

Manual for data analysis of SEC-MALS/RI (Wyatt DAWN HELEOS II (2023.01.27))

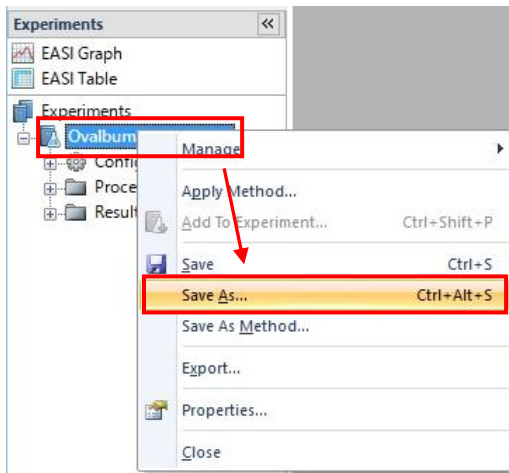
0 Saving data file (In case Astra 8.1.2 is already running and user want to continue the analysis after the SEC-MALS measurement.)

0-1 Saving data file

In the workspace area, right-click [Experiments] → [Sample name] and select [Save As]. After entering the save folder and file name, click [OK] to save the file (file type: ASTRA (*.afe8)).

Before save the data, create a folder under "C:\ASTRA DATA", [e.g. "C:\ASTRA DATA\20230220".]

[Then skip step 1 and proceed to step 2.]

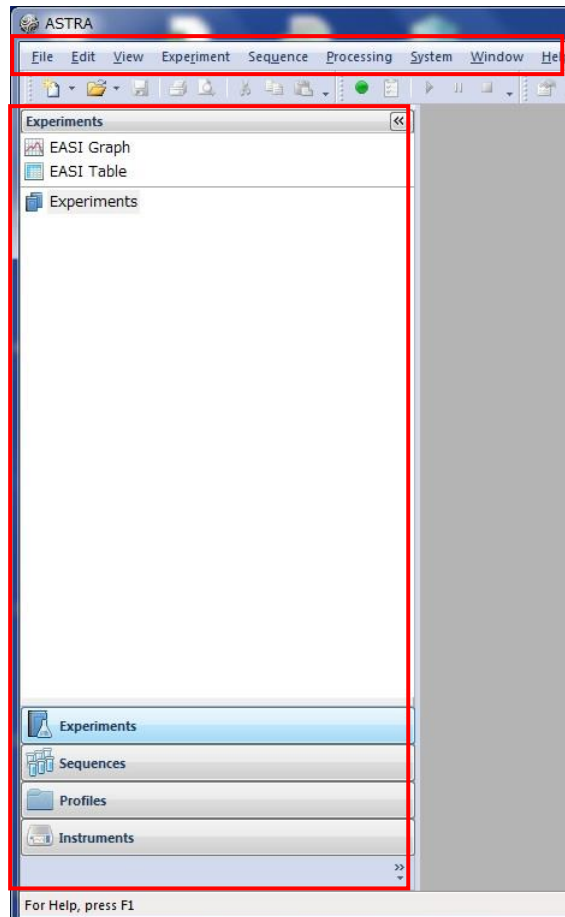


1 Starting Astra 8.1.2, Opening the data file

1-1 Starting Astra 8.1.2

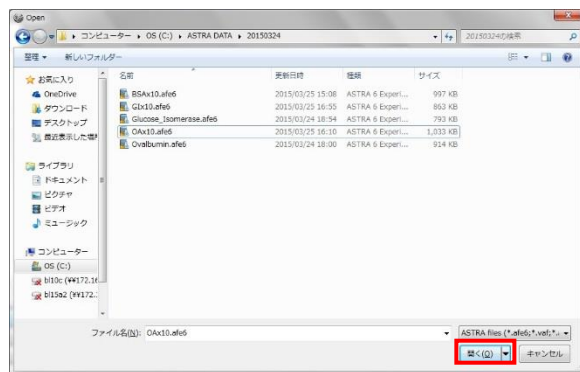
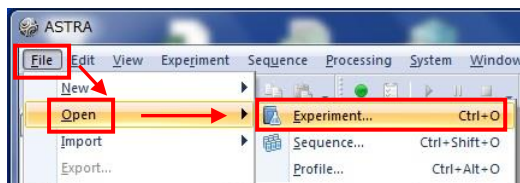
Click the Astra 8.1.2 shortcut icon on the desktop.

Menu bar
Workspace



1-2 Opening the experiment file.

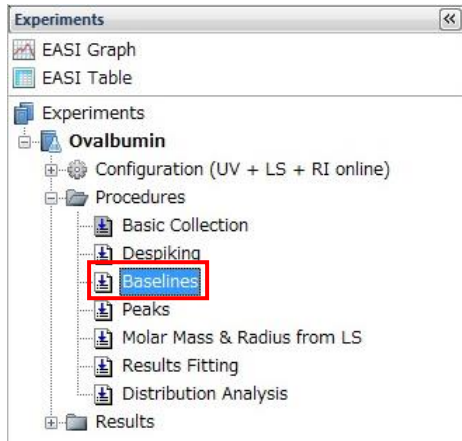
Select [File] → [Open] → [Experiment] from the menu bar to open the "Open" window, select an Experiment file (.afe6 or afe8) and click [開く (O) Open]



