

How to adapt complex calculations in Clarity

This application note shows how to perform calculations in Clarity to avoid using external tools (Microsoft Excel, OpenOffice/LibreOffice Calc). An example of setting custom calculations in Clarity to evaluate an artemisinin assay and the content of impurities in artemisinin is included.

Introduction

Clarity Chromatography Software covers a wide range of standard chromatographic calculations of concentrations of unknown substances in samples based on calibration curves. However, it is also well suited to perform calculations based on external equations. Performing calculations (for example, from pharmacopoeias) in Clarity is more convenient than using external calculation tools (such as MS Excel), and it is also preferred by auditing bodies. This application note demonstrates the capabilities of Clarity Chromatography Software in relation to the creation of customized calculations.

Tools for custom calculations in Clarity

Let's imagine that we need to determine an assay of artemisinin, including the content of impurities. For these purposes, we will use two equations stated in pharmacopoeia and use them in Clarity. The first equation is mandatory for the determination of an assay of artemisinin, while the other equation is used for the determination of the contents of impurities.

Percent content, C, of artemisinin

$$C = \frac{PA_T \times m_R \times C_R}{PA_R \times m_T}$$

PA_T Peak area of artemisinin in the test solution
m_R Mass of artemisinin in the reference solution in mg
PA_R Peak area of artemisinin in the reference solution
m_T Mass of test substance in mg
C_R Concentration of the reference substance

Fig. 1 – Equation for artemisinin assay determination.

$$I = \frac{PA_T \times m_R \times Z_i \times 100}{PA_R \times m_T}$$

PA_T Peak area of artemisinin, 9-epi-artemisinin or any unspecified impurity in the test solution
m_R Mass of artemisinin in the reference solution in mg
Z_i UV response factor

impurity	UV response factor (Z _i)
Artemisinin	0.03
9-epi-artemisinin	0.8
unspecified	1.00

PA_R Peak area of artemisinin in reference solution
m_T Mass of test substance in mg

Total the values obtained for any unspecified impurity
 Total the values obtained for all impurities
 Disregard values < 0.05% (reporting limit)

Fig. 2 – Equation for determination of the content of impurities

The first equation defines how to determine the percentual content (assay) of artemisinin (Fig. 1). Fig. 3 displays the result calculated in Clarity. In order to be able to perform such calculations in Clarity you need to prepare relevant calibration. In this case, the calibration is called "Assay," and its detailed setting can be reviewed in Fig. 6. It is necessary to insert a specific amount (mass) of the test substance into the Amount ① field on the right side of the Chromatogram window. The correctness of the calculations in Clarity was verified against the same calculations done in MS Excel. The result sheet from MS Excel can be downloaded from the following link: [Custom-calculations-in-Clarity-data.zip](#).

