# Agilent 7100 Capillary Electrophoresis System in Chromeleon 7

## Technical Note

This technical guide describes the configuration and use of the G7100A Capillary Electrophoresis System in Chromeleon 7

# Contents

# Introduction and Compatibility Overview

This guide describes how to configure and use the 7100 Capillary Electrophoresis (CE) System in Thermo Scientific's Chromeleon 7 environment (see Table 1).

All established techniques are feasible, such as the use of direct and indirect detection modes, use of special electrolyte systems (containing micelles or gel matrices) and also capillary isoelectric focusing (cIEF) or capillary electrochromatography (CEC). The application of external high pressure is supported.

For data analysis, Chromeleon offers processing tools for peak integration and calculation of concentrations.

Table 1  Supported and unsupported CE configurations in Chromeleon 7.2

| Chromeleon Version | Agilent ICF/ Agilent LC Driver | Supported CE |
|---|---|---|
| 7.2.6<br>7.2.7<br>and higher | A.02.04<br>A.02.14 | Supported<br>● G7100A Capillary Electrophoresis, with the internal components:<br>● G7150A CE Mainframe<br>● G7151A DAD<br>● min. Firmware B.06.73 (older firmware does not support the use of RFID Tags for the DAD UV-Lamp)<br>Not supported<br>● G1600 Capillary Electrophoresis |
| 7.2 SR5<br>7.2 SR5 MUa<br>7.2 SR5 Mub<br>7.2 SR5 MUc<br>7.2 SR5 Mud<br>and higher<br>MU´s | A.02.04<br>A.02.14 | Supported<br>● G7100A Capillary Electrophoresis, with the internal components:<br>● G7150A CE Mainframe<br>● G7151A DAD<br>● min. Firmware B.06.73 (older firmware does not support the use of RFID Tags for the DAD UV-Lamp)<br>Not supported<br>● G1600 Capillary Electrophoresis |

NOTE    Ensure that the Agilent LC/CE modules in the system *meet or exceed* the minimum firmware requirements specified by the 3rd-party CDS software vendor and Agilent's firmware set/firmware interoperability requirements. Agilent recommends using the latest available firmware set.

http://www.agilent.com/en-us/firmwareDownload?whid=69761

# Set up and Configuration of CE in Chromeleon 7

## CE Instrument set up

**1** Close Chromeleon.

**2** The CE uses LAN communication. Connect the CE to the control computer. Connect via switch or hub, if another instrument is connected to the control computer.

**3** Switch the instrument on.

## The CE Driver

The CE driver *is part of* the LC Driver and not listed as a separate component. The components must be present in **Control Panel > Programs and Features**:

| Name | Publisher | Installed On | Size | Version |
|---|---|---|---|---|
| Agilent Instrument Control Framework - GC/HS Drivers A.03.02 | Agilent Technologies | 11/9/2017 | 388 MB | 3.2.103 |
| Agilent Instrument Control Framework - LC Drivers A.02.14 | Agilent Technologies | 11/9/2017 | 224 MB | 2.14.115 |
| Agilent Instrument Control Framework A.02.04 | Agilent Technologies | 11/9/2017 | 99.4 MB | 2.4.124 |

**Figure 1** Components in Programs and Feature

## Configuration Steps for CE in Chromeleon

To run CE in Chromeleon, the configuration of the CE instrument in the Instrument Controller is required.

**1** Ensure that the Chromeleon Services Manager is running.

**2** Open the Instrument Configuration Manager using the offered link or via **Start > All Programs > Thermo Chromeleon 7 > Instrument Configuration Manager**.

**3** In the Instrument Configuration Manager, select a PC and add an instrument using the **Add Instrument** icon.

**4** Enter an instrument name and click **OK**.

**5** Right-click the new instrument and select **Add Module…**.

**6** Select **Agilent** in the manufacturers list on the left, and select **LC System** in the list of modules on the right.
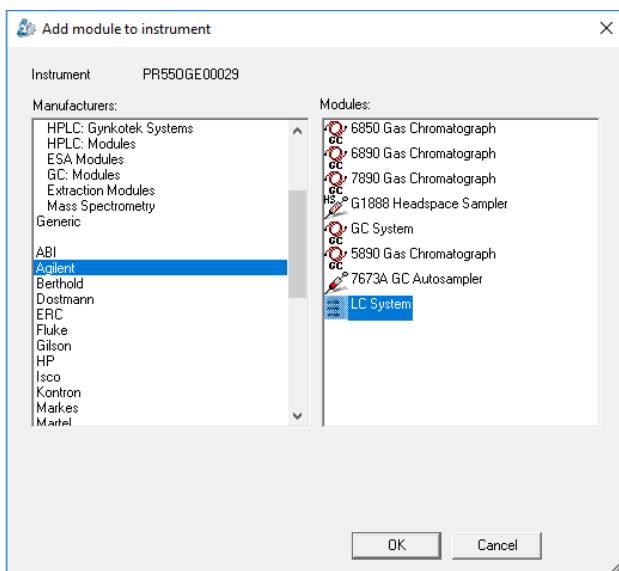
**7**   Click **OK**.



**Figure 2**   Instrument Configuration Manager

**8**   In the **Agilent LC System Configuration** menu, select **Agilent 7100 CE** and click **Auto Configure**.

**9**   In the screen, enter the IP address of the CE instrument and click **OK**. The instrument is detected and the CE and DAD appear on the right side of the configuration window.
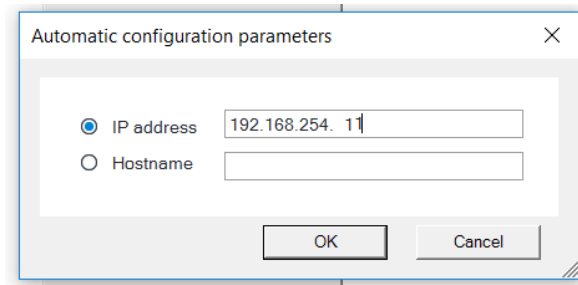


**Figure 3**   IP address to connect

NOTE    The default IP address is 192.168.254.11. Please refer to the *G7100 User Manual* if an IP address change is required.

NOTE    The 7100 Capillary Electrophorese includes a DAD detector. There is only one IP address for the instrument. When setting up the 7100 in a manual process, select the 7100 and the corresponding DAD (see Figure 4). In communication settings, the hostname/IP address for the CE and DAD are identical.



**Figure 4**   Manual setup CE/DAD
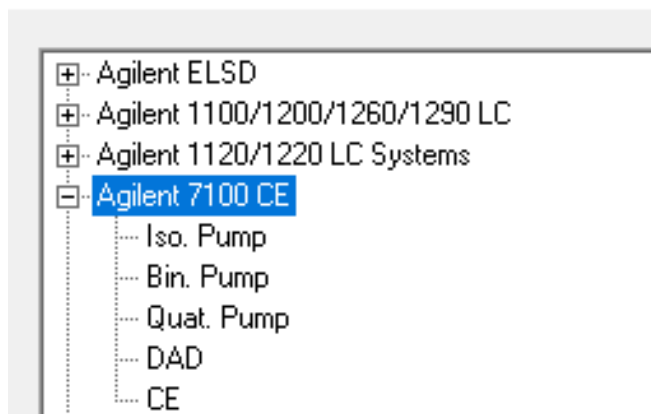
**10** To review or change the configuration, double-click the appropriate module or click **Configure** at the bottom of the screen.

• Defining the **pressure unit** (see also page 24).

<table><tr><td>NOTE</td><td>The pressure unit needs to be defined in bar. Using psi is not supported.</td></tr></table>

• Defining **the temperature control mode** (temperature control switched on by default)

Defining the **Analog In Signal** (see also chapter Known Limitations on page 27)



**Figure 5** Configuration of the CE

**11** After the modules are selected the **Signals (2D)** tab opens. See page 8 for further details.

**12** Click **OK** to leave all configuration screens.

**13** Click **File** to save the configuration.

**Editing the CE Configuration in Chromeleon**

**1** Double-click on the newly added system. The configuration editor opens with several tabs.

    **a** **General**: The **General** tab of the LC System Configuration offers:

- Selection of the 3D Data Acquisition License to enable spectra acquisition
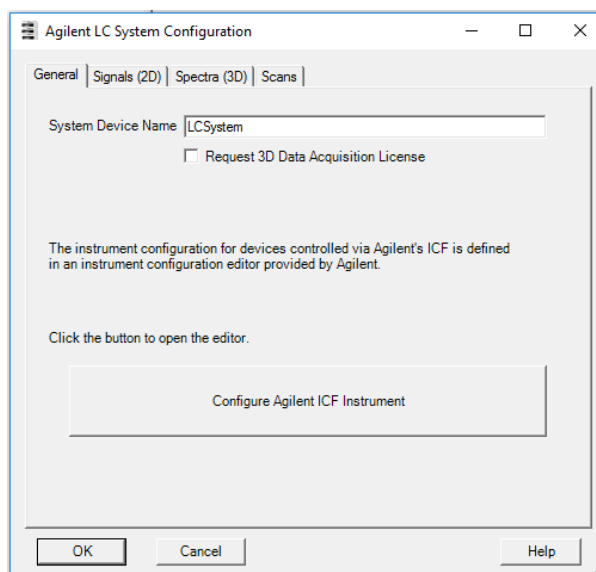- Change of the System Device Name, for example, to CE7100



**Figure 6**  Agilent LC System Configuration

    **b** **Signals (2D)**: Offers the possibility to review the signal mapping.

    **c** **Spectra 3D**: Review the spectra signal mapping. Only available with a Chromeleon **3D acquisition license**.

    **d** **Scans**: Only available for VWD.

# Set up and Configuration of CE in Chromeleon 7

**Agilent LC System Configuration**

General | Signals (2D) | Spectra (3D) | Scans

| | Type | Name | Monitor Signal | Chrom/Aux Signal | Min. Value | Max. Value | Unit | Factor |
|---|---|---|---|---|---|---|---|---|
| ▶ | UV | DAD_Signal_A | DAD: Signal A [mAU] | DAD: Signal A, 254 nm/Bw:4 nm Ref 360 nm/Bw:100 nm [mAU] | -5000 | 5000 | mAU | 1.000 |
| | UV | DAD_Signal_B | DAD: Signal B [mAU] | DAD: Signal B, 210 nm/Bw:4 nm Ref 360 nm/Bw:100 nm [mAU] | -5000 | 5000 | mAU | 1.000 |
| | UV | DAD_Signal_C | DAD: Signal C [mAU] | DAD: Signal C, 214 nm/Bw:4 nm Ref 360 nm/Bw:100 nm [mAU] | -5000 | 5000 | mAU | 1.000 |
| | UV | DAD_Signal_D | DAD: Signal D [mAU] | DAD: Signal D, 230 nm/Bw:4 nm Ref 360 nm/Bw:100 nm [mAU] | -5000 | 5000 | mAU | 1.000 |
| | UV | DAD_Signal_E | DAD: Signal E [mAU] | DAD: Signal E, 260 nm/Bw:4 nm Ref 360 nm/Bw:100 nm [mAU] | -5000 | 5000 | mAU | 1.000 |
| | UV | DAD_Signal_F | DAD: Signal F [mAU] | DAD: Signal F, 273 nm/Bw:4 nm Ref 360 nm/Bw:100 nm [mAU] | -5000 | 5000 | mAU | 1.000 |
| | UV | DAD_Signal_G | DAD: Signal G [mAU] | DAD: Signal G, 280 nm/Bw:4 nm Ref 360 nm/Bw:100 nm [mAU] | -5000 | 5000 | mAU | 1.000 |
| | UV | DAD_Signal_H | DAD: Signal H [mAU] | DAD: Signal H, 250 nm/Bw:100 nm Ref 360 nm/Bw:100 nm [mAU] | -5000 | 5000 | mAU | 1.000 |
| | Aux | DAD_UVLamp_AnodeVoltage | DAD: UV Lamp Anode Voltage [V] | DAD: UV Lamp Anode Voltage [V] | -1024 | 1024 | V | 1.000 |
| | Aux | CE_Voltage | CE: Voltage [KV] | CE: Voltage [KV] | -30 | 30 | kV | 1.000 |
| | Aux | CE_Current | CE: Current [µA] | CE: Current [µA] | -300 | 300 | µA | 1.000 |
| | Aux | CE_Power | CE: Power [W] | CE: Power [W] | -9 | 9 | W | 1.000 |
| | Aux | CE_LeakCurrent | CE: Leak Current [µA] | CE: Leak Current [µA] | -300 | 300 | µA | 1.000 |
| | Aux | CE_InternalPressure | CE: Internal Pressure [mbar] | CE: Internal Pressure [mbar] | -1000 | 1000 | mbar | 1.000 |
| | Aux | CE_ReplenishLevelPres | CE: Replenish Level Pressure [mbar] | CE: Replenish Level Pressure [mbar] | -1000 | 1000 | mbar | 1.000 |
| | Aux | CE_Vacuum | CE: Vacuum [mbar] | CE: Vacuum [mbar] | -1000 | 1000 | mbar | 1.000 |
| | Aux | CE_InjectionPressure | CE: Injection Pressure [mbar] | CE: Injection Pressure [mbar] | -100 | 100 | mbar | 1.000 |
| | Aux | CE_ExternalPressure | CE: External Pressure [bar] | CE: External Pressure [bar] | -16000 | 16000 | bar | 1.000 |
| | Aux | CE_TrayTemperature | CE: Tray Temperature [°C] | CE: Tray Temperature [°C] | -60 | 60 | °C | 1.000 |
| | Aux | CE_CassetteTemperature | CE: Cassette Temperature [°C] | CE: Cassette Temperature [°C] | -60 | 60 | °C | 1.000 |

OK    Cancel    Help

**Figure 7** Mapping of the signals

**2** Click **OK**.

**3** Save the configuration.

# Using the CE in Chromeleon

### The CE Status Dashboard in Chromeleon – Direct Control

**1** Start Chromeleon and open the **Agilent LC System** ePanel.

**2** The CE Status window displays all available modules with their status information. Details can be seen by hovering over the status bar with the mouse.
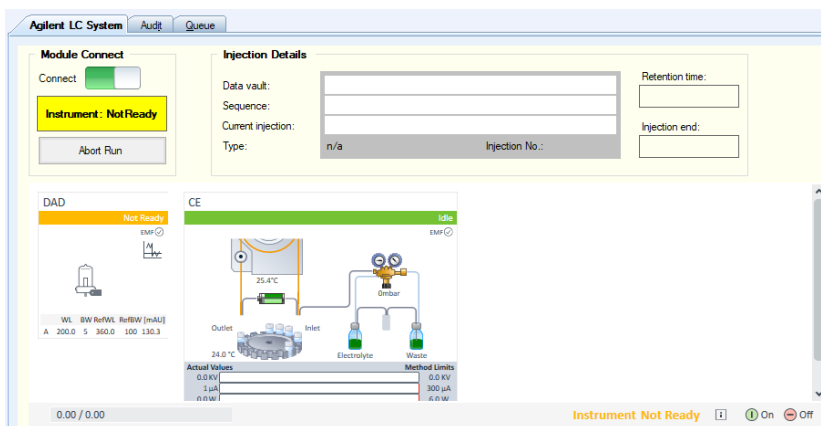


**Figure 8** CE dashboard status information

**3** Right-click on the lamp icon and select **Switch on** to show the green **Idle** state of the DAD part after a short ignition time.

**Using the CE in Chromeleon**

**4** The given space does not allow adjusting the CE status window to full size, therefore a vertical scrollbar is present to adjust the required section of the status dashboard.

