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BAF_S01_DIONEX Ultimate 3000 HPLC

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Protocol status: Working
We use this protocol and it's working

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Abstract

Description of HPLC operation and a standard step-by-step for reversed-phase chromatography of peptide mixtures on a 5 um C-18 column 300A, 150 x 2 mm.

Materials

ACN - Fisher chemical A955-4, Acetonitrile, optima LC/MS
Water - Fisher Chemical, W6-4, Optima LC/MS
TFA - Pierce™, Trifluoroacetic Acid, part number: 28903.
MeOH - Fisher Chemical, Methanol, Optima™ LC/MS Grade, part number: A456-4.
Pipette tips - Fisher Brand, yellow, part number: 02-681-151
Glass Amber vials - Thermo, 6PK1655

Before start

Buffers:
A - 0.1% TFA H_20
B - 0.1% TFA 95% ACN
C (and syringe) - 10% MeOH
Sonicate the buffers for 20 min.
*These are all prepared in the bottles provided that sit on top of the equipment.

Start up the HPLC:

1 Prepare 10% methanol for seal wash and injector wash

2 Check solvents, prepare new if needed and see that lines are not likely to come out of solutions

3 Check that the waste bottle is not full.

4 Check if all components are turned on

5 Open the CHROMELEON 7.0 application. Check if all components are connected.

6 Once the application opens, click on the PUMP tab

6.1 This tab will display the module status, information on the left and right pumps and the pump pressure

7 If pump seal wash does not run, turn it on under commands

8 After the pump seal wash has finished, open the cover of the loading pump and twist open the purge valve

9 Once the purge valve is open, turn on the motor and purge by clicking the empty box to the left of the words. This box will then turn green showing that it is indeed on

10 Purge solvents. Allowing a long purge may reduce the period of pressure fluctuations.

11 For autosampler, prime syringe, and wash syringe.

12 Once the purge finished, close purge valves.

13 Start a low flow rate with 5% B, at 0.100 ml/min, and let it run for 10 min. Increase the flow rate by 0.50 ml/min until reaching 0.250 mL/min. Let isocratic flow running for 20 min.

14 **TIPS:**
When lines from solvent bottles are dry, it helps to use a syringe with a fine tip to suck liquid through.
Attaching a syringe with a tapered tip to the end of the waste line works to suck air bubbles through the lines.
If pumps have sat for long in acetonitrile, check valves may be sticky. Storing pumps in propanol may help, but pressures are high. A pump purge is probably needed. Washing out propanol takes a long time.

Preparing samples:

15 Prepare a new amber glass vial of a fresh blank containing 1.5 mL of 0.1% TFA and put it in the first spot on the autosampler rack you will be loading samples.

16 Dilute the peptide mixture down into 0.1% TFA to a max of 30 ug of peptide mixture or 3 ug/peptide/injection. Centrifuge sample at top speed for 5 min. Collect supernatant to a new amber glass vial.

Running samples

17 Make sure the module is connected. this is the section under MODULE STATUS on the PUMP page

18 Turn on the UV - it will take 10-20 min to warmup.

19 Prepare your samples by diluting them to the correct dilution and putting them in labeled amber vials.

20 Load blank and samples into the autosampler rack on a known order.

21 In the software you will need to create a sequence to run all samples and blanks automatically.

Creating a sequence run

22 In the main window, click CREATE then SEQUENCE

23 In the pop-up, click UVA_U3000, then click NEXT

24 In this new pop-up, make sure the injection volume is set to 100 uL and that the start position is correct. click NEXT

24.1 In our example, the start position is RA1 (Red row A spot 1) for blank but this could be green or blue also. depending on which rack you load

25 Make sure the correct method is loaded, click NEXT

26 Make sure AUTOMATICALLY is selected, click NEXT

27 Make sure the GENERAL SEQUENCE SETTINGS match what you need and then click FINISH

28 A small pop-up window will then appear asking the project name. If adding to an existing project, make sure to select that correct folder.If creating a new project, type in the appropriate project name in OBJECT and click SAVE

29 The DATA window will then open for your project. This is where you will create your sequence

30 In the first sample spot, name it BLANK 1 and change the TYPE from the drop down arrow to BLANK and make sure the position is correct: RA1

31 Create a second sample line, name this appropriately for your first sample (sample 1), and change the TYPE from the drop-down arrow to UNKNOWN. Make sure the position is correct here as well, in our example, sample 01 is on RA2 position.

32 Continue creating your sequence in the 'blank, sample, blank, sample...' pattern until all of your samples are added. Make sure all of your positions are correct (RA1, RA2, RA1, RA3, RA1..) as well as sample type is correct as well (blank = blank, sample = unknown).

32.1 You will always start with a blank AND end with a blank. This will prevent carry over among samples.

33 When your sequence is complete, click START at the top of the window

34 Allow the sequence to run to completion. The HPLC will automatically stop when sequence is complete.

Saving data

35 Double click the first section on the sequence, UV_VIS_1, this will enlarge the chromatography specific to that sample and open the DATA PROCESSING page

36 When DATA PROCESSING is open, click REPORT DESIGNER in the bottom left corner

37 In REPORT DESIGNER, you can customize your chromatogram as much as needed: axis limits, peaks labels, retention time, peaks limits and so on.

38 Once you get your preferred design, click the green LIZARD head in the top left corner and then click EXPORT

39 This will open a new, small, pop-up window. in this window make sure CURRENT INJECTION is selected under the APPLY TO section

40 Make sure the parent folder matches your lab/where you need the data saved to and make sure export format = PDF

41 Finally, click SAVE and repeat for all remaining samples and blanks