Clarity Extensions

GPC Extension

Extensions **ENG**

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Phone: +420 251 013 400
Fax: +420 251 013 401
clarity@dataapex.com
www.dataapex.com

DataApex Ltd.
Petrzilkova 2583/13
158 00 Prague 5
The Czech Republic

Author: DM

Contents

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

Instrument (blue text) marks the name of the window, to which the text refers.

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 (when you already are in the topic describing the window).

WORK1 (capitals) indicates the name of the file and/or directory.

ACTIVE (capital italics) marks the state of the station or its part.

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

Note: Notifies the reader of possibly interesting information.

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

Marks the problem statement or trouble question.

Description: Presents any closer information on the problem, describes its causes etc.

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

1 GPC - Gel Permeation Chromatography

The **Clarity GPC** is an optional **Extension** for the **Clarity Chromatography Station** (from version 2.3). The **Clarity Chromatography Station** can acquire data from any **GPC** system with standard analog output. Any **Clarity** Instrument can use *GPC mode*.
GPC Extension provides interactive and automated **GPC** analysis including recalibration and **GPC** reporting, thus simplifying the retrieval of **GPC** data. It also allows flow rate and multi-detector delay corrections and includes *Narrow*, *Broad* and *Broad on Narrow* calibrations.
GPC Extension is also compatible with **Clarity Offline** software.

2 Specification

The **GPC 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 A28).

3 Installation

The **GPC Extension** is enabled by appropriate user code entered during installation or later by using the *Help - User Code* command from the Clarity main window.

To switch an Instrument to *GPC mode*, choose the **GPC** or **GPC-PDA** option in the *Instrument Type* field in the System Configuration dialog. The given Instrument may be later switched between the *GPC mode* and *standard chromatography mode* by using the *Setting - GPC mode* menu command in the Instrument window.

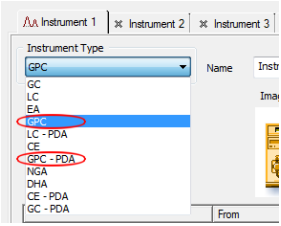


Fig 1: Switching to GPC mode

4 Key Features

The **GPC Extension** brings the following features to the **Clarity** station:

- **Evaluation** - the same chromatograms can be evaluated both in standard and **GPC** mode.
- **GPC Integration** - separate integration tables for **GPC** and standard evaluation are used. There are extensive possibilities for modifying chromatograms. The chromatogram can be changed by entering global parameters or interactively, through the direct graphical modification of the baseline.
- **Overlay** - simultaneous display of a virtually unlimited number of chromatograms. Molecular weight and cumulative molecular weight distributions can be easily compared.
- **GPC Calibration** - *Narrow* or *Broad* standard calibration methods (for more details see the chapter **Basic principles and terms** on pg **5**). combined with flow rate correction and *Universal* calibration. *Multiple broad* standards can be used.
- **GPC Results Table** - display of molecular weight averages together with peak details for active signal. Multiple peaks can be evaluated from one chromatogram.
- **GPC Summary result tables** - display and print of selected results from all simultaneously displayed chromatograms.

The following standard **Clarity** features will also help the user to make the best of the **GPC** analysis in **Clarity**:

- **Measuring** - simultaneous data acquisition from up to four sixteen-detector chromatographs (4×16 configuration).
- **User calculations** - the user can define custom calculations in the **Result Table** and **Summary Table**. With the use of the integrated editor you can create your own columns from the original ones and use individual mathematical functions.
- **Reports** - user selectable report sections, WYSIWYG formatting of Graphs and Tables.
- **Batch** - automatic batch processing, display, export or print of any number of chromatograms.
- **Automated measurement support** - sequence tables for any set of samples with or without an autosampler.
- **Postrun** - automatic display, print, export and start of other programs after completing the measurement.
- **Fraction Collectors** - **GPC** offers support for fraction collectors controlled by **Collect**/**Waste** and **Next** signals.

4.1 Basic principles and terms

- **Gel Permeation Chromatography** (**GPC**) or **Size Exclusion Chromatography** (**SEC**) is a specialized chromatography technique for determination of molecular weight distribution in polymers. Sample of polymer solution, containing mixture of molecules differing in size, is separated on column. Under conditions used for this chromatography mode, the molecules are separated according to size, the larger molecules emerging first and the smaller later. With appropriate polymer standards with known molecular weights, calibration curve describing the dependence between the molecular weight and elution volume can be constructed.
- **Narrow standard** calibration is the most commonly used, when polymer standards with narrow molecular weight distribution are available. Calibration curve is constructed from detected peak maximum retention times and known *Mp* values. Alternatively, *(Mw.Mn)^1/2* values can be used, if *Mp* values are not declared.
- **Broad standard** calibration is used, when only polymer standards with broad molecular weight distribution are available. **Clarity GPC Extension** uses three types of **Broad standard** calibration:
    - **Broad linear** - assuming linear calibration curve for broad peak concerned. Linear calibration equation is calculated from the standard peak elution profile and declared *Mn* and *Mw* values. Multiple Broad Standards can be used to construct a resulting calibration curve.
    - **Broad integral** - a calibration curve is constructed from the standard peak elution profile and a table of declared cumulative molecular weight distribution values. Multiple Broad Standards can be used to construct a calibration curve.
    - **Broad on narrow** - appropriate *K* and *Alpha* values are sought for polymer standard with declared *Mn* and *Mw* values, to fit the standard peak elution profile to existing narrow standard calibration curve.
- **Flow rate correction** - the elution volumes are calculated from retention time and flow rate. Small variations in flow rate have tremendous effect on the correctness of calculated molecular weights. A low molecular weight compound (flow rate marker) can be added to the standards and samples, the retention times in different chromatograms can than be normalized to common base. Flow rate correction can be used with every calibration type.
- **Universal calibration** - the separation of polymer molecules on column is governed by molecular size, not molecular weight. The polymer molecule size is besides its weight dependent on the molecule structure (linear, branched, starlike) and conformation (given by solvent and temperature). A dependence (Mark-Houwink equation) can be used to calculate

molecular weights for molecules of the same size, provided the constants *K* and *Alpha* of the Mark-Houwink equation are known for both polymers. Universal calibration can be used with every calibration type, except **Broad on narrow**.

5 GPC Extension description

After installation, new functions of the **GPC Extension** will be available. Only features changed or added to the **Clarity** standard mode are listed and described here.

5.1 Instrument window

In the Instrument window the new *Setting - GPC Mode* menu command enables the switching between **Standard** and **GPC Mode** on the selected Instrument. The **GPC Mode** is indicated by the "GPC:" inscription preceding the Instrument and Method names in the Instrument window title bar.

Note: When opening an Instrument set as the **GPC** in the System Configuration dialog, it is opened in the **GPC Mode**.

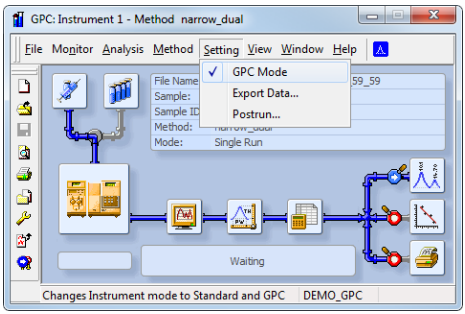


Fig 2: GPC Instrument window

5.2 Single Analysis dialog

The new fields named *K* and *Alpha* and a new *Load K & Alpha* button emerge in the Single Analysis dialog. The *K* and *Alpha* fields enable to enter the Mark-Houwink parameters used for universal calibrations. The default values *14.1* and *0.7* are valid for linear polystyrene in tetrahydrofurane at 25 °C. The *Load K & Alpha* button enables entering those values from user editable table in the K & Alpha dialog.
If you import chromatograms using the *File - Import Chromatogram…* menu in the Chromatogram window, values entered in the *K* and *Alpha* fields will be saved in the imported chromatograms.

