

HPLC Operating Instructions

Version 1.0

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Terminology

- 1) A **method** is a file that instructs the instrument and software how to analyze the sample and process the data once it has been collected.
- 2) A **batch** is a file that contains a sequence of samples to be run or that have been run previously. An **acquisition batch** is used to collect new data, while a **postrun batch** is used to reprocess previously run data. It is very important to distinguish between these two types of batches.
- 3) **Level** refers to a calibration level. For example, if there are 4 different calibration standards for a given batch, that batch has 4 calibration levels.
- 4) **LC Real Time Analysis** is the window to use for data collection, as well as method editing, batch editing, and report format editing. Changes made within LC Real Time Analysis will affect the instrument. This is also referred to as “on-line” mode.
- 5) **LC Analysis Editor** is the window to use for method editing, batch editing, and report format editing while the instrument is running. Changes made within LC Analysis Editor will not affect the instrument. This is also referred to as “off-line” mode.
- 6) **Postrun Analysis** is the window to use for viewing and reprocessing data.
- 7) **Browser** is the window to use for viewing multiple data files simultaneously.
- 8) Files ending in **.jcm** are method files, those ending in **.jcb** are batch files, those ending in **.jcd** are data files, and those ending in **.jcr** are report files.
- 9) A **project** is a folder that contains related data, method, batch, and report files.
- 10) The **Assistant Bar** is the vertical bar on the left-hand side of the screen that contains icons for common actions.

Startup

- 1) If the instrument is not on, turn it on by pushing the power buttons for the controller, detector, autosampler, and pump. Turn on the computer. When the security window opens, just click *Enter*; there is no password.
- 2) To operate in LC Real Time Analysis (“on-line” mode) click *Analysis (Instrument 1)* from the top LCsolution window. Analysis (Instrument 1) is represented by a computer icon. This is the mode to be in when running samples.
- 3) To operate in LC Analysis Editor (“off-line” mode) click *Offline editor (Instrument 1)* from the top LCsolution window. Offline editor (Instrument 1) is represented by a piece of paper icon. This is the mode to be in if you want to edit methods, batches, etc. while the instrument is running.
- 4) To view and/or reprocess data, click *Postrun analysis* from the top LCsolution window. Postrun analysis can also be accessed within LC Real Time Analysis or LC Analysis Editor.

- 5) To see multiple samples in one display, click *Browser* from the top LCsolution window.
- 6) The manual is always available under *Help* → *Online Manual*.

Method Development

- 1) Click the *Data Acquisition* icon (if in LC Real Time Analysis) or the *Method Development* icon (if in LC Analysis Editor) from the Assistant Bar. You may have to hit the *Top* button to access the icon.
- 2) There are *Normal* and *Advanced* options to view the parameters for method development. Click *Advanced*.
- 3) The following tabs in *Advanced* contain information to be modified when creating a method: *Data Acquisition*, *LC Time Prog.*, *Pump*, and *PDA*. The *Controller*, *Autosampler*, and *Auto Purge* tabs usually do not need to be modified.
 - a. *Data Acquisition* – the run time of the method (*LC Stop Time*) is entered here. The detector start and end times (*PDA Start Time* and *PDA End Time*) are also entered here. The button *Apply to all acquisition time* can be pressed to have the same times entered for sample analysis and data collection.
 - b. *LC Time Prog* – the main parameters of the method are entered here.
 - i. *Time* – the time the action is to occur is entered here.
 - ii. *Module* – this is what is controlled at the indicated time; the three choices are pumps, controller, and autosampler.
 - iii. *Action* – this is where the action of the module is specified, there are several choices for each module.
 - iv. *Value* – this specifies the quantitative amount of the *Action*.

For example, a published method requires an initial mobile phase composition of 45% solvent A and 55% solvent C. From 2 minutes to 25 minutes, the method calls for a gradient that ends with 100% solvent C. From 25 minutes to 40 minutes the mobile phase is held constant at 100% solvent C and the analysis ends. This method would be set up in the software as follows:

- i. The *LC Stop Time* under the *Data Acquisition* tab will be 50 minutes. The *PDA End Time* will be 40 minutes. The extra 10 minutes at the end of the run are to let the mobile phase composition equilibrate back to the starting composition for the next run. This is not an actual part of the analysis, so the PDA is not set to collect this data.
- ii. The initial mobile phase composition is set in the *Pumps* tab, described in part c., below.
- iii. The first line in *LC Time Prog* would read *Time = 2.00, Module = Pumps, Action = C. Conc., Value = 55*
- iv. The second line in *LC Time Prog* would read *Time = 25.00, Module = Pumps, Action = C. Conc., Value = 100*
- v. The third line in *LC Time Prog* would read *Time = 40.00, Module = Pumps, Action = C. Conc., Value = 100*

- vi. The fourth line in LC Time Prog would read *Time* = 50.00, *Module* = Pumps, *Action* = C. Conc., *Value* = 55
 - vii. The fifth line in LC Time Prog would read *Time* = 50.00, *Module* = Controller, *Action* = Stop, *Value* is n/a in this case
- A *Controller – Stop* line should be near or at the end of every method. The gradient can be seen graphically by clicking the *Draw curve* button.
- c. *Pump* – the flow and pump parameters are entered here. In *Mode*, always select *Low Pressure Gradient*, even if isocratic flow is desired, because this selection gives more options which include isocratic flow. Set the total flow desired for the run under *Total Pump A flow*. Set the desired initial starting mobile phase composition by entering values in *Solvent A conc.*, *Solvent B conc.*, *Solvent C conc.*, and *Solvent D conc.* *Solvent A conc.* is defaulted to be 100 %, and is decreased whenever a value is entered into another solvent channel. For example, if 30 % is entered in *Solvent B conc.* and 30 % is entered in *Solvent C conc.*, then *Solvent A conc.* will automatically be adjusted to 40 %. The pressure limit is also set in this tab; set it high enough to allow routine operation but low enough that the instrument is protected. Somewhere around 3000 is usually good.
 - d. *PDA* – the detector parameters are entered here. PDA stands for photo diode array; this detector can collect multiple wavelengths simultaneously. Click *Start Wavelength* and enter the value of the wavelength to start collecting data, then click *End Wavelength* and enter the value of the wavelength to stop collecting data. The available wavelength range is 190-800 nm.
- 4) Click *Method* → *Data Analysis Parameters (PDA)*. This window contains other parameters that affect the method, including *Integration*, *Identification*, *Quantitative*, *Compound Table*, and *Multi-Chrom*. These parameters are usually modified in LC Postrun Analysis after a sample has been analyzed and the method is modified to suit that sample and future similar samples. However, changes may be made at this point if desired. If changes are made at this point, they can still be made in LC Postrun Analysis as well.
- a. *Integration* controls the parameters that specify how to integrate the peaks in the chromatogram. For time dependent integration parameters, click the *Program* button. Note that the changes are only effective for the selected channel, unless the *Copy to All Channel* button is pressed after making changes.
 - b. *Identification* specifies the parameters for identifying peaks based on the retention time.
 - c. *Quantitative* specifies the calibration information as well as details of the calibration curve, units, and decimal places/significant figures.
 - d. *Compound Table (Param's tab)* lists the identified compound(s), whether it is a standard, reference, or unknown, the channel used to integrate it, the retention time, and the concentrations of the compound at various levels (if applicable).

- e. *Multi-Chrom* lists the desired wavelength channels, and can also be used for a Max Plot, which generates a chromatogram in the specified wavelength range at the position of maximum absorbance.
- 5) Save the method by going to *File* → *Save Method As*, select the project folder to save the method in, and enter a method name to save it.
- 6) When the method has been created, the *Download* button in LC Real Time Analysis must be clicked to download the method to the instrument.

