

Thermo Scientific SII for Xcalibur Method

---- Overview ----

Name: New Instrument Method

Comment:

Run time: 120.000 [min]

Instrument: Ultimate-3000-Nano on 6bfcy23

Description:

---- Script ----

```
initial      Instrument Setup
              Sampler.LowDispersionMode: Off
              Sampler.WashSpeed: 4.000 [µl/s]
              Sampler.WashVolume: 50.000 [µl]
              Sampler.PunctureDepth: 8.000 [mm]
              Sampler.SampleHeight: 2.000 [mm]
              Sampler.WasteSpeed: 4.000 [µl/s]
              Sampler.DispenseDelay: 2.000 [s]
              Sampler.DispSpeed: 2.000 [µl/s]
              Sampler.DrawSpeed: 0.050 [µl/s]
              Sampler.DrawDelay: 5.000 [s]
              Sampler.RinseBetweenReinjections: Yes
              Sampler.FlushVolume: 5.000 [µl]
              Sampler.TransVialPunctureDepth: 8.000 [mm]
              Sampler.TransLiquidHeight: 2.000 [mm]
              Sampler.TransportVialCapacity: 6000
              Sampler.LastTransportVial: R1
              Sampler.FirstTransportVial: R1
              Sampler.InjectMode: ulPickUp
              Sampler.LoopWashFactor: 2.000
              Sampler.PumpDevice: "LoadingPump"
              Sampler.TempCtrl: On
              Sampler.Temperature.Nominal: 5.0 [°C]
              Sampler.ReadyTempDelta: None
              Sampler.Temperature.LowerLimit: 4.0 [°C]
              Sampler.Temperature.UpperLimit: 45.0 [°C]
              PumpModule.LoadingPump.%A.Equate: "0.1%FA"
              PumpModule.LoadingPump.%B.Equate: "50%MEOH"
              PumpModule.LoadingPump.%C.Equate: "0.1FA"
              PumpModule.LoadingPump.Pressure.LowerLimit: 0 [bar]
              PumpModule.LoadingPump.Pressure.UpperLimit: 200 [bar]
              PumpModule.LoadingPump.MaximumFlowRampUp: 31 [µl/min²]
              PumpModule.LoadingPump.MaximumFlowRampDown: 31 [µl/min²]
              PumpModule.NC_Pump.%A.Equate: "0.1%FA"
              PumpModule.NC_Pump.%B.Equate: "100%ACN/0.1%FA"
              PumpModule.NC_Pump.Pressure.LowerLimit: 10 [bar]
              PumpModule.NC_Pump.Pressure.UpperLimit: 900 [bar]
              PumpModule.NC_Pump.MaximumFlowRampUp: 0.300 [µl/min²]
              PumpModule.NC_Pump.MaximumFlowRampDown: 0.300 [µl/min²]
              ColumnOven.TempCtrl: On
              ColumnOven.Temperature.Nominal: 40.0 [°C]
```

# Instrument Method: uPU-90mingradTOP20-highload\_Luciano

Thermo Scientific SII for Xcalibur Method

```

ColumnOven.Temperature.LowerLimit: 25.0 [°C]
ColumnOven.Temperature.UpperLimit: 75.0 [°C]
ColumnOven.EquilibrationTime: 0.5 [min]
ColumnOven.ReadyTempDelta: 0.5 [°C]
ColumnOven.ValveLeft: 1_2
0.000 [min] Inject Preparation
Wait Sampler.Ready And PumpModule.LoadingPump.Ready And PumpModule.NC_Pump.Ready And Colu
0.000 [min] Inject
Sampler.Inject
0.000 [min] Start Run
PumpModule.LoadingPump.LoadingPump_Pressure.AcqOn
PumpModule.NC_Pump.NC_Pump_Pressure.AcqOn
PumpModule.NC_Pump.NC_Pump_Flow.AcqOn
ColumnOven.ColumnOven_Temp.AcqOn
PumpModule.NC_Pump.NC_Pump_Flow_LeftBlk.AcqOn
PumpModule.NC_Pump.NC_Pump_Flow_RightBlk.AcqOn
0.000 [min] Run
PumpModule.LoadingPump.Flow.Nominal: 20.000 [µl/min]
PumpModule.LoadingPump.%B.Value: 0.0 [%]
PumpModule.LoadingPump.%C.Value: 0.0 [%]
PumpModule.LoadingPump.Curve: 5
PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
PumpModule.NC_Pump.%B.Value: 3.0 [%]
PumpModule.NC_Pump.Curve: 5
3.000 [min]
PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
PumpModule.NC_Pump.%B.Value: 3.0 [%]
PumpModule.NC_Pump.Curve: 5
ColumnOven.ValveLeft: 10_1
90.000 [min]
PumpModule.LoadingPump.Flow.Nominal: 20.000 [µl/min]
PumpModule.LoadingPump.%B.Value: 0.0 [%]
PumpModule.LoadingPump.%C.Value: 0.0 [%]
PumpModule.LoadingPump.Curve: 5
PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
PumpModule.NC_Pump.%B.Value: 45.0 [%]
PumpModule.NC_Pump.Curve: 5
91.000 [min]
PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
PumpModule.NC_Pump.%B.Value: 80.0 [%]
PumpModule.NC_Pump.Curve: 5
105.000 [min]
PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
PumpModule.NC_Pump.%B.Value: 80.0 [%]
PumpModule.NC_Pump.Curve: 5
106.000 [min]
PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
PumpModule.NC_Pump.%B.Value: 3.0 [%]

```

Thermo Scientific SII for Xcalibur Method

```
PumpModule.NC_Pump.Curve: 5
110.000 [min]
ColumnOven.ValveLeft: 1_2
120.000 [min]
PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
PumpModule.NC_Pump.%B.Value: 3.0 [%]
PumpModule.NC_Pump.Curve: 5
120.000 [min] Stop Run
PumpModule.LoadingPump.LoadingPump_Pressure.AcqOff
PumpModule.NC_Pump.NC_Pump_Pressure.AcqOff
PumpModule.NC_Pump.NC_Pump_Flow.AcqOff
ColumnOven.ColumnOven_Temp.AcqOff
PumpModule.NC_Pump.NC_Pump_Flow_LeftBlk.AcqOff
PumpModule.NC_Pump.NC_Pump_Flow_RightBlk.AcqOff
```

## Method Summary

### Method Settings

Application Mode: **Peptide**  
Method Duration (min): **120**

### Global Parameters

#### Ion Source

Use Ion Source Settings from Tune: **True**  
FAIMS Mode: **Not Installed**

#### MS Global Settings

Infusion Mode: **Liquid Chromatography**  
Expected LC Peak Width (s): **20**  
Advanced Peak Determination: **True**  
Default Charge State: **2**  
Internal Mass Calibration: **Off**

### Experiment#1 [ID high load (more than 500 ng)]

Start Time (min): **0**  
End Time (min): **120**

#### Master Scan:

#### Full Scan

Orbitrap Resolution: **120000**  
Scan Range (m/z): **200-2000**  
RF Lens (%): **40**  
AGC Target: **Custom**  
Normalized AGC Target (%): **300**  
Maximum Injection Time Mode: **Auto**  
Microscans: **1**  
Data Type: **Profile**  
Polarity: **Positive**  
Source Fragmentation: **Disabled**  
Scan Description:

## Filters:

### MIPS

Monoisotopic peak determination: **Peptide**

Relax restrictions when too few precursors are found: **True**

### Intensity

Filter Type: **Intensity Threshold**

Intensity Threshold: **5.0e3**

### Charge State

Include charge state(s): **2-6**

Include undetermined charge states: **False**

### Dynamic Exclusion

Dynamic Exclusion Mode: **Custom**

Exclude after n times: **1**

Exclusion duration (s): **20**

Mass Tolerance: **ppm**

Low: **10**

High: **10**

Exclude isotopes: **True**

Perform dependent scan on single charge state per precursor only: **False**

### Data Dependent

Data Dependent Mode: **Number of Scans**

Number of Dependent Scans: **25**

### Scan Event Type 1:

#### Scan:

### ddMS<sup>2</sup>

Multiplex Ions: **False**

Isolation Window (m/z): **1.4**

Isolation Offset: **Off**

Collision Energy Mode: **Fixed**

Collision Energy Type: **Normalized**

HCD Collision Energy (%): **30**

Orbitrap Resolution: **15000**  
TurboTMT: **Off**  
Scan Range Mode: **Define First Mass**  
First Mass (m/z): **110**  
AGC Target: **Standard**  
Maximum Injection Time Mode: **Custom**  
Maximum Injection Time (ms): **50**  
Microscans: **1**  
Data Type: **Centroid**  
Scan Description: