

Supporting Information

A Comparative Analysis of Methods for Quantitation of Sugar During the Corn-to-Ethanol Fermentation Process

*Sarah R. Bilskey, Samantha A. Olendorff, Karolina Chmielewska, Kevin R. Tucker **

Corresponding Author:
Kevin Tucker, Ph.D.
Assistant Professor
Department of Chemistry
Southern Illinois University Edwardsville
44 Circle Dr.; Box 1692
Edwardsville, IL 62026
(O) 618-650-5868
kevtuck@siue.edu

Table S1. Concentration values for the components in the NCERC fermentation standards. Eight standards were made as well as a check standard.

Concentrations of Standards (g/L)									
Analyte	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Chk. Std.	Std8
DP4+	42.7	38.5	21.4	10.7	4.27	2.14	0.427	29.9	0.214
DP3	40.5	36.5	20.3	10.1	4.06	2.03	0.406	28.4	0.203
DP2	44.4	40.0	22.2	11.1	4.44	2.22	0.444	31.1	0.222
Glucose	45.5	41.0	22.8	11.4	4.55	2.28	0.455	31.9	0.228
Lactic Acid	20.3	18.2	10.1	5.07	2.03	1.01	0.203	14.2	0.101
Glycerol	15.5	14.0	7.76	3.88	1.55	0.776	0.155	10.9	0.0786
Acetic Acid	5.24	4.72	2.62	1.31	0.524	0.262	0.0524	3.67	0.0262
Ethanol	200.	180.	100.	50.0	20.0	10.0	2.00	140	1.00

Table S2. Agilent 6460 LC-MS instrumental parameters for preliminary analysis of ion identification of sugars by full scan and SIM as indicated.

Agilent 6460 Source Parameters	
Source	ESI
Heated Drying Gas	Temperature (°C): 275 Flow (L/min): 11
Nebulizer	Pressure (psi): 45
Sheath Gas	Temperature (°C): 375 Flow (L/min): 11
Capillary	Voltage (V): +4000
Nozzle	Voltage (V): 0
Agilent 6460 Mass Spectrometer Acquisition	
Event	Time (sec): 0.4438 (full scan); 0.1350 (SIM)*
Q1 Prerod Bias (Fragmentor)	Voltage (V): 60
Q3 Prerod Bias (CAV)	Voltage (V): 7
Polarity	(+/-): Positive

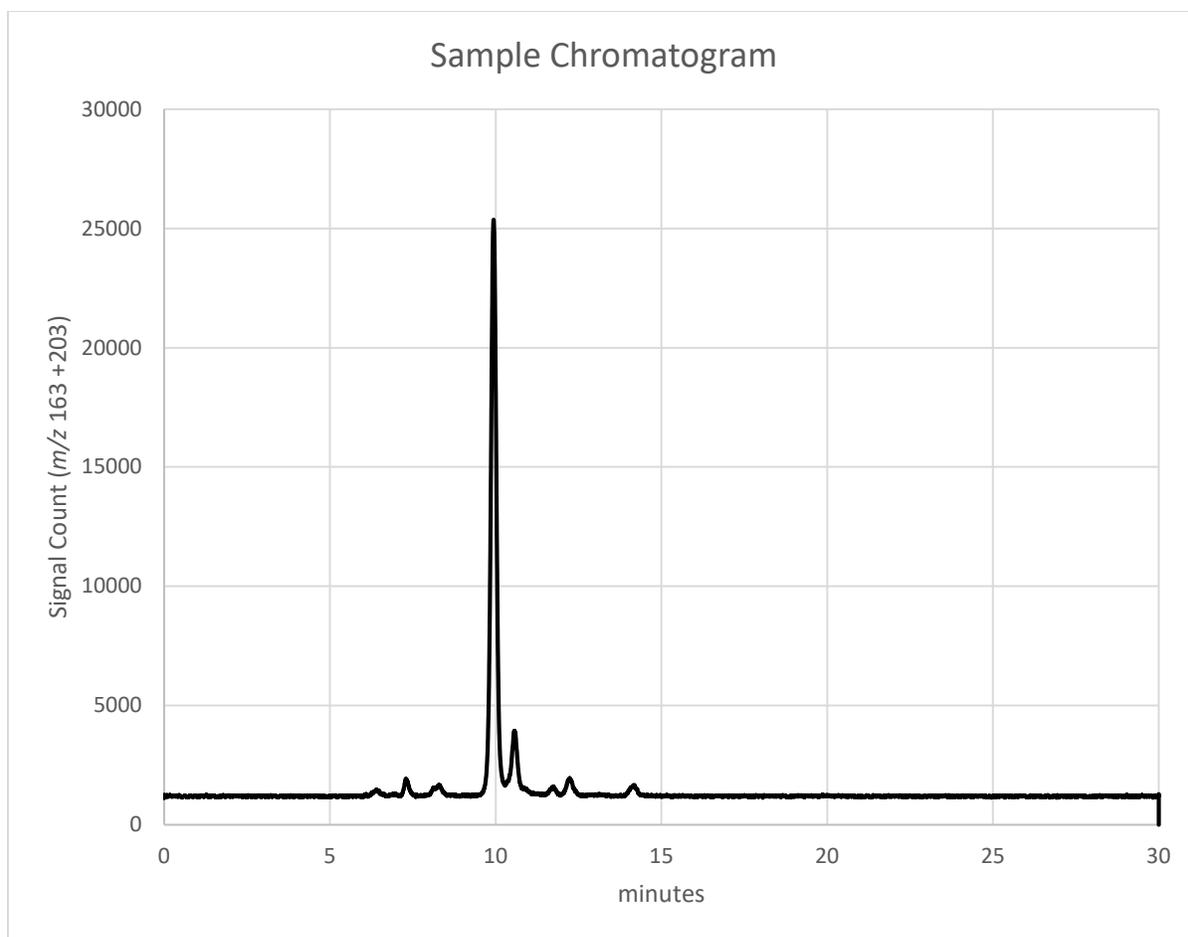


Figure S1. Sample chromatogram from the analysis on a fermentation sample at T38 using the sum of signals from m/z 's 163 and 203. Multiple peaks are detected due to the presence of various monomeric sugars. Glucose is time matched at ~10 minutes and is the largest peak.

