method name	used for which samples	pages
HCD_105min_T20_grad3_TB	QuaNPA MAA bead and protein input optimizations	19-22
HCD_2H5M_T20_HI	DDA method for Hela SILAC benchmark	15-18
HCD_105min_DIA_26iw_TB	DIA method 1 for Hela SILAC benchmark	2-5
HCD_105min_DIA_3xhr_MS1_27iw_MS2_TB	DIA method 2 for Hela SILAC benchmark	6-10
HCD_90min_grad3_DIA_28iw_TB	DIA method for IFNg time course NSP samples	11-14

Instrument Method: HCD_105min_DIA_26iw_TB

Thermo EASY-LC method print for QExactive HF

Sample pickup:

Volume [μ l] : 1.00 Flow [μ l / min] : 20.00 Volume [µl]

Sample loading:

Volume [μ l] : 22.00 Flow [μ l / min] : (unspecified) Max. pressure [Bar] : 800.00

Gradient:

ss] Duration	n [mm:ss] F.	low [nl/min]	Mixture [%B]
:00	00:00	300.00	3.00
:00	04:00	300.00	8.00
:00	02:00	300.00	10.00
:00	68:00	300.00	32.00
:00	12:00	300.00	50.00
:00	01:00	300.00	100.00
:00	07:00	300.00	100.00
:00	01:00	300.00	3.00
:00	10:00	300.00	3.00
	ss] Duration :00 :00 :00 :00 :00 :00 :00 :00 :00 :0	:00 00:00 :00 04:00 :00 02:00 :00 68:00 :00 12:00 :00 01:00 :00 07:00 :00 01:00	:00 00:00 300.00 :00 04:00 300.00 :00 02:00 300.00 :00 68:00 300.00 :00 12:00 300.00 :00 01:00 300.00 :00 07:00 300.00 :00 01:00 300.00 :00 01:00 300.00

Pre-column equilibration:

Volume [µ1] : 12.00 Flow [µ1 / min] : (unspecified) Volume [µl]

Max. pressure [Bar]: 800.00

Analytical column equilibration:

Volume [μ l] : 3.50 Flow [μ l / min] : (unspecified) Max. pressure [Bar] : 600.00

Autosampler wash:

cobal Settings se lock masses seck mass injection strom. peak width (FWHM) me sthod duration set mized Tolerances (+/-) sek Masses seclusion seclusion seclusion settral Loss ses Tags smamic Exclusion Experiments Sill MS — SIM secret seclusion seclusion Experiments Sill MS — SIM secret seclusion Experiments Sill MS — SIM secret seclusion Sill MS — SIM secret seclusion Sill MS — SIM secret seclusion Sill MS — SIM secret seclusion Sill MS — SIM secret seclusion Sill MS — SIM secret seclusion Sill MS — SIM secret seclusion Sill MS — SIM secret seclusion Sill MS — SIM secret seclusion Sill MS — SIM secret seclusion Sill MS — SIM secret seclusion Sill MS — SIM secret seclusion Sill MS — SIM secret seclusion Sill MS — SIM secret seclusion Sill MS — SIM secret seclusion Sill MS — SIM secret seclusion Sill MS — SIM secret seclusion Sill MS — SIM secret secret seclusion Sill MS — SIM secret sec	best 20 105.00 105.00 0 to 105 positive 0.0 1 60,000 3e6	min
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ethod duration stomized Tolerances (+/-) sock Masses soclusion sutral Loss sass Tags smamic Exclusion Experiments all MS — SIM smeral smitime solarity sesource CID still MS — SIM scroscans sesolution GC target sximum IT smber of scan ranges san range sectrum data type EA smeral	105.00	min
sthod duration stomized Tolerances (+/-) ock Masses sociusion sutral Loss sas Tags vnamic Exclusion Experiments All MS — SIM smeral sutrine olarity n-source CID sociul MS — SIM scroscans sociution GC target sximum IT smber of scan ranges san range occtrum data type EA smeral	0 to 105 positive 0.0 1 60,000	min
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Experiments Exper	positive 0.0 1 60,000	
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Experiments All MS — SIM Eneral Entime Clarity Ensource CID Entil MS — SIM Erroscans Esolution EC target Eximum IT Ember of scan ranges Evan range Evectrum data type EA Eneral	positive 0.0 1 60,000	
all MS - SIM eneral intime clarity n-source CID ill MS - SIM croscans esolution GC target aximum IT imber of scan ranges can range electrum data type EA eneral	positive 0.0 1 60,000	
all MS - SIM eneral intime clarity n-source CID ill MS - SIM croscans esolution GC target aximum IT imber of scan ranges can range electrum data type EA eneral	positive 0.0 1 60,000	
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clarity 1-source CID 111 MS - SIM 1.croscans	positive 0.0 1 60,000	
n-source CID all MS - SIM croscans esolution GC target eximum IT ember of scan ranges ean range electrum data type EA eneral	0.0 1 60,000	eV
all MS — SIM Coroscans Esolution GC target Eximum IT Ember of scan ranges Ean range Dectrum data type EA	1 60,000	ev
croscans esolution GC target eximum IT ember of scan ranges can range eectrum data type EA eneral	60,000	
esolution GC target eximum IT ember of scan ranges ean range eectrum data type CA eneral	60,000	
GC target aximum IT umber of scan ranges can range pectrum data type CA eneral		
aximum IT umber of scan ranges can range pectrum data type can can can can can can can can can ca		
can range pectrum data type A Eneral	20	ms
pectrum data type Andrew Denomination CA Can be denominated by the second of th	1	
<u>CA</u> eneral	400 to 1000	m/z
- neral	Profile	
	0	
antime Dlarity	0 to 105	mın
n-source CID	positive 0.0	ΩV
efault charge state	2	CV
ZA	_	
croscans	1	
esolution	30,000	
GC target	1e6	
ximum IT	50	ms
oop count	26	
XX count	1	
XX isochronous ITs solation window	on 23.3	m / =
OTACTON WINDOW	23.3	ш/ 2