2 Setting baselines

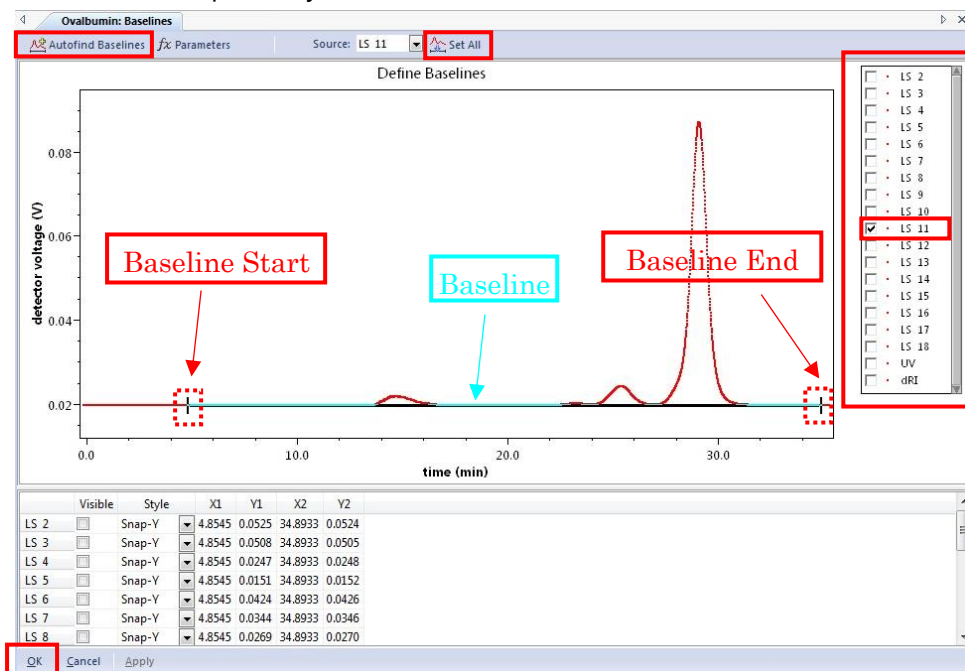
2-1 Baselines tab

In the workspace, double-click [Procedures] → [Baselines] to display the Baselines tab.



2-2 Specifying the Baseline range

Check the LS11 box to display the data from the 90° LS detector (on the right side of the graph area.) Click [Autofind Baselines] to automatically set the baseline range. Then drag the left and right edges of the baseline (light blue) to set the start and end of the baseline respectively.



2-3 Confirmation of Baseline range

Click [Set All] to apply the baseline range of LS11 set in step 2-2 to all LS data. Then check whether the baseline range is appropriate for each of the LS1~18, UV and RI data, and make adjustments to each data.

The displayed data could be changed by selecting the check boxes on the right side, After checking and adjusting all data, click [OK].

***Common operations on the graph screen**

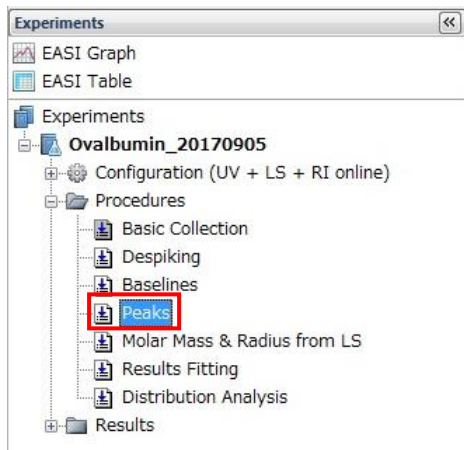
[Ctrl]+left drag : select a rectangle area and enlarge it.

[Ctrl]+right click : go back one step.

3 Specifying the peak range.

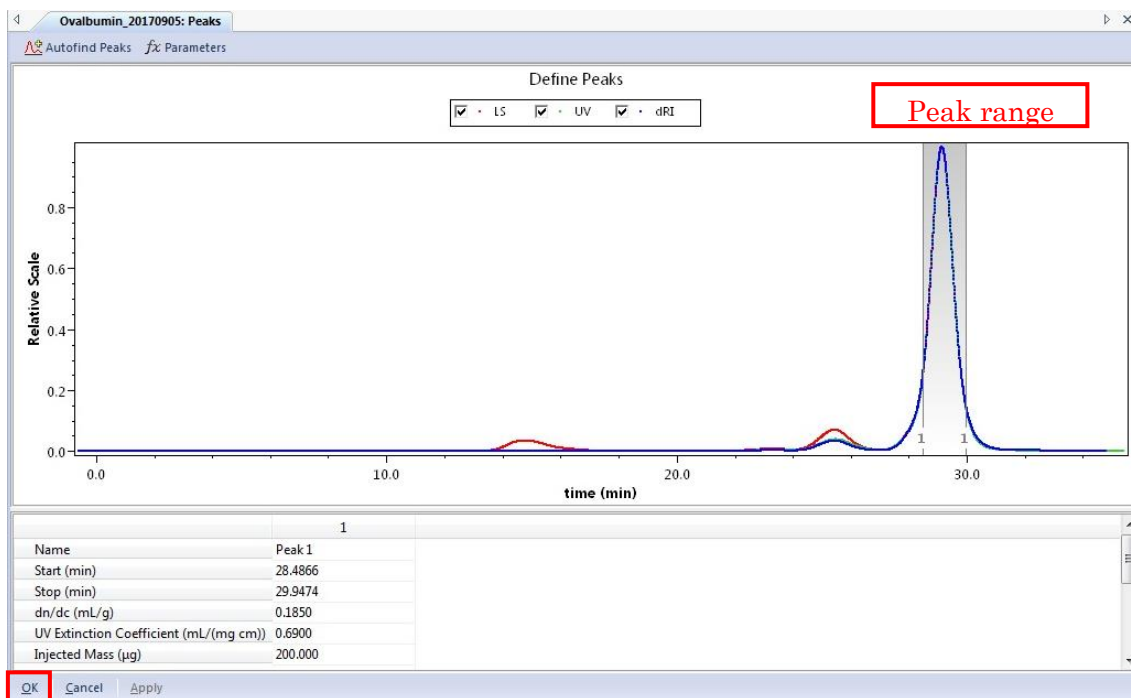
3-1 Peaks tab.

In the workspace, double-click [Procedures] → [Peaks] to display the Peaks tab.



3-2 Specifying the peak range.

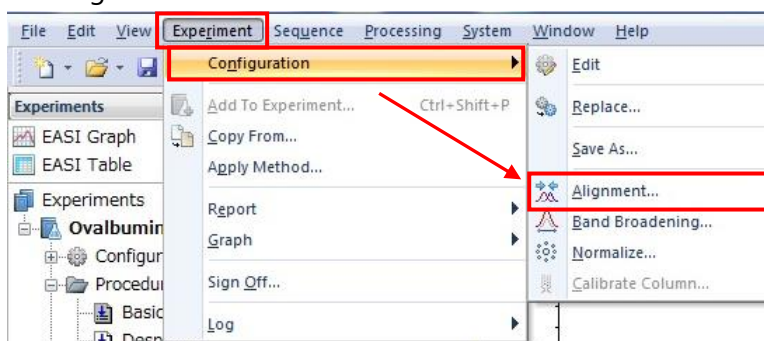
Click on the displayed peak range to select it. And the specified peak range can be adjusted by dragging the left and right edge of the range respectively. Either delete the peak range with the [Delete] key and specify the peak range by dragging. After adjusting the peak range, click [OK].



4 Alignment (Correction for misalignment of peak positions of LS, UV and dRI detectors.)

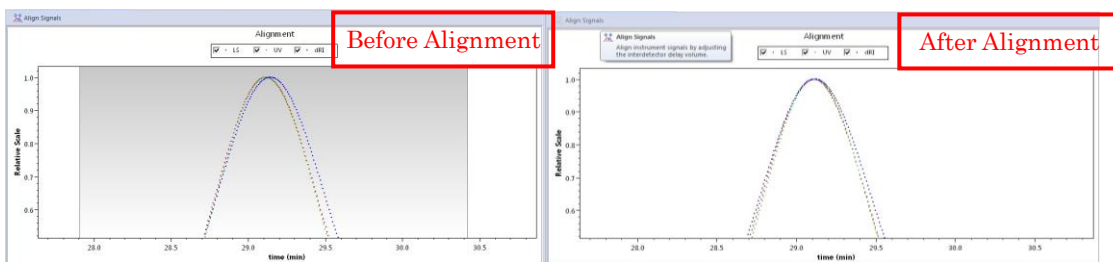
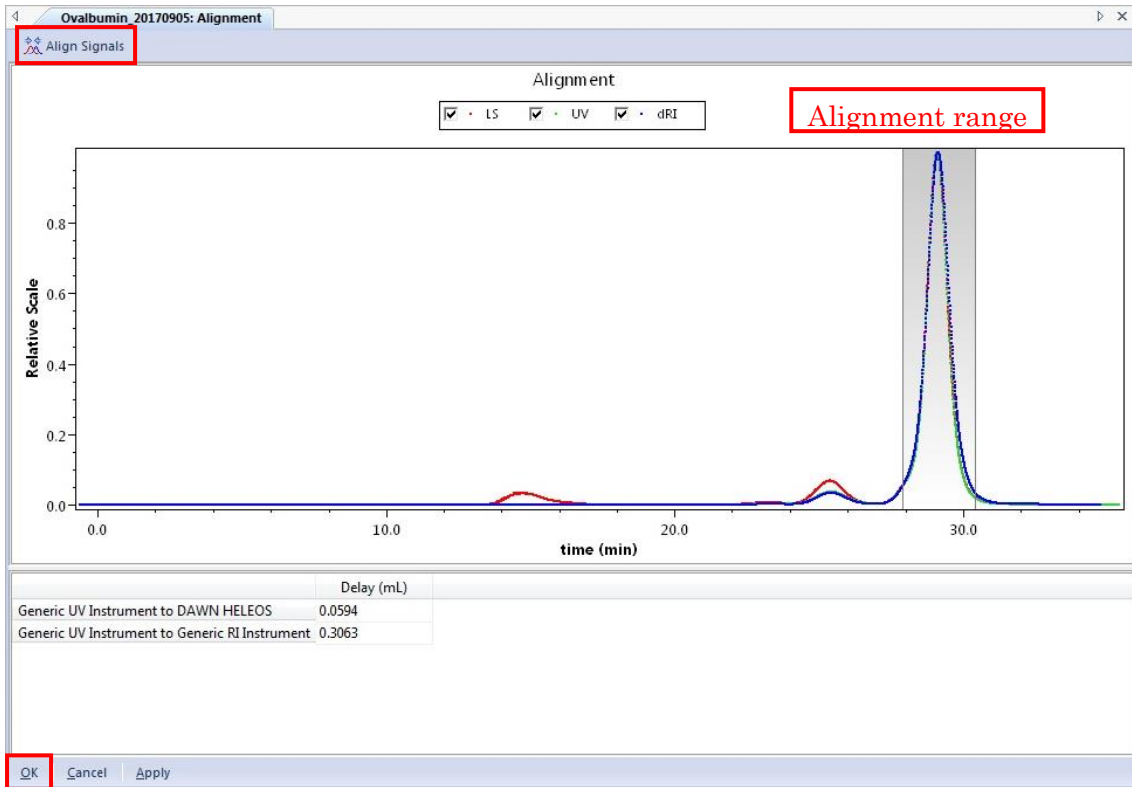
4-1 Alignment tab

Select [Experiments] → [Configuration] → [Alignment] in the menu bar to display the Alignment tab.



4-2 Execution of Alignment

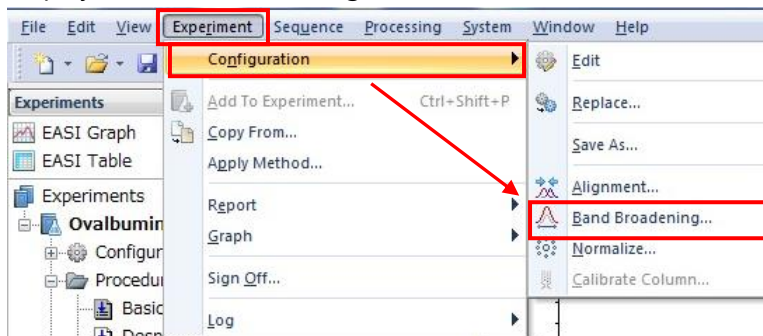
Adjust the Alignment range by Dragging between the start of the peak and the end of the peak. [If necessary zoom-up the display range]. Click [Align Signals] and then confirm that the positions of each peak (LS, UV, dRI) are aligned. After confirmation, click [OK].



