

**Quantification of carbohydrates in human serum using gas
chromatography-mass spectrometry with stable isotope-labeled
internal standard method**

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Table.S1. The ion pairs of carbohydrates of the silylation and acylation protocol.

Carbohydrate	Retention time (min)	Precursor Ion (m/z)	Product ion (m/z)	Collision energy (eV)	Carbohydrate	Retention time (min)	Precursor Ion (m/z)	Product ion (m/z)	Collision energy (eV)
2-Deoxy-D-Ribose	9.96	147	73	14	2-Deoxy-D-Ribose	7.32	141	97	8
D-(+)-Arabinose	10.9	160	73	14	D-(+)-Arabinose	4.52	114	84	6
L-(-)-Fucose	11.63	117	73	8	L-(-)-Fucose	4.62	128	83.8	6
D-(-)-Fructose	13.81	174	133	12	D-(-)-Fructose	14.76	99	70.9	12
D-(+)-Galactose	14.19	160	73	14	D-(+)-Galactose	16.67	84	41.8	12
D-Galacturonic acid sodium salt	15.28	73	45	10	D-Galacturonic acid sodium salt	-	-	-	-
D-(+)-Glucose	14.34	160	45	30	D-(+)-Glucose	17.5	126	97.8	8
D-Glucuronic acid	15.14	147	73	14	D-Glucuronic acid	23.36	84	56.1	10
α -Lactose monohydrate	29.06	160	131.9	6	α -Lactose monohydrate	-	-	-	-
D-(+)-Mannose	14.11	160	73	14	D-(+)-Mannose	20.4	143	96.9	10
D-(-)-Ribose	11.06	160	73	10	D-(-)-Ribose	15.77	199	97	8
D-(+)-Xylose	10.8	307	73	20	D-(+)-Xylose	4.84	84	53.9	12
D-Arabinose-1- ¹³ C	10.9	161	73	12	D-Arabinose-1- ¹³ C	4.52	115	84.9	5
D-Fructose-1- ¹³ C	13.85	174	146	10	D-Fructose-1- ¹³ C	14.76	100	71.9	10
D-Galactose-1- ¹³ C	14.19	161	73	14	D-Galactose-1- ¹³ C	16.45	85	42.8	10
D-Glucose- ¹³ C ₆	14.33	162	73	14	D-Glucose- ¹³ C ₆	17.5	130	99.7	10
D-Mannose-1- ¹³ C	14.1	161	73	14	D-Mannose-1- ¹³ C	20.35	144	98.1	10
D-Ribose-1- ¹³ C	11.05	161	73	12	D-Ribose-1- ¹³ C	15.77	200	97.8	10
D-Lactose-1- ¹³ C monohydrate	29.06	161	75	22	D-Lactose-1- ¹³ C monohydrate	-	-	-	-

Table.S2. Experimental design, independent factors, levels of silylation protocol.

Temperature (°C)	Time (min)	Extracted Solvent	BSTFA/Pyridine
50	80	acetonitrile	1:0
37	40	acetonitrile	3:1
20	60	acetonitrile	1:1
50	40	methanol	1:3
20	80	acetonitrile/methanol	1:0
37	40	acetonitrile	3:1
65	80	acetonitrile/methanol	1:3
65	0	methanol	1:0
20	80	methanol	1:3
65	80	methanol	3:1
37	0	acetonitrile	1:0
65	0	acetonitrile	1:3
50	80	acetonitrile	1:0
20	0	acetonitrile/methanol	1:0
50	0	acetonitrile/methanol	1:1
50	40	methanol	1:3
50	0	acetonitrile/methanol	1:1
65	40	acetonitrile/methanol	1:0
20	20	acetonitrile/methanol	1:3
20	0	methanol	1:1
65	40	acetonitrile	1:1
20	60	methanol	1:0
37	60	acetonitrile/methanol	1:1
65	40	acetonitrile	1:1
37	40	acetonitrile	3:1

Table.S3. Experimental design, independent factors, levels of acylation protocol.

Temperature-AA (°C)	AA/Pyridine	Temperature-MA (°C)	Concentration -MA (mg/mL)
20.00	1:1	120.00	2.50
50.00	1:1	20.00	2.50
37.00	1:1	65.00	10.00
70.00	3:1	65.00	2.50
70.00	1:3	120.00	10.00
50.00	1:19	20.00	2.50
70.00	1:1	100.00	2.50
37.00	1:3	100.00	1.00
70.00	3:1	65.00	2.50
20.00	3:1	100.00	2.50
70.00	1:1	100.00	1.00
100.00	1:3	20.00	10.00
37.00	1:1	65.00	10.00
20.00	1:3	20.00	10.00
70.00	1:19	20.00	1.00
20.00	1:1	100.00	5.00
20.00	1:19	120.00	2.50
50.00	1:19	100.00	10.00
50.00	1:3	65.00	10.00
20.00	1:19	120.00	10.00
70.00	1:1	20.00	10.00
50.00	1:3	65.00	10.00
37.00	1:3	100.00	1.00
20.00	1:1	100.00	1.00
20.00	1:1	20.00	1.00
70.00	1:19	100.00	1.00
20.00	1:3	65.00	1.00
50.00	1:3	65.00	10.00
37.00	1:19	20.00	10.00
50.00	1:1	65.00	1.00
50.00	1:3	65.00	10.00

