Chiral HPLC User Manual

Never work on the machine if you have not been trained to operate the machine.

Step 1: Starting the Analytical Instruments and Logging into "LC Real Time Analysis"

- **Preparing the analytical instruments**
    1) Ensure that all of the units of the analytical instruments (pump, autosampler, column oven, and detector) are connected to the system controller.
    2) Turn the power on for each of the units. Each unit will be initialized.
    3) Confirm that there are no error messages displayed on any of the units. Usually a red light indicates a problem.

- **Logging into "LC Real Time Analysis"**

Start "LC Launcher" and login to "LC Real Time Analysis". Ensure that you have started the analytical instruments prior to starting LCsolution. LCsolution will not recognize the analytical instruments when these are started after LCsolution start up.
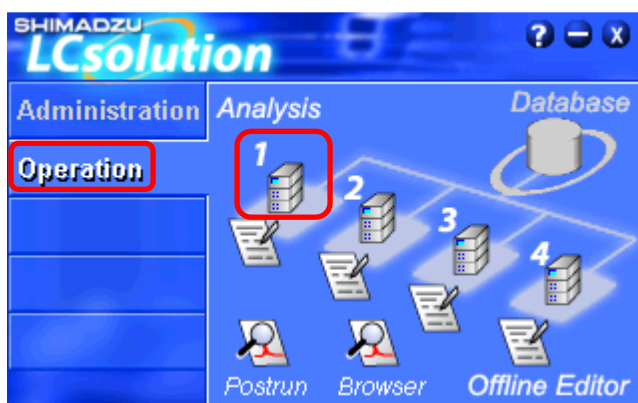
1) " Double-click (LCsolution). LCsolution Launcher will start.

2) Click the [Operation] tab on the left side of the screen. The [Operation] menu will appear.

3) Click on (Analysis) of the instrument you wish to use.

 - Analysis

4) Type your user ID and password, then click [OK]. This is not necessary, click [OK].

Step 2: Performing Batch Analysis

- **Creating a Batch Table**

  Set the schedule for continuous analysis.

  1) Click the (Batch Processing) icon in the assistant bar in "LC Real Time Analysis".

      - Batch Processing

     The batch table will be displayed.

  2) Or Select [New Batch File] from the [File] menu.

  3) Enter the batch analysis schedule in the batch table. The main items to be entered into the batch table are as follows:

     The first row of your Batch Table must always contain a column wash. The following line will contain an analysis of your sample. The last line will again contain a wash of the column with mobile phase (80:20, heptane/isopropanol). The machine allows analysis of pure substances only. One spot on TLC and pure NMR spectrum (No reaction mixture). It is not recommended to separate strongly polar species like carboxylic acid, amines, quaternary ammonium salts and other species with strong binding to silicagel for obvious problems related to extremely long elution time. Also, bigger content of residual solvents like DCM, DMF, DMSO harm the coated type column.

     Before you start check the volume of solvent! If you need new bottle of HPLC solvent, inform Michal Urban.

Parameters of instrument.

PDA: wavelength range = 190-350 nm (max. wavelength range = 190-800 nm).

Temperature: T (standard) = 25°C, T (min.) of oven = 17°C, T (max.) = 40°C.

Sample preparation for analysis.

Sample: Use 1.5 mL vial with septum, 1 mg your compound in 1 ml isopropanol (or mobile phase, no salts and insoluble particles. Filtration of sample in DCM through the small plug of silicagel with cotton in Pasteur pipette is sufficient.

Solvents – HPLC quality: 1) for easily soluble samples, you can use the heptane/isopropanol mixture (80/20), 2) for more polar molecules you can use pure isopropanol, 3) if you are not successful, few drops of EtOAc can help.

Analysis	Vial#	Tray Name	Inj. Volume	Sample Type	Analysis Type	Method File	Data File	Level#
1	1	1	10	0:Unknown	IT QT	Method1.lcm	Unknown1.lcd	0
2	2	1	10	0:Unknown	IT QT	Method1.lcm	Unknown2.lcd	0
3	3	1	10	0:Unknown	IT QT	Method1.lcm	Unknown3.lcd	0
4			1	0:Unknown	IT QT			0

