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Nucleoside analysis with high performance liquid chromatography (HPLC) V.1

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We use this protocol and it's working

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Abstract

This protocol details the detection of modified nucleosides using HPLC.

Troubleshooting

Prepare samples and solutions

1 Prepare the following **standards** at 100 µM by dissolving each in a small volume of HPLC-grade methanol and bringing up the volume as necessary with MQ water. Filter each solution through a 0.22 µm syringe filter (we used SLGSV255F - Millipore-Sigma), and keep solutions at 4 °C prior to HPLC analysis.

Note

We purchased standards from Cayman Chemical Company — catalog numbers are listed in parentheses after each compound.

DNA standards:
2'-deoxyadenosine (27315); 2'-deoxyguanosine (9002864); 2'-deoxycytidine (34708); 2'-deoxyuridine (27803); thymidine (20519); 5-hydroxymethyl-2'-deoxyuridine (23381)

RNA standards:
adenosine (21232); guanosine (27702); cytidine (29602); 5-methyluridine (or ribothymidine) (27986); uridine (20300)

2 Prior to HPLC analysis, adjust the total **experimental nucleoside sample** volume to 100 µL by diluting with MQ water and then centrifuge each sample at 21000 × g for 15 min to remove any debris.

Note

In our initial experiments, we ran ~100 ng of sample per run (injecting 10 µL of the diluted 100 µL total volume). You may need to adjust this dilution to achieve sufficient signal depending on your sample composition.

STEP CASE

30 minute binary gradient 6 steps

Using a 30 minute-long binary gradient to resolve nucleoside peaks.

3 Prepare solutions A and B. Filter solution A through a 0.22 µm filter (we used 10040-440 VWR) and then adjust pH with 1 M HCl.

Note

Solution A: 20 mM ammonium acetate buffer, pH 5.4 (A2706-100ML Millipore-Sigma)

Solution B: HPLC-grade methanol (646377-4L Millipore-Sigma)

Run samples

4 Equilibrate the column with solution A. Once fully equilibrated, inject 10 µL of one of the **standards**.

Note

HPLC column: Thermo Scientific Hypersil ODS (C18) - length 250 mm, 4.6 mm ID, 3 µm particle size - cat. no. 30103-254630

Instrument: Agilent 1220 HPLC instrument (G4290C), VWD detector

5 Begin the gradient at 100% solution A, then ramp up to 25% solution B over 16 min, then ramp down to 0% solution B in 1 min, and finally stay at 0% solution B for 13 min (30 min total method time). Flow rate for the entire run should be 0.5 mL per min. Detect elution of compounds at 260 nm.

6 Run in triplicate to obtain an average retention time and a standard deviation. Repeat for each **standard**.

7 Make sure the HPLC column is re-equilibrated in solution A, then inject 10 µL of your **experimental sample**. Run the same program used for the standards. Perform in triplicate to obtain an average retention time and a standard deviation.

Analysis

8 Extract peak elution times from the HPLC software report. Common calculations can include calculating average elution time and standard deviation, if you have ran replicates. We also often plot the A_{260} signal to look at the shape of individual peaks.