

Standard Operating Procedure

Agilent 1260 High Performance Liquid Chromatograph (Standard Configuration)

Introduction

Liquid chromatography (LC) is a separation process used to isolate the individual components of various mixture. The technique can be used to determine the qualitative nature and quantitative concentration of various species within a sample at the nanogram level.

Purpose

The purpose of this document is to introduce the user to the routine operation of Agilent 1260 High Performance Liquid Chromatograph (1260 HPLC) when using either the Diode Array (DAD), Fluorescence (FLD), and Refractive Index (RID) detectors. This document also outlines the special safety concerns introduced when using the instrument.

Scope

This procedure applies to established procedures of analysis using the standard configuration (shown below) for the 1260 HPLC. It is assumed that the mobile phase, HPLC column, and detector are available*. Individuals must complete a hands-on one-on-one training session with a TRACES staff member.

Referenced Documents

- Agilent ChemStation ed.07/09 (p/n G2070-91126)
- Agilent 1260 Infinity II Quaternary System: Quick Start (p/n G7111-90300)
- Agilent 1260 Infinity Capillary HPLC System: System Manual (p/n G1376-90013)
- Agilent 1200 Series Quaternary Pump: User Manual (p/n G1311-90011)
- The LC Handbook Guide to LC Columns and Method Development (p/n 5990-7595EN)
- Perkin Elmer Analytical Methods for Atomic Absorption Spectroscopy rev.D (p/n 0303-0152)

Responsibilities

1. TRACES Users

- 1.1. All Users must obtain training with TRACES Staff prior to system operation. It is the responsibility of the User to ensure they have a good understanding of the instrument and all operation protocols.
- 1.2. If additional training sessions are needed it is the responsibility of the User to schedule these with TRACES Staff.
- 1.3. User must ensure that the mobile phase, HPLC column, and detector required for the HPLC analysis are available in TRACES*.
- 1.4. Instrument time must be booked by the User via the online booking system prior to system operation.

2. TRACES Staff

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- 2.1. It is the responsibility of TRACES Staff to provide introductory and, if required, subsequent training to all users.

Equipment (Standard Configuration)

- Agilent 1260 Infinity Quaternary HPLC*
 - .1. Mobile Phase: Methanol, Water, Acetonitrile
 - .2. Detector: DAD, FLD, RID
 - .3. HPLC Column: Agilent Poroshell 120 EC-C18 HPLC Column

Safety

IMPORTANT:

- All the organic solvents used for HPLC analysis are flammable
 - Take the appropriate precautions
- Seek assistance from TRACES Staff if you have any concerns

Initial Setup

1. Turn on the seven LC modules (Regardless of requirement)
2. Ensure all the solvents have a minimum of 500 mL
 - a. Anticipate the amount of solvent you for the completion of experiment. Speak to the TRACES Lab Manager to confirm the amounts if you are unsure.
 - b. Establish the identity and channel of all the solvents needed
3. Ensure the HPLC waste bottle is empty (or at least $\frac{3}{4}$ empty)
4. Login to the computer with the appropriate password and username
 - a. Click on Instrument 1 online
5. Confirm that all the modules you require are not greyed out.
 - a. Power down all greyed-out modules.

HPLC Setup Section

6. Purge Solvents
 - a. Open the Purge Valve by turning it counterclockwise
 - b. RCM (right click mouse) in the Quat Pump module
 - i. Click on Method
 1. Increase flow to 5 mL/min
 2. Purge each channel for 5-10min
 3. Click Okay
 - c. RCM in the Quat Pump module
 - i. Click Control
 1. Turn pump to 'On'
7. Load a Method
 - a. Click on Method and Load a method from the list provided
 - i. If you wish to use this loaded method (skip to #9)