5 Band Broadening (Corrections for peak broadening in LS, UV and dRI detectors.)

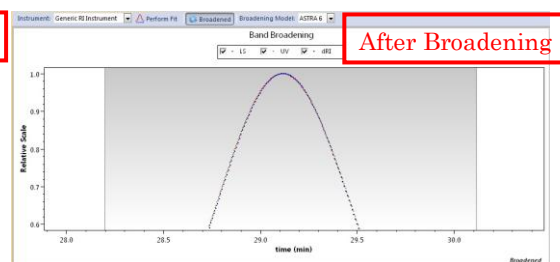
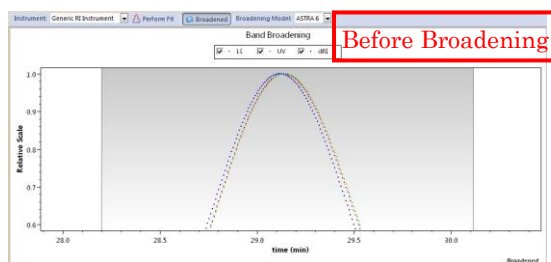
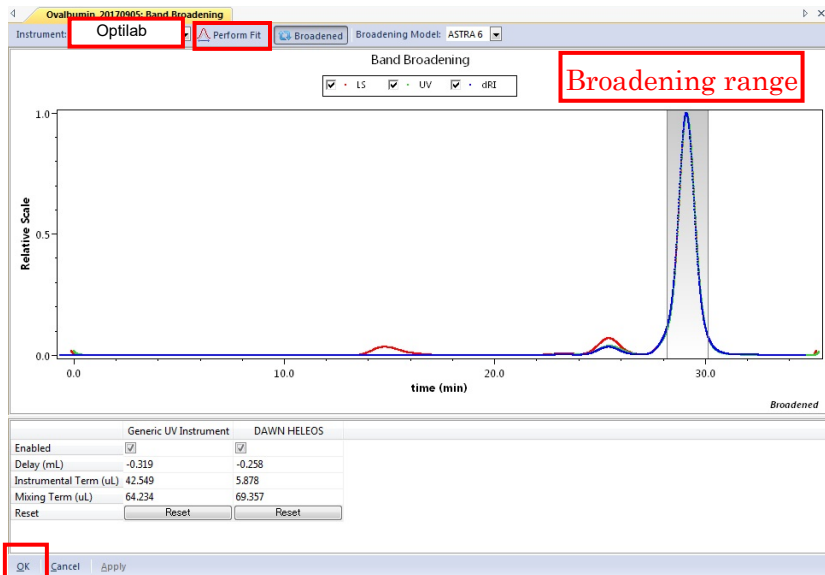
5-1 Band Broadening tab

In the menu bar, select [Experiments] → [Configuration] → [Band Broadening] to display the Band Broadening tab.



5-2 Execution of Band Broadening

Ensure that dRI data (Optilab) is displayed. Specify the correction range by dragging a range that includes entire peak. [If necessary zoom-up the display range]. Click [Perform Fit] and confirm that the width and offset of each peak (LS, UV, dRI) have improved. After confirmation, click [OK].

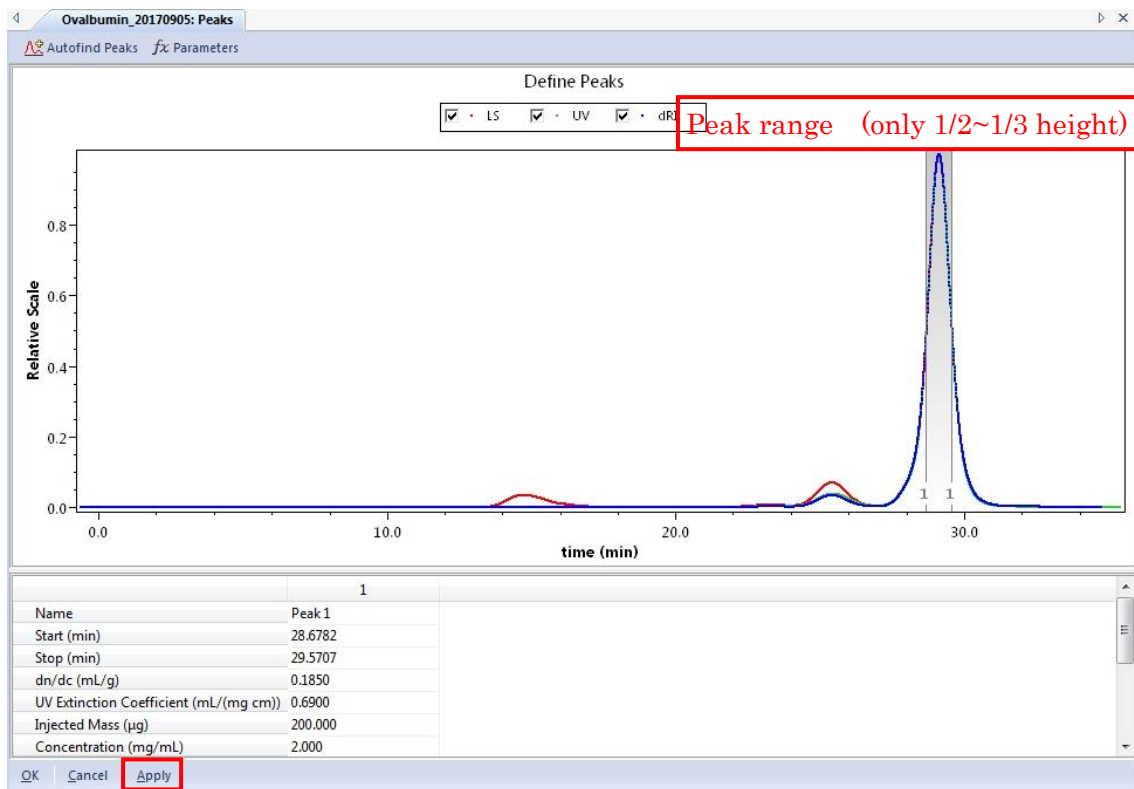


6 Normalize (Correction of intensity of multi angle LS detectors.)

Perform with standard sample before your own samples. Requires re-calibration for every solvent because the correction factors depend on the refractive index of the solvent.

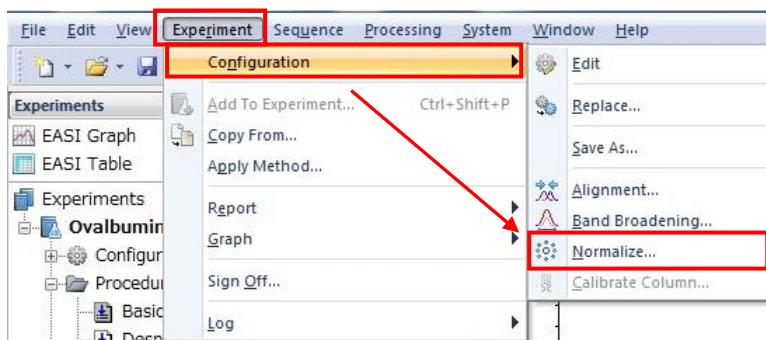
6-1 Re-specifying the peak range

In the workspace, double-click [Procedures] → [Peaks] to display the Peaks tab. Narrow the peak range to 1/2~1/3 of their peak height, and click [Apply].



6-2 Normalize tab

In the menu bar, select [Experiments] → [Configuration] → [Normalize] to display the Normalize tab.

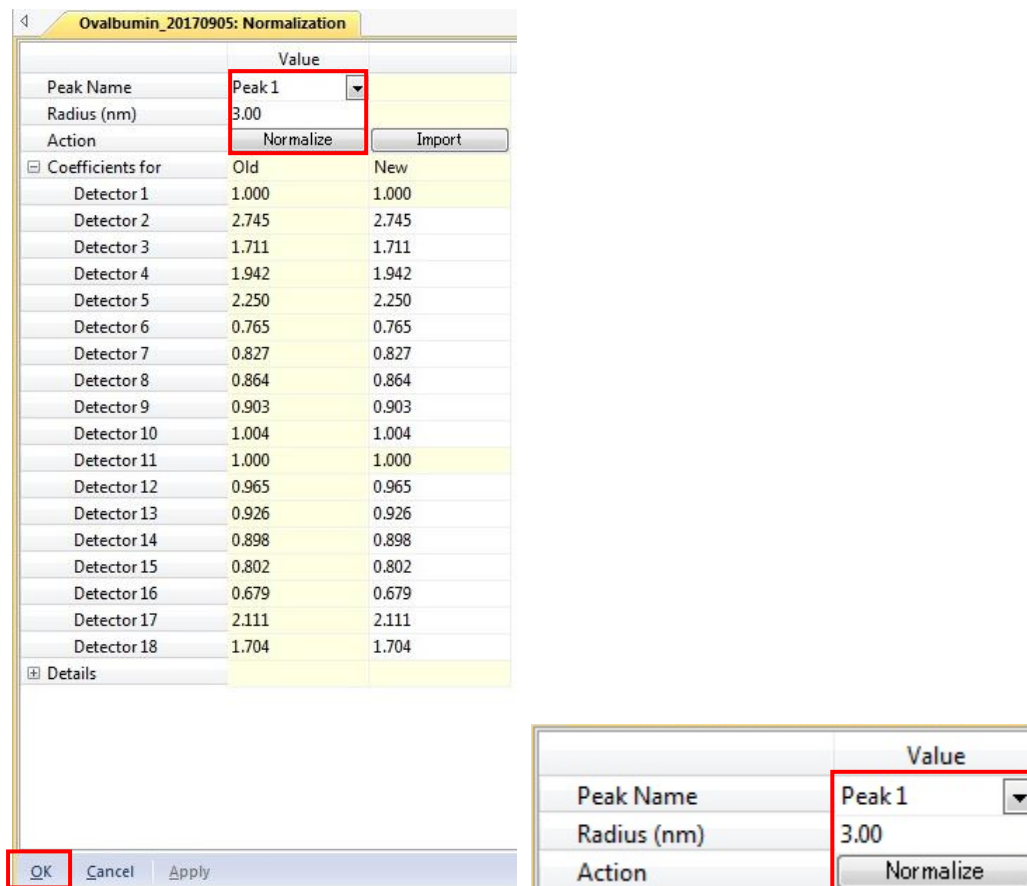


6-3 Normalize

Identify the peak to be calibrated in Peak Name and enter the approximate radius of the sample in Radius. [BSA: 3.5 nm, OA: 3.0 nm?]

Click [Normalize] to calculate new coefficients, then click [OK].

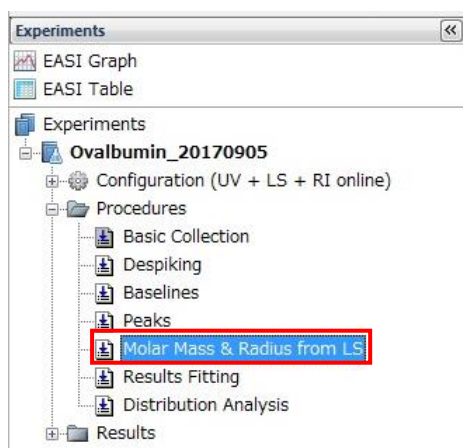
Open the Peak tab, restore the modified peak range and click [OK].

