225-10855

Shimadzu LCMSsolution

for
LCMS-2010 / LCMS-QP8000α

Operation Guide

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

SHIMADZU

Shimadzu Corporation

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Kyoto, Japan

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Introduction

Thank you very much for purchasing the LCMSsolution software for Shimadzu liquid chromatography / mass spectrometry workstations (hereafter called “LCMSsolution”).

LCMSsolution allows you to control the liquid chromatograph (hereafter called “LC”) and the Mass Spectrometer (hereafter called “MS”) from your personal computer, acquire chromatograms and other different kinds of data, and reanalyze the acquired data under different parameters on your personal computer.

This manual is the tutrial in the most simplified analysis procedure using LCMSsolution which helps you to catch more knowledge in other volumes or further actual operations.

The “Operation manual” and “Administration manual” are attached as separate volumes.

The Operation manual has been put together in order to familiarize you with the basic knowledge required to operate LCMSsolution. Be sure to read it thouroughly before using this software. After reading the manual, keep it in a safe place so that it can be accessed whenever necessary.

The Administration manual covers the information useful for system administration such as the support features for GLP/GMP or FDA 21CFR Part11, a set of regulations for electronic records and electronic signature. For more information on the functions of LCMSsolution, refer to this on-line manual.

This manual assumes that the reader is knowledgeable of basic operations of Windows®2000. For the operation of Windows®2000, refer to the instruction manual that comes with that product.

This manual sometimes explains commonly for LabSolutions series. And some explanations may use the drawings come from sister products like LCsolution, if it does not cause misunderstanding in the range of explanations.

Using the instruction manual

Kinds of instruction manuals

The LCMSsolution package contains the following information that describes the operational procedures and functions.

Name	Media	Description
Operation guide for LCMSsolution	Printed Document	Provides tutrial on mostly basic analysis procedure using LCMSsolution.
Operation manual for LCMSsolution	Printed Document	Explains the operational procedures for data acquisition and analysis using LCMSsolution.
Administration manual for LCMSsolution	Printed Document	Explains the operational procedures and basic idea of system administration and data management using LCMSsolution.
On-line help	LCMSsolution program	Provides detailed information on parameters and setting ranges. This is accessible from the Help menu in LCMSsolution. (For using the on-line help, refer to section *"14.1.1 Using Help"* in the Operation manual.)
Operation guide for LCMSsolution (PDF version)	CD-ROM disk for installation	Provides the operation guide volume of the instruction manual as a PDF file so that it can be viewed on your personal computer. The general table of contents is available, including other instruction manuals (PDF versions). It allows you to use each instruction manual via the hyperlink.
Operation manual for LCMSsolution (PDF version)	CD-ROM disk for installation	Provides the operation volume of the instruction manual as a PDF file so that it can be viewed on your personal computer. It is accessible from the Help menu in LCMSsolution. (For using this PDF, refer to section *"14.1.2 Using the Online Manual"* in the Operation manual.)
Administration manual for LCMSsolution (PDF version)	CD-ROM disk for installation	Provides the administration volume of the instruction manual as a PDF file so that it can be referred to on-line whenever operations related to system administration are needed. The general table of contents is available, including all the instruction manuals (PDF versions). It allows you to use each instruction manual via the hyperlink.

Legends for instruction manual

This manual uses the following legends:

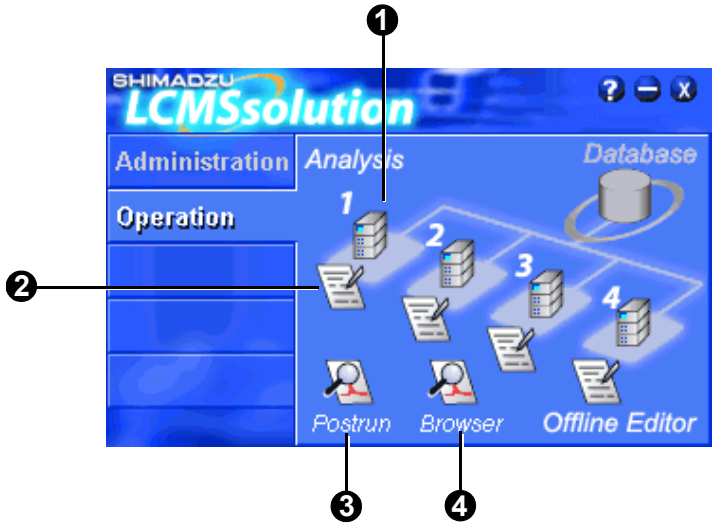
Legend	Meaning
■	Shows additional informations around the topic.
	Points the reference informations.
	Gives you tips.
< >	Shows a window or view name; e.g., <Data Acquisition> window or <Method> view.
[ ]	Shows a parameter, tab, column, cell, bar name, menu command , that can be selected from the menu bar.
[ ]-[ ] command	Shows a sequence of selecting the menu in the first [ ] and then selecting the command in the second [ ]. For example, [File]-[Print] command means that you should click on the File menu and then select the Print command from the displayed list of commands.

Contents

1 Making Preparations for Analysis

1.1 Basics of LCMSsolution

<LCMSsolution Launcher> - [Operation] menu icon



No.	Icon	Name	Description
1		Analysis	Starts the application for configuring and controlling the system and making a single-run or batch analysis. (Starts <LCMS Analysis> in the Online mode)
2		Offline Editor	Starts the application for editing any method file or batch file not in use during the analysis. (Starts <LCMS Analysis> in the Offline mode)
3		Postrun	Starts the application for loading the acquired analysis data to create a calibration curve or perform data processing.
4		Browser	Starts the application for browsing multiple analysis data together or analyzing data together.

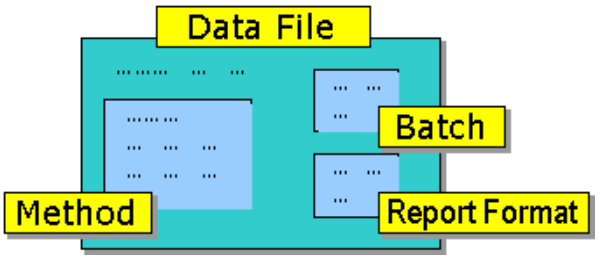
Files used in LCMSsolution

Extension	Name	Description
.lcm	Method File	Analysis condition, Data processing conditions, QA/QC settings, calibration curve information, and system configuration
.lcr	Report Format File	Report formats
.lcb	Batch File	Batch tables and batch settings
.lcd	Data File	Chromatograms, mass spectrums, peak tables, identification/quantitation results, report format, tuning results, methods, and batch table

☞ [Admin Manual]: "4.1 Important File Concepts for Operation"

Data structure in LCMSsolution

The data in the LCMSsolution is retained in data files, consisting various types of records and parameters such as the system configuration, fine-tuning result, system conditions, and analysis conditions that have been used to acquire and analyze data. This structure enables you to browse each data file for monitoring conditions and analysis parameters, thereby ensuring the traceability of data. This means that if a single data file is available, an analysis can be made again.



The method contained in the data file is a copy of the method file that was used to acquire and analyze data. Therefore, when any method parameter in the data file opened via <Data Analysis> is modified, the method contained in the data file is modified rather than the method file.

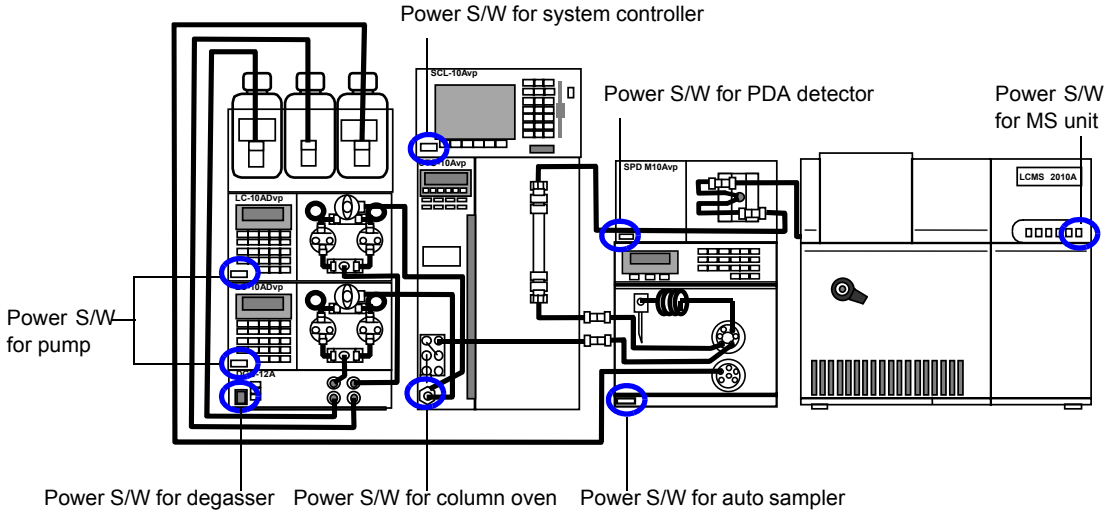
☞ [Admin Manual]: "4.1 Important File Concepts for Operation"

1.2 Starting the LCMSsolution

This document assumes the following system configuration as an example to describe the procedure for an analysis:
High-pressure Gradient LCMS plus PDA (= Photo Diode Array) Detectors System

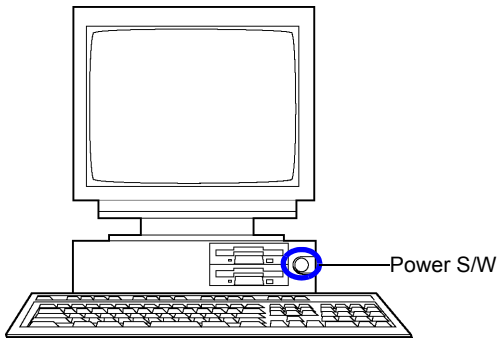
Pump	LC-10ADvp = 2 units
Auto sampler	SIL-10ADvp
Column oven	CTO-10A(C)vp
PDA detector	SPD-M10Avp
Mass spectrometer	LCMS-2010A

1 Check that the LC and MS units are On.



2 Check that nitrogen gas is sent to the MS unit.

3 Turn On the personal computer and peripheral devices to start Windows.



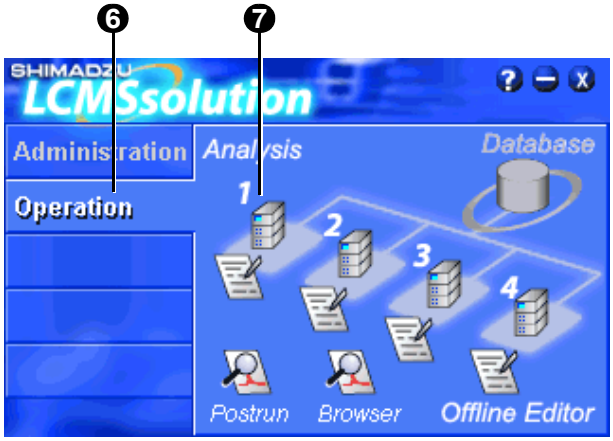
4 Enter your user ID to log on.

5 Double-click the [LCMSsolution] icon  displayed on the Windows desktop.
<LCMSsolution Launcher> will be started.

6 Select [Operation] menu.

7 Click the [Analysis] icon  .

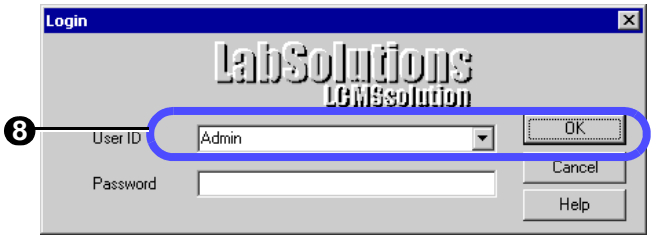
The <Login> screen will appear.



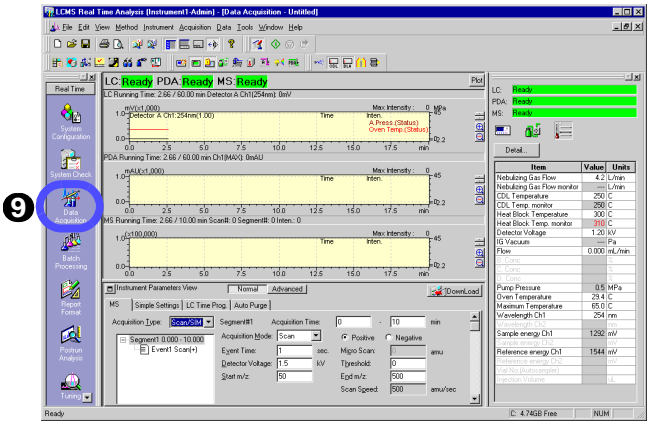
8 Select “Admin” and click the [OK] button.

The LCMS analysis program will be started with the <LCMS Analysis> main window displayed.

☞ [Admin Manual]: “2.4 Registering (Changing/Deleting) Users”, “2.5.2 Changing Passwords”



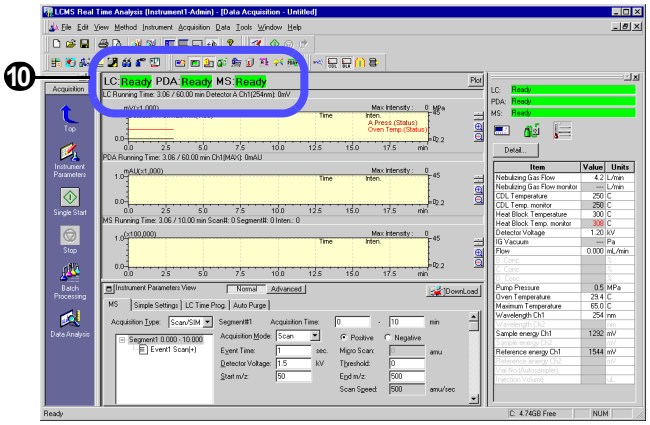
9 Click the [Data Acquisition] icon  .



10 Check that “Ready” is displayed.

If “Not Connected” is displayed, properly complete <System Configuration>.

☞ [Operation Manual]: “14.5 Configuring System”



Description of <Data Acquisition> window

- Toolbar

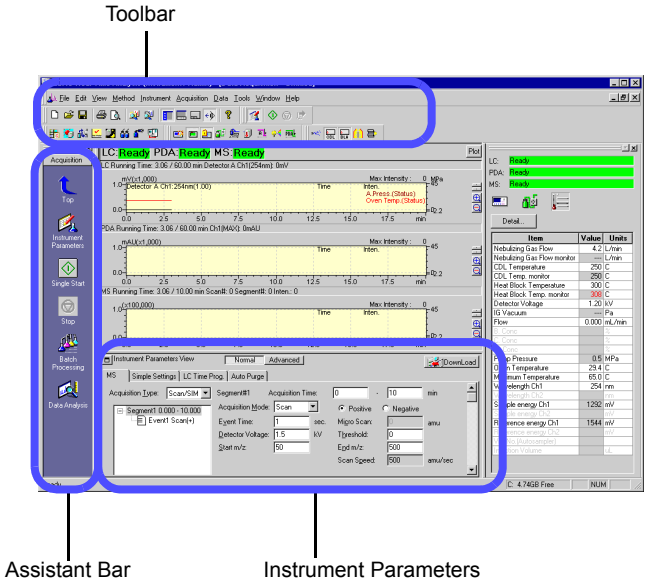
Among the functions available on the Menu bar, the frequently used ones and the functions to directly control the analyzer are assigned to this bar.

- Assistant Bar

The icons to operate the application in accordance with the general analysis flow are assigned to this bar.

- Instrument Parameters

A pane is displayed showing the parameters for the system set up on <System Configuration>.
Set those parameters for data acquisition.



2 Qualitative Processing (Single-run Analysis)

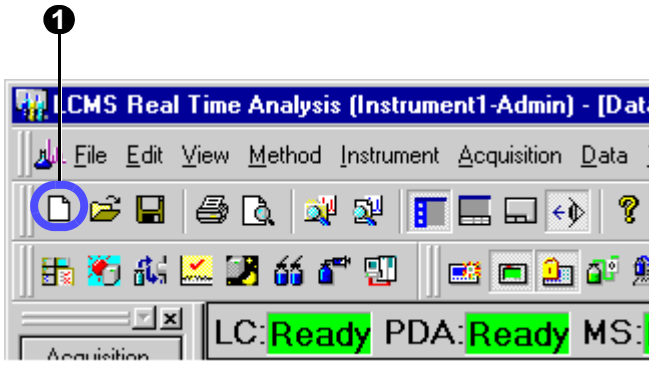
Set the parameters for the LC and MS units on the <Data Acquisition> window and then make an analysis. This document assumes an example of analysis under the following analytical conditions to specifically describe the procedure for the analysis.

Column	Shim-pack VP-ODS 150mm x 2.0mm i.d. 5µm (Equivalent to Shimadzu P/N 228-34937-94)
Mobile phase	Binary Gradient mode Pump A = Water, Pump B = Acetonitrile
Sample	Papaverine 0.5, 1, 5, 25, 50 ng/µL (Shimadzu P/N 225-06613-05)

2.1 Creating a new method file

1 Click the [New] button .

A new method file will be opened.



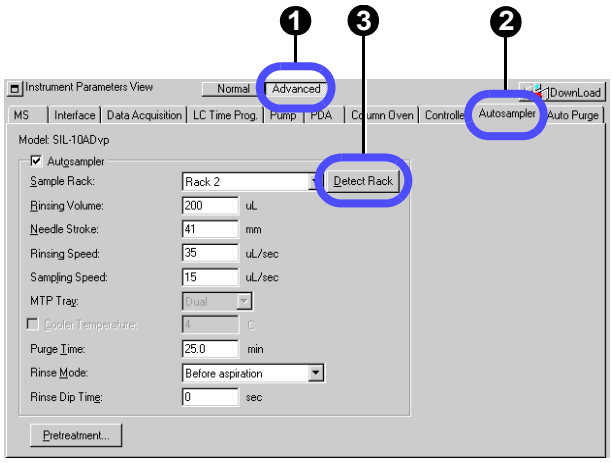
2.2 Setting the LC parameters

2.2.1 Detecting the auto sampler rack

1 Click [Advanced] button.

2 Select the [Autosampler] tab.

3 Click [Detect Rack] button.



2.2.2 Setting the LC parameters

[Operation Manual]: "4.2.1 Setting the LC Parameters"

1 Click [Normal] button

2 Select the [Simple Settings] tab.

3 Enter "6" min in [LC Stop Time].

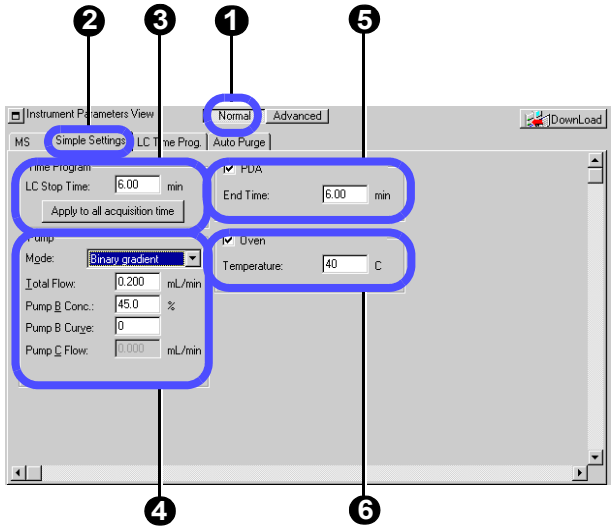
If you click [Apply to all acquisition time] button after entering [LC Stop Time], [End Time] of all the detectors become the same.

4 Enter values for the pump parameters.

Mode	Binary gradient
T.Flow	0.2mL/min
B.Conc	45%

5 Enter "6" min in [End Time] of PDA.

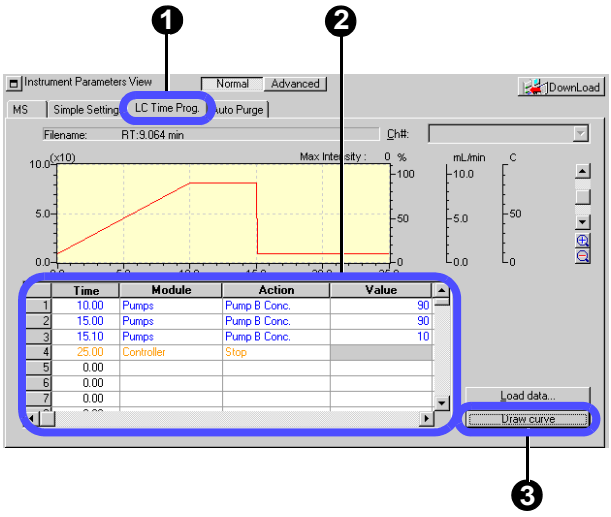
6 Enter "40" °C for the oven temperature.



Be sure to enter a value in [Stop/End Time] (measurement end time) in steps 3 and 5.

Entering the gradient mode conditions

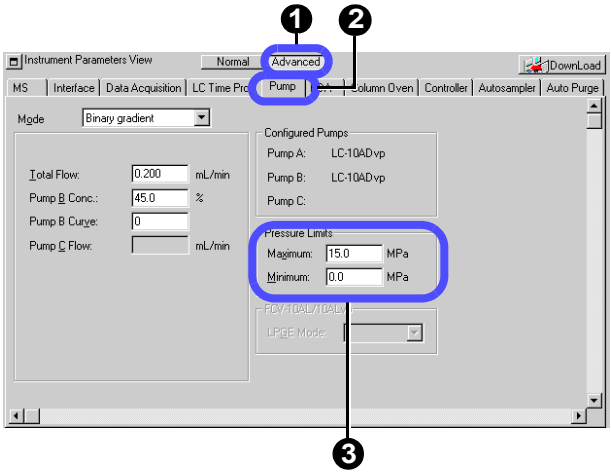
This document describes the procedure for setting up the pumps by assuming that liquid is sent in the gradient mode at a constant mixture ratio of the mobile phase.
To change the gradient mode conditions, perform the following steps:



1. Select the [LC Time Prog.] tab.
2. Enter values in [Time], [Module], [Action], and [Value] for the time program as shown on the right side.
3. Click [Draw curve] button.
   The entered time program will be displayed as a graph.

Setting the pressure limit of a pump

If the column or the like is in an improper state, an error may occur because of exceeding pump's upper pressure limit. In this case, change the upper pressure limit by performing the following steps:



1. Click [Advanced] button.
2. Select [Pump] tab.
3. Enter "15" MPa in [P.Max].

The default value for [P.Max] is 10 MPa.

2.3 Setting the MS parameters

To set the MS (mass spectrometer) parameters, perform the following steps:

[Operation Manual]: "4.2.2 Setting the MS Parameters"

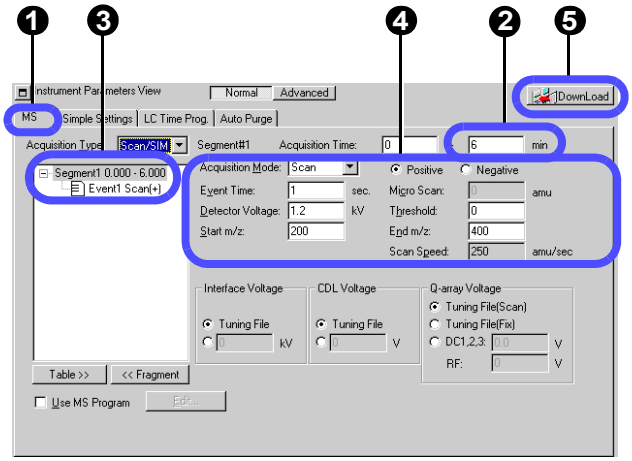
1 Select the [MS] tab.

2 Enter "6" min in [Acquisition Time].

3 Select an event.

4 Set the parameters for the selected event.

Detector voltage	1.2kV
Measurement start m/z	200
Measurement end m/z	400



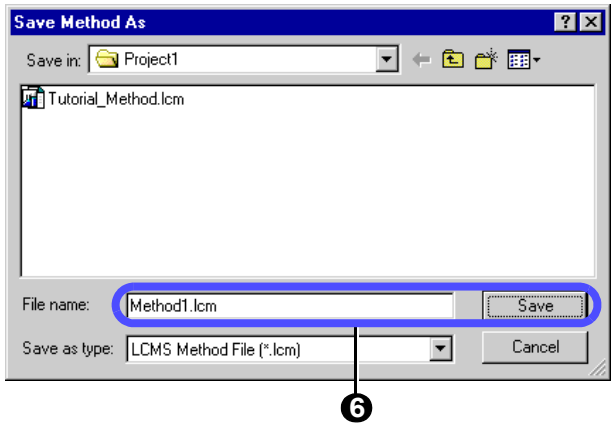
5 Click [DownLoad] button.

The instrument parameters will be transferred to the unit.

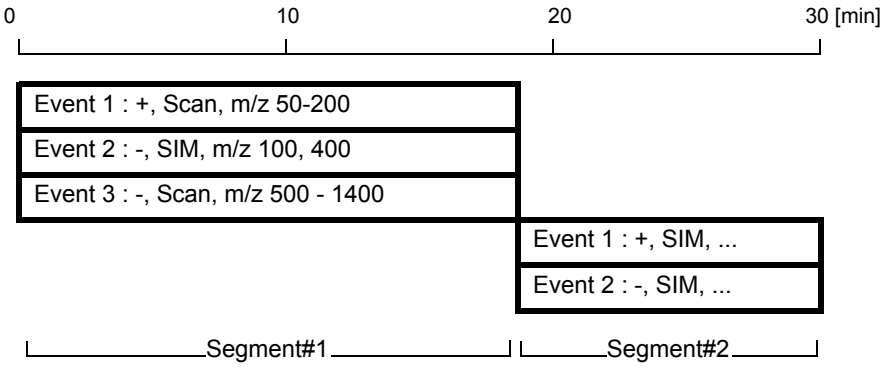
The dialog box will be opened allowing you to save the settings (method).

6 Enter "Method1.lcm" for the file name and click [Save].

The method file will be saved and the set parameters will be transferred to the unit.



Segment and Event



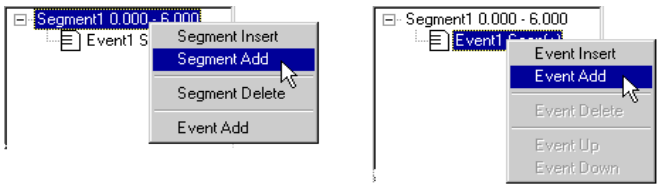
The LCMS-2010A provides the capability to allow you to change the analysis conditions in each specified time range during an analysis. The analysis conditions (a set of analysis conditions) in the specified time range are called a “Segment”. Multiple MS conditions may be specified for each segment and each of those conditions is called an “Event”.
Additions of segments and events allow you to specify more complicated MS analysis conditions. This document assumes that an analysis is made under a single MS condition.

If multiple events are specified within the same segment, an analysis will be made under the condition specified for the event time and then the next event will occur. When the final event specified in the segment is finished, the first event will be resumed again. Thus, the cycle (Event#1 → Event#2 → Event#3 → Event#1... for Segment#1 in the above example) will be repeated for the time specified for the segment.

After the time specified for the segment has elapsed, similar operations will be performed for the next specified segment.

If the “Polarity” (“Positive” or “Negative”) is changed, 400 msec is required for this change. This means that the time of the event after the polarity has been changed becomes shorter practically by 400 msec. Therefore, increase or decrease the event time as necessary.

To add/delete any segment/event, right-click the appropriate segment/event in the event tree and select the desired option from the pop-up menu displayed.



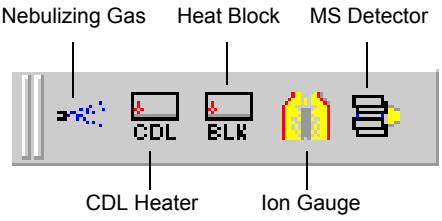
2.4 Starting the operation of the instrument

Before starting an analysis, click the “Instrument Control bar” button at the top of the screen to start the operation of the analyzer. It will take about 20 minutes until the operation becomes stable enough.

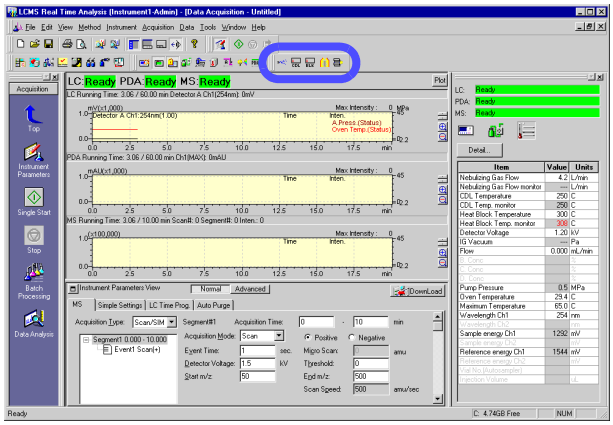
2.4.1 Starting the control of the MS unit

1 Click the following five buttons: [Open/Close Nebulizing Gas], [CDL On/Off], [Heat Block On/Off], [IG On/Off] (= Ion Gauge On\Off), and [MS Detector On/Off].

The MS unit will start operating.



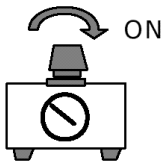
When an analysis is made using the APCI for the interface, the [APCI On/Off] button  is added.



2 For the LCMS-2010A, turn clockwise the knob for the drying gas controller to set the pressure.

For the LCMS-2010A-ESI: 0.1 MPa
For the LCMS-2010A-APCI: 0.02 MPa

Turn the knob clockwise.

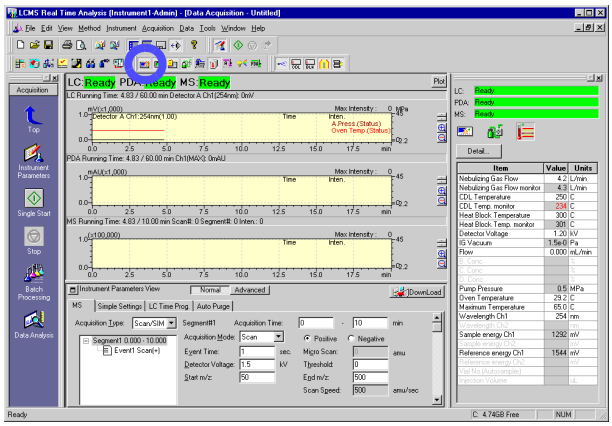


2.4.2 Starting the operation of the LC unit

1 Click [Instrument On/Off] button.

The LC unit will start operating under the conditions specified in the method file.

HPLC Instrument

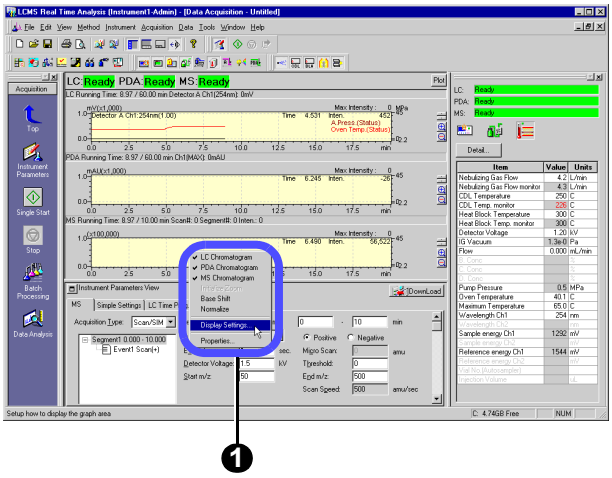


2.4.3 Selecting a graph to be displayed in the <Chromatogram> view

The <Chromatogram> view allows you to specify the types and ranges of axes for the graph to be displayed.

[Operation Manual]: "11.2 Customizing Windows"

1 Right-click anywhere on the graph and select the [Display Settings] menu.



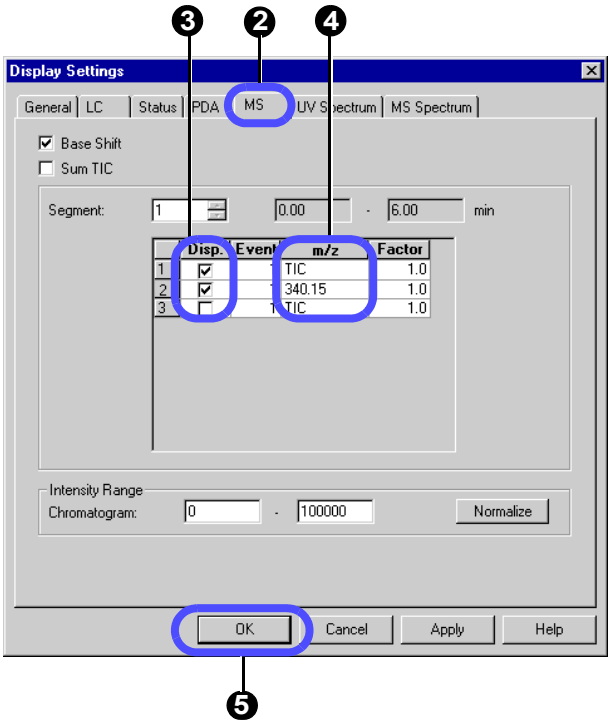
2 Select the [MS] tab.

Enter values for m/z and other parameters for the mass chromatogram to be displayed.

3 Tick the check boxes on the 1st and 2nd rows.

4 Enter 340.15 on the 2nd row of the m/z column.

In this example, the mass chromatogram will be displayed according to TIC and m/z = 340.15.



