

Supplementary Table 2 Procedure of sample preparations for matrix effects, calibration curves, QC samples and urine samples.

(A) Sample set for investigation of matrix effects

		(1) Neat standard	(2) Urine	(3) Standard spiked urine
100 μ L of Mixture containing sample matrix and analytes	50 μ L of Matrix solution	Surrogate matrix (Water)	Sample matrix (Urine)	Sample matrix (Urine)
	50 μ L of standard solution	50 ng/mL Analytes in ethanol/water (1:1, v/v)	Ethanol/water (1:1, v/v)	50 ng/mL Analytes in ethanol/water (1:1, v/v)
50 μ L of IS solution		33 ng/mL of 3 β -sulfooxy-7 β -hydroxy-23- <i>nor</i> -5-cholenoic acid in ethanol/water (1:1, v/v)		
350 μ L of Dilution solution		Water		
↓ Centrifugation				
Analysis (200 μ L of sample aliquot)				

(B) Sample set for investigation of analytical method validation

		(1) Calibration solutions	(2) Urine (N=6)	(3) Standard spiked urine (N=6)
100 μ L of Mixture containing sample matrix and analytes	50 μ L of Matrix solution	Surrogate matrix (Water)	Sample matrix (Urine from healthy subject)	Sample matrix (Urine from healthy subject)
	50 μ L of standard solution	0.3-1000 ng/mL Analytes in ethanol/water (1:1, v/v)	Ethanol/water (1:1, v/v)	2, 50, 800 ng/mL Analytes in ethanol/water (1:1, v/v)
50 μ L of IS solution		33 ng/mL of 3 β -sulfooxy-7 β -hydroxy-23- <i>nor</i> -5-cholenoic acid in ethanol/water (1:1, v/v)		
350 μ L of Dilution solution		Water		
↓ Centrifugation				
Analysis (200 μ L of sample aliquot)				

(C) Sample set for analysis of sample urine

		(1) Calibration sample	(2) Sample urine
100 μ L of Mixture containing sample matrix and analytes	50 μ L of Matrix solution	Surrogate matrix (Water)	Sample matrix (Urine)
	50 μ L of standard solution	0.3-1000 ng/mL analytes in ethanol/water (1:1, v/v)	Ethanol/water (1:1, v/v)
50 μ L of IS solution		33 ng/mL of 3 β -sulfooxy-7 β -hydroxy-23- <i>nor</i> -5-cholenoic acid in ethanol/water (1:1, v/v)	
350 μ L of Dilution solution		Water	
↓ Centrifugation			
Analysis (200 μ L of sample aliquot)			