

Instrument Method: DIA_10-05-2022A

Thermo Scientific SII for Xcalibur Method

---- Overview ----

Date listed as part of the instrument method name may differ if additional drugs are added to method. The main instrument parameters (LC flow parameters, source parameters, etc) will not change.

Name: New Instrument Method

Comment:

Run time: 11.800 [min]

Instrument: Ultimate_3000_04072021 on f19rt72w10

Description:

---- Script ----

```

initial      Instrument Setup
              ColumnOven.TempCtrl: On
              ColumnOven.Temperature.Nominal: 40.0 [°C]
              ColumnOven.EquilibrationTime: 0.5 [min]
              ColumnOven.ReadyTempDelta: 2.0 [°C]
              Sampler.InjectWash: Both
              Sampler.WashSpeed: 8.000 [µl/s]
              Sampler.WashVolume: 80.000 [µl]
              Sampler.SampleHeight: 2.000 [mm]
              Sampler.WasteSpeed: 8.333 [µl/s]
              Sampler.DispenseDelay: 0.000 [s]
              Sampler.DispSpeed: 8.333 [µl/s]
              Sampler.DrawSpeed: 0.330 [µl/s]
              Sampler.DrawDelay: 1.000 [s]
              Sampler.InjectMode: Normal
              Sampler.PumpDevice: "Pump"
              Sampler.LoopWashFactor: 2.000
              Sampler.TempCtrl: On
              Sampler.Temperature.Nominal: 15.0 [°C]
              Sampler.ReadyTempDelta: 2.0 [°C]
              Sampler.Temperature.LowerLimit: 4.0 [°C]
              Sampler.Temperature.UpperLimit: 45.0 [°C]
              PumpModule.Pump.%A.Equate: "H2O/Ammonium formate/Formic acid"
              PumpModule.Pump.%B.Equate: "MeOH"
              PumpModule.Pump.%C.Equate: "Rear Seal Wash"
              PumpModule.Pump.%D.Equate: "H2O"
              PumpModule.Pump.Pressure.LowerLimit: 5 [bar]
              PumpModule.Pump.Pressure.UpperLimit: 800 [bar]
              PumpModule.Pump.MaximumFlowRampUp: 6.000 [ml/min²]
              PumpModule.Pump.MaximumFlowRampDown: 5.998 [ml/min²]
0.000 [min] Inject
              Sampler.Inject
0.000 [min] Start Run
              ColumnOven.ColumnOven_Temp.AcqOn
              PumpModule.Pump.Pump_Pressure.AcqOn
0.000 [min] Run
              PumpModule.Pump.Flow.Nominal: 0.700 [ml/min]
              PumpModule.Pump.%B.Value: 5.0 [%]
              Comment: %A.Value 95.0 [%]
              PumpModule.Pump.%C.Value: 0.0 [%]
              PumpModule.Pump.%D.Value: 0.0 [%]

```

Thermo Scientific SII for Xcalibur Method

PumpModule.Pump.Curve: 5
0.200 [min]
PumpModule.Pump.Flow.Nominal: 0.700 [ml/min]
PumpModule.Pump.%B.Value: 5.0 [%]
Comment: %A.Value 95.0 [%]
PumpModule.Pump.%C.Value: 0.0 [%]
PumpModule.Pump.%D.Value: 0.0 [%]
PumpModule.Pump.Curve: 5
2.500 [min]
PumpModule.Pump.Flow.Nominal: 0.700 [ml/min]
PumpModule.Pump.%B.Value: 15.0 [%]
Comment: %A.Value 85.0 [%]
PumpModule.Pump.%C.Value: 0.0 [%]
PumpModule.Pump.%D.Value: 0.0 [%]
PumpModule.Pump.Curve: 5
9.500 [min]
PumpModule.Pump.Flow.Nominal: 0.700 [ml/min]
PumpModule.Pump.%B.Value: 95.0 [%]
PumpModule.Pump.%C.Value: 0.0 [%]
PumpModule.Pump.%D.Value: 0.0 [%]
PumpModule.Pump.Curve: 5
9.700 [min]
PumpModule.Pump.Flow.Nominal: 0.700 [ml/min]
PumpModule.Pump.%B.Value: 95.0 [%]
Comment: %A.Value 5.0 [%]
PumpModule.Pump.%C.Value: 0.0 [%]
PumpModule.Pump.%D.Value: 0.0 [%]
PumpModule.Pump.Curve: 5
9.800 [min]
PumpModule.Pump.Flow.Nominal: 0.700 [ml/min]
PumpModule.Pump.%B.Value: 5.0 [%]
Comment: %A.Value 95.0 [%]
PumpModule.Pump.%C.Value: 0.0 [%]
PumpModule.Pump.%D.Value: 0.0 [%]
PumpModule.Pump.Curve: 5
11.800 [min] Stop Run
ColumnOven.ColumnOven_Temp.AcqOff
PumpModule.Pump.Pump_Pressure.AcqOff