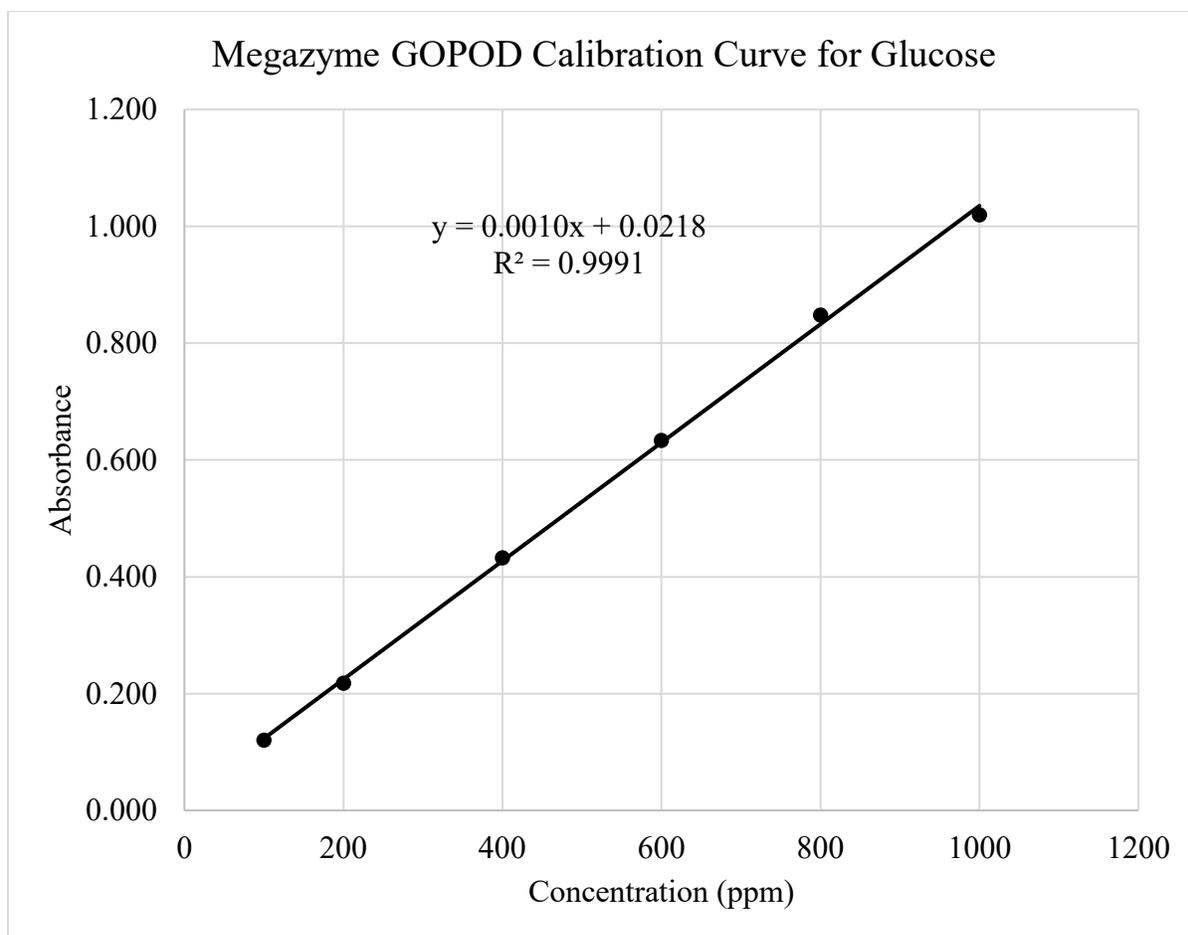


Figure S2. Megazyme GOPOD calibration curve for the analysis of glucose. Standards containing glucose, maltose, and maltotriose range from 100 - 1000 ppm.

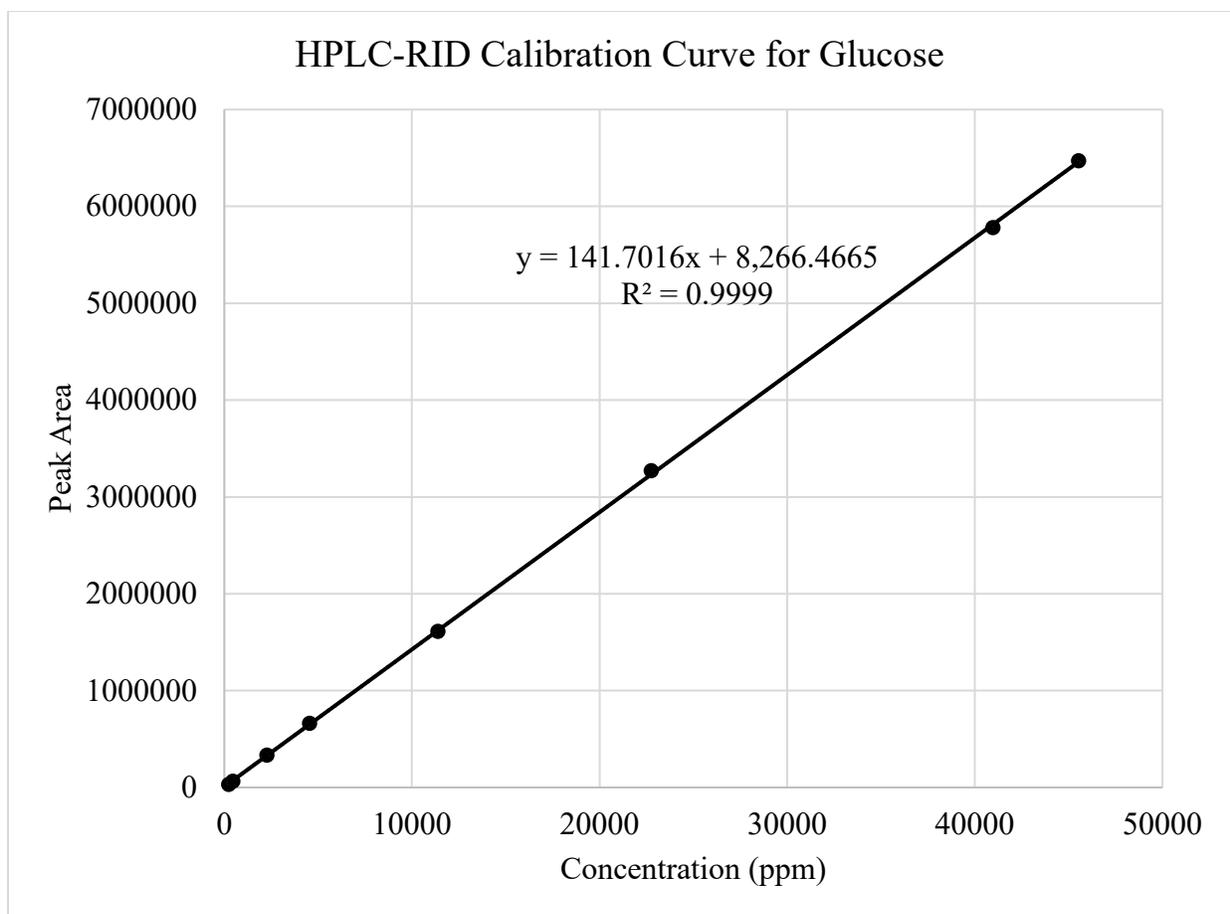


Figure S3. HPLC-RID calibration curve for the analysis of glucose. NCERC fermentation standards were analyzed from 0.02% - 5%.

