Method of Q Exactive

OVERALL METHOD SETTINGS

Global Settings

Use lock masses best

Lock mass injection ―

Chrom. peak width (FWHM) 15 s

Time

Method duration 20.00 min

Customized Tolerances (+/-)

Lock Masses ―

Inclusion ―

Exclusion ―

Neutral Loss ―

Mass Tags ―

Dynamic Exclusion ―

Experiment

FULL MS / DD-MS² (TOPN)

General

Runtime 0 to 20 min

Polarity positive

In-source CID 0.0 eV

Default charge state 1

Inclusion ―

Exclusion ―

Tags ―

Full MS

Microscans 1

Resolution 140,000

AGC target 3e6

Maximum IT 200 ms

Number of scan ranges 1

Scan range 150 to 1500 m/z

Spectrum data type Profile

dd-MS² / dd-SIM

Microscans 1

Resolution 17,500

AGC target 1e5

Maximum IT 50 ms

Loop count 3

MSX count 1

TopN 3

Isolation window 4.0 m/z

Isolation offset 0.0 m/z

Scan range 200 to 2000 m/z

Fixed first mass ―

(N)CE / stepped (N)CE nce: 10, 30, 100

Spectrum data type Profile

dd Settings

Minimum AGC target 8.00e3

Intensity threshold 1.6e5

Apex trigger ―

Charge exclusion unassigned, 3 - 8, >8

Multiple charge states all

Peptide match ―

Exclude isotopes on

Dynamic exclusion 30.0 s

If idle .. do not pick others

Setup

TUNEFILES

General

Switch Count 0

Base Tunefile C:\Xcalibur\methods\Ben\12\_2\_2020\_Positive\_Metabolomics\_Tune.

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 False

Start in 1-2 False

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

(no entries)

EXCLUSION LIST

(no entries)

NEUTRAL LOSSES

(no entries)

MASS TAGS

(no entries)

HPLC method

---- Overview ----

Name: New Instrument Method

Comment:

Run time: 20.000 [min]

Instrument: GD69132\_1 on gd69132

Description:

---- Script ----

initial Instrument Setup

ColumnOven.TempCtrl: Off

ColumnOven.Cooler\_TempCtrl: Off

Sampler.InjectWash: BeforeInj

Sampler.WashSpeed: 8.333 [µl/s]

Sampler.WashVolume: 100.000 [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: 2.000 [µl/s]

Sampler.DrawDelay: 3.000 [s]

Sampler.InjectMode: Normal

Sampler.PumpDevice: "Pump"

Sampler.LoopWashFactor: 2.000

Sampler.TempCtrl: On

Sampler.Temperature.Nominal: 4.0 [°C]

Sampler.ReadyTempDelta: 5.0 [°C]

Sampler.Temperature.LowerLimit: 4.0 [°C]

Sampler.Temperature.UpperLimit: 45.0 [°C]

PumpModule.Pump.%A.Equate: "1mM am ac, 0.1%FA"

PumpModule.Pump.%B.Equate: "80% ACN, 1mM am ac, 0.1%FA"

PumpModule.Pump.%C.Equate: "C"

PumpModule.Pump.%D.Equate: "ACN 100%"

PumpModule.Pump.Pressure.LowerLimit: 0 [bar]

PumpModule.Pump.Pressure.UpperLimit: 1034 [bar]

PumpModule.Pump.MaximumFlowRampUp: 6.000 [ml/min²]

PumpModule.Pump.MaximumFlowRampDown: 6.000 [ml/min²]

0.000 [min] Equilibration

PumpModule.Pump.Flow.Nominal: 0.200 [ml/min]

PumpModule.Pump.%B.Value: 0.0 [%]

PumpModule.Pump.%C.Value: 0.0 [%]

PumpModule.Pump.%D.Value: 0.0 [%]

PumpModule.Pump.Curve: 5

0.000 [min] Inject Preparation

Wait ColumnOven.Ready And Sampler.Ready And PumpModule.Pump.Ready

0.000 [min] Inject

Sampler.Inject

0.000 [min] Start Run

ColumnOven.ColumnOven\_Temp.AcqOn

ColumnOven.Cooler\_Temp.AcqOn

PumpModule.Pump.Pump\_Pressure.AcqOn

0.000 [min] Run

PumpModule.Pump.Flow.Nominal: 0.200 [ml/min]

PumpModule.Pump.%B.Value: 0.0 [%]

PumpModule.Pump.%C.Value: 0.0 [%]

PumpModule.Pump.%D.Value: 0.0 [%]

PumpModule.Pump.Curve: 5

2.000 [min]

PumpModule.Pump.Flow.Nominal: 0.200 [ml/min]

PumpModule.Pump.%B.Value: 0.0 [%]

PumpModule.Pump.%C.Value: 0.0 [%]

PumpModule.Pump.%D.Value: 0.0 [%]

PumpModule.Pump.Curve: 5

13.000 [min]

PumpModule.Pump.Flow.Nominal: 0.200 [ml/min]

PumpModule.Pump.%B.Value: 60.0 [%]

PumpModule.Pump.%C.Value: 0.0 [%]

PumpModule.Pump.%D.Value: 0.0 [%]

PumpModule.Pump.Curve: 5

15.000 [min]

PumpModule.Pump.Flow.Nominal: 0.200 [ml/min]

PumpModule.Pump.%B.Value: 100.0 [%]

PumpModule.Pump.%C.Value: 0.0 [%]

PumpModule.Pump.%D.Value: 0.0 [%]

PumpModule.Pump.Curve: 5

16.000 [min]

PumpModule.Pump.Flow.Nominal: 0.200 [ml/min]

PumpModule.Pump.%B.Value: 0.0 [%]

PumpModule.Pump.%C.Value: 0.0 [%]

PumpModule.Pump.%D.Value: 0.0 [%]

PumpModule.Pump.Curve: 5

20.000 [min]

PumpModule.Pump.Flow.Nominal: 0.200 [ml/min]

PumpModule.Pump.%B.Value: 0.0 [%]

PumpModule.Pump.%C.Value: 0.0 [%]

PumpModule.Pump.%D.Value: 0.0 [%]

PumpModule.Pump.Curve: 5

20.000 [min] Stop Run

ColumnOven.ColumnOven\_Temp.AcqOff

ColumnOven.Cooler\_Temp.AcqOff

PumpModule.Pump.Pump\_Pressure.AcqOff