Electronic Supplementary Information

Multifunctional-Separation-Mode Ion Chromatography Method for Determining Major Metabolites during Multiple Parallel Fermentation of Rice Wine

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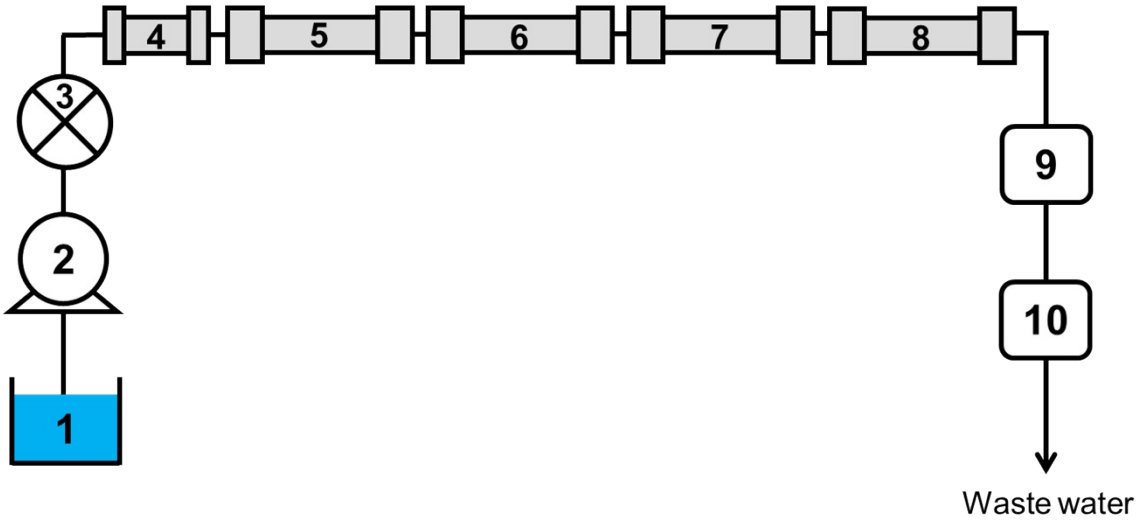

Figure S1. Schematic illustration of the proposed chromatographic system. 1: eluent bottle; 2: pumps for eluent; 3: sample injector; 4: guard column; 5, 6, 7, and 8: separation columns; 9: conductivity detector; and 10: refractive index detector.