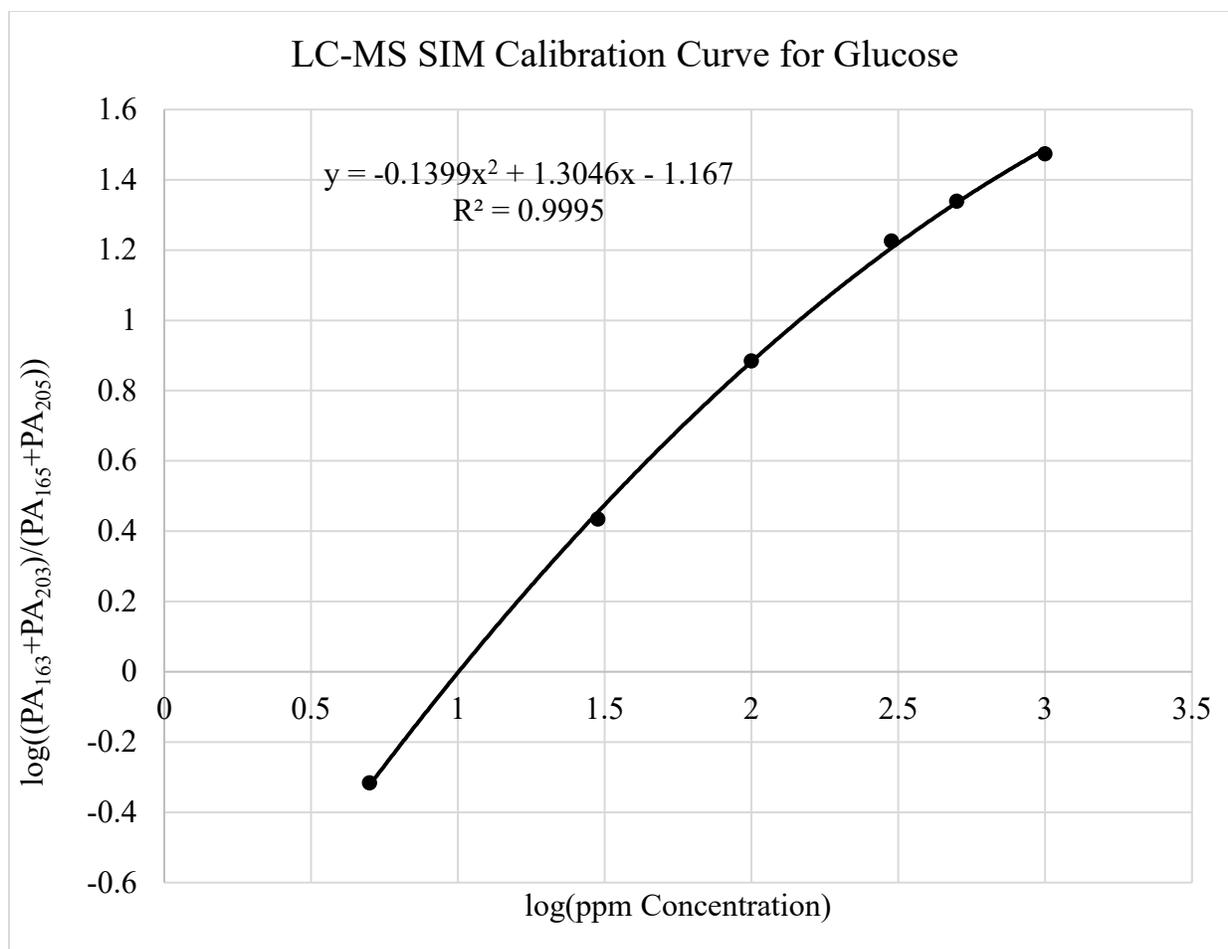


Figure S4. LC-MS SIM calibration curve for the analysis of glucose. Standards containing glucose, maltose, maltotriose and deuterated internal standard range from 5 - 1000 ppm; deuterated internal standard of concentration 10 ppm. The data is plotted on a log-log scale to show a quadratic relationship between peak area and concentration. The y-axis indicates which peak areas (PA) and mass (in subscript) were used for the calibration.

Table S3. Quantitation results for glucose by the Megazyme GOPOD analysis. The dilution factor, diluted concentration, and original concentration for samples T14 - T62 for three flask fermentations are shown. The standard deviation and percent RSD are representative of three technical replicates for each sample. Dashes (-) in the diluted sample concentration column indicate that the sample was not diluted prior to analysis. Concentrations of undiluted T62 samples were unable to be determined by this analytical method.

Megazyme GOPOD Analysis Results for Glucose					
Dilution Factor	Time (hr)	Flask	Diluted Conc. (ppm)	Original Conc. (ppm)	% RSD
300x	14	1	247 ± 3	74300 ± 900	1.00
		2	255 ± 2	76700 ± 500	0.56
		3	247 ± 5	74300 ± 1600	1.77
75x	26	1	295 ± 7	22100 ± 500	2.03
		2	325 ± 6	24400 ± 500	1.55
		3	272 ± 5	20400 ± 400	1.57
4x	38	1	546 ± 7	2190 ± 30	1.09
		2	617.7 ± 1.4	2471 ± 6	0.22
		3	600 ± 10	2410 ± 40	1.41
-	50	1	-	216 ± 3	0.99
		2	-	314 ± 12	3.19
		3	-	291 ± 5	1.58
-	62	1	-	< LOQ	-
		2	-	< LOQ	-
		3	-	< LOQ	-
				Average % RSD	1.41

Table S4. Quantitation results for glucose by the HPLC-RID analysis. The dilution factor, diluted concentration, and original concentration for samples T14 - T38 for three flask fermentations are shown. The standard deviation and percent RSD are representative of three technical replicates for each sample. Dashes (-) in the diluted sample concentration column indicate that the sample was not diluted prior to analysis. Concentrations of undiluted T50 and T62 samples were unable to be determined by this analytical method.

HPLC-RID Analysis Results for Glucose					
Dilution Factor	Time (hr)	Flask	Diluted Conc. (ppm)	Original Conc. (ppm)	% RSD
10x	14	1	15922 ± 18	159220 ± 180	0.11
		2	16047 ± 12	160470 ± 120	0.07
		3	15890 ± 20	158900 ± 200	0.14
10x	26	1	4341 ± 6	43410 ± 60	0.15
		2	4391 ± 8	43910 ± 80	0.19
		3	4268 ± 2	42680 ± 20	0.06
-	38	1	-	6377 ± 14	0.22
		2	-	7242 ± 7	0.10
		3	-	7059 ± 5	0.06
-	50	1	-	< LOQ	-
		2	-	< LOQ	-
		3	-	< LOQ	-
-	62	1	-	< LOD	-
		2	-	< LOD	-
		3	-	< LOD	-
				Average % RSD	0.12

Table S5. Quantitation results for glucose by the LC-MS SIM analysis. The dilution factor, diluted concentration, and original concentration for samples T14 - T62 for three flask fermentations are shown. The standard deviation and percent RSD are representative of three technical replicates for each sample.

LC-MS SIM Analysis Results for Glucose					
Dilution Factor	Time (hr)	Flask	Diluted Conc. (ppm)	Original Conc. (ppm)	% RSD
200	14	1	468 ± 4	93500 ± 900	0.93
		2	458 ± 10	91600 ± 1900	2.09
		3	481 ± 5	96200 ± 900	0.95
100	26	1	324 ± 4	32400 ± 400	1.37
		2	333 ± 9	33300 ± 900	2.57
		3	242 ± 5	24200 ± 500	2.09
100	38	1	24.33 ± 0.12	2433 ± 12	0.51
		2	28.4 ± 0.3	2840 ± 30	0.93
		3	29.3 ± 0.2	2940 ± 20	0.82
10	50	1	46.2 ± 0.8	462 ± 8	1.65
		2	58.3 ± 1.4	583 ± 14	2.35
		3	58.9 ± 0.5	589 ± 5	0.92
10	62	1	11.18 ± 0.04	111.8 ± 0.4	0.39
		2	13.15 ± 0.12	131.5 ± 1.2	0.93
		3	12.6 ± 0.3	126 ± 3	2.37
				Average % RSD	1.39

Table S6. Characteristic ions identified for dextrose, maltose, and maltotriose by LC-MS full scan analysis of pure standards.

Identification of Characteristic Ions by LC-MS			
Analyte	RT (min)	Characteristic Ions (<i>m/z</i>)	Formula
Maltotriose (DP3)	7.60	522.0, 487.0, 505.0, 527.0	[M+H ₂ O] ⁺ , [M+H-H ₂ O] ⁺ , [M+H] ⁺ , [M+Na] ⁺
Maltose (DP2)	8.51	360.0, 341.9, 364.9	[M+H ₂ O] ⁺ , [M] ⁺ , [M+Na] ⁺
Glucose (Glc)	10.17	162.9, 198.0, 179.9, 202.9	[M+H-H ₂ O] ⁺ , [M+H ₂ O] ⁺ , [M] ⁺ , [M+Na] ⁺

Table S7. Summary for the ion identification analysis for glucose, maltose, and maltotriose in the fermentation broth sample. The mass-to-charge ratios (m/z) and their respective retention times (RT) are listed. *Only one major overlapping peak for the identification of glucose was identified, as indicated by dashes (-) in the Peak 2 column for glucose.

Analyte	Formula	Ion (m/z)	Peak 1		Peak 2	
			RT (min)	Abundance	RT (min)	Abundance
Glucose	[Glc+H-H ₂ O] ⁺	162.9	10.39	2714.89	-	-
	[Glc+H ₂ O] ⁺	198.0		2145.64		-
	[Glc•] ⁺	179.9		835.63		-
	[Glc+Na] ⁺	202.8		453.96		-
Maltose	[DP2•] ⁺	341.9	8.65	1193.02	8.80	1054.34
	[DP2+H ₂ O] ⁺	360.0		4239.54		3717.19
	[DP2+Na] ⁺	364.8		388.57		355.57
Maltotriose	[DP3+H] ⁺	504.9	7.90	247.66	7.84	210.83
	[DP3+H ₂ O] ⁺	522.1		371.50		330.19
	[DP3+Na] ⁺	527.0		ND		ND
	[DP3+H-H ₂ O] ⁺	486.8		226.85		ND

Table S8. Comparison of the retention time (RT) for the identified analytes when analyzing the standards versus analyzing the sample by LC-MS full scan.

Retention Time Comparison of Standard and Sample Analytes by LC-MS			
	Standard	Sample	
Analyte	RT (min)		RT difference (min)
Maltotriose	7.60	7.90	0.30
Maltose	8.51	8.65	0.14
Glucose	10.17	10.39	0.22

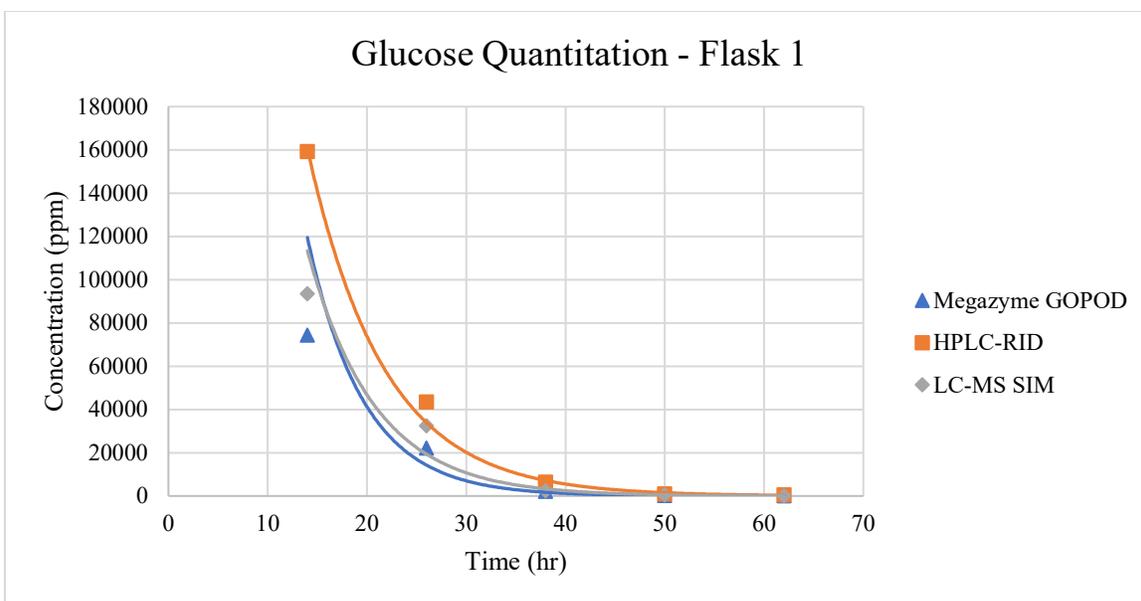


Figure S5. Exponential decay of glucose concentration during corn-to-ethanol fermentation for Flask 1 from T14 to T62. The plotted values are of raw data values, of which some were not reported due to results outside of the calibration.

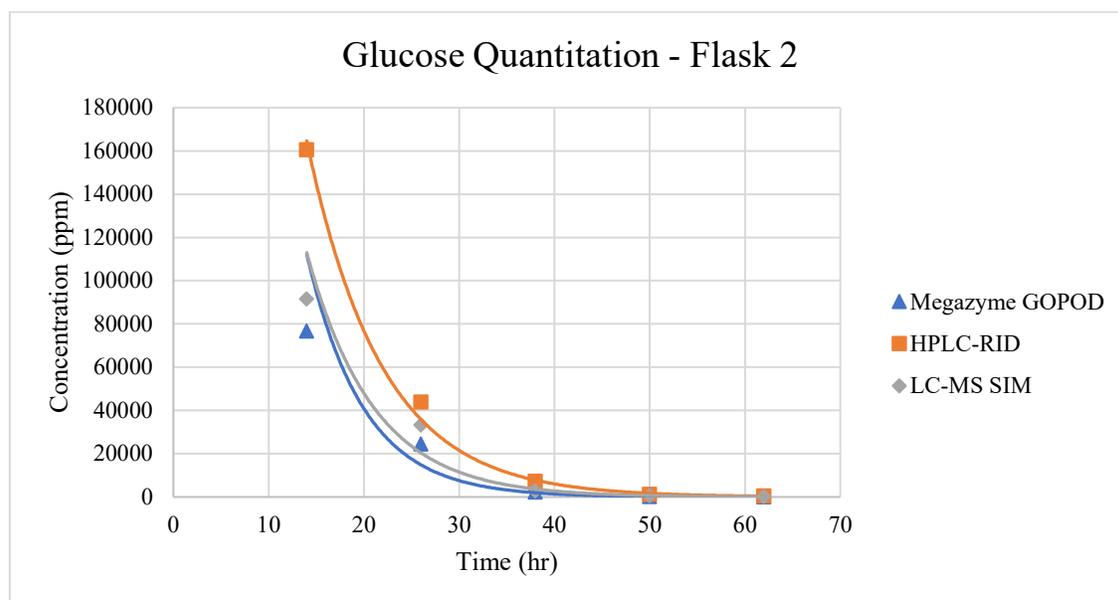


Figure S6. Exponential decay of glucose concentration during corn-to-ethanol fermentation for Flask 2 from T14 to T62. The plotted values are of raw data values, of which some were not reported due to results outside of the calibration.

