

Elucidating the Fine Structure of Fructan Isomers in Agave using Cyclic Ion Mobility Mass Spectrometry

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Introduction

Blue agave plants store a complex mixture of fructose oligo- and polysaccharides (FOS and fructans). For each Degree of Polymerization (DP), there is a variety of isomers. These compounds are related to stress tolerance and serve various industrial purposes, including tequila production. Due to their complexity, studies using chromatographic methods have been limited. The Collision Cross Section (CCS) –derived from Ion Mobility Mass Spectrometry (IMS)– is a physicochemical parameter that depends on an ion's size and structure. It has been successfully employed to distinguish between isomeric molecules. In this work, we compare experimental CCS values (CCSe) obtained from agave fructans with predicted CCS values (CCSt). Our findings indicate that with the CCS, we can identify the structures of fructan isomers.

Schematic

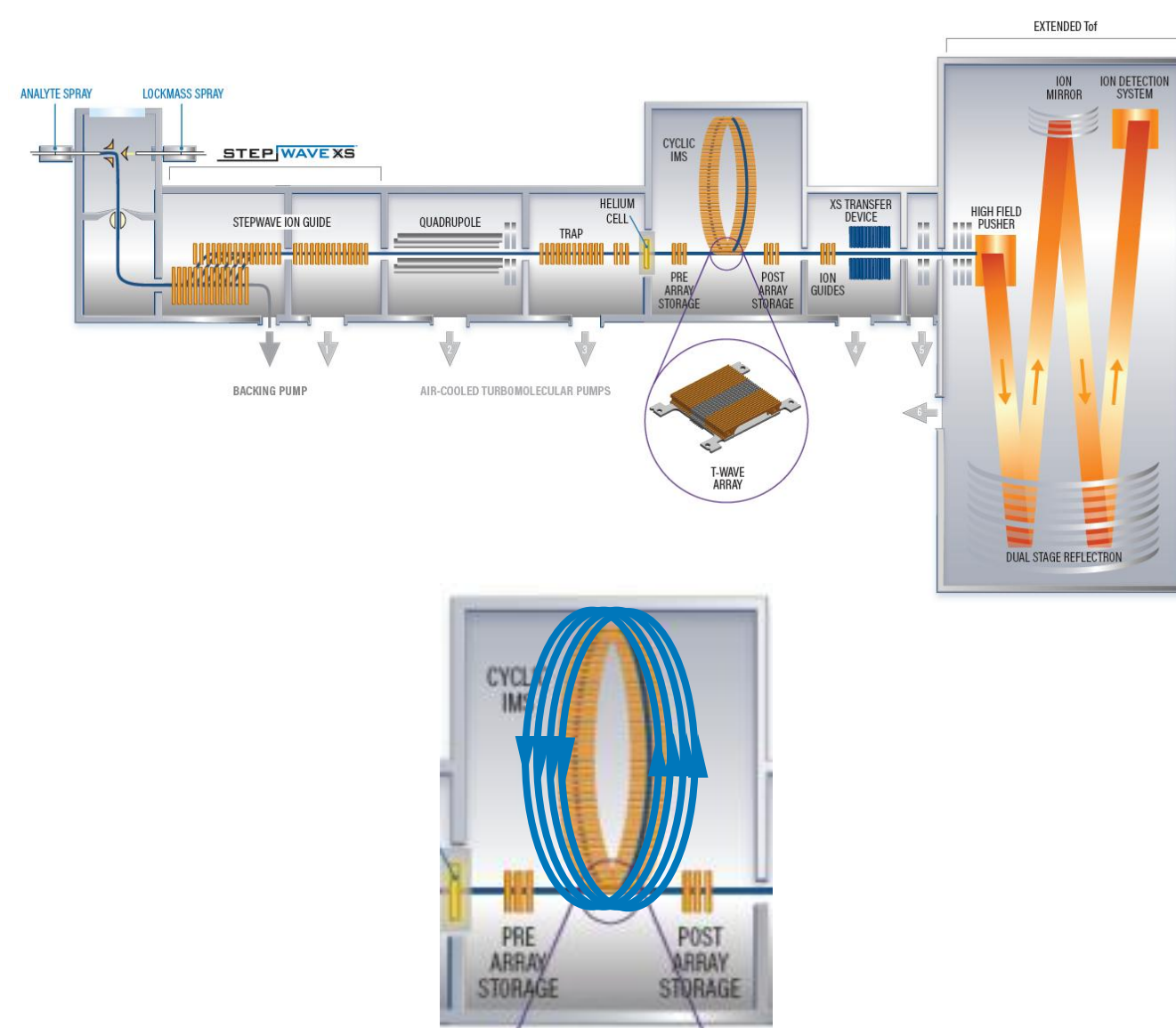


Figure 1. Schematic of the SELECT SERIES Cyclic IMS and its ion optics; schematics of a multipass experiment.

