

Detailed methods

Rat brain samples were collected and snap frozen in liquid nitrogen. Samples were later powderised with a mortar and pestle in a Cryo-Cooler. Powder was homogenised and lysed in lysis buffer (8M of urea and 50mM ammonium bicarbonate, pH 7.8) and sonicated in a Bioruptor Twin bath sonicator (settings: sonicated at high for 15 cycles of 30 seconds on and 30 seconds off; repeated once). Lysates were quantified with a NanoDrop, adjusted to 1mg and 1% Protein A added as a digestion control (final volume 160uL). Samples were reduced for 30 min and alkylated for 45 min (6mM DTT and 22mM iodoacetamide final concentration, respectively) in an Eppendorf thermomixer at 1400rpm at 21°C in the dark. Samples were digested with Promega Trypsin/Lys-C mix at a 1:50 sample to enzyme concentration. The enzyme mix was resuspended in 50mM ammonium bicarbonate (pH 7.8) and added to the samples for 4 hours in an Eppendorf thermomixer at 1400rpm at 21°C in the dark. Samples were then diluted 1:8 with 50mM of ammonium bicarbonate (pH 7.8) for a final concentration of $\leq 1\text{M}$ urea and left for a further 16 hours. The digests were acidified to 1% formic acid, pH 3 and spun in a centrifuge for 30mins. The supernatants were removed to be desalted with an Empore C¹⁸ cartridge and eluted into an aliquot of retention time standards. Following vacuum centrifugation, samples were resuspended in 0.1% formic acid and pooled to form one homogenous digest. The pooled digest was stored at -80°C until analysis.

Three aliquots of the sample were diluted to form three stock solutions: 0.2 mg/ml, 0.4 mg/ml and 0.6 mg/ml; and loaded with the following conditions.

LC methods are as follows:

Buffer A: 0.1% formic acid

Buffer B: 99.9% acetonitrile, 0.1% formic acid

Load 10uL for 8 min at 8 uL/min, 100% Buffer A.

Gradient 5uL/min:

Time (min)	Buffer A (%)	Buffer B (%)
0	98	2
1	98	2
5	92	8
65	95	25
75	60	40
79	6	95
84	6	95
89	98	2
95	98	2

IDA conditions are as follows:

SWATH-MS conditions are as follows: