Technical Overview

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Peak Evaluation and Quantification in Agilent WinGPC Software HPLC Mode

Introduction

Many measurements and evaluations made with Agilent WinGPC software focus on molar mass distributions and mean values. However, depending on the analysis, peak area, retention time, or signal height can also be important. When determining monomer residues, for example, the measurements can be evaluated in high-performance liquid chromatography (HPLC) mode. The samples can be analyzed simultaneously in HPLC and gel permeation chromatography and size-exclusion chromatography (GPC/SEC) mode, as the HPLC results do not affect the molecular weight distribution window and all molecular weight distribution results are displayed as usual. To quantify the signals (that is, to determine the concentration), the detector must first be calibrated; in other words, the response factor must be determined.

This technical overview explains how to use the HLPC mode in WinGPC software.

High-performance liquid chromatography analysis

Select an injection or a sample that should be analyzed in HPLC mode. Activate HPLC mode in WinGPC software either by clicking the **HPLC** icon (Figure 1) or by clicking **Options** > **HPLC-Analysis** > **Height** or **Area** in the Elugram window. The sample peaks then become filled with color (Figure 2).

Figure 1. HPLC icon.

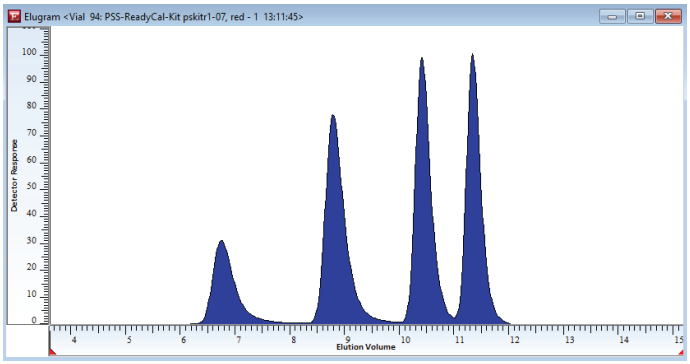

Figure 2. HPLC mode applied to a ReadyCal Standard polystyrene. The peaks are filled with color.

By clicking **Options** > **Peaklist sort for** in the Elugram window, the automatic peak search is started, which integrates all peaks found within the integration limits. All peaks are then sorted according to the specified option (in this example, Area) and labeled with capital letters (A, B, C, etc.), as shown in Figure 3.