Each component of the instrument is represented by an icon on the CE status dashboard. Right-click on an icon to access direct control. A context menu appears and an action can be performed. Some contextual actions require further user interaction.
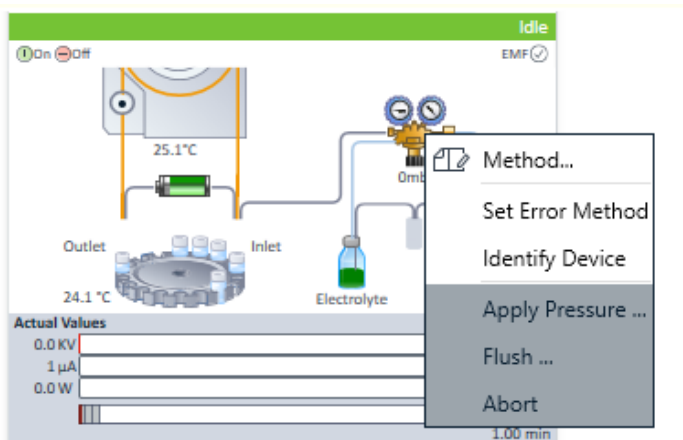
Click **OK** to trigger the action.



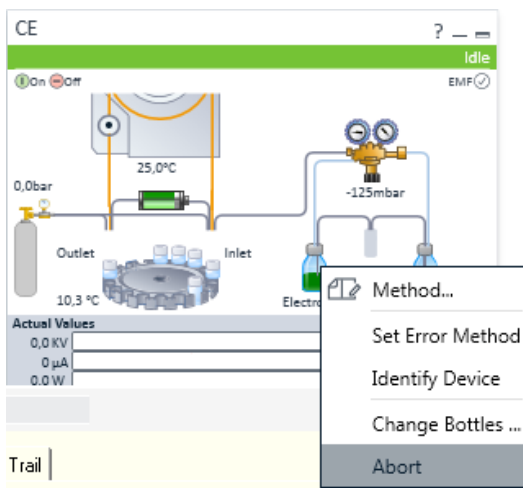**Figure 9**  Example of direct control - actions available for manometer icon



**Figure 10** Example of direct control - actions available for bottle icon

## Using the CE in Chromeleon

Following major actions are accessible via the CE Status dashboard only. This list does not claim to be exhaustive (for examples, see Figure 9 and Figure 10):

- **Change Cassette**
- **Switch on lamp/Switch off lamp**
- **Set Inlet Vial**
- **Set Replenish Vial**
- **Unload Replenish Lifter**
- **Unload Inlet Lifter**
- **Set Outlet Vial**
- Unload Outlet Lifter
- Flushing Capillary by **Flush**
- Injection by **Apply Pressure**
- **Apply Voltage**
- **Replenish Vials**
- **Change Bottles**
- **Get Vial**

All actions are performed immediately and the changes are reflected in the graphical user interface (GUI). The system is in **not ready** state (yellow), while performing these actions. After the action finishes, the instrument resumes an **idle** state (green). Any direct change of a parameter in the direct control menu (control and/or method) does not change the current instrument method.

NOTE    Using Monitor Baseline, it is possible to see the detector signal and all other selected channels while an action is running, e.g. flushing the capillary.

## Tabs on the ePanel

### On-line Plot

The **On-line plot** offers live continuous data-plot information once the acquisition starts.

To see the signals in the **On-line plot**, you must define them.

1   Right- click the **On-line plot** and select **Properties**

2   In **Appearance > Signals**, move the mouse to the right side of the signals line. Three dots appear. Click the three dots to open the **Signals** window. The available signals are listed.
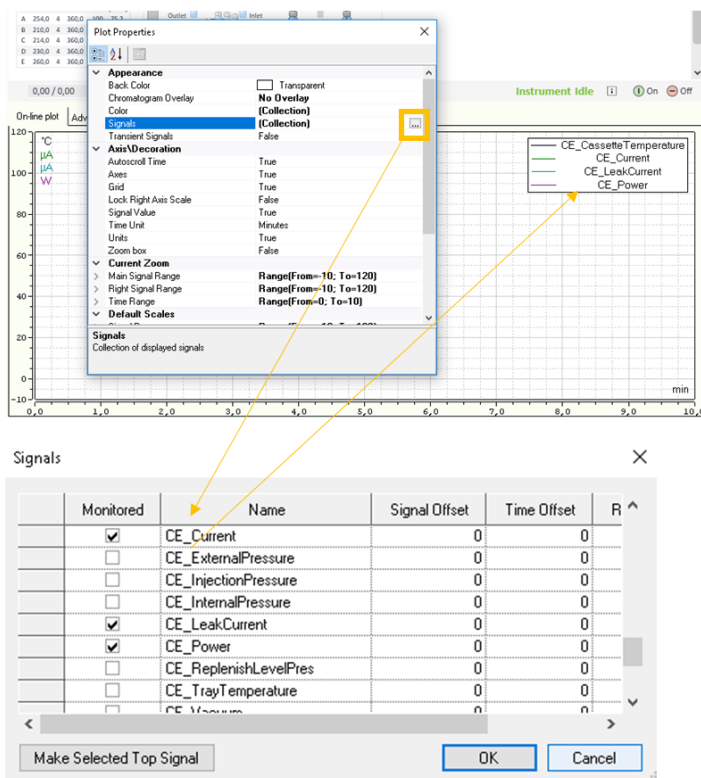
3   Select the check boxes of the signals you need.



**Figure 11** On-line plot signals

4   To see live data outside a run, use **Monitor Baseline**. The command can be found in the head of the Chromeleon Console.

### Advanced

The **Advanced** tab is the generic Chromeleon tab for ICF controlled modules.

- The **Injection Control** informs about the selected injection location.
- As CE does not support **Overlapped Injection**, this feature is found in the CE screen but can only be used in conjunction with an Agilent LC.
- The **Volume Specification** allows the user to define whether the injection location is taken from the method or from the injection list. Agilent supports the **VolumeFromInjectionList** only.
- As **Injection Mode Standard** is supported, **External** has not been tested with CE as there are no external injection possibilities.
- **Type of Run**

### Reconfigure Modules

The **Reconfigure Module** tab offers the possibility to change the **CE Mode** and to make use of external pressure for example.

The CE modi and the set up and use are described in the sections Indirect detection and Application of external pressure in this document (page 24 onwards).

Please note that the combination with MS (**MS installed**) was not tested with this integration.
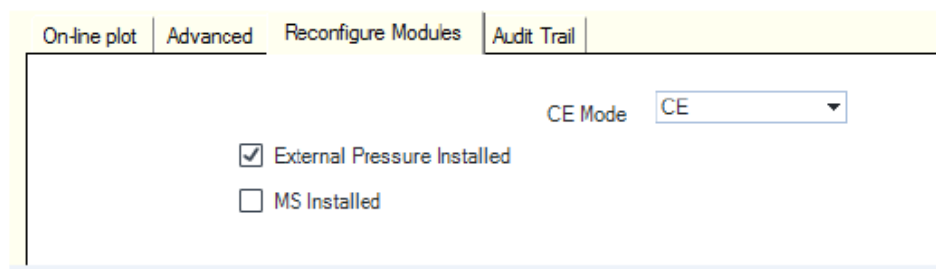


**Figure 12**     Reconfigure Modules

### Audit Trail

Among other things, the **Audit trail** also delivers instrument related information. All parameters set on the CE/DAD, changes, or actions performed are listed in the **Audit trail**. For more information on the **Audit Trail**, refer to the Chromeleon help.

## Creating an Instrument Method

The instrument method contains all the parameters necessary to perform the sample acquisition.

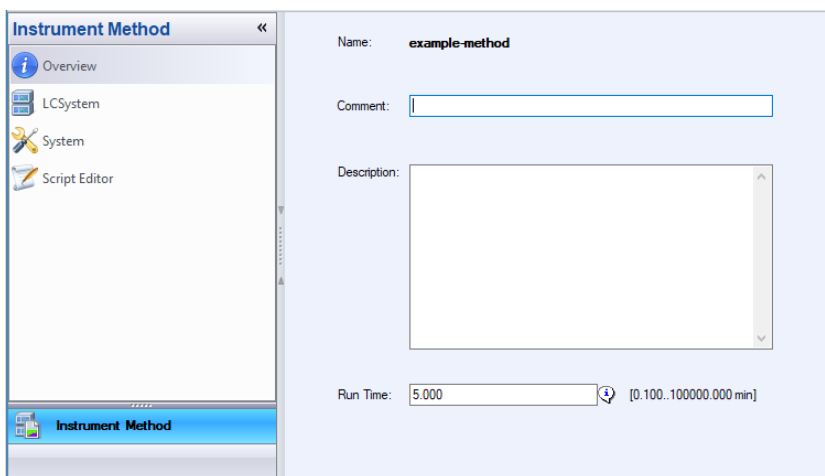**1** Open the instrument method editor, for example via **Create > Instrument Method**.



**Figure 13** The Navigation pane in the Instrument Method Editor

**2** Enter a run time and select the diagnostic channels.

**3** Click **Next**.

**4** Save the instrument method under a dedicated name. This method can later be changed, reopened or used in a sequence.

The instrument method editor offers a navigation pane with several views.

- Using the method wizard, the Navigation pane appears at the end of method creation

- Editing an existing method the Navigation pane is shown first

**a** **Overview**: In the **Overview** view it is possible to enter comments or descriptions e.g. after changing a method. It is also possible to set the run time

**b** LCSystem: The LCSystem view provides access to all method parameters of the CE system, one tab per module, CE and DAD. Select the appropriate tab to enter the method parameters. For details on the parameters refer to the online help and the CE or DAD user manual. For more explanations, see Setting the CE parameters in the LCSystem tab on page 18.

**c** **System**: Offers **General Settings**, where you can define the **Run Time** and select **Diagnostic Channels**.



**Figure 14**     General Settings in the System tab

**d** **Script Editor**: Shows available commands for the method

| | Time | Command | Value | |
|---|---|---|---|---|
| 0 | {Initial Time} | Instrument Setup | | |
| 1 | 0.000 | Inject Preparation | | |
| 2 | | Wait | LCSystem.Ready | |
| 3 | 0.000 | Inject | | |
| 4 | | LCSystem.CE.Inject | Blank=Inject | |
| 5 | 0.000 | Start Run | | |
| 6 | | LCSystem.CE_CassetteTemperature.AcqOn | | |
| 7 | | LCSystem.CE_ExternalPressure.AcqOn | | |
| 8 | | LCSystem.CE_InjectionPressure.AcqOn | | |
| 9 | | LCSystem.CE_InternalPressure.AcqOn | | |
| 10 | | LCSystem.CE_LeakCurrent.AcqOn | | |
| 11 | | LCSystem.CE_ReplenishLevelPres.AcqOn | | |
| 12 | | LCSystem.CE_TrayTemperature.AcqOn | | |
| 13 | | LCSystem.CE_Vacuum.AcqOn | | |
| 14 | | LCSystem.DAD_UVLamp_AnodeVoltage.AcqOn | | |
| 15 | | LCSystem.DAD_Signal_A.AcqOn | | |
| 16 | | LCSystem.CE_Voltage.AcqOn | | |
| 17 | | LCSystem.CE_Current.AcqOn | | |
| 18 | | LCSystem.CE_Power.AcqOn | | |
| 19 | 0.000 | Run | Duration = 5.000 [min] | |
| 20 | 5.000 | Stop Run | | |
| 21 | | LCSystem.CE_CassetteTemperature.AcqOff | | |
| 22 | | LCSystem.CE_ExternalPressure.AcqOff | | |
| 23 | | LCSystem.CE_InjectionPressure.AcqOff | | |
| 24 | | LCSystem.CE_InternalPressure.AcqOff | | |
| 25 | | LCSystem.CE_LeakCurrent.AcqOff | | |
| 26 | | LCSystem.CE_ReplenishLevelPres.AcqOff | | |
| 27 | | LCSystem.CE_TrayTemperature.AcqOff | | |
| 28 | | LCSystem.CE_Vacuum.AcqOff | | |
| 29 | | LCSystem.DAD_UVLamp_AnodeVoltage.AcqOff | | |
| 30 | | LCSystem.DAD_Signal_A.AcqOff | | |
| 31 | | LCSystem.CE_Voltage.AcqOff | | |
| 32 | | LCSystem.CE_Current.AcqOff | | |
| 33 | | LCSystem.CE_Power.AcqOff | | |
| 34 | End | | | |

**Figure 15** Script Editor

### Setting the CE Parameters in the LCSystem Tab

*The CE method Screen*

The CE method screen is available in the **Agilent LC Method** System tab and contains all parameters used to adjust the separation, meaning the home vials can be defined as well as **Cassette Temperature**, **Voltage**, and **Stoptime**. Press F1 on the keyboard to access the online help, offering explanations for each method parameter and their specifications.



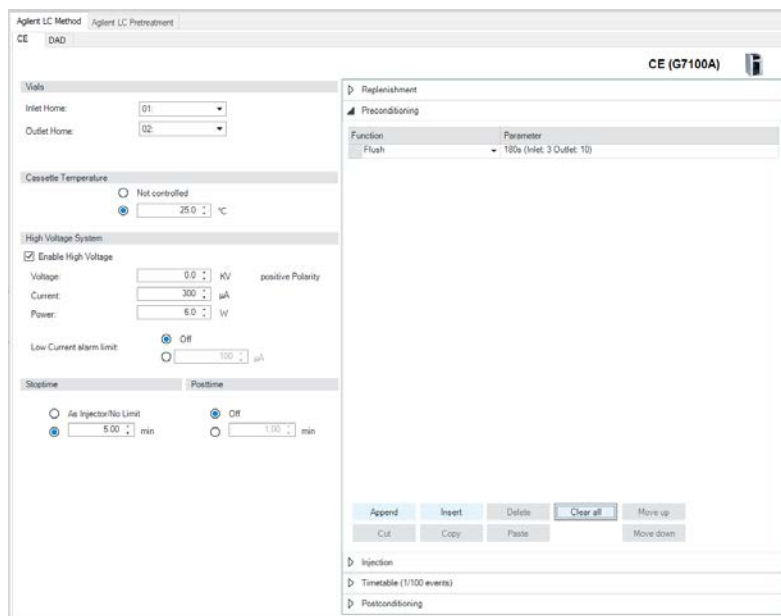**Figure 16**    CE method screen

NOTE    Enter a **Stoptime** for the CE and/or the DAD. For run execution, a **Run Time** is specified in the **Overview** tab; ensure that both values are synchronized.

The method´s **Post Time** is independently added after the **Stoptime**, and no data is recorded during the post time. The next sequence run starts after the **Post Time** of the previous run is finished.

If an entered parameter is out of range, a warning sign is displayed. When hovering with the mouse pointer over the parameter, the message displays the possible range, e.g. **Voltage is out of range [-30kV to 30 kV]**.
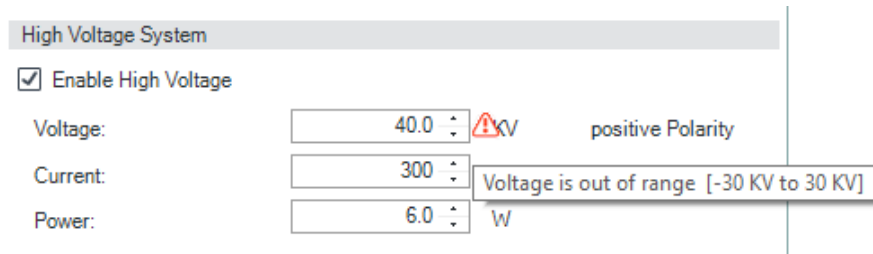


**Figure 17**      Out of range handling

The right side of the method screen offers additional features. The online help explains each of the features:

- **Replenishment**
- **Preconditioning**
- **Injection**
- **Timetable**
- **Postconditioning**

## Using the CE in Chromeleon

*The DAD method screen*

On the left side, the DAD method offers the main method parameters to acquire up to eight signals. Refer to the online help to learn more about each parameter. Remember to set the stop time either to CE or to the same time as the Chromeleon run time (time specified in the overview tab of the Chromeleon instrument method).

The right side offers additional method parameters, e.g. for spectra collection and a timetable, in order to change parameters during a run.

Unlike with other Agilent DAD, there is no VIS-lamp available.



**Figure 18** DAD method screen

### Data Acquisition

In Chromeleon a sequence can either hold a single or multiple injection lines, additional injections can be appended to an existing sequence.

### Sequence Execution

It is possible to chain sequences. The queue shows the status of each chained sequence: **Finished**, **Running**, **Prepared** or **Interrupted**.

1 Click **Create > Sequence** to open the **New Sequence Wizard**.

2 Select the Instrument and click **Next**.

3 Fill in the following entries:

- Pattern for Injection Name
- Number of vials (1...50)
- Injections per vial (1...999)
- Start Position (1...50)
- Injection Volume (1.0)

4 In the screen **Methods & Reporting** select the following:

- Instrument Method
- Processing Method
- Report Template
- View Settings
- Channel

5 Save the sequence.

---

NOTE — The parameter **Inj. Vol (μl)** requires the value 1.0. This value is *not* used for the injection, but it is reported. The instrument method parameters (for example: pressure and time) are used for the injection.

(Instead of volume, the quantifiable parameters are pressure × time for hydrodynamic injection or voltage × time for electrokinetic injection).

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In the resulting sequence table additional parameters can be entered. The details of the injections have to be added to the sequence table, depending on the required workflow.

After any change to an injection, the sequence needs to be saved for the change to take effect.

| NOTE | If a vial is missing, the current run is stopped and the whole sequence and queue is aborted. |

It is possible to stop the run at any time. Then the sequence is stopped immediately, and the run is assigned the status **Interrupted**.

Click **Resume** to continue the sequence.

| NOTE | A shutdown of the CE/DAD is not supported. |

### Replenishment and Conditioning

**Replenishment** allows to automatically change the buffer of a vial either in serial or parallel mode.

**Preconditioning** and **Postconditioning** offers conditioning of the capillary.

These settings are method settings and therefore expected each time the method is running. If this is not wanted, make sure to generate methods with and without replenishment and/or pre/postconditioning.

During multiple analyses the buffer must be refreshed after a certain number of runs. Typically, the exchange of the buffer is done every 3 - 10 runs. Depending on the stability of the buffer, it is sometimes necessary to refresh it before each run.

| NOTE | Using the replenishment as a part of the method, the replenishment is carried out for each run where this is part of the method. |

If you want the refresh to be done less, ensure to create at least two methods.

Example:

Run 1 method A with replenishment

Run 2 method

Run 3 method

Run 4 method with replenishment

Run 5 method

Run 6 method

Run 7 method with replenishment

Run 8 method

Run 9 method

## Using the CE in Chromeleon

The replenishment system provides a quick way to change the buffer automatically. The system removes the used buffer from the vials and transfers it into the waste bottle. Then the vials are filled with fresh buffer from the electrolyte reservoir.

Refer to the G7100 User manuals on the requirements for the bottles, as only specific bottles can be used. For example, they need to be pressure-stable.

The waste bottle should be a 500 ml bottle, whereas the electrolyte bottle can also be a 100 ml bottle. These are both included in the accessory kit.

Following replenishment tasks can be set up in the CE replenishment table in the Instrument method:

- **Empty vial**: empties a vial into the waste bottle
- **Fill vial**: fills a vial to a user-selectable level from the electrolyte reservoir
- **Clean tubes**: flushes the replenishment system to clean the tubes
- **Replenish vial**: empties a vial into the waste and fills it up from the electrolyte reservoir
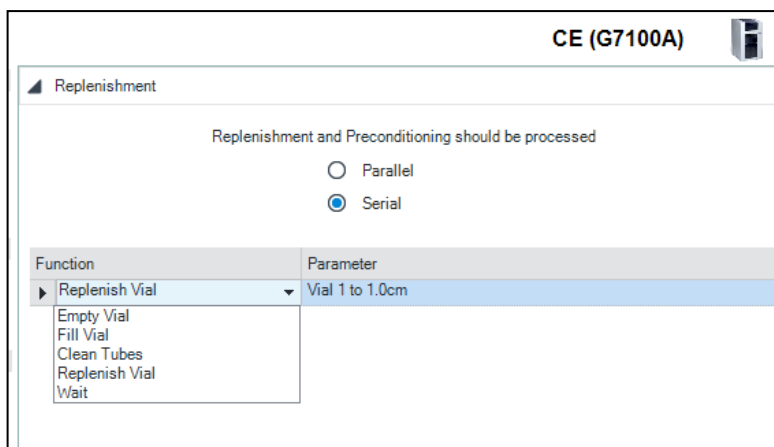- **Wait**: waits for a specified time



**Figure 19**  Replenishment Table

It is possible to execute replenishment in parallel, which decreases the overall analysis time as replenishment and pre- or postconditioning are executed simultaneously. For parallel replenishment, the vials for the first run must be filled with the correct solutions, because the replenishment starts with **Empty Vial**.

NOTE    Using parallel replenishment, the method shows Serial and not Parallel after the reopening. However, the execution will be performed parallel and the audit trail also shows the parallel replenishment correctly.

NOTE    To efficiently clean the replenishment system after use, the special functions **Flush Tubes** and **Clean Level Sensor** should be performed. This advanced cleaning is only available in the Agilent LabAdvisor software.

### Indirect Detection

The indirect detection mode allows the application of indirect photometric detection of non-UV absorbing analytes like inorganic cations, inorganic anions and organic acids and small carbohydrates.

To attain indirect photometric detection, an ionic compound with a high UV absorbance intensity is used as the background electrolyte (BGE). During the separation any non-absorbing analyte causes a reduction of the high background signal, resulting in negative peaks.

Chromeleon can record and calculate positive and negative signals. If most of the analytes are non-absorbing, the detector signals for signal and reference wavelength can be inverted, so that most of the peaks are positive.

### Application of External Pressure

For some special applications using high viscosity buffer solution (e.g. a gel matrix in the CGE and cIEF), the capillary can be conditioned using external high pressure to ensure that the capillary is filled in adequate time.

Two additional modes are available for configuration:

- **CEC**: Capillary Electrochromatography allows high pressure to be applied on both electrolyte vials in the run during the high-voltage application to prevent outgassing and bubble formation.