Methods

Agave fructans were water-extracted from a 6-year-old *Agave tequilana* stem. This extract was separated using Size Exclusion Chromatography (SEC) on a BioGel P2 (Bio-Rad) column at 0.4 mL/min, using ultra-pure water as the eluant, and collecting 3 mL fractions. Fraction DP composition was confirmed with a SYNAPT™ HDMS System (Waters Corporation) and lyophilized. Ion Mobility data was acquired using a SELECT SERIES™ Cyclic™ IMS System (Cyclic Instrument, Waters Corporation). Chromatographic separation was performed using an ACQUITY™ UPLC™ System with an Atlantis™ Premier BEH™ Column C18 AX, 1.7 μm, 2.1 x 150 mm. CCSe were obtained from a Major Mix calibration. CCSt data were obtained from possible fructan structures, *ab initio* modelled, 3D-optimised, and calculated using the Trajectory Method in IMoS v 1.10 (Coots, J., et al. 2020).

Results

We concentrated on a DP4 component from one of the extracts. Multipass IMS on the Cyclic Instrument yielded 6 components (Figure 2) after 6 passes. Additional slicing experiments for the first and second groups yielded almost baseline separation after 12 additional passes (Figures 3 and 4). These figures also show the assignment of the possible structures based on this information. The match was performed by assigning the lowest ATD to the lowest CCSt and propagating the trend accordingly. We obtained one commercially available isomer (Nystose) and used LC-IMS separation (Figure 5). To our surprise, the last peak at 4.2 minutes contains 3 isomers with different ADT. The first 2 peaks at 4.2 minutes (with lower ATD) overlap with peaks 1 and 2 of Figure 2. The third peak matches Nystose. Table 1 summarises our analysis of the CCSe and CCSt based on multipass infusion experiments. We are confident that we have identified Nystose based on the same retention time, MSMS data (not shown) and comparable CCS value.

