Integration Algorithms in Clarity

Introduction

This document presents:

- a comparison of integration algorithms (IAs) available in *Clarity*,
- a generally proposed workflow while integrating chromatograms,
- description of functions that are available only in the *Wave* algorithm.

Integration algorithms are a crucial component of any chromatography software. They identify peaks in a raw chromatography curve, assign integration marks (starts, ends, apexes) and determine the peak baseline. Although it is the responsibility of the operator to verify correct peak integration after the analysis is finished, an automated peak detection facilitated by the integration algorithm can significantly streamline the workflow.

Clarity, from version 9.0, offers two integration algorithms, the original algorithm renamed to „*Legacy*" and a newly introduced algorithm named „*Wave*" which evolved from an experimental algorithm present since version 7.0. The *Wave* algorithm will be regularly updated with new functions, settings and bug fixes of discovered issues (updates for *Legacy* will only be released if a critical bug is discovered). Changes in IAs are always versioned and distinguished by records in the audit trail.

Choosing an integration algorithm

Users can select either the *Legacy* or *Wave* integration algorithm for peak detection. The selection drop-down has been relocated to the *Integration* tab in both the *Chromatogram* window and the *Method Setup* dialog, highlighting the interconnection between the algorithm and the integration table.

The introduction of the *Wave* algorithm does not affect existing results or methods, as they will remain set to *Legacy* unless manually altered. If a method was created using an older version of any of the integration algorithms and was not changed after Clarity update to newer version, latest version of the same algorithm will be used in any newly measured chromatograms. This change will be logged in these chromatograms upon creation. To address potential issues, DataApex aims to use the algorithm version with the most bug fixes. Users aren't required to resave their methods, but if they do, the change will be logged in the method audit trail.

One way to apply the integration algorithm to multiple chromatograms simultaneously is through the *Batch* dialog (not applicable to the *Clarity Lite* version).

General Comparison

Neither algorithm can be universally deemed superior; some situations may warrant the use of one over the other. Below are some examples, presuming that at least *Global Peak Width* and *Global Threshold* operations were performed on the signal. If you encounter any issues with either of the integration algorithms, please inform DataApex via support@dataapex.com.

Advantages of Wave IA:

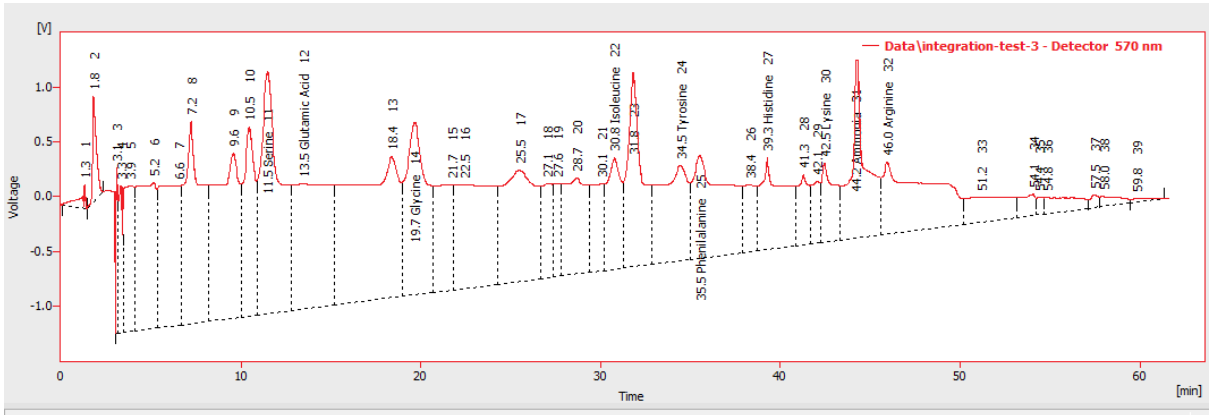
- Handling of negative spikes/peaks in regard to baseline placement.
- Handling of shifting baseline/signal.
- Separation of unresolved peaks.

Disadvantages of Wave IA:

- More sensitive to noise, requiring the use of *Bunching* or another filter in some cases.
- Incorrect integration when peaks of significantly different widths are present (typical for GPC or EA).

Negative spikes/peaks

When a chromatogram contains significant negative peaks or spikes which should not be evaluated, the default integration in the *Wave* algorithm will produce satisfactory results, while the *Legacy* algorithm will not. (In *Legacy* this problem could be solved by applying several Integration Intervals.)



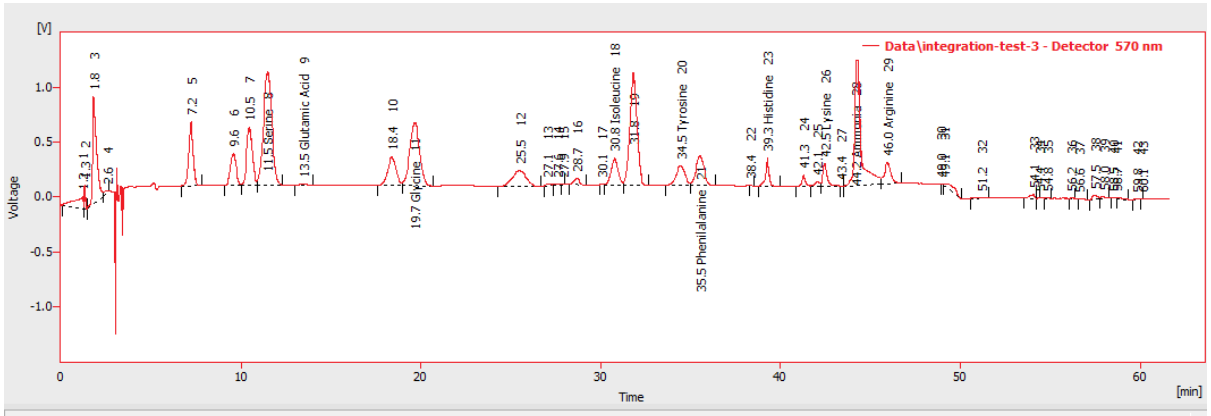
Integration Algorithm (Common for All Signals) Legacy