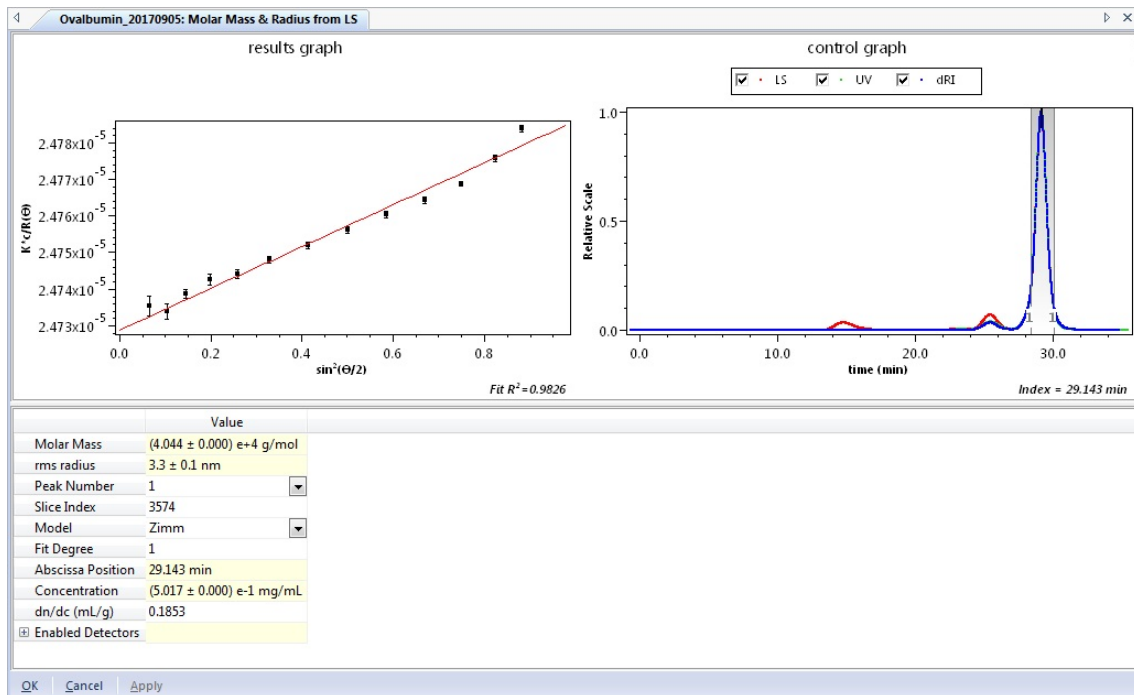


7 Selection of LS detectors to be used

7-1 Molar Mass & Radius from LS tab

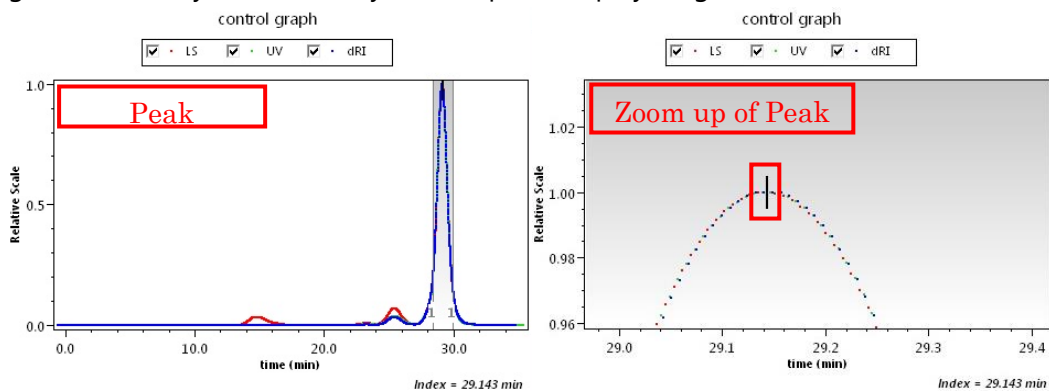
In the workspace, double-click [Procedures] → [Molar Mass & Radius from LS] to display the Molar Mass & Radius from LS tab.





7-2 Selection of peak apexes in the control graph

In the control graph, move the blue bar symbol to the apex of the peak by left-clicking and using the cursor keys. (If necessary zoom-up the display range).



7-3 Selection of LS detectors to be used

Click [+] in [Enabled Detectors] in the lower column of the screen to open the LS detector selection field.

Each point in the results graph corresponds to the result of each LS detector,

To improve Molar Mass and the correlation coefficient at the bottom of the results graph, it is necessary to add or exclude LS points that deviate from the approximate straight line by trial and error.

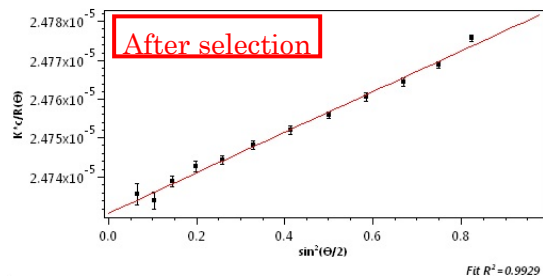
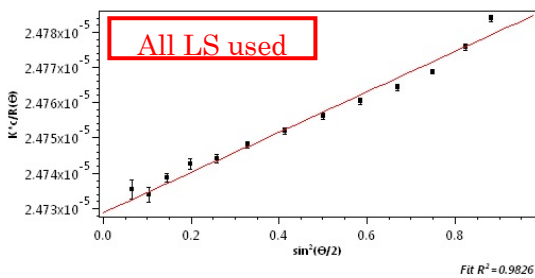
After getting the reasonable results, click [OK].

