# Differentiating Isomers using High Resolution Mass Spectrometry Coupled with MS$^n$, Collision Induced Dissociation, and Ultraviolet Photodissociation

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## ABSTRACT

**Purpose:** Differentiating isomers poses an analytical challenge, especially if they cannot be resolved chromatographically. While tandem mass spectrometry can distinguish structurally distinct isomers based on unique fragments, there isn't always a Higher-energy Collisional Dissociation (HCD) fragment that is diagnostic when the structures are similar. Here we demonstrate how using MS$^n$ fragmentation, Collision Induced Dissociation (CID), and Ultraviolet Photodissociation (UVPD) can be used to obtain diagnostic fragments for structurally similar isomers.

**Methods:** A set of six sugar phosphate isomers: glucose-1-phosphate, galactose-1-phosphate, glucose-6-phosphate, galactose-6-phosphate, mannose-6-phosphate and fructose-6-phosphate were analyzed individually by LC-MS. Spectra were collected using HCD, CID, MS$^n$, and UVPD then evaluated for diagnostic fragments.

**Results:** Each of the sugar-phosphate standards exhibited at lest one fragment unique to that isomer at that RT.

## INTRODUCTION

Sugar phosphates, sugar molecules with a covalently bound phosphate, are an important intermediate in carbohydrate metabolism and a building block for oligonucleotides. This class of compounds can be structurally very similar with poorly resolved chromatograms and similar mass spectra making characterizing what compounds are present difficult. In this work we use a range of different fragmentation options available on the Thermo Scientific™ Orbitrap™ IQ-X™ Tribrid™ mass spectrometer to investigate individual sugar phosphate standards to identify unique fragments that are diagnostic of a specific compound.

## MATERIALS AND METHODS

**Materials:**

Each sugar (glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, mannose-6-phosphate, galactose-1-phosphate and galactose-6-phosphate from Sigma-Aldrich) was prepared at 1mM in 60:40 Acetonitrile:Water as well as a mixture of all six sugar standards at 0.5 mM in quenched plasma (plasma after an acetonitrile protein crash).

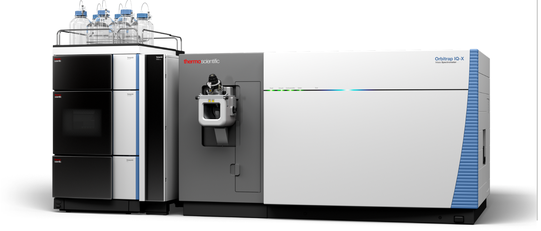
**Test Method:**

LC-MS: All samples were run on a Thermo Scientific™ Vanquish Horizon™ LC system using a ZIC - pHILIC column (Sigma Aldrich) with a mobile phase of A : 5mM Ammonium carbonate+0.1% NH4OH in 100% Water, B : 100% Acetonitrile using a Gradient of 80% ACN to 20% in 20 mins. The detector was a Thermo Scientific Orbitrap IQ-X Tribrid mass spectrometer run in positive ion mode using DDA MS$^n$, HCD, CID, and UVPD fragmentation.

**Data Analysis:**

Chromatograms and mass spectra were processed using Thermo Scientific™ Freestyle™ software. Structural predictions of the fragments were generated using Thermo Scientific™ Mass Frontier™ software.

**Figure 1. Thermo Scientific Horizon Vanquish LC and Orbitrap IQ-X mass spectrometer**

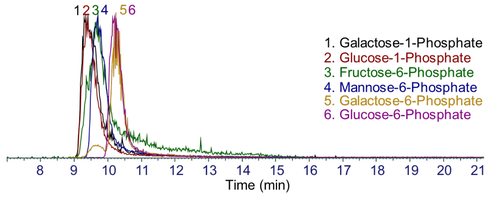


## RESULTS

**Chromatography**

Running each standard individually, it was found that four of the six sugar phosphates eluted with significant chromatographic overlap while the remaining two eluted at a slightly later retention time. In each case, a peak at for the protonated (m/z 261.0370) and sodiated adducts (m/z 283.0189) was observed.

**Figure 2. Overlaid XIC chromatogram for the six protonated sugar phosphate standards**




**Ultraviolet Photodissociation diagnostic peaks**

UVPD spectra for each standard were investigated for peaks that were diagnostic, present for only one standard at that retention time. The first four standards were compared for similar peaks as were the last two that eluted after 10 minutes.

**Figure 3. Diagnostic UVPD fragment for protonated adduct of galactose-1-phosphate**

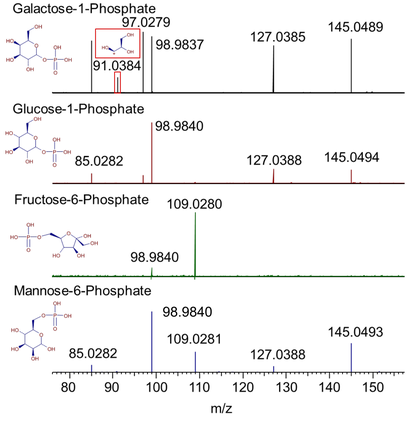



**Figure 4. Diagnostic UVPD fragment for sodiated adduct of glucose-1-phosphate. The region of importance has been scaled up for visibility.**

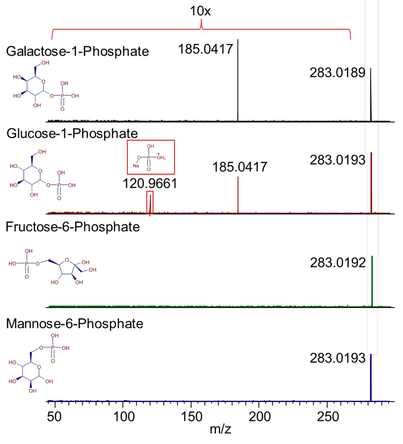
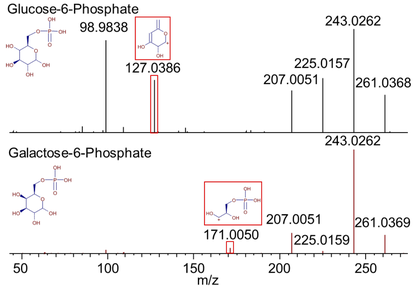



**Figure 5. Diagnostic UVPD fragments for proton adduct of glucose-6-phosphate and galactose-6-phosphate.**




**MS$^n$ CID diagnostic peaks**

HCD MS$^2$ and CID MS$^3$ spectra for each standard were investigated for peaks that were diagnostic for fructose-6-phosphate and mannose-6-phosphate.

**Figure 6. Diagnostic HCD MS$^2$ fragments for sodium adduct of fructose-6-phosphate and mannose-6-phosphate.**

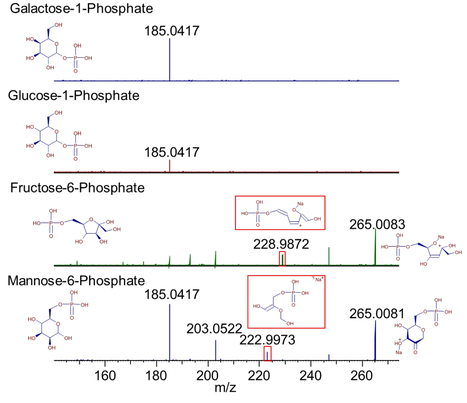
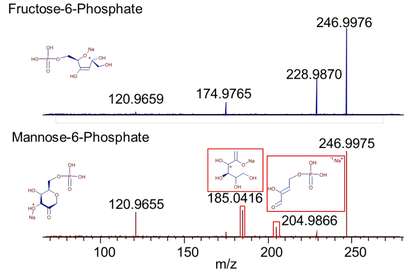



**Figure 7. Diagnostic CID MS$^3$ fragments of the MS$^2$ precursor at m/z 265.0081 from mannose-6-phosphate.**




**Analysis of sugar phosphate mixture in plasma**

The six sugar phosphate standards were spiked into a sample of plasma in order to show that the indicated diagnostic fragments could be observed when multiple isomers are present and co-isolated in a complex matrix using the ion trap as a mass detector with increased sensitivity.

**Figure 8. Diagnostic UVPD MS$^2$ fragment collected in the ion trap for sodium adduct of glucose-1-phosphate. The region of importance has been scaled up for visibility.**

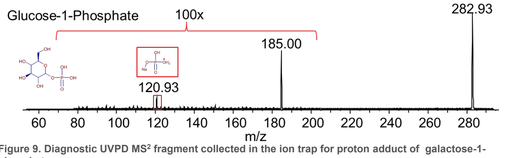
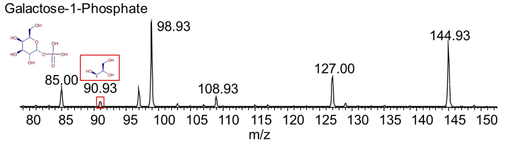



**Figure 9. Diagnostic UVPD MS$^2$ fragment collected in the ion trap for proton adduct of galactose-1-phosphate.**




## CONCLUSIONS

Six isomeric sugar phosphate compounds were investigated using HCD, MS$^n$, CID, and UVPD fragmentation for diagnostic fragments

- Four coeluting isomers as well as a second set of two coeluting isomers could be clearly differentiated using the fragmentation options available on the Orbitrap IQ-X
- Even when mixed together in a complex matrix, these diagnostic fragments were visible using the high sensitivity of the ion trap

## ACKNOWLEDGEMENTS

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## TRADEMARKS/LICENSING



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