

SERVICE How To

Model Number: A2PREP	Originator: Petro van Poppel	Topic Instrument Setup Instructions
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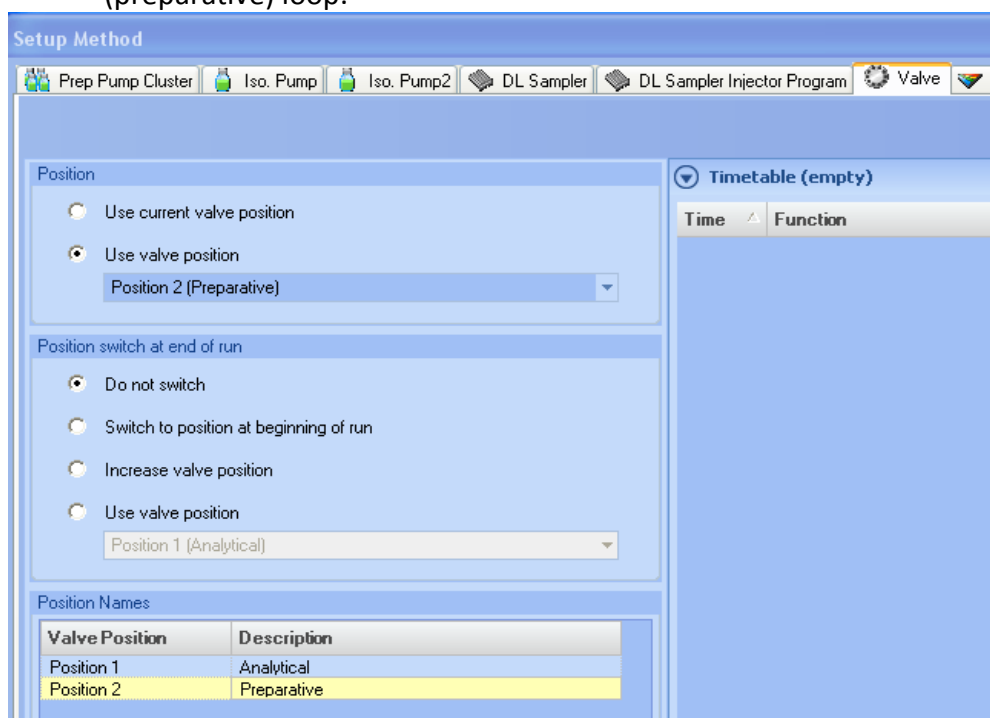
Instrument Setup

You will need:

- Solvent A: 100% water
- Solvent B: 100% ACN
- Standard Combined 1260 Analytical/Preparative System

Procedure:

1. Turn On all modules, prime and purge the pumps for 1 minute at 100mL/min.
2. Create and run a Purge method for the Dual Loop sampler. Use the following details to create the method:
 - a. Use the purge syringe command and purge at least 5 x the dual loop autosampler.
 - b. Select the preparative flow path in the method and ensure that the purge method starts with an injection of a blank in the upper (preparative) loop.



- c. Write an injector program and inject a blank of 4000 μL of ACN to purge the injection needle.

Setup Method

Prep Pump Cluster DL Sampler DL Sampler Injector Program Valve MWD VWD Fraction Collector Instrument Curves

☒ Use Injector Program

Function	Parameter
Valve	Switch valve to "Mainpass"
Valve	Switch valve to "Bypass"
Draw	Draw 4000 μL from sample with default speed using default offset
Eject	Eject default volume to seat with default speed using default offset
Valve	Switch valve to "Mainpass"
Inject	Inject
Wait	Wait 1.5 min
Valve	Switch valve to "Bypass"
Wait	Wait 1 min
Valve	Switch valve to "Mainpass"

Rectangular Snip

- d. Write the following gradient to purge both loops.

