The conditions of HILIC-HRMS

1. The reagent and instruments used in HILIC-HRMS:

Reagent	Manufacturer	Cat No.	Specifications
water	Fisher Scientific	W6-4	LC-MS/4L
methanol	Fisher Scientific	A456-4	LC-MS/4L
acetonitrile	Fisher Scientific	A955-4	LC-MS/4L
Formic acid	Fisher Scientific	A117-50	LC-MS/50ml
ammonium acetate	Sigma-Aldrich	238074-25G	25g
N_2	Produced by the N2 generator		99%

Instruments	Manufacturer	Cat No.
Rapid Separation LC	ThermoFisher Scientific	Dionex™ UltiMate™ 3000
hybridquadrupole Orbitrap mass spectrometer	ThermoFisher Scientific	Q Exactive™
Vortex mixer	Hangzhou Miou Instrument Co., Ltd.	Mix-3000
Centrifuge	Eppendorf	5180 R
Centrifugalvacuum evaporator	ThermoFisher Scientific	Savant™ SpeedVac™
1.5ml Centrifuge Tubes	Corning	Axygen MCT-150-C
2ml LC Vials	DIKMA	5320
250ul LC Vials Insert	ANPEL	VDAP-4025-6297E-100
UPLC RP Column	Waters	ACQUITY UPLC CSH

		C18(1.7um 2.1mm*100mm)
300ul Pipet-Lite	RAININ	LAB-300
1200ul Pipet-Lite	RAININ	LAB-1200

Thermo Scientific™ Dionex™ UltiMate™ 3000 Rapid Separation LC (RSLC) system performed UHPLC separations with reversed phase C18 column or hydrophilic interaction liquid chromatography column using the gradient conditions shown in Table 1 and Table 2.

2. RP separation for lipid

For C18 separation, mobile phase A was acetonitrile/water (60/40) and mobile phase B was isopropanol/ acetonitrile (90/10); both A and B contained 0.1% formic acid and 10mmol/L ammonium acetate. The column was a HSS T3 column (2.1 x 100 mm, 1.8 µm, waters) operated at 45 °C. The flow rate was 300 µL/min and the injection volume was 1 µL.

Table1: The gradient conditions for reversed phase C18 separation for lipid

Time(min)	A (v %)	B (v %)
0	100	0
2	70	30
9	30	70
11	5	95
12	0	100
14	0	100
14.1	100	0
16	100	0

3. HILIC separation

For HILIC separation, mobile phase A was acetonitrile and mobile

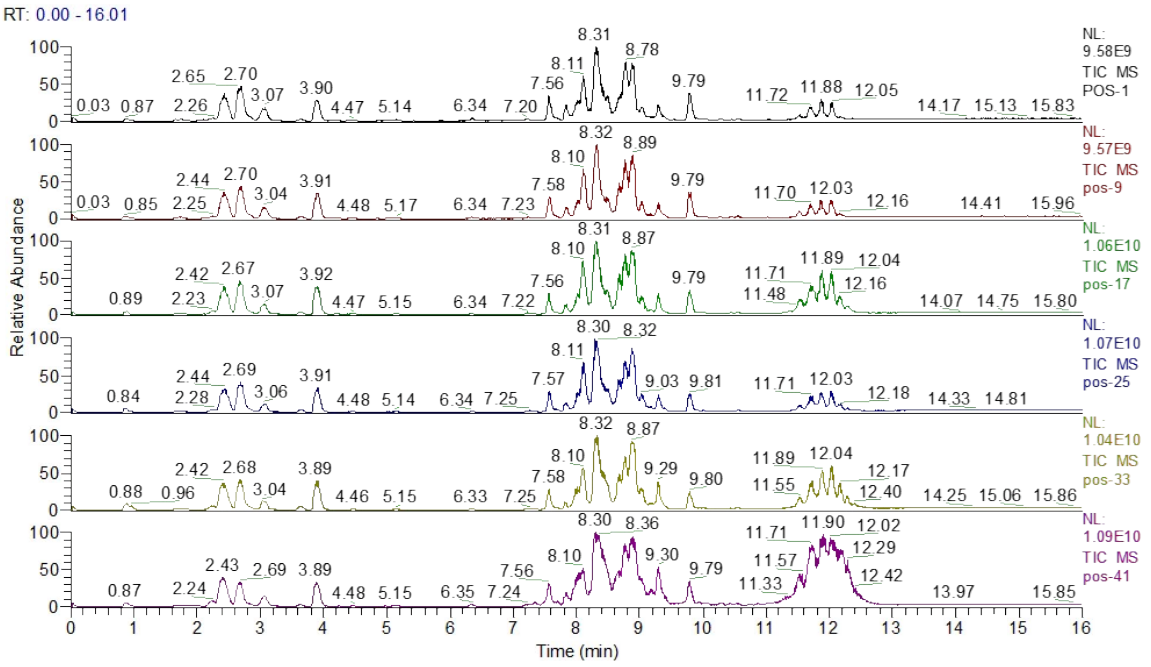
phase B was water; both A and B contained 0.1% formic acid and 10mmol/L ammonium acetate. The column was a BEH Amide column (2.1 x 100 mm, 1.7 μm, waters) operated at 40 °C. The flow rate was 300 μL/min and the injection volume was 1 μL.