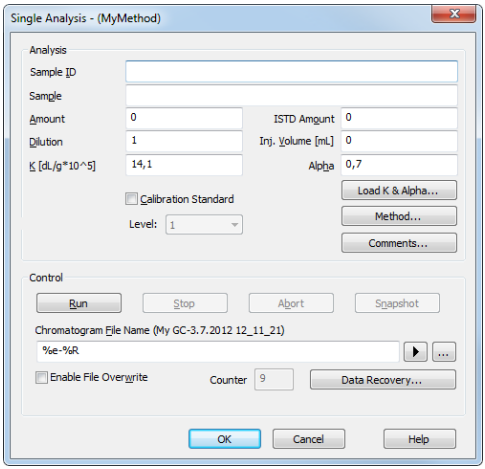
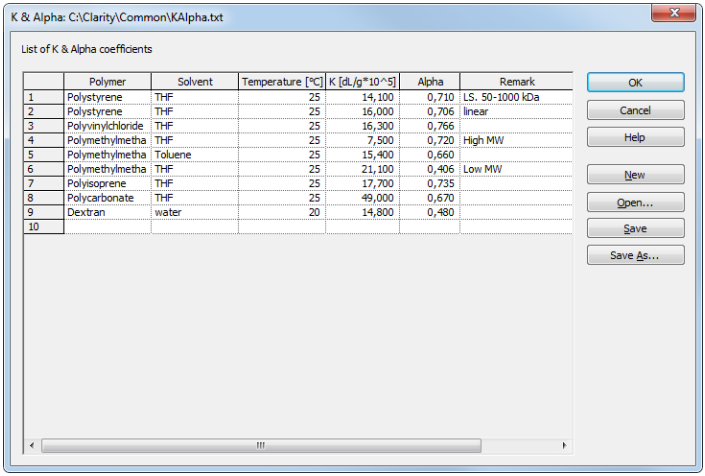


Fig 3: Single Analysis

5.2.1 K & Alpha

The K & Alpha dialog can be opened using the *Load K & Alpha* button in the Single Analysis dialog or using the *Sequence - Select K Alpha...* command (icon) from the Sequence window when the given **Clarity** Instrument is switched to the **GPC mode**. To load the values into the Single Analysis dialog or Sequence window, click the row number besides the compound whose parameters you want to load and then use the *OK* button.



	Polymer	Solvent	Temperature [°C]	K [dL/g*10^5]	Alpha	Remark
1	Polystyrene	THF	25	14,100	0,710	LS. 50-1000 kDa
2	Polystyrene	THF	25	16,000	0,706	linear
3	Polyvinylchloride	THF	25	16,300	0,766	
4	Polymethylmetha	THF	25	7,500	0,720	High MW
5	Polymethylmetha	Toluene	25	15,400	0,660	
6	Polymethylmetha	THF	25	21,100	0,406	Low MW
7	Polyisoprene	THF	25	17,700	0,735	
8	Polycarbonate	THF	25	49,000	0,670	
9	Dextran	water	20	14,800	0,480	
10						

Fig 4: K & Alpha

5.3 Sequence window

In the Sequence window in the **GPC mode** new columns for entering *K* and *Alpha* values are available. The values can also be entered from editable table using the *Select K Alpha...* command (icon) available in local or *Sequence* menu. *GPC Std Type* and *GPC Std No* columns serve for automatic recalibration of **GPC** calibration during sequence runs. The columns that should be displayed or hidden can be, same as in the standard Sequence window, set by using the *Hide Column(s)* and *Show Hidden Column(s)* commands from local menu (right mouse click in table area).

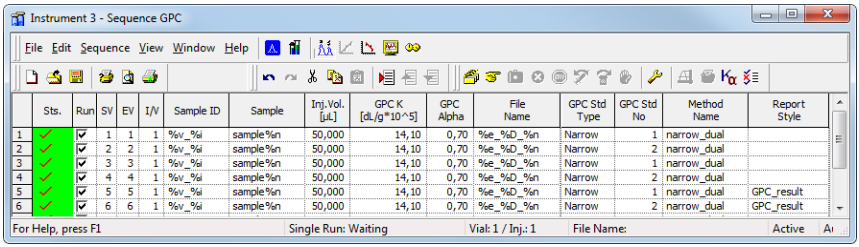


Fig 5: Sequence window

Note: Only relevant parts of report for the actual mode selected (**Standard** or **GPC**) will be printed from the Report Style selected in Sequence.

5.4 Method Setup dialog

New GPC Integration, GPC Calculation, GPC Advanced and GPC Ranges tabs appear in the Method Setup dialog. Different parameters for **Standard** and **GPC** mode can be set for automatic chromatogram integration and calculations after acquisition. However, the evaluation and display of results for measured chromatograms is possible only according to the mode (**GPC** or **Standard**) selected.

Caution: Performing *Reprocess by Instrument Method* or *Complete Reprocessing* in the Batch dialog will always reprocess both the **Standard** and **GPC** part of the method.

5.4.1 GPC Integration

The Method Setup - GPC Integration dialog is similar to the Method Setup - Integration dialog, with the exception of three differences:

- The *Solvent Peak* operation excludes selected integrated peaks from the evaluation. This is useful for low molecular weight impurities, which need to be integrated to get the correct baseline, however their results are not desired. **GPC** mode identifies the solvent peaks differently than the **Standard** mode - peaks are marked as solvent if they are found in the selected intervals.
- The *Flow Marker Peak* designates a peak to be used for flow rate corrected calculations. Only one peak in the whole chromatogram can be used as a flow marker, the last occurrence of this operation in the **Integration Table** will be used.

Note: The *Use Flow Rate Correction* check box must be checked in the used calibration in addition to the selected *Flow Marker* , otherwise the correction will not be performed.

- The *Group* operation is not available in **GPC** mode. To get averaged values for multimodal MW distribution polymer, integrate the peaks as a single one using the *Baseline Lock* and *Add Positive* functions.

  It is possible to set different settings in both dialogs to have the chromatogram evaluated both in the **Standard** mode and in the **GPC** mode.

5.4.2 GPC Calculation

The Method Setup - GPC Calculation dialog is analogical to the Method Setup - Calculation dialog. The *Parameters* and *Report in Result Table* sections are disabled in GPC Calculation dialog.
It is possible to set different settings in both dialogs to have the chromatogram evaluated as both **Standard** mode and in **GPC** mode.

5.4.3 GPC Advanced

The Method Setup - GPC Advanced dialog is identical to the Method Setup - Advanced dialog.
It is possible to set different settings in both dialogs to have the chromatogram evaluated as both **Standard** mode and in **GPC** mode.

5.4.4 GPC Ranges

GPC Ranges tab serves for the calculation of peak area percentage for defined ranges of molecular weights or average molecular weight for selected area percentage ranges. The type of the ranges (either *Percent* or *MW*) can be selected using the *Ranges Type* field. The column headers in the table then correspond to the *Ranges Type* selected.

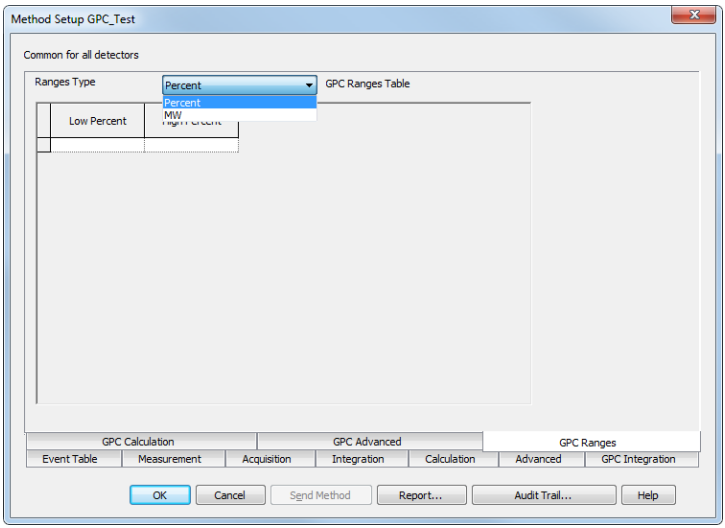


Fig 6: Method Setup - GPC Ranges

Low Percent
Sets the fraction start in % of the peak area from the peak start. Displayed when *Percent Ranges Type* is set.

High Percent

Sets the fraction end in % of the peak area from the peak start. Displayed when *Percent Ranges Type* is set.

High MW

Sets the MW range start for the given fraction. Displayed when *MW Ranges Type* is set.

Low MW

Sets the MW range end for the given fraction. Displayed when *MW Ranges Type* is set.

Note: For example, for the last 10% of the peak area enter set the *Ranges Type* to *Percent* and the values in the range *90* to *100*.

Multiple ranges of the same type can be set. The resulting average molar weight is calculated by the following algorithm:

$$MW = \frac{\Sigma(response \times M^2)}{\Sigma(response \times M)}$$

5.5 Chromatogram window

The Chromatogram window is the central window (opened by clicking the icon) for displaying, modifying and evaluating chromatograms. In the *Graph* pane, alternatively a Chromatogram , MW Distribution or Cumulative MW Distribution tab can be displayed using the appropriate tabs.

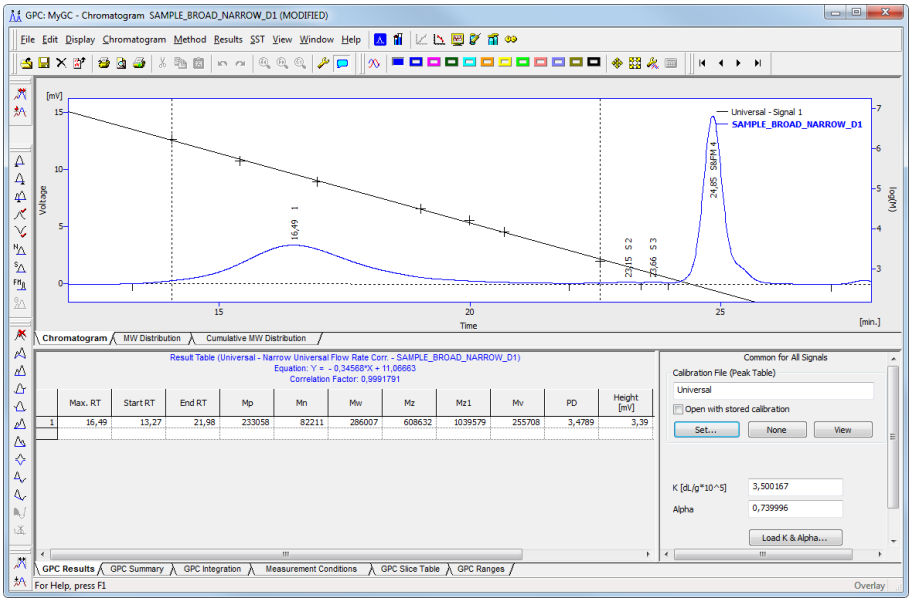


Fig 7: Chromatogram

In the *Tables* pane, any of the GPC Results, GPC Summary, GPC Integration, Measurement Conditions, GPC Slice Table and GPC Ranges tabs can be displayed using the appropriate tabs or commands from the *Method* and *Results* menus.

5.5.1 Chromatogram

The chromatogram or chromatograms are displayed in the *Graph* pane of the Chromatogram window on the Chromatogram tab. The window properties including the *Overlay* and *Graph Properties* are essentially the same as in **Standard** mode and are described in the **Clarity User** and **Clarity Reference Guides**. The *Peak Name* and *Group* peak tags are not available in **GPC** mode.

The GPC calibration curve overlaid above the chromatogram curve in the Chromatogram tab for active signal or its points can be switched off in the Graph Properties - GPC Options dialog (available from local menu). The

Log M axis of the curve is fixed regardless of the zoom in the Chromatogram window.

5.5.1.1 Graph Properties - GPC Options

The GPC Options tab of the Graph Properties dialog is used to customize the appearance of the curves displayed on the Chromatogram tab of the Chromatogram window.

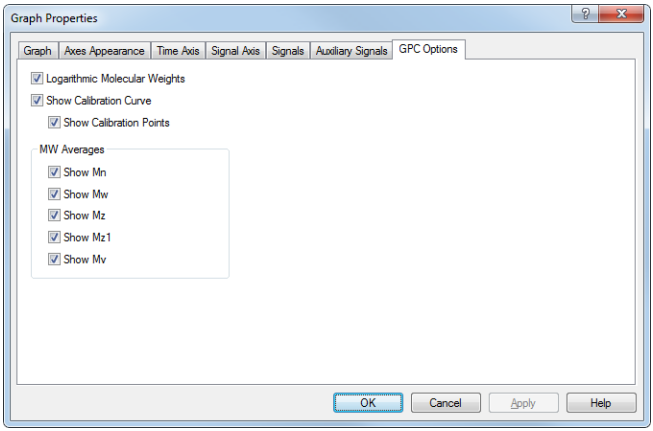


Fig 8: Graph Properties - GPC Options

Logarithmic Molecular Weights

Sets the format of the molecular weight axis (right Y-axis) showing the units for the overlayed GPC calibration curve. If checked, shows the units as the logarithm of the MW, while unchecked displays the non-linear axis in MW.

Show Calibration Curve

Switches the display of the **GPC calibration curve** overlayed over the signal graph on the Chromatogram tab of the Chromatogram window on and off.

Show Calibration Points

Switches the display of the calibration points for the **GPC calibration curve** overlayed over the signal graph on the Chromatogram tab of the Chromatogram window on and off.

MW Averages

Switches the display of the particular points in the active signal graph displayed on the Chromatogram tab of the Chromatogram window. More information on these points can be get in the chapter **"GPC Results"** on pg **18**.

5.5.2 MW Distribution

The MW Distribution tab in the *Graph* pane of the Chromatogram window shows the molecular weight distribution of the active peaks from the (possibly overlaid) signals or chromatograms. In signals with multiple evaluated peaks, the peak is set active by clicking on its row in the **Result Table** for the respective signal. The retention time of the active peak is indicated at the end of its legend in the *Graph* pane.

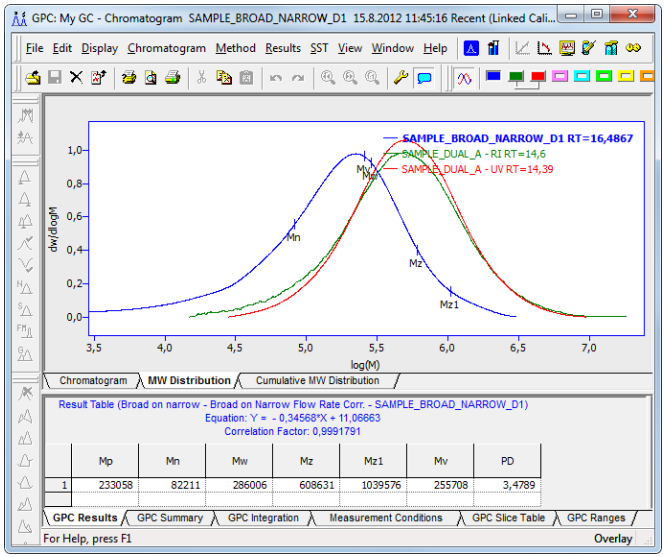


Fig 9: Chromatogram - MW Distribution

5.5.3 Cumulative MW Distribution

The Cumulative MW Distribution tab in the *Graph* pane of the Chromatogram window shows the cumulative molecular weight distribution (in %) of the active peaks from the (possibly overlaid) signals or chromatograms. In signals with multiple evaluated peaks, the peak is set active by clicking on its row in the **Result Table** for the respective signal.

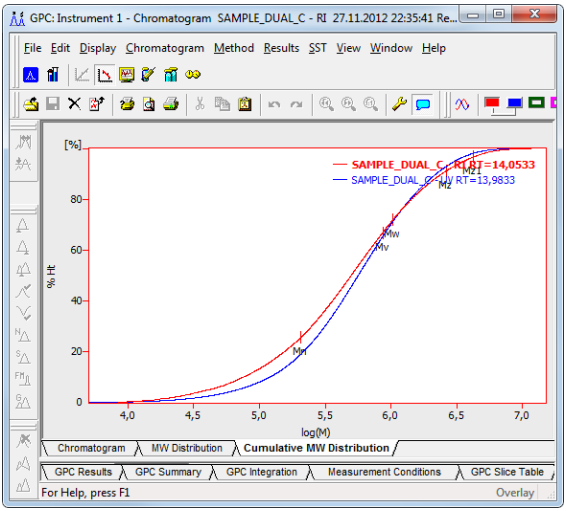


Fig 10: Chromatogram - Cumulative MW Distribution

Note: By integrating multiple peaks as single one it is possible to get a single molecular weight distribution curve for multi-modal polymer samples.

5.5.4 GPC Results

The results for the active chromatogram (signal) can be found in the **Result Table** on the GPC Results tab.
On the right side of the tab the used calibration file and Mark-Houwink parameters can be set or changed.
The following columns can be displayed in tables:

Tab 1: List of columns available in the GPC extension

Column name	Description
Max. RT	Retention time of peak maximum
Start RT	Retention time of peak integration start
End RT	Retention time of peak integration end
Mp	Molecular Weight at peak maximum
Mn	Molecular Weight number average
Mw	Molecular Weight weight average
Mz	Molecular Weight Z average
Mz1	Molecular Weight Z+1 average
Mv	Molecular Weight viscosity average
PD	Polydispersity (Mw/Mn)
Flow Rate Correction	Flow rate correction factor
Height [mV]	Peak height
% Height	Percentage of total height of evaluated peaks
Area [mV.s]	Peak area
% Area	Percentage of total area of evaluated peaks

Additional columns can be displayed or calculated using the *Setup Columns...* and *User Columns - Add...* commands from the local menu.

5.5.5 GPC Summary

Using the GPC Summary tab, a **Summary Result Table** for all displayed signals is accessible. Its appearance can be customized using the *Setup Columns...* and *Summary Options...* commands from the local menu.

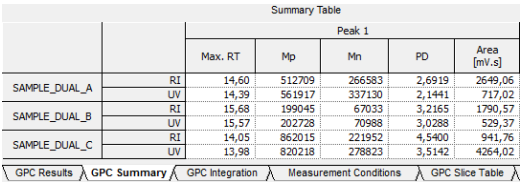


Fig 11: GPC Summary

5.5.6 GPC Integration

In the **Integration Table** on the GPC Integration tab all operations used for the **GPC** peak integration are displayed and editable. The operations can be entered directly into the table or interactively effected on the chromatogram in the *Graph* pane using the *Peak*, *Baseline* and *Integration* toolbars.

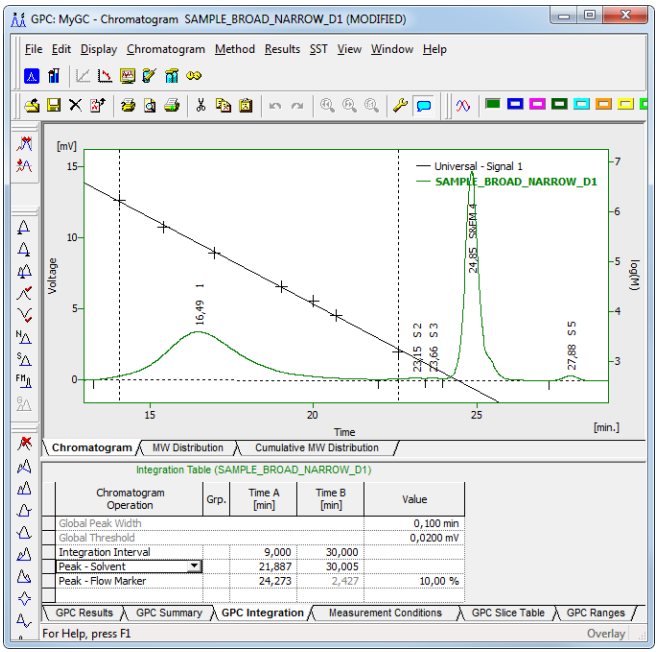


Fig 12: Chromatogram - GPC Integration

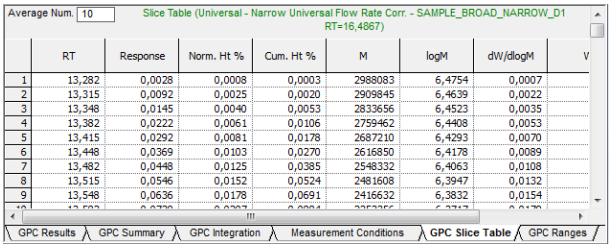
Most operations are common for **Standard** and **GPC** mode and are described in the **Clarity User** and **Clarity Reference Guides**. See See "GPC Integration" on page 11 for the list of differences.

5.5.7 Measurement Conditions

The Measurement Conditions tab is common both for **Standard** and **GPC** mode.

5.5.8 GPC Slice Table

The GPC Slice Table tab displays the **Cumulative Molecular Weight Distribution Table** for the active peak. In signals with multiple evaluated peaks, the peak is set active by clicking on its row in the **Result Table** on the GPC Results tab for the respective signal. The table appearance can be modified using the *Setup Columns...* command from the local menu.


Average Num. 10 Slice Table (Universal - Narrow Universal Flow Rate Corr. - SAMPLE_BROAD_NARROW_D1 RT=16,4867)

	RT	Response	Norm. Ht %	Cum. Ht %	M	logM	dW/dlogM	V
1	13,282	0,0028	0,0008	0,0003	2988083	6,4754	0,0007	
2	13,315	0,0092	0,0025	0,0020	2909845	6,4639	0,0022	
3	13,348	0,0145	0,0040	0,0053	2833656	6,4523	0,0035	
4	13,382	0,0222	0,0061	0,0106	2759462	6,4408	0,0053	
5	13,415	0,0292	0,0081	0,0178	2687210	6,4293	0,0070	
6	13,448	0,0369	0,0103	0,0270	2616850	6,4178	0,0089	
7	13,482	0,0448	0,0125	0,0385	2548332	6,4063	0,0108	
8	13,515	0,0546	0,0152	0,0524	2481608	6,3947	0,0132	
9	13,548	0,0636	0,0178	0,0691	2416632	6,3832	0,0154	

GPC Results | GPC Summary | GPC Integration | Measurement Conditions | GPC Slice Table | GPC Ranges

Fig 13: Chromatogram - GPC Slice Table

The number of slices depends on the peak length and *Peak Width* value used in the **Integration Table** on the GPC Integration tab. The slices can be averaged by entering a number in the range <*1*, *100*> to the *Average Num.* field in the table title area. For the integrated peak, following values are available for the specified time intervals (slices):

RT
Retention time of the slice (averaged).

Response
Slice peak height (averaged).

Norm. Ht
Normalized slice height (summed).

Norm. Ht %
Percentage of the slice height from total of all slices height (summed to give total 100%).

Cum. Ht
Cumulative slice height (averaged).

Cum. Ht % Graph
Cumulative percentage of the slice height from the total of all slice heights in the inverse order (increasing with increasing M) (averaged).

Cum. Ht %
Cumulative percentage of the slice height from the total of all slices heights (averaged).

M
The molecular weight corresponding to the slice retention time (averaged).

Log M
The logarithm of the molecular weight corresponding to the slice retention time (averaged).

dW/dlog M
Normalized distribution of slice molecular weights used for the graph in the MW Distribution tab.

W
Normalized slice height used for molecular weight distribution calculation.

Outside Calib
Flag marking whether the slice is inside or outside of the used calibration retention time range. Outside of the range gives value *1*, while inside the range gives value *0*.

5.5.9 GPC Ranges

This tab presents the same parameters as on the Method Setup - GPC Ranges tab (for more details see also chapter **GPC Ranges** on pg **12**) along with the computed results. For *MW mode*, *Result Percent* field is calculated, while for *Percent mode*, *Result MW* is shown.

Result MW
Displays the average of MW values for the percent range set.

Result Percent
Displays the peak area percentage for the MW range set.

Note: The *GPC Ranges Table* settings are stored in the chromatogram. You can change them on the GPC Ranges tab for the current chromatogram. Alternatively you can change them in the template method and batch reprocess the respective chromatograms with this method.

Note: Multiple ranges of the same type can be set. For MW ranges not containing any peak points no value is calculated.

Note: For chromatograms with multiple peaks the table is shown for the active peak (as selected in the **Result Table** on the GPC Results tab). Its retention time is indicated in table header.

5.6 GPC Calibration window

The GPC Calibration window (opened by clicking the icon which replaces the icon when in the **GPC** mode) is used for creating, modifying and displaying calibration curves.

