Supporting Information

LC-MS in Combination with DMBA Derivatization for Sialic Acid Speciation and Distribution Analysis in Fish Tissues

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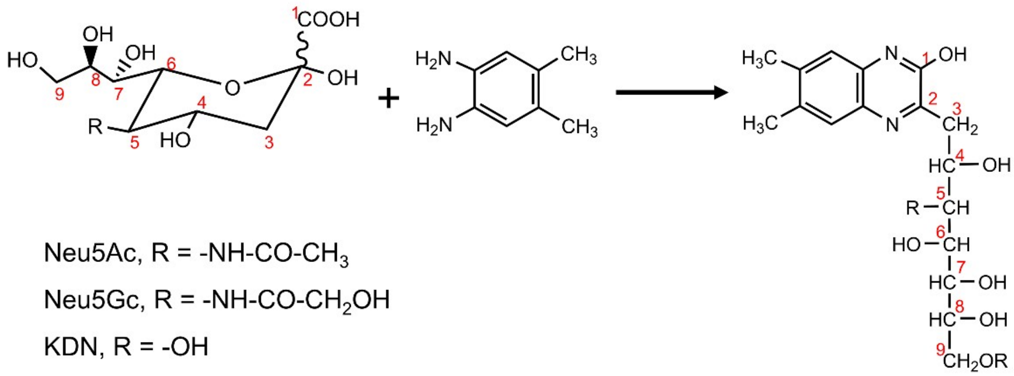


Fig. S1. The schematic diagram of DMBA derivatization of sialic acid.

