

Creating instrument methods

■ [QuickStart](#) > [View Method](#) > [Instrument](#) > [View Instrument Method window](#)

Before you can acquire and analyze data, you must configure an instrument system. You then create an instrument method, to specify the control parameters for that instrument system. After you create an instrument method, you must then add it as part of a method set.

In addition to creating an instrument method, you can also perform these instrument method tasks:

- Modify an instrument method
- Create an instrument method without specifying a chromatographic system
- Delete an instrument method
- Add an instrument to an existing instrument method
- Set the gradient parameters for the 2695, 2695D, and the 2795 systems test
- Set PDA instrument method parameters
- Specify the correct sampling rate
- Extinguish the detector lamp automatically in a method

To create an instrument method:

1. From the View Instrument Method window, click File > New.
2. For each instrument you must configure, in the Instrument list, click the instrument's icon.
3. On each tab, specify the parameters relevant for the selected instrument.
4. Click Save, and enter a name for the instrument method.

Alternative: When using the Pro interface, you can save an instrument method within a method set automatically. From the File menu, click Save with Method Set.

To modify an instrument method:

1. From the View Instrument Method window, click File > Open.
2. Select the method you want to open, and then click Open.
3. Click the icon of the instrument whose parameters you want to modify.
4. On each of the tabs that appear, modify the settings, as required.
5. Save your changes:

- To save changes to an existing instrument method, click File > Save.
- To save changes to a new instrument method, click File > Save As.

Tip: If you modify the percentage of a solvent in a gradient, the software automatically updates the percentage for the remaining solvents when you tab to the next solvent cell.

Creating an instrument method without specifying a chromatographic system

You can create an instrument method without specifying an existing, configured chromatographic system. Doing so is useful when you change the instrumentation of your systems frequently. You can choose and set up any combination of Empower-supported instruments, and create an instrument method for future use. It is also useful when creating instrument methods on computers not physically connected to chromatographic systems.

To create an instrument method without specifying a chromatographic system:

1. In the navigation pane, click Browse Project.
2. Click Method tab, and then click File > New Method > Instrument Method.
 - If you have multiple chromatographic systems configured, the Select System dialog box appears. Click Cancel, to open the Instrument Method Editor without any instruments in the Instruments and Active Channels tree.
 - If one chromatographic system is configured, the Instrument Method Editor opens with the configured instruments in the Instruments and Active Channels tree. To change the instruments within the method, click the instrument icon, to select it, and then right-click and select Turn Off or Delete.
 - If no chromatographic system in your project is configured, the Instrument Method Editor opens without any instruments in the Instruments and Active Channels tree.
3. Create an instrument method, as described earlier in this topic.

To delete an instrument or turn it on or off:


Right-click the instrument's icon, and then click the menu item to perform your task:

- Delete an instrument.
- Turn on an instrument.
- Turn off an instrument.

Restriction: Do not turn off Ethernet devices from the instrument method. Doing so can disrupt a run. If an Ethernet device is not needed, do not include it when creating a chromatographic system.

Tip: For modules with associated data channels, such as the busSAT/IN module, activate or deactivate data channels by powering-on or powering-off the device. Do the same for all similar devices, activating or deactivating channels in the instrument tabs, as necessary.

To add an instrument to an existing instrument method:

1. In the navigation pane, click Browse Project.
2. Click Method tab, and then double-click the instrument method that you want to change.
3. Click Add Instrument .
4. In the Add Instruments dialog box, select the instrument, and then click OK.