Setup Method

Prep Pump Cluster Iso. Pump Iso. Pump2 DL Sampler DL Sampler Injector Program Valve DAD AFC Cluster Instrument Curves

Flow: 10.000 mL/min

Solvents:

A: 98.0 % Water

B: 2.0 % ACN

Pressure Limits:

Min: 0.00 bar Max: 400.00 bar

Stoptime: ☐ As Injector/No Limit ☒ 4.00 min

Posttime: ☐ Off ☒ 1.00 min

Timetable (3/97 events)

Time [min]	A [%]	B [%]	Flow [mL/min]	Max. Pressure Limit [bar]
0.00	98.0	2.0	10.000	400.00
0.01	2.0	98.0
2.00	2.0	98.0
2.01	98.0	2.0

- e. Stop method after 4 minutes.
- f. Save method as "Purge Injector Method"

Standard Analytical Method

3. Create a Standard Analytical method, with the following details:
 - a. Flow of 1.5mL per minute, Gradient from 2% to 98% organics, 6 minute runtime.

Setup Method

Prep Pump Cluster | Iso. Pump | Iso. Pump2 | DL Sampler | DL Sampler Injector Program | Valve | DAD | AFC Cluster | Instrument Curves

Flow
1.500 mL/min

Solvents
A: 98.0 % Water
B: 2.0 % ACN

Pressure Limits
Min: 0.00 bar Max: 400.00 bar

Stopline
☐ As Injector/No Limit
☒ 6.00 min

Posttime
☒ Off
☐ 1.00 min

Timetable (5/97 events)

Time [min]	A [%]	B [%]	Flow [mL/min]	Max. Pressure Limit [bar]
0.00	98.0	2.0	1.500	400.00
0.33	98.0	2.0
3.53	2.0	98.0
4.53	2.0	98.0
4.55	98.0	2.0
6.00	98.0	2.0

- b. Inject 5µL of test sample while using a plug (80%water/20% CAN).

Setup Method

Prep Pump Cluster | Iso. Pump | Iso. Pump2 | DL Sampler | DL Sampler Injector Program | Valve | DAD | AFC Cluster | Instrument Curves

Injection Mode
Use Loop: Lower (50 µL)
☒ Fill loop partially with 10.00 µL
☐ Overfill loop by factor 1.0
☐ Standard injection
☒ Injection with needle wash

Needle wash
Mode: Flush Port
Time: 10.0 sec
Location:
Repeat: 3 times

Stopline
☒ As Pump/No Limit
☐ 1.00 min

Posttime
☒ Off
☐ 1.00 min

Advanced

Auxiliary
Draw speed: 5.000.0 µL/min
Eject speed: 5.000.0 µL/min
Draw position: 0.0 mm
Equilibration time: 1.2 sec
☒ Vial/Well bottom sensing

High throughput
☐ Enable overlapped injection
0.00 min

Plug Settings
☒ Draw Plug before and after the sample
Plug Volume: 5.0 µL
Draw Plug from:
☐ Air
☒ Location Vial 1

- c. Use at least a 10 seconds needle wash and the injection cleaning (10x) function to reduce carryover.
 - d. Set the flow path to analytical.

Setup Method

Prep Pump Cluster | Iso. Pump | Iso. Pump2 | DL Sampler | DL Sampler Injector Program | **Valve** | DAD

Position

☐ Use current valve position

☒ Use valve position

Position 1 (Analytical)

Position switch at end of run

☒ Do not switch

☐ Switch to position at beginning of run

☐ Increase valve position

☐ Use valve position

Position 1 (Analytical)

Position Names

Valve Position	Description
Position 1	Analytical
Position 2	Preparative

Timetable (empty)

Time	Function
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- e. Set the UV absorption to 254 nm use a bandwidth of 30 nm.
- f. Switch the fraction collector for analytical scouting runs “off”.

Setup Method

Prep Pump Cluster | Iso. Pump | Iso. Pump2 | DL Sampler | DL Sampler Injector Program | Valve | DAD | **AFC Cluster** | Instrument Curves

Fraction Trigger Mode

☒ Off

☐ Peak-based max. peak duration 0.5 min

☐ Time-based with number of fractions 1

☐ Time-based with timeslices 0.10 min

Peak Detector

Detector	Unit	Mode	Up Slope (/s)	Down Slope (/s)	Threshold	Upper Threshold
G131SC-DE A0000003	mAU	Off	5.00	5.00	5.000	3000.000
G1390A-PP 00000028	mV	Off	5.00	5.00	5.000	3000.000

☐ Use MSD for mass-based Fraction Collection

Fraction is collected when a peak is detected by

☐ all peak detectors

☒ at least one peak detector

Stoptime

☒ As Pump/Injector

☐ 1.00 min

Posttime

☒ Off

☐ 1.00 min

Advanced

Rinse Fraction Collector Needle

☐ At the start of collection

☐ Between fraction collection

Auxiliary

Max. fill volume per location

☒ as configured

☐ 0.50 mL

Timetable (empty)

Load...

