Trapped ion mobility separation (TIMS) of Glucose-6-Phosphate and Fructose-6-Phosphate

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Introduction

Evaluating central carbon metabolism in living systems is challenging due to the dynamic range of metabolite concentrations. Among sugars and organic acids, phosphorylated sugars glucose-6-phosphate (G6P) and fructose-6-phosphate (F6P) are biologically relevant isomeric compounds. Although there are effective chromatographic separation methods [1], it is often difficult to separate in high throughput studies. In this study, G6P and F6P are rapidly separated based on ion mobility on a Bruker timsTOF Pro 2. Trapped ion mobility spectrometry (TIMS) enables rapid separation of isomeric species based on size and shape in the gas phase for high-throughput applications

Here we applied ion mobility separation using TIMS as a 4th dimension of separation and annotation tool using collisional cross section (CCS). Each metabolite was isolated with baseline mobility peak separation of G6P and FP6 with definitive CCS values. This enables rapid identification and quantitation of G6P And F6P in biological matrices using rapid flow injection analysis and short chromatographic gradients

Methods

G6P and F6P were purchased from Sigma-Aldrich (St Louis, MO, USA), LC -MS grade water was purchased from Fisher Scientific. A 1 mg/mL stock solution was prepared in LC-MS grade water further diluted to 10 µg/mL working solution. Data was acquired with a Bruker Elute UHPLC on Phenomenex Kinetex F5 (150 x 2.1 mm, 2.6 µm, 100Å) with column temperature at 40ºC. Mobile phase A was 0.1% formic acid in H_2O and B was 0.1% formic acid in acetonitrile. Gradient conditions are 0% B at 1 to 2 min, up to 95% at 8 min with a 2 min hold and equilibrated for 5 min on a VIP-HESI timsTOF Pro 2 with TIMS enabled in ESI negative mode. A previously established 4D-Metabolomics method with a mass range of 20 – 1300 m/z and mobility range of $1/K_0$ 0.55 – 0.85 V·s/cm^2 and ramp time of 300 ms was used. Other acquisition parameters were further optimized by adjusting ion mobility resolution mode, accumulation time, duty cycle. Mass and CCS calibration were performed prior to data analysis. Data processing was conducted in Bruker Data Analysis 5.3 software.

Results

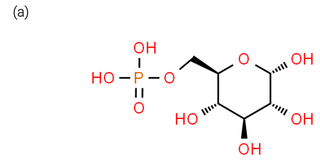

Figure 1. Structures of (a) Glucose-6-Phosphate (b) Fructose-6-Phosphate

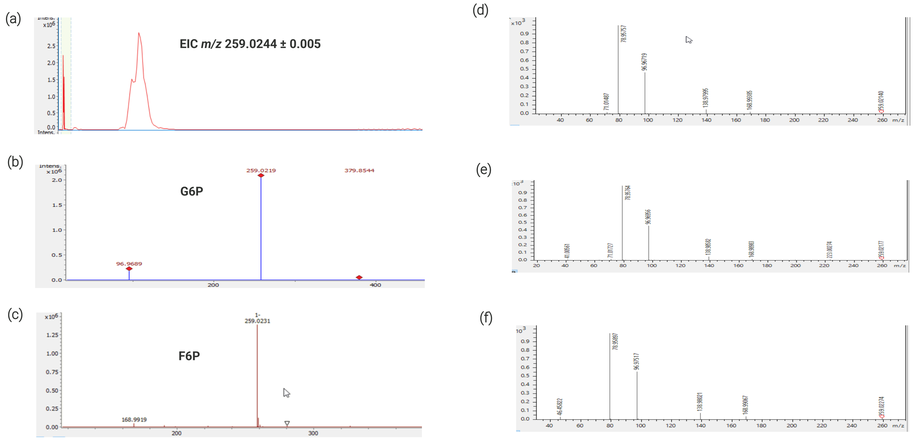

Figure 2. Results of (a) Extracted ion chromatogram of G6P and F6P mixture, (b) mass spectra of G6P alone, (c) mass spectrum of F6P alone (d) MS/MS spectrum of G6P and F6P mixture (e) MS/MS spectrum of G6P (f) MS/MS spectrum of F6P.