    A connected external pressure supply is required for this mode. When this mode is selected, additional pressure options are available in the instrument method:

    - **High pressure flush** in **Preconditioning**, **Injection** and **Postconditioning**.
    - External pressure as method set point and timetable entry.
- **CE/p**: additional external pressure connected

### Configure CEC or CE/p Mode

If an external pressure source is available (pressure 2 - 12 bar) and connected to the CE device, these modes can be configured in the **Reconfigure Modules** tab, which can be found below the CE status dashboard. Select the required CE Mode.
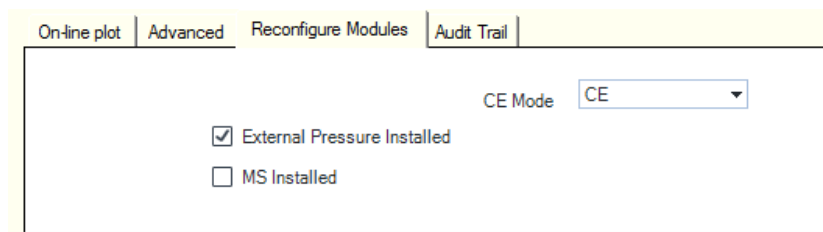


**Figure 20**    Reconfigure Modules

When the **External Pressure Installed** check box is selected, the gas cylinder is shown in the CE status dashboard accessible via the **Instrument Status** tab. High pressure can now be applied by right-clicking on the gas cylinder.
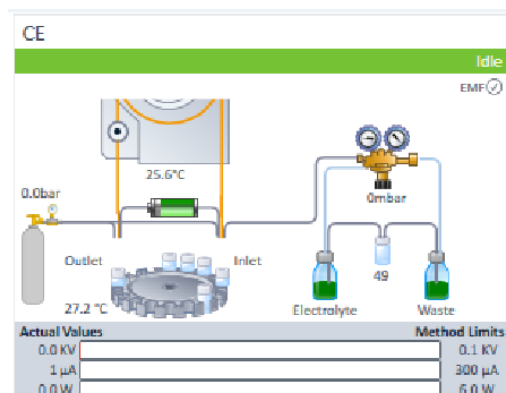


**Figure 21**    Access to external pressure

## Method Impact for CEC or CE/p Mode

- If the box **External Pressure Installed** is checked, the instrument method offers high pressure settings as additional method parameters on the right-hand CE method screen:
  - **Preconditioning**
  - **Injection**
  - **Timetable**
  - **Postconditioning**
- If **CEC** is selected, the instrument method offers an additional initial value **Pressure** on the left-hand CE method screen. Selecting **External**, the pressure is applied to both home vials during run execution (see Figure 19).
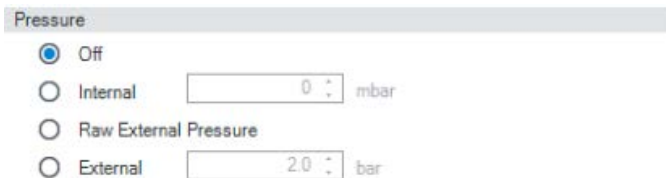


**Figure 22**    Applying external pressure – new menu
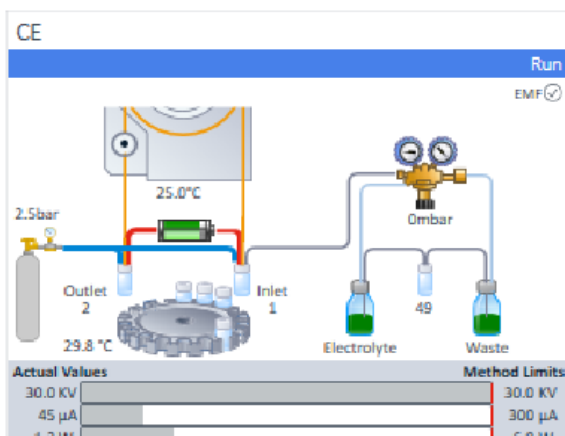


**Figure 23**    Applying external pressure during a run

# Known Limitations

### Control and Action Menus

All actions and direct controls can only be accessed via the CE Status dashboard. There are no menu items for instrument control in the Chromeleon menu bar.

### Fraction Collection

The system offers Set up for Peak Fraction Collection whether a fraction collector is present or not.

### User Vials

User vials are not available in this integration.

### Capillary Catalog, Capillary Handling

A capillary catalog database is not available in this integration. Workaround: Add the capillary information to the Method Comments.

### Sample Diagram

A sample diagram is not available in this integration.

### Vial Table

A vial table is not available in this integration.

### Calibration Curve options

The following Calibration Curves are unavailable in Chromeleon:

- Mobility correction
- Calibration Type cIEF
- Calibration for determination of isoelectric points or molecular weights

## Features Not Supported by CE Driver in Chromeleon Environment

- **Pressure Unit** configuration/PSI Mode

  Selecting PSI as pressure unit, the CE status dashboard shows the pressure in PSI, while the online plot and the resulting Aux Traces on the Chromatogram offer the values in mbar/bar. The correct pressure is applied. PSI mode is not supported

- **Analog In**

  The feature **Analog In** has not been explicitly tested, but the functionality is present.

- **MS installed**

  The feature **MS installed** has not been tested.



**Figure 24**     MS installed

# APPENDIX - Examples

## Example: Running one CE separation

For testing the functions of the 7100 CE and Chromeleon 7.2, an instrument method can be created. An example for equipment and method parameters can be summarized as follows:

| | |
|---|---|
| Capillary: | Fused silica capillary 50 µm ID, 48.5 cm total (40 cm effective), ext. Light path (G1600-60232) |
| Electrolyte: | 20 mM Borate (from IQ-Kit: 5063-6514) |
| Home vials: | 1 and 2 (filled with electrolyte solution) |
| Sample: | 4-Hydroxyacetophenone solution 1 mmol/l (from IQ-Kit: 5063-6514) |

**NOTE**     Dilute the sample 1:10 prior to run.

| | |
|---|---|
| Voltage: | +30 kV |
| Temperature: | 25°C |
| Stop Time: | 5 min |
| Preconditioning: | 180 s (from electrolyte vial 3 into waste vial) |
| Injection: | line 1: 50 mbar for 10 s (from injection vial to outlet vial) line 2: 50 mbar for 5 s (from inlet vial to outlet vial) |
| DAD: | Signal: 200 nm (BW 5 nm) Reference: 360 nm (BW 100 nm) Peakwidth: >0.025 min (10 Hz) |
| DAD Timetable: | 0.5 min Balance |

**NOTE**     Ensure that the Chromeleon **Run Time** is the same as the one defined in the **Stoptime** of the CE/DAD method.

For the sequence table, only one line is needed and the created instrument method has to be chosen.

While a separation is running, the selected channels (for example: **Signal A**, **Current**,…) can be seen in the online plot.
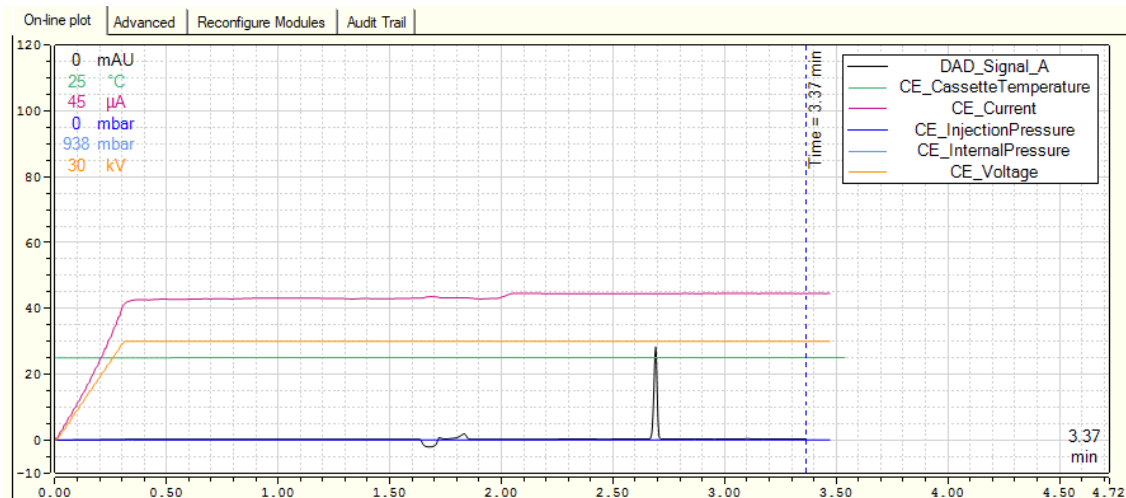
## APPENDIX - Examples