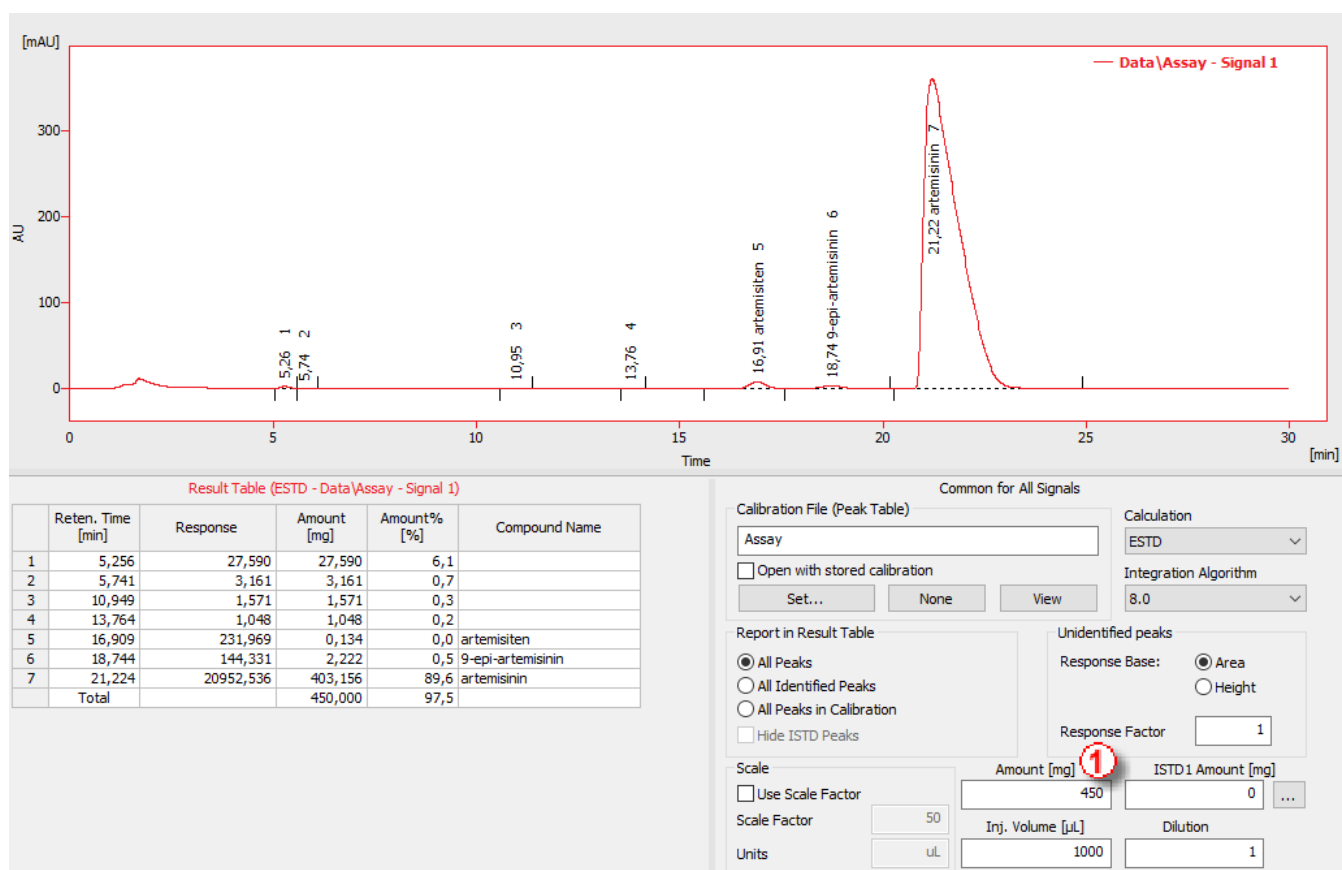


Fig. 3 – Customized assay calculation

The equation in Fig. 2 defines the determination of the percentage content of artemisiten, 9-epi-artemisinin, and all other unspecified impurities. This equation introduces response factors that can be set in the calibration file as correction factors ② (Fig. 4). The correction factors are to be set in the respective calibration file; in this case, the calibration file is called "Impurities." As the impurities content calculation is related to artemisinin, it is necessary to add this relation into the calibration file. This can be done when applying the "Calculated By" ③ column and selecting artemisinin as the calculation basis. See the setting of the related calibration file in Fig. 4.

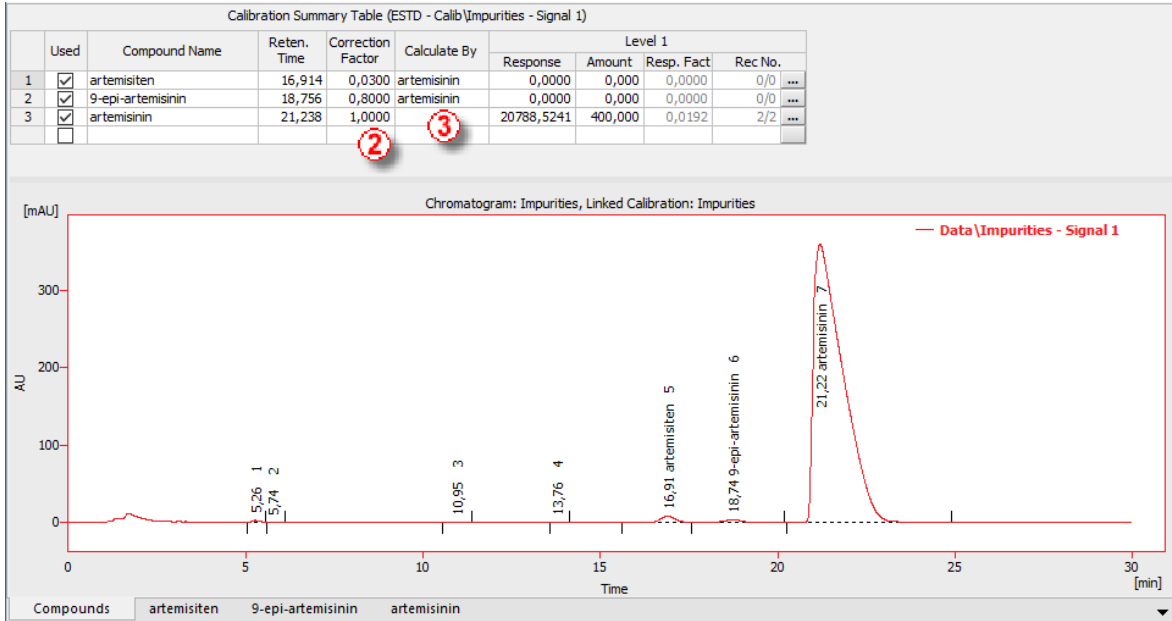


Fig. 4 – Calibration for determination of percentual content of impurities.

This calibration should be linked to the sample chromatogram, and it is necessary to amend a few more settings within the chromatogram window to perform the calculations within Clarity in correspondence with the given equations. The adjustments should be inserted in the calculation pane on the right side of the result table. It is necessary to fill in the sample weight in the Amount ① field. To select artemisinin as the calculation basis of the given equation, it is necessary to copy the response factor of artemisinin from the chromatogram Result Table (Response Factor column ④ a) to the Response Factor field ④ b) in the Calculation pane. The described setting is shown in Fig. 5.

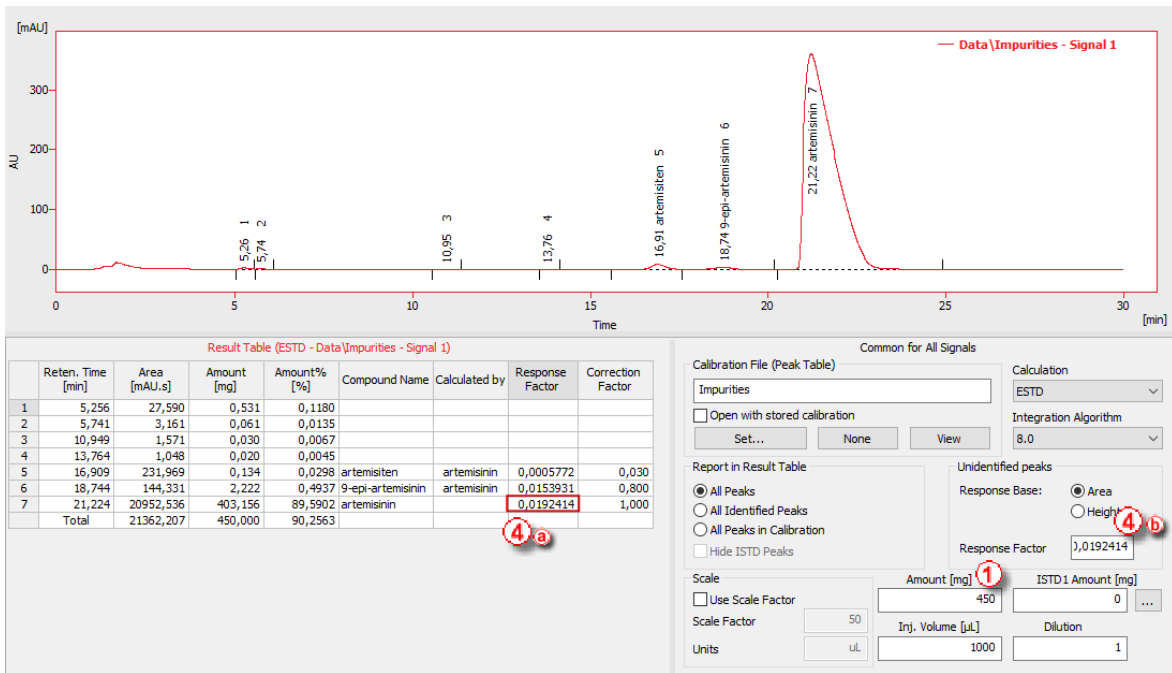


Fig. 5 – Calculated results for percent content determination of the impurities

The correctness of the calculations in Clarity was again verified against the same calculations done in MS Excel. The results can be reviewed in Fig. 7 or in the corresponding Excel sheet, which can be downloaded from the following link: [Custom-calculations-in-Clarity-data.zip](#).