Integration Table

Data\integration-test-3 - Detector 570 nm, Integration Algorithm: Legacy (9.0 rev. 1)

Chromatogram Operation	Time A [min]	Time B [min]	Value
Global Peak Width			0.234 min
Global Threshold			1.1442 mV
Global Filter - Bunching			6

Figure 1. Integration by Legacy IA, it considers the negative spike to be a start of the peak (or peak cluster).



Integration Algorithm (Common for All Signals) Wave

Integration Table

Data\integration-test-3 - Detector 570 nm, Integration Algorithm: Wave rev. 1

Chromatogram Operation	Time A [min]	Time B [min]	Value
Global Peak Width			0.234 min
Global Threshold			1.1442 mV
Global Filter - Bunching			6
Global Baseline Slope			0.000 mV/min

Figure 2. Integration by Wave IA with the same parameters.

Shifting signal/baseline

Similar to the negative peaks/spikes, the same situation may arise with a shifting baseline where the peaks should be evaluated to that baseline. *Legacy* algorithm will provide unsatisfactory integration. In *Legacy* it could be solved by applying *Baseline Valley* to several places and combining it with *Integration Interval*.

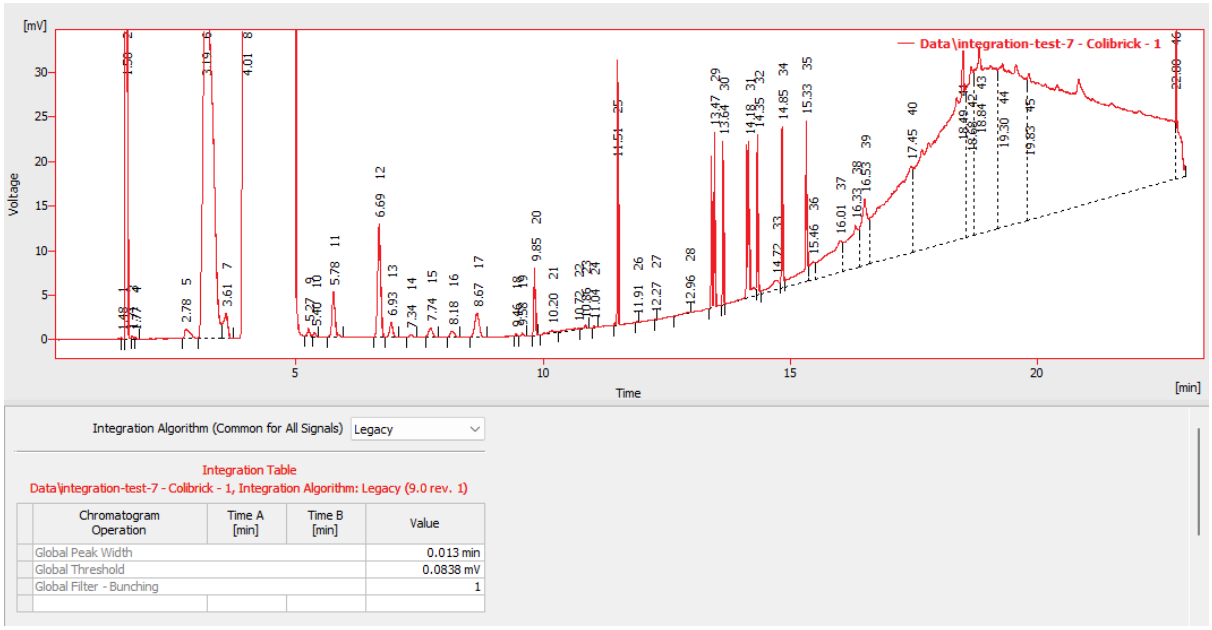


Figure 3. Integration by Legacy IA.

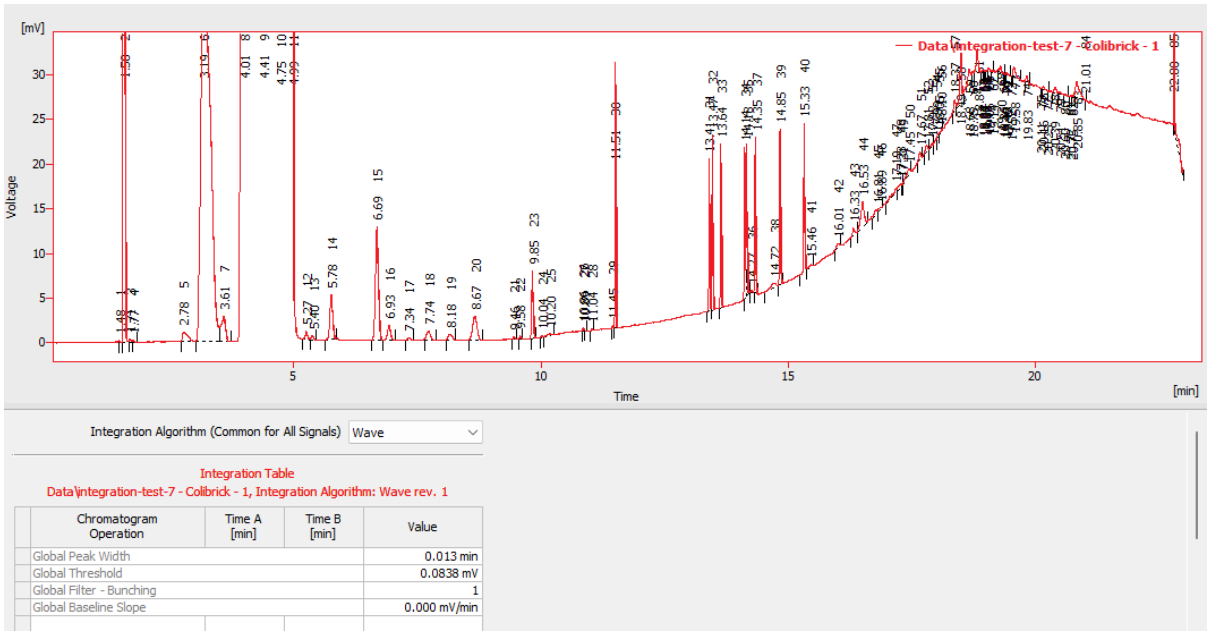
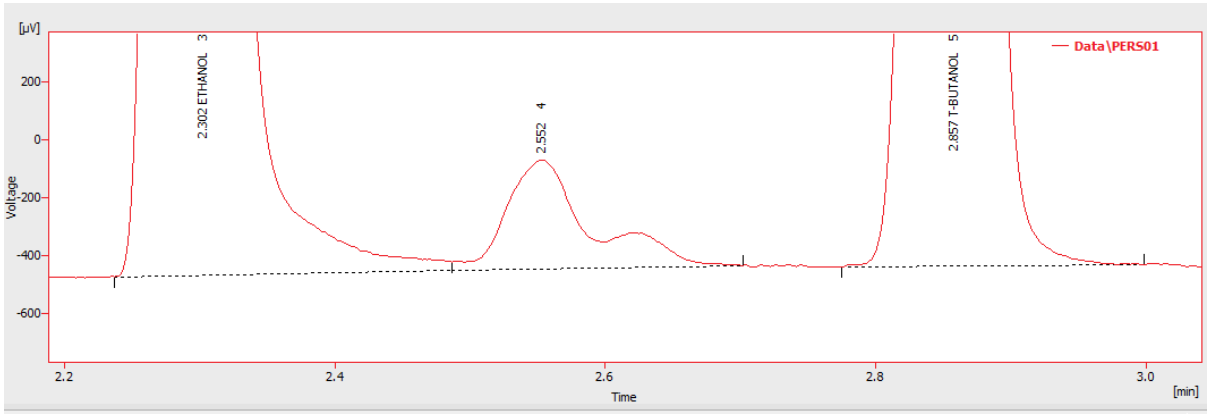


Figure 4. Integration by Wave IA with the same parameters, fairly high number of peaks is caused by low value of Global Threshold.

Unresolved Peaks

In *Legacy* algorithm if a peak does not fulfill some of its criteria (it is too narrow or the valley to other non-resolved peaks is too low) the peak gets joined to a neighboring bigger peak, whereas *Wave* algorithm evaluates the valley depths much better and provides the peaks separated automatically, even in cases where the small peak does not fulfill some of the criteria. Small impurities thus do not influence the area of the bigger peak of interest.

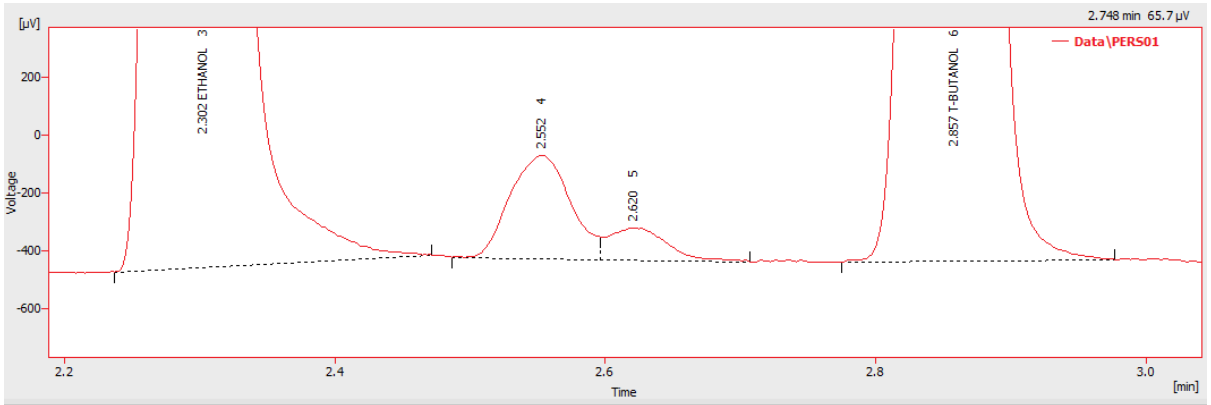


Integration Algorithm (Common for All Signals) Legacy

Integration Table
Data\PERS01, Integration Algorithm: Legacy (9.0 rev. 1)

Chromatogram Operation	Time A [min]	Time B [min]	Value
Global Peak Width			0.020 min
Global Threshold			0.0300 mV
Global Filter - Bunching			1

Figure 5. Integration by Legacy IA.



