

**SUPPORTING INFORMATION**

**Differentiation of Disaccharide Isomers via Diagnostic-Ion-Weighted Spectral  
Library Searching using Data from Tandem MS Analysis of Chloride  
Adducts**

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## 1. User Interface of a MySQL 8.0.43

All 15 disaccharide isomers were analyzed by MS/MS in negative-ion mode using chloride adducted ions. The resulting spectra were converted to mzML format and subsequently uploaded to a MySQL database. Each isomer was acquired in triplicate, with a one-minute acquisition time per run. All runs were performed at 30% collision-induced dissociation (CID) energy and stored as tables within the disaccharide scheme. For each isomer, the corresponding table contains m/z values, signal intensities, and the assigned set of diagnostic fragment ions.

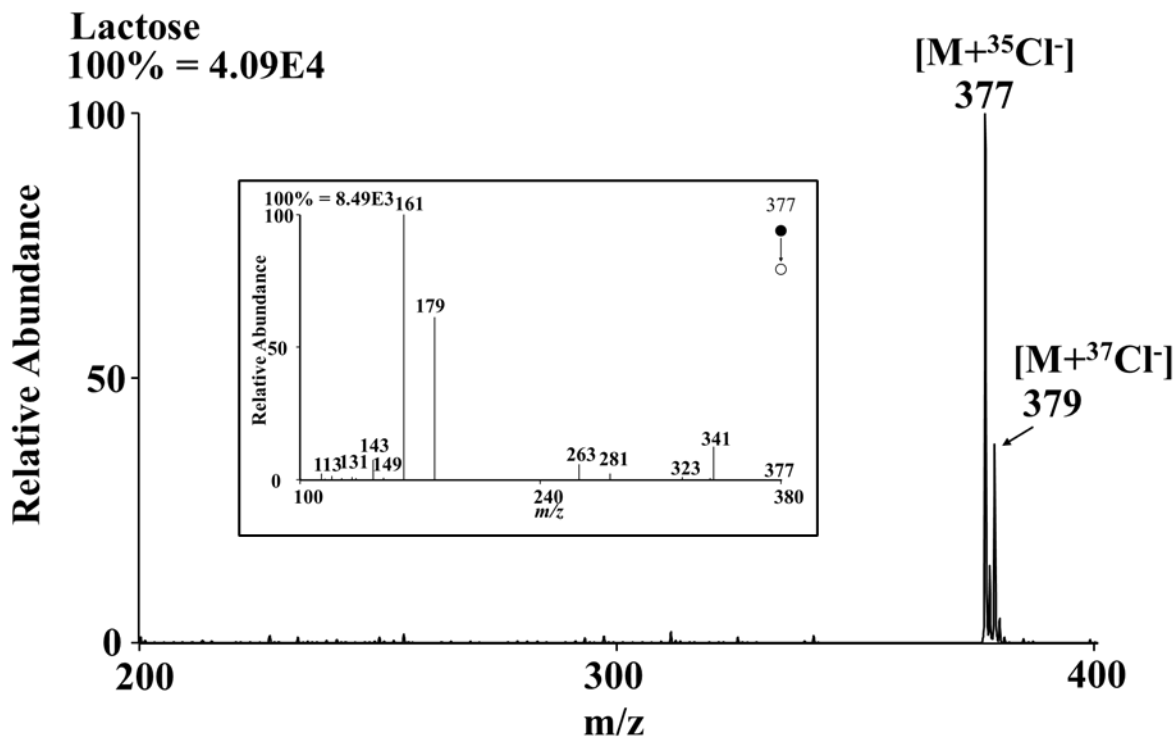
The selected saccharide isomer contains data on m/z, signal intensity, and diagnostic fragment ions.

moverz	signal_intensity	diagnose_ion_moverz	number_index
0	0	115	1
100.748	0.0391129	215	2
0	0	263	3
0	0	221	4
0	0	245	5
0	0	0	6
0	0	0	7
0	0	0	8
0	0	0	9
0	0	0	10
0	0	0	11
0	0	0	12
0	0	0	13
0	0	0	14

**Figure S1.** User interface utilizing MySQL 8.0.43 for spectral library storage platform containing m/z values, intensities, and diagnostic fragment ions.

