How to adapt difficult calculations in Clarity

Application example of setting customized calculations in Clarity for evaluating Artemisinin assay and content of impurities in Artemisinin. This application note illustrates how to calculate results directly in Clarity therefore avoiding the use of external calculation tools such as Microsoft Excel or OpenOffice/LibreOffice Calc.

Introduction

Clarity chromatography station covers wide range of standard chromatographic calculations of concentrations of the unknown substances in samples based on calibration curve. However it is also well suited to perform calculations based on external equations. Performing the 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 creation of customized calculations.

Tools for custom calculations in Clarity

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

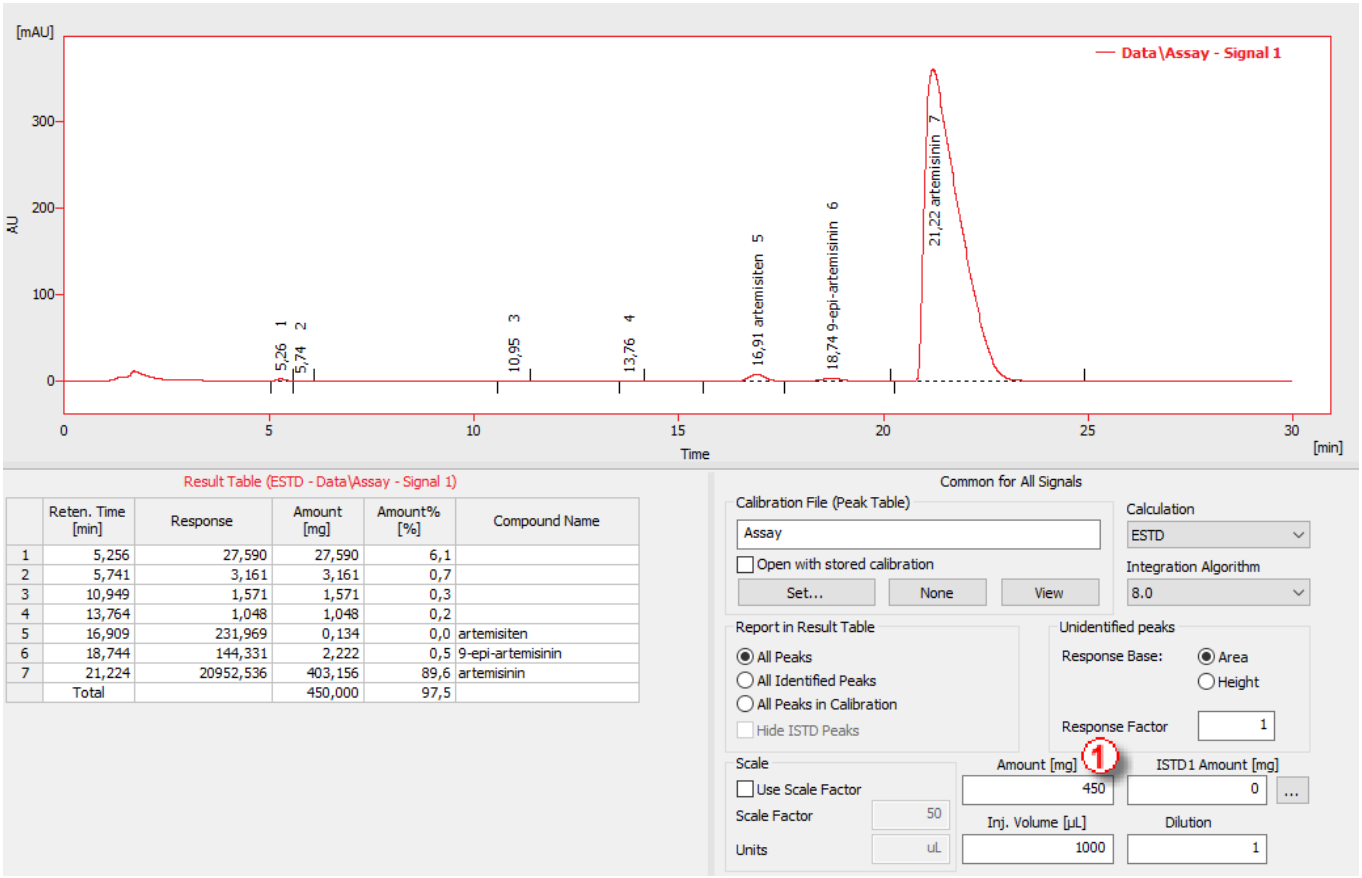
impurity	UV response factor (Z_i)
Artemisiten	0.03
9-epi-artemisinin	0.8
unspecified	1.00