Integration Algorithm (Common for All Signals) Wave

Integration Table
Data\PERS01, Integration Algorithm: Wave rev. 1

Chromatogram Operation	Time A [min]	Time B [min]	Value
Global Peak Width			0.020 min
Global Threshold			0.0300 mV
Global Filter - Bunching			1
Global Baseline Slope			0.000 mV/min

Figure 6. Integration by Wave IA with the same parameters.

Significant noise

In some cases, the signal noise may be very significant and the set threshold value in the *Wave* algorithm will be so low that the peak will be split into several peaks. In the example below you can see that neither of IA provides with just *Global Peak Width* and *Global Threshold* and further operations must be used.

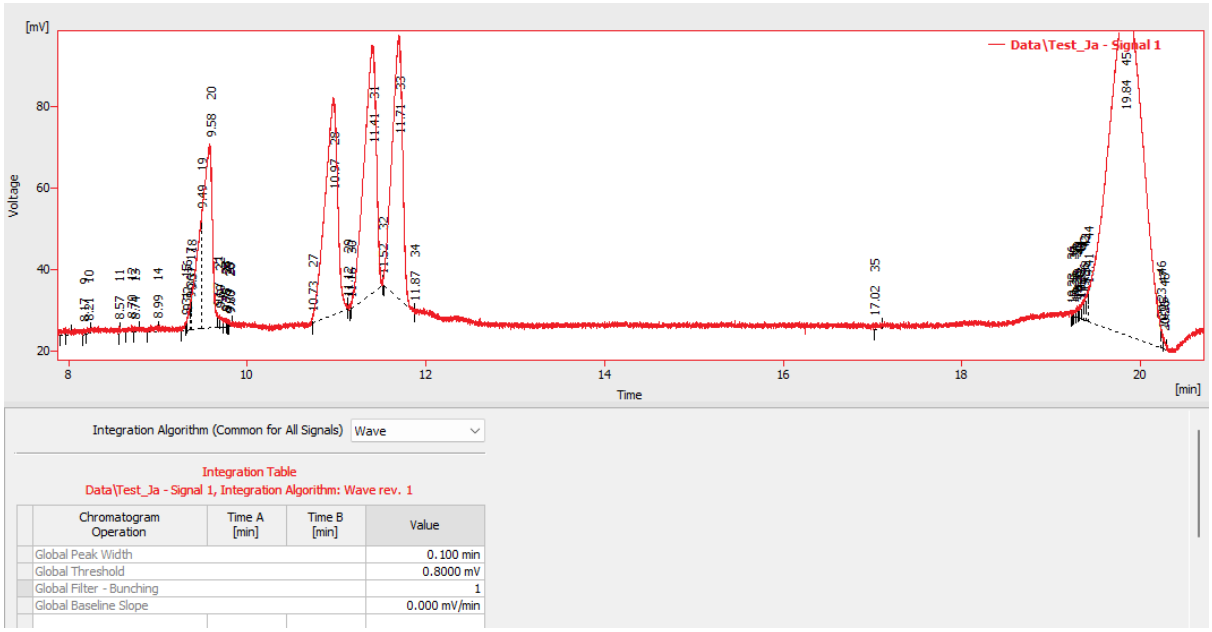


Figure 7. Integration by Wave IA.

