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Supplementary Information

Unsaturated fatty-acids based HPLC fingerprint in combination with quantitative analysis of multi-components by single-marker for classification of *Rana chensinensis* ovum

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Table S1. UPLC-Q/TOF-MS analysis of seven compounds in RCO

Compound	MS Fragmentation	Molecular Formula	Proposed Compound
1	301.2160 $[M–H]^–$,257.2265 $[M\text{-}COOH]^–$	$C_{20}H_{30}O_2$	EPA
2	277.2155 $[M–H]^–$,259.2408 $[M\text{-}COOH]^–$	$C_{18}H_{30}O_2$	ALA
3	327.2336 $[M–H]^–$,283.2520 $[M\text{-}COOH]^–$	$C_{22}H_{32}O_2$	DHA
4	303.2335 $[M–H]^–$,285.2548 $[M–H–H_2O]^–$,259.2430 $[M\text{-}COOH]^–$	$C_{20}H_{32}O_2$	ARA
5	329.2500 $[M–H]^–$,285.2607 $[M\text{-}COOH]^–$	$C_{22}H_{34}O_2$	DPA
6	279.2343 $[M–H]^–$,261.2255 $[M\text{-}COOH]^–$	$C_{18}H_{32}O_2$	LA
7	281.2483 $[M–H]^–$,263.1652 $[M–H–H_2O]^–$	$C_{18}H_{34}O_2$	OA

Table S2. The precision, stability, repeatability and accuracy of the HPLC analysis method for RCO

UFAs	Precision RSD%	Stability RSD%	Repeatability RSD%	Recovery	
				Mean%	RSD%
EPA	0.68	1.85	3.08	100.93	2.79
ALA	0.44	1.87	1.97	104.75	4.99
DHA	0.29	1.89	3.24	96.20	5.20
ARA	0.42	1.91	3.17	104.68	3.07
DPA	0.92	1.99	3.10	96.62	5.38
LA	0.25	1.73	3.08	100.31	2.61
OA	0.21	1.96	3.07	104.47	4.22

Table S3. The similarity data matrix of the 11 common peaks fingerprint

NO.	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	R(11)
S1	1	0.950	0.982	0.974	0.978	0.969	0.971	0.991	0.971	0.96	0.944	0.948	0.949	0.975	0.938	0.948	0.972	0.943	0.912	0.970
S2	0.950	1	0.987	0.994	0.988	0.994	0.991	0.979	0.989	0.979	0.976	0.980	0.988	0.982	0.975	0.983	0.984	0.978	0.981	0.991
S3	0.982	0.987	1	0.995	0.992	0.993	0.991	0.996	0.995	0.987	0.973	0.974	0.978	0.984	0.972	0.975	0.993	0.967	0.958	0.992
S4	0.974	0.994	0.995	1	0.995	0.999	0.997	0.994	0.996	0.989	0.983	0.986	0.987	0.992	0.979	0.986	0.993	0.979	0.976	0.997
S5	0.978	0.988	0.992	0.995	1	0.995	0.997	0.996	0.993	0.980	0.980	0.982	0.989	0.997	0.977	0.983	0.988	0.961	0.967	0.995
S6	0.969	0.994	0.993	0.999	0.995	1	0.999	0.991	0.993	0.989	0.986	0.990	0.988	0.993	0.982	0.989	0.992	0.976	0.980	0.998
S7	0.971	0.991	0.991	0.997	0.997	0.999	1	0.993	0.994	0.990	0.991	0.993	0.991	0.997	0.986	0.993	0.994	0.966	0.981	0.999
S8	0.991	0.979	0.996	0.994	0.996	0.991	0.993	1	0.993	0.982	0.974	0.975	0.978	0.992	0.970	0.975	0.990	0.960	0.953	0.992
S9	0.971	0.989	0.995	0.996	0.993	0.993	0.994	0.993	1	0.99	0.984	0.982	0.989	0.987	0.986	0.984	0.995	0.961	0.972	0.996
S10	0.960	0.979	0.987	0.989	0.98	0.989	0.99	0.982	0.99	1	0.991	0.991	0.980	0.978	0.99	0.988	0.997	0.957	0.980	0.993
S11	0.944	0.976	0.973	0.983	0.98	0.986	0.991	0.974	0.984	0.991	1	0.998	0.990	0.985	0.998	0.997	0.991	0.941	0.991	0.993
S12	0.948	0.980	0.974	0.986	0.982	0.99	0.993	0.975	0.982	0.991	0.998	1	0.989	0.987	0.993	0.998	0.990	0.952	0.992	0.994
S13	0.949	0.988	0.978	0.987	0.989	0.988	0.991	0.978	0.989	0.980	0.990	0.989	1	0.989	0.992	0.994	0.988	0.947	0.986	0.993
S14	0.975	0.982	0.984	0.992	0.997	0.993	0.997	0.992	0.987	0.978	0.985	0.987	0.989	1	0.978	0.987	0.986	0.955	0.970	0.994
S15	0.938	0.975	0.972	0.979	0.977	0.982	0.986	0.970	0.986	0.990	0.998	0.993	0.992	0.978	1	0.994	0.991	0.932	0.989	0.990
S16	0.948	0.983	0.975	0.986	0.983	0.989	0.993	0.975	0.984	0.988	0.997	0.998	0.994	0.987	0.994	1	0.991	0.949	0.992	0.994
S17	0.972	0.984	0.993	0.993	0.988	0.992	0.994	0.990	0.995	0.997	0.991	0.990	0.988	0.986	0.991	0.991	1	0.957	0.977	0.997
S18	0.943	0.978	0.967	0.979	0.961	0.976	0.966	0.960	0.961	0.957	0.941	0.952	0.947	0.955	0.932	0.949	0.957	1	0.949	0.966
S19	0.912	0.981	0.958	0.976	0.967	0.980	0.981	0.953	0.972	0.980	0.991	0.992	0.986	0.970	0.989	0.992	0.977	0.949	1	0.983
R(11)	0.970	0.991	0.992	0.997	0.995	0.998	0.999	0.992	0.996	0.993	0.993	0.994	0.993	0.994	0.990	0.994	0.997	0.966	0.983	1

