LabSolutions™ CL
Version 1.40
Data Acquisition & Processing Theory Guide
Overview

Last updated: June 6, 2022

Table of Contents

1. Overview 2
2. Peak Integration Parameters 2
   2.1 Chromatopac 2
   2.2 i-PeakFinder 4
3. Identification Parameters 6
   3.1 Window Method and Band Method 6
   3.2 Absolute/Relative Retention Time Methods 7
   3.3 Absolute Retention Time Method 8
   3.4 Relative Retention Time Method 8
4. Quantitative Methods 8
5. Calibration Curve 9
   5.1 Linear 9
   5.2 Point to Point 10
   5.3 Quadratic and Cubic 10
   5.4 Mean RF 11
   5.5 Exponential 11
   5.6 Manual RF (Linear, Exponential) 12

Notice

- All rights reserved, including those to reproduce this manual or parts thereof in any form without permission from Shimadzu Corporation.
- The information in this document subject to change without notice and does not represent a commitment on the part of the vendor.
- Any errors or omissions which may have occurred in this manual despite the utmost care taken in its production will be corrected as soon as possible, although not necessarily immediately after detection.
- Original version is approved in English.

©2022 Shimadzu Corporation. All rights reserved.

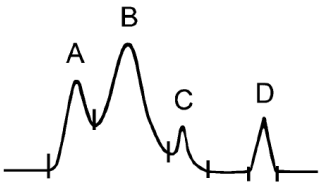
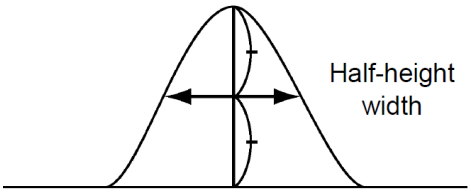
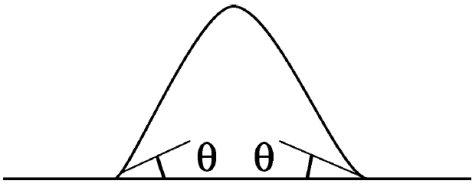
1. Overview

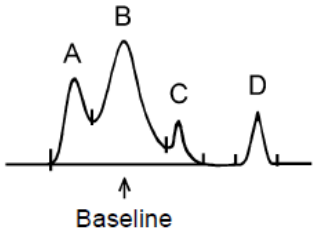
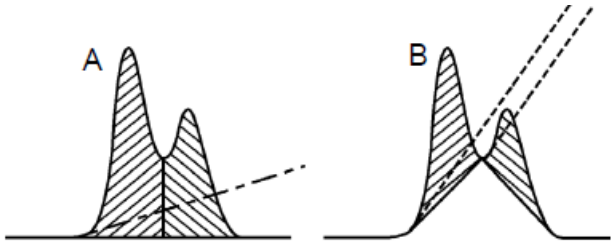
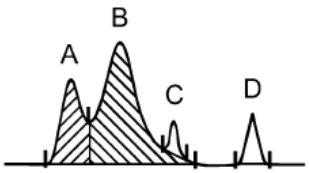
This document shows the overview about the algorithms for analyzing chromatogram. For details, please refer the *Data Acquisition & Processing Theory Guide.*

2. Peak Integration Parameters

2.1 Chromatopac

This section describes the peak integration parameters (Chromatopac) and how peaks are integrated using these parameters.

<table>
<tr><th>Peak Integration Flow</th><th>Parameter and Description</th></tr>
<tr><td rowspan="3">Detecting Peaks
Peak start, peak end, and peak top are first detected on chromatograms according to the [Width] and [Slope] values. Setting these parameters properly detects only necessary peaks and excludes noise.

</td><td>[Width]
Specifies the minimum half-height width of the peaks detected during analysis. Peaks with smaller half-height width are regarded as noise.

</td></tr>
<tr><td>[Slope]
Specifies the slope used as a reference for detecting peaks. The point where a graph slope exceeds (falls under) the set value is recognized as peak start (end).