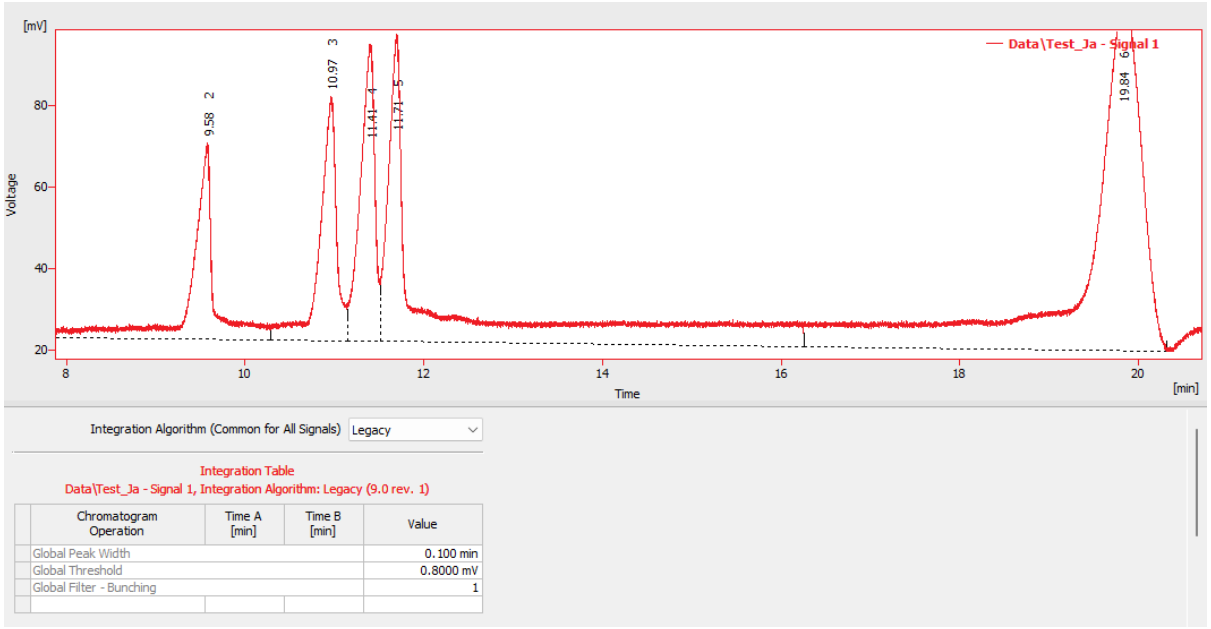
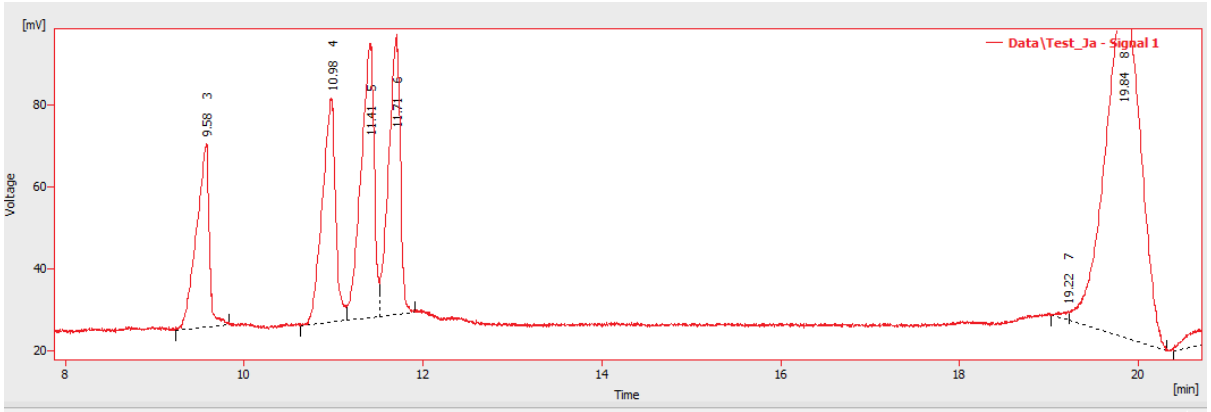


Figure 8. Integration by Legacy IA with the same parameters.

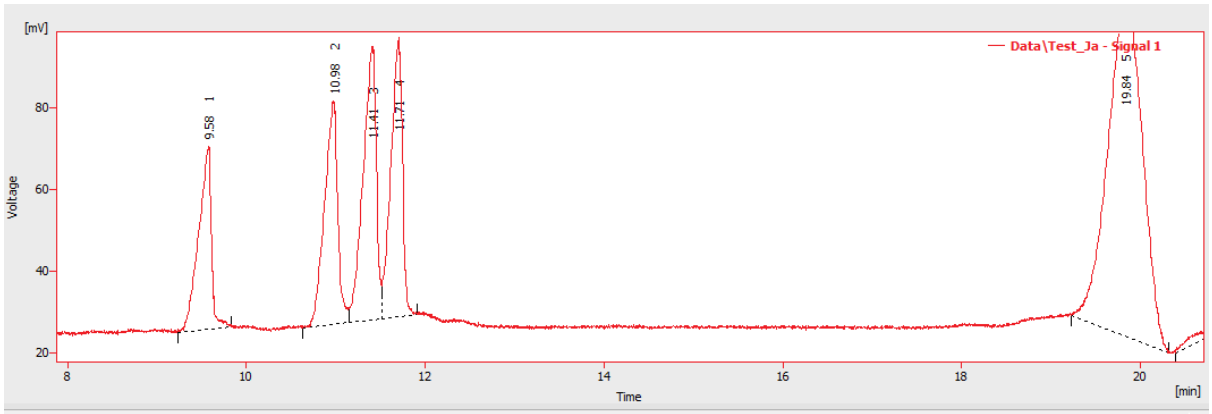


Integration Algorithm (Common for All Signals) Wave

Integration Table
Data\Test_Ja - Signal 1, Integration Algorithm: Wave rev. 1

Chromatogram Operation	Time A [min]	Time B [min]	Value
Global Peak Width			0.100 min
Global Threshold			0.8000 mV
Global Filter - Bunching			5
Global Baseline Slope			0.000 mV/min

Figure 9. Integration by Wave IA after applying Global Bunching. In some cases, small peaks at the start/end of a bigger one may still remain.



Integration Algorithm (Common for All Signals) Wave

Integration Table
Data\Test_Ja - Signal 1, Integration Algorithm: Wave rev. 1

Chromatogram Operation	Time A [min]	Time B [min]	Value
Global Peak Width			0.100 min
Global Threshold			0.8000 mV
Global Filter - Bunching			5
Global Baseline Slope			0.000 mV/min
Min. Area	0.000	0.000	15.000 mV.s

Figure 10. Integration by Wave IA after applying Global Bunching and Minimal Area parameters.

Peaks with significantly different peak widths

Sometimes chromatograms have very different peak widths; in a situation where some peaks cover a significant portion of the chromatogram (e.g., EA or GPC) *Wave* IA may fail to properly evaluate the wide peaks. Currently it is recommended to integrate these chromatograms using *Legacy* IA.