*Method of Q Exactive*Overall method settings**Global Settings**

Use lock masses	off
Lock mass injection	-
Chrom. peak width (FWHM)	6 s

Time

Method duration	11.80 min
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Customized Tolerances (+/-)

Lock Masses	-
Inclusion	5.0 ppm
Exclusion	-
Neutral Loss	-
Mass Tags	-
Dynamic Exclusion	-

*Experiments*Full MS - SIM**General**

Runtime	0 to 11.2 min
Polarity	positive
In-source CID	0.0 eV

Full MS - SIM

Microscans	1
Resolution	70,000
AGC target	1e6
Maximum IT	200 ms
Number of scan ranges	1
Scan range	100 to 500 m/z
Spectrum data type	Centroid

DIA**General**

Runtime	0 to 11.2 min
Polarity	positive
In-source CID	0.0 eV
Default charge state	1

DIA

Microscans	1
Resolution	17,500
AGC target	2e5
Maximum IT	auto
Loop count	1
MSX count	3
MSX isochronous ITs	on
Isolation window	100.0 m/z

Isolation offset	0.0 m/z
Fixed first mass	-
(N)CE / stepped (N)CE	nce: 50
Spectrum data type	Centroid

Setup**Tunefiles****General**

Switch Count 0
Base Tunefile C:\Xcalibur\methods\HESI_700uL_PosNeg_2022.mstune

Contact Closure**General**

Used False
Start in Closed True
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 µL/min
Inner diameter 2.303 mm
Volume 250 µL

Divert Valve A**General**

Used True
Start in 1-2 True
Switch Count 2

Element 1

At 0.60 min
Switches to 1-6

Element 2

At 10.23 min
Switches to 1-2

Divert Valve B**General**

Used False
Start in 1-2 True
Switch Count 0

4 entries

Mass [m/z]	Formula [M]	Species	CS [z]	Polarity	Start [min]	End [min]	(N)CE	MSX	ID	Comment
150.00000				Positive					1	
250.00000				Positive					2	
350.00000				Positive					3	
450.00000				Positive					1	

Exclusion List

6 entries

Mass [m/z]	Formula [M]	Species	CS [z]	Polarity	Start [min]	End [min]	Comment
391.28560		+ H		Positive			
199.00400				Positive			
224.12800				Positive			
210.09700				Positive			
240.10800				Positive			
195.12300				Positive			

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Thermo Scientific SII for Xcalibur Method

----- Overview -----

Name: New Instrument Method

Comment:

Run time: 7.800 [min]

Instrument: Ultimate_3000_04072021 on f19rt72w10

Description:

----- Script -----

```
initial      Instrument Setup
              Sampler.InjectWash: Both
              Sampler.WashSpeed: 8.000 [µl/s]
              Sampler.WashVolume: 80.000 [µl]
              Sampler.SampleHeight: 2.000 [mm]
              Sampler.WasteSpeed: 8.333 [µl/s]
              Sampler.DispenseDelay: 0.000 [s]
              Sampler.DispSpeed: 8.333 [µl/s]
              Sampler.DrawSpeed: 3.300 [µl/s]
              Sampler.DrawDelay: 1.000 [s]
              Sampler.InjectMode: Normal
              Sampler.PumpDevice: "Pump"
              Sampler.LoopWashFactor: 2.000
              Sampler.TempCtrl: On
              Sampler.Temperature.Nominal: 15.0 [°C]
              Sampler.ReadyTempDelta: 2.0 [°C]
              Sampler.Temperature.LowerLimit: 4.0 [°C]
              Sampler.Temperature.UpperLimit: 45.0 [°C]
              ColumnOven.TempCtrl: On
              ColumnOven.Temperature.Nominal: 40.0 [°C]
              ColumnOven.EquilibrationTime: 0.5 [min]
              ColumnOven.ReadyTempDelta: 2.0 [°C]
              ColumnOven.Cooler TempCtrl: Off
              PumpModule.Pump.%A.Equate: "%A"
              PumpModule.Pump.%B.Equate: "%B"
              PumpModule.Pump.%C.Equate: "%C"
              PumpModule.Pump.%D.Equate: "%D"
              PumpModule.Pump.Pressure.LowerLimit: 5 [bar]
              PumpModule.Pump.Pressure.UpperLimit: 800 [bar]
              PumpModule.Pump.MaximumFlowRampUp: 6.000 [ml/min²]
              PumpModule.Pump.MaximumFlowRampDown: 5.998 [ml/min²]
0.000 [min]  Inject Preparation
              Wait Sampler.Ready And ColumnOven.Ready And PumpModule.Pump.Ready
0.000 [min]  Inject
              Sampler.Inject
0.000 [min]  Start Run
              ColumnOven.ColumnOven_Temp.AcqOn
              PumpModule.Pump.Pump_Pressure.AcqOn
0.000 [min]  Run
              PumpModule.Pump.Flow.Nominal: 0.700 [ml/min]
              PumpModule.Pump.%B.Value: 5.0 [%]
```

Thermo Scientific SII for Xcalibur Method

PumpModule.Pump.%C.Value: 0.0 [%]
PumpModule.Pump.%D.Value: 0.0 [%]
PumpModule.Pump.Curve: 5
0.200 [min]
PumpModule.Pump.Flow.Nominal: 0.700 [ml/min]
PumpModule.Pump.%B.Value: 5.0 [%]
PumpModule.Pump.%C.Value: 0.0 [%]
PumpModule.Pump.%D.Value: 0.0 [%]
PumpModule.Pump.Curve: 5
2.500 [min]
PumpModule.Pump.Flow.Nominal: 0.700 [ml/min]
PumpModule.Pump.%B.Value: 15.0 [%]
PumpModule.Pump.%C.Value: 0.0 [%]
PumpModule.Pump.%D.Value: 0.0 [%]
PumpModule.Pump.Curve: 5
5.200 [min]
PumpModule.Pump.Flow.Nominal: 0.700 [ml/min]
PumpModule.Pump.%B.Value: 95.0 [%]
PumpModule.Pump.%C.Value: 0.0 [%]
PumpModule.Pump.%D.Value: 0.0 [%]
PumpModule.Pump.Curve: 5
5.700 [min]
PumpModule.Pump.Flow.Nominal: 0.700 [ml/min]
PumpModule.Pump.%B.Value: 95.0 [%]
PumpModule.Pump.%C.Value: 0.0 [%]
PumpModule.Pump.%D.Value: 0.0 [%]
PumpModule.Pump.Curve: 5
5.800 [min]
PumpModule.Pump.Flow.Nominal: 0.700 [ml/min]
PumpModule.Pump.%B.Value: 5.0 [%]
PumpModule.Pump.%C.Value: 0.0 [%]
PumpModule.Pump.%D.Value: 0.0 [%]
PumpModule.Pump.Curve: 5
7.800 [min] Stop Run
ColumnOven.ColumnOven_Temp.AcqOff
PumpModule.Pump.Pump_Pressure.AcqOff

*Method of Q Exactive*Overall method settings**Global Settings**

Use lock masses	off
Lock mass injection	-
Chrom. peak width (FWHM)	6 s

Time

Method duration	7.80 min
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Customized Tolerances (+/-)

Lock Masses	-
Inclusion	5.0 ppm
Exclusion	-
Neutral Loss	-
Mass Tags	-
Dynamic Exclusion	-

*Experiment*PRM**General**

Runtime	0 to 7.8 min
Polarity	positive
In-source CID	0.0 eV
Default charge state	1
Inclusion	on

MS²

Microscans	1
Resolution	17,500
AGC target	2e5
Maximum IT	50 ms
Loop count	1
MSX count	1
MSX isochronous ITs	on
Isolation window	2.0 m/z
Isolation offset	0.0 m/z
Fixed first mass	-
(N)CE / stepped (N)CE	nce: 50
Spectrum data type	Centroid

*Setup*Tunefiles**General**

Switch Count	0
Base Tunefile	C:\Xcalibur\methods\HESI_700uL_PosNeg_2022.mstune

General

Used False
 Start in Closed True
 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 μ L/min
 Inner diameter 2.303 mm
 Volume 250 μ L