- g. Monitor the Solvent Compositions.
- h. If an MSD is part of the system, follow steps i to k.
- i. Set the correct parameters for the ion source.

MSD Spray Chamber

Method Spray Chamber: API-ES ON

Installed Spray Chamber:

Parameters

	Positive	Negative
Capillary Voltage (V):	4000	3000
Corona Current (µA):	N/A	N/A
Charging Voltage (V):	N/A	N/A

Temperatures, Pressure, and Flow

	Actual	Setpoint	Maximum
Drying Gas Flow (l/min):		12.0	13.0
Nebulizer Pressure (psig):		50	60
Drying Gas Temperature (°C):		350	350
Vaporizer Temperature (°C):		N/A	N/A
Sheath Gas Temperature (°C):		N/A	N/A
Sheath Gas Flow (l/min):		N/A	N/A

Time Table

Time (min)	Parameter	Value
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j. Set the parameters of scan speed and scan range.

Set Up MSD Signals

MSD Control: ☒ Use MSD
StopTime: noLimit
FIA Disabled

General

Tune File: atunes.tun
Ion Source: API-ES
Peakwidth: 0.070 min
Cycle Time: 1.11 sec/cycle

☐ Ultra Fast Scan
☒ Time Filter
Scan Data Storage: Condensed

Active Signals:
☒ 1 Positive TIC
☒ 2 Negative TIC
☐ 3
☐ 4

MSD Signal Settings

Signal: 1 Mode: Scan % cycle time: 50.0

Polarity: Positive

Time(min)	On/Off	Mass Range Low	Mass Range High	Frag-mentor	Gain	Thres-hold	Step size
1 0.00	<input checked="" type="checkbox"/>	125.00	725.00	70	1.00	150	0.10

Signal: 2 Mode: Scan % cycle time: 50.0

Polarity: Negative

Time(min)	On/Off	Mass Range Low	Mass Range High	Frag-mentor	Gain	Thres-hold	Step size
1 0.00	<input checked="" type="checkbox"/>	125.00	725.00	70	1.00	150	0.10

k. Disable Fraction Collection.

Fraction Collection

EC Mode

☒ None
☐ TIC
☐ Use method target masses
☐ Use sample target masses
☐ Import mass info from

MS Signals

Signal	Monitor	Polarity	Mode
Signal 1	<input type="checkbox"/>	Positive	Scan
Signal 2	<input type="checkbox"/>	Negative	Scan
Signal 3	<input type="checkbox"/>		
Signal 4	<input type="checkbox"/>		

Detectors

☒ MS only
☐ Other only
☐ MS and other
☐ MS or other
☐ Time based

Positive Adducts

☐ M
☐ M+H(1)
☐ M+NH4(18)
☐ M+Na(23)
☐ M+K(39)
☐ M+
☐ M+
☐ M+

Charge State

☐ 2 ☐ 3

Negative Adducts

☐ M
☐ M+H(-1)
☐ M+Cl- (35,37)
☐ M+formate- (45)
☐ M+TFA- (113)
☐ M+
☐ M+
☐ M+

Charge State

☐ 2 ☐ 3

Method Target Masses

Edit Mass:

l. Set the parameters for data processing.

Signal Details: Labor

Available Signals

MSD2 TIC, MS File [Add to Method]

Insert Row Append Row Delete Row

Signal Description	Start	End	Delay	Align	Peak 1	Peak 2	Align Window	
DAD1 A, Sig=254,30 Ref=off	0.000	0.000	0.000	Anchor Signal	0.000	0.000	0.000	No
MSD1 TIC, MS File	0.000	0.000	0.000	Align to Anchor	0.000	0.000	0.000	No
MSD2 TIC, MS File	0.000	0.000	0.000	Align to Anchor	0.000	0.000	0.000	No

OK Cancel Help

m. Set the correct integration parameters (start/stop value, peak width/height and area reject).