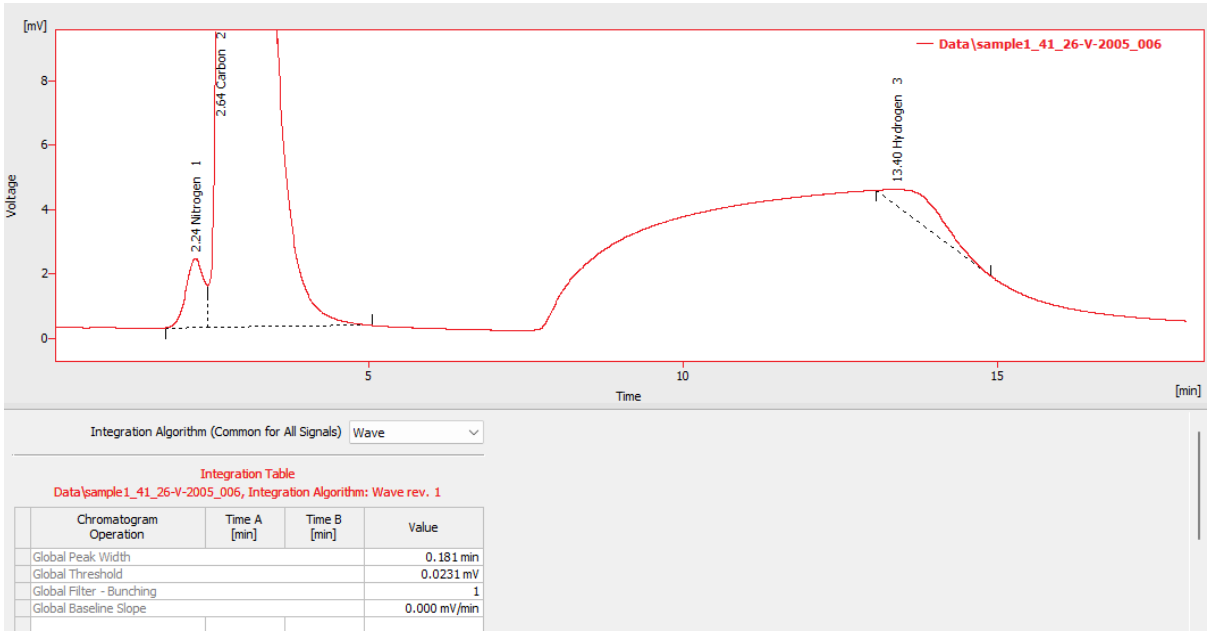


Figure 11. Integration by Wave IA.

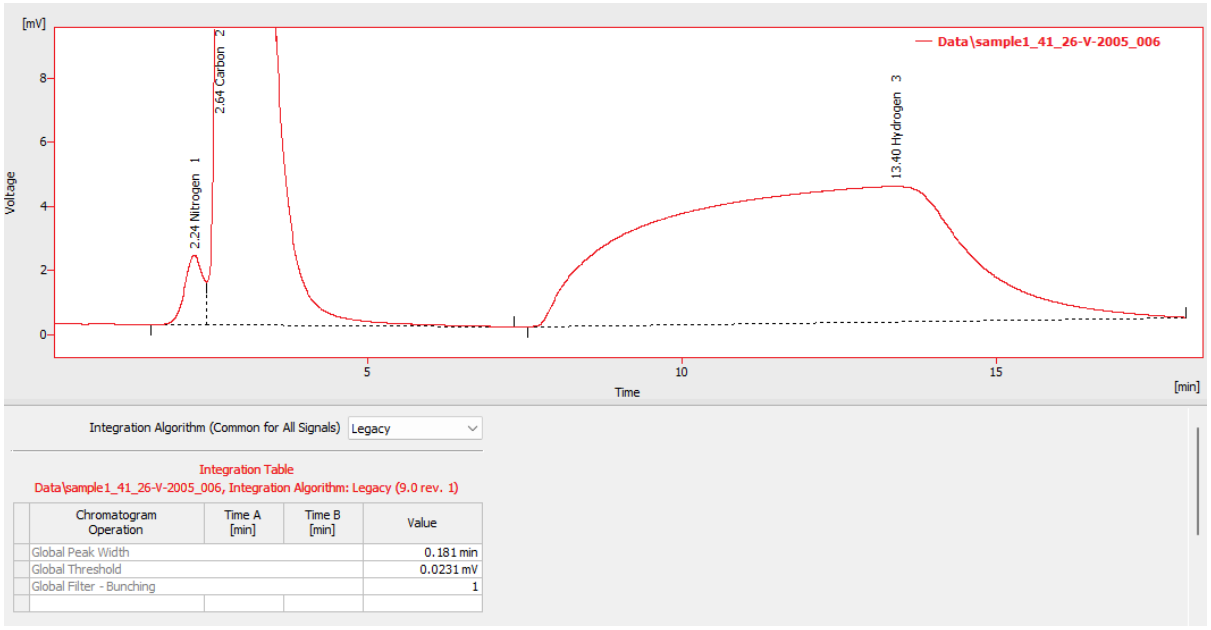


Figure 12. Integration by Legacy IA with the same parameters.

Peaks cut off in the middle

Sometimes random peaks in the *Wave* algorithm get integrated in a weird manner, being cut off. This happens only in the *Wave* IA on a random basis (in a series of chromatograms most peaks are ok, but occasional chromatogram may have one or more peaks cut-off). The phenomenon is a result of filtering the data in combination with the signal curve course, there is no 100% solution in the *Wave* algorithm (applying *Global Bunching* or other filter helps in majority of cases), switching to *Legacy* algorithm helps.

