

Supporting Information for

High-resolution metabolomics of 50 neurotransmitters and tryptophan metabolites in feces, serum, and brain tissues using UHPLC-ESI-Q Exactive mass spectrometry

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Number of pages: 5

Number of figures: 2

Number of tables: 2

Table of Contents

Figure S1. Overview of sample processing for addressing wide dynamic ranges of compounds: Page S2

Figure S2. Barplots of analytical recovery in sample matrices as indicated by SIL standards: Page S3

Table S1. Sample pretreatment procedures for individual compounds: Page S4

Table S2. Analyte recovery rates in sample matrices: Page S5

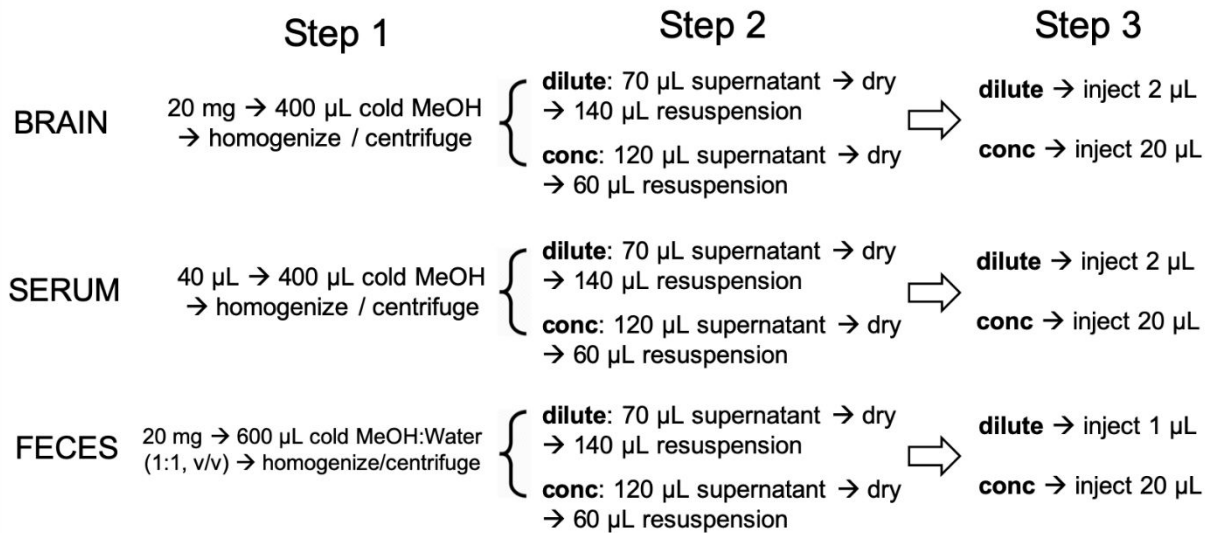


Figure S1. Overview of sample processing procedures. ‘conc’ stands for ‘concentration’.

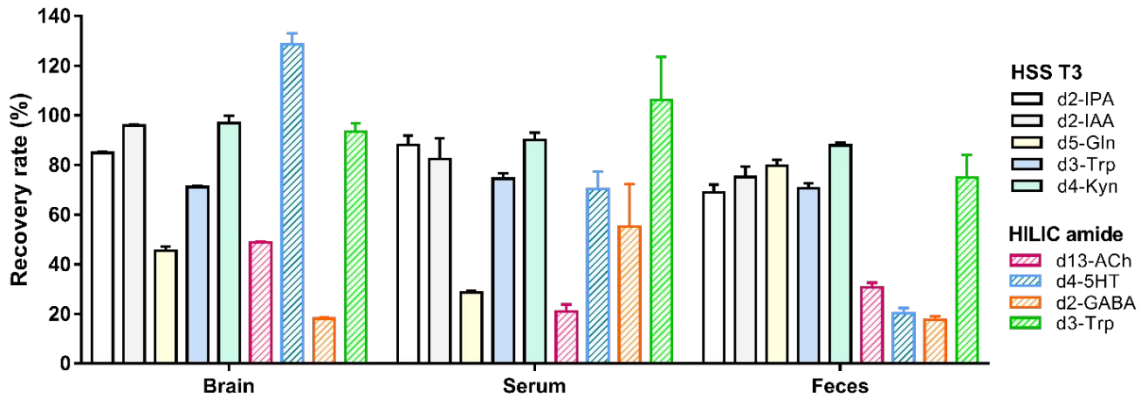


Figure S2. Barplots of analytical recovery in the three sample matrices (Mean \pm SEM, n=4).

Table S1. Sample adaptation to maintain within linearity of assay.

Compound Name	Brain	Serum	Feces
3-Aminopiperidine-2,6-dione	*conc	conc	conc
5-HIAA	conc	conc	conc
5-Hydroxy-N ω -methyltryptamine	conc	conc	conc
Acetylcholine	*dilute	dilute	dilute
Choline	dilute	dilute	dilute
Histamine	conc	conc	conc
Kynurenic acid	conc	conc	conc
L-Methionine	dilute	dilute	dilute
L-Proline	conc	dilute	conc
L-Pyroglutamic acid	dilute	dilute	conc
L-Tyrosine	dilute	dilute	conc
Nicotinic acid	conc	conc	dilute
N α -Acetyl-L-glutamine	conc	conc	conc
Serotonin	conc	dilute	conc
Trimethylamine N-oxide	dilute	dilute	dilute
Tyramine	conc	conc	conc
γ -Aminobutyric acid	dilute	conc	conc
2-Phenethylamine	conc	conc	conc
2-Pyrrolidinone	dilute	dilute	dilute
3-Hydroxyanthranilic acid	dilute	dilute	conc
4-Methoxyindole	conc	conc	conc
AMFK	conc	conc	conc
Anthranilic acid	conc	dilute	conc
Coumarin	conc	conc	conc
Hippuric acid	conc	conc	conc
Indole	conc	conc	conc
Indole-3-acetic acid	conc	conc	conc
Indole-3-acetonitrile	conc	conc	conc
Indole-3-acrylic acid	dilute	dilute	dilute
Indole-3-carboxaldehyde	conc	dilute	dilute
Indole-3-carboxylic acid	conc	conc	conc
Indole-3-ethanol	conc	conc	conc
Indole-3-lactic acid	conc	dilute	dilute
Indole-3-propionic acid	conc	dilute	conc
Kynurenine	conc	dilute	conc
L-Arginine	dilute	dilute	dilute
L-Glutamic acid	dilute	dilute	dilute
L-Phenylalanine	dilute	dilute	dilute
L-Tryptophan	dilute	dilute	dilute
Melatonin	conc	conc	conc
Methyl IAA	conc	conc	conc
N-(2-Phenylacetyl)glycine	conc	dilute	dilute
N-Acetylserotonin	conc	conc	conc
N-Methylphenethylamine	dilute	conc	conc
N-Methyltryptamine	conc	conc	conc
Nudifloramide	conc	dilute	dilute
p-Coumaric acid	conc	conc	conc
Phenylacetyl L-glutamine	conc	conc	conc
Tryptamine	conc	conc	conc
Xanthurenic acid	conc	conc	conc

* specific procedures of ‘*dilute*’ and ‘*conc*’ (short for ‘concentration’) can be found in Figure S1.

These adaptations in sample preparation are conducted to address the vast dynamic range of endogenous levels so that the analytes could be quantified within the linearity of the assay.

Table S2. Analyte recovery in the three sample matrices indicated by spiked SIL internal standards.

% Recovery	HILIC amide				HSS T3				
Sample	<i>d13-ACh</i>	<i>d4-5HT</i>	<i>d2-GABA</i>	<i>d3-Trp</i>	<i>d2-IPA</i>	<i>d2-IAA</i>	<i>d5-Gln</i>	<i>d3-Trp</i>	<i>d4-Kyn</i>
Brain_1	48.96	131.68	18.83	96.49	84.77	96.51	49.96	70.07	98.14
Brain_2	47.28	116.06	16.85	89.49	84.62	95.22	45.84	70.55	91.37
Brain_3	48.36	137.19	17.11	86.33	84.36	95.59	41.86	72.34	93.20
Brain_4	50.02	129.40	19.62	101.32	85.94	96.59	44.29	71.72	105.04
Serum_1	22.90	60.42	13.12	94.68	91.37	100.20	27.46	77.06	93.79
Serum_2	12.59	67.86	45.63	85.35	93.94	80.39	30.86	77.04	81.46
Serum_3	23.42	91.35	95.11	157.96	90.16	60.49	27.81	75.78	92.43
Serum_4	24.99	61.12	66.56	86.72	76.85	88.34	28.10	68.32	92.77
Feces_1	33.07	15.01	14.02	48.97	69.63	82.14	78.20	70.56	91.44
Feces_2	25.11	18.49	19.89	79.34	77.65	82.81	84.10	75.08	85.19
Feces_3	31.59	25.85	20.30	91.04	63.74	66.78	83.02	65.07	88.29
Feces_4	32.94	21.32	16.24	80.55	65.32	69.02	73.89	71.78	86.66