

# Transferring HPLC Peaks into Agilent WinGPC Software for Multi-Area Analysis

#### Introduction

When analyzing complex polymer mixtures or broad peaks with several maxima or fractions, the Agilent WinGPC multi-area analysis function can be valuable. With this tool, an elugram can be "cut" into up to eight areas. Statistics including molar mass averages, area, and area% (related to the overall area) can be calculated for each area in a single step.<sup>1</sup> To obtain the same results, integration limits would usually have to be set manually for each fraction in the elugram window and separate evaluations performed.

With Agilent WinGPC Software version 1.0, the start and end volumes of peaks found in HPLC mode can be transferred to the multi-area analysis tool and used as area definitions. This feature simplifies the workflow while still allowing for manual fine-tuning. This technical overview highlights the combination of the HPLC and multi-area analysis modes in an example analysis.

## **Experimental**

#### HPLC mode

In HPLC mode, open a measurement with several peaks (in this example: a polystyrene Agilent ReadyCal mixture) and apply a baseline. In the elugram window, activate HPLC mode by clicking **Options**, **HPLC Analysis**, then **Area**, or simply click the HPLC mode icon (Figure 1A). WinGPC will locate the peaks and create the peak list. This can be done manually by clicking **Options**, **Peaklist sort for**, and then **Time**, for example (Figure 1B).



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Agilent WinGPC , Build 10478, PC-GPC-SW018, Instance #1



**Figure 1.** (A) Elugram window showing how HPLC mode can be activated, either through the Options drop-down menu or by clicking the HPLC mode icon; (B) elugram window showing how to manually adapt peak list using the Options drop-down menu

The peak list with the HPLC results can be reviewed: click **Options**, **Peaklist send to**, then **Editor** and open the WinGPC Data Editor by clicking the icon<sup>2</sup> (Figure 2).

📝 Dat	a Editor							×
Row	Name	xPMax [ml]	yPMax [V]	MPMax [Da]	Area (V*ml)	Area [%]	Conc. [g/l]	^
1	Peak: A	6.22867E+0	2.45064E+0	3.54425E+7	1.90841E+0	1.38531E+1	0.00000E+0	
2	Peak: B	8.04533E+0	9.71492E+0	3.19173E+7	3.97369E+0	2.88450E+1	0.00000E+0	
3	Peak: C	9.94533E+0	1.06585E+1	2.86050E+7	3.95656E+0	2.87206E+1	0.00000E+0	
4	Peak: D	1.10453E+1	1.21535E+1	2.68468E+7	3.93736E+0	2.85813E+1	0.00000E+0	~
<								>

Figure 2. Agilent WinGPC Data Editor window

To transfer the peak borders found by the WinGPC integrator to the multi-area analysis evaluation, click **Options**, **Peaklist send to**, then **Multi-area settings**. The multi-area analysis mode will be activated automatically, and the Properties of multi-area evaluation dialog box will appear (Figure 3). Here, up to eight areas of the elugram can be defined, each with the parameters from and to. In this example, the parameters from and to have been applied for areas 1 to 6, corresponding to the peaks A to F found in the HPLC mode. Areas 7 and 8 are not defined.

