SOP: SP022.4
Modified: 8/7/19 by DH

Preparation of Alditol Acetate Derivatives

Materials and Reagents:

1. Samples
2. Rhamose standard (note 1)
3. Fucose standard
4. Ribose standard
5. Arabinose standard
6. Xylose standard
7. Mannose standard
8. Galactose standard
9. Glucose standard
10. Myo-inositol standard
11. Scyllo-inositol standard
12. Trifluoroacetic acid, concentrated
13. Water, Burdick & Jackson HPLC-grade (MilliQ, ETF)
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
20. Chloroform, Burdick & Jackson HPLC-grade
21. Savant speed-vac
22. 13 x 100 mm glass tubes w/ lids (twice as many as number of samples plus standard)
23. Capillary pipettor, 0-100 µl
24. Glass capillary pipets, 100 µl
25. Heat block, 120°C
26. Air bath
27. Glass Pasteur pipets
28. Rubber Pasteur pipet bulb
29. Dessicator
30. Vortex
31. Countertop centrifuge
32. Capillary pipettor, 0-10 µl
33. Glass capillary pipets, 10 µl

Protocol:

1._____ Clean the 13x100 mm glass tubes with acetone and let air dry.

2._____ Transfer sample into a cleaned 13 x 100 mm glass tube (note 2). Run samples in triplicate.

3._____ Combine 25 µg of each sugar standard, except scyllo-inositol, in a new 13 x 100 mm glass tube. Run standards in triplicate for LLP, duplicate for $PIM_{1,2}$.

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

5._____ Add 250 µl of 2M TFA to each sample and the standard (notes 4 and 5).

6._____ Cap each tube tightly and place in 120°C heat block for two hours.

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

8._____ Add 10 µg scyllo-inositol to each standard and sample (note 6).

9._____ Spin all tubes briefly in clinical centrifuge at 3K rpm to pull down solutions.

10._____ Completely dry the contents of each tube on the air bath (note 7).

11._____ Add 200 µl of methanol to each sample.

12._____ Completely dry on air bath.

13._____ Repeat steps 11 and 12.

14._____ Make sodium borodeuteride solution and add 250 µl to each sample (notes 8 and 9).

15._____ Cap each tube and let sit on benchtop for at least 2 hours. This step can go overnight and is a good stopping point for the day (note 10).

16._____ Add two drops of glacial acetic acid to each sample (note 11).

17._____ Add 200 µl of 10% acetic acid in methanol solution to each sample (note 12).

18._____ Dry completely on air bath.

19._____ Repeat steps 17 and 18.

20._____ Add approximately 500 µl of methanol to each sample (note 13).

21._____ Completely dry on air bath.

22._____ Repeat steps 20 and 21.

23._____ Add 200 µl of acetic anhydride to each sample. Centrifuge as in step 9.

24._____ Cap each tube and heat at 120°C for two hours in a heating block.

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

26._____ Completely dry on air bath.

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

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

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

30._____ Centrifuge at 3K rpm, 4°C for 5 minutes.

31._____ Transfer the lower, organic layer from each sample into new acetone washed 13 x 100 mm glass tubes. Discard water layer (note 14).

32._____ Completely dry on air bath.

33._____ Sample is now ready for GC analysis. Resuspend each in 200 µl chloroform and transfer to appropriate vials, containing 250 µl capacity spring or molded glass inserts.

Notes:

1. All standards are 1 mg/ml in endotoxin-free (ETF) water.
2. Use 100-200 μg for confirmation of $PIM_{1,2}$. See recent LAM/LM/PIM6 work-ups to know what volumes of LLP to derivatize.
3. See SOP SP005 for use of savant
4. Use 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.
5. Use only glass capillary pipets to transfer liquids from this step on, as the GC will detect plastic components from Pipetman tips.
6. Scyllo-inositol is used as an internal standard for the GC to calculate the amount of neutral sugar in the sample.
7. See Air Bath SOP SP031.
8. The sodium borodeuteride solution is 10 mg of sodium borodeuteride in 1 ml of 1 M ammonium hydroxide in ethanol. Briefly bath sonicate to get into solution.. Sodium borodeuteride is extremely hygroscopic and must be kept in a desiccator. Make the solution fresh each time.
9. 1 M ammonium hydroxide in ethanol must be made fresh every two months. To make, add 6.6 ml of concentrated ammonium hydroxide to 93.4 ml of absolute ethanol; as ammonium hydroxide is caustic, make in a chemical fume hood.
10. The reduction reaction is complete in one hour, but overnight reduction provides the best results.
11. The addition of glacial acetic acid should cause the sample to bubble and fizz, indicating the required excess of reducing agent was present.
12. Make 10% acetic acid in methanol as any other standard v/v solution; glacial acetic acid is caustic, so make in chemical fume hood.
13. To get precipitates into solution, flick each tube a few times.
14. 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.