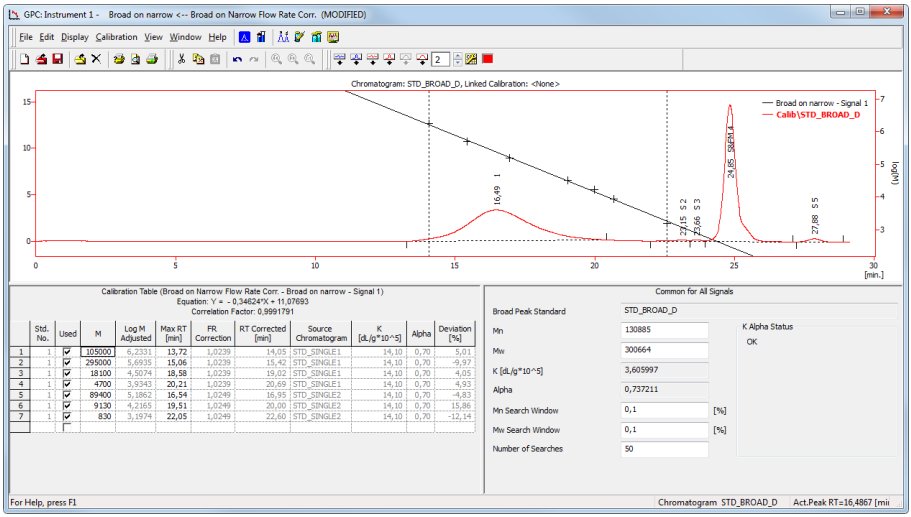


Fig 14: GPC Calibration

In the *Graph* pane of the GPC Calibration window the standard chromatogram with the calibration curve is displayed, same as in the standard Calibration window. Its appearance can be modified using the Graph Properties dialog accessible through the *Properties...* command from the local menu.

The **Calibration Summary Table** is displayed in the *Table* pane of the Calibration window. Its appearance depends on the selected *Calibration Type*.

5.6.1 GPC Calibration Options

When creating a new calibration, the GPC Calibration Options dialog enabling to set the new *Calibration Type* will be displayed first.

Caution: Be aware that the *Calibration Type* can not be changed later.

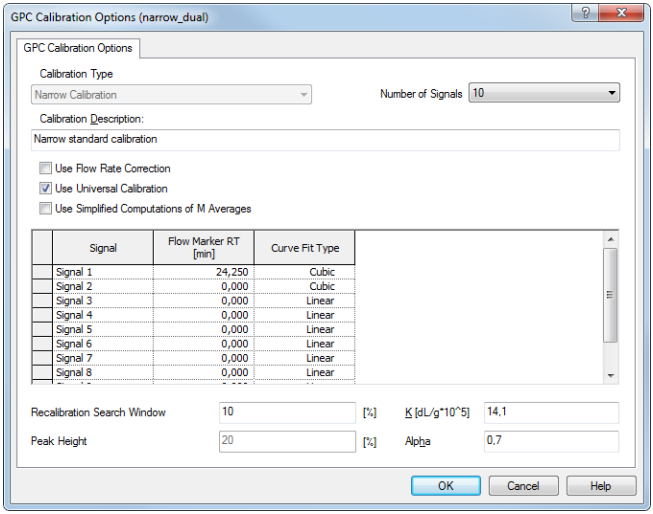


Fig 15: GPC Calibration Options

The following calibration types can be selected:

- *Narrow Calibration*
- *Multiple Broad Integral Calibration*
- *Multiple Broad Linear Fit Calibration*
- *Broad On Narrow Calibration*

The type of calibration used is indicated in the **Calibration Table** title line as well as in the chromatogram **Result Table** title line.

Use Flow Rate Correction

Using this checkbox will amend the calibration curve and calculations respectively, if the *Flow Marker Peak* was identified for standards and sample. The use is indicated in the **Calibration Table** title line as well as in the chromatogram **Result Table** title line by the **Flow Rate Corr.** inscription. In such case the calibration curve is constructed for each chromatogram separately.

Use Universal Calibration

This checkbox will amend the calibration curve and calculations according to the Mark-Houwink parameters entered for standards and sample. The use is indicated in the **Calibration Table** title line as well as

in the chromatogram **Result Table** title line by the inscription **Universal**. In such case the calibration curve is constructed for each chromatogram separately.

Note: The Alpha value is used for calculation of Mv (viscosity M), regardless of state of this checkbox (see formula below)

Use Simplified Computations of M Averages

Checking this checkbox will cause the M averages being computed in the simplified manner rather then the standard manner. The differences in the equations are shown below:

Note: The differences in the M average computation results using simplified or standard calculations will only be present with non-linear calibrations.

Simplified calculations of the M averages

$$M_n = \frac{\sum_{i=1}^{n} H_i}{\sum_{i=1}^{n} \frac{H_i}{M_i}}$$

$$M_w = \frac{\sum_{i=1}^{n} H_i * M_i}{\sum_{i=1}^{n} H_i}$$

$$M_z = \frac{\sum_{i=1}^{n} H_i * {M_i}^2}{\sum_{i=1}^{n} H_i * M_i}$$

$$M_{z1} = \frac{\sum_{i=1}^{n} H_i * {M_i}^3}{\sum_{i=1}^{n} H_i * {M_i}^2}$$

$$M_v = \sqrt[\alpha]{\frac{\sum_{i=1}^{n} H_i * {M_i}^{\alpha}}{\sum_{i=1}^{n} H_i}}$$

where *i* is the number of the peak slice, *n* total number of peak slices, H_i is the normalized height of the peak slice, M_i molecular weight of the peak slice based on the calibration curve and α is the Mark-Houwink constant.

Standard calculations of M averages

$$M_n = \frac{\sum_{i=1}^{n} H_i * D_i}{\sum_{i=1}^{n} \frac{H_i * D_i}{M_i}}$$

$$M_w = \frac{\sum_{i=1}^{n} H_i * M_i * D_i}{\sum_{i=1}^{n} H_i * D_i}$$

$$M_z = \frac{\sum_{i=1}^{n} H_i * {M_i}^2 * D_i}{\sum_{i=1}^{n} H_i * M_i * D_i}$$

$$M_{z1} = \frac{\sum_{i=1}^{n} H_i * {M_i}^3 * D_i}{\sum_{i=1}^{n} H_i * {M_i}^2 * D_i}$$

$$M_v = \sqrt[\alpha]{\frac{\sum_{i=1}^{n} H_i * {M_i}^{\alpha} * D_i}{\sum_{i=1}^{n} H_i * D_i}}$$

where *i* is the number of the peak slice, *n* total number of peak slices, H_i is the normalized height of the peak slice, M_i molecular weight of the peak slice based on the calibration curve, α is the Mark-Houwink constant and D_i stands for the derivation of the calibration's inverse function at the retention time of the peak slice (T_i):

$$D_i = \frac{\delta T_i}{\delta \log M_i}$$

Note: Checking the *Use Simplified Computations of M Averages* checkbox will also influence the calculation of the calibration curve for the *Multiple Broad Linear* and *Broad on Narrow* calculation methods as both of them use some of the M averages values for the calculation.

Number of Signals

The value in this field determines for how many signals the calibration curves are constructed and displayed. The value is increased automatically according to the number of signals in chromatograms used for calibration.

Flow Marker RT [min]

The fields in this column are filled with values from the first standard chromatogram and are used as a base for all subsequent standards and samples. The field can be edited and the value is then applied as a base for all chromatograms (including the first one).

Curve Fit Type

This column is used to set the curve fit type for the particular signal. The *Curve Fit Type* can be selected from polynomial regression with **n** value between *1* and *5*.

Recalibration Search Window

Sets the maximum deviation (in %) of the *Standard Peak* retention time from the stored value to perform the recalibration.

Peak Height

The value (set in %) determines the position of points used for the construction of calibration curve in case multiple broad standards with Hamielec linear fit are used.

K

This field sets one of the Mark-Houwink equation parameters used for universal calibration. Default values are used to display the calibration curve if no chromatogram is opened (otherwise the *K* and *Alpha* from the actual chromatogram are used).

Alpha

This field sets one of the Mark-Houwink equation parameters used for universal calibration. Default values are used to display the calibration curve if no chromatogram is opened (otherwise the *K* and *Alpha* from the actual chromatogram are used).