Vial	Enter the vial number of the sample injected by the autosampler. If no sample is being injected, enter "-1". This means washing the column. (equilibration)
Inj. Volume	Enter the injection volume (unit: µL). The injection is always 1 µL per 1 mg of your substance.
Sample Type	Select the sample type. Select [Standard] for the sample in order to create the calibration curve, and select [Unknown] to quantify the sample. For the first standard sample for creating a calibration curve, enable [Initialize Calibration Curve].
Analysis Type	Specify whether to perform analysis processing. [IT] means to perform peak integration and [QT] means to perform quantitative calculation.
Method File	Specify the method file to be used for the analysis. To select a method, click the arrow in the method row. All methods are already created on disk D. You select your method and chiral column (eg IC_80: 20_1ml_25C). This indicates: IC column, mobile phase 80:20 (heptane / isopropanol), flow rate 1 ml / min, temperature 25 ° C.
Data File	Name the data that is to be saved as the analysis results. When the file name is entered without a path, the data will be created in the currently browsed project folder. Save the analysis results to disk D in your folder.
Level#	Enter the calibration level number when using the standard sample.

4) Name the batch table and save it. Click the (Save) button in the toolbar and save it under a new name. The content of the batch table will be saved.

 - save button

Usually you start with racemic sample.

- **Performing Batch Analysis**

Next, you will perform analysis based on the batch table settings.

1) While the batch table is open in "LC Real Time Analysis", click (Batch Start) in the [Batch] assistant bar.

 - Batch Start

The batch analysis will be performed one by one starting from the first line.

Note:
When pausing the batch analysis, subsequent batch tables can be modified while continuing the current analysis.

To pause the batch analysis at the line that is currently being analyzed or to restart the paused batch analysis, click the (Pause/Restart) icon in the [Batch] assistant bar.

 - Pause/Restart

To stop the batch analysis, click the (Stop) icon in the [Batch] assistant bar.

 - Stop

2) Viewing the peak integration results during batch analysis.

Using the snapshot feature allows you to temporarily display the data in [Postrun Analysis] and to view the peak integration results. This function is useful when you want to make sure the data going through the batch analysis are being integrated in the manner that you intended with the specified method. During the analysis, click the (Snapshot) icon in the [Acquisition] assistant bar while [Data Acquisition] is active.

 - Snapshot

The "Snapshot" will start.

3) Checking the progress of the batch analysis

The progress of the batch analysis can be checked in the output window. Once the batch analysis has been completed, check the messages to see if there were any errors during the analysis. When the batch analysis is unsuccessful, check the content from the log.

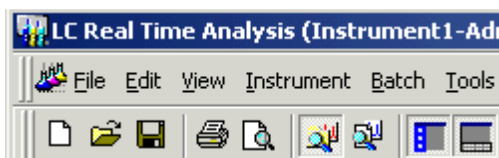| Message	SubMessage	Date	
Start Batch Processing (Data Acquisition)	C:\Data\ProjectX\Batch1.lcb : 1	8/1/2002	
No peak has been detected.	Detector A (C:\Data\ProjectX\Std1.lcd)	8/1/2002	
No peak has been detected.	PDA (C:\Data\ProjectX\Std1.lcd)	8/1/2002	
No peak has been detected.	Detector A (C:\Data\ProjectX\Std2.lcd)	8/1/2002	

Message / LogFile

Step 3: Viewing Data

When using LCsolution, the basic way to manage files is to put all related files such as data files, method files, and batch files in the same "Project Folder". Use the [Data Explorer] to manage projects effectively with LCsolution.
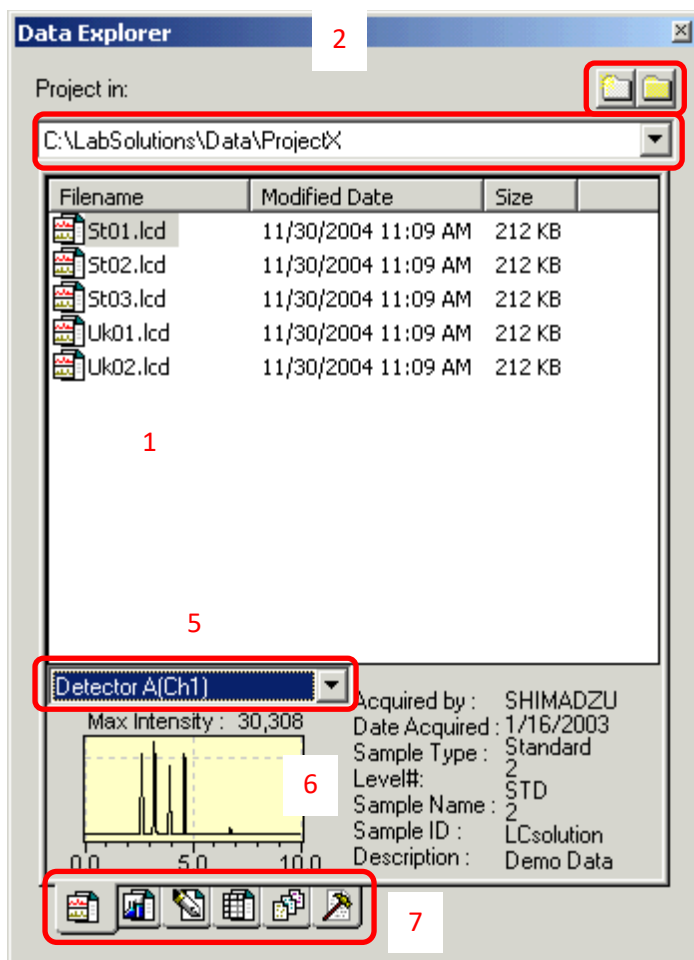
- **Displaying [Data Explorer]**