Sample Analysis

- 1) To run a single sample, click the *Single Start* icon from the Assistant Bar, fill in the requested information, then click *OK*.
- 2) To run multiple samples, an acquisition batch table is created (“Analysis” is shown in blue on the batch table). Click *Batch Processing* from the Assistant Bar. Open a previous batch to be modified or select *File* → *New Batch* to create a new batch file. Or (optional) the Batch Table Wizard can be used by clicking the *Wizard* icon. If using the Wizard, follow the prompts to create a new batch table.
- 3) Enter the information requested in the batch table. *Tray name* is always 1. *Sample name* and *Sample ID* are redundant and one of them can be ignored if desired. *Sample Type* is usually either “Unknown” or “Standard”. *Analysis Type* is always “IT QT”. *Level #* refers to a calibration level and thus is only used for standards. *ISTD Amt.* can be ignored if not using internal standards. If report generation is desired immediately after the sample has finished running, check the box in *Report Output* and enter the appropriate report format file under *Report Format File*. Likewise, if a summary report is desired, click the appropriate Start and End points under *Summary Type* and select the proper report format file under *Summary Report Format File*.
- 4) The first sample in the batch table is usually a blank (or rinse) to equilibrate the system before running actual samples. The last sample in the batch table should be a shutdown to turn off the lamps to the detector and the solvent flows when the batch is finished.
- 5) Make sure the waste bottle has sufficient empty volume to collect the waste from the analyses. If a new bottle is required, remove the waste line from the full bottle and place in the new bottle. Cap the full bottle and inform the lab manager for disposal.
- 6) Save the batch file by clicking *File* → *Save Batch File* or *File* → *Save Batch File As*.
- 7) Click *Batch Start* when ready to run the batch.
- 8) While a sample is running, *Data* → *Snapshot* (in LC Real Time Analysis) can be clicked to see data collected to that point. Samples in the batch that are already completed are available for viewing and reprocessing in LC Postrun Analysis.

Data Analysis

- 1) Open LC Postrun Analysis, either from the top LCSolution window or from within LC Real Time Analysis or LC Analysis Editor.

- 2) Open the desired sample. Only one sample can be open at a time in a given LC Postrun Analysis window, but multiple LC Postrun Analysis windows can be open at the same time.
- 3) Any of the windows in LC Postrun Analysis can be maximized by clicking the “square” icon in the upper left-hand corner of each window. To reduce the size of the window back to the original, re-click the icon. The windows can also be manually enlarged and reduced by grabbing the edges with the mouse pointer and manipulating them. Specific window layouts can be saved by clicking *Layout* → *Save Layout*.
- 4) The Contour View shows the chromatogram in pseudo-three dimensions. Certain properties of this view can be changed by selecting *View* → *Contour View*. The plot usually needs to be rescaled (this is done by right-clicking in the window and selecting *Display Settings*, then adjusting the intensity values) to see the peaks properly.
- 5) *Click View* → *3D image* to see the sample in “true” three dimensions.
- 6) The Chromatogram View shows time vs. intensity at a set wavelength. Certain properties of this view can be changed by selecting *View* → *Chromatogram View*. The *Peak* toggle button moves the retention time line through the various integrated peaks. The *Channel* toggle button cycles the screen through the various specified channels (these are specified in the *Multi-Chrom* tab within *Data Analysis Parameters*). The channel labeled “Extract” is linked to the position of the two drag lines in the Contour View. As the time and/or wavelength drag line is varied, the chromatogram displayed in the Chromatogram View changes. The drag lines in the Contour View can be moved by “grabbing” the arrow at the end of the line with the mouse pointer and moving it to the position of your choice, or by using the left/right and up/down toggle buttons. These buttons are also useful for fine adjustments.
- 7) The Calibration Curve View displays the calibration curve for each channel. Certain properties of this view can be changed by selecting *View* → *Calibration Curve View*.
- 8) The Spectrum View shows wavelength vs. intensity at a set time. Certain properties of this view can be changed by selecting *View* → *Spectrum View*. This view is linked to the Chromatogram View and the Contour View. As these views are changed, the Spectrum View automatically updates to reflect the changes. The Spectrum View is useful to find the wavelength of maximum absorbance at a specific time.
- 9) The Purity View gives information on the purity of the compound peaks. This view is not used currently.
- 10) The Compound Table View lists the compounds specified in the Method. Various parameters of the Compound Table can be modified by clicking *Table Edit* and then selecting the desired action. A new Compound Table can be created, if desired, by clicking *Table Edit* → *Wizard*; note, however, that this will over-write the existing compound table. The *Param*’s tab displays the parameters used to define the peaks, while the *Results* tab lists results from the analysis based on those parameters. To make changes to the Compound Table, the table must be in *Edit* mode, but to save any changes to the table, it must be in *View* mode.