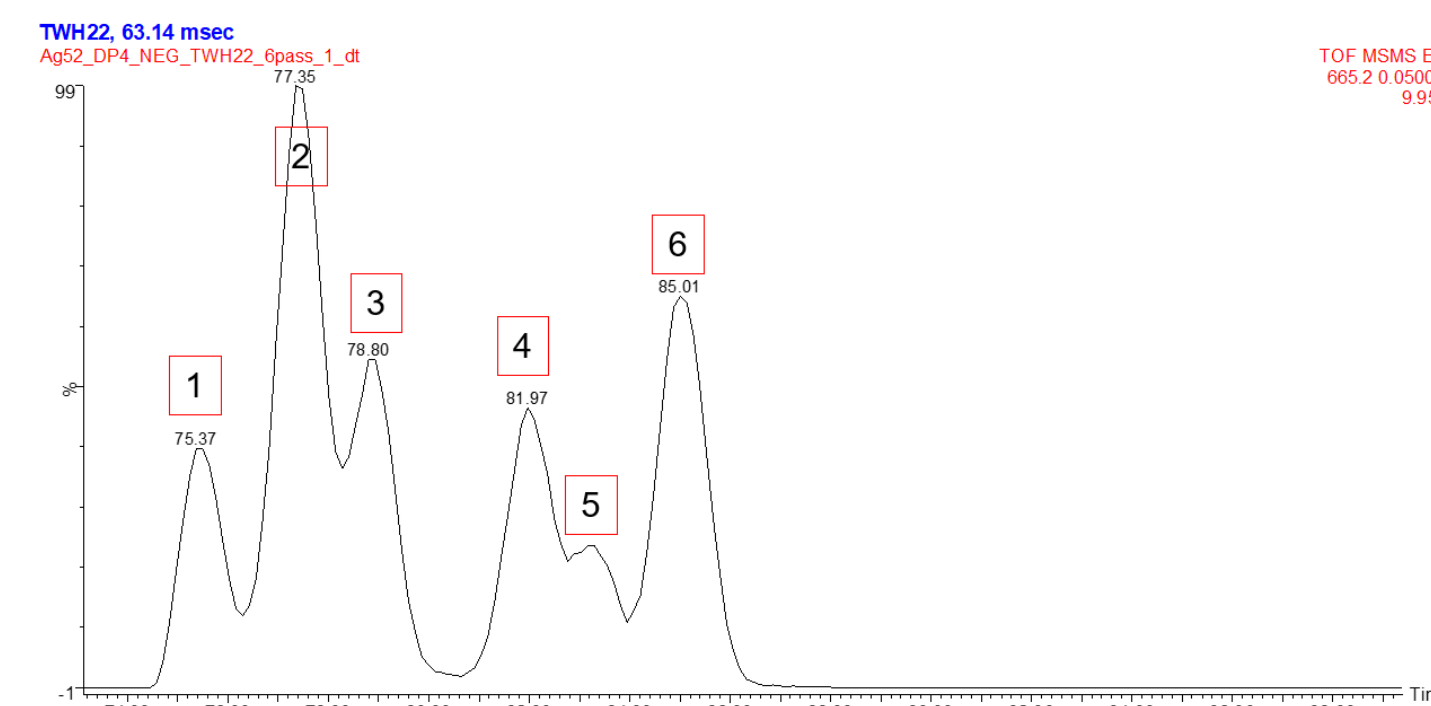


Figure 2. Infusion of sample Ag52 in MSMS mode with low collision energy for DP4 (665 m/z) after 6 passes

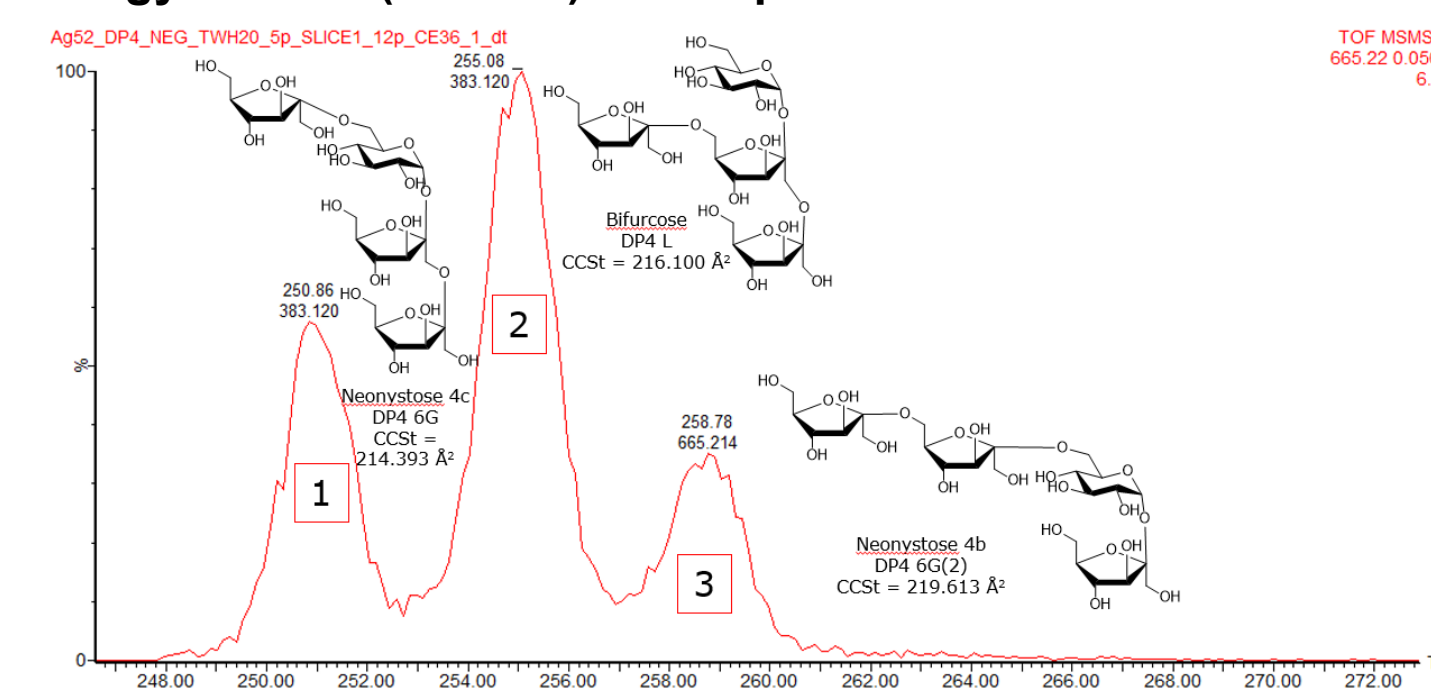


Figure 3. Slicing of the first three peaks of sample Ag52 (from Figure 2) in MSMS mode with low collision energy for DP4 (665 m/z), and 12 new passes.