Conclusion

We have demonstrated that Clarity is equipped with powerful tools for creating customized calculations, which can be based on difficult external equations. This approach can be utilized in order to avoid the use of external calculation tools, which may not be accepted by, e.g., auditing bodies in specific industries, such as in the pharmaceutical industry.

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Excel

Calibration - Double injection of standard solution

	Reten. Time [min]	Area [mAU.s]	Response (Area)
1 st STD	21,24	20785,0545	20788,5241 = ϕ
2 nd STD	21,24	20791,9937	

Sample - Data from chromatogram

Reten. Time [min]	Area [mAU.s]	Amount [mg]	Amount% [%]	Compound Name
1	5,256	27,590	27,59	6,13
2	5,741	3,161	3,161	0,70
3	10,949	1,571	1,571	0,35
4	13,764	1,048	1,048	0,23
5	16,909	231,970	0,134	0,03 Artemisiten
6	18,744	144,331	2,222	0,49 9-epi-artemisinin
7	21,224	20952,536	403,156	89,59 artemisinin
Total		21362,207	450	97,52

Annotations: C_R (400), m_R (400), PA_R (20788,5241), PA_T (20952,536), m_T (450), C (89,52)

Clarity

Instrument 1 - Calibration Assay <-- ESTD

Used	Compound Name	Reten. Time	Left Window	Right Window	Resp. Factor	Response	Amount	Resp. Fact.
1	artemisenin	16,914	0,200 min	0,200 min	0,0000	0,0000	0,0	0,00000000
2	9-epi-artemisinin	18,756	0,200 min	0,200 min	0,0000	0,0000	0,0	0,00000000
3	artemisinin	21,238	0,200 min	0,200 min	0,0000	20788,5241	400,0	0,0192414

Chromatogram: Impurities, Linked Calibration: Impurities

Annotations: PA_R , m_R , PA_T , m_T , C

Reten. Time [min]	Response	Amount [mg]	Amount% [%]	Compound Name
1	5,256	27,590	27,59	6,13
2	5,741	3,161	3,16	0,70
3	10,949	1,571	1,57	0,35
4	13,764	1,048	1,05	0,23
5	16,909	231,969	0,13	0,03 artemisenin
6	18,744	144,331	2,22	0,49 9-epi-artemisinin
7	21,224	20952,536	403,15	89,59 artemisinin
Total		450,00	97,53	

Annotations: PA_T , m_T , C

Calculation: Amount [mg] = 450, ISTD1 Amount = 0, Scale Factor = 50, Inj. Volume [mL] = 1, Dilution = 1, Units = μ L

Original equation

Percent content, C, of artemisinin

$$C = \frac{PA_T \times m_R \times C_R}{PA_R \times m_T}$$

PA_T Peak area of artemisinin. in the test solution
 m_R Mass of artemisinin in the reference solution in mg
 PA_R Peak area of artemisinin in the reference solution
 m_T Mass of test substance in mg
 C_R Concentration of the reference substance

Fig. 6 – Artemisinin assay determination – calculation scheme

Excel

Calibration - Double injection of standard solution

Reten. Time [min]	Area [mAU.s]	Response (Area)
1 st STD	21,24	20785,055
2 nd STD	21,24	20791,994
		20788,5241

Sample - Data from chromatogram

Reten. Time [min]	Area [mAU.s]	Amount [mg]	Amount% [%]	Compound Name	Calculated	Response	Correction Factor	Compound Name
1	5,256	27,590	0,1180				0,019241	
2	5,741	3,161	0,0135				0,019241	
3	10,949	1,571	0,0067				0,019241	
4	13,764	1,048	0,0045				0,019241	
5	16,909	231,970	0,134	Artemisiten	artemisinin	0,000577	0,03	Artemisiten
6	18,744	144,331	0,4937	9-epi-artemisinin	artemisinin	0,015393	0,8	9-epi-artemisinin
7	21,224	20952,536	89,5902	API	artemisinin	0,019241	1	artemisinin
Total		21362,207	450					

Original equation

Calculation Percent content, I, of artemisiten, 9-epi-artemisinin and of any unspecified impurity

$$I = \frac{PA_T \times m_r \times Z_i \times 100}{PA_A \times m_r}$$

PA_T Peak area of artemisiten, 9-epi-artemisinin or any unspecified impurity in the test solution

m_r Mass of artemisinin in the reference solution in mg

Z_i UV response factor

impurity	UV response factor (Z _i)
Artemisiten	0,03
9-epi-artemisinin	0,8
unspecified	1,00

PA_A Peak area of artemisinin in reference solution

m_r Mass of test substance in mg

Total the values obtained for any unspecified impurity
Total the values obtained for all impurities
Disregard values < 0.05% (reporting limit!)

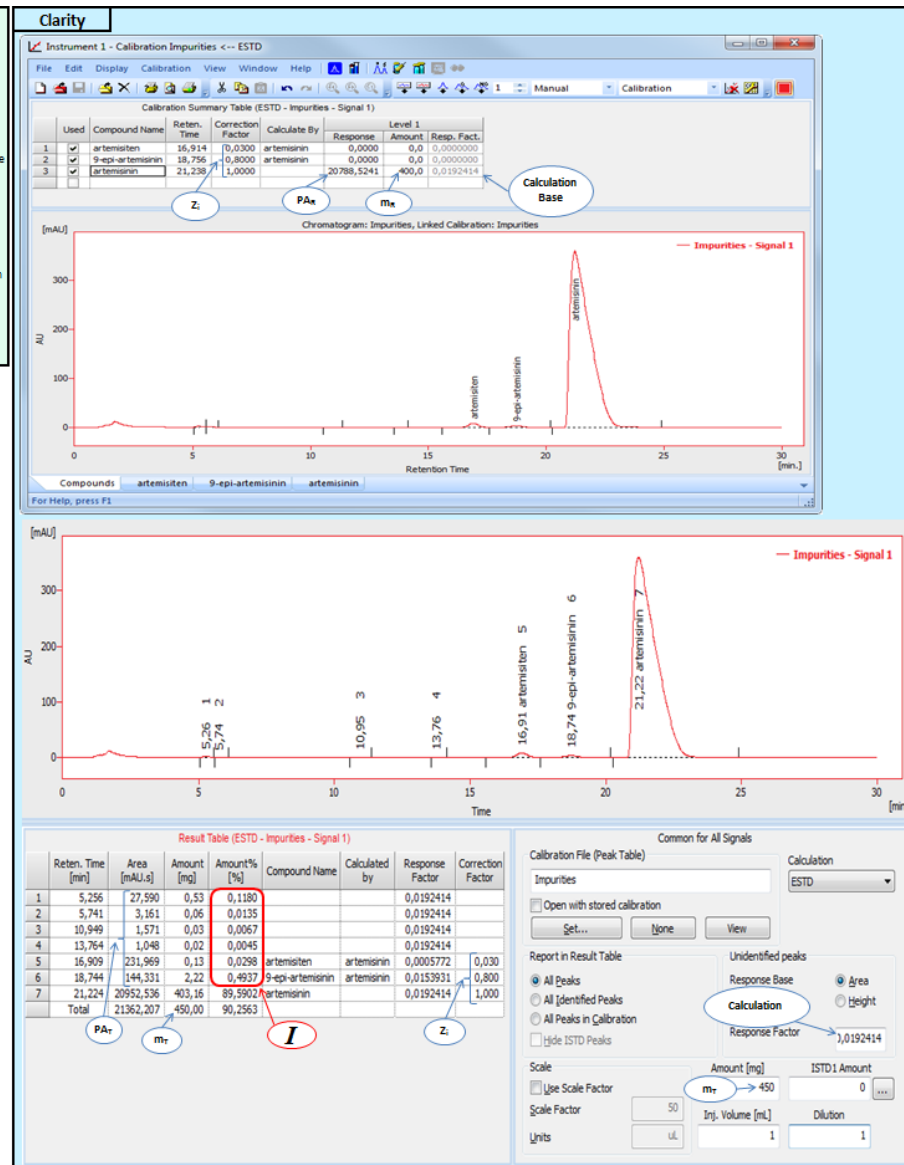


Fig. 7 – Percentual content of impurities determination – calculation scheme