Note: AA: The acylation using acetic anhydride; MA: The methylboronation using the methylboronic acid.

Table.S4. Demographic and clinical chemistry characteristics of 68 subjects (Mean \pm SD).

Parameters	Control(n=34)	Gastric carcinoma (n=33)
Age, years	57.00 \pm 6.13	61.00 \pm 12.08
Sex, F/M	15/19	14/20
Height, cm	174.00 \pm 8.63	165.21 \pm 9.09
Weight, kg	78.00 \pm 9.82	66.38 \pm 14.36
Waistline, cm	90.00 \pm 6.19	83.82 \pm 7.94
BMI, kg/m ²	25.70 \pm 2.24	24.34 \pm 3.87
DBP, mmHg	128.00 \pm 18.35	132.27 \pm 36.50
SBP, mmHg	88.00 \pm 22.83	89.65 \pm 18.84
ALT, U/L	13.00 \pm 6.69	14.91 \pm 11.13
AST, U/L	18.00 \pm 18.40	19.82 \pm 14.21
TBIL, μ mol/L	17.40 \pm 3.68	14.40 \pm 5.44
BUN, mmol/L	6.15 \pm 1.29	6.39 \pm 1.54
CRE, μ mol/l	96.00 \pm 20.40	83.32 \pm 59.66
TG, mmol/L	0.61 \pm 0.45	0.92 \pm 0.35
CHO, mmol/L	3.55 \pm 0.96	3.11 \pm 0.80
HDL-C, mmol/L	0.28 \pm 0.30	0.69 \pm 0.33
LDL-C, mmol/L	1.03 \pm 0.75	1.94 \pm 0.67
GLU, mmol/L	4.05 \pm 0.44	5.24 \pm 0.50

Note: SBP: systolic blood pressure, DBP: diastolic blood pressure, BMI: body mass index, TG: triglycerides, AST: aspartate aminotransferase, ALT: cereal third transaminase, TBIL: total bilirubin, BUN: blood urea nitrogen, CRE: serum creatinine, CHO: total cholesterol, HDL-C: high density lipotein cholesterol, LDL-C: low density lipoprotein cholesterol, GLU: blood glucose.

Table.S5. The model coefficients computed based on radial basis functions with silylation and acylation protocol.

Silylation			Acylation		
Carbohydrates	R ²	Lack of fit	Carbohydrates	R ²	Lack of fit
2-Deoxy-D-Ribose	0.8789	0.9920	2-Deoxy-D-Ribose	0.9416	1.0000
Arabinose	0.9596	0.9980	Arabinose	0.9265	0.5560
Fucose	0.9303	0.9850	Fucose	0.9175	0.2830
Fructose	0.9430	0.9890	Fructose	0.9645	1.0000
Galactose	0.9222	1.0000	Galactose	0.9520	0.6410
Glucose	0.9797	1.0000	Glucose	0.8669	0.7110
Galacturonic acid	0.9039	0.9440			
Lactose	0.8505	0.9940			
Glucuronic acid	0.8820	0.9990	Glucuronic acid	0.9188	1.0000
Mannose	0.9565	1.0000	Mannose	0.8812	0.0860
Ribose	0.9301	0.9530	Ribose	0.8816	0.9310
Xylose	0.9650	1.0000	Xylose	0.9588	1.0000

Table.S6. The recovery of serum spiked with known amounts of carbohydrates in silylation protocol.