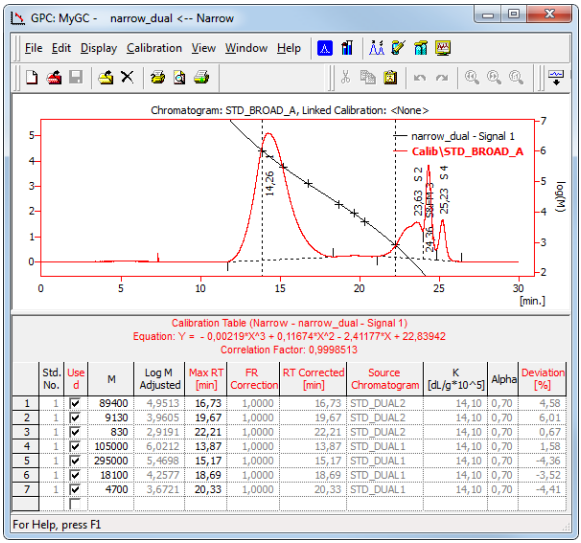
Caution: Change in this value will have effect only after the recalibrating by the *Set Broad Peak* command.

Note: Some fields may be unavailable or not editable depending on the calibration type selected.

Note: The *K* and *Alpha* fields can be edited and then the calibration curve in the Calibration window will be displayed according to the modified values (independently to the display in the Chromatogram window).

5.6.2 Narrow Calibration

Narrow calibration can be used if narrow molecular weight distribution standards (polydispersity < 1.2) are available for given polymer. Calibration curve is constructed from detected peak maximum retention times and known **Mp** values. Alternatively, **(Mw.Mn)^1/2** values can be used, if **Mp** values are not declared.



	Std. No.	Used	M	Log M Adjusted	Max RT [min]	FR Correction	RT Corrected [min]	Source Chromatogram	K [dL/g*10^5]	Alpha	Deviation [%]
1	1	☑	89400	4,9513	16,73	1,0000	16,73	STD_DUAL2	14,10	0,70	4,58
2	1	☑	9130	3,9605	19,67	1,0000	19,67	STD_DUAL2	14,10	0,70	6,01
3	1	☑	830	2,9191	22,21	1,0000	22,21	STD_DUAL2	14,10	0,70	0,67
4	1	☑	105000	6,0212	13,87	1,0000	13,87	STD_DUAL1	14,10	0,70	1,58
5	1	☑	295000	5,4698	15,17	1,0000	15,17	STD_DUAL1	14,10	0,70	-4,36
6	1	☑	18100	4,2577	18,69	1,0000	18,69	STD_DUAL1	14,10	0,70	-3,52
7	1	☑	4700	3,6721	20,33	1,0000	20,33	STD_DUAL1	14,10	0,70	-4,41

Fig 16: GPC Calibration (Narrow)

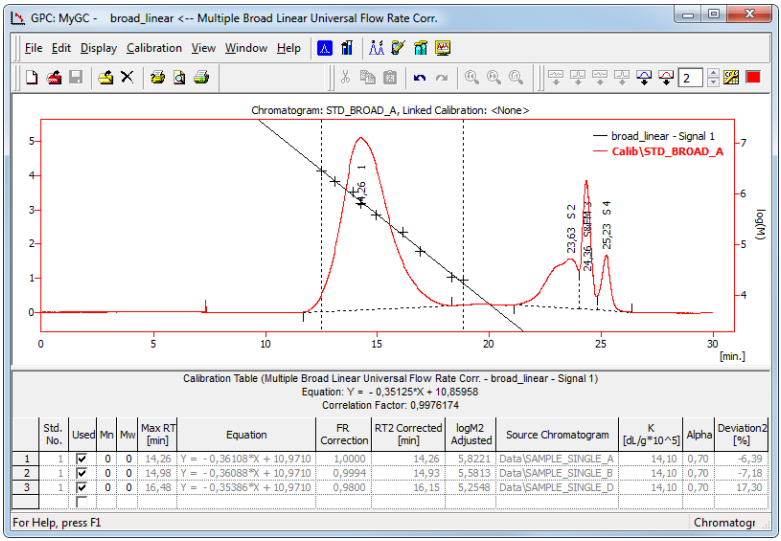
After selecting the calibration type, a standard chromatogram can be opened. The *Add All Narrow Peaks* icon will transfer all integrated peaks data to the **Calibration Summary Table**, setting a new standard number for each standard chromatogram opened and creating a new line for each peak in the standard. The *Add Narrow Peak* icon will perform this operation peak by peak.

For multi-signal standards the *Set RT To All Narrow Peaks* icon is used for transferring the *RT* on subsequent signals to calibration for peaks where *RT* is not used. Operation is performed only for peaks with *Std. No.* value equal to current *Std. No.* indicated on the **Window** toolbar of the Calibration window. This command can also be used for recalibration by selected standard. In this case *RT* of standard peak must match *Std. No.* values as described and also match *Recalibration Search Window* of peak being recalibrated (for more details see the chapter **GPC Calibration Options** on pg **23**). The *Set RT To Narrow Peak* icon performs this operation peak by peak.

5.6.3 Multiple Broad Linear Calibration

This method utilizes **Broad Standards** with solely known *Mn* and *Mw* values. The Hamielec method assumes that the calibration can be approximated by straight line in the area of **Broad Peak** being calibrated. The software uses numeric Newton method for calculating *A* and *B* parameters of linear calibration curve of the given peak. Accuracy of *Mn* and *Mw* values calculated is about 1 e-5 in comparison to *Mn* and *Mw* values documented for the standard. Multiple Broad Peaks can be used subsequently to extend the area of calibration reliability.

Three points for each peak are used to construct a resulting calibration curve. One point is peak *Max RT*, the others are computed by *Peak Height* value entered in the GPC Calibration Options dialog.



	Std. No.	Used	Mn	Mw	Max RT [min]	Equation	FR Correction	RT2 Corrected [min]	logM2 Adjusted	Source Chromatogram	K [dL/g*10^5]	Alpha	Deviation2 [%]
1	1	☑	0	0	14,26	Y = - 0,36108*X + 10,9710	1,0000	14,26	5,8221	Data\SAMPLE_SINGLE_A	14,10	0,70	-6,39
2	1	☑	0	0	14,98	Y = - 0,36088*X + 10,9710	0,9994	14,93	5,5813	Data\SAMPLE_SINGLE_B	14,10	0,70	-7,18
3	1	☑	0	0	16,48	Y = - 0,35386*X + 10,9710	0,9800	16,15	5,2548	Data\SAMPLE_SINGLE_D	14,10	0,70	17,30

Fig 17: GPC Calibration (Broad Linear)

After selecting the calibration type, a standard chromatogram can be opened. The *Add Broad Peak* icon will transfer the integrated peak data to the **Calibration Summary Table**, setting a new standard number and creating a new line for each peak.

For multi-signal standards the *Set Broad Peak* icon is used for transferring the peak data on subsequent signals to calibration. Operation is performed only for peaks with *Std. No.* value equal to current *Std. No.* indicated on the **Window** toolbar of the Calibration window.

Note: The last **Broad Peak** is temporarily remembered and used in subsequent recalculations. Current **Broad Peak** is indicated in right lower corner of the Calibration window.

5.6.4 Multiple Broad Integral calibration

The standard to be used will have a table detailing the **% Cumulative Weight Fraction** (*Percent [%]* in **Clarity**) and **Molecular Weight** (*M*) values supplied by the manufacturer of the standard. Multiple Broad Peaks can be used subsequently to extend the area of calibration reliability.

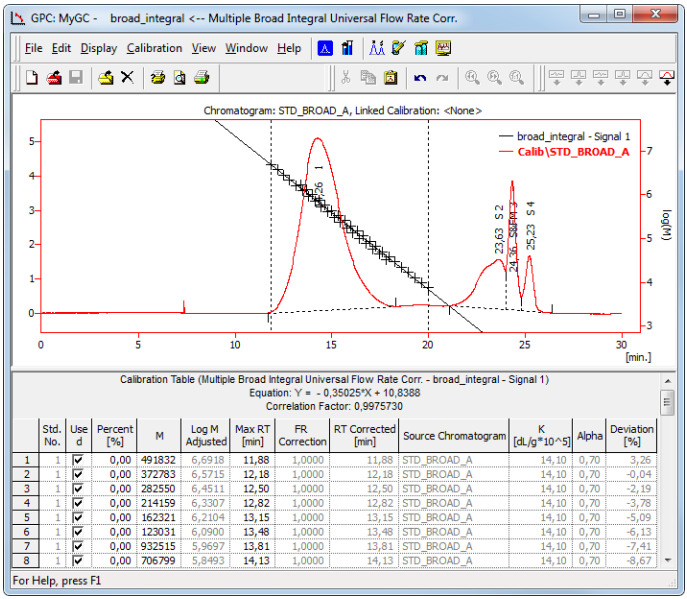


Fig 18: GPC Calibration (Broad Integral)

After selecting the calibration type the *Percent [%]* and *M* parameters must be entered manually. Standard chromatogram should be opened next. The *Set Broad Peak* icon will recalculate the *RT* values according to the data entered into the **% Cumulative Weight Fraction** field using the peak being calibrated. Operation is performed only for rows with *Std. No.* value equal to current *Std. No.* indicated in the **Window** toolbar of the Calibration window.

