

LCMS Parameters	
LC Model	Thermo Scientific™ Vanquish™ LC Systems
MS Instrument	Thermo Scientific™ Q Exactive™ HF hybrid quadrupole-Orbitrap mass spectrometer
LC Column	Agilent Zorbax Eclipse Plus C18 (2.1 x 100 mm, 1.8 μm)
Guard Column	Agilent Zorbax Eclipse Plus C18 (2.1 x 5 mm, 1.8 μm)
Mobile Phase A	H ₂ O with 0.1 % formic acid
Mobile Phase B	Acetonitrile with 0.1 % formic acid
Ionization Source	Heated ESI
Flow Rate	0.4 mL/min
Injection Volume	1 μL
Capillary Temperature	320 °C
Spray Voltage, PI	3.8 kV
Spray Voltage, NI	3.5 kV
LC Gradient 1	2%(0min)-2%(5min)-35%(20min)- 100%(40min)-100%(50min)-2%(55min)-2%(60min)
LC Gradient 2	0%(0min)-0%(5min)-10%(15min)-100%(25min)-100%(30min)-2%(35min)-2%(40min)
AGC Target (Full MS)	1.00E+06
Max Injection Time	50 msec
Resolution	45000
Scan Range (m/z)	100 -1500

Note: Methanol (mostly), acetonitrile, or acetone was used to prepare LCMS samples depending on the solvent compatibility. All samples were tested in triplicate. Additionally, formic acid (0.1%) was added to the mobile phase as an additive for both positive and negative ion detection modes. All organic solvents and water utilized for sample preparation and mobile phase are of LCMS grade quality. LC gradient 1 is applied to most samples, while LC gradient 2 is specifically employed for the analysis of RM64 and RM96, which are strong hydrophilic analytes with low logP values.