

# HPLC Operation – Outline

## Version 1.2

### 8/7/07

#### **General HPLC instructions:**

- always put a shutdown method at the end of the batch and make sure there is enough mobile phase to analyze the samples; it is very hard on the instrument and column if they run dry
- make sure the waste bottle has sufficient empty volume to collect the waste from the analyses; if not get a new waste bottle
- the manual is always available under *Help* → *Online Manual*

#### **To operate in LC Real Time Analysis (connected to the instrument):**

Click *Analysis (Instrument 1)* from the LCsolution top window

#### **To operate in LC Analysis Editor (not connected to the instrument):**

Click *Offline editor (Instrument 1)* from the LCsolution top window

#### **For post-run analysis (to analyze/reprocess data):**

Click *Postrun analysis* from the LCsolution top window

- Post-run analysis can also be accessed within LC Real Time Analysis and LC Analysis Editor

#### **For viewing multiple files simultaneously:**

Click *Browser* from the LCsolution top window

#### **For method development:**

- Data Acquisition (LC Real Time Analysis) or Method Development (LC Analysis Editor)
  - o Advanced
    - Data Acquisition
      - LC Stop Time
      - PDA Start Time
      - PDA End Time
    - LC Time Prog.
      - Time, module, action, value
    - Pump

- Mode (always use low pressure gradient)
- Total Pump A flow
- Solvent A conc. is defaulted to 100 %
- Solvent B conc.
- Solvent C conc.
- Solvent D conc.
- Pressure limits
- PDA
  - Start wavelength
  - End wavelength
- Controller (use default settings)
- Autosampler (use default settings)
- Auto Purge (use default settings)

#### Method→Data Analysis Parameters (PDA)

- Integration
- Identification
- Quantitative
- Compound table (usually generated in Postrun Analysis)
- Multi-Chrom (to set specific PDA wavelengths)

**Important!** the *Download* button must be clicked while in LC Real Time Analysis to download the method to the instrument

#### **For sample analysis:**

Acquisition batch processing- this creates a table of samples to be analyzed (Acquisition batch table – “Analysis” shown in blue on batch table)

- Create within LC Real Time Analysis or LC Analysis Editor
- can use Wizard if desired
- enter blanks, calibration samples, and unknown samples as desired
- last sample should be a shutdown
- can specify report format to automatically generate report, if desired
- hit Batch Start when ready to run the batch
- while samples are running, can hit Data→ Snapshot in LC Real Time Analysis to see partial data from a run that is in progress

#### **For data analysis and reprocessing:**

LC Postrun analysis→ open sample (only one sample can be open at a time, but multiple LC Postrun analysis windows can be open at the same time)

- Contour view – usually need to rescale to see peaks properly
  - Can go to *View*→ *3D image* to see in “true” 3 dimensions
- Chromatogram view (time vs. intensity at set wavelength)
  - Peaks

- Channels
  - Extracted (current position of extraction cursors in Contour View)
  - Max Plot (generates a chromatogram at the wavelength position of maximum absorbance)
  - 1 (registered in Multi Chromatogram table)
  - 2 (registered in Multi Chromatogram table)
  - Etc.
- Calibration curve view (separate curve for each channel)
- Spectrum view (wavelength vs. intensity at set time)
- Purity view (not currently used)
- Compound table view
  - View
  - Edit
  - Params
  - Results
- *Method*→ *Data Analysis Parameters* is accessible within LC PostRun Analysis to allow method changes to be made
- *View*→ *Peak Table* for list of integrated peaks by channel
- Use *Save Data File* or *Save Data File As...* if changes are only desired for a single data file
- Use *Save Method As...* if you want to update the method with changes, which can then be applied to future analyses (and current analyses as well if batch processed in Postrun)
- Postrun batch processing - updates data files with new integration information saved in the updated method (Postrun batch table – “Postrun” shown in blue on batch table)
  - Create within LC Postrun analysis
  - **very** important to distinguish this batch processing function from the analysis batch processing function!
  - Postrun batch processing can be performed multiple times as the method changes
- Report formats can be created for specific types of samples or existing formats can be used
  - Reports can be generated automatically from the batch table or generated post-run
  - Reports can be saved as PDF files
- The Browser can be used to view multiple files simultaneously
  - *LC Quant Browser* for viewing quantitated data (peak areas, peak heights, etc.)
  - *Data Browser* for viewing graphical data (spectrums, contour views, etc.)