Area 1	Area 2	Area 3	Area 4	Preset Unit
				🔾 min
from : 5.52867	from: 7.562	from : 9.52033	from : 10.6453	(€ ml
to : 7.562	to: 9.29533	to : 10.6453	to: 11.962	🔘 g/mol
) min	⊖ min	⊖ min	⊖ min	Ref. Area
Evaluation 1 for :	Evaluation 2 for :	Evaluation 3 for :	Evaluation 4 for :	O Evaluat, 1
🗹 Area1 🛛 🗹 Min	🗌 Area1 🛛 🗹 Mn	🗌 Area1 🛛 🗹 Min	🗌 Area1 🛛 🗹 Mn	O Evaluat. 2
Area2 Mw	Area2 Mw	Area2 Mw	Area2 Mw	O Evaluat. 3
Area3 ⊻Mz Area4 √My	□ Area3 ☑ Mz □ Area4 ☑ Mv	Marea3 MMz Area4 MMy	Area3 MZ Area4 MV	O Evaluat. 4
Area5 🗹 D	Area5 🔽 D	🗌 Area5 🛛 🗹 D	🗌 Area5 🗹 D	C Evaluat. 5
Area6 ⊻[n]	Area6 [n]	Area6 [n]	Area6[n]	O Evaluat. 0
Area8 Mp	Area8 Mp	Area8 Mp	□ Area8 ☑ Mp	O Evaluat. 8
A				Area MWD
► 8%	M H <sup>2</sup>	☑ A <sup>3</sup>	▲ A <sup>4</sup>	
Area 5	Area 6	Area 7	Area 8	Arres Freibe
				for elugram:
rom :	from :	from :	from :	draw
o:	to:	to:	to :	🗹 auto search
nin ⊖ ml ⊖ g/mol	◉ min ◯ ml ◯ g/mol	● min () ml () g/mol	◉ min ◯ ml ◯ g/mol	Search within
Evaluation 5 for :	Evaluation 6 for :	Evaluation 7 for :	Evaluation 8 for :	5 [%]
Area1 Mn	Area1 Mn	Area1 Mn	Area1 Mn	
Area2 MW	Area2 Mw	Area3 Mz	Area2 MW	
Area4 Mv	Area4 🗌 Mv	Area4 Mv	🗌 Area4 🗌 Mv	Open
_Area5U ]∆rea6[n]	∐Area5 ∐D Dárea6 ∏ín]	∐Area5 ∐D Area6 ∏[n]	∐Area5 ∐D Dárea6 ∏ín]	
Area7 Vp	Area7 Vp	Area7 Vp	Area7 Vp	Save
Area8 Mp	Area8 Mp	Area8 Mp	Area8 Mp	Clear all
□ A%	□ A%	□ A%		Cancel

Figure 3. Properties of multi-area evaluation dialog box

The results WinGPC calculates are specified in each case, including molar mass averages ( $M_n$ ,  $M_w$ , ...), area (A), and area% (A%). The user can choose the specific area that WinGPC should evaluate.

The default setting is the (single) area defined above the evaluation. However, several areas can be selected, combined, and evaluated simultaneously. Similarly, the integration limits can be freely set in the elugram window, as well as which fraction of the chromatogram will be evaluated to give the results in the MWD window.

The overall area that is used as a reference for area% calculations can be selected on the right. The overall "area MWD" is predefined and can be controlled by the integration limits in the elugram window. WinGPC can draw the area limits in the elugram to make it easier to see if the chromatogram has been "cut" into the desired fractions (Figure 4).



Figure 4. Elugram displaying area limits

If areas are manually defined, the option auto search should be selected to let WinGPC search for minima around from and to within the allowed deviation (the default is 5%) and correct the parameters, if necessary. Click **OK** to leave the properties dialog box and activate the multi-area analysis evaluation. The defined areas will be plotted in the elugram if this option has been selected previously. The multi-area analysis mode can be accessed at any time through the raw data menu: click **Options**, **Analysis**, then **Multi-area create** or **Multi-area settings**. Here, borders can be manually adjusted and new areas can be created. These areas can also be deleted by entering zero for both the parameters from and to. Multi-area activate toggles the mode, and Multi-area results displays the results evaluated by the WinGPC, corresponding to the selected settings.

When multi-area mode is active, the related results will be added automatically as a second page to the standard printout from the raw data, elugram, or MWD window (click **Print** or **File**, then **Print**). The MWD window ASCII report (click **File**, then **Save ASCII Report**) also contains the multi-area results. Multi-area results and area definitions are also represented as ReportDesigner variables and allow for individually designed reports.

### Conclusion

Agilent WinGPC multi-area analysis mode is the combination of up to eight single evaluations (i.e., integration limits are set in the elugram and results appear in the MWD window, eight times in sequence, in one step). When combined with HPLC mode, the multi-area analysis allows a user to seamlessly integrate the automatic peak search function leading to a more streamlined way to process complex data.

### References

- 1. Appl. #17, WinGPC Newsletter 04/2001
- 2. cf. Appl. #51, WinGPC Newsletter 01/2012

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