

LabSolutions

Data Acquisition & Processing Theory Guide

Read the instruction manual thoroughly before you use the product.
Keep this instruction manual for future reference.

Introduction

Read this Instruction Manual thoroughly before using the product.

Thank you for purchasing Shimadzu analytical instrument workstation “LabSolutions” (hereafter referred to as “the software” or “LabSolutions”).

This manual describes the procedures for operating this product. Read this manual thoroughly before using the product and operate the product in accordance with the instructions in this manual.

Also, keep this manual for future reference.

This manual assumes that the reader is knowledgeable of basic operations of Windows. For the operation of Windows, refer to the instruction manual that comes with that product.

Important

- If the user or installation location changes, ensure that this Instruction Manual is transferred with the product.
- If this manual is lost or damaged, immediately contact your Shimadzu representative to request a replacement.
- To ensure safe operation, contact your Shimadzu representative for product installation, adjustment, or re-installation (after the product is moved).

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Instruction Manuals

■ List of Instruction Manuals

Name	Content
Getting Started Guide	This manual follows an actual data acquisition procedure to describe basic methods of operation for first-time users. Read this manual to learn basic operations of the software.
Operators Guide	This manual describes overall operations and handy functions in more details, such as the software's system configuration, data analysis, batch processing, confirmation of data acquisition results, and report functions.
System Users Guide	This manual describes system administration and data management of the software. Refer to this manual as necessary.
Installation & Maintenance Guide	This manual describes installation and maintenance of the software.
Data Acquisition & Processing Theory Guide	This manual describes peak detection and quantitation of sample components. Refer to this manual as necessary.
Help	Clicking the on-screen [Help] button or pressing the [F1] key displays a description of on-screen parameters, answers to specific questions or solutions to various problems. Also, clicking the [Help] button on the error message window displays the details of the error or solutions to the error. Refer to Help before contacting us.

■ Indications Used in Instruction Manuals

Cautions and Notes are indicated using the following conventions, and the following symbols are used in this manual:

Indication	Meaning
 CAUTION	Indicates a potentially hazardous situation which, if not avoided, may result in minor to moderate injury or equipment damage.
 NOTE	Emphasizes additional information that is provided to ensure the proper use of this product.
 Reference	Indicates the location of related reference information.
[]	Indicates the names of buttons, menu options, setting options, windows/sub-windows, and icons that are displayed in a window. Example: Click [OK].

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1. Period:

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2. Description:

If a product/part failure occurs for reasons attributable to Shimadzu during the warranty period, Shimadzu will repair or replace the product/part free of charge (including USB dongles). However, in the case of products which are usually available on the market only for a short time, such as personal computers and their peripherals/parts, Shimadzu may not be able to provide identical replacement products.

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- (2) In no event will Shimadzu's liability to you, whether in contract, tort (including negligence), or otherwise, exceed the amount you paid for the product.

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- 3) Product use in combination with hardware or software other than that designated by Shimadzu
- 4) Computer viruses leading to device failures and damage to data and software, including the product's basic software
- 5) Power failures, including power outages and sudden voltage drops, leading to device failures and damage to data and software, including the product's basic software
- 6) Turning OFF the product without following the proper shutdown procedure leading to device failures and damage to data and software, including the product's basic software
- 7) Reasons unrelated to the product itself
- 8) Product use in harsh environments, such as those subject to high temperatures or humidity levels, corrosive gases, or strong vibrations
- 9) Fires, earthquakes, or any other act of nature, contamination by radioactive or hazardous substances, or any other force majeure event, including wars, riots, and crimes
- 10) Product movement or transportation after installation
- 11) Consumable items
Note: Recording media such as floppy disks and CD/DVD-ROMs are considered consumable items.

* If there is a document such as a warranty provided with the product, or there is a separate contract agreed upon that includes warranty conditions, the provisions of those documents shall apply.

* Warranty periods for products with special specifications and systems are provided separately.

* **The license cannot be reissued if you lose the USB dongle provided with the product.**



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1

Data Processing Parameters

This chapter describes the data processing parameters and equations used to analyze data in this software. Data processing parameters are displayed in the [Method View] of the Data Analysis window.



This chapter consists of the following sections:

- "1.1 Peak Integration Parameters" P.1
- "1.2 Identification Parameters" P.14
- "1.3 Quantitative Methods" P.22
- "1.4 Accuracy and Deviation Between the Standard Concentrations" P.28

1.1 Peak Integration Parameters

Peak integration processes such as target peak detection, baseline fluctuation compensation, unresolved peak separation, and unnecessary peak rejection, are executed according to the peak integration parameters stored in the method files.

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

The following describes the three different approaches for setting peak integration parameters:

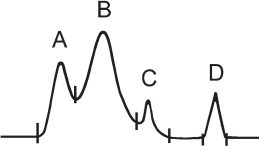
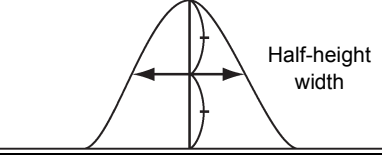
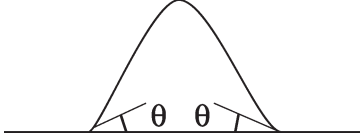
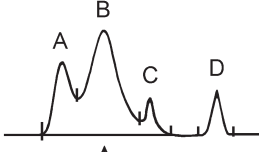
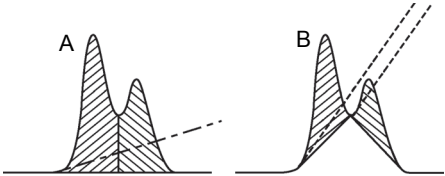
Name	Description
Peak Integration Parameters	Specifies the basic parameters for integrating peaks across the entire chromatogram. These parameters are set in the [Method View] - [Integration] tab.
Integration Time Program	Changes peak integration parameters at specified times on the chromatograms. This program is accessed by clicking the [Program] button on the [Peak Integration] tab of [Method view], to display the [Integration Time Program] sub-window.
Manual Peak Integration	Manually specifies peak integration parameters for each individual peak. The set parameters are only applied to the current data file. Right click on the [Chromatogram View] in the Data Analysis window to display the [Manual Integration Bar].

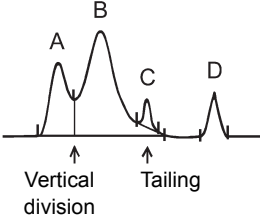
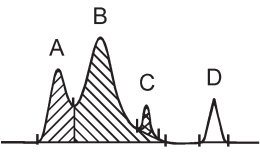
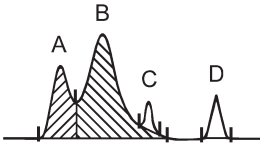
Reference

For details on how to set the peak integration parameters, refer to the Operator's Guide.

1.1.1 Peak Integration Parameters and Operation Flow

Use the following steps to execute peak integration. The table below describes how specific peak integration parameters relate to each integration process.

Peak Integration Flow	Parameter	Function < >: Default Value []: Setting Range	Description
<p>Step 1: Peak Detection</p>  <p>Peak start, peak end, and peak top are first detected on chromatograms according to the [Width] and [Slope] values. Set these parameters properly to detect only the necessary peaks and exclude noise.</p>	[Width]	The minimum width at half-height of peaks <3> (Unit: sec) [0.04 to 200]	<p>Specifies the minimum width at half-height detected during analysis. Peaks with smaller widths at half-height are regarded as noise.</p> 
	[Slope]	Peak detection sensitivity (defines peak start or peak end) <1000> (Unit: $\mu\text{V}/\text{min}$) [0 to 4×10^{11}]	<p>Specifies the slope used as a reference for detecting peaks. The point where a graph slope exceeds the set value is recognized as the peak start and where it falls under the set value is recognized as peak end.</p>  <p>Executing the Slope test to obtain a suitable [Slope] value from baseline noise signals.</p> <p>Reference "1.1.4 Slope Test" P.6</p>
	[T.DBL]	Time to change peak detection parameters <1000> (Unit: min) [0 to 1000]	<p>Doubles the minimum width at half-height ([Width]), and halves the peak detection sensitivity ([Slope]) at each set value $\times 2^n$ ($n=0$ to 14). This parameter facilitates the integration of peaks as they become broader with time. If "0" is set, these parameters are adjusted automatically.</p> <p>Reference "1.1.7 [T.DBL] (Time to Change the Peak Detection Parameters)" P.12</p>
<p>Step 2: Process the Baseline (Determine how to integrate overlapped peaks)</p>  <p>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.</p>	[Drift]	Define to slope of the baseline <0> = Auto-process (Unit: $\mu\text{V}/\text{min}$) [-10^7 to 10^7]	<p>Determines how to integrate overlapped peaks. When a valley of overlapped peaks exists above the baseline slope (Drift) the peaks are divided by a vertical line (A). When the valley exists below the baseline slope, the baseline is drawn through the valley (B).</p>  <p>If "0" is set, the software automatically determines how to process the baseline. A negative value can also be set.</p> <p>Reference "1.1.5 [Drift] (Slope to Define Baseline)" P.8</p>

Peak Integration Flow	Parameter	Function <>: Default Value []: Setting Range	Description
<p>Step 3: Integrate Unresolved Peaks</p>  <p>In principle, unresolved peaks are automatically divided by a vertical line. Depending on the conditions, some peaks are recognized appearing on another peak's tail.</p>	None	Auto-process	-
<p>Step 4: Calculate Peak Area</p>  <p>Peak area is calculated (integrated) for each identified peak.</p>	None	Auto-process	-
<p>Step 5: Remove Unnecessary or Small Peaks</p>  <p>Peaks with areas or heights lower than the set value can be hidden from the screen and peak reports.</p>	[Min Area/ Height]	The minimum peak area and height that is displayed <1000> (Unit: counts) [0 to 10 ⁷]	Select "Area" or "Height" to define the small peaks to be hidden (from the screen, peak report, etc.) in [Calculated by] on the [Integration] tab. Use the following unit. Area (μV·sec), Height (μV)

1.1.2 [Width] (The Minimum Width at Half-Height)

The [Width] is the most fundamental parameter among all peak integration parameters. The software detects peaks according to the [Width] value, and performs peak integration under the conditions most suitable for the peaks.

In [Width], specify the minimum width at half-height (width at 50% of peak height) for peaks detected during analysis. Set the value equal to or slightly smaller than the width at half-height of the sharpest peak in the chromatogram.

[Width] is measured in sec.

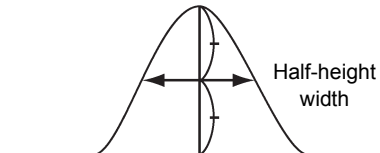


Fig.1-1 Half-Height Width



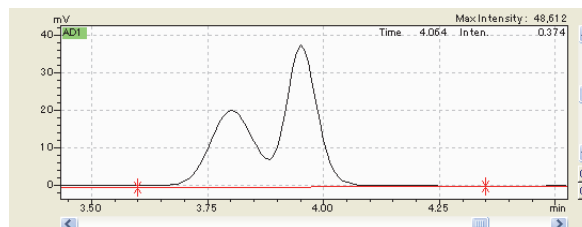
NOTE

Noise width is normally smaller than peak width. By setting the [Width] of the target peak, peaks with smaller widths at half-height are excluded as noise.

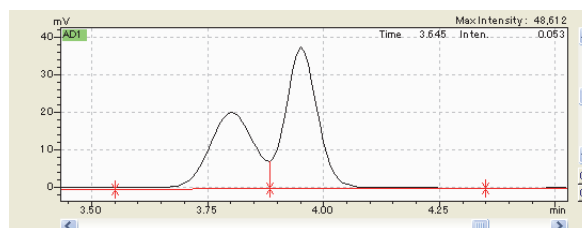
■ [Width] Example

The following shows the comparison between the [Width] settings of 30 and 10 for the same chromatogram.

- [Width] = 30: Only one peak is detected.



- [Width] = 10: Two peaks are detected.



1.1.3 [Slope] (Peak Detection Sensitivity Determining Peak Start/End)

This software detects peaks (i.e., determines peak start/end) according to the graph slope. When θ is set to [Slope] as shown in [Fig.1-2](#), the peak start is detected when the graph slope exceeds the angle θ . Conversely, the peak end is detected when the graph slope falls under the angle θ .

If [Slope] is set to a large value, the software detects only sharp peaks. When a smaller value is used, the software detects broader peaks, as well as a lot of noises in some cases.

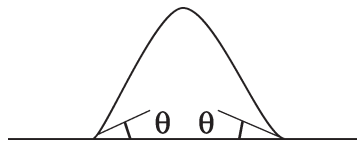
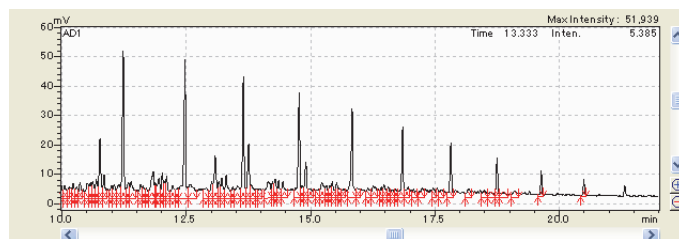


Fig.1-2 Peak Detection and [Slope]

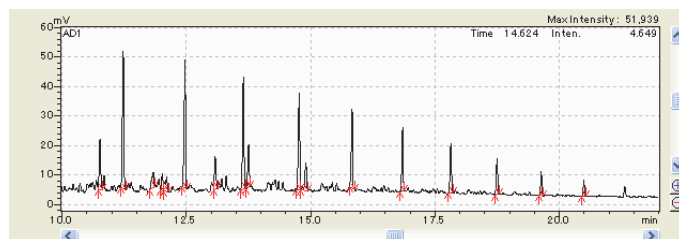
■ [Slope] Example

The following shows the comparison between the [Slope] settings of 1000 and 100000 for the same chromatogram.

- [Slope] = 1000: Noise peaks are detected as minute peaks.



- [Slope] = 100000: Only peaks with inclination larger than the [Slope] value are detected.



1.1.4 Slope Test

The Slope test feature automatically calculates the [Slope] value from baseline noise and drift on chromatograms.

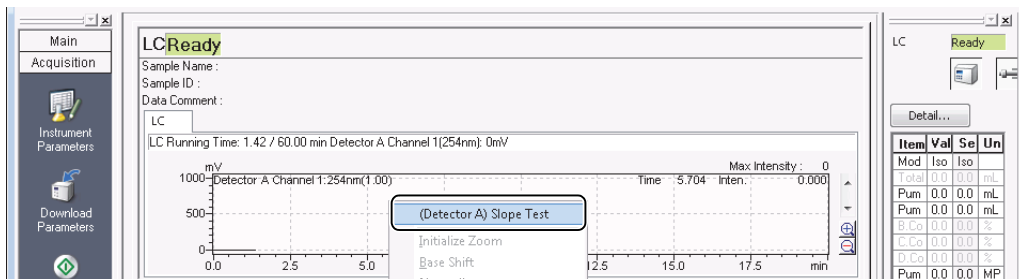
There are two methods for obtaining the slope value: (1) using the baseline before analysis when detector signals are displayed, and (2) using the baseline on chromatograms already obtained.

■ Slope Test Before Analysis

1 Right-click on the chromatogram in the [Data Acquisition] window. Click [Slope Test] on the displayed menu.

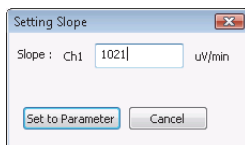
Or click [Slope Test] from the [Acquisition] menu in the [Data Acquisition] window.

The Slope test begins. The test is executed for either 10 times the [Width] value or 10 seconds, whichever is longer.



2 The [Setting Slope] sub-window is displayed. After verifying the value in the [Slope] box, click [Set to Parameter].

The Slope test result ([Slope] value in the [Setting Slope] sub-window) is saved to the peak integration [Slope] parameter. Close the [Setting Slope] sub-window. Click [Cancel] to close the [Setting Slope] sub-window without saving.



NOTE

The Slope test often obtains a different slope value for each execution. Therefore, it is recommended to slightly increase the value in the [Slope] box before saving the parameter.

NOTE




The value obtained in the Slope test is based on the baseline before analysis, and does not reflect the baseline drift which occurs during analysis. Therefore, the following must be observed:

- DO NOT use the Slope test result for gradient LC and programmed temperature GC analyses where a large baseline drift is expected. In such cases, manually set the [Slope] value larger than the drift value so that the baseline will not be detected as a peak.

Reference

Refer to the Operator's Guide for details on how to calculate the Slope value from the baseline (Slope Test).

■ Slope test after analysis

- 1 In the [Postrun Analysis] window, open a data file that contains chromatogram data.
- 2 Click  **Edit** in the [Method View].
The software goes into the edit mode.
- 3 Select the [Integration] tab, and click  **Program** (**Program**).
The [Integration Time Program] sub-window is displayed.
- 4 Click  **(Slope Test)** in the [Integration Time Program] sub-window.
Moving the cursor onto the chromatogram displays a vertical line.
- 5 **Move the cursor to the desired starting point for Slope test on the baseline, and click the mouse button. Choose a section where no peak, no temporary noise, and no drift is observed.**
A vertical line appears at the starting point.
- 6 **Move the cursor to the desired test ending point, and click the mouse button.**
A vertical line appears at the ending point. Specify the ending point so that the test period is longer than 10 times the [Width] value or at least 10 seconds.
A suitable slope value is automatically calculated according to the noise within the specified baseline section. The [Slope Test] sub-window is then displayed.
- 7 **After verifying the displayed value, click [Set to Parameter].**
The [Slope Test] sub-window is closed. The Slope test result is set as the [Slope] parameter in the [Method View] - [Integration] tab page. Click [Cancel] to close the [Slope Test] sub-window without saving the value.
Once the [Slope] value is changed, the software re-executes peak integration.

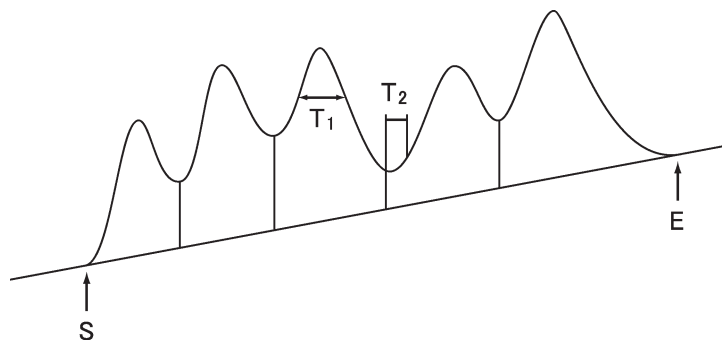
1.1.5 [Drift] (Slope to Define Baseline)

Use the [Drift] parameter to specify the base fluctuation. If [Drift] is set to "0", the baseline is automatically processed according to predetermined rules. If the [Drift] parameter is small, adjacent peaks are regarded as unresolved peaks, and divided by a vertical line at the peak valley.

■ Automatic Baseline Correction ([Drift] = 0)

When [Drift] is set to "0", baseline is corrected according to the following rules shown in *Fig. 1-3*. When the time width of the valley (T_2) is smaller than the estimated width at half-height of the previous peak (T_1), the peaks are unresolved and divided by a vertical line. (2) When T_2 is larger than T_1 , T_2 is processed as baseline.

- $T_1 > T_2$: Regarded as unresolved peaks and divided vertically.



- $T_1 < T_2$: Regarded as separated peaks.

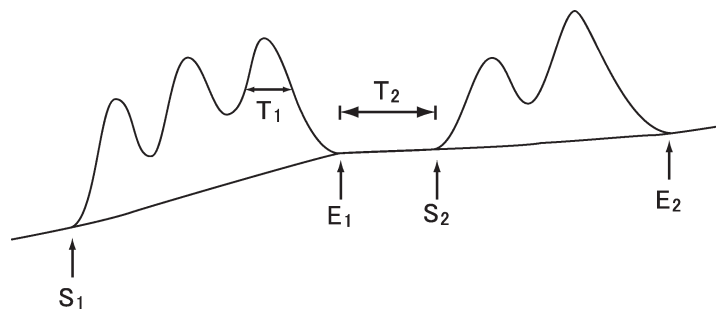


Fig.1-3 Automatic Baseline Correction ([Drift] = 0)

■ Baseline Correction Using [Drift]

A valley with even a small width (T_2) can be processed as a baseline point by setting a value other than “0” for [Drift].

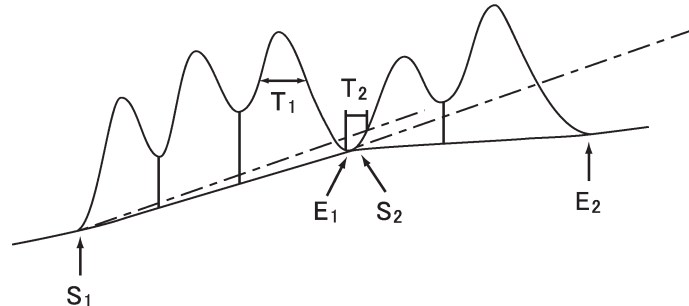


Fig.1-4 Baseline Correction with [Drift] Not Equal to “0”

A slope defined by the [Drift] value (a dashed line) is virtually drawn from the peak start (S_1) as shown in Fig.1-4. If the peak end (E) is positioned below the slope, the baseline is drawn from peak start (S) to peak end (E) diagonally.

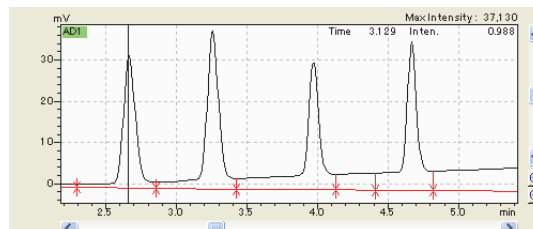
NOTE

When setting the [Drift] parameter to values other than “0”, use a value larger than the actual baseline drift observed during analysis. If the value is smaller, the peak end will never fall below the assumed slope, resulting in all of the peaks being recognized as unresolved peaks.

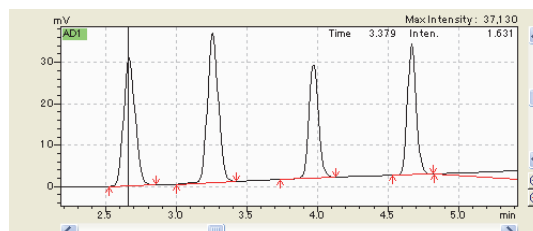
■ [Drift] Example

The following is a comparison between a [Drift] setting of 100 and 5000 for the same chromatogram.

- [Drift] = 100: Baseline is drawn with peaks vertically divided.



- [Drift] = 5000: Baseline is drawn with peaks completely separated.



1.1.6 Integrating Unresolved Peaks

Unresolved peaks may appear when peaks are detected according to [Width] and [Slope] and the baseline is corrected with [Drift].

The software automatically determines whether to divide the peaks vertically as overlapped peaks (vertical division), or to process them as a small peak appearing on the other peak's tail (tailing).

■ Vertical Division

In principle all unresolved peaks are divided by a vertical line at the peak valley.

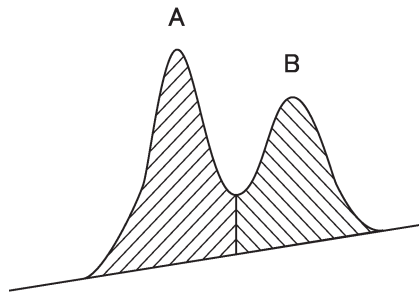


Fig.1-5 Vertical Division

■ Tailing

The software determines whether or not the accompanying peak is located on a tailing peak, using two peak height ratio, width ratio, ratio of valley height and peak height, etc.

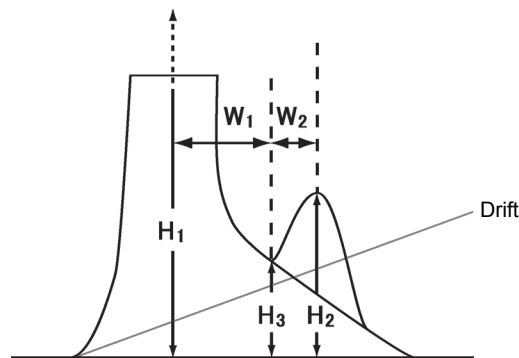


Fig.1-6 Conditions for Tailing

If all of the conditions below are satisfied, the accompanying peak is regarded as being on the tail.

Valley height H_3 is above the Drift line (including when Drift = 0).

$H_1 / H_2 > 10$ The accompanying peak is sufficiently smaller than the main peak.

$W_1 / W_2 > 3$ The accompanying peak is sufficiently narrower than the main peak.

$H_2 / H_3 < 100$ The accompanying peak start is high relative to the peak height.

If any of the following conditions is met, the accompanying peak is not regarded as being on the tail.

The valley height H_3 fell below the Drift line.

[Drift] is set to 0 and the software detects the end of unresolved peaks.

$H_1 / H_2 < 10$ There is not sufficient height difference between the peaks.

■ Example of Unresolved Peak Integration

Fig. 1-7 shows an example of automatic unresolved peak integration.

Peak A is processed as a main tailing peak. Peaks B and C are processed as being located on peak A's tail. Note that unresolved peaks on the tail such as Peak C are always divided vertically. Whether or not one peak is on the other's tail is no longer examined.

Peaks E, F, and G are unresolved, but not located on the tail of the main peak and are therefore, divided vertically.

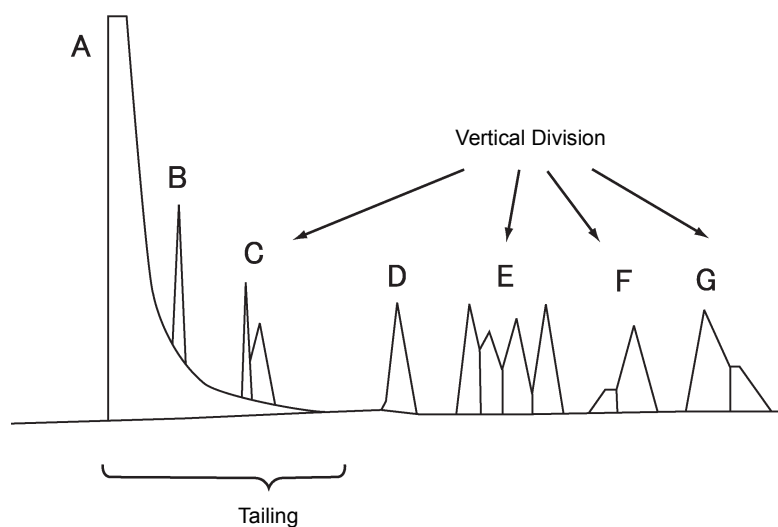


Fig. 1-7 Unresolved Peak Integration



NOTE

The following peak marks can be used to indicate various integration status. These peak marks can be displayed on the peak table and chromatogram peak top either on the screen or in the reports:

- S: Main tailing/leading peak
- T: Peak on a tail
- L: Peak on a head
- V: The second or later peaks of unresolved peaks (divided vertically)
- H: Peak whose baseline is corrected as horizontal
- M: Peak integrated manually
- E: Error peak (due to over flow or under flow)

1.1.7 [T.DBL] (Time to Change the Peak Detection Parameters)

When [T.DBL] is set, the software detects peaks by increasing [Width] by 2 times and decreasing [Slope] by 1/2, at each [T.DBL] value $\times 2^n$ ($n = 0$ to 14). If [T.DBL] is set to 0, [Width] and [Slope] are adjusted according to a predetermined ratio. To stop these parameters from changing during analysis, specify a time longer than the analysis. This parameter is usually left as 1000 (default).



NOTE

It is recommended that these parameters NOT be changed during analysis. Use a time longer than the analysis.

■ Keeping Peak Detection Parameters Constant

In gradient LC and programmed temperature GC analyses, peaks do not become broader with time. Set the [T.DBL] value longer than analysis time to have the [Width] and [Slope] parameters remain constant.

■ Changing Peak Detection Parameters Using [T.DBL]

Set [T.DBL] to 0 to automatically integrate peaks that become broader over time. [T.DBL] increases the [Width] by 2 times and decreases the [Slope] by 1/2 at the specified time (see Fig.1-8).

This parameter change is repeated at the time specified in the [T.DBL] parameter (see Fig.1-8).

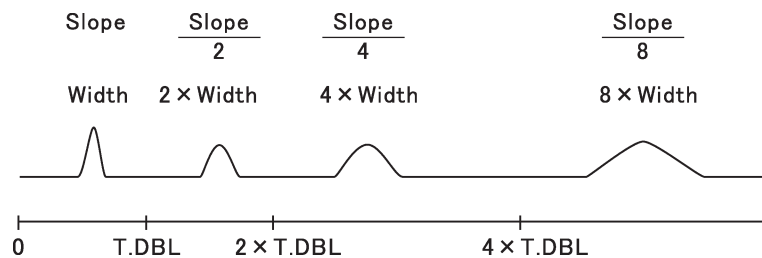


Fig.1-8 Changing [Width] and [Slope] at the [T.DBL] time



NOTE

The [Width] parameter can be changed up to 15 times per analysis. This number includes cases of changing the value using either [Width] or [T.DBL] in the integration time program.

Set the [T.DBL] value to a time when a peak width is twice that of the first peak width. Use the following calculation to determine the [T.DBL] value if no such peak (width twice that of the first peak) is present in the chromatogram.

Example:

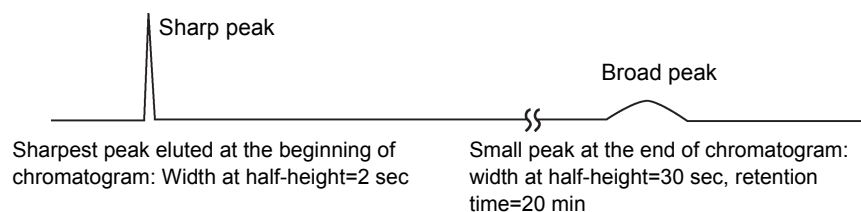


Fig.1-9 T.DBL Calculation

1 Use the width at half-height of the sharpest peak eluted at the beginning of the chromatogram for the [Width] parameter.

In this example, 2 sec.

2 Measure the retention time and width at half-height of a small peak eluted at the end of chromatogram.

In this example, retention time = 20 min and width at half-height = 30 sec.

Since the peak width has increased by 15 times in 20 minutes,

The time required to double the peak width = $20 \text{ min} \div 15 \text{ times} \times 2 = \text{Approximately } 2.7 \text{ min}$

The general equation will be,

$$\text{T.DBL} = [\text{Width}] \text{ value} \times \frac{\text{retention time late peak}}{\text{width at half-height late peak}} \times 2$$

■ Adjusting [Width] and [Slope] Automatically ([T.DBL] = 0)

If [T.DBL] is set to 0, [Slope] and [Width] values are automatically adjusted as peaks become broader. In isocratic LC and isothermal GC analyses, the peak width increases over time. High peak detection sensitivity (low [Slope] value) is not necessary in the beginning because early-eluting peaks have a large slope. The width of later peaks becomes broader and the software automatically decreases the [Slope] value which increases the peak detection sensitivity. Similarly, the minimum width at half-height is low at the beginning and is increased as peaks become broader.



Fig.1-10 Chromatogram Suitable for Auto-setting by [T.DBL]

NOTE

Set [T.DBL] to a value longer than the analysis time (instead of 0) in the following cases.

- (1) When peaks do not become broader with time as in gradient LC analyses or temperature-programmed GC analyses.
- (2) When a sharp peak appears after a broad peak, as in [Fig.1-11](#).

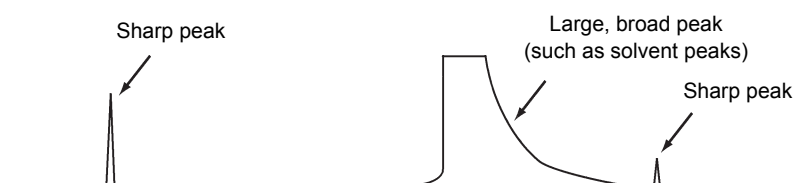


Fig.1-11 Chromatogram NOT Suitable for [T.DBL]=0

1.2 Identification Parameters

The software uses the values in the compound table and the identification parameters to identify the detected peaks.

This section describes the identification parameters, and how they are used to identify the detected peaks.

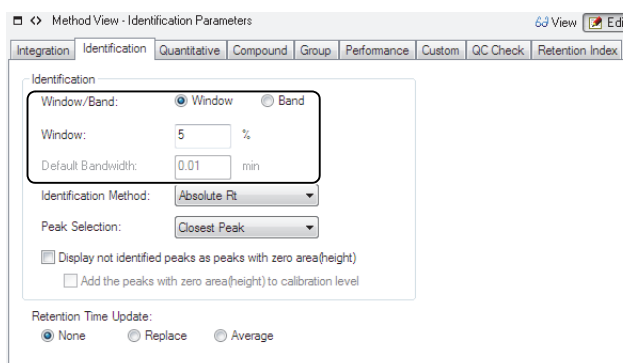
Reference

To set the identification parameters, refer to the Operator's Guide.

1.2.1 Window Method and Band Method

A peak identification allowance window can be set to allow identification of a peak even if it deviates from the retention time in the compound table. The peak is identified as the peak in the compound window as long as the deviation falls within the time allowance.

There are two methods for setting a time allowance: [Window] or [Band]. Click a radio button to select one of the methods.



■ Window Method

The Window method defines the time allowance in proportion to the peak retention time. The longer the retention time, the larger the absolute time allowance (see [Fig. 1-12](#)). Use this method to specify the time allowance for all peaks at once. Depending on the value, multiple peaks may be detected within the same time allowance.

The Window method is useful for isocratic LC and isothermal GC analyses, where peak width and retention time fluctuation increase over time.

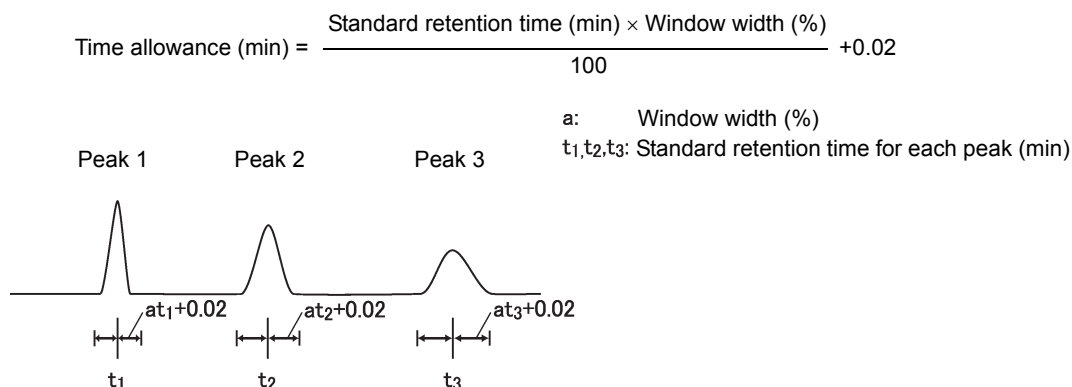


Fig.1-12 Window Method

Band Method

The Band method defines a different allowance for each peak. Use this method to specify the optimum time allowance for each peak (see Fig.1-13).

The Band method is useful for gradient LC and temperature programmed GC analyses, where peak width and retention time remain comparatively unchanged.

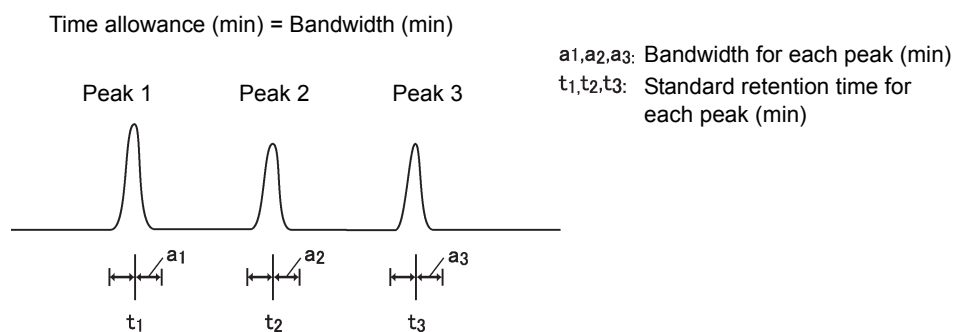


Fig.1-13 Band Method



NOTE

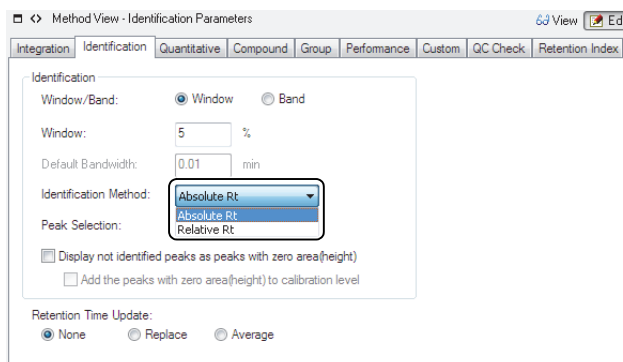
The [Band] parameter can be set in the [Table Style] sub-window of the [Compound Table].

The [Default Band Time] value on the [Identification] tab is used as the default band time for all of the peaks.

1.2.2 Absolute/Relative Retention Time Methods

This software identifies peaks based on retention time. The retention time can be specified as absolute retention time (counted from analysis start) or relative retention time (corrected using a reference peak).

Select [Absolute Rt] or [Relative Rt] for the [Identification Method].



Absolute Retention Time

The software identifies target peaks according to the equation below. It uses 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

Relative Retention Time

The software identifies peaks after correcting any deviation to the retention time that may have occurred due to changes in the analysis conditions. First, a preset reference peak is identified using the absolute retention time. Target peaks are then identified according to the equation below.

This method is useful for consecutive analyses, where the retention time varies over time, and for analyses where the time error is significant at the start of the analysis.

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

- T : Standard retention time of target peak
- T₁ : Standard retention time of reference peak
- t : Measured retention time of target peak
- t₁ : Measured retention time of reference peak
- w : Time allowance of target peak

Use multiple reference peaks to more accurately correct the retention times. If a target peak exists between two reference peaks (see Fig.1-14), the peak is identified using both reference peaks according to the equation below.

$$\left| T - \left\{ \frac{t - t_1}{t_2 - t_1} \times (T_2 - T_1) + T_1 \right\} \right| < W$$

- T : Standard retention time of target peak
- T₁ : Standard retention time of reference peak 1
- T₂ : Standard retention time of reference peak 2
- t : Measured retention time of target peak
- t₁ : Measured retention time of reference peak 1
- t₂ : Measured retention time of reference peak 2
- w : Time allowance of target peak

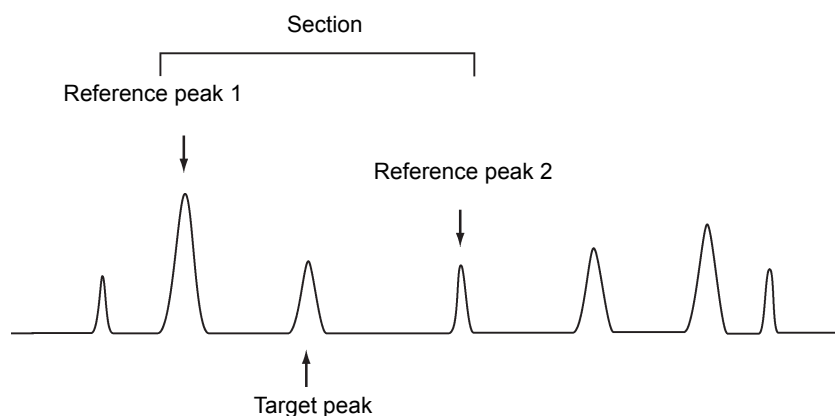


Fig.1-14 Relative Retention Time Method



NOTE

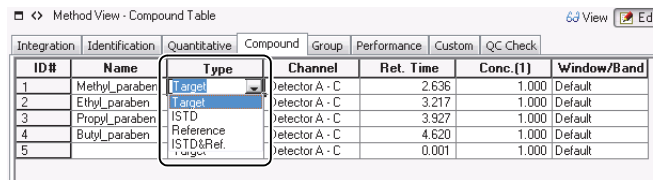
The relative retention time is obtained by the following equation:

$$RRT = \frac{t_1 - t_0}{t_2 - t_0}$$

- RRT : Relative retention time
- t₁ : Retention time of target peak
- t₂ : Retention time of reference peak
- t₀ : Unretained peak time

Reference Peak and ISTD Peak

Select reference peaks to identify peaks with the relative retention time method. Select ISTD to quantitate peaks with the internal standard method.



ID#	Name	Type	Channel	Ret. Time	Conc.(1)	Window/Band
1	Methyl_paraben	Target	Detector A - C	2.636	1.000	Default
2	Ethyl_paraben	Target	Detector A - C	3.217	1.000	Default
3	Propyl_paraben	ISTD	Detector A - C	3.927	1.000	Default
4	Butyl_paraben	Reference	Detector A - C	4.620	1.000	Default
5		ISTD&Ref.	Detector A - C	0.001	1.000	Default

Whether using the Window or Band method, the reference and ISTD peak is identified as the largest (height or area) peak in the time allowance.

If there is a larger peak than the intended peak within the time allowance, the peak may be mistakenly identified as the reference (or ISTD) peak, leading to incorrect identification and quantitation of other peaks.

The misidentification can be avoided by, (1) selecting the largest (height or area) peak as the reference or ISTD peak, or (2) using a smaller time allowance so that peaks larger than the intended peak do not fall within the time allowance.

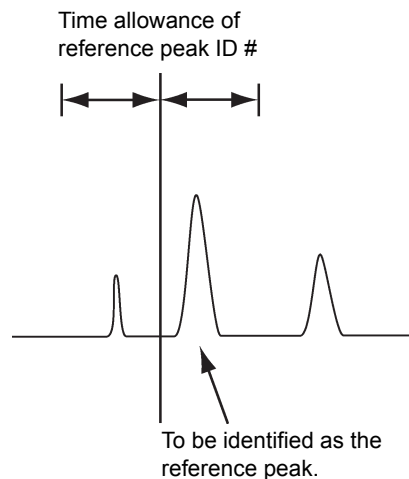


Fig. 1-15 Reference Peak Identification

1.2.3 Identification of Adjacent Peaks

■ Multiple Peaks in the Same Time Allowance

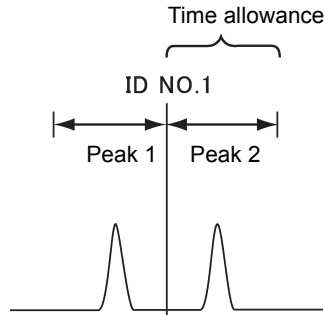
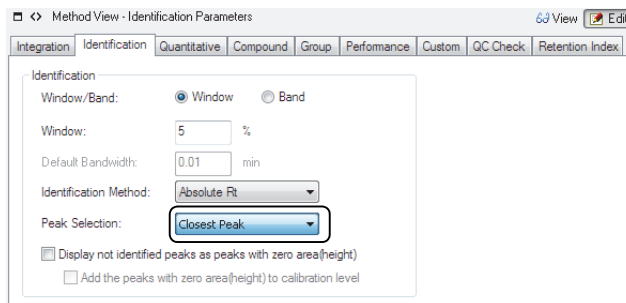


Fig.1-16 Multiple Peaks in the Same Time Allowance

Use the [Peak Selection] drop-down menu to identify the peaks when multiple peaks exist within the same time allowance. Select from: [All Peaks], [Closest Peak], [Largest Peak], [Similarity] (PDA), [All + Similarity] (PDA), [Closest + Similarity](PDA) and [Largest + Similarity] (PDA).



Parameter	Description
All Peaks	Identifies all peaks as compounds.
Closest Peak	Selects and identifies the peak having the retention time closest to the standard retention time set to the Compound Table.
Largest Peak	Selects and identifies the peak having the largest peak area (or height) from among the peaks in the peak allowable width. Whether to use peak area or peak height depends on the setting of [Calculated by] in [Quantitative] tab.
Similarity (PDA)	Calculates the similarity between the spectrum at the retention time of the peak in the peak allowable width and the standard spectrum registered to the Compound Table, and identifies the peak with the largest similarity value as the compound.
All + Similarity (PDA)	Identifies all peaks within the allowable peak width that have a similarity value greater than or equal to the threshold value.
Closest + Similarity (PDA)	Selects the peak having the retention time closest to the standard retention time specified in the compound table, and also identifies it if the peak has a similarity value greater than or equal to the threshold value.
Largest + Similarity (PDA)	Selects the peak having the largest peak area (or height) from among the peaks with allowable peak widths, and also identifies it if the peak has a similarity value greater than or equal to the threshold value. Whether to use peak area or peak height depends on the setting of [Calculated by] in the [Quantitative] tab.

**NOTE**

For identification of ISTD and reference peaks, the software always picks up the largest (highest) peak within the time allowance, even though [Peak Selection] is set to [All Peaks], [Closest Peak], or [Similarity] (PDA).