Carbohydrates	Background value (µg/mL)	Added (µg/mL)	Found (µg/mL)	Recovery (%)	Average Recovery (%)	CV%
2-Deoxy-D-Ribose	0.11	0.08	0.2±0.02	119.90	114.32	8.00
		0.10	0.23±0.01	114.89		4.14
		0.13	0.25±0.02	108.18		9.24
		0.50	1.20±0.11	101.41		8.74
Arabinose	0.70	1.00	1.77±0.01	107.73	105.62	0.76
		1.50	2.31±0.07	107.73		3.25
		1.00	2.37±0.05	98.17		5.00
Fructose	1.39	2.00	3.04±0.12	82.49	93.11	0.77
		3.00	4.56±0.02	98.67		4.79
		0.10	0.28±0.00	118.28		1.63
Fucose	0.17	0.20	0.35±0.01	93.85	99.47	2.43
		0.30	0.42±0.01	86.29		2.57
		0.50	1.44±0.08	112.60		5.89
Galactose	0.87	1.00	1.91±0.07	103.40	102.31	3.68
		2.00	2.69±0.32	90.95		11.84
		8.00	19.03±0.56	84.29		2.96
		10.00	21.50±0.08	84.16		84.16
Galacturonic acid	10.39	12.00	24.58±0.28	84.04	84.16	1.15
		300.00	1107.94±18.82	94.69		1.70
		600.00	1375.67±31.73	92.01		94.45
Glucose	824.13	800.00	1586.61±12.41	96.65	94.45	8.72
		0.25	0.60±0.01	81.57		1.95
		0.50	0.85±0.05	90.71		84.54
Glucuronic acid	0.39	0.75	1.00±0.04	81.32	84.54	4.45
		0.02	0.05±0.003	94.86		2.31
		0.03	0.06±0.003	101.81		95.20
Lactose	0.03	0.03	0.06±0.003	101.81	95.20	1.76
		0.04	0.08±0.01	88.94		4.76
Mannose	3.03	1.50	4.78±0.21	116.34	105.20	4.79

		3.00	5.78 ± 0.22	91.51		6.31
		4.50	7.88 ± 0.28	107.76		2.39
		0.50	1.25 ± 0.11	107.02		9.09
Ribose	0.72	0.75	1.68 ± 0.18	120.18	118.24	11.07
		1.00	1.99 ± 0.19	127.52		9.71
		0.75	1.53 ± 0.01	112.60		0.88
Xylose	0.85	1.00	1.77 ± 0.05	103.40	102.12	2.96
		1.25	1.87 ± 0.06	90.95		3.05

Table.S7. The precision of Intra-day and Inter-day with silylation protocol.

Carbohydrates ($\mu\text{g/mL}$)	Intra-day(n=6)		Inter-day(n=6)	
	Mean \pm SD	RSD (%)	Mean \pm SD	RSD (%)
2-Deoxy-D-Ribose	0.109 \pm 0.003	2.60	0.125 \pm 0.020	16.30
Arabinose	0.530 \pm 0.005	0.86	0.575 \pm 0.004	0.69
Fructose	3.052 \pm 0.103	3.39	3.027 \pm 0.090	2.98
Fucose	0.155 \pm 0.003	1.79	0.155 \pm 0.002	1.48
Galactose	7.593 \pm 1.234	16.25	9.170 \pm 0.194	2.11
Galacturonic acid	12.485 \pm 1.488	11.92	19.362 \pm 0.078	0.40
Glucose	852.539 \pm 8.901	1.04	869.328 \pm 8.660	0.99
Glucuronic acid	0.726 \pm 0.074	10.14	0.798 \pm 0.009	1.16
Lactose	0.080 \pm 0.005	6.58	0.099 \pm 0.004	3.90
Mannose	1.560 \pm 0.055	3.51	1.729 \pm 0.340	19.64
Ribose	0.242 \pm 0.017	7.12	0.380 \pm 0.030	7.90
Xylose	1.924 \pm 0.086	4.47	1.890 \pm 0.059	3.13

Table.S8. The recovery of serum spiked with known amounts of carbohydrates in acylation protocol.

Carbohydrates ($\mu\text{g/mL}$)	Background value ($\mu\text{g/mL}$)	Added ($\mu\text{g/mL}$)	Found ($\mu\text{g/mL}$)	Recovery (%)	Average Recovery (%)	CV%
2-Deoxy-D-Ribose	0.29	0.10	0.39 \pm 0.01	100.32	97.48	1.64
		0.50	0.74 \pm 0.00	91.54		0.30
		1.00	1.29 \pm 0.01	100.60		0.86
Arabinose	3.42	2.50	6.30 \pm 0.21	115.23	105.40	3.30
		5.00	8.51 \pm 0.23	101.77		2.67
		7.50	10.86 \pm 0.17	99.21		1.52
Fructose	5.12	3.00	8.68 \pm 0.02	118.97	96.24	0.21
		6.00	10.43 \pm 0.10	88.61		1.00
		9.00	12.42 \pm 0.48	81.14		3.84
Fucose	0.13	0.05	0.18 \pm 0.01	100.51	109.39	3.19
		0.10	0.24 \pm 0.02	107.76		6.69
		0.20	0.37 \pm 0.00	119.90		0.24
Galactose	1.55	1.00	2.67 \pm 0.08	112.11	107.76	3.00
		1.50	3.31 \pm 0.09	117.21		2.59
		3.00	4.37 \pm 0.30	93.96		6.94
Glucose	410.00	200.00	585.46 \pm 8.67	87.73	96.38	1.48
		300.00	725.44 \pm 15.93	105.15		2.20
		500.00	891.38 \pm 26.07	96.28		2.93
Glucuronic acid	15.41	10.00	25.04 \pm 1.24	96.28	103.61	4.95
		15.00	31.31 \pm 0.83	105.94		2.64
		20.00	37.13 \pm 0.96	108.60		2.59
Mannose	5.09	3.00	7.62 \pm 0.78	84.37	96.42	10.19
		6.00	10.70 \pm 0.40	93.41		3.70
		9.00	15.13 \pm 0.47	111.48		3.08
Ribose	0.27	0.10	0.36 \pm 0.02	88.93	93.11	5.54

