

# Demo MDLC method

# 2D method overview

Fraction	Fractionation (Grad2 method)	Salt injection (AS method)	Conc. of NH <sub>4</sub> OAc	pH 10 %B	pH 2 %B	V2 position
0	√	26 min	1000 mM	0-12	/	A / Load
1	√	26 min	1000 mM	12-14	8-20	B / Inject
2	√	26 min	1000 mM	14-16	8-25	A / Load
3	√	26 min	1000 mM	16-19	10-25	B / Inject
4	√	26 min	1000 mM	19-22	10-28	A / Load
5	√	26 min	1000 mM	22-26	12-30	B / Inject
6	√	26 min	1000 mM	26-30	12-32	A / Load
7	√	26 min	1000 mM	30-45	15-35	B / Inject
8	×	/	/	/	20-40	A / Load

# 3D method overview

Fraction	Fractionation (Grad2 method)	Salt injection (AS method)	Conc. of NH <sub>4</sub> OAc	pH 10 %B	pH 2 %B	V2 position
0	√	26 min	20 mM	0-12	/	A / Load
1	√	26 min	20 mM	12-14	5-20	B / Inject
2	×	0 min	100 mM	/	8-20	A / Load
3	×	0 min	100 mM	/	5-20	B / Inject
4	×	0 min	1000 mM	/	8-20	A / Load
5	×	0 min	1000 mM	/	5-20	B / Inject
6	√	26 min	20 mM	14-16	8-20	A / Load
7	√	26 min	20 mM	16-19	8-22	B / Inject
8	×	0 min	100 mM	/	10-25	A / Load
9	×	0 min	100 mM	/	8-22	B / Inject
10	×	0 min	1000 mM	/	10-25	A / Load
11	×	0 min	1000 mM	/	8-22	B / Inject
12	√	26 min	20 mM	19-22	10-25	A / Load
13	√	26 min	20 mM	22-26	12-28	B / Inject
14	×	0 min	100 mM	/	15-32	A / Load
15	×	0 min	100 mM	/	12-28	B / Inject
16	×	0 min	1000 mM	/	15-32	A / Load
17	×	0 min	1000 mM	/	12-28	B / Inject
18	√	26 min	20 mM	26-30	15-32	A / Load
19	√	26 min	20 mM	30-45	20-35	B / Inject
20	×	0 min	100 mM	/	20-35	A / Load
21	×	0 min	100 mM	/	20-35	B / Inject
22	×	0 min	1000 mM	/	20-35	A / Load
23	×	0 min	1000 mM	/	20-35	B / Inject
24	×	/	/	/	20-35	A / Load

Eksigent demo method

## Eksigent Injection sequence

Run Table: 2D dualtrap\_PPRP\_20121220\_\_8F\_90mingrad\_HENRY.ini

Seq #	Run	Autosampler			LC		hi
		Method	Tray	Vial	Method	Channel	
1	<input checked="" type="checkbox"/>	2D Dualtrap AS_90mingrad	2	A01	2D Dualtrap Grad2_0	Gradient 2	Q
2	<input checked="" type="checkbox"/>				2D Dualtrap LoadingPump	Loading Pump	Q
3	<input checked="" type="checkbox"/>				2D_C1_P1_90min	Gradient 1	Q
4	<input checked="" type="checkbox"/>	2D Dualtrap AS_90mingrad	2	A01	2D Dualtrap Grad2_1_90min	Gradient 2	Q
5	<input checked="" type="checkbox"/>				2D Dualtrap LoadingPump_90mingrad	Loading Pump	Q
6	<input checked="" type="checkbox"/>				2D_C1_P1_90min	Gradient 1	Q
7	<input checked="" type="checkbox"/>	2D Dualtrap AS_90mingrad	2	A01	2D Dualtrap Grad2_2_90min	Gradient 2	Q
8	<input checked="" type="checkbox"/>				2D Dualtrap LoadingPump_90mingrad	Loading Pump	Q
9	<input checked="" type="checkbox"/>				2D_C2_P1_90min	Gradient 1	Q
10	<input checked="" type="checkbox"/>	2D Dualtrap AS_90mingrad	2	A01	2D Dualtrap Grad2_3_90min	Gradient 2	Q
11	<input checked="" type="checkbox"/>				2D Dualtrap LoadingPump_90mingrad	Loading Pump	Q
12	<input checked="" type="checkbox"/>				2D_C3_P1_90min	Gradient 1	Q
13	<input checked="" type="checkbox"/>	2D Dualtrap AS_90mingrad	2	A01	2D Dualtrap Grad2_4_90min	Gradient 2	Q
14	<input checked="" type="checkbox"/>				2D Dualtrap LoadingPump_90mingrad	Loading Pump	Q
15	<input checked="" type="checkbox"/>				2D_C4_P1_90min	Gradient 1	Q
16	<input checked="" type="checkbox"/>	2D Dualtrap AS_90mingrad	2	A01	2D Dualtrap Grad2_5_90min	Gradient 2	Q
17	<input checked="" type="checkbox"/>				2D Dualtrap LoadingPump_90mingrad	Loading Pump	Q
18	<input checked="" type="checkbox"/>				2D_C5_P1_90min	Gradient 1	Q
19	<input checked="" type="checkbox"/>	2D Dualtrap AS_90mingrad	2	A01	2D Dualtrap Grad2_6_90min	Gradient 2	Q
20	<input checked="" type="checkbox"/>				2D Dualtrap LoadingPump_90mingrad	Loading Pump	Q

## Eksigent Injection sequence (continue)

Run Table: 2D dualtrap\_PPRP\_20121220\_\_8F\_90mingrad\_HENRY.ini

Seq #	Run	Autosampler			LC		In
		Method	Tray	Vial	Method	Channel	
19	<input checked="" type="checkbox"/>	2D Dualtrap AS_90mingrad	2	A01	2D Dualtrap Grad2_6_90min	Gradient 2	Q
20	<input checked="" type="checkbox"/>				2D Dualtrap LoadingPump_90mingrad	Loading Pump	Q
21	<input checked="" type="checkbox"/>				2D_C6_P1_90min	Gradient 1	Q
22	<input checked="" type="checkbox"/>	2D Dualtrap AS_90mingrad	2	A01	2D Dualtrap Grad2_7_90min	Gradient 2	Q
23	<input checked="" type="checkbox"/>				2D Dualtrap LoadingPump_90mingrad	Loading Pump	Q
24	<input checked="" type="checkbox"/>				2D_C7_P1_90min	Gradient 1	Q
25	<input checked="" type="checkbox"/>	2D Dualtrap AS_90mingrad	2	A01	2D Dualtrap Grad2_8_90min	Gradient 2	Q
26	<input checked="" type="checkbox"/>				2D Dualtrap LoadingPump_90mingrad	Loading Pump	Q
27	<input checked="" type="checkbox"/>				2D_C8_P1_90min	Gradient 1	Q
28	<input type="checkbox"/>						
29	<input type="checkbox"/>						
30	<input type="checkbox"/>						
31	<input type="checkbox"/>						
32	<input type="checkbox"/>						
33	<input type="checkbox"/>						
34	<input type="checkbox"/>						
35	<input type="checkbox"/>						
36	<input type="checkbox"/>						
37	<input type="checkbox"/>						
38	<input type="checkbox"/>						

## Autosampler method

**Autosampler Settings**

**Autosampler Procedure**

Name:

**System Configuration**

Eksigent AS-2

1	External Events		Start Gradient 1		Start LC
2	External Events		Start Gradient 2		Start G
3	External Events		Start Loading Pump		Start Lc
4	Wait	00:30:00			Pause 1
5	Valve		Injector Load		Valve F
6	Aspirate	12 uL	Reagent-1	Speed: 1 Height: 3	Pick-up
7	Wait	00:00:05			Pause 1
8	Aspirate	0 uL	Reagent-1	Speed: 1 Height: 3	Pick-up
9	Aspirate	8 uL	Sample	Speed: 1 Height: 4	Pick-up
10	Wait	00:00:05			Pause 1
11	Aspirate	0 uL	Sample	Speed: 1 Height: 4	Pick-up

Change into (17, 3) when injecting 20 and 100 mM salt in 3D method

## Autosampler method (continue)

**Autosampler Settings**

Autosampler Procedure

Name
2D Dualtrap AS\_90mingrad
Save

System Configuration

Eksigent AS-2
edit

X »	10	Wait	00:00:05						Pause 1
	11	Aspirate	0 uL	Sample	Speed:	1	Height:	4	Pick-up
	12	Aspirate	5 uL	Reagent-1	Speed:	1	Height:	3	Pick-up
	13	Wait	00:00:05						Pause 1
	14	Aspirate	0 uL	Reagent-1	Speed:	1	Height:	3	Pick-up
	15	External Events		Start Loading Pump					Start Lo
	16	Valve		Injector Inject					Switch
	17	Dispense	25 uL	Waste	Speed:	5	Height:	0	Dispens
	18	Wait	01:40:00						Pause 1
	19	END							

Test on A1
Stop!
Print
ToPDF
Audit
OK
Cancel



# LC Method Settings



## Selected Method

Name 2D Dualtrap LoadingPump\_90mingrad



Save

Print

Summary

Run Conditions

Gradient Profile

Gradient Table

	Time (min)	Qa (μL/min)	Event
<input checked="" type="checkbox"/> <input type="checkbox"/>	1	0	3
	2	140	3
	3		
	4		
	5		
	6		
	7		
	8		
	9		
	10		
	11		
	12		
	13		

## Flow Mode

☒ Isocratic

Delete

View Audit Trail

OK

Cancel

# LC Method Settings

## Selected Method

Name

Save

Print

Summary

Run Conditions

Gradient Profile

Gradient Table

	Time (min)	% A	% B	Event
<input checked="" type="checkbox"/> <input type="checkbox"/>	1	0	92	8
	2	1	92	8
	3	91	80	20
	4	96	80	20
	5	106	50	50
	6	111	50	50
	7	116	95	5
	8	140	95	5
	9			
	10			
	11			
	12			
	13			

## Flow Mode

☒ Conserved flow

☐ Independent flow

## Profile Editor

Total flowrate:

nL/min

Change these number accordingly to the fraction and save as individual methods

Delete

View Audit Trail

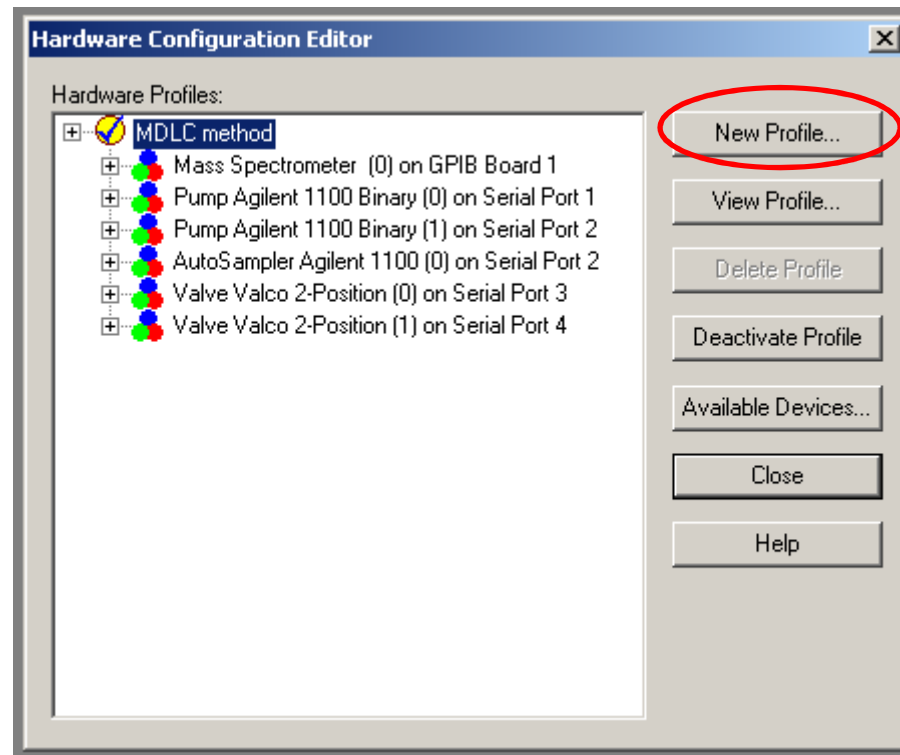
OK

Cancel

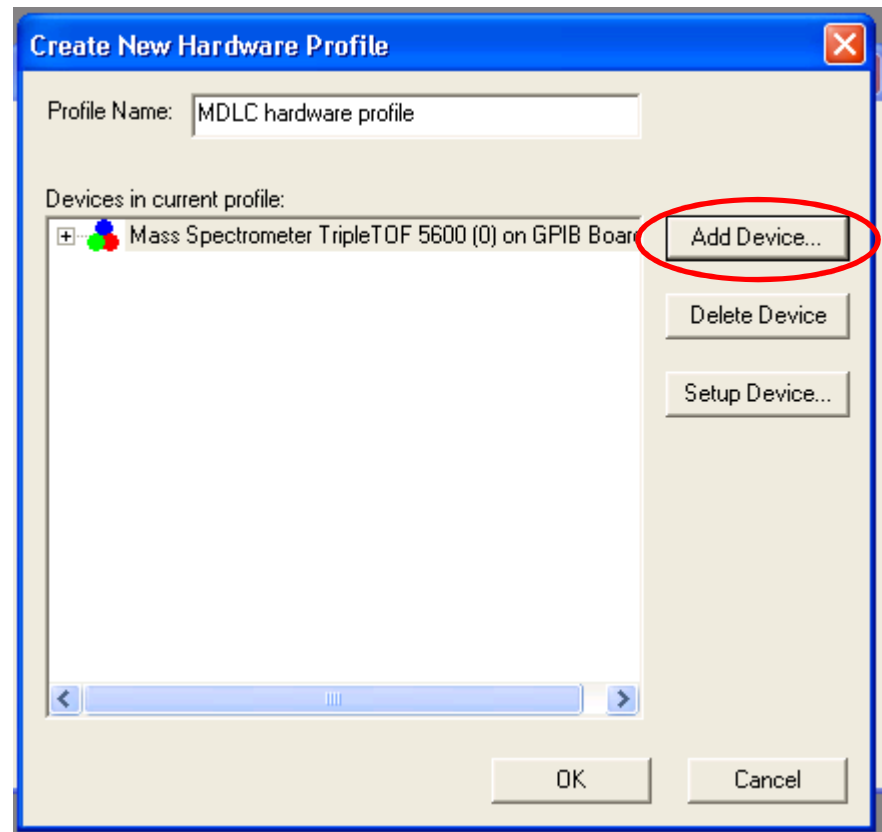
Agilent demo MDLC method

# Hardware profile setup guide

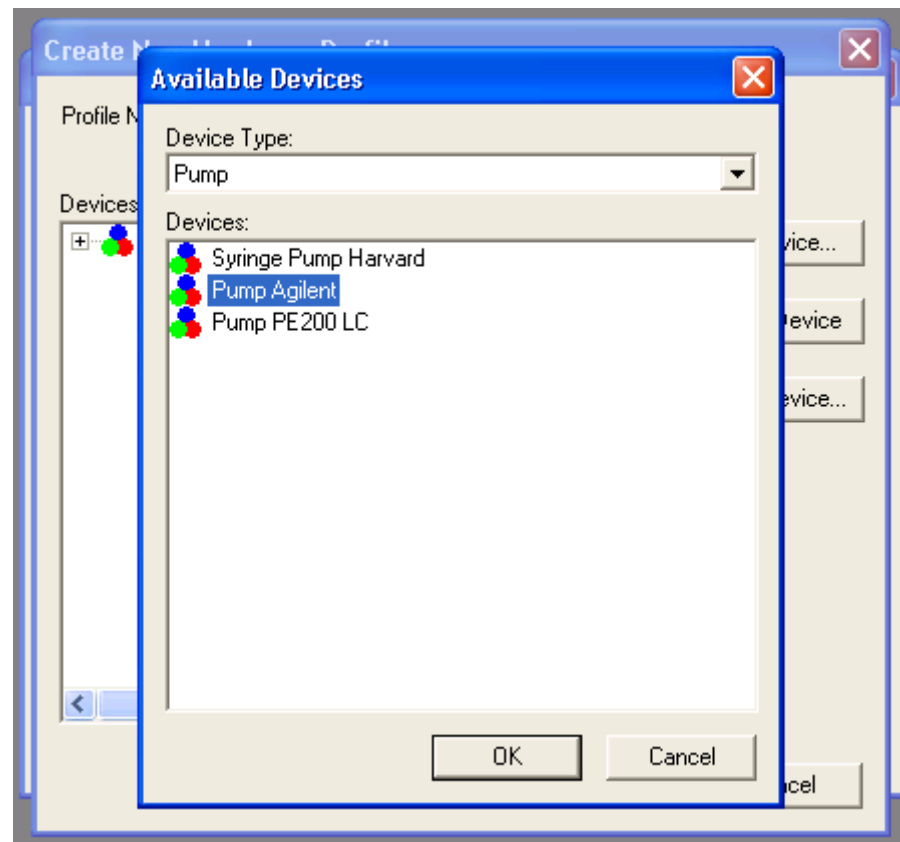
- Click “New Profile”



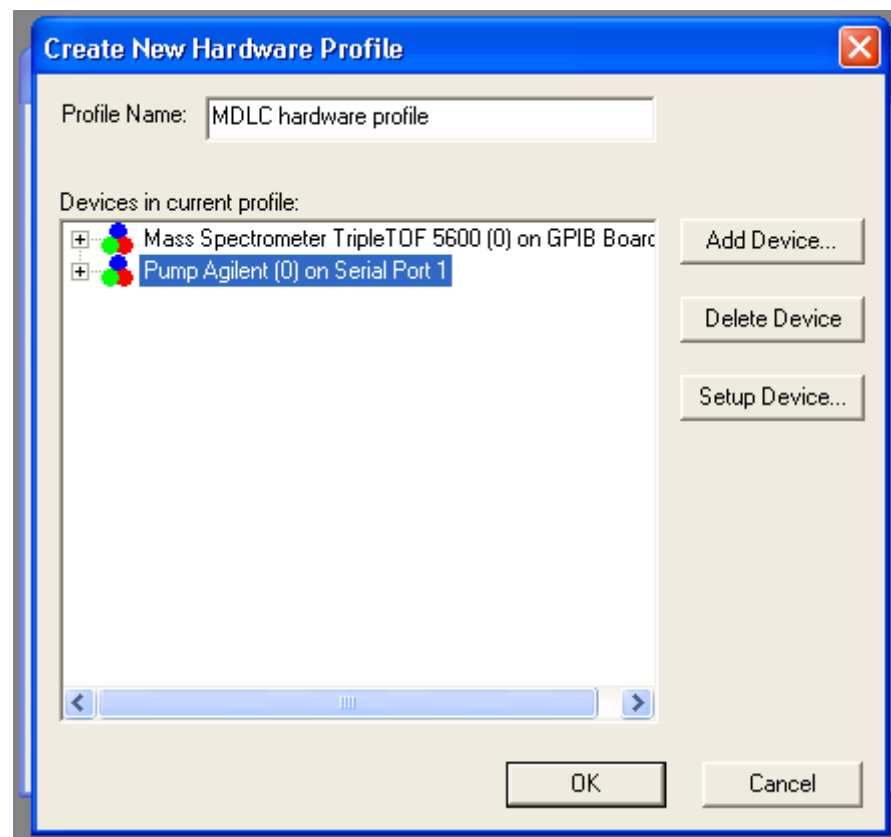
- After adding the mass spectrometer (as instructed as the manufacturer), click “Add Device)