Table2: The gradient conditions for HILIC separation of polar metabolites

Time(min)	A (v %)	B (v %)
0	95	5
1	95	5
7	50	50
12	50	50
12.1	95	5

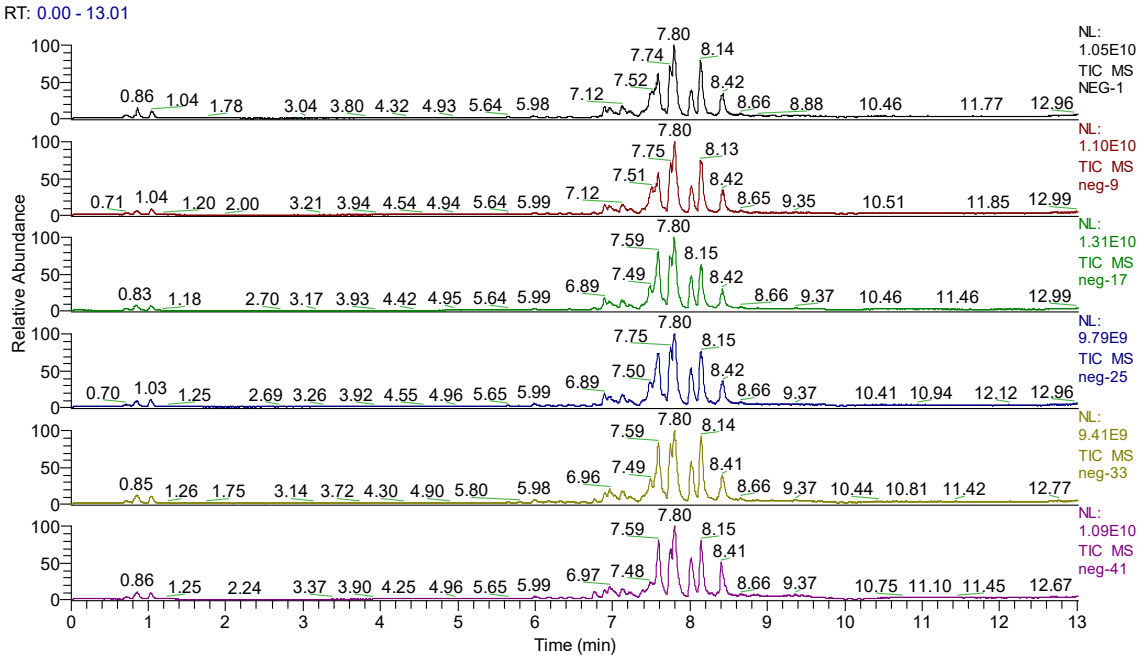
The ion flow chromatogram

1. Reversed phase chromatography positive ion electrospray extracted ion chromatograms



2. Reversed phase chromatography negative ion electrospray

extracted ion chromatograms:



3. HydrophilicM interaction liquid chromatography column (positive mode)