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 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_R Peak area of artemisinin in reference solution

m_T Mass of test substance in mg

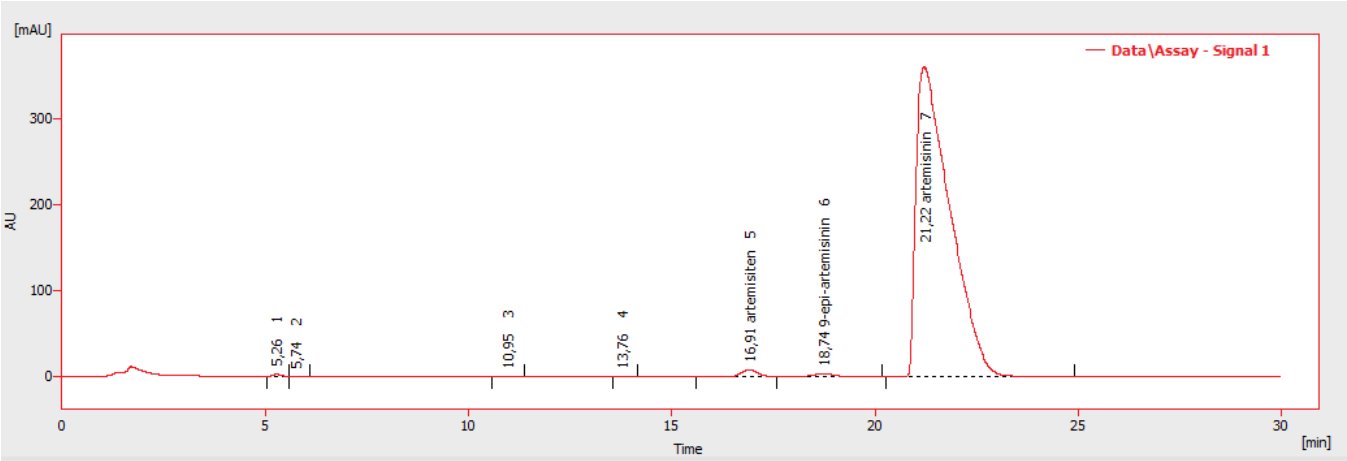
Total the values obtained for any unspecified impurity

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.



Result Table (ESTD - Data\Assay - Signal 1)

	Reten. Time [min]	Response	Amount [mg]	Amount% [%]	Compound Name
1	5,256	27,590	27,590	6,1	
2	5,741	3,161	3,161	0,7	
3	10,949	1,571	1,571	0,3	
4	13,764	1,048	1,048	0,2	
5	16,909	231,969	0,134	0,0	artemisiten
6	18,744	144,331	2,222	0,5	9-epi-artemisinin
7	21,224	20952,536	403,156	89,6	artemisinin
	Total		450,000	97,5	

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.