  Clicking the (Toggle Data Explorer) button in the toolbar toggles the [Data Explorer] window on and off.

   - Toggle Data Explorer

1	The list of files will be displayed. These files can be loaded by either double-clicking them or by dragging and dropping them onto another window. • Files can be arranged by file name, analysis date, and update date. Right-click on the area where the file names are displayed and select [Arrange Icons] from the displayed menu. In the submenu, select the criteria for arrangement. • To show additional information such as the sample name, right-click on the file name and then click [Show File Info.] from the displayed menu.
2	By double-clicking the title bar, you can toggle between the floating display and the docked display (in which the [Data Explorer] window will be incorporated in the LCsolution window).
3	Creates a new project folder, and copies and browses the existing project folders.
4	Previously used project folders can be listed and selected.
5	Selects the detector for the chromatogram to be displayed.
6	You can preview the content of the file that is focused. Partial sample information can also be displayed. Right-click on the file name, and click [Data Preview] from the displayed menu to toggle the preview on or off.
7	Files can be displayed by file type. To display all the files, select [All Files].

- **Converting the Data Format**

  You can convert LCsolution data files to the ASCII format and the data files from other LC Workstations (manufactured by Shimadzu) to the LCsolution format.
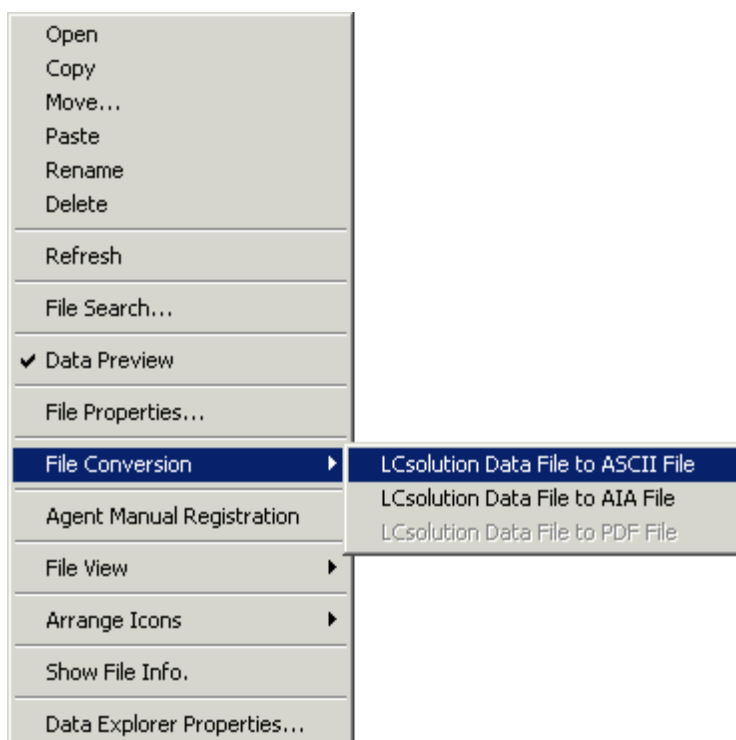
  Right-click on the file name and select [File Conversion] from the displayed menu, and from the submenu, select the type of file to be converted to.

- **Viewing Data**

  View the data by starting "LC Data Analysis".