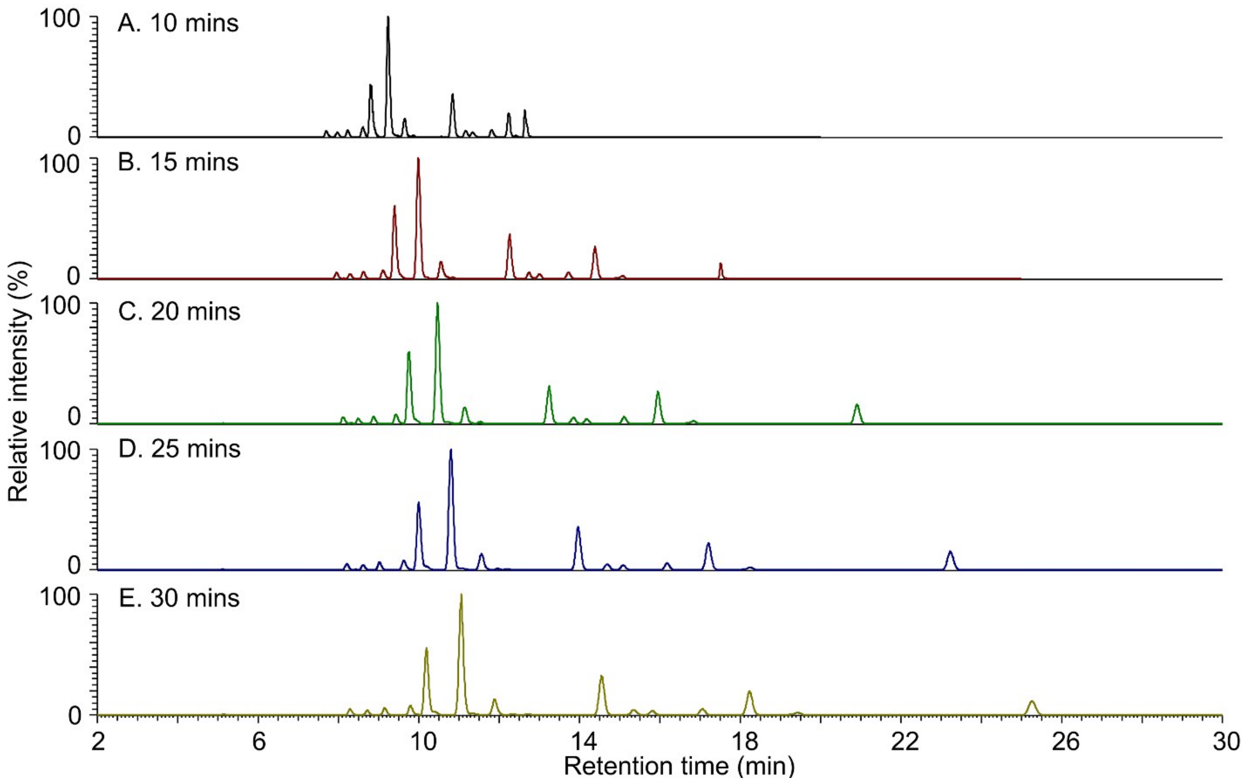


Fig. S2. The effect of LC gradients on the separation of different sialic acids. XICs are the sum of ions at *m/z* 452.20, 468.20, 494.21 and 536.22. The mobile phase B was increased from 10 % at 3 min to 20 % at different time. (A) 10 min; (B) 15 min; (C) 20 min; (D) 25 min; (E) 30 min.

