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ELECTRONIC SUPPORTING INFORMATION

Two-Dimensional Isomer Differentiation Using Liquid Chromatography-Tandem Mass Spectrometry with In-Source, Droplet-Based Derivatization

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TABLE OF CONTENTS

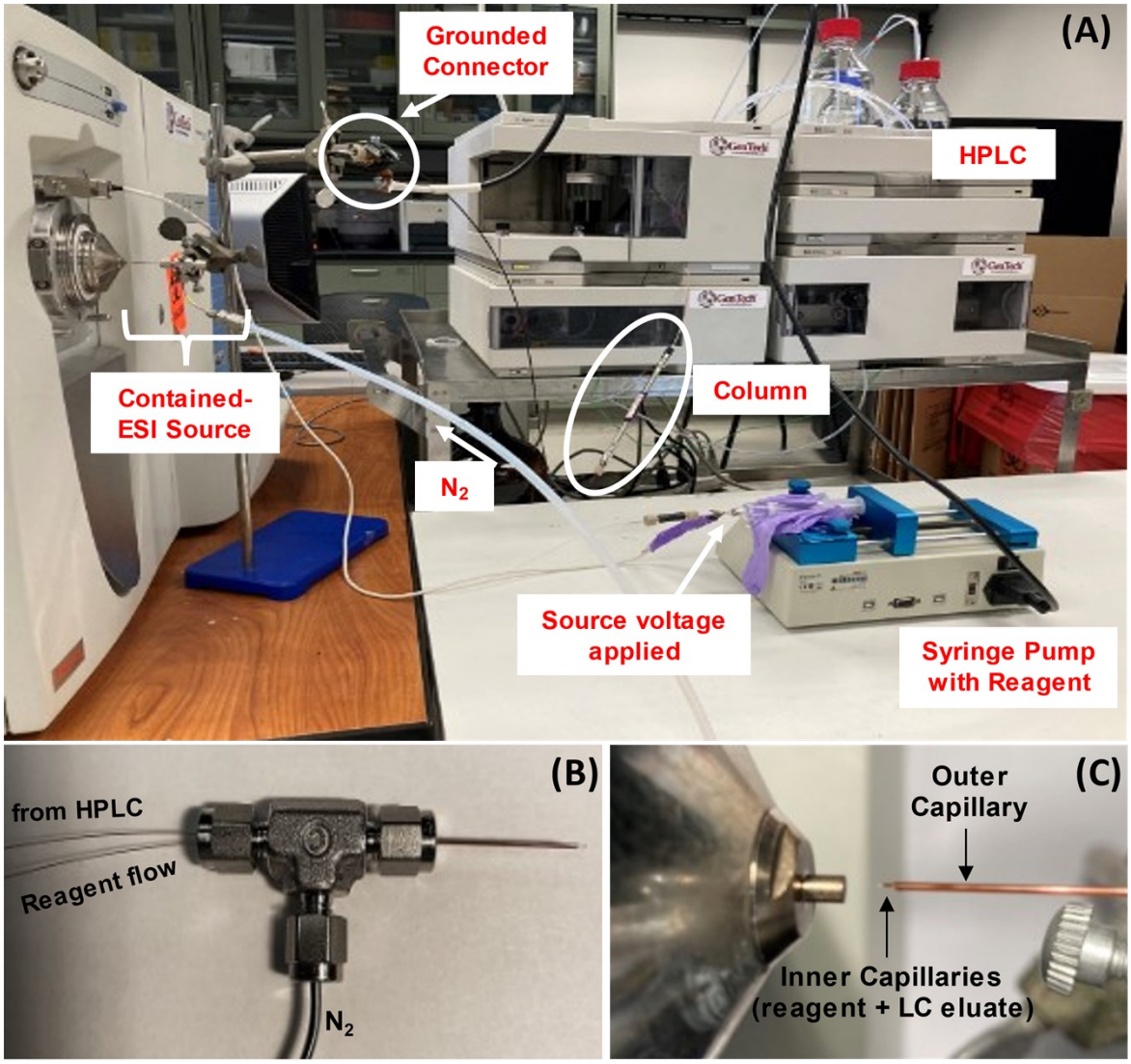

Figure S1. (**A**) Image showing the LC-contained-ESI-MS setup. LC eluate exiting the column passes through a grounded connector before entering the contained-ESI source. The derivatization reagent (PBA) is delivered coaxially to the contained-ESI source by a syringe pump. Voltage is applied to the reagent stream using an alligator clip connected to the stainless-steel needle of the reagent syringe to generate electrospray. (**B**) Image of the contained-electrospray ion source showing coaxial introduction of the HPLC eluent and reagent streams. (**C**) The inner capillaries containing the eluate and reagent streams both extend ~1 mm beyond the outer capillary and converge at the tip of the emitter.

Table S1. LC-contained-ESI-MS/MS method parameters for the analysis of sucrose isomers using in-source phenylboronic acid derivatization.

<table>
<tr><th colspan="2">LC-contained-ESI-MS/MS System and Method Parameters for Sucrose Isomer Differentiation</th></tr>