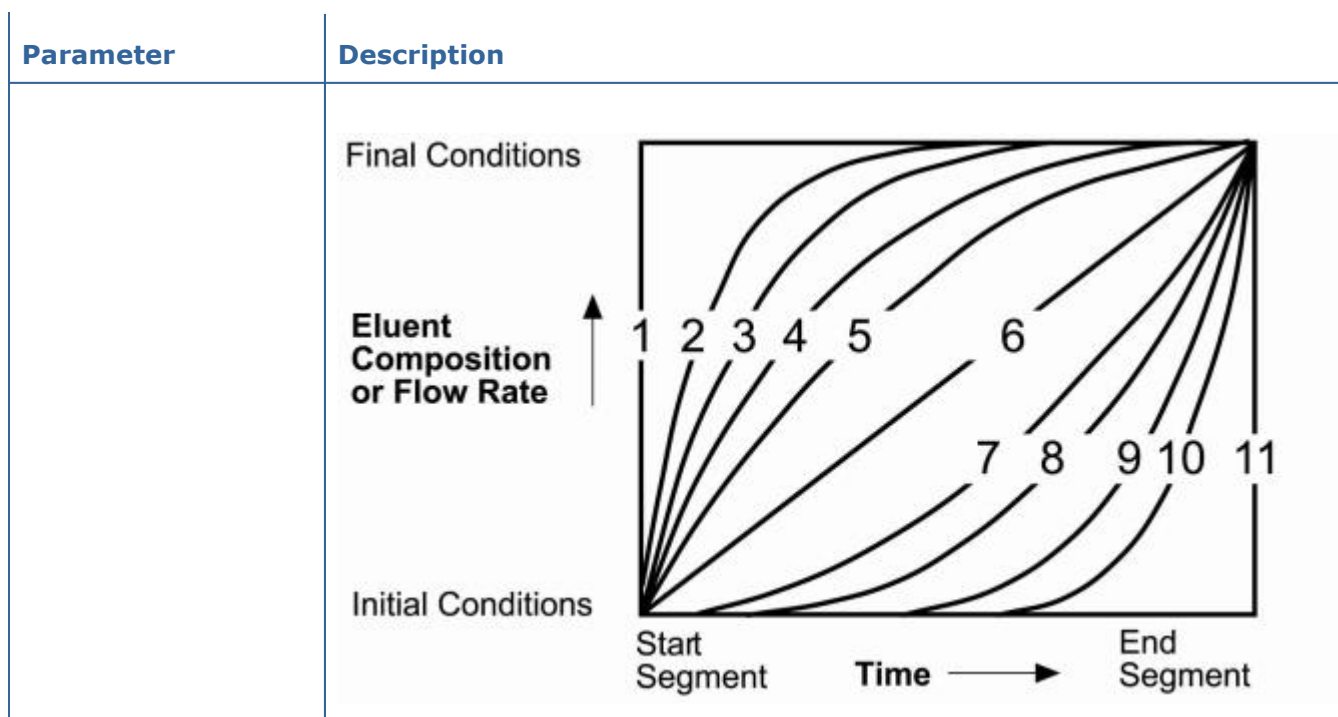
Tip: You can add or delete an instrument from the method without changing the actual system configuration.

Setting the gradient parameters for the 2695, 2695D, 2795, 600 or PCM systems

You can set gradient parameters according to the guidelines in this table.

Gradient parameters:

Parameter	Description
Time	Defines when conditions for the row take effect. The Time column for the first row is disabled (to represent a fixed default time value of 0.00 that establishes initial conditions for the gradient run). Entries for rows other than row 1: 0.00 to 650.00 minutes.
Flow	Total flow rate for the system.
%A, %B, %C, %D	Percentage of solvent flow from each reservoir. The total of all solvents in each gradient segment must equal 100%. The system calculates individual flow rates when you enter the percentage value for each solvent.
Curve	Rate of change of solvent composition and flow rate over time, based on the curve number and the length of the gradient segment. The gradient curve profile that you specify in each row of the Gradient table affects both solvent composition and flow rate. Curves are specified by number. Choices: 1 to 11.



Adjusting solvent and flow rate percentages in the gradient table

As you specify flow rate percentages to create a gradient, the software automatically calculates the flow rate values for the solvent column to the right, so that the total flow rate percentage across all solvent columns is 100%.

If you modify a value in a solvent column, the software adjusts the solvent value in the column to the right.

When you modify a solvent percentage, and 2 or more (non-zero) solvent values appear in columns to the right, the software adjusts only the first column to the right (to a value that makes the total percentage equal to 100%). Any subsequent columns to the right revert to 0%.