## 2. Full MS in Negative Chloride Adducted Mode

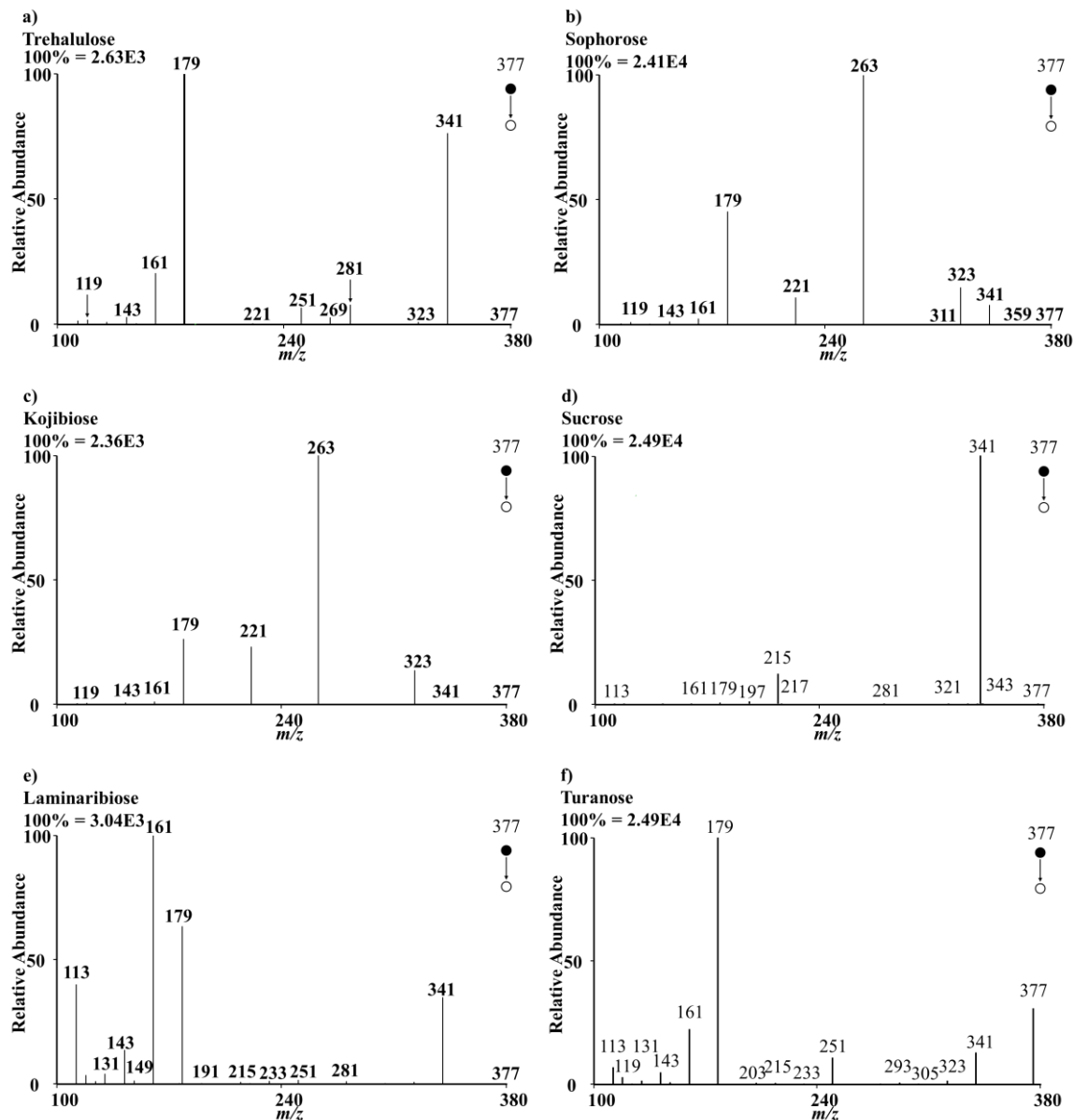
The full MS spectra of each disaccharide isomer was acquired in negative-ion mode, utilizing chloride adduction. An example spectrum is shown below for lactose (MW 365 Da), where the chloride adduct appears at  $m/z$  377, corresponding to the  $[M+^{35}\text{Cl}]^-$ . The chloride-37 isotopic adduct is also observed at  $m/z$  379. The relative signal intensities of these two peaks exhibit the expected  $\sim 3:1$  isotopic abundance ratio.