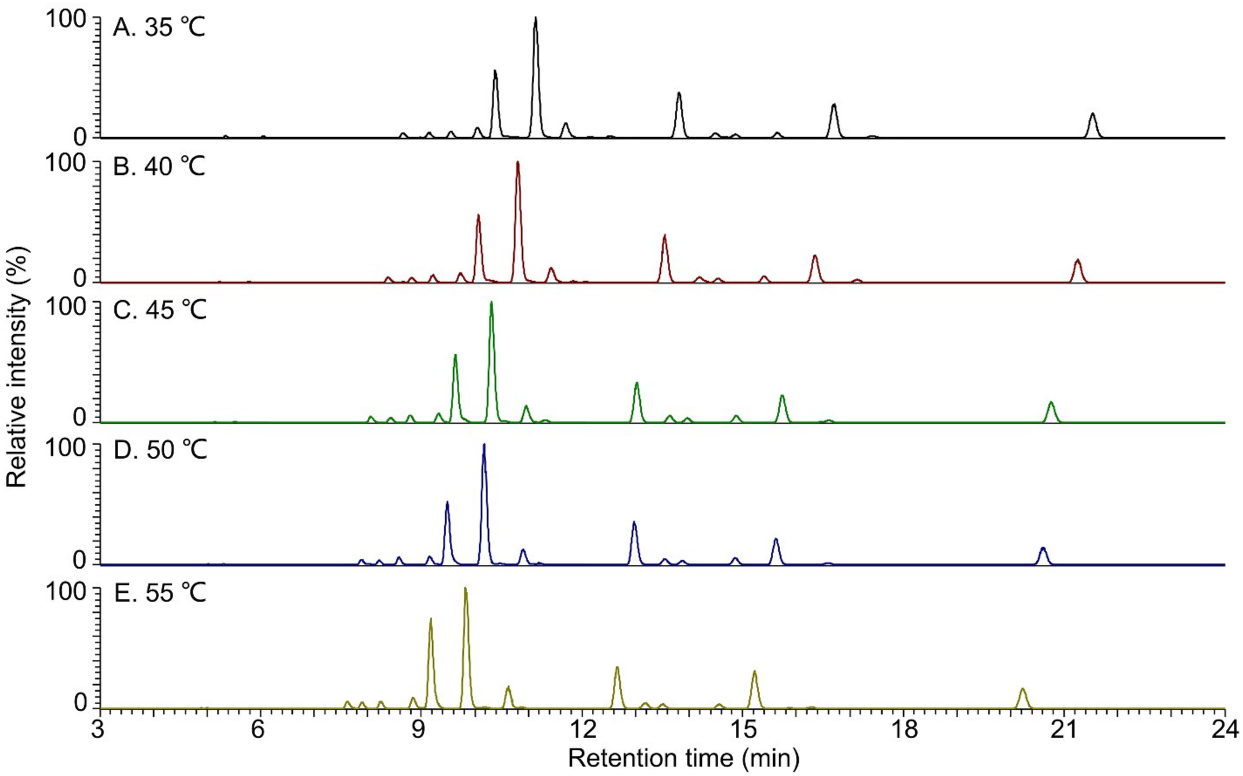


Fig. S3. The effect of column temperature on the separation of sialic acids. The XICs are the sum of ion at *m/z* 452.20, 468.20, 494.21 and *m/z* 536.22. (A) 35 ℃; (B) 40 ℃; (C) 45 ℃; (D) 50 ℃; (E) 55 ℃.

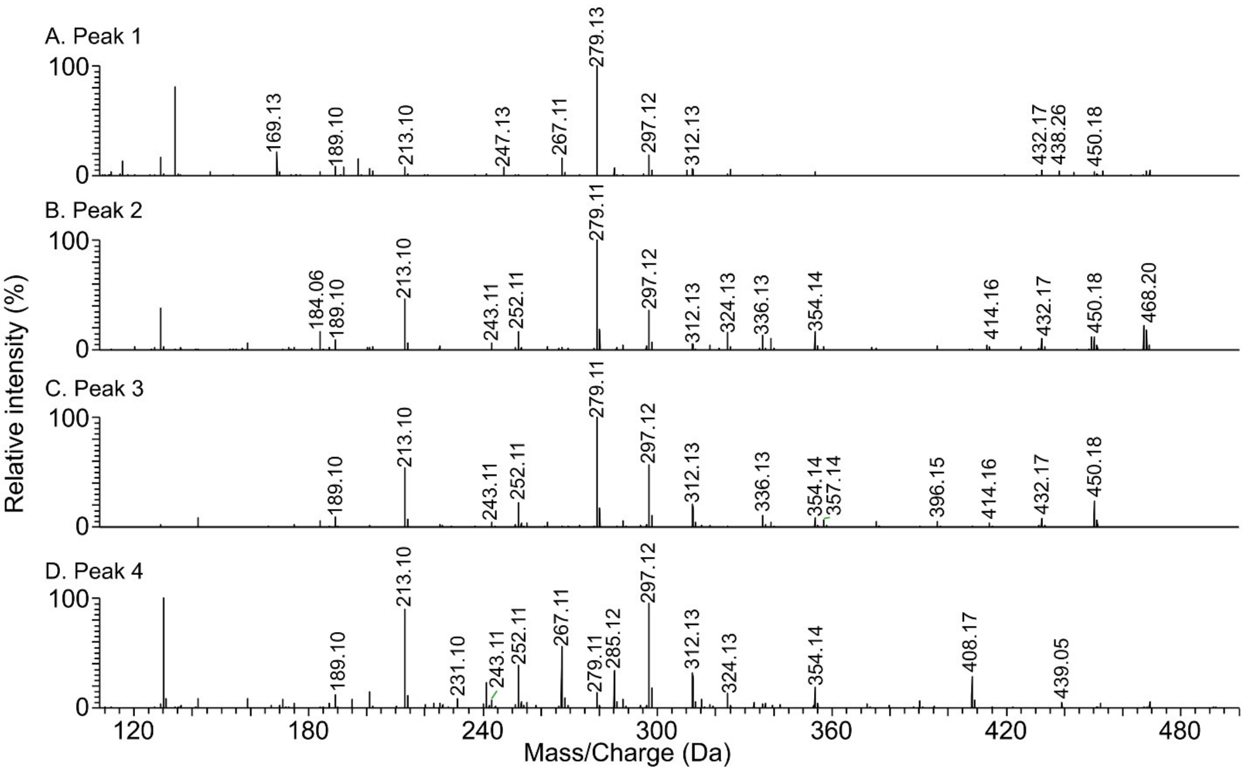


Fig. S4. LC-MS/MS analysis of DMBA-labeled mono-*O*-acetylated *N*-glycolylneuraminic acids from crucian carp blood. (A) MS/MS spectrum extracted at Peak 1; (B) MS/MS spectrum extracted at Peak 2; (C) MS/MS spectrum extracted at Peak 3; (D) MS/MS spectrum extracted at Peak 4.

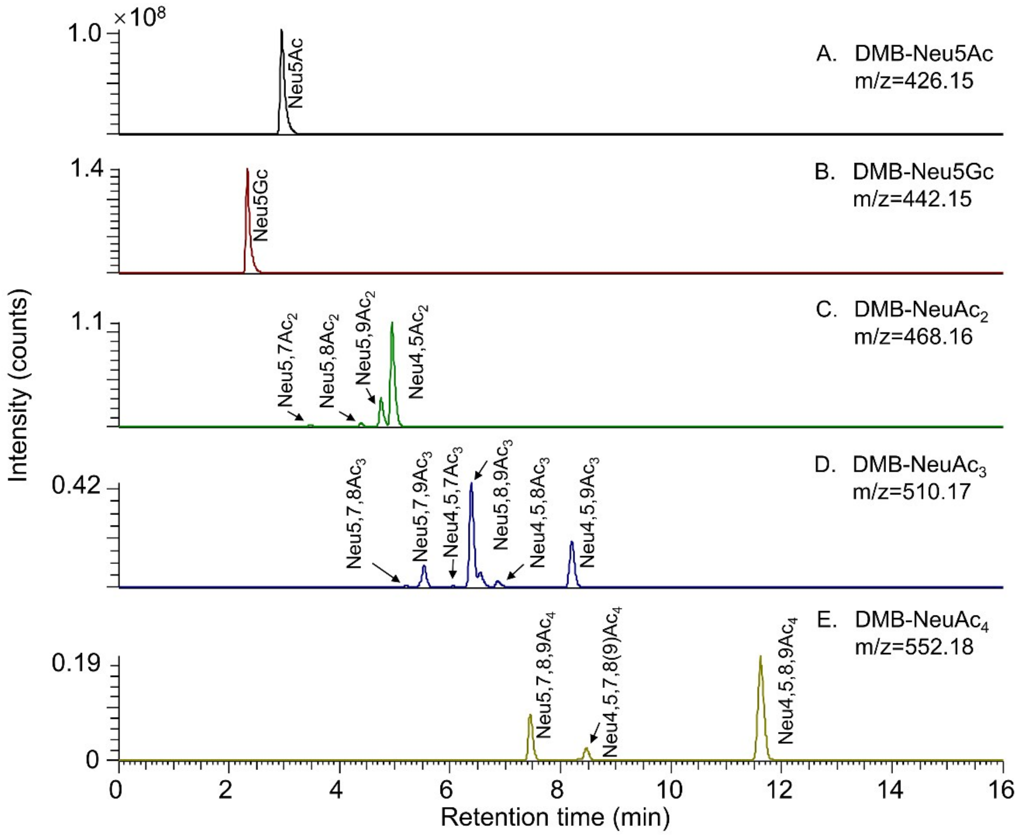
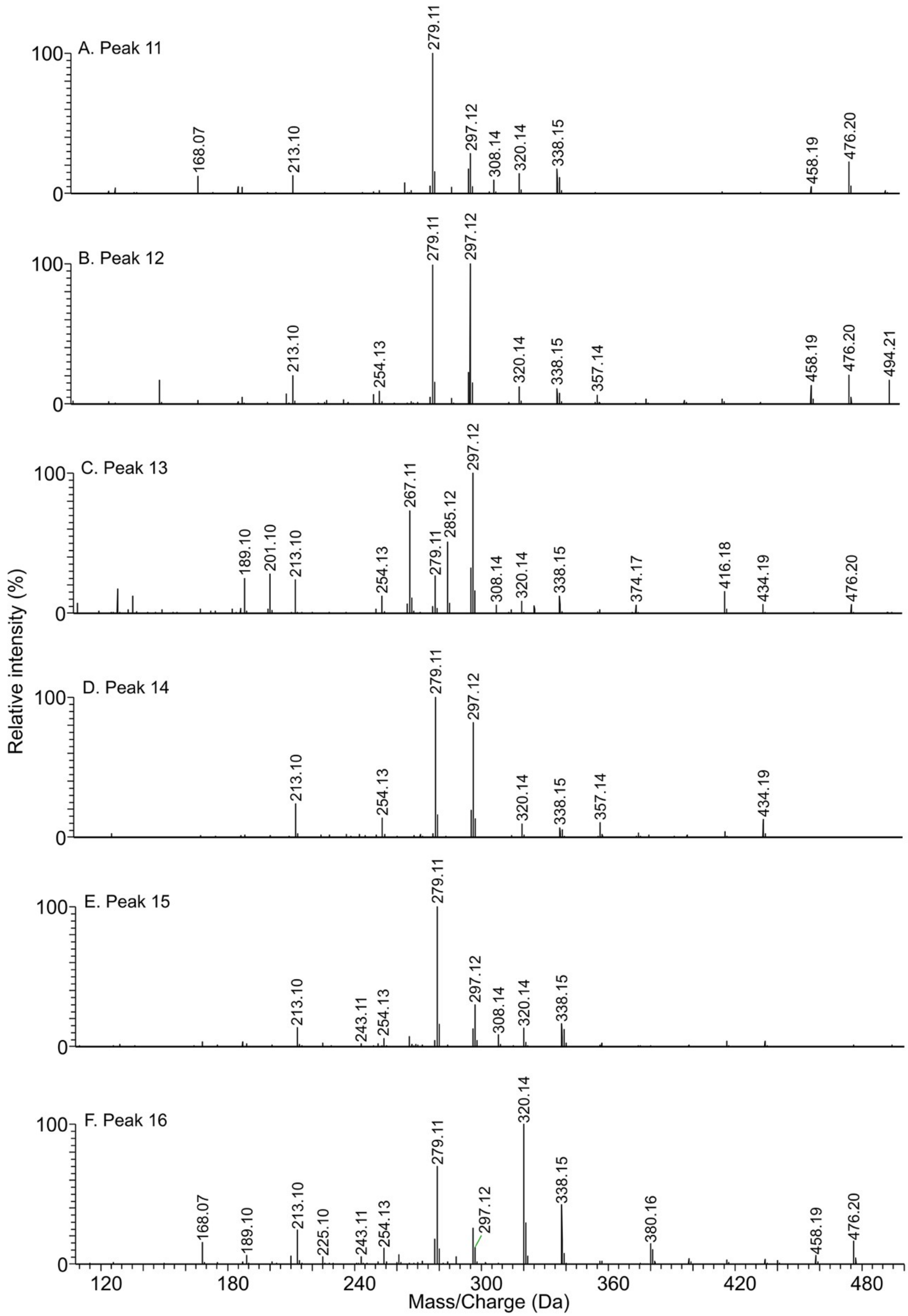


