Revision 2

Instrument Method: DIA_10-05-2022A

```
Thermo Scientific SII for Xcalibur Method

---- Overview ----
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 [%]
```

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.

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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
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			

```
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 [%]
```

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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
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	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	0.50 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

24 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
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

Instrument Method: Screening_updated 08-03-2023

```
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
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 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 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 [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			