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- ii. To create a new method, use the loaded method as a template
 - iii. Save the Method under a different name (go to #8)
8. Create a Method
- a. Click on the Instrument Module windows to change a parameter
 - b. Quat Pump
 - i. RCM in the Column Comp. module
 - ii. Click on Method
 - 1. Set eluent composition, gradient and flow rate
 - c. Column Heating
 - i. RCM in the Temperature Column Compartment (TCC) module
 - ii. Click on Method
 - 1. ID the HPLC Column and the parameters
 - 2. Set the Temperature Control for the HPLC column loaded
 - 3. Ramping temperature is possible in the Timetable area
 - d. ALS
 - i. RCM on the Sampler module
 - 1. Click on Method
 - a. Set the Injection Volume
 - e. Detectors
 - i. DAD
 - 1. RCM in the DAD module
 - a. Click Method
 - i. Set the characteristic wavelength(s) and reference wavelength (if necessary)
 - ii. Set the peak width: default values 5-20 Hz
 - iii. Spectrum stored: All in Peak
 - iv. Zero Offset: 5% (default)
 - v. Slit: 2nm (default)
 - vi. Auto balance during the Pre-run
 - vii. UV Lamp on between 190-400nm
 - viii. Vis lamp on between 400-950nm
 - b. Timetable
 - i. Change signal channels or wavelengths during a run
 - c. Lamps ON
 - i. RCM in the DAD module
 - ii. Click Control
 - iii. Lamps
 - 1. UV –On (if necessary)
 - 2. Vis –On (if necessary)
 - 3. At Power Off (default)
 - a. Do not use
 - ii. FLD
 - 1. RCM in the FLD module
 - a. Click Method
 - i. Signal to be set:

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1. Excitation:200-1200nm
 2. Emission: 200-1200nm
 - ii. Set the peak width: default values 2-5 Hz
 - b. Click Control
 - i. On
 - ii. Multiplier Factor:1.00
 - iii. RID

CAN NOT be in-line with the other detectors OR with the Fractionator

 1. RCM in the RID module
 - a. Click Method
 - i. Optical Unit Temperature set
 - ii. Set the peak width: default values 2-20Hz
 - iii. Polarity (+) is default value
 - iv. Auto zero Before Analysis: On
 - v. Automatic Recycling After Analysis: Off
 - b. Click Control
 - i. On
 - f. Save Method
 - i. Use a different name from any of the loaded methods
9. Sequence*
 - a. Sequence Parameter
 - i. Set a new subdirectory for EVERY new sequence
 1. The data may be overwritten
 2. Change to prefix/counter and set a base name to id samples
 3. Sequence output: As specified by method
 - b. Sequence Table
 - i. Transcribe for each sample:
 1. Vial number
 2. Sample name
 3. Method*
 4. Injections/Vial
 5. Sample Type: Set to sample
 - ii. Filldown Wizard can be used*
 - iii. Press Okay
10. Method>Save
 - a. Use an exclusive method name

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HPLC Analysis Run Section

11. Analysis Run*

- a. Single Run
 - i. Select 'Run Control'
 - ii. Sample Info...
 - iii. Populate Required Area
 1. Run Method
- b. Multiple Run
 - i. Run Sequence
 1. Select 'Run Control'
 - a. Run 'Sequence'

12. Print Report

- a. Press **Okay** to print to the default printer
- b. If the need is to auto print the Report go to #16(c)(i)
- c. If the Report is unsatisfactory go to #16(b) and alter accordingly

Data Evaluation Section

13. Click on Instrument Offline

14. Click **Data Analysis** tab

15. Click on File>Load Signal

- a. Load the data file of interest

16. Reports

- a. Print (default parameters)
- b. Specify Report (specify parameters to print)
 - i. Determine Qualitative Results Info
 1. Calculate: **Percent**
 2. Based On: **Area**
 3. Sorted by: **Signal**
 - ii. Report Style: **Short**
 1. **Add Chromatogram Output** box checked
 - iii. Report Layout for Uncalibrated Peaks
 1. **With Calibrated Peaks** box checked
- c. Destination
 - i. **Printer** box checked
 1. Reports are auto printed at the end of a run
 - a. Ensure a default printer is available
 - ii. **Screen** box checked

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1. Reports are shown on the screen at the end of run where they can be printed
2. The subsequent run report will remove the preceding run report from the screen
- iii. **File** box checked
 1. **File Setting**
 - a. Check the Unique pdf file box
 - b. Check the desired file format
 - i. Files are saved in the xxxx.D file format

17. Integration*

- a. Using the CS Integrator
 - i. Integrate Manually
 1. Using Integration Events
- b. Auto Integrate

18. Calibration*

- a. Calibration Table
 - i. Set the Analyte Name, Level and Concentration
 - ii. Calibrate

19. Method>Save

- a. This will lock the Report/Integration/Calibration Results, to the specific method for successive analysis. All default values will be saved for future analysis.

Baseline:

On instrument control diagram: hit **on** to start the ovens, pumps and lamps. Wait until all sections are ready (diagram color from yellow to green). Observe the baseline in **Online Plot (View-> Online Signals-> Signal Window 2)**; it should be stabilized before you start running samples.

*The TRACES Manager will provide full details during hands-on training.