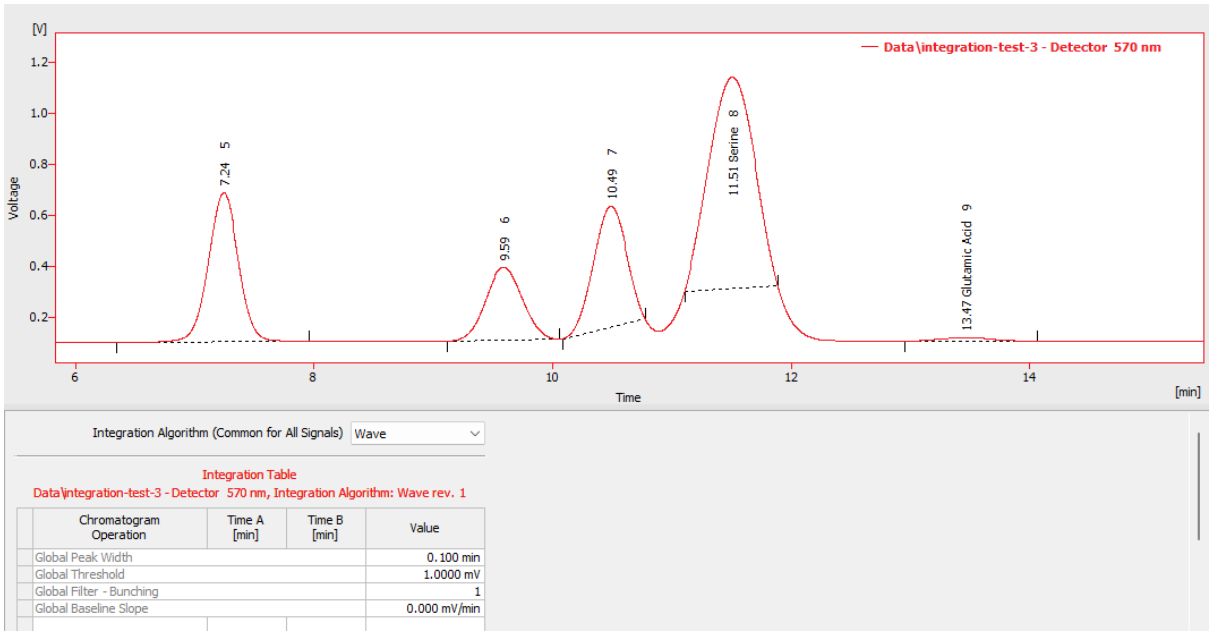


Figure 13. Integration by Wave IA.

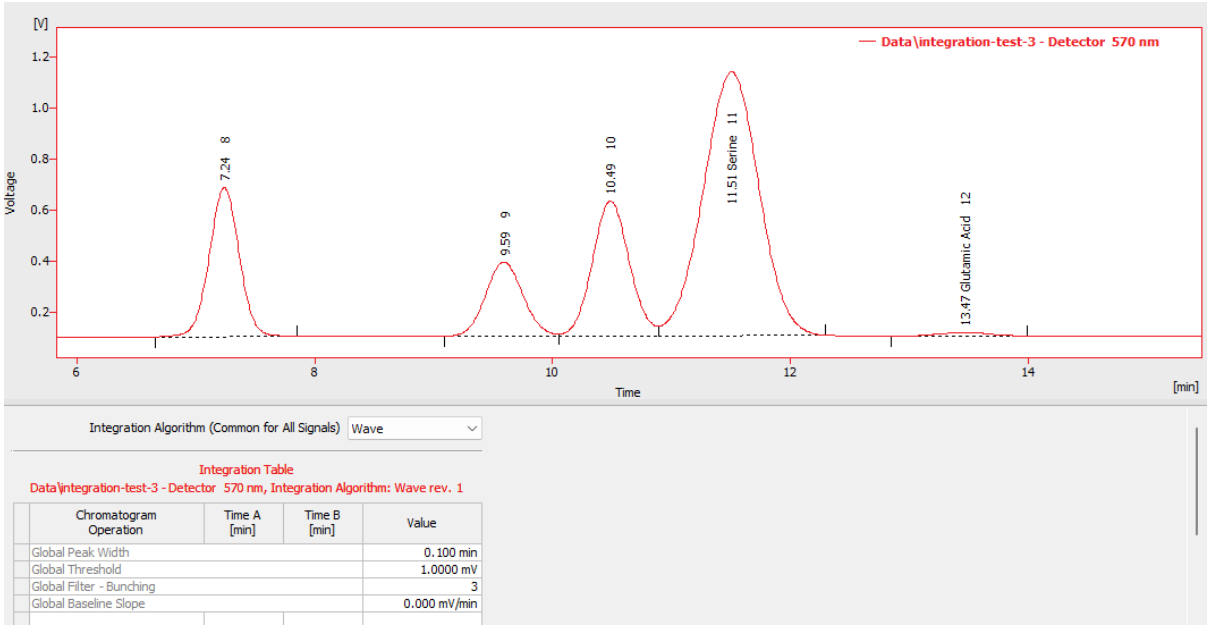


Figure 14. Integration by Wave IA after applying Global Bunching.