Examples:

- If you modify the value in the rightmost cell, then %A is adjusted to reflect 100% minus the newly entered value, adjusting the remaining values to 0%

Flow	A	B	C	D
Start	50%	30%	8%	12%
You modify solvent D to 10%. Empower updates solvents B and C:				
	90%	0%	0%	10%

- If you modify a value in a cell other than the rightmost cell, and the sum of the adjusted cell and the cells to its left (if any) do not exceed 100, then the cell immediately to the right of the modified cell is adjusted to reflect 100% minus the sum of its neighbors to the left, and the remaining cells are set to 0%.

Flow	A	B	C	D
Start	50%	50%	0%	0%
You modify solvent B to 30%. Empower updates solvents C and D:	50%	30%	20%	0%
You modify solvent C to 10%. Empower updates solvent D:	50%	30%	10%	10%
You modify solvent B to 25%. Empower updates solvents C and D:	50%	25%	25%	0%

- If you modify a cell other than the rightmost cell, and the sum of the modified cell and the cells to its left (if any) exceed 100%, then the value of A adjusts to equal the sum of 100%.

Flow	A	B	C	D
Start	50%	50%	0%	0%
You change solvent B to 80%. Empower updates solvent A:	20%	80%	0%	0%
You change solvent B to 70%. Empower updates solvent C:	20%	70%	10%	0%
You change solvent C to 90%. Empower updates solvents A and B:	10%	0%	90%	0%

Setting PDA instrument method parameters

When creating a PDA instrument method that specifies long run times, consider adopting the following settings on the General tab, to reduce data storage requirements.

- Spectral resolution – Use only the minimum Resolution necessary to identify the spectral features of interest. If spectral features are sharply defined, use a small Resolution value. If spectral features are broadly defined, use a larger Resolution value. The default value is 1.2.
- Wavelength range – Avoid wavelength areas where the solvent strongly absorbs, or where compounds of interest do not absorb.

Tip: You must set the wavelength range to a value greater than the resolution value to collect data.

- Sampling rate – To accurately perform peak purity, acquire at least 12 spectra across the peak. Be aware that acquiring more than 30 spectra per peak does not improve peak purity accuracy and may degrade signal-to-noise ratios. For 2D data, acquire at least 15 points across the narrowest peak of interest.

Determining the correct sampling rate

Empower calculates a Points Across Peak value for each integrated peak in the chromatogram. Empower 2 and Empower 3 calculate the value differently than does Empower software (build 1154) or Millennium³² software. The calculation Empower 2 and Empower 3 use is the index of the data point closest to the end time minus the index of the data point closest to the start time. This is an improved calculation because it is accurate for peaks that have non-uniform data rates.

Tip: The Points Across Peak value appears in the Peaks table, at the bottom of the Main window of Empower's Review component. If the Points Across Peak field is not visible, right-click inside the table, and then click Table Properties. Click the Columns tab, and then scroll down to find the Points Across Peak field. Clear the check box, and then click OK.

If the Points Across Peak value for the narrowest peak of interest is less than 15, specify a higher sampling rate in the *instrument method*. If the Points Across Peak value for the narrowest peak of interest is greater than 30, specify a lower sampling rate in the *instrument method*.

Set the sampling rate to the lowest value required to achieve 15 or more points across the narrowest peak. Excessively high sampling rates can overwhelm the system, providing more data than what is necessary for your analysis.

To modify the sampling rate:

1. From the View Instrument Method window, click the icon for your detector.
2. Click the Channel 1 tab, and modify the sampling rate.
3. Click File > Save.

Tip: If the calculated optimum sampling rate is not available in the list for your detector, use the next largest value.

4. Reacquire the sample using the modified *instrument method*.
5. Optimize integration based on the narrowest peak listed in the 2D Channels table.

Configuring a method to automatically extinguish the detector lamp

You can extinguish the detector lamp at a specific time after the last injection. Doing so can help extend lamp life and reduce flow-cell maintenance.

To program a lamp-off event:

In the Events table of the *instrument method*, specify when, during a run, to extinguish the detector lamp.

Recommendation: If you plan to use the detector within four hours of its last use, do not extinguish the lamp, as no benefit results from doing so.

After completing runs using multi-solvent reservoir pumps, switch to a non-buffered solvent. Ensure that the new solvent is miscible with the previous solvent.

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- » [Setting user access to methods](#)
- » [Modifying method sets](#)
- » [Grouping methods in a set to analyze data](#)