		0.20	0.45±0.02	89.06		3.88
		0.30	0.57±0.01	101.33		1.88
		0.50	1.26±0.06	95.42		4.41
Xylose	0.78	1.00	1.79±0.07	100.74	94.40	3.72
		2.00	2.52±0.02	87.04		0.77

Table.S9. The precision of Intra-day and Inter-day with acylation protocol.

Carbohydrates ($\mu\text{g/mL}$)	Intra-day (n=6)		Inter-day(n=6)	
	Mean \pm SD	RSD (%)	Mean \pm SD	RSD (%)
2-Deoxy-D-Ribose	0.059 \pm 0.001	1.18	0.060 \pm 0.001	2.46
Arabinose	3.988 \pm 0.073	1.82	3.928 \pm 0.051	1.30
Fructose	1.965 \pm 0.018	0.92	1.769 \pm 0.153	8.67
Fucose	0.099 \pm 0.001	1.35	0.100 \pm 0.001	1.27
Galactose	3.546 \pm 0.045	1.28	3.574 \pm 0.044	1.22
Glucose	565.837 \pm 10.456	1.84	567.227 \pm 11.181	1.97
Glucuronic acid	17.445 \pm 0.044	0.25	17.365 \pm 0.433	2.49
Mannose	4.991 \pm 0.039	0.77	4.963 \pm 0.066	1.32
Ribose	0.686 \pm 0.000	0.05	0.687 \pm 0.001	0.08
Xylose	3.522 \pm 0.005	0.12	3.588 \pm 0.033	0.92

Table.S10. The stability of carbohydrates derivatives at different temperature within five days by silylation protocol.

Carbohydrates	Room temperature (n=3)		4 °C (n=3)		-40 °C (n=3)	
	Mean±SD	RSD%	Mean±SD	RSD%	Mean±SD	RSD%
2-Deoxy-D-Ribose	0.109±0.004	3.99	0.122±0.018	14.63	0.116±0.004	3.53
Arabinose	0.549±0.002	0.40	0.574±0.004	0.75	0.560±0.044	7.85
Fructose	3.069±0.081	2.65	2.980±0.032	1.06	2.920±0.103	3.53
Fucose	0.155±0.000	0.27	0.154±0.001	0.48	0.154±0.001	0.41
Galactose	9.992±1.382	13.83	8.927±0.066	0.74	8.392±1.853	22.08
Galacturonic acid	12.343±8.367	67.78	15.371±6.982	45.42	18.431±1.534	8.32
Glucose	854.925±2.009	0.23	865.280±4.235	0.48	820.163±14.860	1.81
Glucuronic acid	0.729±0.059	8.03	0.791±0.020	2.57	0.765±0.017	2.25
Lactose	0.078±0.035	44.25	0.098±0.005	5.34	0.080±0.011	13.71
Mannose	2.639±1.673	63.41	1.438±0.539	37.50	2.931±1.824	62.22
Ribose	0.244±0.128	52.36	0.403±0.017	4.31	1.371±1.881	137.21
Xylose	1.923±0.096	4.97	1.917±0.126	6.59	1.757±0.088	5.00

Table.S11. The stability of carbohydrates derivatives at different temperature within five days by acylation protocol.

Carbohydrates	Room temperature (n=3)		4 °C (n=3)		-40 °C (n=3)	
	Mean±SD	RSD%	Mean±SD	RSD%	Mean±SD	RSD%
2-Deoxy-D-Ribose	0.095±0.083	87.84	0.059±0.001	0.89	0.060±0.006	9.94
Arabinose	7.466±6.185	82.84	3.882±0.107	2.75	4.390±1.091	24.84
Fructose	1.272±3.611	283.75	0.473±0.099	20.92	0.995±1.808	181.61
Fucose	0.088±0.017	19.59	0.076±0.003	4.05	0.073±0.006	8.09
Galactose	2.556±1.314	51.41	3.461±0.041	1.18	5.539±3.656	65.99
Glucose	560.689±7.788	1.38	552.322±18.185	3.29	561.526±15.830	2.81
Glucuronic acid	37.027±22.998	62.11	19.935±0.088	0.44	19.541±0.933	4.77
Mannose	6.078±0.709	11.66	7.308±0.042	0.58	6.256±1.091	17.44
Ribose	0.676±0.041	6.02	0.602±0.004	0.61	0.656±0.065	9.94
Xylose	1.959±0.406	20.73	1.832±0.022	1.19	1.779±0.243	13.64

Table.S12. Quantitative determinations after the derivatization of carbohydrates in serum using silylation and acylation protocols.

Carbohydrate	Acylation	Silylation	P value
2-Deoxy-D-Ribose	0.096±0.044	0.639±0.286	<0.001
Arabinose	7.968±2.717	0.297±0.275	<0.001
Fructose	2.447±1.645	1.365±0.217	<0.001
Fucose	0.240±0.154	0.601±0.193	<0.001
Galactose	5.577±5.989	20.119±2.033	<0.001
Glucose	876.038±221.279	797.535±110.166	0.073
Glucuronic acid	17.074±7.782	0.392±0.242	<0.001
Mannose	10.569±4.987	8.463±3.187	0.06
Ribose	0.327±0.255	0.325±0.509	0.959
Xylose	1.037±0.290	0.138±0.038	<0.001

Table.S13. Quantitative analysis of carbohydrates profiles of the two groups.

Carbohydrate	Control (n=34)	Gastric carcinoma (n=33)	P value
2-Deoxy-D-Ribose	0.085±0.036	0.090±0.029	0.697
Arabinose	7.85±2.57	7.72±2.35	0.840
Fructose	132.08±52.95	2.30±1.43	0.004
Fucose	0.20±0.11	0.22±0.13	0.654
Galactose	4.52±3.28	4.69±3.19	0.840
Galacturonic acid	21.35±3.11	13.02±4.36	< 0.001
Glucose	837.31±245.79	868.58±220.35	0.710
Glucuronic acid	21.01±10.83	17.28±7.80	0.281
Lactose	0.14±0.44	0.21±0.27	0.676
Mannose	13.28±5.35	10.45±5.03	0.116
Ribose	0.82±1.12	0.31±0.24	0.036
Xylose	1.11±0.44	0.99±0.19	0.419

Table.S14. Abbreviations and acronyms.

Abbreviations	Full name
AA	Acetic anhydride
ANN	Artificial neural network
BMI	Body weight index
BSTFA	N, O- bis (Trimethylsilyl)trifluoroacetamide
CV	Coefficient of variation
EI	Electron bomb ionization
GC	Gas chromatography
LC	Liquid chromatography
LOQ	Limit of quantification
MA	Methylboronic acid
ME	Matrix effect
MS	Mass spectrometry
RSD	Relative standard deviation
RSM	Response surface methodology
RT	Retention time
SBP	Systolic blood pressure
SIL-ISM	Stable isotope-labeled internal standard method
S/N	Signal/noise
TC	Total cholesterol
TG	Triglycerides
TMS	Trimethylsilane
UPLC	Ultra performance liquid chromatography

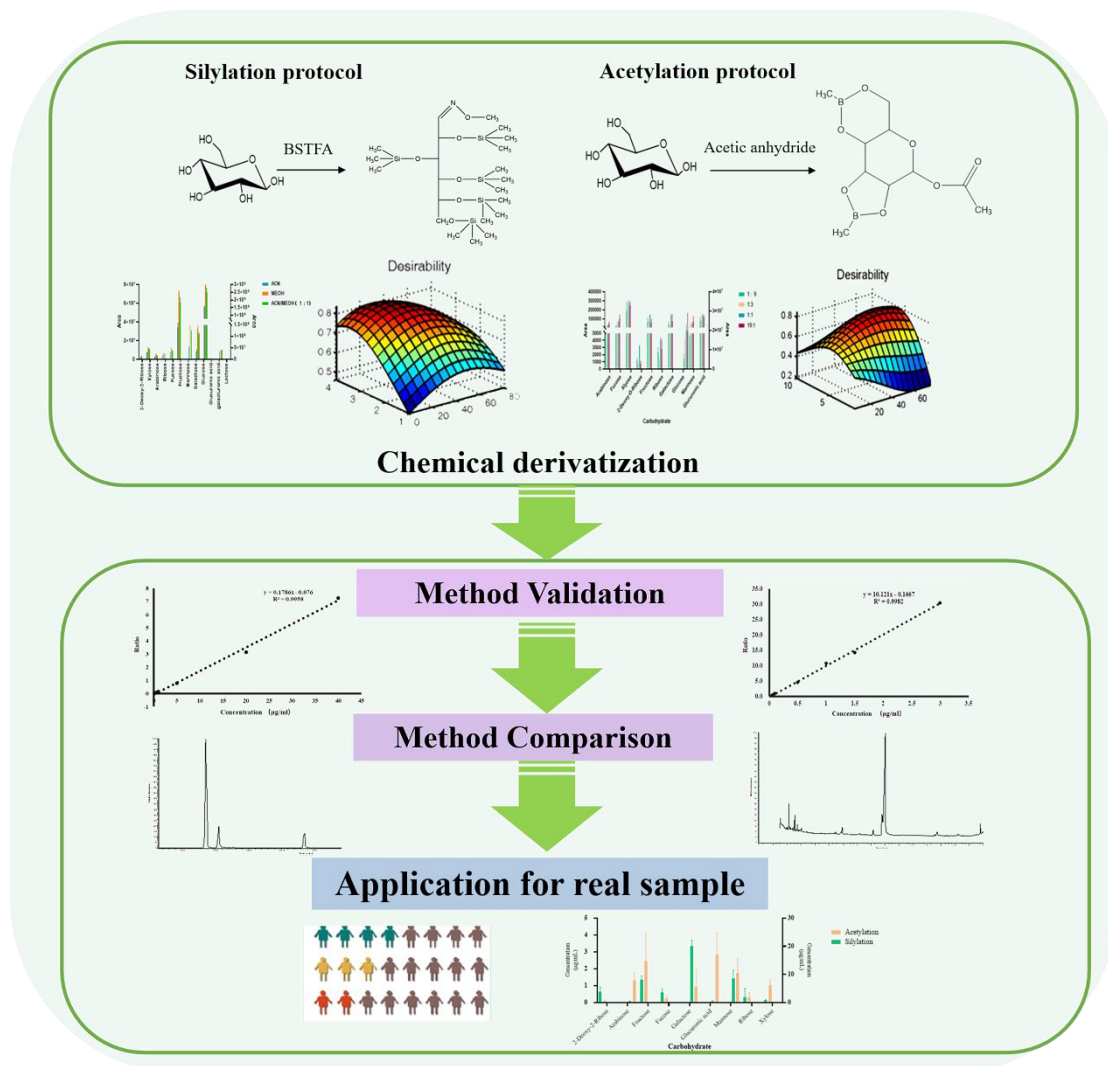


Figure.S1. The workflow of this study.