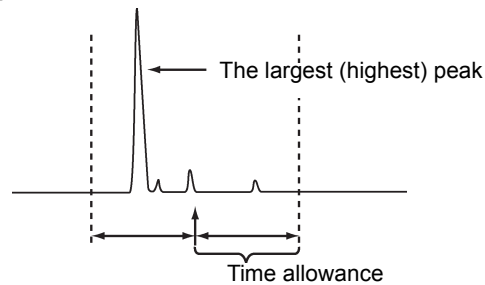


Fig.1-17 Identifying ISTD Peak and Reference Peak

1

■ One Peak in Overlapping Time Allowances and ID #s

When multiple time allowances with different ID numbers overlap, and a peak falls within the overlapped section, the software identifies the peak in a different manner depending on the Window or Band method.

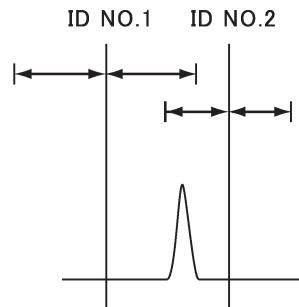


Fig.1-18 One Peak in Two Time Allowances

- Window method
The peak is identified as the ID No. whose standard retention time is the closest to the peak retention time. In the example in [Fig. 1-18](#), the peak is identified as ID No. 2.
- Band method
The peak is identified with the smallest ID No. In the example in [Fig. 1-18](#), the peak is identified as ID No. 1.

■ Adjacent Peaks with Different ID #s

When peaks are very close to each other as shown in [Fig. 1-19](#), it may be difficult to specify the time allowance for each peak. To identify and quantitate those peaks, use the Window method and set each peak retention time to the same value.

The peaks will be identified in the order of resolution. The [Peak Selection] value becomes invalid with the Window method. Therefore, if an unnecessary peak is detected, the peaks and ID numbers may become inconsistent, resulting in incorrect identification.

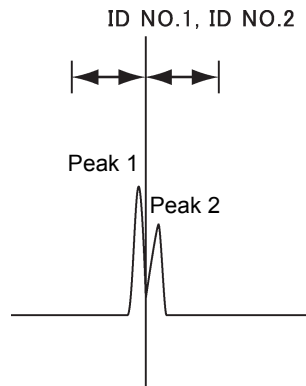
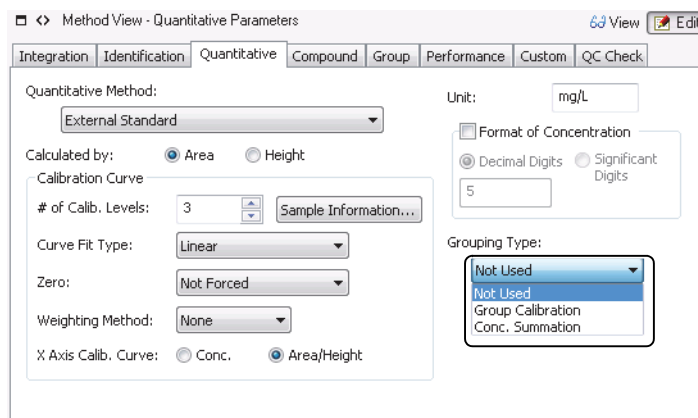


Fig.1-19 Adjacent Peaks

1.2.4 Grouping

Grouping is the process of grouping peaks by types, such as homologues and isomers, to perform calculations for each group.

There are two types of grouping: [Group Calibration] and [Conc. Summation].



■ Group Calibration

The software first obtains the sum of area (or height) of the grouped peaks. Then the calibration curve is created and quantitation is executed for the group.

Area/height per group is the sum of the areas/heights of the compounds in the same group.

■ Conc. Summation

The software first creates the calibration curve for each grouped compound, and quantitates the peaks individually. Then the concentrations of all grouped compounds are summed to obtain the group concentration.

1.3 Quantitative Methods

This software uses the following six types of quantitative methods.

The equations and rounding methods applied to each quantitative method is explained below.

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 the standard and unknown sample. This method gives stable results and eliminates injection errors. (1) A specific amount of ISTD substance is added to the standard sample that contains a know quantity of the target component. (2) The standard sample is analyzed and a calibration curve is created from the area (height) ratio and component concentration ratio between the target and ISTD peak. (3) The area ratio between the target and ISTD peaks is obtained from the unknown sample that contains the same ISTD substance. (4) Using the concentration ratio acquired from the calibration curve above (step 2), the target component is quantitated.
External Standard (Absolute Calibration Curve)	Creates a calibration curve expressing the relationship between standard concentration 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	An unknown sample with an added known amount of standard component and unknown sample without that is addition are measured under same conditions. The standard component is quantitated using the difference of two obtained peak areas (heights). This method is useful for analyses where the relative sensitivity of the target component varies with solvent composition or coexisting components (as in head space GC).

1.3.1 Quantitative Methods and Equations (with Dilution Factor)



NOTE

Set [Dilution Factor] to either [Apply] or [Not Used] in the [Data Processing Setting] sub-window in the [System Settings] window.

■ Area/Height on the X-Axis

1

Quantitative Method	One Point	Two Points	Content
Area Normalization	-	-	Content (%) = $\frac{A_i}{\sum A_i} \times 100$
Corrected Area Normalization	$F1 = \frac{C_1}{A_1}$	$F1 = \frac{C_1 - C_2}{A_1 - A_2}$ $F2 = C_2 - F1 \times A_2$	Content (%) = $\frac{F1_i A_i - F2_i}{\sum (F1_i A_i + F2_i)} \times 100$
Internal Standard	$F1 = \frac{C_1}{C_{isn_1}} \times \frac{A_1}{A_{isn_1}}$	$F1 = \left(\frac{C_1}{C_{isn_1}} - \frac{C_2}{C_{isn_2}} \right) \times \left(\frac{A_1}{A_{isn_1}} - \frac{A_2}{A_{isn_2}} \right)$ $F2 = \frac{C_2}{C_{isn_2}} - F1 \times \frac{A_2}{A_{isn_2}}$	Content = $\left(\frac{F1_i A_i}{A_{isn}} + F2_i \right) \times \frac{W_{isn}}{W_{spl}} \times DFACT$
External Standard (Absolute Calibration Curve)	$F1 = \frac{C_1}{A_1}$	$F1 = \frac{C_1 - C_2}{A_1 - A_2}$ $F2 = C_2 - F1 \times A_2$	Content = $\frac{F1_i A_i + F2_i}{W_{spl}} \times DFACT$
Corrected Area Normalization with Scale Factor	$F1 = \frac{C_1}{A_1}$	$F1 = \frac{C_1 - C_2}{A_1 - A_2}$ $F2 = C_2 - F1 \times A_2$	Content (%) = $\frac{F1_i A_i - F2_i}{\sum (F1_i A_i + F2_i)} \times DFACT$
Standard Addition	-	$F1 = \frac{C_1 - C_2}{A_1 - A_2}$ $F2 = C_2 - F1 \times A_2$	Content = $\frac{F2_i}{W_{spl}} \times DFACT$

■ Concentration on the X-Axis

Quantitative Method	One Point	Two Points	Content
Area Normalization	-	-	Content (%) = $\frac{A_i}{\sum A_i} \times 100$
Corrected Area Normalization	$F1 = \frac{A_1}{C_1}$	$F1 = \frac{A_1 - A_2}{C_1 - C_2}$ $F2 = A_2 - F1 \times C_2$	Content (%) = $\frac{(A_i - F2_i)/F1_i}{\sum (A_i - F2_i)/F1_i} \times 100$
Internal Standard	$F1 = \frac{A_1/A_{isn_1}}{C_1/C_{isn_1}}$	$F1 = \left(\frac{A_1}{A_{isn_1}} - \frac{A_2}{A_{isn_2}} \right) / \left(\frac{C_1}{C_{isn_1}} - \frac{C_2}{C_{isn_2}} \right)$ $F2 = \frac{A_2}{A_{isn_2}} - F1 \times \frac{C_2}{C_{isn_2}}$	Content = $\frac{(A_i/A_{isn} - F2_i)}{F1_i} \times \frac{W_{is}}{W_{spl}} \times DFACT$
External Standard (Absolute Calibration Curve)	$F1 = \frac{A_1}{C_1}$	$F1 = \frac{A_1 - A_2}{C_1 - C_2}$ $F2 = A_2 - F1 \times C_2$	Content = $\frac{A_i - F2_i}{F1_i} \times \frac{DFACT}{W_{spl}}$
Corrected Area Normalization with Scale Factor	$F1 = \frac{A_1}{C_1}$	$F1 = \frac{A_1 - A_2}{C_1 - C_2}$ $F2 = A_2 - F1 \times C_2$	Content (%) = $\frac{(A_i - F2_i)/F1_i}{\sum (A_i - F2_i)/F1_i} \times DFACT$
Standard Addition	-	$F1 = \frac{A_1 - A_2}{C_1 - C_2}$ $F2 = A_2 - F1 \times C_2$	Content = $\frac{F2_i}{F1_i} \times \frac{DFACT}{W_{spl}}$

- A_1 : Peak area (height) of Standard 1
- A_2 : Peak area (height) of Standard 2
- C_1 : Concentration (component amount) of Standard 1
- C_2 : Concentration (component amount) of Standard 2
- A_{isn_1} : Peak area (height) of Nth ISTD peak in Standard 1
- A_{isn_2} : Peak area (height) of Nth ISTD peak in Standard 2
- C_{isn_1} : Concentration (component amount) of Nth ISTD peak in Standard 1
- C_{isn_2} : Concentration (component amount) of Nth ISTD peak in Standard 2
- W_{spl_1} : Sample amount of Standard 1
- W_{spl_2} : Sample amount of Standard 2
- $F1_i$: Slope compensation factor
- $F2_i$: Constant compensation factor
- A_i : Peak area (height)
- A_{isn} : Area (height) of Nth ISTD peak
- W_{spl} : Sample amount
- W_{isn} : Amount of the Nth ISTD
- DFACT : Dilution factor

1.3.2 Quantitative Methods and Equations (without Dilution Factor)

■ Area/Height on the X-Axis

Quantitative Method	One Point	Two Points	Content
Area Normalization	-	-	Content (%) = $\frac{A_i}{\sum A_i} \times 100$
Corrected Area Normalization	$F1 = \frac{C_1}{A_1}$	$F1 = \frac{C_1 - C_2}{A_1 - A_2}$ $F2 = C_2 - F1 \times A_2$	Content (%) = $\frac{F1_i A_i - F2_i}{\sum (F1_i A_i + F2_i)} \times 100$
Internal Standard	$F1 = \frac{C_1 / A_1}{C_{isn_1} / A_{isn_1}}$	$F1 = \left(\frac{C_1}{C_{isn_1}} - \frac{C_2}{C_{isn_2}} \right) / \left(\frac{A_1}{A_{isn_1}} - \frac{A_2}{A_{isn_2}} \right)$ $F2 = \frac{C_2}{C_{isn_2}} - F1 \times \frac{A_2}{A_{isn_2}}$	Content = $\left(\frac{F1_i A_i}{A_{isn}} + F2_i \right) \times \frac{W_{isn}}{W_{sp1}} \times 100$
External Standard (Absolute Calibration Curve)	$F1 = \frac{W_{sp1} \times C_1}{100 \times A_1}$	$F1 = \frac{C_1 \times W_{sp1_1} - C_2 \times W_{sp1_2}}{100(A_1 - A_2)}$ $F2 = \frac{C_2 \times W_{sp1_2}}{100} - F1 \times A_2$	Content = $\frac{F1_i A_i + F2_i}{W_{sp1}} \times 100$
Corrected Area Normalization with Scale Factor	$F1 = \frac{C_1}{A_1}$	$F1 = \frac{C_1 - C_2}{A_1 - A_2}$ $F2 = C_2 - F1 \times A_2$	Content (%) = $\frac{F1_i A_i - F2_i}{\sum (F1_i A_i + F2_i)} \times W_{sp1}$
Standard Addition	-	$F1 = \frac{C_1 \times W_{sp1_1} - C_2 \times W_{sp1_2}}{100(A_1 - A_2)}$ $F2 = \frac{C_2 \times W_{sp1_2}}{100} - F1 \times A_2$	Content = $\frac{F2_i}{W_{sp1}} \times 100$

1

■ Concentration on the X-Axis

Quantitative Method	One Point	Two Points	Content
Area Normalization	-	-	Content (%) = $\frac{A_i}{\sum A_i} \times 100$
Corrected Area Normalization	$F1 = \frac{A_1}{C_1}$	$F1 = \frac{A_1 - A_2}{C_1 - C_2}$ $F2 = A_2 - F1 \times C_2$	Content (%) = $\frac{(A_i - F2_i)/F1_i}{\sum (A_i - F2_i)/F1_i} \times 100$
Internal Standard	$F1 = \frac{A_1/A_{isn_1}}{C_1/C_{isn_1}}$	$F1 = \left(\frac{A_1}{A_{isn_1}} - \frac{A_2}{A_{isn_2}} \right) / \left(\frac{C_1}{C_{isn_1}} - \frac{C_2}{C_{isn_2}} \right)$ $F2 = \frac{A_2}{A_{isn_2}} - F1 \times \frac{C_2}{C_{isn_2}}$	Content = $\frac{(A_i/A_{isn} - F2_i)}{F1_i} \times \frac{W_{is}}{W_{spl}} \times 100$
External Standard (Absolute Calibration Curve)	$F1 = \frac{A_1 \times 100}{C_1 \times W_{spl}}$	$F1 = \frac{100(A_1 - A_2)}{C_1 \times W_{spl_1} - C_2 \times W_{spl_2}}$ $F2 = A_2 - F1 \times C_2 \times W_{spl_2} / 100$	Content = $\frac{A_i - F2_i}{F1_i} \times \frac{100}{W_{spl}}$
Corrected Area Normalization with Scale Factor	$F1 = \frac{A_1}{C_1}$	$F1 = \frac{A_1 - A_2}{C_1 - C_2}$ $F2 = A_2 - F1 \times C_2$	Content (%) = $\frac{(A_i - F2_i)/F1_i}{\sum (A_i - F2_i)/F1_i} \times W_{spl}$
Standard Addition	-	$F1 = \frac{100(A_1 - A_2)}{C_1 \times W_{spl_1} - C_2 \times W_{spl_2}}$ $F2 = A_2 - F1 \times C_2 \times W_{spl_2} / 100$	Content = $\frac{F2_i}{F1_i} \times \frac{100}{W_{spl}}$

- A_1 : Peak area (height) of Standard 1
- A_2 : Peak area (height) of Standard 2
- C_1 : Concentration (component amount) of Standard 1
- C_2 : Concentration (component amount) of Standard 2
- A_{isn_1} : Peak area (height) of Nth ISTD peak in Standard 1
- A_{isn_2} : Peak area (height) of Nth ISTD peak in Standard 2
- C_{isn_1} : Concentration (component amount) of Nth ISTD peak in Standard 1
- C_{isn_2} : Concentration (component amount) of Nth ISTD peak in Standard 2
- W_{spl_1} : Sample amount of Standard 1
- W_{spl_2} : Sample amount of Standard 2
- $F1_i$: Slope compensation factor
- $F2_i$: Constant compensation factor
- A_i : Peak area (height)
- A_{isn} : Area (height) of Nth ISTD peak
- W_{spl} : Sample amount
- W_{isn} : Amount of the Nth ISTD

1.3.3 Rounding

The following table shows the number of significant digits and rounding method applied to CLASS-LC10, CLASS-GC10, CLASS-VP and LabSolutions.

Parameter		CLASS-LC10, CLASS-GC10		CLASS-VP		LabSolutions	
		Internal Calculation	Stored Format	Internal Calculation	Stored Format	Internal Calculation	Stored Format
Peak Integration	Area	48-bit integer (unit: 0.1)	48-bit integer (unit: 0.1)	Double precision	Double precision	Double precision	Double precision
	Sum of Areas	48-bit integer (unit: 0.1)	-	Double precision	-	Double precision	-
	Height	32-bit integer (unit: 1/16)	32-bit integer (unit: 1/16)	Double precision	Double precision	Double precision	Double precision
	Sum of heights	32-bit integer (unit: 1/16)	-	Double precision	-	Double precision	-
Quantitation	Concentration	Single precision	Single precision	Single precision	Single precision	Double precision	Double precision
	Sum of Concentrations	Single precision	-	Single precision	-	Double precision	-
	Quantitative Calculation Factor	Single precision	-	Single precision	Single precision (*1)	Double precision	Double precision
Column Performance	Separation Factor	Single precision	-	Single precision	Single precision	Double precision	Double precision
	Number of Theoretical Plate	Single precision	-	Single precision	Single precision	Double precision	Double precision
	Number of Theoretical Plate/meter	Single precision	-	Single precision	Single precision	Double precision	Double precision
	Capacity Factor (k')	Single precision	-	Single precision	Single precision	Double precision	Double precision
	Resolution	Single precision	-	Single precision	Single precision	Double precision	Double precision
	Tailing Factor	Single precision	-	Single precision	Single precision	Double precision	Double precision
	Tailing Factor (10%)	None	None	Single precision	Single precision	Double precision	Double precision

*1 For CLASS-VP, only the quantitative calculation factors in the original method used for analysis are saved in data files.

NOTE

The parameters internally calculated for output (marked with "-") are not saved in data files.

[Reference]

- Single precision floating
Single-precision values consist of an 8-bit binary exponent, and a 24-bit mantissa. Significant digits are 6 to 7 for decimal format. Available range of exponent part is $10^{\pm 38}$.
- Double precision floating
Double precision values consist of an 11-bit exponent and a 53-bit mantissa. Significant digits are 15 to 16 for decimal format. Available range of exponent part is $10^{\pm 308}$.

Reference

For LabSolutions, the user can set the rounding method and digits of values displayed on the screen and reports.

For detailed procedures, refer to the System User's Guide.

1.4 Accuracy and Deviation Between the Standard Concentrations

The accuracy (Accuracy [%]) and deviation (%Deviation) between the standard concentration in the compound table and that obtained from the calibration curve can be verified in the [Quantitative Results View] of the [Quant Browser] window.

This section describes the equation for obtaining Accuracy [%] and %Deviation.

Reference

For the procedures for setting Accuracy [%] and %Deviation, refer to the help files.

1.4.1 Accuracy [%]

[Accuracy [%]] can be obtained using the following equation:

■ [Sample Type] is [Standard], [Control], or [Unknown (QA/QC)]

$$\text{Accuracy [\%]} = C_r / C_c \times 100$$

C_r : Concentration obtained from calibration curve
 C_c : Concentration set to the relevant level in the compound table

NOTE

Concentration from calibration curve = (Concentration in the compound result table) × (Sample volume in the [Single Run] sub-window or batch table) / Dilution factor

To verify the current sample amount and dilution factor, open the [Sample Info.] tab from the [Properties] sub-window.

■ [Sample Type] is [Spiked]

$$\text{Accuracy [\%]} = C_r / C_s \times 100$$

C_r : Concentration obtained from calibration curve
 C_s : Spiked amount set in the compound table

1.4.2 %Deviation

[%Deviation] can be obtained using the equation described below. The software executes the statistic calculation based on the absolute value of [%Deviation].

■ [Sample Type] is [Standard], [Control], or [Unknown (QA/QC)]

$$\% \text{Deviation} = (C_r - C_c) / C_c \times 100$$

C_r : Concentration obtained from calibration curve
 C_c : Concentration set to the relevant level in the compound table

■ [Sample Type] is [Spiked]

$$\% \text{Deviation} = (C_r - C_s) / C_s \times 100$$

C_r : Concentration obtained from calibration curve
 C_s : Spiked amount set in the compound table

2

Calibration Curve

This chapter describes the types of calibration curves, the equations used in calibration, the standard concentration correction factor and calibration curve creation with multiple standards.

This chapter consists of the following sections:

- "2.1 Calibration Curve Type" P.29
- "2.2 Calibration Curve Correction" P.36

2

2.1 Calibration Curve Type

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

2.1.1 Linear

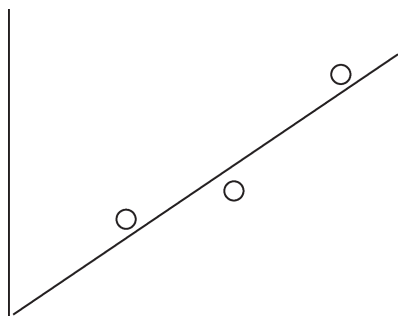
A number (# of Calib. Levels) of standards with different concentrations are analyzed, and a linear calibration curve is drawn using the least square method.

If only one standard concentration level is used, the calibration curve is drawn as a linear line passing through the obtained point and the origin.

If two standard concentration levels are used and the calibration curve does not pass through the origin, the calibration curve is drawn as a linear line passing through the two obtained points.

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

Up to 64 calibration levels can be set (# of Calib. Levels). Up to ten measurements can be averaged for each calibration level.



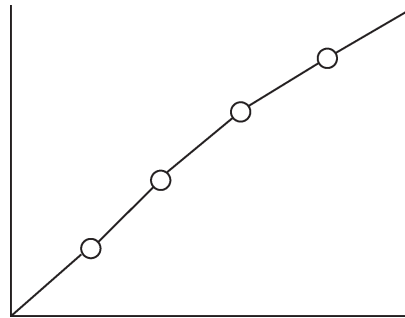
2.1.2 Point to Point

A number ([# of Calib. Levels]) of standards with different concentrations are analyzed, and the calibration curve is drawn by connecting the obtained points.

If only one standard concentration level is used, the calibration curve is drawn as a linear line passing through the obtained point and the origin.

If two or more standard concentration levels are used, the calibration curve is drawn by connecting the obtained points. The calibration curve is always connected to the origin.

Up to 64 calibration levels can be set ([# of Calib. Levels]). Up to ten measurements can be averaged for each calibration level.



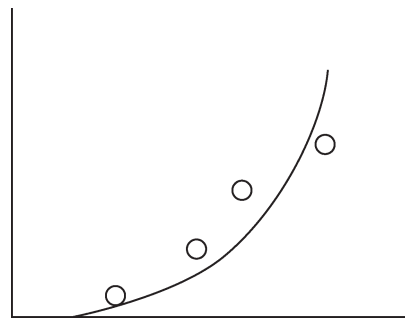
2.1.3 Quadratic and Cubic

A number ([# of Calib. Levels]) of standards with different concentrations are analyzed, and a quadratic or cubic calibration curve is drawn by the least square method.

Three or more standard concentration levels are required for a quadratic calibration curve.

Four or more standard concentration levels are required for a cubic calibration curve.

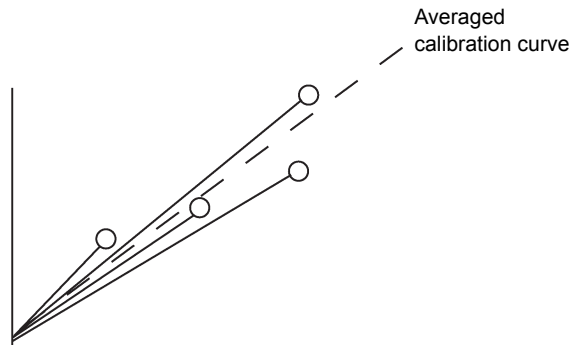
Up to 64 calibration levels can be set ([# of Calib. Levels]). Up to ten measurements can be averaged for each calibration level.



