LCMS Protocol

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1 ABSTRACT

Our LCMS protocol outlines the method we used to construct our lampreyDB database (https://www.lampreydb.com), and it is designed to provide a step-by-step guide for those who wish to follow our methodology. The protocol includes detailed instructions on the instrumentation and chromatographic condi- tions used, as well as information on the mass spectrometry parameters and settings.

2 Materials and Methods

2.1 Extraction

1. Weigh 30 mg of tissue sample accurately into a 2 mL EP tube. Add 20 μL of internal standard (L-2-chlorophenylalanine, 0.3 mg/mL prepared in methanol) and 400 μL of methanol-water (V:V=4:1).
2. Add two small steel balls, pre-cooled in a -20°C refrigerator for 2 minutes, and grind using a Tissuelyser-48 grinding miller (60 Hz, 2 minutes).

3. The resulting extract was briefly vortexed and sonicated at ambient temperature (25-28 ℃) for 10 minutes.
4. , the extracts were centrifuged at 13,000 rpm and 4 ℃ for 10 minutes.
5. 300 μL of the supernatant was transferred into a brown glass vial and dried using a freeze concentration centrifugal dryer.
6. To each sample, 300 μL of a methanol and water mixture (1/4, v/v) was added. The mixture was vortexed for 30 seconds and then placed at -20 °C for 2 hours.
7. The samples were centrifuged at 13,000 rpm and 4 °C for 5 minutes. The resulting supernatants (150 μL) from each tube were collected using crystal syringes, filtered through a 0.22 μm PTFE filter (Acrodisc® CR 13mm; PALL), and transferred to LC vials for LCMS analysis.

2.2 Liquid chromatography-mass spectrometry analysis conditions

The analytical instrument used in this experiment was a Dionex Ultimate 3000 UHPLC system fitted with Q-Exactive quadrupole-Orbitrap mass spectrometer equipped with heated electrospray ionization (ESI) source (Thermo Fisher Scientific, Waltham, MA, USA).

The chromatographic conditions were as follows:

- Chromatographic column: An ACQUITY UPLC HSS T3 column (100 mm × 2.1 mm, 1.8 μm)
- Column temperature: 45°C
- Mobile phase: A: water (containing 0.1% formic acid), B: acetonitrile (containing 0.1% formic acid)
- Flow rate: 0.4 mL/min
- Injection volume: 2 μL

Table 1. The LC elution gradient

Time	A%	B%
0	95	5
2	95	5
4	75	25
8	50	50
10	20	80
14	0	100
15	0	100
15.1	95	5

Time	A%	B%
16	95	5

2.3 Mass Spectrometry Conditions

- Ion source: ESI
- Samples were analysed at both positive and negative ion scanning modes respectively.

Table 2. MS parameters

Parameters	Positive ion mode	Negative ion mode
Spray Voltage (V)	3800	-3000
Capillary Temperature (°C)	320	320
Sheath Gas Flow Rate (Arb)	35	35
Aux gas flow rate (Arb)	8	8
Mass range (m/z)	66.7 - 1000.5	66.7 - 1000.5
Full ms resolution	70000	70000
MS/MS resolution	35000	35000
NCE/stepped NCE	10, 20, 40	10, 20, 40

3 Contact

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