



TMS Derivatization and Analysis by GC-MS

MATERIALS

- 3 M Methanolic-HCl (Supelco, 33355)
- Methanol (Sigma, 322415)
- Acetic Anhydride (Sigma, 539996)
- Pyridine (Sigma, 270970)
- Tri-Sil HTP Reagent (Pierce, TS-48999)
- 1 mg/mL solution of Standard Sugars: Rhamnose, Fucose Ribose, Arabinose, Xylose, Mannose, Galactose, Glucose, N-acetyl Glucosamine, N-acetyl Galactosamine, N-acetyl mannosamine, Glucuronic acid, Galacturonic acid.

1 M Methanolic-HCl:

- Slowly dissolve 1 mL of 3 M Methanolic-HCl with 2 mL of cold Methanol on ice in a glass screw-top tube.

Standard Preparation:

- To prepare the TMS standard place 10 µg of each sugar, into a glass sample tube along with 1 µg of myo-inositol, as an internal standard and lyophilize.

Sample Preparation:

- 100 µg of sample is taken in a glass screw cap tube and 1 µg of myo-inositol is added and lyophilize.

PROCEDURE

1. Add 200 µL of 1 M Methanolic-HCl to the sample and tap gently to mix. Tighten the screw cap, and incubate overnight (16-18 hr) at 80 °C.
2. Cool the sample to room temperature, and then remove the Methanolic-HCl by dry nitrogen flush, and then add 100 µL of methanol to remove residual Methanolic-HCl and bring the sample to dryness using nitrogen flush.
3. Next re-N-acetylate the samples by adding 4:1:1 Methanol:Pyridine:Acetic Anhydride to a final volume of at least 200 µL. Tightly cap the tubes and incubate at 100 °C for 1 hr.
4. Cool the sample again to room temperature and bring to dryness using dry nitrogen flush.
5. Derivatize samples with the addition of 200 µL of Tri-Sil HTP Reagent, recap and heat at 80 °C for 30 min. Cool down samples and remove excess Tri-Sil HTP by dry the samples by dry N₂ flush.



6. Add 1 mL of hexane to each sample and then vortex and sonicate to break up the dried sample. Next, centrifuge the sample for 2 min at 2000 rpm.
7. Filter the sample through a Pasteur pipette packed with glass wool. Prepare the glass wool by first washing with a small amount of hexane.
8. Collect the filtrate and remove the hexane by dry nitrogen flush.
9. Reconstitute the samples in 100-150 μ L of dichloromethane and transfer into a sample vial for analysis by GC-MS.

GC-MS SETTINGS:

Carrier Gas: Helium

Inlet Conditions:

- Temperature: 220 °C
- Pressure: 12.897 psi
- Flow: 22.99 mL/min

GC-MS Transfer Line Temp: 280 °C

Column: DB-5 or Equivalent, 30 m x 0.25 mm x 0.25 μ m

Column Flow: 1.1971 mL/min

Injection Volume: 1 μ L

Run Time: 45.667 min

Conditions	$^{\circ}$ C/min	$^{\circ}$ C	Hold Time (min)	Run Time (min)
Initial		100	0	0
Ramp1	5	120	1	5
Ramp2	3	230	4	45.667