INTEGRATION OF PEAKS AND AREAS WITH CHROMELEON™ 7.2.

#1: THE PEAK DEFINITION

A peak is defined by a clear distinction of the signal noise. The rule is that a signal that is three times higher than the noise (**L**imit **o**f **D**etection) represents a peak that can be identified/integrated and when it is ten times higher should the peak be quantified (**L**imit **o**f **Q**uantification). However, the limits of the peak should be taken into account. Is the peak symmetrical and extends down to the baseline on both sides? Are there any shoulders or riders, and is there actually the course to the baseline between two peaks, or are the peaks not clearly separated? Especially when dealing with shoulders, riders or unresolved peak groups, Chromeleon offers many possibilities to define this automatically.

#2: THE COBRA ALGORITHM

With the Cobra algorithm a representative chromatogram of a sequence can be evaluated. The aim is to exclude the peaks that are not relevant. This determination is made in 4 steps.

❯ Defining the period of time in which integration is taking place (**Inhibit Integration**)

❯ Determining the range of baseline noise (**Baseline Noise Range**)

❯ Determining Peak Limits via Smoothing Width (**Smoothing Width**)

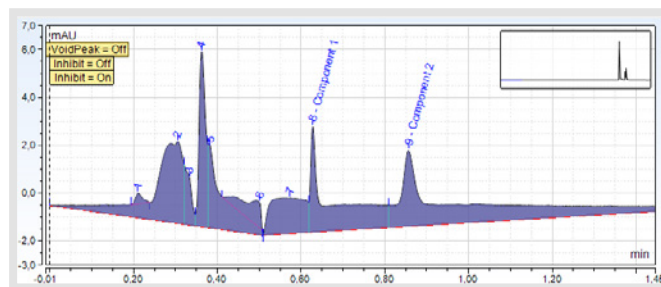❯ Setting the smallest relevant area value (**Minimum Area**)
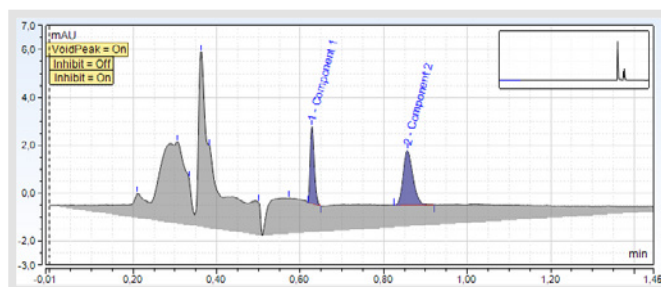

Figure 1: Consider Void Peak Function not active

Figure 2: Consider Void Peak Function active

INTEGRATION AREA: It can be easily controlled by the initial and final time where areas should be integrated. A injection peak, or even a peak occurring through a purge step, can be eliminated by initiating or terminating the integration therefrom. If a component table already exists, a negative injection peak can be detected automatically by the function Consider Void Peak and excluded from integration (Figure 1 and Figure 2)

BASELINE NOISE RANGE: The Cobra algorithm offers the possibility to choose between an automatically defined area where the baseline noise is defined automatically or a manually adjustable area. If the range is set manually, then the range applies to all injections of the sequence associated with the current Processing Method. If the automatic range is selected, Cobra takes over the range in each chromatogram
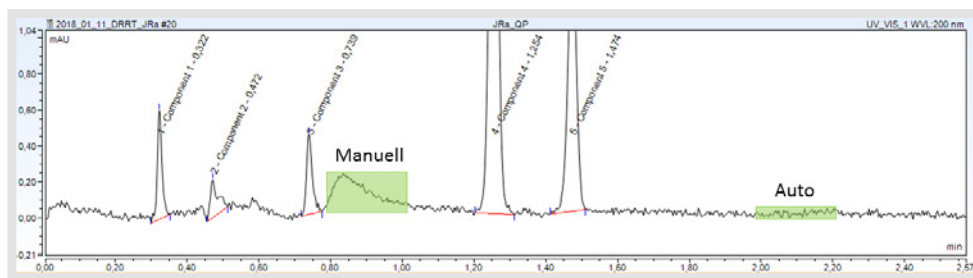

Figure 3: Baseline Noise Range via auto-determination and manual determination

in which the noise is particularly small. It is important to note that the noise should be characteristic. This can sometimes be an advantage as it does not accidentally contain a peak in the range (Figure 3).

SMOOTHING WIDTH: The parameter is crucial for the correct determination of peak start and end. There are three options between these. The value determined by Cobra can be adopted, a value can be entered manually or a peak can be clicked interactively. Then the value of half the peak width at 50% peak height is adopted. Basically, approximately half the peak width at 50% peak height of the narrowest peak of interest should be used as a guide, otherwise the integration is very sensitive or insensitive.