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

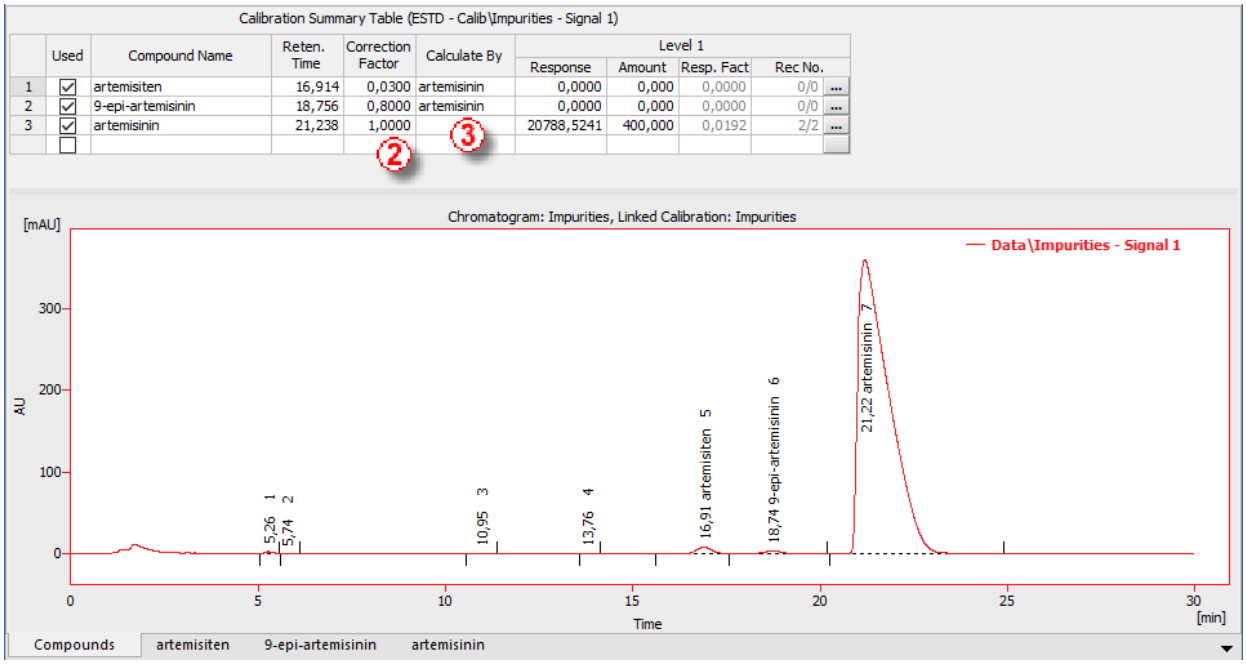
The first equation defines how to determine percent content (assay) of artemisinin, on the figure 3 is displayed result calculated in Clarity. In order to be able to perform such calculations in Clarity you need to prepare related calibration. In this case the calibration is called "Assay" and its detailed setting can be reviewed in the figure 6. It is necessary to insert specific amount (mass) of the test substance into Amount ① field on the right side of 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 following link: Custom-calculations-in-Clarity-data.zip.



	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

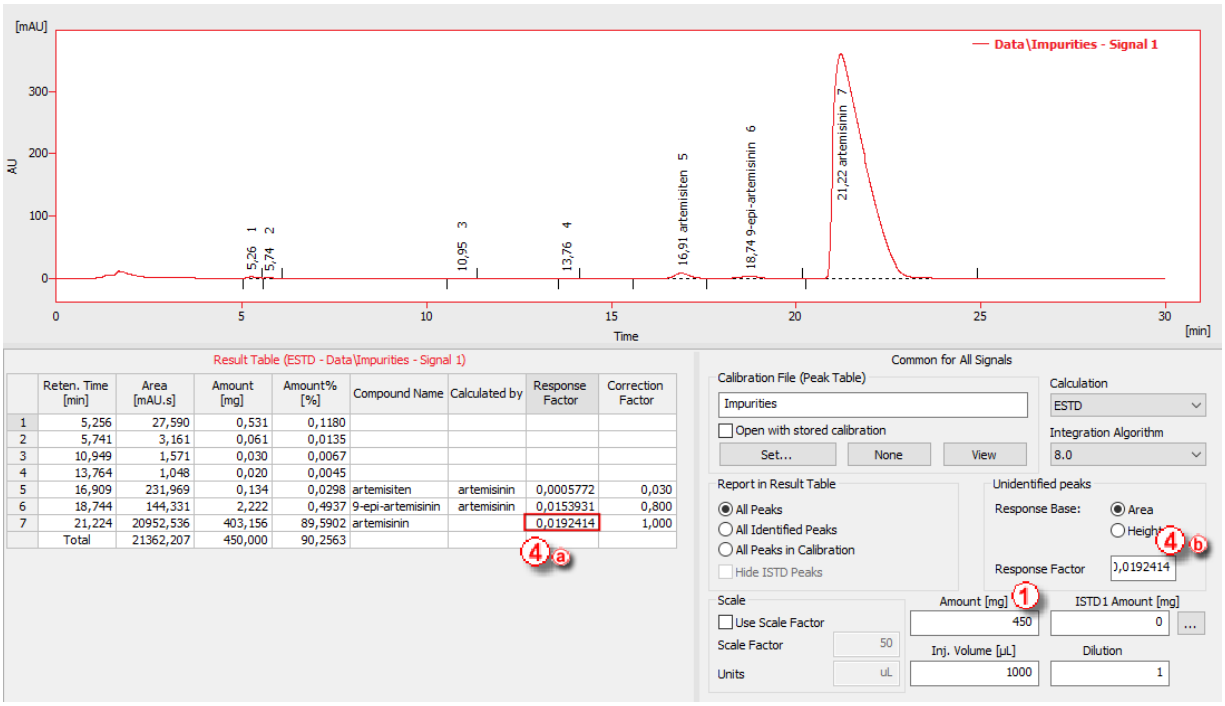
The second equation defines determination of the percentage content of artemisiten, 9-epi-artemisinin and all other unspecified impurities. This equation introduces response factors that can be set in calibration file as correction factors ②. The correction factors are about to be set in the respective 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 calibration file. This can be done when applying "Calculated By" ③ column where is about to be selected artemisinin as a calculation basis. See the setting of the related calibration file on the figure 4.



	Used	Compound Name	Reten. Time	Correction Factor	Calculate By	Level 1 Response	Level 1 Amount	Level 1 Resp. Fact	Level 1 Rec No.
1	☑	artemisiten	16,914	0,0300	artemisinin	0,0000	0,000	0,0000	0/0
2	☑	9-epi-artemisinin	18,756	0,8000	artemisinin	0,0000	0,000	0,0000	0/0
3	☑	artemisinin	21,238	1,0000		20788,5241	400,000	0,0192	2/2
	☐								

Fig. 4 – Calibration for percent content determination of the impurities.

This calibration should be linked to the sample chromatogram and it is necessary to amend a couple more settings within the chromatogram window to make Clarity to calculate in correspondence with given equation. 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. As a calculation basis of the given equation is artemisinin substance it is necessary to copy the response factor of the artemisinin substance from the chromatogram Result Table (Response Factor column ④ⓐ) to the Response Factor field ④ⓑ in the Calculation pane. The described setting is shown on the figure 5.