<tr><td>HPLC</td><td>Agilent 1100</td></tr>
<tr><td>Mass Spectrometer</td><td>Thermo Finnegan LTQ linear ion trap</td></tr>
<tr><td>Software</td><td>Thermo Fisher Scientific Xcalibur 2.2 SP1</td></tr>
<tr><td>HPLC Column</td><td>Waters Acquity BEH Amide, 100 mm x 2.1 mm, 1.7 µm, 300 Å</td></tr>
<tr><td>Column Temperature</td><td>Ambient</td></tr>
<tr><td>Mobile Phase</td><td>Isocratic, 84:16 acetonitrile water (v:v), 0.1% NH_4OH</td></tr>
<tr><td>Injection Volume</td><td>5 µL</td></tr>
<tr><td>Injection Delay</td><td>None</td></tr>
<tr><td>Sample Composition</td><td>80:20 acetonitrile:aqueous</td></tr>
<tr><td>Ion Source Parameters</td><td>
<table>
<tr><th>Parameter</th><th>Setting</th></tr>
<tr><td>Source</td><td>Contained-Electrospray (Type I mode)</td></tr>
<tr><td>Mode</td><td>Negative ion (ESI-)</td></tr>
<tr><td>Reagent</td><td>4 mM Phenylboronic Acid in 1:1 ACN, pH 10 with NH_4OH</td></tr>
<tr><td>Reagent Flow</td><td>5 µL/min</td></tr>
<tr><td>Sheath Gas</td><td>Nitrogen, 20 psi</td></tr>
<tr><td>Source Voltage</td><td>-6.5 kV</td></tr>
</table>
</td></tr>
<tr><td>Mass Spectrometer Settings</td><td>Product Ion Scanning
Inlet capillary temperature = 325 °C
Precursor Ion = m/z 427 ± 1
Collision Energy (%) = 23</td></tr>
<tr><td>Run Time per Injection</td><td>Approximately 40 min</td></tr>
</table>

