SOP: SP022

Preparation of Alditol Acetate Derivatives

Materials and Reagents:

1. Sample, 20 to 50 μgs
2. Rhamose standard, 10 mg/ml in B & J water
3. Fucose standard, 10 mg/ml in B & J water
4. Ribose standard, 10 mg/ml in B & J water
5. Arabinose standard, 10 mg/ml in B & J water
6. Xylose standard, 10 mg/ml in B & J water
7. Mannose standard, 10 mg/ml in B & J water
8. Galactose standard, 10 mg/ml in B & J water
9. Glucose standard, 10 mg/ml in B & J water
10. Myo-inositol standard, 10 mg/ml in B & J water
11. Scyllo-inositol standard, 1 mg/ml in B & J water
12. Trifluoroacetic acid, concentrated, 1 ml ampule
13. Water, Burdick & Jackson HPLC-grade
14. Methanol, Burdick & Jackson HPLC-grade
15. Sodium borodeuteride ($NaBD_4$), solid
16. Ammonium hydroxide, concentrated
17. Ethanol, absolute
18. Acetic acid, glacial
19. Acetic anhydride, 2 ml ampule
20. Chloroform, Burdick & Jackson HPLC-grade
21. Savant speed-vac
22. 13 x 100 mm glass tubes (twice as many as number of samples plus standard)
23. 13 mm PTFE-lined lids (as many as number of samples plus standard)
24. Capillary pipettor, 0-100 μl
25. Glass capillary pipets, 100 μl
26. Heat block, 120^{o}C
27. Air bath
28. Glass Pasteur pipets
29. Rubber Pasteur pipet bulb
30. Dessicator
31. Vortex
32. Benchtop centrifuge
33. Capillary pipettor, 0-10 μl
34. Glass capillary pipets, 10 μl

Protocol:

1. _____ Transfer each sample into a cleaned 13 x 100 mm glass tube.

2. _____ Combine 25 μg of each sugar standard, except scyllo-inositol, in a new 13 x 100 mm glass tube.

3. _____ Completely dry all samples and the neutral sugar standard on the savant (note 1).

4. _____ Add 250 μl of 2M TFA to each sample and the standard (notes 2 and 3).

5. _____ Cap each tube tightly and place in 120^{o}C heat block for two hours.

6. _____ Remove samples from the heat block and let cool to room temperature.

7. _____ Add 10 μg of scyllo-inositol to each sample and the standard (note 4).

8. _____ Completely dry the contents of each tube on the air bath (note 5).

9. _____ Add approximately 100 µl of methanol to each sample (note 6).

10. _____ Completely dry on air bath.

11. _____ Repeat steps 9 and 10.

12. _____ Make $NaBD_4$ solution and add 250 µl to each sample (note 7).

13. _____ Cap each tube and let sit on benchtop overnight (note 8).

14. _____ Add two drops of glacial acetic acid to each sample (note 9).

15. _____ Add 200 µl of 10% acetic acid in methanol solution to each sample (note 10).

16. _____ Dry completely on air bath.

17. _____ Repeat steps 15 and 16.

18. _____ Add approximately 100 µl of methanol to each sample (note 6).

19. _____ Completely dry on air bath.

20. _____ Repeat steps 18 and 19.

21. _____ Add 100 µl of acetic anhydride from ampules to each sample.

22. _____ Cap each tube and heat at 120^{o}C for two hours in a heating block.

23. _____ Remove samples from the heat block and let cool to room temperature.

24. _____ Completely dry on air bath.

25. _____ Add 1 ml of water to each sample.

26. _____ Add 2 ml of chloroform to each sample.

27. _____ Cap each tube and mix by vortexing vigorously.

28. _____ Centrifuge at 2,500 x g, 4^{o}C for five minutes.

29. _____ Transfer the lower, organic layer from each sample into new 13 x 100 mm glass tubes and discard water layer (note 11).

30. _____ Completely dry on air bath.

31. _____ Sample is now ready for GC analysis.

Notes:
1. See SOP SP005 for use of savant
2. Use a fresh ampule of concentrated trifluoroacetic acid (TFA) to make 2M TFA for each alditol acetate preparation. Concentrated TFA is 12.98 M; use 153 µl of acid to every 847 µl of water to make each 1 ml of 2M TFA. Make in a glass container.
3. Use only glass capillary pipets to transfer liquids from this step on, as the GC will detect plastic components from Pipetman tips.

4. Scyllo-inositol is used as an internal standard for the GC to calculate the amount of neutral sugar in the sample.
5. See Air Bath SOP SP031.
6. This approximation is five drops from a glass Pasteur pipet with a rubber bulb. It is not necessary to add a specified amount of methanol, only enough to saturate the sample and remove any residual water from the sample.
7. The $NaBD_4$ solution is 10 mg of $NaBD_4$ in 1 ml of 1 M NH_4OH in ethanol, and must be freshly made prior to use. $NaBD_4$ is extremely hygroscopic and must be kept in a dessicator. 1 M NH_4OH in ethanol must be made fresh every two months. To make, add 6.6 ml of concentrated NH_4OH to 93.4 ml of absolute ethanol; as NH_4OH is caustic, make in a chemical fume hood.
8. The reduction reaction is complete in one hour, but overnight reduction provides the best results.
9. The addition of glacial acetic acid should cause the sample to bubble and fizz, indicating the required excess of reducing agent was present.
10. Make 10 % acetic acid in methanol as any other standard v/v solution; glacial acetic acid is caustic, so make in chemical fume hood.
11. It is important not to contaminate the organic layer with debris from the water layer. To do this, expel several drops of air while passing the Pasteur pipet through the water layer until the tip is in the organic layer. It is better to leave a small amount of organic layer in the tube than to risk water contamination.

Reference:

McNeil, M., D. Chatterjee, S. W. Hunter, and P. J. Brennan. 1989. Mycobacterial glycolipids: isolation, structures antigenicity and synthesis of neoantigens. Methods Enzymology. 179: 215-242.