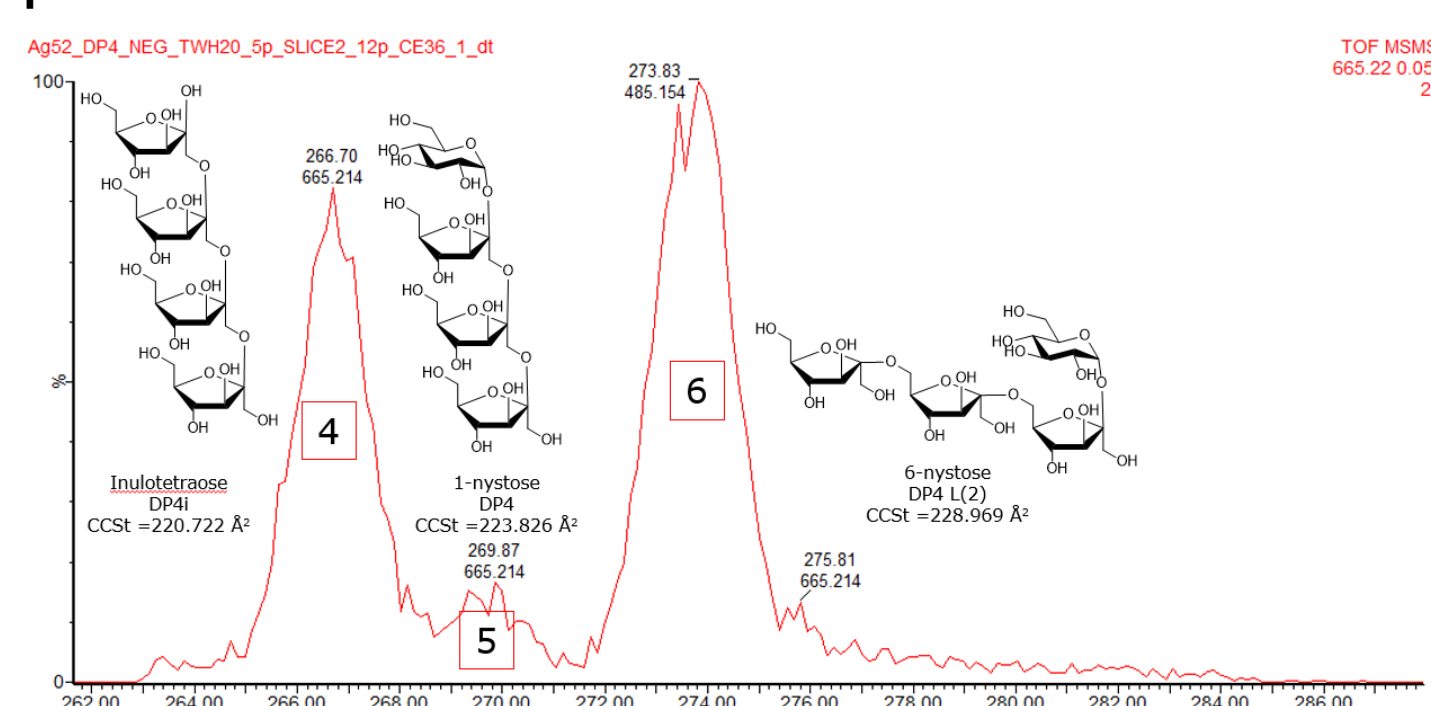


Figure 4. Slicing of the last three peaks of sample Ag52 (from Figure 2) in MSMS mode with low collision energy for DP4 (665 m/z) and 12 new passes.

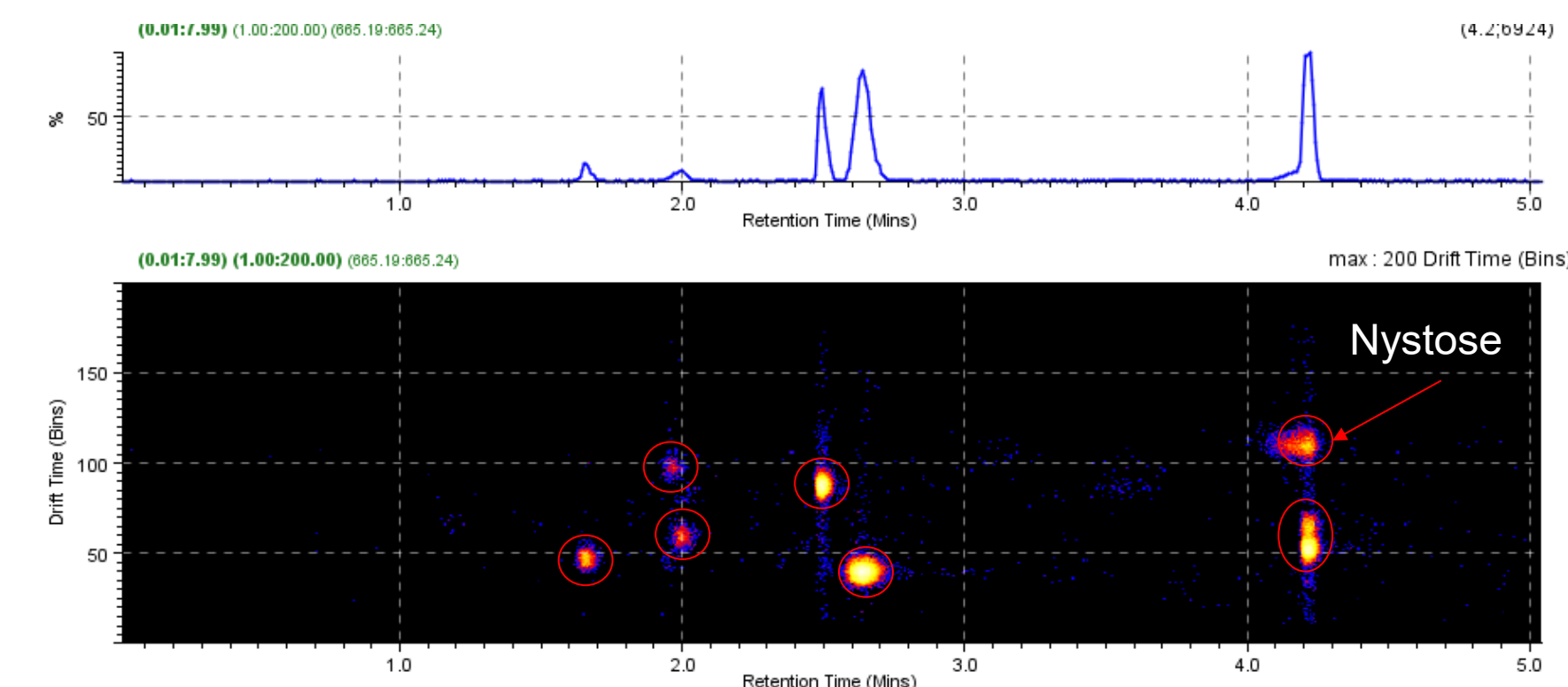


Figure 5. LC-MSMS (665 m/z) of sample Ag52 with CE= 4 eV. The Nystose standard was identified by RT, MS, MSMS, and CCS.

Component	Experimental CCSe (Å²)	Predicted CCSt (Å²)	Difference (%)
Peak 1	219.19	214.4	2.2
Peak 2	221.36	216.1	2.4
Peak 3	223.35	219.6	1.7
Peak 4	227.42	220.7	3.0
Peak 5	229.01	223.8	2.3
Peak 6	231.24	228.9	1.0
Nystose standard	231.16	228.9	1.0

Table 1. Comparison of CCS values. CCSe calculated with linear fit. CCSt calculated as described in the text.

Conclusions

- By infusion only, we see 6 components that can be partially resolved with Ion Mobility after 6 passes.
- By combining LC and Ion Mobility, we can see 8 components.
- One of the peaks matched the standard Nystose. LC, and MSMS were identical, and CCS was within 1% of the predicted value.
- The increased peak capacity of LC-cIMS reveals the presence of more isomers than would be expected from direct cIMS alone.

REFERENCES

1. Coots, J., Gandhi, V., Onakoya, T., Chen, X., & Larriba-Andaluz, C. (2020). A parallelized tool to calculate the electrical mobility of charged aerosol nanoparticles and ions in the gas phase. *Journal of Aerosol Science*, 147(April), 105570. <https://doi.org/10.1016/j.jaerosci.2020.105570>

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