- 11) *Method* → *Data Analysis Parameters* (see **Method Development** for more details) is also accessible within LC Postrun Analysis.
- 12) To see a list of identified and integrated peaks by channel, click *View* → *Peak Table*.
- 13) When the desired changes have been made, the data file can be saved by clicking *File* → *Save Data File* or *File* → *Save Data File As* to re-name the file. **Note:** this saves the changes only to this specific file! If the method is to be updated as well, also click *File* → *Save Method As* to update the method.
- 14) If method changes are to be applied to several samples, postrun batch processing should be used. Open or create a new postrun batch within Postrun Analysis by selecting *Batch Processing* from the Assistant Bar. Make sure the samples to be reprocessed are listed in the batch. Click *Batch Start* to reprocess the samples according to the method(s) specified in the batch. Postrun batch processing can be performed multiple times as the method changes. Note that the previous data file is overwritten when batch reprocessed. If a file has been reprocessed and the originally acquired data file is desired, select *File* → *Rollback to Original Data* to restore the data file.

Browser

- 1) Multiple data files can be viewed simultaneously using the Browser. The Browser is accessed from the top LCsolution window by clicking *Browser*.
- 2) To view quantitated data (e.g. concentrations, peak heights, etc.) click *LC Quant Browser* from the Assistant Bar. To view graphical data (e.g. chromatograms, contour views, etc.) click *Data Browser* from the Assistant Bar.
- 3) *LC Quant Browser*:
 - a. Drag and drop data files (or double-click) into the *Quantitative Result View* to add data files to the table.
 - b. The *Compound Table View* and *Quantitative Result View* are linked; switching compounds in the *Compound Table View* will automatically refresh the *Quantitative Result View*. Likewise, switching filenames in the *Quantitative Result View* will automatically refresh the *Compound Table View*.
 - c. The *Chromatogram View* and *Quantitative Result View* are linked; switching filenames in the *Quantitative Result View* will automatically refresh the *Chromatogram View*.
 - d. View and Edit modes are both available; the data is automatically reflected to the method when switching from Edit to View mode.
 - e. Right-clicking in the *Quantitative Result View* brings up a window that allows other changes to be made.
 - f. Layout information such as the names of the imported method file and data files, the order of the files, and the settings for statistical calculations can be saved as a browsing file by selecting *Layout* → *Save Browsing File*.
 - g. Collective postrun analysis or statistical calculations can be performed on the data:

- i. To change the calibration curve information, click the *Modify Calibration Curve* icon from the Assistant Bar. Be careful! This action modifies the method file.
 - ii. To change the Data Analysis Parameters, click the *Data Analysis Parameters* icon in the Assistant Bar.
 - iii. To view statistical calculations in the Quantitative Result View, click *View* → *Quantitative Result* → *Statistical Result*.
 - iv. To print the quantitative results of all data, click the *Summary Report* icon in the Assistant Bar.
 - v. To print the quantitative results of a single file, click *File* → *Print Quant. Report for Current Data* → *Print*.
- 4) *Data Browser*:
- a. Drag and drop data file(s) into the main window to display the graphical data. The type of data to display is chosen from the *Select Data Type* window that pops up automatically.
 - b. The sample information of the file will appear if the mouse pointer is held on the title of the cell for a few seconds.
 - c. The vertical blue bar is next to the active cell.
 - d. Each cell works in conjunction with the other cells (“display collaboration”) when the thumbtack icon is green. If collaboration is not desired for a particular cell, click the thumbtack icon to disable collaboration for that cell.
 - e. Collaboration with cells from different data files is possible.
 - f. If the file includes data that can be displayed in another format, the display format can be changed by right-clicking on the cell, selecting *Change Data Type* from the displayed menu, and choosing the display format from the sub-menu.
 - g. To adjust the layout of the cells, right-click on a cell and select *Adjust Layout*, then the desired action from the sub-menu.
 - h. To exchange the displayed data between two cells, drag and drop the title of the first cell to the second cell.
 - i. To close a cell, right-click on it and select *Close Data File* from the displayed menu.
 - j. Layouts can be saved by clicking *Layout* → *Save Layout File*.
 - k. To perform a library search for a displayed spectrum, right-click on the spectrum cell and select *Library Search for Current Spectrum* from the displayed menu. The library search is performed based on the search criteria recorded in the method in the data file.

