

# Use Your Autosampler to Make Calibration Standards

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#### Introduction and General Setup

- We describe a feature of many autosamplers that allows the user to perform solution manipulations.
  - Either serial or parallel dilutions can be performed.
  - This approach is useful when:
    - High-value standard solutions must be prepared in a minimum volume with maximum accuracy.
    - Classic analytical dilutions involve large volumes of solvent and multiple volumetric flasks.
- Solutions
  - Stock Solution
    - Must be in compatible solvent
  - Receiving Vial(s)
    - Clean, empty, no cap
      - Vial inserts can be used for small volume dilutions
- HPLC System
  - Disconnect column
    - Use union or send column inlet tubing to waste
  - Program pump to deliver desired dilution solution
  - Set flow to 1.0 mL/min
    - Any flow can be used, but the system pressure should be as low as possible.
  - Equilibrate system with flow through autosampler loop
    - "Mainpass" in Agilent systems

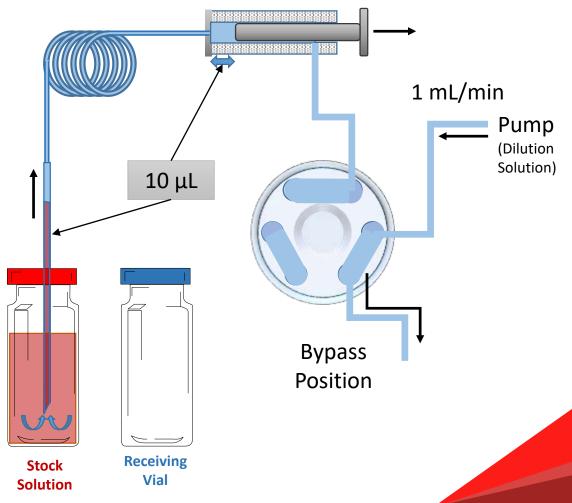


### How Does it Work?

- Step 1: Draw Stock Solution
  - Draw desired volume of stock solution using "Draw" command

#### Example:

Preparation of 10 μg/mL solution from 1,000 μg/mL stock solution by <u>diluting 10</u> μL of stock solution to 1,000 μL with dilution solution. (1:100 Dilution)





### How Does It Work?

#### Step 2: Dilution

 Use "Valve" or "Main In" command to switch injection valve to Mainpass into the receiving vial for a specified time

Stock

Solution

Vial

#### 1 mL/min Pump (Dilution Solution) Mainpass Position The Receiving Vial now contains 1 mL of solution, which includes the original 10 µL of stock solution. (a 1:100 dilution) Receiving

#### Example:

Preparation of 10 μg/mL solution from 1,000 μg/mL stock solution. (1:100 Dilution) Use a time of 60 sec. for this command. At 1 mL/min, this step will divert a total of 1 mL to the receiving vial.



### How Does It Work?

- Step 3:
  - Mix/agitate the receiving vial thoroughly





Receiving Vial After Procedure (Stock Solution Is Blue) Receiving Vial After Shaking



Why? The dilution step pumps the solution into the bottom of the vial, and complete mixing does not occur.

#### Example Injector Program

OpenLab ChemStation C.01.09 – 1:100 Dilution

Setup Method							
💺 Binary Pump	) 🔷 I	HiP Sampler 🧇 HiP Sampler Injector Program 🚀 Column Com	). 🔝 DAI	Needed to reset sampling syringe to home position.			
🗹 Use Injector	Program			This is the volume of stock solution.			
Function		Parameter		Wash options will vary depending on your model.			
Eject - Eject maximum volume to seat with 500 μL/min using default offs			et /				
Draw	-	Draw 10 $\mu$ L from sample with 100 $\mu$ L/min using default offset		This example uses a position relative to the sample.			
Wash	•	Wash needle as specified in the method		Depending on your model, you may have to specify a			
Valve	-	Switch valve to "Main In" from vial+ 10 for 60 s using default offse	t	relative position for tray, row, and column.			
Wash	•	Wash needle as specified in the method					
Inject	•	Inject	_	Wash options will vary depending on your model.			
				Needed to complete the process.			
Show timetable	e graph						
		OK Apply	Ca	incel Help			
			•				

- Other Method Settings
  - Pump

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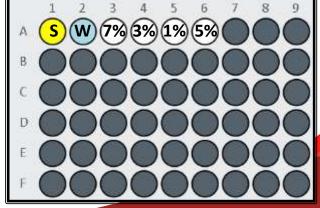
- Flow: 1 mL/min of dilution solution
- Stop Time: 0.1 min.
- Injector
  - Appropriate settings for needle wash
- Column
  - None needed
- Detector
  - Default settings

- Injection/Sequence Settings
  - Set the sample as the location of the Stock solution
    - Or, you can set a fixed location in the program and the sample location is ignored.

