Semi-prep. HPLC - UV4 (purification, Isco - Gynkotek)

Sjef Boeren 2006.12.15 12:48:22 +01'00'

MAKE SURE NEVER TO:
1. pump air or particles through the column.
2. inject when the injector is dry.
3. inject solutions containing particles.
4. apply large (sudden) pressure drops over the column.

Ad.1.: Make sure the degasser is on and filter the eluent trough a 0.2 um filter when necessary. Purge pumps. Make sure you have enough eluent.
Ad.2.: Pump eluent through injector and inject a suitable solvent in injector before injecting onto column.
Ad.3.: Centrifuge all samples (and filtrate when necessary).
Ad.4.: Increment flow rate in small steps (0.1 ml/min) when the prime is closed.

Always:
- Clean injector with a suitable solvent when you stop.
- Keep solvent reservoir bottles and the solvent waste barrel as much closed as possible. Organic solvents are volatile and can be toxic.
- Make up the eluent (e.g. addition of TFA) in the fume cupboard.
- When using water, refresh it daily.

1. System setup.
Switch power on: degasser (if it is not on yet), both pumps, the monitor, magnetic stirrer and computer. Push 'Start' buttons on both pumps. The green lights on the pumps are now burning, pressure = 0 bar. Pump A (H_2O) stands on top of pump B (MeOH).
Check wether the waste barrel is NOT full yet. If so, change it.

2. Purging the pumps.
Open the prime (THIS IS ESSENTIAL).
Use the computer:
Double click the 'Chromeleon' icon.
Click: Workspace, open workspace: HPLC-1_local/Panels/default.
In the Instrument 'Control panel', set the flow to:
Flow: 0.2 ml/min, % B: 50 (%A = 100 - %B)

Change eluent bottles when necessary taking care not to mix old eluent into the new eluent (dry the inlet filter before switching the filter to the new eluent bottle).
Both pumps are now working, pressure should still be approximately 0 bar since the prime is open. Check!

After 2 min, change the flow: Flow: 1.0 ml/min %B: 50.
Increase the flow in steps of 1-2 ml/min to 5.0 ml/min by repeating the last step. Hereafter, decrease the %B to 10%. Check whether all air bubbles are out of the water inlet tube. If not, force them out.
Increase the %B to 90% (always stepwise) and make sure that also the methanol inlet tube is free of airbubbles.
When both inlet lines are free of airbubbles, increase the %B to 100% and decrease the flow to 0.2 ml/min (stepwise!).

3. Close the prime. Increase the flow stepwise to 1 ml/min (keep the percentage of B at 100%). The pressure should now go up, eluent is flowing through the column and the detector.
The pressure should stay below 6 bar for a semi-preperative column. Stepwise increase the flow to 3 ml/min. MAKE SURE THE PRESSURE DOES NOT REACH 6 BAR, if so, decrease the flow and wait until the pressure drops.

4. Switch the lamp of the detector on by **clicking the 'Lamp on/off' button** in the Instrument 'Control panel'. Wait 1 min. until the red led on the front of the detecor changes to green. Click the 'Inject + acqOn' button, inject some water into the injector and inject it onto the system.

Now you have three signals which give you an idea of the status of the system.
A: the pressure. Using a semi-preparative column the pressure is approximately 2 MPa (eluting with methanol at 3 ml/min). For older columns the pressure may be a little bit higher.
B: the detector outlet. When the system is perfectly OK, no airbubbles should be visible.
C: the detector signal. Full screen deflection can be obtained by right clicking in the chromatogram display and selecting 'Autoscale'.
Air bubles in the system can be seen as a wobbling signal. When the signal does not become stable then try blocking the outlet line with your fingers.

Wait until the system is OK (pressure + detector outlet + detector signal. Contact Sjef when the pressure does not drop below 10 MPa).
Then (stepwise!) **increase the flow** to 4 - 10 ml/min for a semi-preparative column **and bring the %B to the values you want to start the acquisition with** in 2 minutes. A convenient way to do this is: click: Control, Flow (or Ctrl+F or the flow button), change 'Type' to 'Ramp', change 'Time' to 2 min., change the '%B end' to the percentage you want it to become and click the 'OK' button.
Wait until the detector signal is stable (the time this takes depends on the type of column and the flow you are using). Change the detector wavelength if necessary. You can bring the signal back to zero by clicking the 'Autozero' button.

The pump system has now been set up and is ready for the first injection.
Click the 'Acquisition off' button.

5. Methods info.
An injection is performed in a so-called batch. Each batch can contain a series of injections called 'Sequence' (sequence of injections). Before each (series of) injection(s) the following method files have to be prepared:
A) Program file (*.PGM): Equipment settings like a gradient for the pumps.
B) Method file (*.QNT): Settings for baseline calculation like: how steep should the signal rise to be regarded as the start of a peak
C) Sequence file (the blue folders): Series of injections measured one after another. A 'Program' and a 'Method' should be given for each injection of a sequence.