The **% Cumulative Weight Fraction** and **Molecular Weight** values are common for all signals. If this type of calibration is to be used for multi signal chromatograms, the *Detector Delay* function should be used to put the signals on a common time base.

Note: The last **Broad Peak** is temporarily remembered and used in subsequent recalculations. Current **Broad Peak** is indicated in right lower corner of the Calibration window.

5.6.5 Broad on Narrow calibration

This method first requires the calibration of the column with a series of **Narrow Standards** and then a calibration with a **Broad Standard** with known *Mn* and *Mw*. It relies on a principle that **Narrow Standards** are used to characterize the shape of the calibration curve, while the **Broad Standard** is then run to compute suitable *K* and *Alpha* using numeric Newton method. Accuracy of *Mn* and *Mw* values calculated is about 1 e-5 compared to *Mn* and *Mw* values documented for the standard.

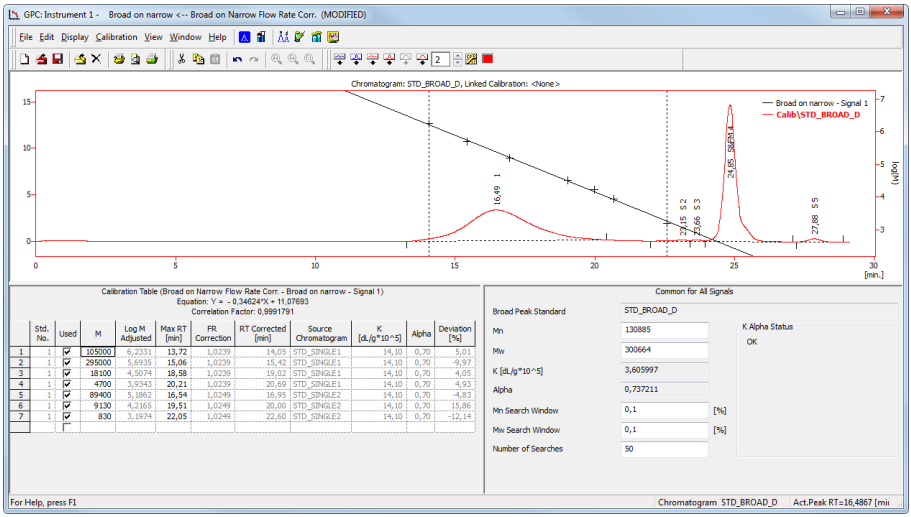


Fig 19: GPC Calibration (Broad on Narrow)

After selecting the calibration type, a narrow standard chromatogram can be opened. The *Add All Narrow Peaks* icon will transfer all integrated peaks data to the **Calibration Summary Table**, setting a new standard number for each standard chromatogram opened and creating a new line for each peak in the standard. The *Add Narrow Peak* icon will perform this operation peak by peak.

For multi-signal standards the *Set RT To All Narrow Peaks* icon is used for transferring the *RT* on subsequent signals to calibration for peaks where *RT* is not used. Operation is performed only for peaks with *Std. No.* value equal to current *Std. No.* indicated on the **Window** toolbar of the Calibration window. This command can also be used for recalibration by selected standard. In this case RT of standard peak must match *Std. No.* values as described and also match *Recalibration Search Window* of peak being recalibrated (for more details see the chapter **GPC Calibration Options** on pg **23**). The *Set RT To Narrow Peak* icon performs this operation peak by peak.

Broad Standard chromatogram can be opened next and *Mn* and *Mw* values should be entered. The *Set Broad Peak*  icon will compute desired *K* and *Alpha* values and display them in appropriate input boxes. The *K Alpha Status* are informs if *K* and *Alpha* values are recalculated properly after last operation in the GPC Calibration. If the Broad Peak was set and the recalculation was performed without an error, the status is marked as *OK*. Otherwise a description is displayed in case the *Broad Peak* was not set or the computation of narrow calibration failed.

5.6.6 GPC Calibration Audit Trail

As the GPC Calibration window is not the same as the normal Calibration window, the events that should be saved into the audit trail log might also differ. On the **Clarity** instrument marked as GPC instrument, a new tab in the Audit Trail Settings dialog appears:

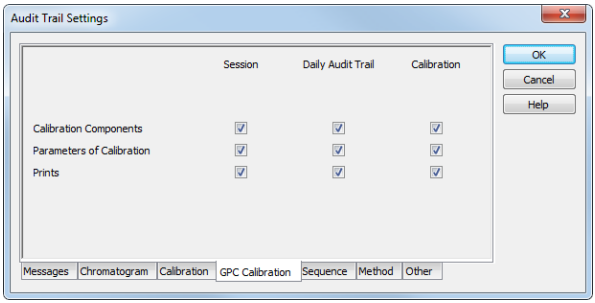


Fig 20: GPC Calibration tab

The options to be enabled or disabled on this tab are the same as the options available on the Calibration tab of the Audit Trail Settings dialog, but relate only to the **GPC Calibration** operations.

5.6.7 Keep in mind that:

- The calibration curve and equation will not be displayed, if the equation could not be constructed using all the points marked as used. Please check that you have entered retention times and molecular weights for all points to be used in the calibration.
- Calibration curve is cut off on nearest local extreme outside the *Minimum RT* and *Maximum RT* to assure it is monotonous on definition interval. Definition interval is checked during all computations.
- If local extreme occurs between the *Minimum RT* and *Maximum RT*, *Invalid points to construct curve* error will be reported. Check the correctness of your data (typing mistake in *Mp* value is a typical problem) or decrease the polynomial fit degree.
- The last **Broad Peak** is temporarily remembered and used in subsequent recalculations. Current **Broad Peak** is indicated in lower-right corner of the Calibration window.
- The **Universal Calibration** cannot be used for **Broad On Narrow** because computed *K* and *Alpha* values will be overwritten by *K* and *Alpha* of the unknown sample. If you know *K* and *Alpha* of the unknown sample, use **Narrow Universal Calibration**.
- When **Broad On Narrow** calibration type is used, the *K* and *Alpha* values fields in the Chromatogram - GPC Results tab are inactive, because these values are loaded from the calibration results.

5.7 Export

To transfer data from **Clarity** to other programs, the simplest way is through the **Windows** clipboard, i.e. using the *Copy* (Ctrl + C) and *Paste* (Ctrl + V) commands on the selected table area.
The *Export Chromatogram* options are the same as in **Standard** mode, (for more details see also chapter Export Chromatogram)see the **Clarity Reference Guide** chapter **Export Chromatogram**.

5.7.1 Export of graphs

The *Chromatogram* (including overlaid calibration curve), *MW Distribution* or *Cumulative MW Distribution* graphs can be transferred to other programs using the *Export*, *Save As Picture to Clipboard* or *Save As Picture to File* commands. The actually displayed graph pane will be copied to clipboard or saved into a file in an *.EMF vector graphic format and can be pasted or inserted to any **MS Office** document. It is advisable to apply all desired formatting to the picture (fonts, sides ratio, etc.) completely in **Clarity** prior to performing the export command.

5.7.2 Export of data

The Export Data dialog accessible using the *Setting - Export Data* command from the Instrument window will change to Export GPC Data in **GPC** mode. The same dialog also opens using the *File - Export - Export Data* command in the Chromatogram window. Settings made in the Export GPC Data dialog will be used during automated processing if the *Export Data* checkbox is checked in the PostRun Setting and Batch dialogs.