	Reten. Time [min]	Area [mAU.s]	Amount [mg]	Amount% [%]	Compound Name	Calculated by	Response Factor	Correction Factor
1	5,256	27,590	0,531	0,1180				
2	5,741	3,161	0,061	0,0135				
3	10,949	1,571	0,030	0,0067				
4	13,764	1,048	0,020	0,0045				
5	16,909	231,969	0,134	0,0298	artemisiten	artemisinin	0,0005772	0,030
6	18,744	144,331	2,222	0,4937	9-epi-artemisinin	artemisinin	0,0153931	0,800
7	21,224	20952,536	403,156	89,5902	artemisinin		0,0192414	1,000
	Total	21362,207	450,000	90,2563				

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

We have again verified the correctness of the calculations in Clarity against the same calculations done in MS Excel. The results can be reviewed on the figure 7 or in available Excel sheet that 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 even based on difficult external equations. This approach can be utilized in order to avoid usage of external calculation tools that may be difficult to be accepted for example by auditing bodies in specific industries such as pharmaceutical industry.

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Excel

Calibration - Double injection of standard solution

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

C_R → 100; 400 → m_R; PA_R

Sample - Data from chromatogram

	Reten. Time [min]	Area [mAU.s]	Amount [mg]	Amount% [%]	Compound Name
1	5,256	27,590	27,590	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	

