

## Method of Q Exactive

### OVERALL METHOD SETTINGS

#### Global Settings

|                          |           |
|--------------------------|-----------|
| Use lock masses          | best      |
| Lock mass injection      | Full MS   |
| Chrom. peak width (FWHM) | 6 s       |
| Time Method duration     | 60.00 min |

#### Customized Tolerances (+/-)

|                   |   |
|-------------------|---|
| Lock Masses       | — |
| Inclusion         | — |
| Exclusion         | — |
| Neutral Loss      | — |
| Mass Tags         | — |
| Dynamic Exclusion | — |

### Experiment

#### FULL MS / DD-MS<sup>2</sup> (TOPN)

##### General

|                      |             |
|----------------------|-------------|
| Runtime              | 0 to 60 min |
| Polarity             | positive    |
| In-source CID        | 0.0 eV      |
| Default charge state | 2           |
| Inclusion            | —           |
| Exclusion            | —           |
| Tags                 | —           |
| Full MS              |             |
| Microscans           | 1           |
| Resolution           | 70,000      |
| AGC target           | 3e6         |

|                             |                         |
|-----------------------------|-------------------------|
| Maximum IT                  | 100 ms                  |
| Number of scan ranges       | 1                       |
| Scan range                  | 400 to 1750 m/z         |
| Spectrum data type          | Profile                 |
| dd-MS <sup>2</sup> / dd-SIM |                         |
| Microscans                  | 1                       |
| Resolution                  | 17,500                  |
| AGC target                  | 1e5                     |
| Maximum IT                  | 100 ms                  |
| Loop count                  | 10                      |
| MSX count                   | 1                       |
| TopN                        | 10                      |
| Isolation window            | 2.0 m/z                 |
| Isolation offset            | 0.0 m/z                 |
| Scan range                  | 200 to 2000 m/z         |
| Fixed first mass            | 50.0 m/z                |
| (N)CE / stepped (N)CE       | nce: 29                 |
| Spectrum data type          | Profile                 |
| dd Settings                 |                         |
| Minimum AGC target          | 1.00e3                  |
| Intensity threshold         | 1.0e4                   |
| Apex trigger                | —                       |
| Charge exclusion            | unassigned, 1, 7, 8, >8 |
| Multiple charge states      | all                     |
| Peptide match               | preferred               |
| Exclude isotopes            | on                      |
| Dynamic exclusion           | 15.0 s                  |
| If idle ..                  | do not pick others      |

## Setup

### TUNEFILES

#### General

Switch Count 0

Base Tunefile C:\Xcalibur\Tune files\ESC-QE-nanotune-180418.mstune

### CONTACT CLOSURE

#### General

Used True

Start in Closed False

Switch Count 0

### SYRINGE

#### General

Used False

Start in OFF True

Stop at end of run False

Switch Count 0

#### Pump setup

Syringe type Hamilton

Flow rate 3.000  $\mu$ L/min

Inner diameter 2.303 mm

Volume 250  $\mu$ L

### DIVERT VALVE A

General Used False

Start in 1-2 True

Switch Count 0

### DIVERT VALVE B

#### General

Used      False

Start in 1-2   True

Switch Count   0

#### LOCK MASSES

| 1 entry | Mass | Polarity | Start | End | Comment | [m/z] | [min] | [min] | 445.12003 | Positive |
|---------|------|----------|-------|-----|---------|-------|-------|-------|-----------|----------|
|---------|------|----------|-------|-----|---------|-------|-------|-------|-----------|----------|

#### Sample pickup:

Volume [μl]      :   18.00

Flow [μl / min]   :   5.00

#### Sample loading:

Volume [μl]      :   20.00

Flow [μl / min]   :   1.00

Max. pressure [Bar] : 400.00

#### Gradient:

| Time [mm:ss] | Duration [mm:ss] | Flow [nl/min] | Mixture [%B] |
|--------------|------------------|---------------|--------------|
| 00:00        | 00:00            | 300           | 0            |
| 35:00        | 35:00            | 300           | 25           |
| 48:00        | 13:00            | 300           | 90           |
| 54:00        | 06:00            | 300           | 90           |
| 60:00        | 06:00            | 300           | 3            |