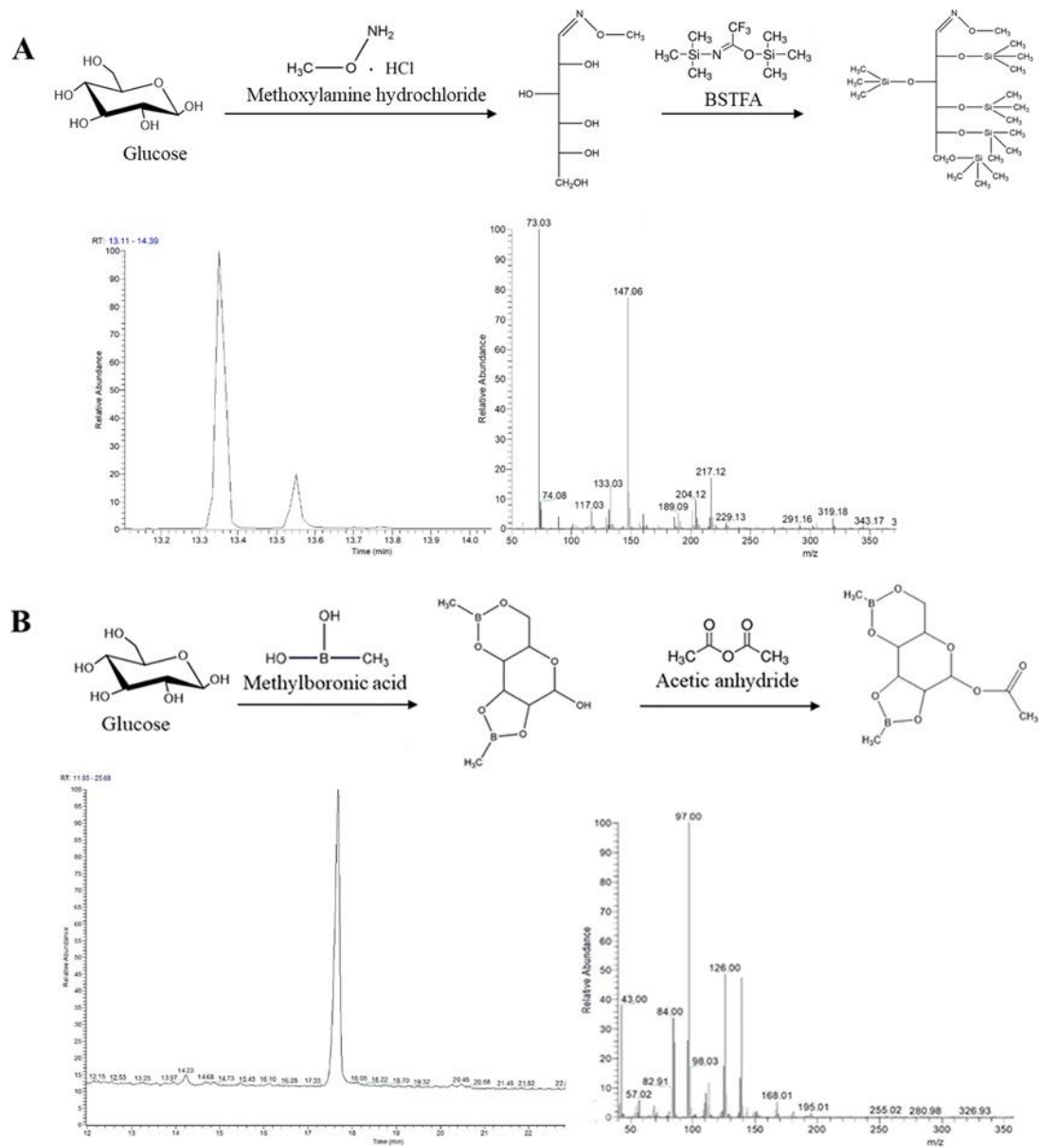


Figure.S2. The chemical reaction, TIC chromatographic peak and mass spectrum peak of glucose obtained with the two protocols described in this study. (A) Silylation protocol. (B) Acylation protocol.

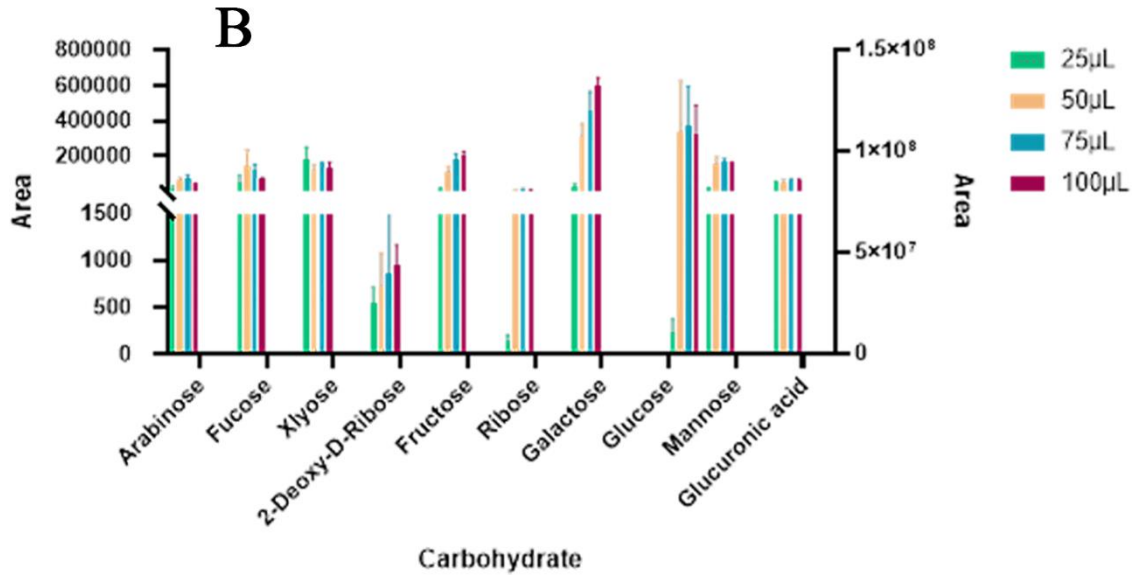
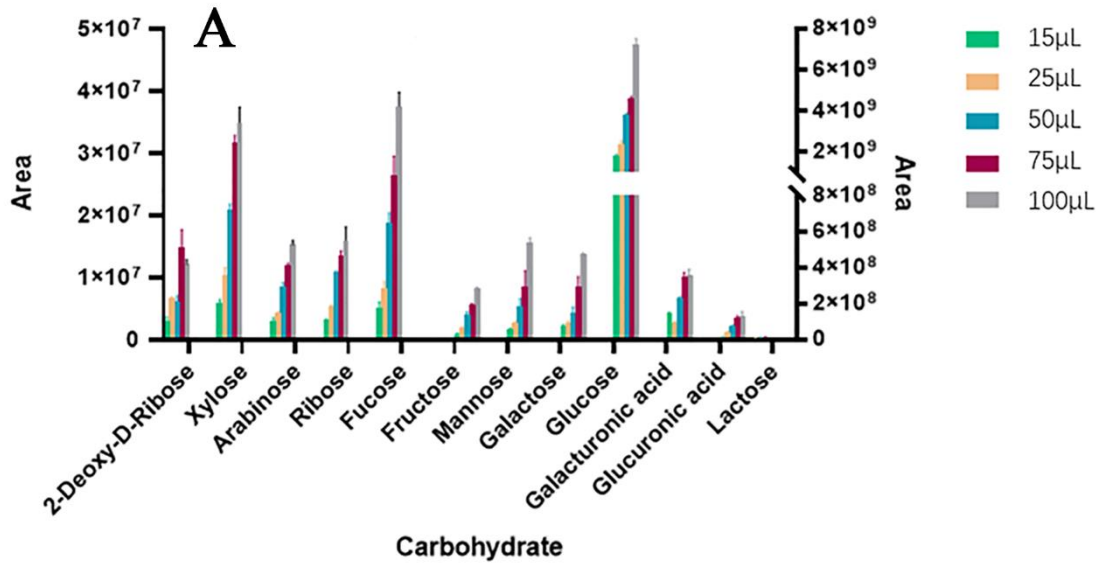


