Table S1. Variable parameters for each of the LC-MS acquisition methods used.

Method	HILIC positive	HILIC negative	Reverse phase positive	Reverse phase negative
Column	Agilent Poroshell 120 HILIC-Z PEEK-lined column, 150 mm length, 2.1 mm diameter, 2.7 µm particle size		Thermo Accucore C18 column, 150 mm length, 2.1 mm diameter, 2.6 µm particle size	
Mobile phase A	10 mM ammonium formate and 0.1 % formic acid in water	10 mM ammonium acetate, pH 9 with ammonium hydroxide and 10 µM medronic acid	0.1 % formic acid in water	
Mobile phase B	9:1 acetonitrile / 10 mM ammonium formate and 0.1 % formic acid in water	85:15 acetonitrile / 10 mM ammonium acetate, pH 9 with ammonium hydroxide and 10 µM medronic acid in water	0.1 % formic acid in 98:2 acetonitrile / water	
Solvent flow rate	0.25 ml/min		0.3 ml/min	
Column temperature	50 °C		40 °C	
Needle wash composition	9:1 acetonitrile / water		5:95 acetonitrile / water	
Gradient, %B	0 min 98 %B 3 min 98 %B 23 min 5 %B 24 min 5 %B 24 min 98 %B	0 min 96 %B 2 min 96 %B 22 min 65 %B 24 min 65 %B 24 min 96 %B	0 min 5 %B 1 min 5 %B 8 min 100 %B 10 min 100 %B 10 min 5 %B	
Re-equilibration time	5 min		4 min	
Total run time	30 min		15 min	
Mass spectrometer polarity	Positive	Negative	Positive	Negative
Ionspray voltage	5500 V	-4500 V	5500 V	-4500 V
Scan range (*m/z*)	50 – 1000	60 – 1600	50 – 1000	60 – 1600
Adducts for peak picking	M+H, M+Na, M+K, M+H-H_2O, M+2H	M-H, M-H-H_2O, M+Cl	M+H, M+Na, M+K, M+H-H_2O, M+2H	M-H, M-H-H_2O, M+Cl
Peak picking retention time limits	1.3 – 24 min		0.9 – 10 min	