MINIMUM AREA: The Minimum Area determines the smallest area value of interest. So far, all peaks have been integrated by the values of the smoothing width and the noise. There are also three options for determining the minimum area. The value determined by Cobra can be adopted, a value can be entered manually or a peak can be clicked interactively. Then the area value of the clicked peak is adopted.

#3: THE SMART PEAKS INTEGRATION ASSISTANT

The Smart Peaks Integration Assistant provides the ability to determine how to handle unresolved peak groups or tabs over defined periods of the chromatogram.

Depending on the complexity of the problem, there are two to five options available:

❭ **Drop Perpendicular** (Figure 4) When plummeting, the solder is cut at the minimum between two maxima. The sensitivity depends directly on the minimum area. Depending on the setting of the smoothing width, shoulders that do not have a minimum can sometimes still be separated via the solder precipitation. The Drop Perpendicular represents the worst-case scenario.
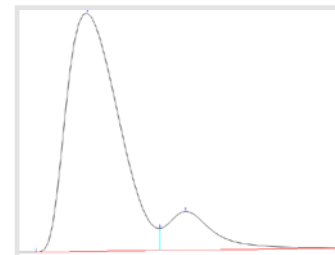

Figure 4: Drop Perpendicular

❭ **Valley to Valley** Determination (Figure 5) In this variant, the baseline is drawn to any existing minimum, if the resulting area is larger than the minimum area. With this parameter e.g. Peaks in a drift can be integrated very well.
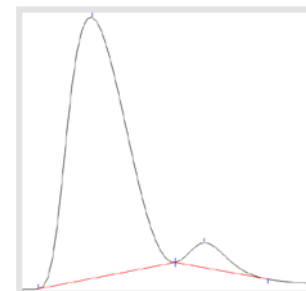
❭ **Tangential Rider Skim at Lower Peak End** (Figure 6) In this tangential ridge separation, the tangent is created at the lower end of the peak and guided to the minimum on the other side of the peak.
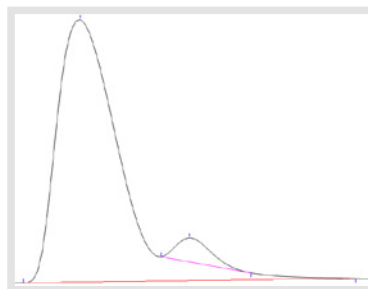

Figure 5: Valley to Valley

❭ **Tangential Rider Skim at Both Peak Ends**
(Figure 7) This variant applies the tangent at both ends of the peak. The areas of the rider or shoulders are usually larger in this variant than in the previously mentioned tangential variant. In general, the peak areas of the smaller peak are always smaller than the real value for the tangential variants, whereas the values for solder precipitation (Figure 4) are too large.
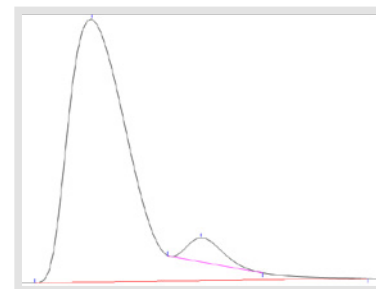

Figure 6: Tangential Rider Skim at Lower Peak End

Figure 7: Tangential Rider Skim at Both Peak Ends

> ❯ **Exponential Rider Skim** (Figure 8) In exponential rider separation, the peak treated as the main peak is based on an exponential curve of the peak line to the baseline. The remaining area is then assigned to the rider or the shoulder. However, it must be taken into account that the applied peaks can raise the reference peak from the peak height and thus also shift the peak maximum. Basically, however, it can be stated that, measured against scientific standards, the exponential variant has the least error and thus comes closest to the real peak areas in terms of the result.
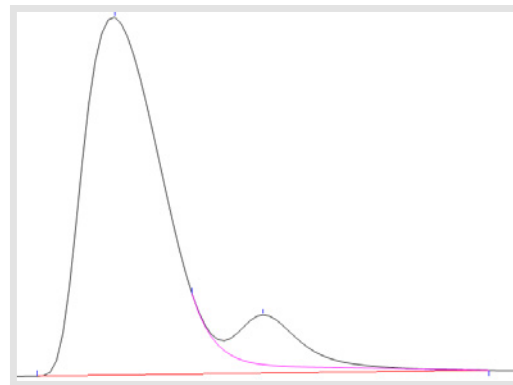
Figure 8: Exponential Rider Skim

#4: OTHER DETECTION PARAMETERS

The addition of further parameters also makes it possible to make further settings. There is a total of 25 parameters that can be used. Three of them will be explained in more detail below. First, the parameters **Baseline Point, Peak Group** and the parameter **Snap Baseline**.