#### Pre-column equilibration:

Volume [μl]      :   0.00

Flow [μl / min]   :   2.00

Max. pressure [Bar] : 350.00

Analytical column equilibration:

Volume [μl] : 3.00

Flow [μl / min] : 1.00

Max. pressure [Bar] : 400.00

Auto-sampler wash:

Flush volume [μl] : 100.00

=== Tune Data: ===:

Spray Voltage (+): 2200.00

Spray Voltage (-): 1500.00

Capillary Temperature (+ or +-): 305.00

Capillary Temperature (-): 250.00

Sheath Gas (+ or +-): 0.00

Sheath Gas (-): 0.00

Aux Gas (+ or +-): 0.00

Aux Gas (-): 0.00

Spare Gas (+ or +-): 0.00

Spare Gas (-): 0.00

Max Spray Current (+): 50.00

Max Spray Current (-): 50.00

Probe Heater Temp. (+ or +-): 350.00

Probe Heater Temp. (-): 350.00

S-Lens RF Level: 56.00

Ion Source: NSI

=== Calibration Data: ===:

Mass Cal. (+) age (d): 4.90

Mass Cal. (-) age (d): 4.89

Isolation Cal. (+) age (d): 99.1

Isolation Cal. (-) age (d): 99.1

Amplifier Gain: 1000.000

Mass Calibration Parameter (0): 7.90000000e+01  
Mass Calibration Parameter (1): 1.00000000e+02  
Mass Calibration Parameter (2): 1.73279398e-09  
Mass Calibration Parameter (3): 1.59220758e+09  
Mass Calibration Parameter (4): -2.30194649e-12  
Mass Calibration Parameter (5): 1.59220758e+09  
Mass Calibration Parameter (6): -2.66331722e-10  
Mass Calibration Parameter (7): 1.55126609e+09  
Mass Calibration Parameter (8): 4.92801661e-13  
Mass Calibration Parameter (9): 1.55126609e+09  
Mass Calibration Parameter (10): -1.14000000e-06  
Mass Calibration Parameter (11): 0.00000000e+00  
Mass Calibration Parameter (12): -1.14000000e-06  
Mass Calibration Parameter (13): 0.00000000e+00  
Mass Calibration Parameter (14): -1.02106533e-12  
Mass Calibration Parameter (15): 1.59220760e+09  
Mass Calibration Parameter (16): -7.54030880e-13  
Mass Calibration Parameter (17): 1.55126610e+09  
Mass Calibration Parameter (18): -6.40724443e-11  
Mass Calibration Parameter (19): 1.43098704e+09  
Mass Calibration Parameter (20): -3.11636511e-11  
Mass Calibration Parameter (21): 1.39090074e+09  
Mass Calibration Parameter (22): 0.00000000e+00  
Mass Calibration Parameter (23): 0.00000000e+00  
Mass Calibration Parameter (24): 0.00000000e+00  
Mass Calibration Parameter (25): 0.00000000e+00  
Mass Calibration Parameter (26): 0.00000000e+00  
Mass Calibration Parameter (27): 0.00000000e+00  
Mass Calibration Parameter (28): 0.00000000e+00  
Mass Calibration Parameter (29): 0.00000000e+00  
Mass Calibration Parameter (30): 4.20000000e+01

|                                  |                |
|----------------------------------|----------------|
| Mass Calibration Parameter (31): | 1.00000000e+00 |
| Mass Calibration Parameter (32): | 1.00000000e+00 |
| Mass Calibration Parameter (33): | 0.00000000e+00 |
| Mass Calibration Parameter (34): | 2.57694532e+01 |
| Mass Calibration Parameter (35): | 1.17147754e+06 |
| Mass Calibration Parameter (36): | 1.17147754e+05 |
| Mass Calibration Parameter (37): | 1.28000000e+03 |
| Mass Calibration Parameter (38): | 2.56000000e+02 |
| Mass Calibration Parameter (39): | 1.60034644e+09 |
| Mass Calibration Parameter (40): | 6.00000000e+00 |
| Mass Calibration Parameter (41): | 1.38066190e+02 |
| Mass Calibration Parameter (42): | 7.00989448e+02 |
| Mass Calibration Parameter (43): | 1.95087652e+02 |
| Mass Calibration Parameter (44): | 5.89713307e+02 |
| Mass Calibration Parameter (45): | 5.24264964e+02 |
| Mass Calibration Parameter (46): | 3.59733856e+02 |
| Mass Calibration Parameter (47): | 1.22199064e+03 |
| Mass Calibration Parameter (48): | 2.35626093e+02 |
| Mass Calibration Parameter (49): | 1.42197786e+03 |
| Mass Calibration Parameter (50): | 2.18429370e+02 |
| Mass Calibration Parameter (51): | 1.62196509e+03 |
| Mass Calibration Parameter (52): | 2.04520553e+02 |
| Mass Calibration Parameter (53): | 1.00000000e+00 |
| Mass Calibration Parameter (54): | 1.00000000e+00 |
| Mass Calibration Parameter (55): | 1.00000000e+00 |
| Mass Calibration Parameter (56): | 2.57422190e+01 |
| Mass Calibration Parameter (57): | 9.76791655e+05 |
| Mass Calibration Parameter (58): | 9.76791655e+04 |
| Mass Calibration Parameter (59): | 2.40000000e+03 |
| Mass Calibration Parameter (60): | 2.56000000e+02 |
| Mass Calibration Parameter (61): | 1.60034674e+09 |

|                                  |                |
|----------------------------------|----------------|
| Mass Calibration Parameter (62): | 8.00000000e+00 |
| Mass Calibration Parameter (63): | 2.65147903e+02 |
| Mass Calibration Parameter (64): | 5.05903486e+02 |
| Mass Calibration Parameter (65): | 5.14284397e+02 |
| Mass Calibration Parameter (66): | 3.63252270e+02 |
| Mass Calibration Parameter (67): | 1.27999721e+03 |
| Mass Calibration Parameter (68): | 2.30252154e+02 |
| Mass Calibration Parameter (69): | 1.37999083e+03 |
| Mass Calibration Parameter (70): | 2.21753275e+02 |
| Mass Calibration Parameter (71): | 1.47998444e+03 |
| Mass Calibration Parameter (72): | 2.14131002e+02 |
| Mass Calibration Parameter (73): | 1.57997805e+03 |
| Mass Calibration Parameter (74): | 2.07244311e+02 |
| Mass Calibration Parameter (75): | 1.67997166e+03 |
| Mass Calibration Parameter (76): | 2.00982013e+02 |
| Mass Calibration Parameter (77): | 1.77996528e+03 |
| Mass Calibration Parameter (78): | 1.95255127e+02 |
| Mass Calibration Parameter (79): | 0.00000000e+00 |
| Mass Calibration Parameter (80): | 0.00000000e+00 |
| Mass Calibration Parameter (81): | 0.00000000e+00 |
| Mass Calibration Parameter (82): | 0.00000000e+00 |
| Mass Calibration Parameter (83): | 0.00000000e+00 |
| Mass Calibration Parameter (84): | 0.00000000e+00 |
| Mass Calibration Parameter (85): | 0.00000000e+00 |
| Mass Calibration Parameter (86): | 0.00000000e+00 |
| Mass Calibration Parameter (87): | 0.00000000e+00 |
| Mass Calibration Parameter (88): | 0.00000000e+00 |
| Mass Calibration Parameter (89): | 0.00000000e+00 |
| Mass Calibration Parameter (90): | 0.00000000e+00 |
| Mass Calibration Parameter (91): | 0.00000000e+00 |
| Mass Calibration Parameter (92): | 0.00000000e+00 |