**Figure 25**   Online plot during the run

After the run, the injection time is shown in the line of the sequence table and the **Status** of the run changes from **Running** to **Finished**. The resulting electropherogram can be obtained in the **Data** screen by double-clicking on the run.
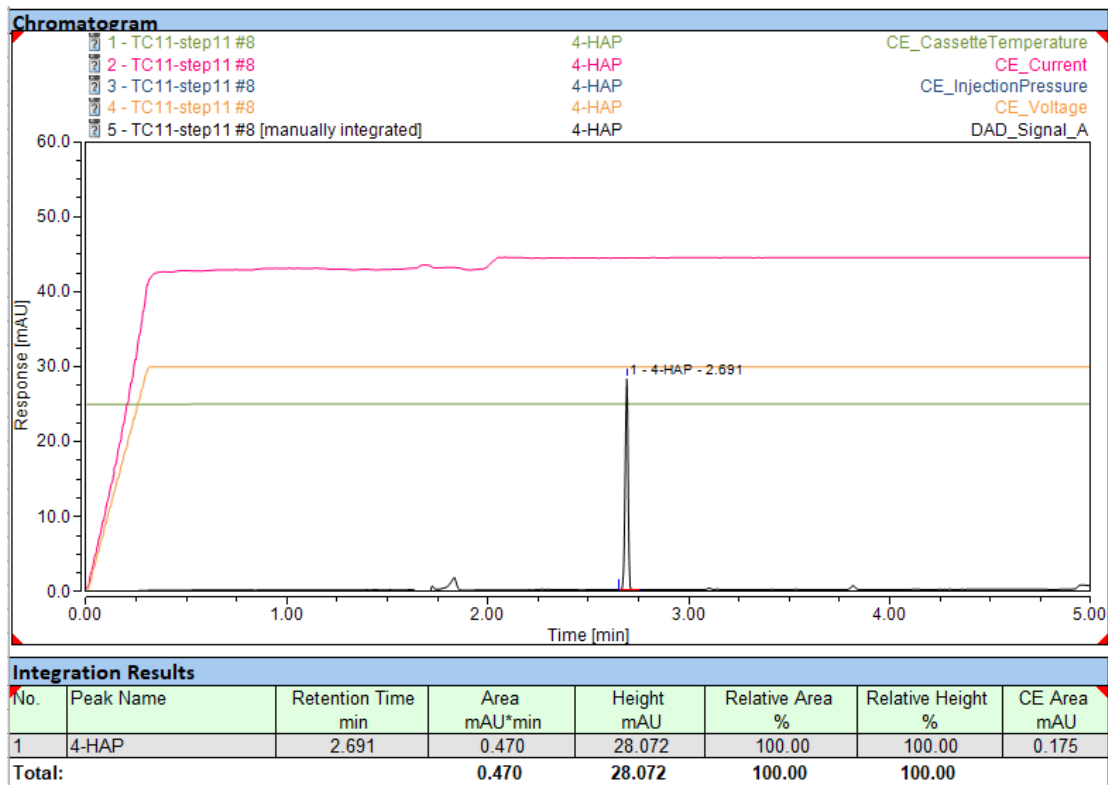
## APPENDIX - Examples



**Figure 26**    Resulting electropherogram

## APPENDIX - Examples

## Example: Multiple Injections

For testing multiple injections in one sequence, the instrument method and the experimental setup described in section Example: Running one CE separation on page 29 can be used. See the sequence table and the overlay of the resulting electropherograms (Figures 23 and 24 below).



**171205-injection precision**

| # | DAD_Signal_A | | Name | Type | Level | Position | Volume [IN/A] | Instrument Method | Processing Method | Status | Inject Time |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | 4-HAP | Unknown | | 20 | 1.0 | method0815 | Basic Quantitative | Finished | 12/5/2017 11:29:12 A... |
| 2 | | | 4-HAP | Unknown | | 20 | 1.0 | method0815 | Basic Quantitative | Finished | 12/5/2017 11:37:37 A... |
| 3 | | | 4-HAP | Unknown | | 20 | 1.0 | method0815 | Basic Quantitative | Finished | 12/5/2017 11:46:02 A... |
| 4 | | | 4-HAP | Unknown | | 20 | 1.0 | method0815 | Basic Quantitative | Finished | 12/5/2017 11:54:28 A... |
| 5 | | | 4-HAP | Unknown | | 20 | 1.0 | method0815 | Basic Quantitative | Finished | 12/5/2017 12:02:53 P... |
| 6 | | | 4-HAP | Unknown | | 20 | 1.0 | method0815 | Basic Quantitative | Finished | 12/5/2017 12:11:18 P... |
| 7 | | | 4-HAP | Unknown | | 20 | 1.0 | method0815 | Basic Quantitative | Finished | 12/5/2017 12:19:44 P... |
| 8 | | | 4-HAP | Unknown | | 20 | 1.0 | method0815 | Basic Quantitative | Finished | 12/5/2017 12:28:09 P... |
| 9 | | | 4-HAP | Unknown | | 20 | 1.0 | method0815 | Basic Quantitative | Finished | 12/5/2017 12:36:35 P... |
| 10 | | | 4-HAP | Unknown | | 20 | 1.0 | method0815 | Basic Quantitative | Finished | 12/5/2017 12:44:59 P... |
| 11 | | | 4-HAP | Unknown | | 20 | 1.0 | method0815 | Basic Quantitative | Finished | 12/5/2017 12:53:25 P... |
| 12 | | | 4-HAP | Unknown | | 20 | 1.0 | method0815 | Basic Quantitative | Finished | 12/5/2017 1:01:50 PM... |

Click here to add a new injection

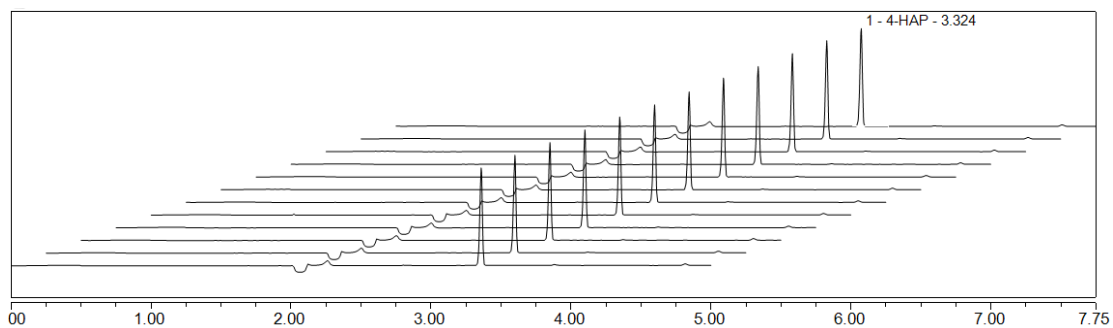**Figure 27**   Sequence table for 12 injections



**Figure 28**   Overlay of 12 consecutive injections

## APPENDIX - Examples

The set of twelve injections was used to calculate system precision. The peak areas, migration times, peak heights and CE Areas were calculated using a Chromeleon processing method and are summarized in the following figure.

