

Thermo Scientific SII for Xcalibur Method

---- Overview ----

Name: New Instrument Method

Comment:

Run time: 120.000 [min]

Instrument: W120-OE4_1 on w120-oe4

Description:

---- Script ----

```
initial      Instrument Setup
              Sampler.LowDispersionMode: Off
              Sampler.WashSpeed: 4.000 [µl/s]
              Sampler.WashVolume: 100.000 [µl]
              Sampler.PunctureDepth: 8.000 [mm]
              Sampler.SampleHeight: 0.000 [mm]
              Sampler.WasteSpeed: 4.000 [µl/s]
              Sampler.DispenseDelay: 2.000 [s]
              Sampler.DispSpeed: 2.000 [µl/s]
              Sampler.DrawSpeed: 0.200 [µl/s]
              Sampler.DrawDelay: 5.000 [s]
              Sampler.RinseBetweenReinjections: Yes
              Sampler.FlushVolume: 5.000 [µl]
              Sampler.TransVialPunctureDepth: 8.000 [mm]
              Sampler.TransLiquidHeight: 5.000 [mm]
              Sampler.TransportVialCapacity: 99999
              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: 3.0 [°C]
              Sampler.Temperature.LowerLimit: 4.0 [°C]
              Sampler.Temperature.UpperLimit: 45.0 [°C]
              PumpModule.LoadingPump.%A.Equate: "%A"
              PumpModule.LoadingPump.%B.Equate: "%B"
              PumpModule.LoadingPump.%C.Equate: "%C"
              PumpModule.LoadingPump.Pressure.LowerLimit: 0 [bar]
              PumpModule.LoadingPump.Pressure.UpperLimit: 500 [bar]
              PumpModule.LoadingPump.MaximumFlowRampUp: 31 [µl/min²]
              PumpModule.LoadingPump.MaximumFlowRampDown: 31 [µl/min²]
              PumpModule.NC_Pump.%A.Equate: "%A"
              PumpModule.NC_Pump.%B.Equate: "%B"
              PumpModule.NC_Pump.Pressure.LowerLimit: 0 [bar]
              PumpModule.NC_Pump.Pressure.UpperLimit: 800 [bar]
              PumpModule.NC_Pump.MaximumFlowRampUp: 0.300 [µl/min²]
              PumpModule.NC_Pump.MaximumFlowRampDown: 0.300 [µl/min²]
              ColumnOven.TempCtrl: On
              ColumnOven.Temperature.Nominal: 35.0 [°C]
```

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```

ColumnOven.Temperature.LowerLimit: 25.0 [°C]
ColumnOven.Temperature.UpperLimit: 75.0 [°C]
ColumnOven.EquilibrationTime: 0.5 [min]
ColumnOven.ReadyTempDelta: 2.0 [°C]
ColumnOven.ValveRight: 1_2
0.000 [min] Equilibration
PumpModule.LoadingPump.Flow.Nominal: 30.000 [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: 2.0 [%]
PumpModule.NC_Pump.Curve: 5
0.000 [min] Inject Preparation
Wait Sampler.Ready And PumpModule.LoadingPump.Ready And PumpModule.NC_Pump.Ready And ColumnOven.Ready
0.000 [min] Inject
Sampler.Inject
0.000 [min] Start Run
ColumnOven.ColumnOven_Temp.AcqOn
PumpModule.LoadingPump.LoadingPump_Pressure.AcqOn
PumpModule.NC_Pump.NC_Pump_Flow.AcqOn
PumpModule.NC_Pump.NC_Pump_Flow_LeftBlk.AcqOn
PumpModule.NC_Pump.NC_Pump_Flow_RightBlk.AcqOn
PumpModule.NC_Pump.NC_Pump_Pressure.AcqOn
0.000 [min] Run
PumpModule.LoadingPump.Flow.Nominal: 30.000 [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: 2.0 [%]
PumpModule.NC_Pump.Curve: 5
3.000 [min]
PumpModule.LoadingPump.Flow.Nominal: 30.000 [µl/min]
PumpModule.LoadingPump.%B.Value: 0.0 [%]
PumpModule.LoadingPump.%C.Value: 0.0 [%]
PumpModule.LoadingPump.Curve: 5
ColumnOven.ValveRight: 10_1
PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
PumpModule.NC_Pump.%B.Value: 2.0 [%]
PumpModule.NC_Pump.Curve: 5
4.000 [min]
PumpModule.LoadingPump.Flow.Nominal: 5.000 [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: 4.0 [%]

```

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```
PumpModule.NC_Pump.Curve: 5
106.000 [min]
PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
PumpModule.NC_Pump.%B.Value: 30.0 [%]
PumpModule.NC_Pump.Curve: 5
107.000 [min]
PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
PumpModule.NC_Pump.%B.Value: 78.0 [%]
PumpModule.NC_Pump.Curve: 5
109.000 [min]
PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
PumpModule.NC_Pump.%B.Value: 78.0 [%]
PumpModule.NC_Pump.Curve: 5
110.000 [min]
PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
PumpModule.NC_Pump.%B.Value: 2.0 [%]
PumpModule.NC_Pump.Curve: 5
112.000 [min]
PumpModule.LoadingPump.Flow.Nominal: 5.000 [µl/min]
PumpModule.LoadingPump.%B.Value: 0.0 [%]
PumpModule.LoadingPump.%C.Value: 0.0 [%]
PumpModule.LoadingPump.Curve: 5
113.000 [min]
PumpModule.LoadingPump.Flow.Nominal: 30.000 [µl/min]
PumpModule.LoadingPump.%B.Value: 0.0 [%]
PumpModule.LoadingPump.%C.Value: 0.0 [%]
PumpModule.LoadingPump.Curve: 5
114.000 [min]
ColumnOven.ValveRight: 1_2
120.000 [min] Stop Run
ColumnOven.ColumnOven_Temp.AcqOff
PumpModule.LoadingPump.LoadingPump_Pressure.AcqOff
PumpModule.NC_Pump.NC_Pump_Flow.AcqOff
PumpModule.NC_Pump.NC_Pump_Flow_LeftBlk.AcqOff
PumpModule.NC_Pump.NC_Pump_Flow_RightBlk.AcqOff
PumpModule.NC_Pump.NC_Pump_Pressure.AcqOff
```

Method Summary

Method Settings

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

Global Parameters

Ion Source

Ion Source Type: **NSI**
Spray Voltage: **Static**
Positive Ion (V): **2200**
Negative Ion (V): **600**
Ion Transfer Tube Temp (°C): **275**
Use Ion Source Settings from Tune: **False**
FAIMS Mode: **Not Installed**

MS Global Settings

Infusion Mode: **Liquid Chromatography**
Expected LC Peak Width (s): **30**
Advanced Peak Determination: **True**
Default Charge State: **1**
Enable Xcalibur AcquireX Ab method modifications: **False**
Internal Mass Calibration: **User-defined Lock Mass**
Mode: **Scan-to-Scan**
Mass Tolerance (ppm): **15**
Lock Mass Injection: **False**
Current Lock Mass: **Current**

m/z	Polarity
445.12003	Positive

Experiment #1 [MS]

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

Master Scan:

Full Scan

Orbitrap Resolution: **60000**
Scan Range (m/z): **380-1400**
RF Lens (%): **40**
AGC Target: **Custom**
Normalized AGC Target (%): **300**
Maximum Injection Time Mode: **Custom**
Maximum Injection Time (ms): **45**
Microscans: **1**
Data Type: **Profile**
Polarity: **Positive**
Source Fragmentation: **Disabled**
Scan Description:

Filters:

Intensity

Filter Type: **Intensity Threshold**
Intensity Threshold: **1.0e5**

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): **30**
Mass Tolerance: **ppm**
Low: **10**
High: **10**
Exclude isotopes: **True**
Perform dependent scan on single charge state per precursor only: **True**

MIPS

Monoisotopic peak determination: **Peptide**
Relax restrictions when too few precursors are found: **True**

Data Dependent

Data Dependent Mode: **Cycle Time**

Time between Master Scans (sec): 2

Scan Event Type 1:

Scan:

ddMS²

Multiplex Ions: **False**

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

Isolation Offset: **Off**

Collision Energy Type: **Normalized**

HCD Collision Energies (%): **26**

Orbitrap Resolution: **15000**

TurboTMT: **Off**

Scan Range Mode: **Auto**

AGC Target: **Custom**

Normalized AGC Target (%): **100**

Maximum Injection Time Mode: **Custom**

Maximum Injection Time (ms): **22**

Microscans: **1**

Data Type: **Centroid**

Scan Description: