Use Your Autosampler to Make Calibration Standards

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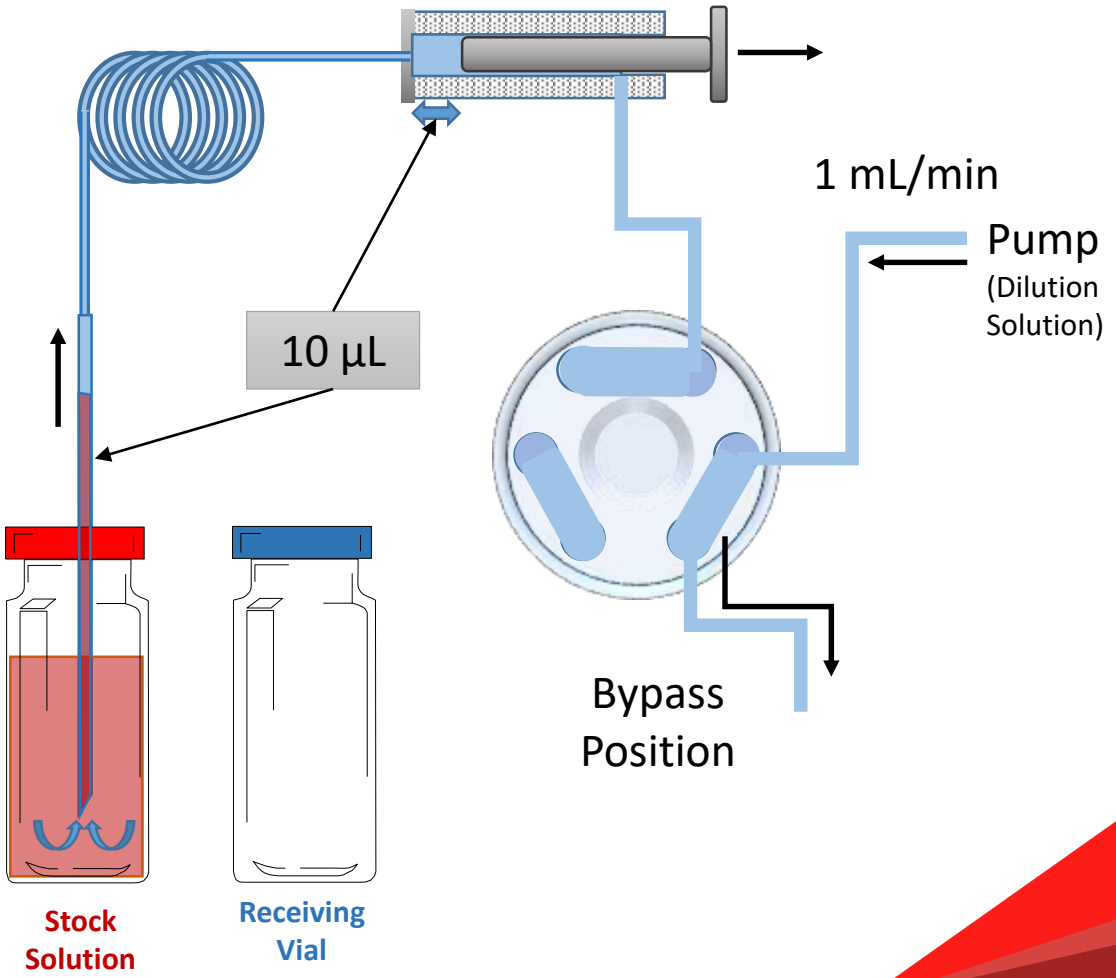
Introduction and General Setup

- 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?