5 Click [OK] button.

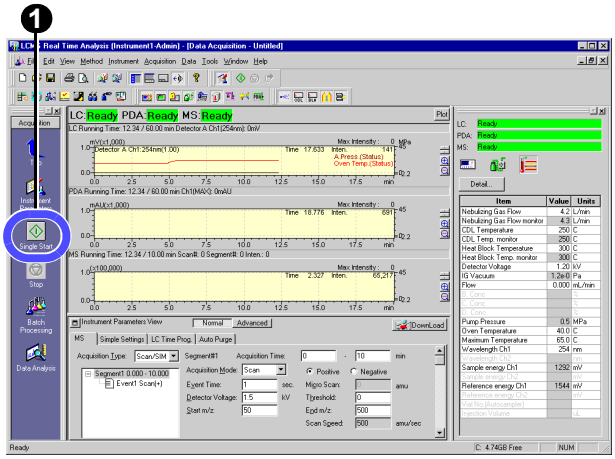
To leave the <Display Settings> window open, click [Apply] button.

2.5 Acquiring data through a single-run analysis

To make a single-run analysis under the conditions specified in "2.2 Setting the LC parameters" and "2.3 Setting the MS parameters", perform the following steps:

1 Click the [Single Start] icon  .

The <Single Run> window will be displayed.

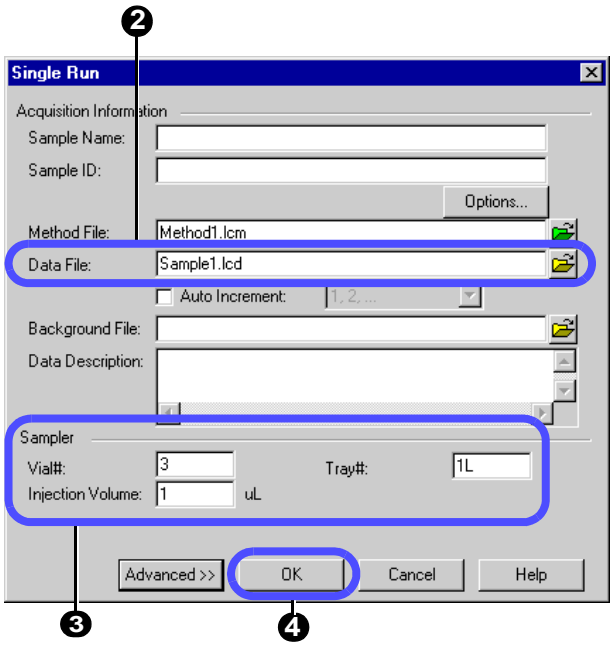


☞ [Operation Manual]: "4.3 Starting a Single-run Analysis"

2 Enter "Sample1.lcd" for the data file name to be created.

3 Enter vial number "3" and injection amount "1".

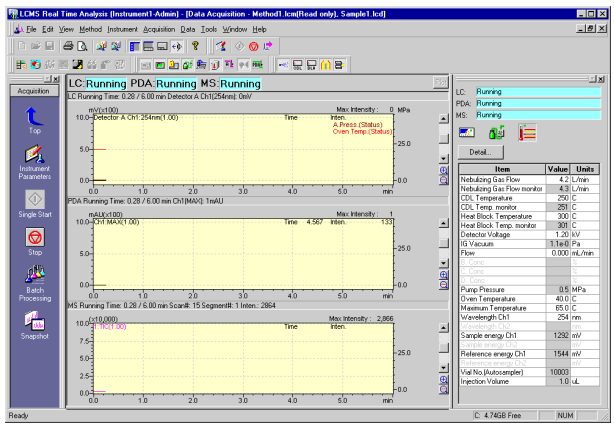
In this example, previously fill 5 ng/µL of papaverine into vial No. 3 of the auto sampler, and inject 1 µL from that vial.



4 Click [OK] buttom.

The single-run analysis will be started.

After the [Acquisition Time] specified in the method file has elapsed, the analysis is finished automatically.

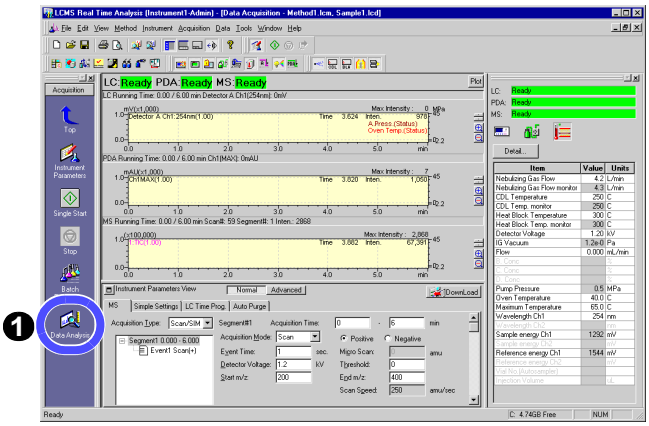


2.6 Performing qualitative processing on <MS Data Analysis>

2.6.1 Starting the <MS Data Analysis>

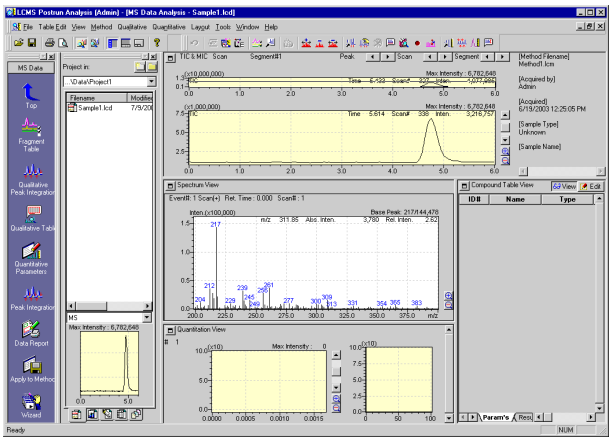
After the single-run analysis has been finished, perform data analysis as follows:

1 Click the [Data Analysis] icon .
<MS Data Analysis> will be started.
The last acquired data will be loaded and then displayed.

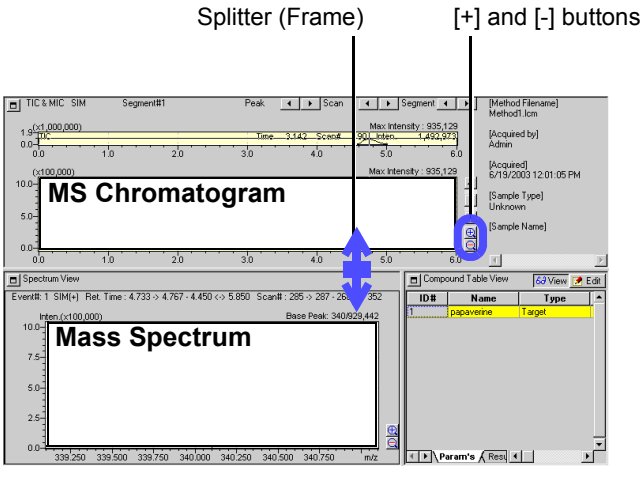


[Operation Manual]: "5.1 Operation in the <MS Data Analysis> Window"

When the data file is first opened, only TIC is displayed in the <Chromatogram> View.



Dragging the cursor on each graph will allow you to enlarge that area.
Right-clicking anywhere on each graph will allow you to select the [Initialize Zoom] or [Undo Zoom] option.



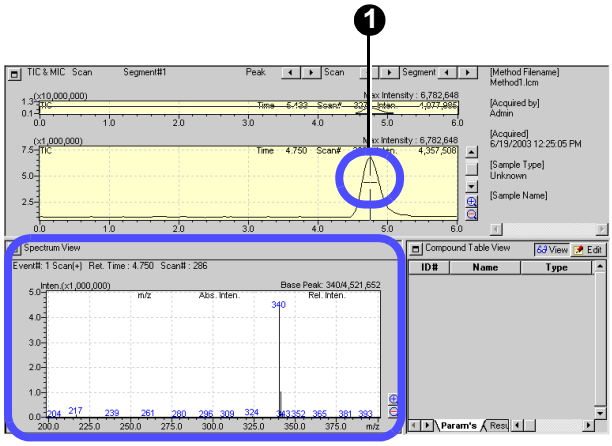
Clicking the [+] or [-] button will allow you to increase or decrease the level of the intensity axis.

Dragging the cursor on the splitter (frame) will allow you to change the aspect ratio of each view.

2.6.2 Displaying a mass spectrum

1 Double-click anywhere on the chromatogram.

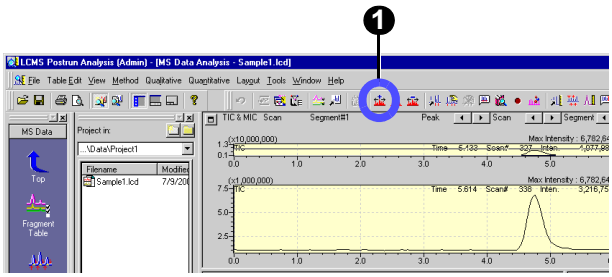
The cut-out cursor will be moved to that time.
The mass spectrum for the cut-out cursor position in the <Chromatogram> View will be displayed in the <Spectrum> View.



Averaging the mass spectrum

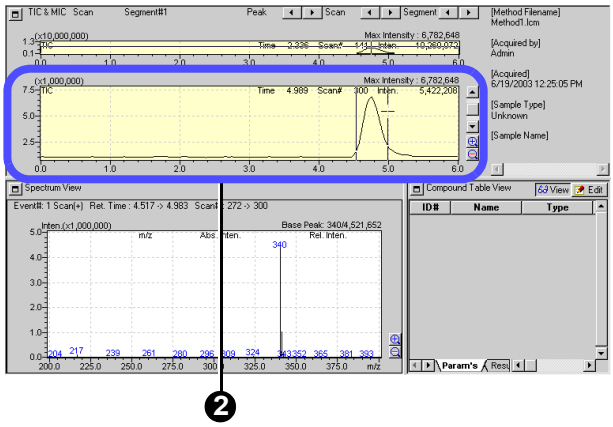
Averaging the mass spectrum will allow you to obtain a clearer spectrum.

1 Click the [Average Spectrum] button on the Toolbar.



2 Drag the cursor on the chromatogram to define the area you want to average.

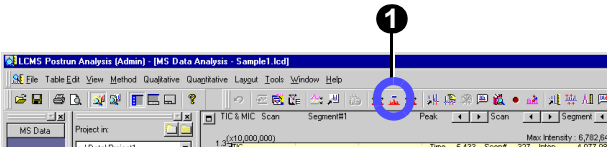
The averaged spectrum in the defined time range (between 4.517 and 4.983 min in this example) will be displayed.



Performing subtractive processing of a mass spectrum

If the background mass spectrum is subtracted from the averaged spectrum, an even clearer spectrum can be obtained.

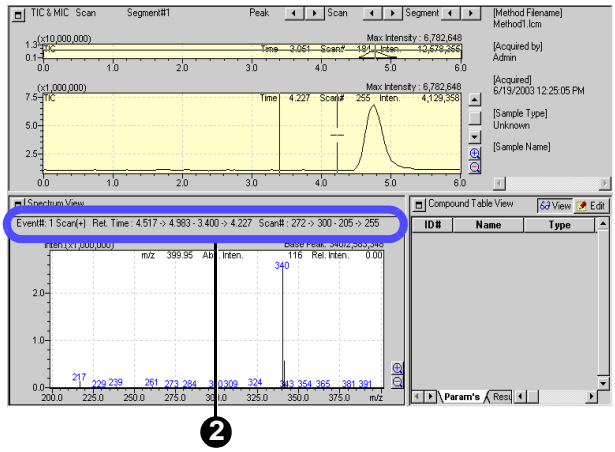
1 With the averaged spectrum displayed, click the [Average & Subtract Spectrum] button on the Toolbar.



2 Drag the cursor on the chromatogram to define the area you want to subtract.

The spectrum obtained by subtracting the background will be displayed.

The information displayed above the spectrum graph indicates that the averaged spectrum for retention time between 3.400 and 4.227 min has been subtracted from that for retention time between 4.517 and 4.983 min.

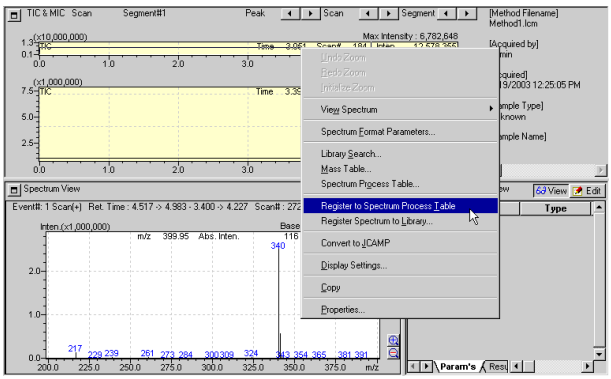


Registering the averaged/subtracted spectrum in the “Spectrum Process Table”

If you register the averaged/subtracted spectrum in the spectrum processing table, you will be able to reproduce that spectrum easily on a later day.

1 Right-click anywhere on the spectrum graph and select [Register to Spectrum Process Table].

The averaged/subtracted mass spectrum will be registered.

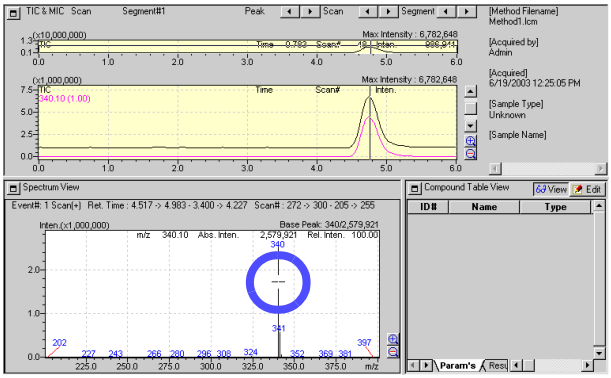


Alternatively, it can also be registered by clicking the button on the Toolbar.

2.6.3 Displaying a mass chromatogram

1 Double-click a mass spectrum peak.

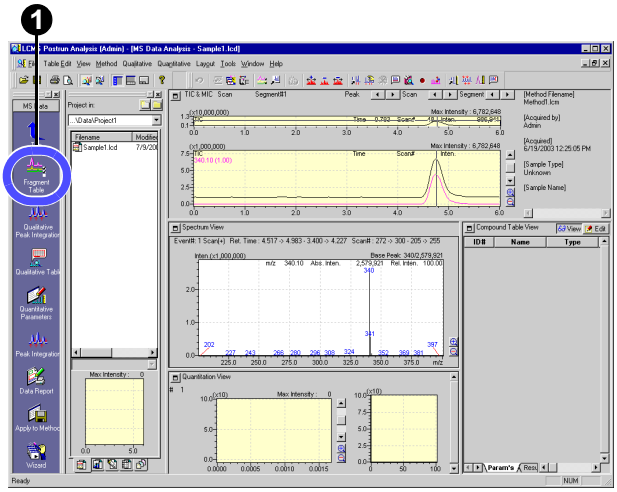
A mass chromatogram will be additionally displayed in the <Chromatogram> View.
The settings for the mass chromatogram are registered in the <Fragment Table> window.



Opening the <Fragment Table> window

1 Click the [Fragment Table] icon  .

The <Fragment Table> window will be displayed.

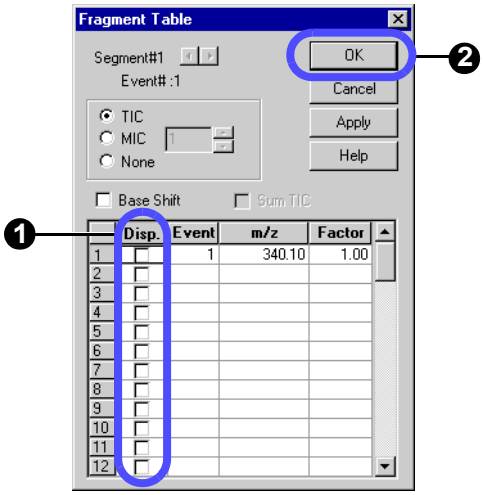


Deleting the erroneously registered chromatogram

1 Remove a tick mark from the check box in the [Disp.] column on <Fragment Table> window.

2 Click [OK] button.

The window will be closed and the chromatogram will be hidden.

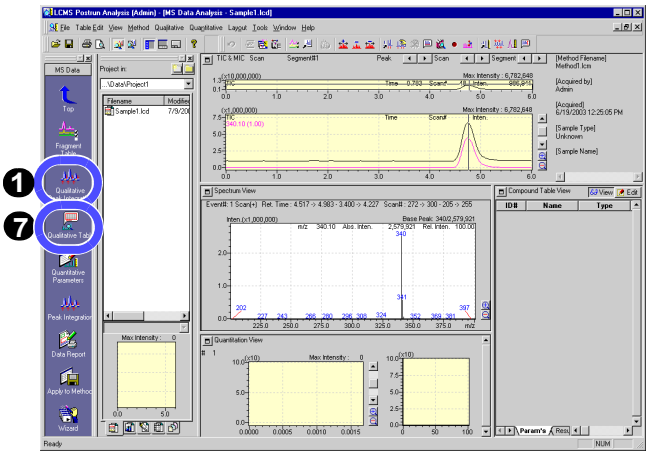


2.7 Performing peak integration (peak detection)

In this example, change the integration conditions in a single-run analysis and then perform peak integration again as follows:

1 Click the [Qualitative Peak Integration] icon  .

The <Qualitative Peak Integration> window will be displayed.