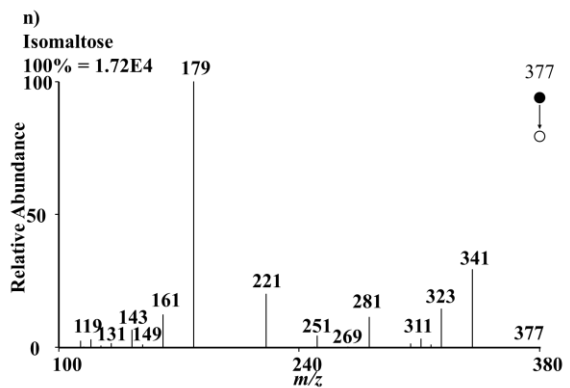
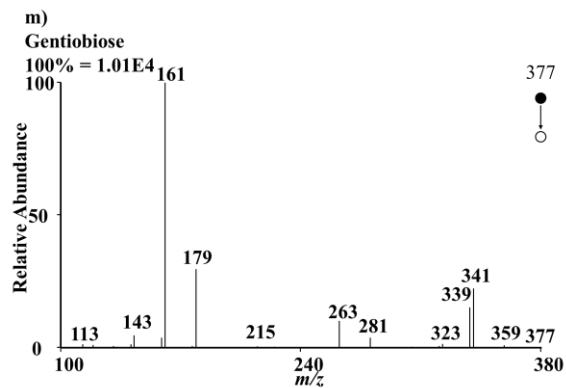
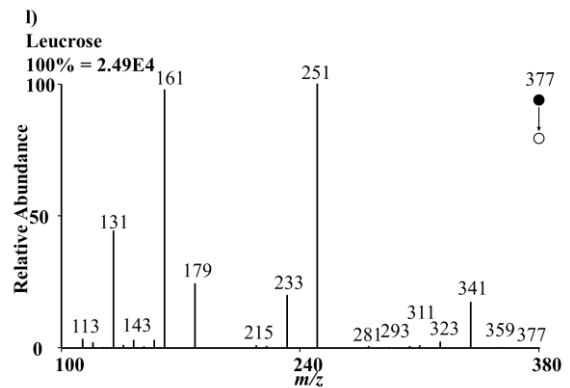
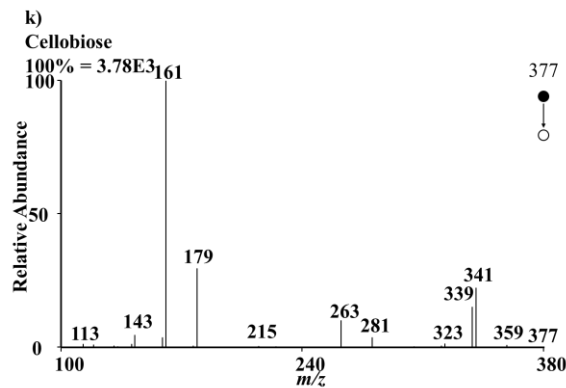
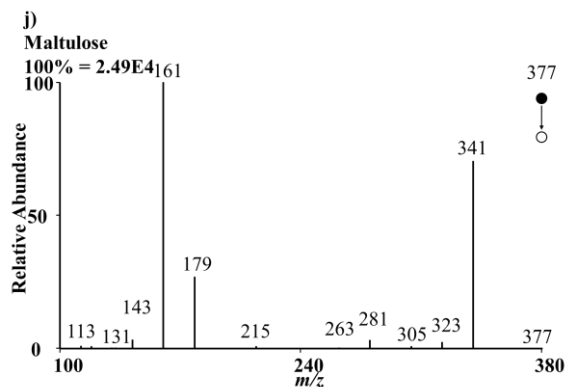
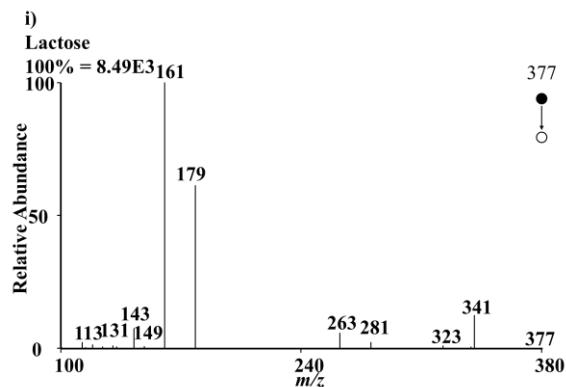
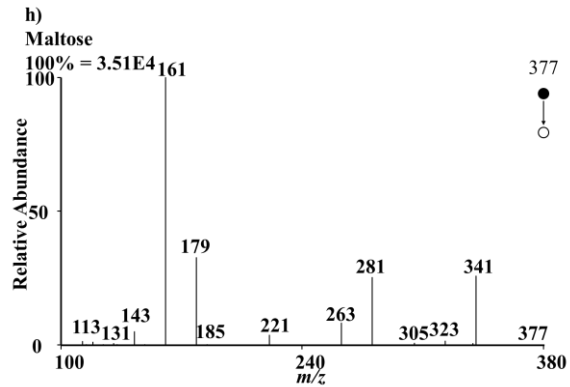
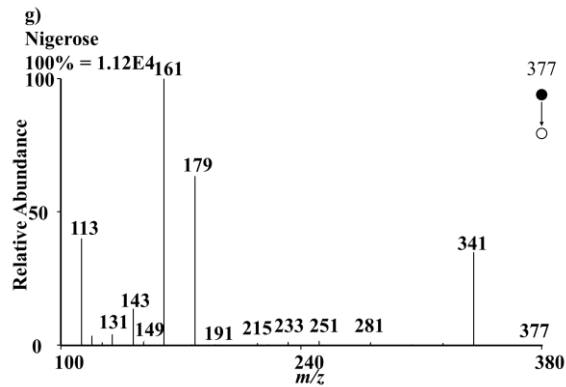