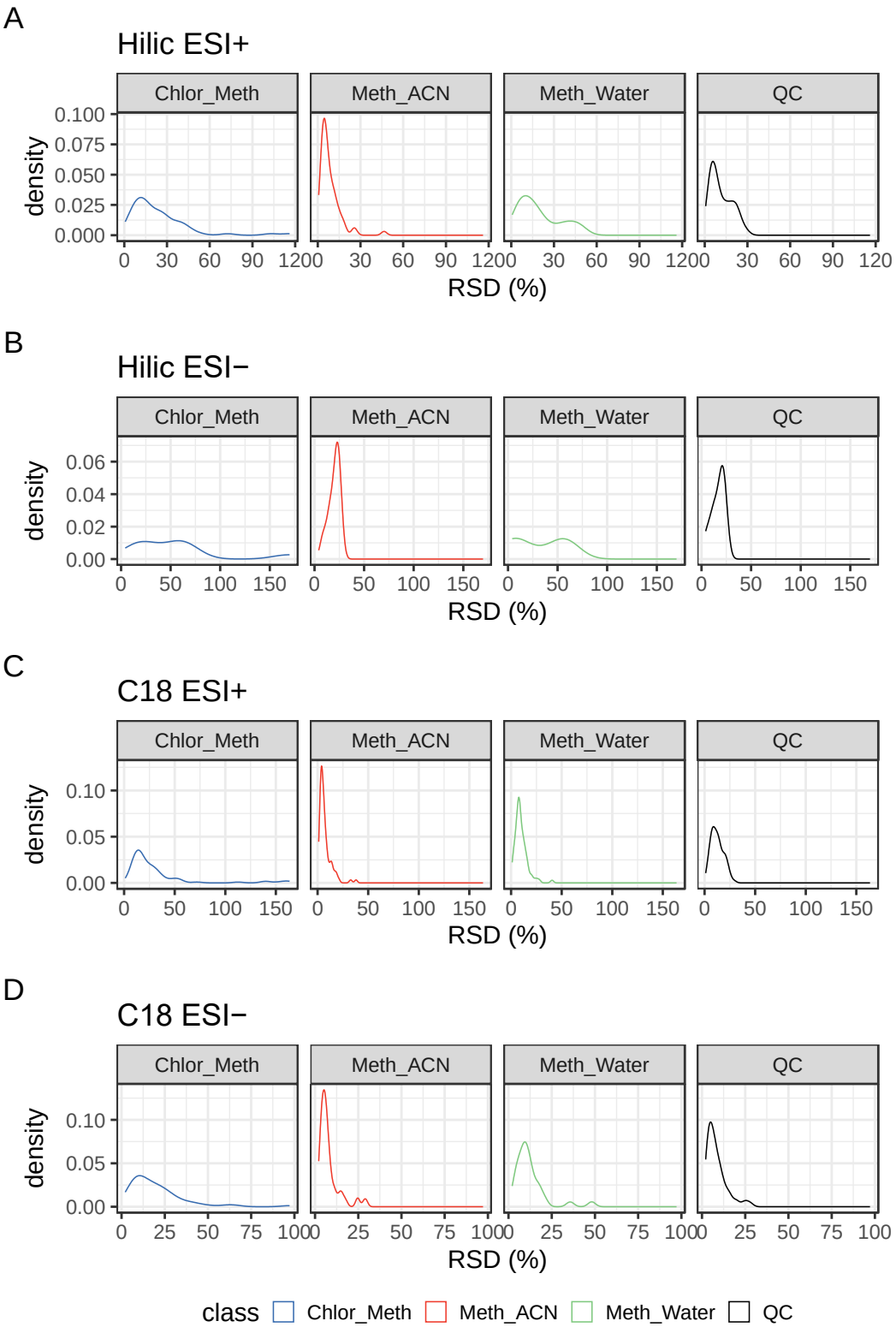

Figure S1. Density plot for all metabolites RSD values across the four experiments and divided by extraction protocol to evaluate extraction reproducibility. The figure also shows RSD for the QC samples to assess injection replicability.