	Value
Molar Mass	(4.044 ± 0.000) e+4 g/mol
rms radius	3.3 ± 0.1 nm
Peak Number	1
Slice Index	3574
Model	Zimm
Fit Degree	1
Abscissa Position	29.143 min
Concentration	(5.017 ± 0.000) e-1 mg/mL
dn/dc (mL/g)	0.1853
<input checked="" type="checkbox"/> Enabled Detectors	

	Value
Detector 8	<input checked="" type="checkbox"/>
Detector 9	<input checked="" type="checkbox"/>
Detector 10	<input checked="" type="checkbox"/>
Detector 11	<input checked="" type="checkbox"/>
Detector 12	<input checked="" type="checkbox"/>
Detector 13	<input checked="" type="checkbox"/>
Detector 14	<input checked="" type="checkbox"/>
Detector 15	<input checked="" type="checkbox"/>
Detector 16	<input type="checkbox"/>
Detector 17	<input type="checkbox"/>
Detector 18	<input type="checkbox"/>

results graph

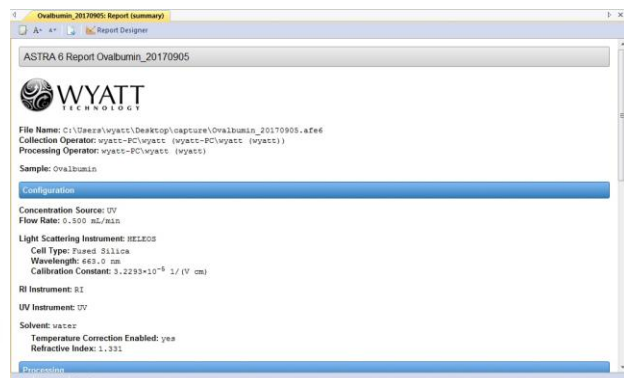
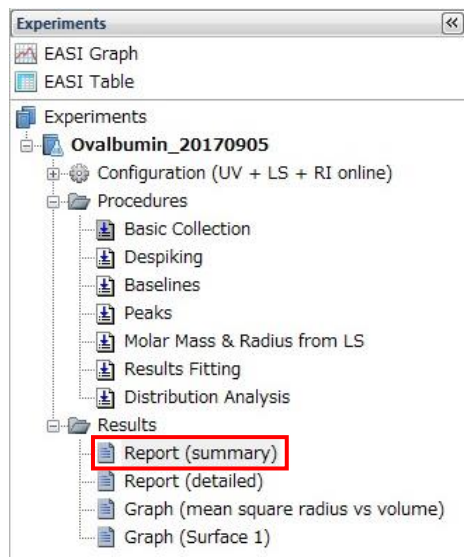
results graph



8 Report (output results with csv and PDF)

8-1 Displaying Report files

In the workspace, double-click [Results] → [Report (summary)] to display the Report (summary) tab.



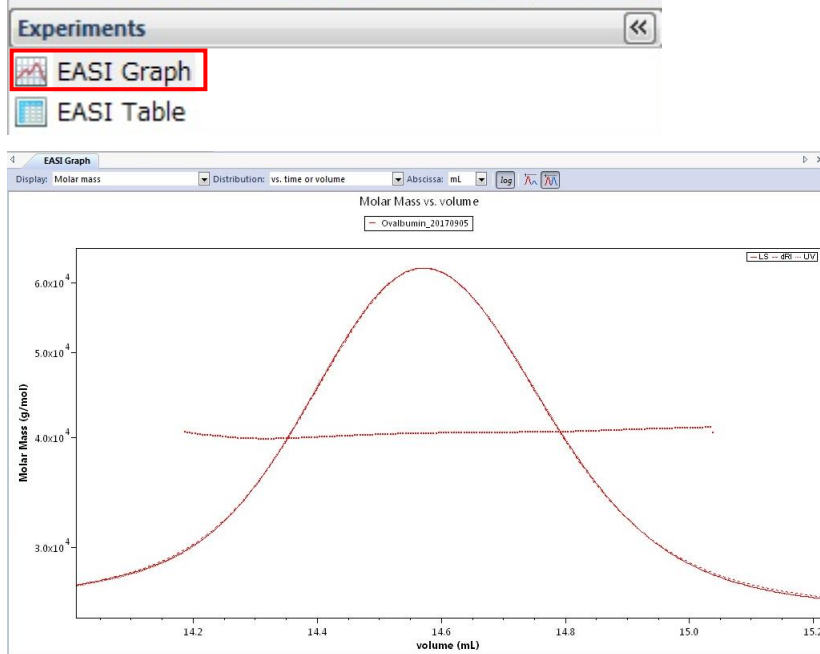
8-2 Saving Report file

In the menu bar, select [File] → [Print] to display the Print window. Select Microsoft

Print to PDF in the printer selection and click Print to output the Report file as PDF.

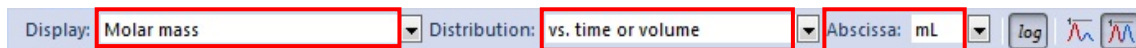
8-3 Saving csv text file

In the workspace, select [EASI Graph] to display the EASI Graph tab.



8-4 Checking the settings.

In the upper part of the EASI Graph tab, set that Display: "molar mass", Distribution: "vs. time or volume" and Abscissa: "mL".



8-5 "EASI Graph options" window

Right-click on the EASI Graph tab and select [Edit] to open "EASI Graph Options" window.

