumentation with no modifications.
Pressure-driven preconcentration method	
In-capillary SPE	A short zone with large, porous particles is placed inside the capillary. Analyte extraction and elution are like HPLC[26].

Precision of sample introduction in CE/MS

In contrast to spectrophotometric detection, detector response in ESI-MS depends on many factors. These factors include the ion formation process, ion capture and introduction, magnetic and electric field focusing, and the fragmentation process. In practice, a reproducibility of solute response for an ion better than 10 % is regarded as excellent.

The reproducibility of CE sample introduction by pressure injection is significantly better in practice for many CE instruments. The use of an internal standard in both the standard and in the unknown sample will eliminate variability of the sample injection process. EOF variability and compound mobility may cause fluctuations in peak area. This also applies to MS detection. Ideally, when an isotope of the compound to be determined (for example, deuterated) is available, this will definitively improve the precision of the determination.

Schappler et al. have described a delayed standard injection method for improving quantitative reproducibility in CE/MS in comparison with the ECHO method used in LC/MS[27]. They worked with insulin samples. In this approach, the standard with known concentration was injected first, followed by a zone of BGE. Next, the sample with the unknown was injected and the run was started. These authors found the reproducibility of sample introduction to be unsatisfactory, which caused them to add an internal standard to both the standard and the unknown sample to enable calibration of the injection variability. In combination, these measures achieved an overall precision of 2 % for the determination of insulin in formulations.

9.6 The CE/MS interface – the ion source

As shown in Figure 3 and Figure 4, the triple tube sprayer is connected to a housing, which is attached to the MS instrument and enables the positioning of the sprayer in front of the MS inlet. The housing with the sprayer and all other parts required in the ionization process is called the ion source. The housing is needed to provide a safe electrical environment for the voltages involved in the CE separation and solute ionization processes. Further, the housing enables to set the temperature, isolate the spray from disturbances in the lab environment (air draft, temperature variation), and dispose of eluent waste in a controlled and safe way.

The ion sources used in Agilent CE/MS Systems are the same as used for LC/MS. Instead of the standard LC sprayer, the triple tube interface sprayer is used in CE/MS. Switching between an Agilent LC/MS and CE/MS System only requires exchanging the sprayer (except for adding the sheath solvent pump, see Chapter 15 "Preparing the Agilent 1260 Series Pump and Vacuum Degasser").

9.7 Types of ionization – ESI, APCI, and APPI

There are two principally different versions of Agilent ion sources: the standard ionization sources and the Jet Stream source. In the latter source, a hot sheath gas narrows the spray plume, which is of particular importance for the higher flow rates used in LC/MS. More details are available in the Agilent Technologies Technical Overview "Agilent Jet Stream Thermal Gradient Focusing Technology," publication number 5990-3494EN.

The standard ionization source comes in different models, which support four different atmospheric pressure ionization techniques:

- Electrospray ionization (ESI)
- Photoionization (APPI)
- Chemical ionization (APCI)
- Dual-mode ionization (combining APCI and ESI in one source)

To use the CE/MS sprayer on Agilent ion sources for APPI and APCI, an adapter piece is required. This adapter is available free-of-charge on request. Please contact your Agilent representative.

By design, the Agilent Jet Stream source is only available for ESI. Hence, in principle, there are five LC/MS ion sources available for application in CE/MS. There are, however, a few experimental constraints that will be dealt with in detail in Chapter 10.

A detailed description of the ionization modes is beyond the scope of this guidebook. Interested readers can refer to textbooks on MS.

Since CE separations largely deal with charged, ionizable, and/or highly polar substances, the user will usually choose to use ESI. APPI and APCI sources are better suited for the ionization of neutral and low polar molecules, and have shown particular advantages in LC/MS, for example, when acting as a mass-flow-sensitive detector instead of being concentration-sensitive like ESI. Further, both APPI and APCI are less susceptible to matrix effects or ion suppression. Therefore, these ionization methods may be more accurate.

In CE/MS, APPI and APCI sources have been used in special cases. For example, Markides[28, 29] et al. and others[30, 31] have described that CE/APPI-MS) is a viable and feasible alternative to electrospray ionization. This is especially for a MEKC separation since the ionization process is not hampered by the surfactants present. De Jong et al.[32] have published comparable results with CE/APCI-MS. Both groups worked with the Agilent ion sources for APPI and APCI. For more details, refer to the cited literature.

9.8 Sheath solvent composition and delivery

As explained in section 9.2 "Composition of the BGE," the sheath solvent plays a crucial role in the Agilent triple tube sprayer CE/MS interface. The sheath solvent enables a liquid electrical connection to the spray needle electrode for the CE separation and the voltage for electrospray formation. For proper operation of the interface, the sheath solvent must be delivered at a constant flow rate (1 to 10 µL/min). In this way, the electrospray can be maintained independent of the magnitude and direction of EOF. Low sheath solvent viscosity, low surface tension, and high vapor pressure result in a highly robust and stable electrospray.

Besides the two fundamental functions of the sheath solvent mentioned previously, extra prospects expanding the versatility of CE/MS become available using a sheath solvent. For example, by selecting a low or high pH sheath solvent, the charge state of the analytes will become unambiguously positive (cations) or negative (anions). In this way, it will be possible to separate molecules as anions but ionize them in electrospray as neutral, polar molecules, or as cations.

More recently work has been published describing more elaborate chemical reactions in the spray resulting in enhanced charging of proteins[33].

Alternatively, the sheath solvent may contain low concentrations of reference compounds to calibrate the mass axis or additives that help in ionization with APPI.

Recommended sheath solvents

The sheath solvent is usually composed of a 50:50 mixture of a volatile organic acid or base in water and an organic modifier such as methanol or isopropanol. These solvents facilitate spray formation. The use of acetonitrile in the sheath solvent is discouraged. Swelling of the protective polyimide layer on the outside of the fused silica CE capillary has been observed because of long-term exposure to acetonitrile, especially at high concentrations. Therefore, acetonitrile in the sheath solvent may lead to clogging of the separation capillary by polyimide remains, which deteriorates spray formation and to eventual breakage of the exposed fused silica capillary in the sprayer.

In addition, acetonitrile is an aprotic solvent and does not assist protonation, hence lower signal intensity is recorded with this solvent compared with alcohols in the sheath solvent.

Low molecular weight organic acids such as formic or acetic acid are used in the sheath solvent for CE/MS detection of cations. Alternatively, ammonium hydroxide or tertiary amines are applied in the sheath solvent for CE/MS of anions. The sheath solvent may also contain a volatile buffer such as ammonium acetate or ammonium formate for low, neutral, or high pH buffering. Table 2 gives an overview of recommended sheath solvents.

Table 2. Overview of recommended sheath solvents.

Molecular Charge	Recommended Sheath Solvents*
Cations (Positive ESI)	50 % methanol or isopropanol with: 0.1 to 1 % formic or acetic acid 5 to 20 mM ammonium formate or acetate
Anions (Negative ESI)	50 % methanol or isopropanol with: 5 to 20 mM ammonium formate or acetate/methanol or isopropanol 1:1 0.1 to 1 % ammonium hydroxide/methanol or isopropanol 1:1**
Remarks	* The solvents are listed in order of increasing pH. ** Ammonium hydroxide solution may lose ammonia and change pH. In general, it is recommended to refresh the sheath solvent once every week.

Reference mass compounds may be added to sheath solvent when a TOF or Q-TOF mass spectrometer is used instead of using the reference mass sprayer in LC/MS mode. Agilent recommends using purine and HP-0921 of the Agilent Reference Mass Mixture (p/n G1969-85001) for the reference mass. Add each to the methanol solution used in the sheath solvent.

Especially when using APPI, substances that become easily photo-ionized and can transfer their charge to the analytes are added to the sheath solvents. Such additives are called dopants, for example, acetone or anisole.

Sheath solvent delivery

As mentioned before, the sheath solvent is delivered at flow rates of 1 to 10 µL/min. Many groups have reported that, in general, the ESI interface can be regarded as a concentration sensitive detector. Therefore, the peak area is inversely proportional with the sheath solvent flow rate and the sheath solvent flow rate would be set as low as possible. As a rule of thumb, a flow rate of 4 to 6 µL/min of the sheath solvent is regarded optimal. At higher flow rate of the sheath solvent, the dilution of the solute will increase and therefore the sensitivity will decrease.

Given that a volumetric flow rate of 5 µL/min may be 50 to 100 times higher than the EOF, it would be expected that the solute concentration is diluted 50 to 100-times on leaving the CE separation capillary. In practice though, it has been observed that the favorable (for ESI) composition of the sheath solvent offsets part of the dilution effect.

Apart from the dilution by the sheath solvent, the typical sheath solvent flow rate is too large to enjoy the benefit of CE/MS as a nanoflow-rate separation method. Real nanoflow rates of 50 nL/min and below comprises a nano-electrospray ionization process with strong enhancement of response.

The benefits of the co-axial sheath solvent delivery are summarized as follows:

- Provides a stable wet electrical connection with the spray tube with a common ground connection for CE and ESI voltage
- Renders the spray formation independent of the presence and magnitude electro-osmotic flow
- Solvent sheath composition can be chosen to deliver optimal conditions for spray formation therefore mitigating the dilution effect
- Achieves a stable spray with pneumatic assistance
- Enables in-spray chemistry such as (de)protonation, derivatization, or charge transfer (APPI), and can deliver mass calibration standards

Agilent recommends to deliver the sheath solvent by split flow using an Agilent 1260 Infinity II Isocratic Pump instead of a syringe pump (see Figure 3 and Figure 4). Syringe pumps can supposedly deliver the required flow rate (and at lower cost) but experience has shown that the flow rate stability of syringe pumps is unacceptable, resulting in extra noise and ghost peaks during MS detection. Further, the 1260 Infinity II Isocratic Pump includes degassing of the sheath solvent and provides for longer and unattended operation.

9.9 Operational parameters – nebulizing gas pressure, drying gas temperature and flow rate

As explained in Chapter 6 "Coupling Liquid-Phase Separations with Mass Spectrometry," pneumatic assistance of the spraying process has been an essential ingredient to obtain a stable and robust spray. In practice, it has been found by several groups that the nebulizing gas flow exerts a slight suction on the liquid in the CE capillary to the extent that a hydraulic flow towards the exit of the capillary becomes established[34, 35]. Because hydraulic flow in a capillary forms a parabolic solvent flow velocity profile, extra zone broadening will occur and a reduction of the efficiency of the analyte zones is observed.

Precautions need to be taken to minimize this effect and also to avoid that the capillary becomes emptied during manipulation of the sample and BGE vial.

For example, the hydraulic flow can be reduced by applying negative pressure on the inlet vial. In practice, the negative pressure would be increased in steps of 10 mbar until the efficiency of the peaks is re-established as determined by UV detection, until the EOF is back to the original value[36].

The use of narrow capillaries with inside diameters of 50 µm or less and/or longer lengths of the capillary will also reduce this effect. In addition, it is desirable to make sure that during the injection process, the nebulizing gas flow is reduced to avoid drawing in a bubble during the injection cycles.

It is recommended to time-program a low nebulizing gas pressure during the CE sample injection process.

To assist the evaporation process in the source, a heated gas is delivered by the MS towards the spray. This is the drying gas and will be typically between 100 to 200 °C. One precaution needs to be mentioned. At low flow rates, the drying gas flow rate should be set to low values since a too strong drying gas flow rate will destabilize the small spray fume.

9.10 Electrospray ionization voltage

Since the triple tube sprayer is grounded, the voltage for electrospray ionization is delivered by the mass spectrometer. Consequently, when analyzing positively charged ions from the spray, the electrospray voltage has to be set at negative value. In contrast, for analysis of negatively charged ion from the spray, the voltage will be set to a positive value.

In practice, the system will require you to select a positive or negative ion detection mode of analysis. It is important to realize that in LC/MS or CE/MS systems where the electrospray voltage is applied to the spray needle, this will be an opposite state. For positive ion MS, the electrospray voltage will be negative and vice versa.

Another important consequence of this configuration is the construction materials of the MS inlet capillary (see Figure 6). To make sure that the ES voltage remains insulated from the MS, this capillary is made of glass in Agilent mass spectrometers. Otherwise, this capillary is a metal capillary.

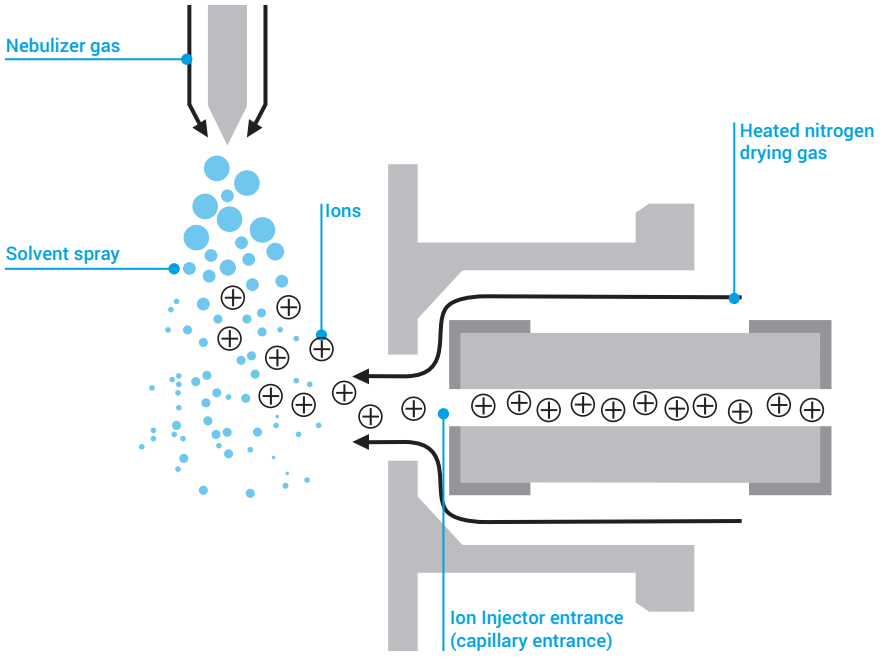


Figure 6. Role of drying gas.

Part 2 Practice of CE/MS

Part two focuses on the practical of CE/MS. Starting with the general setup including software configuration, the chapter continuous with a detailed section around the installation of Agilents Triple Tube Sprayer.

The final sections are highlighting some CE/MS method examples and guidelines for maintenance and trouble shooting.

10 Practice of CE/MS

The Agilent 7100 Capillary Electrophoresis instrument can be coupled with Agilent Time of Flight, Triple Quadrupole, or Quadrupole Time of Flight Series LC/MS systems. A representative picture of one of these systems is shown in Figure 7.

Agilent InfinityLab LC/MSD Series systems are controlled through Agilent OpenLab CDS (ChemStation Edition) software (M8301AA) complemented with the CE (M8501AA) and MS (M8361AA) drivers for OpenLab CDS. Other Agilent mass spectrometers (6200, 6400, and 6500 Series systems) are typically controlled through the Agilent MassHunter software.

In the following paragraphs, common and specific aspects of setup and connection of the 7100 CE instrument with 6000 Series LC/MS systems will be described in detail. The pictorial visualization of hydraulic and electrical connections is given here for Agilent InfinityLab LC/MSD Series systems. Deviations from the actual situation in the Agilent Time of Flight, Triple Quadrupole, or Quadrupole Time of Flight Series LC/MS systems are given here but they are not essentially different.

Since the configuration of the Agilent InfinityLab LC/MSD Series system with the OpenLab CDS (ChemStation Edition) software differs significantly from the MassHunter CE/MS Systems, their setup is described in separate chapters.

- Chapter 11 “General Setup of Agilent CE/MS Systems”
- Chapter 12 “Setting up an Agilent CE/MS System with Agilent OpenLab CDS (ChemStation Edition)”
- Chapter 13 “Setting up an Agilent CE/MS System with Agilent MassHunter Software”

Figure 7. Agilent 7100 Capillary Electrophoresis system and Agilent G6495B Triple Quadrupole LC/MS system.

Descriptions of the initial setup of the 7100 CE system and the Agilent InfinityLab LC/MSD, Time of Flight, Triple Quadrupole, and Quadrupole Time of Flight Series LC/MS systems are not given here. These setups are described in detail in different documents such as the user guide for the 7100 CE system and the individual 6000 Series LC/MS System installation guides (publication numbers can be found on the Agilent website at www.agilent.com).

If your Agilent CE/MS adapter kit and CE/MS sprayer kit are going to be installed by an Agilent service engineer or an Agilent-certified service provider, proceed to Chapter 14 “Installing the Capillary and the CE/MS Cassette.”

All accessories and documentation needed to interface the 7100 CE system to a non-Agilent mass spectrometer are the responsibility of the respective vendor and are not provided here.

11 General Setup of Agilent CE/MS Systems

11.1 Leveling the CE and MS instruments

When setting up the CE/MS system, the two instruments should be positioned close together to minimize the total length of the separation capillary connecting them. Placing the Agilent CE on a height adjustable cart simplifies positional adjustment or changeover to an HPLC system (Figure 8).

In general, the buffer level in the capillary inlet vial should be at an equal height to the buffer level in the capillary outlet vial to avoid siphoning. In the 7100 CE instrument, the capillary in the inlet vial ends 365 mm above the bench level. Adding to this the buffer fill height of 15 mm yields a total height level of 380 mm. The outlet capillary in the triple tube sprayer must end at the same height as the buffer level in the inlet vial. If this height is below the inlet vial, a siphon flow towards the outlet will occur. Conversely, the capillary would become emptied when the capillary height in the sprayer is higher than the level of the buffer in the inlet vial.

Placing the 7100 CE instrument and Agilent InfinityLab LC/MSD Series on the same bench results in the outlet capillary end in the sprayer being 70 mm lower than the inlet capillary end. A cart or a table that is lower than the bench height with the MS and which is adjustable in height simplifies the setup for leveling. Alternatively, elevate the MS by 70 mm on the bench.

For other Agilent LC/MS models, the height of the sprayer may differ and is given in Figure 9.

The 7100 CE instrument has a T-shaped sign on the right side of the cover (Figure 10), which indicates the position of the electrode in terms of height and can serve as reference for an adjustment.

Figure 8. Flexible desk (ionBench BCHLC4575) offering simple height adjustment for a CE instrument. Photo courtesy of ionBench (3 route de Chamvres, 89300 Joigny, France).

	MS model	Hight different X [mm] to G1600/G7100
SQ	G6125CA, G6125BA, G6135CA, G6135BA	70
TOF	6230	26
QTOF	G6500 Series	55
QqQ	G6400 Series	55

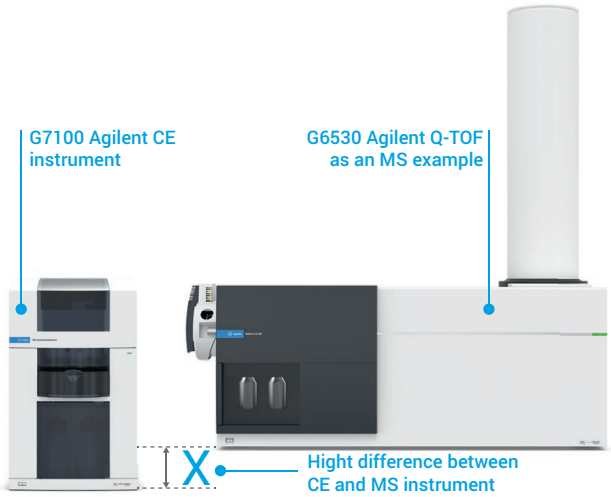


Figure 9. Height of the sprayer above bench level for Agilent LC/MS instruments.

11.2 Preparing the CE/MS outlet of the 7100 CE instrument

The 7100 CE instrument has a movable closure (or latch) at the right on the right side of the outer face that can be slid down (Figure 10). The opening allows the user to install the CE/MS capillary cassette.

To prepare the instrument, do the following:

1. Open the instrument lid.
2. Bend the movable closure (or latch) slightly inwards.
3. Move the closure (or latch) down until the mechanical stop.

Note: Do not take the movable closure (or latch) out of the instrument.

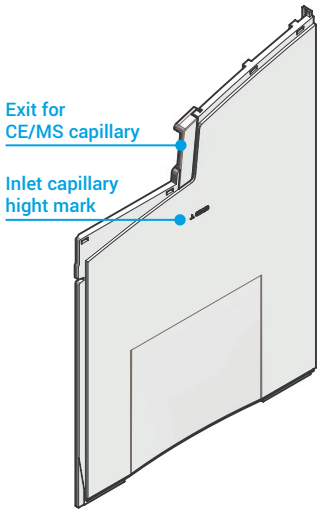


Figure 10. Right side of the Agilent 7100 CE instrument.

11.3
Connections at the back panel of the 7100 CE instrument

Installing the ground cable

A cable for grounding both instruments to a common electrical ground is provided in the CE/MS adapter kit.

1. Connect one end with a cable lug to a screw of the electronics box in the back of the 7100 CE instrument (Figure 11).
2. Connect the other end with a crocodile clip to a metal part of the ion source or to the installed ESI sprayer.

Establishing the CAN connection to the Agilent InfinityLab pump

1. Plug a CAN cable to the CAN slot of the electronics box in the back of the 7100 CE instrument (Figure 11).
2. Connect the other end to the CAN slot of the isocratic pump once located in the vicinity.

Note: Do not confuse LAN and CAN adaptor slots on the CE. Mixed up connections might damage the electronics.

Establishing the remote-control connection to MS instrument

1. Plug a remote start control cable in the respective slot of the electronics box in the back of the 7100 CE instrument (Figure 11) labeled with the word Remote.
2. Connect the other end of the remote cable to the respective slot of the MS instrument (Figure 12, similar for other 6000 MS Series).

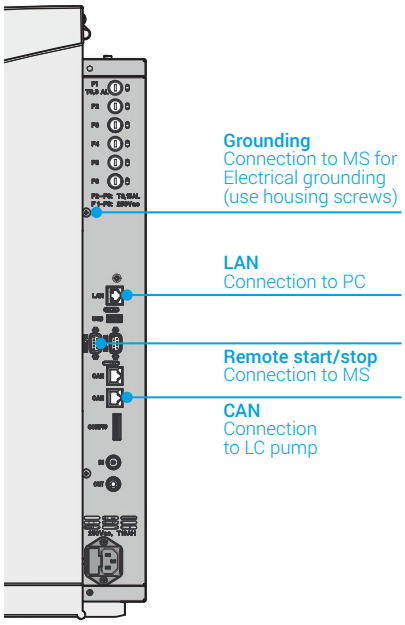


Figure 11. Connections on the rear side of the Agilent 7100 CE instrument.

Preparing the LAN communication of the CE/MS system

Typically, the PC controlling the CE/MS system harbors two LAN cards. One is for control of the 7100 CE instrument. Often the other one connects the computer to the local area network.

1. Plug a LAN cable to the respective slot of the electronics box in the back of the 7100 CE (Figure 12) labeled with the label LAN.
2. Plug a LAN cable to the respective slot in the MS instrument (Figure 12).
3. Connect both instrument LAN cables to the network hub.
4. Connect the network hub to the respective LAN card of the control PC.

The following IP addresses apply for setup with default addresses. See the respective installation for deviating setups, for example, when a BootP service is used.

The default IP address for the MS is 192.168.254.12

The default IP address for the CE is 192.168.254.11

The default IP address for the PC is 192.168.254.1 with subnet 255.255.255.0 and gateway 192.168.254.1 (which is the second LAN card in the acquisition PC, preconfigured on the recovery images).

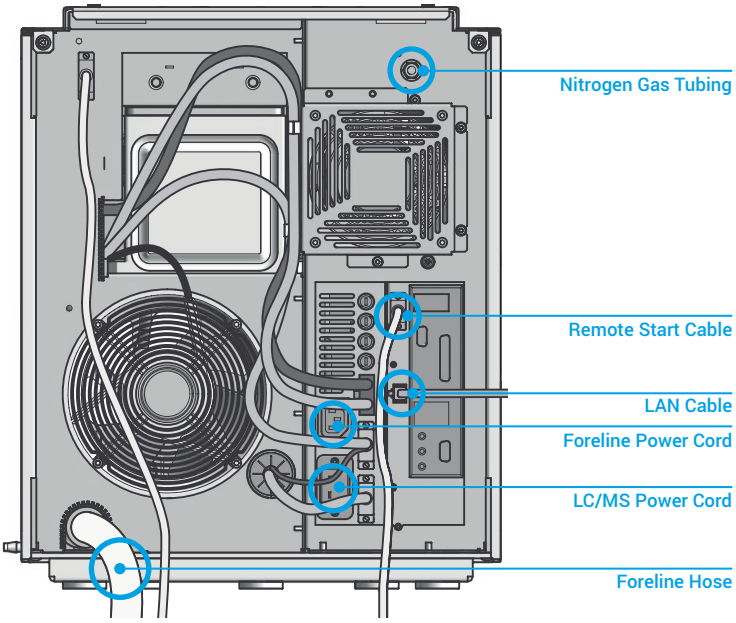


Figure 12. Rear side of an Agilent InfinityLab LC/MSD system as an example (refer to your MS user manual).

12 Setting up an Agilent CE/MS System with Agilent OpenLab CDS (ChemStation Edition) Software

The details provided here apply to the setup of Agilent CE/MS System with OpenLab CDS (ChemStation Edition) software. For experimental details of coupling the 7100 CE instrument with Agilent Time of Flight, Triple Quadrupole, or Quadrupole Time of Flight Series LC/MS systems, refer to Chapter 13 "Setting up an Agilent CE/MS System with Agilent MassHunter Software."

12.1 Installation of the Agilent CE/MS ChemStation software add-on module

OpenLab CDS (ChemStation Edition) software for CE/MS can be ordered with a PC or separately for use with another PC. Use the instructions given in the ChemStation installation guide or in the Agilent InfinityLab LC/MSD Series system installation guide if the complete bundle was not ordered. Agilent CE/MS ChemStation software allows you to control the 7100 CE instrument, manage CE/MS methods, set up sequences for CE/MS runs, acquire CE/MS data, and run data analysis.

The following steps describe the installation, configuration, and licensing steps for setup of OpenLab CDS (ChemStation Edition) software.

1. Install the software by running the application from the DVD supplied with the system.
2. Run the system configuration checker.
3. Run the OpenLab CDS Installation Wizard (make sure that you include I/O Libraries when given the choice of products to install)
4. Connect to Agilent.Subscribenet.com and use the authorization codes to obtain the license numbers.

License numbers are provided through the SubscribeNet that can be accessed using the Internet address Agilent.Subscribenet.com. The CE/MS installation requires an installed I/O Library. After the installation is complete, the Instrument Editor is displayed.

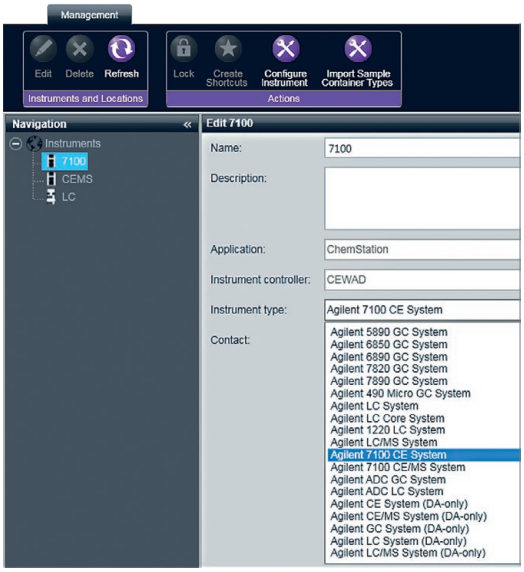


Figure 13. Agilent OpenLab CDS C.01.x (ChemStation Edition) Instrument Configuration screen.

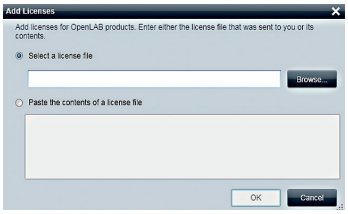


Figure 14. Licenses setup screen (Agilent.SubscribeNet.com).

12.2 Configuring the 1260 Infinity II Isocratic Pump

When the pump is connected by a CAN cable to the 7100 CE instrument, it can be configured in ChemStation either by automatic configuration or by selectively adding the pump in the configuration editor. Automatic configuration is recommended.

1. From the OpenLab Control Panel, select Configure Instrument.
2. Go to the Instrument Configuration screen, Figure 15. This either happens automatically during ChemStation startup or requires you to select Instrument configuration from the Instrument menu. The IP address of the 7100 CE instrument will be set automatically.
3. Review if the outcome is in line with Figure 16. Four modules are available in this example (CE, DAD, isocratic pump, and MSD).
4. Launch the instrument session (online).

Note: Always configure the DAD with the system even if you wish to remove it later from the ChemStation user interface. See section 17.3 “Method setup for CE/MS analysis without DAD” for more information.

Optionally: If you wish to selectively add the isocratic pump from the configuration editor, you need to do the following:

5. Select the IsoPump icon in the Configurable Modules list, see Figure 17.
6. Click the arrow to move the module to Selected Modules.
7. Select the IsoPump icon in the Selected Modules list.
8. Click Configuration.
9. Under Communication, enter the serial number of the pump
10. Click OK.

You will have to close and restart the online copy of ChemStation to activate the settings if you started editing the configuration from the Instrument > Instrument configuration menu. An option to change the instrument mode without the need for a restart of the ChemStation exists if you select Instrument > More CE > Instrument configuration.

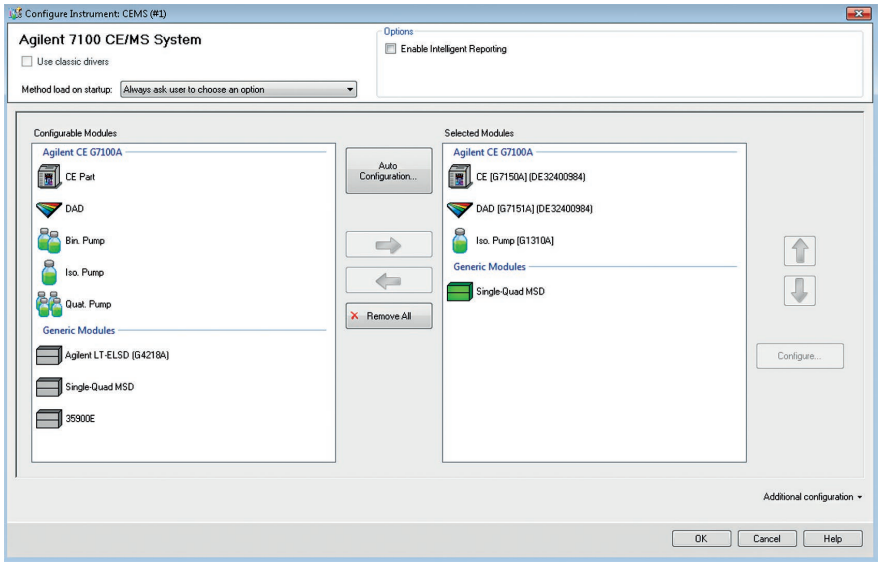


Figure 15. Agilent 7100 CE/MS system configuration.

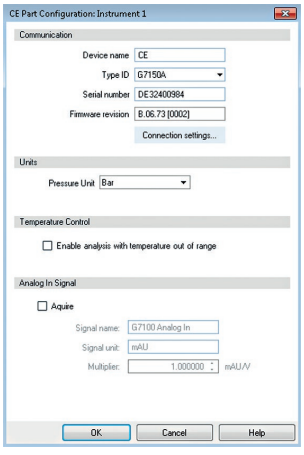


Figure 16. Configuration of the Agilent 7100 CE instrument.

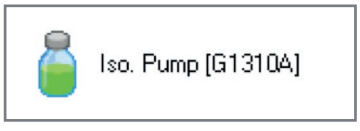


Figure 17. Select the IsoPump icon in the Configurable Modules list.

12.3
Configuring the MS

1. In the Configuration Editor, select Single-Quad MSD, see Figure 18.
2. In the Configure Selected Module dialog, click OK to accept the given IP address.
3. Close the Configuration Editor.
4. Next time you start the online ChemStation for this instrument, an MSD configuration dialog will be displayed, see Figure 19.
5. In the Configured Modules list, select the default module and click Remove.
6. In the Available Modules list, select the instrument model you have and click Add.
7. Click OK.

See the MS manual for information on how to start the instrument, how to equilibrate temperature and vacuum, and how to autotune before the first CE/MS experiments.

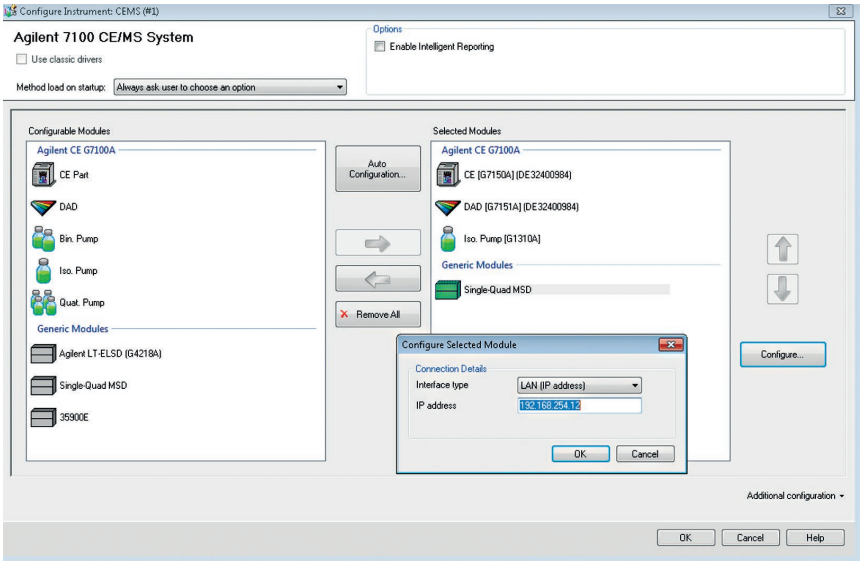


Figure 18. Selecting Single-Quad MSD in the Configuration Editor.

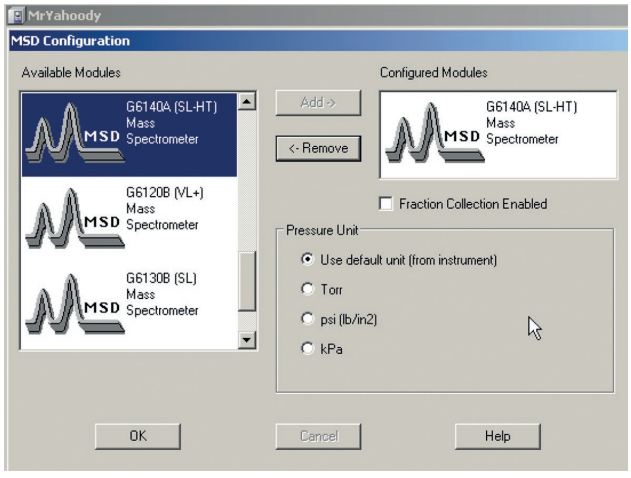
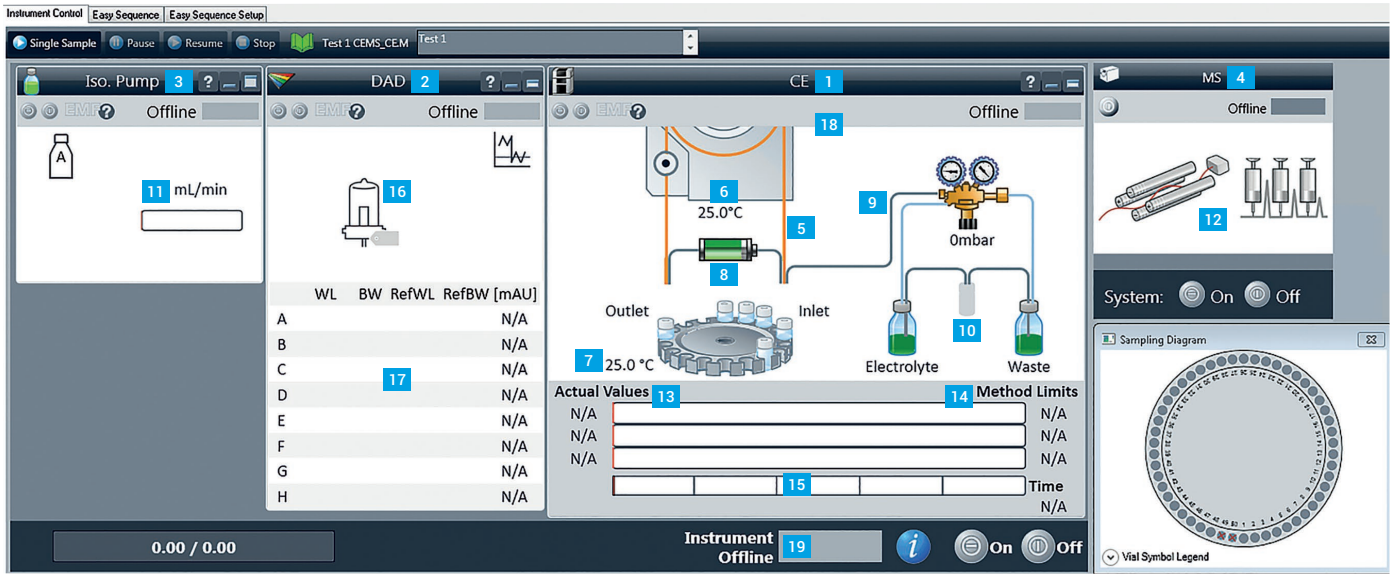


Figure 19. MSD configuration.

12.4 Explanation of the graphical user interface

The core components of the Agilent CE instrument in a CE/MS system are shown in the CE control diagram that is also called the dashboard, see Figure 20. All other components of the CE/MS system such as the DAD, pump, or MS are represented by an individual diagram. The current analysis mode is displayed in the top line of the CE control diagram. The analysis modes that can be selected under instrument configuration are CE, CE+p, CEC, or CE/MS. Changes in the current CE mode for your instrument can be made under Instrument > More CE > Instrument Configuration (see Figure 20).



1	CE control diagram	8	High voltage power supply	15	Method steps and progress
2	DAD control diagram	9	Internal pressure	16	D2 lamp
3	Isocratic pump	10	Replenish station	17	Detector wavelengths/spectra
4	MS	11	Pump actuals	18	Instrument mode
5	Inlet lift/loaded vial	12	MS parameter access	19	Overall instrument status
6	Cassette temperature	13	Actual electrical values		
7	Tray/Tray temperature	14	Electrical parameter from method		

Figure 20. User interface for a CE/MS system with CE, DAD, pump, and MS.

12.5 Setting the CE mode

Despite the configuration explained above, you might wish to conduct analysis in other CE modes such as the normal CE mode (for example, for zone electrophoresis), CE+p (for gel applications), or for CEC. If so, it is not required to go to the 7100 CE Configuration menu that requires a restart of the ChemStation to activate the setting, see Figure 15. You can navigate to a configuration-selective dialog through Instrument > More CE > CE Configuration, which does not require you to restart the ChemStation (see Figure 21).

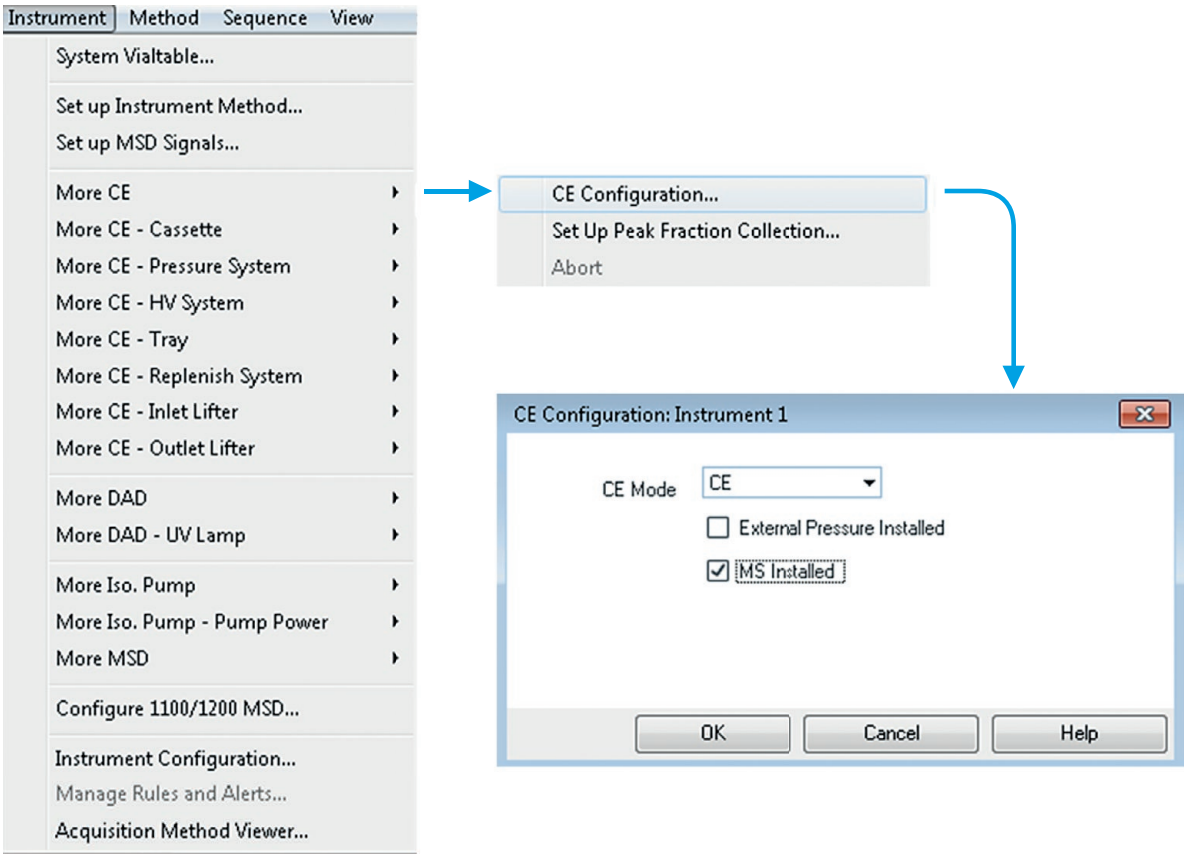


Figure 21. Selective configuration of CE mode.