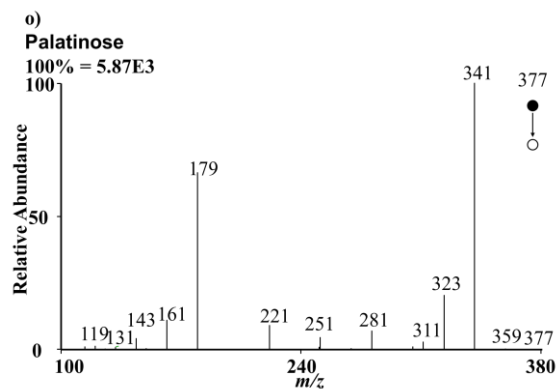
**Figure S2.** Full MS analysis of lactose in negative-ion mode via chloride adduction using nESI. Inset shows MS/MS analysis of the peak at  $m/z$  377. Inset shows MS/MS at  $m/z$  377.

### 3. Tandem Mass Spectra of Negative Chloride-Adducted Disaccharide Isomers

The tandem mass spectra for all disaccharide isomers analyzed in negative-ion mode via CID of the chloride adduct are shown below. All disaccharides were isolated at  $m/z$  377 using an isolation width of  $m/z$  1. The fragment ions can be traced through the various fragmentation pathways described in Figure S3 above.







**Figure S3.** Individual MS/MS for **a)** Trehalose **b)** Sophorose **c)** Kojibiose **d)** Sucrose **e)** Laminaribiose **f)** Turanose **g)** Nigerose **h)** Maltose **i)** Lactose **j)** Maltulose **k)** Cellobiose **l)** Leucrose **m)** Gentiobiose **n)** Isomaltose **o)** Palatinose each at concentration of 20  $\mu$ M.