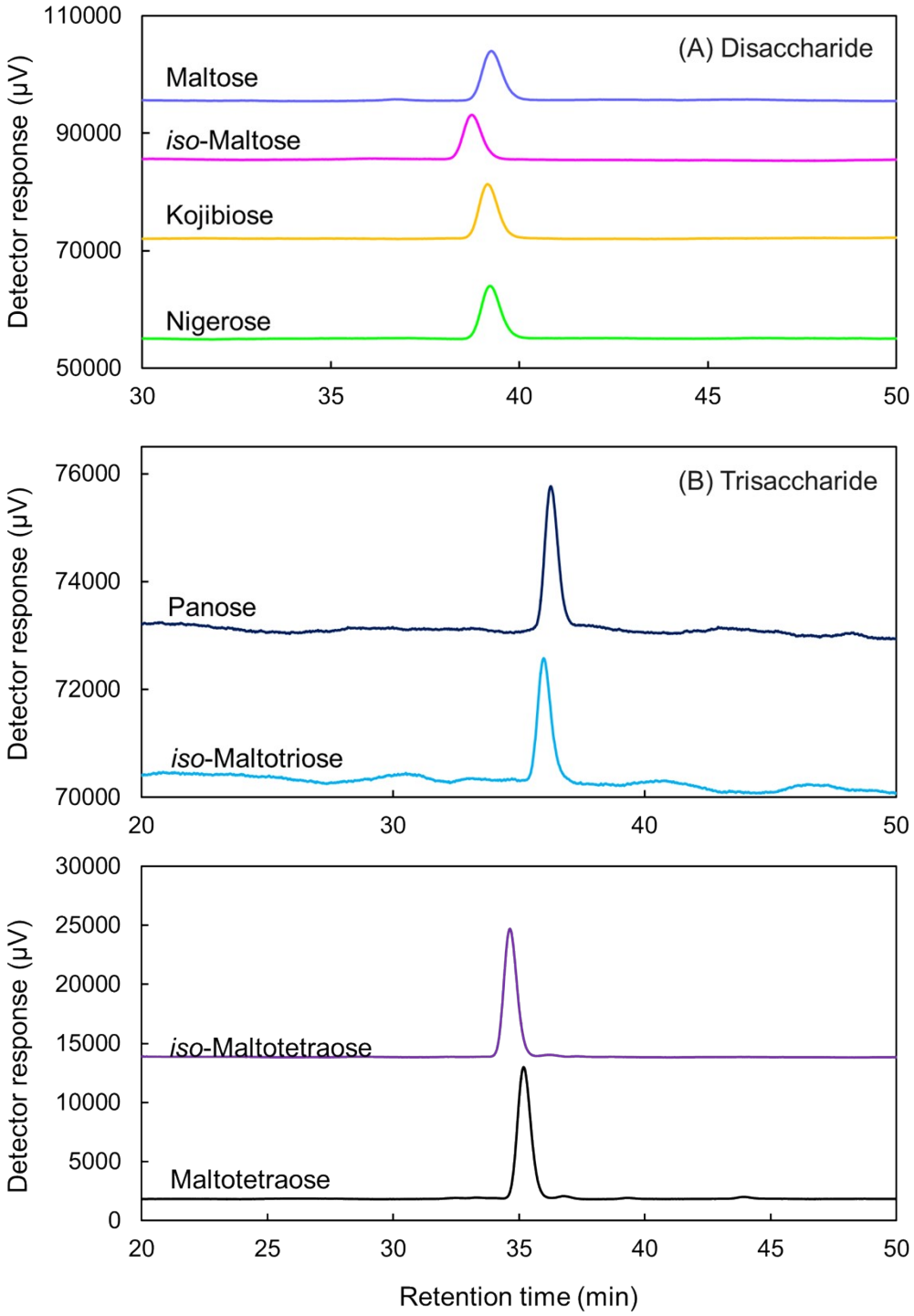

Figure S2. Comparison of the peak areas for (A) four disaccharides, (B) two trisaccharides, and (C) two tetrasaccharides.

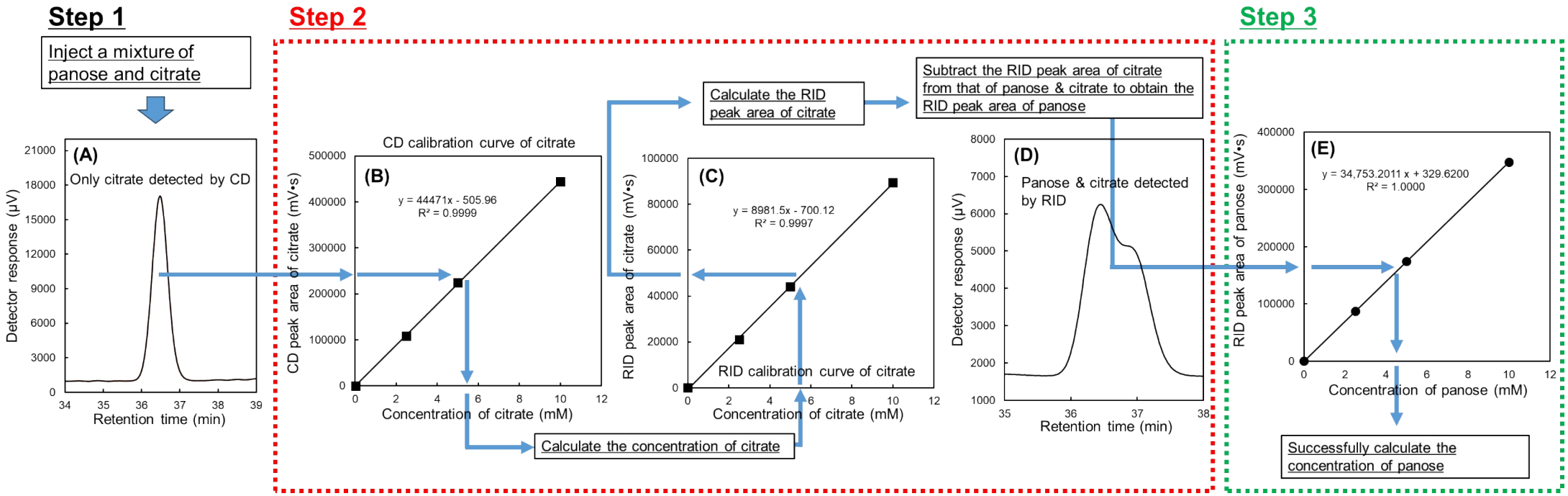

Figure S3. Graphical depiction of the steps involved in calculating the concentrations for overlapping peaks: (A) chromatogram of citrate detected using the conductivity detector (CD), (B) calibration curve of citrate detected using the CD, (C) calibration curve of citrate detected using the refractive index detector (RID), (D) chromatogram of panose and citrate detected using the RID, and (E) calibration curve of panose detected using the RID.