### Case Study 1: Consumer Product Ingredient

- Method
  - Prepare 4 dilutions of a single stock solution in methanol for analysis of different products
    - 7%, 3%, 1%, and 5%
  - Pump Flow = 1 mL/min methanol
  - Locations
    - Stock Solution in P1-A1
    - Wash vial in P1-A2
    - 7% in P1-A3
    - 3% in P1-A4
    - 1% in P1-A5
    - 5% in P1-A6

Use Injector Pr	02000							
inction	Parameter							
Eject			i80 µL/min using detauit atto					
Draw			h 200 µL/min using default of					
Valve			n "P1-A3" for 68 ‡ using defa	iestio fue	7%			
Wash		e in location "P1-A2" 2 tin			, ,0			
End		a seat with default speed		10 M 10				
Draw Valve	and the second second second		h 200 µL/min using default of					
Wash		in location "PT-A2" 2 tin	n "P1-A4" for 60 s using defa	ra moler	3%			
Eiect		o seal with default speed			0 /0			
Draw				fort -				
Value	Draw 10 µL from location "P1.41" with 200 µL/min using default offset     Switch valve to "Main In" from location "P1.45" for 50 ± using default offset							
Wash	Switch valve to "Main In" from location "PTIA5" for 50 ± using default offset     Wash needs in location "PTIA5" 2 times							
Eject	Elect 10 µL to seat with default speed using default offset							
Draw	+ Draw 50 µL	from location "P1-A1" with	h 200 µL/min using default of	faet				
Valve	- Switch valve	to "Main In" from locatio	n "P1-A6" for 60 = using defa	sult officer				
Wash	· Wash needs	in location "P1-A2" 2 tin	nes		5%			
Inject	+ Insect							
Append	Insert	Delma	Clear al Hover up					
Gu	Copy	Patte	More Deve					
his for database	2014 - C							
how timetable	(Jidui)		1 1999	400007	142427			
		OK.	Apply	Cancel	Help			



## Case Study 1: Results

Three autosampler preparations of the 7% standard

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• Minimum 8 injections of each standard

	Avg Area
Prep 1	1574.65
Prep 2	1578.40
Prep 3	1581.96
Avg	1578.3
SD	3.65
RSD	0.23

 Compare average area with manually prepared standard

	Avg Area	Prep Time	Solvent Used
Manual Prep	1577.650	20 min	15 mL
Autosampler Prep	1578.337	12 min	400 mL
% Difference	0.04%		

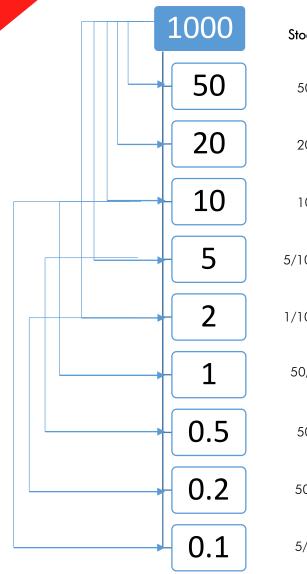
• Compare Preparation Levels

Level, %	Avg Area	RF
7.00	1574.008	224.9
3.00	670.017	223.3
1.00	222.654	222.7
5.00	1115.356	223.1
	Avg	223.0
	SD	0.345
	RSD	0.15

Conclusions:

- Autosampler preparations showed excellent precision
- The Autosampler and Manual methods were very close
- Linearity was excellent across all dilutions (similar Response Factors)
- The method passed all validation tests and was adopted for future use.

