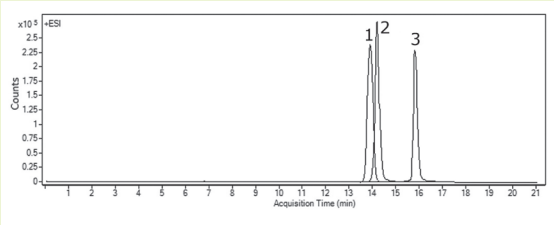
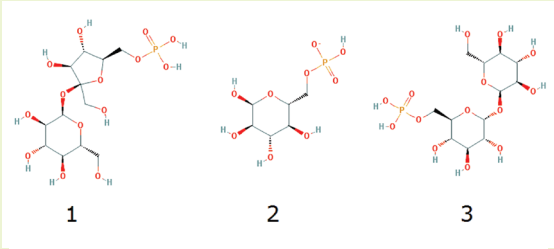
Phosphorylated Sugars: S6P, G6P, and T6P

Central metabolic intermediates in plants





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.

Method Conditions

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

Catalog No.: 70000-15P-2

Dimensions: 2.1 x 150 mm

Solvent: A: DI H_2O / 10 mM ammonium acetate, pH=6.0
B: 90% acetonitrile / 10% DI H_2O / 10 mM ammonium acetate, pH=6.0

Gradient:

time (min.)	%B
0	85
3	85
13	80
15	80
18	75
20	60
21	85

Injection vol.: 1 µL

Flow rate: 0.4 mL/min

Detection: ESI – NEG - Agilent 6210 MSD TOF mass spectrometer

Sample: 15 microM G6P, 20 microM S6P, and 20 microM T6P were prepared in 10% DI H_2O / 10% methanol / 80% acetonitrile

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 $[M-H]^-$

t_0: 0.9 min

Discussion

Sugar phosphates are very highly polar metabolites and may be difficult to retain using traditional reversed phase columns. In addition, S6P and T6P are isobaric compounds so it is very important to separate these two phosphorylated sugars. G6P is easily distinguished by its m/z. The three phosphorylated sugars were separated when the Cogent Diamond Hydride column was used. 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. The developed method can be used in studies of biosynthetic processes.

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