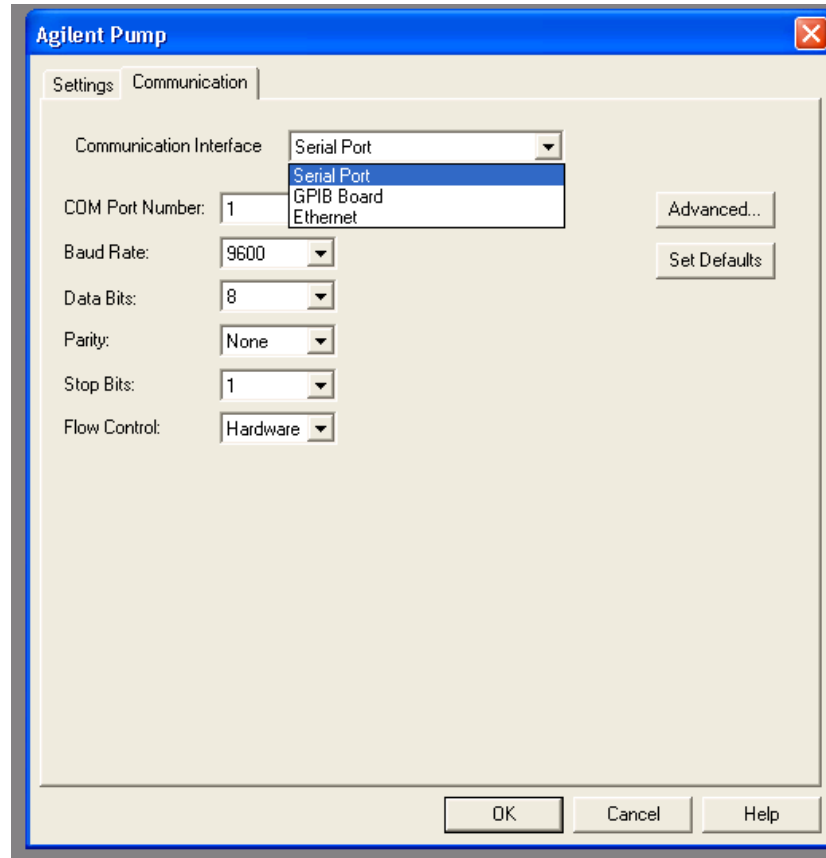
- Choose “Pump” from the drop down list and click “PumpAgilent”, then “ok”



- Click “Setup Device”

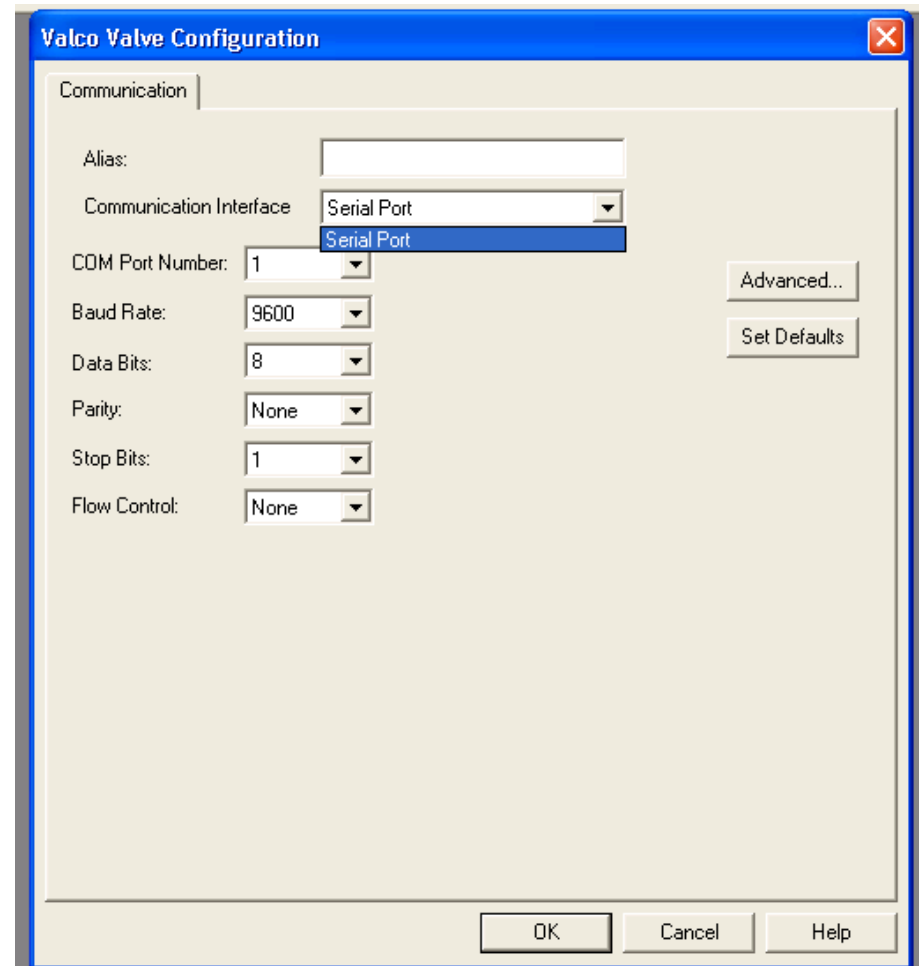
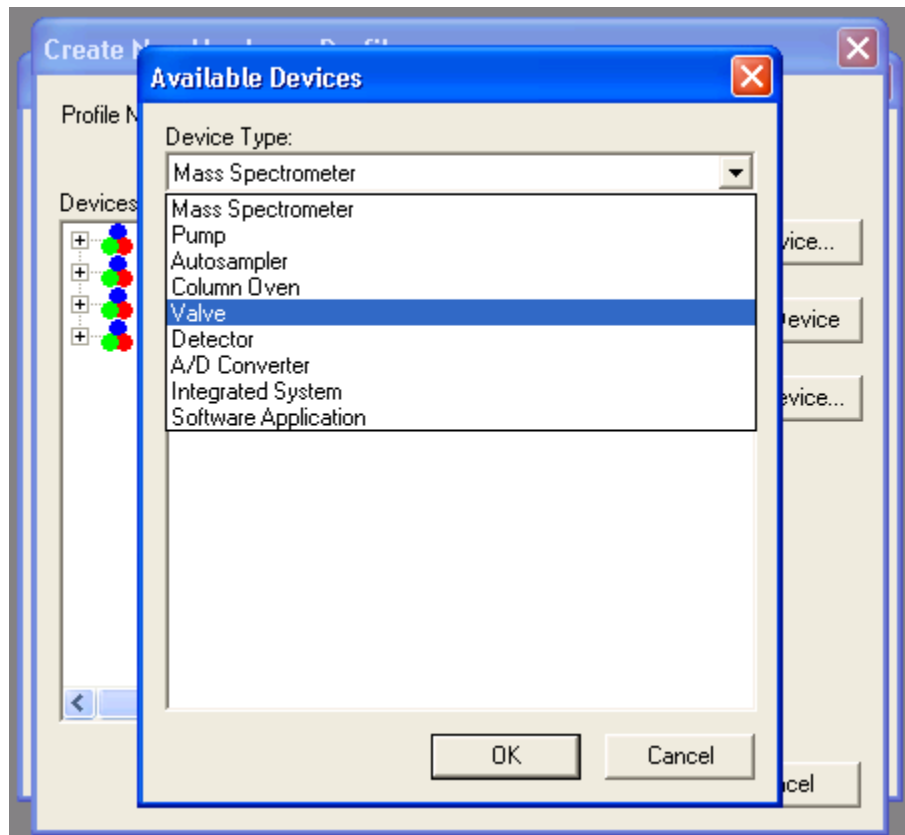


- Choose the communication interface and the port number as the LC is connected. Serial port is suggested



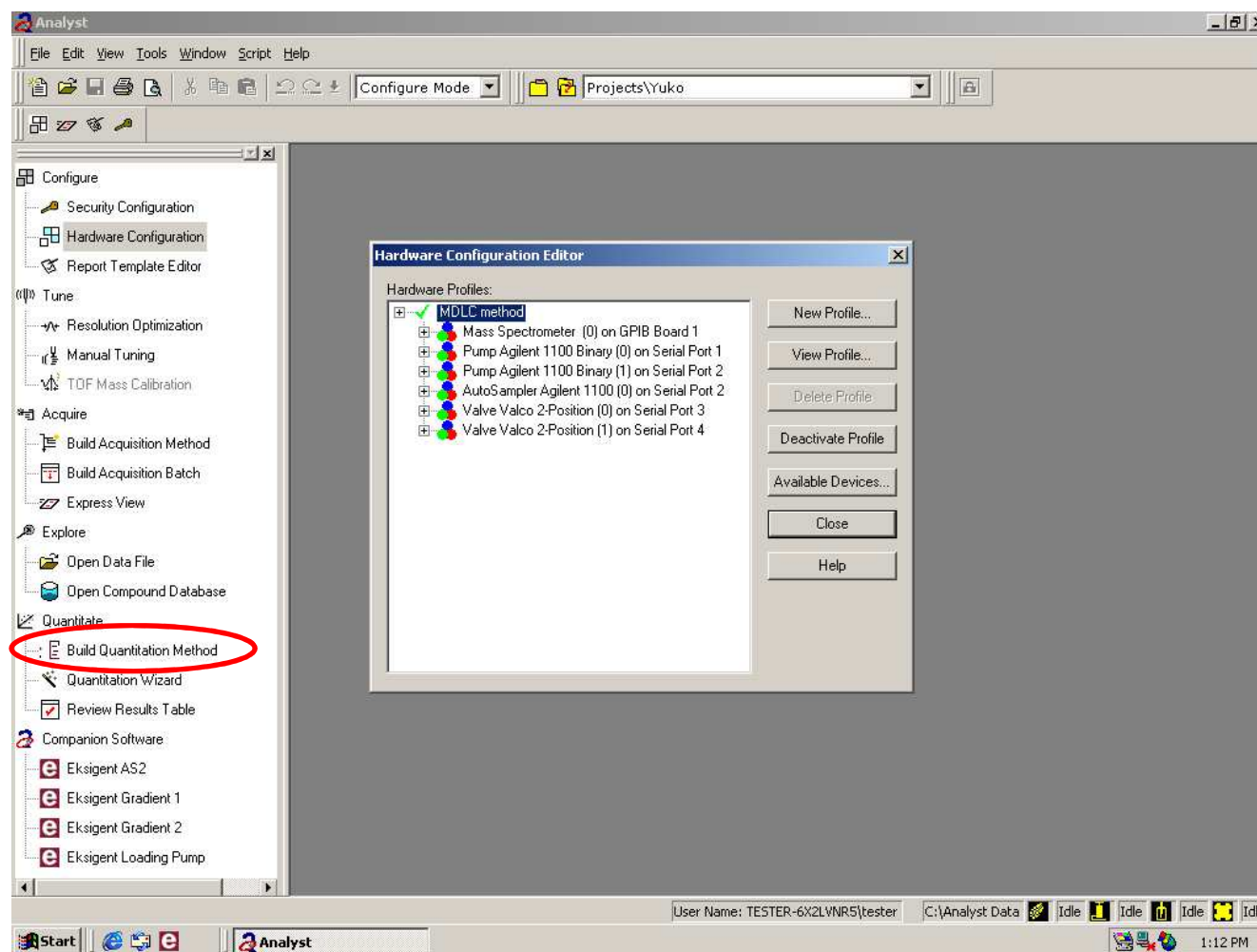
- Add other Agilent components in a similar manner

- Valves are added in a similar manner. Choose “Valve” from the drop down list, and choose serial port as communication interface



# Setting MDLC method for Agilent system

- Double click “Build Acquisition method”



# pH 10 gradient pump method

Analyst - [Acquisition Method: ]

File Edit View Acquire Tools Explore Window Script Help

Acquire Mode SBS

Configure

- Security Configuration
- Hardware Configuration
- Report Template Editor
- Tune and Calibrate
  - Instrument Optimization
  - Manual Tuning
- Acquire (1)
  - Method Wizard
  - Build Acquisition Method
  - Build Acquisition Batch
- Explore
  - Open Data File
  - Open Compound Database
- Companion Software
  - Formula Finder
  - MultiQuant 2.1
  - PeakView

Acquisition method

- Mass Spectrometer 140.010 mins
  - Period 140.000 mins
    - TOF MS (+)
    - Product Ion (+) IDA
- Agilent 1200 Capillary LC Pump (140)
  - Equilibrate (0.0 mins)
  - Run (140.0 mins)
- Agilent 1200 NanoFlow Pump (140.0)
  - Equilibrate (0.0 mins)
  - Run (140.0 mins)
- Agilent 1100 Autosampler
- Integrated Valco Valve
- Integrated Valco Valve

LC Pump Gradient Limits Limits (Advanced) Micro Mode

	Total Time (min)	Flow Rate (µl/min)	A (%)	B (%)
0	0.00	1.00	88.0	12.0
1	1.00	1.00	88.0	12.0
2	6.00	1.00	86.0	14.0
3	30.00	1.00	86.0	14.0
4	30.10	0.00	86.0	14.0
5	140.00	0.00	86.0	14.0

Change the gradient according to the fraction

Add Step Remove Step Show Graph >>

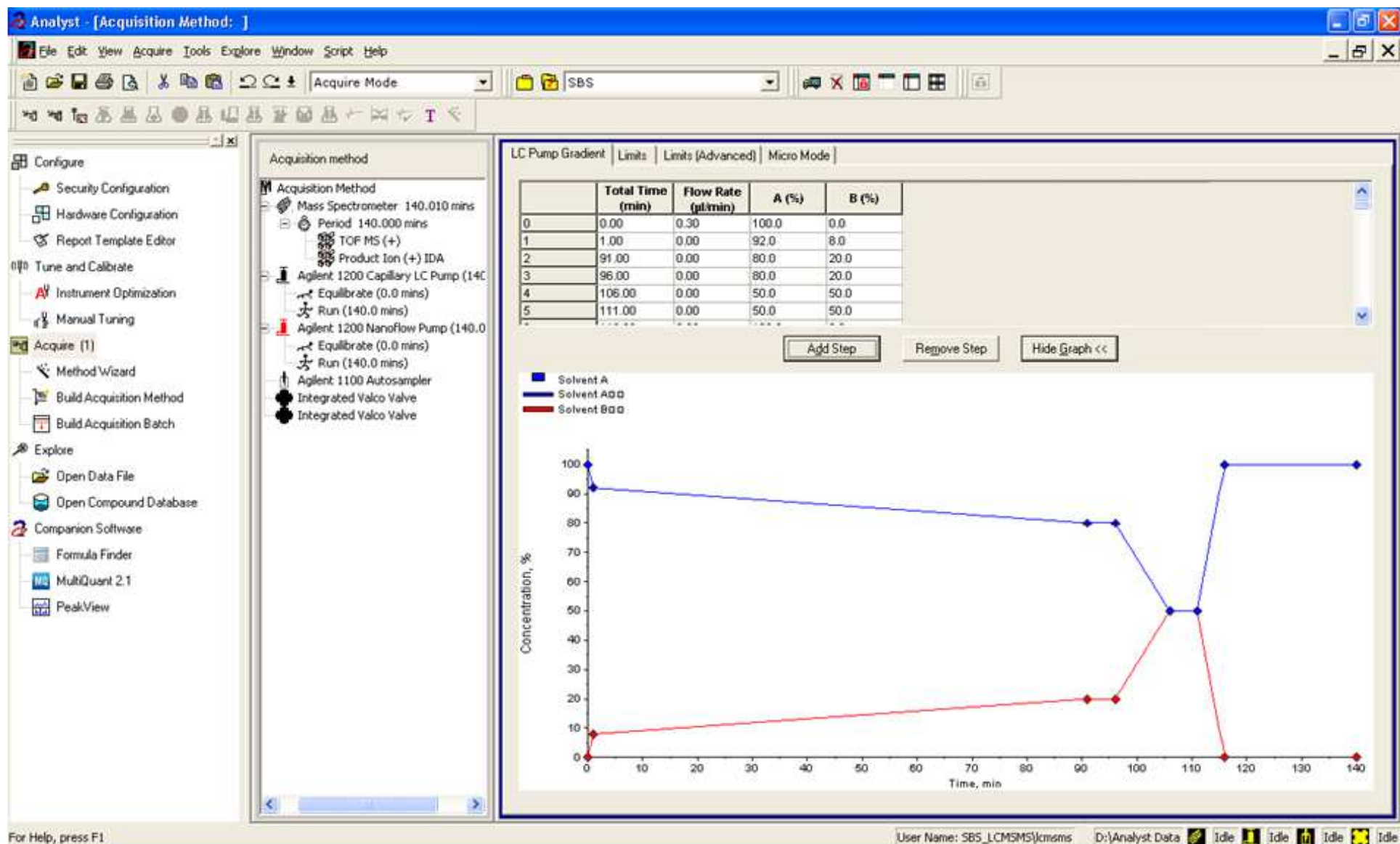
pH 10 gradient pump method when there is no fractionation

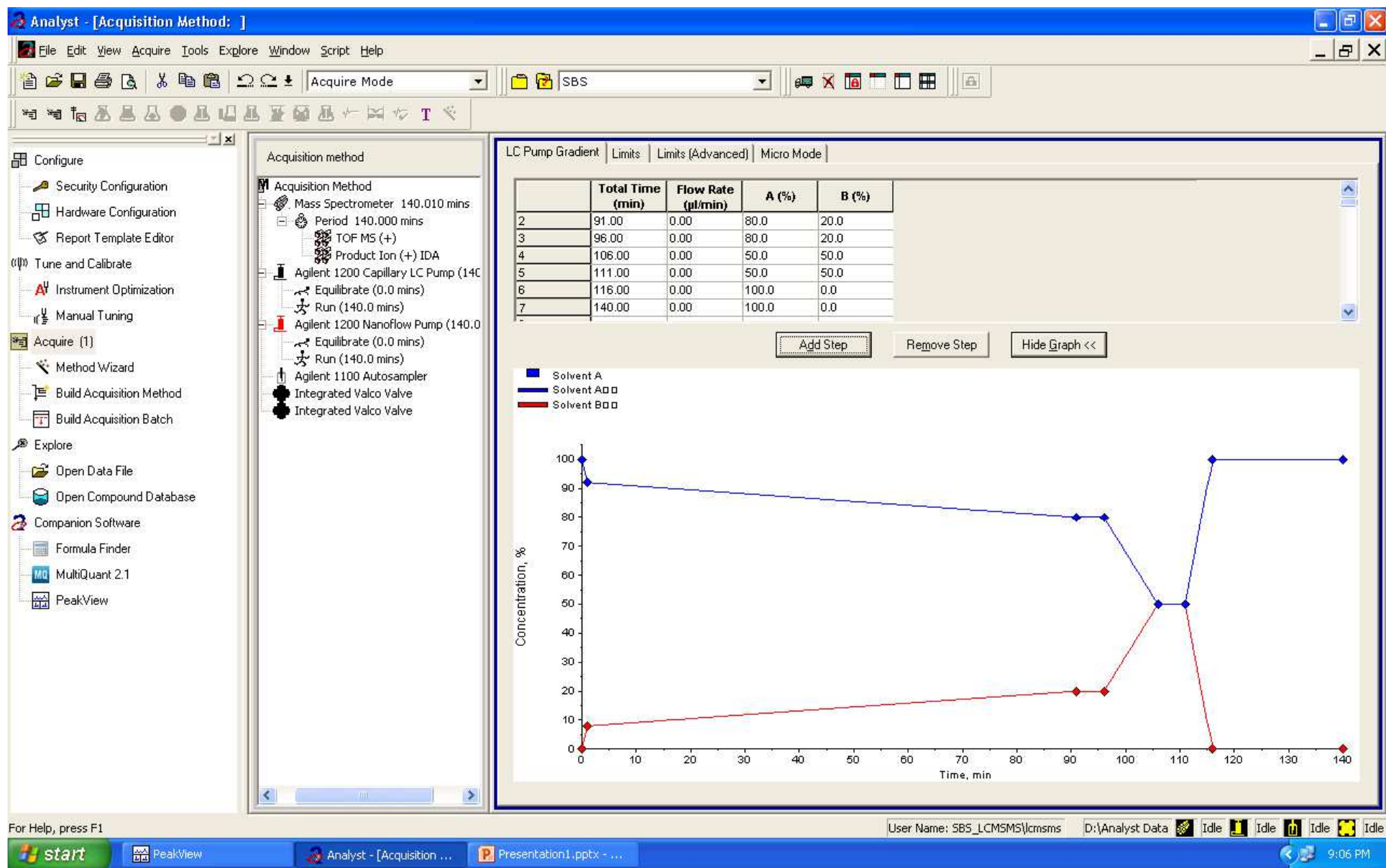
	Total Time (min)	Flow Rate (µl/min)	A (%)	B (%)
0	0.00	1.00	86.0	14.0
1	1.00	0.00	86.0	14.0
2	139.00	0.00	86.0	14.0
3	140.00	1.00	86.0	14.0

For Help, press F1

User Name: SBS\_LCMSMS\lcmsms D:\Analyst Data Idle Idle Idle Idle

# pH 2 gradient pump method





# Autosampler method (3D 20 mM/2D 1000 mM)

The screenshot displays the Analyst software interface for configuring an Autosampler method. The main window is titled "Analyst - [Acquisition Method: ]". The left sidebar contains a "Configure" section with options like Security Configuration, Hardware Configuration, Report Template Editor, Tune and Calibrate, Instrument Optimization, Manual Tuning, Acquire (1), Method Wizard, Build Acquisition Method, Build Acquisition Batch, Explore, Open Data File, Open Compound Database, Companion Software, Formula Finder, MultiQuant 2.1, and PeakView.

The central pane shows the "Acquisition method" tree with the following structure:

- Acquisition Method
  - Mass Spectrometer 140.010 mins
    - Period 140.000 mins
      - TOF MS (+)
        - Product Ion (+) IDA
    - Agilent 1200 Capillary LC Pump (14C)
      - Equilibrate (0.0 mins)
      - Run (140.0 mins)
    - Agilent 1200 Nanoflow Pump (140.0)
      - Equilibrate (0.0 mins)
      - Run (140.0 mins)
    - Agilent 1100 Autosampler
      - Integrated Valco Valve
      - Integrated Valco Valve

The right pane shows the "Agilent Autosampler Properties" dialog box. The "Advanced Properties" tab is active. It includes the following settings:

- Automatic Delay Volume Reduction: ☐ Enabled, Sample Flush-Out Factor: 5.0
- Equilibration Time (sec): 2
- Enable Vial/Well Bottom Sensing: ☐
- Use Custom Injector Program: ☒

The "Custom Injector Program" section contains a table with the following data:

Line #	Function	Position	Source	Location	Time (sec)	Offset (mm)
7	VALVE	BYPASS	SAMPLE	Vial 1	1	DEF.

The "Program Table" section lists the following steps:

- 1: INJECT
- 2: WAIT 30.00 min.
- 3: VALVE mainpass
- 4: DRAW 3.0 µl from sample, max. speed, def. offset
- 5: INJECT
- 6: WAIT 30.00 min.
- 7: VALVE bypass

The status bar at the bottom indicates "User Name: SBS\_LCMSMS\lcmsms" and "D:\Analyst Data".

# Autosampler method (3D 100 mM)

The screenshot displays the Analyst software interface for configuring an Autosampler method. The window title is "Analyst - [Acquisition Method: ]". The menu bar includes File, Edit, View, Acquire, Tools, Explore, Window, Script, and Help. The toolbar shows various icons for file operations and acquisition settings.

The left sidebar contains a "Configure" section with the following options:

- Security Configuration
- Hardware Configuration
- Report Template Editor
- Tune and Calibrate
  - Instrument Optimization
  - Manual Tuning
- Acquire (1)
  - Method Wizard
  - Build Acquisition Method
  - Build Acquisition Batch
- Explore
  - Open Data File
  - Open Compound Database
- Companion Software
  - Formula Finder
  - MultiQuant 2.1
  - PeakView

The main panel is divided into two sections: "Acquisition method" and "Agilent Autosampler Properties".

**Acquisition method**

- Mass Spectrometer 140.010 mins
  - Period 140.000 mins
    - TOF MS (+)
    - Product Ion (+) IDA
- Agilent 1200 Capillary LC Pump (14C)
  - Equilibrate (0.0 mins)
  - Run (140.0 mins)
- Agilent 1200 Nanoflow Pump (140.0)
  - Equilibrate (0.0 mins)
  - Run (140.0 mins)
- Agilent 1100 Autosampler
  - Integrated Valco Valve
  - Integrated Valco Valve

**Agilent Autosampler Properties**

Automatic Delay Volume Reduction:  
☐ Enabled Sample Flush-Out Factor: 5.0  
Equilibration Time (sec): 2  
☐ Enable Vial/Well Bottom Sensing  
☒ Use Custom Injector Program

Custom Injector Program

Line #	Function	Position	Source	Location	Time (sec)	Offset (mm)
1	VALVE	MAINPASS	SAMPLE	Vial 1	1	DEF.

Program Table

- 1: VALVE mainpass
- 2: DRAW 3.0 µl from sample, max. speed, def. offset
- 3: INJECT
- 4: WAIT 30.00 min.
- 5: VALVE bypass

Buttons: Change, Insert, Append, Copy, Paste, Delete

For Help, press F1

User Name: SBS\_LCMSMS\lcsmms D:\Analyst Data Idle Idle Idle Idle

# Autosampler method (3D 1000 mM)

The screenshot displays the Analyst software interface for configuring an Autosampler method. The main window is titled "Analyst - [Acquisition Method: ]". The left sidebar contains a "Configure" section with options like Security Configuration, Hardware Configuration, Report Template Editor, Tune and Calibrate, Instrument Optimization, Manual Tuning, Acquire (1), Method Wizard, Build Acquisition Method, Build Acquisition Batch, Explore, Open Data File, Open Compound Database, Companion Software, Formula Finder, MultiQuant 2.1, and PeakView.

The central panel shows the "Acquisition method" tree with the following structure:

- Acquisition Method
  - Mass Spectrometer 140.010 mins
    - Period 140.000 mins
      - TOF MS (+)
        - Product Ion (+) IDA
    - Agilent 1200 Capillary LC Pump (14C)
      - Equilibrate (0.0 mins)
      - Run (140.0 mins)
    - Agilent 1200 Nanoflow Pump (140.0)
      - Equilibrate (0.0 mins)
      - Run (140.0 mins)
    - Agilent 1100 Autosampler
      - Integrated Valco Valve
      - Integrated Valco Valve

The right panel shows the "Agilent Autosampler Properties" and "Advanced Properties" tabs. The "Advanced Properties" tab is active, displaying the "Custom Injector Program" section. The "Program Table" is as follows:

Line #	Function	Amount (µl)	Source	Location	Speed (µl/min)	Offset (mm)
1	VALVE mainpass					
2	DRAW 8.0 µl from sample, max. speed, def. offset	8.0	SAMPLE	Vial 1	MAX	DEF.
3	INJECT					
4	WAIT 30.00 min.					
5	VALVE bypass					

The status bar at the bottom indicates "User Name: SBS\_LCMSMS\lcsmms" and "D:\Analyst Data".

# V1 method

The screenshot displays the Analyst software interface for configuring an acquisition method. The left sidebar contains a 'Configure' section with various options, including 'Acquire (1)'. The main panel shows the 'Integrated Valco Valve Method Properties' dialog. The 'Valve Type' is set to 'Diverter'. The 'Position Name for Step0' is set to 'A'. The 'Total Time (mi)' and 'Position' table is shown, with entries for steps 1 through 4. The 'Total Time (mi)' values are 0.0, 1.0, 6.0, and 140.0, and the 'Position' values are A, B, A, and A respectively. The 'V1 method without fractionation' text is overlaid in red. The 'Add Entry' and 'Remove Entry' buttons are at the bottom of the table.

Analyst - [Acquisition Method: ]

File Edit View Acquire Tools Explore Window Script Help

Acquire Mode SBS

Configure

- Security Configuration
- Hardware Configuration
- Report Template Editor
- Tune and Calibrate
- Instrument Optimization
- Manual Tuning
- Acquire (1)
- Method Wizard
- Build Acquisition Method
- Build Acquisition Batch
- Explore
- Open Data File
- Open Compound Database
- Companion Software
- Formula Finder
- MultiQuant 2.1
- PeakView

Acquisition method

- Acquisition Method
  - Mass Spectrometer 140.010 mins
    - Period 140.000 mins
      - TOF MS (+)
        - Product Ion (+) IDA
  - Agilent 1200 Capillary LC Pump (140.0 mins)
    - Equilibrate (0.0 mins)
    - Run (140.0 mins)
  - Agilent 1200 Nanoflow Pump (140.0 mins)
    - Equilibrate (0.0 mins)
    - Run (140.0 mins)
  - Agilent 1100 Autosampler
  - Integrated Valco Valve

Integrated Valco Valve Method Properties

Valve Type: Diverter

Change Position Names:

Position Name for Step0: A

To use the valve as an injector, select the Synchronization Mode, "Manual Sync with Valve".

To use the valve as a diverter, select any other Synchronization Mode.

(To change the Synchronization Mode, click on "Acquisition Method" tab.)

	Total Time (mi)	Position
1	0.0	A
2	1.0	B
3	6.0	A
4	30.0	B
5	140.0	A
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		

V1 method without fractionation

	Total Time (mi)	Position
1	0.0	A
2	1.0	B
3	139.0	B
4	140.0	A

Add Entry Remove Entry

For Help, press F1

User Name: SBS\_LCMSMS\lcmsms D:\Analyst Data Idle Idle Idle Idle Idle

# V2 method

Analyst - [Acquisition Method: ]

File Edit View Acquire Tools Explore Window Script Help

Acquire Mode SBS

Configure

- Security Configuration
- Hardware Configuration
- Report Template Editor
- Tune and Calibrate
  - Instrument Optimization
  - Manual Tuning
- Acquire [1]
  - Method Wizard
  - Build Acquisition Method
  - Build Acquisition Batch
- Explore
  - Open Data File
  - Open Compound Database
- Companion Software
  - Formula Finder
  - MultiQuant 2.1
  - PeakView

Acquisition method

- Acquisition Method
  - Mass Spectrometer 140.010 mins
    - Period 140.000 mins
      - TOF MS (+)
      - Product Ion (+) IDA
  - Agilent 1200 Capillary LC Pump (140.0)
    - Equilibrate (0.0 mins)
    - Run (140.0 mins)
  - Agilent 1200 Nanoflow Pump (140.0)
    - Equilibrate (0.0 mins)
    - Run (140.0 mins)
  - Agilent 1100 Autosampler
  - Integrated Valco Valve
  - Integrated Valco Valve

Integrated Valco Valve Method Properties

Valve Type: Diverter

Change Position Names:

Position Name for Step0: A

To use the valve as an injector, select the Synchronization Mode, "Manual Sync with Valve".

To use the valve as a diverter, select any other Synchronization Mode.

(To change the Synchronization Mode, click on "Acquisition Method" tab.)

	Total Time (m)	Position
1	0.0	B
2	140.0	B
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		

Add Entry Remove Entry

Position B for odd number fractions; Position A for even number fractions.

For Help, press F1

User Name: SBS\_LCMSMS\lcsmms D:\Analyst Data Idle Idle Idle Idle