The parameter **Baseline Point** inserts a baseline point exactly at the selected position, which means that at this point imaginary course of the baseline and baseline are

Figure 9: Drift integration without inserted parameters (top) and no integration with inserted parameter (bottom).

merged. For example, this parameter can be used to specify a peak end or beginning. It is also possible to eliminate such a strong drift integration (Figure 9).
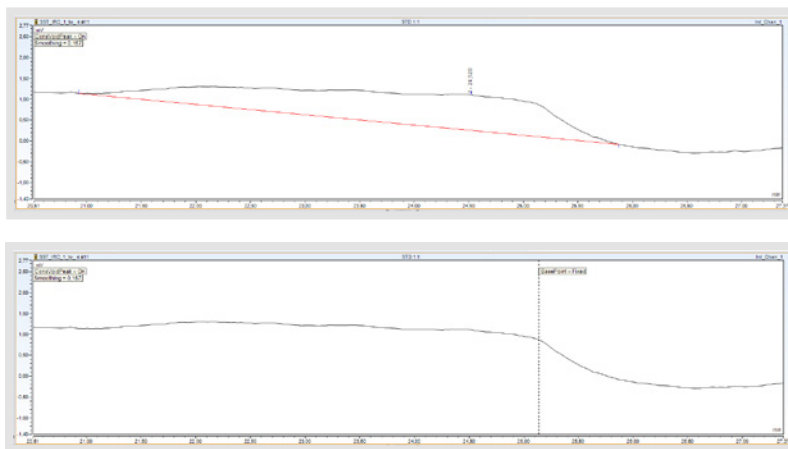
The **Peak Group** parameter defines the beginning and end of a group of peaks, which are then integrated and evaluated as one surface (Figure 10). The option Auto can be selected as option or Fixed. With the Fixed option, the start / end of the peak group is set exactly where the parameter was set. For the Auto option, Chromeleon will set a suitable start / end for the parameter.
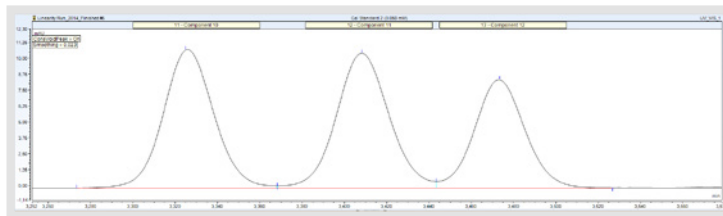
The **Snap Baseline** parameter is directly linked to the Minimum Area, which was already covered in Cobra Detection. If the Minimum Area is set to 0, then the Snap Baseline parameter has the same effect on both „On" and „Off". In doing so, the baseline is lurked at the level at which the parameter was set and all peaks are integrated into this virtual baseline with solder precipitation (Figure 11).
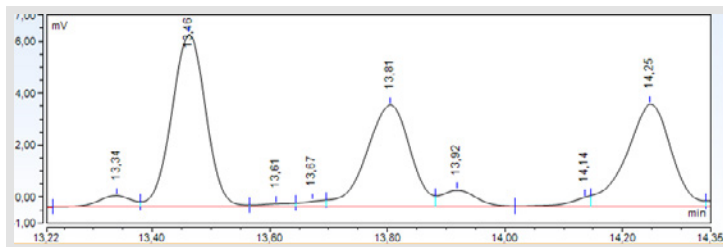
If we set the Minimum Area to a higher value and selected On for Snap Baseline, all peaks with a sufficiently large peak area are integrated with the Valley to Valley algorithm (Figure 12).
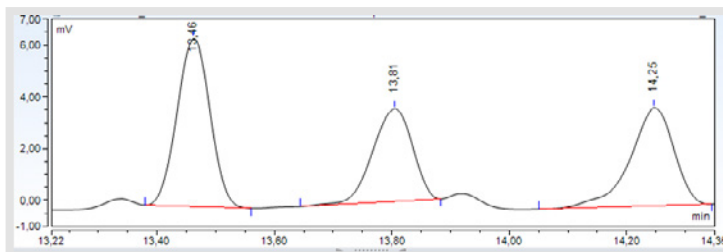
If we set the parameter Snap Baseline to „Off", the original level of the baseline is reapplied and all peaks with a sufficiently large peak area are integrated with this level with solder precipitation (Figure 13).
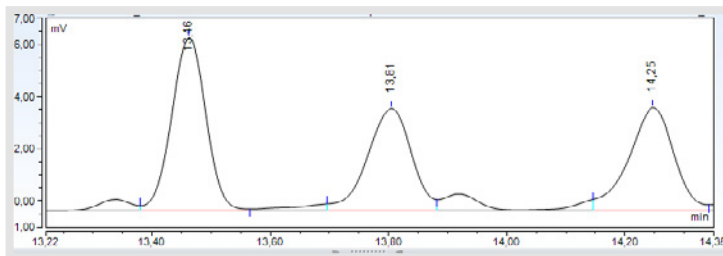

Figure 10: Integration of the 3 peaks as a peak group with an output area.

Figure 11: Integration with a minimum area of 0 and an inserted snap baseline parameter.

Figure 12: Integration with a Minimum Area greater than 0 and Snap Baseline to On.

Figure 13: Integration with a Minimum Area greater than 0 and Snap Baseline set to „Off".

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