Reports

- 1) Existing report format files may be used for samples or new report format files may be created as desired. Reports can be generated automatically by specifying the report format files to be used in the batch table (see the **Sample Analysis**

- section). Reports can also be easily generated post-run. Reports can be saved as .PDF files.
- 2) To access report formats, click *Report Format* on the Assistant Bar within LC Real Time Analysis or LC Analysis Editor. The *Top* icon may have to be clicked first to see the *Report Format* icon. Open an existing report format file, if desired, by clicking *File* → *Open Report Format File*. A new report format file is automatically opened when *Report Format* is clicked, but *File* → *New Report Format File* can also be selected if a new file is desired at another time.
 - 3) To create/modify a report format file, there are several drop-down menus at the top of the screen to choose from, as well as icons for several of the more commonly desired features (these icons duplicate actions found in the drop-down menus). *Item* is a good place to start, as this is where common report features can be added. Select the desired feature by selecting *Item* → (*Common, General, LC/PDA Common, PDA, or Figure*) → (the desired item). For example, *Item* → *LC/PDA Common* → *Calibration Curve* is a feature of most reports. Once the feature is selected, drag the mouse pointer over the report page to create a box in which the feature will be displayed. Once the box has been drawn on the page, a *Properties* window pops up that should be modified as desired. The properties of the feature can be modified at any time by right-clicking in the feature box and selecting *Properties*. Likewise, the feature can be modified at any time by right-clicking in the feature box and selecting the desired action. The feature can also be rescaled at any time by selecting it, grabbing a corner of the box with the mouse pointer, and moving it to the desired location.
 - 4) To insert or delete pages in a report format file, click *Page* → *Insert* or *Page* → *Delete*. To move to different pages within the report format file, click *Page* → *Move* → (desired page).
 - 5) To add text as a header or footer, click *View* → *Header/Footer* and add the desired text.
 - 6) Various page layout features are located in the *Layout* drop-down menu.
 - 7) To view data in a report format file without actually generating a report (this is useful for report format file creation), open the report format file and drag a data file into it from the Data Explorer window. The report format file should be populated with the data from the file.
 - 8) To generate a report for a single file, open the desired file in LC Postrun Analysis. If the Assistant Bar is not displayed, click *View* → *Assistant Bar* to make it visible. Click *PDA Data Analysis*, then click *Data Report*. The data will be displayed in the report format file in which it was collected (this will be the default report format file if none was specified during acquisition). If a different report format file is desired, click *File* → *Open Report Format File* and select the desired report format file. The report can then be printed to a .PDF file if desired. To save a new report format file with the data, open it as just described, click *Return*, then click *File* → *Save Data File*.
 - 9) To generate a report for multiple files, postrun batch processing is the easiest procedure. From the Assistant Bar, click *Batch Processing*. Set up a batch with the desired files from which to generate reports, and specify the proper report format files. If only report generation is desired from the batch (for example, the

- method has not changed so the data files do not need to be reprocessed) then *Quantitative Integration* and *Quantitative Calculation* can be de-selected under *Analysis Type*. Click *Batch Start* and the reports will be generated.
- 10) To save reports as .PDF files, click *File* → *Print*, make sure “FinePrint pdfFactory” is selected, then click *OK*. The report will then be displayed in a pdfFactory window, from where it can be viewed and/or saved by clicking *View PDF* and *Save*, respectively.