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BAF_Protocol_015_ 3-NPH derivatization of short chain fatty acid

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Protocol status: Working
We use this protocol and it's working

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Abstract

Short Chain Fatty Acid derivitization before MS analysis.

Materials

1. 3-Nitrophenylhydrazine hydrochloride (3-NPH) ⟶ Sigma N21804-5G
2. Butylated hydroxytoluene (BHT) ⟶ Sigma PHR1117-1g
3. *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (ED) ⟶ Sigma E1769- 5g
4. Pyridine ⟶ Sigma 270970 - 100 mL
5. Valeric acid ⟶ Sigma 75054-1mL
6. Isovaleric acid ⟶ Sigma 78651- 1mL
7. Hexanoic acid (caproic acid) ⟶ Sigma 21529 - 1mL
8. 4-Methylvaleric acid (Isocaproic acid) ⟶ Sigma 277827 - 1mL
9. Butyric acid ⟶ Sigma 19215 - 5 mL
10. Isobutyric acid ⟶ Sigma 46935-U - 500mg
11. Lactic acid ⟶ Sigma L6661 - 100 mL
12. Acetic acid ⟶ Sigma 338826 - 25 mL
13. Column: Waters ACQUITY UHPLC BEH C18 1.7 um, 2.1 × 150 mm - 186002353
14. Thermo Optima 0.1% FA (formic acid) in water - LS118-4
15. Thermo Optima Methanol - A456-212
16. Thermo Autosampler vials 0.25 mL – 14-823-136
17. Thermo Orange caps – 14-823-380
18. Fisherbrand Standard Pipette Tips (200 uL - Yellow) – 2707502
19. Thermo Orbitrap ID-X - FETD1-10001
20. Thermo Vanquish Duo UHPLC

Samples

1 Refer to BAF_Protocol_008 Metabolomics: Soluble Metabolite Extraction for soluble metabolite extraction of a sample of interest and dry the extracted metabolites.

Derivatization reagents: prepare all fresh

2 Reagent powder storage: EDC is stored at -20C, all others are at room temperature. Prepare working solutions on small glass botles.

3 **250 nM 3-NPH solution** as derivatization agent in 50% methanol:
→ dissolve 47.4 mg of 3-NPH in 1 mL of 50% methanol

4 **2 mg/mL BHT** in 100% methanol:
→ dissolve 2 mg in 1 mL methanol.

5 **150 mM EDC** (stored at -20C) in 100% methanol:
→ dissolve 28.75 mg in 1 mL methanol.

6 **7,5% pyridine** in 75% methanol.
→ add 750 uL in 9.25 mL of 75% methanol.

Standards and standard curve

7 Prepare a stock solution of each standard at 10 mg/mL in 75% MeOH. Use the stock solution to generate a 1000 ug/mL

8 Prepare standard curve:

	A	B	C	D	E	F
	Working solution (ug/mL)	**Working solution vol (uL)**	**Diluent vol (uL)**	**Final Conc. (ug/mL)**	**ng/mL**	**Dilution level**
	1000	1000	0	1000	1E6	
	100	100	900	100	100,000	L1

	A	B	C	D	E	F
	10	100	900	10	10,000	L2
	1	100	900	1	1000	L3
	0.1	100	900	0.1	100	L4
	0.01	100	900	0.01	10	L5
	0.001	100	900	0.001	1	L6

Derivatization reaction

9 Solubilize metabolite extracts in 100 µL of 75% methanol
Add 50 µL of each into a clean new Eppendorf tube

10 Add 50 µL of each standard curve level into a clean new Eppendorf tube

11 To each sample or standard add:
50 µL of 150 mM EDC,
50 µL of 7,5% pyridine,
Incubate in thermomixer at 30 ºC

12 To quench the reaction, add 50 µL of BHT

13 Dilute with 250 µL of water making a total of volume of 500 µL and to achieve 40% methanol at final sample.

14 Samples are ready to be analyzed by LC-MS/MS. Use 40% methanol as blank runs.

LC-MS/MS parameters - Vanquish UPLC

15 Column: Acquity UPLC BEH C-18 1.7 um 2.1 × 150 mm (Waters)

Solvent A: 0.1% FA in water
Solvent B: 0.1% FA in 90% methanol
Flow = 0.250 ml/min.
Gradient:
0-1 min 50%B; 1-5 min 70% B; 5-8 min 98% B; 8-13 98% B; 13-13.1 min 15%B.

Mass spectrometer parameters - Orbitrap ID-X

16 **Full scan parameter**
MS1 Res = 120,000
Range = 67-1000
RF Lens = 60
AGC = 25%
Max IT (ms)= 50
Polarity = Negative

PRM parameters:
CE=30
Res: 50,000
Isolation window: 1.5
AGC: 50
MaxIT (ms): 86
Polarity = Negative

Targeted masses:

Molecule Name	Molecule Formula	Precursor Adduct	Precursor m/z (detected exact mass)	Precursor charge	CE
Iso/valeric acid	C5H10O2	[M-H]	236.0726	-1	30
Iso/caproic acid	C6H12O2	[M-H]	250.0864	-1	30
Propionic acid	C3H6O2	[M-H]	208.0452	-1	30
Lactic acid	C5H10O2	[M-H]	224.0379	-1	30
Iso/butyric acid	C4H8O2	[M-H]	222.0589	-1	30
Acetate	C2H4O2	[M-H]	194.0312	-1	30

Analyze data using Skyline.

Protocol references

Han J, Gagnon S, Eckle T, Borchers CH. Metabolomic analysis of key central carbon metabolism carboxylic acids as their 3-nitrophenylhydrazones by UPLC/ESI-MS. Electrophoresis. 2013 Oct;34(19):2891-900. doi: 10.1002/elps.201200601. Epub 2013 Jul 8. PMID: 23580203; PMCID: PMC4033578.