DataApex
Clarity™
CHROMATOGRAPHY SOFTWARE

CE EXTENSION

Clarity Extension ENG

Code/Rev.: M062/100A
Date: 2025-05-12

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To facilitate the orientation in the **CE Extension** manual and **Clarity** chromatography station, different fonts are used throughout the manual. Meanings of these fonts are:

Open File (italics) describes the commands and names of fields in **Clarity**, parameters that can be entered into them or a window or dialog name.
WORK1 (capitals) indicates the name of the file and/or directory.
ACTIVE (capital italics) marks the state of the station or its part.
Chromatogram (blue underlined) marks clickable links referring to related chapters.

The bold text is sometimes also used for important parts of the text and the name of the **Clarity** station. Moreover, some sections are written in format other than normal text. These sections are formatted as follows:

Note: Notifies the reader of relevant information.

Caution: Warns the user of possibly dangerous or very important information.

Marks the problem statement or trouble question.

Description: Presents more detailed information on the problem, describes its causes, etc.

Solution: Marks the response to the question, presents a procedure how to remove it.

1 CE - Capillary Electrophoresis

The **Clarity CE** is an optional **Extension** for the **Clarity Chromatography Station** (from version 2.4.4). The **Clarity Chromatography Station** can acquire data from any **CE** system with standard analog output. Any **Clarity** Instrument can use *CE mode*.

CE Extension provides specific terminology, evaluation by time corrected area and peak identification by peak start or end (additionally to the peak apex).

CE Extension is also compatible with **Clarity Offline** software.

2 Specification

The **CE Extension** is an optional, fully integrated part of **Clarity** software. It can be ordered as a part of new datastation or as an Extension to existing datastation (p/n A31).

3 Installation

The CE Extension is activated by entering an appropriate user code during the installation of **Clarity** or later using the *Help - User Code…* command from the ***Clarity*** main window.

To enable the CE Extension on an ***Instrument***, you have to set the corresponding instrument type. To set the instrument type, click the ... button in the ***System Configuration*** dialog.

In the invoked *Instrument Type Setting* dialog, select the CE or CE-PDA option.

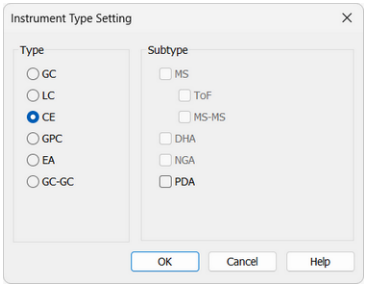

Fig. 1: Switching to the CE mode

4 Key Features

The **CE Extension** adds the following features to the **Clarity** station:

- CE-specific terminology
- evaluation by time corrected area
- peak identification by peak start or end (as an addition to the peak apex)
- Apparent and Effective mobility calculations.

4.1 CE-specific terminology

Several terms used in chromatography were substituted by the equivalent terms used in the capillary electrophoresis. Moreover, some of the information fields on the *Method Setup - Measurement* and *Chromatogram - Measurement Conditions - Instrument* tabs were renamed to better match the **CE** terminology.

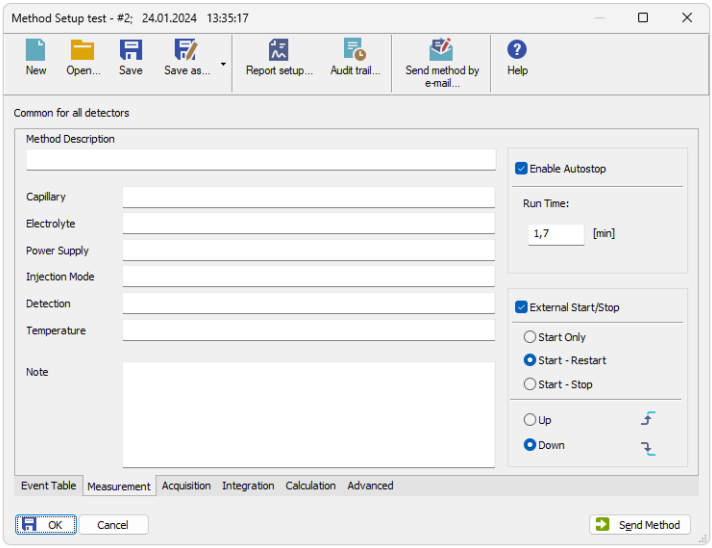

Fig. 2: Method Setup - Measurement

Tab. 1: Substituted CE terms

Default mode	CE mode
Chromatogram	Electropherogram
Retention time (RT)	Migration Time (MT)

Tab. 2: Renamed existing informational fields:

Default mode	CE mode
Column	Capillary
Mobile Phase	Electrolyte

Default mode	CE mode
Flow Rate	Power supply
Pressure	Injection Mode

4.2 Setting of the separation parameters

In *CE Mode*, ***Method Setup*** - *Advanced* tab allows you to adjust the separation parameters in the *Capillary Settings*.

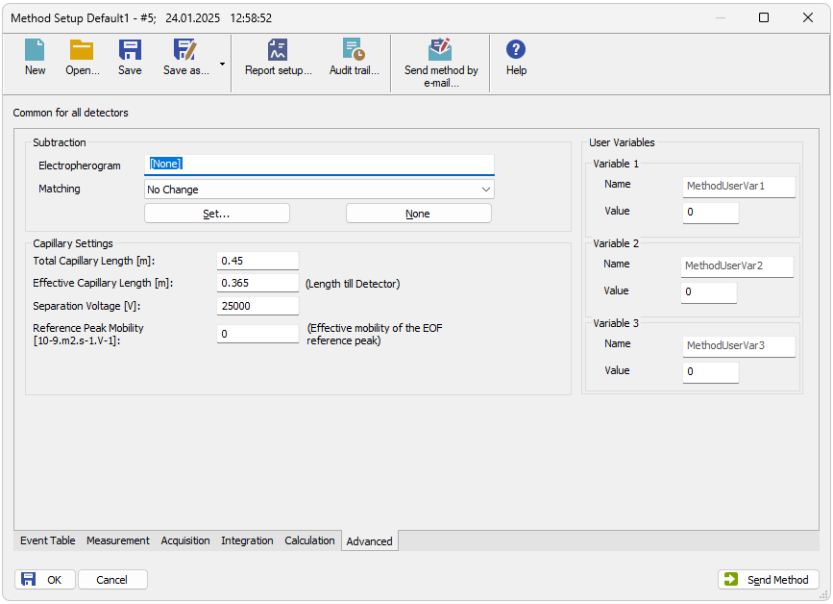

Fig. 3: Capillary setting in the Method Setup - Advanced

Capillary Settings

Section displayed only when the ***Instrument*** is in *CE mode*. It is also displayed in the Chromatogram window - Performance tab.

Total Capillary Length [m]

Length of the capillary.

Effective Capillary Length [m]

Effective capillary length from injection end to the detector position.

Separation Voltage [V]

Separation voltage. Allows you to enter also negative values.

Reference Peak Mobility [$10^{-9}.m^2.s^{-1}.V^{-1}$]

Effective mobility of the EOF reference peak.

User Variables

Sets *Method User Variables* which can be used for *User Columns* calculations. Up to three independent variables can be set.

Name

Sets the name of the variable. If the field is left empty, default name *MethodUserVar1-MethodUserVar3* would remain filled in.

Value

Sets the numerical value of the variable.

4.3 Peak Identification

As peaks in **CE** are often asymmetrical, in some cases the position of peak start is less influenced by analyte concentration and thus better suited for compound identification.

The **CE** module allows the peaks to be also identified by peak start or peak end time in addition to the standard peak apex.

The **Reten. Time** column is changed to the *Apex MT* column and new *Start MT*, *End MT* and *Identify By* columns are available in the ***Calibration Summary Table*** in *Calibration* window and in the **Result Table** and **Summary Table** in the ***Electropherogram*** window.

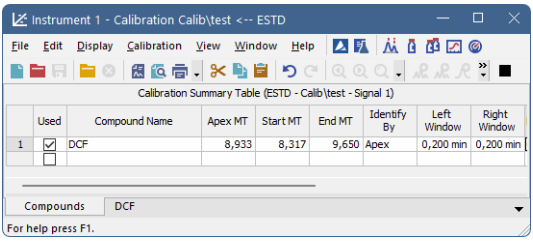

Fig. 4: Calibration

Note: When a new compound is created there are pre-filled values for *Apex MT*, *Start MT* and *End MT* with default value 0, for *Identify By* is applied default value *Apex*. Default values for all other columns are described in **Clarity** Help in chapter **Calibration**.

4.4 Quantification

The migration times in **CE** are significantly influenced by experimental conditions. As the peak area is dependent on the speed by which the peak migrates through detector cell, the time corrected area is in some cases more suitable for quantification then the area alone.

New option is available for the *Response Base (RB)* columns in the ***Calibration*** or ***Electropherogram*** windows - besides the *Area (A)* or *Height (H)* the *Time Corrected Area (C)* can be selected individually for any compound in the calibration table.

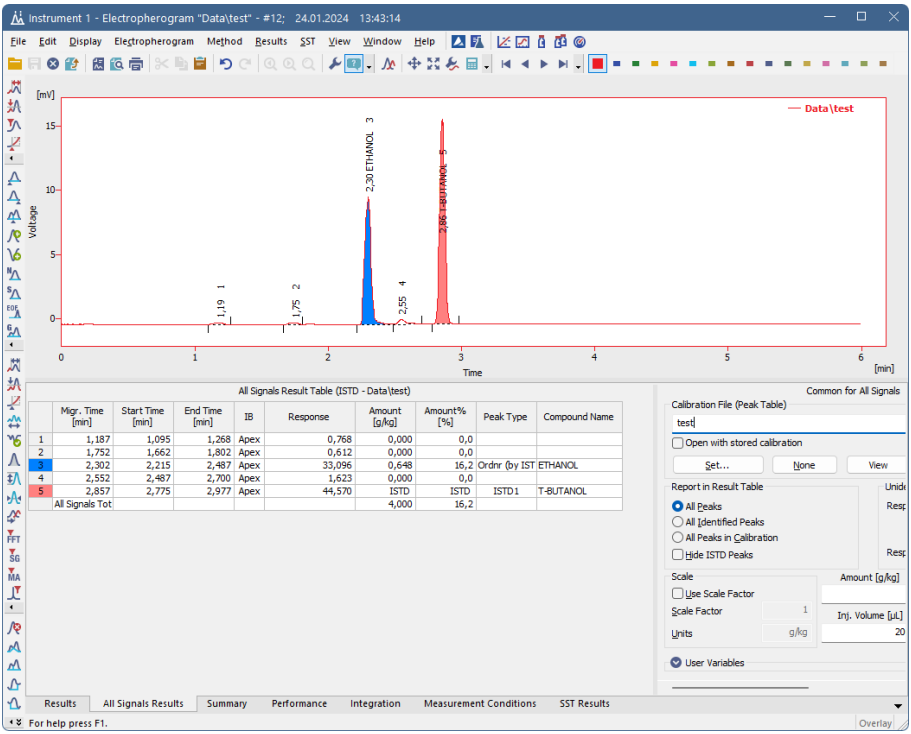

Fig. 5: Electropherogram

It is possible to add the *EOF Marker Peak* in *Electropherogram* using the EOF button (located in the vertical toolbar) or using the the *Peak - EOF marker* option in the *Integration table*. In case of multiple *EOF Marker Peaks* are entered, the last one will be used.

In case you need to display the *Apparent* or *Effective Mobility* in the *Result Table*, use the pop-up menu of the *Result Table* and select the *Setup Columns* command. The *Setup Columns* dialog allows you to display those hidden columns.

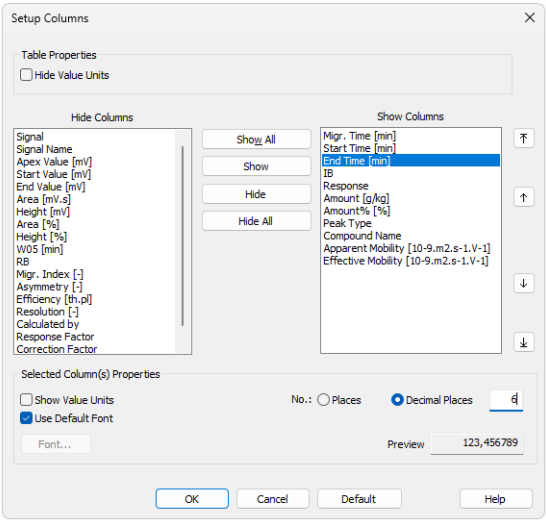

Fig. 6: Results - Setup Columns in CE Mode

4.5 Instrument Control

Clarity is prepared for development of control modules for **CE** instruments. **CE** *Instrument Type* was added to the *System Configuration* dialog and new *Inlet Vial (InV)* and *Outlet Vial (OutV)* columns are available in the **Sequence Table**.

Software development kit (**SDK**) is available for instrument manufacturers to develop control modules for their **CE** instruments. The control module will add a tab to the *Method Setup* dialog and can also add a pane in the *Device Monitor* window.

4.6 Mobility Calculations

The *CE Mode* allows you to calculate the *Apparent* and *Effective Mobility*. It is displayed in the *Electropherogram* window - *Performance* tab.

In case the *Performance* tab is not displayed, you can display it using the menu command *Results - Performance Table*.

To calculate the *Effective Mobility*, *EOF Marker Peak* has to be selected. (EOF Marker Peak can be assigned to appropriate peak in chromatogram through EOF Integration operation .)

The separation parameters can be predefined for all chromatograms using the same method in the *Method Setup* dialog - *Advanced* tab, see the chapter **"Setting of the separation parameters"** on pg. **5**.