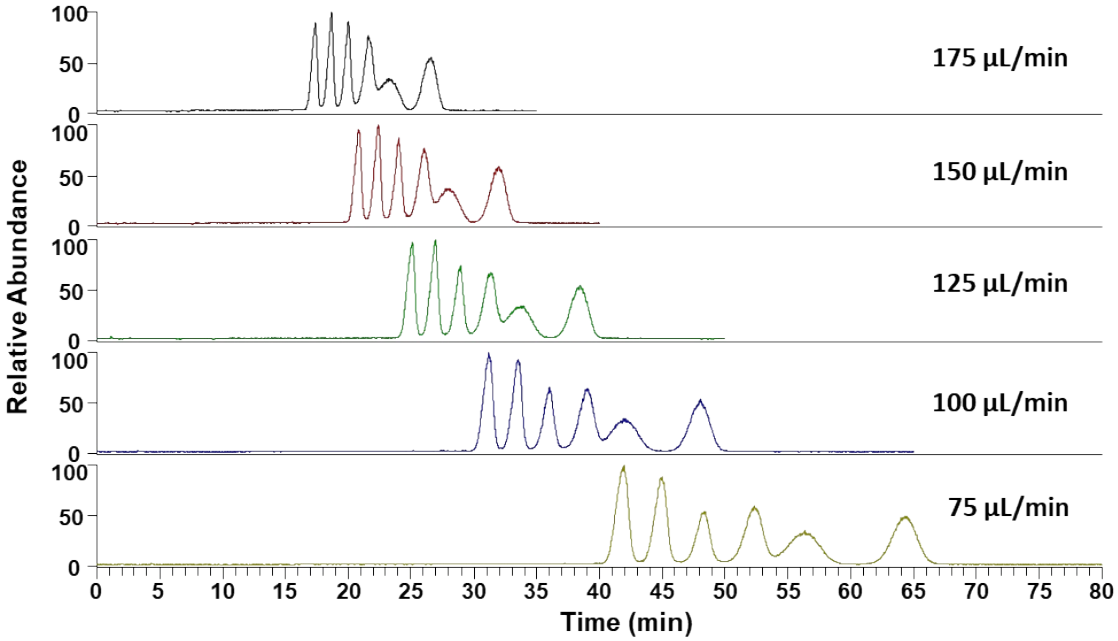

Figure S2. Chromatograms showing separation of a 10 µM mixture of the six disaccharide isomers at various flow rates. Data were acquired using the optimized method parameters shown in Table S1 except for the LC flow rate, which was varied.

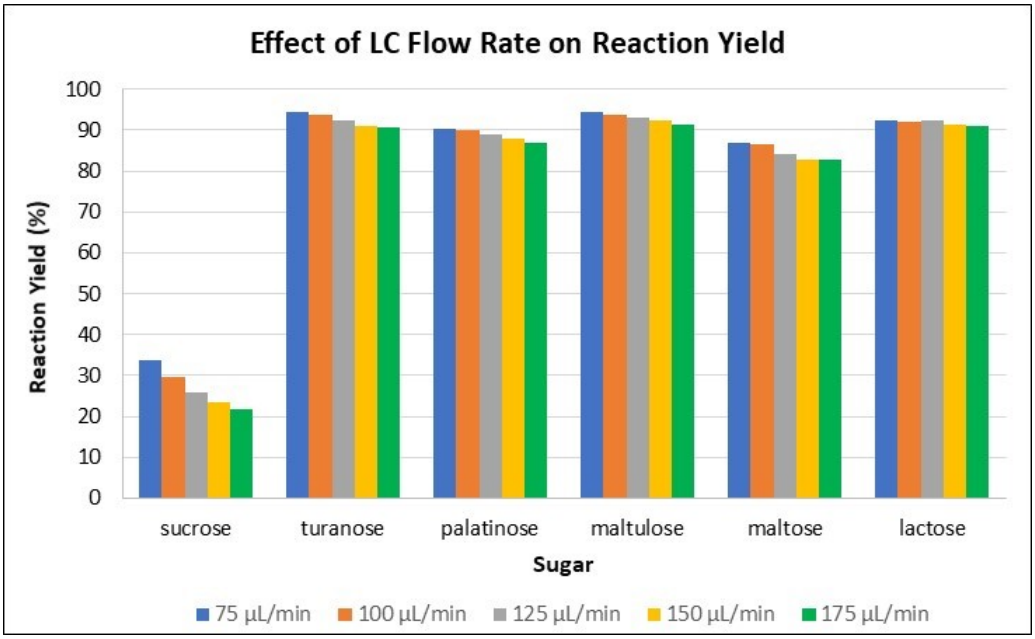

Figure S3. Effect of LC flow rate on PBA-sugar reaction yield. The MS was operated in single ion monitoring (SIM) mode for the deprotonated precursor ions of both the derivatized species (*m/z* 427) and the underivatized species (*m/z* 341). Reaction yield for this experiment was defined as the ratio of the peak area for the derivatized ion to that of the underivatized ion, expressed as a percentage.