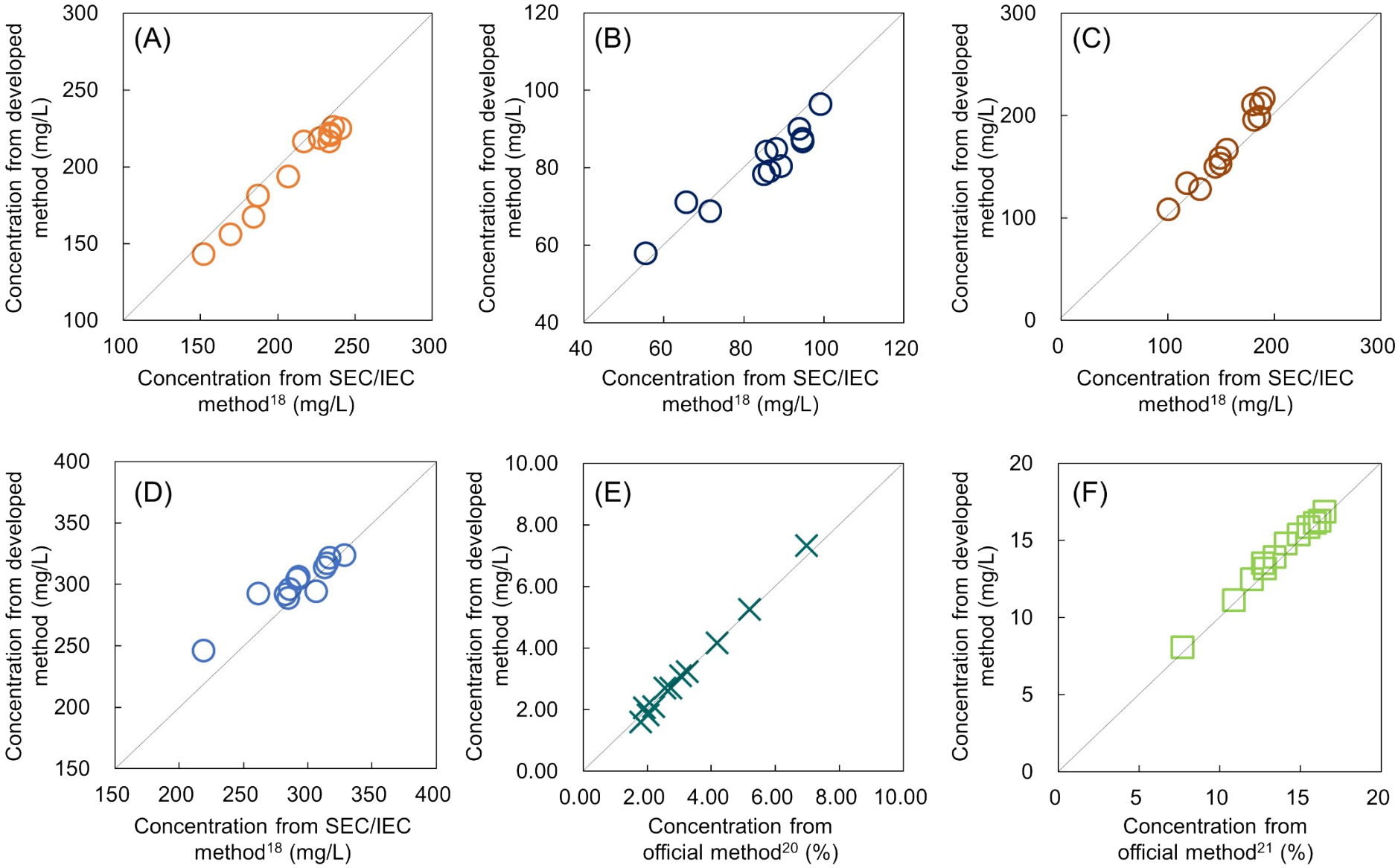

Figure S4. Comparison of concentrations determined using our developed method and other methods: (A) pyruvate, (B) citrate, (C) L-malate, (D) succinate, (E) glucose, and (F) ethanol. The experimental conditions are the same as those in Figure 3. SEC: size-exclusion chromatography; IEC: ion-exclusion chromatography.