2.1.4 Mean RF

A number ([# of Calib. Levels]) of standards with different concentrations are analyzed, and each of the obtained points is connected to the origin. The slope coefficients of all of the linear lines are averaged.

Up to 64 calibration levels can be set ([# of Calib. Levels]). Up to ten measurements can be averaged for each calibration level.



2.1.5 Exponential

A number ([# of Calib. Levels]) of standards with different concentrations are analyzed, and a linear calibration curve is drawn in a natural log-natural log graph.

If two standard concentration levels are used and the calibration curve does not pass through the origin, the calibration curve is drawn as a linear line passing through the two obtained points.

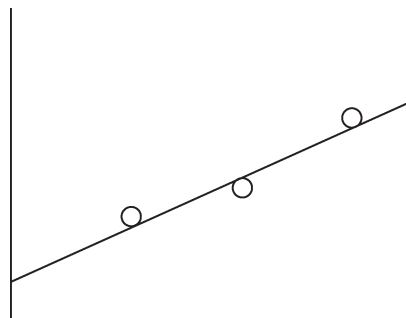
In other cases, the calibration curve is drawn as a linear line using the least square method.

Up to 64 calibration levels can be set ([# of Calib. Levels]). Up to ten measurements can be averaged for each calibration level.



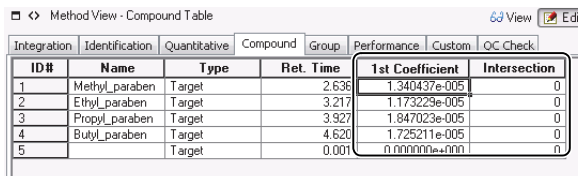
NOTE

- Standards at 2 or more levels are required to draw the natural log-natural log graph.
- [Zero] can not be set to [Force Through] since there is no origin.
- Set the [Curve Fit Type] to [Linear].



2.1.6 Manual RF (Linear, Exponential)

An arbitrary calibration curve can be manually defined by entering the slope (linear coefficient) and intercept into the compound table. The software then quantitates the peak with the specified calibration curve.



ID#	Name	Type	Ret. Time	1st Coefficient	Intersection
1	Methyl_paraben	Target	2.636	1.340437e-005	0
2	Ethyl_paraben	Target	3.217	1.173229e-005	0
3	Propyl_paraben	Target	3.927	1.847023e-005	0
4	Butyl_paraben	Target	4.620	1.725211e-005	0
5		Target	0.001	0.000000e+000	0

Only the calculated curve is displayed in the Manual RF calibration curve graph without a calibration point. The calibration curve is created regarding X and Y as natural log axes for the Manual RF (Exponential).

2.1.7 Quantitation Using the Calibration Curve for Another Component

If it is difficult to prepare a standard for a specific component, such as an impurity, a calibration curve of a component with similar relative sensitivity to the detector can be used to quantitate the target component concentration.

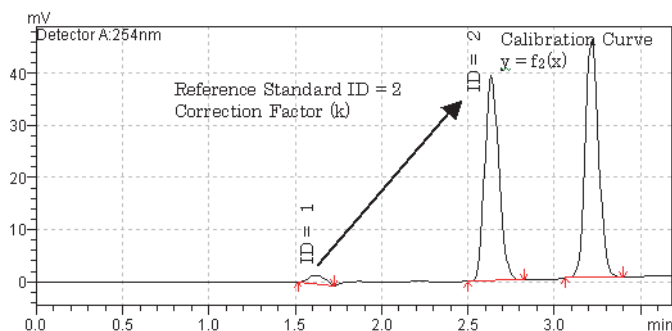



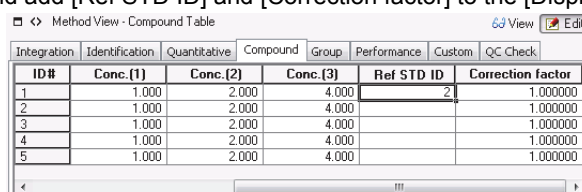
Fig.2-1 Impurity Content Calculation

The following parameters are used for quantitation with another component calibration curve:

Parameter	Description
Reference Standard ID (Ref STD ID)	Specify the ID No. of the prepared standard peak. Based on the standard calibration curve, the concentration of a component without a standard can be quantitated. Enter "-1" (negative one) to hide the ID No. component row from the quantitative result table. In this case, the ID No. row displays "Only reference".  NOTE Be careful NOT to refer two reference standard ID #s to each other. (e.g. ID # 1 = 2, ID # 2 = 1)
Correction Factor	Specify the factor to compensate for the sensitivity difference between the relevant peak and reference standard peak. Correction Factor = (Peak area (height) per unit weight of the component specified by the reference standard ID) / (Peak area (height) per unit weight of quantitated component)

NOTE

As default, [Ref STD ID] and [Correction factor] are not displayed in the [Compound] tab of [Method View]. To calculate impurity concentrations, navigate to [Compound] tab of [Method View]. Then select [Table Style] from the View menu, and add [Ref STD ID] and [Correction factor] to the [Display Items].



ID#	Conc.(1)	Conc.(2)	Conc.(3)	Ref STD ID	Correction factor
1	1.000	2.000	4.000	2	1.000000
2	1.000	2.000	4.000		1.000000
3	1.000	2.000	4.000		1.000000
4	1.000	2.000	4.000		1.000000
5	1.000	2.000	4.000		1.000000

Equations

$$C_i = f(A_i \times \text{Corr})$$

f : Calibration curve equation for a compound designated as the reference standard ID

C_i : Peak concentration (content)

A_i : Peak area (height, area ratio, or height ratio)

Corr : Correction factor

NOTE

- Correction factor is applied to the peak area (height, area ratio, or height ratio), instead of to the quantitative result (concentration).
- This equation is available for all quantitative methods using a calibration curve. However, the [Correction factor] value is not used for internal standards ([ISTD] or [ISTD & Ref.]).

2

2.1.8 Least Square Method and Weighted Least Square Method

This section describes the least square method for collinear approximations (linear expression: $Y = F1 \cdot X + F2$). The contribution rate and correlation coefficient for collinear approximations can be obtained using the following equations (even where $F1$ and $F2$ are not determined).

Even though the explanation is omitted here, the least square method for quadratic and cubic approximations can be expressed with matrix operations.

Least Square Method

The least square method for collinear approximations can be expressed using the following equations:

$$F1 = \frac{N \times \sum XY - \sum X \times \sum Y}{N \times \sum X^2 - \sum X \times \sum X}$$

$$F2 = \frac{\sum X^2 \times \sum Y - \sum XY \times \sum X}{N \times \sum X^2 - \sum X \times \sum X}$$

Contribution rate:

$$r^2 = \frac{(\sum XY - \frac{1}{N} \times \sum X \times \sum Y)^2}{\left\{ \sum X^2 - \frac{(\sum X)^2}{N} \right\} \left\{ \sum Y^2 - \frac{(\sum Y)^2}{N} \right\}}$$

Correlation coefficient:

$$r = \frac{(\sum XY - \frac{1}{N} \times \sum X \times \sum Y)}{\sqrt{\left\{ \sum X^2 - \frac{(\sum X)^2}{N} \right\} \left\{ \sum Y^2 - \frac{(\sum Y)^2}{N} \right\}}}$$

x and y values vary according to quantitative methods as follows:

X-axis is Area/Height

- With dilution factor

Quantitative Method	X	Y
Corrected Area Normalization (including that with scale factor)	A_i	C_i
Internal Standard	A_i/A_{is_i}	C_i/C_{is_i}
External standard (Absolute Calibration Curve) Standard Addition	A_i	C_i

- Without dilution factor

Quantitative Method	X	Y
Corrected Area Normalization (including that with scale factor)	A_i	C_i
Internal Standard	A_i/A_{is_i}	C_i/C_{is_i}
External standard (Absolute Calibration Curve) Standard Addition	A_i	$C_i \times W_{spl_i}/100$

X-axis is Concentration

- With dilution factor

Quantitative Method	X	Y
Corrected Area Normalization (including that with scale factor)	C_i	A_i
Internal Standard	C_i/C_{is_i}	A_i/A_{is_i}
External standard (Absolute Calibration Curve) Standard Addition	C_i	A_i

- Without dilution factor

Quantitative Method	X	Y
Corrected Area Normalization (including that with scale factor)	C_i	A_i
Internal Standard	C_i/C_{is_i}	A_i/A_{is_i}
External standard (Absolute Calibration Curve) Standard Addition	$C_i \times W_{spl_i}/100$	A_i

N : The number of calibration points

A_i : Peak area (height)

C_i : Peak concentration (component amount)

A_{is_i} : Internal standard peak area (height)

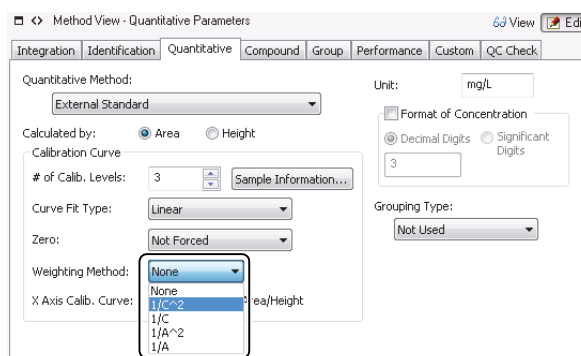
C_{is_i} : Internal standard peak concentration (component amount)

W_{spl_i} : Sample amount

(Subscript i indicates the i th standard.)

Weighted Least Square Method

Usually, the least square method assumes the same degree of error at each concentration level, and obtains the calibration curve by assuming that the sum of the squares of the deviation at each level is the least possible value. However, this method can create relatively large errors at low concentrations. To solve this problem, the calibration curve is calculated so that the sum of the squares of deviation of the ratio between each level and its concentration becomes the least possible value, equating the relative error at each level in relation to the concentration. This is called weighting.



The weighted least square method for collinear approximations is expressed by the following equations.

$$F1 = \left(\sum W_{XY} - \frac{\sum W_X \times \sum W_Y}{\sum W} \right) \left(\sum W_{X^2} - \frac{(\sum W_X)^2}{\sum W} \right)$$

$$F2 = \frac{\sum W_i Y_i}{\sum W_i} - F1 \times \frac{\sum W_i X_i}{\sum W_i}$$

Contribution rate:

$$r^2 = \frac{\left(\sum W_{XY} - \frac{\sum W_X \times \sum W_Y}{\sum W} \right)^2}{\left\{ \sum W_{X^2} - \frac{(\sum W_X)^2}{\sum W} \right\} \left\{ \sum W_{Y^2} - \frac{(\sum W_Y)^2}{\sum W} \right\}}$$

Correlation coefficient:

$$r = \frac{\left(\sum W_{XY} - \frac{\sum W_X \times \sum W_Y}{\sum W} \right)}{\sqrt{\left\{ \sum W_{X^2} - \frac{(\sum W_X)^2}{\sum W} \right\} \left\{ \sum W_{Y^2} - \frac{(\sum W_Y)^2}{\sum W} \right\}}}$$

W signifies "weight".

Four types of [Weighting Method] are available: [1/C], [1/C²], [1/A], and [1/A²].

Parameter	Description
1/C	Weights as much as 1/C. "C" signifies concentration (component amount).
1/C ²	Weights as much as 1/C ² . "C" signifies concentration (component amount).
1/A	Weights as much as 1/A. "A" signifies [Area] or [Height] set in [Calculated by] in the [Quantitative] tab page.
1/A ²	Weights as much as 1/A ² . "A" signifies [Area] or [Height] set in [Calculated by] in the [Quantitative] tab page.

Set [1/C²] or [1/A²] to weight in proportion to the concentration. The effect of weighting becomes smaller when [1/C] or [1/A] is set. Normally, use [1/C] or [1/C²] when the Y-axis is concentration, and use [1/A] and [1/A²] when the Y-axis is area/height.



NOTE

X and Y values vary with quantitative methods. For the definitions of X and Y, refer to "X-axis is Area/Height" P.34, and "X-axis is Concentration" P.34 in "Least Square Method".

2.2 Calibration Curve Correction

This section describes the procedures to create calibration curves (1) by applying a compensation factor to standard concentrations in the compound table, and (2) by using multiple standards.

2.2.1 Calibration Curves Using Standard Concentration Factors

To create calibration curves, the software uses true concentrations calculated by multiplying each concentration level by the [Std Conc Factor]. Conventionally, using standards with concentrations of 1, 0.5, 0.25, and 0.125 prepared from a 0.998 stock solution, each concentration was multiplied by 0.998, and the values were individually entered into the compound table. All of the concentration values had to be replaced, each time a new stock solution was prepared.

If [Std Conc Factor] is used, only the [Std Conc Factor] value needs to be changed not the concentrations in the compound table.

Parameter	Description
Std Conc Factor	Calibration curves are created based on concentrations derived by multiplying each level concentration by the stock standard concentration.

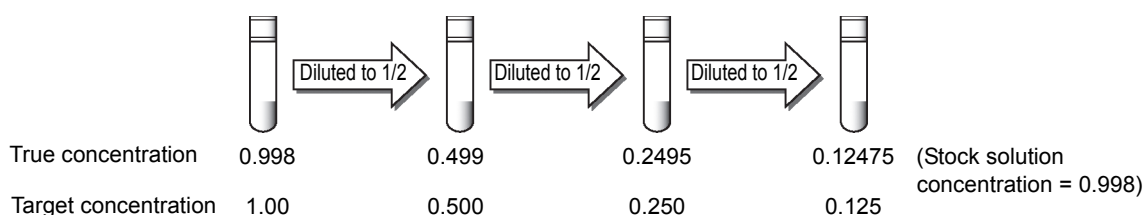


Fig.2-2 Example of Using [Std Conc Factor]

Conventional Compound Table Parameters

ID #	Compound Name	Conc. (1)	Conc. (2)	Conc. (3)	Conc. (4)
1	Something	0.12475	0.2495	0.499	0.998

Compound Table Parameters with [Std Conc Factor]

ID #	Compound Name	Conc. (1)	Conc. (2)	Conc. (3)	Conc. (4)	Std Conc Factor
1	Something	0.125	0.25	0.5	1	0.998



NOTE

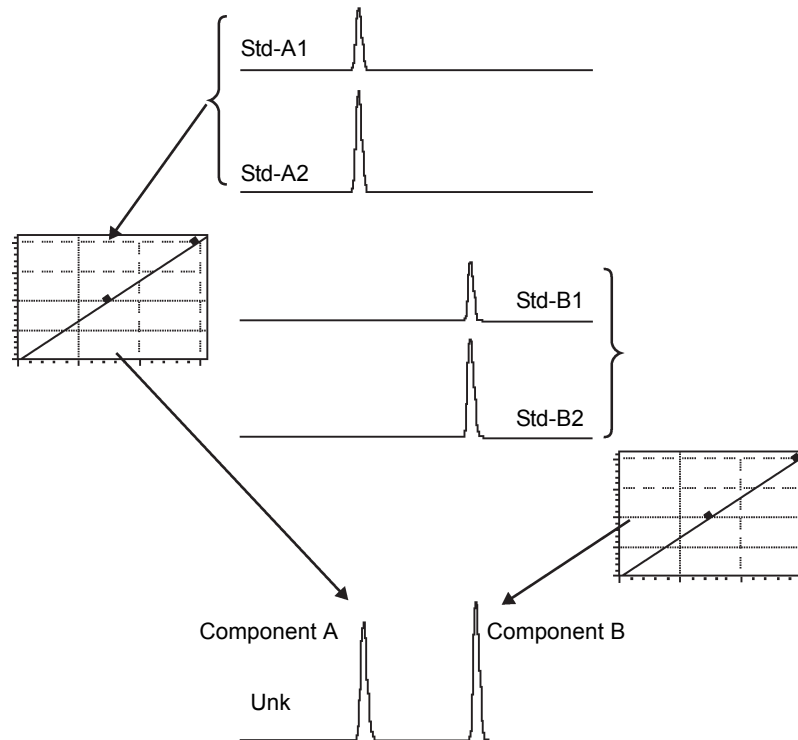
As default, [Std Conc Factor] is not displayed in the [Compound] tab of [Method View]. Navigate to [Compound] tab of [Method View]. Then select [Table Style] from the View menu, and add [Std Conc Factor] to the [Display Items]. This causes the [Std Conc Factor] column to be visible in the Compound Table.

■ [Std Conc Factor] Example

Commercially-available, traceable standard solutions come with not only the concentration value, but also the f value (factor) to correct the concentration. Enter the concentrations of the prepared standard into the compound table [Conc.] columns, and enter the f value into the [Std Conc Factor] column. This setting allows obtaining true concentrations automatically, without time-consuming manual calculations (See [Fig.2-2](#)).


2.2.2 Calibration Curves for Multiple Target Components

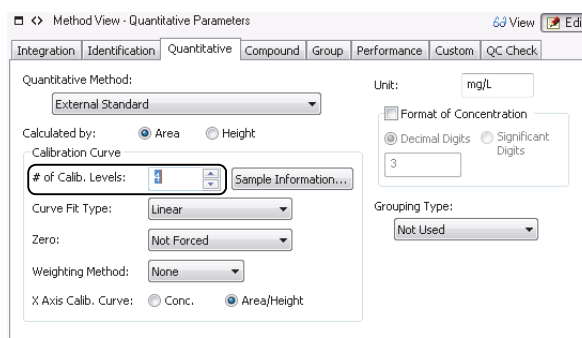
To quantitate multiple components in an unknown sample, a separate calibration curve must be created for each component. Having acquired standard data Std-A1 and Std-A2 for Component A, and Std-B1 and Std-B2 for Component B, (1) create the calibration curves for Components A and B in the same method file, and (2) quantitate the concentrations (contents) of components A and B in the already acquired unknown sample data (Unk). The detailed procedure follows. If the retention time of target component is known, this operation can be set in the [Realtime Batch] window.



- 1 Double click the unknown sample data file (Unk) containing the retention time data for target components A and B, in the [Data Explorer] sub-window of the [Postrun Analysis] program.

The Data Analysis window is displayed, and the data file (Unk) is loaded to the software.



- 1 Click  (Edit) on the [Method View].
The software goes into the edit mode.
- 2 Click the [Quantitative] tab.
Set [# of Calibration Levels] to 4.



- Click the [Compound] tab.
Specify the concentrations (1) to (4) for components A and B in the compound table.
Press the [Delete] key to select [Not Used].

Method View - Compound Table View Edit

ID#	Name	Ret. Time	Conc.(1)	Conc.(2)	Conc.(3)	Conc.(4)
1	Compound A	2.636	10.000	20.000	Not Used	Not Used
2	Compound B	3.217	Not Used	Not Used	10.000	20.000

- Click  (View) on the [Method View].
The software goes into the view mode.
- Click the  (Apply to Method) icon in the [Data Analysis] assistant bar.
The [Save Method As] sub-window is displayed.
Verify the file name, and follow the screen instructions. The set parameters are then applied to the method file.

2 Click [Main] on the assistant bar.

The [Main] assistant bar for the [Postrun Analysis] program is displayed.

3 Click the (Postrun Batch) icon on the [Main] assistant bar.

The [Postrun Batch] window is displayed.

- Create the batch table using the method file saved in the procedure above.

Postrun	Sample Name	Sample Type	Method File	Data File	Level#
1	STD	1:Standard(I)	Calib.lcm	Std-A1.lcd	1
2	STD	1:Standard	Calib.lcm	Std-A2.lcd	2
3	STD	1:Standard	Calib.lcm	Std-B1.lcd	3
4	STD	1:Standard	Calib.lcm	Std-B2.lcd	4
5	UNK	0:Unknown	Calib.lcm	UNK.lcd	1

- Specify the sample types.
Enter Std-A1 in the first row and set the sample type to "Standard-Initialize Calibration Curve (I)". Set Std-A2, Std-B1, and Std-B2 in the subsequent rows to "Standard-Add Calibration Level".
- Specify the level numbers.
Set Std-A1, Std-A2, Std-B1, and Std-B2 to level numbers 1, 2, 3, and 4, respectively.

4 Click the (Start Postrun Batch) icon on the [Postrun Batch] assistant bar.

The postrun batch process begins.

The calibration curve for Component A is created by the level 1 and 2 data. The calibration curve for Component B is created by the level 3 and 4 data. By applying the method file to the unknown sample data, the software quantitate the concentrations (component amounts) of Components A and B, based on the created calibration curves.

3

Equations

This chapter describes the various calculation equations used in this software.

This chapter consists of the following sections:


- *"3.1 Noise/Drift Calculation Parameters" P.39*
- *"3.2 QA/QC Parameters" P.42*
- *"3.3 Column Performance Equations" P.45*
- *"3.5 Methods for Calculating Peak-to-Valley Ratio" P.54*
- *"3.6 Background Compensation Processing" P.55*
- *"3.7 AART Retention Time Calculation Method" P.57*

3

3.1 Noise/Drift Calculation Parameters

This section describes parameters and equations used to obtain the detection and quantitative limit from the noise values in measured chromatograms.

■ To verify the current noise and drift parameters

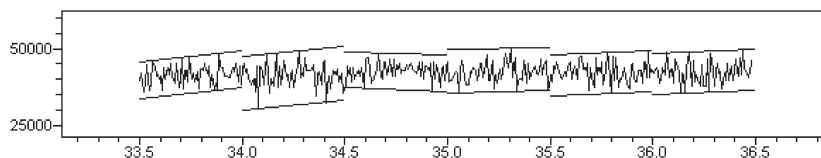
- 1 Open the [Data Analysis] window and load the desired chromatogram.
- 2 Select the [Integration] tab in [Method View].
- 3 Click  (Edit) and then click the [Noise/Drift Calculation ...] button. The [Noise/Drift Calculation Settings] sub-window is displayed.

3.1.1 Noise Calculation Methods

There are three methods for calculating noise: ASTM, rms, and EP.

■ ASTM

The noises in this system is obtained with the ASTM method as follows.



The figure above shows the image of obtaining the noise level for every 0.5 minute from 33.5 minutes to 36.5 minutes.

The data is divided with 0.5 minute intervals and parallel lines are obtained for each section as shown in the figure.

[Calculation procedure]

- (1) The slope of the approximated linear line for all of the data points within each section is calculated by the least square method.
- (2) Two parallel lines are drawn by shifting the slope upward and downward, so that all data points within the section fall between two lines.
- (3) The distance between the two parallel lines in the intensity axis direction is defined as the noise width for the section.

The noise level is the mean value of the Y-axis distance between the parallel lines for all sections.