</td></tr>
<tr><td>[T.DBL]
Increases the minimum half-height width (=[Width]) by two times, and decreases peak detection sensitivity (=[Slope]) to 1/2 at each set value × 2^n (n=0 to 14). This parameter facilitates the integration of chromatogram peaks becoming broader with time.
If "0" is set, these parameters are adjusted automatically.</td></tr>
<tr><td>Processing the baseline
(Determining how to integrate overlapped peaks)</td><td>[Drift]
Determines how to integrate overlapped peaks. As shown in the left figure below, when a valley of overlapped peaks exists above</td></tr>
</table>

 To calculate (= integrate) peak area, a baseline is assumed between the first peak start and the last peak end of overlapped peaks. Set the [Drift] value as appropriate when the baseline fluctuation is significant.	the slope (= set value) starting from the first peak start, the peaks are divided by a vertical line. When the valley exists below the slope, the baseline is drawn through the valley, as shown in the figure at right. If “0” is set, the software automatically determines how to process the baseline. A negative value can also be set. 
Integrate Unresolved Peaks	**-**
Calculate Peak Area	**-**
Removing unnecessary, small peaks  Peaks with areas smaller or heights lower than the set value can be hidden from the screen and peak reports.	**[Min Area/Height]** Selecting "Area" or "Height" as a reference to define the small peaks to be hidden (from the screen, peak report, etc.) can be set in [Calculated by] on the [Integration] tab page. The unit of counts: Area (uV·sec), Height (uV)

2.2 i-PeakFinder

This section describes the main functions in the order internal processes are performed in the i-PeakFinder algorithm.

Process	Parameter and Description
Noise estimation The noise value to be used as the threshold value for peak detection is estimated. The estimated noise values are calculated for a certain time interval, rather than just one value for the entire chromatogram.	**[Nose Estimation Off / Noise Estimation On]** Noise values are not estimated during the time range from Off to On. Noise estimation is not performed in this time range. Instead, a value calculated from the estimated noise values in the range on both sides where Noise Estimations On is used.
	[Noise Estimation Range] When this parameter is specified, the noise standard deviation is calculated in the range and used in the entire chromatogram.
Peak detection	**[Detection Threshold] NR.Level=** Peaks of which estimated peak height is greater than the threshold value are detected using (estimated noise value) × (setting value) as the threshold value.
	[Minimum Half Width] Width Min= Peaks of which half width is equal to or above the setting value are detected.
	[Maximum Half Width] Width Max= Peaks of which half width is equal to or below the setting value are detected.
	[Integration Range] Peaks out of the specified time range are not detected.
	[Integration Off / Integration On] Peaks are not detected in the time range between Off and On. This parameter is merged with [Integration Range] on the time program for processing.
Adjustment of peak detection points The positions of the peak start and end points are adjusted.	**[Peak Baseline Height]** The positions of the peak start and end points are adjusted.
	[Peak Base Start=] A position near the position calculated by multiplying the half width at half maximum (HWHM) of the peak by the setting value is set as the peak start point.

	[Peak Base End=] A position near the position calculated by multiplying the half width at half maximum (HWHM) of the peak by the setting value is set as the peak end point.
Overlapping peak processing Unseparated continuous peaks are processed.	**[Unify Peaks by Separation Width] Separation Width=** When the resolution of two peaks is below the setting value, peaks are unified to the larger peak.
	[Detection Threshold of Unseparated Small Peak] NR.Level(Shoulder)= This is a detection threshold (NR.Level) used to redetermine the bulging part of the peak as a peak.
	[Shoulder Peak Detect Off / On] Shoulder peaks are not detected in the time range from Off to On.
	[Unify Peaks by Shoulder Ratio] Min.Shoulder Ratio%= The shoulder peak is unified to the main peak when the ratio of the shoulder peak height and main peak height (%) is below the setting value.
	[Unify Peaks by Depth Ratio] Min.Depth Ratio%= Peaks on both sides of a valley are unified when the depth of the valley between adjacent unseparated (vertical division) peaks is equal to or less than (height of the lower peak top next to the valley) × (setting value) / 100.
	[Tailing Off] When this checkbox is selected, automatic tailing is not performed.
Baseline processing Baseline of unseparated continuous peaks are processed.	**[Baseline Type(Vertical Division)]** All peaks are vertically divided. Peaks processed with tailing process are also vertically divided.
	[Baseline Type(Base to Base)] Baseline separation is performed on all peaks.
	[Baseline Type(Baseline Length)] Max.Baseline Length= When the baseline length is more than the setting value, baseline separation is performed on valleys in peaks and the baseline is bent.
	[Baseline Type(Valley/Height Ratio)] Min.VB Ratio%= When the ratio (%) of the height of the valley in unseparated peaks and the maximum height of

