

## Acquisition Method Info

Method Name ACN\_Test\_27\_ink.wash.m  
Method Path C:\Masshunter\methods\User\Katharina\ACN\_Test\_27\_ink.wash.m  
Method Description Default Method

## Device List

HiP Sampler  
Binary Pump  
Column Comp.  
DAD  
QQQ

## MS QQQ Mass Spectrometer

Ion Source AJS ESI Tune File atunes.tune.xml  
Stop Mode No Limit/As Pump Stop Time (min) 1  
Time Filter On Time Filter Width (min) 0.07

## Time Segments

Index	Start Time (min)	Scan Type	Ion Mode	Div Valve	Delta EMV	Store
1	0	MS2 Scan	ESI+Agilent Jet Stream	To MS	0	No
2	1	MRM	ESI+Agilent Jet Stream	To MS	200	Yes
3	3	MRM	ESI+Agilent Jet Stream	To MS	200	Yes
4	4.5	MRM	ESI+Agilent Jet Stream	To Waste	200	No

## Time Segment 1

## Scan Segments

Segment Name	Start Mass	End Mass	Scan Time	Frag (V)	Cell Acc (V)	Polarity
	100	1000	500	380	5	Positive

## Scan Parameters

Step Size (amu)	Data Stg	Threshold
0.1	Profile	0

## Source Parameters

Parameter	Value (+)	Value (-)
Gas Temp (°C)	290	290
Gas Flow (l/min)	14	14
Nebulizer (psi)	20	20
SheathGasHeater	300	300
SheathGasFlow	11	11
Capillary (V)	3000	3000
VCharging	1500	1500

## Time Segment 2

## Scan Segments

Cpd Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)	Cell Acc (V)	Polarity
Cortisol	No	363.22	Widest / Wide (6490)	121.1	Wide / Unit (6490)	100	380	28	5	Positive
Cortisol	No	363.22	Widest / Wide (6490)	90.9	Wide / Unit (6490)	100	380	60	5	Positive
Cortisone	No	361.2	Widest / Wide (6490)	163	Wide / Unit (6490)	100	380	24	5	Positive
Cortisone	No	361.2	Widest / Wide (6490)	121.1	Wide / Unit (6490)	100	380	36	5	Positive
d8Corticosterone	Yes	355.2	Widest / Wide (6490)	337	Wide / Unit (6490)	100	380	9	5	Positive
d8Corticosterone	Yes	355.2	Widest / Wide (6490)	125.1	Wide / Unit (6490)	100	380	25	5	Positive

## Scan Parameters

Data Stg      Threshold  
Centroid      0

## Source Parameters

Parameter	Value (+)	Value (-)
Gas Temp (°C)	290	290
Gas Flow (l/min)	14	14
Nebulizer (psi)	20	20
SheathGasHeater	300	300
SheathGasFlow	11	11
Capillary (V)	3000	3000
VCharging	1500	1500

## Ion Funnel Parameters

Pos High Pressure RF	150	Neg High Pressure RF	150
Pos Low Pressure RF	60	Neg Low Pressure RF	60



## Time Segment 3

## Scan Segments

Cpd Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)	Cell Acc (V)	Polarity
7-keto-27-hydroxycholesterol	No	417.34	Wide / Unit (6490)	417.3	Wide / Unit (6490)	100	380	0	5	Positive
D6-7-beta-27-hydroxycholesterol	Yes	407.38	Wide / Unit (6490)	389.1	Wide / Unit (6490)	100	380	20	5	Positive
D6-7-beta-27-hydroxycholesterol	Yes	407.38	Wide / Unit (6490)	159.1	Wide / Unit (6490)	50	380	24	5	Positive
7-alpha-27-hydroxycholesterol	No	383.3	Wide / Unit (6490)	383.3	Wide / Unit (6490)	100	380	0	5	Positive
7-alpha-27-hydroxycholesterol	No	383.3	Wide / Unit (6490)	159	Wide / Unit (6490)	150	380	20	5	Positive
7-beta-27-hydroxycholesterol	No	383.3	Wide / Unit (6490)	159	Wide / Unit (6490)	100	380	20	5	Positive

## Scan Parameters

Data Stg Centroid Threshold  
0

## Source Parameters

Parameter	Value (+)	Value (-)
Gas Temp (°C)	290	290
Gas Flow (l/min)	14	14
Nebulizer (psi)	20	20
SheathGasHeater	300	300
SheathGasFlow	11	11
Capillary (V)	3000	3000
VCharging	1500	1500

## Ion Funnel Parameters

Pos High Pressure RF	150	Neg High Pressure RF	150
Pos Low Pressure RF	60	Neg Low Pressure RF	60

## Time Segment 4

## Scan Segments

Cpd Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)	Cell Acc (V)	Polarity
Compound 1	No	350	Wide / Unit (6490)	200	Wide / Unit (6490)	200	380	0	5	Positive