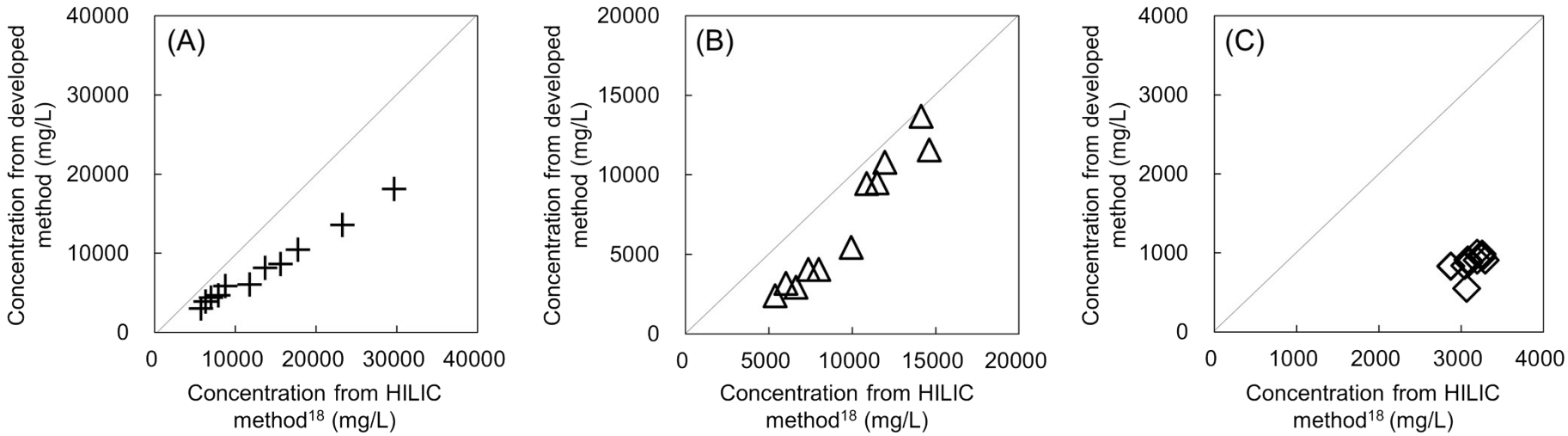

Figure S5. Comparison of concentrations determined using our developed method and other methods: (A) disaccharides, (B) trisaccharides, and (C) tetrasaccharides.

The experimental conditions for the developed method are the same as those in Figure 3. HILIC: hydrophilic interaction liquid chromatography.