Figure.S3. Effects of serum volume on the derivatization efficiency. (A) Silylation protocol; (B) Acylation protocol.

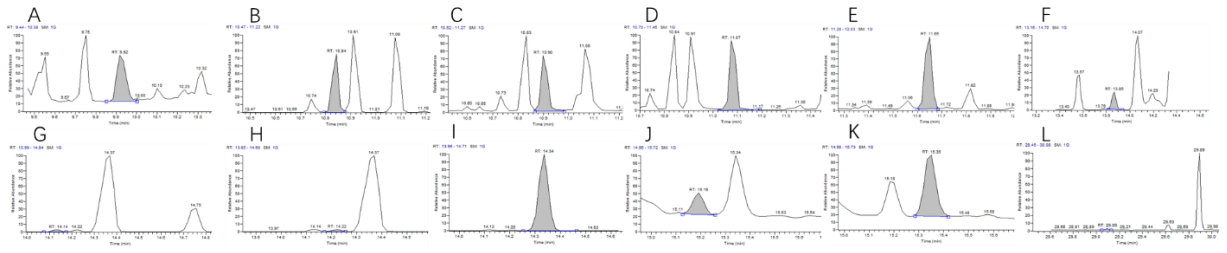


Figure.S4. SRM chromatograms for silylation in serum. (A) 2-Deoxy-D-Ribose (B) Xylose (C) Arabinose (D) Ribose (E) Fucose (F) Fructose (G) Mannose (H) Galactose (I) Glucose (J) Glucuronic acid (K) Galacturonic acid (L) Lactose.

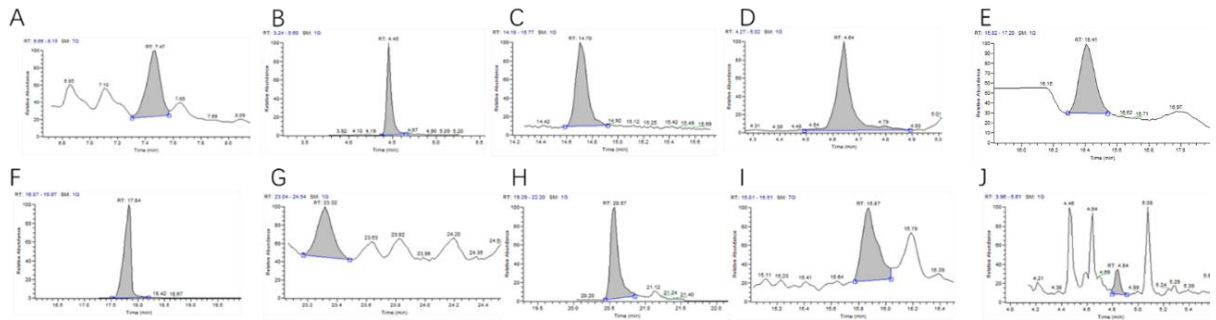


Figure.S5. SRM chromatograms for acylation in serum. (A) 2-Deoxy-D-Ribose (B) Arabinose (C) Fructose (D) Fucose (E) Galactose (F) Glucose (G) Glucuronic acid (H) Mannose (I) Ribose (J) Xylose