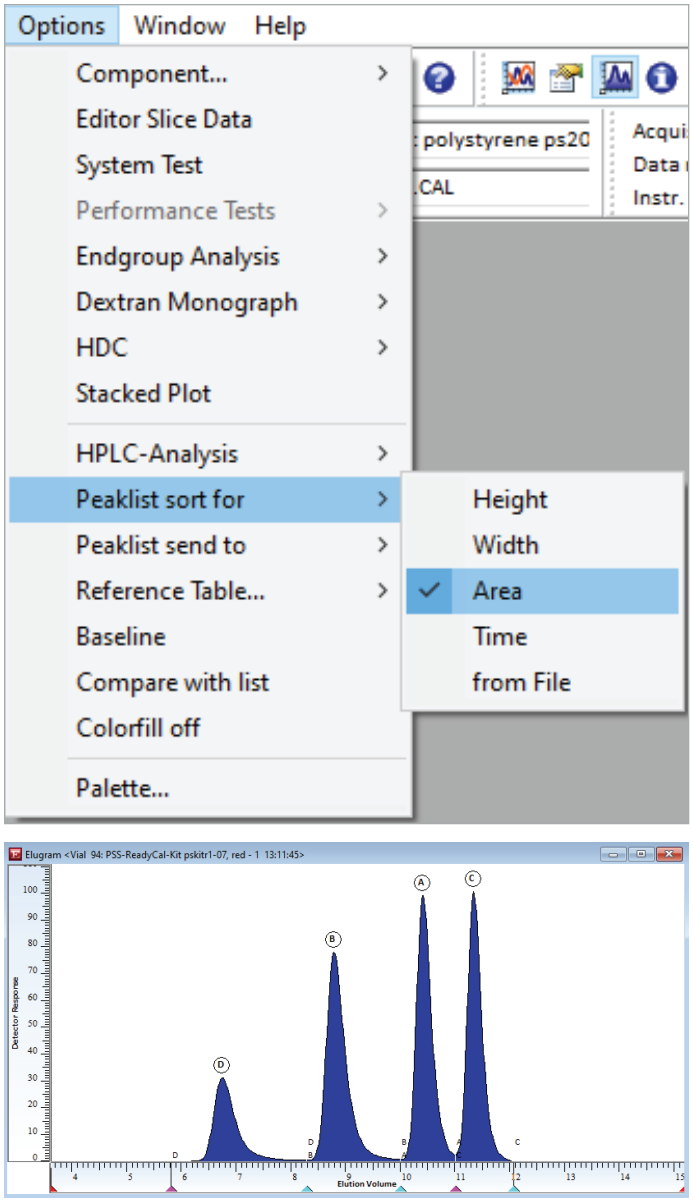

Figure 3. HPLC mode applied with the option **Peaklist sort for** > **Area** to a ReadyCal Standard. The peaks are now filled with color and labeled with capital letters.

Note: The adjustment of the left limit (pink marker) and the right limit (light-blue marker) on the X-axis can be achieved by clicking and dragging with the left (pink marker) or right mouse button (light-blue marker).

Manual deletion or addition of peaks

To delete unwanted components and peaks, right-click the peak label (A, B, C, etc.) in the Elugram window (Figure 4) and click **OK** in the dialog box.

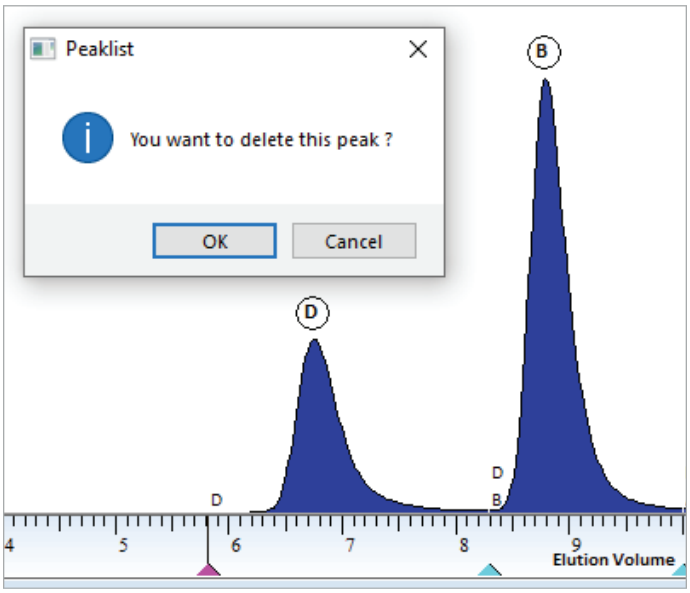

Figure 4. Right-clicking the peak letter in the Elugram window opens a dialog box that enables the deletion of the selected peak.

To add a component, right-click the X-axis beneath the desired peak and select **Insert peak** (Figure 5).

Finally, the peak limits can be adjusted as described in the "High-performance liquid chromatography analysis" section.

Note: To get an overview of the whole peaklist that is generated in the background, click **Options** > **Peaklist send to** > **Editor**. The Data Editor window is displayed, showing the peaklist of the most recently evaluated sample in the Elugram window (Figure 6).

All peaks are labeled with default names. Concentrations are not yet calculated, because the detector has not yet been calibrated. However, the elution volume (xPMax), peak height (yPMax), and area are provided.

