

# **CE/MS Analysis**

Optimization Guide





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#### **Manual Structure**

In this booklet the theoretical background as well as practical tips is given to use the Agilent CE/MS System.

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## In This Guide

In this booklet the theoretical background as well as practical tips is given to use the Agilent CE/MS System.

## **Contents**

1	Agilent CE/MS System 7
	Device Overview 8
	Device Preparation 9
2	CE/MS Method Optimization 11
	CE/MS Analysis Conditions 12
	Capillary Preparation 13
	Buffer Selection 20 Sheath Liquid Preparation 22
	Sheath Liquid Preparation 22 Applied Voltage Setting 25
	Ion Source Parameters 26
	Optimization of the Ion Introduction Voltage and Fragment Voltage 27
3	Explanation of Analysis Conditions 29
	Explanation of Analysis Conditions 30
	Measurement of Cations (Positively Charged Compounds) 31
	Measurement of Anions (Negatively Charged Compounds) 33
4	CE/MS Basic Analysis Conditions 39
	Introduction to Basic Analysis Conditions 40
	Cation Analysis Conditions 41
	Anion Analysis Conditions 50
5	Maintenance 55
	Maintenance 56
	Sprayer 58
	Diamond Column Cutter 59
	CE Main Body 60
	MS Main Body 61

#### 6 Troubleshooting 63

Troubleshooting 64

#### 7 CE Configuration 71

Conducting CE/MS analysis without using (lighting) a UV lamp 72 Conducting CE/MS analysis using the G7100 Instrument 74

#### **Contents**

## **Device Overview**

Figure 1 on page 8 shows a Capillary Electrophoresis—Mass Spectrometer (CE/MS) System configuration diagram. The CE/MS system consists of devices for CE, MS and a pump for sending sheath liquid. The setup of the PC and software that control the various devices may vary according to the type of MS connected. Because each ion source section of the Agilent Technologies MS has a common structure, the technical details related to the CE/MS described in this document can be applied to any type of MS.

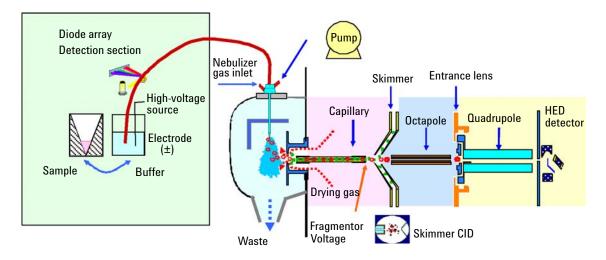


Figure 1 CE/MS system configuration diagram (The MS in the figure is a G1946B Quadrupole MS)

## **Device Preparation**

#### **Device Connections**

Confirm that the various devices and the computer are correctly connected, that the software is included and that the system starts normally. For details of the connection method see the respective manuals.

#### **Device Installation**

Set up the system so that the right side of the CE main body is close to the position of the MS ion source.

In the case of CE/MS, the height of the CE electrode and the height of the tip of the MS sprayer must be equal. Because the height of the MS ion source varies depending on the MS model, pay attention to the height of the stand on which the CE is set up. Check the installation height with Agilent Technologies before connecting the system for the first time.

If the difference in height between the positions of the MS (tip of sprayer) and the CE (electrode) is around 1 cm, the syphoning effect is negligible. However, if there is a large difference in height, a syphoning effect is created in the cappilaries. As the syphoning effect increases, inflow of air into the capillaries is generated, creating problems such as the current value not being supplied and improper sample injection.

#### 1 Agilent CE/MS System

**Device Preparation** 

#### **MS Tuning**

Although a CE/MS sprayer is used for CE/MS analysis, during MS tuning (all MSs) and precise mass calibration (TOF and QTOF only), a LC/MS sprayer is used. Set up the LC/MS sprayer and connect the PEEK piping and nebulizer gas piping from the MS. Perform tuning (or calibration). After tuning is completed, return to the CE/MS spray; connect the capillaries, sheath piping and nebulizer gas piping, and start the CE/MS analysis.

MS sensitivity in tuning may be compromised if the LC/MS sprayer is not clean, and we therefore recommend washing it on a regular basis as part of maintenance. (In particular, if LC/MS operation frequency is low, there are few chances for solvent to flow into the LC/MS sprayer and rinse, and so it easily becomes dirty.)



# CE/MS Method Optimization

CE/MS Analysis Conditions 12
Capillary Preparation 13
Capillary Length 13
Simultaneous Uptake of MS and DAD 13
Capillary Cutting 16
Capillary Installation on Sprayer 18
Buffer Selection 20
Recommended Buffers 20
Use of Nonvolatile Buffers and Various Additives 20
Buffers and Separation Behavior 21
Sheath Liquid Preparation 22
Sheath Liquid Optimization 22
Cautions Regarding Sheath Liquid During CE/TOF and CE/QTOF 2
Applied Voltage Setting 25
Ion Source Parameters 26
Optimization of the Ion Introduction Voltage and Fragment Voltage 27

## **CE/MS Analysis Conditions**

CE-MS The following parameters should be optimized when CE/MS analysis is conducted.

#### CE Aspect 1

Mainly setting the separation conditions

- Separation capillaries (type, length)
- Phoresis buffer (pH, type, concentration)

#### **MS** Aspect

Mainly setting the detection conditions

- MS parameters (Capillary Voltage, Fragmentor Voltage, etc.)
- Sheath liquid and spray chamber parameters (type, flow rate, nebulizer pressure, dry gas flow rate, dry gas temperature, etc.)

Optimize these parameters and perform CE/MS analysis. Agilent offers highly flexible conditions for cation analysis and anion analysis. Refer to "CE/MS Basic Analysis Conditions" on page 39.

**Capillary Preparation** 

## **Capillary Preparation**

## **Capillary Length**

1 Cut commercial capillaries, sold in meter units, to the required length, and use.

#### NOTE

The minimum capillary length usable is 60 cm. A total length of 100 cm is the recommended standard length.

**2** Change the capillary length as needed for the separation.

NOTE

In principle, length should be between 60 and 120 cm long.

## Simultaneous Uptake of MS and DAD

In CE, the distance from the capillary entrance to the detector becomes the effective length.

#### NOTE

In simultaneous uptake from DAD and MS, because their effective lengths are different, there are significant differences in the degree of separation between DAD and MS, and comparison of the data is problematic. It has also been confirmed that, when 1 M formic acid is used as a buffer, the light energy from the DAD UV lamp may cause disintegration of the sample. We therefore recommend using CE/MS analysis and not DAD.

By changing the configuration of the ChemStation software that controls the CE CE/MS analysis can be conducted without switching on the UV lamp. In this case, alignment interface also becomes unnecessary. (The setting details are provided below) Refer to Figure 2 on page 14 for the capillary installation method.

#### 2 CE/MS Method Optimization

**Capillary Preparation** 

If simultaneous uptake of DAD and MS is performed, create a DAD detection window 40 cm from the capillary entrance, and prepare a capillary with a total length of 100 cm. In such a case, the effective length to DAD should be 40 cm and the effective length to MS should be 100 cm (Figure 4 on page 15).

#### NOTE

See Figure 3 on page 14 for an example of capillary installation on the alignment interface and of cartridge installation. This installation method is an example of the installation recommended when a capillary is cut out of a bulk capillary. Although another installation method has been described for the CE/MS capillary marketed by Agilent (Figure 5 on page 15), excessive force is applied to the capillary when using that method, and under certain analysis conditions, there have been many cases of damage to the capillary.

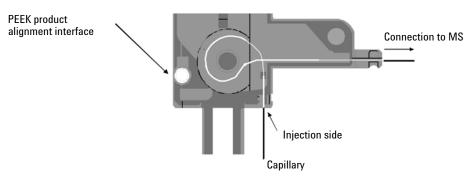


Figure 2 Capillary installation method (not using DAD)

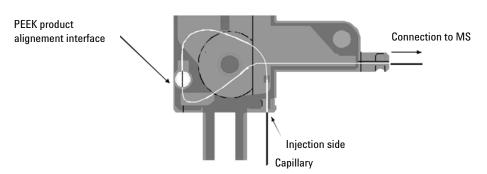


Figure 3 Capillary installation method (using DAD) (an example in which a capillary produced from bulk capillaries was used)

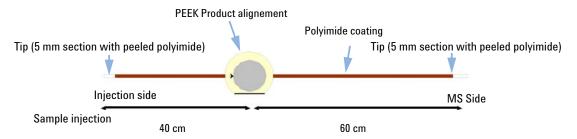


Figure 4 Alignment interface installation (when using DAD)

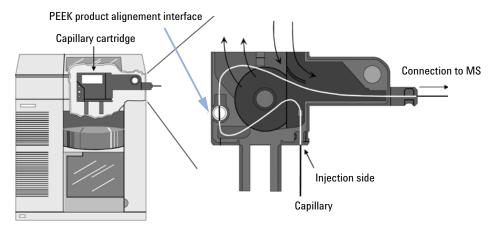


Figure 5 An installation method in which the capillary is easily damaged

#### 2 CE/MS Method Optimization

**Capillary Preparation** 

## **Capillary Cutting**

#### WARNING

#### Sharp capillaries

Wear an eye shield when handling capillaries.

#### WARNING

#### Inflammable solvents

- → Pay careful attention to not cause fires or burns when handling fire.
- 1 In order to achieve stable nebulizing, the capillary tip, that becomes the sprayer side, must be smooth.

#### NOTE

Cutting the capillary with a special diamond blade capillary cutter (part number: 5183-4669) is highly recommended; use a magnifying glass to confirm that a smooth section is obtained (Figure 6 on page 17).

- **2** Next, cut the capillary to the required length.
- **3** Burn both tips of the cut capillary with a lighter, and peal off 5 to 7 mm of the brown polyimide coating (Figure 7 on page 17).

#### NOTE

If using a coated capillary, where the capillary inner wall is coated with a polymer, etc., peel off only the sprayer side. If using a coated capillary, shorten the burning time as much as possible. Extended burning melts the polymer and may cause blockage of the capillary. Wipe off the blackened, scorched sections with methanol and acetone, peel until they are clean, and carefully remove. When handling the capillary, note that the strength of the capillary tip, from which the polyimide coating was peeled, is decreased.

#### NOTE

If analysis is done without removing the polyimide coating of the capillary on the sprayer side, the sheath liquid causes the polyimide coating to swell and expand to the sprayer tip, so that stable nebulizing cannot be done. This results in problems such as decreased sensitivity and current value abnormalities.



Figure 6 Capillary cutting examples (confirm with a magnifying glass)

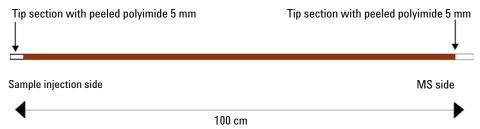


Figure 7 Example of a capillary for CE/MS analysis

**Capillary Preparation** 

## **Capillary Installation on Sprayer**

Install when the sheath liquid solution sending is stopped. If the capillary is installed or removed while sheath liquid is being sent, it will cause leakage of the sheath liquid into the sprayer.

NOTE

Note that leakage of the sheath liquid will cause problems in analysis. (Refer to Troubleshooting below for details.)

#### WARNING

#### **Sharp capillaries**

Wear an eye shield when handling capillaries.

#### WARNING

#### High voltage

During analysis, high voltage is applied to the capillary which may cause electrical shock.

- → Cover and insulate the PTFE tube in the exposed section of the capillary.
- → Make sure that no voltage is applied when working in contact with the capillary and sprayer part.
- 1 Carefully install in the sprayer, so as not to damage the cut surface of the capillary.
- 2 Since dirt on the hands, etc., is detected as background, we recommend that you thoroughly wipe the outside of the capillary with isopropanol (IPA) during installation before installing it in the sprayer.
- **3** Examine the sprayer tip with a magnifying glass, confirm protrusion of the capillary and then install it in the device.

## NOTE

For detailed method of installation on the sprayer, see the manual "Installing the Agilent CE ESI-MS Sprayer Kit (G1607A)" or the "Agilent Capillary Electrophoresis System Manual". The manuals assume 2 scales, but because a gap may be produced when the PEEK screw is fixed, we highly recommend that you check the protrusion of the tip. Protrusion of about 1/4 to 1/3 of the capillary diameter, i.e., of about  $100~\mu m$ , is ideal. Firmly tighten the PEEK screw that fixes the capillary and confirm that there is no leakage of the sheath liquid when the capillary is fixed.

#### NOTE

For safety, cover the exposed section of the capillary (between the CE main body and the sprayer) with a PTFE protecting tube.

## **Buffer Selection**

#### **Recommended Buffers**

Buffers recommended for use in CE/MS are volatile acid-base types such as formic acid, ammonium formate, acetic acid and ammonium acetate. Analysis buffers for CE, marketed by our company (Organic Acid Buffer, Plating Bath Buffer, Basic Anion Buffer, Cation Buffer, Inorganic Anion Buffer, etc.), are not recommended for use in CE/MS analysis. The reason for this will be explained in the following section.

#### **Use of Nonvolatile Buffers and Various Additives**

We recommend the use of volatile buffers in CE/MS. As in LC/MS, nonvolatile buffers, such as phosphoric acid buffer and boric acid buffer, cause dirt in the electrospray chamber and the MS, block ionization and result in a decrease in sensitivity.

Moreover, although additives, such as various surfactants, reagents for optical resolution, etc., are sometimes added to the buffer in order to improve separation in CE, the use of such additives is also the cause of dirt in the chamber and a decrease in sensitivity, and we recommend avoiding such additives as much as possible, or using as low concentrations as possible. If additives are used, cleaning the ion source and maintenance of the MS capillary become essential, and care must be taken to increase the frequency of these oprations.

In the absence of a phoresis buffer, if nonvolatile sodium hydroxide and phosphoric acid are used for rinsing the capillary (for conditioning), the nebulizer pressure should be set to 0 psi, and the sheath liquid drained.

NOTE

We recommend setting the capillary voltage to 0 V, and — to the extent possible — not inserting it into the MS main body. Even if such measures are taken, frequency of ion source cleaning and MS capillary maintenance should be increased.

## **Buffers and Separation Behavior**

CE separation is significantly affected by buffer pH. Better CE separation is qualitatively and quantitatively advantageous. Because it also minimizes the effects of ionization suppression, it is advantageous for MS detection. Although many ionic compounds can be analyzed under the basic conditions recommended by Agilent Technologies, when these conditions are optimized, by taking into consideration the characteristics of the compound to be measured and ionization mode selection, the analysis conditions, such as the buffer pH, are also optimized. Moreover, because higher concentration of the phoresis buffer can be advantageous for the stacking effect (an effect caused by the difference in the strengths of the electric fields between the sample zone and the phoresis buffer, which causes the sample to become concentrated in the capillary immediately after its injection and sensitivity to increase), a good peak shape can be obtained.

NOTE

If the buffer concentration is too high, the MS detection sensitivity is compromised.

## **Sheath Liquid Preparation**

## **Sheath Liquid Optimization**

In CE, the flow rate of the buffer in the capillary, caused by the generation of an electroosmotic flow, is very low, on the order of several hundred nl/min. For this reason, in order to conduct stable CE/MS analysis, the system is devised so that the sheath liquid is sent from outside the capillary to supplement the flow rate. As a sheath liquid, we recommend using a  $50\,\%$  methanolic solution as the base material. If an acid base is to be added to the sheath liquid, an acid base with volatility similar to that of the buffer is preferable; we recommend the use of acetic acid, formic acid or ammonium.

Generally, if a 50 % methanolic solution alone, or 5 mM ammonium acetate in a 50 % methanolic solution is used, either the ESI-Positive or -Negative modes can be measured with good sensitivity. If sufficient sensitivity cannot be obtained at the ESI-Positive mode, reexamine after changing to formic acid or acetic acid in a range of 0.1 to 1 %. For the ESI-Negative mode, reexamine after changing 5 to 10 mM ammonia water. However, if a coated capillary is used, after completing analysis with a high-pH sheath liquid, we recommend that you replace it with a neutral sheath liquid or with a water/methanol solution. This is done in order to prevent deterioration of the coating by the high-pH sheath liquid.

Table 1 on page 23 shows the composition of a standard sheath liquid. In addition to methanol, ethanol, isopropanol, etc., can also be used as the organic solvent added. Since acetonitrile is particularly pre to causing deterioration of the capillary polyimide coating, we recommend not using it.

The flow rate of the sheath liquid is variable within a range of 4 to 10  $\mu$ l/min (0.4 to 1.0 ml/min in the LC pump settings). If the quantity of the sheath liquid is high, stable measurements can be conducted, but because the sample element is diluted, sensitivity decreases somewhat. It is thought that sensitivity increases at a low flow of 4 to 6  $\mu$ l/min, but because sensitivity is readily impacted by the smoothness of the capillary cut surface and by the set up in the sprayer, conversely problems such as decreased sensitivity and difficulties in conducting stable analysis frequently occur. Therefore, analysis conducted at a sheath liquid flow of 8  $\mu$ l/min is thought to yield the best values, in terms of both stability and sensitivity.

Moreover, we recommend placing the CE/MS system, including the sheath liquid pump, in a location where it will not be affected by temperature changes. If the temperature of the installation environment varies considerably, the viscosity of the sheath liquid will change, causing the pressure of the sheath liquid solution sending pump to oscillate and the flow rate to change.

Although the sheath liquid can be continuously used, as a result of the recycling function, as a rule it should be replaced with a new sheath liquid every day, if possible, and at least every week. This is done in order to prevent dirt accumulation in the sheath liquid as a result of recycling. Moreover, when using a high-pH sheath liquid, because evaporation of the ammonia tends to cause changes in the pH, we recommend preparing a new sheath liquid every day. Before analyzing the sheath liquid, conduct preliminary deaeration of the sheath liquid, and pass the sheath liquid through a degasser. If deaearation is insufficient, spike-like signals appear on the electroferrogram, caused by air bubbles contained in the sheath liquid; increased changes in the pressure of the sheath liquid pump reduce reproducibility (migration time, area value) and prevent stable analysis.

Furthermore, when removing a capillary, make sure that solution sending of the sheath liquid is stopped. Note that leakage of the sheath liquid in the sprayer will cause leakage current.

**Table 1** Example of a standard sheath liquid composition

Analysis object (ionization method)	Main usable sheath liquids	
Cationic (ESI-Positive)	50 % methanol 5 mM ammonium formate in 50 % methanol 5 mM ammonium acetate in 50 % methanol 0.1 to 1 % acetic acid in 50 % methanol 0.1 to 1 % formic acid in 50 % methanol	
Anionic (ESI-Negative)	50 % methanol 5 mM ammonium formate in 50 % methanol 5 mM ammonium acetate in 50 % methanol 1 to 10 mM acetic acid in 50 %methanol	

## Cautions Regarding Sheath Liquid During CE/TOF and CE/QTOF

If measurements are conducted as CE/TO and CE/QTO, we recommend using analysis conditions whereby the reference mass is put in the sheath liquid, without using a reference mass sprayer, as described below.

- Detach the reference mass sprayer and close the nitrogen gas supply port for the reference sprayer in the MS main body
- If the reference sprayer is not detached, set the nebulizer pressure of the reference sprayer to 0 psi and edit the analysis method whereby the check on Use Bottle A was removed (only QTOF is possible)

#### NOTE

We recommend using Purine and HP-0921 of the Agilent Reference Mass Mixture (G1969-85001) for the reference mass. Add each to the 50 % methanolic solution after ultrasonic deaeration (standard: add 20 to 100  $\mu l$  each to 500 ml50 % methanolic solution) and use as a sheath liquid. A degasser must be used. Pay close attention to contamination of the sheath liquid, and remember to prepare a new batch every day. If ammonium formate, ammonium acetate or ammonia is added to the sheath liquid, the added ions may tend to exit.

**Applied Voltage Setting** 

## **Applied Voltage Setting**

#### **Applied Voltage**

#### NOTE

We recommend using a Time Program for the voltage during analysis, and applying it with in gradients so that it reaches the set value within 0.1 to 0.3 min.

By using an electric field program that gradually increases the voltage at the beginning of analysis, it is possible to prevent sudden voltage application to the capillary. As a rule, keep the upper limit of the voltage applied to 300 to  $350~\rm V/cm$ .

Pay particular attention when using a high-concentration (e.g., 1 M) formic acid buffer. In some cases, such buffers may cause sudden application of high voltage and result in damage to the capillary. Damaged capillaries may result in the following problems: current not flowing during analysis, current value dropping significantly during analysis and capillary breakage. If a capillary is damaged, replace the capillary and lower the applied voltage value. When using a 1 M formic acid buffer, set the upper limit value to 300 V/cm.

If the length of the capillary is shortened, pay attention to the voltage set value. A safety mechanism will go into operation when the current value during analysis exceeds the uppler limit value of the CE/MS device, which is  $\pm 50~\mu A$ . The device automatically lowers the applied voltage value, so that the flow value does not exceed  $\pm 50~\mu A$ . Even though analysis is not interrupted when the safety mechanism is operating, because the voltage applied is not the applied voltage value set in the method, stable data cannot be collected. Reexamine the capillary length or the applied voltage value.

In order to verify the voltage value and current value during analysis, when there appear to be problems with analysis, carry out troubleshooting by checking **CurrentVoltage** of **Store Data**, which is set in the ChemStation software controlling the CE, monitoring values by displaying the current value on the online plot,etc.

## **Ion Source Parameters**

Parameters that can be used to optimize the spray chamber include the nebulizer pressure, dry gas temperature and dry gas flow rate. First, conduct analysis using the recommended values provided in Table 2 on page 26

 Table 2
 Recommended values for CE/MS spray chamber parameters

Parameters	CE/MS recommended values
Sheath liquid flow	8 – 10 µl/min
Nebulizer pressure	10 psi <sup>1</sup>
Dry gas flow	10 I/min
Dry gas temperature	200-300 °C
Vcap	4000 V (pos),3500 V (neg)

When injecting the sample, if the nubulizer pressure is to be set to 0 psi, set it using the time program (ChemStation) or time segment (MassHunter). During the preconditioning and injection processes, set the nebulizer pressure to 0 psi. Mass

# Optimization of the Ion Introduction Voltage and Fragment Voltage

Optimization of the ion introduction voltage and fragment voltage is based on the same concept as optimization in LC/MS. When measuring low molecular weight compounds, such as amino acids and organic acids, as the measurement subjects of CE, the standard fragment voltage is 100 V. Optimize the fragment voltage as needed. Also optimization of other parameters is based on the same concept as optimization in LC/MS.

## 2 CE/MS Method Optimization

Optimization of the Ion Introduction Voltage and Fragment Voltage



## **Explanation of Analysis Conditions**

Explanation of Analysis Conditions 30

Measurement of Cations (Positively Charged Compounds) 31

Measurement of Anions (Negatively Charged Compounds) 33



## **Explanation of Analysis Conditions**

Since there are differences in the speed (electrophoretic mobility) in which ionic compounds undergo electrophoresis, separation is achieved while the compounds are in electrophoretic movement. The degree of electrophoretic movement is proportional to the charge of the solute and in reverse proportion to the ionic radius. For example, an ion with a small ionic radius and a high charge would have high mobility, while an ion with a large ionic radius and a small charge would have limited mobility. Since the charge of a solute depends to a large degree on the pH of the buffer used for the measurement, buffer pH can be seen the most important parameter in controlling separation.

## **Measurement of Cations (Positively Charged Compounds)**

Generally, in the measurement of cations, buffers in the low-pH zone (between 2 and 5) are used in order to increase the ionicity of the measurement subject. Many cations can be analyzed using an inner-wall untreated fused silica capillary and a formic acid buffer. Table 3 on page 32 shows examples of capillaries and buffers generally used in cation analysis.

Figure 8 on page 31 shows separation under such analysis conditions and a typical example of the electroferrogram.

If the phenomenon of sample adsorption to the capillary inner wall (the detected peak shows marked tailing, etc.) occurs, even though a low-pH buffer is used, an organic solvent such as methanol/acetonitrile should be added to about 0 to 30 % of the buffer, or a capillary whose inner wall is coated with a hydrophilic polymer (Polyvinyl Alcohol-Coated (PVA) Capillaries) should be used. Moreover, when a coated capillary is used, note that it is within the pH-proof range of the capillary, described in the attached manual.

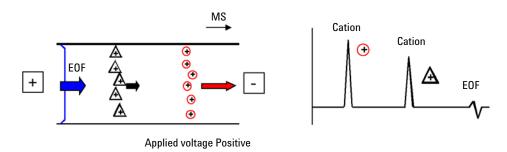


Figure 8 Examples of separation (left) and an electroferrogram (right)

#### 3 Explanation of Analysis Conditions

**Measurement of Cations (Positively Charged Compounds)** 

 Table 3
 Examples of capillaries and buffers used for cation analysis

Capillary	Main usable buffers	
Inner wall untreated fused silica capillary	0.1 to 1 M formic acid 0.1 to 1 M acetic acid 10 to 50 mM ammonium formate 10 to 50 mM ammonium acetate (basically acidic to neutral buffers)	
Polymer coated capillary (PVA etc.)	Buffers other than formic acid (basically acidic to neutral buffers) (Note the pH-tolerance range of each capillary. For PVA, pH 2.5 to 9.5)	

Conduct separation at a "Positive" applied voltage. Because the MS side is grounded, it serves as a relative cathode.

## **Measurement of Anions (Negatively Charged Compounds)**

Generally, in the measurement of anions, buffers in the neutral to high-pH zone (between 6 and 10) are used in order to increase ionicity. If a fused silica capillary is used, an electroosmotic flow (hereinafter, EOF) is created; the higher the pH of the buffer the faster the EOF.

The EOF flow direction (from the anode side to the cathode side) and the direction of the anion migration (from the cathode side to the anode side) are opposite to each other. Although anions tend to migrate to the anode, because the EOF electrophoretic mobility is one digit or more higher, they are carried by the EOF to the cathode side. Cations, for which the directions of the electrophoretic migration and of the EOF are the same, move the fastest to the cathode side.

Although neutral materials do not separate, they are all carried at the same speed as the EOF. Anions migrate in the opposite direction to the EOF, but they are transported by the EOF and move most slowly to the cathode side. For this reason, when you set the MS side so that it serves as a relative cathode (i.e., the applied voltage is "positive"), the order of migration, by which materials are detected, is cations-EOF (neutral materials)-anions.

However, because ions with a small ionic radius and a high charge migrate in the opposite direction to the EOF, they cannot be detected at the MS side, which is the cathode side. For this reason, there is also a method that uses a coated capillary, in which the EOF is decreased, sets the MS side as a relative anode (i.e., the applied voltage is "negative"), and so causes all the anions to migrate to the same MS side (anode).

Bringing all these together results in the following 2 analysis conditions.

**Measurement of Anions (Negatively Charged Compounds)** 

#### Using an inner wall untreated fused silica capillary

Because a high-pH buffer is used and the analysis conditions are such that anions are swept in the opposite direction to the migration original direction by a fast EOF, these conditions are generally suitable for analysis of anions with little mobility. If cations simultaneously exist in the sample, those cations will be detected before the EOF. Figure 9 on page 34 shows separation under such analysis conditions and a typical example of the electroferrogram. Although anions migrate in the direction of the anode, because the EOF mobility is greater, they are carried by the EOF to the MS side.

These analysis conditions are advantageous because readily available and inexpensive fused silica capillaries are used and the cost performance is therefore good, the buffer pH is not restricted, and capillary durability is high.

The buffer pH has significant effect on the separation and peak shape. Adjust the buffer pH so that the measurement subject gains ionicity. Neutral substances, that have no ionicity, do not separate and are detected as they settle at the EOF position.

NOTE

If the type and number of functional groups of the measurement subject suggest that it has no (or low) ionicity, or if it is a compound whose separation seems to be achieved because of LC reverse phase distribution, we recommend analysis by means of LC/MS.

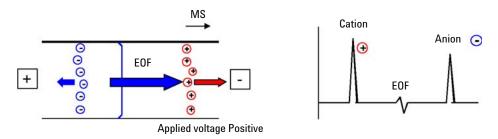


Figure 9 Examples of separation (left) and an electroferrogram (right)

Moreover, although transported by the EOF, anions with relatively high mobility (such as citric acid and oxalic acid) tend to be detected after the analysis time and to have inferior peak shape.

#### NOTE

In order to improve the peak shape, we recommend the analysis conditions whereby the coated capillary, introduced in "Using PVA-coated capillaries", is used.

On the other hand, anions with very high mobility, that move opposite the EOF (some inorganic anions, etc.), are not transported to the MS side and so are not detected in MS. We recommend the analysis conditions of "Using PVA-coated capillaries" also when analyzing such anions.

#### **3** Explanation of Analysis Conditions

**Measurement of Anions (Negatively Charged Compounds)** 

#### **Using PVA-coated capillaries**

With this type of coated capillary, EOF is hardly generated even when nearly neutral buffers are used. Such capillaries are suitable, therefore, for the analysis of anions with relatively high mobility. When fused silica capillaries are used, stable analysis can be conducted even with buffers in the neutral zone (pH 6 to 8), where EOF speed is prone to be unstable.

NOTE

Note, however, that the usable buffer pH is limited, the capillaries are generally expensive, and the durability of the capillaries is somewhat inferior.

Figure 10 on page 37 shows separation under such analysis conditions and a typical example of an electroferrogram.

Even with a coated capillary, there is slight flow of EOF from the MS side to the sample injection side (in other words, in the counter-flow direction to the sample injection side). For this reason, in the electrospray state, the EOF flows in the direction of the MS side and becomes more unstable than in the conditions described above ("Using an inner wall untreated fused silica capillary"), and there are instances in which stable CE/MS analysis cannot be conducted. Therefore, in order to conduct stable analysis, we recommend using a time program and continuously applying minimal pressure (between 20 and 50 mbar) during analysis from the sample injection side of the capillary. Furthermore, the CE current value is stable under analysis conditions in which the sheath liquid contains a salt, such as ammonium formate or ammonium acetate.

Moreover, capillaries whose inner wall is positively charged are also available commercially, but our company does not handle that type of capillary.

Generally, polymer coated capillaries have inferior durability compared with the standard fused silica capillaries, and they tend to easily become blocked. Examination of the sample preprocessing method and of the capillary washing method is important. Moreover, because specific adsorption phenomena (ion adsorption, complex formation) may occur, based on the polymer characteristics, carefully read the manuals pertaining to polymer coated capillaries.

Table 7 on page 50 shows examples of capillaries and buffers generally used in anion analysis.

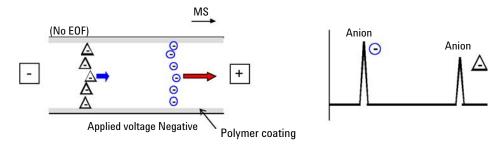


Figure 10 Examples of separation (left) and an electroferrogram (right)

 Table 4
 Examples of capillaries and buffers used for anion analysis

Capillary	Main usable buffers	
Inner wall untreated fused silica capillary	10 to 50 mM ammonium acetate 10 to 50 mM ammonium formate	
Applied Voltage Positive	(basically neutral to alkaline buffers)	
Polymer-coated capillary (PVA etc.) <sup>1</sup> Applied Voltage Negative During analysis make sure to apply pressure of 20 to 50 mbar!	10 to 50 mM ammonium acetate 10 to 50 mM ammonium formate 10 to 50 mM ammonium carbonate (basically neutral to alkaline buffers) Note the pH-tolerance range of each capillary. For PVA, pH 2.5 to 9.5)	

Note that the use of a high buffer for an extended period will cause peeling of the polymer coating in the capillaries.

#### 3 Explanation of Analysis Conditions

**Measurement of Anions (Negatively Charged Compounds)** 

**Anion Analysis Conditions** 

50

#### 4 CE/MS Basic Analysis Conditions Introduction to Basic Analysis Conditions

## **Introduction to Basic Analysis Conditions**

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 shown. Furthermore, the data provided in this document was collected by the following quadrupole MS. (Quinine sulfate (G1946D)), amino acids (G6530A), organic acid (G6530A).

## **Cation Analysis Conditions**

#### **Cation Basic Analysis Conditions Amino Acid Example**

The analysis conditions shown here (Table 5 on page 42) are very general analysis conditions that can be used to measure almost all basic compounds. Figure 11 on page 43 and Figure 12 on page 44 show the analysis results of amino acids. Since a low-pH buffer, with pH 1.9, was used, the basic compounds show cationicity. In addition to amines, almost all amino acids and peptides that have an isoelectric point showing cationicity. In other words, all compounds with functional groups that show basicity and with cationicity under conditions of pH 1.9 can be meausred under these analysis conditions. To optimize separation of isomers and improve the peak shape, see "Explanation of Analysis Conditions" on page 29.

**Cation Analysis Conditions** 

 Table 5
 CE/MS analysis conditions (for cation analysis)

<CE> Capillary: fused silica capillary (50 μm

i.d., Total length 100 cm

Without UV detection window

Buffer: 1 M Formic acid

Voltage: Positive 0 to 30 kV(Gradient)

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

(Inhome Vial)

Time programming: 0.3 min Voltage +30 kV

<MS> Polarity: ESI-Positive

Capillary voltage: 4000 V

Fragmentor voltage: 100 V

Drying gas, Temp: N<sub>2</sub>10 I/min., 300 °C

Neb gas press.: 10 psi

Sheath liq.: 5 mM Ammonium acetate in

50 % Methanol

Flow rate: 8 µl/min

Dilution of amino acid standard mixture (part number: 5061-3330) to 100 µM.

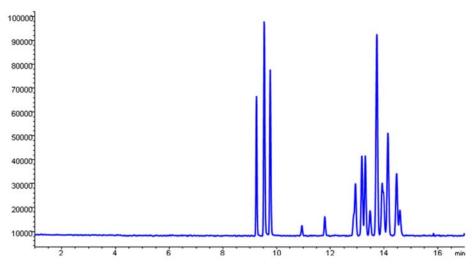


Figure 11 Mass electroferrogram (SIM) of amino acids Std

**Cation Analysis Conditions** 

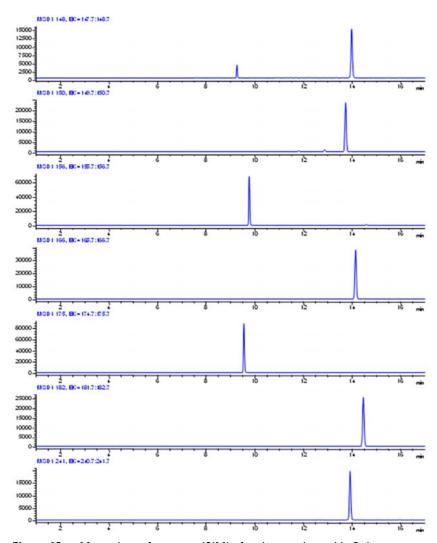


Figure 12 Mass electroferrogram (SIM) of various amino acids Std

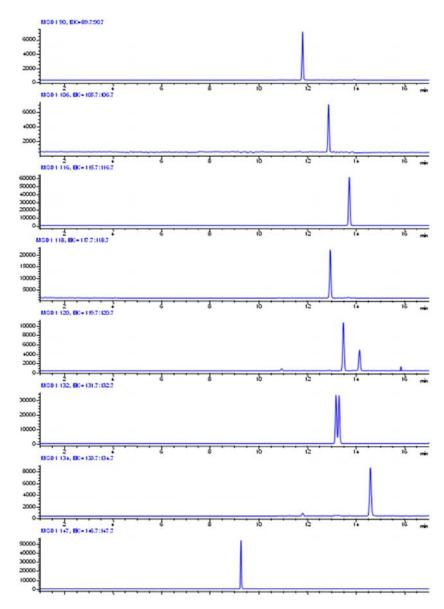


Figure 13 Mass electroferrogram (SIM) of various amino acids Std

**Cation Analysis Conditions** 

#### **Example of Quinine Sulfate Analysis**

The following is an example in which quinine sulfate was analyzed under analysis conditions for cations.

The analysis conditions are shown in Table 6 on page 47. The total ion electroferrogram obtained is shown in Figure 14 on page 48. The mass electroferrogram extracted at m/z = 325, which is the pseudomolecular ion of quinine sulfate [M+H], is shown in Figure 15 on page 48. The mass spectrum of quinine sulfate is shown in Figure 16 on page 49. As reference, the current values of the capillary of the capillary electrophoresis unit, used in this analysis, are shown in Figure 17 on page 49.

 Table 6
 CE/MS analysis conditions (for cation analysis)

<CE> Capillary: fused silica capillary (50 μm

i.d., Total length 100 cm

Without UV detection window

Buffer: 1 M Formic acid

Voltage: Positive 0 to 27 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  $2.0\ s$ 

(Inhome Vial)

Time programming: 0.3 min Voltage +27 kV

<MS> Polarity: ESI-Positive

Scan range: m/z=100-500

Capillary voltage: 3500 V

Fragmentor voltage: 80 V

Drying gas, Temp: N<sub>2</sub>10 I/min., 300 °C

Neb gas press.: 10 psi

Sheath liq.: 5 mM Ammonium acetate in

50 % Methanol

Flow rate: 10 µl/min

**Cation Analysis Conditions** 

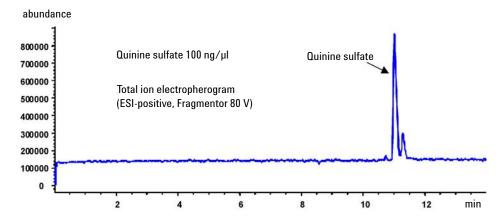
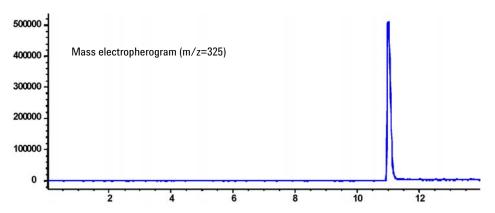


Figure 14 Total ion electroferrogram



**Figure 15** Mass electroferrorgram (m/z = 325)

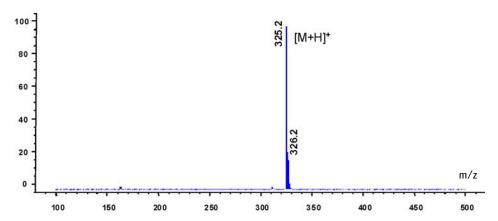


Figure 16 Mass spectrum of quinine sulfate (ESI-Positive, Frag. 80V)

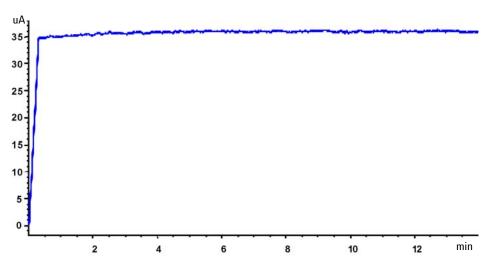


Figure 17 Current values during analysis of quinine sulfate

## **Anion Analysis Conditions**

#### **Anion Basic Analysis Conditions Organic Acid Example**

We will show an example of analysis of an organic acid under anion analysis conditions. (These analysis conditions are not implemented as confirmation of operation during CE/MS standard installation.) These analysis conditions (Table 7 on page 50) can be applied for analysis of compounds with anionicity in the pH of the buffer used, such as organic acids and aromatic carboxylic acids.

**Table 7** CE/MS analysis conditions (for anion analysis)

<CE> Capillary: fused silica capillary (50 μm i.d., Total length

80 cm<sup>1</sup> Without UV detection window

Buffer: 20 mM Ammonium formate pH10 (Adjusted with

1 %NH<sub>4</sub>OH)

Voltage: Positive 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 (Inhome Vial)

Time programming: 0.3 min Voltage +30 kV

<MS> Polarity: ESI-Negative

Capillary voltage: 350 V Fragmentor voltage: 100 V

Drying gas, Temp: N<sub>2</sub>10 I/min., 300 °C

Neb gas press.: 10 psi

Sheath lig.: 5 mM Ammonium hydroxide in 50 % Methanol

Flow rate: 8 µl/min

 $<sup>^{1}</sup>$  A 100 cm capillary may be used

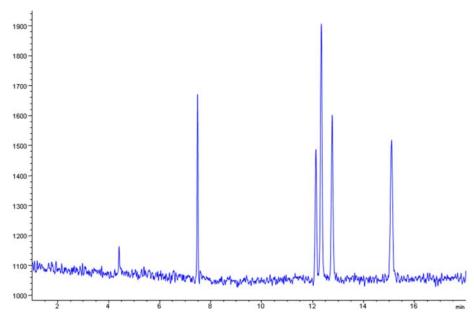


Figure 18 Mass electroferrogram (SIM) of organic acids (in the order of lactic acid, succinic acid, malic acid, tartaric acid and citric acid) organic acid 1 mg/l Std

<CE>

**Anion Analysis Conditions** 

#### p-Toluenesulfonic Acid Analysis

An analysis example of p-toluenesulfonic acid is shown with reference to the basic analysis conditions of anions.

The analysis conditions are shown in Table 8 on page 52. The total ion electroferrogram obtained is shown in Figure 19 on page 53. The mass electroferrogram extracted at m/z = 171, which is the pseudomolecular ion of toluenesulfonic acid [M-H], is shown in Figure 20 on page 53. The mass spectrum of toluenesulfonic acid is shown in Figure 21 on page 54. As reference, the current values of the capillary of the capillary electrophoresis unit, used in this analysis, are shown in Figure 22 on page 54.

Table 8	CE/MS	analysis	conditions	(for	p-toluenes	ulfonic	acid)
---------	-------	----------	------------	------	------------	---------	-------

Capillary: fused silica capillary (50 µm i.d., Total length

60 cm)

Without UV detection window

Buffer: 20 mM Ammonium acetate, pH 9.0 (Adjusted

with 1 % NH<sub>4</sub>OH)

Voltage: Positive 0 to 20 kV (Gradient)

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 (Inhome Vial)

Time programming: 0.3 min Voltage +20 kV

<MS> Polarity: ESI-Negative

Scan range: m/z=70-300
Capillary voltage: 3500 V
Fragmentor voltage: 100 V

Drying gas, Temp: N<sub>2</sub>10 I/min, 300 °C

Neb gas press.: 10 psi

Sheath lig.: 5 mM Ammonium acetate in 50 % Methanol

Flow rate: 10 µl/min

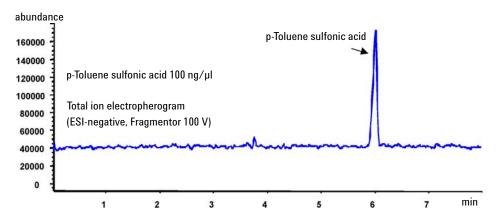
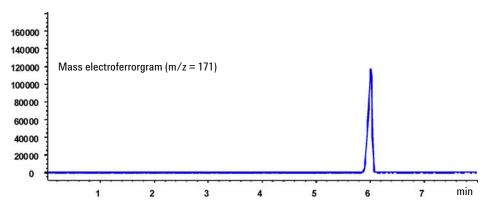


Figure 19 Total ion electroferrogram



**Figure 20** Mass electroferrorgram (m/z = 171)

**Anion Analysis Conditions** 

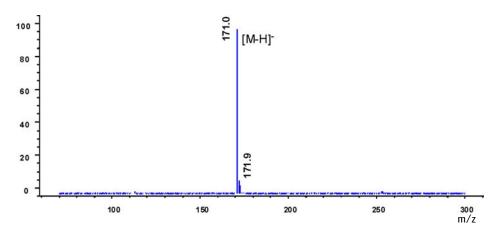


Figure 21 Mass spectrum of toluenesulfonic acid (ESI-Negative, Frag. 100V)

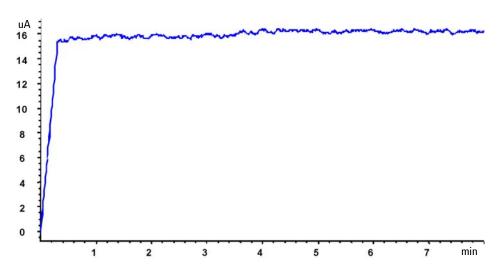
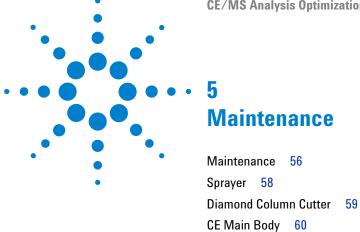


Figure 22 Current value during toluenesulfonic acid analysis

MS Main Body 61



#### Maintenance

#### When Analysis is Immediately Restarted

#### NOTE

If measurement starts a day or several days after the end of analysis, it can be done in the same state as at the end of analysis. However, if both a coated capillary and a high-pH sheath liquid are used, because of the possibility that the alkalinity of the sheath liquid will damage the coating, we recommend replacing the sheath liquid with a 50 % methanol aqueous solution.

#### NOTE

Furthermore, if sending of the sheath liquid is stopped because of Shutdown (Standby) after the end of analysis, we recommend stopping after the inside of the capillary is washed and the tip of the sprayer is washed with sheath liquid. When blank analysis or a method for washing is prepared and then implemented at the conclusion of serial analysis, take care that dirt from the sample does not remain in the sprayer.

1 Wash the capillary with buffer and ultrapure water and conduct blank analysis for about 30 minutes, to flush the sample matrix out of the capillary and wash the sprayer tip with sheath liquid.

#### 0R

Alternatively, after the end of analysis, do not immediately stop sending the sheath liquid, but keep it flowing at a flow rate of 4 to 6  $\mu$ l/min, as an effective measure against adhesion of dirt in the sprayer. In such a case, set the nebulizer pressure to 0 psi and the Vcap to 0 V, and set the fragmentor voltage to 0 V without putting the device on Standby.

## When Analysis is Not Immediately Restarted

- 1 Wash the inside of the capillary with buffer, ultrapure water, etc.
- **2** Replace the sheath liquid with 50 % methanol, and then with isopropanol (IPA), and thoroughly wash the splitter and sprayer.
- **3** Conduct the washing while the capillary is installed in the sprayer.

#### NOTE

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

## Sprayer

If problems that seem to be caused by the sprayer, occur, wash the sprayer and replace the inner needle. Refer to the Troubleshooting section below to determine the cause of problems.

Sprayer maintenance is based on the operation frequency. Exchangeable parts include 2 gaskets (part number: G1607-20030 (1/pk)) and a needle (part number: G1607-60041). Replace the gaskets every 3 to 6 months, or if excessivespike noise continues to be detected, even after the sprayer is washed. If examination with a magnifying glass reveals distortion and corrosion (rust) of the needle tip, replace it with a new needle.

NOTE

Dirt inside the sprayer and inside the needle may cause problems. Immerge the side of the sprayer tip in a solvent (acetone, IPA, MeOH, etc.) and conduct ultrasonic washing. (If a conical flask is used, it can be washed without the tip touching the bottom of the container.) When immerging the tip, do not immerge the entire sprayer in the solvent. Doing so will cause the sealing material inside the sprayer to deteriorate.

NOTE

Because the tip of the sprayer is very delicate, handle it with great care, so as not to cause deformation of the tip. Although deformation of the needle only can be handled by replacing it with a new needle, if the sprayer itself is deformed, sensitivity significantly decreases, in which case each of the main bodies has to be replaced.

NOTE

Because background increases after the gaskets and needle are replaced, use for analysis after the passages are thoroughly washed. We recommend that new gaskets and needles always be ready in reserve.

#### **Diamond Column Cutter**

In order to obtain a smooth capillary cut surface with a diamond column cutter (part number: 5183-4669), make sure that you use it correctly. Although the diamond blade is made of hard material, the blade section is very delicate. If the diamond blade is damaged by impact from a fall or application of excessive force while pressing on the capillary, it will not perform as originally intended.

NOTE

Use the blade only after carefully reading the manual.

The diamond blade is a disposable product. If you cannot obtain a smooth capillary cut surface, replace the blade with a new exchange diamond blade (part number: 5183-4670). Alternatively, blade sharpness can be easily maintained by thoroughly spraying with compressed air at each cutting and removing fragments from the blade.

See the attached diamond column cutter manual for details of how to replace the diamond blade.

#### Maintenance CE Main Body

## **CE Main Body**

- Wash the CE electrodes and prepuncher about once every 2 weeks.
- Wash with water and then with methanol, thoroughly dry and install.

#### NOTE

When washing the electrodes, never use the ultrasonic washer; doing so will cause the internal sealing material to deteriorate. Avoid drying with warm air.

• Also wash the insulation plates, where the electrodes are fixed, and the capillary cartridge, about once a month. Or else, wipe dirt off with a wipe soaked in water once a week.

#### NOTE

Because the CE is analysis equipment to which high voltage is applied, it readily collects charged dust from the environment. Take care to keep it clean.

Install washed parts in the device only after thoroughly drying them.

For detailed maintenance of the CE, refer to the manual attached to the CE.

## MS Main Body

After conducting ordinary EC/MS analysis, it is sufficient to lightly wipe the ion source (spray chamber). However, if a nonvolatile additive was added to the buffer, the spray chamber readily becomes dirty, and gentle washing is therefore necessary. The degree of uncleanliness may necessitate washing the MS capillary.

Maintenance of the MS side includes washing the MS capillary, replacing the nitrogen gas purifier and changing the oil of the rotary pump; refer to the manual attached to the MS for other maintenance details of the MS. We recommend that the nitrogen gas purifier be replaced at least once a year, and if possible every six months (depending on the installation environment, especially if a nitrogen generator is used).

#### 5 Maintenance

MS Main Body



## Troubleshooting

#### Troubleshooting 64

Instability of MSD Signal (No Signal Optained) 64

The CE Current Does Not Flow 65

MS Signal Sensitivity (S/N) is Low 65

MS Background is High 66

Instability of MSD signal (Reproducibility of Migration Time Not Obtained. In Serial Analysis, Migration Time Becomes Shorter or Longer) 66

Instability of MS Signal (Reproducibility of Migration Time Not Obtained. Changes are Irregular) 67

The Current Value During Analysis is Unstable 68

MSD Sensitivity Dropped 68

Instability of MSD Signal (Spike Noise is Frequently Confirmed,

Intermittent Noise, etc.) 69

## 6 Troubleshooting Troubleshooting

## **Troubleshooting**

Troubleshooting applies only to systems connected to the CE and MS manufactured by Agilent Technologies. Because the causes for problems may be complex, always refer to the entire troubleshooting section.

### **Instability of MSD Signal (No Signal Optained)**

**Table 9** Instability of MSD signal (no signal optained)

Potential Cause	Suggested action
MS problem	Conduct MS tubing and check if a signal is detected by the MS unit.
Inappropriate CE/MS analysis conditions	Conduct analysis under standard analysis conditions and STD and confirm. Confirm that the buffer and sheath liquid compositions and pH match the analysis conditions. Set the CE separation conditions / MS detection conditions taking into account sample properties and ionicity.
Inappropriate setting of analysis conditions	Confirm that there is no error in the settings. (in particular, the analysis time settings, applied voltage polarity and ionization polarity) If the analysis stops during the process, confirm that there is no problem with the current value, etc.
The sheath liquid does not flow. The sheath flow is too low	Increase the flow of the sheath liquid. A flow of 8 to 10 µl/min is recommended. Confirm that the splitter for the sheath liquid is not blocked. If a blockage is confirmed, replace with a new splitter.
Insufficient deaeration of sheath liquid.	Preliminary deaeration of the sheath liquid is recommended. Use a degasser.

#### The CE Current Does Not Flow

Table 10 The CE current does not flow

Potential Cause	Suggested action
Air bubbles form in the capillary	Flush again and reanalyze
Plugged capillary	If the problem is not resolved by flushing & reanalysis, replace with a new capillary. If you find that there is no problem with the STD and that the blockage was caused only by the sample, reexamine the method of preprocessing the sample.
Broken capillary	The DAD detection window section or MS side tip section are broken. Replace with a new part. Damage is found although externally nothing is broken (cracking).
Deterioration of a coated capillary	Replace with a new capillary Coated capillaries tend to get blocked more easily than uncoated capillaries. If you find that there is no problem with the STD and that the blockage was caused only by the sample, reexamine the method of preprocessing the sample.
The CE and MS installation heights do not match.	If the CE side is too high, air may enter the capillary when the vial is replaced.

## MS Signal Sensitivity (S/N) is Low

Table 11 MS signal sensitivity (S/N) is low

Potential Cause	Suggested action
Spray section inappropriate (cut surface, settings, dirt, deformation)	Use a capillary with a smooth cut surface. Accurately install it in the spray. Wash the sprayer. Confirm the needle position, etc. If corrosion, deformation or dirt are seen in the spray needle, replace it with a new needle. To confirm, conduct MS tuning and check if a signal is detected by the MS unit.

**Troubleshooting** 

### **MS Background is High**

Table 12 MS background is high

Potential Cause	Suggested action	
Dirty spray or capillary	Identify the cause and wash or replace as needed. Details are	
Dirty solvents / water / reagents in buffer or sheath liquid	provided in another section. Pay attention to dirty system / water / solvents / containers / gas. TOF and QTOF users in particular should always maintain cleanliness.	
Dirty pump / degasser for sheath, dirty nitrogen gas		

# Instability of MSD signal (Reproducibility of Migration Time Not Obtained. In Serial Analysis, Migration Time Becomes Shorter or Longer)

**Table 13** Instability of MSD signal (Reproducibility of migration time not obtained. In serial analysis, migration time becomes shorter or longer)

Potential Cause	Suggested action  Thoroughly flush using the buffer of the analysis conditions. Break in with STD or no injection for at least 5 to 10 rounds and then analyze.	
The capillary does not reach equilibrium		
Effect of sample matrix	If good reproducibility has been confirmed during STD analysis, consider effects of the sample matrix. Examine the preprocessing, separation conditions and preconditioning contents.	
Change in buffer pH	Reproducibility of repeated analysis may be poor because the buffer capacity of the buffer is insufficient. Reexamine the analysis conditions.	

## Instability of MS Signal (Reproducibility of Migration Time Not Obtained. Changes are Irregular)

**Table 14** Instability of MS signal (Reproducibility of migration time not obtained. Changes are irregular)

Potential Cause	Suggested action
Unsuitability of the CE injection section, spray section	CE maintenance (prepuncher, electrodes, cassette washing, insulating plate washing) is essential. If there is any suspicion about the spray, perform maintenance (replacement and washing of needle / gaskets). Accurately install the capillary, and use a sufficiently balanced capillary.  If there is still no improvement, it is necessary to check analysis conditions, sample properties and equipment problems.  For this purpose, analyze and confirm STD under basic cation analysis conditions (measuring amino acids in 1 M formic acid) or anion analysis conditions (measuring organic acids in 20 mM ammonium formate, pH 10) recommended by our company in this document.  If, despite having done the above, an irregular migration time is confirmed, consult our call center.

## The Current Value During Analysis is Unstable

 Table 15
 The current value during analysis is unstable.

Potential Cause	Suggested action
The current value gradually drops. Leakage current	Thoroughly wash and dry the electrodes, prepuncher, insulating plates and capillary cartridge.
The current value gradually drops. The migration buffer is deteriorating	Make a fresh migration buffer. Storing the migration buffer in a sealed refrigerator is recommended. Note that high-pH buffers are especially susceptible to changes in composition and pH.
Leakage current caused by sheath liquid leakage from the sprayer	The sheath liquid may be causing liquid leakage in the sprayer. Replace the gaskets and accurately assemble them.
Decrease in current because of sheath liquid counter-flow.	Change analysis conditions to apply pressure of 20 to 50 mbar during analysis. Add an acid base to the sheath liquid.
Capillary deterioration	Replace with a new capillary.

### **MSD Sensitivity Dropped**

 Table 16
 MSD sensitivity dropped.

Potential Cause	Suggested action
The polyimide coating at the tip of the capillary was not peeled off. The polyimide coating prevents stable nebulizing.	Burn the polyimide coating, wipe with methanol and remove
Capillary deterioration	Replace with a new capillary.
Buffer, sheath liquid deterioration	Prepare fresh.
Dirty CE/MS sprayer	Dirt might have adhered to the sprayer. Perform sprayer maintenance.
Dirty spray chamber, MS	Perform MS capillary, etc., maintenance.

## Instability of MSD Signal (Spike Noise is Frequently Confirmed, Intermittent Noise, etc.)

**Table 17** Instability of MSD signal (Spike noise is frequently confirmed. Intermittent noise, etc.

Potential Cause	Suggested action
Insufficient deaeration of sheath liquid	Preliminary deaeration of the sheath liquid is recommended. Use a degasser.
Leakage of sheath liquid from sprayer, incomplete adjustment of sprayer	The sheath liquid may be causing liquid leakage in the sprayer. Replace the gaskets and accurately assemble them.

#### **6** Troubleshooting

**Troubleshooting** 



## **CE Configuration**

Conducting CE/MS analysis without using (lighting) a UV lamp 72
Conducting CE/MS analysis using the G7100 Instrument 74

## Conducting CE/MS analysis without using (lighting) a UV lamp

1 Confirm that a long GBIB cable connects the PC and the bottom row at the back of the CE (mainframe). At the same time, confirm that a short GBIB cable connects the upper row at the back of the CE (DAD) with its bottom row (mainframe).

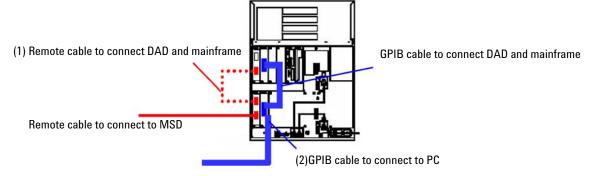


Figure 23 Connection to the back of the CE (when DAD is not used)

- **2** Remove the remote cable that connects the top row at the back of the HPCE (DAD) with its bottom row (mainframe).
- **3** The remote cable from the MSD connects to the bottom row at the back of the HPCE (mainframe). When connecting the upper row (DAD) side with the MSD, note that the ChemStation does not recognize the HPCE.

NOTE

The operation is not needed for the trap.

**4** In the Configuration Editor, set after deleting the "3D-CE DAD" of the "GPIB Address 19". (Example in Figure 24 on page 73)

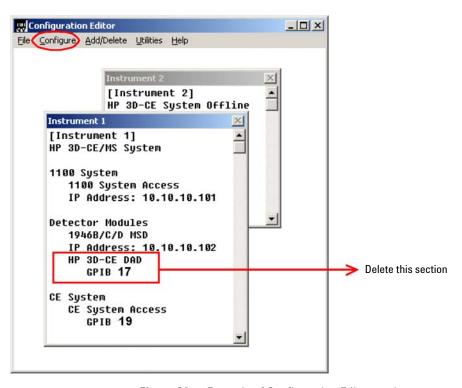


Figure 24 Example of Configuration Editor settings

NOTE

Since the IP address and settings of the device are different for each customer, record them in advance.

## **Conducting CE/MS analysis using the G7100 Instrument**

1 Confirm that a LAN cable connects the PC to the back of the CE (mainframe, right side seen from front).

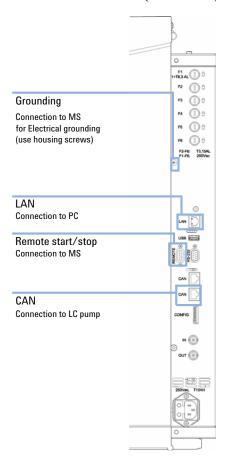


Figure 25 Connection to the back of the G7100 CE

#### **CE Configuration** 7

Conducting CE/MS analysis using the G7100 Instrument

#### www.agilent.com

### In This Book

The manual describes the following:

- Introduction to the System
- Optimization
- Conditions
- Maintanance
- Troubleshooting
- Configuration

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G7100-90001