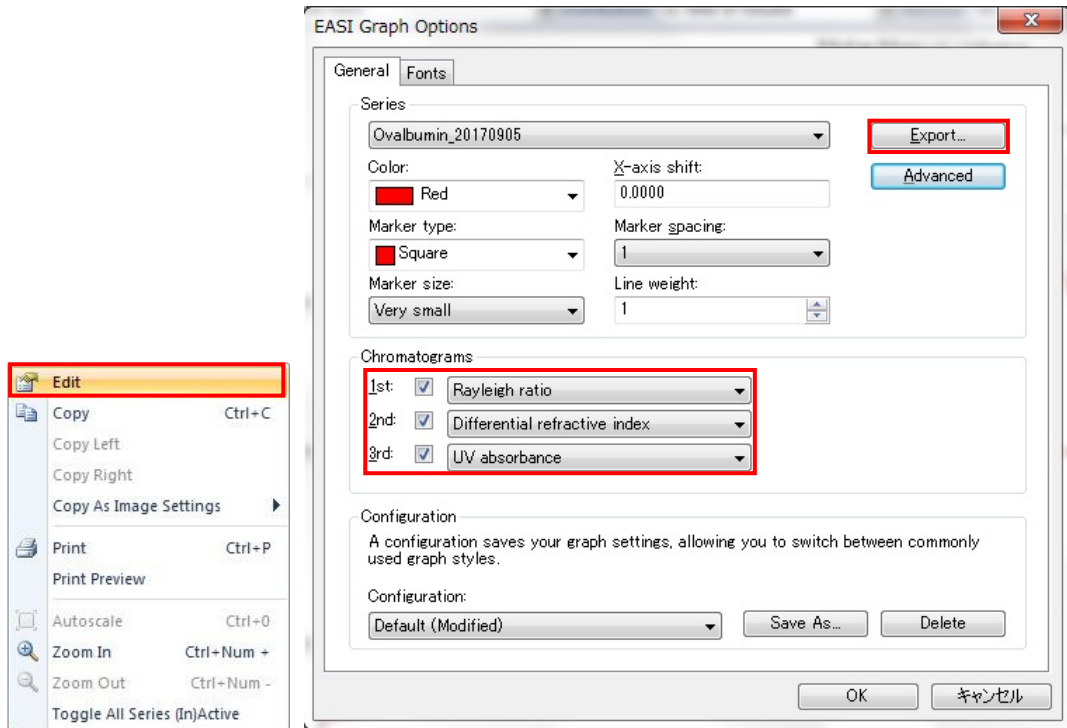
In "Chromatograms" area,

- Check 1st box and select "Rayleigh ratio"

- Check 2nd box and select "Differential refractive index"

- Check 3rd box and select "UV absorbance".

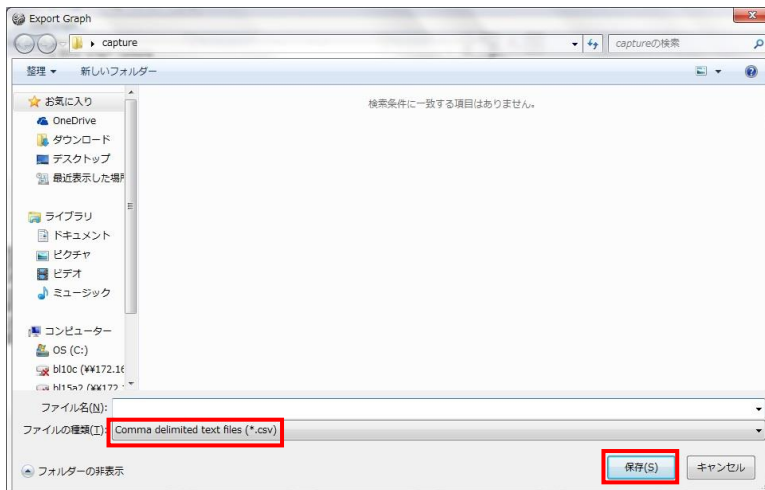
And then click [Export].



8-6 Saving results with csv format

Select csv format as file type (even if Excel format is selected, the output file could not be opened with excel and other editor software), enter folder and file name and click "[保存(S)]:Save" to save results.

Users can copy data to their own USB memory from the MALS PC.



test_20170905.csv - Excel

ファイル ホーム 挿入 ページレイアウト 数式 データ 校閲 表示 PDF

MS Pゴシック 11 A⁺ A⁻ 折り返して全体を表示する 標準

B I U 折り返して全体を表示する セルを結合して中央揃え %

グループボード フォント 配置 数値

J8

	A	B	C	D	E	F	G	H
1	volume (mL)	sample	volume (mL)	sample	volume (mL)	sample	volume (mL)	sample
2	-0.059398	0.0001071	-0.306267	0.0005638	0	0.0002876	14.186734	40554.512
3	-0.055303	0.0001144	-0.302172	0.0006783	0.0040949	0.0003035	14.190829	40511.008
4	-0.051208	0.0001211	-0.298077	0.0007615	0.0081898	0.0003191	14.194924	40467.2
5	-0.047113	0.0001269	-0.293982	0.0008302	0.0122847	0.0003343	14.199019	40423.212

Csv file after revising column name.

test_20170905.csv - Excel

ファイル ホーム 挿入 ページレイアウト 数式 データ 校閲 表示 PDF

MS Pゴシック 11 A⁺ A⁻ 折り返して全体を表示する 標準

B I U 折り返して全体を表示する セルを結合して中央揃え %

グループボード フォント 配置 数値

J1

	A	B	C	D	E	F	G	H
1	volume_LS (mL)	sample_LS	volume_dRI (mL)	sample_dRI	volume_UV (mL)	sample_UV	volume_Mw (mL)	sample_Mw
2	-0.05939805	0.000107095	-0.30626687	0.000563827	0	0.000287631	14.18673416	40554.51154
3	-0.05530316	0.00011441	-0.30217198	0.000678262	0.004094893	0.000303489	14.19082905	40511.00755
4	-0.05120827	0.000121081	-0.29807709	0.000761477	0.008189786	0.000319081	14.19492394	40467.19968
5	-0.04711338	0.000126862	-0.2939822	0.000830174	0.012284679	0.00033434	14.19901884	40423.21238
6	-0.04301848	0.00013155	-0.2898873	0.000862627	0.016379571	0.000349209	14.20311373	40380.00528

In csv file.

Column A:	volume (ml) [volume value of LS]
Column B:	xxx (sample name) [signal of LS]
Column C:	volume (ml) [volume value of dRI]
Column D:	xxx (sample name) [signal of dRI]
Column E:	volume (ml) [volume value of UV]
Column F:	xxx (sample name) [signal of UV]
Column G:	volume (ml) [volume value of Mw]
Column H:	xxx (sample name) [Mw]

Data are arranged every two columns. (Signals of LS, dRI and UV are scaled.)

[It is better to rename each data name of the 1st row to make it easier to understand later].