### Summary

**Sequence Details**

| | | | |
|---|---|---|---|
| Name: | 171205-injection precision | Created On: | 05/Dec/17 11:24:56 |
| Directory: | | Created By: | ChromtestPC1 |
| Data Vault: | ChromeleonLocal | Updated On: | 12/Dec/17 19:53:49 |
| No. of Injections: | 12 | Updated By: | ChromtestPC1 |

**By Component** 4-HAP

| No. | Peak Name | Ret.Time min DAD_Signal_A 4-HAP | Area mAU*min DAD_Signal_A 4-HAP | Height mAU DAD_Signal_A 4-HAP | CE Area mAU DAD_Signal_A 4-HAP | Rel.Area % DAD_Signal_A 4-HAP | Peak Type DAD_Signal_A 4-HAP |
|---|---|---|---|---|---|---|---|
| 1 | 4-HAP | 3.358 | 0.650 | 30.733 | 0.194 | 100.00 | BMB* |
| 2 | 4-HAP | 3.349 | 0.649 | 30.772 | 0.194 | 100.00 | BMB* |
| 3 | 4-HAP | 3.349 | 0.648 | 30.823 | 0.193 | 100.00 | BMB* |
| 4 | 4-HAP | 3.349 | 0.645 | 30.814 | 0.192 | 100.00 | BMB* |
| 5 | 4-HAP | 3.348 | 0.646 | 30.897 | 0.193 | 100.00 | BMB* |
| 6 | 4-HAP | 3.348 | 0.645 | 30.763 | 0.193 | 100.00 | BMB* |
| 7 | 4-HAP | 3.344 | 0.644 | 30.882 | 0.192 | 100.00 | BMB* |
| 8 | 4-HAP | 3.339 | 0.648 | 31.191 | 0.194 | 100.00 | BMB* |
| 9 | 4-HAP | 3.338 | 0.642 | 30.852 | 0.192 | 100.00 | BMB* |
| 10 | 4-HAP | 3.332 | 0.641 | 30.939 | 0.192 | 100.00 | BMB* |
| 11 | 4-HAP | 3.327 | 0.642 | 31.024 | 0.193 | 100.00 | BMB* |
| 12 | 4-HAP | 3.324 | 0.639 | 30.855 | 0.192 | 100.00 | BMB* |

**Figure 29**  Results

A good relative standard deviation (Excel: stdev.s) of 0.5 % for the areas and around 0.3 % for the migration times and CE area were obtained. These satisfactory values are achieved by a special self-regulating injection procedure, which significantly improves the injection precision. For more information please refer to the *Agilent CE User Manual G7100-90000 Rev. B*.

## Example: Indirect Detection Using the Plating Bath Buffer

An instrument method can be created using the Plating Bath Buffer for testing the indirect detection functions. The following reagents and parameters can be used:

| | |
|---|---|
| Capillary: | Fused silica capillary 50 µm ID, 80.5 cm total (72 cm effective) (G1600-62211) |
| Electrolyte: | Plating Bath Buffer (Agilent: 5064-8236) |
| Home vials: | 1 and 2 (filled with electrolyte solution) |
| Sample: | Suitable Plating Bath Mixture (laboratory made) |
| Voltage: | -30 kV |
| Temperature: | 25°C |
| Stoptime: | 15 min |
| Preconditioning: | 240 s (from electrolyte vial 3 into waste vial) |
| Injection: | line 1:from injection vial to outlet vial: 50 mbar for 10 s |
| | line 2: from inlet vial to outlet vial: 50 mbar for 5 s |
| DAD channel A: | Signal: 350 nm (BW 20 nm) |
| | Reference: 275 nm (BW 10 nm) |
| DAD channel B: | Signal: 275 nm (BW 10 nm)R |
| | Reference: 350 nm (BW 20 nm) |
| | Peak width: >0.025 min (10 Hz) |
| Timetable: | 2.0 min Balance |

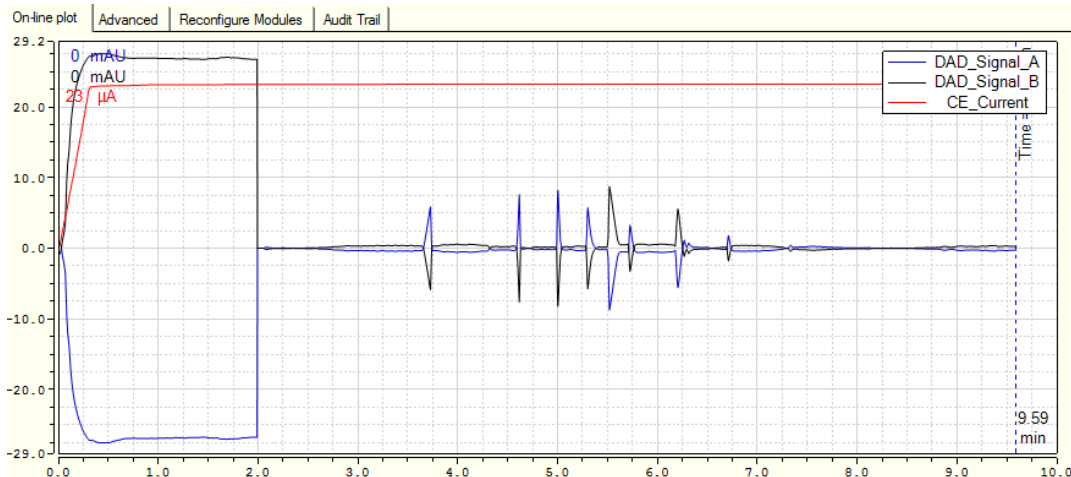Comparing the reversed wavelength the peaks can be shown as positive or negative peaks.



**Figure 30**    Indirect detection online plot

It is evident from the electropherogram in the Figure below that the selected wavelength values result in positive peaks for the non-UV-absorbing analytes: sulfate, malate, hypophosphite, phosphite and lactate. The nickel-complex has its own UV- intensity and results in a negative peak. The figure also shows that the integration and evaluation of the peak areas are possible for positive and negative peaks in the same signal.
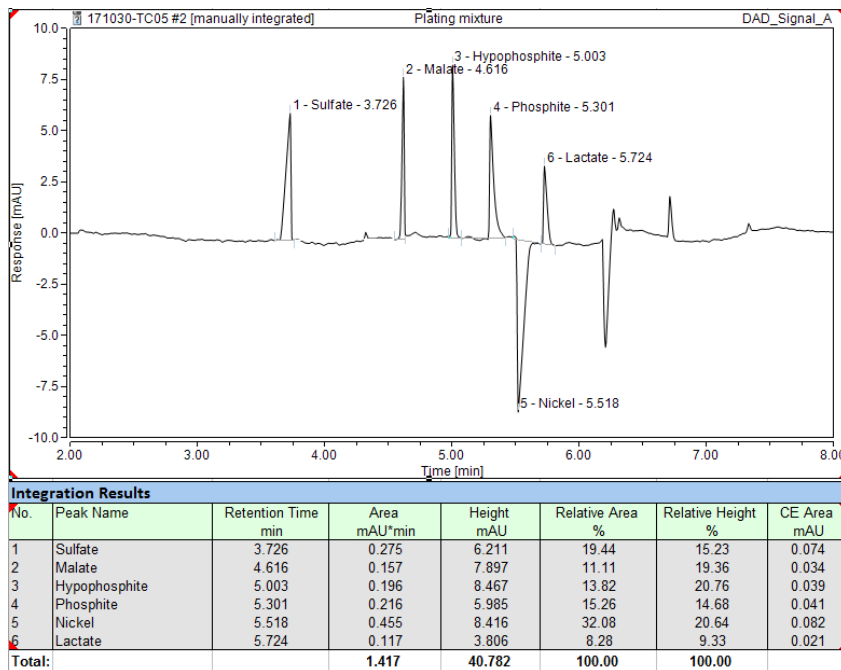


**Integration Results**

| No. | Peak Name | Retention Time min | Area mAU*min | Height mAU | Relative Area % | Relative Height % | CE Area mAU |
|---|---|---|---|---|---|---|---|
| 1 | Sulfate | 3.726 | 0.275 | 6.211 | 19.44 | 15.23 | 0.074 |
| 2 | Malate | 4.616 | 0.157 | 7.897 | 11.11 | 19.36 | 0.034 |
| 3 | Hypophosphite | 5.003 | 0.196 | 8.467 | 13.82 | 20.76 | 0.039 |
| 4 | Phosphite | 5.301 | 0.216 | 5.985 | 15.26 | 14.68 | 0.041 |
| 5 | Nickel | 5.518 | 0.455 | 8.416 | 32.08 | 20.64 | 0.082 |
| 6 | Lactate | 5.724 | 0.117 | 3.806 | 8.28 | 9.33 | 0.021 |
| Total: | | | 1.417 | 40.782 | 100.00 | 100.00 | |

**Figure 31**    Integration and reporting of positive and negative peaks

**Agilent**