## Scan Parameters

Data Stg Centroid Threshold  
0

## Source Parameters

Parameter	Value (+)	Value (-)
Gas Temp (°C)	290	290
Gas Flow (l/min)	14	14
Nebulizer (psi)	20	20
SheathGasHeater	300	300
SheathGasFlow	11	11
Capillary (V)	3000	3000
VCharging	1500	1500

## Ion Funnel Parameters

Pos High Pressure RF	150	Neg High Pressure RF	150
Pos Low Pressure RF	60	Neg Low Pressure RF	60

## Chromatograms

Chrom Type	Label	Offset	Y-Range
TIC	TIC	0	10000000

## Instrument Curves

Actual

Name: HiP Sampler Model: G4226A

**Auxiliary**

Draw Speed	100.0 µL/min
Eject Speed	100.0 µL/min
Draw Position Offset	0.0 mm
Wait Time After Drawing	2.0 s
Sample Flush Out Factor	5.0
Vial/Well bottom sensing	Yes

**Injection**

Injection Mode	Injection with needle wash
Injection Volume	1.00 µL
Needle Wash	
Needle Wash Location	Flush Port
Wash Time	10.0 s

**High throughput**

Automatic Delay Volume Reduction	No
Overlapped Injection	
Enable Overlapped Injection	No

**Valve Switching**

Valve Movements	0
Valve Switch Time 1	
Switch Time 1 Enabled	No
Valve Switch Time 2	
Switch Time 2 Enabled	No
Valve Switch Time 3	
Switch Time 3 Enabled	No
Valve Switch Time 4	
Switch Time 4 Enabled	No

**Stop Time**

Stoptime Mode	As pump/No limit
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**Post Time**

Posttime Mode	Off
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Name: Binary Pump Model: G4220A

Flow 0.500 mL/min  
 Use Solvent Types Yes  
 Stroke Mode Synchronized  
 Low Pressure Limit 0.00 bar  
 High Pressure Limit 1000.00 bar  
 Max. Flow Ramp Up 100.000 mL/min<sup>2</sup>  
 Max. Flow Ramp Down 100.000 mL/min<sup>2</sup>  
 Expected Mixer Jet Weaver V35 Mixer

#### Stroke A

Automatic Stroke Calculation A Yes

#### Stop Time

Stoptime Mode Time set  
 Stoptime 8.00 min

#### Post Time

Posttime Mode Time set  
 Posttime 1.00 min

#### Solvent Composition

	Channel	Ch. 1 Solv.	Name 1	Ch2 Solv.	Name 2	Selected	Used	Percent
1	A	5.0 % ACN in Water V.02		5.0 % Methanol in Water V.02	0.1% FA	Ch. 1	Yes	55.00 %
2	B	95.0 % ACN in Water V.02		95.0 % Methanol in Water V.02	0.1%FA	Ch. 1	Yes	45.00 %

#### Timetable

	Time	A	B	Flow	Pressure
1	4.00 min	3.00 %	97.00 %	--- mL/min	--- bar
2	4.50 min	20.00 %	80.00 %	--- mL/min	--- bar
3	7.00 min	20.00 %	80.00 %	--- mL/min	--- bar
4	8.00 min	55.00 %	45.00 %	--- mL/min	--- bar

Name: Column Comp. Model: G1316C

Valve Position Position 1 (Port 1 -> 2)  
 Ready when front door open Yes

#### Left Temperature Control

Temperature Control Mode Temperature Set  
 Temperature 65.0 °C

##### Enable Analysis Left Temperature

Enable Analysis Left Temperature On Yes  
 Enable Analysis Left Temperature Value 0.8 °C

#### Right Temperature Control

Right temperature Control Mode Combined

##### Enable Analysis Right Temperature

Enable Analysis Right Temperature On Yes  
 Enable Analysis Right Temperature Value 0.8 °C

#### Stop Time

Stoptime Mode As pump/injector

#### Post Time

Posttime Mode Off

#### Timetable

Name: DAD Model: G4212A

Peakwidth > 0.1 min (2 s response time) (2.5 Hz)  
Slit 4 nm  
UV Lamp Required No  
Analog Output  
Analog Zero Offset 5 %  
Analog Attenuation 1000 mAU

Signals  
Prepare Mode  
Margin for negative Absorbance 100 mAU  
Autobalance  
Autobalance Prerun No  
Autobalance Postrun No

Spectrum  
Spectrum Store None  
Stoptime  
Stoptime Mode As pump/injector  
Posttime  
Posttime Mode Off

Timetable  
Signals  
Signal table

	Acquire	Signal
1	No	Signal A
2	No	Signal B
3	No	Signal C
4	No	Signal D
5	No	Signal E
6	No	Signal F
7	No	Signal G
8	No	Signal H