#### 4. Relative standard deviation (RSD)

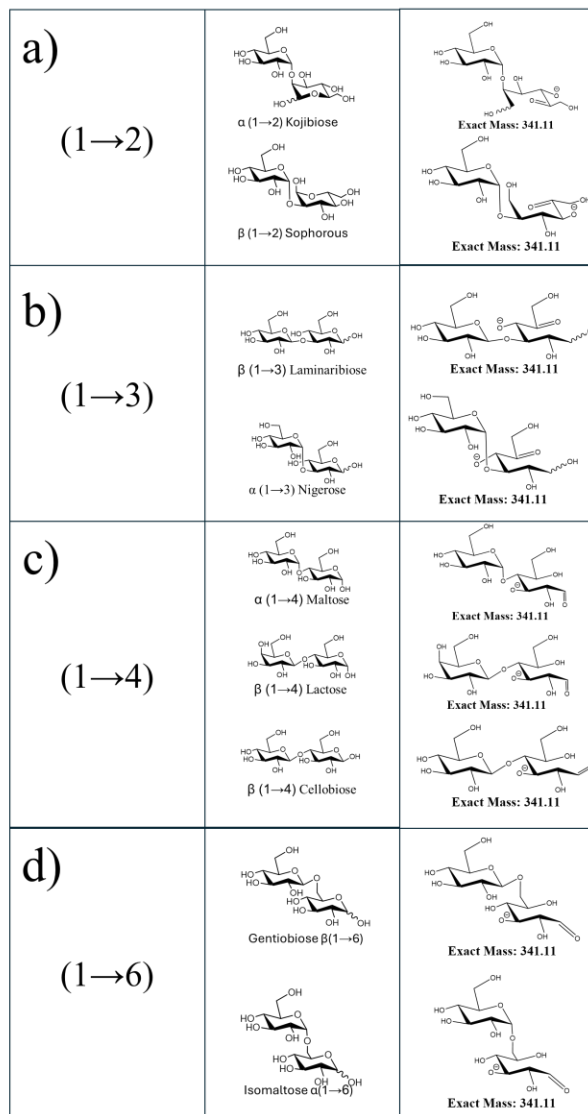
To evaluate reproducibility and method stability, relative standard deviation (RSD) analysis was performed on the diagnostic-ion-weighted score ( $S_D$ ) value of each isomer against the reference library. The RSD analysis was conducted using three independent replicate analysis. The results show that all RSD values were below 30%, indicating that the method characterizes saccharide isomer with good precision and reproducibility.

Reference Library	trehalulose	0.00	0.01	7.22	0.07	0.02	13.18	0.17	0.29	29.99	0.17	0.17	0.23	0.33	9.69	0.06
	sophorose	6.52	0.01	0.14	1.80	1.79	3.95	14.52	4.88	10.39	4.54	5.29	0.72	0.46	3.13	2.91
	kojibiose	0.06	0.01	0.01	2.19	0.19	0.14	0.11	0.28	0.07	0.19	0.16	0.20	0.15	0.15	0.16
	sucrose	0.25	0.00	0.81	0.00	1.45	0.30	0.09	0.35	0.12	0.39	0.32	0.77	0.31	0.01	0.63
	turanose	0.07	0.08	0.22	0.73	0.00	0.14	0.23	0.09	0.11	0.14	0.14	0.16	0.23	0.07	0.06
	maltose	0.14	0.33	22.31	0.09	0.17	2.43	0.03	0.04	0.12	0.01	0.00	0.01	0.03	0.19	0.22
	maltulose	0.09	0.44	1.05	0.37	0.35	0.21	0.00	0.05	0.14	0.11	0.10	0.16	0.14	0.46	0.24
	leucrose	5.44	0.05	0.21	0.85	0.13	0.12	0.05	23.79	4.86	0.12	0.11	0.14	0.12	0.12	0.17
	palatinose	0.13	0.36	0.30	0.28	0.46	9.75	0.03	0.50	0.02	0.30	5.78	0.46	0.21	0.31	0.32
	laminaribiose	0.77	0.93	0.93	0.39	0.72	0.13	0.00	0.61	0.63	0.01	0.03	0.13	0.07	0.82	0.83
	nigerose	13.93	0.30	6.93	1.11	2.71	3.75	0.09	0.99	23.79	0.01	4.68	0.08	0.08	7.04	6.69
	lactose	0.20	0.44	13.58	2.68	0.39	0.05	0.25	0.04	0.10	0.03	0.05	0.02	0.12	0.30	0.46
	cellobiose	0.29	1.02	1.18	0.71	0.16	0.33	0.08	0.13	0.39	0.03	0.03	0.01	0.05	0.22	0.23
	gentibiose	0.25	0.13	0.35	1.62	0.20	1.23	0.88	3.58	0.40	0.34	0.34	0.31	0.67	0.01	0.08
	isomaltose	0.01	0.03	0.05	0.03	0.00	0.09	0.07	0.11	0.03	0.03	0.04	0.06	0.10	0.01	0.01
		trehalulose	sophorose	kojibiose	sucrose	turanose	maltose	maltulose	leucrose	palatinose	laminaribiose	nigerose	lactose	cellobiose	gentibiose	isomaltose
	Unknown Compounds															

**Figure S4.** Relative standard deviation (RSD) of triplicate measurements for different disaccharide isomers, provided in percentages (%).