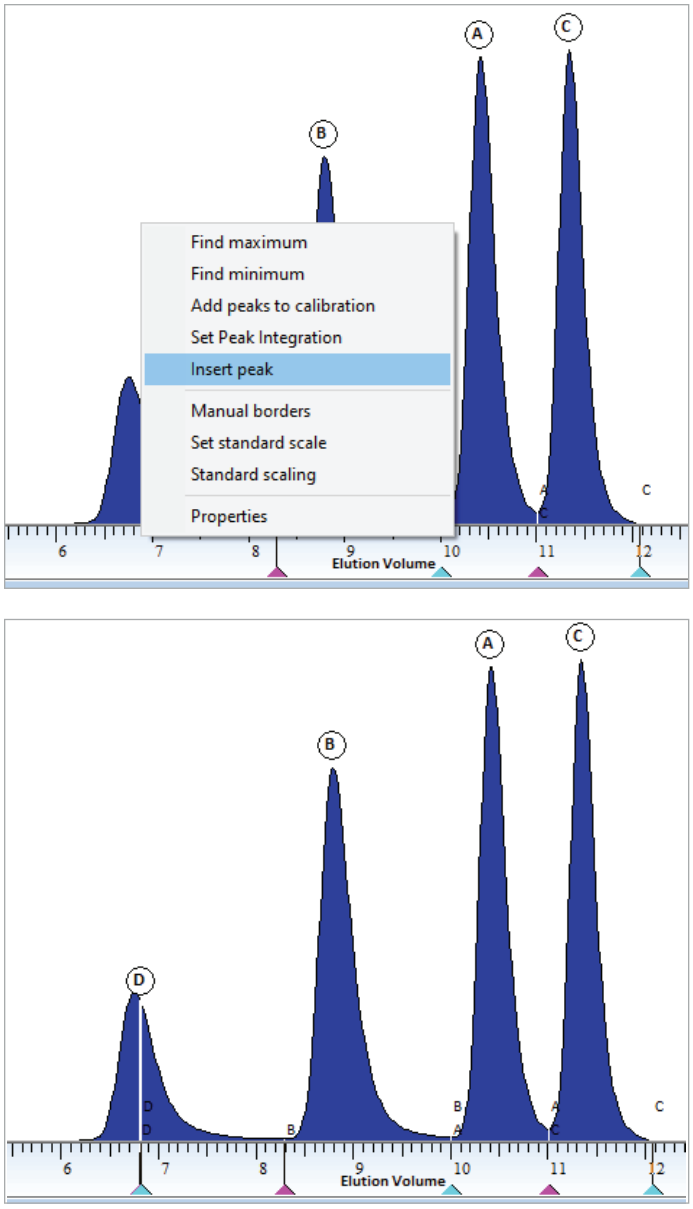

Figure 5. Manual insertion of a new peak (in this example, peak D). The peak limits still need to be adjusted.

Data Editor

Row	Name	xPMax [ml]	yPMax [V]	MPMax [Da]	Area [V*ml]	Area [%]	Conc. [g/l]	Conc. [%]	V-Min [ml]	V-Max [ml]	V-Del [ml]
1	Peak: A	1.04087E+1	1.10376E+1	2.78508E+7	3.75624E+0	2.94085E+1	0.00000E+0		1.00087E+1	1.10087E+1	1.00000E+0
2	Peak: B	8.79200E+0	8.67021E+0	3.05722E+7	3.71838E+0	2.91121E+1	0.00000E+0		8.29200E+0	1.00087E+1	1.71667E+0
3	Peak: C	1.13420E+1	1.11787E+1	2.63914E+7	3.55601E+0	2.78409E+1	0.00000E+0		1.10087E+1	1.20753E+1	1.06667E+0
4	Peak: D	6.75867E+0	3.49416E+0	3.43756E+7	1.74200E+0	1.36385E+1	0.00000E+0		5.80867E+0	8.29200E+0	2.48333E+0

Figure 6. Data Editor window showing the peaklist.

Reference table creation

To identify peaks, create the reference table by clicking **Options** > **Reference Table...** > **Create** in the Elugram window. Here, you can enter a meaningful name for each component (Figure 7).

