

SUPPLEMENTARY INFORMATION

An optimized LC-HRMS untargeted metabolomics workflow for multi-matrices investigations in the three-spined stickleback

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Figure S1 : Optimized analytical workflow for the multi-matrices LC-HRMS untargeted metabolomic analysis of three-spined stickleback

Figure S2: Intensities of internal standard metabolites (normalized to the maximal response) analyzed by LC(RPLC)-(ESI+)- or LC(HILIC)-(ESI-)- HRMS after extraction of 50 mg or 25 mg of fish with 2.5 mL or 7 mL of MeOH/H₂O/heptane.

Figure S3 : Intensities of internal standard metabolites (normalized to the maximal response) analyzed by LC(RPLC)-(ESI+)- or LC(HILIC)-(ESI-)- HRMS after extraction of 50 mg or 25 mg of liver with 2.5 mL or 7 mL of MeOH/H₂O/heptane.

Figure S4 : Intensities of internal standard metabolites (normalized to the maximal response) analyzed by LC(RPLC)-(ESI+)- or LC(HILIC)-(ESI-)- HRMS after extraction of 50 mg or 25 mg of brain with 2.5 mL or 7 mL of MeOH/H₂O/heptane.

Figure S5 : Intensities of internal standard metabolites (normalized to the maximal response) analyzed by LC(RPLC)-(ESI+)- or LC(HILIC)-(ESI-)- HRMS after extraction of 50 mg or 25 mg of gills with 2.5 mL or 7 mL of MeOH/H₂O/heptane.

Figure S6 : Intensities of internal standard metabolites (normalized to the maximal response) analyzed by LC(RPLC)-(ESI+)- or LC(HILIC)-(ESI-)- HRMS after extraction of 50 mg of fish with 2.5 mL of different proportions of MeOH/H₂O/heptane.

Figure S7. Intensities of internal standard metabolites (normalized to the maximal response) analyzed by LC(RPLC)-(ESI+)- or LC(HILIC)-(ESI-)- HRMS after extraction of 50 mg of liver with 2.5 mL of different proportions of MeOH/H₂O /heptane.

Figure S8. Intensities of internal standard metabolites (normalized to the maximal response) analyzed by LC(RPLC)-(ESI+)- or LC(HILIC)-(ESI-)- HRMS after extraction of 50 mg of brain with 2.5 mL of different proportions of MeOH/H₂O /heptane.

Figure S9. Intensities of internal standard metabolites (normalized to the maximal response) analyzed by LC(RPLC)-(ESI+)- or LC(HILIC)-(ESI-)- HRMS after extraction of 25 mg of gills with 2.5 mL of different proportions of MeOH/H₂O /heptane.

Table S1: Internal standards employed and adducts used during the analytical development

Figure S1 : Optimized analytical workflow for the multi-matrices LC-HRMS untargeted metabolomic analysis of three-spined stickleback

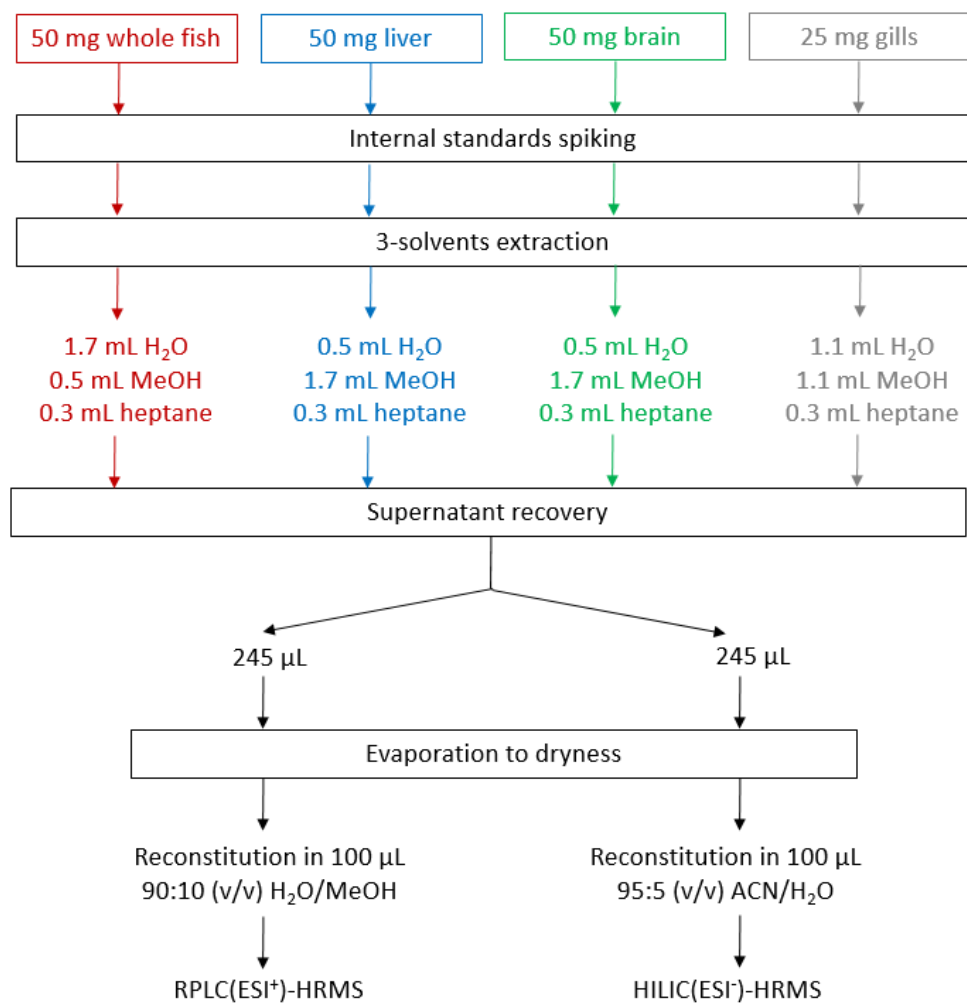


Figure S2. Normalized intensities of internal metabolites analyzed by LC(RPLC)-(ESI+)- or LC(HILIC)-(ESI)- HRMS after extraction of 50 mg or 25 mg of fish with 2.5 mL or 7 mL of MeOH/H₂O/heptane.

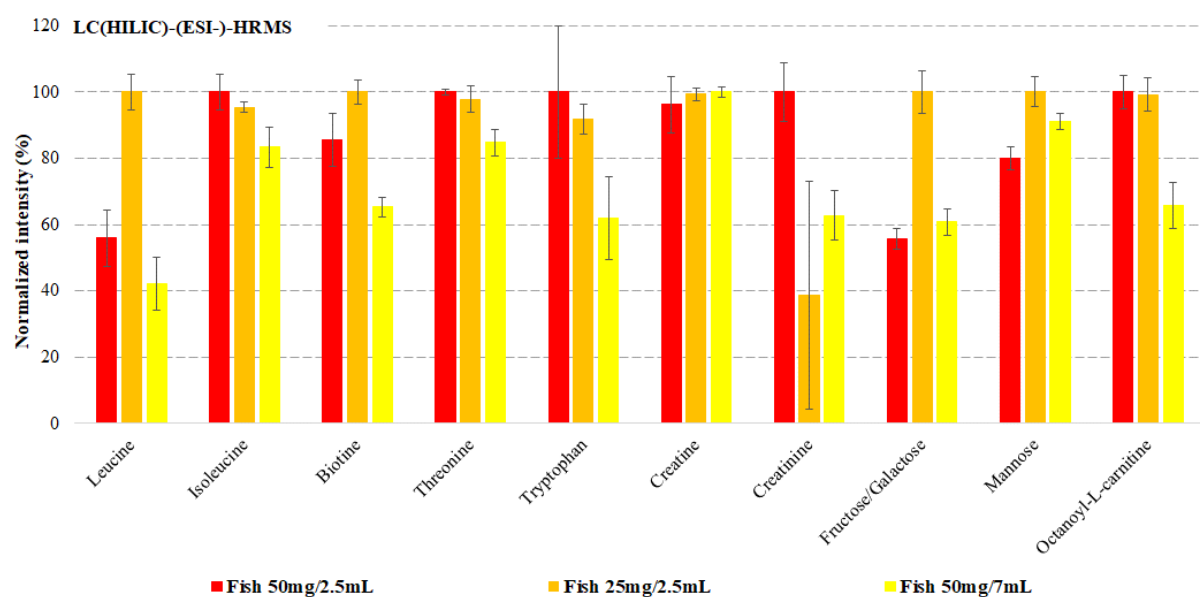
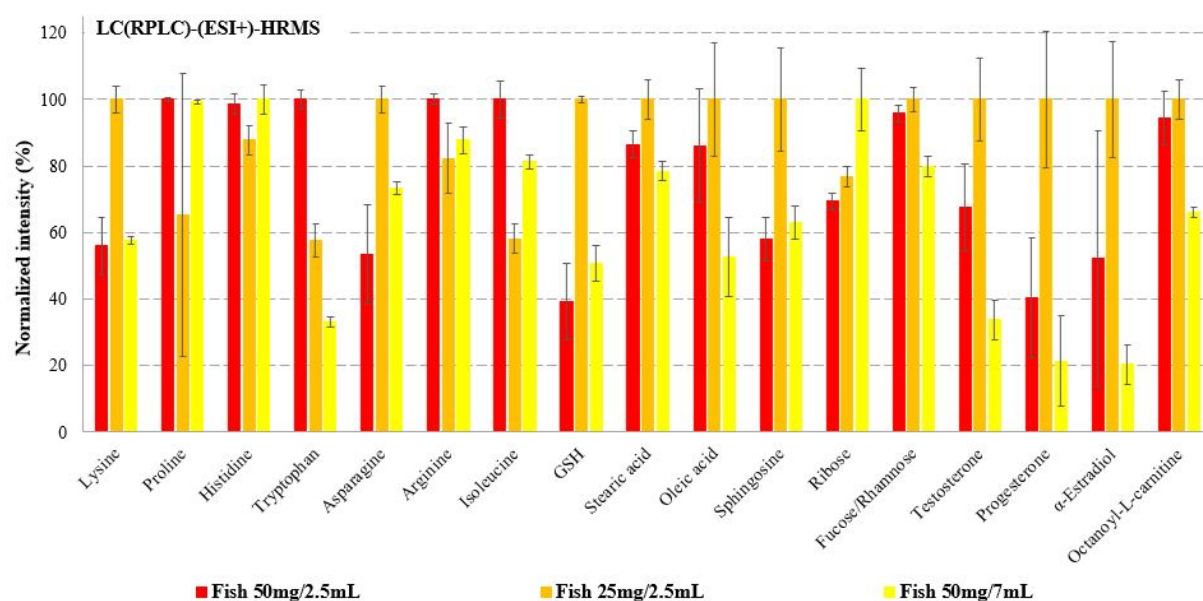


Figure S3. Normalized intensities of internal metabolites analyzed by LC(RPLC)-(ESI+)- or LC(HILIC)-(ESI-)- HRMS after extraction of 50 mg or 25 mg of liver with 2.5 mL or 7 mL of MeOH/H₂O/heptane.

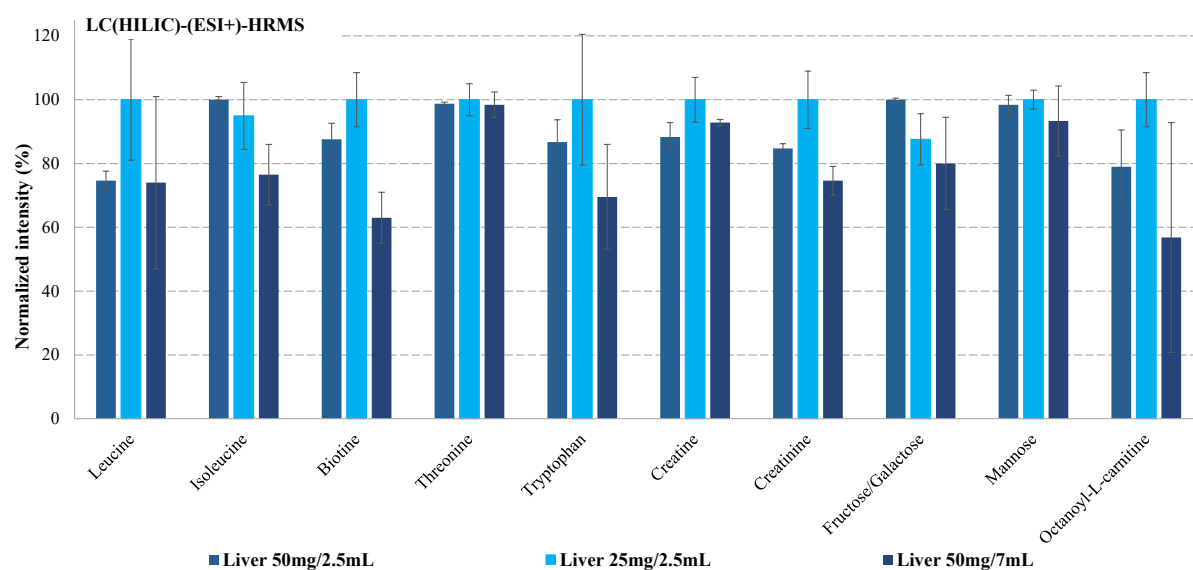
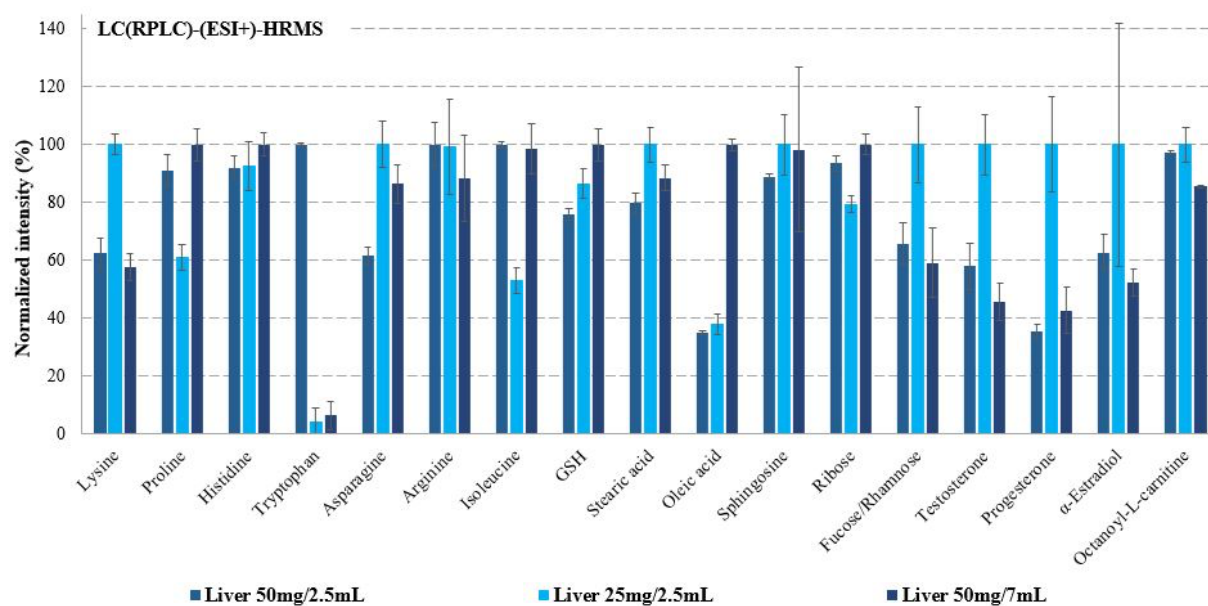


