

Thermo Scientific SII for Xcalibur Method

---- Overview ----

Name: New Instrument Method

Comment:

Run time: 141.000 [min]

Instrument: NanoLC on b200-pc102

Description:

---- Script ----

```
initial      Instrument Setup
              ColumnOven.TempCtrl: On
              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.Temperature.Nominal: 35.0 [°C]
```

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```

ColumnOven.ValveLeft: 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.ValveLeft: 10_1
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.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
PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
PumpModule.NC_Pump.%B.Value: 2.0 [%]
PumpModule.NC_Pump.Curve: 5
ColumnOven.ValveLeft: 10_1
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: 7.0 [%]
PumpModule.NC_Pump.Curve: 5
124.000 [min]
PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
PumpModule.NC_Pump.%B.Value: 38.0 [%]
PumpModule.NC_Pump.Curve: 5
PumpModule.LoadingPump.Flow.Nominal: 5.000 [µl/min]
PumpModule.LoadingPump.%B.Value: 0.0 [%]
PumpModule.LoadingPump.%C.Value: 0.0 [%]

```

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```
PumpModule.LoadingPump.Curve: 5
125.000 [min]
PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
PumpModule.NC_Pump.%B.Value: 98.0 [%]
PumpModule.NC_Pump.Curve: 5
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.ValveLeft: 1_2
130.000 [min]
PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
PumpModule.NC_Pump.%B.Value: 98.0 [%]
PumpModule.NC_Pump.Curve: 5
131.000 [min]
PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
PumpModule.NC_Pump.%B.Value: 2.0 [%]
PumpModule.NC_Pump.Curve: 5
141.000 [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): **141**

### 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): **30**  
Advanced Peak Determination: **True**  
Default Charge State: **2**  
Internal Mass Calibration: **Off**

### Experiment#1 [Orbitrap HCD - High Load (greater than 500 ng)]

Start Time (min): **0**  
End Time (min): **141**

#### Master Scan:

#### MS OT

Detector Type: **Orbitrap**  
Orbitrap Resolution: **120000**  
Mass Range: **Normal**  
Use Quadrupole Isolation: **True**  
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: **Profile**  
Polarity: **Positive**  
Source Fragmentation: **Disabled**  
Scan Description:

### Experiment#2 [tMSn]

Start Time (min): **0**  
End Time (min): **141**

#### Master Scan:

#### tMS<sup>2</sup> OT HCD

MS<sup>n</sup> Level (n): **2**  
Multiplex Ions: **False**  
Isolation Mode: **Quadrupole**  
Activation Type: **HCD**  
Collision Energy Mode: **Fixed**  
HCD Collision Energy Type: **Normalized**  
HCD Collision Energy (%): **28**  
Detector Type: **Orbitrap**  
Orbitrap Resolution: **30000**  
TurboTMT: **Off**  
Mass Range: **Normal**  
Scan Range Mode: **Auto**  
RF Lens (%): **40**  
AGC Target: **Custom**  
Maximum Injection Time Mode: **Custom**  
Maximum Injection Time (ms): **54**  
Microscans: **1**  
Data Type: **Profile**  
Polarity: **Positive**  
Source Fragmentation: **Disabled**  
Use EASY-IC™: **False**  
Loop Control: **All**  
Dynamic Retention Time: **Off**  
Scan Description:  
Time Mode: **Unscheduled**

#### Mass List Table

Mass List Table						
Compound	Formula	Adduct	m/z	z	Isolation Window (m/	Normalized AGC Target

					z)	(%)
			405.9344	2	12	1000
			417.4396	2	13	1000
			428.4446	2	11	1000
			438.4492	2	11	1000
			448.4537	2	11	1000
			458.4583	2	11	1000
			467.9626	2	10	1000
			476.9667	2	10	1000
			485.9708	2	10	1000
			494.9749	2	10	1000
			503.979	2	10	1000
			512.4829	2	9	1000
			520.9867	2	10	1000
			529.4906	2	9	1000
			537.9944	2	10	1000
			546.4983	2	9	1000
			554.502	2	9	1000
			563.0058	2	10	1000
			571.5096	2	9	1000
			579.5133	2	9	1000
			588.0172	2	10	1000
			596.521	2	9	1000
			604.5247	2	9	1000
			613.0286	2	10	1000
			622.0326	2	10	1000
			630.5365	2	9	1000
			639.0404	2	10	1000
			647.5442	2	9	1000
			656.0481	2	10	1000
			665.0521	2	10	1000

			674.0563	2	10	1000
			683.0604	2	10	1000
			692.0645	2	10	1000
			701.0686	2	10	1000
			710.5729	2	11	1000
			720.5774	2	11	1000
			730.582	2	11	1000
			740.5866	2	11	1000
			751.0913	2	12	1000
			762.0963	2	12	1000
			773.1013	2	12	1001
			784.1063	2	12	1002
			795.6116	2	13	1000
			808.1172	2	14	1000
			820.6229	2	13	1000
			833.1285	2	14	1000
			846.6347	2	15	1000
			860.6411	2	15	1000
			875.648	2	17	1000
			891.6552	2	17	1000
			908.6629	2	19	1000
			926.6711	2	19	1000
			945.1795	2	20	1000
			965.6888	2	23	1000
			988.6993	2	25	1000