13 Setting up an Agilent CE/MS System with Agilent MassHunter Software

The driver for the Agilent 7100 CE instrument was integrated in Agilent MassHunter software starting with version B.05.01 for TOF/QTOF and version B.06.00 for QQQ. The configuration of the entire CE/MS system is done through the Configuration tool.

1. Click Start > All Programs > Agilent > MassHunter Workstation > Acq Tools > Instrument Configuration.
2. In the Instrument Configuration screen (see Figure 22):
   a. If you want to change the name of the instrument, type a new name under Instrument name.
   b. Select the Mass Spectrometer check box.
   c. Click the option for your MS instrument.
   d. Select the Agilent LC (1100/1200/1260/1290) / CE check box.
   e. Click Agilent CE System Access.
   f. Click Device Config and configure the CE system (see Figure 23).
   g. Click OK.
3. When configuration is completed, click OK.

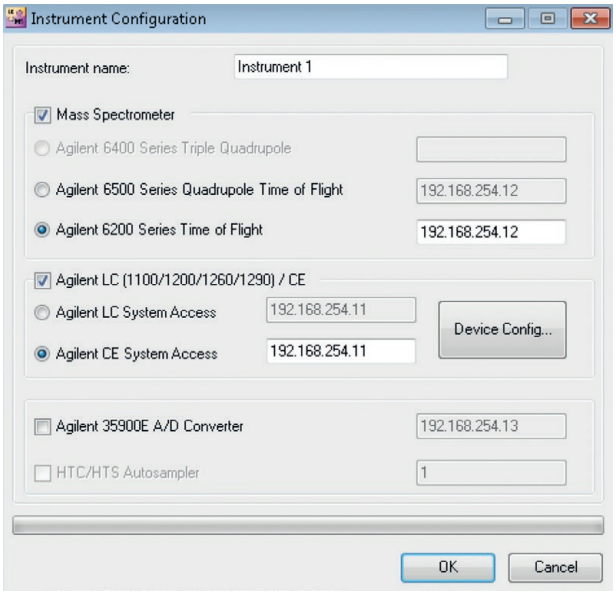


Figure 22. Instrument Configuration screen.

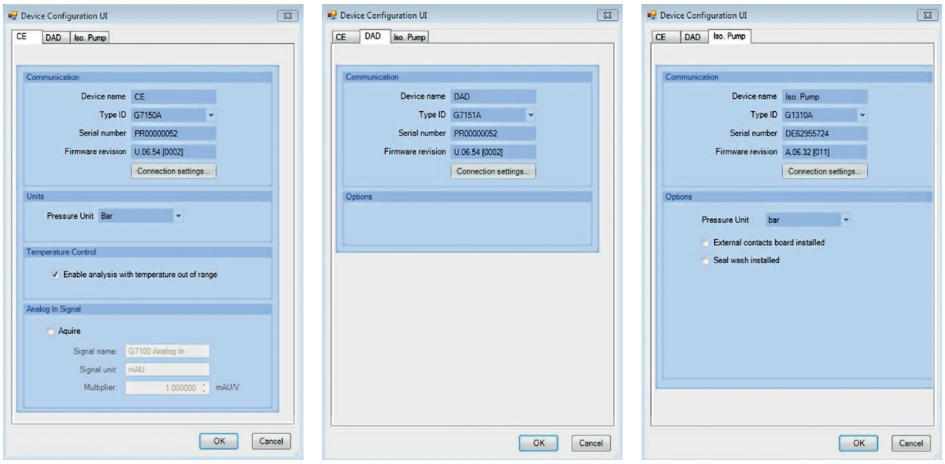


Figure 23. Screens for device configuration of the CE instrument, DAD, and isocratic pump.

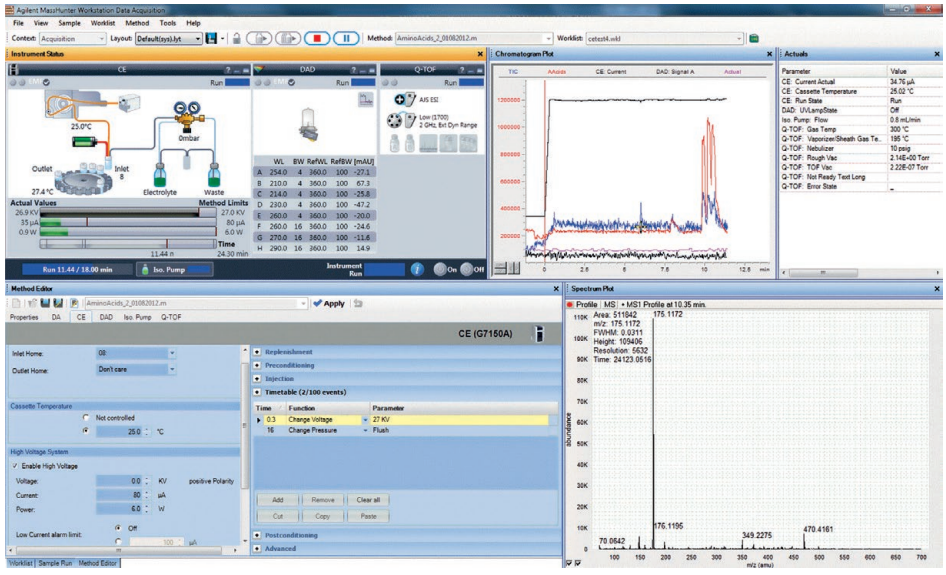


Figure 24. Graphical CE/MS system user interface under MassHunter.

The setup of the graphical user interface for the CE instrument is identical to OpenLab CDS (ChemStation Edition) (see Figure 24). The same is true for the method settings screens that can be accessed through the Method Editor. CE and MS signals such as TIC, EIC, UV-traces, and signals of other external detectors (for example, LIF) and parameters such as CE current and voltage can be displayed in the Chromatogram Plot. Online MS spectra and the setup of the MS instrument and other LC modules (such as the isocratic pump) are handled as in the standard MassHunter LC/MS software.

Changes of the current CE mode for your instrument can be made under Tools > Device Configuration (see Figure 25).

When running a CE/MS worklist, it is important to be aware about the Worklist Run Parameter Wait Time for Ready. This parameter defines the maximum time in minutes to wait for each device to be ready for injection once the method setpoints are sent to the instrument.

If a device is not ready within the specified time, then the run stops with an error. This prevents the system from waiting indefinitely when there is a problem. Be sure to set this value long enough to account for all the time required by capillary preconditioning. There is a "not ready timeout." By default, this is often 10 minutes. But when the preconditioning takes longer, the system will stop without a real error message.

To change the Wait Time for Ready parameter, you need to do the following (see Figure 26):

1. In the Agilent MassHunter Workstation Data Acquisition software, click Worklist > Worklist Run Parameters…
2. The Wait Time for Ready (min) is on the tab Page 1 at the bottom under Run Settings.
3. Type in your desired value.
4. Click OK.

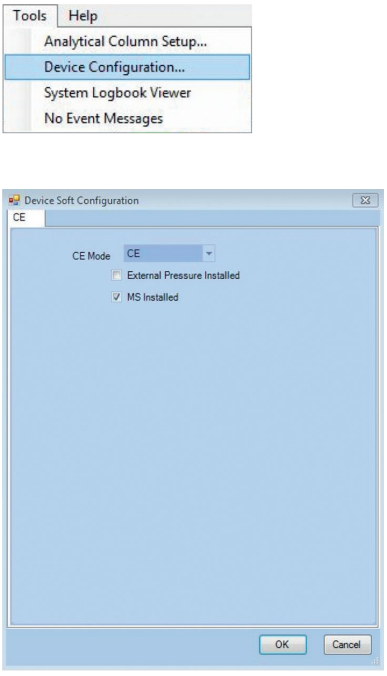


Figure 25. Selective configuration of the CE Mode.

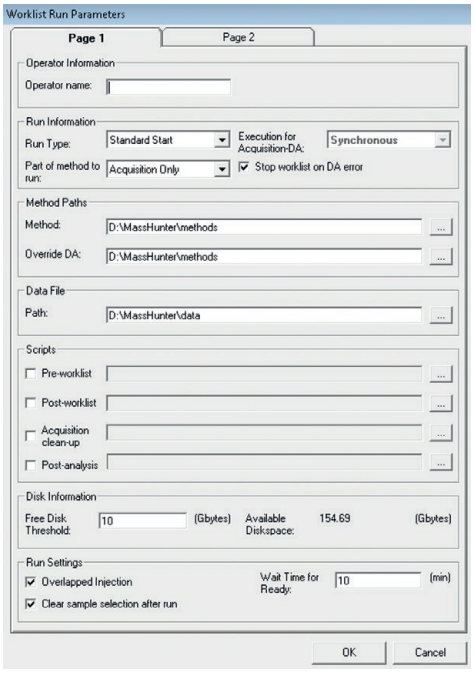


Figure 26. Worklist Run Parameters screen.

14 Installing the Capillary and the CE/MS Cassette

14.1 Preparing the capillary

Bare fused silica capillaries for CE/MS with 50 µm inside diameter and a length of 125 cm are available from Agilent (p/n G1600-67311, 2 per pack). The capillaries are available with a UV window (to use UV detection together with MS) or without a UV window (for MS only analysis). Agilent CE/MS capillaries have the polyimide outside coating removed at both capillary ends. Besides a typical initial alkaline conditioning step, which is described later, in principle the capillary can be used without further adaptation. However, for two reasons, adaptation of the capillary length might be desired; faster analysis times and/or skipping DAD detection.

To achieve a faster run time, a shorter capillary connection to the Agilent mass selective detector can be established. Up to 45 cm can be cut off from the long end of the above CE/MS capillary with a remaining minimum length of about 80 cm. Be careful not to shorten the capillary too much. Shorter length will hamper the installation of the capillary in the sprayer and the insertion of the sprayer in the MS source.

Be aware the shorter the capillary the higher the electrical field strength is. In CE/MS, the DAD is closer to the inlet (high voltage) than under normal CE conditions. In the area of the detector, the material is at electrical ground. This means the voltage difference (from inside the capillary to the detector body) is relatively high in relation to normal CE conditions and therefore the risk of arcing events, which can damage the capillary, is high. Please use voltage ramps at the start of runs.

To skip DAD detection, you might want to prepare a capillary from a capillary reel (for example, 160-2650-5, 50 µm, 5 m fused silica) which has no window for UV detection. Such a capillary is not led through the DAD cell compartment and therefore special settings in the DAD part of the method are used (see section 17.3 “Method setup for CE/MS analysis without DAD”). A typical length for this approach is 70 to 100 cm.

Cutting and preparation of the fused silica separation capillary

Fused silica capillaries can be cut to the desired length with a diamond-blade capillary cutter (CE column cutter with rotating diamond blade, p/n 5183-4669). It is highly recommended to use this tool.

The importance of a smooth flat end of the CE capillary is well documented in the literature. Use a magnifying glass to confirm that a smooth section is obtained. The shape and stability of the spray depend on the quality of the cut. Ordinary scoring devices gather and rip the coating of the capillary. Jagged edges are left that prevent a perfect spray and can act as adsorptive sites for sample components (Figure 27). For details, see the manual supplied with the CE column cutter.

Warning: To avoid injury, always wear safety glasses when handling the capillary and the cassette.

Relevant details for cutting preparation of the capillary for CE/MS are as follows:

1. Clean the surface thoroughly with acetone.
2. Open the locking wheel.
3. Insert the capillary in the CE column cutter while pressing the blade release button (red dot).
4. Fix the capillary with the locking wheel.
5. Release the blade release button (red dot).
6. Cut by turning the wheel 2 to 6 times in one direction until the capillary breaks, avoid tension during cutting.
7. Inspect the end to ensure it is flat and orthogonal to the capillary direction.

Figure 27. Capillary cutting examples.

If a CE column cutter is not available, you might follow the procedure using a simple scoring device (ceramic scribe column cutter, p/n 5181-8836, 4 per pack) as described below.

1. Place the capillary over a large radius surface under slight tension.
2. Hold the scribe at an angle of approximately 30° to the capillary.
3. Draw the edge of the scribe across the capillary penetrating the polyimide.
4. Pull the capillary horizontal until it breaks. If the capillary will not break, the polyimide has not been cut. Repeat above steps.

In addition, it is important to remove the outside polyimide coating on the separation capillary inlet and outlet side. High content of an organic solvent in the BGE and/or in the sheath solvent, especially acetonitrile, will cause the polyimide to swell and detach from the capillary outside. On the inlet side, this will prohibit the introduction of a narrow sample zone. On the outlet side, in the sprayer, stable spray formation may be hindered. This will result in decreased sensitivity and instable CE and ESI currents.

Remove the polyimide from the capillary tips by using the window etching tool (590-3003, acidic treatment) or by burning away with a micro torch. Melting of the fused silica must be avoided. If using a coated capillary in the CE separation, shorten the burning time as much as possible or refrain from using a micro torch (see Figure 28).

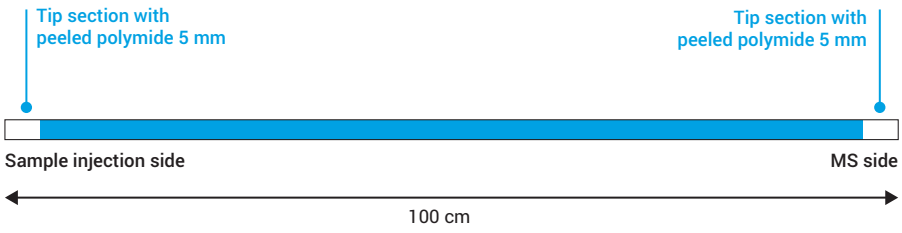


Figure 28. Example of a CE/MS capillary with polyimide-free tips.

14.2 Installing the capillary in the CE/MS cassette

As explained in section 9.4 "Electric field and polarity," the DAD detection zone in Agilent CE/MS systems is relatively close to the inlet (high voltage) compared with standard CE operating conditions. In the detection cell compartment, all walls are on electrical ground.

Therefore, in this configuration, the voltage difference (from inside the capillary to the detector body) is relatively higher in relation to normal CE conditions. Although fused silica is an excellent insulator, still a risk of arcing exists, which should be avoided. For that reason, an alignment interface made from an insulating material, PEEK must be used. Do not use the standard alignment interface types used for CE with DAD (which have a metal rim).

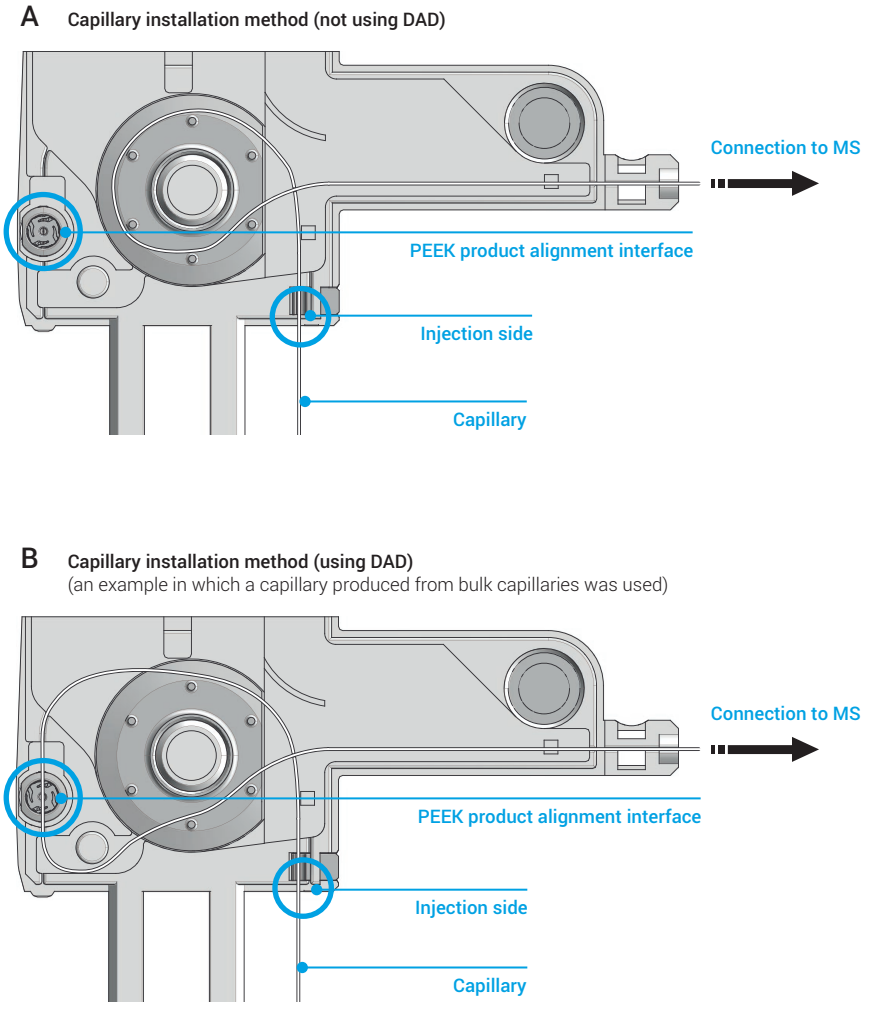


Figure 29. Position of the capillary in the CE/MS cassette.

Procedures for inserting the capillary into the alignment interface, installing this in a cassette and inserting the cassette into the CE are described in the User Manual for the 7100 CE System (p/n G7100-90000).

- In contrast to the regular CE mode, a nonmetal (PEEK) alignment interface is used.
- A different capillary cassette is used for CE/MS applications, allowing the CE capillary to exit the cassette
- When using a DAD, the point of detection is at 21.6 cm from the inlet.
- The overall length of the capillary must be sufficient to reach the MS. The minimal total length of the CE capillary to exit the CE instrument should be 80 cm to allow UV detection.

1. Insert the capillary as shown in Figure 29B if it should pass the UV window. Alternatively, when skipping UV detection, mount the capillary as in Figure 29A and decrease the total capillary length until the MS detector.
2. Cut the PTFE tube to an appropriate length to cover the exposed capillary between the end of the CE/MS cassette and the inlet of the MS. Place the PTFE tube over the capillary and screw it on tightly at the CE/MS cassette.
3. Insert the CE/MS cassette in the same position as for the regular CE mode. Make sure that the long part of the capillary stays outside the instrument after closing the top cover and is not damaged.

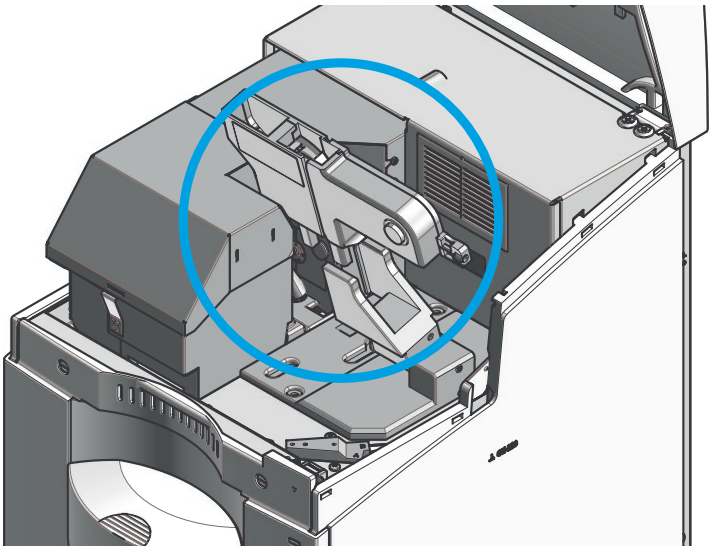


Figure 30. Inserting the CE/MS cassette.

15 Preparing the Agilent 1260 Infinity II Pump and Vacuum Degasser

15.1 Hydraulic connections

The following steps are required if the pump and degasser are used.

1. Install the pump and the degasser (optional) as described in their installation documentation. Otherwise an infusion pump must be installed and connected.
2. The sheath flow splitter (included in the Agilent CE/MS sprayer kit) must be connected to the pump outlet. The following section describes the setup.
3. Install the flow splitter (p/n G1607-60000) in the isocratic pump. Put the two rubber plugs (p/n 1520-0401) from the Agilent CE/MS sprayer kit into the sheet metal as shown below in Figure 32 and use the two screws (p/n 0515-0982) also included to mount the splitter.
4. Connect the tubing labeled pump to the pump outlet, Figure 31. The waste tubing may be drawn back into the sheath liquid bottle to reuse the solvent.
5. Leave the tubing labeled Out disconnected from the sprayer but place its end in a beaker. It will be connected later.
6. Fill the sheath liquid in solvent bottle A of the pump. Connect the tubing of bottle A to the degasser (Figure 34). Open the purge valve and flush the pump at 2 mL/min (100 %A) for at least 10 min. Reduce the flow rate to 0.4 mL/min and close the purge valve. The splitter splits the sheath liquid in the ratio 1:100, so the sheath liquid flow rate will be 4 µL/min. If a degasser is not used, the sheath liquid must be degassed before use, and the flush time reduced. The backpressure at 0.4 mL/min is approximately 40 bar.

15.2 Setting the pump parameters

- Flow: 0.400 mL/min (1:100 flow splitter = 0.004 mL/min)
- %B: 0
- Active channel: A
- Maximum pressure/flow: 400 bar

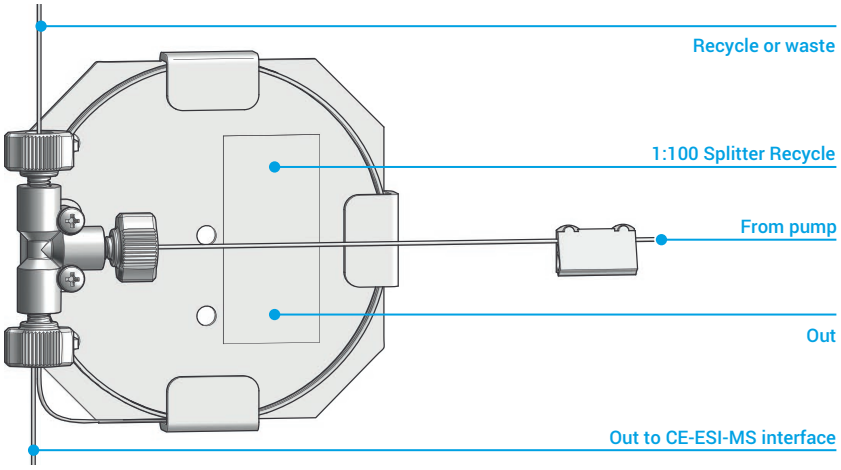


Figure 31. The sheath flow splitter.

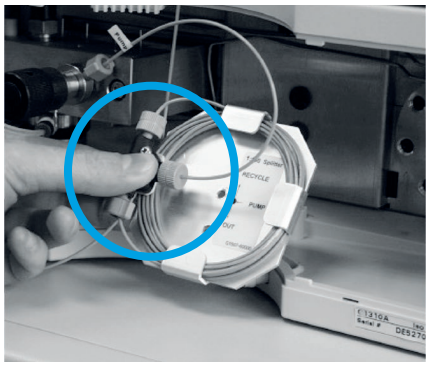


Figure 32. Installing the flow splitter.

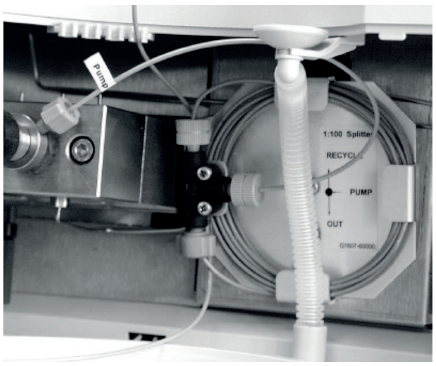


Figure 33. Connections of the flow splitter.

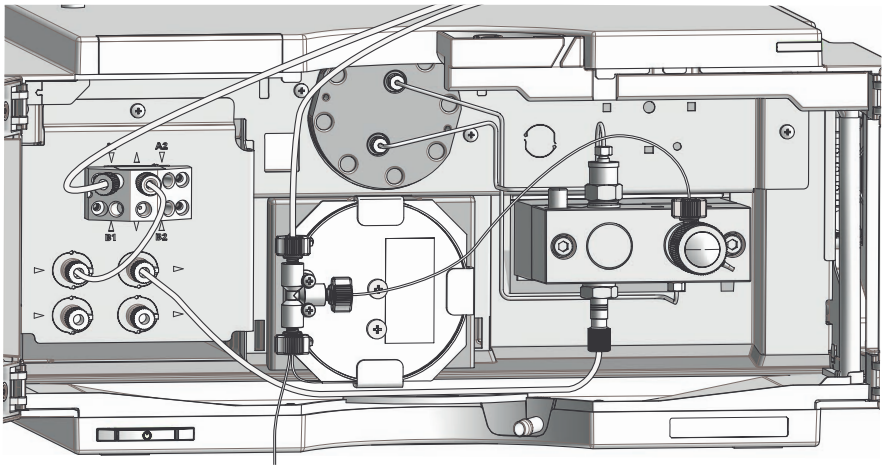


Figure 34. Connection of the splitter unit into an Agilent 1260 Infinity II Isocratic Pump with integrated Degasser

16 Installing the Triple Tube Sprayer

Chapter 16, Chapter 17, and Chapter 18 describe the setup of a CE/MS system with a Agilent InifinityLab LC/MSD Series system and OpenLab CDS (ChemStation Edition) software for instrument control. Examples of setpoints for ion source and data acquisition parameters of Agilent Time of Flight, Triple Quadrupole, and Quadrupole Time of Flight Series LC/MS systems can be found in Agilent Application Notes.

16.1 Sources for CE/MS

At this point, essential information pertaining to the triple tube spray is given to enable use of the proper sprayer with the available sources. Given the different ionization modes that are available for Agilent LC/MS systems (see section 9.7 "Types of ionization – ESI, APCI, and APPI"), constraints in the use of the CE triple tube sprayer in the available sources exist as a consequence of their development over the past years. Table 3 shows the compatibility of sources and sprayer.

Figure 35 shows the design differences between the earlier A and newer B version of the sprayer.

In reconfiguring an LC/MS system to a CE/MS system, it is imperative to be aware of the above compatibility matrix and select the proper source and sprayer.

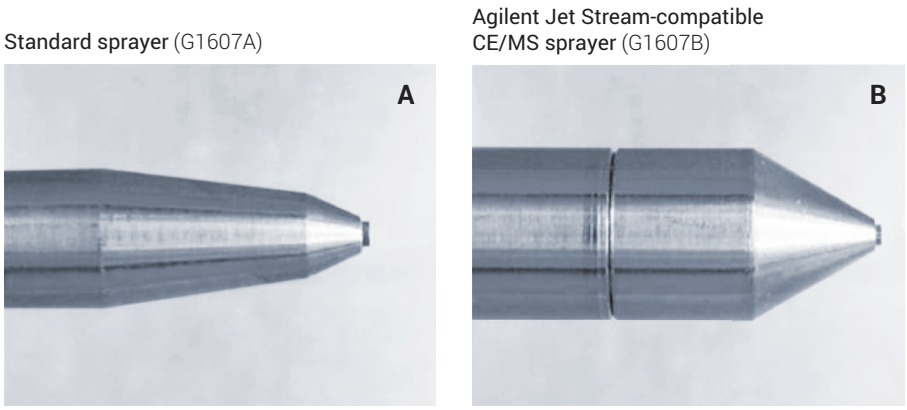


Figure 35. A and B versions of the CE/MS sprayer .

Table 3. Compatibility of ionization mode/source and sprayer

Ionization Mode/Source	Sprayer		Comments
	G1607A	G1607B	
Electrospray (AP-ESI, G1948B)	Yes	Yes	
Agilent Jet Stream (G1958A)	No	Yes	G1607A cannot be used with Jet Stream sources
Chemical Ionization (APCI, G1947B)	Yes	Yes	Requires metal spacer, available on request from Agilent
Photoionization (APPI, G1971B)	Yes	Yes	Requires metal spacer, available on request from Agilent
Dual-Mode Ionization (CI+ESI, G1978B)	Yes	Yes	

16.2 Inserting and adjusting the CE capillary in the CE/ESI-MS sprayer

The API electrospray source must be installed, see appropriate documentation. It is assumed that the Agilent LC/MS system has been successfully tuned for single-charged molecules.

1. Remove the protective plastic tube from the sprayer tip. Turn the adjustment screw counterclockwise (+ direction) until its mechanical stop. Then turn two complete turns clockwise (– direction).
2. Open the fitting for the CE capillary and insert the capillary. Fix the capillary so that it still can be moved up or down. The capillary should be aligned flat with the sprayer tip. You can use your finger nail.
3. Finally tighten the fitting screw tight enough to keep the capillary in place. The relative position of the capillary in the sprayer must be kept over all the next manual steps until the run, otherwise misalignment will happen with subsequent low performance.
   Avoid touching the sprayer tip while handling the sprayer. The ESI needle is fragile. Keep the protective sleeve on as long as possible and protect it again as soon as possible. Exchange ESI needle if it was found to be damaged.

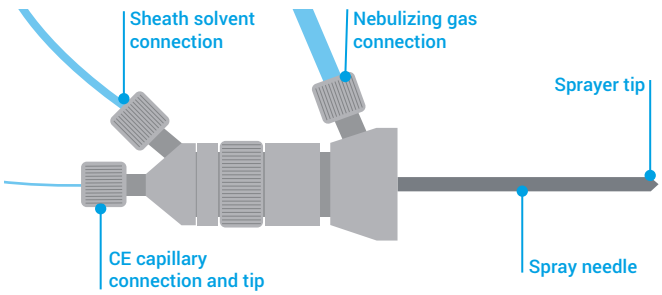


Figure 36. The Agilent CE/ESI-MS sprayer.

4. Turn the adjustment screw a quarter of a turn counterclockwise (two marks in + direction). Finally, the capillary should protrude approximately 0.1 to 0.2 mm out of the sprayer tip.
5. Check the length of the protruding capillary carefully using a microscope, it should be one third of the od of the capillary, see Figure 40. The outside diameter of the capillary is 0.365 mm.
   Despite the normal adjustment with two marks in + direction the optimum might be different for other spray conditions, such as sheaths liquid with different viscosity. Try four marks in + direction, for example, if you see an irregular baseline or sputtering effects.

16.3 Inserting the CE/ESI-MS sprayer

1. Connect the nebulizing gas and the sheath liquid to the lower part of the CE-ESI sprayer.
2. Remove the cover of the ion source. Carefully insert the CE sprayer into the electrospray chamber of the MSD. Do not touch the electrospray chamber with the sprayer tip because it can damage the sprayer tip easily. Do not hold the sprayer at the adjustment screw while inserting it into the ion source as it can misalign the sprayer.
3. Mount the ion-source cover included in the Agilent CE/MS sprayer kit.

16.4 CE capillary conditioning

Before first use, a new bare fused silica separation capillary should be properly conditioned. A procedure including a 5 min, 1 N NaOH flush, followed by a 10 min flush with water, and a 20 min flush with run buffer is appropriate for the analysis of the test sample. If this conditioning is skipped, the overall performance is reduced and especially the migration time stability will be affected. The conditioning can be carried out manually, or all steps can be programmed into a method and included in a sequence.

An example vial table is given in Table 4. Vial table entries can be made under Instrument > System vial table. Load vials in the following positions of the tray of the CE instrument.

Table 4. Vial positions for capillary conditioning and checkout

Position	Content
1	1 N NaOH
2	Water
3	Run buffer (10 mM ammonium acetate, pH 6.9, 0.04 % methanol)
4	Flush buffer (same as run buffer)
5	Test sample (1 mg/mL quinine sulfate dehydrate)

Figure 37. CE/ESI-MS sprayer adjustment screw.

Figure 38. CE/ESI-MS sprayer capillary insertion.

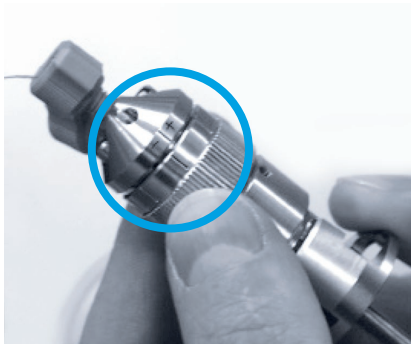
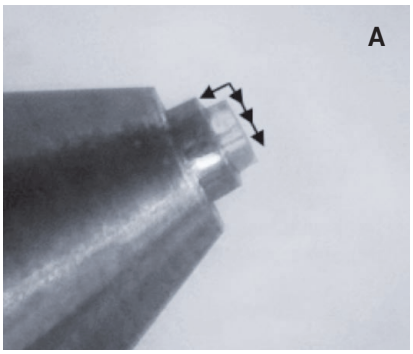
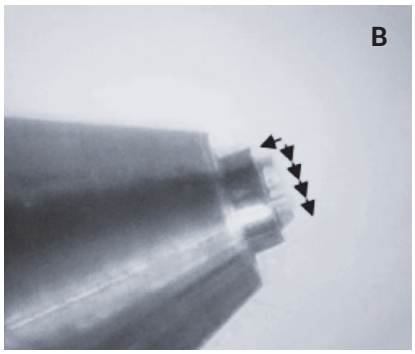


Figure 39. CE/ESI-MS sprayer capillary adjustment.





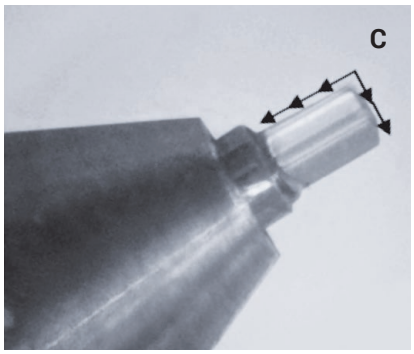


Figure 40. Protruding capillary from ESI needle, good (A, B), bad (C).

Figure 41. Nebulizing gas connection.

Figure 42. CE/ESI-MS sprayer insertion to MS source.

Figure 43. CE/ESI-MS sprayer completely installed.

The conditioning can be done while the sprayer assembly is removed from the source and is placed over a beaker to collect the waste. This is especially recommended when harsher conditions (concentration HCl, > 1 M formic acid or > 1 M NaOH) are used in the preconditioning procedure. Condition capillaries according to their specifications. Please be aware most coated capillaries can become irreversibly damaged by this.

To minimize contamination of buffer vials with conditioning solutions (such as NaOH) which might stick to the outside of the capillary or electrode, it is recommended to dip the inlet electrode in a large dedicated vial filled with buffer before letting the instrument load the actual inlet and outlet home vials with run buffer. This precaution can be programmed into the timetable by a combination of the commands “load vial” and “wait.”

16.5 CE current check

After the installation of the sprayer assembly and conditioning of the separation capillary as described before, a constant CE current under electrophoresis conditions is expected. Therefore, before you start a sample analysis, you might want to do a rapid test to confirm the adjustment of capillary as well as the sheath liquid supply is working. The CE current is a simple indicator for proper installation. The target of this conductivity checkout under ESI conditions is the electrophoresis part and not the MS part. The test steps can simply be executed from the CE/MS System diagram by clicking the relevant representation.

The ESI Voltage is off during this check.

Establish a flow of 0.4 mL/min for the isocratic pump.

1. Load a vial with run buffer to the inlet lift.
2. Flush buffer into the capillary for 5 minutes.
3. Apply 15 kV for 10 minutes.
4. Monitor the current in the online plot of the ChemStation.

The CE instrument does an automated ramp of 6 kV/s and is therefore after 3 s at 15 kV. After a short time, the current should become stable as well. The absolute current will vary with the actual capillary length. It will be in the range of 1 to 2 µA for the ammonium acetate buffer system and an 80 to 125 cm, 50 µm id fused silica capillary. See signal for current during a checkout run in Figure 44.

Be aware that substances inside the sprayer body, pump, degasser, or the tubing might be washed out over time and might affect CE/MS experiments for quite a while until they are completely washed out. Also, the outside of the capillary, which is exposed to the sheath liquid stream inside the sprayer, might be washed out over a comparable long period. The long timeframe for equilibration is due to the low flow rate for the sheath liquid.

16.6
CE/MS Check-out method

Please load the method def_CE.M in the instrument context to the online ChemStation and program the method parameter shown in Table 5. The instrument mode should be CE/MS with no low current alarm limit.

Establish and run a sequence with five consequent injections from the vial with the test sample. Due to the equilibration of the system the first run of the sequence may not be successful and should be discarded. The following runs should result in UV and MS traces like those shown in Figure 45.

MS spectra taken from the peak in the MS trace should be like the one shown in Figure 46. The CE current should be approximately 4 to 7 µA (dependent on capillary length and actual buffer concentration).

Table 5. CE/MS analysis condition for test sample.

CE	Capillary	Fused silica capillary (50 µm id, total length 80–125 cm, UV detection window, conditioned with NaOH)
	Buffer	10 mM ammonium acetate, pH 6.9, 0.04 % methanol
	Voltage	0 kV
	Temperature	20 °C
	Replenishment	None
	Preconditioning	5 min flush from vial 4, run buffer
	Sample	Agilent checkout sample (quinine sulfate)*
	Injection	Pressure 50 mbar for 2.0 s, vial 5, test sample
		Pressure 50 mbar for 2.0 s, vial 3, run buffer
	Time programming	0.3 min voltage +27 kV
	Stop time	10 min
DAD	Signal	254.16 nm
	Reference	450.80 nm
	Spectra	190–450 nm, every second
	Autobalance	Prerun
	Stop time	As CE
	Lamps on for acquisition	UV lamp
Pump	Flow	0.4 mL/min
	Stop time	No limit
	Solvent A	100 %, 5 mM NH_4Ac, pH 6.9 in 50 % MeOH
	Pressure	0–400 bar

MS	Use MSD	Enabled
	Stop time	No limit
	Ion source	AP ESI
	Scan range	m/z=100–500
	Time filter	Enabled
	Data storage	Condensed
	Peak width	0.12 min
	Signal 1	
	Mode	Scan
	Range	m/z=100–500
	Polarity	Positive
	Fragmentor voltage	65 V
	Threshold	50
	Step size	0.01
	Signal 2	
	Mode	SIM
	Polarity	Positive
	Cycle time	50 % cycle time
	SIM ion	325
	Fragmentor voltage	65 V
	Spray chamber	
	Gas temperature	130 °C (max 350 °C)
	Drying gas	10.0 l/min (max 13.0)
	Nebulizer pressure	10 psi (60 max)
	V_{Cap}	4000 V

**Experienced users may want to use other pure standards like amino acids.*

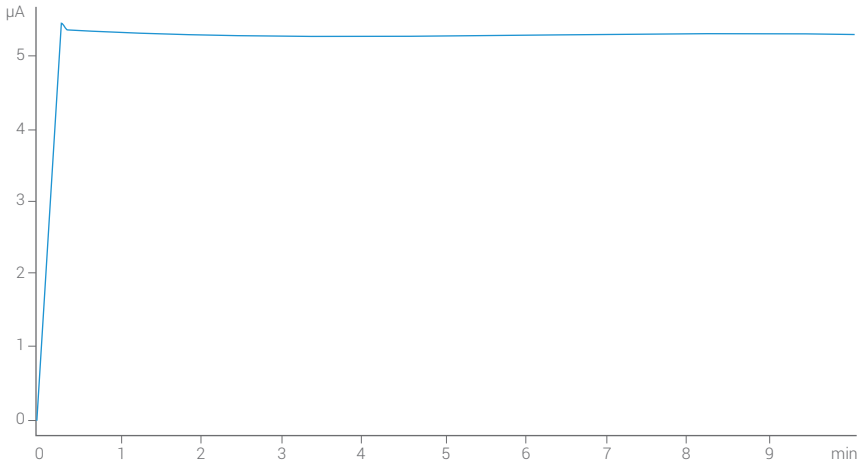


Figure 44. Current stability during a checkout run at 27 kV.

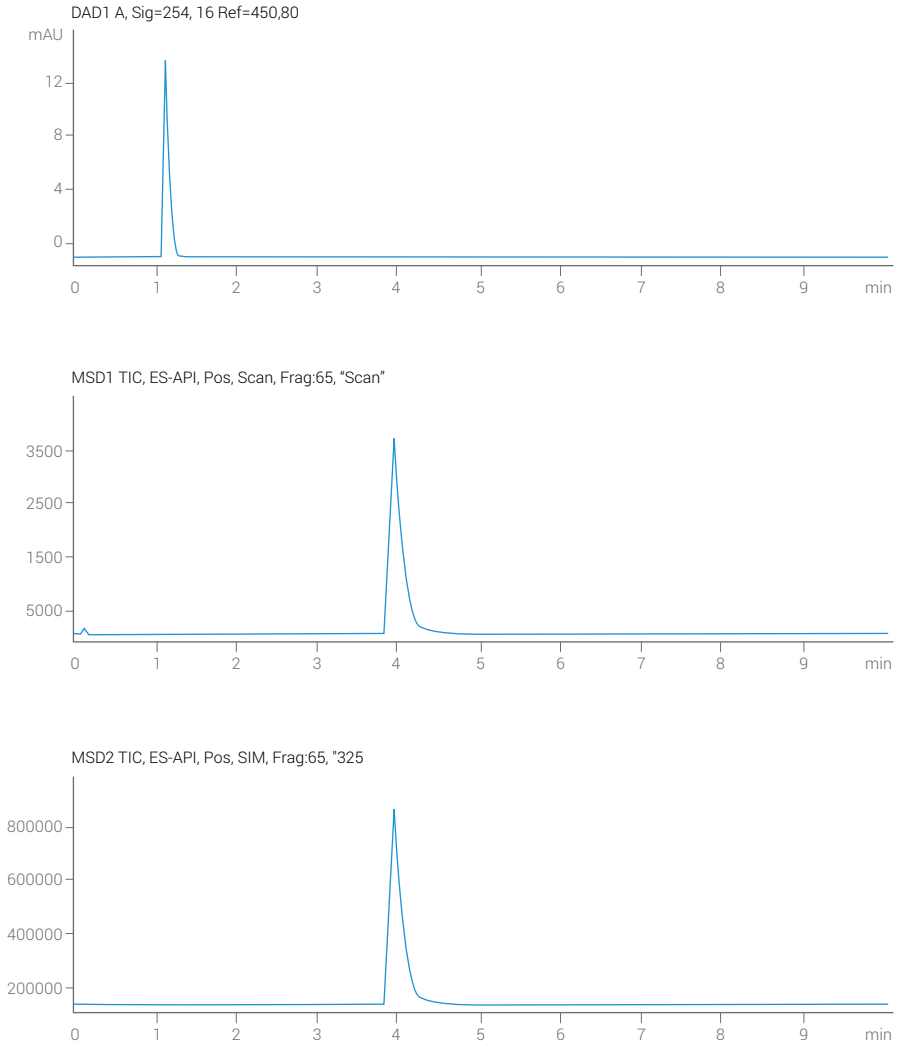


Figure 45. UV, scan and SIM traces for MS.

16.7 Storing the CE/MS capillary, sheath flow splitter, and CE/MSD sprayer needle after use

To avoid plugging of the capillary, the sheath flow splitter and the CE/MSD sprayer needle need to be cleaned when not in use. Clean the sheath flow splitter and CE/MSD sprayer needle as follows.

1. Replace the sheath liquid bottle with a bottle filled with water.
2. Leave the CE/MS capillary, the sheath flow splitter, and the nebulizing gas connected.
3. Prime the pump and flush for 10 min, pump water through the sheath flow splitter and CE/MSD sprayer needle.
4. Replace the water in the sheath liquid bottle with isopropanol.
5. Prime the pump and flush for 10 min, pump isopropanol through the sheath flow splitter and CE/MSD sprayer needle.

Store the CE/MS capillary as follows.

1. Flush the capillary with water for 10 min.
2. Insert an empty vial with a cap into the tray and flush the capillary with air for 10 min.
3. The capillary can now be removed and stored.

Heat damage of the sprayer needle might come from MS drying gas when no sheath liquid is flowing. Do not leave the instrument idle at high drying gas temperatures with no sheath liquid. If so, remove the ESI sprayer from the source. When not in use, leave the sprayer in the nebulizer adjustment fixture (p/n GT430-20470).

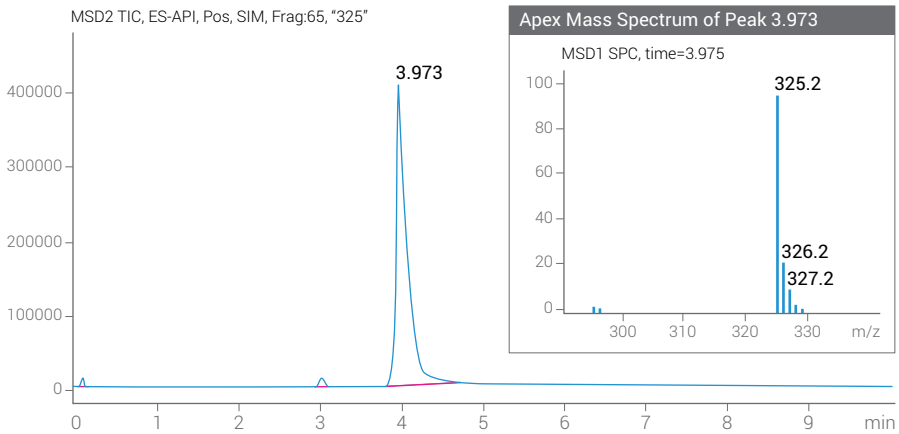


Figure 46. MS Spectrum from MS-Scan Trace.

17 Method Setup for CE/MS Analysis with DAD

17.1 CE/MS method setup

The parameters given in this section are typical for the analyzed test sample (quinine sulfate). However, they might have to be adapted when other samples are analyzed.

Refer to the section "How to Use Your Agilent CE Instrument for Capillary Electrophoresis Separations" in the User Manual for the 7100 CE System (p/n G7100-90000) for a general introduction of method parameters for applications running in CE mode. The parameters do match as well for setting up methods in CE/MS mode (Figure 47).

The Setup Instrument Method screen allows you to access the system parts that used a modern RC.net driver. In contrast, system parts that are controlled by so-called classic drivers are not showing up in the screen. The Agilent InfinityLab LC/MSD Series system has a classic driver. The MS part of a method is therefore programmed differently, see section 17.2 "Setup of the MS part of a CE/MS method."

For a CE/MS system including a pump, a tab with parameters of the pump will be displayed (Figure 48). Refer to the manual of the isocratic pump for details of parameters that can be modified. The parameter of major relevance for CE/MS application is the flow rate. Remember that the actual flow rate used in the sprayer is reduced by an installed 1:100 splitter.

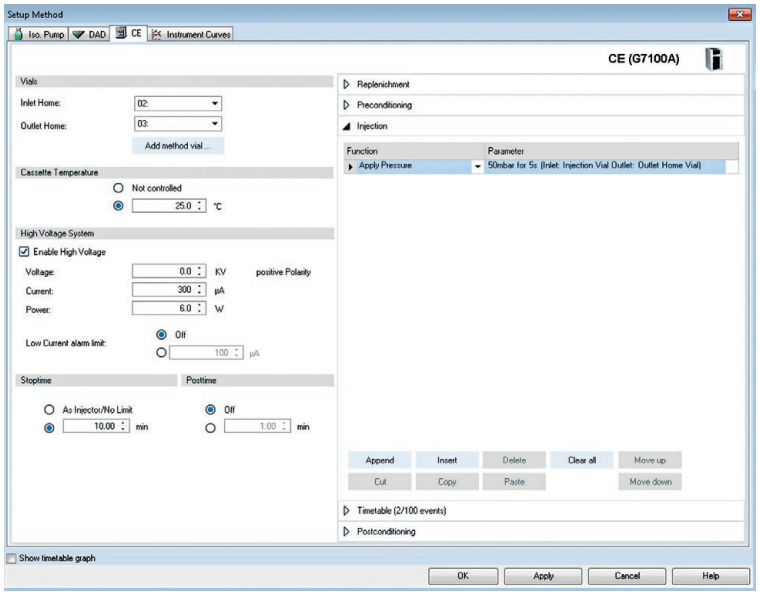


Figure 47. CE/MS method setup for CE parameter.

Another tab in the Setup Instrument Method screen gives access to instrument curves that can be recorded for CE, DAD, and pump parameters (Figure 49).

In CE/MS mode, leak current cannot be used for troubleshooting, neither from online plot nor from an instrument curve. This contrasts with electrophoresis in CE, CE+p, or CEC mode because the capillary ends in a different device, namely the MS source, and the current goes to ground there instead of returning to the HV power supply. Therefore, no data can be recorded.

Wash inlet electrode while no outlet vial is defined can cause air being introduced into the separation capillary. Make sure flush is applied afterwards otherwise missing conductivity and arcing events might occur.

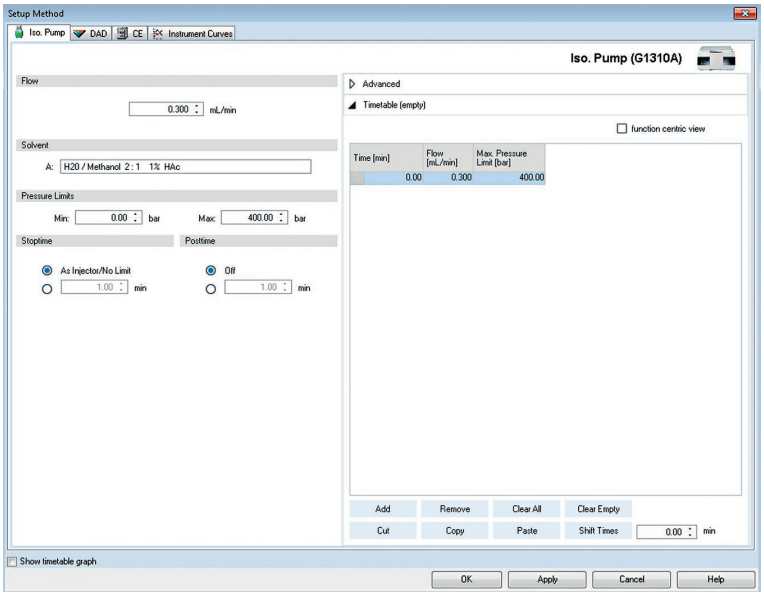


Figure 48. CE/MS method setup for pump parameter.

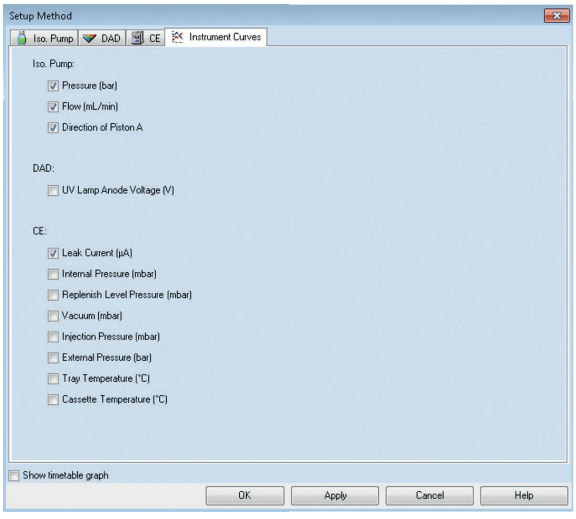


Figure 49. CE/MS Method Instrument curves selection.

17.2
Setup of the MS part of a CE/MS method

The Agilent InfinityLab LC/MSD Series system is controlled by a classic ChemStation driver and needs programming in a different screen than the CE instrument, DAD, and pump, which are controlled by RC.net drivers. You can access the relevant screens under Instrument > More MSD or by clicking the icon in the system control diagram.

Table 6. Typical settings for conventional spray chamber parameters.

Parameter	Value
Drying gas	6–10 L/min
Drying gas temperature	100–300 °C
Nebulizing gas	10–20 psi
HV (positive mode)	3.5–4 kV
HV (negative mode)	3–3.5 kV

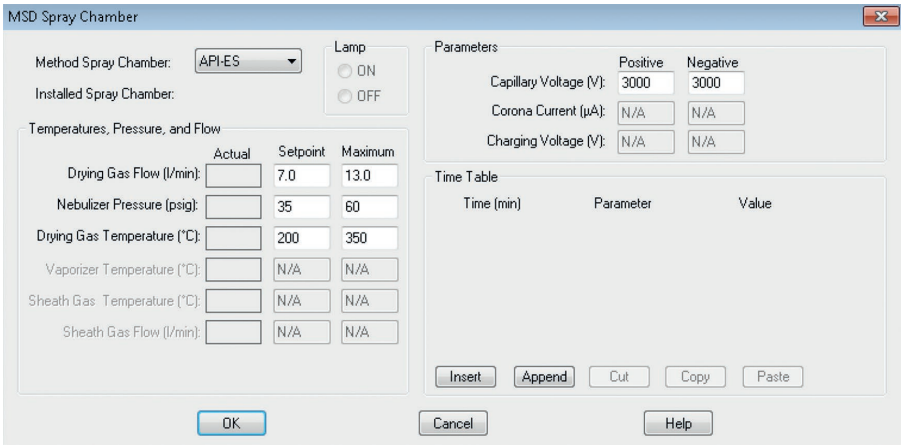


Figure 50. MS method parameter for the spray chamber.

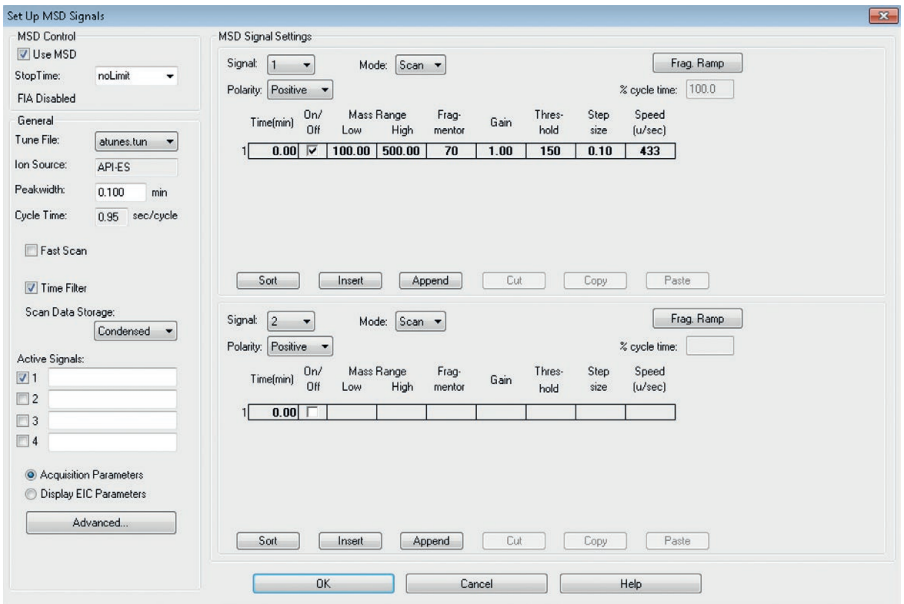


Figure 51. Method parameter for MSD signals

17.3 Method setup for CE/MS analysis without DAD

If analyses will be run with the UV lamp switched off and without recording of DAD signals, the parameters for the DAD part of the method can be set as follows. These settings will inactivate the normal cassette detection algorithm of the instrument, which would require the UV lamp to be switched on and an alignment interface to be in place. The settings below must be used if a capillary without UV window is inserted in an alternative way as shown in section 14.2, figure 29A.

1. Access the DAD method parameters from Setup Instrument Method screen or by clicking the DAD control interface that gives access to the DAD part of the current method only.
2. Clear the check box for UV lamp in the Lamps on required for acquisition section.
3. Clear the check boxes for Prerun/Postrun in the Autobalance section.
4. Clear all check boxes for Use Signal in the Signals section.
5. Click OK and save the method.
6. Modify any other parameters of the method independently according to your needs.

The UV lamp is not automatically switched off when a method with the above settings is loaded. Make sure that the lamp is not switched on manually or automatically when you want to avoid UV light passing the cassette in runs with such methods.

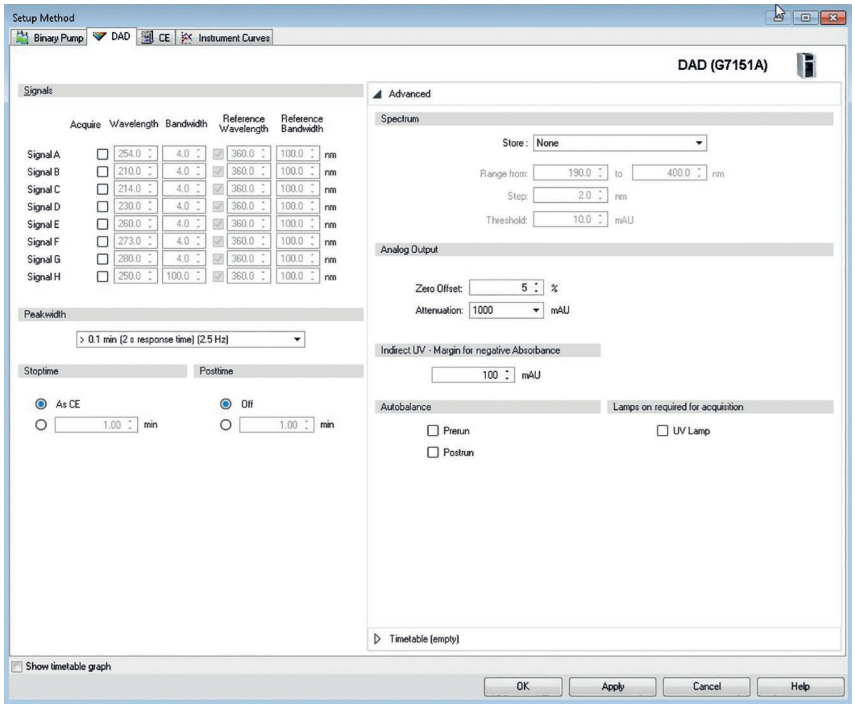


Figure 52. Parameters for CE/MS analysis with UV lamp off.

17.4 Removing the DAD from the control diagram

If the CE/MS system will be exclusively used for analysis without DAD detection, the DAD user interface can be removed completely from the control diagram by eliminating it from the configuration. However, it is recommended to leave the DAD in the configuration to enable easy switching between the different modes CE, CE+p, CEC, or CE/MS.

Components of the Agilent 7100 CE instrument in a CE/MS system are shown in the CE control diagram that is also known as the dashboard, see Figure 53.

All other components of the CE/MS system such as DAD, pump, or MS are represented by an individual diagram. The current analysis mode is displayed in the top line of the CE control diagram. The analysis modes that can be selected under instrument configuration are CE, CE+p, CEC, or CE/MS. Changes in the current CE mode for your instrument can be made under Instrument > More CE > Instrument Configuration, see Figure 21.

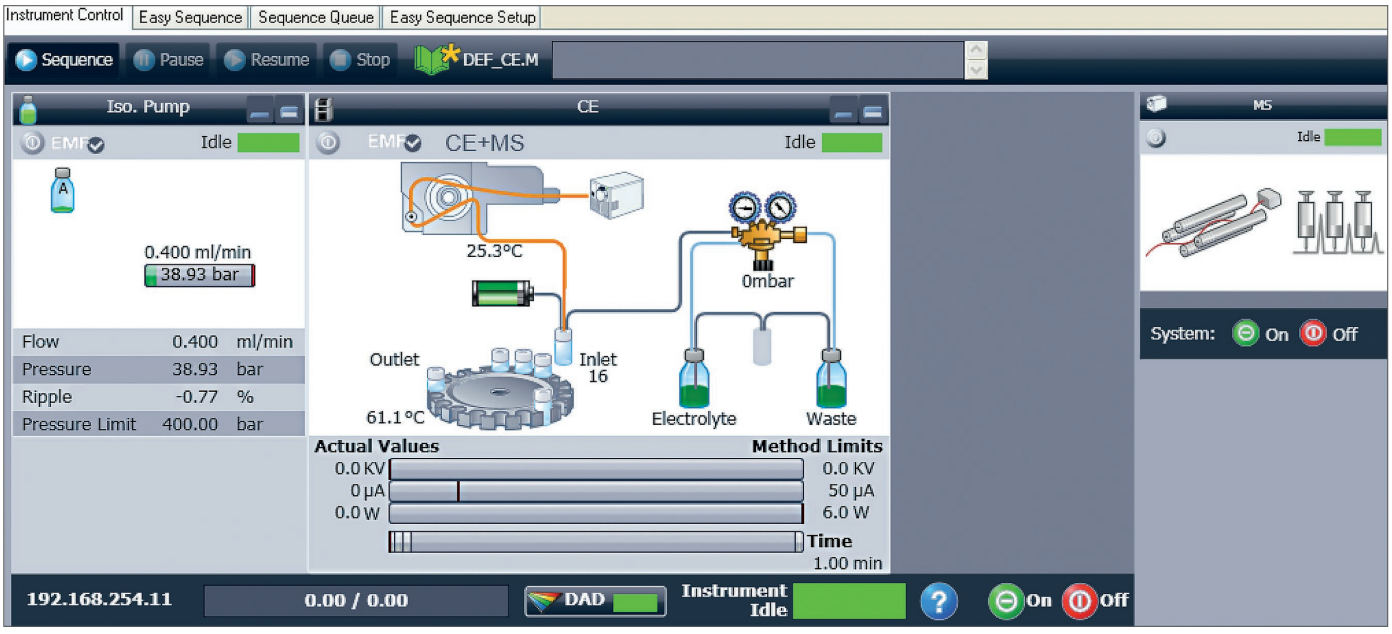


Figure 53. System diagram with minimized DAD control interface.

17.5 Explanation of the graphical user interface

The core components of the Agilent CE instrument in a CE/MS system are shown in the CE control diagram that is also called the dashboard, see Figure 20. All other components of the CE/MS system such as the DAD, pump, or MS are represented by an individual diagram. The current analysis mode is displayed in the top line of the CE control diagram. The analysis modes that can be selected under instrument configuration are CE, CE+p, CEC, or CE/MS. Changes in the current CE mode for your instrument can be made under Instrument > More CE > Instrument Configuration (see Figure 21).

You might minimize the DAD user interface by clicking the icon in the top right corner of the DAD user interface. Later a DAD icon is in the same line as the overall status icon (Figure 53).

Follow the steps below to establish certain parameters (Figure 52) in the DAD before you eliminate the DAD completely from the instrument configuration. These parameters will be applied to the DAD, they are kept until they are changed by a method and are not directly accessible from the system diagram after elimination of the DAD from the configuration.

1. Do the following while the DAD is still configured in an online copy of the ChemStation connected to the respective instrument.
2. Load the CE default method.
3. Access the method parameters by selecting the item Set up Instrument Method from the Instrument menu. This displays the CE Method Setup screen with access to all part of a method. Another way is to simply click the DAD control interface, which gives access to the DAD part of the current method only.
4. Clear the check box for UV lamp in the Lamps on required for acquisition section.
5. Clear the check boxes for Prerun/Postrun in the Autobalance section.
6. Clear all check boxes for Use Signal in the Signal section.
7. Click OK to apply the settings.

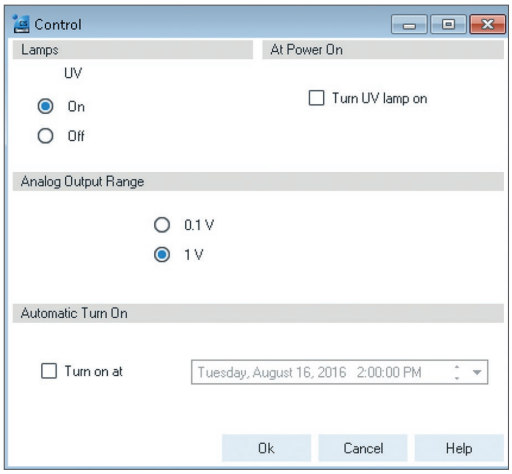


Figure 54. Parameters in DAD control.

8. Save the method under a different name to the CE/MS method folder.
9. In the Method and Run Control of the ChemStation, select Instrument > More DAD / Control... > Control to open the DAD control screen (Figure 54).
10. Clear the check box for Turn UV lamp on in the section At Power On
11. Clear the check box for UV lamp on in the section Lamps
12. Ensure that no entry is made in the section Automatic Turn On.
13. Click OK.
14. Make sure that the UV lamp is switched off.

As a side effect of these established parameters in the DAD, you might see deviating status information in Agilent Lab Advisor software in comparison to the ChemStation. In Lab Advisor, an instrument configured without DAD will show a not ready for the DAD, due to the UV lamp being switched off, and will show also an overall not ready status although the system is ready for ChemStation to start a run (Figure 55).

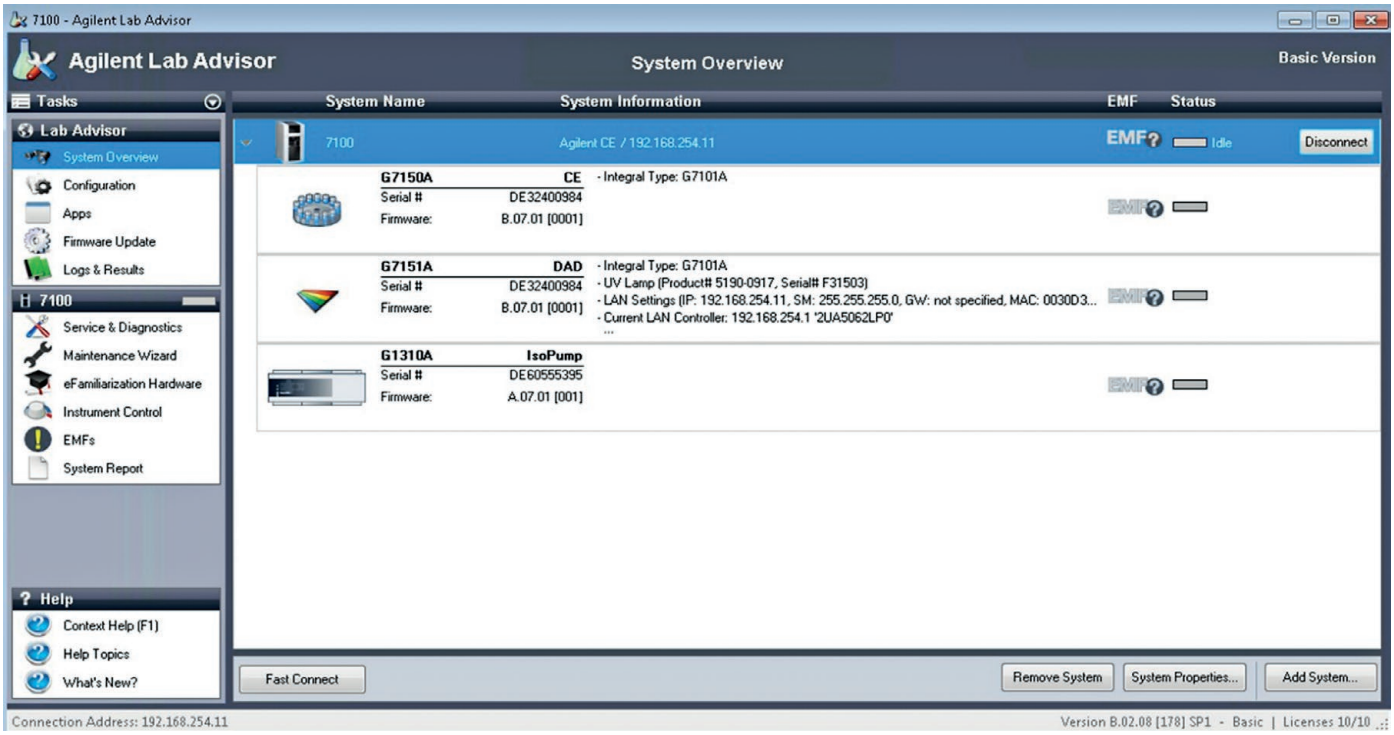


Figure 55. Agilent Lab Advisor system overview.

18 Methods and Applications of CE/MS

In the following sections, two more advanced CE/MS methods for analysis of cations and of anions are described. The setup information described here pertains to the Agilent InfinityLab LC/MSD Series system with Agilent OpenLab CDS software (ChemStation Edition) software for instrument control. Examples of setpoints for source parameters and data acquisition parameters for using Agilent Time of Flight, Triple Quadrupole, and Quadrupole Time of Flight Series LC/MS can be found in Agilent Application Notes.

Analyze a standard substance before analyzing a real sample, using CE/MS analysis, and confirm that the device and spray are in order. As an example, analysis of amino acids under cation analysis conditions and analysis of malic acid under anion analysis conditions are given. The data from the amino acids and organic acid samples provided in this document was collected with a quadrupole MS.

18.1 Method for CE/MS analysis of cations

The analysis conditions shown here (Table 7) are general analysis conditions that can be used to measure almost all basic compounds. Figure 56, Figure 57, and Figure 58 show the analysis results of with the amino acids sample.

Since a low-pH buffer (pH 1.9) is used and all amino acids used have an isoelectric point higher than 1.9, they are present as cations in the sample and during separation. In general, all compounds with basic functional groups can be measured under these analysis conditions. A positive voltage is applied for the CE separation.

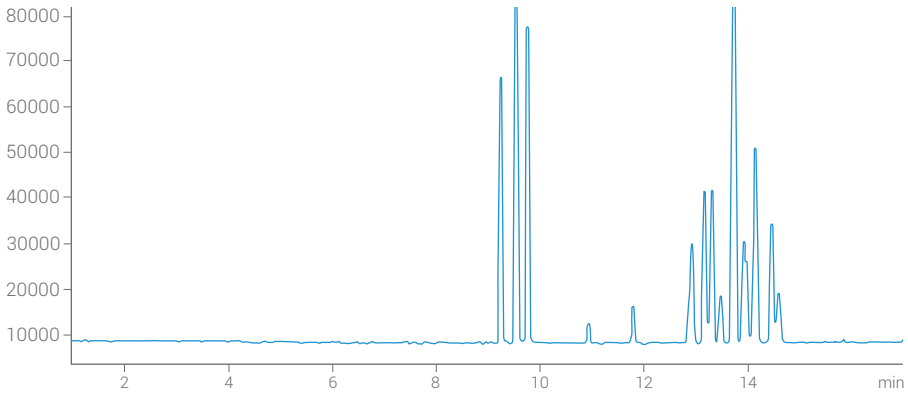


Figure 56. 100 µm amino acid standard mixture (p/n 5061-3330).

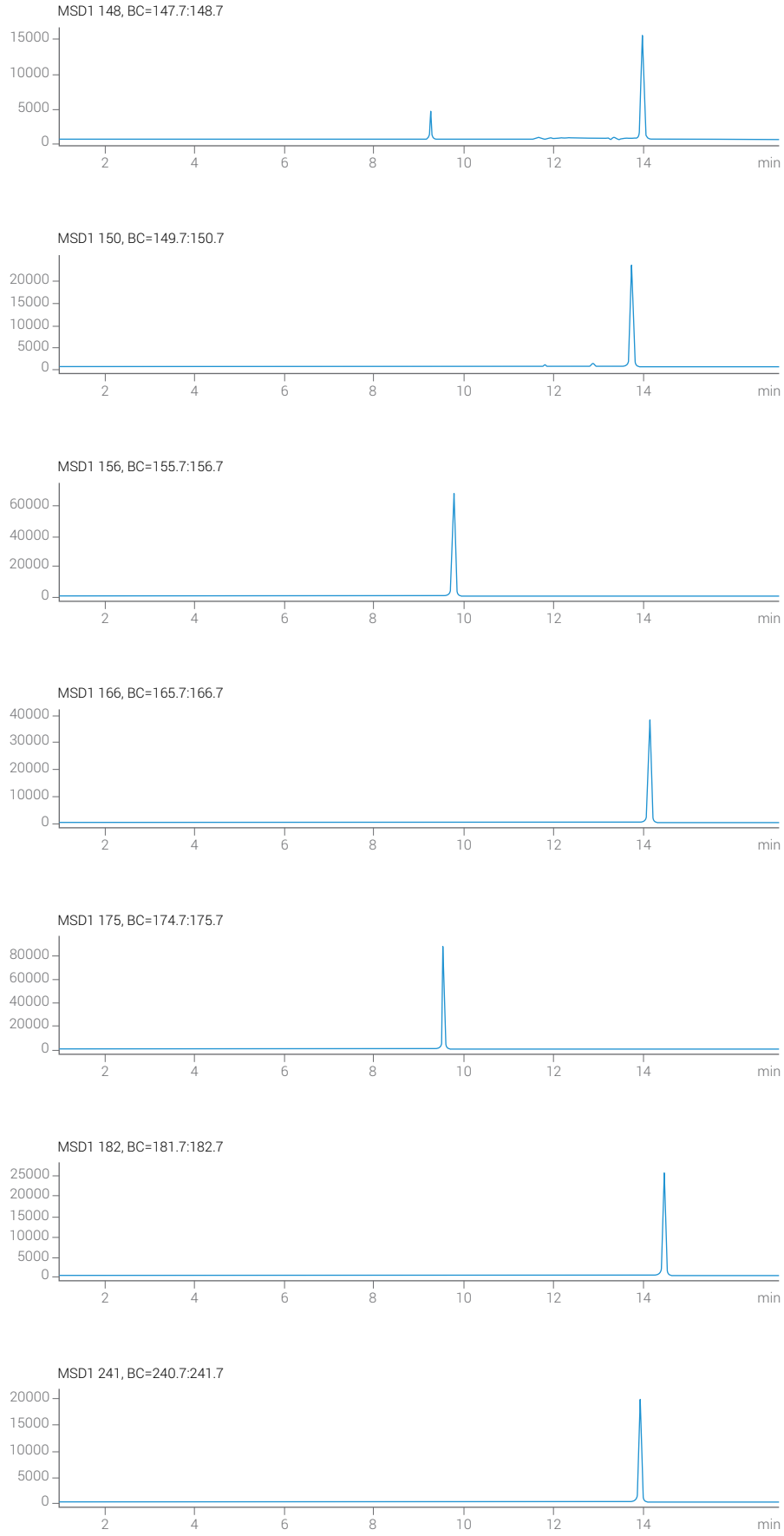


Figure 57. Mass electropherogram (SIM) of various amino acid standards.

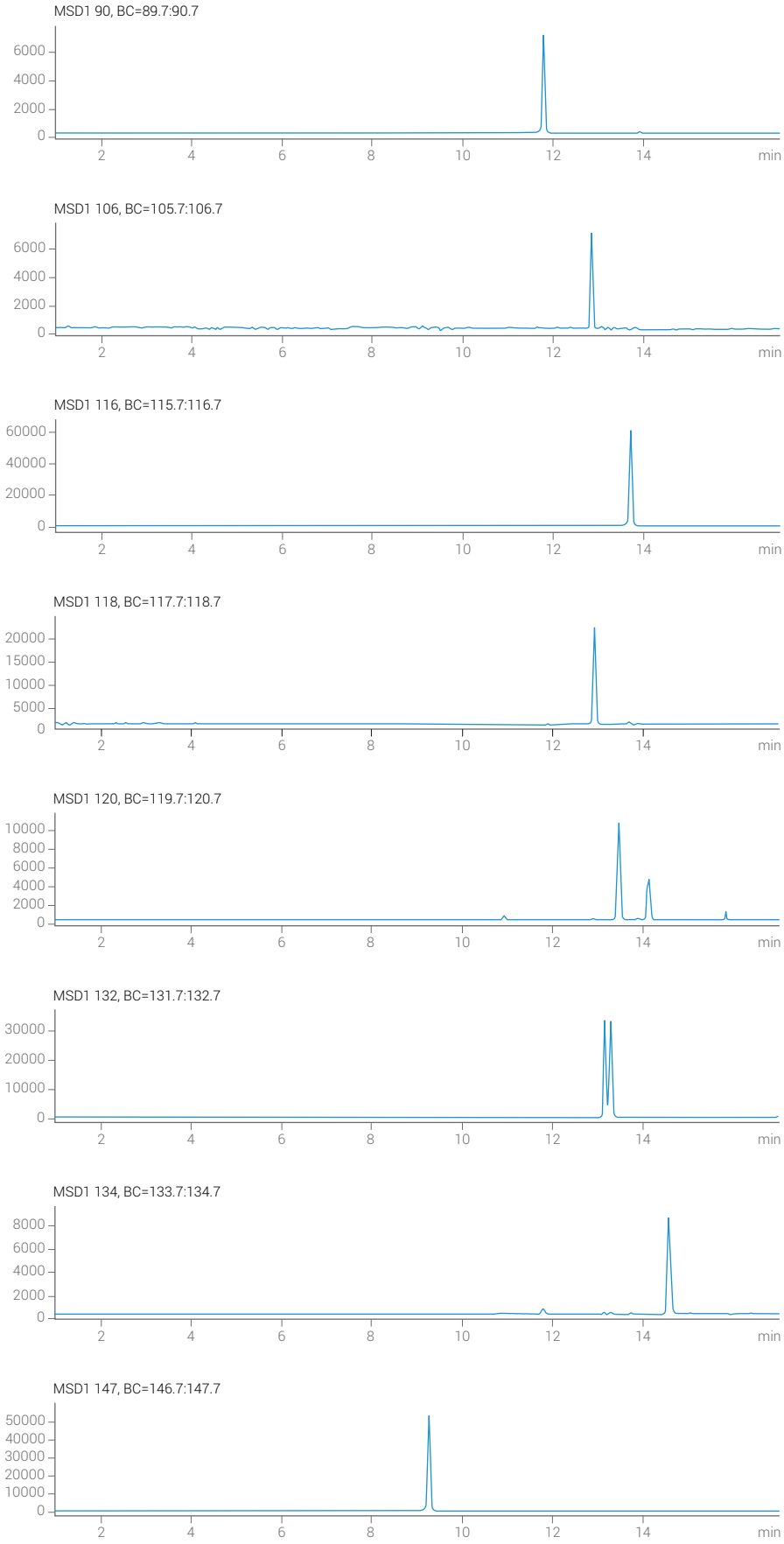


Figure 58. Mass electropherogram (SIM) of various amino acid standards.

Table 7. CE/MS analysis conditions (for cation analysis).

CE Capillary	Fused silica capillary, 50 µm id, total length 100 cm Without UV detection window
Buffer	1 M formic acid
Voltage	Positive 30 kV
Temperature	20 °C
Preconditioning	Flush with buffer for 5 min
Injection pressure	50 mbar for 8.0 s (sample)
Pressure	50 mbar for 2.0 s (inlet vial)
Time programming	0.3 min
Voltage	+30 kV
MS Polarity	ESI positive
Capillary voltage	4000 V
Fragmentor voltage	100 V
Drying gas, temperature	N_2 10 L/min, 300 °C
Nebulizer gas pressure	10 psi
Sheath liquid	5 mM ammonium acetate in 50 % methanol
Flow rate	8 µL/min
Sample	Diluted amino acid standard mixture (p/n 5061-3330) to 0.1 nmol/µl

18.2 Method for CE/MS analysis of anions

Anion analysis conditions: organic acids

The following is an example of analysis of organic acids. The analysis conditions (Table 8) can be applied for analysis of such compounds, which are anions at the pH of the BGE used, such as aliphatic and aromatic carboxylic acids and other weakly acidic substances.

Table 8. CE/MS analysis conditions (for anion analysis).

CE capillary	Fused silica capillary, 50 µm id, total length 80 cm (a 100 cm capillary may be used) Without UV detection window
Buffer	20 mM ammonium formate pH 10 (adjusted with 1 % NH_4OH)
Voltage	0 to +30 kV (gradient)
Temperature	20 °C
Preconditioning	Flush with buffer for 4 min
Injection	Pressure 50 mbar for 8.0 s (sample)
Pressure	50 mbar for 2.0 s (inlet vial)
Time programming	0.3 min voltage +30 kV
MS polarity	ESI negative
Capillary voltage	350 V
Fragmentor voltage	100 V
Drying gas, temperature	N_2 10 L/min, 300 °C
Nebulizer gas pressure	10 psi
Sheath liquid	5 mM ammonium hydroxide in 50 % methanol
Flow rate	8 µL/min
Sample	Organic acids test mixture, p/n 8500-6900, containing 1 mg/L of lactic acid, succinic acid, malic acid, tartaric acid, and citric acid

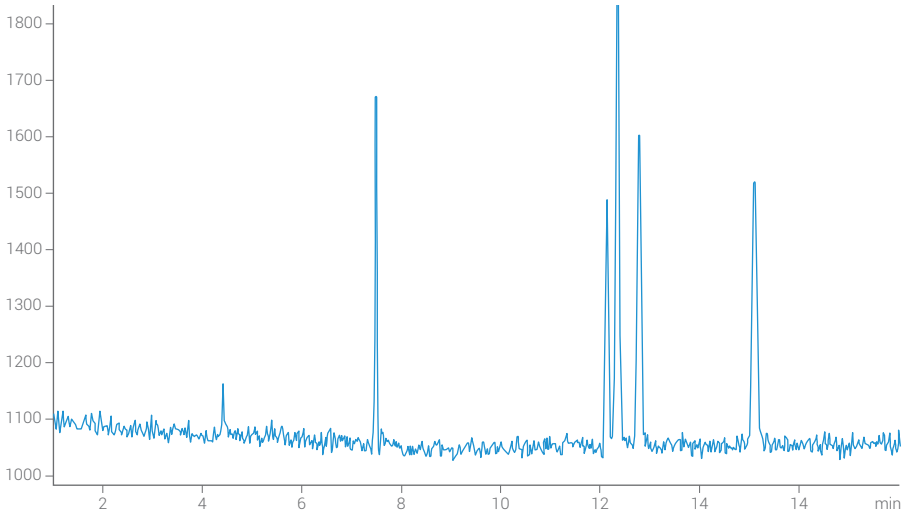


Figure 59. CE separation of 1mg/L lactic acid, succinic acid, malic acid, tartaric acid, and citric acid.

Anion analysis conditions: p-toluenesulfonic acid

The analysis of p-toluene sulfonic acid is shown as a further example of the analysis of anions under basic conditions. The conditions are shown in Table 9. The total ion electropherogram obtained is shown in Figure 60. The mass electropherogram extracted at ***m/z*** = 171, which is the pseudomolecular ion of p-toluene sulfonic acid [M-H], is shown in Figure 60. The mass spectrum of toluene sulfonic acid is shown in Figure 61. As a reference, the current values of the capillary electrophoresis unit, used in this analysis, are shown in Figure 61.

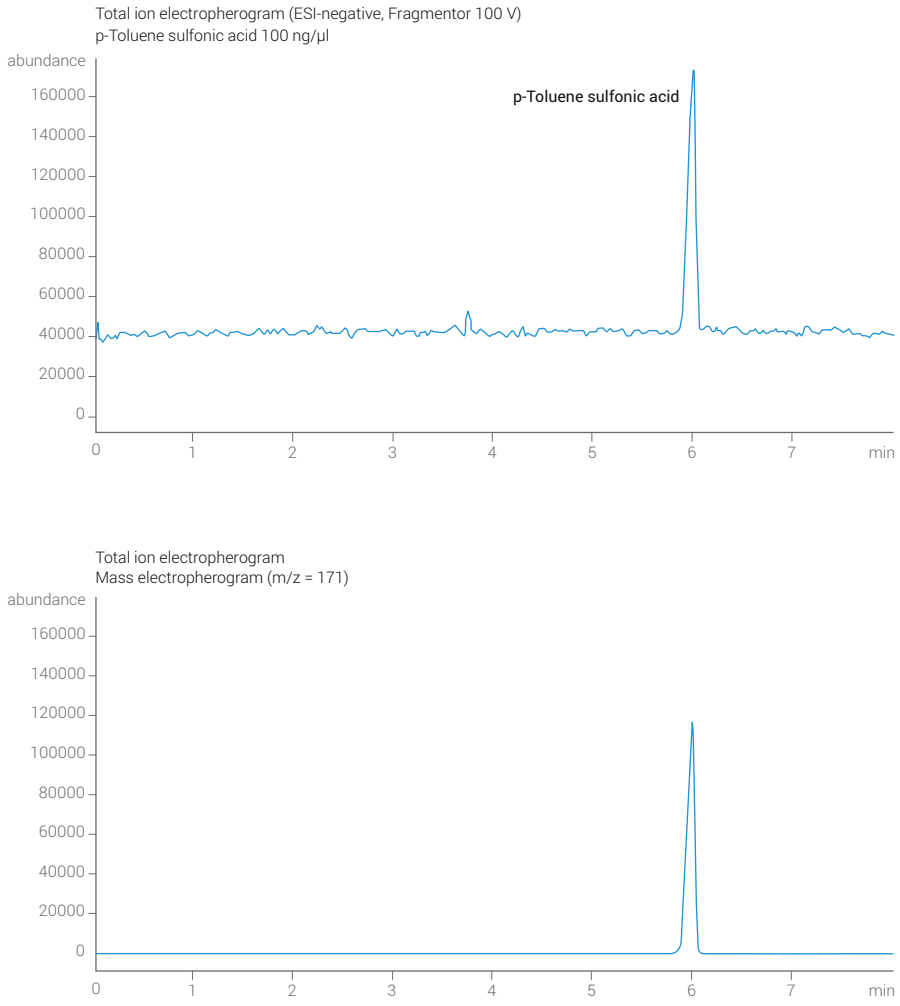


Figure 60. Upper electropherogram: Total ion electropherogram of 100 ng/µl p-Toluene sulfonic acid solution. Lower electropherogram: Extracted ion electropherogram (m/z = 171), showing the pseudomolecular ion of toluenesulfonic acid.

Table 9. CE/MS analysis conditions (for p-toluenesulfonic acid).

CE Capillary	Fused silica capillary (50 µm id, total length 60 cm) Without UV detection window
Buffer	20 mM ammonium acetate, pH 9.0 (adjusted with 1 % NH_4OH)
Voltage	Positive 20 kV
Temperature	25 °C
Preconditioning	Flush with buffer for 4 min
Injection	Pressure 50 mbar for 6.0 s (sample)
Pressure	50 mbar for 2.0 s (inlet vial)
Time programming	0.3 min voltage +20 kV
MS polarity	ESI-Negative
Scan range	***m/z*** =7 0–300
Capillary voltage	3500 V
Fragmentor voltage	100 V
Drying gas, temperature	N_2 10 L/min, 300 °C
Nebulizer gas pressure	10 psi
Sheath liquid	5 mM ammonium acetate in 50 % methanol
Flow rate	10 µL/min
Sample	p-toluenesulfonic acid monohydrate standard CAS number: 6192-52-5

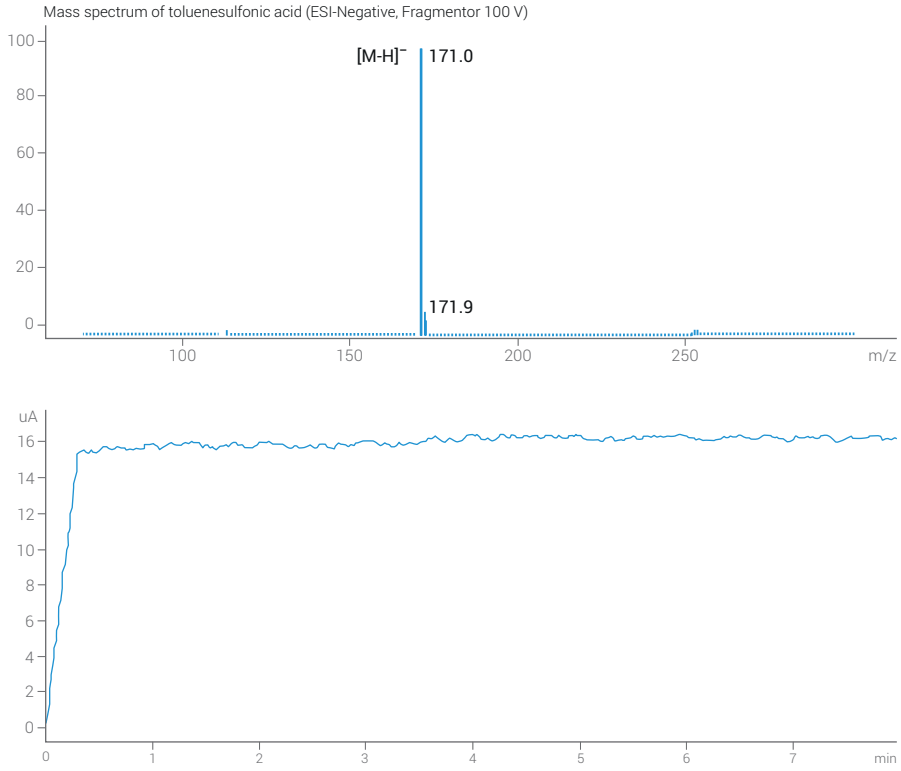


Figure 61. Upper electropherogram: Mass spectrum of toluenesulfonic acid. Lower trace: Current value during toluenesulfonic acid analysis.

19 Maintenance

Capillary electrophoresis is a true microliter- or rather a nanoliter-scale separation method. The volume of a separation capillary, 1000 x 0.05 mm is approximately 2 µL. Sample volume typically is in the order of 2 to10 nL while the osmotic flow will be in the range of 100 to 300 nL/min.

These dimensions mandate the use of solvents, buffer salts, reagents, and additives of the utmost purity in the BGE and sheath solvent. As well as cleanliness and inertness of sample microvials, and of all tools used during sample preparation, a clean and dust-free lab environment is recommended. A controlled temperature in the lab is of advantage given that the separation capillary must bridge the space between CE exit and CE sprayer and is exposed to the lab environment.

In addition, you need to be aware that mass spectrometers are sensitive detectors. Any contamination brought into the separation part of the system will be picked up and seen by the mass spectrometer, which may cause false identifications and interpretations leading to wrong answers.

Given these remarks, the following are detailed guidelines pertaining to keep the Agilent CE/MS system in optimal shape. Further details are also given in the installation guide for the CE/MS sprayer kit. Maintenance information for the 7100 can be found in Chapter 8 "Maintenance" of the User Manual for the 7100 CE System (p/n G7100-90000) and in the respective manuals of the Agilent LC/MS systems.

This troubleshooting section applies only to systems connected to the CE and MS manufactured by Agilent.

19.1 Storing the CE/MS capillary, the sheath flow splitter, and the CE/MS sprayer needle

Daily practice

If the CE and MS systems are in an idle state (that is, waiting for the next analysis), keep the sheath solvent flow, the nebulizing gas, and drying gas running. If you stop measuring for the day or a longer period, follow the next recommendations. To avoid plugging of the capillary, the sheath flow splitter and the CE/MSD sprayer needle need to be cleaned when not in use.

Cleaning the sheath flow splitter and the CE/MS sprayer needle

1. Replace the sheath liquid bottle with a bottle filled with water.
2. Leave the CE/MS capillary, the sheath flow splitter and the nebulizing gas connected.
3. Prime the pump and flush for 10 min, pump water through the sheath flow splitter and CE/MS sprayer needle at 0.4 mL/min.

4. Replace the water in the sheath liquid bottle with a bottle filled with isopropanol.
5. Prime the pump and flush for 10 mins, pump isopropanol through the sheath flow splitter and CE/MS sprayer needle.

Clean and storing the CE/MS capillary

1. Flush the capillary with water for 10 min followed by a flush with methanol.
2. Insert an empty vial with a cap into the sample tray and flush the capillary with air for 10 min by applying pressure to the empty vial.
3. Remove the capillary and store in a safe place, the capillary ends can be sealed with a septum or similar.

Inspect and store the sprayer assembly

1. After the separation capillary is removed, check for corrosion of the metal sprayer tube and/or remove deposits. Use a magnifying glass or a microscope if available.
2. If the needle shows corrosion and/or is not symmetrical and flat anymore, it should be replaced.
3. Instructions to replace the needle and/or the gasket (p/n G1607-20030) follow instructions in section 22.1 "Assembly of the CE/MS sprayer kit" and/or in the installation guide for the CE/MS sprayer kit.
4. Leave the sprayer in the nebulizer adjustment fixture (GT430-20470) with sheath solvent and nebulizing gas connected.

A detailed description of sprayer needle maintenance is given for the LC/MS sprayers in the Nebulizer Adjustment Fixture User Guide (p/n G1960-90470). Relevant details of the operations described apply as well for the CE/MS sprayer.

The sprayer needle might become damaged by the hot drying gas (200 to 300 °C) when no sheath liquid is flowing. Do not leave the instrument idle at high drying gas temperatures with no sheath liquid. If so, remove the ESI sprayer from the source.

If you leave an acid, such as formic acid, adhered to the sprayer, it will cause corrosion of the sprayer needle. The cleaning operation is important because dirty needles cause problems such as decreased sensitivity, abnormal current values, and damage to the capillary.

20 Troubleshooting

20.1 Symptoms related to CE current

During the run, CE current is unstable, shows spikes, or is too low.

Cause	Remedy
Poor electrical contact at sprayer tip.	Adjust axial position of the CE capillary in the CE/ESI-MS sprayer (see section 16.2 "Inserting and adjusting the CE capillary in the CE/ESI-MS sprayer" and Figure 62).
Bubble formation at the spray needle (by electrolysis).	See section 9.4 "Electric field and polarity." Increase the sheath solvent flow rate to flush out electrolysis gas. When operating in reversed-polarity mode, the sprayer needle becomes the cathode. Replace the standard sprayer needle with the platinum sprayer needle (p/n G7100-60041) to avoid corrosion of the stainless-steel needle.
Poorly degassed sheath solvent and/or sheath solvent flow rate fluctuates (for example, with a motorized syringe pump).	Use the recommended isocratic pump with online degassing.
There is a problem with the CE capillary. Small gas bubbles or particles are present in the capillary. If the DAD is used, the capillary may be broken in the alignment interface.	Flush the capillary strongly and do the current test (see section 16.5 "CE current check"). Inspect the capillary in the alignment interface under a strong magnifying glass or microscope.

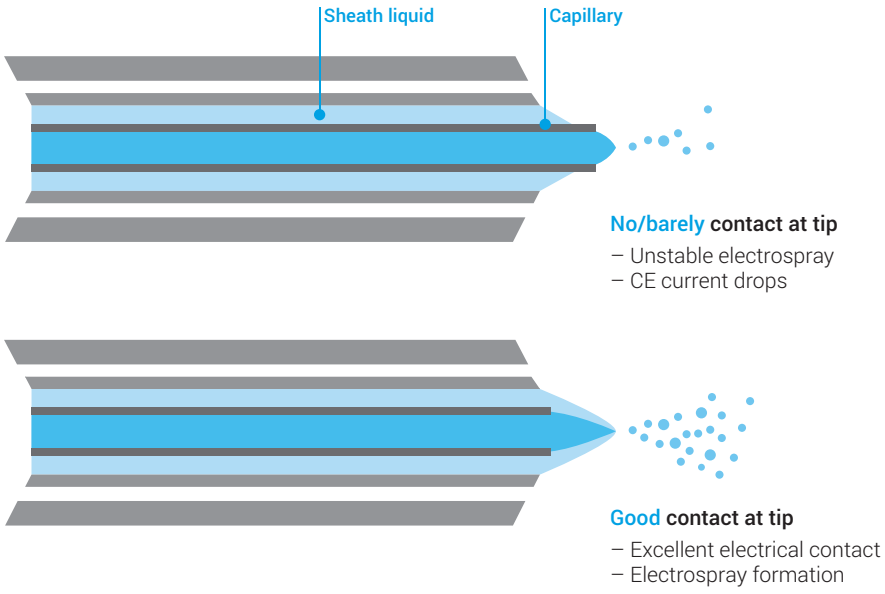


Figure 62. Center tube CE capillary; middle tube spray needle with sheath solvent; outer tube nebulizing gas tube.

CE current breaks down after sample injection

Cause	Remedy
A liquid gap is formed in the BGE by the suction effect from by nebulizing gas flow.	Time program the nebulizing gas pressure during the injection time to a low value; use a longer or narrower id separation capillary, 50 µm id is preferred.
A liquid gap is formed by siphoning towards the capillary outlet during sample introduction sequence.	Check the level height of the inlet vial and the height of the sprayer. See section 11.1 "Leveling the CE and MS instruments."

20.2 Symptoms related to the MS signal

The baseline MS signal (TIC) varies strongly

Cause	Remedy
The sheath solvent flow rate is unstable, is fluctuating, and/or is poorly degassed.	Use the recommended isocratic pump with online degassing.
Polyimide of the CE capillary has swollen or has become detached (see Figure 63).	Avoid the use of high concentration of acetonitrile (>50 %) in BGE and/or sheath solvent. Use Agilent standard CE capillaries or remove polyimide at the CE capillary ends.

Peak shapes are distorted

Cause	Remedy
The inlet or outlet tip of the separation capillary is damaged or not flat.	Cut the capillary ends with the cutter (see section 14.1 "Preparing the capillary") or use a new capillary. Pretreat a new capillary properly.
Incompatibility of sheath solvent and background electrolyte. Precipitations or emulsion formed.	Check mixing behavior of BGE and sheath solvent.
Corrosion of the spray needle (only with cationic capillary and when the spray needle is the anode).	Replace stainless-steel spray needle with platinum spray needle (p/n G7100-60041).

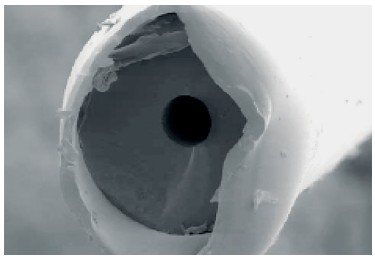


Figure 63. Polyimide of the CE capillary has swollen or has become detached.

Decreased migration times and wider peaks

Cause	Remedy
Hydraulic flow exists towards the capillary outlet.	Check the level height of the inlet vial and the height of the sprayer to avoid siphoning. See section 11.1 “Leveling the CE and MS instruments.” Reduce the nebulizing gas pressure to avoid the suction effect. See section 21.1 ”Measurement and reduction of the suction effect.”

Poor reproducibility or drift of migration times

Cause	Remedy
Effect of temperature fluctuations in the lab on the nonthermostatted part of the CE separation capillary.	Make sure to use the PTFE capillary from the CE sprayer kit or operate in an air-conditioned lab.
BGE in inlet vial has changed.	Replenish the contents of the inlet vial after 3 to 5 injections or use a fresh BGE vial.

Poor reproducibility of peak heights (TIC)

Cause	Remedy
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 since poorly cut or damaged.	Use an internal standard.

No peaks or low abundance of peaks is observed

Cause	Remedy
The solutes are absorbed by the separation capillary wall.	Check presence of peaks and separation with DAD.

Peaks are observed in blank runs (carryover)

Cause	Remedy
When injecting electrokinetically, the level of the sample solution in the vial reaches the electrode. The sample solution can enter the annular space between CE capillary and electrode inner wall. Remaining sample solution gets into the BGE/next sample vial and is introduced with the next injection.	Make sure that the level of the sample solvent in the sample vial remains below the electrode end. Do pressure injection. Use the short electrode (p/n G7100-60033) with pressure injections so that the electrode does not touch the sample. Use the Wash Inlet Electrode command in the sample injection sequence, see the User Manual for the 7100 CE System (p/n G7100-90000).

20.3
Symptoms – ESI Current

ESI current is not stable or too low

Cause	Remedy
Too high drying gas flow rate.	Reduce drying gas flow rate as much as possible.

Too high background MS current

Cause	Remedy
Dirty spray needle or CE capillary or sheath liquid pump.	Flush capillary and clean the spray needle or the sheath liquid pump (extensive details for inspecting the spray needle are given in the user manual (p/n G1960-90470).
Dirty BGE or sheath solvent.	Use utmost cleanliness in all parts of your system.

21 Advanced Method Development Information

21.1 Measurement and reduction of the suction effect

The occurrence of a hydrodynamic flow in the CE capillary during CE/MS using the triple tube sprayer has been reported by several groups (see section 9.9 "Operational parameters – nebulizing gas pressure, drying gas temperature and flow rate"). This flow is caused by low pressure that arises at the outlet of the CE capillary because the velocity of the nebulizing gas flow and of the sheath solvent is higher than the velocity of the solvent moving in the capillary. Therefore, the pressure on the liquid in the inlet vial is higher than on the outlet side. This phenomenon is called the Bernoulli effect.

As already explained in earlier sections, the presence of a hydraulic flow in the CE capillary is undesired since a parabolic flow velocity profile will form which results in broadening of the solute zone. In addition, this flow becomes superimposed on the EOF and the electrophoretic solute velocity and reduces the separation time, the example in Figure 64 illustrates the effect[37].

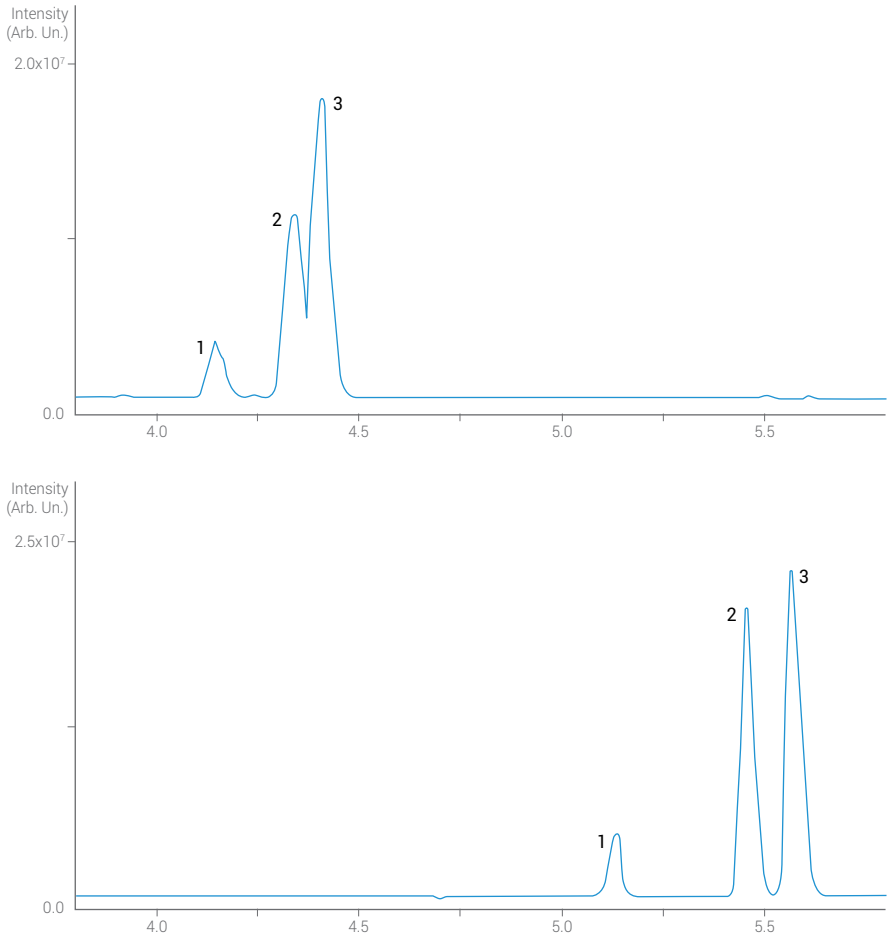


Figure 64. CE/MS separation of an enkephalin test mixture of 50 µg/ml using a PB-PVS-coated capillary. Top electropherogram shows the result without applying negative pressure at the inlet vial, lower electropherogram shows the result utilizing 50 mbar at the inlet vial (reproduced from reference 37 with permission).

In Figure 64, the result of the CE/MS separation of an enkephalin test mixture (GGFM-NH2 (1), YGGFL (2) and YAGFM (3) at 50 µg/mL) using a PB-PVS-coated capillary and a BGE of 500 mM formic acid (pH 2.5) is shown. The top trace A shows the result without applying negative (or compensating) pressure at the inlet vial. The lower trace B was obtained by applying –50 mbar pressure at the inlet vial. As can be clearly observed, the migration time increased but also the efficiency of separation improved since the adverse effect of hydraulic flow on band broadening has been suppressed. The plate numbers for the three peaks increased from 15,000–50,000 to 100,000–200,000.

The following describes a brief procedure that enables to establish the magnitude of the compensating pressure that needs to be applied on the inlet side to effectively suppress the effect of hydraulic flow during CE/MS. This experiment is carried out without applying the electrical field for CE separation. Hence, there is no EOF nor electrophoretic mobility of solutes. Since there is no separation, a sample containing a single marker substance should be used. The procedure is illustrated by the following equations and in Figure 65.

The velocity of the marker measured is obtained from:

$$\upsilon_0 = \frac{L_{tot}}{t_{mig}}$$

If a pressure (ΔP) is applied to the inlet vial a flow is generated given by:

$$\upsilon_P = \frac{\Delta P \cdot K_d}{\eta \cdot L_{tot}}$$

The total flow velocity now is the sum of the hydraulic flow generated by the under pressure at the outlet side (υ_0) and the over pressure applied on the inlet side.

$$\frac{L_{tot}}{t_{mig}} = \upsilon_0 + \upsilon_P = \upsilon_0 + \frac{\Delta P \cdot K_d}{\eta \cdot L_{tot}}$$

In three separate runs, the migration time of the marker is measured as a function of a constant pressure applied to the inlet vial. The inverse of the migration time is now plotted versus the pressure applied to the inlet vial.

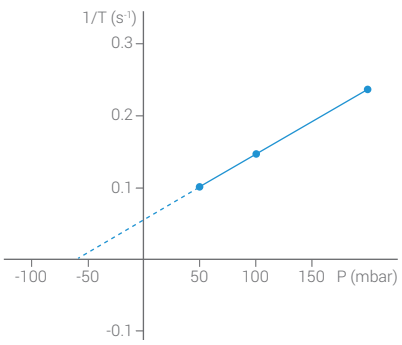


Figure 65. Plot of migration time of an EOF marker versus pressure on the inlet vial.

This will provide a straight line, which crosses the X-axis at the value of the negative pressure that will balance the under pressure at the CE/MS sprayer outlet side. The exact value of this pressure can be obtained by linear regression and calculating the intercept. In the example given, about –50 mbar will be required to balance the negative pressure on the outlet side.

21.2 Influence of sheath solvent composition

During CE, sample ions and co-ions of the BGE exit the capillary at the sprayer tip, and simultaneously, counterions from the sheath solvent enter the capillary and migrate towards the injection side while at the same time BGE ions enter the capillary on the inlet side[38].

With low or absent EOF and if the BGE contains different counterions from the sheath solvent, a moving ionic boundary may develop inside the capillary. This process is schematically shown in Figure 66.

Depending on the respective electrophoretic mobilities and *pKa* values of these ions, the boundary between the original BGE and the buffer formed by the replacement of its counterions by the sheath solvent counterions may be either sharp or diffuse. In addition, the pH and conductivity of the new BGE behind the moving ionic boundary may differ significantly from the original BGE. This differing BGE will cause band shape distortion.

To counteract this phenomenon, match the counterion in the buffer and the sheath regarding mobility and *pKa*. Preferably, they should be the same. A slight pressure difference between inlet and outlet can be created, for example, as described before and/or use the Bernoulli effect to reduce the pressure on the outlet side. Or, just pressurize the inlet to induce a hydrodynamic flow velocity that exceeds the velocity of the moving boundary. Alternatively, consider operation conditions that generate an EOF.

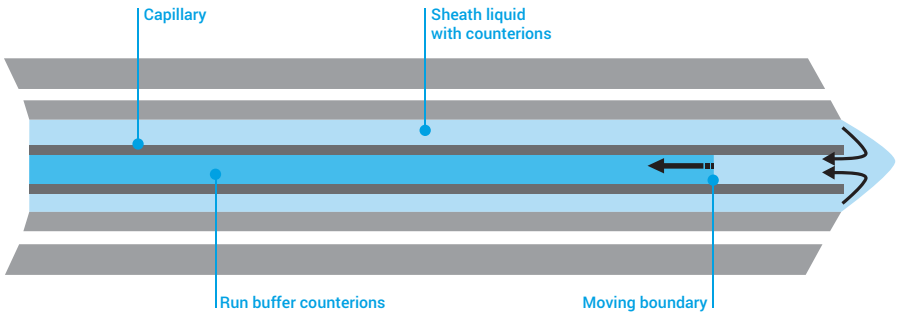


Figure 66. The counterions of the BGE and the sheath solvent are assumed to be different, which results in propagation of the sheath solvent counterions into the CE capillary.

22 Reference Information

22.1 Assembly of the CE/MS sprayer kit

In general, the CE-MS sprayer needs little maintenance. But it may happen that from time-to-time or when a problem occurs that some parts need to be replaced. The parts shown in Figure 67 and Table 10 can be exchanged by the user.

Table 10. Parts for maintenance or repair.

Item Number	Description	Part number
1	Sprayer body	No part number
2	Sprayer head	No part number
3a, 3b	Gasket	G1607-20030
4	O-ring	0905-1022
5	Sprayer needle	G1607-60041
6	Spring	5022-2140
7	Screw body	G1607-20029*
8a, 8b	Seal holding screw	G1607-20022*
9	Protection tube for the sprayer	0890–0581
Not shown	PEEK fitting and ferrule capillary and nebulizing gas	0100-1543
Not shown	PEEK ferrule capillary	5022-2141

**Please contact your Agilent service representative for replacement.*

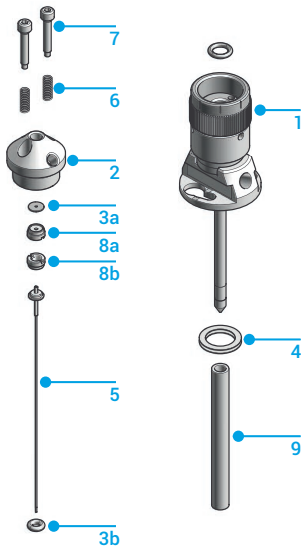


Figure 67. The Agilent CE/MS sprayer.

Exchanging the sprayer needle (item 5)

1. Disconnect the nebulizing gas, the sheath liquid tube, and the CE/MS capillary from the sprayer.
2. Remove the CE/MS sprayer from the MSD and protect the sprayer with the genuine PTFE tube.
4. Open the two body screws (7). Be careful not to lose the attached springs (6), see Figure 68A.
5. Unscrew the sprayer head (2) from the sprayer body (1), see Figure 68B.
6. Open the seal holding screw (8b) of the sprayer body.
7. Remove the defective sprayer needle (5).
8. Slide a new gasket (3b) over the new sprayer needle.
9. Carefully insert the new needle to the sprayer body.
10. Insert the seal holding screw (8b) again but do not over tighten it.
11. Screw the sprayer head (2) onto the sprayer body (1).
12. Check if the sprayer needle protrudes about 0.1 to 0.2 mm (about one third of its diameter) from the sprayer tip (Figure 71. Protruding ESI needle, good (A), bad (B, C)). Use the nebulizer adjustment fixture (p/n G1960-67470) and the

corresponding pocket microscope (p/n G1960-67470), which are part of the accessories kit of the Agilent MSD. If necessary, re-adjust the sprayer needle as described later.

13. Carefully assemble the connections, adjust the capillary as described earlier and reinstall it into the MSD.
14. Use the test sample to verify the proper function.
15. Allow sheath liquid to rinse away surface contaminations from the newly inserted material.

If the seal holding screw is over tightened, tension is on the ESI needle and the needle path through the sprayer is can become curved. Thus, the needle might exit in a way that it touches the rim at the exit. Do not over tighten the seal holding screw. Loosen the screw, turn the needle and careful retighten it if the needle touches the rim. New Agilent CE/MS sprayers are pre-adjusted. Do not open the hex-key screws on the body part.

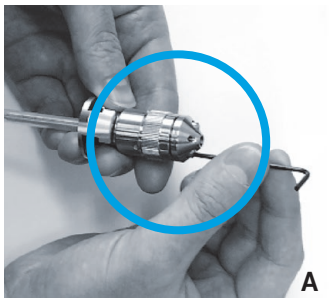
Re-adjusting the sprayer needle

New Agilent CE/MS Sprayers are pre-adjusted. The sprayer needle should protrude from the sprayer tip about 0.1 to 0.2 mm. This setting gives highest sensitivity and baseline stability. During normal operation, there is no need to re-adjust the sprayer needle.

Only re-adjust the CE/MS sprayer if the sprayer needle position differs from the settings above.

1. Turn the adjustment screw counterclockwise (+ direction) until its mechanical stop.
2. Open the three hex-key screws on the lower adjustment ring by 0.5 turn. The lower adjustment ring is from metal and has flat surface.
3. During the following adjustment procedure, press the sprayer head and body parts together to remove backlash (Figure 70)
4. Turn the lower adjustment ring counterclockwise until the sprayer needle is flat with the sprayer tip.
5. Turn the lower adjustment screw a quarter of a turn clockwise.
6. Tighten the three hex-key screws on the lower adjustment ring. Check that the sprayer needle now protrudes about 0.1 to 0.2 mm (about one third of its diameter) from the sprayer tip, see Figure 71. This can be done by using the nebulizer adjustment fixture (p/n G1960-67470) and the corresponding pocket microscope (p/n G1960-67470) which are part of the accessories kit of the Agilent MSD.

If necessary, re-adjust the sprayer needle. Despite the normal adjustment with 0.1 to 0.2 mm, the optimum might be different for other spray conditions or individual ESI sprayers. If you see an irregular baseline or sputtering effects, try 0.3 mm.



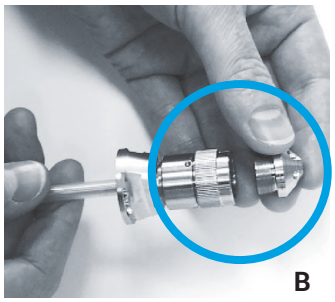


Figure 68. Removing the sprayer head from the sprayer body.

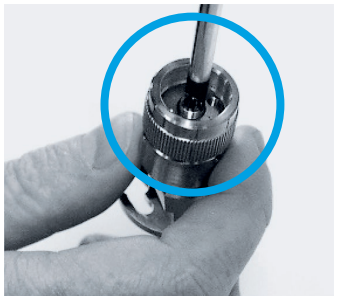


Figure 69. Accessing the gasket in the sprayer body.

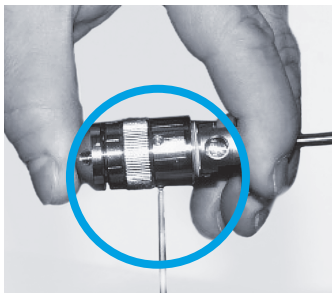


Figure 70. Handling of sprayer during needle adjustment.

22.2 Sprayer maintenance

In general, the CE/MS sprayer needs little maintenance. But it may happen that from time-to-time or if a problem occurs that some parts need to be replaced. For problems that might be related to the sprayer the following options are reasonable:

- Re-adjustment of the sprayer such as inner needle, capillary, adaption flow rates
- Cleaning followed by re-adjustment
- Replacement of parts as the sprayer such as needle and gaskets

Replacement, cleaning, and adjustment procedures are described below and in earlier chapters. See Chapter 20 “Troubleshooting” to determine the root cause of problems. The parts shown in Table 11 can be exchanged for maintenance.

Table 11. Parts for maintenance or repair (see Figure 72).

Item Number	Description	Part Number
1	Sprayer body	No part number
2	Sprayer head	No part number
3a, 3b	Gasket	G1607-20030
4	O-ring	0905-1022
5	Spray needle, stainless steel	G1607-60041
6	Spring	5022-2140
7	Screw body	G1607-20029*

8a, 8b	Seal holding screw	G1607-20022*
9	Protection tube for the sprayer	0890-0581
Not shown	PEEK fitting and ferrule capillary and nebulizing gas	0100-1543
Not shown	PEEK ferrule capillary	5022-2141
5*	Platinum Electrospray needle	G7100-60041

**Please contact your Agilent service representative for replacement.*

Platinum electrospray needle assembly for CE/MS

This platinum needle (p/n G7100-60041) is built into the CE electrospray interface (ESI) sprayer (p/n G1607-60002) as replacement part. It differs from the standard (stainless-steel) ESI sprayer needle (p/n G1607-60041) in the material but has slightly different physical dimensions that require fine-tuning of flow gas and liquid flow rates compared to the standard needle. The platinum ESI sprayer needle offers more robustness and a longer lifetime especially in applications with reversed CE polarity. Reversed polarity leads to oxidative processes at the capillary exit and from this corrosion metal ion are set free, which might affect the analysis negatively (for example, anion detection in metabolomics).

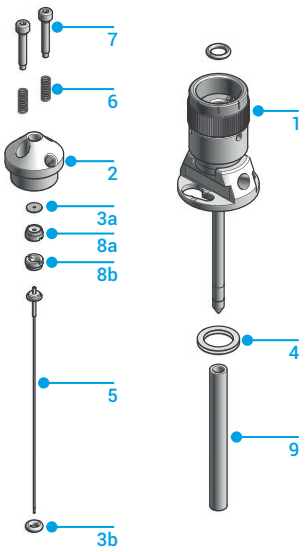


Figure 71. The Agilent CE/MS sprayer.

Replacing the sprayer gasket

A leaky gasket (item 3a, 3b, p/n G1607-20030) can generate an unstable MSD signal. The gasket can be damaged if the flow rate of the sheath liquid exceeds 200 µL/min. A set of gaskets is provided with the Agilent CE/MS sprayer kit or can be ordered separately. Parts needed for gasket replacement:

- Hex key 2 mm
- Hex key 1.3 mm
- Gasket (p/n G1607-20030)

1. Disconnect the nebulizing gas, the sheath liquid tube, and the CE/MS capillary from the sprayer.
2. Remove the CE/MS sprayer from the MS.
3. Slide protective tube over the sprayer tip.
4. Open the two body screws (7). Be careful not to lose the attached springs (6).
5. Unscrew the sprayer head (2) from the sprayer body (1).
6. Using the special screw driver (5022-2142), open the seal holding screw (8a) in the bottom of the sprayer head. Remove gasket and any particles trapped here.
7. Replace the gasket (3a) with a new one.
8. Insert the seal holding screw (8a).
9. Screw the sprayer head (2) onto the sprayer body (1)
10. Carefully assemble the connections, adjust the capillary as described earlier and reinstall it into the MS.
11. Use the test sample to verify the proper function.
12. Allow sheath liquid to rinse away surface contaminations from the newly inserted material.

Replacing the sprayer needle

Periodically check the sprayer tip for erosion under a microscope. Use the nebulizer adjustment fixture (p/n G1960-67470) and the corresponding pocket microscope (p/n G1960-67470), which are part of the accessories kit of the MS). Erosion may take place due to oxidative processes during applications. It might also be due to heat damage from the MS drying gas.

You can also examine the sprayer tip by applying a higher sheath flow. Set the pump to 5 mL/min to generate a sheath flow of 50 µL/min. At this flow it is possible to see the spray cone. The cone must be symmetrical. If the tip shows some erosion or the cone isn't symmetrical, the sprayer needle (5) needs to be exchanged.

Heat damage of the sprayer needle might come from MS drying gas when no sheath liquid is flowing. Do not leave the instrument idle at high drying gas temperatures with no sheath liquid. If so, remove the ESI sprayer from the source.

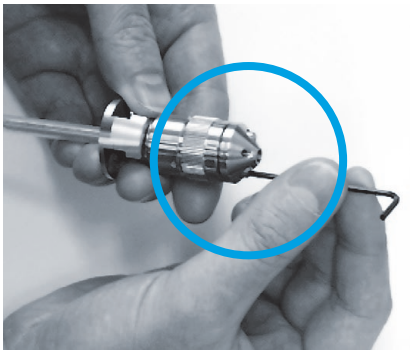


Figure 72. Unscrewing the sprayer head.

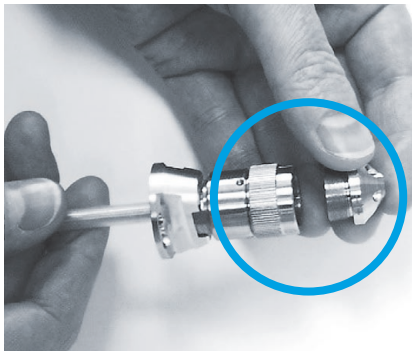


Figure 73. Removing the sprayer head.

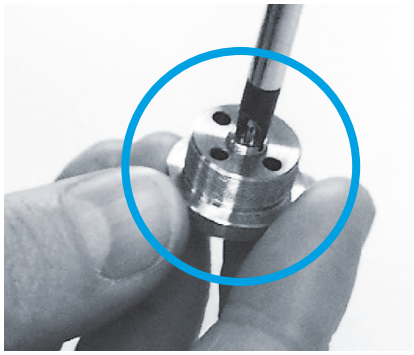


Figure 74. Accessing to gasket in sprayer head.

22.3 Preparation of buffers and test sample

The following solutions must be prepared. Note that all solvents used for CE should be filtered through a 0.2 µm filter before use.

- 1 N sodium hydroxide (initial conditioning of the capillary)
- 1 mL water (initial conditioning of the capillary)
- 10 mM ammonium acetate pH 6.9 (running buffer)
- 5 mM ammonium acetate in 50 % methanol (sheath liquid, prepare fresh at least once a week)
- 1 mg/mL quinine sulfate dihydrate in water (test sample, prepare fresh before use)

Test sample

1. Weigh 1 mg of the test sample in a 2 mL Eppendorf tube.
2. Add 1 mL water.
3. Stir until the compound is completely dissolved (place the vial for 30 min in a mixer or for 15 min in an ultrasonic bath).
4. Prepare a CE vial with the test solution (1 mL in a glass vial or 500 µL in a polypropylene (PP) vial).

Solvents used for initial conditioning

1. Prepare one vial with 1 N sodium hydroxide (300 µL in a PP vial).
2. Prepare one vial with water (1 mL in glass vial or 500 µL in PP vial).

Running buffer

1. Dilute the content of one 5 mL ampoule (containing 1.9 g ammonium acetate in 20:80 v:v methanol/water) in 245 mL water. This dilution gives a stock solution of 100 mM ammonium acetate pH 6.9, 0.4 % (v:v) methanol.
2. Dilute a certain amount of the stock solution to 1:10.
3. Prepare two vials (1 mL in glass vials or 500 µL in PP vials) with running buffer.

Sheath liquid

1. Mix 225 mL of CE grade water with 250 mL of methanol.
2. Add 25 mL of the 100 mM ammonium acetate stock solution.
3. Stir thoroughly.
4. Fill the sheath liquid in solvent bottle A of the pumping system. Where a syringe pump is used to deliver the sheath liquid, fill a syringe.

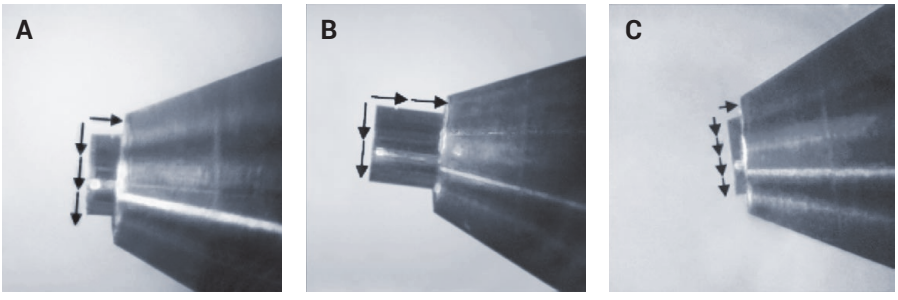


Figure 75. Protruding ESI needle, good (A), bad (B, C).

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