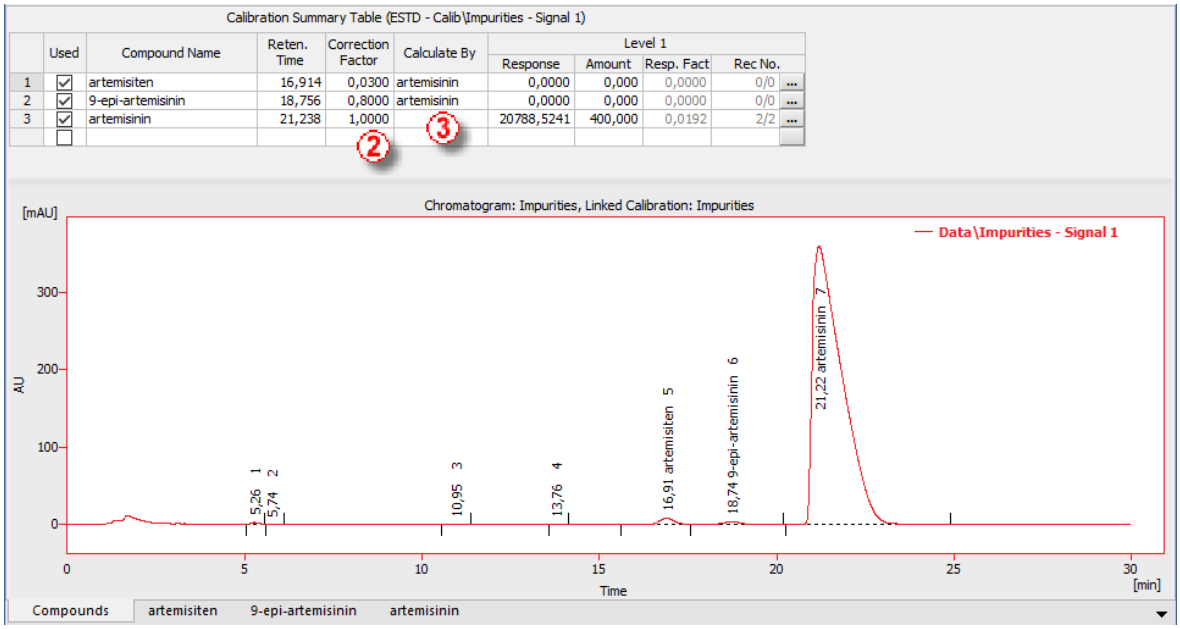


Fig. 4 – Calibration for determination of percentual content of impurities.

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ⓐ) to the Response Factor field ④ⓑ in the Calculation pane. The described setting is shown in Fig. 5.