Edit Integration Events

Method Manual Events ☐

OK Cancel

Initial Events For All Signals:

Integration Events	Value
Tangent Skim Mode	Standard
Baseline Correction	Advanced
Tail Peak Skim Height Ratio	0.00
Front Peak Skim Height Ratio	0.00
Skim Valley Ratio	20.00
Peak to Valley Ratio	500.00

Specific Events For Signal:

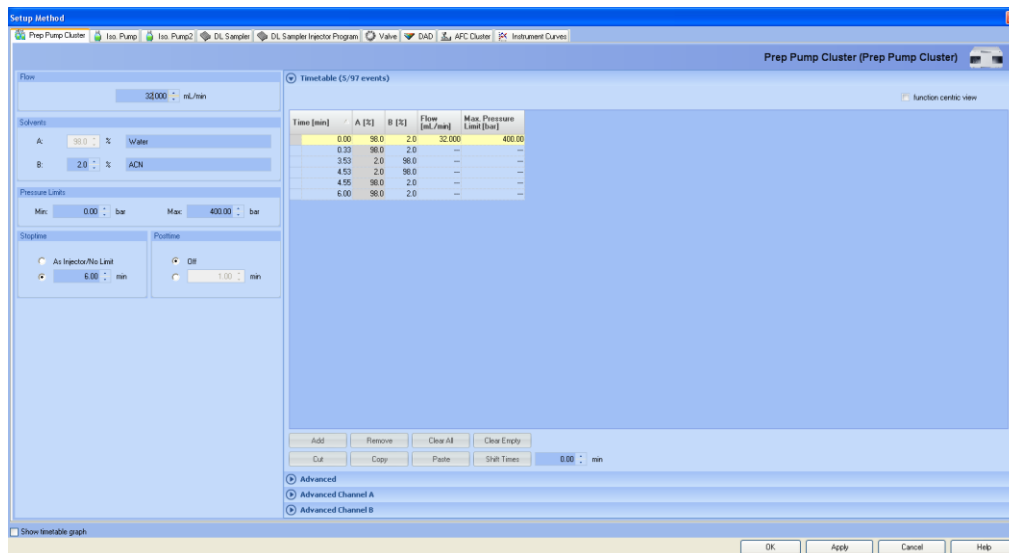
MSD1 TIC Specific

Time	Integration Events	Value
Initial	Slope Sensitivity	100000
Initial	Peak Width	0.08
Initial	Area Reject	1000
Initial	Height Reject	100000
Initial	Shoulders	OFF
1.500	Integration	ON
5.500	Integration	OFF

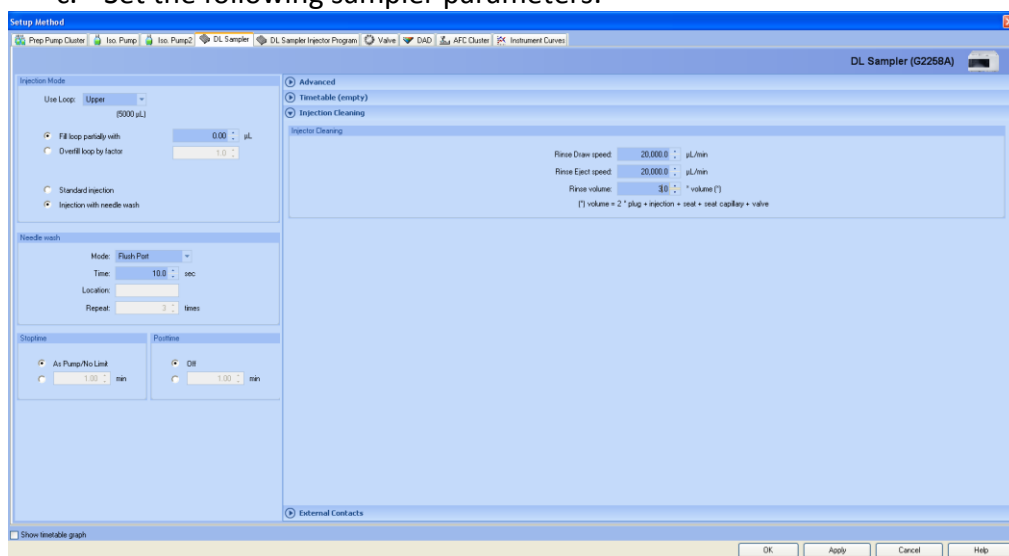
n. Save the method as "Analyt_Basis_Short" and create a sequence of 3 injections.