2 Select the [Integration] tab.

3 Select “Detail” for the integration method.

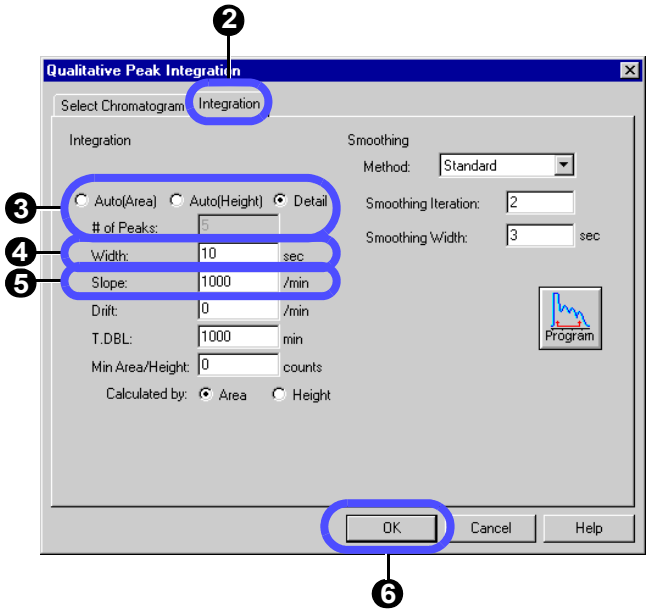
If you select Auto (Area) or Auto (Height), peaks in the number close to the entered maximum number of peaks will be detected.

4 Enter “10” sec in Width.

If you specify the minimum width of peaks to be detected, the noise peak will be eliminated.
Peaks will be detected to the extent that the half-width value is one forth the Width value.

5 Enter “1000” /min for the Slope value.

This is the parameter that determines the start and end points of the peak.
When the absolute value of the gradient of the chromatogram becomes this value, the start and end points of the peak are determined there.

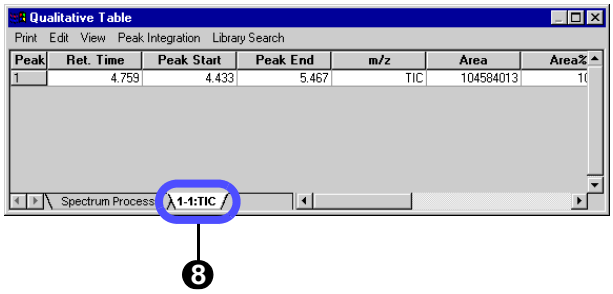


6 Click [OK] button.

The postrun will be carried out using the qualitative integration parameters you have set.

7 Click the [Qualitative Table] icon  .

The <Qualitative Table> window will be displayed.



8 Select the [TIC] tab.

The integration result will be displayed.

The [Spectrum Process] tab allows you to check the registered averaged spectrum.

Simple procedure for setting the integration parameters

Temporarily enter a little smaller values for Width and Slope and then double them, and see how peaks are detected*. In the example given in this document, first enter Width 10 and Slope 1000 and then Width 20 and Slope 2000.

* If the Width value is excessively increased, no minute noises will be detected as peaks.
If the Slope value is excessively increased, no moderate changes in the baseline will be detected as peaks.

Repeat the above steps and when the unnecessary peaks become undetectable, adopt the integration parameter at that point.

Checking data with <Data Explorer>

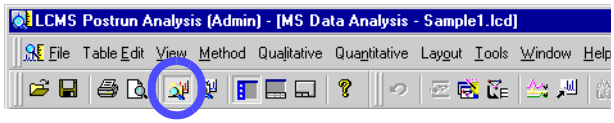
The LCMSsolution manages the data files, method files, batch files, and other related files in "Project Folders". <Data Explorer> allows you to manage the project of the LCMSsolution more effectively.

Project folders may be freely created, copied, or handled with <Data Explorer> of the LCMSsolution and the standard Explorer of Windows.

[Operation Manual]: "13.2 Managing Files Effectively"
[Admin Manual]: "6.1.1 Customizing Data Explorer Display Data"

1 Click the [Data Explorer] button .

This will toggle between displaying and hiding <Data Explorer>.

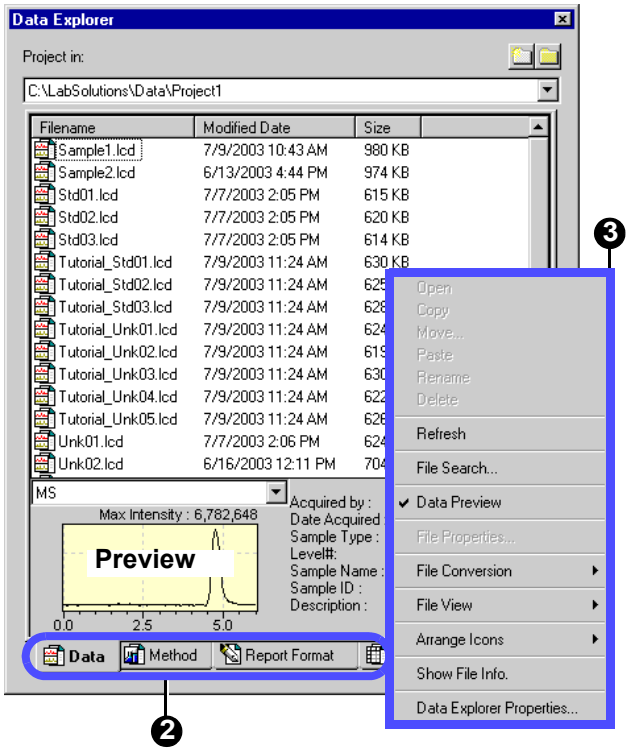


2 Change the display for each file type.

Double-clicking the file or dragging and dropping it to the window will allow you to load the file.

3 Right-click anywhere on the file icon.

A popup menu will appear.



Data Preview
The highlighted data file can be previewed.
Part of the sample information can also be checked.

Show File Info.
When "Detail" for [File View] is selected, the sample name and other additional information will also be displayed as the file information.

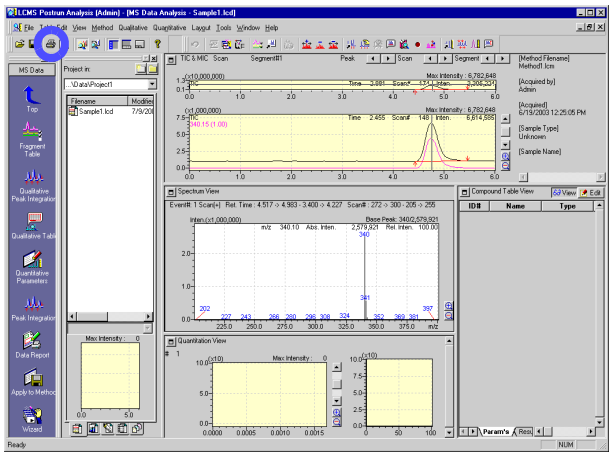
2.8 Printing out the analysis result

To print out the result of qualitative processing, perform the following steps.

2.8.1 Printing out a “Graph Image”

Print out the chromatogram and MS spectrum displayed on the screen as follows:

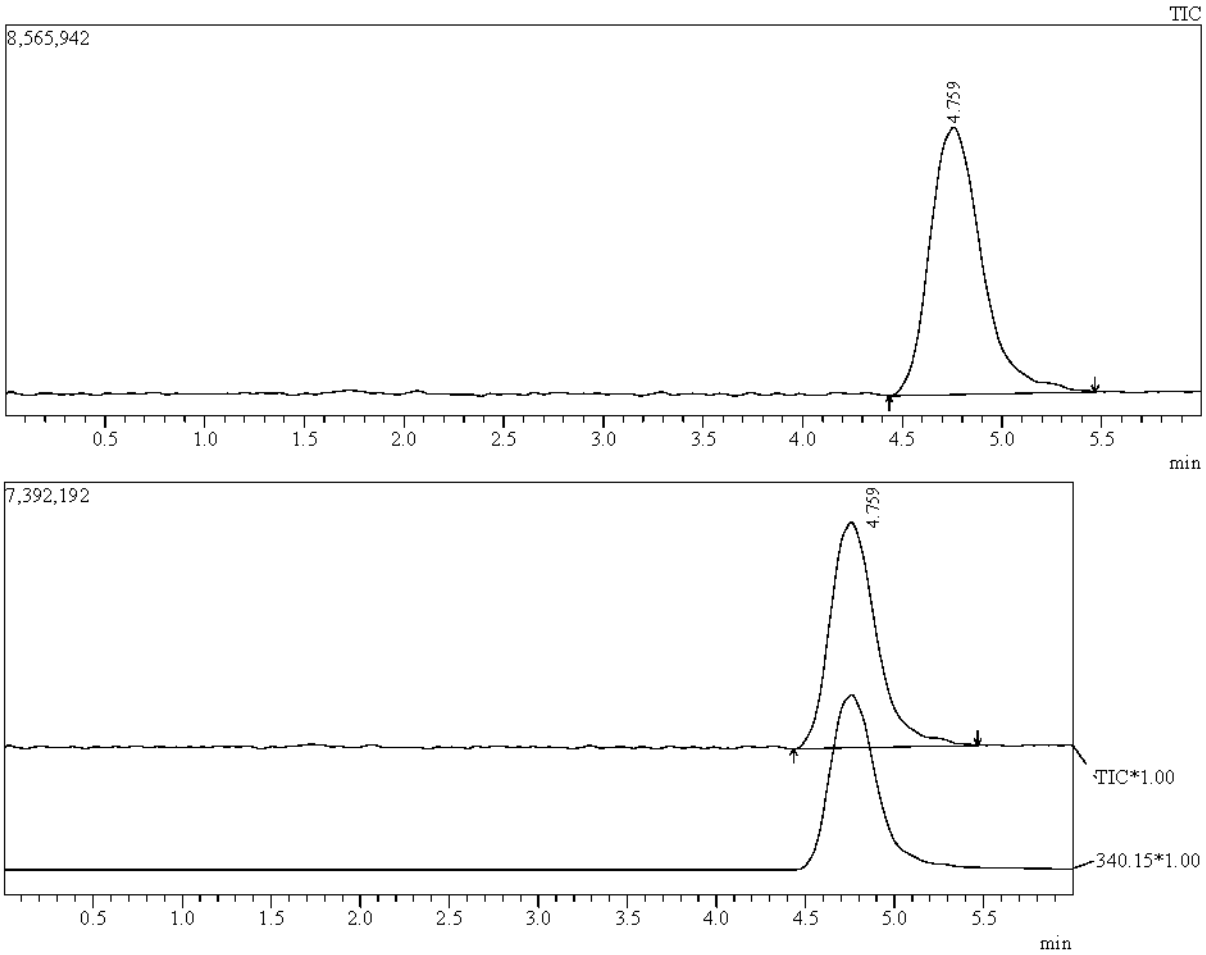
1 Click the [Print] button .
[Print Image] will be carried out.



Example of printing out a graph image

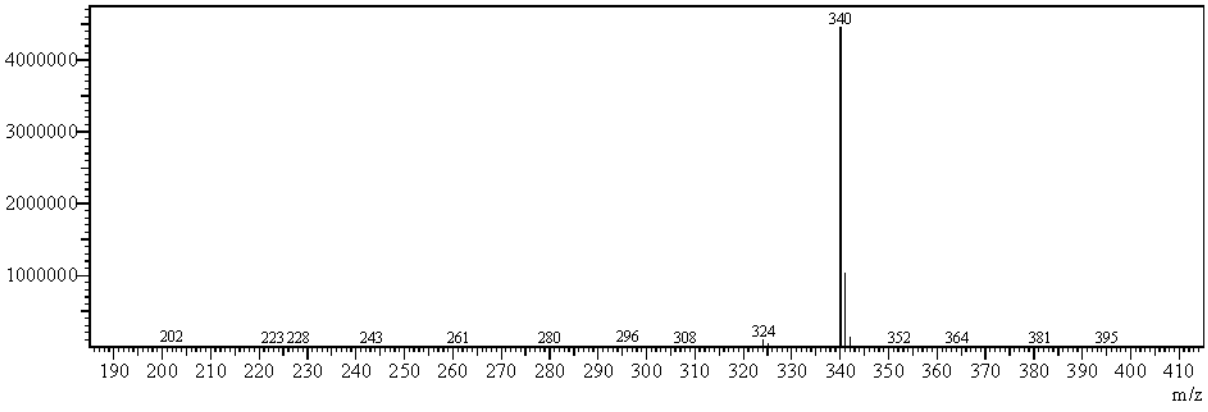
==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>



<Spectrum>

Retention Time:4.767(Scan#:287)
Max Peak:106 Base Peak:340.10(4457918)
Spectrum:Averaged 4.750-4.783(286-288)
Background:Calc Polarity:Pos Segment1 - Event1

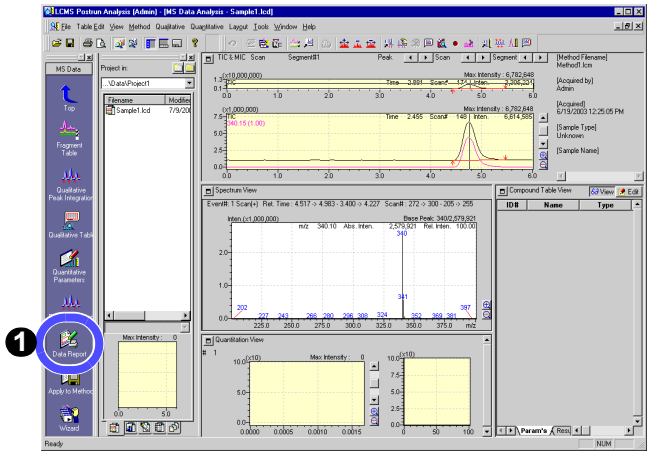


2.8.2 Selecting a layout for printing

<Data Report> allows you to print out a report image in the report format edited in the layout edit pane.
In this example, load the preinstalled report format file “Sample1.lcr” to print out a graph image.

[Operation Manual]: “10.2 Reprinting Data Processing Results”

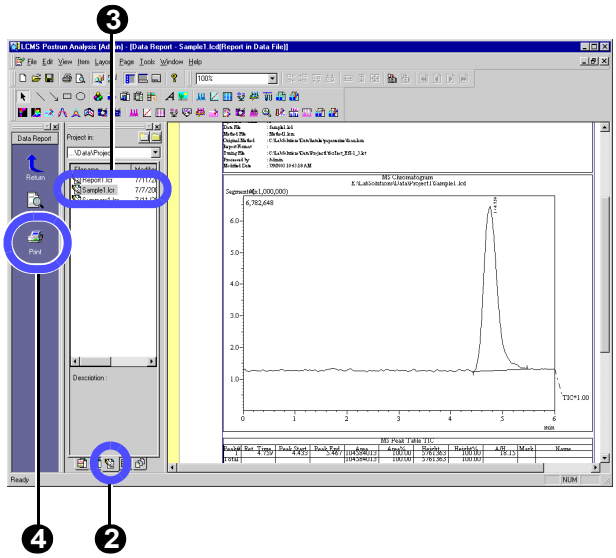
1 Click the [Data Report] icon  .
The data report will be displayed.



2 Select the [Report Format] tab with <Data Explorer>.

3 Drag and drop the file icon to the layout edit pane located on the right side.
The “Sample1.lcr” report format will be displayed.

4 Click the [Print] icon  .
The report in the layout edit pane will be printed out.

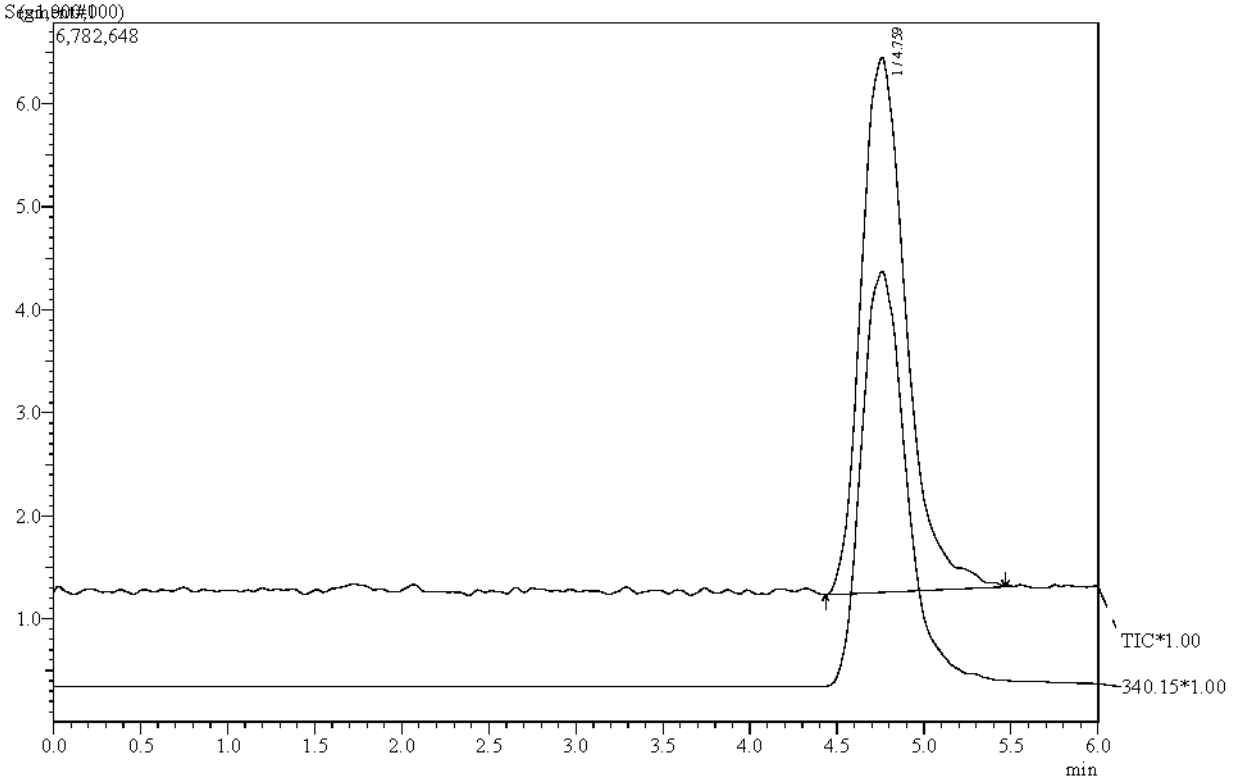


Example of using the report format file for printing

Sample Information

Acquired by	: Admin
Date Acquired	: 6/19/2003 12:25:05 PM
Sample Type	: Unknown
Level#	: 0
Sample Name	:
Sample ID	:
ISTD Amount	: [1]=1 [2]=1 [3]=1 [4]=1 [5]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial#	: 2
Injection Volume	: 1
Data File	: Sample1.lcd
Method File	: Method1.lcm
Original Method	: C:\LabSolutions\Data\Project1\Method1.lcm
Report Format	:
Tuning File	: C:\LabSolutions\Data\Project1\ESI-1_5.lct
Processed by	: Admin
Modified Date	: 7/9/2003 10:43:18 AM

MS Chromatogram
C:\LabSolutions\Data\Project1\Sample1.lcd



MS Peak Table TIC

Peak#	Ret. Time	Peak Start	Peak End	Area	Area%	Height	Height%	A/H	Mark	Name	ID#	vent
1	4.759	4.433	5.467	104584013	100.00	5761363	100.00	18.15		Papaverine	1	1-1
Total				104584013	100.00	5761363	100.00					

3 Quantitative Processing (Batch Analysis)

3.1 Creating a “Compound Table”

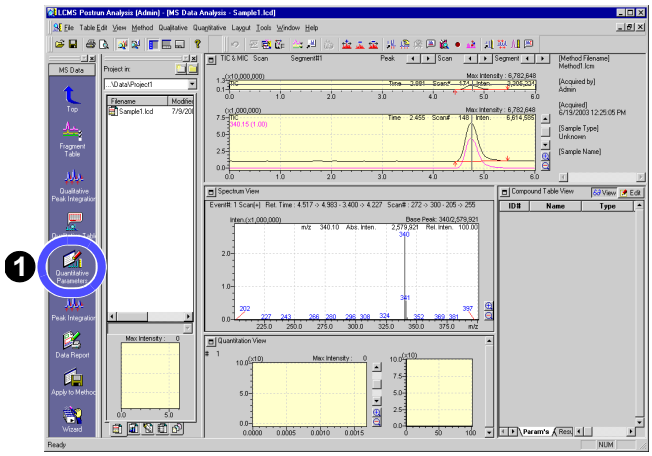
In the quantitative processing, the concentration of the compound contained in an “Unknown Sample” is calculated by creating a “Calibration Curve” with a “Standard Sample” of a known concentration, which contains the same compound as that being quantitatively analyzed.
In this example, inject 1 µL of a standard sample containing 0.5, 1, and 5 ng/µL of papaverine to create a calibration curve. Simulate the quantitative processing to analyze 0.75 ng/µL of papaverine as an unknown sample.

[Operation Manual]: “5.5.2 Editing a “Compound Table””, “5.5.4 Using <Compound Table Wizard>”

3.1.1 Setting the quantitative parameters in <MS Data Analysis>

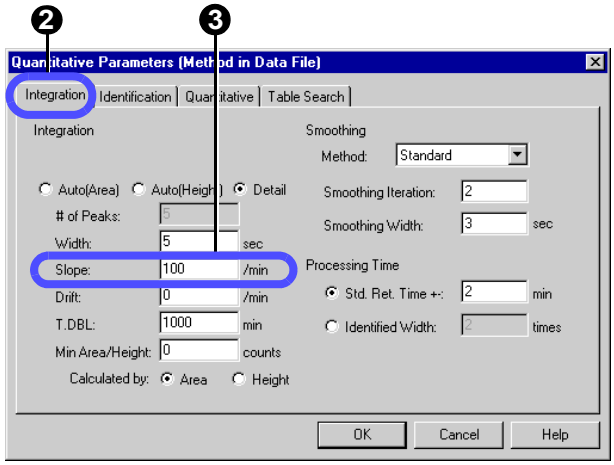
Set the quantitative parameters in the following steps using the papaverine data (Sample1.lcd) that has been loaded to <MS Data Analysis> in the previous chapter.

1 Click the [Quantitative Parameters] icon .



2 Select the [Integration] tab.

3 Enter “100” /min for Slope.
In principle, enter a value equivalent to 1/2000 the targeted peak height.
If no peak is detected, reduce the Slope value by half.

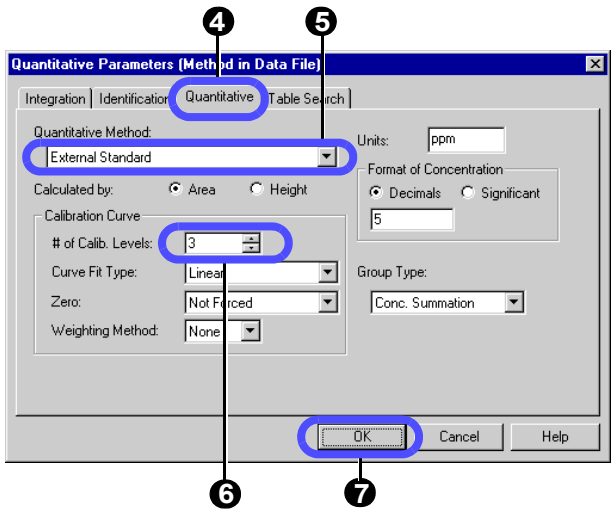


4 Select the [Quantitative] tab.

5 Select “External Standard” for [Quantitative Method].

6 Enter “3” for [# of Calib. Levels].

7 Click [OK] button.



3.1.2 Creating a “Compound Table”

To complete the quantitative settings for each compound, set “Compound Table” to [Edit Mode].

1 Click [Edit] button  in the <Compound Table> View.

2 Enter values in the “Compound Table”.

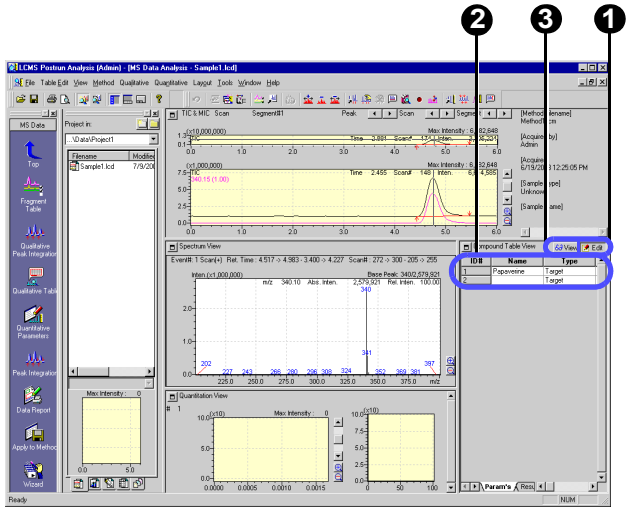
Name	Type	m/z	Ret. Time	Conc. 1	Conc. 2	Conc. 3
Papaverine	Target	340.15	4.800	0.5	1	5

If you click a peak in the <Chromatogram> View with the [Ret. Time] cell highlighted, the retention time for that chromatogram peak will be entered automatically.

If you click a peak in the <Spectrum> view with the [m/z] cell highlighted, the m/z value for that spectrum peak will be entered automatically.

3 Click [View] button  .

The edited settings will be established.

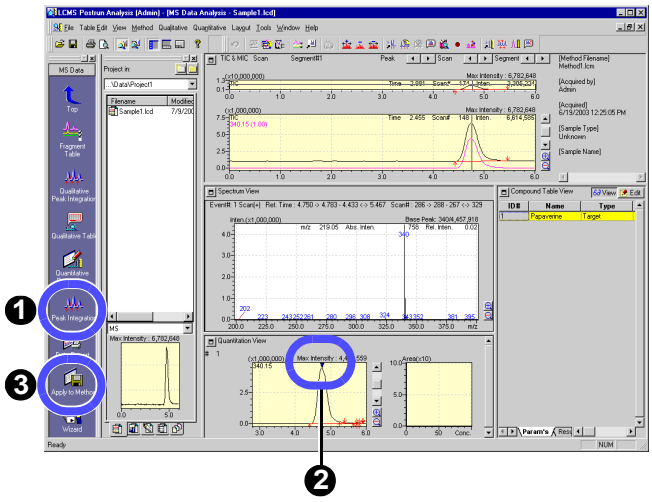


■ Checking and saving the quantitative parameters/compound table

1 Click the [Peak Integration] icon  .

2 Check for the identification mark (▼) on the chromatogram peak.

The identification mark is given to the identified peak.
The peak has the (↑) and (↓) marks at the starting and end points, respectively.
If the peak integration fails, adjust the Slope value among the integration parameters.

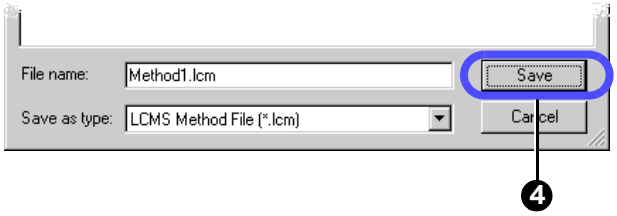


3 Check that the peak has been identified properly, and then click the [Apply to Method] icon  .

The Save dialog box will be opened.

4 Check that “Method1.lcm” is selected for the file name, and then click [Save] button.

The method file will be overwritten.

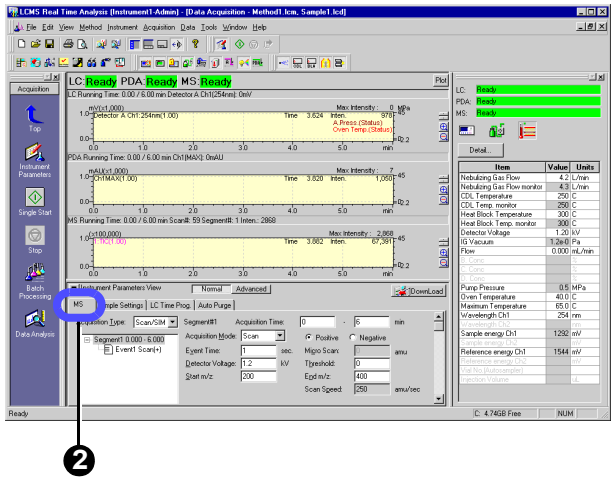


3.2 Creating a SIM Table

The SIM (Selected Ion Monitoring) mode is the analysis mode that selects ion bofore data acquisition, and acquires the selected ions only. Therefore, the sensitivity is higher than the SCAN mode that acquires broader range of m/z values.
In this example, use the mass number specified in "3.1 Creating a "Compound Table"" to change the parameters at data acquisition, so that the quantitative analysis can be made in the SIM mode at higher detection sensitivity.

1 Return to the <LCMS Analysis> - <Data Acquisition> screen.

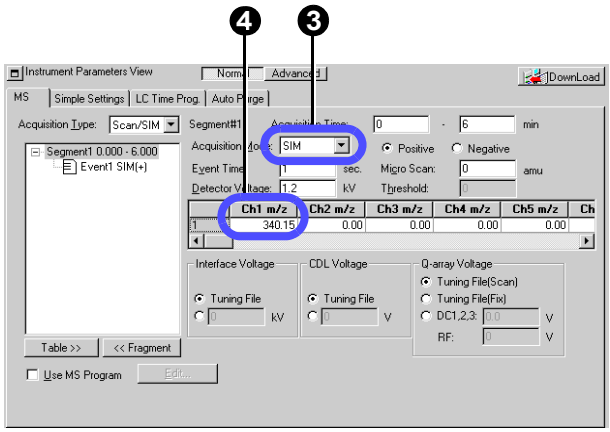
2 Click the [MS] tab of the <Instrument Parameters> View.



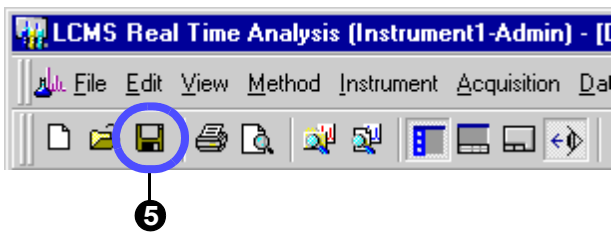
3 Select "SIM" for the analysis mode.

The setting for the measured m/z value will be changed from entering a range to entering an individual m/z value.

4 For Ch1, enter "340.15" for the m/z value for papaverine in the compound table.



5 Click the [Save] button to save the method file.



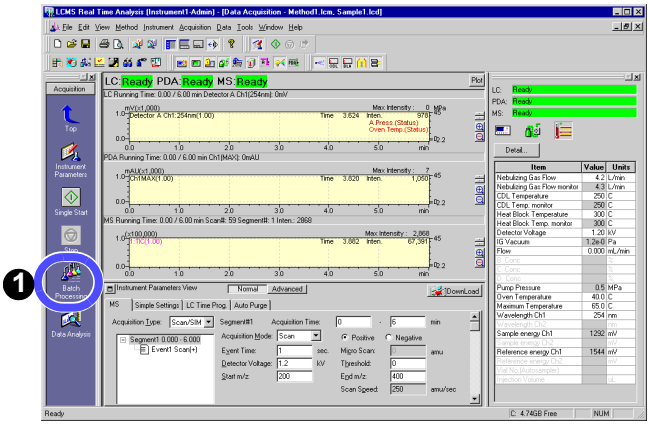
3.3 Creating a “Batch Table”

To make an batch analysis (continuous analysis), use the created method file to set up the batch table.

1 Click the [Batch Processing] icon  .

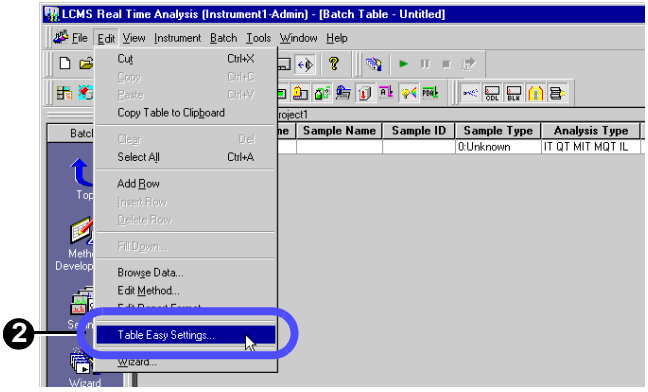
The <Batch Table> window will be displayed.

Create the batch table assigning the 1st to 3rd rows to standard samples and the fourth row to an unknown sample.



2 Choose the [Edit]-[Table Easy Settings] menu.

The <Table Easy Settings> window will be displayed.



