Agilent ChemStation

Understanding Your ChemStation for CE Systems

Agilent Technologies

Notices

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Agilent Technologies
Hewlett-Packard-Strasse 8
76337 Waldbronn

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Software Revision

This guide is valid for B.04.xx revisions of the Agilent ChemStation software, where xx refers to minor revisions of the software that do not affect the technical accuracy of this guide.

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Safety Notices

CAUTION

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A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

In This Guide...

This guide describes specific features of ChemStation for CE systems, such as the vial table, migration time-based calibrations, mobility correction, report styles, and calculations.

Contents

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CE Agilent ChemStation Specific Functions in Method and Run Control View

Vial Table

NOTE The **Vial Table** functionality is available in the online ChemStation session only.

The **Vial Table** is a table that associates vials in the vial tray with samples, and, more importantly, with task specific vials such as buffers, flush vial, clean tubes vial, and the waste. The **Vial Table** is linked to the Sequence Table. When a sequence is loaded, the information from the Sequence Table is copied into the Vial Table. However, Vial Table entries are not transferred back into the Sequence Table. The **Vial Table Advanced Settings** dialog box is displayed when you choose the **Advanced** button in the **Vial Table**. It allows you to enable warnings of conflicts between the **Vial Table** and the method or sequence and the use of symbolic names. You must select **Enable vial table checks and warnings** to check for conflicts between the **Vial Table** and the method and sequence.

When a method or a sequence is loaded, a consistency check is made between the vial allocations in the **Vial Table** and those in the method or sequence. If there are any vial conflicts they can easily be resolved by using the **Conflict** tables.

NOTE Position 49 in the vial tray is used for the needle wash vial and position 50 is left empty to allow the vial lift to return. These positions are not available in the **Vial Table**.

The **Used in** column of the Vial Table enables the usage of the vial to be specified. There are five valid entries for the **Used in** fields:

Don't Care	No consistency check is made
Method	The vial is referenced in the method
Sequence	The vial is referenced in the Sequence Table
System	This is a special vial belonging to the system configuration. The **Name** must be one of the following symbolic names:

- **@INLET** the inlet vial

- **@OUTLET** the outlet vial
- **@FLUSH** the flush vial
- **@WASTE** the waste vial
- **@clean tubes** the vial used for cleaning the replenishment tubes
- **@USER X**(where as X can be 1 to 10) the sequence placeholder

  This option allows the specification of individual vial numbers for the symbolic names used in the method. This enables the user to specify different vials for Inlet Home, Outlet Home, Replenishment, Preconditioning, Postconditioning, etc. for each line in the sequence.

Not Used There is no vial in this position

Method Conflict Table

The **Method Conflict Table** is displayed when you load a method that has vials defined that conflict with the vials defined in the Vial Table. The **Method Conflict Table** has two sides; the left half contains an image of the **Vial Table**; the right half shows the conflicting vials.

To resolve the conflicts you can select to either replace (single arrow) or to move the vial from the method to the next free position in the **Vial Table** (double arrow). This can be done for each conflicting vial in the table.

When user-defined vials (with symbolic names @User1, @User2, etc.) are used the conflict test cannot be performed on these vials, because without the sequence information it cannot be decided whether a conflict exists or not.

Sequence Conflict Table

The **Sequence Conflict Table** is displayed when you set up or load a sequence that has vials defined that conflict with the vials defined in the Vial Table. The **Sequence Conflict Table** has two sides; the left half contains an image of the **Vial Table**; the right half shows the conflicting vials.

To resolve the conflicts you can select to overwrite the **Vial Table** information with the information from the **Sequence Table**, or if the conflict is caused by a system entry it cannot be overwritten. You can select to close the **Sequence Conflict Table** without resolving the conflicts.

When user-defined vials (in the columns User1, User2, etc.) are used the conflict test cannot be performed on these vials, because without the method information it cannot be decided whether a conflict exists or not.

Method Simulation

You can use the simulation function to check your method. During simulation the diagram reflects the actions that would be performed during the method, i.e. the vials specified in the method are shown in the lifts, power and voltage applied is shown as it would during an actual run. Simulation is faster than running an analysis - each step takes about 3 seconds. A step is defined by a change in the CE diagram.

To start simulation, load the method you want to simulate and select **Simulation** from the **Instrument** menu.

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Peak Top Type

Unlike LC, GC, or MS peaks, it is quite normal for CE peaks to be asymmetrical. Because of this, is very important to have the ability to select integration parameters that will yield the highest level of accuracy and reproducibilty in your quantitation results.

The following peak top types are available when selecting **Peak Top Type** in the **Integration** drop-down menu:

Highest Point

- select when the peak is triangular, and
- when you work with different concentrations

Parabolic Interpolation

- use for tailing, unseparated peaks

Center of Gravity

- provides more accurate calculations with triangular shaped peaks
- samples with similar concentrations

Gauss Fit

- use for symmetric peaks

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Calibration Tables

Calibration Types

There are four different calibrations types available in the drop-down list for your Calibration Table:

- **Standard Calibration**
- **Protein Molecular Weight Calibration**
- **DNA Base-Pair Calibration**
- **Capillary Isoelectric Focusing Calibration**

Standard Calibration

Standard Calibration is based on peak area or peak height. When you select **Standard Calibration** you have the option to **Calculate Signals Separately** or **Calculate with Corrected Areas**.

Calculate Signals Separately is selected when you want to ensure that, in the calculation of Norm% reports, the amount percent of separately reported signals add up to 100% for each signal. When **Calculate signals separately** is deselected, the amount percent of all signals add up to 100%. Selecting **Calculate signals separately** is a prerequisite for sorting by signal in the calibration table.

Select **Calculate with Corrected Areas** to make a correction to the peak area based on the migration time. In this mode, the area is divided by the migration time which can improve reproducibility in quantitative analysis when migration times are unstable.

In addition to the Standard Calibration, there are 3 capillary electrophoresis specific calibrations that are migration time based one signal. If the data file contains multiple signals, then only one signal, defined by the signal description in the calibration method, must be selected and is extracted from the data file. The format of the calibration table is dependent on the calibration type selected.

Quantitation tasks may now be performed based on biopolymer size calibration (Ferguson Plot) for SDS-Protein.

Protein Molecular Weight Calibration

The **Protein molecular weight calibration** requires a calibration standard with components of known molecular weights and a reference peak. The calibration equation is:

$$\log(MW) = k_1 \cdot (t_{ref}/t) + k_0$$

where:

MW is the molecular weight

t_{ref} is the migration time of the reference peak

t is the migration time

k_0 and k_1 are the coefficients of the linear equation

The calibration table contains the Name, Migration Time, t_{ref}/t (relative migration time), Molecular Weight and *log(MW)* for each component.

DNA Base-Pair Calibration

The **DNA base-pair calibration** is similar to the **protein molecular weight calibration**, but operates without a reference peak; it requires a calibration standard with a known number of base pairs. The calibration equation is:

$$\log(\#\mathrm{BP}) = k_1 \cdot 1/t + k_0$$

where:

#BP is the number of base pairs

t is the migration time

k_0 and k_1 are the coefficients of the linear equation

The calibration table contains the Name, Migration Time, *1/t*, Base Pairs and *log(Base Pairs)* for each component.

Capillary Isoelectric Focusing Calibration

The **capillary isoelectric focusing calibration** (cIEF) requires a calibration standard with standard proteins of known isoelectric points (pI). The calibration equation is:

$$pI = k_1 \cdot t + k_0$$

where:

pI is the isoelectric point

t is the migration time

k_0 and k_1 are the coefficients of the linear equation

The calibration table contains the Name, Migration Time and *pI* (isoelectric point) for each component.

Migration Time Based Calibrations

Using Migration Time Based Calibrations in a Sequence

Migration time-based calibrations and recalibrations can be included in a sequence, but only explicit calibrations and cyclic recalibrations are supported; bracketed recalibration is not supported. There is no Sequence Summary report with migration time-based calibrations.

Report Styles for Migration Time Based Calibrations

The report styles available for migration time based calibrations are limited to **Short** (quantitative text results) and **Full** (header, sample information, instrument conditions, logbook, quantitative results and peak purity plot).

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Calibration using Mobility Correction

Introduction

Slight changes in buffer composition, run temperature or viscosity, as well as adsorption to the capillary wall, can influence the Electro Osmotic Flow (EOF) and cause it to be unstable. The resulting change in the EOF can create a rather high standard deviation of migration times. Corrections for mobility can significantly reduce the effect of run-to-run migration time shifts by monitoring the migration time of a mobility reference peak and in turn significantly increasing the migration time reproducibility.

The mobility reference peak should be chosen with the following priorities:

- Select peak with the highest signal
- Select the most isolated peak
- The EOF marker or internal standard can also be used as the mobility reference peak
- Enlarge the search window to always find the mobility reference peak
- If several peaks fall in the search window, the peak with the highest signal is automatically chosen as the mobility reference peak.

There are two mobility correction types available:

Effective Mobility Correction

Effective Mobility Correction uses the effective mobilities of all peaks and requires the availability of the voltage ramp data together with the electropherogram. In addition, working with effective mobility correction allows the true effective mobilities for all sample components to be determined.

Relative Mobility Correction

Relative Mobility Correction can operate in the absence of voltage data and would then assume a constant voltage for all measurements.

Effective Mobility Calculations

In addition to a reference peak the requirements for effective mobility correction include a neutral marker which corresponds to the velocity of the EOF. Some commonly used markers and their associated wavelengths are:

Table 1 Commonly Used EOF Markers

Compound	Wavelength
1-Propanol	210nm
Acetone	330nm
Acetonitrile	190nm
Benzene	280nm
Guanosine	252nm
Mesityl oxide	253nm
Methanol	205nm
Phenol	218nm
Pyridine	315nm
Tetrahydrofuran	212nm
Uracil	259nm

Voltage over time data and the capillary dimensions are either saved with the data file or they can manually be entered during the calibration table setup. Storing the voltage data during the run does this most accurately. Make sure to also store the capillary dimensions with the method. To reprocess signals that have been acquired without voltage data/capillary dimensions, enter the voltage and ramp time manually in the “Voltage and Capillary Dimensions” group of the dialog box.

From the data the effective mobility for each component is determined.

General

The apparent mobility of a sample peak is defined by the equation:

$$\mu_{app} = (l \cdot L)/(t \cdot V(t))$$

where

l is the effective length of the capillary (the length from the point of injection to the point of detection)

L is the total capillary length

$V(t)$ is the average voltage from time 0 to the migration time t of the peak

The average voltage is calculated from either the measured voltage or from the voltage ramp specified in the method using the following equations:

If $t < t_R$ then

$$V(t) = V/(2 \cdot t_R) \cdot t$$

If $t > t_R$ then

$$V(t) = V \cdot (1 - t_R/(2 \cdot t))$$

where

t is the migration time of the peak

t_R is the ramp time

V is the end voltage

The equation for mobility can be simplified by introducing a coefficient:

$$k(t) = (l \times L)/V(t)$$

The relative or apparent mobility is then

$$\mu_{app} = k(t)/t$$

Effective or real mobility is

$$\mu_{\mathrm{real}} = \mu_{app} - \mu_{EOF}$$

where

μ_{app} is the apparent mobility of any peak

μ_{EOF} is the apparent mobility of a neutral marker

Components with lower velocity than the EOF (usually anions) will result in negative values for the effective mobility.

Calibration

The real mobility of a sample peak to be used as the mobility reference peak in future measurements is calculated using the migration time of a neutral marker (μ_{EOF}):

$$\mu_{\mathrm{real}\,ref} = \mu_{appref} - \mu_{EOF} = k(t_{ref})/t_{ref} - k(t_{EOF})/t_{EOF}$$

The effective mobilities of all peaks are then calculated and stored as expected mobilities:

$$\mu_{\mathrm{real}N} = \mu_{appN} - \mu_{EOF} = k(t_N)/t_N - k(t_{EOF})/t_{EOF}$$

The calibration table then contains the measured migration time and the calculated real mobility for each compound in the columns for the expected migration time and the expected mobility.

Mobility Calculation

The actual value of μ_{EOF} is calculated using the Mobility Reference Peak:

$$\mu_{EOFact} = \mu_{appref} - \mu_{\mathrm{real}\,ref} = k(t_{ref})/t_{ref} - \mu_{\mathrm{real}\,ref}$$

The expected migration time for each peak is then adjusted:

$$t_{new\,\mathrm{exp}N} = k(t_{old\,\mathrm{exp}N})/(\mu_{\mathrm{real}N} + \mu_{EOFact})$$

The calculated values are used for peak identification and replace the values within the calibration table.

Recalibration

The migration time of the mobility reference peak is used to calculate the actual value of μ_{EOF} :

$$\mu_{EOFact} = \mu_{appref} - \mu_{\mathrm{real}\,ref} = k(t_{ref})/t_{ref} - \mu_{\mathrm{real}\,ref}$$

The expected migration time for every peak is adjusted:

$$t_{new\,\mathrm{exp}N} = k(t_{old\,\mathrm{exp}N})/(\mu_{\mathrm{real}N} + \mu_{EOFact})$$

and the mobilities are updated:

$$\mu_{\mathrm{real}N} = \mu_{appN} - \mu_{EOFact}$$

During a calibration run the expected values for the migration time as well as the real mobility values are updated in the calibration table.

Relative Mobility Calculations

Migration time correction based on relative mobilities can also be performed. In this case neither an EOF marker, voltage, nor capillary dimensions are needed. The software still corrects migration time shifts but does not display mobility values.