	unseparated peaks is below the setting value, baseline separation is performed on the valley.
	[Base to Base On / Base to Base Off] Baseline separation is performed on unseparated peaks in the time range from On to Off.
	[HORIZ Baseline On /HORIZ Baseline Off] A horizontal baseline is forcibly drawn in the time range from On to Off.
Peak filter processing Unnecessary peaks are removed using parameters.	**[Min.Aspect] Min.Aspect Ratio=** Peaks of which height/width ratio is smaller than the setting value are removed.
	[Min. Area] Min.Area= Peaks of which area is smaller than the setting value are removed.
	[Min Height] Min.Height= Peaks of which height is less than the setting value are removed.
	[Max. Peaks] The specified number of peaks with the largest area or height are kept and other peaks are removed.
	[Relative to Main Peaks] Peaks of which area or height relative to the maximum peak is smaller than the set value is removed.

3. Identification Parameters

The software identifies detected peaks according to the values set in the compound table, as well as in identification parameters. The user can define the time allowance for peak identification.

3.1 Window Method and Band Method

Even though a peak's measured retention time deviates from the retention time set in the compound table, the software identifies the peak as such, as long as the deviation falls within the time allowance specified. There are two methods for setting the time allowance: [Window] (= Time Window) and [Band] (= Time Band).

■Window Method

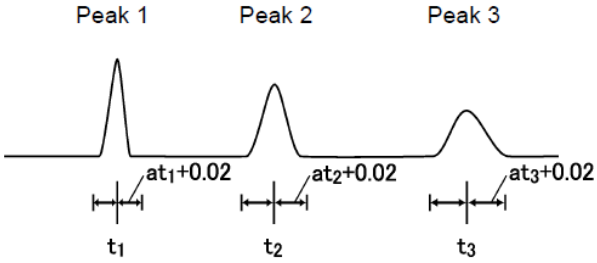
In the Window method, the time allowance is defined in proportion to each peak retention time. The longer the retention time of each peak, the larger the absolute time allowance for the peak becomes, as shown in

below. Using this method, the user can specify the time allowance for all peaks at once. However, multiple peaks may be detected within the same time allowance depending on the set value. The Window method is useful for isocratic LC analyses, where peak width and retention time fluctuation increase in later peaks, and it is therefore necessary to increase the time allowance.

Time allowance (min) = (Standard retention time (min) × Window width (%) / 100) + 0.02

a: Window width (%)

t_1, t_2, t_3: Standard retention time for each peak (min)



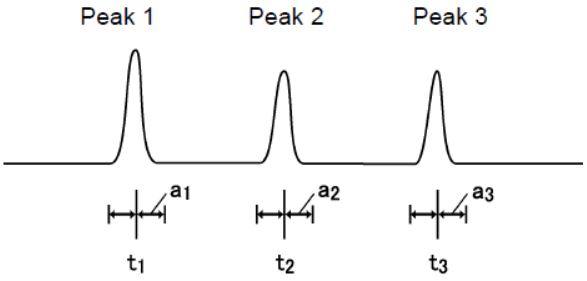
■Band Method

In the Band method, the time allowance is defined for each peak. Using this method, the user can individually specify the time allowance optimum for each peak, as shown in below.
The Band method is useful for gradient LC analyses, where peak width and retention time remain comparatively unchanged, and peaks appear adjacently.

Time allowance (min) = Bandwidth (min)

a_1, a_2, a_3: Bandwidth for each peak (min)

t_1, t_2, t_3: Standard retention time for each peak (min)



3.2 Absolute/Relative Retention Time Methods

This software identifies peaks based on the retention time. The user can specify whether the retention time is to be counted from analysis start (absolute retention time method), or to be corrected using a reference peak (relative retention time method).

3.3 Absolute Retention Time Method

In this method, the software identifies target peaks according to the equation below, using the preset standard retention time and time allowance for each peak. It is not necessary to specify a reference peak. This method is generally used for peak identification.

$$|T - t| < W$$

T: Standard retention time of target peak

t: Measured retention time of target peak

W: Time allowance of target peak

3.4 Relative Retention Time Method

In this method, the software identifies peaks after relatively correcting the retention time deviation due to analysis condition change. A preset reference peak is first identified using the absolute retention time method. Target peaks are then identified according to the equation below.

This method is useful for consecutive analysis, where the retention time varies with use frequency, as well as for analysis where the time error is significant at sample injection (i.e., analysis start).

$$\left| T - \frac{T_1}{t_1} \times t \right| < W$$

T: Standard retention time of target peak

T_1: Standard retention time of reference peak

t: Measured retention time of target peak

t_1: Measured retention time of reference peak

W: Time allowance of target peak

4. Quantitative Methods

This software uses the following six types of quantitative methods. The table below shows the overview of about the methods.

Quantitative Method	**Description**
Area Normalization	The ratio of each individual peak area (height) in relation to all of the peak areas (heights).
Corrected Area Normalization	Using standard samples (or from literature), the sensitivity correction factor is first obtained for all components to be detected. Using the factor, the peak area (height) measured from unknown sample is corrected. Then the ratio of the corrected peak area (height) in relation to all peak area (height) is obtained.

Internal Standard	Target peaks are quantitated by adding an ISTD substance to both of the standard and unknown sample, as below. This method can obtain stable results with injection errors eliminated. (1) A certain amount of ISTD substance is added to the standard containing the target component with known quantity. (2) The standard is measured, and the calibration curve of area (height) ratio and concentration (component amount) ratio (between target and ISTD) is created. (3) The area ratio (between target and ISTD) is obtained from the measured unknown sample with the same ISTD substance added. (4) Using the concentration (component amount) ratio acquired from the calibration curve above, the target component is thus quantitated from the sample and ISTD amounts.
External Standard (Absolute Calibration Curve)	By creating a calibration curve expressing the relation between standard concentration (component amount) and peak area (height), the target component is quantitated by applying unknown sample peak area (height) to the calibration curve.
Corrected Area Normalization with Scale Factor	The content of each component is obtained by calculating the total quantitation value as the dilution factor (or sample volume, if no dilution factor is set) instead of 100.
Standard Addition	Unknown sample with a known amount of objective component and unknown sample without addition are measured in the same conditions. The objective component is then quantitated using the difference of two obtained peak areas (heights). This method is useful for analyses where the target component relative sensitivity varies with solvent composition or coexisting components.

5. Calibration Curve

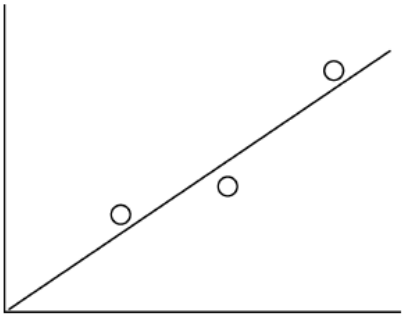
This software can utilize the following seven types of calibration curves: [Linear], [Point to point], [Quadratic], [Cubic], [Mean RF], [Exponential], [Manual RF].

5.1 Linear

Standards with different concentrations up to the [# of Calib. Levels] are individually analyzed, and a linear calibration curve is drawn by the least square method.

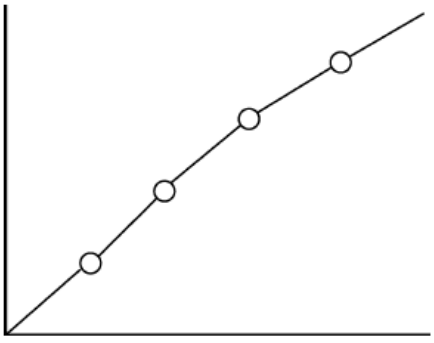
When using a standard at one concentration level, the calibration curve is drawn as a simple linear line passing through the obtained point and the origin.

When using the standard at two concentration levels, and when the calibration curve does not go through the origin, the calibration curve is drawn as a simple linear line passing through two obtained points.
In other cases, the calibration curve is drawn as a linear line using the least square method.
The user can set the [# of Calib. Levels] up to 64, and obtain each level's calibration point by averaging up to ten measurements.



5.2 Point to Point

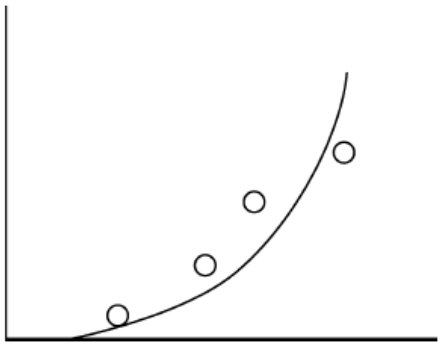
Standards with different concentrations up to the [# of Calib. Levels] are individually analyzed, and the calibration curve is drawn by connecting the obtained points.
When using a standard at one concentration level, the calibration curve is drawn as a simple linear line passing through the obtained point and the origin.
When using the standard at two or more concentration levels, the calibration curve is drawn by connecting those obtained points.
The user can set the [# of Calib. Levels] up to 64, and obtain each level's calibration point by averaging up to ten measurements.



5.3 Quadratic and Cubic

Standards with different concentrations up to the [# of Calib. Levels] are individually analyzed, and a quadratic or cubic calibration curve is drawn by the least square method.
To draw a quadratic calibration curve, it is required to set calibration points at three or more levels.
To draw a cubic calibration curve, it is required to set calibration points at four or more levels.

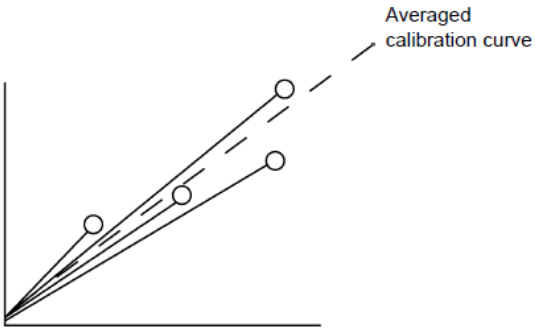
The user can set the [# of Calib. Levels] up to 64, and obtain each level's calibration point by averaging up to ten measurements.



5.4 Mean RF

Standards with different concentrations up to the [# of Calib. Levels] are individually analyzed, and each of the obtained points is connected to the origin. The slope coefficients of all linear lines are then simply averaged.

The user can set the [# of Calib. Levels] up to 64, and obtain each level's calibration point by averaging up to ten measurements.



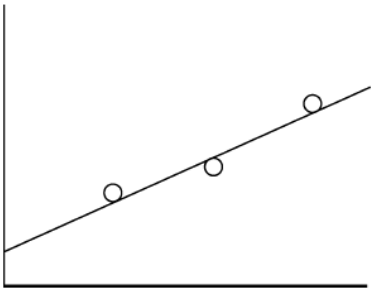
5.5 Exponential

Standards with different concentrations up to the [# of Calib. Levels] are individually analyzed, and a linear calibration curve is drawn in a natural log-natural log graph.

When using standards at two concentration levels, the calibration curve is a simple linear line passing through two obtained points.

In other cases, the calibration curve is drawn as a linear line using the least square method.

The user can set the [# of Calib. Levels] up to 64, and obtain each level's calibration point by averaging up to ten measurements.



5.6 Manual RF (Linear, Exponential)

The user can manually define an arbitrary calibration curve by entering the slope (= linear coefficient) and intercept in the compound table. The software then quantitates the peak by the specified calibration curve. In the Manual RF method, the calibration curve graph displays only the calculated curve with no calibration point. Particularly for the Manual RF (Exponential), the calibration curve is created regarding X and Y as natural log axes.