



DMB-Labeling and Analysis of Sialic Acids

MATERIALS

- 4,5-Methylenedioxy-1,2-phenylenediamine dihydrochloride (DMB) (Sigma, A89804) (Store in desiccator at -20 °C)
- Sodium Cyanoborohydride (Sigma, 156159)
- Dimethyl Sulphoxide (Sigma, 276855)
- 2-Mercaptoethanol (Sigma, M6250)
- 4N Acetic Acid (HOAc)
- Glacial Acetic Acid (HOAc)
- Sodium Hydrosulfite (Sigma, 157953) (Store in desiccator)
- Acetonitrile (Fisher, A998-4)
- 50% Methanol (Use HPLC Grade Methanol)

Preparation of 1 mL of 250 mM Sodium Hydrosulfite:

- 51.21 mg of Sodium Hydrosulfite

Dissolve the Sodium Hydrosulfite in 1 mL of Milli-Q water and vortex to mix. Store at -20 °C and though right before use as Sodium Hydrosulfite will decay in water.

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 °C

PROCEDURE:

1. Dissolve 50 µg of sample and dissolve in 100 µL of Milli-Q water and place in a 1 mL screw top microfuge tube. If the analysis is to be performed on a liquid sample use no more than 100 µL.
2. For Hydrolysis, add an equal volume of 4 N HOAc to give a final concentration of 2 N and place at 80 °C for 3 hours.
3. Following hydrolysis place the sample in a pre-washed 10k MWCO spin filter and spin at 14,000g for 20 min. Add 200 µL of Milli-Q water to the spin filter and centrifuge again.
4. Remove the filter from the spin filter and dry the samples under vacuum using a speed vac.
5. Re-suspend the dried samples in 50 µL of Milli-Q water and 50 µL of DMB reagent (See table below on how to prepare). Place the samples on a heat block at 50 °C for 2.5 hrs to derivatize the samples.



DMB Reagent Preparation	# of Samples				
	5	10	15	20	30
DMB (mg) (Stored at -20°C)	0.87	1.74	2.60	3.48	5.20
MilliQ H ₂ O (μL)	162.3	324.6	486.9	649.2	973.8
Glacial HOAc (μL)	44.3	88.6	132.9	177.2	265.8
2-Mercaptoethanol (μL)	29.1	58.2	87.3	116.4	174.6
0.25M Sodium Hydrosulfite (μL)	39.6	79.3	118.9	158.6	237.9
Total Volume	275	550	825	1100	1650

6. After derivatization allow the sample tubes to cool to room temperature and then place in a centrifuge and spin at 14,000 rpm for 1 min to bring down any condensation on the sides or top of the sample tube.
7. Samples must be stored away from light at -20 °C.
8. Fluorescence detection is very sensitive and 1 pmole of DMB-sialic acids can be easily detected. Injection of 0.2 μg of BSM I-S is sufficient to see sialic acids.

HPLC OF DMB LABELED SIALIC ACIDS:

- Colum:
 - Dionex Acclaim 120 C18 column 5 μm, 4.6 x 250mm with 4.6 x 10 mm Guard
- Solvents:
 - A: Milli-Q Water
 - B: Acetonitrile
 - C: 50% Methanol
 - Initial conditions of 79% A, 7% B, and 14% C at 1.0 mL/min
- Fluorescence Detector:
 - Excitation: 373 nm
 - Emission: 448 nm
 - Gain: 4
 - Sensitivity: Med
- Gradient Settings:

Time (min)	Water	ACN	50% MeOH
0	79%	7%	14%
40	75%	11%	14%
50	75%	11%	14%
60	40%	60%	0%
65	40%	60%	0%
67	79%	7%	14%
80	79%	7%	14%