



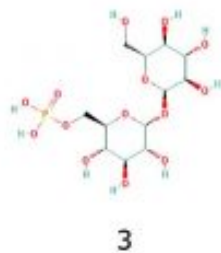
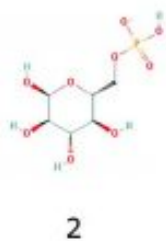
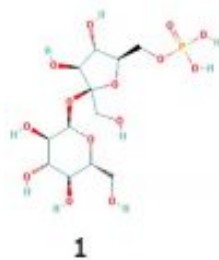
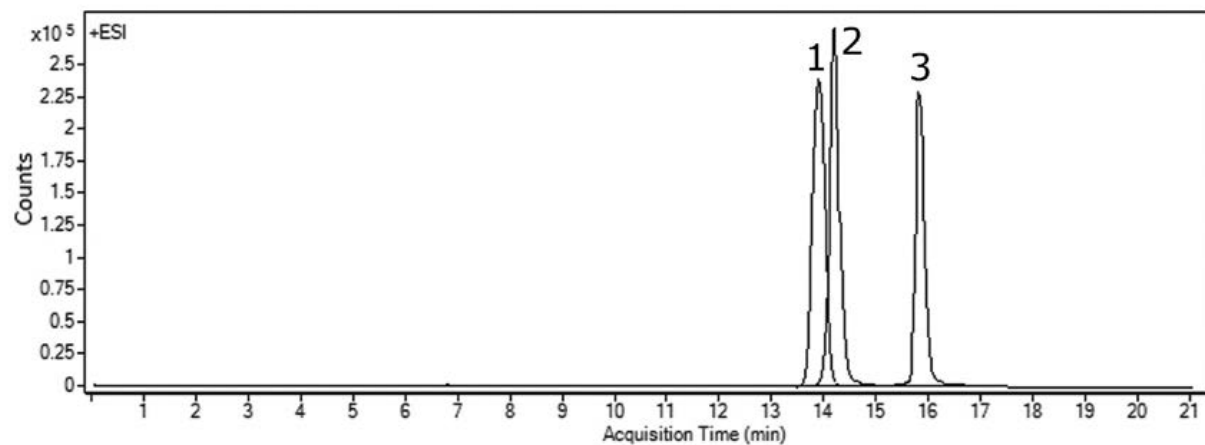
Phosphorylated Sugars Analyzed by LCMS - AppNote

Sucrose-6-phosphate, Glucose-6-phosphate, & Trehalose-6-phosphate

Central Metabolic Intermediates in Plants

Sugar Phosphates are very highly polar Metabolites and may be difficult to retain using traditional Reversed Phase Methods. In addition, S6P and T6P are Isobaric Compounds so it is very important to separate these two Phosphorylated Sugars for MS Detection. G6P is easily distinguished by its mass to charge ratio (m/z).

These three Phosphorylated Sugars were separated using this Method even though the separation is quite challenging, because the structures of two out of three compounds are very similar. Note that a very shallow Gradient is used in this separation.



Peaks:

1. S6P: sucrose-6-phosphate, 421.0753 m/z [M-H]⁻
2. G6P: glucose-6-phosphate, 259.0224 m/z [M-H]⁻

3. T6P: trehalose-6-phosphate, 421.0753 m/z [M-H]-

Method Conditions

Column: Cogent Diamond Hydride™, 4µm, 100Å

Catalog No.: 70000-15P-2

Dimensions: 2.1 x 150mm

Mobile Phase:

A: DI Water / 10 mM Ammonium Acetate, pH=6.0

B: 90% Acetonitrile / 10% DI Water / 10 mM Ammonium Acetate, pH=6.0

Gradient:

Time (minutes)	%B
0	85
3	85
13	80
15	80
18	75
20	60
21	85



Flow rate: 0.4 mL/minute

Detection: ESI - NEG - Agilent 6210 MSD TOF Mass Spectrometer

Injection vol.: 1 μ L

Sample Preparation: 15 μ M G6P, 20 μ M S6P, and 20 μ M T6P were prepared in 10% DI Water / 10% Methanol / 80% Acetonitrile

t₀: 0.9 minutes

Note: Starch produced by Plants is an essential material in the human diet. T6P has a function in promoting biosynthetic processes of Starch in Plants, but the exact mechanism is still unknown. The ability to quantify levels of T6P in Plant tissue is of crucial importance in studies of the regulatory role of T6P in Carbon use. The low quantity of T6P in plant matrices makes its detection and quantification a very challenging analytical problem. The developed method can be used in studies of biosynthetic processes.



Attachment

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