Waters Micromass LCT Premier Mass Spectrometer

Specifications

Resolution in Positive Ion (V Mode)

Infuse mellitin at a concentration of 5 pmol/µL in 50:50 acetonitrile:water + 0.2% formic acid, using a flow rate of 5 µL/min. Ensure that the intensity of the peak at 712 Da is <300 counts/s.

The peak at 712 Da should be resolved such that the mass divided by the peak width at half height is greater than 5000 when using a scan time of 1 s and an inter-scan delay of 0.1 s. At least 30 s of data should be summed.

Resolution in Positive Ion (W Mode)

Infuse mellitin at a concentration of 5 pmol/µL in 50:50 acetonitrile:water + 0.2% formic acid, at a flow rate of 5 µL/min. Ensure that the intensity of the peak at 712 Da is <300 counts/s.

The peak at 712 Da should be resolved such that the mass divided by the peak width at half height is greater than 10,000 when using a scan time of 1 s and an inter-scan delay of 0.1 s. At least 30 s of data should be summed.

Resolution in Negative Ion (V Mode)

Infuse raffinose at a concentration of 500 pg/µL in 50:50 acetonitrile:water, at a flow rate of 10 µL/min. Ensure that the intensity of the peak at 503 Da is <300 counts/s.

The peak at 503 Da should be resolved such that the mass divided by the peak width at half height is greater than 5,000 when using a scan time of 1 s and an inter-scan delay of 0.1 s. At least 30 s of data should be summed.

Resolution in Negative Ion (W Mode)

Infuse raffinose at a concentration of 500 pg/µL in 50:50 acetonitrile:water at a flow rate of 10 µL/min. Ensure that the intensity of the peak at 503 Da is < 300 counts/s.

The peak at 503 Da should be resolved such that the mass divided by the peak width at half height is greater than 10,000 when using a scan time of 1 s and an inter-scan delay of 0.1 s. At least 30 s of data should be summed.

Sensitivity in Positive Ion

In V mode, infuse 50 pg/µL of leucine enkephalin in 50:50 acetonitrile:water + 0.1% formic acid at a flow rate of 5 µL/min. Using a scan rate of 1 s, an inter-scan delay of 0.1 s and a mass range of 100 to 1000 Da, check that the resolution at 556 Da is greater than 5000 and the intensity is greater than 440 counts/s.

Switch the instrument to W mode. Check that the resolution is greater than 10,000 and the intensity is greater than 110 counts/s.

Sensitivity in Negative Ion

In V mode, infuse 500 pg/µL of raffinose in 50:50 acetonitrile:water at a flow rate of 10 µL/min. Using a scan rate of 1 s, an inter-scan delay of 0.05 s and a mass range of 100 to 1000 Da, check the resolution at 503 Da is greater than 5000 and the intensity is greater than 240 counts/s.

Switch the instrument to W mode. Check that the resolution is greater than 10,000 and the intensity is greater than 60 counts/s.

Mass Calibration Accuracy

Infuse 50 pg/µL of sodium formate at a flow rate of 5 µL/min into the analyte probe. In V mode, ensure that the intensity is less than 300 counts/s and that the resolution is greater Mass than 5000. Run a calibration using the parameters listed in Table E-1, with no smoothing and no background subtraction, and using a centroid top value of 80%.

Mass Calibration Accuracy Parameters:

Scan Type	Continuum
Scan Range	150-900 Da
Scan Time	5 s
Inter-Scan Delay	0.1 s
Acquisition Time	1 min

Save the calibration and then perform an acquisition for 1 minute using the same parameters. Combine and center the data, using 4 channels and a centroid top value of 80%.Measure at least 6 peaks and calculate the mass error (ppm) for each peak. Using the formula below, calculate the RMS error. The RMS error should be < 5 ppm.

$$\text{RMS error} = \sqrt{\frac{\Sigma(\text{MassError})^2}{n}}$$

Mass Measurement Accuracy

Infuse 50 pg/µL of sodium formate at 5 µL/min into the analyte probe. In W mode, ensure that the intensity is less than 300 counts/s and that the resolution is greater than 10,000. Run a calibration using the parameters listed in Table E-1. Create a calibration with no smoothing and no background subtraction, using a centroid top value of 80%.Save the calibration and then perform the following acquisition.

Infuse 50 pg/µL of leucine enkephalin into the reference probe and 500 pg/µL of raffinose into the ESI probe. Ensure that both probes give an intensity of <200 counts/s and that the resolution is greater than 10,000.

Acquire data in LockSpray mode for 25 minutes, using the following parameters:

Scan Type	Continuum
Scan Range	100-1000 Da
Scan Time	1 s
Inter-Scan Delay	0.1 s
Acquisition Time	25 min
Reference Scan Frequency	5
Reference Cone Voltage	100 V

Combine 12 scans at each of the following times: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 minutes.

Using the Mass Measure feature on each of the spectrums, create a centered spectrum using lockmass 556.2771 Da. Calculate the RMS error using the formula below. The m/z value of raffinose is 527.1588 Da.

The calculated RMS error should be <3 ppm.

$$\text{RMS error} = \sqrt{\frac{\Sigma(\text{MassError})^2}{n}}$$

Chromatographic Signal to Noise

Note: This test should only be performed if there is already a suitable HPLC system installed.

Infuse 50 pg/µL of leucine enkephalin at a flow rate of 5 µL/min. Tune the ion beam in V mode and check that the resolution is greater than 5000.

Purge the HPLC using 75:25 methanol:water and 5 mM ammonium acetate. Connect the 10-µL sample loop, injection port, and waste tube to the Rheodyne injector. Connect a Waters Atlantis dC18 column (2.1 x 30 mm; 3 µM) between the injector and the probe. Condition the column for approximately 20 minutes.

Set up the following sample list acquisition parameters:

Sample List Acquisition Parameters:

Scan Type	Continuum
Scan Range	150-900 Da
Scan Time	2 s
Inter-Scan Delay	0.1 s
Acquisition Time	30 min

Make five injections of 1 pg/µL reserpine in 75:25 methanol: water and 5 mM ammonium acetate, at 5 minute intervals.

Using the 609 Da mass chromatogram, calculate the peak to peak, 1 SD signal to noise ratio for each peak. The average signal-to-noise value should be >100.