General

Just as in the effective mobility calculations, the coefficient

$$k(t) = (l \cdot L)/V(t)$$

is used in the relative mobility calculations to describe the relationship between mobility and migration time:

$$\mu_{app} = k(t)/t$$

The difference is that in the equations for Relative Mobility, k appears in both numerator and denominator of a fraction; this means that the capillary dimension can be eliminated. The factor k is calculated as

$$k(t) = 1/V(t)$$

where $V(t)$ is the average voltage from time 0 to the migration time of the peak.

When the voltage parameter is set to **Ignore**, **k** is a constant and can be eliminated from the equations for the expected migration time (see below).

The following equations describe the general case for $k = k(t)$, although the software takes all cases into account when calculating k.

Calibration

A mobility reference peak is identified and its migration time (t_{refcal}) is stored. The expected migration times (t_{expcalN}) of all other peaks are saved.

Mobility Calculation

After detection of the reference peak, the expected migration time for each peak is adjusted according to the actual migration time of the mobility reference peak:

$$t_{new\,\mathrm{exp}N} = \frac{k(t_{old\,\mathrm{exp}N})}{(k(t_{\mathrm{exp}\,calN})/t_{\mathrm{exp}\,calN} - k(t_{refcal})/t_{refcal} + k(t_{refact})/t_{refact})}$$

Then, the migration time of the reference peak from the last calibration run is updated:

$$t_{refcal} = t_{refact}$$

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Other CE-specific features

Special Report Styles for Capillary Electrophoresis

The following report style has been added to the Agilent ChemStation for CE systems:

CE Mobility

CE Mobility comprises quantitative text results, especially the apparent mobility. To use this report style, you need to supply the information on the capillary used before acquisition and you store the voltage signal. The apparent mobility is calculated according to the following formula.

$$\mu_{app} = \frac{l \cdot L}{t \cdot V}$$

Where

l is effective capillary length (cm)

L is total capillary length (cm)

t is migration time (min)

V is voltage (kV)

If effective mobility correction (see “Effective Mobility Calculations” on page 21) is activated, the peak type column in simple reports (external standard reports for example) is replaced by a mobility column. The CE mobility report prints effective instead of apparent mobilities.

Corrected Peak Areas

The Agilent ChemStation for CE systems allows you to use corrected peak areas instead of the normal area calculations. These areas are used in standard calibration and reports.

To activate this feature, select **Calculate with Corrected Areas** to make a correction to the peak area based on the migration time. In this mode, the area is divided by the migration time which can improve reproducibility in quantitative analysis when migration times are unstable.

The corrected area is calculated according to the following formula:

$$A_c = \frac{A}{60 \cdot t}$$

Where

Ac is corrected peak area (mAU)

A is peak area (mAU·sec)

t is migration time (min)

This corrected area is sometimes also referred to as normalized area.

System Suitability for Capillary Electrophoresis

Capacity Factor k’

In capillary electrophoresis the capacity factor k’ value can’t be calculated automatically for all operation modes. Refer to the manual *High Performance Capillary Electrophoresis:* A Primer for the formulas respectively. The values listed in the reports are only valid for the Agilent ChemStation for LC 3D systems since the Agilent ChemStation for CE systems uses the same algorithms as the Agilent ChemStation for LC 3D systems.

CE-MSD

Background Subtraction

When you select the **Subtract Background** (BSB) menu item, the most recently selected mass spectrum is subtracted from each point in the current electropherogram. The resulting data is saved in the same directory and with the same name as the original data file; however, the file extension is changed to .BSB.

The new data file becomes the current data file and the background subtracted electropherogram is displayed. A record of the number of background subtractions that have been performed is kept in the Operator item of the data file header.

If you view a tabular listing of BSB data, you may observe differences due to the precision of data representation.

NOTE

The HELP text files in the LC/MSD refer only to LC parameters and not CE. Some features that are available in the LC/MSD software are either not available or not applicable to CE/MSD applications but are used in LC. The function **peak matching** is not applicable for CE-MS and is therefore not active. In CE-MS, UV and MS detection occurs at different effective lengths of the separation capillary. Because of the different resolution at different effective lengths, peak matching is not possible.

Method Subdirectories for different CE Modes

Methods in CE are dependent on CE mode selected. Therefore they are stored in different subdirectories in the Method subdirectory:

CE	Stores methods for CE mode
CEC	Stores methods for the CEC mode
CEp	Stores methods for the CE plus pressure mode
CEMS	Stores methods for the CE MS mode.
CEMSp	Stores methods for the CE MS plus pressure mode.

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In This Book

Agilent ChemStation for CE systems provides a number of features specifically designed for CE systems to optimize operation and data analysis for this specific instrument technique. This guide describes these features, such as the vial table, migration time-based calibrations, mobility correction, report styles, and calculations.

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