

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 [ul/s]
              Sampler.WashVolume: 100.000 [ul]
              Sampler.PunctureDepth: 8.000 [mm]
              Sampler.SampleHeight: 0.000 [mm]
              Sampler.WasteSpeed: 4.000 [ul/s]
              Sampler.DispenseDelay: 2.000 [s]
              Sampler.DispSpeed: 2.000 [ul/s]
              Sampler.DrawSpeed: 0.200 [ul/s]
              Sampler.DrawDelay: 5.000 [s]
              Sampler.RinseBetweenReinjections: Yes
              Sampler.FlushVolume: 5.000 [ul]
              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 [ul/min²]
              PumpModule.LoadingPump.MaximumFlowRampDown: 31 [ul/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 [ul/min²]
              PumpModule.NC_Pump.MaximumFlowRampDown: 0.300 [ul/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: 76.0 [%]
PumpModule.NC_Pump.Curve: 5
109.000 [min]
PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
PumpModule.NC_Pump.%B.Value: 76.0 [%]
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: **False**  
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: **120000**  
Scan Range (m/z): **350-1400**  
RF Lens (%): **40**  
AGC Target: **Custom**  
Normalized AGC Target (%): **300**  
Maximum Injection Time Mode: **Custom**  
Maximum Injection Time (ms): **45**  
Microscans: **1**  
Data Type: **Centroid**  
Polarity: **Positive**  
Source Fragmentation: **Disabled**  
Scan Description:

## Experiment #2 [tMS2]

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

## Master Scan:

### tMS<sup>2</sup>

Multiplex Ions: **False**  
Isolation Offset: **Off**  
Collision Energy Type: **Normalized**  
HCD Collision Energies (%): **28**  
Orbitrap Resolution: **30000**  
TurboTMT: **Off**  
Scan Range Mode: **Auto**  
RF Lens (%): **40**  
AGC Target: **Custom**  
Normalized AGC Target (%): **1000**  
Maximum Injection Time Mode: **Custom**  
Maximum Injection Time (ms): **54**  
Microscans: **1**  
Data Type: **Centroid**  
Polarity: **Positive**  
Source Fragmentation: **Disabled**  
Loop Control: **All**  
Dynamic RT: **Off**  
Time Mode: **Unscheduled**  
Scan Description:

## Mass List Table

Mass List Table					
Compound	Formula	Adduct	m/z	z	Isolation Window (m/z)
		(no adduct)	406.9348	2	14
		(no adduct)	419.9408	2	14
		(no adduct)	432.4465	2	13
		(no adduct)	444.4519	2	13
		(no adduct)	455.9572	2	12
		(no adduct)	466.9621	2	12
		(no adduct)	477.9671	2	12
		(no adduct)	488.4719	2	11
		(no adduct)	498.4764	2	11
		(no adduct)	508.9813	2	12
		(no adduct)	519.486	2	11
		(no adduct)	529.4906	2	11
		(no adduct)	538.9949	2	10
		(no adduct)	548.4992	2	11
		(no adduct)	558.5038	2	11
		(no adduct)	568.5083	2	11
		(no adduct)	578.0126	2	10
		(no adduct)	587.517	2	11
		(no adduct)	597.5215	2	11
		(no adduct)	607.526	2	11
		(no adduct)	617.5306	2	11
		(no adduct)	627.5352	2	11
		(no adduct)	637.5397	2	11
		(no adduct)	647.5442	2	11
		(no adduct)	657.5488	2	11
		(no adduct)	668.0535	2	12

		(no adduct)	678.5584	2	11
		(no adduct)	689.0631	2	12
		(no adduct)	700.0681	2	12
		(no adduct)	711.0731	2	12
		(no adduct)	722.0781	2	12
		(no adduct)	734.0836	2	14
		(no adduct)	746.5892	2	13
		(no adduct)	759.095	2	14
		(no adduct)	771.6006	2	13
		(no adduct)	784.6065	2	15
		(no adduct)	798.6129	2	15
		(no adduct)	812.6193	2	15
		(no adduct)	827.1259	2	16
		(no adduct)	842.6329	2	17
		(no adduct)	859.1404	2	18
		(no adduct)	876.6484	2	19
		(no adduct)	895.657	2	21
		(no adduct)	915.6661	2	21
		(no adduct)	937.1759	2	24
		(no adduct)	960.6865	2	25
		(no adduct)	986.6984	2	29
		(no adduct)	986.6984	2	29