Basic integration workflow

It is recommended to set at least the *Integration Interval* and global parameters before resorting to other operations. This strategy suffices for most chromatograms and such integration is then applicable to all similar samples. The recommended order of operations is as follows (refer to the *User Guide* for a detailed description):

1. **Set Integration Interval(s).**
   Some areas of the chromatograms are not relevant to the analysis and may cause wrong integration of the rest of the chromatogram if left in operation e.g., sudden steep signal rises/drops when lamps are switched on, mobile phase compositions suddenly changed etc. Make sure that the *Integration Interval* operation does not define interval starting or ending inside of a peak slope, in either IA the expectation is that the chromatogram starts and ends on a baseline.

2. **Set Global Peak Width.**
   If the chromatogram has peaks with significantly differing peak widths, it may be useful to apply *Local Peak Width* operation later.

3. **Use Global Bunching operation;**
   Global Bunching is calculated based on the chromatogram frequency and the set *Global Peak Width*, so it should be applied after setting the *Global Peak Width*.

4. **Set Global Threshold;**
   Threshold value to omit the lowest peaks depends on the noise on the signal curve. Since *Global Bunching* acts as a signal filter, the automatically suggested *Global Threshold* value will be different after applying *Global Bunching* higher than 1.

Exclusive features in Wave algorithm

The *Wave* IA contains some *Integration Table* functions that are not available in the *Legacy* IA. If these functions are present in the *Integration Table*, they will become invisible (not performed) when switching to the *Legacy* IA, but will remain if the algorithm reverts to *Wave*.

Peak – Show and Peak - Hide

The *Peak - Show* and *Peak - Hide* functions provide a convenient way to manage visibility of specific peaks in your data. These tools allow you to tailor your display by either showing or hiding specific peaks.
By default, the *Wave* algorithm might automatically ignore some of the minor peaks identified during integration. However, completely eliminating these peaks could potentially affect the baseline's trajectory. Therefore, these functions offer a flexible way to handle unwanted peaks without completely removing them.
Both the *Peak - Show* and *Peak - Hide* features are complemented by the *Baseline - Lock* function. This tool completely removes all peaks that have their maxima within a set time interval. This could affect other peaks with maxima outside the specified window, particularly if they share the same baseline.

To address this, the *Peak - Hide* function has been designed within the *Wave* algorithm. It merely hides peaks within the selected time interval, instead of eradicating them. These peaks are taken into account when constructing a joint baseline but are excluded from the final calculation and the *Result Table*. The counterpart function *Peak - Show* allows you to visualize a previously hidden peak or to display peaks that were automatically hidden by the algorithm.

In scenarios where a region of the chromatogram contains hidden peaks and the user executes the *Peak – Add Positive/Negative* operation, all automatically generated but concealed peaks within the defined time interval are replaced by the newly added peak. This could lead to the cancellation of hidden peaks in areas where the manually added peak is not displayed due to baseline crossing limitations.

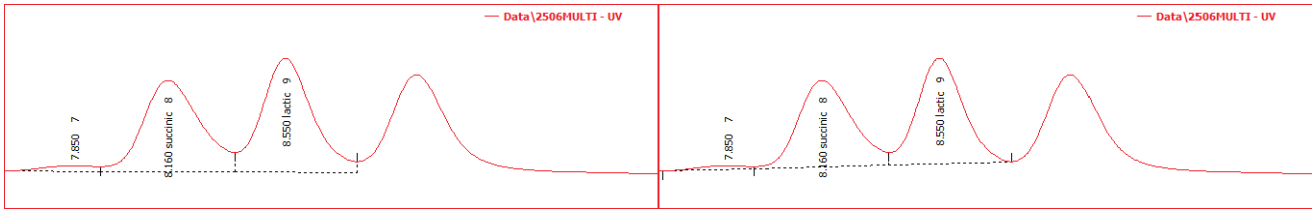


Figure 15. Difference between Peak Hide (left) and Baseline Lock (right).

Global/Local Baseline Slope

Automatic peak generation on a standard signal curve typically comprises three parts: *Peak Start*, *Peak Apex*, and *Peak End*. The highest signal point determines the *Peak Apex*'s location, while the positioning of *Peak Starts* and *Peak Ends* can vary, potentially being either closer or farther from the *Peak Apex*, based on user preference. Different settings can be more effective depending on the situation.

Prior to the introduction of the *Wave IA*, the automatic determination of *Peak Starts* and *Peak Ends* was governed by the instantaneous peak slope, calculated using the *Global/Local Threshold* parameter. If users wanted the automatically generated peak borders to be closer to the Peak Apex, they had to increase the Threshold value. However, this resulted in raising the detection limit for peaks on the baseline, which could prevent the identification of smaller peaks.

The *Wave IA* presents an alternative. It allows users to maintain their chosen *Threshold* value while separately defining the chromatographic curve slope for the detection of *Peak Starts* and *Peak Ends*, through the use of the *Global Baseline Slope / Local Baseline Slope* operations. This parameter is not mandatory; users can retain the original slope calculation method by leaving the *Global Baseline Slope* value set to *0*. Any value other than zero will cause a shift in the positions of the *Peak Starts* and *Peak Ends*.

You can find an approximate value for this function using the *Chromatogram – Show Slope/Level* function in the *Chromatogram* window menu. However, note that altering the *Baseline Slope* value may result in the separation of peak clusters into individual peaks or the merging of separate peaks, and some peaks may not be integrated at all.

For specific alterations to the start and/or end positions of individual peaks, the *Peak – Start* and *Peak – End* operations remain useful. These functions override the automatically calculated positions for peak start and peak end.