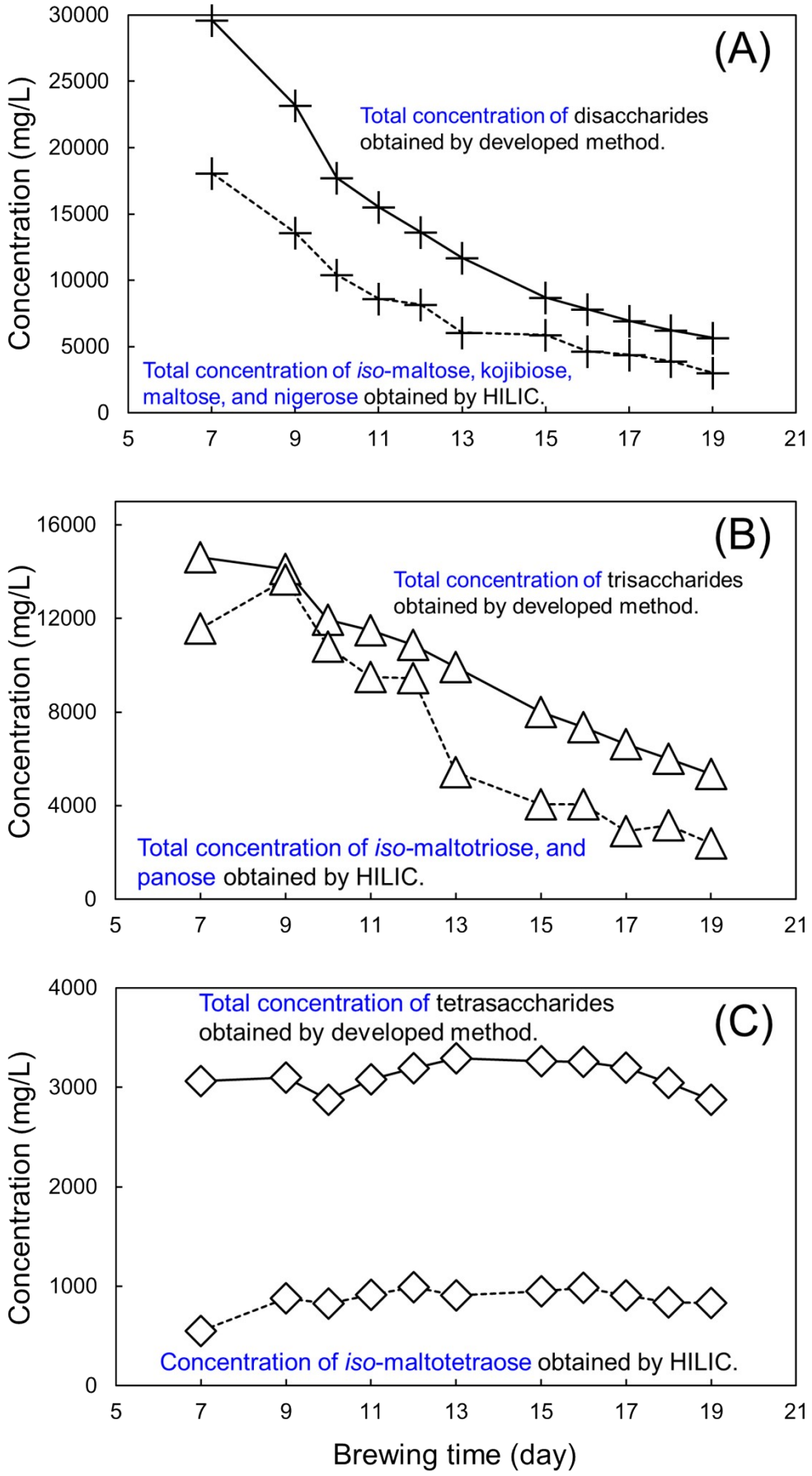

Figure S6. Comparison of the changes in the total concentrations of disaccharides, trisaccharides, and tetrasaccharides determined using our developed method and the total concentrations of disaccharides (*iso*-maltose, kojibiose, maltose, and nigerose), trisaccharides (*iso*-maltotriose and panose), and tetrasaccharides (*iso*-maltotetraose) quantitated using the HILIC method. The experimental conditions for the developed method are the same as those in Figure 3.

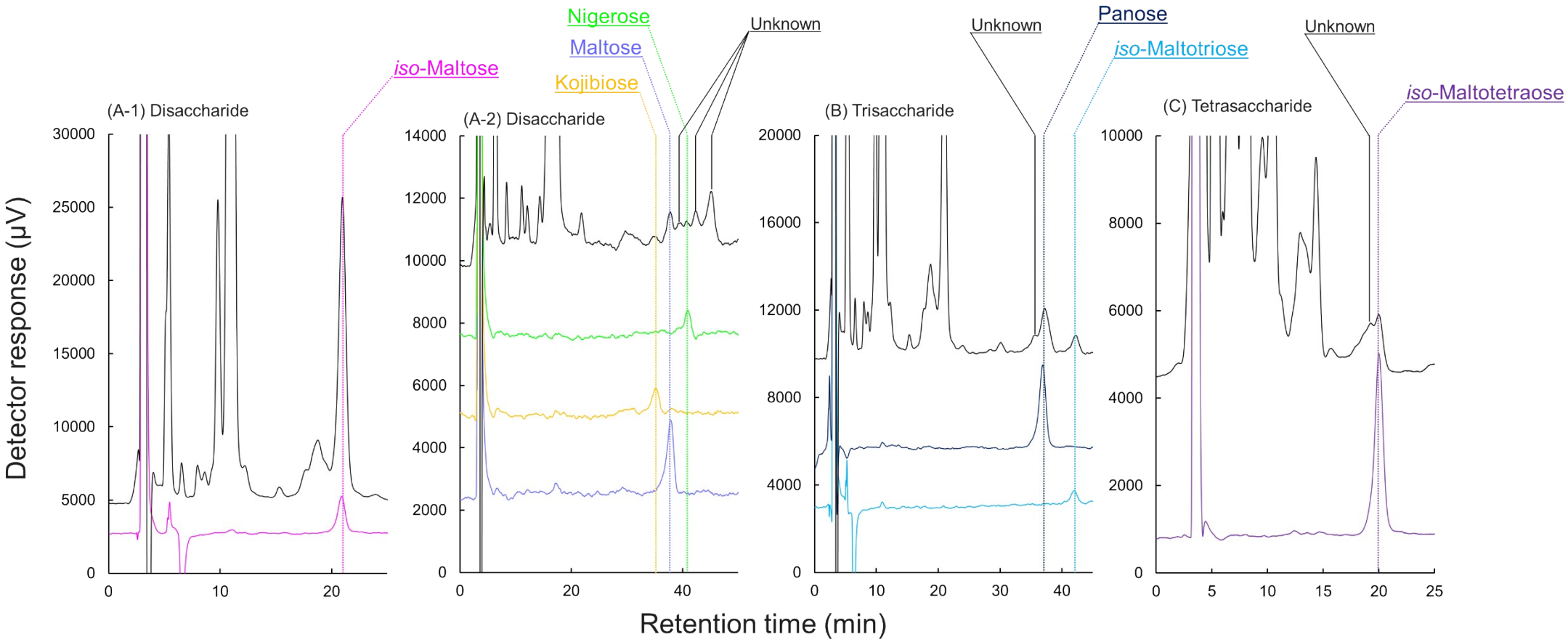

Figure S7. Separation of unknown oligosaccharides using HILIC: (A-1, A-2) disaccharides, (B) trisaccharides, and (C) tetrasaccharides.

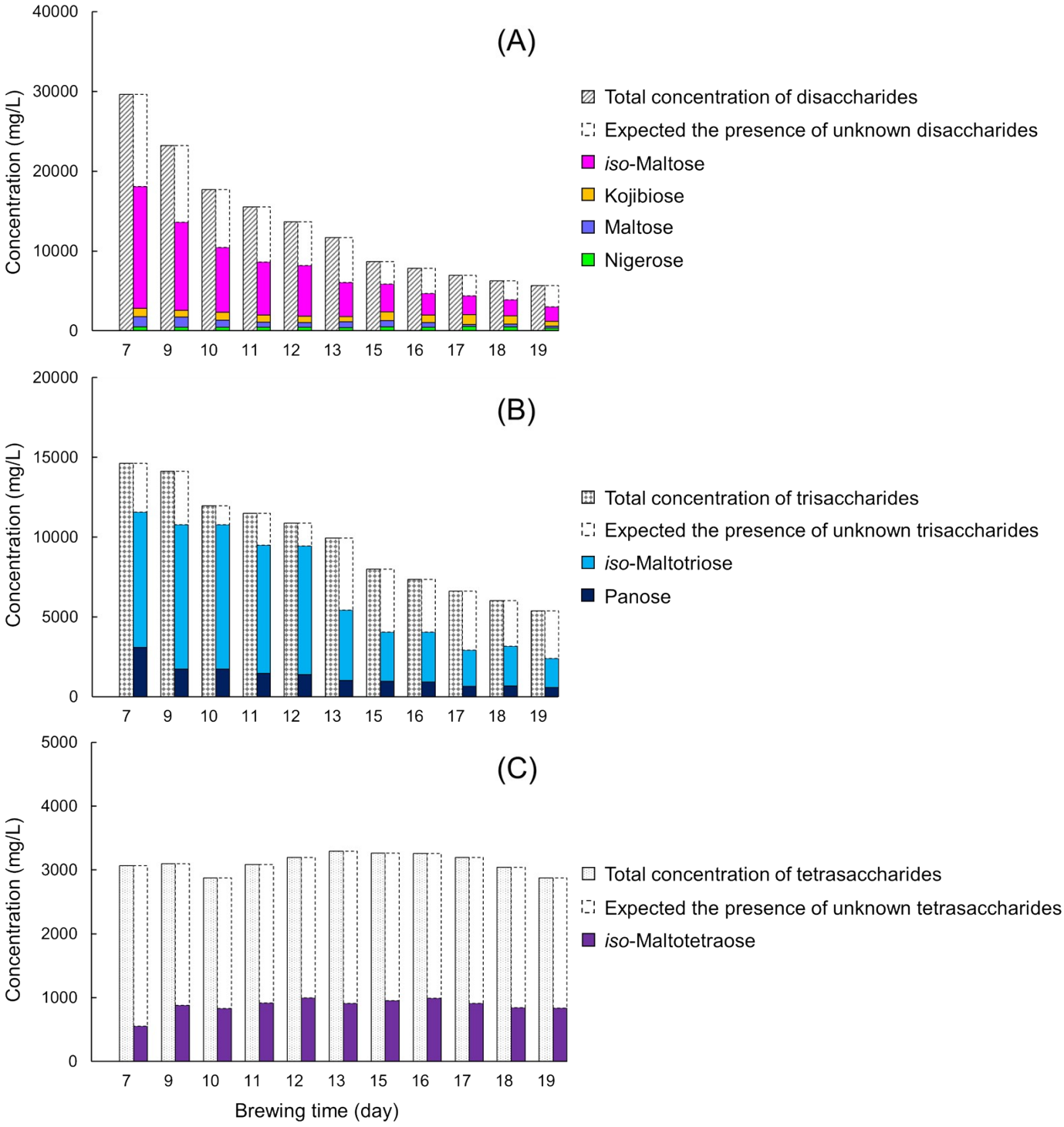

Figure S8. Comparison of the changes in the total concentrations of disaccharides, trisaccharides, and tetrasaccharides determined using our developed method and the expected concentrations of unknown oligosaccharides when considering the total concentrations of disaccharides (*iso*-maltose, kojibiose, maltose, and nigerose), trisaccharides (*iso*-maltotriose and panose), and tetrasaccharides (*iso*-maltotetraose) quantitated using the HILIC method. The experimental conditions for the developed method are the same as those in Figure 3.

Table S1. List of reagents.

Chemical reagent	Reagent grade	Distributor
Citrate	Wako special grade	FUJIFILM Wako Pure Chemical Co. (Osaka, Japan)
L-Malate	Wako 1st grade	FUJIFILM Wako Pure Chemical Co. (Osaka, Japan)
Fumarate	—	FUJIFILM Wako Pure Chemical Co. (Osaka, Japan)
Succinate	Wako special grade	FUJIFILM Wako Pure Chemical Co. (Osaka, Japan)
α-Ketoglutarate	Wako special grade	FUJIFILM Wako Pure Chemical Co. (Osaka, Japan)
Phosphate	Guaranteed reagent	FUJIFILM Wako Pure Chemical Co. (Osaka, Japan)
Phthalate	Wako special grade	FUJIFILM Wako Pure Chemical Co. (Osaka, Japan)
Glucose	Guaranteed reagent	FUJIFILM Wako Pure Chemical Co. (Osaka, Japan)
Xylose	Wako special grade	FUJIFILM Wako Pure Chemical Co. (Osaka, Japan)
Mannitol	Guaranteed reagent	FUJIFILM Wako Pure Chemical Co. (Osaka, Japan)
Arabinose	—	FUJIFILM Wako Pure Chemical Co. (Osaka, Japan)
Galactose	Wako special grade	FUJIFILM Wako Pure Chemical Co. (Osaka, Japan)
Maltose	Wako 1st grade	FUJIFILM Wako Pure Chemical Co. (Osaka, Japan)
Kojibiose	For biochemistry	FUJIFILM Wako Pure Chemical Co. (Osaka, Japan)
Glycerol	Wako 1st grade	FUJIFILM Wako Pure Chemical Co. (Osaka, Japan)
Ethyl *α*-D-glucoside	For food analysis	FUJIFILM Wako Pure Chemical Co. (Osaka, Japan)
Ethanol	Guaranteed grade	FUJIFILM Wako Pure Chemical Co. (Osaka, Japan)
iso-Maltose	—	Hayashibara Co. (Okayama, Japan)
Panose	Guaranteed grade	Hayashibara Co. (Okayama, Japan)
Maltotetraose	—	Hayashibara Co. (Okayama, Japan)
iso-Citrate	—	Sigma-Aldrich Co. (St. Louis, MO, USA)
Nigerose	—	Sigma-Aldrich Co. (St. Louis, MO, USA)
Pyruvate	—	Tokyo Chemical Industry Co. (Tokyo, Japan)
iso-Maltotetraose	HPLC grade	Tokyo Chemical Industry Co. (Tokyo, Japan)
cis-Aconitate	—	Alfa Aesar (Haverhill, MA, USA)
iso-Maltotriose	—	Combi-Blocks (San Diego, CA, USA)

Table S2. Analyte performance under the optimal conditions determined in this study.

Analyte	Retention time (min)		RSD (%)*				LOD** (μM, % for ethanol)		LOQ*** (μM, % for ethanol)		Linearity range (mM, % for ethanol)	Correlation coefficient		Additional recovery (%)	
			Retention time		Peak area										
	CD	RID	CD	RID	CD	RID	CD	RID	CD	RID		CD	RID	CD	RID
Pyruvate	33.3	N.D.	0.0172	—	0.122	—	18.3	—	55.5	—	1.25-10	0.999	—	103	—
Citrate	36.4	36.9	0.0274	0.0313	0.349	0.288	13.3	46.7	40.4	142	1.25-10	0.999	0.999	102	98.7
iso-Citrate	37.8	38.2	0.0458	0.109	0.710	0.222	28.1	153	93.6	465	1.25-10	0.999	0.999	99.1	97.8
Malate	42.1	42.5	0.0137	0.0235	0.281	0.252	24.2	73.9	73.2	224	1.25-10	0.999	0.999	99.2	99.5
Succinate	50.3	50.8	0.230	0.238	0.545	0.853	28.5	102	86.4	309	1.25-10	0.999	0.999	102	99.6
Fumarate	48.8	49.5	0.0516	0.0117	0.817	0.418	27.1	172	82.1	521	1.25-10	0.999	0.999	103	—
Glucose	N.D.	43.7	—	0.0606	—	0.485	—	25.3	—	76.7	10-500	—	0.999	—	103
Maltose	N.D.	39.1	—	0.0443	—	0.485	—	15.6	—	47.2	1.25-10	—	0.999	—	102
iso-Maltose	N.D.	38.6	—	0.0150	—	0.600	—	22.5	—	68.2	1.25-10	—	0.999	—	101
Kojibiose	N.D.	39.0	—	0.0513	—	0.221	—	22.1	—	66.8	1.25-10	—	0.999	—	98.8
Nigerose	N.D.	39.1	—	0.0391	—	0.206	—	31.6	—	94.1	1.25-5	—	0.999	—	101
Panose	N.D.	36.3	—	0.0159	—	0.646	—	30.7	—	93.0	1.25-10	—	0.999	—	99.1
iso-Maltotriose	N.D.	36.0	—	0.0279	—	0.574	—	23.7	—	71.8	1.25-10	—	0.999	—	102
iso-Maltotetraose	N.D.	35.1	—	0.0914	—	0.468	—	12.0	—	36.4	1.25-10	—	0.999	—	104
Ethanol	79.6	80.3	—	0.00720	—	0.633	—	0.036	—	0.109	1.0-15	—	0.999	—	98.4