A

Data Editor

Row	Detector	Peakmax.[ml]	max. Deviation [%]	Response	Name
1	1.00000E+0	1.04087E+1	5.00000E+0	1.00000E+0	Peak: A
2	1.00000E+0	8.79200E+0	5.00000E+0	1.00000E+0	Peak: B
3	1.00000E+0	1.13420E+1	5.00000E+0	1.00000E+0	Peak: C
4	1.00000E+0	6.75867E+0	5.00000E+0	1.00000E+0	Peak: D

B

Data Editor

Row	Detector	Peakmax.[ml]	max. Deviation [%]	Response	Name
1	1.00000E+0	1.04087E+1	5.00000E+0	1.00000E+0	Peak: Alpha
2	1.00000E+0	8.79200E+0	5.00000E+0	1.00000E+0	Peak: Bravo
3	1.00000E+0	1.13420E+1	5.00000E+0	1.00000E+0	Peak: Charlie
4	1.00000E+0	6.75867E+0	5.00000E+0	1.00000E+0	Peak: Delta

Figure 7. Data Editor window showing the reference table with (A) default names and (B) adjusted peak names.

The Detector column in the reference table corresponds to the number of the detector used in the Agilent WinGPC method (this entry cannot be edited). The default maximum tolerance for the deviation between elution volume found and $Peak_{max}$ (xPMax) is 5% when WinGPC software tries to identify an unknown peak. This value can be adjusted in the peaklist. The default value of the response factor is currently 1. To save the reference table, click **File** > **HPLC Ref. export** in the Data Editor window (Figure 8). This makes the generated peaklist available for future evaluations.

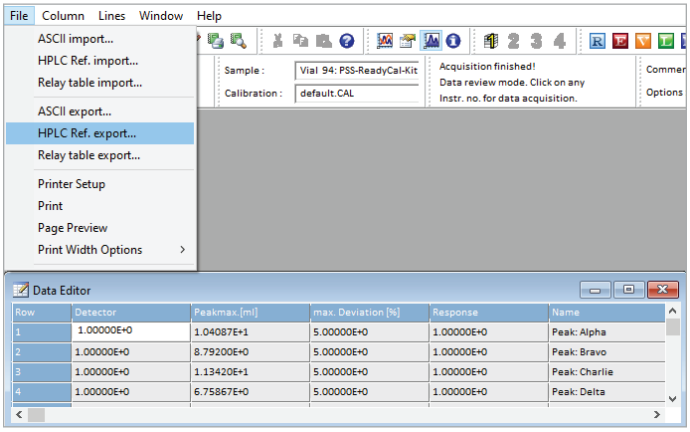

Figure 8. HPLC Reference table export from the Data editor window.

Data evaluation

To evaluate a sample of your choice, HPLC mode must be activated by either clicking the HPLC icon (Figure 1), or by clicking **Options** > **HPLC - Analysis** > **Height** or **Area** in the Elugram window. Click **Options** > **Peaklist sort for** to start the automatic peak search, which integrates all peaks found within the integration limits. Then, in the Elugram window, click **Options** > **Reference Table** > **Load**, and apply the reference table to the actual measurement and identify the peak, as shown in Figure 9. Identified components will be labeled in yellow in the Elugram window. The name and elution volume found are added.

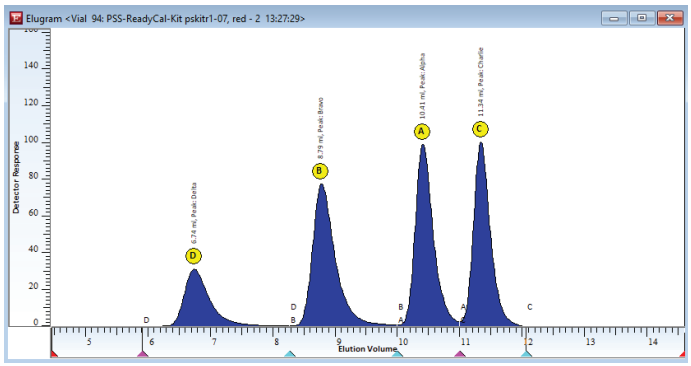

Figure 9. All identified peaks get a yellow-colored label, and the found names and elution volumes are added.

The peaklist will also be updated with the corresponding name taken from the reference table and the preliminary concentration (Area [V*ml]), calculated with a response factor of 1 (Figure 10).