Isolation offset $0.0 \, \text{m/z}$ Fixed first mass 200.0 m/z (N)CE / stepped (N)CE nce: 27 Spectrum data type Profile

Setup

Tunefiles

General

Switch Count 0

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

Contact Closure

General

Used False Start in Closed True Switch Count

Syringe

General

Used False
Start in OFF True
Stop at end of run False Switch Count

Pump setup

Syringe type Hamilton
Flow rate 3.000 µL/min
Inner diameter 2.303 mm Volume 250 µL

Divert Valve A

General

Used False Start in 1-2 True Switch Count 0

Divert Valve B

General

Used False Start in 1-2 True Switch Count 0

Lock Masses

1 entry

Mass Polarity Start End Comment [m/z] [min] [min]

445.12003 Positive

Inclusion List

26 entries

#13.65000 Positive #13.65000 Positive #158.25000 Positive #160.285000 Positive #17.45000 Positive #17.45000 Positive #18.25000 Positive			Species		Polarity			(N) CE	MSX	ID	Comment
### Positive Positive ### Positive Positive #### Positive Positive ### Positive Positi	[m/z]	[M]		[z]		[min]	[min]				
### 15000 #### 15000 #### 15000 #### 15000 #### 15000 #### 15000 #### 15000 #### 15000 #### 15000 ##### 15000 ##########											
R80.55000 Positive R02.85000 Positive R02.85000 Positive R047.45000 Positive R09.75000 Positive R09.75000 Positive R09.05000 Positive R09.050000 Positive R09.05000 Positive											
Positive	458.25000				Positive						
Positive	480.55000				Positive						
347.45000 Positive 369.75000 Positive 392.05000 Positive 314.35000 Positive 358.95000 Positive 381.25000 Positive 203.55000 Positive 225.85000 Positive 248.15000 Positive 292.75000 Positive 315.05000 Positive 365.05000 Positive 367.35000 Positive 381.95000 Positive 304.25000 Positive 304.885000 Positive	502.85000				Positive						
669.75000 Positive 692.05000 Positive 614.35000 Positive 636.65000 Positive 658.95000 Positive 603.55000 Positive 625.85000 Positive 648.15000 Positive 692.75000 Positive 692.75000 Positive 693.7.35000 Positive 681.95000 Positive 694.25000 Positive 604.25000 Positive 648.85000 Positive	525.15000				Positive						
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14.35000 Positive 36.65000 Positive 381.25000 Positive 303.55000 Positive 325.85000 Positive 348.15000 Positive 370.45000 Positive 392.75000 Positive 315.05000 Positive 337.35000 Positive 337.35000 Positive 381.95000 Positive 3904.25000 Positive 3948.85000 Positive	569.75000				Positive						
736.65000 Positive 758.95000 Positive 703.55000 Positive 703.55000 Positive 725.85000 Positive 770.45000 Positive 792.75000 Positive 792.75000 Positive 793.735000 Positive	592.05000				Positive						
## Positive Positive	614.35000				Positive						
881.25000 Positive 903.55000 Positive 925.85000 Positive 948.15000 Positive 920.75000 Positive 9315.05000 Positive 9317.35000 Positive	636.65000				Positive						
703.55000 Positive 725.85000 Positive 748.15000 Positive 770.45000 Positive 792.75000 Positive 815.05000 Positive 837.35000 Positive 839.65000 Positive 881.95000 Positive 904.25000 Positive 926.55000 Positive 928.85000 Positive	558.95000				Positive						
Positive	681.25000				Positive						
748.15000 Positive 770.45000 Positive 792.75000 Positive 815.05000 Positive 837.35000 Positive 881.95000 Positive 904.25000 Positive 926.55000 Positive 948.85000 Positive	703.55000				Positive						
770.45000 Positive 792.75000 Positive 815.05000 Positive 837.35000 Positive 859.65000 Positive 881.95000 Positive 904.25000 Positive 926.55000 Positive 948.85000 Positive	725.85000				Positive						
792.75000 Positive 815.05000 Positive 837.35000 Positive 859.65000 Positive 881.95000 Positive 904.25000 Positive 926.55000 Positive 948.85000 Positive	748.15000				Positive						
### Positive #### Positive ##### Positive ##### Positive ##### Positive ##### Positive ###### Positive ####### Positive ####### Positive ####################################	770.45000				Positive						
337.35000 Positive 359.65000 Positive 381.95000 Positive 304.25000 Positive 326.55000 Positive 348.85000 Positive	792.75000				Positive						
859.65000 Positive 881.95000 Positive 904.25000 Positive 926.55000 Positive 948.85000 Positive	815.05000				Positive						
881.95000 Positive 904.25000 Positive 926.55000 Positive 948.85000 Positive	837.35000				Positive						
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948.85000 Positive	904.25000				Positive						
	926.55000				Positive						
971 15000 Positive	948.85000				Positive						
	971.15000				Positive						

Instrument Method: HCD_105min_DIA_3xhr_MS1_27iw_MS2_TB

Thermo EASY-LC method print for QExactive HF

Sample pickup:

Volume [μ l] : 1.00 Flow [μ l / min] : 20.00 Volume [µl]

Sample loading:_

Volume [μ l] : 22.00 Flow [μ l / min] : (unspecified)

Max. pressure [Bar] : 800.00

Gradient:

Time [mm:ss]	Duration [mm:ss]	Flow [nl/min]	Mixture [%B]
00:00	00:00	300.00	3.00
04:00	04:00	300.00	8.00
06:00	02:00	300.00	10.00
74:00	68:00	300.00	32.00
86:00	12:00	300.00	50.00
87:00	01:00	300.00	100.00
94:00	07:00	300.00	100.00
95:00	01:00	300.00	3.00
105:00	10:00	300.00	3.00

Pre-column equilibration:

Volume [µl]

Volume [µ1] : 12.00 Flow [µ1 / min] : (unspecified)

Max. pressure [Bar] : 800.00

Analytical column equilibration:

Volume [μ l] : 3.50 Flow [μ l / min] : (unspecified) Max. pressure [Bar] : 600.00

Autosampler wash:

Command	Method of Q Exactive I	nr.	
Use lock masses best Lock mass injection — Chrom. peak width (FWHM) 20 s Fine 105.00 min Customized Tolerances (+/-) — Lock Masses — Inclusion — Exclusion — Neutral Loss — Mass Tags — Dynamic Exclusion — Experiments	Overall method settings		
Lock mass injection	Global Settings		
Chrom. peak width (FWHM)		best	
### Method duration		_	
Method duration 105.00 min Customized Tolerances (+/-) - Lock Masses - Inclusion - Exclusion - Neutral Loss - Mass Tags - Dynamic Exclusion - Experiments Miles Sim Microscans 1 Resolution DIA General Experiments DIA General Experiments Automosphale DIA General Experiments Automosphale DIA Microscans Colspan="2">Automosphale DIA Microscans <td></td> <td>20</td> <td>S</td>		20	S
Customized Tolerances (+/-)		105.00	
Lock Masses		105.00	min
Inclusion			
Experiments I ot 105 min Polarity Profile Experiments Experiments Experiments Experiments I ot 105 min Polarity Profile Experiments Experiments Experiments I ot 105 min Polarity Profile Experiments Experiments Experiments I ot 105 min Polarity Profile Experiments Experiments Experiments Experiments I ot 105 min Polarity Profile Experiments Experiments Experiments Experiments Experiments Experiments I ot 105 min Polarity Profile Experiments Ex		_	
Neutral Loss		_	
Mass Tags		_	
Experiments		_	
Full MS - SIM General Runtime		-	
Full MS - SIM General Runtime			
General Runtime 0 to 105 min Polarity positive In-source CID 0.0 eV Full MS - SIM Microscans 1 Resolution 120,000 AGC target 3e6 Maximum IT 20 ms Number of scan ranges 1 Scan range 400 to 1000 m/z Spectrum data type Profile DIA General Runtime 0 to 105 min Polarity positive In-source CID 0.0 eV Default charge state 2 DIA 2 Microscans 1 Resolution 30,000 AGC target 1e6 Maximum IT 50 ms Loop count 9 MSX count 1 MSX count 1 MSX isochronous ITs 0	Experiments		
Runtime Polarity positive In-source CID	Full MS - SIM		
Polarity	General		
In-source CID			min
Full MS - SIM Microscans 1 Resolution 120,000 AGC target 3e6 Maximum IT 20 ms Number of scan ranges 1 Scan range 400 to 1000 m/z Spectrum data type Profile Pr	<u> -</u>	positive	
Microscans 1 Resolution 120,000 AGC target 3e6 Maximum IT 20 ms Number of scan ranges 1 Scan range 400 to 1000 m/z Spectrum data type Profile DIA General 0 to 105 min Runtime 0 to 105 min Polarity positive In-source CID 0.0 eV Default charge state 2 DIA 30,000 Microscans 1 Resolution 30,000 AGC target 1e6 Maximum IT 50 ms Loop count 9 MSX count 1 MSX isochronous ITs on		0.0	eV
Resolution			
AGC target 3e6 Maximum IT 20 ms Number of scan ranges 1 Scan range 400 to 1000 m/z Spectrum data type Profile DIA General Runtime 0 to 105 min Polarity positive In-source CID 0.0 eV Default charge state 2 DIA Microscans 1 Resolution 30,000 AGC target 1e6 Maximum IT 50 ms Loop count 9 MSX count 1 MSX isochronous ITS on			
Maximum IT 20 ms Number of scan ranges 1 Scan range 400 to 1000 m/z Spectrum data type Profile DIA General Runtime 0 to 105 min Polarity positive In-source CID 0.0 eV Default charge state 2 DIA Microscans 1 Resolution 30,000 AGC target 1e6 Maximum IT 50 ms Loop count 9 MSX count 1 MSX isochronous ITS		•	
Number of scan ranges 1 Scan range 400 to 1000 m/z Spectrum data type Profile DIA General Runtime 0 to 105 min Polarity positive In-source CID 0.0 eV Default charge state 2 DIA Microscans 1 Resolution 30,000 AGC target 126 Maximum IT 50 ms Loop count 9 MSX count 11 MSX isochronous ITs 0 n			
Scan range Spectrum data type Profile DIA General Runtime O to 105 min Polarity Positive In-source CID O.0 eV Default charge state DIA Microscans Resolution Second Secon			ms
Spectrum data type DIA General Runtime Polarity In-source CID Default charge state DIA Microscans Resolution AGC target Maximum IT Loop count MSX count MSX isochronous ITs Profile Profile Profile Profile Profile 10 10 11 11 11 11 12 13 14 15 15 16 16 16 16 16 16 16 16			,
DIA General Runtime Polarity In-source CID Default charge state DIA Microscans Resolution AGC target Maximum IT Loop count MSX count MSX isochronous ITs O to 105 min positive 0.0 eV 0	-		m/z
General Runtime 0 to 105 min Polarity positive In-source CID 0.0 eV Default charge state 2 DIA Microscans 1 Resolution 30,000 AGC target 1e6 Maximum IT 50 ms Loop count 9 MSX count 1 MSX isochronous ITs on	Spectrum data type	Profile	
Runtime			
Polarity positive In-source CID 0.0 eV Default charge state 2 DIA Microscans 1 Resolution 30,000 AGC target 1e6 Maximum IT 50 ms Loop count 9 MSX count 1 MSX isochronous ITs on		0	
In-source CID 0.0 eV Default charge state 2 DIA 30,000 Microscans 1 Resolution 30,000 AGC target 1e6 Maximum IT 50 ms Loop count 9 MSX count 1 MSX isochronous ITs on			min
Default charge state 2 DIA Microscans Resolution 30,000 AGC target 1e6 Maximum IT 50 ms Loop count 9 MSX count 1 MSX isochronous ITs on	-	=	077
DIA 1 Microscans 1 Resolution 30,000 AGC target 1e6 Maximum IT 50 ms Loop count 9 MSX count 1 MSX isochronous ITs on			ev
Microscans Resolution AGC target Maximum IT Loop count MSX count MSX isochronous ITs 1 30,000 30,000 30,000 1 1 1 1 1 1 1 1 1 1 1 1		2	
Resolution AGC target Maximum IT Loop count MSX count MSX isochronous ITs 30,000 1e6 50 ms 9 MS on		1	
AGC target 1e6 Maximum IT 50 ms Loop count 9 MSX count 1 MSX isochronous ITs 1			
Maximum IT 50 ms Loop count 9 MSX count 1 MSX isochronous ITs on			
Loop count 9 MSX count 1 MSX isochronous ITs on			ms
MSX count 1 MSX isochronous ITs on			
MSX isochronous ITs on		1	
Isolation window 23.2 m/z		on	
	Isolation window		m/z

		0.0	,	
	solation offset		m/z	
	ixed first mass	200.0	m/z	
	N)CE / stepped (N)CE	nce: 27		
SI	pectrum data type	Profile		
Fı	all MS - SIM			
	eneral			
Rι	untime	to 105	min	
Po	plarity	ositive		
Ιr	n-source CID	0.0	eV	
Fu	ıll MS — SIM			
M	icroscans	1		
Re	esolution	120,000		
A	GC target	3e6		
	aximum IT	20	ms	
Nι	umber of scan ranges	1		
Sc	can range 400	to 1000	m/z	
SI	pectrum data type	Profile		
D.	TA.			
_	eneral			
) to 105	min	
		ositive	111211	
	n-source CID	0.0	ΑV	
	efault charge state	2	CV	
	IA	2		
	icroscans	1		
	esolution	30,000		
	GC target	1e6		
	aximum IT		ms	
	pop count	9	-	
	SX count	1		
	SX isochronous ITs	on		
Is	solation window	23.2	m/z	
Is	solation offset	0.0	m/z	
F	ixed first mass	200.0	m/z	
(1	N)CE / stepped (N)CE	nce: 27		
Sı	pectrum data type	Profile		
F-	11 MC _ CTM			
_	ull MS — SIM eneral			
		to 105	min	
	propriet in the state of the st	ositive 0.0		
	ill MS — SIM	0.0	C V	
	icroscans	1		
	esolution	120,000		
	GC target	3e6		
	aximum IT	20	ms	
	umber of scan ranges	1	111.5	
140				

Scan range	400 to 1000 m/z
Spectrum data type	Profile

DIA

General

Runtime 0 to 105 min Polarity positive In-source CID 0.0 eV Default charge state 2 Microscans 1 30,000 Resolution AGC target 1e6 50 ms Maximum IT Loop count MSX count 1 MSX isochronous ITs on 23.2 m/z

Isolation window $0.0 \, \text{m/z}$ Isolation offset Fixed first mass 200.0 m/z (N)CE / stepped (N)CE nce: 27

Profile Spectrum data type

Setup

Tunefiles

General

Switch Count 0

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

Contact Closure

General

Used False Start in Closed True Switch Count

Syringe

General

Used False Start in OFF True Stop at end of run False Switch Count

Pump setup

Syringe type Hamilton

 $3.000 \mu L/min$ Flow rate Inner diameter 2.303 mm 250 μL Volume

Divert Valve A

General

Instrument Method: HCD_105min_DIA_3xhr_MS1_27iw_MS2_TB False Used Start in 1-2 True Switch Count Divert Valve B General Used False Start in 1-2 True Switch Count Lock Masses 1 entry Mass Polarity Start End Comment [min] [min] [m/z] 445.12003 Positive Inclusion List 27 entries Mass Formula Species CS Polarity Start End (N)CE MSX ID Comment [min] [min] [m/z] [M] [z] 411.00000 Positive 432.60000 Positive 454.80000 Positive 477.00000 Positive 499.20000 Positive 521.40000 Positive 543.60000 Positive 565.80000 Positive 588.00000 Positive 610.20000 Positive 632.40000 654.60000 Positive 676.80000 Positive 699.00000 Positive 721.20000 Positive 743.40000 Positive 765.60000 Positive 787.80000 Positive 810.00000 Positive 832.20000 Positive 854.40000 Positive 876.60000 Positive 898.80000 Positive 921.00000 Positive 943.20000 Positive 965.40000 Positive 987.60000 Positive

Instrument Method: HCD_90min_grad3_DIA_28iw_TB

Thermo EASY-LC method print for QExactive HF

Sample pickup:

Volume [μ l] : 1.00 Flow [μ l / min] : 20.00 Volume [µl]

Sample loading:

Volume [μ l] : 22.00 Flow [μ l / min] : (unspecified) Max. pressure [Bar] : 800.00

Gradient:

Time	[mm:ss]	Duration	[mm:ss]	Flow	[nl/min]	Mixture [%]	В]
	00:00		00:00		300.00	5.	00
	01:00		01:00		300.00	6.	00
	51:00		50:00		300.00	27.	00
	70:00		19:00		300.00	44.	00
	70:10		00:10		300.00	95.	00
	80:00		09:50		300.00	95.	00
	81:00		01:00		300.00	5.	00
	81:10		00:10		300.00	5.	00
	90:00		08:50		300.00	5.	00

Pre-column equilibration:

Volume [µ1] : 12.00 Flow [µ1 / min] : (unspecified)

Max. pressure [Bar]: 800.00

Analytical column equilibration:

Volume [μ l] : 3.50 Flow [μ l / min] : (unspecified) Max. pressure [Bar] : 600.00

Autosampler wash:

	ve HF
Overall method settings	
Global Settings	
Use lock masses	best
Lock mass injection Chrom. peak width (FWHM)	_ 20 s
Time	20 5
Method duration	90.00 mir
Customized Tolerances (+/-)	
Lock Masses	_
Inclusion	_
Exclusion	_
Neutral Loss	_
Mass Tags	_
Dynamic Exclusion	-
Experiments	
Full MS - SIM	
General	
Runtime	0 to 90 mir
Polarity	positive
In-source CID	0.0 eV
Full MS — SIM	1
Microscans Resolution	1 60,000
Resolution AGC target	60,000 3e6
Maximum IT	40 ms
Number of scan ranges	1
Scan range	400 to 1000 m/z
Spectrum data type	Profile
DIA	
General Runtime	0 +- 00
Runtime Polarity	0 to 90 mir positive
In-source CID	0.0 eV
Default charge state	2
DIA	2
Microscans	1
Resolution	30,000
AGC target	1e6
Maximum IT	40 ms
Loop count	26
MSX count	1
MOSZ i a a la company TITI a	on
MSX isochronous ITs Isolation window	22.0 m/z

Isolation offset $0.0 \, \text{m/z}$ Fixed first mass 200.0 m/z (N)CE / stepped (N)CE nce: 27 Spectrum data type Profile

Setup

Tunefiles

General

Switch Count 0

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

Contact Closure

General

Used False Start in Closed True Switch Count

Syringe

General

Used False
Start in OFF True
Stop at end of run False Switch Count

Pump setup

Syringe type Hamilton
Flow rate 3.000 µL/min
Inner diameter 2.303 mm Volume 250 µL

Divert Valve A

General

Used False Start in 1-2 True Switch Count 0

Divert Valve B

General

Used False Start in 1-2 True Switch Count

Lock Masses

1 entry

Mass Polarity Start End Comment [m/z] [min] [min]

445.12003 Positive

Inclusion List

28 entries

		Species		Polarity			(N) CE	MSX	ID	Comment
[m/z]	[M]		[z]		[min]	[min]				
411.00000				Positive						
32.00000				Positive						
153.00000				Positive						
474.00000				Positive						
195.00000				Positive						
516.00000				Positive						
537.00000				Positive						
558.00000				Positive						
579.00000				Positive						
600.00000				Positive						
621.00000				Positive						
642.00000				Positive						
663.00000				Positive						
684.00000				Positive						
705.00000				Positive						
726.00000				Positive						
747.00000				Positive						
768.00000				Positive						
789.00000				Positive						
810.00000				Positive						
831.00000				Positive						
852.00000				Positive						
873.00000				Positive						
894.00000				Positive						
915.00000				Positive						
936.00000				Positive						
957.00000				Positive						
978.00000				Positive						

Instrument Method: HCD_2H5M_T20_HI

Thermo EASY-LC method print for QExactive HF

Sample pickup:

Volume [μ l] : 1.00 Flow [μ l / min] : 20.00 Volume [µl]

Sample loading:

Volume [μ l] : 22.00 Flow [μ l / min] : (unspecified)

Max. pressure [Bar] : 800.00

Gradient:

Time [mm:ss]	Duration [mm:ss]	Flow [nl/min]	Mixture [%B]
00:00	00:00	300.00	3.00
04:00	04:00	300.00	8.00
06:00	02:00	300.00	10.00
74:00	68:00	300.00	32.00
86:00	12:00	300.00	50.00
87:00	01:00	300.00	100.00
94:00	07:00	300.00	100.00
95:00	01:00	300.00	3.00
105:00	10:00	300.00	3.00

Pre-column equilibration:

Volume [μ l] : 12.00 Flow [μ l / min] : (unspecified)

Max. pressure [Bar] : 800.00

Analytical column equilibration:

Volume [μ l] : 2.00 Flow [μ l / min] : (unspecified) Max. pressure [Bar] : 600.00

Autosampler wash:

Method of Q Exactive HF					
Overall method settings					
Global Settings					
Use lock masses	best				
Lock mass injection	_				
Chrom. peak width (FWHM)	20	S			
Time					
Method duration	105.00	min			
Customized Tolerances (+/-)					
Lock Masses	_				
Inclusion	_				
Exclusion	_				
Neutral Loss	_				
Mass Tags	_				
Dynamic Exclusion	_				
The suite of					
Experiment					
Full MS / dd-MS ² (TopN) General					
Runtime	0 to 105	min			
Polarity	positive	IIITII			
In-source CID	0.0	7.7			
Default charge state	2	ev			
Inclusion	_				
Exclusion	_				
Tags	_				
rays Full MS					
Microscans	1				
Resolution	60,000				
AGC target	3e6				
Maximum IT		ms			
	32	IIID			
Number of scan ranges	350 to 1500	m / 7			
Scan range Spectrum data type	Profile	ш/ Д			
dd-MS² / dd-SIM	riolile				
Microscans	1				
Resolution	15,000				
AGC target	19,000 1e5				
Maximum IT	50	ms			
Loop count	20	1110			
MSX count	1				
TopN	20				
Isolation window	2.0	m/7			
Isolation offset		m/z			
Scan range	200 to 2000				
Fixed first mass	110.0				
TIAGU TITSC MASS	110.0	111/ 4			

(N)CE / stepped (N)CE nce: 26 Spectrum data type Profile dd Settings 1.00e3 Minimum AGC target Intensity threshold 2.0e4 Apex trigger unassigned, 1, 5 - 8, >8Charge exclusion Multiple charge states Peptide match preferred Exclude isotopes on Dynamic exclusion 25.0 s If idle .. do not pick others

Setup

Tunefiles

General

Switch Count 0

Base Tunefile C:\Xcalibur\methods\nanosource.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

Pump setup

Syringe type Hamilton
Flow rate 3.000 µL/
Inner diameter 2.303 mm $3.000 \mu L/min$ Volume 250 µL