The sugar phosphate reference standard mixture using LC-VIP-HESI-timsTOF Pro 2 in negative mode co-elute chromatographically yet had baseline mobility peak separation and therefore can be distinguished. The average CCS deviation is 1.6% compared to reported reference values in the CCS Compendium [2].

Table 1. CCS value comparison to published refence data

Name	[M-H]$^-$	Mobility $1/K_0$	CCS (Å) measured	CCS (Å) reported	CCS (Å) Error %
Glucose-6-phosphate	259.0244	0.697	148.7	146.7	1.3
Fructose-6-phosphate	259.0244	0.707	146.9	144.3	1.8

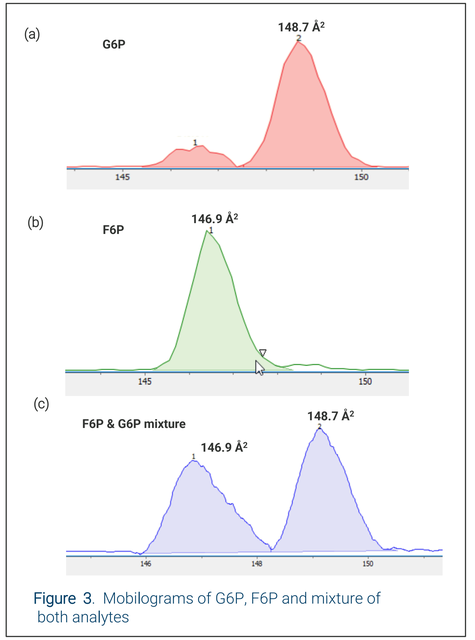

Figure 3. Mobilograms of G6P, F6P and mixture of both analytes

Discussion

Glucose transported into cells undergoes phosphorylation by the enzymes hexokinase and glucokinase. G6P can be shuttled into two major metabolic pathways, glycolysis and the pentose phosphate pathway (PPP). Alternatively, glucose can be converted to fructose and phosphorylated to F6P or converted to glycogen as a mode of energy storage.

Since G6P and F6P are intermediate metabolites in glycolysis and gluconeogenesis that have distinct roles in cellular metabolism, it is important to accurately and sensitively identify them in a wide variety of biological matrices. As both metabolites play a significant role in cellular homeostasis, accurately identifying and quantifying them is crucial in understanding dysregulated metabolic pathways, such as those observed in cancer [3].

However, G6P and F6P have **identical molecular weight and MS/MS fragmentation peak masses (Fig.2)**. It is challenging to separate them based solely on the conventional 3D methods of retention time, accurate mass and fragmentation pattern. Here we applied a **4D-TIMS method for separation and annotation using CCS (Fig.3)**. Each metabolite was isolated using ion mobility, with base peak separation of G6P and FP6 with CCS values of 148.7 Å^2 and 146.9 Å^2, respectively. Hence, we demonstrated fast separation of G6P and F6P based on their size and shape.

References

[1] Wang, Y., et al., "Baseline Separation of Six Hexose Phosphate Isomers by Liquid Chromatography-Mass Spectrometry from Tissues" Bio-protocol Preprint, 2022, bio-protocol.org/prep1541.

[2] Picache, J.A., et al, "Collision cross section compendium to annotate and predict multi-omic compound identities", Chem. Sci., 2019, 10, 983-993.

[3] Song J., et al., "The Multiple Roles of Glucose-6-Phosphate Dehydrogenase in Tumorigenesis and Cancer Chemoresistance", Life, 2022, 12, 271.

Conclusion

- TIMS-MS based method for separation of Glucose-6-phosphate and Fructose-6-phosphate
- Mobilgrams can provide relative quantitation of isoforms in biological matrices .
- This method is compatible with direct infusion or short LC gradients
- Base peak separation of isoforms will distinguish between two analytes by comparing to refence CCS values reported in database or can be validated with refence standards

LC-TIMS-MS/MS Isomer Separation