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**Shodex™**



**HPLC Columns**

# PHOSPHORYLATE SUGARS – SHODEX HILICPAK VT-50



## HILIC Columns for Phosphorylated Sugar Analysis

Various types of phosphorylated saccharides are involved with glycolysis, glyconeogenesis, glycometabolism, and other naturally occurring biological phenomena. One example of a phosphorylated saccharide includes fructose-2,6-bisphosphate the most powerful activator of phosphofruktokinase. Recent research has shown the phosphorylated saccharide intermediates created during glyconeogenesis and glycometabolism can be used in other ways than naturally intended for a number of applications, drastically broadening the interest in phosphorylated saccharide.

Phosphorylated saccharides occupy a central position in carbohydrate metabolism. Numerous sugar interconversions occur when the sugars are linked to a phosphates and di-phosphates. When this occurs, the phosphorylated saccharides can function as glycosyl donors in many transglycosylation reactions, providing a variety of oligosaccharides, glycosides, and polysaccharides.

Phosphorylated sucrose and fructose have been synthesized by the transglycosylation process in plants, glycogen in animal tissues, and chitin. The isolated phosphorylated sucrose has further shown to potentially be effective in experimental drugs. Depending upon which carbon contains the phosphate group, directly effects how the new drugs interact within the body. Phosphorylated fructose has been recently suggested to be a part of the creation of ATP.

While phosphorylated mannose and glucose were found to be potent inhibitors of lysosomal hydrolase endocytosis by fibroblasts, as well as isolated from plants and bacteria. It was only just proposed that phosphorylated mannose is an essential component of the recognition marker on acid hydrolases from the direct evidence for phosphorylated mannose's ability on high uptake forms of lysosomal enzymes. With the knowledge of these few applications, the field has currently been exponentially growing.

Four variations of phosphorylate saccharides were analyzed by a Shodex HILICpak VT-50 2D column under LC-MS conditions. The sample contained two phosphorylated glucose and two phosphorylated fructose, each phosphorylated at a different carbon, providing different biological functions. This analytical

condition can also be used with other detectors including RI, ELSD, and corona CAD.

### Experimental Conditions:

The analysis of glucose-6-phosphate, fructose-6-phosphate, glucose-1-phosphate, and fructose-1-phosphate was accomplished using the Shodex HILICpak VT-50 2D (2.0 mm ID x 150 mm ID, 5 $\mu$ ) a HILIC column suitable for LC/MS. The column temperature was 60 oC and flow rate was 0.3 mL/min. The eluent conditions were 25mM HCOONH<sub>4</sub> aq./CH<sub>3</sub>CN=80/20. An injection volume of 5 $\mu$ L of 1 $\mu$ M of each sugar was used for the experiment. The HPLC system was coupled with an ESI-MS (SIM Negative: m/z 259) detector.

### Results:

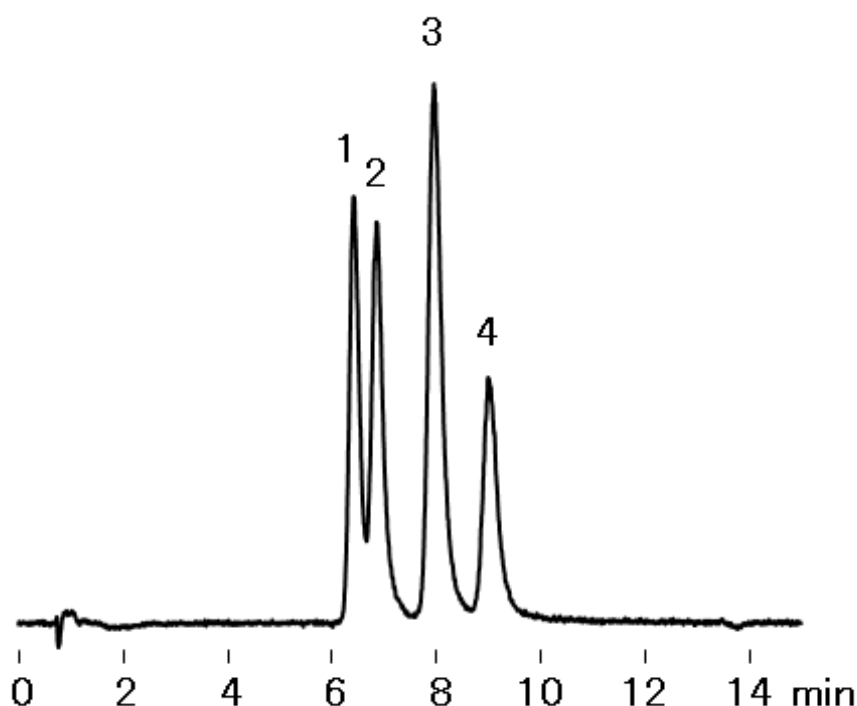
The aqueous sample containing the four phosphorylated sugars was analyzed

successfully using HILIC and MS detection with Shodex HILICpak VT-50 2D (Figure 1). From this single run, each phosphorylated saccharide was prominently separated. The closest peaks were glucose-6-phosphate and fructose-6-phosphate, both eluting between 6-7 minutes.

### Conclusions:

Shodex HILICpak VT-50 2D, a Hydrophilic Interaction Chromatography (HILIC) column is suitable for anionic substance and phosphorylated saccharide analysis, LC/MS detection. The polymer-based packing material provides excellent chemical stability and minimum deterioration over extended periods of time.

Figure 1. The Analysis of Phosphorylated Saccharides using Shodex VT-50 2D.



Column: HILICpak VT-50 2D,  
Column temperature: 60 oC,  
Injection volume: 5  $\mu$ L, Eluent:  
25mM HCOONH<sub>4</sub> aq./CH<sub>3</sub>CN :  
80/20, Flow rate: 0.3 mL/min.  
Detector: ESI-MS (SIM Negative:  
m/z 259). Sample: 1-and 6-  
phosphorylated saccharides.

1. Glucose-6-phosphate (G6P)
2. Fructose-6-phosphate (F6P)
3. Glucose-1-phosphate (G1P)
4. Fructose-1-phosphate (F1P)

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