Divert Valve A

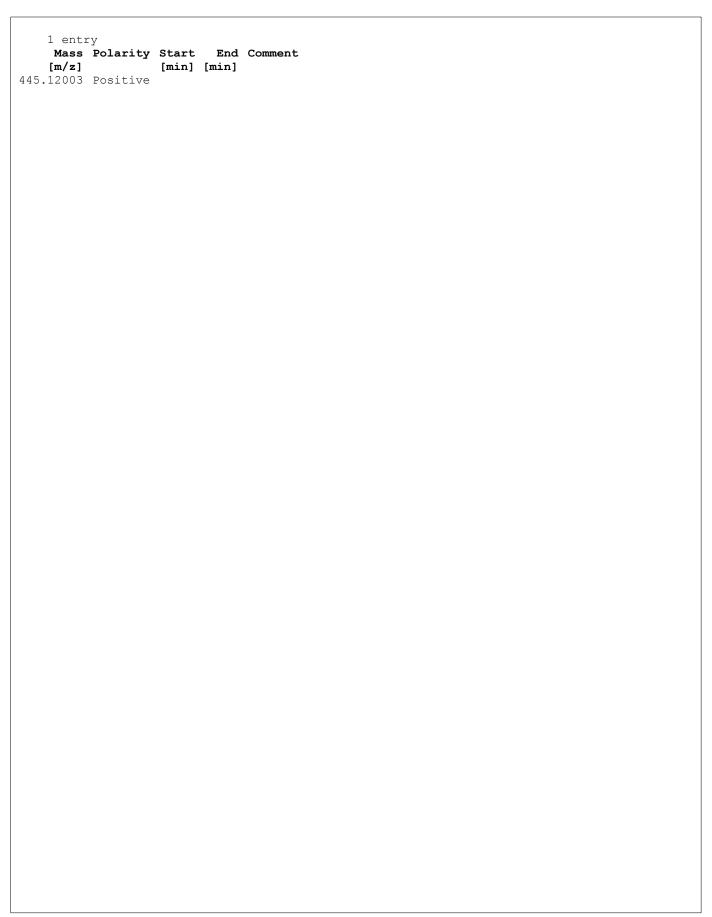
General

False Used Start in 1-2 True Switch Count

Divert Valve B

General

Used False Start in 1-2 True Switch Count 0 Instrument Method: HCD_2H5M_T20_HI



Instrument Method: HCD_105min_T20_grad3_TB

Thermo EASY-LC method print for QExactive_HF

Sample pickup:

Volume [μ l] : 1.00 Flow [μ l / min] : 20.00 Volume [µl]

Sample loading:_

Volume [μ l] : 22.00 Flow [μ l / min] : (unspecified)

Max. pressure [Bar] : 800.00

Gradient:

Time [mm:ss]	Duration [mm:ss]	Flow [nl/min]	Mixture [%B]
00:00	00:00	300.00	4.00
01:00	01:00	300.00	6.00
70:00	69:00	300.00	27.00
85:00	15:00	300.00	44.00
85:10	00:10	300.00	95.00
95:00	09:50	300.00	95.00
95:10	00:10	300.00	5.00
104:50	09:40	300.00	5.00
105:00	00:10	300.00	5.00

Pre-column equilibration:

Volume [µ1] : 12.00 Flow [µ1 / min] : (unspecified)

Max. pressure [Bar]: 800.00

Analytical column equilibration:

Volume [μ l] : 2.00 Flow [μ l / min] : (unspecified) Max. pressure [Bar] : 600.00

Autosampler wash:

Company	Method of Q Exactive HF					
Use lock masses	Overall method settings					
Lock mass injection						
Chrom. peak width (FWHM) Time Method duration Customized Tolerances (+/-) Lock Masses Inclusion Neutral Loss Mass Tags Dynamic Exclusion Experiment Experiment Experiment Experiment Experiment		best				
Method duration 105.00 min Customized Tolerances (+/-) Lock Masses -		_	_			
Method duration 105.00 min Crustomized Tolerances (+/-)		20	S			
Customized Tolerances (+/-) Lock Masses - -		105 00	min			
Lock Masses		103.00	штп			
Inclusion		_				
Exclusion Neutral Loss Mass Tags Dynamic Exclusion Experiment Experiment Experiment Full MS / dd-MS² (TopN) General Runtime Polarity In-source CID Default charge state 1 2 Inclusion Exclusion 2 - Fags 3 - Fags 3 - Fags 4 - Fags 5 - Fags 5 - Fags 6 - Fags 7 - Fags 8 - Full MS Microscans Resolution AGC target Aaximum IT Aaxi		_				
Neutral Loss		_				
Experiment		_				
Experiment		_				
### Experiment Full MS / dd-MS² (TopN)		_				
Full MS / dd-MS² (TopN) General Runtime	•					
Full MS / dd-MS² (TopN) General Runtime	Experiment					
Runtime 0 to 105 min Polarity positive In-source CID 0.0 eV Default charge state 2 Inclusion - Exclusion - Tags - THIN MS - TH	-					
Runtime						
Polarity		0 +- 105				
In-source CID			ШТП			
Default charge state	-	_	77.			
Inclusion			ev			
Exclusion — Tags — Full MS Microscans 1 60,000 AGC target 326 Maximum IT 32 ms Number of scan ranges 350 to 1500 m/z Spectrum data type 4 Profile 4 AG-MS² / dd-SIM Microscans 1 5,000 AGC target 15,000 AGC target 15,000 AGC target 15,000 AGC target 165 Maximum IT 50 ms Resolution 15,000 AGC target 125 Maximum IT 50 ms Loop count 1 50 ms Loop count 20 MSX count 1 1 TopN Isolation window 2.0 m/z Isolation offset 5 0.0 m/z Isolation offset 200 to 2000 m/z Isolation offset 200 to 2000 m/z Isolation and Isolation in Informatical Isolation in Informatical Isolation in Informatical Isolation offset 200 to 2000 m/z Isolation and Isolation in Informatical Isolation Isolation in Informatical Isolation	The state of the s					
Full MS Microscans		_				
Full MS Microscans 1 Resolution 60,000 AGC target 3e6 Maximum IT 32 ms Number of scan ranges 1 Scan range 350 to 1500 m/z Spectrum data type Profile dd-MS² / dd-SIM 1 Microscans 1 Resolution 15,000 AGC target 1e5 Maximum IT 50 ms Loop count 20 MSX count 1 TopN 20 Isolation window 2.0 m/z Isolation offset 0.0 m/z Scan range 200 to 2000 m/z		_				
Microscans Resolution 60,000 AGC target 3e6 Maximum IT 32 ms Number of scan ranges 1 Scan range 350 to 1500 m/z Spectrum data type Profile dd-Ms² / dd-SIM Microscans 1 Resolution 15,000 AGC target 1e5 Maximum IT 50 ms Loop count 20 MSX count 1 TopN 20 Isolation window 2.0 m/z Isolation offset 0.0 m/z Scan range 200 to 2000 m/z	-					
Resolution AGC target Maximum IT Number of scan ranges Scan range Spectrum data type dd-Ms² / dd-SIM Microscans Resolution AGC target Maximum IT I I I I I I I I I I I I		1				
AGC target 32 ms Maximum IT 32 ms Number of scan ranges 1 Scan range 350 to 1500 m/z Spectrum data type 7 Add-SIM 7 Microscans 1 Resolution 15,000 AGC target 150 ms Loop count 150 ms Loop count 170						
Maximum IT 32 ms Number of scan ranges 1 Scan range 350 to 1500 m/z Spectrum data type Profile dd-MS² / dd-SIM 1 Microscans 1 Resolution 15,000 AGC target 1e5 Maximum IT 50 ms Loop count 20 MSX count 1 TopN 20 Isolation window 2.0 m/z Isolation offset 0.0 m/z Scan range 200 to 2000 m/z						
Scan range Spectrum data type dd-MS² / dd-SIM Microscans Resolution AGC target Maximum IT Loop count MSX count IopN Isolation window Isolation offset Scan range 350 to 1500 m/z Profile Mz Profile Mz Profile 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1		32	ms			
Scan range 350 to 1500 m/z Spectrum data type Profile dd-MS² / dd-SIM 1 Microscans 1 Resolution 15,000 AGC target 1e5 Maximum IT 50 ms Loop count 20 MSX count 1 TopN 20 Isolation window 2.0 m/z Isolation offset 0.0 m/z Scan range 200 to 2000 m/z	Number of scan ranges	1				
dd-MS² / dd-SIM Microscans 1 Resolution 15,000 AGC target 1e5 Maximum IT 50 ms Loop count 20 MSX count 1 TopN 20 Isolation window 2.0 m/z Isolation offset 0.0 m/z Scan range 200 to 2000 m/z		350 to 1500	m/z			
Microscans 1 Resolution 15,000 AGC target 1e5 Maximum IT 50 ms Loop count 20 MSX count 1 TopN 20 Isolation window 2.0 m/z Isolation offset 0.0 m/z Scan range 200 to 2000 m/z	Spectrum data type	Profile				
Resolution 15,000 AGC target 1e5 Maximum IT 50 ms Loop count 20 MSX count 1 TopN 20 Isolation window 2.0 m/z Isolation offset 0.0 m/z Scan range 200 to 2000 m/z	dd-MS ² / dd-SIM					
AGC target 1e5 Maximum IT 50 ms Loop count 20 MSX count 1 TopN 20 Isolation window 2.0 m/z Isolation offset 0.0 m/z Scan range 200 to 2000 m/z	Microscans	1				
Maximum IT 50 ms Loop count 20 MSX count 1 TopN 20 Isolation window 2.0 m/z Isolation offset 0.0 m/z Scan range 200 to 2000 m/z	Resolution	15,000				
Loop count 20 MSX count 1 TopN 20 Isolation window 2.0 m/z Isolation offset 0.0 m/z Scan range 200 to 2000 m/z	AGC target					
MSX count 1 TopN 20 Isolation window 2.0 m/z Isolation offset 0.0 m/z Scan range 200 to 2000 m/z			ms			
TopN 20 Isolation window 2.0 m/z Isolation offset 0.0 m/z Scan range 200 to 2000 m/z						
Isolation window 2.0 m/z Isolation offset 0.0 m/z Scan range 200 to 2000 m/z						
Isolation offset 0.0 m/z Scan range 200 to 2000 m/z			,			
Scan range 200 to 2000 m/z						
rixed first mass 110.0 m/z						
	fixed first mass	110.0	m/Z			

(N)CE / stepped (N)CE nce: 26 Spectrum data type Profile dd Settings 1.00e3 Minimum AGC target Intensity threshold 2.0e4 Apex trigger unassigned, 1, 5 - 8, >8Charge exclusion Multiple charge states Peptide match preferred Exclude isotopes on Dynamic exclusion 25.0 s If idle ..

Setup

do not pick others

Tunefiles

General

Switch Count 0

Base Tunefile C:\Xcalibur\methods\nanosource.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

Pump setup

Syringe type Hamilton
Flow rate 3.000 µL/
Inner diameter 2.303 mm $3.000 \, \mu L/min$ Volume 250 µL

Divert Valve A

General

False Used Start in 1-2 True Switch Count

Divert Valve B

General

Used False Start in 1-2 True Switch Count 0