Data Editor

Row	Name	xPMax [ml]	yPMax [V]	MPMax [Da]	Area [V*ml]	Area [%]	Conc. [g/l]	Conc. [%]	V-Min [ml]	V-Max [ml]	V-Del [ml]
1	Peak: A	1.04087E+1	1.10376E+1	2.78508E+7	3.75624E+0	2.94085E+1	0.00000E+0		1.00087E+1	1.10087E+1	1.00000E+0
2	Peak: B	8.79200E+0	8.67021E+0	3.05722E+7	3.71838E+0	2.91121E+1	0.00000E+0		8.29200E+0	1.00087E+1	1.71667E+0
3	Peak: C	1.13420E+1	1.11787E+1	2.63914E+7	3.55601E+0	2.78409E+1	0.00000E+0		1.10087E+1	1.20753E+1	1.06667E+0
4	Peak: D	6.75867E+0	3.49416E+0	3.43756E+7	1.74200E+0	1.36385E+1	0.00000E+0		5.80867E+0	8.29200E+0	2.48333E+0

Figure 10. Updated peaklist with the found names plus elution volumes.

Peak quantification: Response factor determination

To quantify a signal with an unknown concentration, the correct response factor is needed. Measure a concentration series of at least four to five samples prepared from the substance you want to quantify (PS, in this example). It is advised that you do not work with a dilution series. Make sure the correct concentration and injection volume for each sample is entered in the sequence table or sample editor (in the Raw Data window, click **Editor** > **Samples**).

Once the concentration series is measured, evaluate all related sample peaks in HPLC mode, as described in the "High-performance liquid chromatography analysis" section. To activate the overlay, click **Overlay** > **Include curve, Overlay** > **Overlay** in the Elugram window, or click the Overlay icon (Figure 11).

Figure 11. Overlay icon.

Calculate the response factor for components by clicking **Options** > **Reference Table...** > **Response factors** in the Elugram window (Figure 12).

Clicking **To "Ref." File** updates your reference table with the determined response factor. Clicking **Options** > **Reference Table** > **Load...** applies the updated reference table to the measurement. Now, peak A can be identified and quantified.