Fig. S5. LC-MS analysis of DMB-labeled sialic acids from crucian carp blood. (A) XIC at *m/z* 426.15 (Neu5Ac); (B) XIC at *m/z* 442.15 (Neu5Gc); (C) XIC at *m/z* 468.16 ($NeuAc_2$); (D) XIC at *m/z* 510.17 ($NeuAc_3$); (E) XIC at *m/z* 552.18 ($NeuAc_4$).



A. Peak 11
B. Peak 12
C. Peak 13
D. Peak 14
E. Peak 15
F. Peak 16
Relative intensity (%)
Mass/Charge (Da)

60

Fig. S6. LC-MS/MS analysis of DMBA-labeled di-*O*-acetylated *N*-acetylneuraminic acids (*m/z* 494.21) from crucian carp blood. (A) MS/MS spectrum extracted at Peak 11; (B) MS/MS spectrum extracted at Peak 12; (C) MS/MS spectrum extracted at Peak 13; (D) MS/MS spectrum extracted at Peak 14; (E) MS/MS spectrum extracted at Peak 15; (F) MS/MS spectrum extracted at Peak 16.

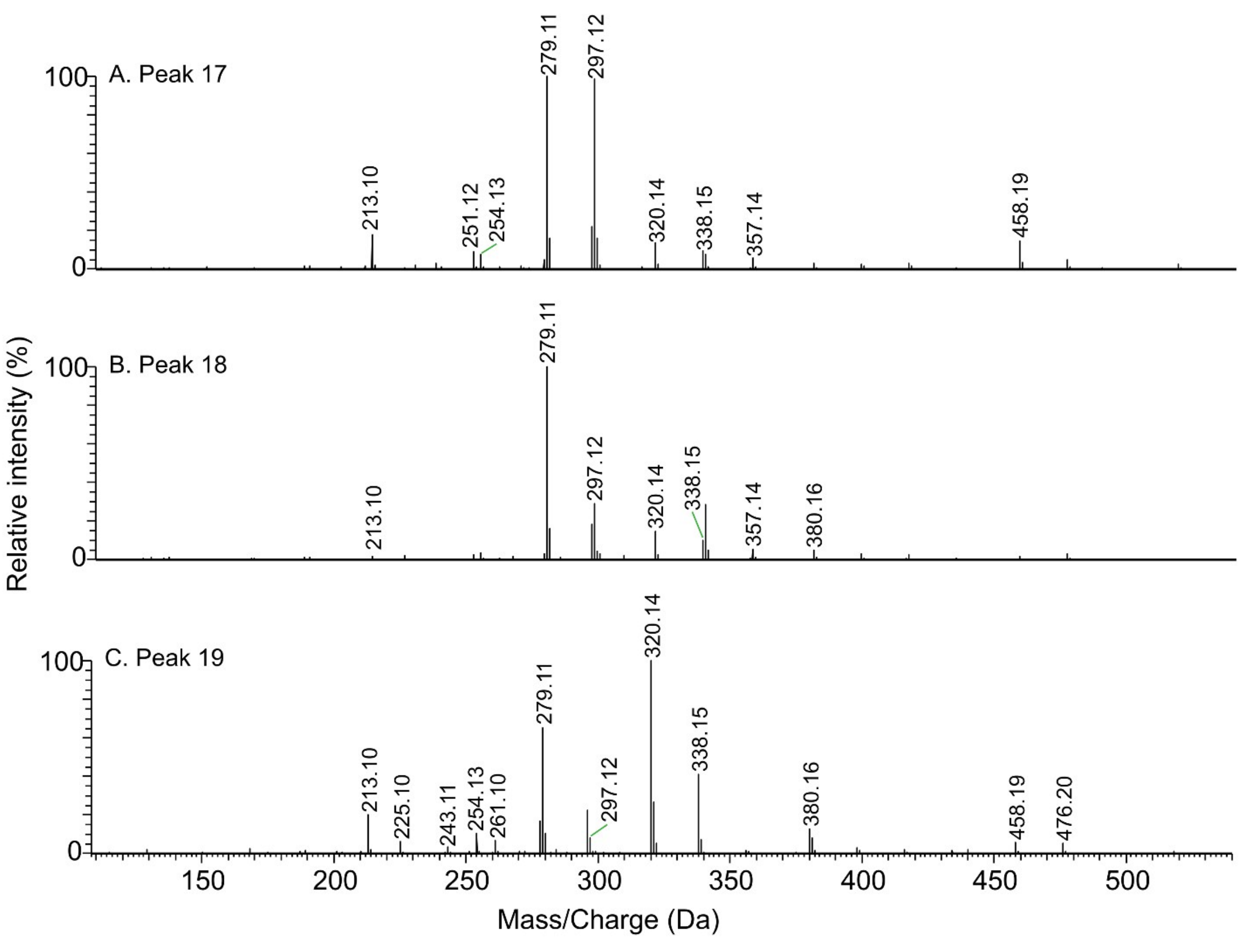


Fig. S7. LC-MS/MS analysis of DMBA-labeled tri-*O*-acetylated *N*-acetylneuraminic acids (*m/z* 536.22) from crucian carp blood. (A) MS/MS spectrum extracted at Peak 17; (B) MS/MS spectrum extracted at Peak 18; (C) MS/MS spectrum extracted at Peak 19.

Table S1. Detected sialic acids in crucian carp blood with their relative retention times and characteristic ions.

Peak No.	Sialic acid	Abbreviation	Retention time (min)	RRT_1	RRT_2	m/z $[M+H]^+$	Characteristic ions
1	7-*O*-Acetyl-*N*-glycolylneuraminic acid	Neu5Gc7Ac	7.49	1.01	0.98	468.20	213.10, 267.11, 279.13, 297.12, 450.18
2	8-*O*-Acetyl-*N*-glycolylneuraminic acid	Neu5Gc8Ac	8.27	1.11	1.09	468.20	213.10, 243.11, 279.11, 297.12, 450.18
3	9-*O*-Acetyl-*N*-glycolylneuraminic acid	Neu5Gc9Ac	8.42	1.13	1.21	468.20	213.10, 243.11, 279.11, 297.12, 450.18
4	4-*O*-Acetyl-*N*-glycolylneuraminic acid	Neu4Ac5Gc	8.79	1.18	1.23	468.20	213.10, 243.11, 267.11, 279.11, 297.12, 408.17
5	*N*-Acetylneuraminic acid	Neu5Ac	7.44	1.00	1.00	410.19	213.10, 243.11, 267.11, 279.11, 285.12, 297.12, 392.18
6	*N*-Glycolylneuraminicacid	Neu5Gc	6.60	0.89	0.84	426.19	213.10, 243.11, 267.11, 279.11, 285.12, 297.12, 408.17
7	7-*O*-Acetyl-*N*-acetylneuraminic acid	$Neu5,7Ac_2$	8.05	1.08	1.12	452.20	201.10, 213.10, 267.11, 279.11, 285.12, 297.12, 434.19
8	8-*O*-Acetyl-*N*-acetylneuraminic acid	$Neu5,8Ac_2$	9.32	1.25	1.40	452.20	213.10, 243.11, 279.11, 297.12, 434.19
9	9-*O*-Acetyl-*N*-acetylneuraminic acid	$Neu5,9Ac_2$	9.64	1.30	1.59	452.20	213.10, 243.11, 279.11, 297.12, 434.19

10	4-*O*-Acetyl-*N*-acetylneuraminic acid	Neu4,5Ac$_2$	10.31	1.39	1.70	452.20	201.10, 213.10, 243.11, 267.11, 279.11, 285.12, 297.12, 392.18
11	7,8-Di-*O*-acetyl-*N*-acetylneuraminic acid	Neu5,7,8Ac$_3$	10.56	1.42	1.77	494.21	213.10, 279.11, 297.12, 476.20
12	7,9-Di-*O*-acetyl-*N*-acetylneuraminic acid	Neu5,7,9Ac$_3$	10.96	1.47	2.03	494.21	213.10, 279.11, 297.12, 476.20
13	4,7-Di-*O*-acetyl-*N*-acetylneuraminic acid	Neu4,5,7Ac$_3$	11.32	1.52	2.51	494.21	213.10, 279.11, 285.12, 297.12, 434.19, 476.20
14	8,9-Di-*O*-acetyl-*N*-acetylneuraminic acid	Neu5,8,9Ac$_3$	13.02	1.75	2.73	494.21	213.10, 243.11, 279.11, 297.12, 434.19
15	4,8-Di-*O*-acetyl-*N*-acetylneuraminic acid	Neu4,5,8Ac$_3$	13.65	1.83	3.28	494.21	213.10, 243.11, 279.11, 297.12
16	4,9-Di-*O*-acetyl-*N*-acetylneuraminic acid	Neu4,5,9Ac$_3$	15.72	2.11	4.75	494.21	213.10, 243.11, 279.11, 297.12, 476.20
17	7,8,9-Tri-*O*-acetyl-*N*-acetylneuraminic acid	Neu5,7,8,9Ac$_4$	14.88	2.00	3.81	536.22	213.10, 279.11, 297.12
18	4,7,8(9)-Tri-*O*-acetyl-*N*-acetylneuraminic acid	Neu4,5,7,8(9)Ac$_4$	16.62	2.23	4.99	536.22	213.10, 279.11, 297.12
19	4,8,9-Tri-*O*-acetyl-*N*-acetylneuraminic acid	Neu4,5,8,9A$_4$	20.75	2.79	9.51	536.22	213.10, 243.11, 279.11, 297.12

Relative retention time (RRT) is the ratio of the retention time of analyte relative to that of Neu5Ac. RRT_1s are the values from DMBA derivatization and obtained in this study. RRT_2s are the values from DMB derivatization that obtained from reference #28.