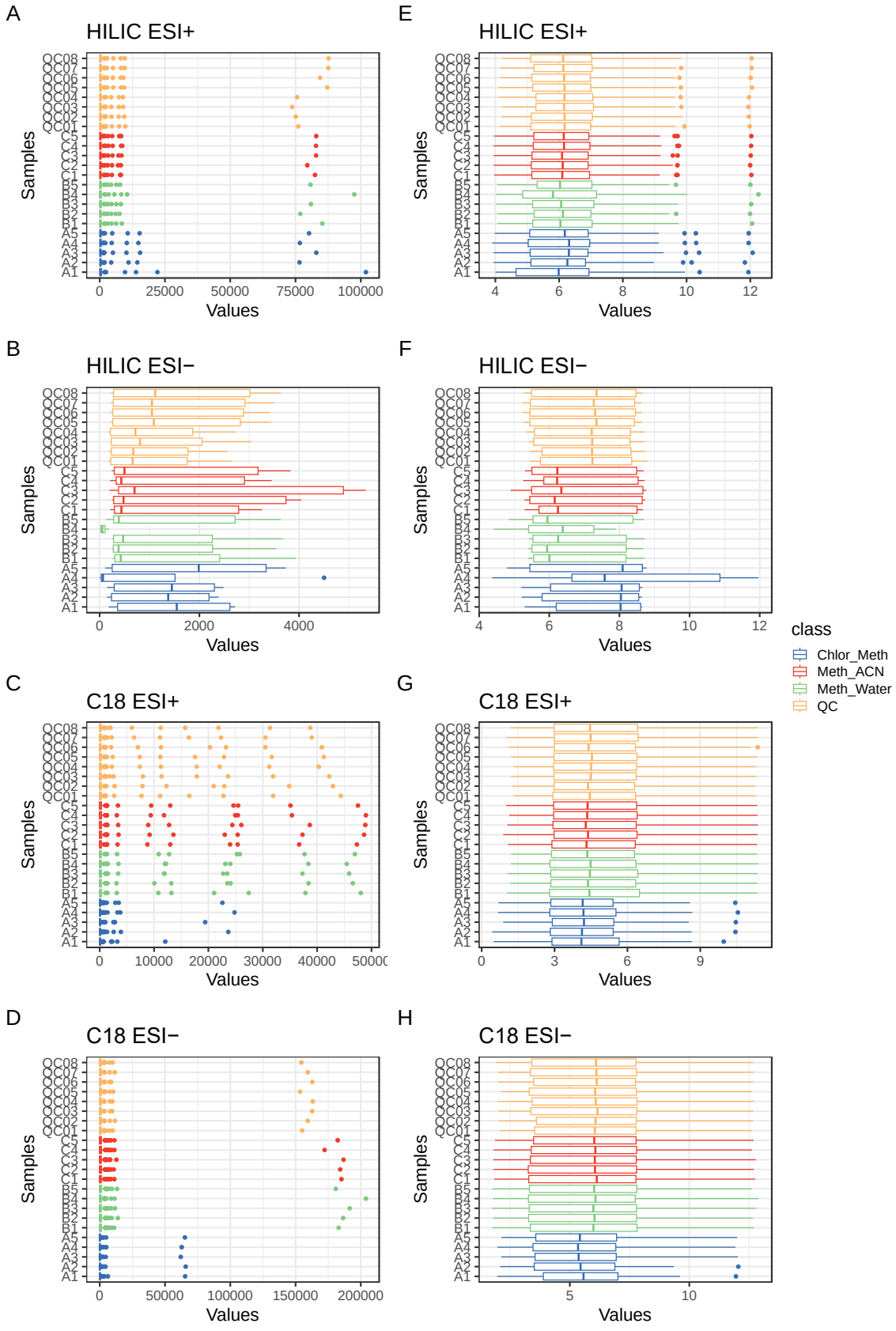

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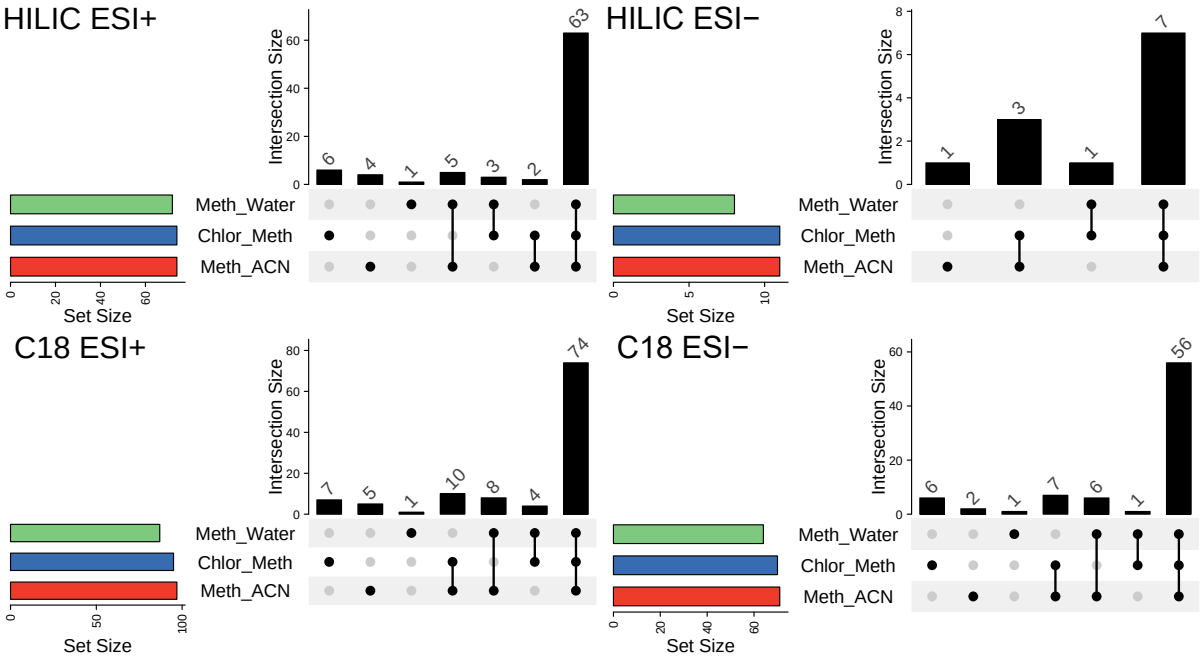
Figure S2. Results for data processing. (A-D) Boxplots of all samples and QCs for the four assays prior to transformation and normalisation. (E-H) Boxplots of all samples and QCs for the four assays after transformation and normalisation.



15 **Figure S3.** UpsetR plot sowing the intersection of compounds across the three extractions for all assays considered in the study. Data were processed separately for each extraction protocol.