

LC-MS/MS conditions positive and negative mode

LC conditions

Injection volume: 5 μ L

Column: Restek Ultra Aqueous C18, 100 x 2.1 mm ID, 3 μ m particle size

Temperature: 35°C

Flow rate: 0.40 mL/min

Positive mode:

Eluent A: water containing 1% formic acid + 1 mM ammonium formate

Eluent B: methanol/water = 95/5 (V/V) containing 1% formic acid + 1 mM ammonium formate

Negative mode:

Eluent A: water containing 0.1% acetic acid + 5 mM ammonium acetate

Eluent B: methanol/water = 95/5 (V/V) containing 0.1% acetic acid + 5 mM ammonium acetate

Gradient:

| Time (min) | %A | %B |
|------------|-----|-----|
| 0 | 100 | 0 |
| 1 | 100 | 0 |
| 2 | 50 | 50 |
| 3 | 50 | 50 |
| 8 | 0 | 100 |
| 10 | 0 | 100 |
| 10.5 | 100 | 0 |
| 15 | 100 | 0 |

MS conditions positive mode

MS system: 5500 QTrap (AB Sciex)

Generic settings:

| | |
|------------------|----------|
| Scan mode | ESI+ |
| Scan type | MRM |
| Scheduled MRM | Yes |
| Polarity | positive |
| Target scan time | 0.3 sec |
| Resolution Q1 | unit |
| Resolution Q3 | unit |
| MR pause | 5 msec |

| | |
|------------------------|--------|
| Curtain gas (CUR) | 40 psi |
| Collision gas (CAD) | medium |
| Temperature (TEM) | 400 °C |
| Ion Source Gas 1 (GS1) | 50 psi |
| Ion Source Gas 2 (GS2) | 50 psi |
| IonSpray Voltage (IS) | 4000 V |
| Exit Potential (EP) | 10 V |

MS conditions negative mode

MS system: 5500 QTrap (AB Sciex)

Generic settings:

| | |
|---------------|----------|
| Scan mode | ESI- |
| Scan type | MRM |
| Scheduled MRM | No |
| Polarity | negative |
| Dwell time | 5 ms |
| Resolution Q1 | unit |
| Resolution Q3 | unit |
| Settling time | 20 msec |
| MR pause | 5 msec |

| | |
|------------------------|---------|
| Curtain gas (CUR) | 40 psi |
| Collision gas (CAD) | medium |
| Temperature (TEM) | 400 °C |
| Ion Source Gas 1 (GS1) | 50 psi |
| Ion Source Gas 2 (GS2) | 50 psi |
| IonSpray Voltage (IS) | -4000 V |
| Exit Potential (EP) | -10 V |