Figure S4. Normalized intensities of internal metabolites analyzed by LC(RPLC)-(ESI+)- or LC(HILIC)-(ESI-)- HRMS after extraction of 50 mg or 25 mg of brain with 2.5 mL or 7 mL of MeOH/H₂O/heptane.

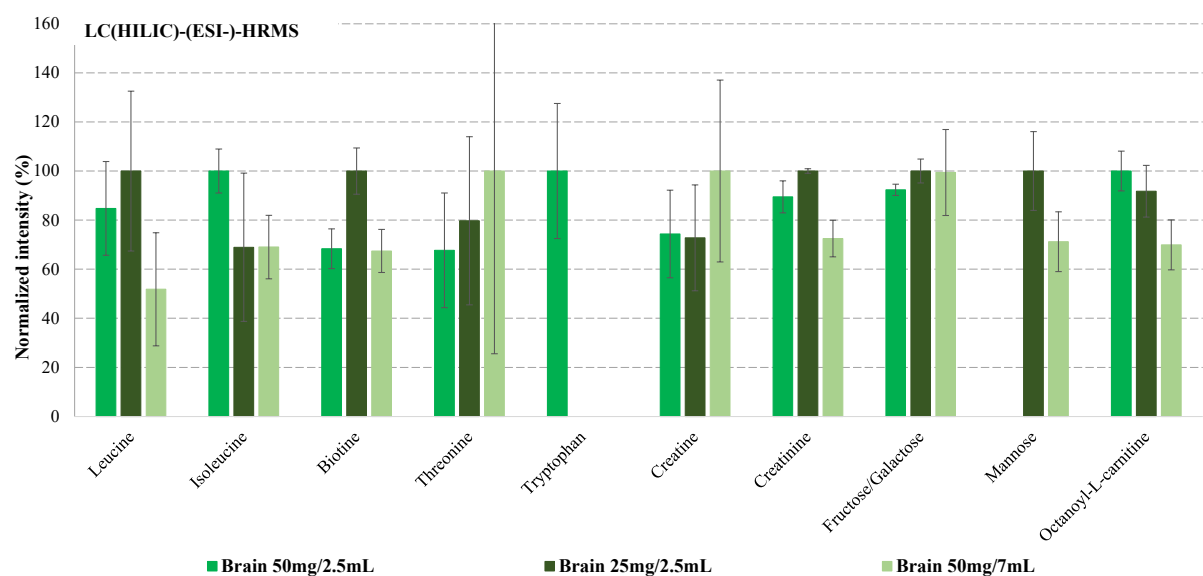
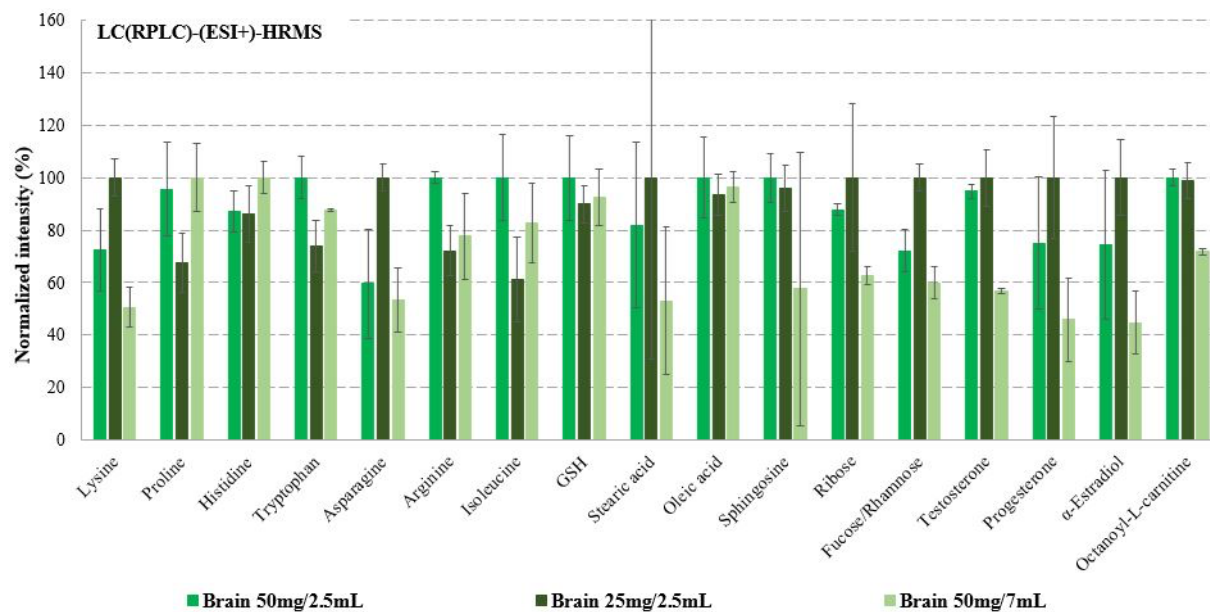


Figure S5. Normalized intensities of internal metabolites analyzed by LC(RPLC)-(ESI+)- or LC(HILIC)-(ESI-)- HRMS after extraction of 50 mg or 25 mg of gills with 2.5 mL or 7 mL of MeOH/H₂O/heptane.

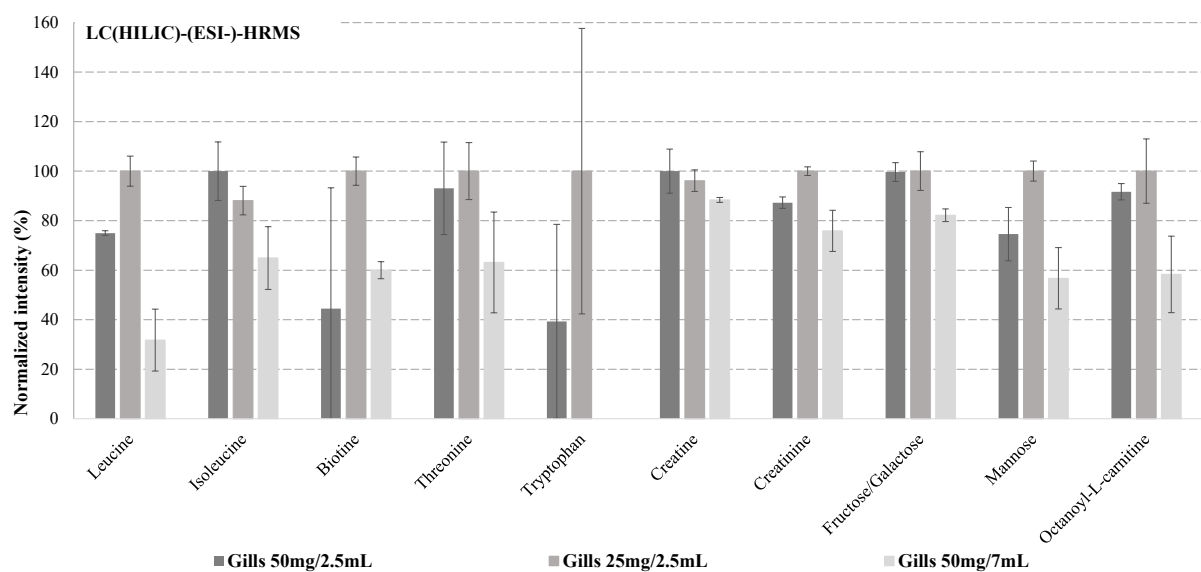
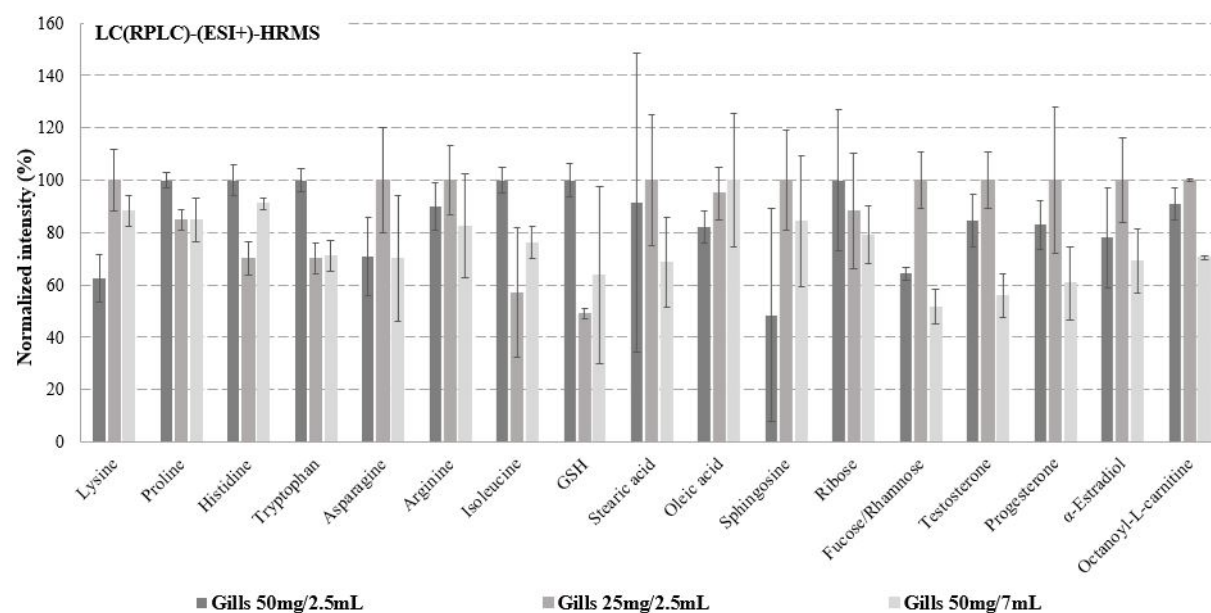


Figure S6. Normalized intensities of internal metabolites analyzed by LC(RPLC)-(ESI+)- or LC(HILIC)-(ESI)- HRMS after extraction of 50 mg of fish with 2.5 mL of different proportions of MeOH/H₂O/heptane.

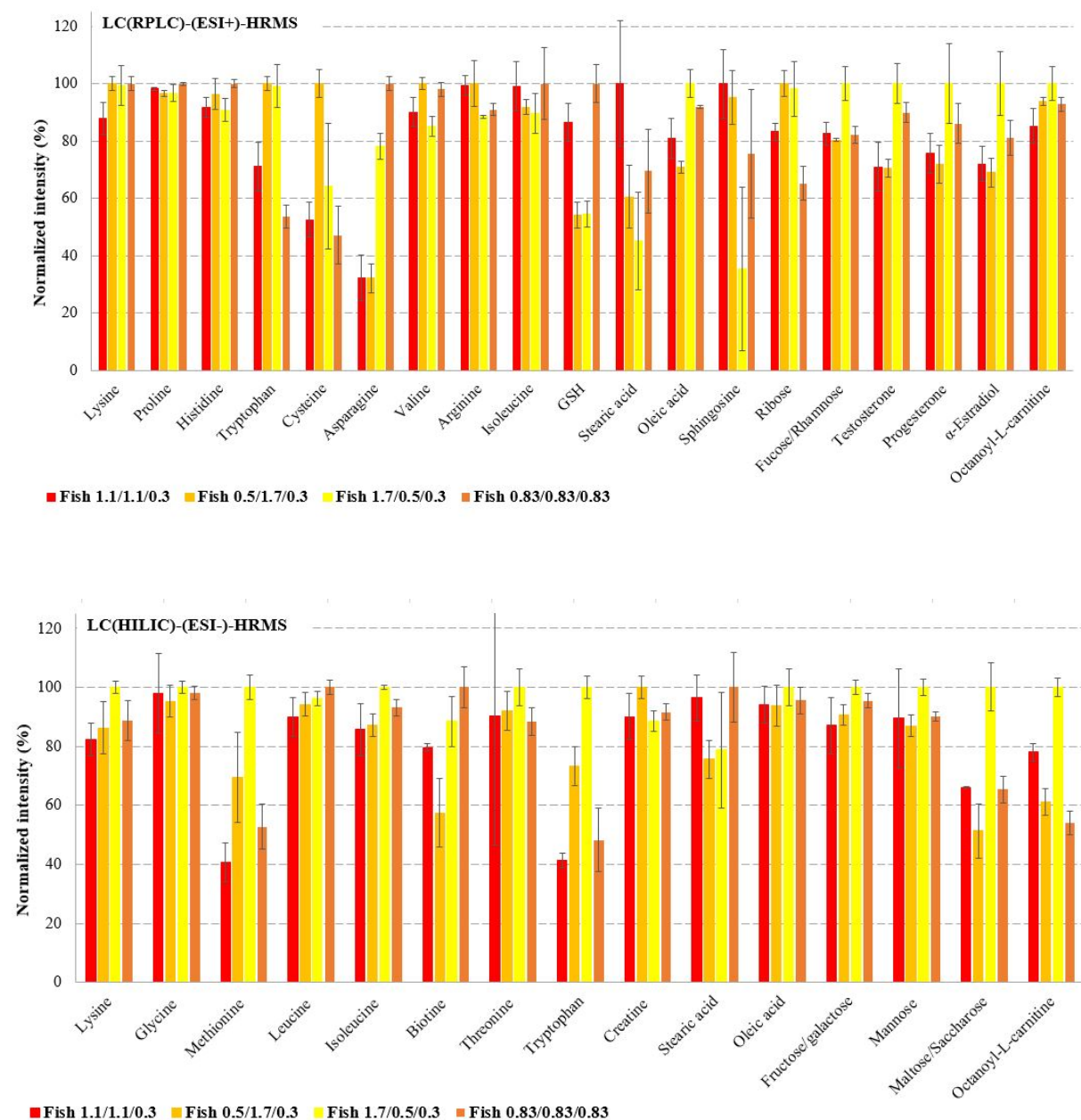


Figure S7. Normalized intensities of internal metabolites analyzed by LC(RPLC)-(ESI+)- or LC(HILIC)-(ESI)- HRMS after extraction of 50 mg of liver with 2.5 mL of different proportions of MeOH/H₂O/heptane.

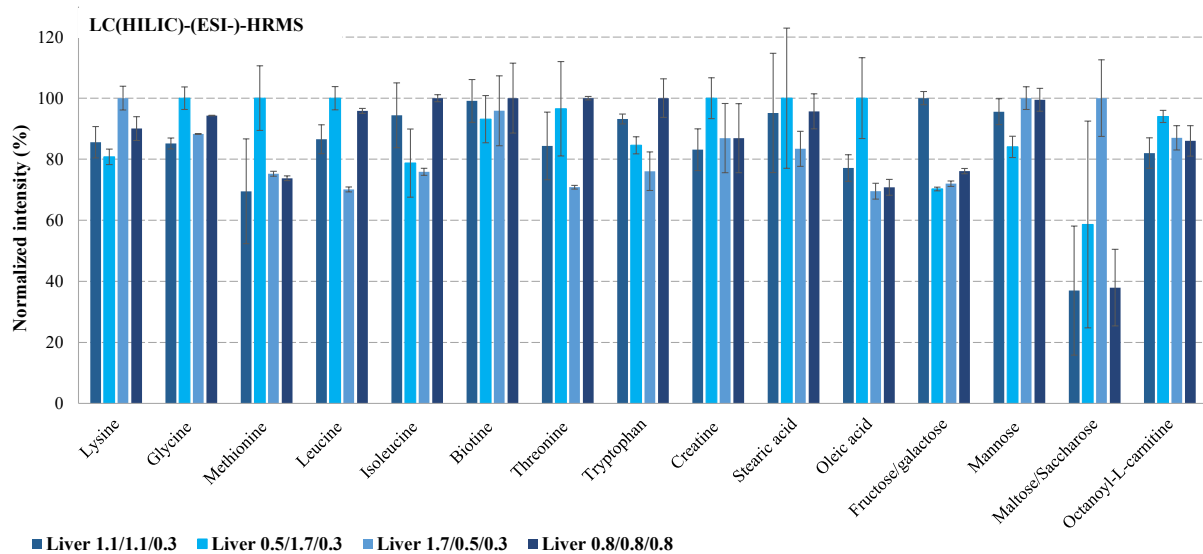
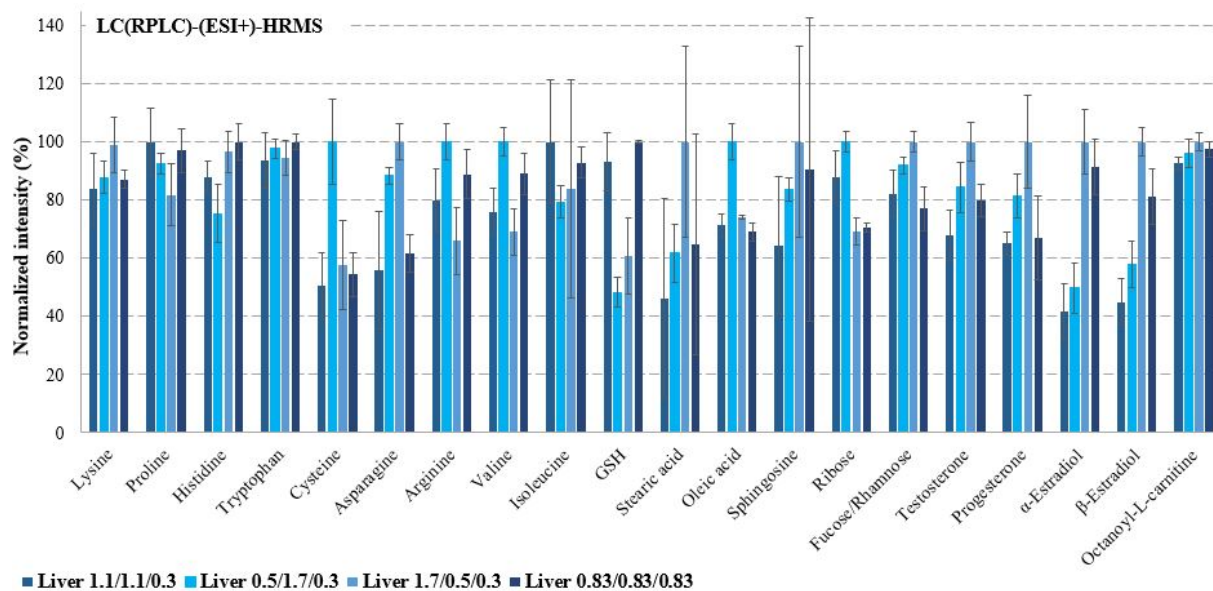


Figure S8. Normalized intensities of internal metabolites analyzed by LC(RPLC)-(ESI+)- or LC(HILIC)-(ESI)- HRMS after extraction of 50 mg of brain with 2.5 mL of different proportions of MeOH/H₂O/heptane.

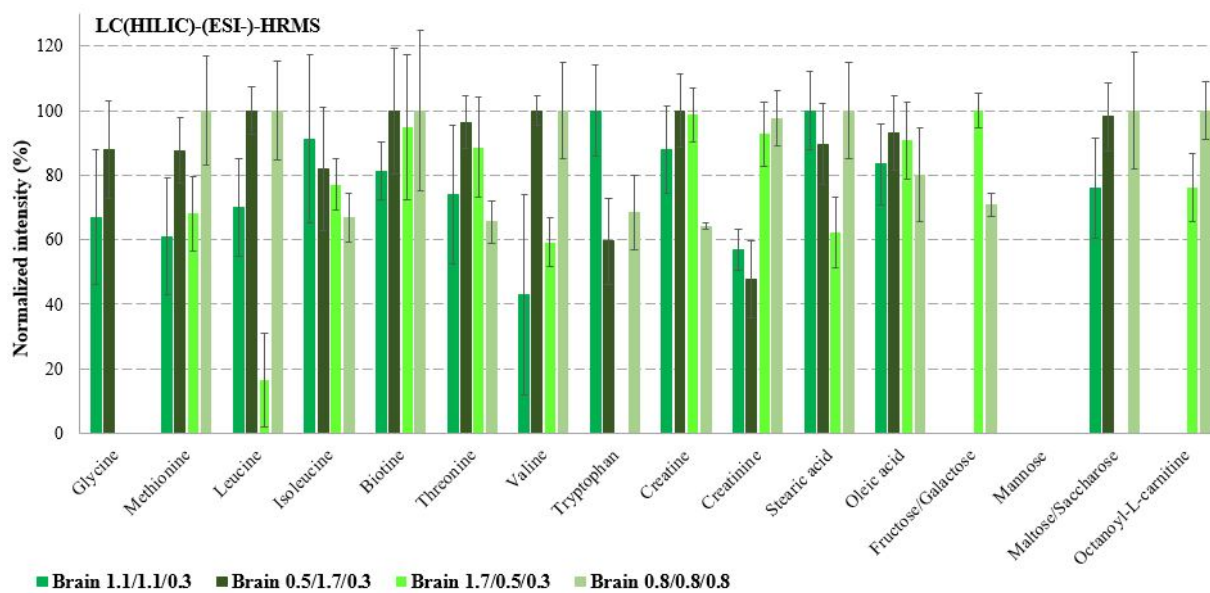
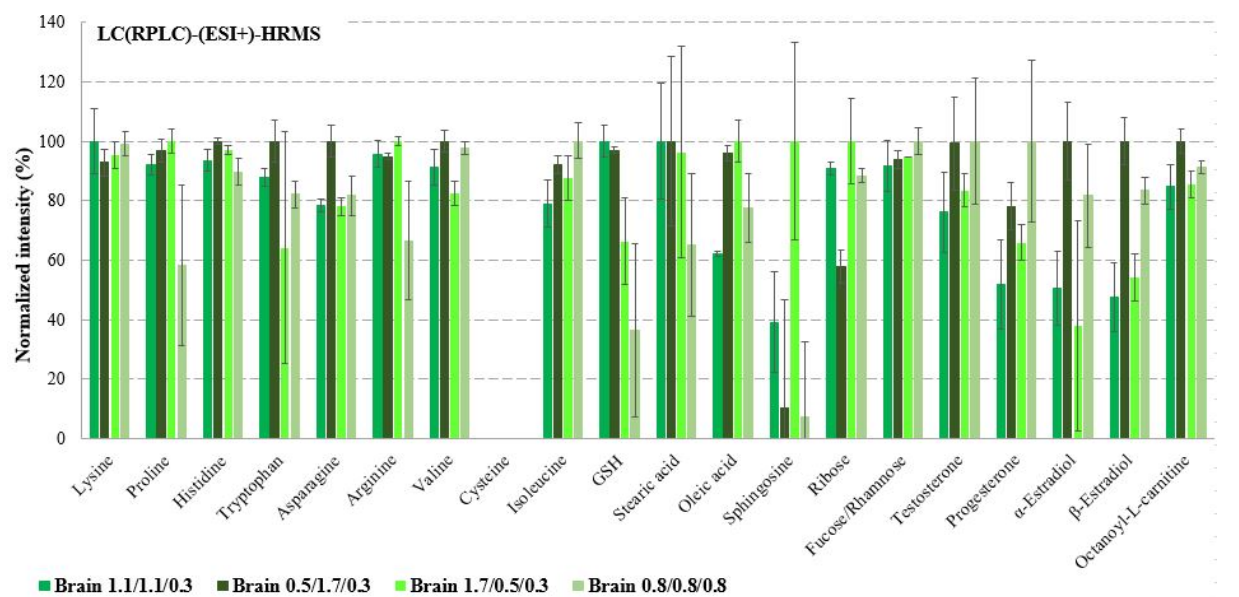


Figure S9. Normalized intensities of internal metabolites analyzed by LC(RPLC)-(ESI+)- or LC(HILIC)-(ESI)- HRMS after extraction of 25 mg of gills with 2.5 mL of different proportions of MeOH/H₂O/heptane.

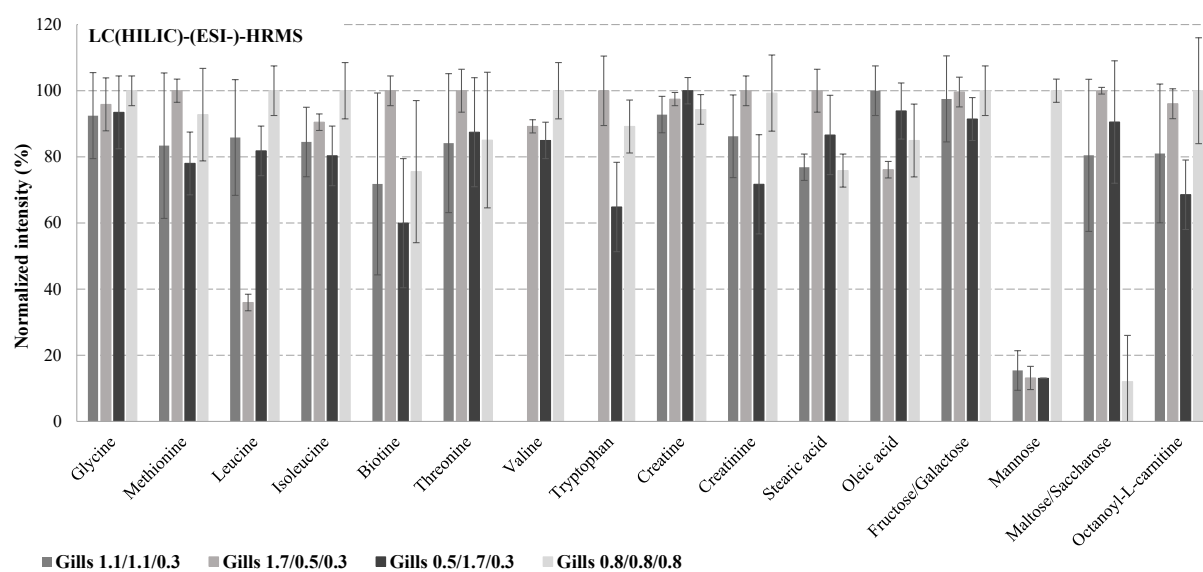
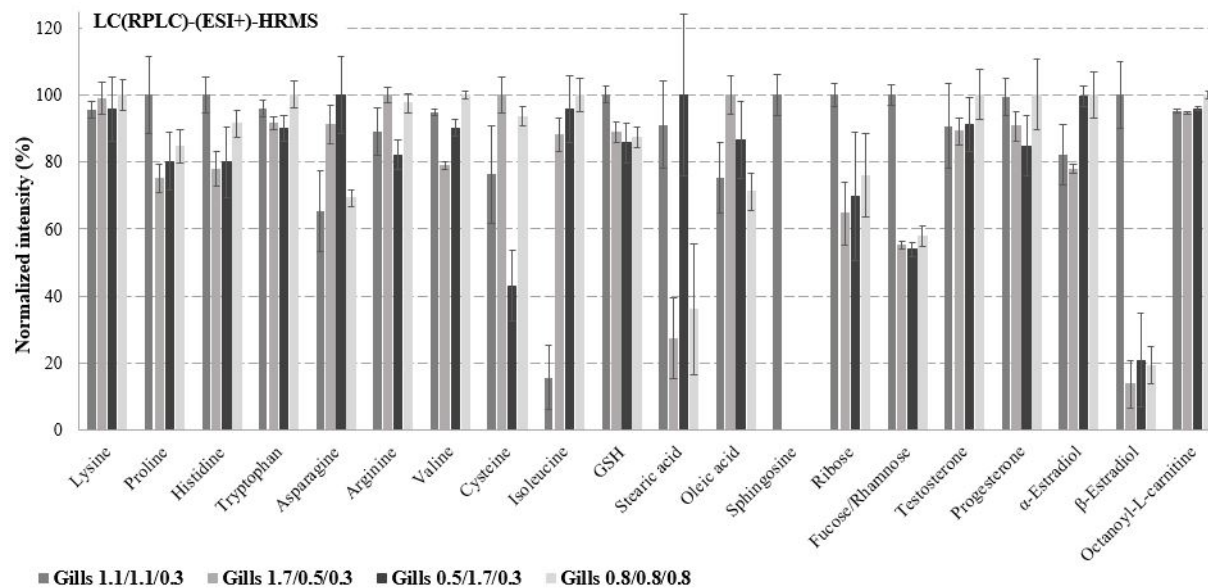


Table S1. Internal standards employed and adducts used during the analytical development

Compounds	Chemical group	Supplier	Purity (%)	Formula	Adduct detected mass (Da)	Chromatographic condition
Stearic acid	Fatty acid	Sigma-aldrich	> 95.0	C ₁₈ H ₃₆ O ₂	[M+H] ⁺ 285.2788 [M-H] ⁻ 283.2631	RPLC HILIC
Sphingosine	Sphingolipid	Sigma-aldrich	> 95.0	C ₁₉ H ₃₇ NO ₂	[M+H] ⁺ 312.2897	RPLC
Oleic acid	Fatty acid	Sigma-aldrich	> 95.0	C ₁₈ H ₃₄ O ₂	[M+H] ⁺ 283.2632 [M-H] ⁻ 281.2475	RPLC HILIC
Palmitic acid	Fatty acid	Sigma-aldrich	98.0	D ₃₁ C ₁₆ O ₂ H	[M+H] ⁺ 288.4421	RPLC
					[M+Na] ⁺ 310.4240	RPLC
					[M-H] ⁻ 286.4264	RPLC
					[M-H] ⁻ 286.4264	HILIC
Cholesterol	Lipid sterol	Sigma-aldrich	99.0	¹³ C ₃ C ₂₄ H ₄₆ O	[M-H] ⁻ 388.3566	HILIC
Valine	Amino acid	Sigma-aldrich	98.0	¹⁵ NC ₅ H ₁₁ O ₂	[M+H] ⁺ 119.0833	RPLC
					[M-H] ⁻ 117.0676	RPLC
					[M+H] ⁺ 119.0833	HILIC
					[M-H] ⁻ 117.0676	HILIC
Glutamine	Amino acid	Sigma-aldrich	98.0	¹⁵ NC ₅ H ₁₀ NO ₃	[M+H] ⁺ 148.0735	RPLC
					[M-H] ⁻ 146.0578	RPLC
					[M-H] ⁻ 146.0578	HILIC
Glycine	Amino acid	Sigma-aldrich	99.0	¹³ CNCO ₂ H ₅	[M+H] ⁺ 77.0427	RPLC
					[M-H] ⁻ 75.0270	RPLC
					[M+H] ⁺ 77.0427	HILIC
					[M-H] ⁻ 75.0270	HILIC
Lysine	Amino acid	Sigma-aldrich	98.0	D ₄ N ₂ C ₆ O ₂ H ₁₀	[M+H] ⁺ 151.1379	RPLC
Methionine	Amino acid	Sigma-aldrich	98.0	C ₅ D ₃ H ₈ NO ₂ S	[M+H] ⁺ 153.0772	RPLC
					[M-H] ⁻ 151.0615	RPLC
					[M+H] ⁺ 153.0772	HILIC

						[M-H] ⁻ 151.0615	HILIC
Leucine	Amino acid	Sigma-aldrich	98.0	¹³ C ₆ H ₁₃ (¹⁵ N)O ₂	[M+H] ⁺ 139.1191	RPLC	
					[M-H] ⁻ 137.1034	RPLC	
					[M+H] ⁺ 139.1191	HILIC	
					[M-H] ⁻ 137.1034	HILIC	
Tryptophan	Amino acid	-	> 95.0	C ₁₁ H ₁₂ N ₂ O ₂	[M+H] ⁺ 205.0972	RPLC	
					[M-H] ⁻ 203.0815	HILIC	
Asparagine	Amino acid	Sigma-aldrich	98.0	C ₄ H ₈ N ₂ O ₃	[M+H] ⁺ 133.0608	RPLC	
Histidine	Amino acid	Sigma-aldrich	> 95.0	C ₆ H ₉ N ₃ O ₂	[M+H] ⁺ 156.0768	RPLC	
Threonine	Amino acid	Fluka	99.0	C ₄ H ₉ NO ₃	[M+H] ⁺ 120.0655	RPLC	
					[M-H] ⁻ 118.0499	HILIC	
Arginine	Amino acid	Fluka	99.5	C ₆ H ₁₄ N ₄ O ₂	[M+H] ⁺ 175.1190	RPLC	
Cysteine	Amino acid	Sigma-aldrich	97.0	C ₃ H ₇ NO ₂ S	[M+H] ⁺ 122.0270	RPLC	
Isoleucine	Amino acid	Sigma-aldrich	98.0	C ₆ H ₁₃ NO ₂	[M+H] ⁺ 132.1019	RPLC	
					[M-H] ⁻ 130.0862	HILIC	
Proline	Amino acid	-	> 95.0	C ₅ H ₉ NO ₂	[M+H] ⁺ 116.0706	RPLC	
Aspartic acid	Amino acid	Sigma-aldrich	98.0	¹⁵ NC ₄ O ₄ H ₇	[M+H] ⁺ 135.0418	RPLC	
					[M-H] ⁻ 133.0262	RPLC	
Creatine	Derivative amino acid	ChemService	> 95.0	C ₄ H ₉ N ₃ O ₂	[M+H] ⁺ 132.0768	RPLC	
					[M-H] ⁻ 130.0611	HILIC	
Creatinine	Derivative amino acid	Sigma-aldrich	> 95.0	C ₄ H ₇ N ₃ O	[M+H] ⁺ 114.0662	RPLC	
					[M-H] ⁻ 112.0505	HILIC	
Lactic acid	Organic acid	Sigma-aldrich	98.0	¹³ CC ₂ H ₆ O ₃	[M+H] ⁺ 91.0345	HILIC	
					[M-H] ⁻ 89.0189	HILIC	
Taurocholic acid	Biliar acid	Clinisciences	99.0	C ₂₆ H ₃₉ D ₅ NO ₇ S	[M+H] ⁺ 520.3225	RPLC	
					[M-H] ⁻ 518.3069	RPLC	
					[M+H] ⁺ 520.3225	HILIC	
					[M-H] ⁻ 518.3069	HILIC	

Octanoyl-L-carnitine	Peptide	Sigma-aldrich	97.0	C ₁₅ D ₃ H ₂₆ NO ₄	[M+H] ⁺ 291.2358	RPLC
					[M+Na] ⁺ 313.2177	RPLC
					[M-H] ⁻ 289.2201	RPLC
					[M+H] ⁺ 291.2358	HILIC
					[M-H] ⁻ 289.2201	HILIC
GSH	Peptide	Sigma-aldrich	98.0	C ₁₀ H ₁₇ N ₃ O ₆ S	[M+H] ⁺ 308.0911	RPLC
Biotine	Vitamin	Sigma-aldrich	98.0	C ₁₀ D ₂ H ₁₄ N ₂ O ₃ S	[M+H] ⁺ 247.1080	HILIC
					[M-H] ⁻ 245.0923	HILIC
α-Estradiol	Hormone	Sigma-Aldrich	99.9	C ₁₈ H ₂₄ O ₂	[M+H] ⁺ 273.1849	RPLC
					[M-H] ⁻ 271.1693	RPLC
β-Estradiol	Hormone	CDN isotopes	99.5	C ₁₈ H ₂₂ D ₂ O ₂	[M+H] ⁺ 275.1975	RPLC
					[M-H] ⁻ 273.1818	RPLC
					[M+H] ⁺ 275.1975	HILIC
					[M-H] ⁻ 273.1818	HILIC
Testosterone	Hormone	CDN isotopes	99.7	C ₁₉ H ₂₆ D ₂ O ₂	[M+H] ⁺ 291.2288	RPLC
					[M+H] ⁺ 291.2288	HILIC
					[M-H] ⁻ 289.2131	HILIC
Progesterone	Hormone	CDN isotopes	98.5	C ₂₁ H ₂₁ D ₉ O ₂	[M+H] ⁺ 324.2883	RPLC
					[M+H] ⁺ 324.2883	HILIC
Estrone	Hormone	CDN isotopes	99.2	C ₁₈ H ₂₀ D ₂ O ₂	[M+H] ⁺ 273.3873	RPLC
					[M-H] ⁻ 271.3716	RPLC
					[M+H] ⁺ 273.3873	HILIC
					[M-H] ⁻ 271.3716	HILIC
Glucose	Sugar	Sigma-aldrich	99.0	¹³ C ₆ H ₁₂ O ₆	[M+H] ⁺ 187.0908	RPLC
					[M-H] ⁻ 185.0751	RPLC
					[M+H] ⁺ 187.0908	HILIC
					[M-H ₂ O-H] ⁻ 167.0646	HILIC
Mannose	Sugar	Sigma-aldrich	99.0	¹³ CC ₅ H ₁₂ O ₆	[M-H] ⁻ 185.0751	HILIC
					[M+H] ⁺ 182.0740	RPLC
					[M-H] ⁻ 180.0584	RPLC
					[M+H] ⁺ 182.0740	HILIC
					[M-H ₂ O-H] ⁻ 162.478	HILIC

						[M-H] ⁻ 180.0584	HILIC
Fucose	Sugar	Sigma-aldrich	98.0	C ₆ H ₁₂ O ₅	[M+H] ⁺ 165.0758	RPLC	
					[M+Na] ⁺ 187.0577	RPLC	
					[M+NH ₄] ⁺ 182.1023	RPLC	
Rhamnose	Sugar	-	> 95.0	C ₆ H ₁₂ O ₅	[M+H] ⁺ 165.0758	RPLC	
					[M+Na] ⁺ 187.0577	RPLC	
					[M+NH ₄] ⁺ 182.1023	RPLC	
Fructose	Sugar	Sigma-aldrich	99.0	C ₆ H ₁₂ O ₆	[M+H] ⁺ 181.0707	RPLC	
					[M-H ₂ O-H] ⁻ 161.0445	HILIC	
Galactose	Sugar	Sigma-aldrich	98.0	C ₆ H ₁₂ O ₆	[M+H] ⁺ 181.0707	RPLC	
					[M-H ₂ O-H] ⁻ 161.0445	HILIC	
Saccharose	Sugar	Merck	> 95.0	C ₁₂ H ₂₂ O ₁₁	[M+H] ⁺ 343.1235	RPLC	
					[M-H ₂ O-H] ⁻ 323.0973	HILIC	
Maltose	Sugar	Sigma-aldrich	99.0	C ₁₂ H ₂₂ O ₁₁	[M+H] ⁺ 343.1235	RPLC	
					[M-H ₂ O-H] ⁻ 323.0973	HILIC	
Ribose	Sugar	Sigma-aldrich	98.0	C ₅ H ₁₀ O ₅	[M+H] ⁺ 151.0601	RPLC	