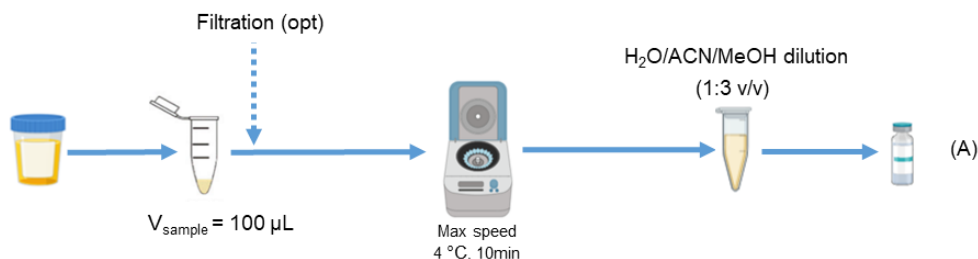


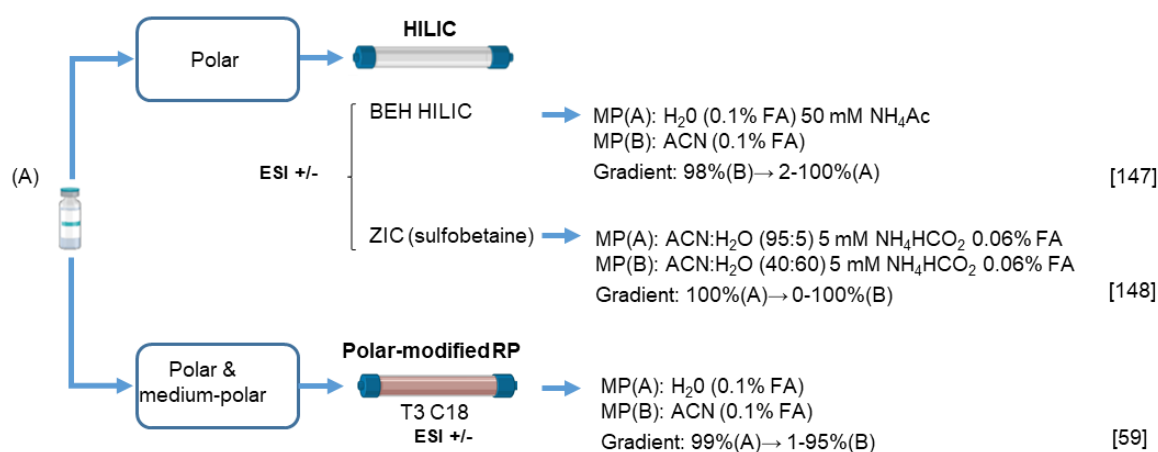
Figure S6. Recommended protocol and technical aspects for urine

URINE

Sample Preparation



LC Configuration



Metabolome coverage

ESI	HILIC (Log P < 1)	RP modified (-3 < Log P < 7)	RP (Log P > -1)
+	289 metabolites: , benzenoidsheterocyclic comp., aas, carbohydrates, amines, acylcarnitines...	677 metabolites: heterocyclic comp., benzenoids, aas, acylcarnitines, organic oxygen, amines...	586 metabolites: heterocyclic comp., benzenoids, steroids, aas, ketoacids, organic oxygen, amines...
-	56 metabolites: aas, carboxylic acids, FAs...	107 metabolites: FAs, phospholipids, eicosanoids, BAs, organic acids...	90 metabolites: FAs, phospholipids, BAs, eicosanoids, organic acids...
+/-	330 metabolites: aas, carboxylic acids, heterocyclic comp., nucleotides, benzenoids, carbohydrates...	585 metabolites: heterocyclic comp., aas, benzenoids, FAs, sterpoids, Bas, nucleotides, carbohydrates...	460 metabolites: benzenoids, heterocyclic comp., FAs, steroids, aas, flavonoids...

aas: amino acids; FAs: fatty acids; BAs: bile acids

Tips

- Perform the optional filtration if the urine is not clear.
- Neutral pH in mobile phases are recommended when HILIC analysis is performed.
- Normalization of urine by creatinine, osmolality, specific gravity or MS total useful signal is strongly recommended.
- Pay speciall attention to creatinine as it can be confounded by sex, muscle mass, ethnicity, or disease states, even within the same individual.