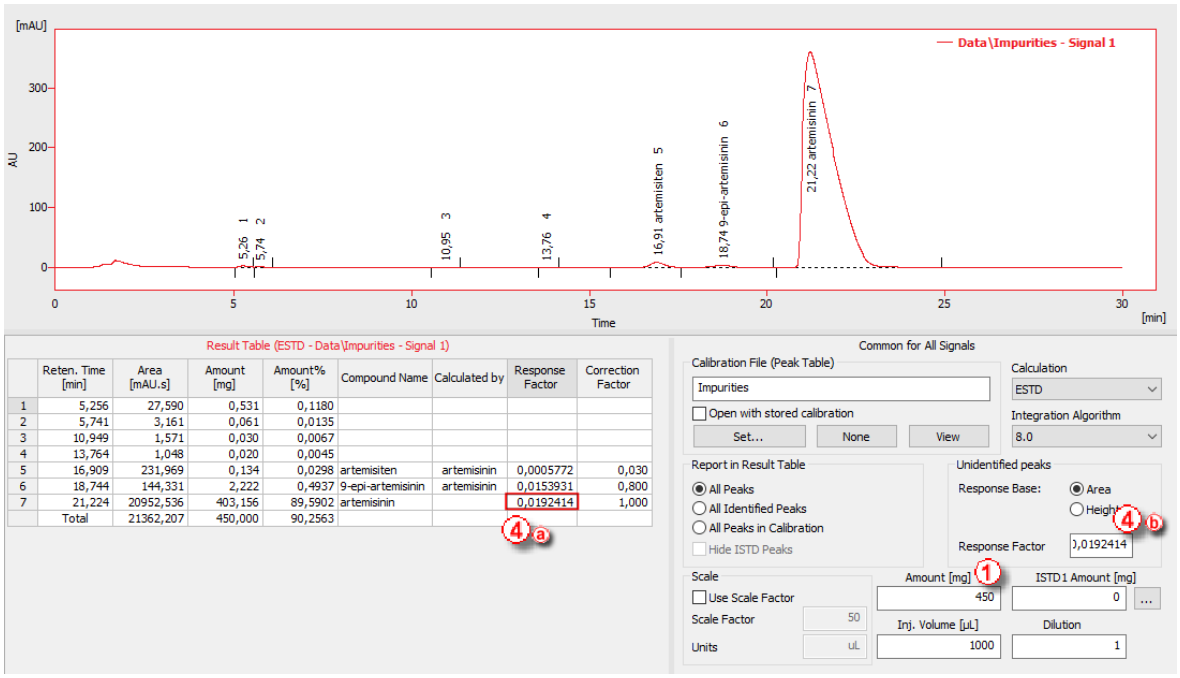


Fig. 5 – Calculated results for percent content determination of the impurities

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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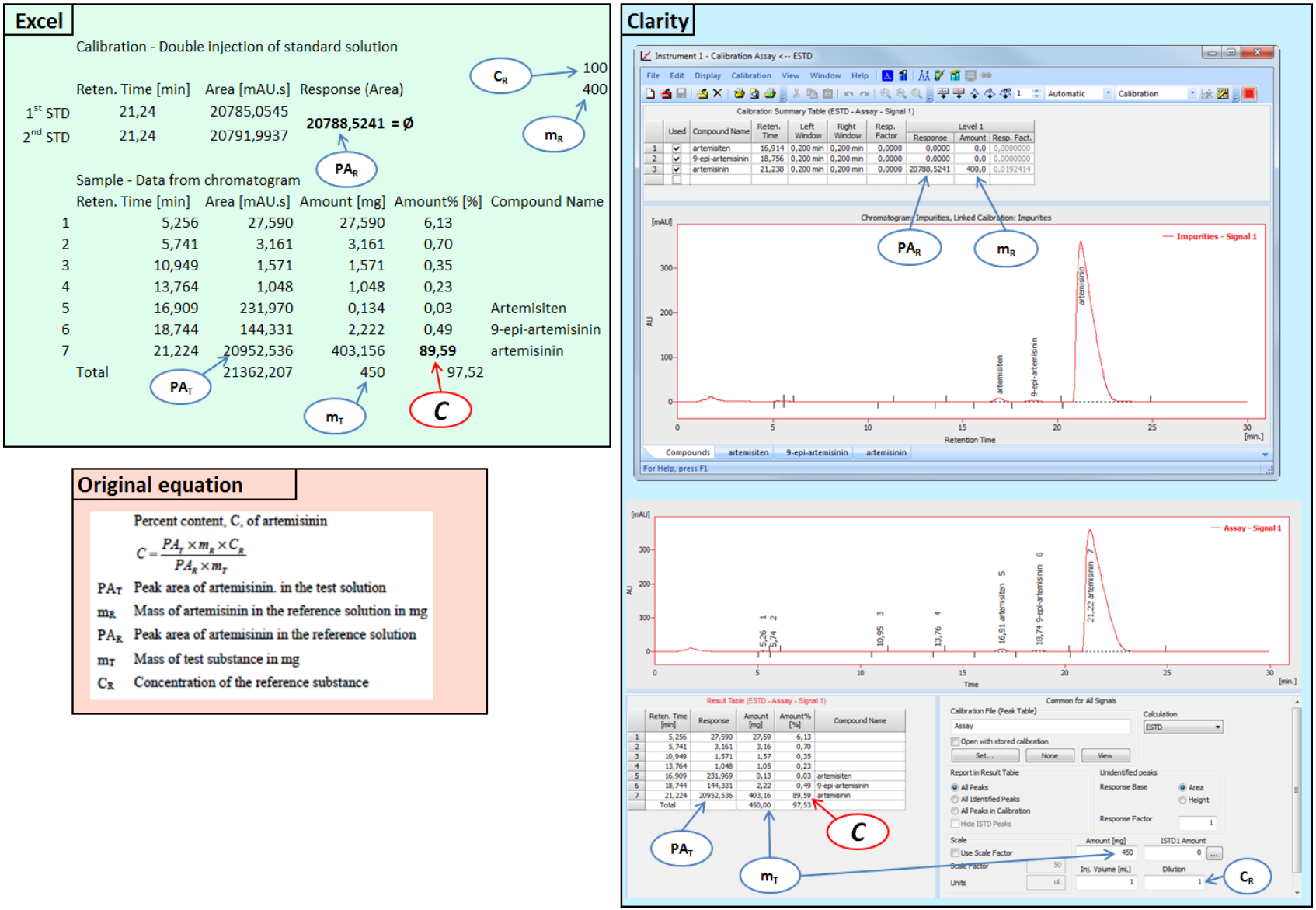

Fig. 6 – Artemisinin assay determination – calculation scheme

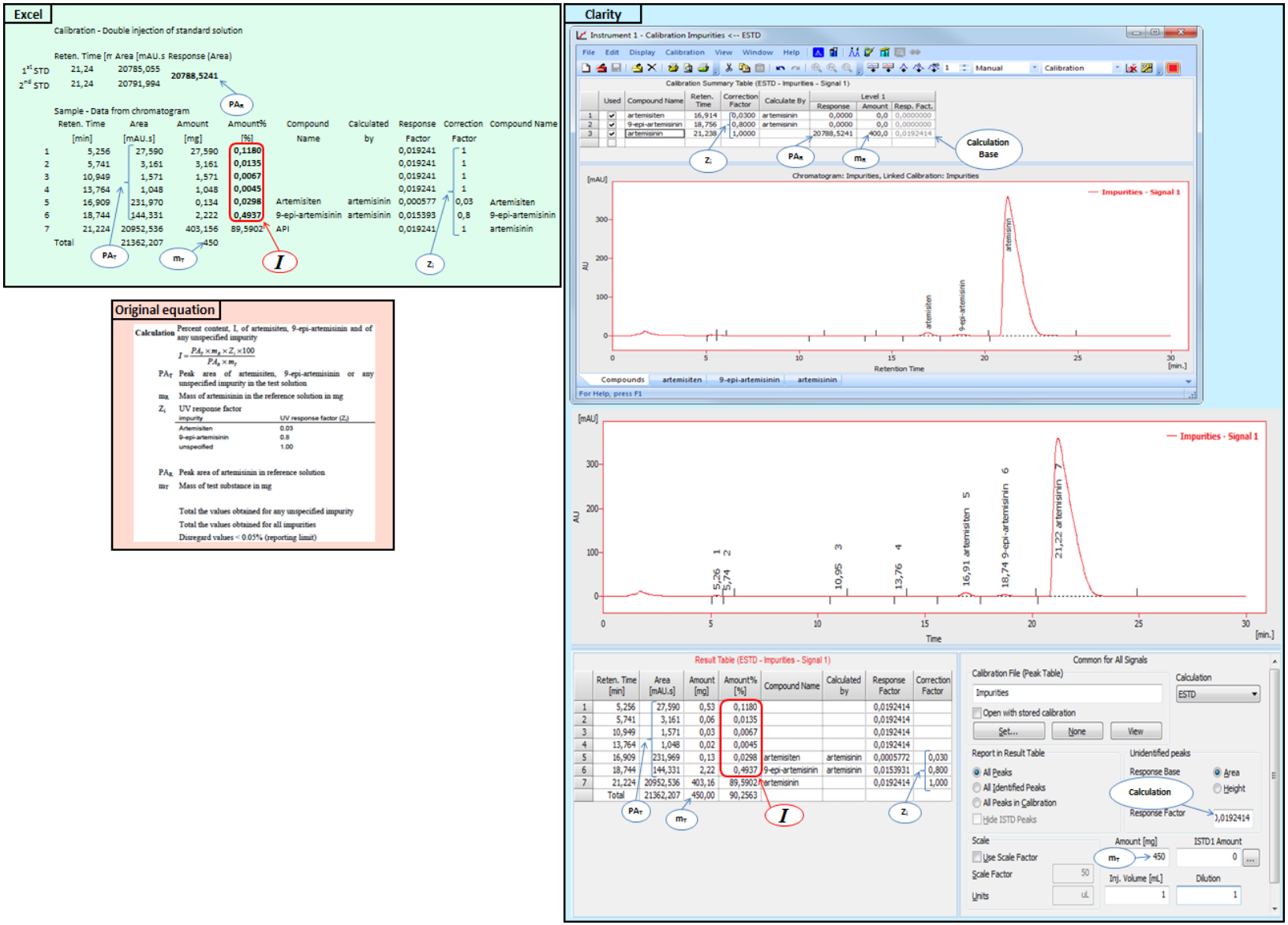

Fig. 7 – Percentual content of impurities determination – calculation scheme