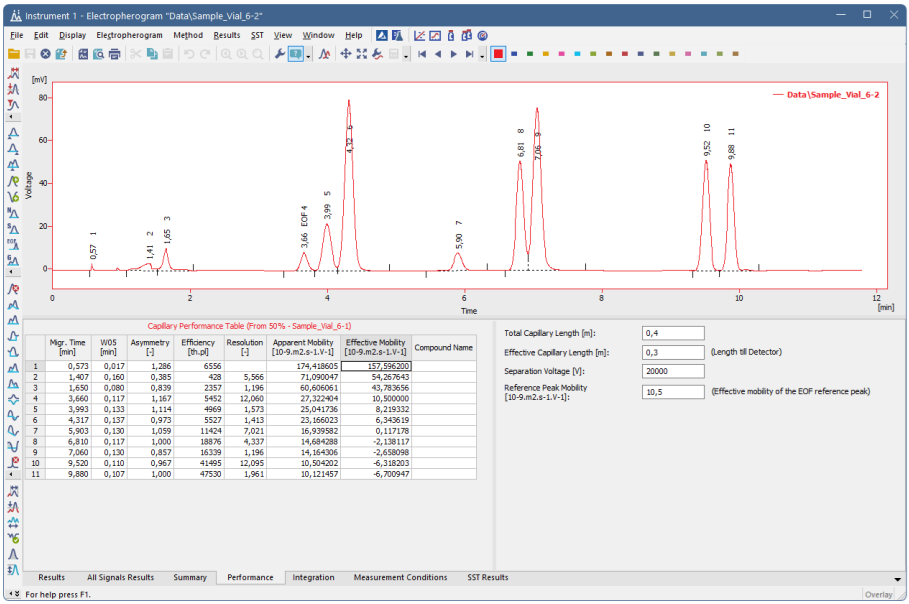

Fig. 7: Performance tab in the Electropherogram window

4.7 Mobility calculation formulas

Apparent mobility of analyte A, $m_{app,A}$ is calculated according to equation (1):

$$m_{app;A} = \frac{L_{eff} L_{tot}}{t_{mig;A} U_{sep}} \qquad (1)$$

L_{eff} - effective capillary length (from injection end to the detector position)

L_{tot} - total capillary length

U_{sep} - applied separation voltage

$t_{mig,A}$ - migration time of the analyte A (in Gaussian peaks = migration time of peak apex).

Effective electrophoretic mobility, $m_{eff,A}$, is defined by eq. (2):

$$m_{eff,A} = m_{app,A} - m_{EOF} \qquad (2)$$

m_{EOF} is mobility of electroosmotic flow (*EOF*) calculated according to eq. (3):

$$m_{EOF} = \frac{L_{eff} L_{tot}}{t_{mig;EOF} U_{sep}} \qquad (3)$$

$t_{mig,EOF}$ - migration time of the electroneutral compound (marker of *EOF*)

Substitution of equations (1) and (3) into equation (2) gives the following equation for effective electrophoretic mobility of analyte A, $m_{eff,A}$:

$$m_{A,eff} = \frac{L_{eff} L_{tot}}{U_{sep}} \left(\frac{1}{t_{mig,A}} - \frac{1}{t_{mig,EOF}} \right) \qquad (4)$$

Alternatively, mobility of *EOF*, m_{eof}, can be determined from the apparent and effective mobilities of a reference analyte R, $m_{app,R}$ and $m_{eff,R}$, respectively.

$$m_{EOF} = m_{app,R} - m_{eff,R} \qquad (5)$$

$m_{app,R}$ is calculated according to eq. (1), where $t_{mig,A}$ is replaced for migration time of reference analyte R, $t_{mig,R}$:

$$m_{app,R} = \frac{L_{eff} L_{tot}}{t_{mig,R} U_{sep}} \qquad (6)$$

Substitution of equations (6) into equation (5) gives the following equation for the electroosmotic flow mobility, m_{EOF}:

$$m_{EOF} = \frac{L_{eff} L_{tot}}{t_{mig,R} U_{sep}} - m_{eff,R} \qquad (7)$$

Effective mobility of reference compound R, $m_{eff,R}$, has to be known from the previous measurement in the same background electrolyte and at the same temperature, at which the apparent mobility of analyte A is measured.

Effective electrophoretic mobility, $m_{eff,A}$, is then calculated according to eq. (2), where $m_{app,A}$ is obtained from eq. (1) and the electroosmotic flow mobility, m_{EOF} from eq. (7).

For the special case, when reference analyte *R* is electroneutral compound (*EOF marker*), i.e. $m_{eff,R} = 0$ and $t_{mig,R} = t_{mig,EOF}$, the eq. (7) becomes identical to eq. (3) and effective mobility of analyte *A* can be calculated according to eq. (4).