3 Select “New” for [Batch Table].

4 Specify “Standard” samples.

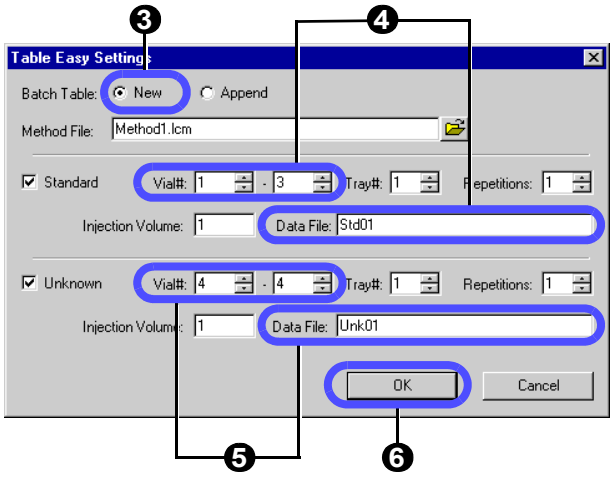
Vial# : “1” - “3”

Data Filename : “Std01”

5 Specify a “Unknown” sample.

Vial# : “4” - “4”

Data Filename : “Unk01”



6 Click [OK] button.

The 4-row batch table will be created.

	Vial#	Inj. Vol	Sample Name	Sample ID	Sample Type	Level#
1	1	1			1:Standard:(I)	1
2	2	1			1:Standard	2
3	3	1			1:Standard	3
4	4	1			0:Unknown	0

7 Tick the check box in the [Report Output] column and enter a file name in the [Report Format File] column.

Set only the fourth row for the unknown sample.

In this example, specify the preinstalled report format file “Report1.lcr”.

Analysis Type	Method File	Data File	Report	Report Format File
IT QT MIT M	Method1.lcm	Std01.lcd	☐	
IT QT MIT M	Method1.lcm	Std02.lcd	☐	
IT QT MIT M	Method1.lcm	Std03.lcd	☐	
IT QT MIT M	Method1.lcm	Unk01.lcd	☑	Report1.lcr

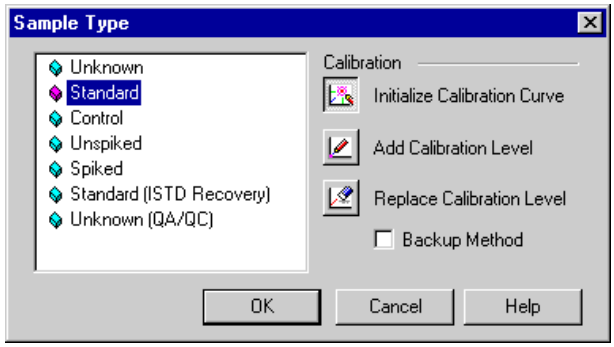
7

If the full path is not specified for any file name, the data will be created in the specified project folder.

The default values are given to the following items of the batch table. No modification is required of those values so far as the operations in this document are concerned.

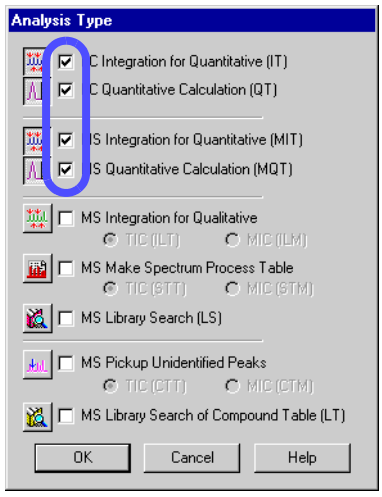
- Sample Type

Clicking this column will display the <Sample Type> window shown on the right side.
Select a sample type from this window.
Select “Standard” for a sample to create/update a calibration curve or “Unknown” for a sample under quantitative analysis.
For the first standard sample to create a calibration curve, enable “Initialize Calibration Curve”.



- Analysis Type

Specify whether analytical processing is performed or not.
Clicking this column will display the <Analysis Type> window shown on the right side.
Tick the desired items.
For example, MIT (= Integration) shows that peak integration will be carried out and MQT (= Quantitative) indicates that quantitative calculation will be performed.



- Level#

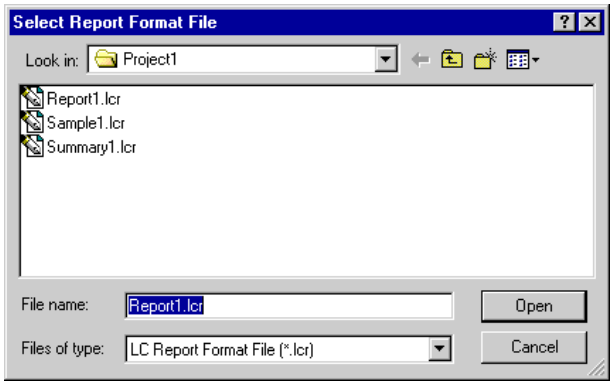
Enter the level of a standard sample.

- Report Output

Ticking the check box will allow you to automatically print out the analysis result report.

- Report Format File

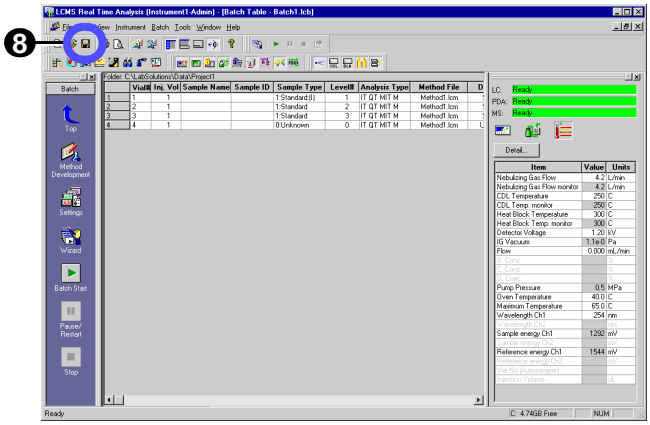
Clicking this column will display the <Select Report Format File> window shown on the right side. The analysis result report will be printed out in the report format specified here.



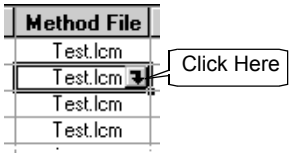
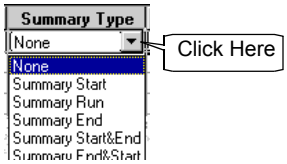
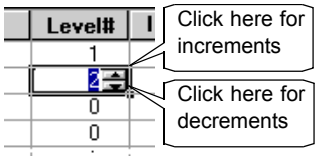
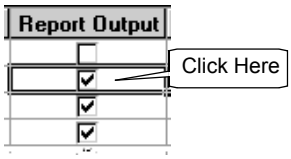
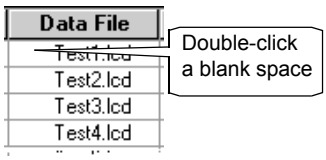
[Operation Manual]: “9.3 Batch Processing Parameters”

8 Click the [Save] button on the Tool-bar.

9 Enter “Batch1.lcb” for the file name.



Entering data in the table cells

Type	Example	Description
Window popup type (for complicated settings)		When you click the button displayed to the right of the cell you have selected, the appropriate window pops up for you to enter data in that cell.
Drop-down list type (for selection from a list)		When you click the button displayed to the right of the cell you have selected, the available options are displayed in a drop-down list. Select the desired option from that list by clicking it.
Spin input type (for input of a specific value)		When you click the upper or lower rectangle mark button displayed to the right of the cell you have selected, the stepped value assigned to that cell is increased or decreased. To enter any value other than the stepped values, directly enter it in the cell.
Check box type (for On/Off input)		Click the check box displayed on the cell to give or remove the tick mark.
Double-click type (for opening the file)		The data file or method file on the selected row of the batch table can be opened from the menu. Alternatively, the same operation can be performed by double-clicking a blank space in the cell.

3.4 Making a batch analysis

Using the batch table created in "3.3 Creating a "Batch Table"" make a batch analysis as follows.

1 Place the sample onto the autosampler.

Vial 1	Solution of 500 ppb papaverine (standard sample)
Vial 2	Solution of 1 ppm papaverine (standard sample)
Vial 3	Solution of 5 ppm papaverine (standard sample)
Vial 4	Unknown sample (to be determined) * In this example, a solution of 0.75 ppm papaverine is used as an unknown sample.

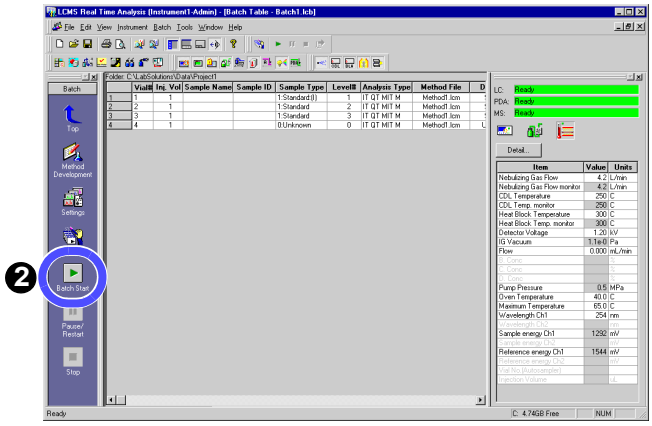
2 Click the [Batch Start] icon  .

During the batch analysis, <Batch Table> and the <Data Acquisition> window are simultaneously displayed in divided screens.

To stop the batch analysis, click the [Stop] icon  .

If the batch analysis is paused, you may change the subsequent batch tables while continuing the analysis of the ongoing measurements.

A snapshot can be performed to check the currently acquired data.
To make a snapshot, click the [Snapshot] icon  on the [Acquisition] Assistant Bar during the analysis.



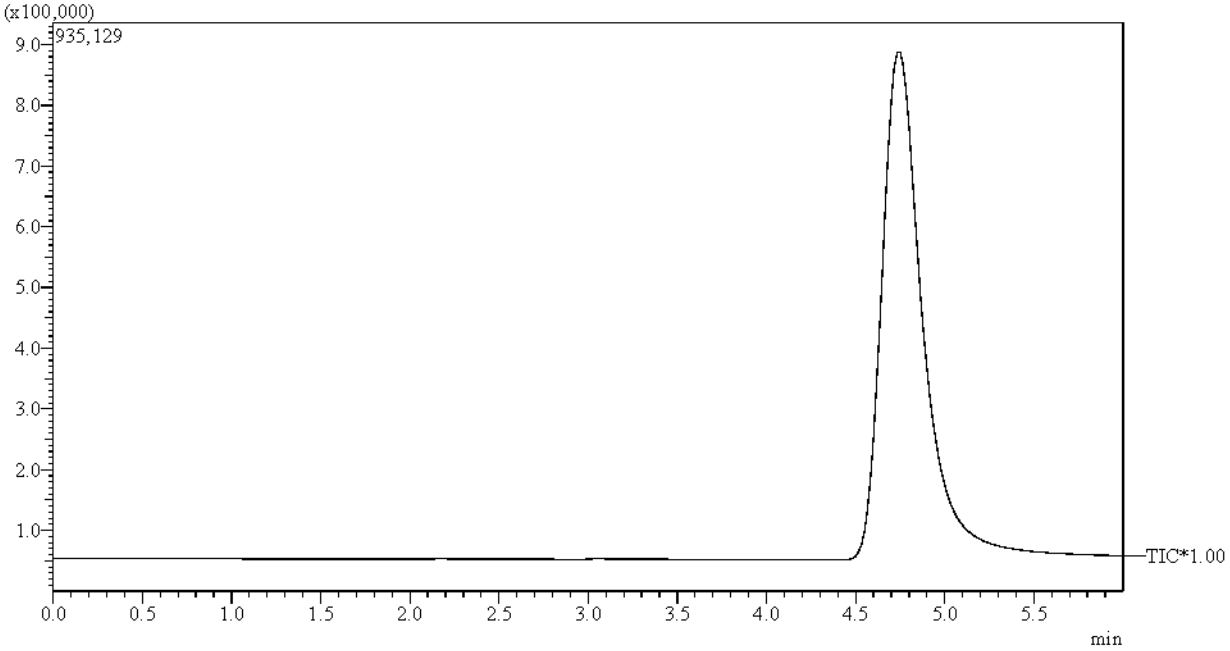
3 After the unknown sample has been analyzed, a report is output.

An example of printing out a report after batch analysis

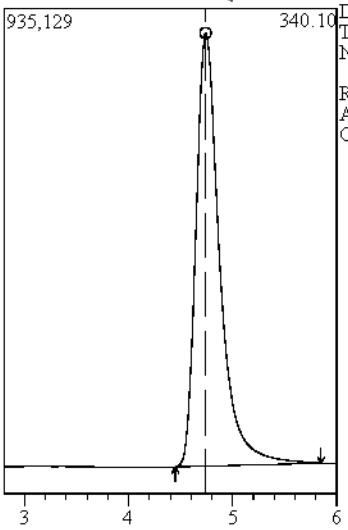
Sample Information

Acquired by	: Admin
Date Acquired	: 6/19/2003 12:01:05 PM
Sample Type	: Unknown
Level#	: 0
Sample Name	:
Sample ID	:
ISTD Amount	: [1]=1 [2]=1 [3]=1 [4]=1 [5]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial#	: 10
Injection Volume	: 1
Data File	: Unk01.lcd
Method File	: Method1.lcm
Original Method	: C:\LabSolutions\Data\Project1\Method1.lcm
Report Format	: Report2.lcr
Tuning File	: C:\LabSolutions\Data\Project1\ESI-1_5.lct
Processed by	: Admin
Modified Date	: 8/8/2003 4:09:20 PM

MS Chromatogram
C:\LabSolutions\Data\Project1\Unk01.lcd



MS Quantitation Graph



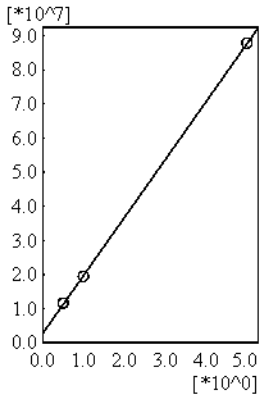
ID#: 1 m/z: 340.10
Type: Target
Name: papaverine

Ret.Time: 4.743
Area: 14816115
Conc.: 0.707ppm

MS Calibration Curve

ID#: 1 m/z: 340.10
Name: papaverine
Function: f(x)=16,977,562.0X + 2,804,692
Rr1=0.9999711 Rr2=0.9999423
Mean RF: 20,058,615.0 RF SD: 2,867,764 RF %RSD: 1·

Curve Fit Type: Linear



#	Conc.(ppm)	Mean Area
1	0.500	11591411.00
2	1.000	19447075.00
3	5.000	87729745.00

Printing out a summary of multiple results from batch analysis

After the batch analysis, print out a “Summary Report” (a simple report of more than one analysis result) as follows.

1 On <Report>, create a format for the summary report containing report items [MS Summary].

[Operation Manual]: “10.3 Creating Report Files”

There are the following two types of summary report items:

- [Concentration]: The results of concentration, area, and height are displayed in a summary.
- [Compound]: The peak information such as concentration and column performance is displayed for each compound.

2 Enter [Summary Type] in <Batch Table>.

Specify “Summary Start” for the top of the data to be output to the summary report, “Summary Run” for the data to be included in the summary report, and “Summary End” for the data on the final line to be included in the summary report.

3 Enter [Summary Report Format File].

Enter a file name to the right of the cell in which you have specified “Summary Start”.

For example, if you complete the following settings, the summary report including the data “Tutorial_Unk01.lcd”, “Turotial_Unk02.lcd”, and “Tutorial_Unk04.lcd” will be printed out in the format “Summary1.lcr” when the batch analysis is finished.