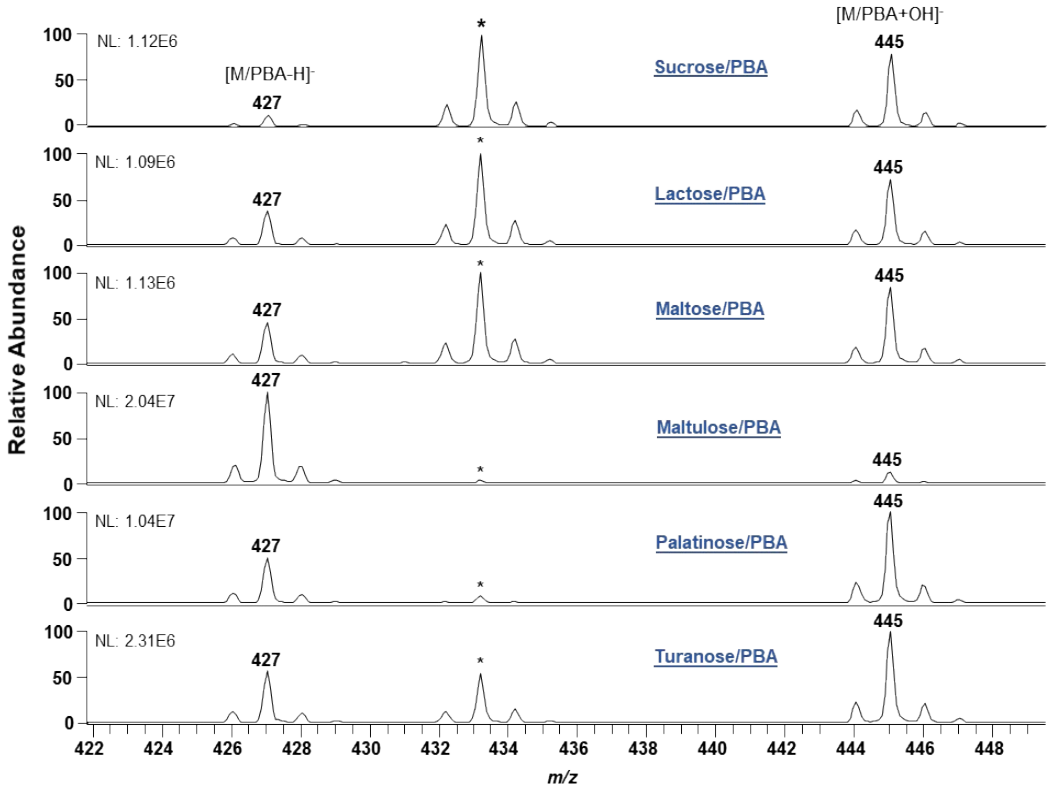

Figure S4. Full scan mass spectra of the six disaccharide isomers showing precursor ions representing both the deprotonated (*m/z* 427) and hydroxylated (*m/z* 445) monosubstituted derivatives. Peaks attributed to the blank or reagent are denoted by (*).

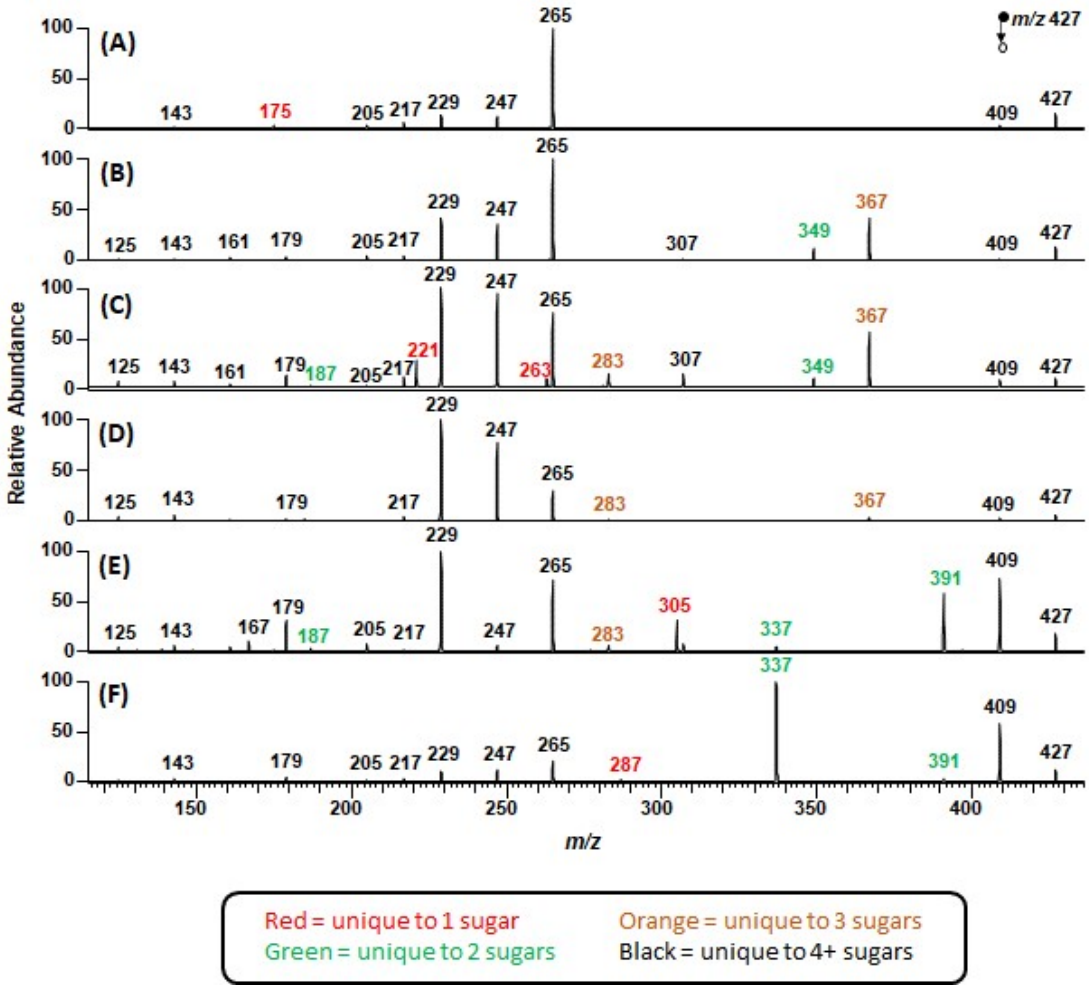

Figure S5. Product ion mass spectra for the six sucrose isomers including (A) sucrose, (B) lactose, (C) maltose, (D) maltulose, (E) palatinose, and (F) turanose following CID of the deprotonated, monosubstituted PBA derivatives (*m/z* 427). Prevalence is color coded indicating unique product ions for certain isomers.

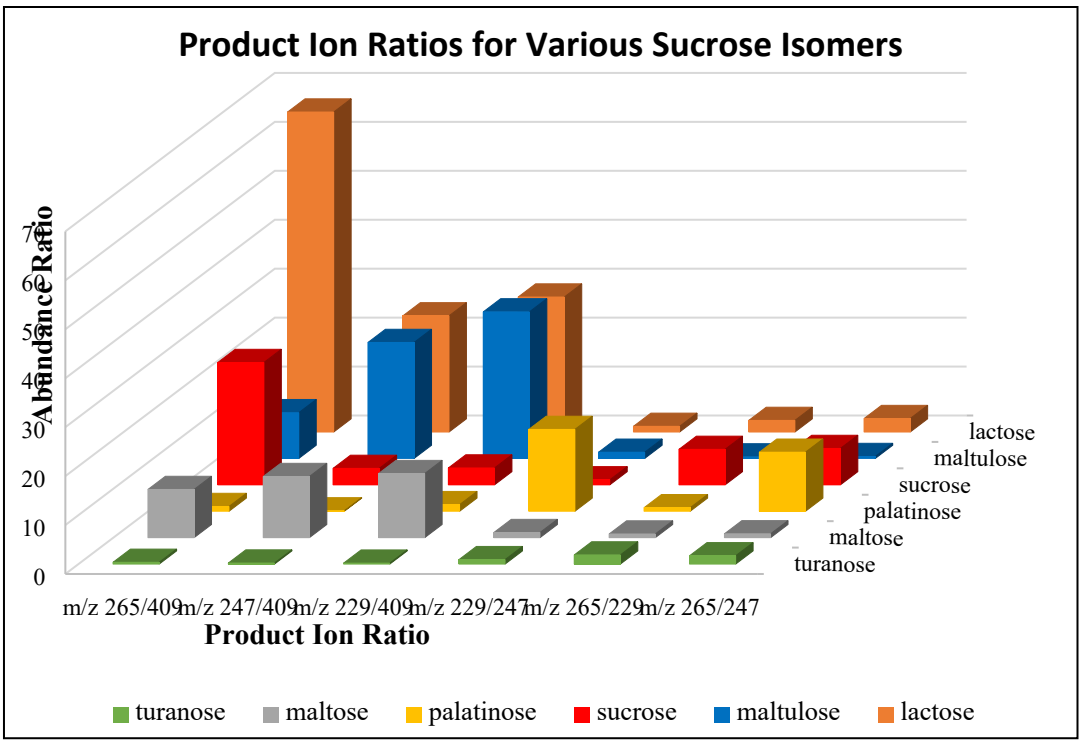

Figure S6. Plot of product ion abundance ratios for each of the six sucrose isomers generated using four product ions common to all of the isomers: *m/z* 229, *m/z* 247, *m/z* 265, and *m/z* 409.

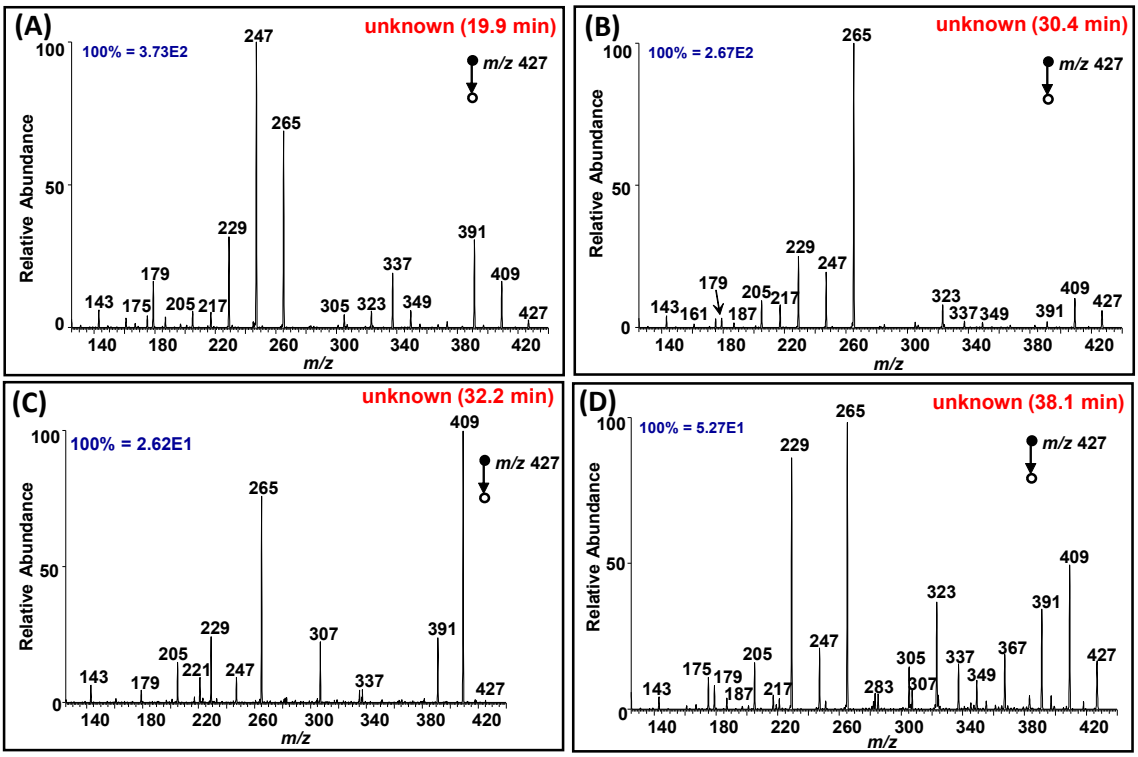

Figure S7. Product ion mass spectra acquired for peaks representing unidentified disaccharides in the honey samples at retention times of (A) 19.9 minutes, (B) 30.4 minutes, (C) 32.2 minutes, and (D) 38.1 minutes.