Instrument Method: DIA_10-05-2022A

```
Thermo Scientific SII for Xcalibur Method
                                Date listed as part of the instrument method name may differ if additional drugs are added to method. The
---- Overview ----
Name: New Instrument Method
                                main instrument parameters (LC flow parameters, source parameters, etc) will not change.
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. Lower Limit: 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/min<sup>2</sup>]
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.AcgOff
            PumpModule.Pump.Pump Pressure.AcqOff
```

Page 2 of 5

Method of Q Exacti	ve
verall method settings	
Global Settings	
Jse lock masses	off
Lock mass injection Chrom. peak width (FWHM)	6 s
lime	
Method duration	11.80 min
Customized Tolerances (+/-)	
Lock Masses Inclusion	
Inclusion Exclusion	5.0 ppm
Neutral Loss	_
Mass Tags	_
Oynamic Exclusion	_
Experiments	
Full MS - SIM	
General	
Runtime	0 to 11.2 min
Polarity	positive
In-source CID Full MS — SIM	0.0 eV
Microscans	1
Resolution	70,000
AGC target	1e6
Maximum IT	200 ms
Number of scan ranges	1
Scan range	100 to 500 m/z Centroid
Spectrum data type	Centroid
<u>DIA</u> 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 100.0 m/z
Isolation window	100.0 m/2

Isolation offset Fixed first mass (N)CE / stepped (N)CE Spectrum data type

 $0.0 \, \text{m/z}$

nce: 50 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
Switch Count 0
Pump setup

Pump setupSyringe typeHamiltonFlow rate3.000 μL/minInner diameter2.303 mmVolume250 μL

Divert Valve A

General Used Start in 1-2 True Switch Count 2

Element 1

0.60 min Αt Switches Element 2
10.23 min Switches to 1-6

Switches to 1-2

Divert Valve B

General False Start in 1-2 True Switch Count 0

Mass Formula [m/z] [M] 00000 00000		CS	POLORITA							
00000		[z]			End [min]	(N) CE	MSX	ID	Comment	
00000		[2]	Positive	[111711]	[IIITII]			1		
			Positive					2		
			Positive					3		
0000			Positive					1		
sion List										
entries Mass Formula	Species	CS	Polarity	Start	End	Comme	nt.			
[m/z] [M]		[z]			[min]	COMMIC				
28560	+ H		Positive							
00400			Positive							
2800			Positive							
9700			Positive							
0800			Positive							
2300			Positive							

```
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. Lower Limit: 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/min<sup>2</sup>]
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
```

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Experiment	_	
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RM emeral entime clarity effault charge state enclusion		
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emeral Intime In		
untime plarity n-source CID efault charge state nclusion		
n-source CID efault charge state nclusion	0 to 7.8 min	
n-source CID efault charge state nclusion	positive	
efault charge state aclusion	0.0 eV	
nclusion	1	
	on	
\mathbf{S}^2		
croscans	1	
esolution	17,500	
GC target	2e5	
aximum IT	50 ms	
pop count	1	
SX count	1	
SX isochronous ITs	on	
solation window	2.0 m/z	
solation offset	0.0 m/z	
ixed first mass	-	
N)CE / stepped (N)CE	nce: 50	
pectrum data type	Centroid	
Setup		
unefiles		
eneral		
witch Count 0		
ase Tunefile C:\Xcalibur\methods\HESI_700uL_PosNeg_2022.mstune		

General

False Used Start in Closed True Switch Count

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 250 μL Volume

Divert Valve A

General

Used True Start in 1-2 True Switch Count 2

Element 1

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

Lock Masses

(no entries)

Inclusion List

١	24 ent	tries							
١	Mass	Formula Species	CS	Polarity	Start	End	(N) CE	MSX ID	Comment
١	[m/z]	[M]	[z]		[min]	[min]			
١	359.04609			Positive	4.46	5.46	50		Alpha-
١									hydroxytriazolam
I	278.19033			Positive	4.49	5.49	50		Amitriptyline
١	468.31084			Positive	4.43	5.43	50		Buprenorphine
١	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

8					
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
205.13354	Positive	1.00	2.00	50	Psilocin
355.21802	Positive	4.00	5.00	50	para-
,					fluorofentanyl
189.13862	Positive	2.00	3.00	50	DMT

```
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 Lock mass injection	off —	
Chrom. peak width (FWHM) Time Method duration	14.80	
Customized Tolerances (+/-) Lock Masses	14.00	ШП
Inclusion Exclusion	5.0	ppm
Neutral Loss Mass Tags	_ _	
Dynamic Exclusion	_	
Experiments		
Full MS — SIM General		
Runtime Polarity	0 to 14.2 positive	min
In-source CID Full MS - SIM	0.0	eV
Microscans Resolution	70,000	
AGC target Maximum IT Number of scan ranges	1e6 200 1	ms
Scan range Spectrum data type	80 to 500 Centroid	m/z
Full MS — SIM General		
Runtime Polarity	0 to 14.2 negative	min
In-source CID Full MS - SIM	0.0	eV
Microscans Resolution	70,000	
AGC target Maximum IT Number of scan ranges	1e6 200 1	ms
Scan range Spectrum data type	80 to 500 Centroid	m/z
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 µL/min
Inner diameter 2.303 mm
Volume 250 µL

<u>Divert Valve A</u>

General

Used True Start in 1-2 True Switch Count 2 Switch 5.
Element 1
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

1 01101											
Mass	Formula	Species	CS	Polarity	Start	End	(N) CE	MSX	ID	Comment	
[m/z]	[M]		[z]		[min]	[min]					
150.00000				Positive					1		
250.00000				Positive					2		
350.00000				Positive					3		

450.00000				Positive				1		
[m/z] 391.28560	ries Formula [M]	Species + H	CS [z]	Positive	Start [min]	End [min]	Comment			
199.00400 224.12800 210.09700 240.10800 195.12300				Positive Positive Positive Positive						