	Analysis Type	Method File	Data File	Summary Type	Summary Report Format
1	IT QT MIT MQT	Tutorial_Method.lcm	Tutorial_Unk01.lcd	Summary Start	Summary1.lcr
2	IT QT MIT MQT	Tutorial_Method.lcm	Tutorial_Unk02.lcd	Summary Run	
3	IT QT MIT MQT	Tutorial_Method.lcm	Tutorial_Unk03.lcd	None	
4	IT QT MIT MQT	Tutorial_Method.lcm	Tutorial_Unk04.lcd	Summary End	

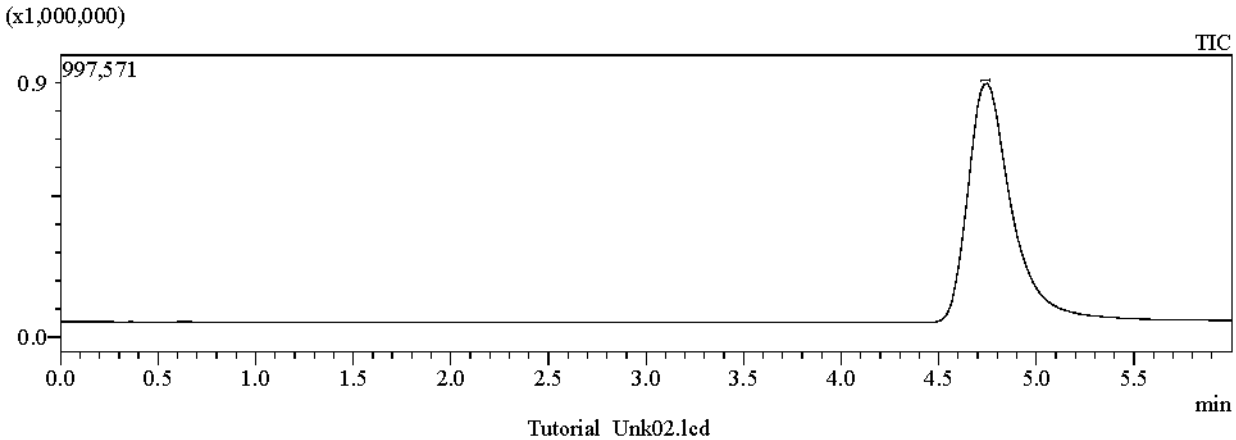
❷ ❸

4 Click the [Batch Start] icon .

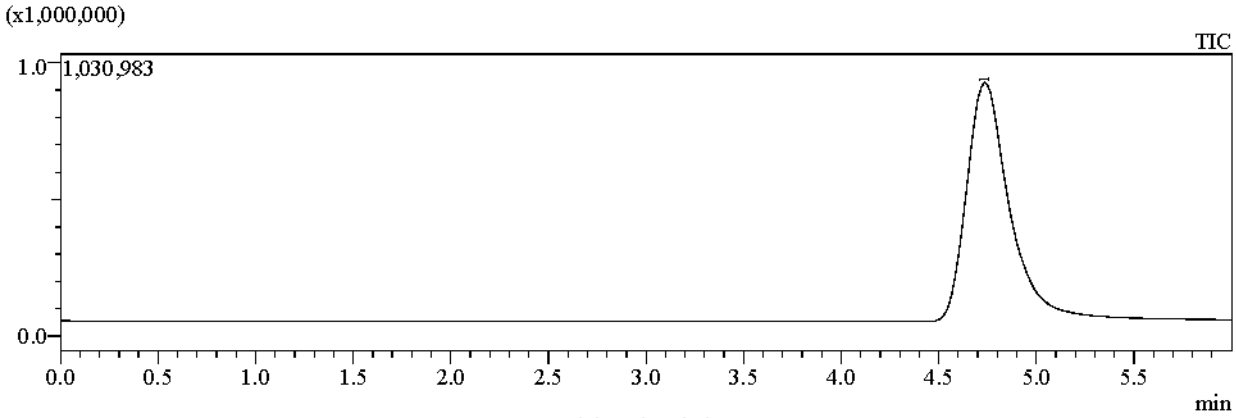
The batch analysis will be made.
After the batch analysis has been finished, the specified summary report file is printed out.

An example of printing out a summary report

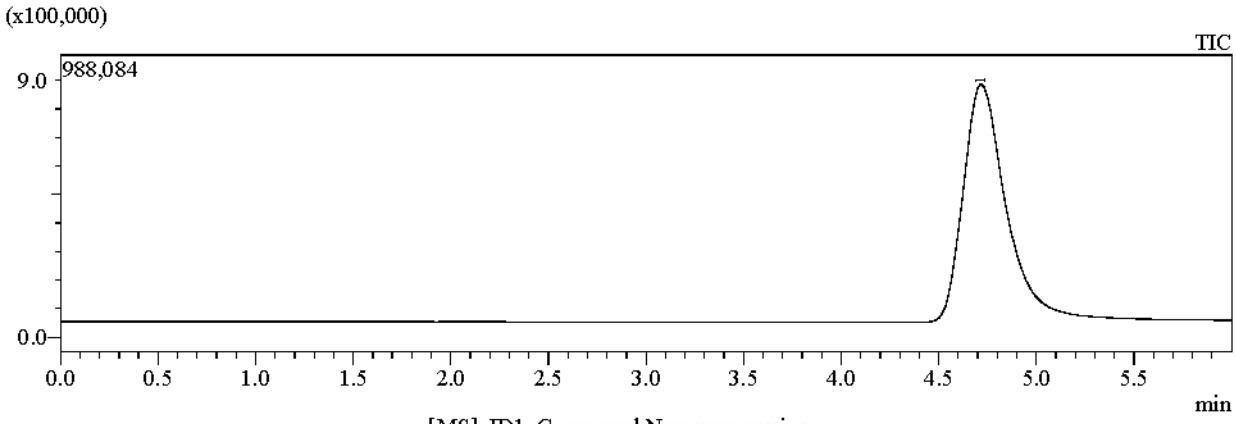
MS Summary(Compound)
Tutorial Unk01.lcd



Tutorial Unk02.lcd



Tutorial Unk04.lcd



[MS] ID1 Compound Name:papaverine

Title	Sample Name	Sample ID	Retention Time	Area	Height	Concentration
Tutorial Unk01.lcd			4.743	11077384	835152	0.746
Tutorial Unk02.lcd			4.737	10886185	846829	0.735
Tutorial Unk04.lcd			4.718	10418105	807638	0.706
Average			4.733	10793891	829873	0.729
%RSD			0.276	3.142	2.425	2.840
Maximum			4.743	11077384	846829	0.746
Minimum			4.718	10418105	807638	0.706
Std. Dev.			0.013	339191	20122	0.021

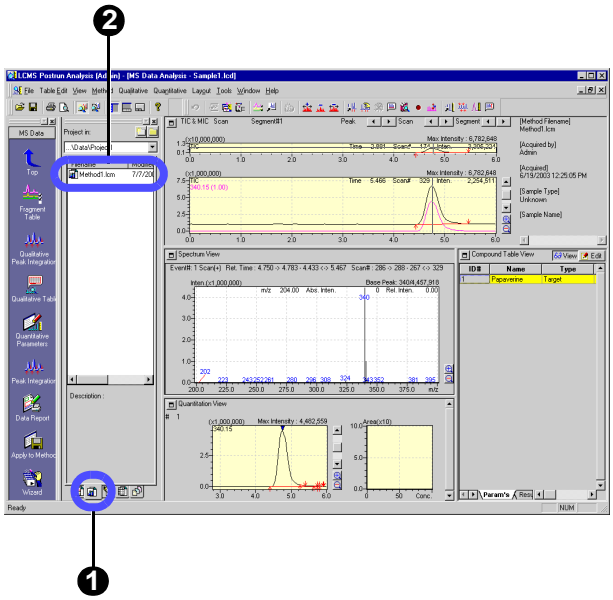
4 Data Analysis

4.1 Checking a “Calibration Curve”

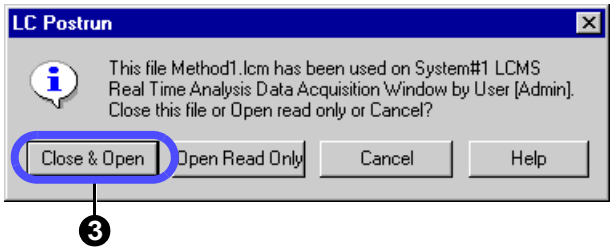
To check and modify the “Calibration Curve” that has been created using the data on the standard sample analyzed in Chapter 3, use <MS Calibration Curve>.

1 Select the [Method] tab of <Data Explorer> displayed in <LCMS Postrun>.

2 Double-click the method file “Method1.lcm”.



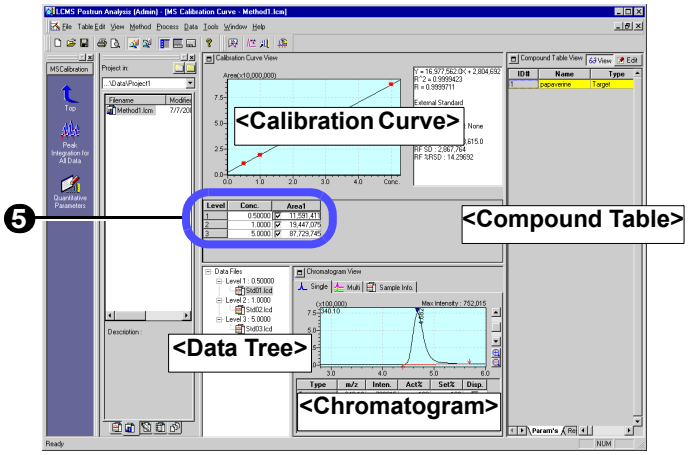
3 Select [Close & Open] button in the selection dialog box.



Since the method file “Method1.lcm” is loaded by <Data Acquisition> in Chapter 3, temporarily close the file.

4 <MS Calibration Curve> will be displayed.

5 Check to see whether all of three area values are registered.



If the area value is 0, no peak integration has been performed.
Adjust the Slope value and then carry out peak integration again.

To change the “Slope” value:
Click the [Quantitative Parameters] icon on the [MSCalibration] Assistant Bar and then change the “Slope” value on the [Integration] tab.

To perform “Peak Integration” again:
Click the [Peak Integration for All Data] icon on the [MSCalibration] Assistant Bar to carry out peak integration.

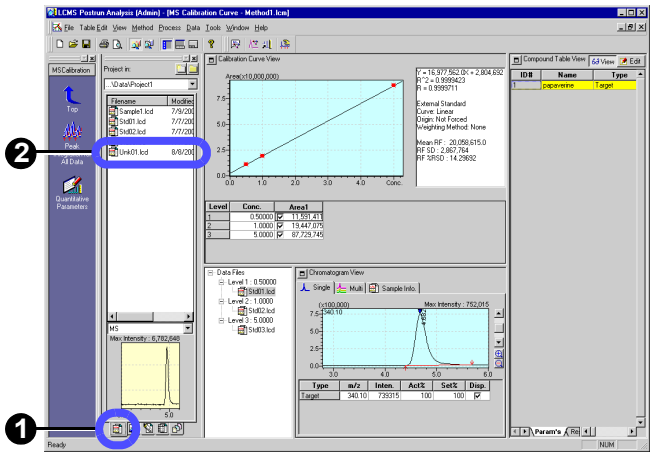
6 Click the [Save] button on the Toolbar.

The modified method file will be saved.

4.2 Checking the quantitative calculation result of an unknown sample

Using the <MS Data Analysis> window, check the data analysis result of the unknown sample analyzed in Chapter 3 as follows.

1 Click the [Data] tab of <Data Explorer> displayed on <LCMS Postrun>.

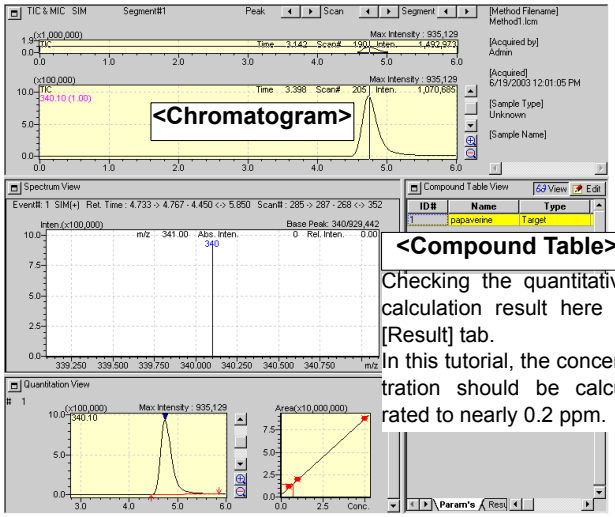


2 Double-click the data file “Unk01.lcd” that has been obtained by analyzing the unknown sample.

<MS Data Analysis> will be displayed with the data file “Unk01.lcd” loaded.

3 If the calibration curve has been changed in “4.1 Checking a “Calibration Curve””, then drag and drop the data file “Method1.lcm” from <Data Explorer> - [Method] tab to <MS Data Analysis>.

The “Calibration Curve Information” will be imported.



Checking the quantitative calculation result here in [Result] tab.
In this tutorial, the concentration should be calculated to nearly 0.2 ppm.

4 Check that the identification mark (▼) is displayed on the chromatogram peak.

If the mark is not displayed, the peak integration has not been completed successfully.
Adjust the Slope value and then carry out the peak integration again.

To change the “Slope” value:
Click the [Quantitative Parameters] icon on the [MS Data] Assistant Bar and then change the “Slope” value on the [Integration] tab.

To perform “Peak Integration” again:
Click the [Peak Integration] icon on the [MSData] Assistant Bar to carry out peak integration.

5 Click the [Save] button on the Toolbar.

The reanalyzed data files will be saved.

4.3 Loading a batch file to the “Quant Browser”

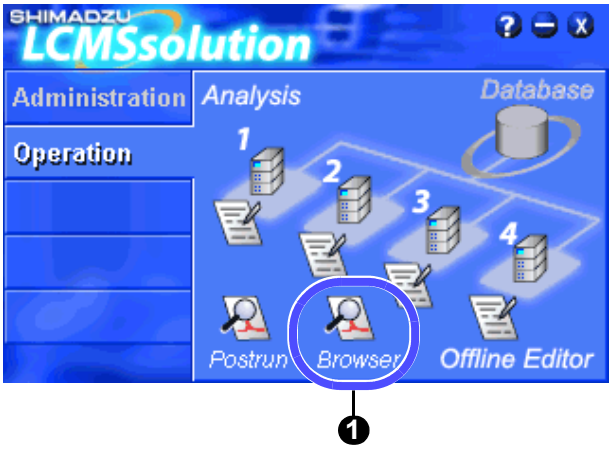
Using of the “Quant Browser” (= Quantitation Browser) allows you to easily re-analyze multiple data.

4.3.1 Displaying the quantitative result from the batch file

1 Click the [LCMS Browser] icon 

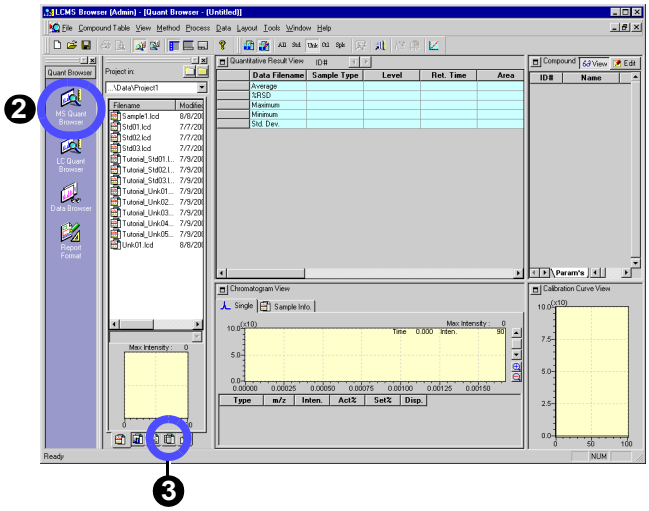
on <LCMSsolution Launcher>.

<LCMS Browser> will be displayed.



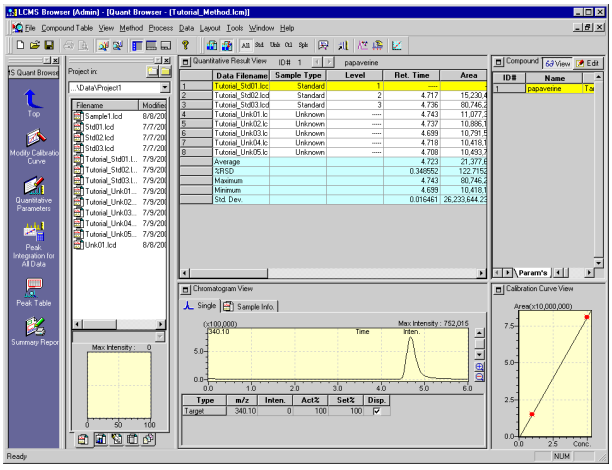
[Operation Manual]: “8.1 Browsing the Quantitative Calc. Results at a Time”

2 Click the [MS Quant Browser] icon 
on the [LCMS Browser] Assistant Bar.



3 Drag and drop the file icon “Tutorial_Batch.lcb” from the [Batch] tab of <Data Explorer> to <Quant Browser>.

All of the sample data (“Tutorial_Std01.lcd” through “Tutorial_Std03.lcd” and “Tutorial_Unk01.lcd” through “Tutorial_Unk05.lcd”) will be loaded.



Alternatively, the data may be loaded by selecting multiple data file from <Data Explorer> and then simultaneously dragging and dropping them.

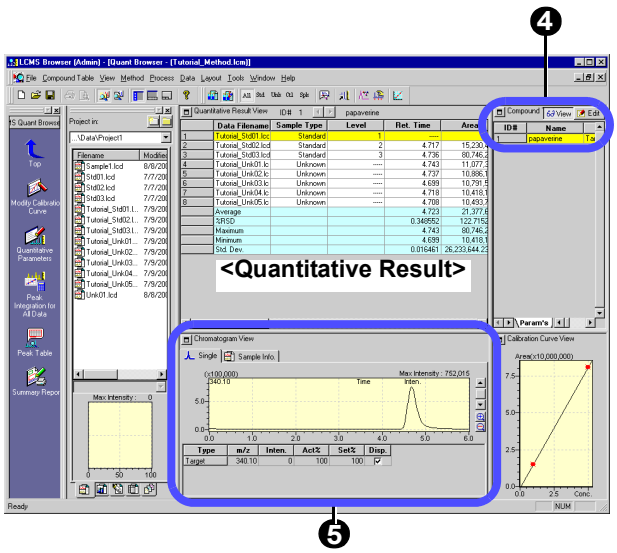
4 Click the compound table.

The quantitative result of the compound on specified row will be displayed.

To delete the data file, right-click the <Quantitative Result> View and then choose [Delete] from the menu displayed.

The calibration curve for the above compound will also be displayed.

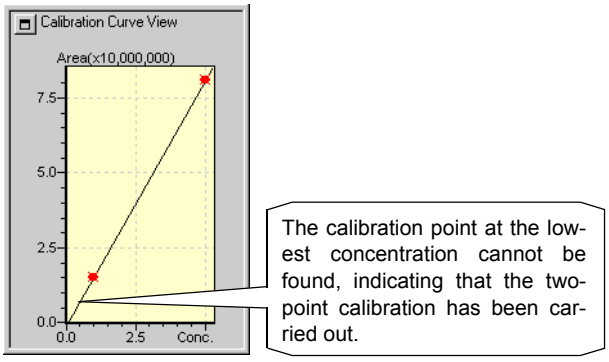
5 Check the chromatogram in the <Chromatogram> View.



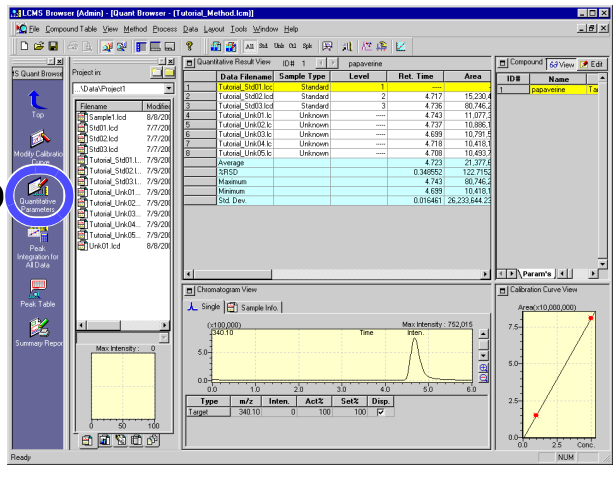
4.3.2 Setting the integration parameters again to retry peak integration

The sample data consists of the quantitative data obtained using three-points absolute calibrations.
However, it shows that the data processing of the standard sample in a low concentration range is failed due to improper integration parameters.

In this example, the data on the highlighted 1st row of the <Quantitative Result> View indicates a failure when the file has been loaded.
If you check the area value, you will find that it is zero.
If you also check the <Chromatogram> View, you will see that the peaks have not been integrated.



1 Click the [Quantitative Parameters] icon  .



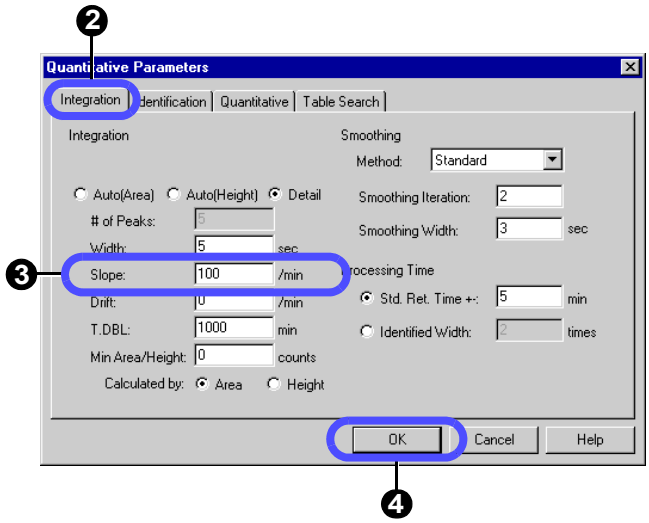
[Operation Manual]: “8.2 Making a Postrun Analysis of Multiple Data”

2 Click the [Integration] tab.

3 Enter “100” /min for the [Slope] value.

If the value is too large, enter a smaller value.

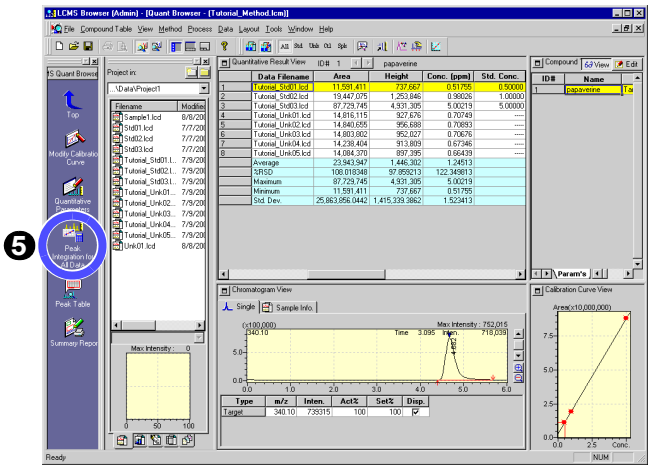
4 Click [OK] button.



5 Click the [Peak Integration for All Data] icon  to retry the peak integration.

The peaks will be detected.

The three-point calibration curve will be displayed.



The proper quantitative value has been obtained.

Quantitative Result View ID# 1 papaverine

	Data Filename	Area	Height	Conc. (ppm)	Std. Conc.
1	Tutorial_Std01.lcd	-----		-----	0.00000
2	Tutorial_Std02.lcd	15,230,436	1,152,1	1.00000	1.00000
3	Tutorial_Std03.lcd	80,746,261	4,833,9	5.00000	5.00000
4	Tutorial_Unk01.lcd	11,077,384	835,1	0.74644	-----
5	Tutorial_Unk02.lcd	10,886,185	846,8	0.73477	-----
6	Tutorial_Unk03.lcd	10,791,516	841,4	0.72899	-----
7	Tutorial_Unk04.lcd	10,418,105	807,6	0.70619	-----
8	Tutorial_Unk05.lcd	10,493,710	799,1	0.71080	-----
	Average	21,377,657	1,445,3	1.37531	
	%RSD	122.715247	103.747,6	116.458396	
	Maximum	80,746,261	4,833,9	5.00000	
	Minimum	10,418,105	799,1	0.70619	
	Std. Dev.	26,233,644.2351	1,499,355.01	1.601667	

Quantitative Result View ID# 1 papaverine

	Data Filename	Area	Height	Conc. (ppm)	Std. Conc.
1	Tutorial_Std01.lcd	11,591,411	737,6	0.51755	0.50000
2	Tutorial_Std02.lcd	19,447,075	1,253,8	0.98026	1.00000
3	Tutorial_Std03.lcd	87,729,745	4,931,3	5.00219	5.00000
4	Tutorial_Unk01.lcd	14,816,115	927,6	0.70749	-----
5	Tutorial_Unk02.lcd	14,840,655	956,6	0.70893	-----
6	Tutorial_Unk03.lcd	14,803,802	952,0	0.70676	-----
7	Tutorial_Unk04.lcd	14,238,404	913,8	0.67346	-----
8	Tutorial_Unk05.lcd	14,084,370	897,3	0.66439	-----
	Average	23,943,947	1,446,3	1.24513	
	%RSD	108.018348	97.8592	122.349813	
	Maximum	87,729,745	4,931,3	5.00219	
	Minimum	11,591,411	737,6	0.51755	
	Std. Dev.	25,863,856.0442	1,415,339.38	1.523413	

Files handled by the Quant Browser

The <Quant Browser> is an application that reanalyzes multiple data using the same method file for data processing.
Files are loaded in accordance with the following rules:

- Method file

Load a method file from the [Method] tab of <Data Explorer>.
If you do not specify a method file, the method file of the first loaded data will be loaded automatically.
If the loaded method file contains calibration curve information, the data file for the standard sample used to create that calibration curve will be loaded.

- Data file

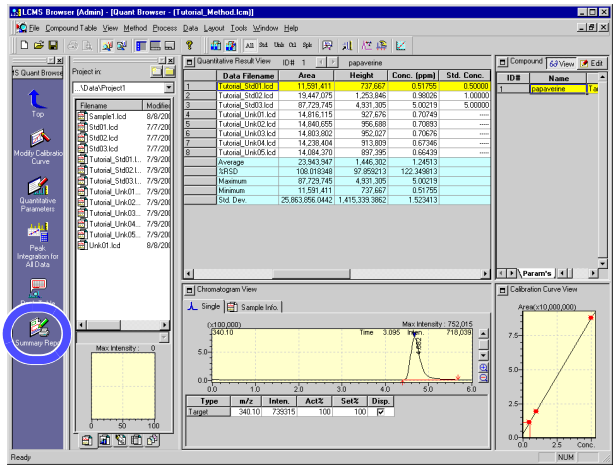
Load a data file or data files from the [Data] tab of <Data Explorer>.
The use of the Toolbar buttons allows you to display the data for each sample type.

4.4 Printing out a summary report from the Quant Browser

<Quant Browser> has the “Summary Report” capability to report all of the loaded data as follows.

1 Click the [Summary Report] icon  .

The image for each compound in the table will be printed out.



An example of printing from the quantitation browser

=== Shimadzu LCMSsolution Quant. Browser Report ===

[MS] ID1 Compound Name:papaverine

Title	Sample Name	Sample ID	Ret.Time	Area	Height	Conc.
Tutorial_Std01.lcd			4.682	11591411	737667	0.518
Tutorial_Std02.lcd			4.717	19447075	1253846	0.980
Tutorial_Std03.lcd			4.736	87729745	4931305	5.002
Tutorial_Unk01.lcd			4.743	14816115	927676	0.707
Tutorial_Unk02.lcd			4.737	14840655	956688	0.709
Tutorial_Unk03.lcd			4.699	14803802	952027	0.707
Tutorial_Unk04.lcd			4.718	14238404	913809	0.673
Tutorial_Unk05.lcd			4.708	14084370	897395	0.664
Average			4.717	23943947	1446302	1.245
%RSD			0.444	108.018	97.859	122.350
Maximum			4.743	87729745	4931305	5.002
Minimum			4.682	11591411	737667	0.518
Std. Dev.			0.021	25863856	1415339	1.523

4.5 Using the Data Browser

Using of the data browser allows you to display multiple data files in various types of formats as follows.

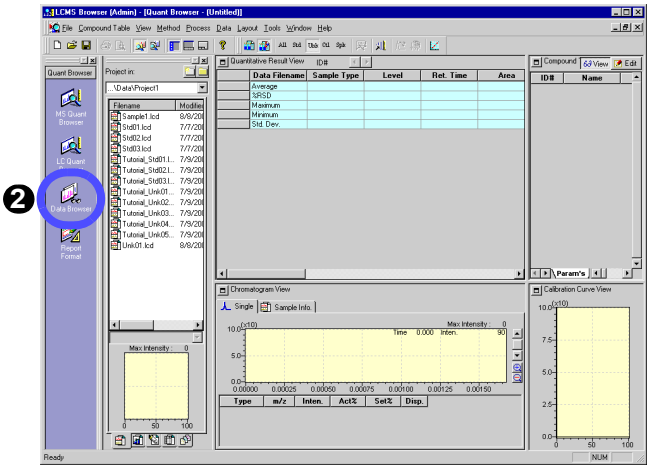
1 Click the [Browser] icon 

from <LCMSsolution Launcher>.

<LCMS Browser> will be started.

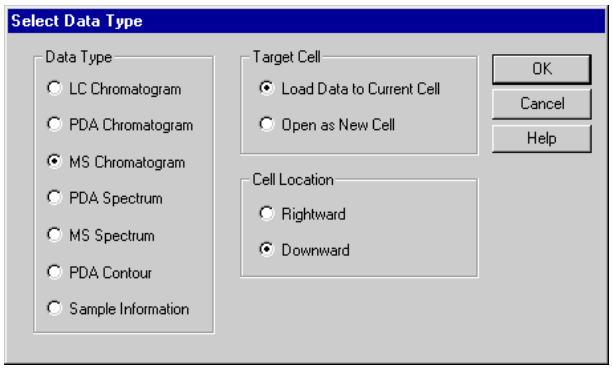
[Operation Manual]: "8.3 Listing Multiple Data"

2 Click the [Data Browser] icon  .



3 Open a data file (multiple data files may be selected) by dragging and dropping it.

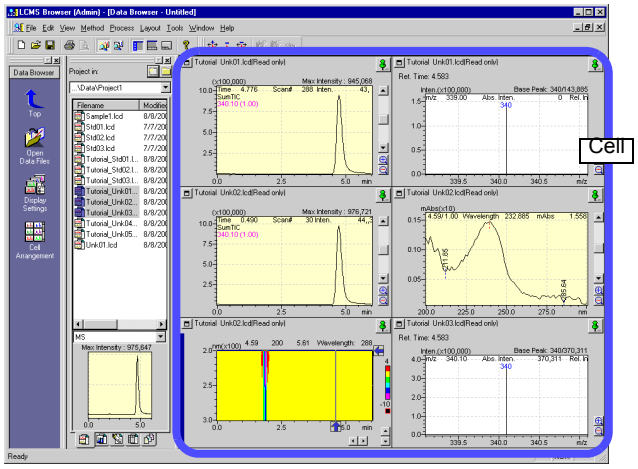
A window will pop up for the user to select the data type displayed, whether to replace data or add a cell, and the direction of adding that cell in the latter case.



A maximum of 64 cells (8 x 8) may be displayed.

If you click the focus pin  located in the upper right corner of each cell so that the pin is displayed as  in multiple cells, then the display of those cells will be changed interlocking with each other.

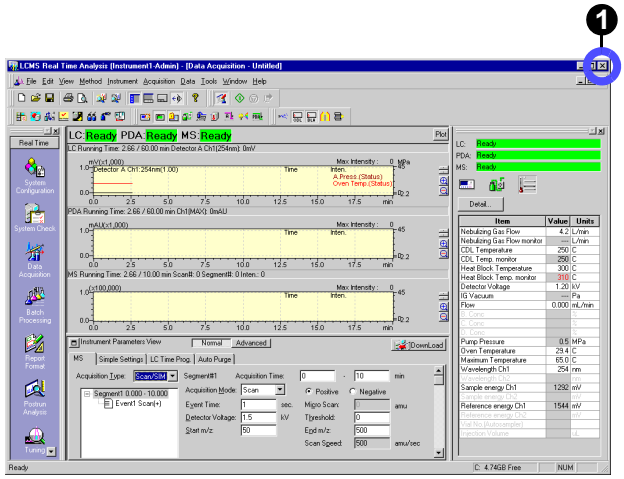
For example, if both cells for an MS chromatogram and a PDA spectrum are “pinned”, then double-clicking the MS chromatogram will display the PDA spectrum for that time.



5 Exiting the LCMSsolution

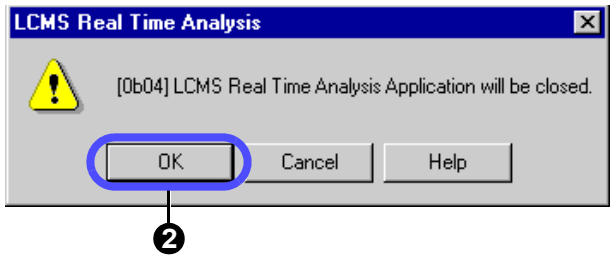
5.1 Existing the LCMSsolution

1 Click ✕ in the upper right corner of the screen.



Alternatively, you may select the [Exit] menu located at the bottom of the [File] menu to exit the LCMSsolution programs.

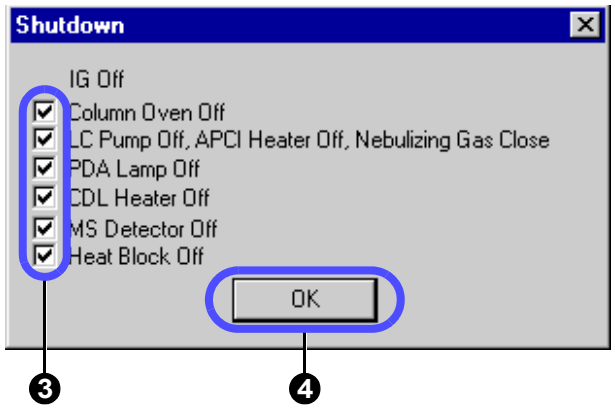
2 When using <LCMS Analysis>, click [OK] button on the confirming dialog box.



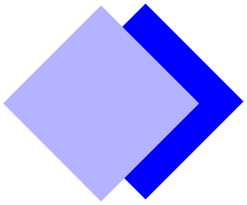
3 For <LCMS Analysis>, the <Shutdown> window is displayed.
Give the tick mark to all the check boxes.

4 Click [OK] button.
The shutdown procedure will be started.

5 Click [No] button in the confirmation dialog box.
For any file that has not been saved, the confirming dialog box is displayed to prompt you to confirm whether the file must be saved when you exit the LCMSsolution.



6 All the LCMSsolution programs will be terminated with Windows shut down.



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