Standard Preparative Method

4. Create a Standard Preparative method, with the following details:
 - a. Flow of 32mL per minute, Gradient from 2% to 98% organics, 6 minute runtime.

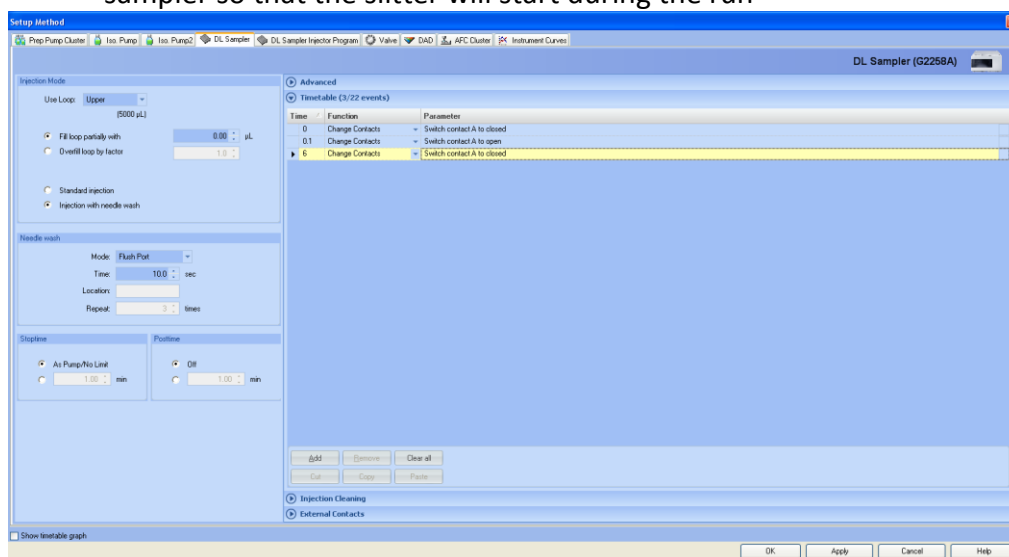


- b. If an MSD is used, set a make-up flow rate of 1.5mL/min, where the make-up solvent contains: 70 % Methanol/ 25% Acetonitrile/4.9 % Water/ 0.1% Formic Acid.
 - c. Set the following sampler parameters.

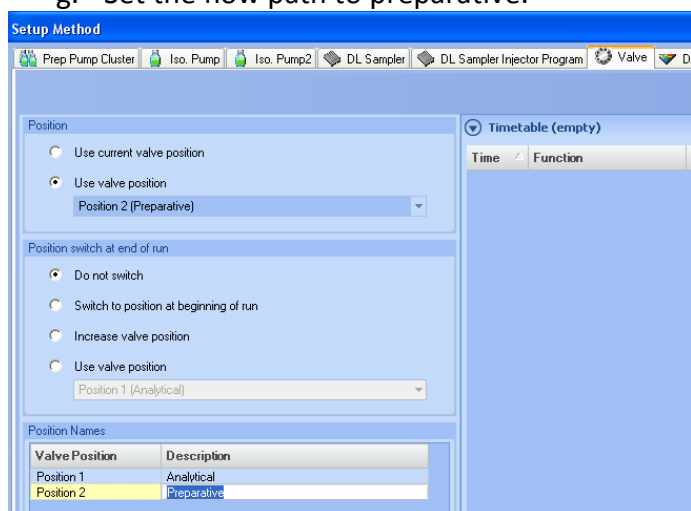


- d. Use at least a 10 seconds needle wash and the injection cleaning (3x) function to reduce carryover.
 - e. Set the active flow split to a split ratio of 1:1000