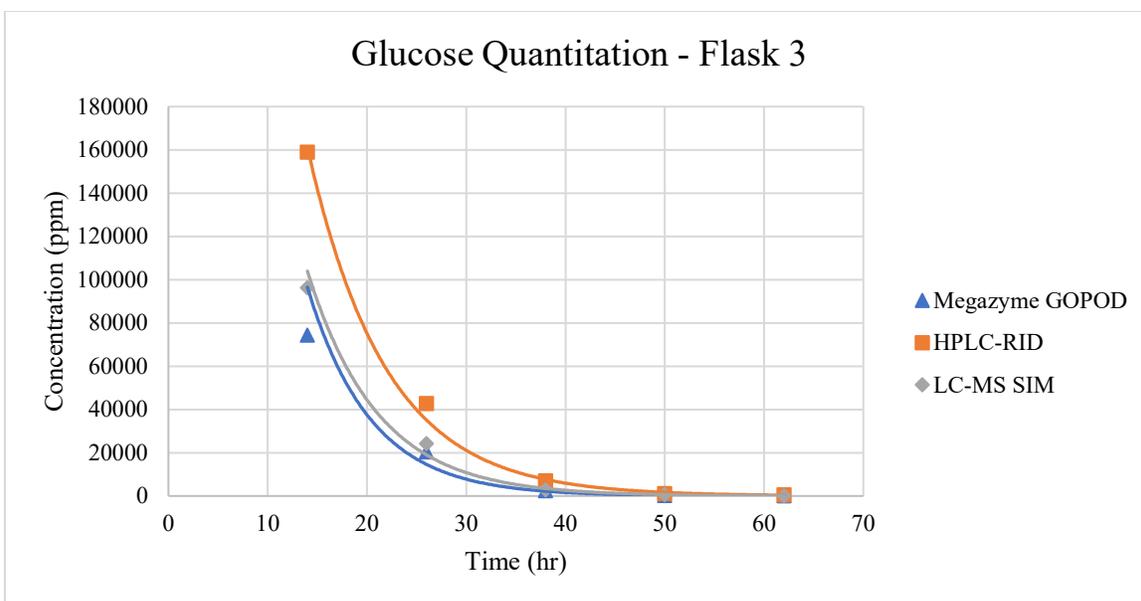


Figure S7. Exponential decay of glucose concentration during corn-to-ethanol fermentation for Flask 3 from T14 to T62. The plotted values are of raw data values, of which some were not reported due to results outside of the calibration.

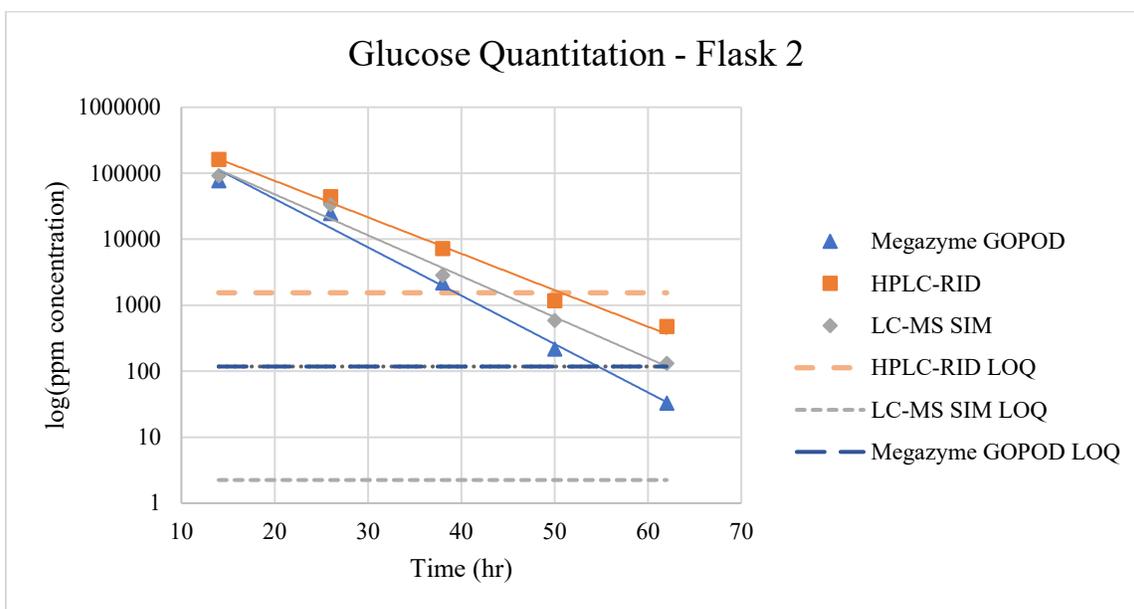


Figure S8. Decay of glucose concentration during the corn-to-ethanol fermentation process for Flask 2 from T14 to T62. The data is in log scale on the y-axis to show the LOQ of the analytical methods tested and the data points which fall below it. The data plotted is of raw data values, of which some were not reported due to results outside of the calibration.

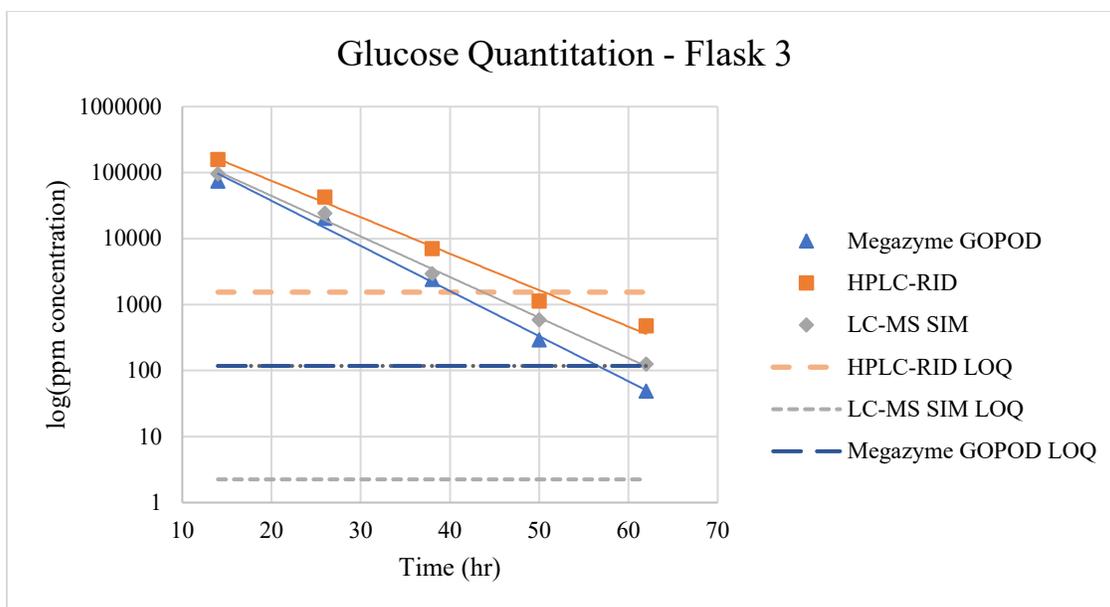


Figure S9: Decay of glucose concentration during the corn-to-ethanol fermentation process for Flask 3 from T14 to T62. The data is in log scale on the y-axis to show the LOQ of the analytical methods tested and the data points which fall below it. The data plotted is of raw data values, of which some were not reported due to results outside of the calibration.

Table S9. Comparison of quantified concentrations of dextrose in biological flask fermentation replicates by paired samples t-test.

Method	Flasks Compared		Samples	p-value
Megazyme GOPOD	F1	F2	T14 - T50	0.127
	F1	F3		0.411
	F2	F3		0.114
HPLC-RID	F1	F2	T14 - T38	0.339
	F1	F3		0.536
	F2	F3		0.067
Agilent LC-MS SIM	F1	F2	T14 - T62	0.077
	F1	F3		0.580
	F2	F3		0.473

Table S10. Comparison of results of the analytical methods for the quantitation of dextrose using the paired samples t-test.

Dextrose Quantitation Method Comparison - Paired Samples t-test (p-values)			
Method	Megazyme GOPOD	HPLC-RID	LC-MS SIM
Megazyme GOPOD			
HPLC-RID	0.000		
Agilent LC-MS SIM	0.000	0.000	

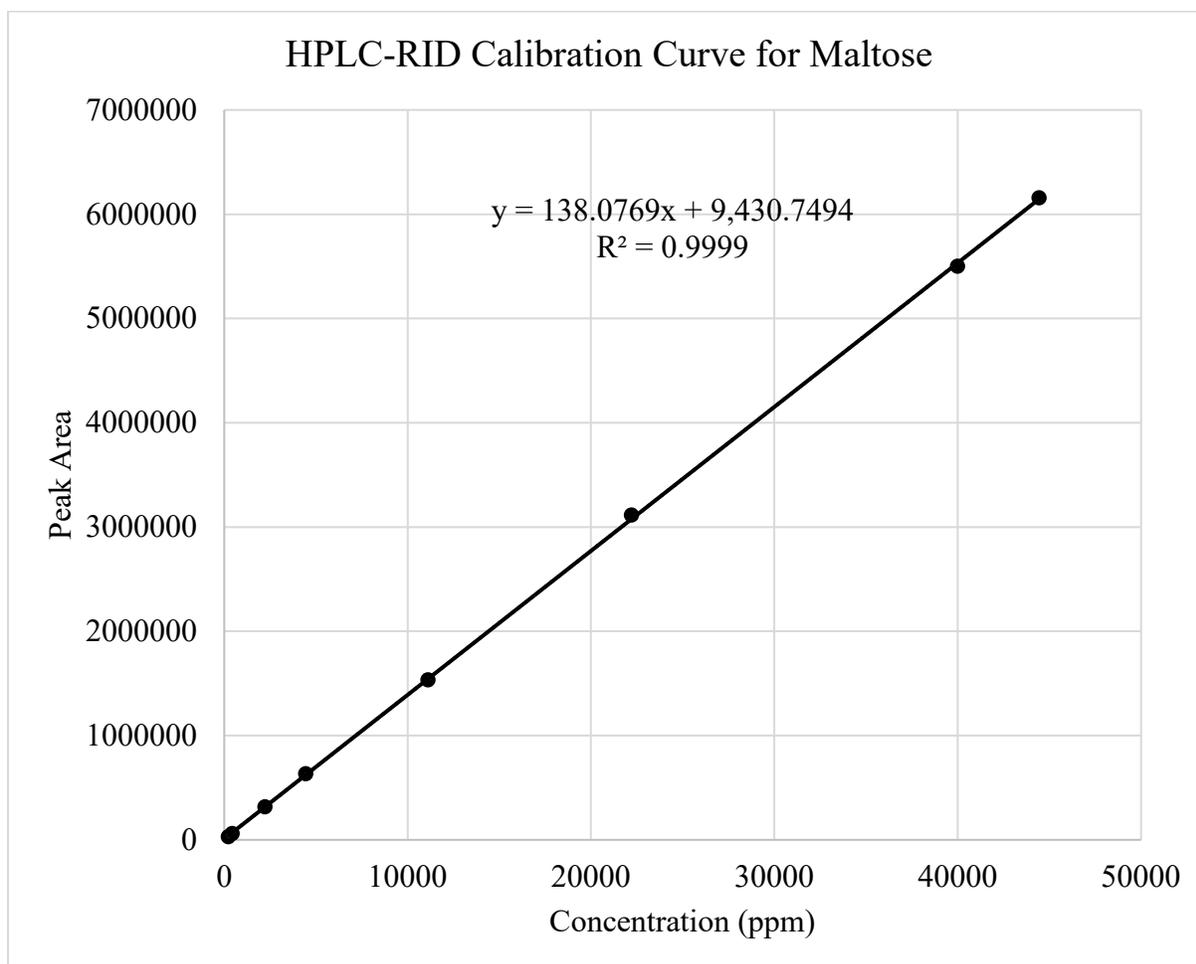


Figure S10: HPLC-RID calibration curve for the analysis of maltose. NCERC fermentation standards were analyzed from 0.02% - 4%.

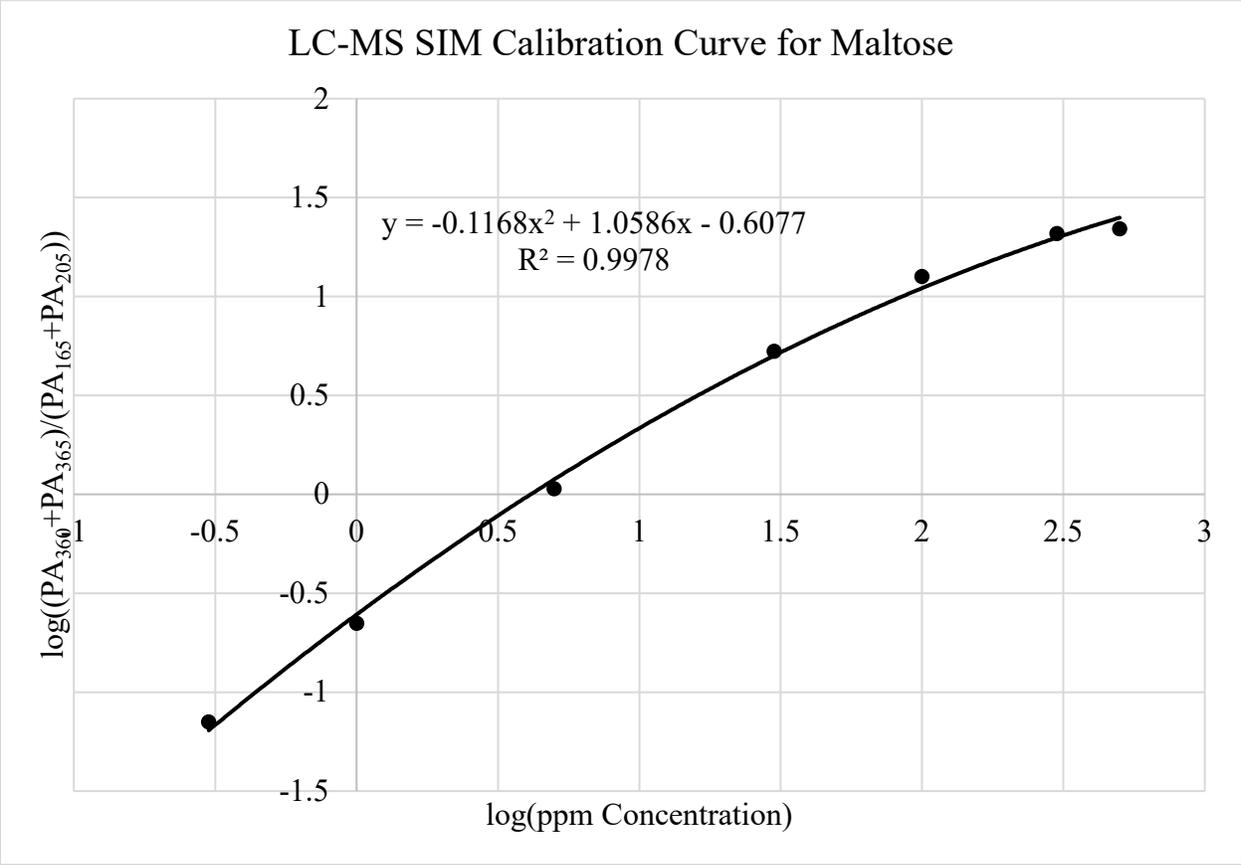


Figure S11: LC-MS SIM calibration curve for the analysis of maltose. Standards containing glucose, maltose, maltotriose and deuterated internal standard range from 0.3 - 500 ppm; deuterated internal standard of concentration 10 ppm. The data is plotted on a log-log scale to show a quadratic relationship between peak area and concentration.

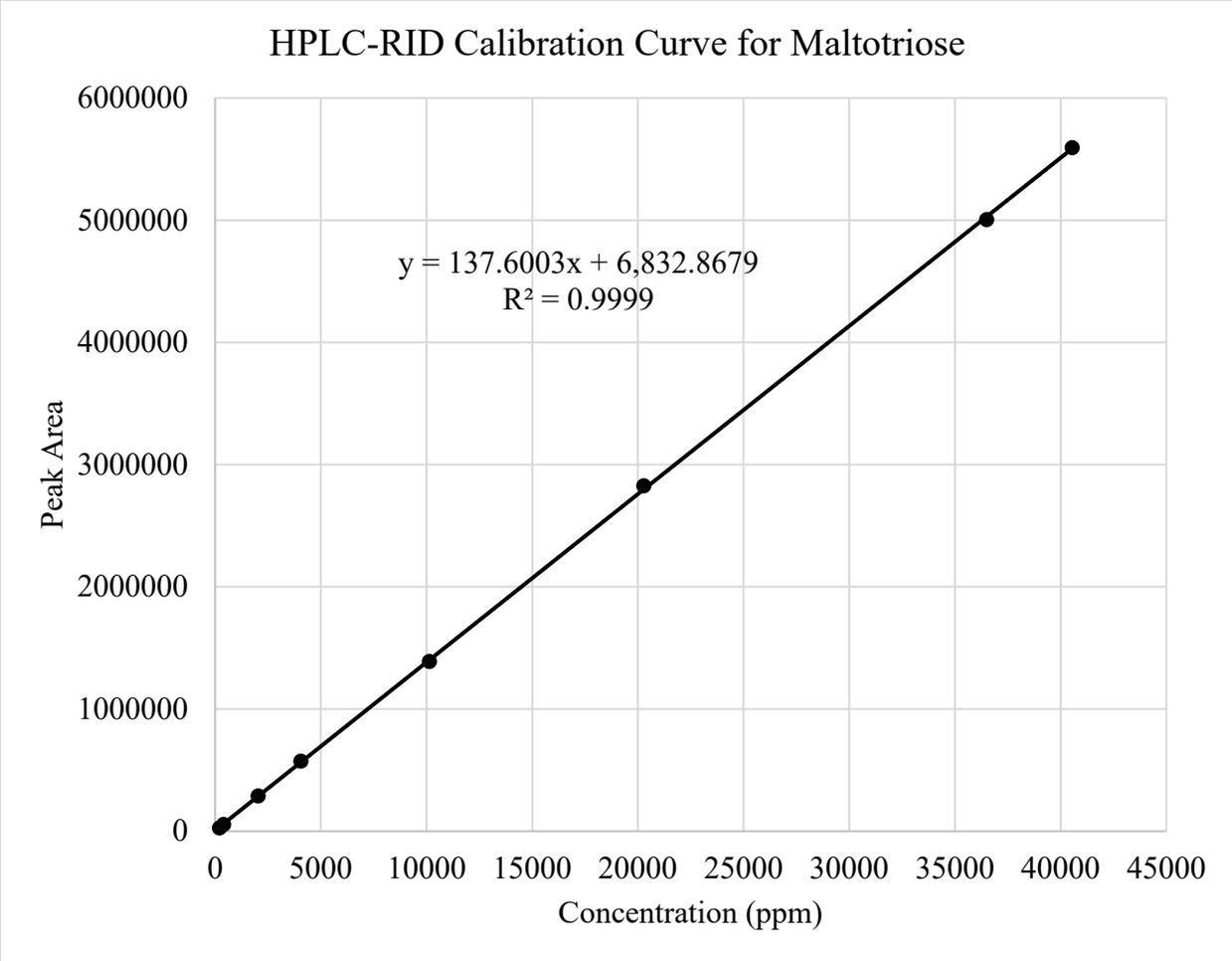


Figure S22: HPLC-RID calibration curve for the analysis of maltotriose. NCERC fermentation standards were analyzed from 0.02% - 4%.