* n = 5, ** S/N = 3.3, *** S/N = 10

		Brewing time (days)														
		3	**5**	**7**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Pyruvate		266	217	222	225	219	226	221	217	209	194	182	167	156	143	129
Citrate		48.4	57.9	68.8	78.4	71.2	79.1	84.3	87.5	84.9	80.5	90.1	96.5	84.9	86.8	88.3
iso-Citrate		N.D.*	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
L-Malate		49.2	101	118	150	131	145	149	156	178	186	181	180	188	190	204
Succinate	mg/L	173	219	262	292	285	286	283	293	312	317	315	307	313	329	334
Fumarate		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Disaccharides		33236	36086	29601	23213	17713	15542	13638	11685	8814	8697	7823	6935	6265	5663	5037
Trisaccharides		9098	13463	14627	14121	11952	11502	10868	9934	7167	7994	7358	6621	6021	5372	4888
Tetrasaccharides		5528	4412	3065	3096	2877	3084	3195	3294	2959	3262	3257	3197	3044	2877	2771
Glucose	mg/L	100475	69754	51902	41763	32553	30358	27403	25560	28216	22006	20281	19176	18002	17290	16204
	%	10.0	6.98	5.19	4.18	3.26	3.04	2.74	2.56	2.82	2.20	2.03	1.92	1.80	1.73	1.62
Ethanol	mg/L	42310	80627	111248	131660	124618	135022	139127	148015	154633	153812	158506	161272	162608	168379	171842
	%	4.23	8.06	11.1	13.2	12.5	13.5	13.9	14.8	15.5	15.4	15.9	16.1	16.3	16.8	17.2

Table S3. Analytical results for 15 brewing-processed rice wine samples.

*N.D.: under the detection limit of the developed method

			Brewing time (days)										
			7	9	10	11	12	13	15	16	17	18	19
Pyruvate	mg/L	SEC/IEC	222	225	219	226	221	217	194	182	167	156	143
		Method 1*	234	241	227	236	234	233	206	187	184	169	152
Citrate		SEC/IEC	68.8	78.4	71.2	79.1	84.3	87.5	80.5	90.1	96.5	84.9	86.8
		Method 1*	71.6	85.0	65.6	86.5	85.7	94.6	89.4	93.9	99.2	88.1	94.7
L-Malate		SEC/IEC	118	150	131	145	149	156	186	181	180	188	190
		Method 1*	134	153	128	150	159	167	199	196	211	212	217
Succinate		SEC/IEC	262	292	285	286	283	293	317	315	307	313	329
		Method 1*	293	304	289	296	292	306	322	317	294	314	324
Glucose	%	SEC/IEC	5.19	4.18	3.26	3.04	2.74	2.56	2.20	2.03	1.92	1.80	1.73
		Method 2**	5.27	4.17	3.23	3.10	2.70	2.70	2.10	1.83	2.07	1.60	1.60
Ethanol		SEC/IEC	11.1	13.2	12.5	13.5	13.9	14.8	15.4	15.9	16.1	16.3	16.8
		Method 3***	11.4	13.4	12.5	13.6	14.0	14.9	16.0	16.2	16.5	16.9	17.3

Table S4. Validation data for organic acids, glucose, and ethanol.

The experimental conditions of the developed method are the same as those in Figure 3.

* Ion-exclusion-mode IC method, ** Enzyme-based 4-aminoantipyrine visual colorimetric method, *** Vibration-type density meter method.

				Brewing time (days)										
				7	9	10	11	12	13	15	16	17	18	19
Disaccharides	mg/L	SEC/IEC		29601	23213	17713	15542	13638	11685	8697	7823	6935	6265	5663
		Method 4****	Total	18101	13592	10443	8623	8167	6077	5876	4676	4395	3908	3028
			Maltose	1308	1288	890	633	586	726	769	572	226	338	252
			iso-Maltose	15285	11031	8123	6651	6323	4293	3515	2692	2359	2014	1834
			Kojibiose	1031	844	970	896	833	673	1098	955	1272	1070	581
			Nigerose	477	429	461	442	426	385	494	457	539	487	361
Trisaccharides		SEC/IEC		14627	14121	11952	11502	10868	9934	7994	7358	6621	6021	5372
		Method 4****	Total	11569	13671	10790	9500	9452	5444	4052	4063	2928	3171	2397
			Panose	3101	2182	1732	1458	1396	1019	978	912	655	678	575
			iso-Maltotriose	8468	11490	9058	8041	8056	4425	3074	3151	2272	2493	1821
Tetrasaccharides		SEC/IEC		3065	3096	2877	3084	3195	3294	3262	3257	3197	3044	2877
		Method 4****	*iso*-Maltotetraose	552	879	829	916	994	910	952	988	912	839	836

Table S5. Validation data for oligosaccharides.

**** HILIC-mode chromatographic method.