## 5. Isomer with different compositions and configuration

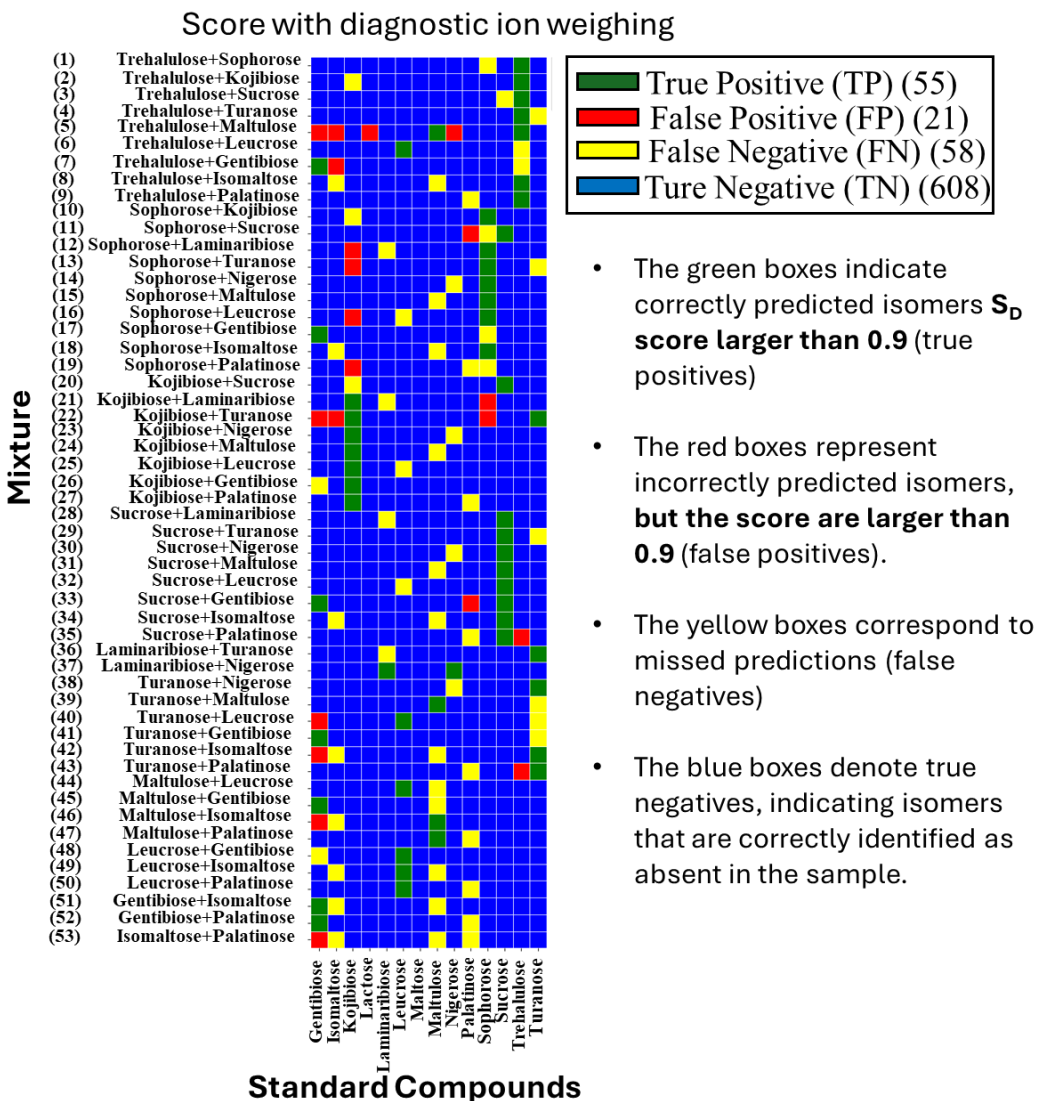
The isomers with identical linkage position are most likely to yield similar MS/MS spectra. This similarity is primarily due to shared intermediate formed during the collisional induced dissociation (CID). As shown in the figures below, for the (1→2) linkage, kojibiose and sophorose generate common intermediate fragments, with difference arising from the configuration of glycosidic bonds ( $\alpha$  versus  $\beta$ ).



