Analysis of Sialic Acids by HPAEC-PAD

MATERIALS

- 4N Acetic Acid (HOAc)
- 50% NaOH (Fisher, SS254-1)
- 50% Isopropanol
- Sodium Acetate Trihydrate (NaOAc) (VWR, JT3462-1)
- Heat Block
- Glass Culture Tube, 13 x 100 mm, with Teflon lined Cap (Pyrex, 9826-13)

Preparation of 1L of 100 mM NaOH with 5 mM NaOAc:

- 0.7 g of Sodium Acetate Trihydrate
- 5.2 mL of 50% NaOH

Dissolve the Sodium Acetate Trihydrate in Milli-Q water that has been filtered through a 0.2 µm filter. Then add the 50% NaOH and bring the volume to 1 L. Thoroughly mix the solution and sparge the solution with Helium for 15 min to remove dissolved gases.

Preparation of 1L of 100 mM NaOH with 250 mM NaOAc:

- 34 g of Sodium Acetate Trihydrate
- 5.2 mL of 50% NaOH

Dissolve the Sodium Acetate Trihydrate in Milli-Q water that has been filtered through a 0.2 µm filter. Then add the 50% NaOH and bring the volume to 1 L. Thoroughly mix the solution and sparge the solution with Helium for 15 min to remove dissolved gases.

Preparation of 4 mL of 4 N Acetic Acid:

- 920 µL of Glacial Acetic Acid

In a glass vial dissolve 920 µL of glacial acetic acid in 3080 µL of Milli-Q water. Store at 4 ^{o}C

PROCEDURE:

1. Dissolve 500 µg of sample and dissolve in 200 µL of Milli-Q water and place in a glass reaction tube. If the analysis is to be performed on a liquid sample use no more than 200 µL for analysis.

2. To the sample add an equal volume of 4N HOAc, the final concentration of HOAc should be 2N, and tightly cap the reaction tube.

   NOTE: Avoid salt buffers such as Tris, NaCl, and NaOH as this can alter the separation and retention times of the sample components

3. Hydrolyze the sample on a heating block at 80 ^{o}C for 3hr.

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4. Once hydrolysis is complete remove the sample tubes from the heating block and allow them to cool to room temperature. Centrifuge the samples at 2000 rpm for 2 min to bring down any condensation on the sides and cap of the tube.

5. Next, evaporate the samples under a flow of dry nitrogen while applying a low amount of heat to the sample tubes.

6. When the samples are dry add 100 µL of 50% IPA, vortex and evaporate the samples a second time.

7. Resuspend the dried samples in Milli-Q water. Use a volume appropriate for the amount of sample that will be injected into the HPLC.

NOTE: If this is an unknown sample resuspend in 100 µL of Milli-Q water and inject up to 50% of the sample. Never inject 100% of the sample unless you know that it is a very small amount of material as a large amount of sample will hamper accurate quantitation of monosaccharides.

NOTE: It is essential to remove any protein from the sample using spin filtration to prevent contamination and blockage of the column, guard column and their inlet frits with protein.

HPAEC-PAD ANALYSIS OF SIALIC ACIDS:

Using Dionex ICS-3000

- Colum:
  - Dionex CarboPac PA1 column 4 mm x 250 mm, 4µm, with a 4 mm x 50 mm Guard
- Solvents:
  - A: Water
  - B: 100 mM NaOH with 5 mM NaOAc
  - C: 100 mM NaOH with 250 mM NaOAc
  - Initial conditions of 16% B at 1.0 mL/min
- Pulsed Amperometric Detector:
  - Waveform: Standard Quad
- Gradient Settings:

Time	%A	%B	%C
0	80	10	10
20	30	10	60
25	10	10	80
30	10	10	80
31	80	10	10
50	80	10	10

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