

## 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) 0.1  
 Data Stg Profile  
 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

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 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 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	3.00 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
---------------	------------------

### Post Time

Posttime Mode	Off
---------------	-----

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

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

**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