**Figure S5.** Same linkage isomers can yield shared CID intermediates. (a) 1→2 linkage: kojibiose vs. sophorose (b) 1→3 linkage: laminaribiose vs. nigerose (c) 1→4 linkage: maltose, lactose, and cellobiose (d) 1→6 linkage: isomaltose vs. gentiobiose.

## 6. Binary Sample Analysis with Diagnostic Ions

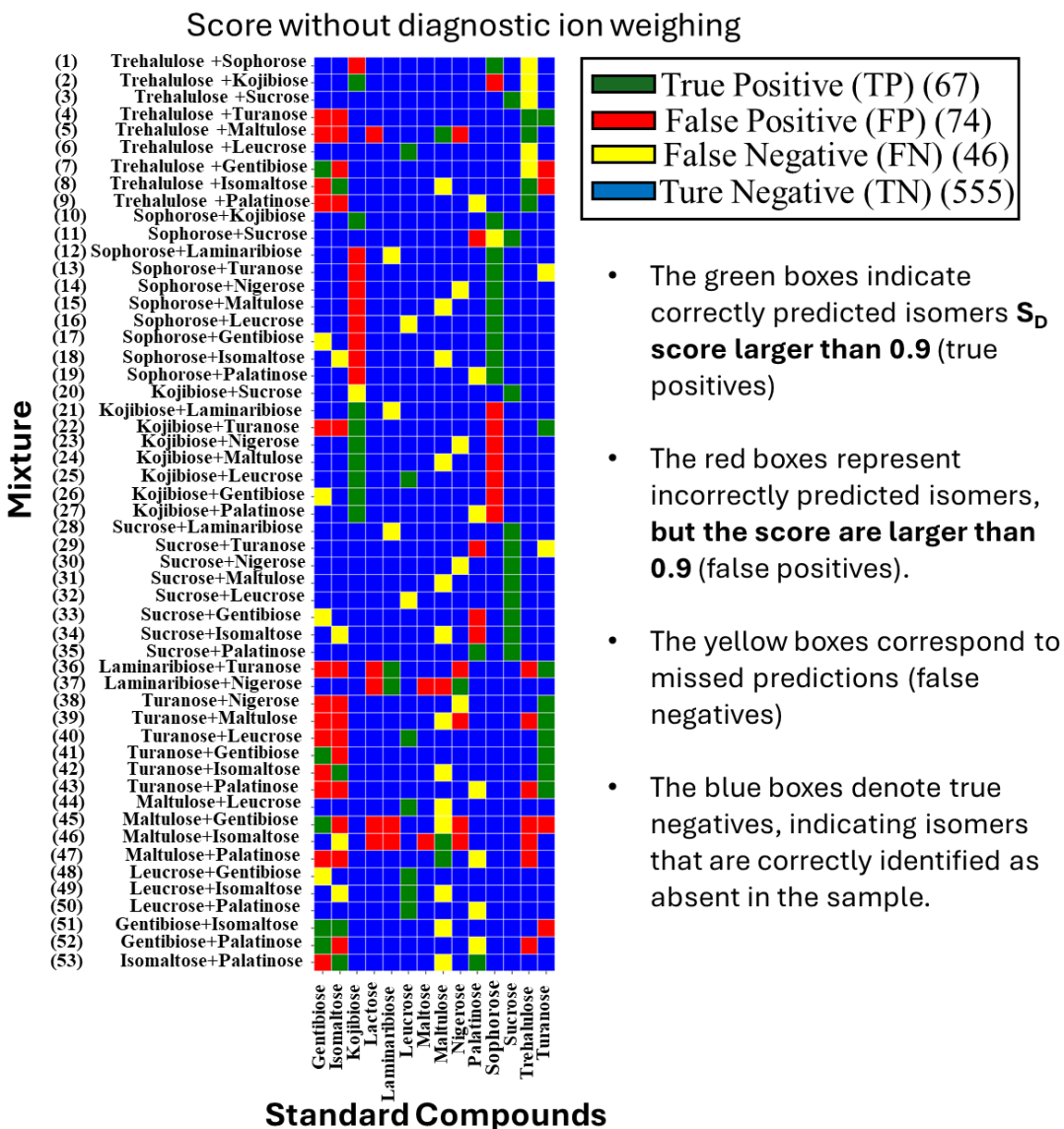
We prepared 53 different binary mixture samples to evaluate the ability of diagnostic-ion-weighted algorithm to identify the correct isomers in a binary mixture.



**Figure S6.** Binary sample analysis using diagnostic-ion-weighted scoring algorithm. Green indicates true positives with the  $S_D$  score exceeding 0.9, red indicates false positives, yellow indicates false negatives, and blue represents true negatives.

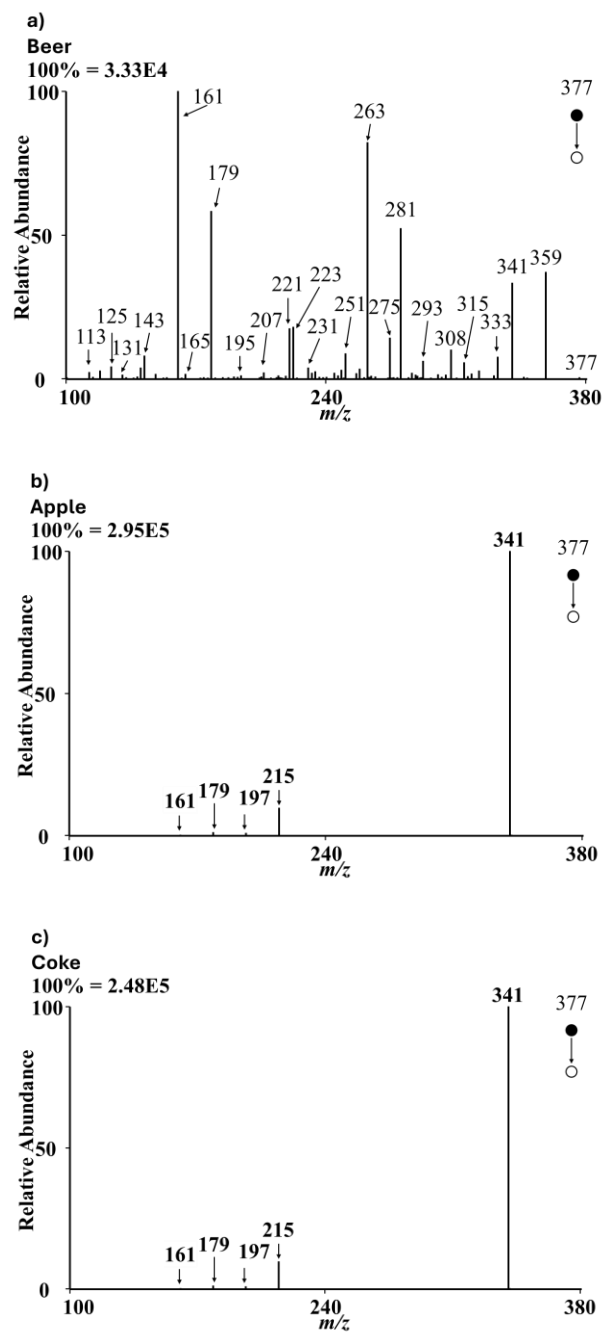
## 7. Binary Sample Analysis without Diagnostic Ions

We prepared 53 different binary mixture samples to evaluate the ability of conventional scoring algorithms to identify the correct isomers in a binary mixture.



**Figure S7.** Binary sample analysis without using diagnostic-ion-weighted scoring algorithm. Green indicates true positives with the  $S_D$  score exceeding 0.9, red indicates false positives, yellow indicates false negatives, and blue represents true negatives.

## 8. MS/MS spectra of real samples: apple juice, coca cola, and beer.



**Figure S8.** Individual negative-mode MS/MS spectra of chloride-adducted ions from real samples: (a) beer, (b) apple, and (c) Coke.