|                                   |                |
|-----------------------------------|----------------|
| Mass Calibration Parameter (93):  | 0.00000000e+00 |
| Mass Calibration Parameter (94):  | 0.00000000e+00 |
| Mass Calibration Parameter (95):  | 0.00000000e+00 |
| Mass Calibration Parameter (96):  | 0.00000000e+00 |
| Mass Calibration Parameter (97):  | 0.00000000e+00 |
| Mass Calibration Parameter (98):  | 0.00000000e+00 |
| Mass Calibration Parameter (99):  | 0.00000000e+00 |
| Mass Calibration Parameter (100): | 0.00000000e+00 |
| Mass Calibration Parameter (101): | 0.00000000e+00 |
| Mass Calibration Parameter (102): | 0.00000000e+00 |
| Mass Calibration Parameter (103): | 0.00000000e+00 |
| Mass Calibration Parameter (104): | 0.00000000e+00 |
| Mass Calibration Parameter (105): | 0.00000000e+00 |
| Mass Calibration Parameter (106): | 0.00000000e+00 |
| Mass Calibration Parameter (107): | 0.00000000e+00 |
| Mass Calibration Parameter (108): | 0.00000000e+00 |
| Mass Calibration Parameter (109): | 0.00000000e+00 |
| Mass Calibration Parameter (110): | 0.00000000e+00 |
| Mass Calibration Parameter (111): | 0.00000000e+00 |
| Mass Calibration Parameter (112): | 0.00000000e+00 |
| Mass Calibration Parameter (113): | 0.00000000e+00 |
| Mass Calibration Parameter (114): | 0.00000000e+00 |
| Mass Calibration Parameter (115): | 0.00000000e+00 |
| Mass Calibration Parameter (116): | 0.00000000e+00 |
| Mass Calibration Parameter (117): | 0.00000000e+00 |
| Mass Calibration Parameter (118): | 0.00000000e+00 |
| Mass Calibration Parameter (119): | 0.00000000e+00 |
| CTCD Scale (+):                   | 2.035          |
| CTCD Scale (-):                   | 3.884          |
| CTCD Para (+):                    | 0.800          |
| CTCD Para (-):                    | 0.500          |

Detect Delay: 5.50  
Relais Delay: 5.00  
Res.-Dep. Delay: 10.00  
Quad DC (+,0): 3.600  
Quad DC (+,1): 0.000  
Quad DC (-,0): -4.772  
Quad DC (-,1): 0.000  
Quad OTK (+,0): -24.983  
Quad OTK (+,1): -0.056  
Quad OTK (-,0): 13.110  
Quad OTK (-,1): 0.077  
Quad Para A: 0.000  
Quad Para B: 0.000  
Quad Para C: 0.000  
CLT GND Voltage (+): 21.60  
CLT GND Voltage (-): 14.20  
CLT Offset Voltage (+): 1650.0  
CLT Offset Voltage (-): 1650.0  
CLT Push Voltage (+): 110.0  
CLT Push Voltage (-): 110.0  
CLT Pull Voltage (+): 280.0  
CLT Pull Voltage (-): 280.0  
Lens 6 Voltage (+): 670.0  
Lens 6 Voltage (-): 670.0  
Z-Lens 3 Voltage (+): 230.0  
Z-Lens 3 Voltage (-): 230.0  
De-Inject Voltage (+): 40.0  
De-Inject Voltage (-): 40.0  
De-Measure Voltage (+): 470.0  
De-Measure Voltage (-): 470.0  
CE-Inject Voltage (+): 3800.0

CE-Inject Voltage (-): 3800.0

LowSNQuanLevel: 1

=== Configuration Data: ===:

Preamplifier Protect Mode: 0

TMP Sweep Mode: 1

Temp Sensor Mode: 0

=== Identification: ===:

SW Version: 2.9-290033/2.9.0.2926