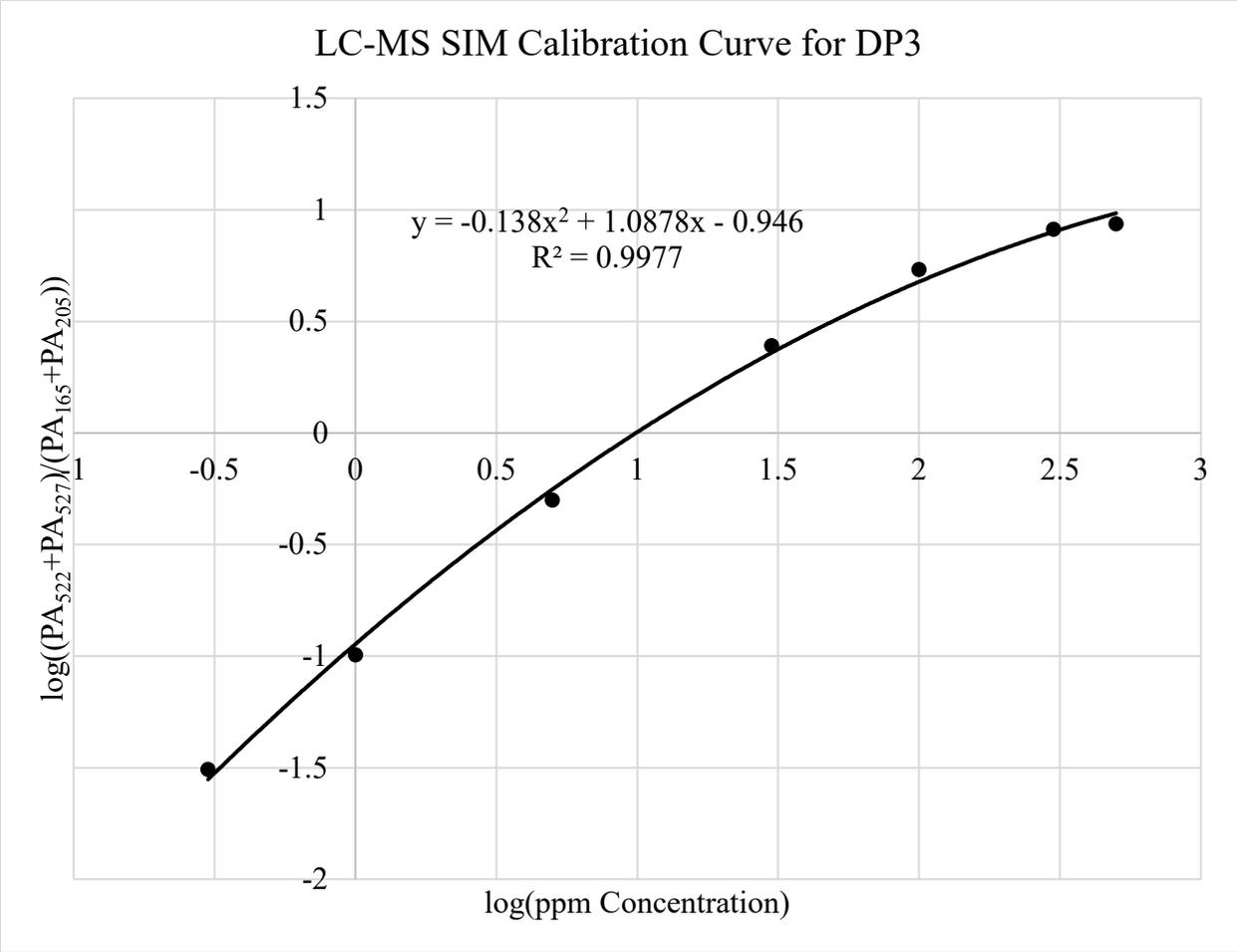


Figure S33: LC-MS SIM calibration curve for the analysis of maltotriose. Standards containing glucose, maltose, maltotriose and deuterated internal standard range from 0.3 - 500 ppm; deuterated internal standard of concentration 10 ppm. The data is plotted on a log-log scale to show a quadratic relationship between peak area and concentration.

Table S11: Quantitation results for DP2 by the HPLC-RID analysis. The dilution factor, diluted concentration, and original concentration for samples T14 - T62 for three flask fermentations are shown. The standard deviation and percent RSD are representative of three technical replicates for each sample. Dashes (-) in the diluted sample concentration column indicate that the sample was not diluted prior to analysis. Not reportable (NR) indicates that the concentration of the diluted sample fell outside of the quantifiable range for the analyte and therefore no calculated concentration can be reported.

HPLC-RID Analysis Results for DP2					
Dilution Factor	Time (hr)	Flask	Diluted Conc. (ppm)	Original Conc. (ppm)	% RSD
10x	14	1	< LOD	NR	-
		2	< LOD	NR	-
		3	< LOD	NR	-
-	26	1	-	1660 ± 3	0.19
		2	-	1645 ± 3	0.16
		3	-	1621 ± 5	0.31
-	38	1	-	< LOQ	-
		2	-	< LOQ	-
		3	-	< LOQ	-
-	50	1	-	< LOQ	-
		2	-	< LOQ	-
		3	-	< LOQ	-
-	62	1	-	< LOQ	-
		2	-	1664 ± 6	0.33
		3	-	< LOQ	-
				Average % RSD	0.22

Table S12: Quantitation results for DP2 by the LC-MS SIM analysis. The dilution factor, diluted concentration, and original concentration for samples T14 - T62 for three flask fermentations are shown. The standard deviation and percent RSD are representative of three technical replicates for each sample. Not reportable (NR) indicates that the concentration of the diluted sample fell outside of the quantifiable range for the analyte and therefore no calculated concentration can be reported.

LC-MS SIM Analysis Results for DP2					
Dilution Factor	Time (hr)	Flask	Diluted Conc. (ppm)	Original Conc. (ppm)	% RSD
200	14	1	157 ± 3	31500 ± 600	1.96
		2	184 ± 6	36900 ± 1200	3.35
		3	168 ± 5	33600 ± 1000	2.87
100	26	1	21.2 ± 0.6	2120 ± 60	2.64
		2	22.4 ± 0.7	2240 ± 70	3.27
		3	18.2 ± 0.3	1830 ± 30	1.80
100	38	1	15.39 ± 0.11	1539 ± 11	0.72
		2	17.5 ± 0.3	1750 ± 30	1.61
		3	17.01 ± 0.18	1701 ± 18	1.06
10	50	1	> Highest Cal.	NR	-
		2	470 ± 20	4800 ± 200	4.26
		3	> Highest Cal.	NR	-
10	62	1	489 ± 2	4890 ± 20	0.43
		2	> Highest Cal.	NR	-
		3	494 ± 17	4940 ± 170	3.37
				Average % RSD	2.28

Table S13: Quantitation results for DP3 by the HPLC-RID analysis. The dilution factor, diluted concentration, and original concentration for samples T14 - T62 for three flask fermentations are shown. The standard deviation and percent RSD are representative of three technical replicates for each sample. Dashes (-) in the diluted sample concentration column indicate that the sample was not diluted prior to analysis. Not reportable (NR) indicates that the concentration of the diluted sample fell outside of the quantifiable range for the analyte and therefore no calculated concentration can be reported.

HPLC-RID Analysis Results for DP3					
Dilution Factor	Time (hr)	Flask	Diluted Conc. (ppm)	Original Conc. (ppm)	% RSD
10x	14	1	< LOD	NR	-
		2	< LOD	NR	-
		3	< LOD	NR	-
-	26	1	-	2234 ± 11	0.49
		2	-	2410 ± 8	0.35
		3	-	2400 ± 15	0.64
-	38	1	-	< LOQ	-
		2	-	< LOQ	-
		3	-	< LOQ	-
-	50	1	-	< LOQ	-
		2	-	< LOQ	-
		3	-	< LOQ	-
-	62	1	-	< LOQ	-
		2	-	< LOQ	-
		3	-	< LOQ	-
				Average % RSD	0.49

Table S14: Quantitation results for DP3 by the LC-MS SIM analysis. The dilution factor, diluted concentration, and original concentration for samples T14 - T62 for three flask fermentations are shown. The standard deviation and percent RSD are representative of three technical replicates for each sample.

LC-MS SIM Analysis Results for DP3					
Dilution Factor	Time (hr)	Flask	Diluted Conc. (ppm)	Original Conc. (ppm)	% RSD
200	14	1	8.49 ± 0.03	1698 ± 7	0.41
		2	9.2 ± 0.2	1840 ± 40	2.17
		3	9.34 ± 0.08	1868 ± 15	0.81
100	26	1	67 ± 2	6700 ± 200	3.20
		2	81.6 ± 1.3	8160 ± 130	1.63
		3	67 ± 2	6700 ± 200	3.07
100	38	1	15.85 ± 0.08	1585 ± 8	0.51
		2	22.43 ± 0.06	2243 ± 6	0.26
		3	21.25 ± 0.19	2125 ± 19	0.89
10	50	1	138 ± 5	1380 ± 50	3.96
		2	140 ± 6	1400 ± 60	4.10
		3	149 ± 4	1490 ± 40	2.81
10	62	1	92 ± 2	920 ± 20	2.19
		2	108 ± 5	1080 ± 50	4.29
		3	106 ± 4	1060 ± 40	3.62
				Average % RSD	2.26