### Case Study 2: DNPH Derivative



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tock/Total Vol. from Source

#### 50/1000 from 1000

20/1000 from 1000

10/1000 from 1000

5/1000 from 1000

1/1000 from 1000

50/500 from 10

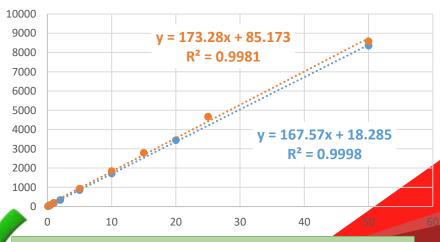
50/500 from 5

50/500 from 2

5/500 from 10

				<u> </u>			/
	Autosam	pler (Prog	ıram)		Manual		
Lvl	Amt	Area	RF		Amt	Area	RF
1	0.1	17.231	172.3		0.1	22.258	222.6
2	0.2	34.431	172.2		0.5	103.12	206.2
3	0.5	85.098	170.2		1	196.42	196.4
4	1	171.45	171.4		5	933.34	186.7
5	2	343.7	171.8		10	1845.2	184.5
6	5	859.47	171.9		15	2792.7	186.2
7	10	1724.8	172.5		25	4672.4	186.9
8	20	3449.2	172.5		50	8587.4	171.7
9	50	8359.5	167.2				
		Avg	171.3			Avg	192.7
		SD	1.71			SD	15.6
		RSD	1.00			RSD	8.11

#### Autosampler Manual



Better linearity and more consistent response factors (RF) for the autosampler method.

# Case Study 3: Making Mixtures Prepare a mixture of three components, each diluted 1:25 from three individual stock solutions.

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Setup Method							
	🗇 HiP Sampler 🧇 HiP Sampler Injector Program 🗬 Column Comp. 🤜		Sample location in Sequence				
			Table is P1-A1				
Use Injector P	rogram						
Function	Parameter				location is relative to equale		
Eject	<ul> <li>Eject maximum volume to seat with 500 μL/min using default offset</li> </ul>				Location is relative to sample,		
Draw (1)	<ul> <li></li></ul>			2	one column over (P1-A2)		
Wash	➡ Wash needle as specified in the method						
Draw	∠ Vertice Join America Contraction (0,0,0,1) with 200 μL/min using default offse	ət			location is relative to sample		
Wash	<ul> <li>Wash needle as specified in the method</li> </ul>			3	Location is relative to sample,		
Draw	<ul> <li>Draw 20 μL from location+ (0,0,0,2) with 200 μL/min using default offse</li> </ul>			one row down (P1-B1)			
Wash	<ul> <li>Wash needle as specified in the method</li> </ul>						
Valve	<ul> <li>Switch valve to "Main In" from location+ (0,0,1,0) for 60 s using default offset 3 4</li> </ul>				Duran flow rate is 0.50 ml /min		
Wash	<ul> <li>Wash needle as specified in the method</li> </ul>		4	Pump flow rate is 0.50 mL/min			
Inject	ect 🗸 Inject				so total volume is 500 $\mu$ L		
Show limetable							
	OK Apply	Cancel	Help				
		Valve.	tuun	_	for uzeOfficet		
113	4 5 6 7 8 9	O FRIEND	O Sandie		60 1 a 🛞 Disfault		
	200000	O Main Stol	O Location		O 0.0.7 Her		
		O lister	C Sed				
		🛞 Mair In	C Ai				
			O VW+				
			81055611000cm 10#	ray Plat	e Rov Colume		
			🛞 Sanple+	0.1			
, 000		<ul> <li>The Second Second</li></ul>	ample-I	- 0	ption allows you to		
	Conductory	specify a position relative to the Sample location. You specify the change for					
	Conclusion:						
Y	ou can make individual stock solutions						
		late, R	ow.	and Column.			
	and then make your combined standards						

as needed.

Limitations

- Small dilution factors (e.g., 1:2) are limited if large volumes are required.
  - Vial inserts would be required
    - e.g., 50 / 100

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- UHPLC systems often have smaller injection limits
  - Requires use of inserts
- Some UHPLC pumps may not be as reliable at the low pressures observed with this method
- Mixing/agitation is required, so complete automation is not possible (yet).
  - Future modifications may solve this problem.



### Summary/Advantages

- Programmable autosamplers can be used to prepare mixtures and/or calibration standards with high accuracy
  - Options will vary across models and software versions, but the general procedure is the same.
- Solvent use is significantly reduced
- Use of volumetric glassware is significantly reduced
  - Only preparation of stock solution is required
- Smaller amounts of standards are required
  - No need for storage of dilutions.
  - Make what you need when you need it.
- Completion time is usually less than the corresponding manual method, and does not require staff time during the program (in most cases).



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- Improve efficiency and productivity with better methods and processes, and
- Have access to a range of support options.