The divided section can be specified on [Noise/Drift Calculation Settings].

■ rms

The rms noise refers to the standard deviation of the measurement points detected within a specified baseline section.

■ EP

First, a time range is selected that is 20 times the width at half-height of the target peak. This time range is selected so that the retention time of the target peak is in the center. The blank solution is analyzed to obtain its chromatogram. The above time range is applied to the blank chromatogram. The EP noise is then obtained as follows.

[Calculation procedure]

- (1) The slope of the approximated linear line for all of the blank data points within the time range is calculated by the least square method.
- (2) Two parallel lines are drawn by shifting the slope upward and downward, so that all data points within the section fall between the two lines.
- (3) The distance between the two parallel lines in the intensity axis direction is defined as the noise width of the time range, and half the distance is defined as the EP noise.

3.1.2 Detection/Quantitation Limit Coefficients

Detection limit refers to the minimum amount of a target component that can be qualitatively detected. It is not necessary to be able to quantitate the target component. The obtained value does not need to have the accuracy and precision required for quantitation. The detection limit is the minimum required amount of a component that can be recognized as a peak on LC/GC chromatograms.

Quantitation limit refers to the minimum required amount of a target component that can be accurately and precisely quantitated.

Three methods of determining the detection and quantitative limit are described below. The method to be applied varies according to the instrument used for the analysis.

- Visual judgment
- Signal-to-noise relationship
- Standard deviation and calibration curve slope

The second and third methods are normally adopted for LC and GC analyses.

This software uses the signal-to-noise relationship to determine the detection and quantitation limits. Specify the detection and quantitation limit coefficients in the [Noise/Drift Calculation Settings] sub-window.

$$DL = \alpha \times \text{Conc} \times N/S$$

DL: Detection limit
 α : Coefficient (normally 3 to 3.3)
 Conc: Peak concentration
 N: Noise
 S: Peak height

$$QL = \alpha \times \text{Conc} \times N/S$$

QL: Quantitation limit
 α : Coefficient (normally 10)
 Conc: Peak concentration
 N: Noise
 S: Peak height



NOTE

Detection and quantitation limits are expressed in concentrations. Therefore, to obtain those values, it is necessary to create the calibration curve and determine each peak concentration.



Reference

Use QA/QC functions to obtain the detection and quantitation limits using the standard deviation and calibration curve slope. Refer to the help files for details on the QA/QC functions.

3.1.3 Drift Settings

First, define the calculation section by specifying its start and end times in the [Noise/Drift Calculation Settings] sub-window. The software calculates the slope of the approximated linear line for data points within the section, using the least square method. The variation per hour is then calculated from the slope, to obtain the drift value.

3.2 QA/QC Parameters

Various QA/QC parameters and criteria are available to evaluate the validity of the analytical system.

This section explains the content of the QA/QC parameters and equations used.

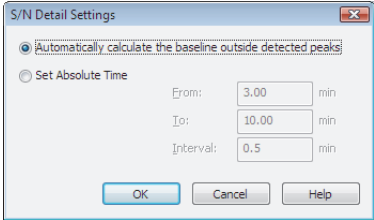
Select [QA/QC Parameters] from the [Method] menu of the [Data Acquisition] window. The [QA/QC] sub-window is displayed.

3.2.1 Common Items

Item	Equation	
Averaging	$\bar{x} = \frac{\sum_{i=1}^N X_i}{N}$	\bar{x} : Mean x_i : Data N: Number of the data
Standard Deviation	$s = \sqrt{\frac{\sum_{i=1}^N (x_i - \bar{x})^2}{N-1}}$	s: Standard deviation x_i : Data \bar{x} : Mean data N: Number of the data
Relative Standard Deviation (%)	Relative standard deviation (%) = $\frac{s \times 100}{\bar{x}}$	s: Standard deviation \bar{x} : Mean data


3.2.2 Calibration

Item	Equation	
Correlation Coefficient	$r = \frac{\sum_{i=1}^N \{(x_i - \bar{x})(y_i - \bar{y})\}}{\sqrt{\left\{ \sum_{i=1}^N (x_i - \bar{x})^2 \right\} \left\{ \sum_{i=1}^N (y_i - \bar{y})^2 \right\}}}$	r: Correlation coefficient x_i : Concentration (Concentration ratio) \bar{x} : Mean x_i y_i : Area (Area ratio) or height (height ratio) \bar{y} : Mean y_i N: Number of the data
Residual Sum of Squares	Residual sum of squares = $\sum_{i=1}^N (y_i - \hat{y})^2$	y_i : Area (Area ratio) or height (height ratio) \hat{y} : Calibration curve slope *1 N: Number of the data
Residual SD of Regression Line	$S_{y/x} = \sqrt{\frac{\sum_{i=1}^N (y_i - \hat{y})^2}{N-2}}$	$S_{y/x}$: Residual SD of regression line y_i : Area (Area ratio) or height (height ratio) \hat{y} : Slope of calibration curve *1 N: Number of the data
Residual SD of Y Intercepts	$S_y = S_{y/x} \left\{ 1 + 1/N + \bar{x}^2 / \sum (x_i - \bar{x})^2 \right\}^{1/2}$	S_y : Residual standard deviation of y intercept $S_{y/x}$: Residual SD of regression line x_i : Concentration (Concentration ratio) \bar{x} : Mean x_i N: Number of the data

Item	Equation	
Response Factor	$RF = \frac{S}{Conc}$	RF : Response factor S : Signal Conc : Concentration
	When the internal standard method is used: $RF = \frac{S \times Conc_{ISTD}}{Conc \times S_{ISTD}}$	RF : Response factor S : Signal Conc : Concentration S _{ISTD} : Signal of the ISTD Conc _{ISTD} : Concentration of the ISTD
S/N	<p>The S/N ratio denotes the ratio between signals and noises. The noise in this system is obtained with the ASTM method as follows.</p> <p>Reference</p> <p>For details on the ASTM method for calculating noise, refer to "ASTM" at "3.1.1 Noise Calculation Methods". However, the time interval to be used with the ASTM method here is set in the [S/N Detail Settings] sub-window. If [Automatically calculate the baseline outside detected peaks] is selected, chromatogram data is automatically divided into 0.5 minute intervals and calculated.</p> 	
Detection Limit	The detection limit is obtained by the following equation. $DL = S_B \times \alpha / f$	DL : Detection limit S _B : Standard deviation of signal for zero concentration α : Coefficient (normally 3 to 3.3) f : Slope of calibration curve *1
	According to the method, select the standard deviation of signals for zero concentration from the next: Residual SD of regression line, Residual SD of Y intercepts, and S/N. When using an internal standard, the detection limit is calculated from signal ratio and concentration ratio, and the result is expressed as concentration ratio. To obtain detection limits based on noise, set the Quantitative Parameters [Calculated by] to [Height], and [X Axis of Calib. Curve] to [Conc.].	
Quantitative Limit	The quantitative limit is obtained by the following equation. $QL = S_B \times \alpha / f$	DL : Quantitative limit S _B : Standard deviation of signal for zero concentration α : Coefficient (normally 10) f : Slope of calibration curve *1
Deviation (%)	Signifies the deviation of the calibration point at each level from the calibration curve. [Deviation (%)] is obtained by the following equation: $Deviation_N = \frac{f(S_N) - Conc_N}{Conc_N} \times 100$	Deviation _N : Deviation (%) at level N f(S _N) : Concentration function based on calibration point signal intensity S at level N Conc _N : Specified concentration at level N

*1: Use a linear calibration curve not passing through the origin.

3.2.3 Quality Control

Item	Equation	
Mean Deviation (%)	$\text{Deviation}_{\text{Mean}} = \frac{\text{Conc}_{\text{Mean}} - \text{Conc}_{\text{True}}}{\text{Conc}_{\text{True}}} \times 100$	$\text{Deviation}_{\text{Mean}}$: Mean Deviation (%) $\text{Conc}_{\text{Mean}}$: Mean concentration $\text{Conc}_{\text{True}}$: True concentration value
Method Detection Limit	$\text{MDL} = s \times t(N-1, \alpha)$	MDL : Method detection limit s : Standard deviation of the concentration $t(N-1, \alpha)$: $\alpha \times 100\%$ confidence interval with N-1 degree of freedom
Detection limit	$\text{DL} = S \times \alpha$	DL : Detection limit S : Standard deviation of the concentration α : Coefficient (normally 3 - 3.3)
Quantitative limit	$\text{QL} = S \times \alpha$	QL : Quantitative limit S : Standard deviation of the concentration α : Coefficient (normally 10)
Deviation (%) Confidence Interval	Obtains the 95% confidence interval of the deviation (%). Range of confidence interval: $\text{Deviation}_{\text{Mean}} - \frac{S_A}{\sqrt{N}} t(N-1, 0.025)$ $-\text{Deviation}_{\text{Mean}} + \frac{S_A}{\sqrt{N}} t(N-1, 0.025)$	$\text{Deviation}_{\text{Mean}}$: Deviation (%) S_A : Standard deviation of accuracy (%) t : Number of data $(N-1, 0.025)$: t static with N-1 degree of freedom, significance level 0.025.
<p>[Check confidence interval] and [Check upper and lower limits] are available for determining whether 0 is involved in the confidence interval. These methods are specified on [Accuracy (%) Confidence Interval Detail].</p> <p> NOTE The upper and lower limits of the confidence interval are not verified when selecting [Check confidence interval].</p>		


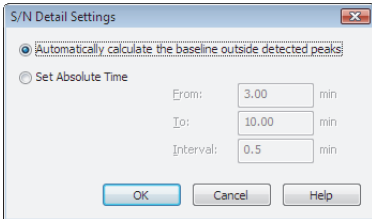
3.2.4 Recovery

Item	Equation	
Recovery	<ul style="list-style-type: none"> Calculating from the spike amount $R = \frac{\text{Conc} - \text{Blank}}{\text{SpikeAmount}}$	R : Recovery Blank : Blank SpikeAmount : Spike amount
	<ul style="list-style-type: none"> Calculating with standard (ISTD recovery) $R_i = \frac{A_i/A_{i0}}{\bar{A}_i/\bar{A}_{i0}}$	R_i : Recovery of the compound i A_i : Area of the compound i \bar{A}_i : Mean area of the compound i in sample specified as [Standard (ISTD Recovery)] sample type $i0$: Recovery standard

3.2.5 Degradation Check

Item	Equation	
Breakdown	$\text{breakdown} = \frac{\text{Area}_{\text{rest}}}{\text{Area}_{\text{analyte}} + \text{Area}_{\text{rest}}} \times 100$	breakdown : Breakdown rate (%) Area _{analyte} : Area of analyte Area _{rest} : Total area in the same group except analyte

3.2.6 Noise/Drift Check

Item	Equation
Noise (ASTM)	<p> Reference</p> <p>For details on the ASTM method for calculating noise, refer to "ASTM" at "3.1.1 Noise Calculation Methods". However, the time interval to be used with the ASTM method here is set in the [S/N Detail Settings] sub-window. If [Automatically calculate the baseline outside detected peaks] is selected, chromatogram data is automatically divided into 0.5 minute intervals and calculated.</p> 
Noise (rms)	The rms noise refers to the standard deviation of the measurement point signals detected within a specified baseline section.
Noise (EP)	(1) First, a time range is selected that is 20 times the width at half-height of the target peak. This time range is selected so that the retention time of the target peak is in the center. (2) The blank solution is analyzed to obtain its chromatogram. (3) The above time range is applied to the blank chromatogram. (4) The slope of the approximated linear line for all of the blank data points within the time range is calculated by the least square method. (5) Two parallel lines are drawn by shifting the slope upward and downward, so that all data points within the section fall between the two lines. (6) The distance between the two parallel lines in the intensity axis direction is defined as the noise width of the time range, and half the distance is defined as the EP noise.
Drift	(1) The slope of the approximated linear line for all of the data points in a specified section is obtained by the least square method. (2) The variation per hour is calculated from the slope to obtain the drift value.

3

3.3 Column Performance Equations

■ USP

Item	Equation	
Number of Theoretical Plate	$N = 16 \times \left(\frac{t_R}{W}\right)^2$	N : Number of theoretical plate t _R : Retention time W : Peak width. The peak width is the time between two points at the intersections straight lines through the points of inflection to the left and right of a peak and the baseline.
HETP	$H = \frac{L \times 1000}{N} \text{ (}\mu\text{m)}$	H : HETP L : Column length (mm) N : Number of theoretical plate

Item	Equation	
Tailing Factor	$S = \frac{W_{0.05}}{2 \times a_{0.05}}$	S : Tailing factor W _{0.05} : Peak width at 5% peak height a _{0.05} : Width of the front half of the peak (from the start point to the apex) at 5% peak height
Resolution	$R = 2 \times \frac{t_R - t_{Rp}}{W + W_p}$	R : Resolution t _R : Retention time t _{Rp} : Retention time of the previous peak W : Peak width. The peak width is the time between two points at the intersections straight lines through the points of inflection to the left and right of a peak and the baseline. W _p : Peak width for previous peak
Capacity Factor (k')	$k' = \frac{t}{t_0} - 1$	t : Retention time t ₀ : Unretained peak time
Separation Factor	$a = \frac{k'_2}{k'_1} = \frac{t_2 - t_0}{t_1 - t_0}$	a : Separation factor k' ₁ : Capacity factor (k') of the previous peak (Peak 1) k' ₂ : Capacity factor (k') of the test peak (Peak 2) t ₁ : Retention time of Peak 1 t ₂ : Retention time of Peak 2 t ₀ : Unretained peak time

**NOTE**

“Unretained Peak Time” refers to the time when a compound with no adsorption to the stationary phase is eluted. To use the first-eluted peak as the reference, set [Unretained Peak Time] in the [Performance] tab to [1st Peak Time].

■ USP2

Item	Equation	
Number of Theoretical Plate	$N = 5.54 \times \left(\frac{t_R}{W_{0.5}}\right)^2$	N : Number of theoretical plate t _R : Retention time W _{0.5} : Peak width at 50% peak height
HETP	$H = \frac{L \times 1000}{N} \text{ (}\mu\text{m)}$	H : HETP L : Column length (mm) N : Number of theoretical plate
Tailing Factor	$S = \frac{W_{0.05}}{2 \times a_{0.05}}$	S : Tailing factor W _{0.05} : Peak width at 5% peak height a _{0.05} : Width of the front half of the peak (from the start point to the apex) at 5% peak height
Resolution	$R = \frac{2 \times (t_R - t_{Rp})}{1.70 \times (W_{0.5} + W_{p0.5})}$	R : Resolution t _R : Retention time t _{Rp} : Retention time of the previous peak W _{0.5} : Peak width at 50% peak height W _{p0.5} : The previous peak width at 50% peak height
Capacity Factor (k')	$k' = \frac{t}{t_0} - 1$	t : Retention time t ₀ : Unretained peak time

Item	Equation	
Separation Factor	$a = \frac{k'_2}{k'_1} = \frac{t_2 - t_0}{t_1 - t_0}$	a : Separation factor k' ₁ : Capacity factor (k') of the previous peak (Peak 1) k' ₂ : Capacity factor (k') of the test peak (Peak 2) t ₁ : Retention time of Peak 1 t ₂ : Retention time of Peak 2 t ₀ : Unretained peak time

■ JP, EP, BP, and DAB

Item	Equation	
Number of Theoretical Plate	$N = 5.54 \times \left(\frac{t_R}{W_{0.5}}\right)^2$	N : Number of theoretical plate t _R : Retention time W _{0.5} : Peak width at 50% peak height
HETP	$H = \frac{L \times 1000}{N} \text{ (}\mu\text{m)}$	H : HETP L : Column length (mm) N : Number of theoretical plate
Tailing Factor	$S = \frac{W_{0.05}}{2 \times a_{0.05}}$	S : Tailing factor W _{0.05} : Peak width at 5% peak height a _{0.05} : Width of the front half of the peak (from the start point to the apex) at 5% peak height
Resolution	$R = 1.18 \times \frac{t_R - t_{Rp}}{W_{0.5} + W_{p0.5}}$	R : Resolution t _R : Retention time t _{Rp} : Retention time of the previous peak W _{0.5} : Peak width at 50% peak height W _{p0.5} : The previous peak width at 50% peak height
Capacity Factor (k')	$k' = \frac{t}{t_0} - 1$	t : Retention time t ₀ : Unretained peak time
Separation Factor	$a = \frac{k'_2}{k'_1} = \frac{t_2 - t_0}{t_1 - t_0}$	a : Separation factor k' ₁ : Capacity factor (k') of the previous peak (Peak 1) k' ₂ : Capacity factor (k') of the test peak (Peak 2) t ₁ : Retention time of Peak 1 t ₂ : Retention time of Peak 2 t ₀ : Unretained peak time

■ JP2

In accordance with revisions authorized by the Japan Pharmacopoeia in April 1996, the number of theoretical plates is calculated with 5.55 for JP2.

Item	Equation	
Number of Theoretical Plate	$N = 5.55 \times \left(\frac{t_R}{W_{0.5}}\right)^2$	N : Number of theoretical plate t _R : Retention time W _{0.5} : Peak width at 50% peak height
HETP	$H = \frac{L \times 1000}{N} \text{ (}\mu\text{m)}$	H : HETP L : Column length (mm) N : Number of theoretical plate
Tailing Factor	$S = \frac{W_{0.05}}{2 \times a_{0.05}}$	S : Tailing factor W _{0.05} : Peak width at 5% peak height a _{0.05} : Width of the front half of the peak (from the start point to the apex) at 5% peak height
Resolution	$R = 1.18 \times \frac{t_R - t_{Rp}}{W_{0.5} + W_{p0.5}}$	R : Resolution t _R : Retention time t _{Rp} : Retention time of the previous peak W _{0.5} : Peak width at 50% peak height W _{p0.5} : The previous peak width at 50% peak height
Capacity Factor (k')	$k' = \frac{t}{t_0} - 1$	t : Retention time t ₀ : Unretained peak time
Separation Factor	$a = \frac{k'_2}{k'_1} = \frac{t_2 - t_0}{t_1 - t_0}$	a : Separation factor k' ₁ : Capacity factor (k') of the previous peak (Peak 1) k' ₂ : Capacity factor (k') of the test peak (Peak 2) t ₁ : Retention time of Peak 1 t ₂ : Retention time of Peak 2 t ₀ : Unretained peak time



■ EMG

Item	Equation
Number of Theoretical Plate	$N = 41.7 \times \frac{\left(\frac{t_R}{W_{0.1}}\right)^2}{\frac{b_{0.1}}{a_{0.1}} + 1.25}$ <p> N : Number of theoretical plate t_R : Retention time $W_{0.1}$: Peak width at 10% peak height $a_{0.1}$: Width of the front half of the peak (from the start point to the apex) at 10% peak height $b_{0.1}$: Width of the back half of the peak (from the apex to the end point) at 10% peak height </p>
HETP	$H = \frac{L \times 1000}{N} \text{ (}\mu\text{m)}$ <p> H : HETP L : Column length (mm) N : Number of theoretical plate </p>
Tailing Factor	$S = \frac{W_{0.05}}{2 \times a_{0.05}}$ <p> S : Tailing factor $a_{0.05}$: Width of the front half of the peak (from the start point to the apex) at 5% peak height $W_{0.05}$: Peak width at 5% peak height </p>
Resolution	$R = 2.15 \times \frac{t_R - t_{Rp}}{W_{0.1} + W_{p0.1}}$ <p> R : Resolution t_R : Retention time t_{Rp} : Retention time of the previous peak $W_{0.1}$: Peak width at 10% peak height $W_{p0.1}$: The previous peak width at 10% peak height </p>
Capacity Factor (k')	$k' = \frac{t}{t_0} - 1$ <p> t : Retention time t_0 : Unretained peak time </p>
Separation Factor	$a = \frac{k'_2}{k'_1} = \frac{t_2 - t_0}{t_1 - t_0}$ <p> a : Separation factor k'_1 : Capacity factor (k') of the previous peak (Peak 1) k'_2 : Capacity factor (k') of the test peak (Peak 2) t_1 : Retention time of Peak 1 t_2 : Retention time of Peak 2 t_0 : Unretained peak time </p>



■ EMG (50%)

Item	Equation	
Number of Theoretical Plate	$N = 41.7 \times \frac{\left(\frac{t_R}{W_{0.1}}\right)^2}{\frac{b_{0.1}}{a_{0.1}} + 1.25}$	N : Number of theoretical plate t_R : Retention time $W_{0.1}$: Peak width at 10% peak height $a_{0.1}$: Width of the front half of the peak (from the start point to the apex) at 10% peak height $b_{0.1}$: Width of the back half of the peak (from the apex to the end point) at 10% peak height
HETP	$H = \frac{L \times 1000}{N} \text{ (}\mu\text{m)}$	H : HETP L : Column length (mm) N : Number of theoretical plate
Tailing Factor	$S = \frac{W_{0.05}}{2 \times a_{0.05}}$	S : Tailing factor $a_{0.05}$: Width of the front half of the peak (from the start point to the apex) at 5% peak height $W_{0.05}$: Peak width at 5% peak height
Resolution	$R = 1.18 \times \frac{t_R - t_{Rp}}{W_{0.5} + W_{p0.5}}$	R : Resolution t_R : Retention time t_{Rp} : Retention time of the previous peak $W_{0.5}$: Peak width at 50% peak height $W_{p0.5}$: The previous peak width at 50% peak height
Capacity Factor (k')	$k' = \frac{t}{t_0} - 1$	t : Retention time t_0 : Unretained peak time
Separation Factor	$a = \frac{k'_2}{k'_1} = \frac{t_2 - t_0}{t_1 - t_0}$	a : Separation factor k'_1 : Capacity factor (k') of the previous peak (Peak 1) k'_2 : Capacity factor (k') of the test peak (Peak 2) t_1 : Retention time of Peak 1 t_2 : Retention time of Peak 2 t_0 : Unretained peak time

■ Area/Height

Item	Equation	
Number of Theoretical Plate	$N = 16 \times \left(\frac{t_R}{W}\right)^2$	<p>N : Number of theoretical plate t_R : Retention time W : Peak width (where $W = 4 \times \sigma$)</p> <p> NOTE σ^2, signifying peak shape (smoothness), is a dispersion term for the probability density function when assuming peak shapes can be approximated by normal distribution curves. Peak width is defined as peak top $\pm 2\sigma$ ($= 4\sigma$), which includes almost the entire peak area ($= 95.44\%$). By transforming the normal distribution probability density function formula, σ can be obtained by the following equations:</p> $\sigma = \frac{1}{\sqrt{2\pi}} \times \left(\frac{A}{Ht}\right) \quad A: \text{Area}, \quad Ht: \text{Height}$ $\approx 0.399 \times \left(\frac{A}{Ht}\right)$
HETP	$H = \frac{L \times 1000}{N} \text{ (}\mu\text{m)}$	<p>H : HETP L : Column length (mm) N : Number of theoretical plate</p>
Tailing factor	$S = \frac{W_{0.05}}{2 \times a_{0.05}}$	<p>S : Tailing factor $W_{0.05}$: Peak width at 5% peak height $a_{0.05}$: Width of the front half of the peak (from the start point to the apex) at 5% peak height</p>
Resolution	$R = 2 \times \frac{t_R - t_{Rp}}{W + W_p}$	<p>R : Resolution t_R : Retention time t_{Rp} : Retention time of the previous peak W : Peak width (where $W = 4 \times \sigma$) W_p : The previous peak width</p> <p> NOTE σ^2, signifying peak shape (smoothness), is a dispersion term for the probability density function when assuming peak shapes can be approximated by normal distribution curves. Peak width is defined as peak top $\pm 2\sigma$ ($= 4\sigma$), which includes almost the entire peak area ($= 95.44\%$). By transforming the normal distribution probability density function formula, σ can be obtained by the following equations:</p> $\sigma = \frac{1}{\sqrt{2\pi}} \times \left(\frac{A}{Ht}\right) \quad A: \text{Area}, \quad Ht: \text{Height}$ $\approx 0.399 \times \left(\frac{A}{Ht}\right)$
Capacity Factor (k')	$k' = \frac{t}{t_0} - 1$	<p>t : Retention time t_0 : Unretained peak time</p>
Separation Factor	$a = \frac{k'_2}{k'_1} = \frac{t_2 - t_0}{t_1 - t_0}$	<p>a : Separation factor k'_1 : Capacity factor (k') of the previous peak (Peak 1) k'_2 : Capacity factor (k') of the test peak (Peak 2) t_1 : Retention time of Peak 1 t_2 : Retention time of Peak 2 t_0 : Unretained peak time</p>

■ User defined

Item	Equation	
Number of Theoretical Plate	$N = c \times \left(\frac{t_R}{W}\right)^2$	<p>N : Number of theoretical plate c : Coefficient t_R : Retention time W : Peak width at (the value set in User Defined)% height of peak</p> <p> NOTE Set [Peak Width at] in the [Performance] tab to define the coefficient. When the [Peak Width at] value is n, the coefficient c is calculated by the following equation:</p> $c = 8 \times \log_c \frac{100}{n}$
HETP	$H = \frac{L \times 1000}{N} \text{ (}\mu\text{m)}$	<p>H : HETP L : Column length (mm) N : Number of theoretical plate</p>
Tailing Factor	$S = \frac{W}{2 \times a}$	<p>S : Tailing factor W : Peak width at (the value set in User Defined)% peak height a : Width of the front half of the peak (from the start point to the apex) at (the value set in User Defined)% peak height</p>
Resolution	$R = c \times \frac{t_R - t_{Rp}}{W + W_p}$	<p>R : Resolution c : Coefficient t_R : Retention time t_{Rp} : Retention time of the previous peak W : Peak width at (the value set in User Defined)% peak height W_p : The previous peak width</p> <p> NOTE Set [Peak Width at] in the [Performance] tab to define the coefficient. When the [Peak Width at] value is n, the coefficient c is calculated by the following equation:</p> $c = \sqrt{2 \times \log_c \frac{100}{n}}$
Capacity Factor (k')	$k' = \frac{t}{t_0} - 1$	<p>t : Retention time t₀ : Unretained peak time</p>
Separation Factor	$a = \frac{k'_2}{k'_1} = \frac{t_2 - t_0}{t_1 - t_0}$	<p>a : Separation factor k'₁ : Capacity factor (k') of the previous peak (Peak 1) k'₂ : Capacity factor (k') of the test peak (Peak 2) t₁ : Retention time of Peak 1 t₂ : Retention time of Peak 2 t₀ : Unretained peak time</p>

■ Tailing factor(10%)

Item	Equation	
Tailing factor(10%)	$S = \frac{W_{0.1}}{2 \times a_{0.1}}$	<p>S : Tailing factor W_{0.1} : Peak width at 10% peak height a_{0.1} : Width of the front half of the peak (from the start point to the apex) at 10% peak height</p>

3.4 Calculation of Relative Retention Time

The relative retention time can be calculated using the following equation, where the reference peak can be selected either from the specified ID peak or specified retention time.

$$\text{Relative retention time} = \frac{t_1 - t_0}{t_2 - t_0}$$

t_1 : Retention time of target peak
 t_2 : Retention time of reference peak
 t_0 : Unretained peak time

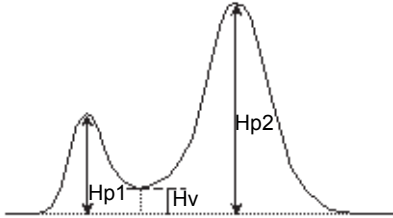
To calculate the relative retention time, display [Performance] tab in [Data Analysis Parameters] window from [Method] menu of [Data Acquisition] window ([Performance] tab in [Method View] of [Data Analysis] window), and then specify the following parameters.

The screenshot shows the 'Data Processing Parameters' dialog box with the 'Performance' tab selected. The 'Calc. Method' section includes options for USP, EP, EMG (50%), USP2, BP, Area/Height, JP, QAB, and EMG, with 'User Defined' checked. The 'Unretained Peak Time' section has '1st Peak Time' selected and 'Set Time' set to 0 min. The 'Column Length' is 150 mm. The 'Calculate identified peaks only' checkbox is unchecked. The 'Calculation of Relative Retention Time' checkbox is checked. The 'Reference Peak' is set to ID# 1. The 'Unretained Peak Time correction' checkbox is unchecked. The 'Copy to All Channels' button is visible. The 'User' section includes fields for Method Name, Number of Theoretical Plate, Peak Width at, Coefficient, Tailing Factor, and Resolution, all with input fields set to 0.

Parameter	Description
Calculate of Relative Retention Time	When this box is checked, Relative Retention Time is calculated.
Reference Peak	Select [ID#] or [Retention Time] of the reference peak to specify. After Selecting a method, set the ID number or the retention time in the box on the right. <ul style="list-style-type: none"> • 1) ID# : Sets the ID number. • 2) Retention Time : Sets the retention time in minutes.
Unretained Peak Time correction	When this box is checked, Unretained Peak Time is used. The Unretained Peak Time is the same as that of the column performance.

3.5 Methods for Calculating Peak-to-Valley Ratio

This software calculates the peak-to-valley ratio for each peak in relation to the previous peak. The lower peak height of the target peak height and previous peak height is used as the peak height for the numerator of the peak-to-valley ratio.



($H_{p1} < H_{p2}$)

Peak-to-Valley ratio = H_{p1} / H_v

($H_{p1} > H_{p2}$)

Peak-to-Valley ratio = H_{p2} / H_v

Hp1: previous peak height

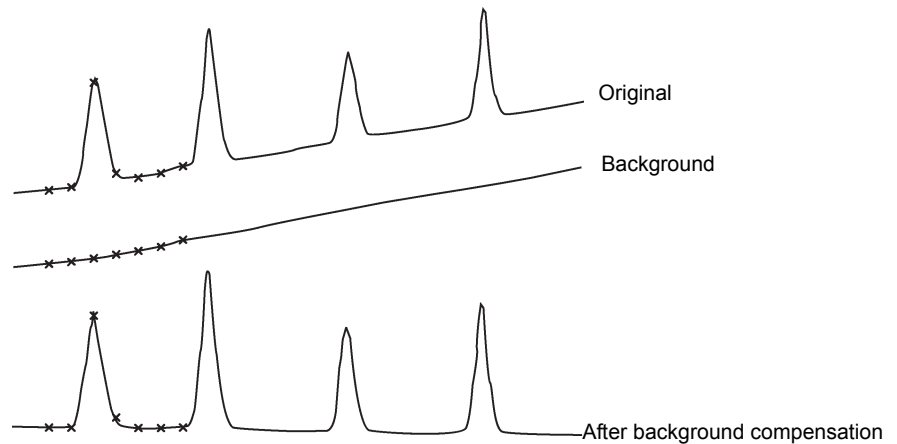
Hp2: target peak height

Hv: valley height between the target peak and the previous peak

3.6 Background Compensation Processing

■ Identical Start, End, and Sampling Rate/Interval

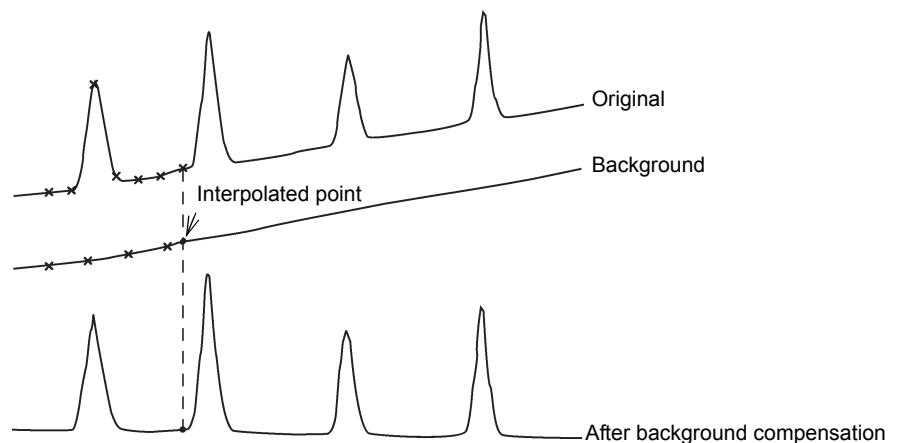
The background chromatogram is subtracted from the original chromatogram.



3

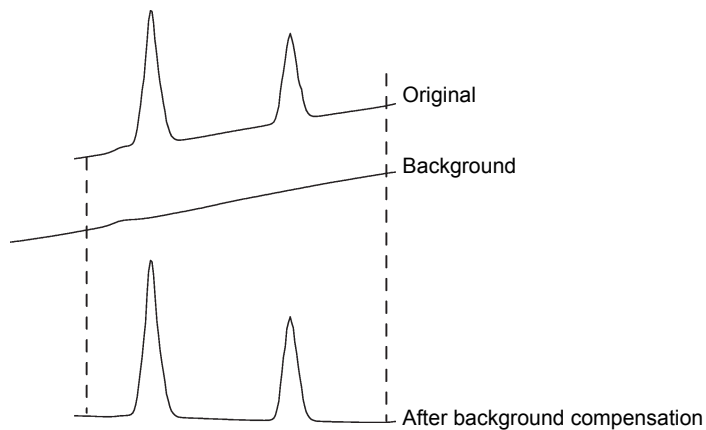
■ Different Sampling Rate/Interval

The background intensities at the points corresponding to the original chromatogram sampling points are interpolated from the background sampling points before/after to the interpolated point. The background chromatogram is then subtracted from the original chromatogram.



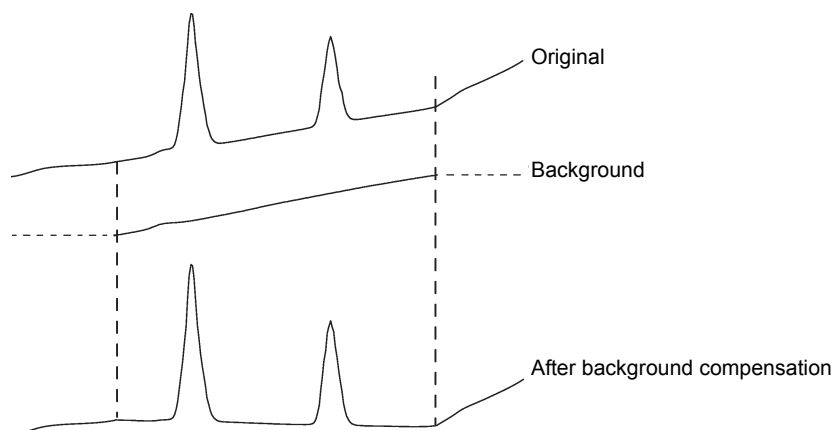
■ Same Length or Longer Background Chromatogram

Only the section of the background chromatogram that corresponds to the original chromatogram is subtracted.



■ Shorter Length Background Chromatogram

The starting and ending points of the background chromatogram are extrapolated. Then, the background chromatogram is subtracted from the original chromatogram.



3.7 AART Retention Time Calculation Method

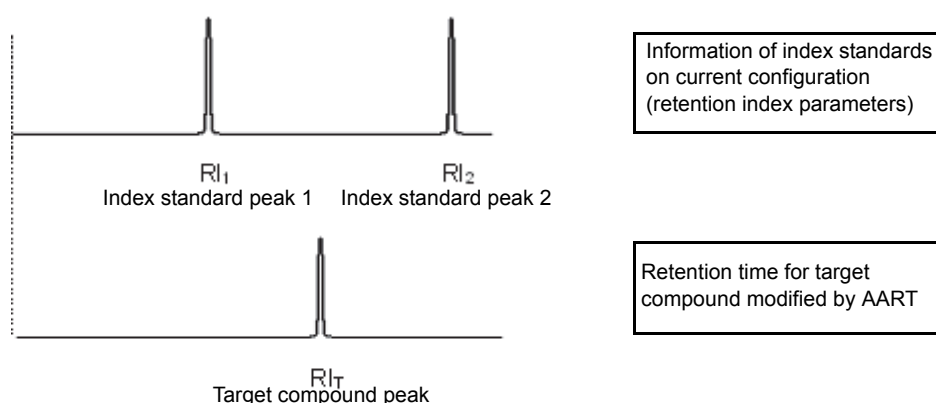
In gas chromatography, the peak retention time is greatly affected by the column type, the temperature, and the flow rate of the carrier gas. The retention index is an indicator of the retention time of a compound peak obtained via a proportional calculation based on the peak retention time of index standards (e.g., n-alkanes). The retention indexes for many compounds have been made public and can be used for the identification of unknown compounds.

AART calculates the set times based on retention indexes of target compounds and the identification results of index standards such as n-alkanes set in Retention Index parameters.

3

3.7.1 Calculation Method for Retention Times in Compound Table

1. Calculation Method when Target Compound Peak Is between the Index Standard Peaks



$$RT_T = RT_1 + (RT_2 - RT_1) \times \frac{RI_T - RI_1}{RI_2 - RI_1}$$

RI_T, RT_T : Retention index and retention time of target compound peak

RI_1, RT_1 : Retention index and retention time of index standard whose retention index approaches that of target compound peak most closely from below

RI_2, RT_2 : Retention index and retention time of index standard whose retention index approaches that of target compound peak most closely from above

Eq.1 Formula for calculating retention time when target compound peak is between index standard peaks

Example: For the identified results shown below, the retention time corresponding to a retention index of 1530 is calculated as follows:

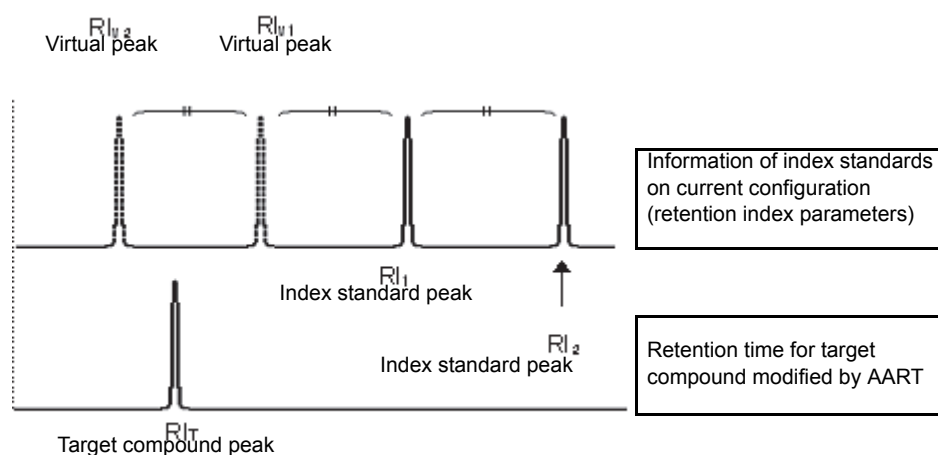
$$\begin{aligned}
 RT_T &= RT_{C15} + (RT_{C16} - RT_{C15}) \times \frac{RI_T - RI_{C15}}{RI_{C16} - RI_{C15}} \\
 &= 8.292 + (9.354 - 8.292) \times \frac{1530 - 1500}{1600 - 1500} \\
 &\approx 8.611
 \end{aligned}$$

Identified Results of Data File				
ID#	Proc	Name	Ret. Time	Ret. Index
1	<input checked="" type="checkbox"/>	C13	2.072	1183
2	<input checked="" type="checkbox"/>	C14	2.732	1195
3	<input checked="" type="checkbox"/>	C15	3.659	1300
4	<input checked="" type="checkbox"/>	C16	4.803	1400
5	<input checked="" type="checkbox"/>	C17	6.065	1500

Fig.3-1 Example of identified results for n-alkane data

2. Calculation Method when Target Compound Peak is not between the Index Standard Peaks

If there is no index standard whose retention index is smaller (or larger) than that of the target compound peak, calculation of the retention time is based on the existence of virtual index standards at intervals equal to those between the retention times and retention indexes of the two index standards closest to the retention index of the target compound peak. The retention time calculated in this way is only a reference value and may differ greatly from the actual retention time.



In this example, no compound peak is eluted faster than the target compound peak (retention index RI_T) and the retention indexes of the index standards which are closest to that of the target compound peak are RI_1 and RI_2 . We assume that there are index standards (RI_{V1} , RI_{V2} , RI_{V3} ...) eluted faster than RI_1 at intervals equal to the interval between RI_1 and RI_2 and the interval between the corresponding retention times.

If the retention index of the target compound peak is between RI_{V1} and RI_{V2} , the retention time of the target compound peak (RT_T) is calculated as follows:

$$RT_T = RT_{v2} + (RT_{v1} - RT_{v2}) \times \frac{RI_T - RI_{v2}}{RI_{v1} - RI_{v2}}$$

Here, RI_{v1} , RI_{v2} , RT_{v1} , and RT_{v2} are defined as follows:

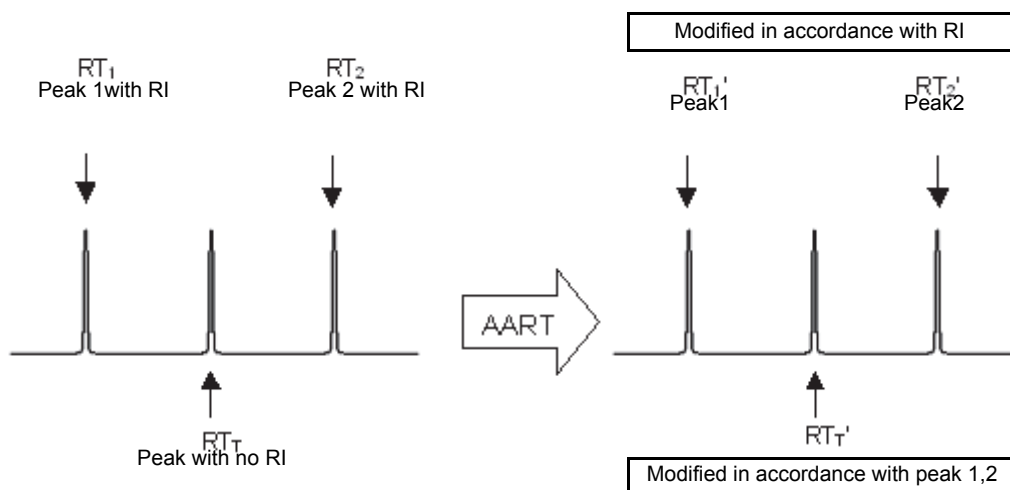
$$RI_{v1} = RI_1 - (RI_2 - RI_1) \quad RI_{v2} = RI_{v1} - (RI_2 - RI_1)$$

$$RT_{v1} = RT_1 - (RT_2 - RT_1) \quad RT_{v2} = RT_{v1} - (RT_2 - RT_1)$$

Eq.2 Formula for calculating retention time when target compound peak is not between index standard peaks

3. Calculation Method when Retention Index of Target Compound is not Set

If the retention index of the target compound is not set, the retention time cannot be calculated using the methods described above in items 1 and 2. In this case, the retention time is calculated using the "pre-modification retention times" of compounds for which the retention indexes are set in the compound table, the "post-modification retention times" obtained using the methods described above in items 1 and 2, and the respective displacements. At this time, the (pre-modification) retention time for the target compound must be set.



$$RT_T' = RT_1' + (RT_2' - RT_1') \times \frac{RT_T - RT_1}{RT_2 - RT_1}$$

Here, the respective variables are defined as follows:

RT_T : Pre-modification retention time of target compound

RT_T' : Post-modification retention time of target compound

RT_1 : Pre-modification retention time of compound whose retention time approaches that of target compound peak most closely from below

RT_1' : Post-modification retention time of compound whose retention time approaches that of target compound peak most closely from below

RT_2 : Pre-modification retention time of compound whose retention time approaches that of target compound peak most closely from above

RT_2' : Post-modification retention time of compound whose retention time approaches that of target compound peak most closely from above

The retention indexes for compounds 1 and 2 are set.

Eq.3 Formula for calculating retention time when retention index of target compound is not set

Example: If retention times set in the compound table of 11.000 and 12.000 minutes are shifted to 10.600 and 11.700 minutes respectively, the calculation for a target compound retention time of 11.300 minutes is as follows:

$$RT_T' = 10.600 + (11.700 - 10.600) \times \frac{11.300 - 11.000}{12.000 - 11.000}$$

$$\approx 10.930$$

If there is no compound 1 (i.e., a compound to the left), then "0" is used for RT_1 and RT_1' .

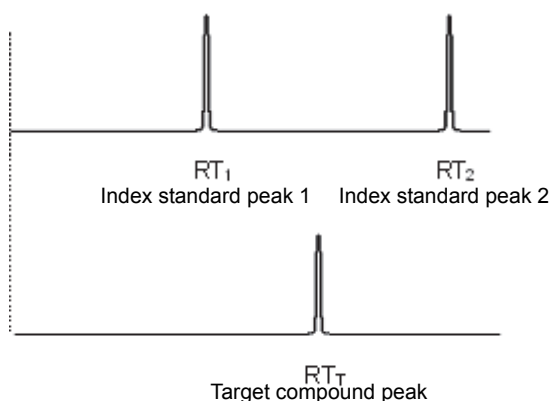
$$RT_T' = RT_2' \times RT_T \div RT_2$$

If there is no compound 2 (i.e., a compound to the right), then "0" is used for RT_2 and RT_2' .

$$RT_T' = RT_1' \times RT_T \div RT_1$$

3.7.2 Calculation Method for Retention Indexes in Compound Table

1. Calculation Method when Target Compound Peak is between the Index Standard Peaks



$$RI_T = RI_1 + (RI_2 - RI_1) \times \frac{RT_T - RT_1}{RT_2 - RT_1}$$

RI_T, RT_T : Retention index and retention time of target compound peak

RI_1, RT_1 : Retention index and retention time of index standard whose retention index approaches that of target compound peak most closely from below

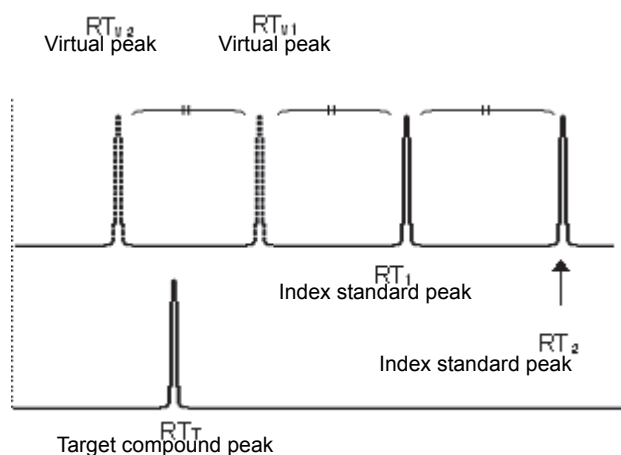
RI_2, RT_2 : Retention index and retention time of index standard whose retention index approaches that of target compound peak most closely from above

Eq 4 Formula for calculating retention index when target compound peak is between index standard peaks

2. Calculation Method when Target Compound Peak is not between the Index Standard Peaks

If there is no index standard whose retention time is smaller (or larger) than that of the target compound peak, calculation of the retention index is based on the existence of virtual index standards at intervals equal to those between the retention times and retention indexes of the two index standards closest to the

retention time of the target compound peak. The retention index calculated in this way is only a reference value and may differ greatly from the actual retention index.



In this example, no compound peak is eluted faster than the target compound peak (retention time RT_T) and the retention times of the index standards which are closest to that of the target compound peak are RT_1 and RT_2 . We assume that there are index standards (RT_{v1} , RT_{v2} , RT_{v3} ...) eluted faster than RT_1 at intervals equal to the interval between RT_1 and RT_2 and the interval between the corresponding retention indexes.

If the retention time of the target compound peak is between RT_{v1} and RT_{v2} , the retention index of the target compound peak (RI_T) is calculated as follows:

$$RI_T = RI_{v2} + (RI_{v1} - RI_{v2}) \times \frac{RT_T - RT_{v2}}{RT_{v1} - RT_{v2}}$$

Here, RT_{v1} , RT_{v2} , RI_{v1} , and RI_{v2} are defined as follows:

$$RT_{v1} = RT_1 - (RT_2 - RT_1) \quad RT_{v2} = RT_{v1} - (RT_2 - RT_1)$$

$$RI_{v1} = RI_1 - (RI_2 - RI_1) \quad RI_{v2} = RI_{v1} - (RI_2 - RI_1)$$

Equation 6 Formula for calculating retention time when target compound peak is not between index standard peaks

4

Peak Purity Algorithms

This chapter describes the peak purity algorithms. Peak purity refers to the index used to evaluate whether a chromatogram peak, obtained by a photodiode array detector, contains multiple components.

This chapter explains:

- the principles for peak purity analyses,
- procedures for setting the parameters and actual applications,
- parameter optimization and limitation items (since the applicable range for purity calculation depends on measurement and sample conditions), and
- validation of the method parameters, allowing more accurate purity calculations.

This chapter consists of the following three sections:

- ["4.1 Basic Principles of Peak Purity Analysis" P.63](#)
- ["4.2 Method Development for Peak Purity Analysis" P.70](#)
- ["4.3 Method Optimization" P.74](#)

4

4.1 Basic Principles of Peak Purity Analysis

In purity analysis, the software calculates the "similarity" between the objective and reference spectra. The peak purity is evaluated by comparing the similarity with a "threshold value" calculated from the noise components. To better understand the purity analysis algorithm, it is important to understand the calculation methods for these parameters.

4.1.1 Calculating the Similarity

This section describes the equations used to calculate the similarity - index of how closely the two spectra match.

To understand the similarity, first consider spectra as sets of absorbances at different wavelengths. In this case, absorbance spectra S1 and S2 are expressed by the following vectors:

$$S1 = (a1(\lambda_1), a1(\lambda_2), \dots, a1(\lambda_n))$$

$$S2 = (a2(\lambda_1), a2(\lambda_2), \dots, a2(\lambda_n))$$

where (λ_1) signifies the absorbance at wavelength (λ_1) .

When focusing on two wavelength data, these two vectors can be graphically represented as shown in Fig.4-1.

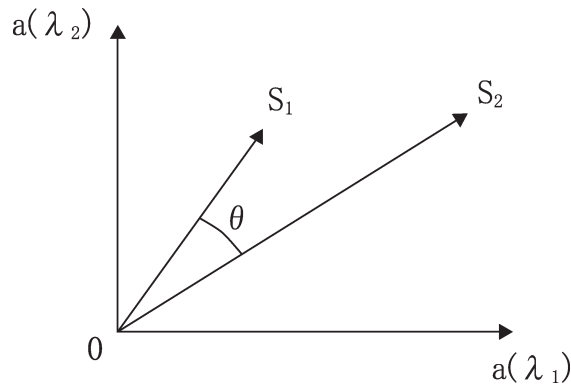


Fig.4-1 Spectra Represented as Vectors

If two spectrum shapes are identical, vectors S1 and S2 should point to the same direction, and the angle θ in Fig.4-1 becomes "0", even though absorbances are different. Since the smaller the angle θ , the greater the similarity between two spectra, the similarity of two spectra (SI) can be obtained by calculating $\cos\theta$. The equation will be as follows:

$$SI = \frac{S_1 \cdot S_2}{|S_1||S_2|} = \cos\theta$$

or

$$SI = \frac{\sum a_1(\lambda_i) \times a_2(\lambda_i)}{\sqrt{\sum a_1(\lambda_i)^2} \times \sqrt{\sum a_2(\lambda_i)^2}}$$

The closer the similarity (SI) to 1, the more closely two spectra match.

4.1.2 Calculating the Threshold

Generally, when two spectra are obtained from a single component peak, the shapes of those spectra should be the same within the peak. In actual measurement, however, the angle θ does not necessarily become "0", because of the effect of the background noise of the detector, noise from mobile phase absorption, etc. Fig.4-2 illustrates the case.

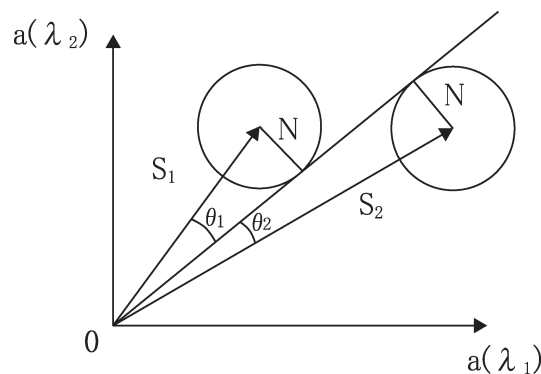


Fig.4-2 Noise Components

In *Fig. 4-2*, circles with radius N indicate the uncertainty due to noise components of size N .

This means that it is possible that the similarity between two peaks is reduced by the amount $\cos(\theta_1 + \theta_2)$ due to the noise.

However, if the angle θ between vectors S_1 and S_2 exceed $(\theta_1 + \theta_2)$, there may exist some factor other than noise components, which makes the two spectrum shapes different.

The value $\cos(\theta_1 + \theta_2)$ here is called "threshold (t)".

The software compares the threshold (t) with obtained similarity, to evaluate whether or not two spectrum shapes match.

Threshold (t) is calculated by the following equation:

$$t = \cos(\theta_1 + \theta_2) = \sqrt{\left(1 - \frac{N^2}{|S_1|^2}\right) \times \left(1 - \frac{N^2}{|S_2|^2}\right)} - \frac{N^2}{|S_1||S_2|}$$

where

$$|S_1| = \sqrt{\sum a_1(\lambda_i)^2}$$

$$|S_2| = \sqrt{\sum a_2(\lambda_i)^2}$$

Assuming the noise spectrum intensity at wavelength λ_i is $no(\lambda_i)$, radius N is obtained as follows:

$$N = \sqrt{\sum no(\lambda_i)^2}$$

4.1.3 Calculating the Peak Purity

This section describes how this software calculates peak purity based on similarity and threshold values, and how the results are displayed.



NOTE

The presence of impurities is determined using similarity (SI), threshold (t), and a purity index, as follows:

$SI \geq t$	Purity index ≥ 0	No impurity is detected
$SI < t$	Purity index < 0	Impurity is detected

This software uses the following three methods to determine peak purity.

- 3-point peak purity method
- N-point peak purity method
- total peak purity method

Note that peaks obtained with a photodiode array detector have spectrum values at each sampling point.

- The 3-point peak purity method uses spectra at three sampling points.
- The N-point peak purity method is an extended 3-point peak purity method that can use spectra from 5, 7, and 9 sampling points.
- Total peak purity method uses the spectra from all of the sampling points.

Even though these methods use a different number of sampling point spectra, they all evaluate purity using the similarity and threshold values, calculated in "[4.1.1 Calculating the Similarity](#)" and "[4.1.2 Calculating the Threshold](#)", respectively.

3-Point Peak Purity Method

This method uses spectra at three specific sampling points on the peak. Fig.4-3 shows the positions of the three sampling points.

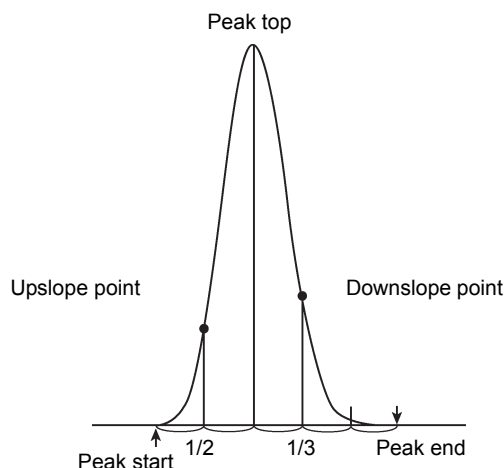


Fig.4-3 Sampling Points for 3-Point Peak Purity Method

The target peak is divided at the peak top for purity evaluation.

The purity of the front half of the peak is evaluated at the middle of the peak start (Upslope point) and at peak top. The purity of the rear half of the peak is evaluated considering the tailing characteristics of chromatogram peaks at the 1/3 point between the peak top and peak end (Downslope point). The reference spectrum is always the peak top spectrum.

This software displays similarity, threshold, and purity index (difference between similarity and threshold) for the upslope point, downslope point, and 3-points (average of the purity indices for the upslope and downslope points).

N-Point Peak Purity Method

This method uses spectra at the sampling points shown below. Since spectra near peak shoulders are also calculated, purity can be evaluated based on a broader region than that used in the 3-point peak purity method. However, if the peak start and end are not properly determined by peak integration purity calculation may be impossible due to the lack of spectrum intensities at those sampling points. Therefore, integration parameters need to be accurately set or the peak needs to be manually integrated.

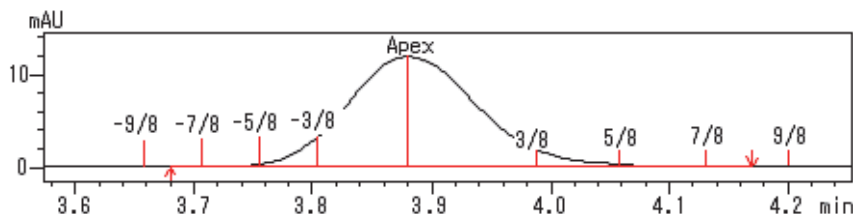


Fig.4-4 Sampling Points for N-point Peak Purity Method

N-point	Sampling Point Used			
5	$\pm 3/8$		$\pm 4/5$	
7	Peak start	$\pm 4/5$	$\pm 3/8$	Peak end
9	$\pm 9/8$	$\pm 7/8$	$\pm 5/8$	$\pm 3/8$

Similarly to the 3-point peak purity method, the software calculates the similarity between spectrum at each sampling point and peak top spectrum, and evaluates the purity for the front and rear halves of the peak.

■ Total peak purity method

This method uses spectra from all of the sampling points on the peak for the purity calculations. Even though the calculation process takes longer than the 3-point and N-point peak purity methods, it allows for more accurate purity evaluations.

Fig.4-5 shows the position of sampling points used for total peak purity method.

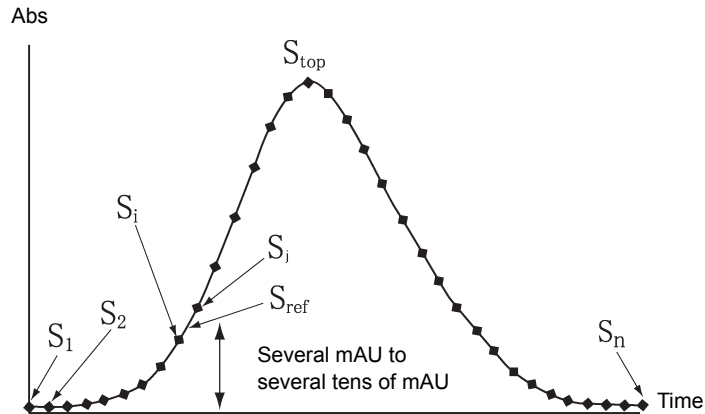


Fig.4-5 Sampling Points for Total Peak Purity Method

Spectra for each of the sampling points (S_1, S_2, \dots, S_n) are compared with the spectrum at the reference point with higher absorbance (S_{ref}), located at a slightly inner area towards the peak top. Each sampling point on the peak has a corresponding reference point. Even though the position of the reference point varies with absorbance level, it is set to give a difference of several mAU to several tens of mAU from the sampling point, based on the maximum absorbance of the spectrum (Max. Plot designated wavelength range). As shown in *Fig.4-5*, the reference spectrum for spectrum S_1 (S_{ref}) is obtained from the adjacent spectra S_i and S_j .

Unlike the previous methods that use the peak top spectrum (S_{top}) as a reference for all sampling points, this method can evaluate the purity by eliminating the effect of spectrum shape difference due to sample concentration variations.

This software calculates the purity at each sampling point using the total peak purity method, and displays the result with retention time along the graph horizontal axis. Two types of graphs are available: a similarity curve, which plots the similarity and threshold value, and a purity curve, which plots the purity index (similarity-threshold). *Fig.4-6* and *Fig.4-7* show examples of these curves.

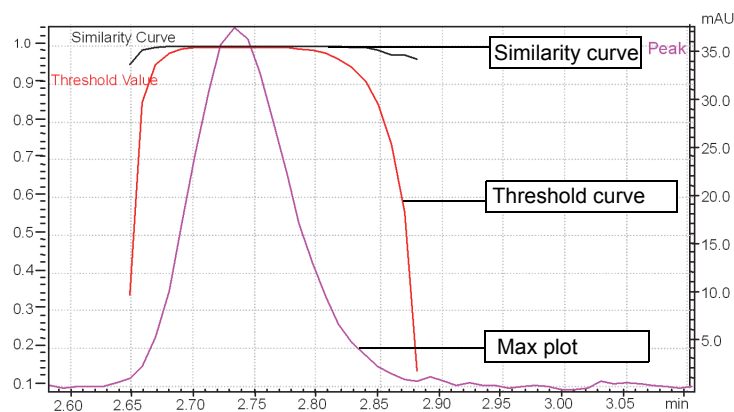


Fig.4-6 Similarity Curve

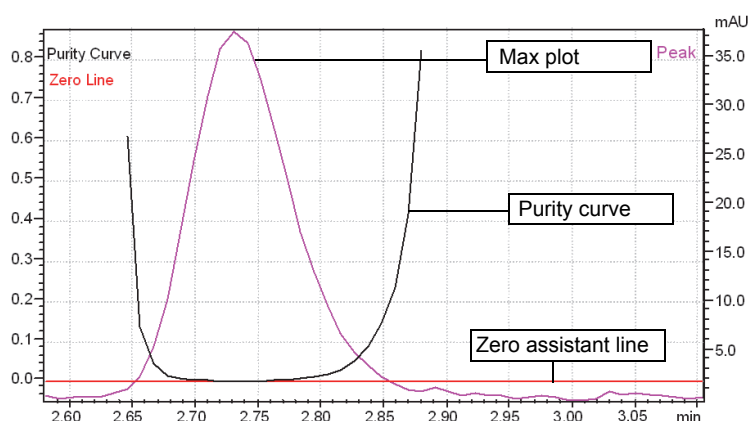


Fig.4-7 Purity Curve

Switch between the Similarity and Purity curve by right-clicking on the graph and selecting [Display Settings] to open the [Purity View (Peak Purity) Display Settings] sub-window.

The software determines which sampling point has the minimum purity index and displays the similarity, threshold, and purity index obtained at that point. These values are called “Peak Purity Index”, “Single Point Threshold”, and “Min Peak Purity Index”, respectively. “Min Peak Purity Index” is the threshold subtracted from the similarity, multiplied a million times for convenience of reference.

If the “Min Peak Purity Index” is a negative value, the retention time for that sampling point is also displayed. This retention time does not indicate the retention time of impurity peak, but the point where the shapes of the two spectra differ the most.

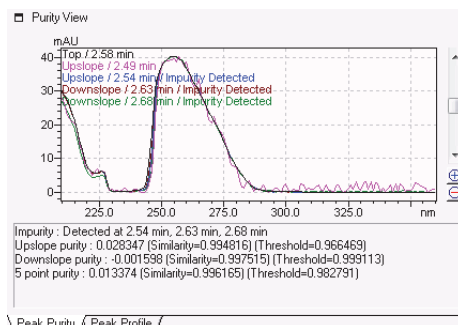
The absolute value of the index does not indicate an impurity amount.

The reference point is defined as the point with a higher absorbance (several mAU to several tens of mAU) than the corresponding sampling point. It is located slightly towards the center of the peak. The wavelength range for the max plot is defined using [From] and [To] in the [Purity] tab of the [PDA Data Analysis] window. See ["4.2 Method Development for Peak Purity Analysis"](#). The software displays the max plot of the peak over the similarity and purity curves. The time axis is defined as the range between peak start and peak end on the relevant chromatogram.

4.1.4 Peak Purity Analysis Result Example

This example uses shows a result using the 5-point peak purity method.

The figure below illustrates the result of the peak evaluation:



Peak top: 2.58 min
Peak start: 2.464 min
Peak end: 2.709 min

The sampling points on the front half of the peak are: Peak top – (Peak top – Peak start) × 4/5 = 2.49 min

Peak top – (Peak top – Peak start) × 3/8 = 2.54 min

The sampling points on the rear half of the peak are: Peak top + (Peak end – Peak top) × 3/8 = 2.63 min

Peak top + (Peak end – Peak top) × 4/5 = 2.68 min

By adding the peak top, a total of 5 points are used for purity calculations.

“Upslope Purity Index” at the bottom of the graph indicates the mean value from two sampling point spectra on the front half of the peak. “Downslope Purity Index” indicates the mean value for the rear half of the peak.

NOTE

- If calculation is impossible at a sampling point due to the lack of spectrum intensity, the sampling point is disregarded from the result. If calculation is impossible at both sampling points, “Cannot be calculated” is displayed.
- “5-point Purity Index” averages the upslope and downslope indexes. If a purity calculation is impossible for either the front or rear half, “Cannot be calculated” is displayed.
- “Impurity: Detected at” is displayed for all retention times where the spectrum similarity falls under the threshold.

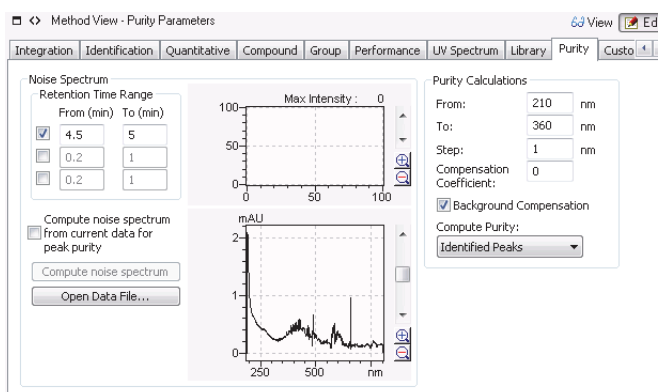
The graph displays the spectra extracted at each sampling point. The label indicates the sampling point where each spectra was obtained (e.g. peak top, upslope, and downslope), as well as its retention time.

NOTE

- “Impurity: Detected at” is displayed when the spectral similarity between the peak top and any sampling point falls under the threshold.
- “Cannot be calculated” is displayed when purity calculation is impossible.

4.2 Method Development for Peak Purity Analysis

This section describes the development of a method for the purity analysis of measured chromatogram peaks. Set the purity calculation parameters according to actual measurement conditions in the [PDA Data Analysis] window - [Method View] - [Purity] tab. Setting appropriate parameters allows for more accurate purity evaluation.



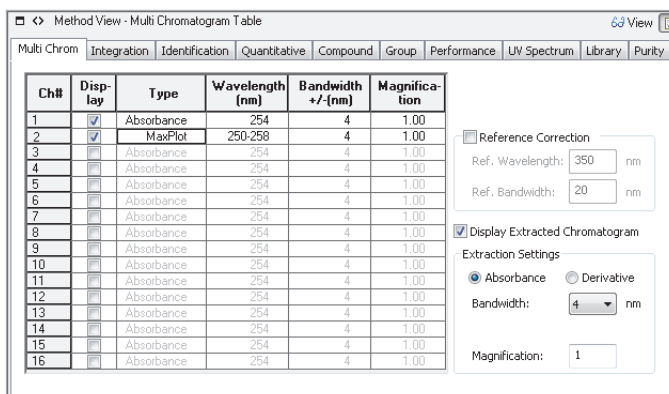
Reference

Refer to "4.3.1 Method Development" P.74 to set method development parameters.

4.2.1 Specifying the Multi-Chromatogram Wavelength

Data obtained with a photodiode array detector are saved as three-dimensional data of wavelength, retention time, and intensity. Extract data at a specific wavelength from the 3-D data to obtain a chromatogram, then save the extraction wavelength in the method file. This type of chromatogram is called a "multi-chromatogram".

The peak start, peak end, and retention time are required to conduct purity analysis for a peak. Therefore, isolate the objective peak by extracting a multi-chromatogram and conducting peak integration on the chromatogram. Select the wavelength for the multi-chromatogram that allows the objective peak to exhibit sufficiently high absorbance.



4.2.2 Calculating the Noise Spectrum

The software calculates noise spectra based on the time specified in [Retention Time Range] on the [Purity] tab.

Noise spectrum intensity at a wavelength λ_i [$no(\lambda_i)$] is calculated as three times the standard deviation (SD) of baseline fluctuations on the multi-chromatogram at λ_i in the [Retention Time Range]. The equation is as follows:

$$no(\lambda_i) = 3 \times SD$$

$$SD = \sqrt{\frac{n \sum a(t_j)^2 - (\sum a(t_j))^2}{n(n-1)}} \quad \text{where}$$

n: The number of sampling points in the set retention time range
a(t_j): Absorbance at time t_j

The noise spectrum is obtained by executing the above calculation for each wavelength.

NOTE

- It is impossible to evaluate peak purity when the intensity of noise spectrum is greater than that of the objective peak spectrum. In the following cases, calculated noise spectrum intensity may differ from the noise value contained in the actual objective peak:
 - The mobile phase composition differs between the period of elution of the objective peak and the retention time range used for noise spectrum calculation.
 - Contaminates are eluted in the retention time range used for noise spectrum calculation.
 - Background noise from hardware greatly differs between noise calculation data and objective peak data. This may be due to lamp replacement, use of different equipment, etc.
- To avoid these problems, set the noise spectrum calculation parameters according to the following items.
 - Use a [Retention Time Range] that includes the objective peak. Otherwise, analyze a blank and calculate the noise spectrum using the time near the objective peak on the blank data, then save the noise spectrum as a method.
 - Display the max plot of the data used for the noise spectrum calculation, and specify a retention time range that does not include contaminants.
 - Select the [Compute noise spectrum from current data for peak purity] check box to always calculate noise spectrum using the objective peak data.
 If this box is checked, the noise spectra will not be saved to the method, and noise spectra in the method becomes unavailable. Noise spectrum is calculated for each purity calculation from the objective peak data, using the set retention time range. This allows for more accurate noise spectrum calculation. If a contamination peak appears in the retention time range, an extremely large noise level is detected, preventing accurate purity evaluation.

4.2.3 Defining the Wavelength Range

Peak purity is calculated within the wavelength range defined by [From] and [To] in [Purity Calculations]. Correct purity calculation may be obstructed when the target substance has no UV absorbance in the range, or when a low-wavelength range is set in which the mobile phase absorbs UV.

4.2.4 Specifying the Compensation Coefficient

This value is normally 0. If a purity evaluation result differs from expected values, the evaluation threshold can be adjusted by specifying a numerical value to this parameter. The evaluation result is displayed after the set value is subtracted from the min peak purity index. Consider the case of a measured peak that obviously contains no impurities or contains purities within the permitted limit as an example. If this peak is evaluated to contain impurities (min peak purity index is negative) due to, for example, spectrum distortion from indeterminate noise effect or from measuring a concentrated sample, change the evaluation result to “no impurity” by specifying [Compensation Coefficient]. (For example, enter a compensation coefficient of -1000, when the min peak purity index is -1000.)

Conversely, consider a purity curve as shown in [Fig.4-8](#), which clearly shows that there is an impurity near 4.09. If this peak is evaluated to contain no impurity, modify the evaluation by specifying the corresponding positive value in [Compensation Coefficient].

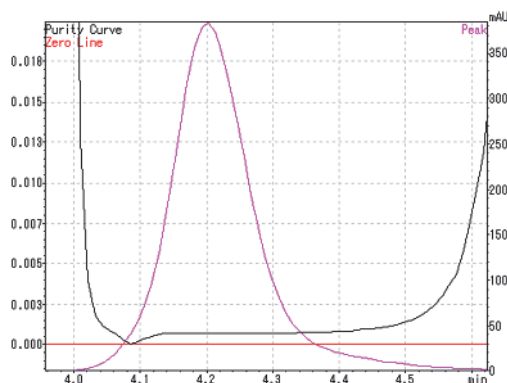


Fig.4-8 Example of Purity Curve Result Output



NOTE

The purity evaluation can be intentionally modified by specifying a [Compensation Coefficient]. Always make sure that the value is not changed in the future by including the [Compensation Coefficient] in the purity evaluation report, enabling the audit trail function. The evaluation result can also be changed when changing the noise spectrum used for purity evaluation. Always confirm that the noise spectrum calculation parameters have not been changed.

4.2.5 Spectrum Background Compensation

If the [Background Compensation] box is checked during purity calculation, background compensation is performed on the spectrum used for purity evaluation. This setting reduces the effect of baseline drift, allowing for more accurate purity evaluation. The background compensation is executed by the following equation:

$$S_{\text{corr}} = S - S_{\text{b}}$$

- S_{corr} : Spectrum after background compensation
- S : Spectrum before background compensation
- S_{b} : Background spectrum

The absorbance at wavelength λ_i after background compensation is calculated by the following equation:

$$a_{\text{corr}}(\lambda_i) = a(\lambda_i) - a_{\text{b}}(\lambda_i)$$

- $a_{\text{corr}}(\lambda_i)$: Absorbance after background compensation
- $a(\lambda_i)$: Absorbance before background compensation
- $a_{\text{b}}(\lambda_i)$: Background absorbance

$a_{\text{b}}(\lambda_i)$ is obtained by the following equation:



NOTE

For the 7 or 9 point peak purity method, the software does not execute background compensation even though [Background Compensation] is set to ON, since peak start/end and sampling points outside the peak are used for calculation.

$$a_{\text{b}}(\lambda_i) = \frac{a_{\text{s}}(\lambda_i)(t_{\text{e}} - t) + a_{\text{e}}(\lambda_i)(t - t_{\text{s}})}{t_{\text{e}} - t_{\text{s}}}$$

- $a_{\text{s}}(\lambda_i)$: Absorbance at peak start
- $a_{\text{e}}(\lambda_i)$: Absorbance at peak end
- t : Retention time for the background-compensated spectrum
- t_{s} : Retention time of peak start
- t_{e} : Retention time of peak end

S_{corr} can be obtained by calculating this across the entire wavelength range.

To improve purity calculation accuracy, always select the [Background Compensation] check box, even though it might take slightly more time for the calculation process.

4.2.6 Setting the [Compute Purity] Options

The purity analysis involves various calculations using three-dimensional data. If multi-chromatograms are specified at multiple channels, and if peaks are detected at each chromatogram, the software response becomes slower during peak integration. This is because each time peak integration is executed, the purity of an extremely large number of peaks is calculated.

To avoid this situation, set the [Compute Purity] options as described below. Select an appropriate parameter in the [PDA Data Analysis] window - [Method View] - [Purity] tab - [Compute Purity].

Parameter	Description
Not Calculated	Select this parameter if purity analysis is not necessary. The processing speed remains high since the software does not calculate the purity at each peak integration. The purity analysis result is not displayed on the peak table or as peak top comments on chromatograms.
Identified Peaks	Select this parameter if the peaks that require purity analysis are already determined. The purity analysis result is only displayed for the peaks identified in the compound table (ID table).
All Peaks	The purity is calculated for all of the detected peaks.

The similarity curves and purity curves are used to calculate the purity for the selected peaks.

4.3 Method Optimization

Due to characteristics of absorption detectors, such as the photodiode array detector, the detector response to the sample concentration may be non-linear for higher-concentration samples. Since the purity analysis calculates the similarity between two spectra, this effect becomes significant even in the concentration region where the calibration curve linearity is not affected. In such concentration regions, a peak without impurity may be wrongly evaluated to contain impurities, since the two spectra are judged to be different due to the spectrum distortion.

This effect becomes even more significant in purity calculations that use the peak top spectrum as a reference. Spectrum distortion is greater for high concentration samples and the similarity decreases more from peak shoulders towards the peak top. This software uses a unique method, such as "*Total peak purity method*", to calculate the purity of the peaks with an absorbance of up to 1 AU, without specifying any compensation parameter.

However, if purity calculation parameters are set incorrectly, purity analysis may be impossible even for peaks with a maximum absorbance of less than 1 AU.

Follow the procedure described below to develop and verify the optimum method.

4.3.1 Method Development

Follow the procedure described below to develop the optimum method.

1

Specify the multi-chromatogram extraction wavelength.

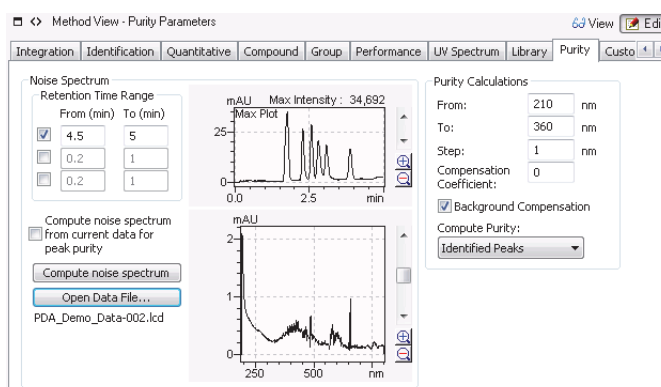
Select the [Multi Chrom] tab and specify the multi-chromatogram extraction wavelengths in the multi-chromatogram table. Refer to the peak top spectrum of the target peak. Specify the wavelength that obtains the highest absorbance for the peak.

Reference

If the target peak has not been completely integrated, set appropriate peak integration parameters according to the Operators Guide.

Refer to "[4.2.1 Specifying the Multi-Chromatogram Wavelength](#)" P.70 for details on how to set multi-chromatogram extraction wavelengths.

2 Set the parameters on the [Purity] tab in the [Method View] of the [PDA Data Analysis] window.



- 1 Specify the retention time range for the noise spectrum calculation.
Click [Open Data File] to open the objective data for purity analysis, or a data file obtained in the same conditions as the objective data.
The max plot is displayed in the upper graph window. The chromatogram can be enlarged by dragging the mouse over the range to be enlarged. Locate a retention time range with no peaks and a flat baseline. Specify this range for the noise spectrum calculation. Refer to ["4.2.2 Calculating the Noise Spectrum"](#) for details.



NOTE

Do NOT specify a value smaller than 0.2 min in the case when some PDA detector models do not contain any signals from 0 to 0.2 min. The default setting is from 0.2 min to 1 min.

- 2 Click [Compute noise spectrum].
The calculated noise spectrum is displayed in the lower graph window. Ensure that the maximum absorbance of the noise spectrum is not greater than the maximum absorbance of the objective peak. If the maximum absorbance of the noise spectrum is larger, the software cannot analyze peak purity using the noise spectrum. Use the following measures in this situation.
 - Redefine the retention time range.
 - Restrict the wavelength range to perform purity calculation only within the range where the noise spectrum absorbance is smaller.
 - Increase the sample concentration.



NOTE

- Three separate retention time ranges can be selected. As default, only the top is selected. To analyze the purity of multiple peaks in a gradient analysis, select multiple areas near the peaks.
- Unless [Compute noise spectrum from current data for peak purity] is selected, the current noise spectrum is saved in the method. The noise spectrum is then used for the subsequent purity analyses for any data obtained using this method, and it is no longer necessary to calculate noise spectrum for each purity analysis. Conversely, to always calculate noise spectrum using the objective data for purity analysis, select the [Compute noise spectrum from current data for peak purity] check box. It is recommended that the check box NOT be selected since purity analysis results can be more easily compared when obtained under the same conditions.

- 3 Specify the wavelength range using [From] and [To] in the [Purity Calculations] section of the [Purity] tab.
Define the wavelength range to perform purity calculations. By referring to the spectra contained in the objective peak for purity analysis, specify the wavelength range where the peak has sufficient absorbance. It is recommended that wavelengths less than 210 nm NOT be included in the region, since the lower wavelengths often detect mobile phase absorption.
- 4 Specify [Step].
The default value is 1. If the step value is changed to 2 or greater, the uncertainty of the purity evaluation may increase, even though the processing speed increases due to the lesser number of calculation points.
- 5 Specify [Compensation Coefficient].
Start with the default setting of 0. Change the value as appropriate according to the purity analysis result. Refer to "[4.2.4 Specifying the Compensation Coefficient](#)" for details.
- 6 Check/uncheck the [Background Compensation] check box.
It is recommended that this check box always be checked. Remove the check only when the processing speed is extremely slow, and when it is certain that its removal does not affect the purity analysis result. Refer to "[4.2.5 Spectrum Background Compensation](#)" for details.
- 7 Specify [Compute Purity].
[Not Calculated] is the default value. Select [Identified Peaks] when the objective peak for purity analysis has already been identified in the compound table. In other cases, select [All Peaks]. Refer to "[4.2.6 Setting the \[Compute Purity\] Options](#)" for details.

4.3.2 Method Validation

This section describes procedures for validating the appropriateness of the method created in "[4.3.1 Method Development](#)".

- 1 **Prepare a standard sample that is known to contain no impurities.**
Prepare the standard sample to a similar concentration so that it will exhibit a similar absorbance to that of the objective peak. If the objective peak absorbance is not consistent, adjust the sample concentration to exhibiting the highest expected absorbance.
- 2 **Analyze the sample with the created method.**
Repeat this analysis five times to verify the method more accurately.
- 3 **Open the result in the [Purity View].**
Double-click the objective peak on the chromatogram in the [PDA Data Analysis] window to display the analysis result in the [Purity View]. Verify the Min Peak Purity Index.
The Min Peak Purity Index can be displayed in the peak top comments, peak table, and compound result table, unless [Purity Index Mode] is set to [None] in the [Purity View(Peak Purity) Display Settings] sub-window.

4.3.3 Interpretation of the Method Validation Result

■ Positive or Min Peak Purity Index Value Equal to 0

Use the method for the subsequent purity analysis, without change.

■ Negative Min Peak Purity Index Value

Due to spectrum distortion because of high sample concentration, background noise, chemical purity of standard samples, etc., the peaks analyzed by the current method may be wrongly evaluated to contain impurities. Enter the min peak purity index with the largest absolute value obtained in the repeated analyses to the [Compensation Coefficient] for the method.

■ Calculation is Impossible

If "Cannot be calculated" is displayed, it indicates that the purity index calculation is impossible because the maximum absorbance of the objective peak is smaller than the noise spectrum absorbance.

In this situation, an accurate purity evaluation is obstructed because the peak obtained at the sample concentration is overwhelmed by the noise. Increase the sample concentration to solve this problem, or review the method parameters. Check the noise spectrum calculation parameters and the wavelength range for the purity calculations. Recalculate the noise spectrum to decrease the noise level. Narrow the wavelength range, selecting only the range where the noise spectrum absorbance is smaller than the objective peak spectrum absorbance.

4

4.3.4 Application Examples

■ Applicable concentration range

A compensation coefficient is required to analyze the purity of high concentration samples. This is because the detector response to sample concentration becomes non-linear at higher concentrations. However, the correct purity evaluation may be obstructed if the compensation coefficient is set inappropriately. Refer to "4.3 Method Optimization".

The following example shows a purity analysis using high concentration samples without a compensation coefficient:

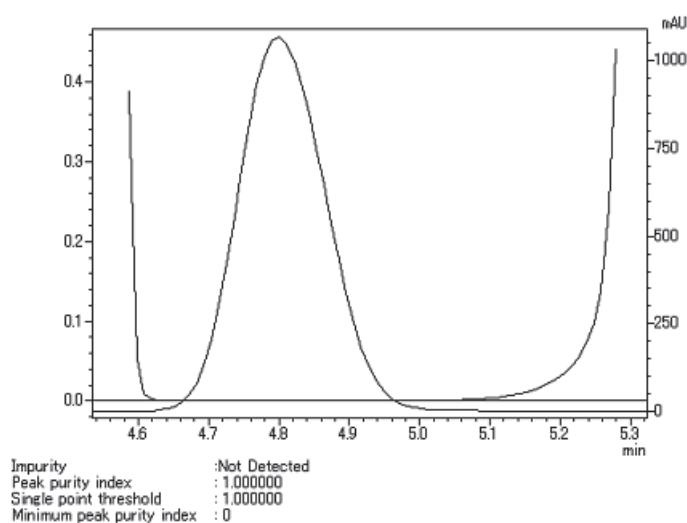


Fig.4-9 Purity Curve of Ethyl Paraben

Fig.4-9 shows the purity curve of the peak obtained by analyzing Ethyl Paraben. Notice that even the peak with a maximum absorbance greater than 1 AU is not affected by spectrum distortion.

Fig.4-10 shows the result of analyzing the same sample after adding 0.1% impurity. Notice that an impurity was detected near 4.47 min.

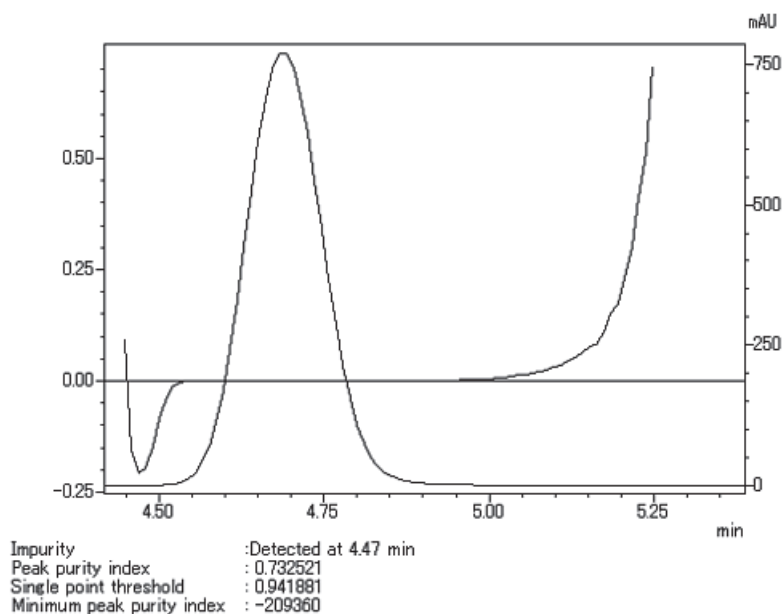


Fig.4-10 Purity Curve of Ethyl Paraben with 0.1% p-Ethyl Aminobenzoate Ester

As shown in the examples above, the purity can be evaluated for a sample with absorbance up to approximately 1 AU, even without any compensation coefficient.

■ Detection Limit for Impurity Content

Whether or not an impurity can be detected by purity analysis depends on the content of the impurity relative to the major component amount. The detection limit for the impurity content varies according to the following factors:

1. Spectral similarity between major component and impurity

The purity analysis is impossible between two components having identical spectral shapes. Even if not identical, if both spectral shapes have similar patterns, the spectrum difference may fall under the threshold. A sufficient absorbance value is required to detect these small differences. The detection limit varies according to the similarity of the two components.

2. Retention time difference between major component and impurity

Purity analysis is impossible for two components with identical retention times because the spectral shapes are identical. The detection limit varies according to the retention time difference of the two components.

3. Absolute impurity content

Even though the spectral shapes of two components greatly differ and the retention time difference is sufficiently large, purity analysis is impossible unless the absolute impurity content reaches the detection limit. Consider analyzing the purity of a peak containing 0.1% impurity. When the absorbance of the major component peak is 1 AU, the impurity absorbance is 1 mAU, which can be detected without any problem. Conversely, when the major component absorbance is 1 mAU, the impurity absorbance becomes 1 nAU, which may be difficult to detect.

The detection limit of the impurity content varies greatly according to analysis conditions and measured sample type. Fig.4-11 shows an example of the purity curve for ethyl paraben containing 0.05% p-ethyl aminobenzoate ester.

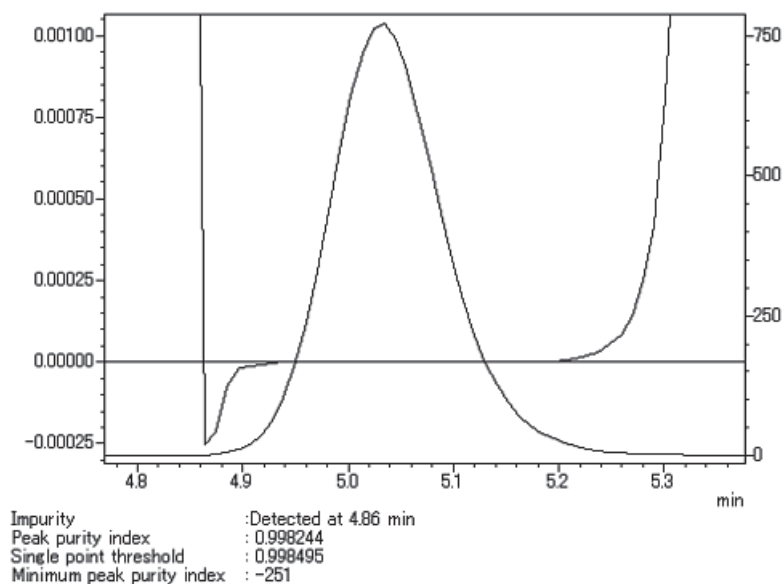


Fig.4-11 A Purity Curve of Ethyl Paraben Containing 0.05% p-Ethyl Aminobenzoate Ester

It was observed from the example that even 0.05% impurity was detected. Next, [Fig.4-12](#) and [Fig.4-13](#) show the UV spectra of p-ethyl aminobenzoate ester and ethyl paraben standard solution, respectively.

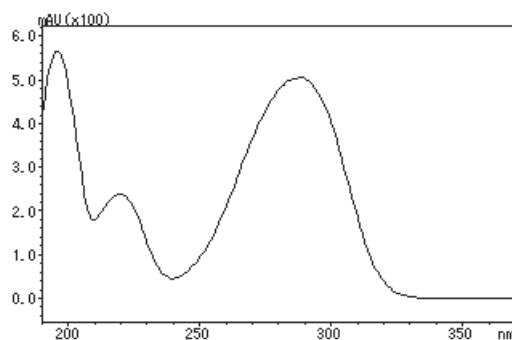


Fig.4-12 UV Spectrum of p-Ethyl Aminobenzoate Ester

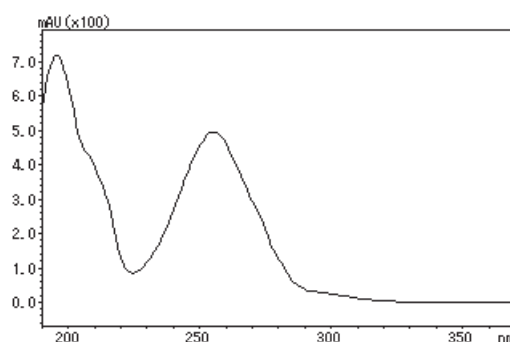


Fig.4-13 UV Spectrum of Ethyl Paraben

The similarity between these spectra became 0.688. The separation factor was 1.053, obtained by analyzing the standard solutions in the same analysis conditions.

Under such conditions, the presence of impurities can be sufficiently detected even for impurity with 0.05% concentration. However, the detection limit is expected to become higher when two component spectra are more similar, or the retention times closer, than is the case in this example.



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