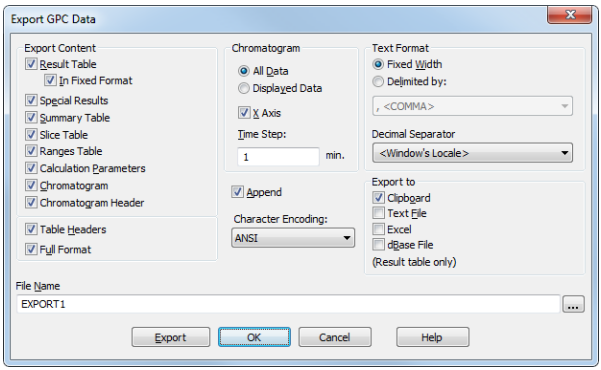


Fig 21: Export GPC Data

Caution: Only commands different from the standard Export Data dialog will be described here.

Result Table
Exports all the columns available for **Result Table**, each peak on separate line.

Slice Table
Exports all the columns available for **Slice Table**, continuously for all peaks. (*Peak No.* will be exported as one of the columns).

Ranges Table
Exports the **Ranges Table**.

Chromatogram
Exports the time/signal values according to the settings in the *Chromatogram* area.

Chromatogram Header
Exports the chromatogram information including the calibration file details.
All other options behave in the same way as in standard mode, (for more details see also chapter Export Data)see the **Clarity Reference Guide** chapter **Export Data**.

5.7.3 Export Summary Table

The summary table is exported as a *.TXT file from the Chromatogram window using the *File - Export - Export Summary Table* command. The export is performed according to the actual setup used for the **Summary Table**. The setup can be changed using the *Setup Columns...* and *Summary Options...* commands from the table local menu.

5.8 Report Setup

While in the **GPC** mode, the *Report Setup* command still opens the Report Setup dialog, though with some changes on particular tabs. Only the Method, Chromatogram, Calibration and Results tabs are different from standard mode and will be described here.

5.8.1 Report Setup - Method

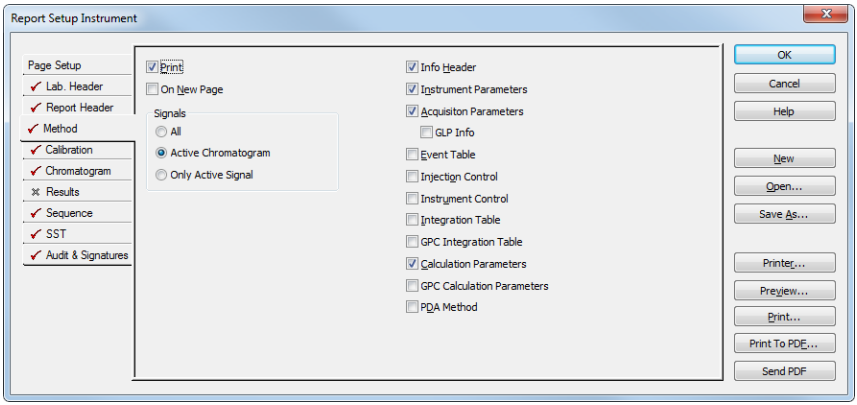


Fig 22: Report Setup - Method (GPC)

The added *GPC Integration Table* and *GPC Calculation Parameters* checkboxes enable the display of corresponding GPC Integration and GPC Calculation tabs in the Method Setup dialog.

5.8.2 Report Setup - Calibration

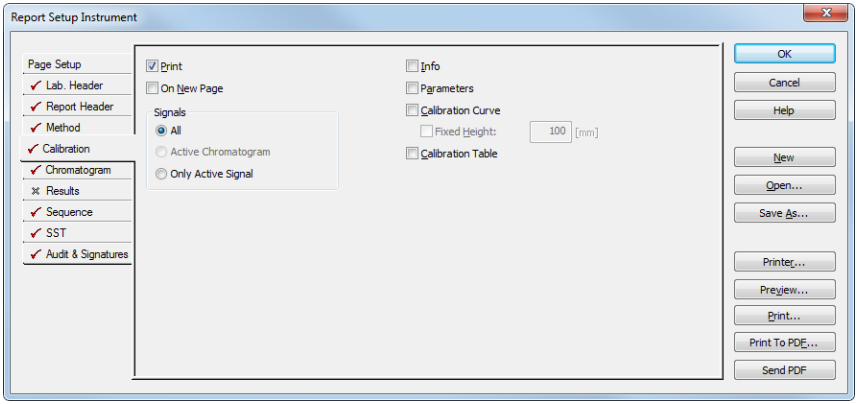


Fig 23: Report Setup - Calibration (GPC)

Calibration Curve

Prints the calibration curve displayed in the Calibration window.

Fixed Height

If the *Fixed Height* checkbox is checked, the chromatogram will be printed with a fixed height instead of the fixed ratio 2:3. The permitted lower height range is *30 mm*, the upper height range is not set - the graph will be scaled to the page height if larger than the page itself.

Calibration Table

Prints the **Calibration Summary Table**. This checkbox is equivalent to the *Summary* checkbox on the standard Report Setup - Calibration tab.
Other options are the same as in standard mode (for more details see also chapter Calibration).

5.8.3 Report Setup - Chromatogram

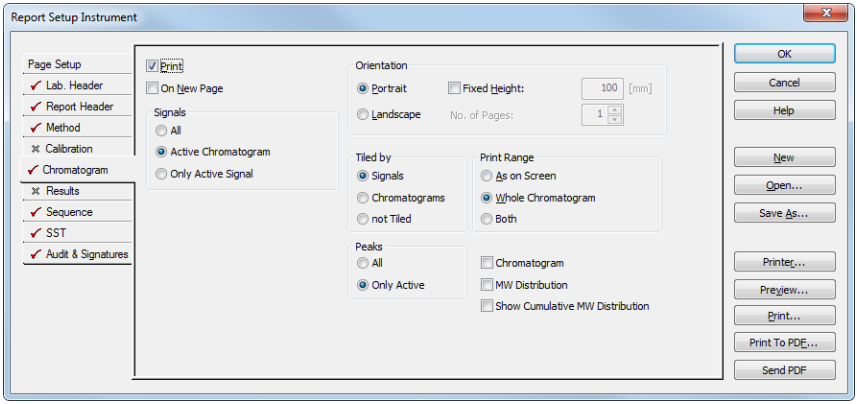


Fig 24: Report Setup - Chromatogram (GPC)

The printing of *Chromatogram*, *MW Distribution* or *Cumulative MW Distribution* can be individually selected by the appropriate checkboxes. All graphs are printed using the common *Orientation* settings.
The *Peaks* area will only be active if the *Tiled by - Signals* option has been selected. It will then print the *MW Distribution* and *Cumulative MW Distribution* graphs for all or only for active peak in each signal.
Other options are the same as in standard mode (for more details see also chapter Chromatogram).

5.8.4 Report Setup - Results

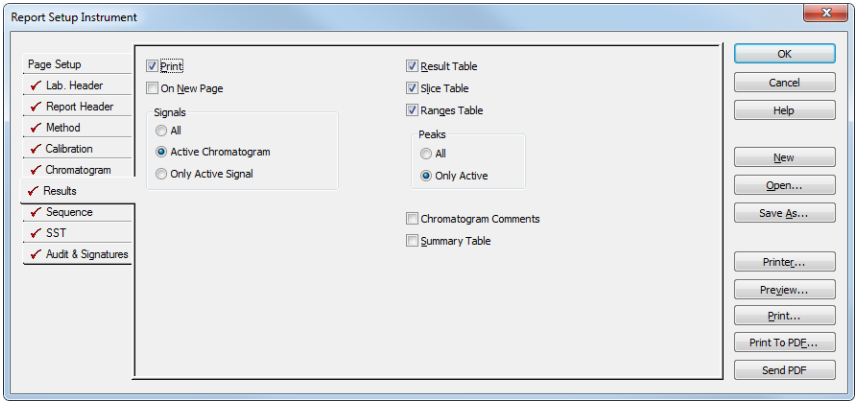


Fig 25: Report Setup - GPC Results

The printing of *Result Table*, *Slice Table*, *Ranges Table* or *Summary Table* can be individually selected by the appropriate checkboxes. The *Slice Table* and *Ranges Table* can be printed for the active peak or for all peaks from the given signal, as set (commonly for both tables) in the *Peaks* area.

Note: The peak is set active by clicking on its row in the **Result Table**. During automatic printing (from Postrun, Sequence or Batch), the first peak is considered as active.

The tables are printed as configured on the display (with the exception of fonts). The setup can be changed using the *Setup Columns...* command from the table local menu, eventually by the *Summary Options...* command for the **Summary Table**.