PA_T; m_T; **C**

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

Clarity

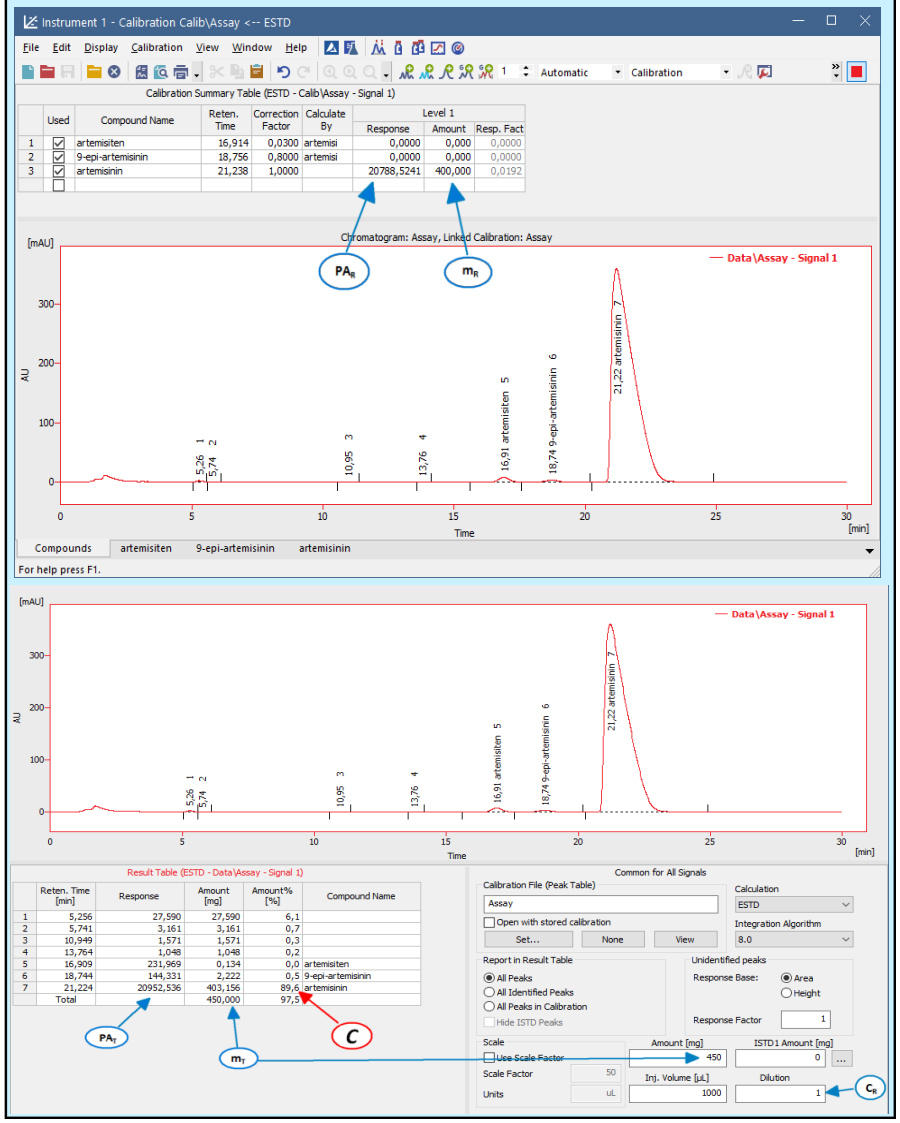

Fig. 6 – Artemisinin assay determination – calculation scheme

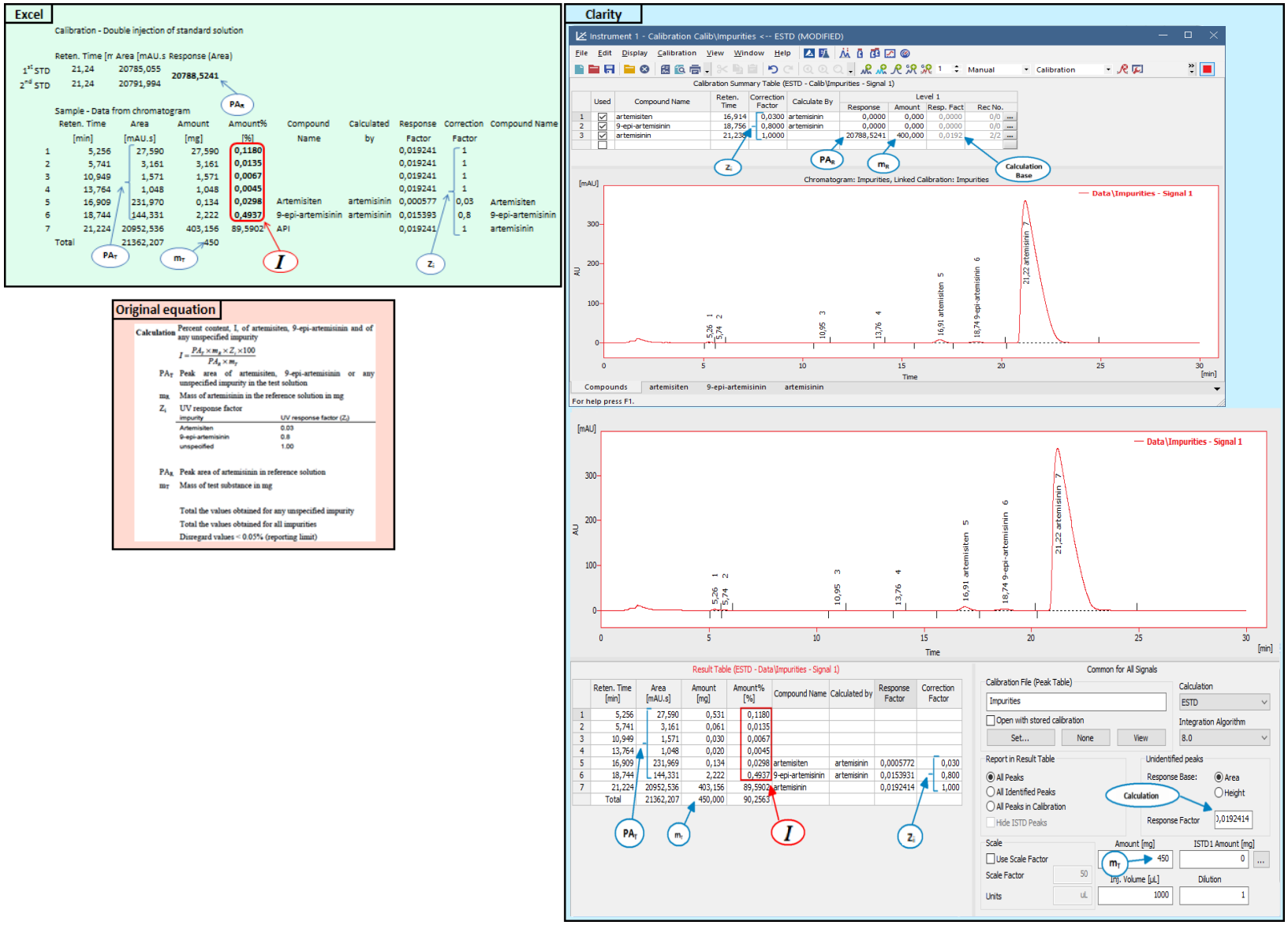

Fig. 7 – Percent content of impurities determination – calculation scheme