Table S4. The similarity data matrix of the seven identified UFAs fingerprint

NO.	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	R(7)
S1	1.000	0.958	0.983	0.977	0.980	0.974	0.975	0.991	0.970	0.960	0.945	0.952	0.952	0.980	0.937	0.953	0.971	0.962	0.920	0.972
S2	0.958	1.000	0.994	0.995	0.990	0.995	0.992	0.985	0.997	0.988	0.979	0.980	0.989	0.982	0.982	0.983	0.992	0.979	0.980	0.993
S3	0.983	0.994	1.000	0.997	0.994	0.997	0.994	0.996	0.995	0.987	0.973	0.977	0.980	0.988	0.972	0.979	0.993	0.984	0.965	0.994
S4	0.977	0.995	0.997	1.000	0.995	0.999	0.997	0.995	0.999	0.992	0.983	0.985	0.987	0.992	0.982	0.986	0.995	0.985	0.977	0.998
S5	0.980	0.990	0.994	0.995	1.000	0.996	0.997	0.997	0.996	0.982	0.980	0.982	0.989	0.998	0.978	0.983	0.990	0.967	0.968	0.995
S6	0.974	0.995	0.997	0.999	0.996	1.000	0.999	0.995	0.998	0.994	0.987	0.990	0.988	0.993	0.985	0.989	0.997	0.980	0.980	0.999
S7	0.975	0.992	0.994	0.997	0.997	0.999	1.000	0.995	0.997	0.994	0.991	0.993	0.991	0.997	0.989	0.993	0.997	0.971	0.982	0.999
S8	0.991	0.985	0.996	0.995	0.997	0.995	0.995	1.000	0.993	0.982	0.974	0.978	0.980	0.996	0.970	0.978	0.990	0.975	0.958	0.993
S9	0.970	0.997	0.995	0.999	0.996	0.998	0.997	0.993	1.000	0.990	0.986	0.987	0.993	0.992	0.986	0.988	0.995	0.980	0.981	0.998
S10	0.960	0.988	0.987	0.992	0.982	0.994	0.994	0.982	0.990	1.000	0.993	0.995	0.983	0.983	0.990	0.993	0.997	0.975	0.989	0.995
S11	0.945	0.979	0.973	0.983	0.980	0.987	0.991	0.974	0.986	0.993	1.000	0.999	0.991	0.986	0.999	0.998	0.992	0.949	0.995	0.992
S12	0.952	0.980	0.977	0.985	0.982	0.990	0.993	0.978	0.987	0.995	0.999	1.000	0.989	0.987	0.997	0.998	0.994	0.955	0.993	0.994
S13	0.952	0.989	0.980	0.987	0.989	0.988	0.991	0.980	0.993	0.983	0.991	0.989	1.000	0.989	0.994	0.994	0.991	0.952	0.988	0.993
S14	0.980	0.982	0.988	0.992	0.998	0.993	0.997	0.996	0.992	0.983	0.986	0.987	0.989	1.000	0.982	0.987	0.990	0.958	0.970	0.995
S15	0.937	0.982	0.972	0.982	0.978	0.985	0.989	0.970	0.986	0.990	0.999	0.997	0.994	0.982	1.000	0.998	0.991	0.948	0.997	0.991
S16	0.953	0.983	0.979	0.986	0.983	0.989	0.993	0.978	0.988	0.993	0.998	0.998	0.994	0.987	0.998	1.000	0.995	0.952	0.993	0.995
S17	0.971	0.992	0.993	0.995	0.990	0.997	0.997	0.990	0.995	0.997	0.992	0.994	0.991	0.990	0.991	0.995	1.000	0.974	0.985	0.999
S18	0.962	0.979	0.984	0.985	0.967	0.980	0.971	0.975	0.980	0.975	0.949	0.955	0.952	0.958	0.948	0.952	0.974	1.000	0.948	0.974
S19	0.920	0.980	0.965	0.977	0.968	0.980	0.982	0.958	0.981	0.989	0.995	0.993	0.988	0.970	0.997	0.993	0.985	0.948	1.000	0.985
R(7)	0.972	0.993	0.994	0.998	0.995	0.999	0.999	0.993	0.998	0.995	0.992	0.994	0.993	0.995	0.991	0.995	0.999	0.974	0.985	1.000

Table S5. The mean values of RCFs based on different influencing factors

Factor	$F_{ALA/EPA}$	$F_{ALA/DHA}$	$F_{ALA/ARA}$	$F_{ALA/DPA}$	$F_{ALA/LA}$	$F_{ALA/OA}$
Column temperature	1.7418	2.3176	1.6621	1.7626	0.7012	0.1522
Flow rate	1.7478	2.3129	1.6585	1.7601	0.6989	0.1510
HPLC instrument system and column	1.7339	2.2903	1.6239	1.7685	0.7010	0.1581

$F_{ALA/i}$: the relative correction factor (RCF) of the internal reference ALA relative to the UFA_i to be measured (i= EPA, DHA, ARA, DPA, LA, and OA).

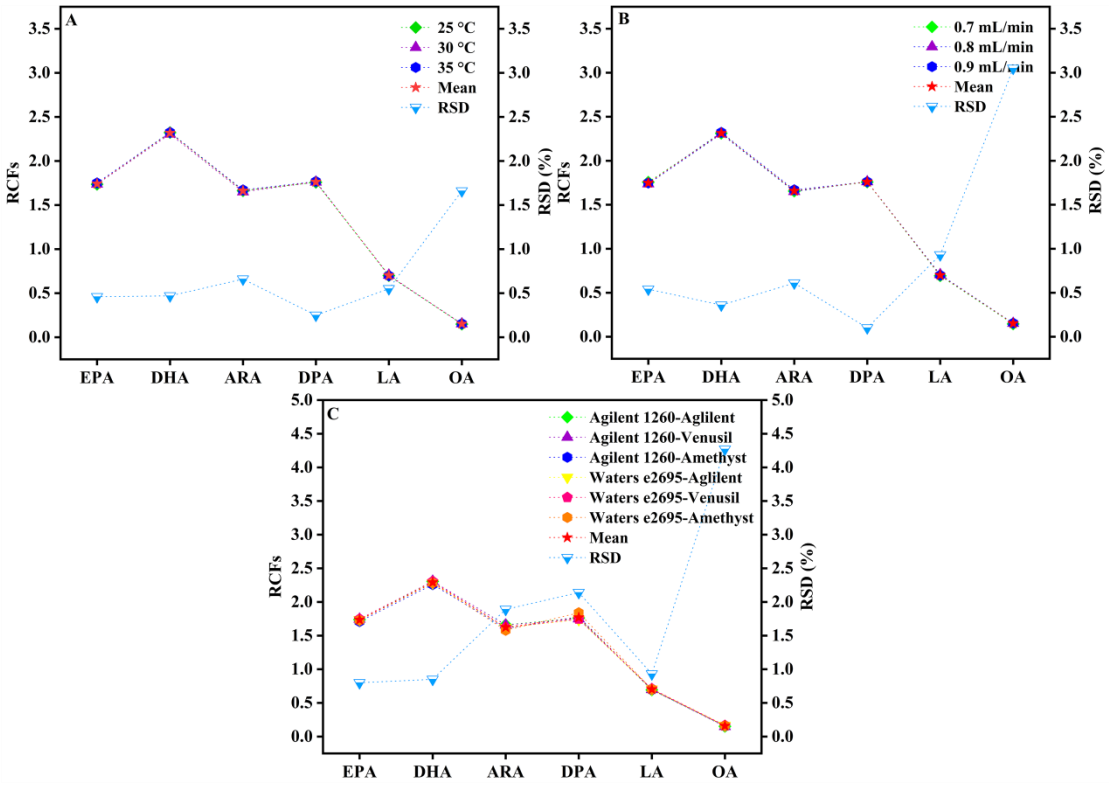

Fig. S1 Effect of different influencing factors on the robustness of RCFs (A) column temperature, (B) flow rate, (C) HPLC instrument system and column.