When you want to do an injection for the first time, ask an experienced user to prepare the methods for you. Methods can be prepared with: 'File, New, select 'Program File or Method File' or copied from HPLC-1_local\Programs and HPLC-1_local\Methods or copied from any sequence (eg HPLC-1_local\Data\Start method\Start.

6. Sample injection.
Click 'File, New, Sequence (using Wizard)'.
1. File information' window: Enter a Sequence name, keep the Timebase = HPLC-1 (select it under 'My computer' when it is not visible). Click 'Next'.
2. Unknown samples' window: Enter a Sample name and the number of injections you want to perform. Press 'Apply'. Click 'Next'.
3. Standard samples' window: Select 'no standards'. Click 'Next'.

4. Methods & Reporting' window':
Select the (gradient) 'Program file = C:\HPLC-1-local\Data\YourName\YourMethod' and 'Quantification method = C:\Data\YourName\Default' and 'Preferred Report = C:\ HPLC-1-local \Reports\Default', 'Preferred channel = UV_VIS-1. Click 'Next'.
5. Enter a 'sequence name + title'. The 'Data source' should be 'HPLC-1_local' and the directory 'Data\your name'. Click 'Next'.

Click 'Batch, edit' (or Ctrl+B or the 'Edit batch' button). Remove any unwanted sequences from the list. Click 'Add' , select your sequence, click Open. Click 'Ready check'. When everything is OK, load your sample in the injector (injector handle in the left position). Click the 'Start' button, inject by turning the injector fast to the right.
When you did put more injections into the same sequence, the next run will automatically follow after the one running, waiting till the injector is turned again (therefore, turn it back before this run is finished). When you did not put another injection in the sequence but you want to do another injection then either: prepare a new sequence, or edit the running sequence.

7. Integration and printing.
Data can be reviewed, reintegrated or replotted most easily from the Browser. Click the 'Browser' icon in the toolbar. The Browser shows the Directories in yellow and sequences in blue (left side), the methods in the selected directory (right side, top) and the injections (right side, bottom). Double-click on an injection name will open the 'Integration' window showing the (integrated) chromatogram. The next wavelength can be seen bij clicking the 'Next Channel' icon in the toolbar. When you are not happy with way the baseline is calculated then you can make manual adjustment of the baseline with the icons on the left-hand side. Automatic recalculation is preferred when you have to (re)calculate the baseline for more then one chromatogram. To do this, click 'View, QNT-editor (or the 'QNT-Editor' icon in the toolbar). Select the 'Detection' tab under the table. Change the settings, e.g. when you have more integrated peaks then you really want, insert a 'Minimum Area' (5) or a Sensitivity' (0.1) and a 'Peak Slice' (0.5) value.
When you are finished, click the 'Integration' icon in the toolbar. When you cannot see the result table, then select the 'show report' icon in the toolbar. Also, make sure the integration tab is selected below the table to observe the peak areas. Click the 'Print' icon in the toolbar, select the 'Integration' sheet, click 'OK'. The chromatogram data can be easily exported by right clicking in the chromatogram and selecting 'Export, chromatogram', Destination = disk: C:\My Documents\export.txt.
Chromatograms can be overlayed in the 'integration' mode or with the QNT-editor by clicking: File, Add overlay. Another way is to select one (or more) runs in the browser, right click and select 'Open, All channels' to see all channels of one run overlayed or select 'Compare, e.g. UV_VIS_1' to overlay one wavelength of both (or more) runs.

8. How to stop.
First, switch off the detector lamp by clicking once on the 'Lamp On/Off' button. The lamp costs fl. 2500,-.
Leave the injector in the inject position. Clean the injector with a proper solvent.
At the end of the day the column has to be cleaned. Gradually go to 100% B (e.g. in 2 min.). Pump elueant B for at least 10 min., then decrease the flow (gradually) to 0.2 ml/min.
Switch off the pumps, the monitor, stirrer and the computer. You may leave the degasser on when the system will be used the next day. Otherwise, switch it off.

Have a nice evening,

Sjef

PGM: kopieer detector detector settings uit goede methode (bijv. protein)
Indien program niet helder is: op verkeerde (oude sys1) timebase aangesloten:

Control, HPLC-1 vanuit editor.

Editor: F2: change name

F9: fill column

PGM en QNT file moeten altijd ook in sequence staan.

Manual opslaan van bijv. een opgenomen Basislijn kan altijd als meting gestopt wordt via ‘Control, Acquisition off’ of via de ‘Acquisition off’ button in de tool bar. De laatste handmatig opgenomen ‘run’ wordt opgeslagen in ‘HPLC-1_local\HPLC-1\manual’.

Replicate ID: identifier number: dezelfde pieken in meerdere runs met hetzelfde Repl.ID kan autom. worden uitgemiddeld.

Reference wavelength: in PGM file. Zet absorptie 0, normaal bij 600 nm maar is variabel.

License key code: RRHJ9HIWMYYS

Uvd 340S/170S interface: 0*0318