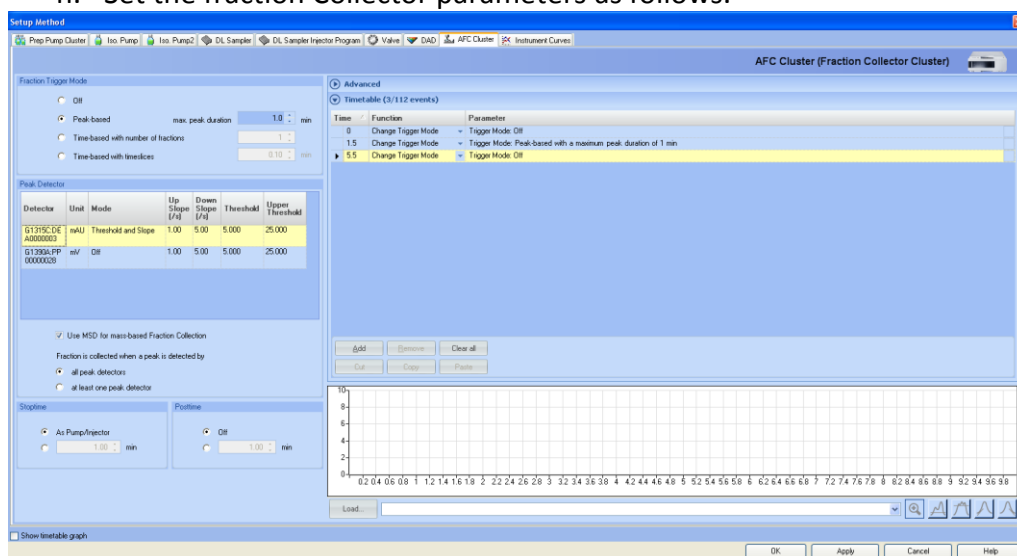
- f. Start and stop the active flow split by an external contact event on the sampler so that the splitter will start during the run



- g. Set the flow path to preparative.



- h. Set the fraction Collector parameters as follows.



- i. Set the UV absorption to 254 nm use a bandwidth of 30 nm.
j. If an MSD is part of the system, follow steps i to k.

k. Set the correct parameters for the ion source.

Fraction Collection

FC Mode

☐ None

☐ TIC

☐ Use method target masses

☒ Use sample target masses

☐ Import mass info from

MS Signals

Monitor: ☒ Signal 1, ☒ Signal 2, ☐ Signal 3, ☐ Signal 4

Polarity: ☒ Positive, ☒ Negative

Mode: ☐ Scan, ☐ Scan

Detectors

☒ MS only

☐ Other only

☐ MS and other

☐ MS or other

☐ Time based

Positive Adducts

☐ M

☒ M+H(1)

☐ M+NH4(18)

☐ M+Na(23)

☐ M+K(39)

☐ M+

☐ M+

☐ M+

Negative Adducts

☐ M

☒ M+H(1)

☐ M+Cl (35,37)

☐ M+formate- (45)

☐ M+TFA- (113)

☐ M+

☐ M+

☐ M+

Method Target Masses

Selected

Edit Mass:

Insert

Remove

Parameters

OK Cancel Help

l. Set the threshold and slope parameters for mass based fraction collection.

Fraction Collection Parameters

Model Type

FC Hardware: Configuration...

Trigger Type:

Peak Timing

Min Peak Width (min): Time Limit (min):

Max Peak Width (min):

MSD m/z Detection

Peak Slope (counts/sec): Collector Delay (min):

Threshold (counts): Mass Window (+/-):

Analog Output Range (kilo counts/volt):

Other Detector

Peak Slope (mv/sec): Collector Delay (min):

Threshold (%): Full Scale (volts):

OK Cancel Help

m. Leave the delay value as is, it would have been adjusted from the purification software (Delay Calibration).

n. Save the method as "Prep_Basis_Short".