  Start "LC Data Analysis" and open the data file acquired during the batch analysis. The name of the data file is the name typed in the batch table. In the batch table, data files can be opened by selecting [Browse Data] in the right-click menu on the batch table.
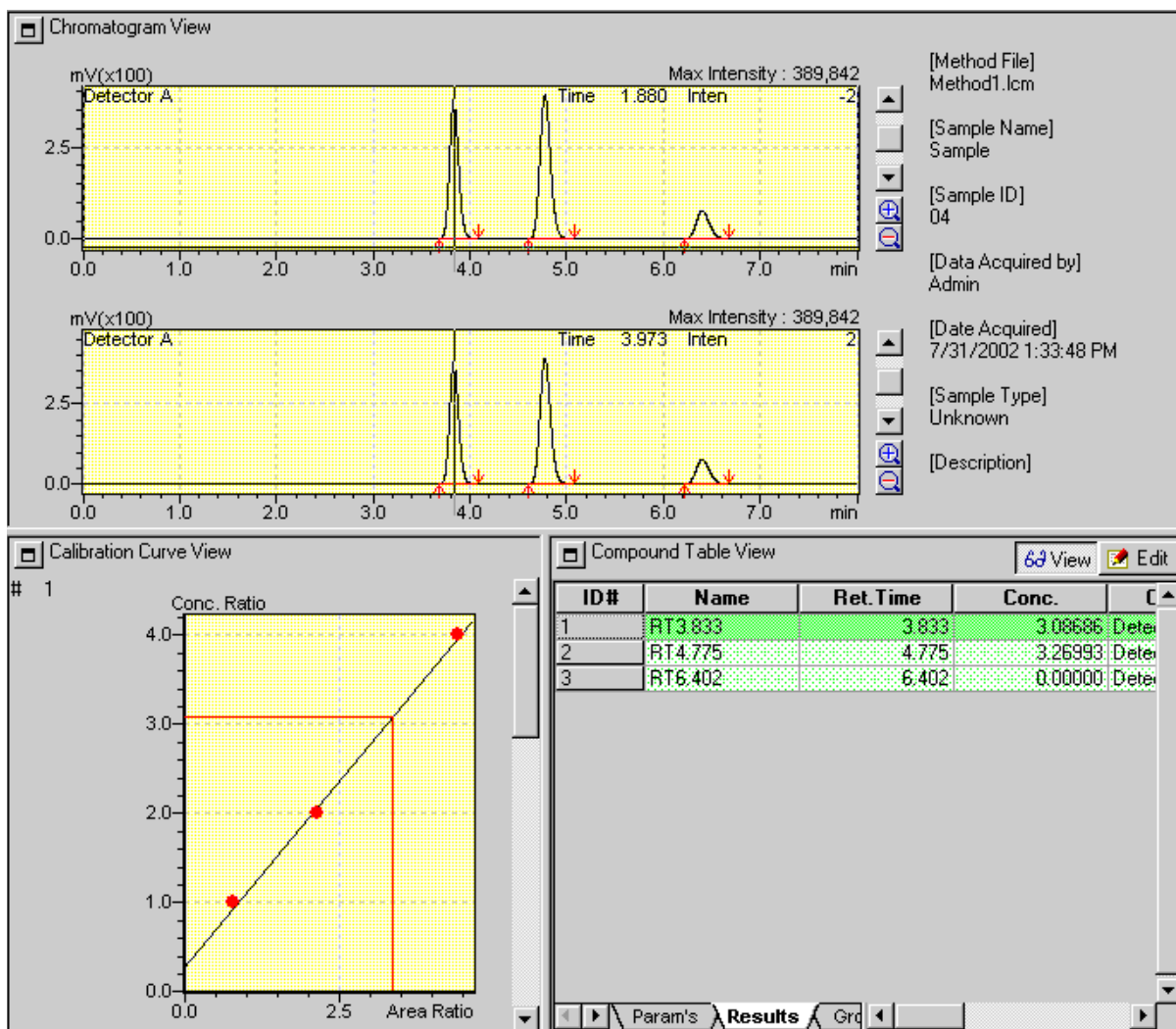
Analysis	Sample Type	Analysis Type	Method File	Data File
1	0:Unknown	IT QT	test.lcm	test.lcd
2	0:Unknown	IT QT		

  Also, double-click the (Data File) icon on the [Data] tab of [Data Explorer] to import the data file and start the "LC Data Analysis".

   - Data File

  In "LC Data Analysis", the calibration curve and the results from identification and quantitative calculation can be viewed.

1	The obtained chromatogram can be viewed in the [Chromatogram View].
2	The information on the calibration curve that was used for quantitative calculations can be viewed in the [Calibration Curve View].
3	The results from the quantitative calculation can be viewed in the [Results] tab of the [Compound Table View].

Step 4: Ending the Analytical Instruments and "LC Real Time Analysis"

Exit the program using the X icon.