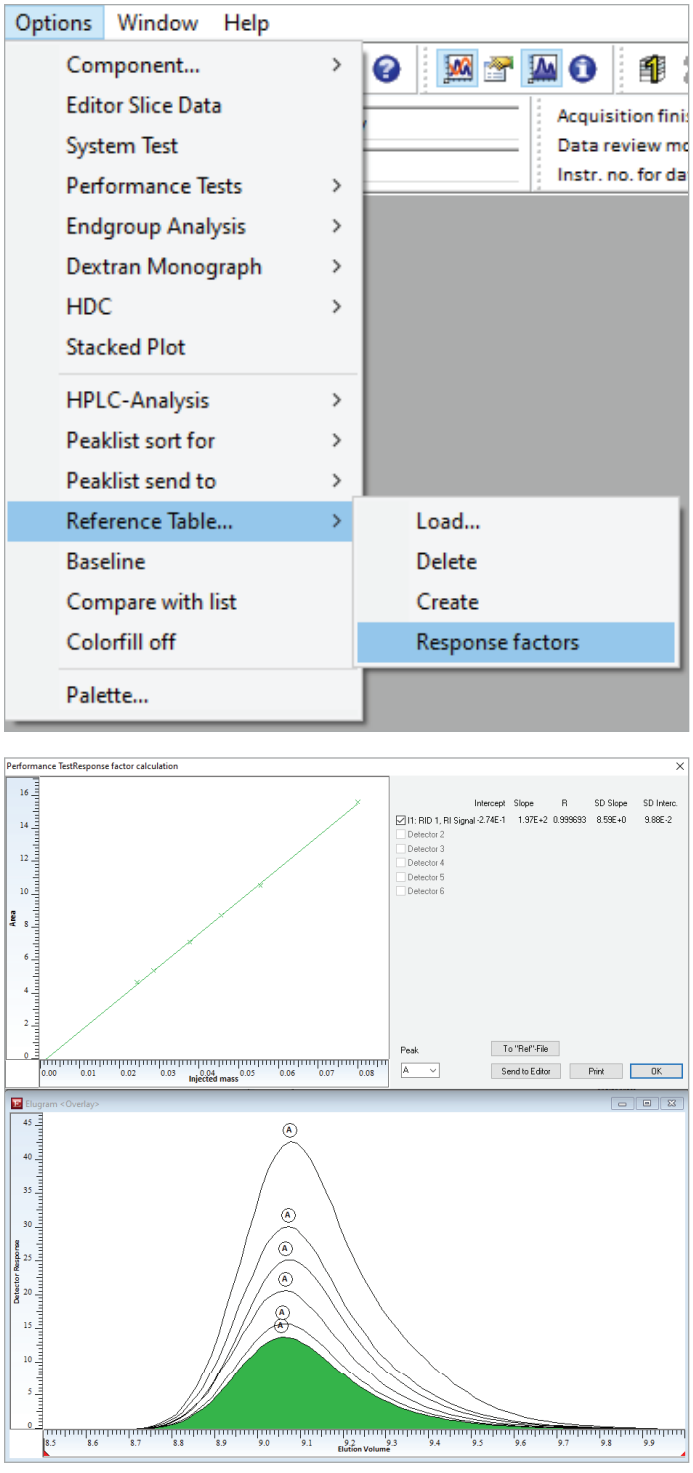

Figure 12. Response factor determination from a concentration series.

Left-click on the yellow label to show the results, as shown in Figure 13. Clicking **Options** > **Editor Peaklist** opens the Data Editor window with the actual HPLC results. The correct concentration, calculated with the determined response factor, is also given.

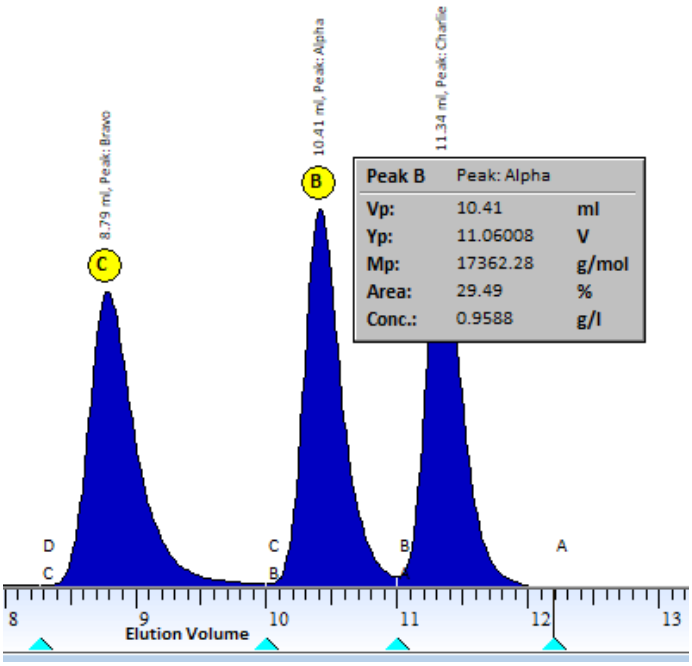

Figure 13. Left-clicking on the yellow label shows the results, in this case for Peak A. The correct concentration, calculated with the determined response factor, is also displayed.

Note: WinGPC software calculates the response factor from the plot as 1/Slope simultaneously for several components (pull-down menu at peak) and all available detectors. Response factor calculation from only one concentration is possible but explicitly not recommended. The plot with all calculated parameters for each component can also be printed out.

Note: To determine response factors from several components at the same time, open the sample editor and enter the concentrations for the maximal four possible components in order of elution: "Comp. 1" elutes first, then "Comp. 2", etc. When starting the peak search in HPLC mode, choose **Peaklist sort for** > **Time** before adding the HPLC evaluations to overlay. WinGPC software assigns the concentrations correctly to the respective peaks for response factor calculations.

Conclusion

Agilent WinGPC software offers versatile capabilities for peak evaluation and quantification. This technical overview provides a foundational understanding of how to effectively use HPLC mode within WinGPC software.

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