Divert Valve A**General**

Used True
 Start in 1-2 True
 Switch Count 2

Element 1

At 0.50 min
 Switches to 1-6

Element 2

At 7.20 min
 Switches to 1-2

Divert Valve B**General**

Used False
 Start in 1-2 True
 Switch Count 0

Lock Masses

(no entries)

Inclusion List

21 entries

Mass [m/z]	Formula [M]	Species	CS [z]	Polarity	Start [min]	End [min]	(N)CE	MSX	ID	Comment
359.04609				Positive	4.46	5.46	50			Alpha-hydroxytriazolam
278.19033				Positive	4.49	5.49	50			Amitriptyline
468.31084				Positive	4.43	5.43	50			Buprenorphine
325.17107				Positive	4.12	5.12	50			Citalopram
318.17000				Positive	3.89	4.89	50			Cocaethylene
276.17468				Positive	4.45	5.45	50			Cyclobenzaprine
290.11030				Positive	4.80	5.80	50			Diazepam-D5

311.15542	Positive	4.15	5.15	50	DM-Citalopram
280.16959	Positive	4.22	5.22	50	Doxepin
375.20670	Positive	4.07	5.07	50	Furanylfentanyl
172.13280	Positive	1.50	2.50	50	Gabapentin
234.14886	Positive	3.66	4.66	50	Methylphenidate
250.18016	Positive	3.70	4.70	30	NDM-Tramadol
414.26389	Positive	3.99	4.99	50	Norbuprenorphine
262.15830	Positive	4.47	5.47	50	Norcyclobenzapri ne
233.16484	Positive	3.50	4.50	50	Norfentanyl
326.21146	Positive	4.42	5.42	50	Norpropoxyphene
264.17468	Positive	4.51	5.51	50	Nortriptyline
221.11050	Positive	3.50	4.50	50	Xylazine
395.23290	Positive	4.18	5.18	50	Carfentanil
291.17030	Positive	3.69	4.69	50	Norcarfentanil

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Thermo Scientific SII for Xcalibur Method

----- Overview -----

Name: New Instrument Method

Comment:

Run time: 14.800 [min]

Instrument: Ultimate_3000_04072021 on f19rt72w10

Description:

----- Script -----

```
initial      Instrument Setup
              ColumnOven.TempCtrl: On
              ColumnOven.Temperature.Nominal: 40.0 [°C]
              ColumnOven.EquilibrationTime: 0.5 [min]
              ColumnOven.ReadyTempDelta: 2.0 [°C]
              Sampler.InjectWash: Both
              Sampler.WashSpeed: 8.000 [µl/s]
              Sampler.WashVolume: 80.000 [µl]
              Sampler.SampleHeight: 2.000 [mm]
              Sampler.WasteSpeed: 8.333 [µl/s]
              Sampler.DispenseDelay: 0.000 [s]
              Sampler.DispSpeed: 8.333 [µl/s]
              Sampler.DrawSpeed: 0.330 [µl/s]
              Sampler.DrawDelay: 1.000 [s]
              Sampler.InjectMode: Normal
              Sampler.PumpDevice: "Pump"
              Sampler.LoopWashFactor: 2.000
              Sampler.TempCtrl: On
              Sampler.Temperature.Nominal: 15.0 [°C]
              Sampler.ReadyTempDelta: 2.0 [°C]
              Sampler.Temperature.LowerLimit: 4.0 [°C]
              Sampler.Temperature.UpperLimit: 45.0 [°C]
              PumpModule.Pump.%A.Equate: "H2O/Ammonium formate/Formic acid"
              PumpModule.Pump.%B.Equate: "MeOH"
              PumpModule.Pump.%C.Equate: "Rear Seal Wash"
              PumpModule.Pump.%D.Equate: "H2O"
              PumpModule.Pump.Pressure.LowerLimit: 5 [bar]
              PumpModule.Pump.Pressure.UpperLimit: 800 [bar]
              PumpModule.Pump.MaximumFlowRampUp: 6.000 [ml/min²]
              PumpModule.Pump.MaximumFlowRampDown: 5.998 [ml/min²]
0.000 [min] Inject
              Sampler.Inject
0.000 [min] Start Run
              ColumnOven.ColumnOven_Temp.AcqOn
              PumpModule.Pump.Pump_Pressure.AcqOn
0.000 [min] Run
              PumpModule.Pump.Flow.Nominal: 0.700 [ml/min]
              PumpModule.Pump.%B.Value: 5.0 [%]
              Comment: %A.Value 95.0 [%]
              PumpModule.Pump.%C.Value: 0.0 [%]
              PumpModule.Pump.%D.Value: 0.0 [%]
```

Thermo Scientific SII for Xcalibur Method

PumpModule.Pump.Curve: 5

0.200 [min]
PumpModule.Pump.Flow.Nominal: 0.700 [ml/min]
PumpModule.Pump.%B.Value: 5.0 [%]
Comment: %A.Value 95.0 [%]
PumpModule.Pump.%C.Value: 0.0 [%]
PumpModule.Pump.%D.Value: 0.0 [%]
PumpModule.Pump.Curve: 5

2.500 [min]
PumpModule.Pump.Flow.Nominal: 0.700 [ml/min]
PumpModule.Pump.%B.Value: 15.0 [%]
Comment: %A.Value 85.0 [%]
PumpModule.Pump.%C.Value: 0.0 [%]
PumpModule.Pump.%D.Value: 0.0 [%]
PumpModule.Pump.Curve: 5

13.500 [min]
PumpModule.Pump.Flow.Nominal: 0.700 [ml/min]
PumpModule.Pump.%B.Value: 95.0 [%]
PumpModule.Pump.%C.Value: 0.0 [%]
PumpModule.Pump.%D.Value: 0.0 [%]
PumpModule.Pump.Curve: 5

13.510 [min]
PumpModule.Pump.Flow.Nominal: 0.700 [ml/min]
PumpModule.Pump.%B.Value: 5.0 [%]
PumpModule.Pump.%C.Value: 0.0 [%]
PumpModule.Pump.%D.Value: 0.0 [%]
PumpModule.Pump.Curve: 5

13.800 [min]
PumpModule.Pump.Flow.Nominal: 0.700 [ml/min]
PumpModule.Pump.%B.Value: 5.0 [%]
PumpModule.Pump.%C.Value: 0.0 [%]
PumpModule.Pump.%D.Value: 0.0 [%]
PumpModule.Pump.Curve: 5

14.800 [min] Stop Run
ColumnOven.ColumnOven_Temp.AcqOff
PumpModule.Pump.Pump_Pressure.AcqOff

*Method of Q Exactive***Overall method settings****Global Settings**

Use lock masses	off
Lock mass injection	-
Chrom. peak width (FWHM)	6 s

Time

Method duration	14.80 min
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Customized Tolerances (+/-)

Lock Masses	-
Inclusion	5.0 ppm
Exclusion	-
Neutral Loss	-
Mass Tags	-
Dynamic Exclusion	-

*Experiments***Full MS - SIM****General**

Runtime	0 to 14.2 min
Polarity	positive
In-source CID	0.0 eV

Full MS - SIM

Microscans	1
Resolution	70,000
AGC target	1e6
Maximum IT	200 ms
Number of scan ranges	1
Scan range	80 to 500 m/z
Spectrum data type	Centroid

Full MS - SIM**General**

Runtime	0 to 14.2 min
Polarity	negative
In-source CID	0.0 eV

Full MS - SIM

Microscans	1
Resolution	70,000
AGC target	1e6
Maximum IT	200 ms
Number of scan ranges	1
Scan range	80 to 500 m/z
Spectrum data type	Centroid

Setup

General

Switch Count 0

Base Tunefile C:\Xcalibur\methods\HESI_700uL_PosNeg_2022.mstune

Contact Closure**General**

Used False

Start in Closed True

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 μ L/min

Inner diameter 2.303 mm

Volume 250 μ L**Divert Valve A****General**

Used True

Start in 1-2 True

Switch Count 2

Element 1

At 0.60 min

Switches to 1-6

Element 2

At 14.20 min

Switches to 1-2

Divert Valve B**General**

Used False

Start in 1-2 True

Switch Count 0

Lock Masses

(no entries)

Inclusion List

4 entries

Mass [m/z]	Formula [M]	Species	CS [z]	Polarity	Start [min]	End (N) [min]	CE	MSX	ID	Comment
150.00000				Positive					1	
250.00000				Positive					2	
350.00000				Positive					3	

450.00000

Positive

1

Exclusion List

6 entries

Mass [m/z]	Formula [M]	Species	CS [z]	Polarity	Start [min]	End [min]	Comment
391.28560		+ H		Positive			
199.00400				Positive			
224.12800				Positive			
210.09700				Positive			
240.10800				Positive			
195.12300				Positive			

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