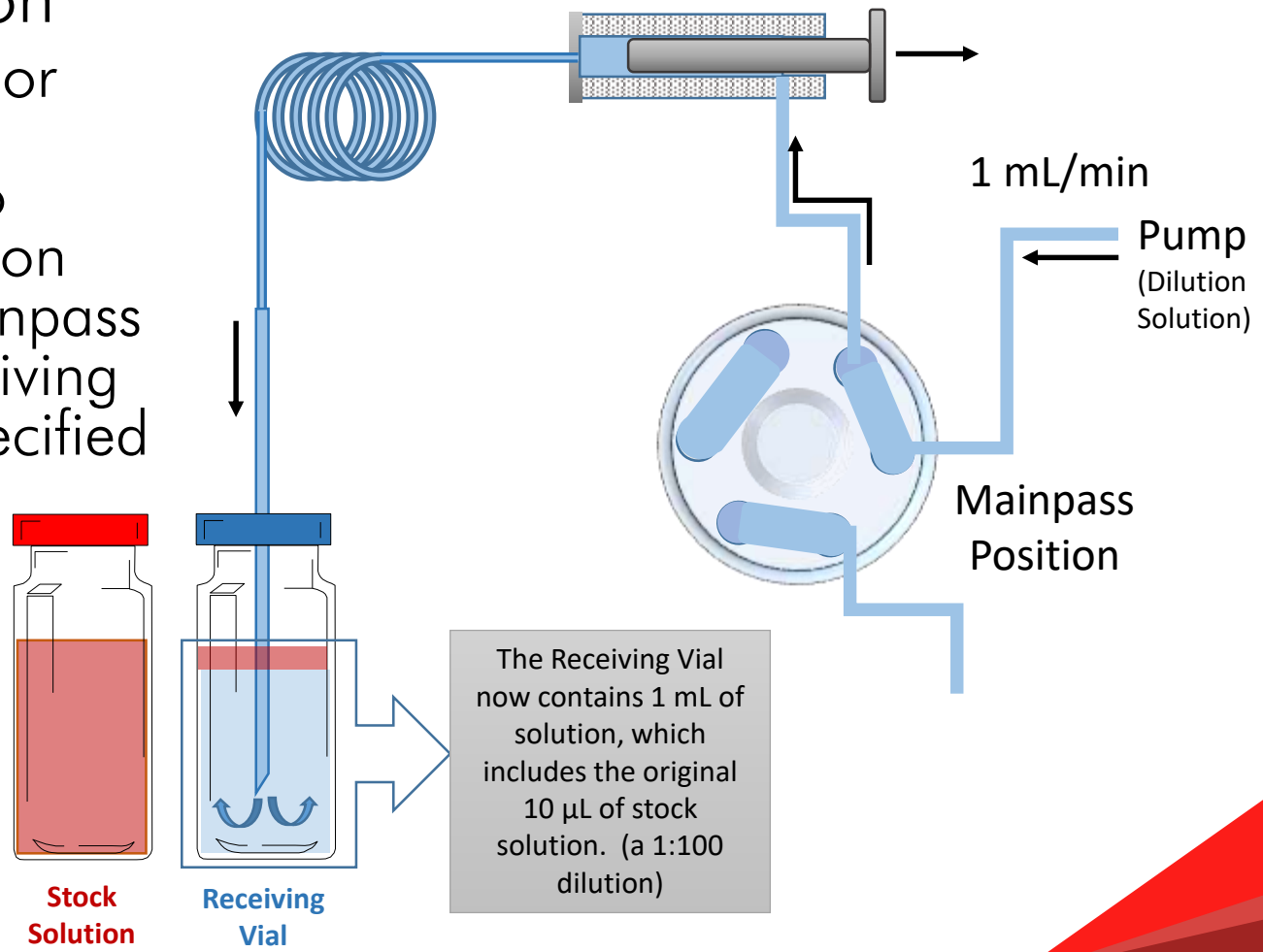
- Step 1: Draw Stock Solution
  - Draw desired volume of stock solution using "Draw" command

Example:
Preparation of 10 µg/mL solution from 1,000 µg/mL stock solution by diluting 10 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



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

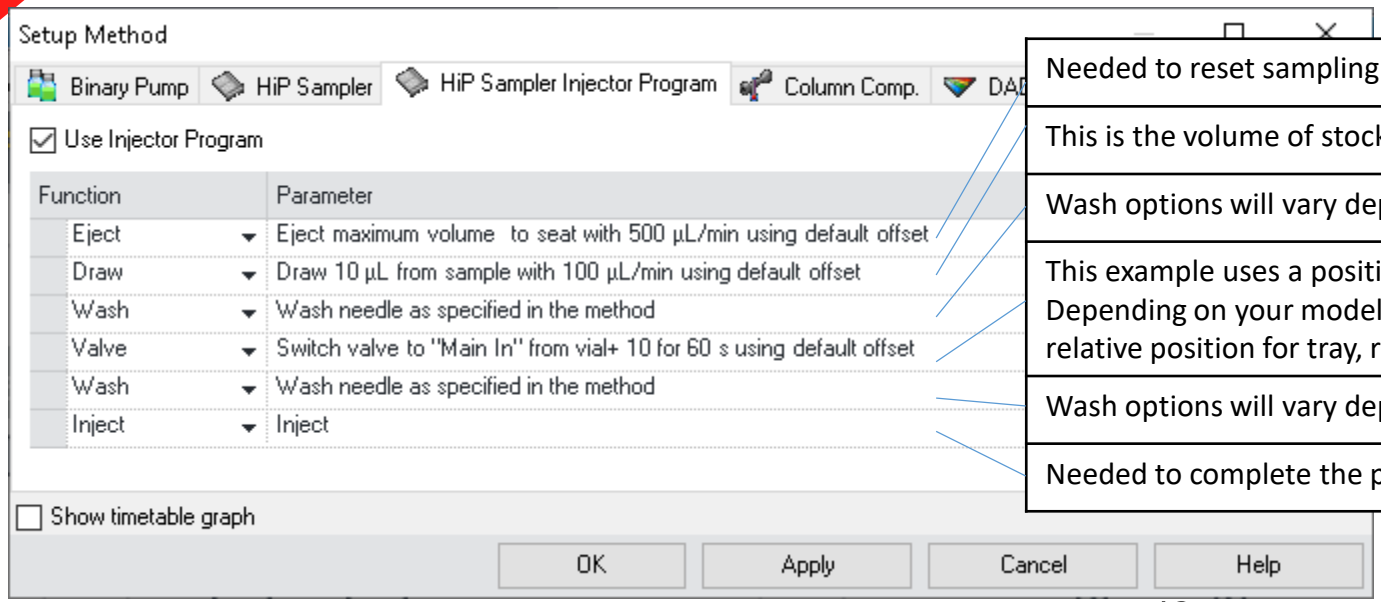
Receiving Vial After Procedure (Stock Solution Is Blue)

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



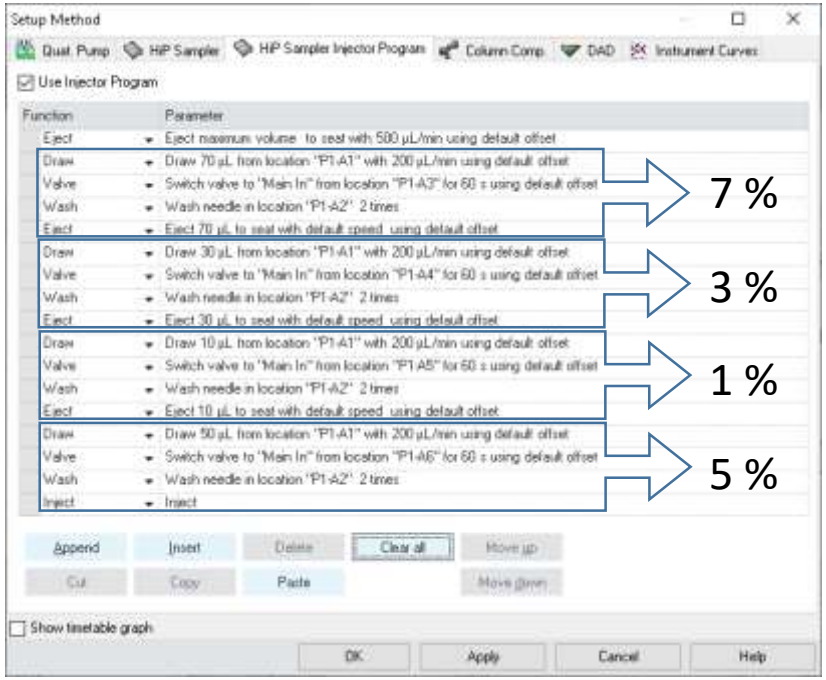
Needed to reset sampling syringe to home position.
This is the volume of stock solution.
Wash options will vary depending on your model.
This example uses a position relative to the sample. Depending on your model, you may have to specify a relative position for tray, row, and column.
Wash options will vary depending on your model.
Needed to complete the process.

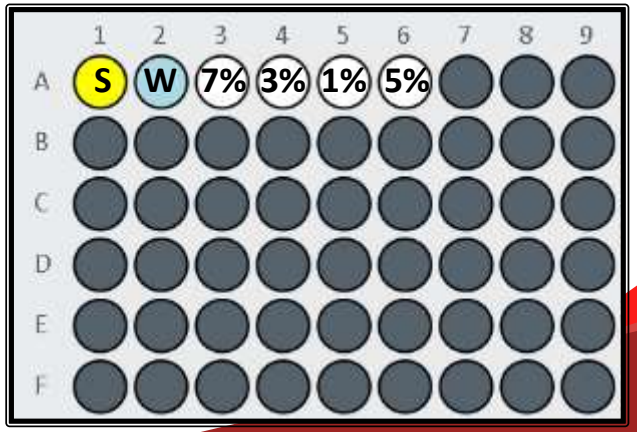
- Other Method Settings
  - Pump
    - Flow: 1 mL/min of dilution solution
    - Stop Time: 0.1 min.
  - Injector
    - Appropriate settings for needle wash
  - Column
    - None needed
  - Detector
    - Default settings

- 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





Case Study 1: Results

- Three autosampler preparations of the 7% standard
  - 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%**		

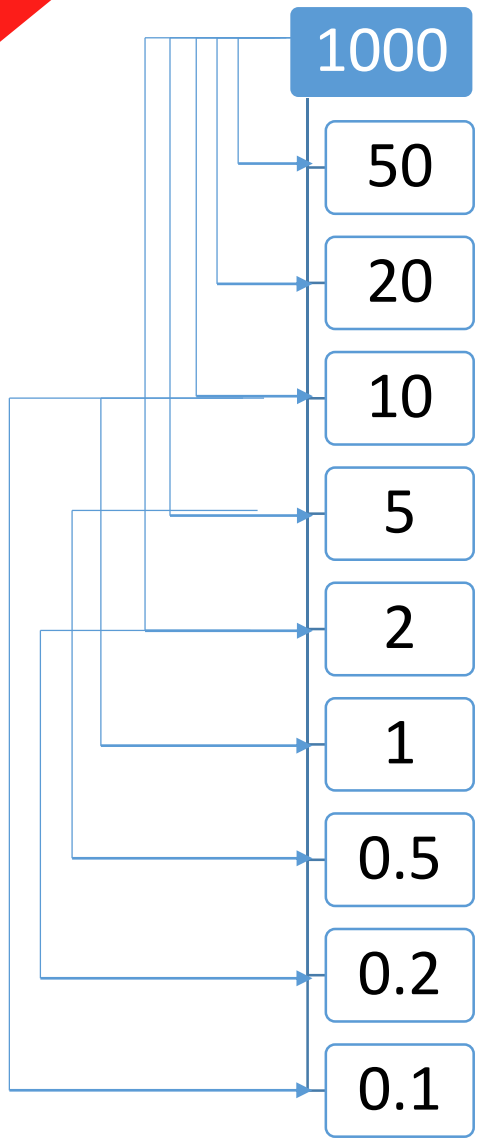
- Compare Preparation Levels

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:

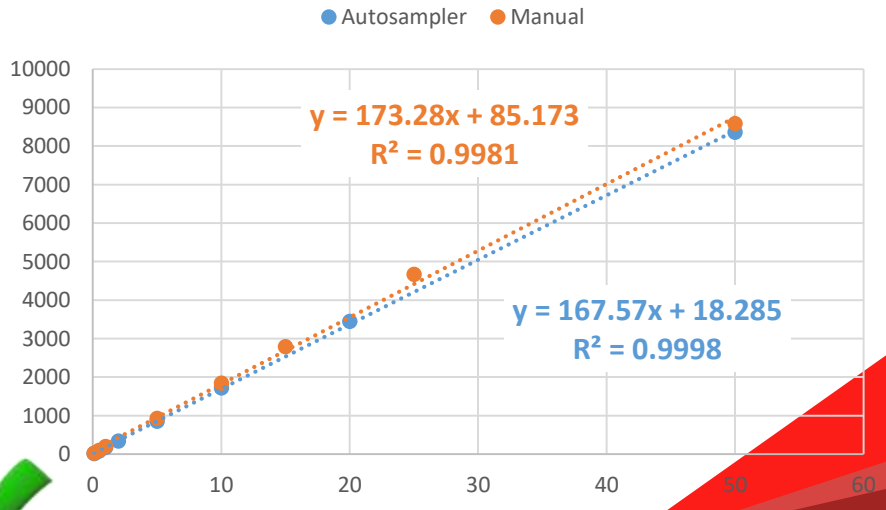
- Autosampler preparations showed excellent precision
- The Autosampler and Manual methods were very close
- Linearity was excellent across all dilutions (similar Response Factors)
- The method passed all validation tests and was adopted for future use.

Case Study 2: DNPH Derivative



Stock/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

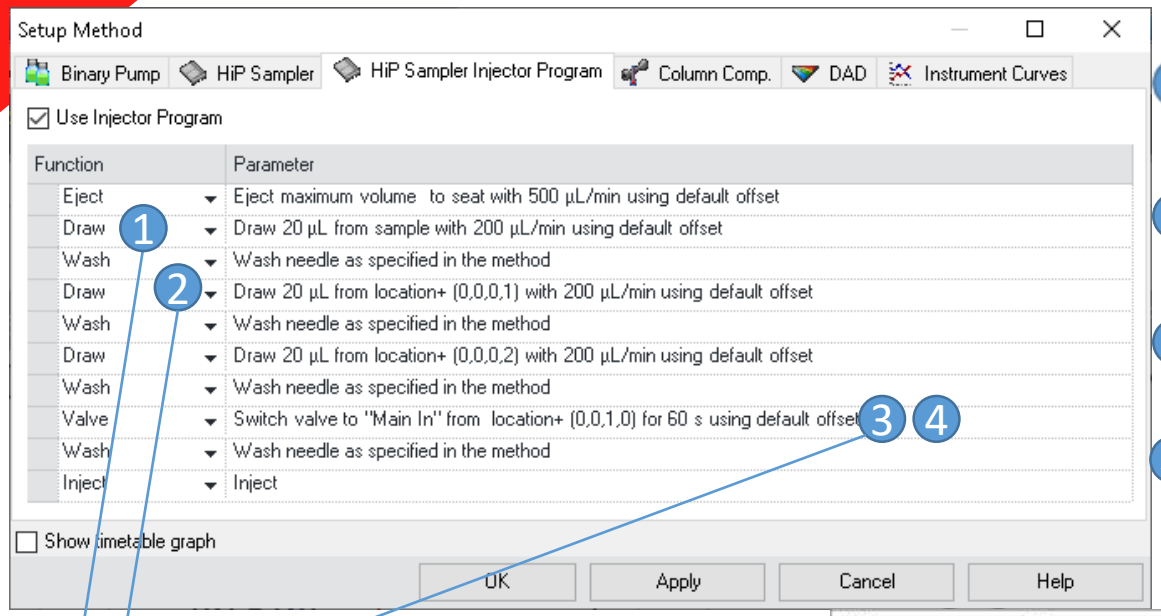
	Autosampler (Program)				Manual		
Lvl	Amt	Area	RF		Amt	Area	RF
1	0.1	17.231	172.3		0.1	22.258	222.6
2	0.2	34.431	172.2		0.5	103.12	206.2
3	0.5	85.098	170.2		1	196.42	196.4
4	1	171.45	171.4		5	933.34	186.7
5	2	343.7	171.8		10	1845.2	184.5
6	5	859.47	171.9		15	2792.7	186.2
7	10	1724.8	172.5		25	4672.4	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



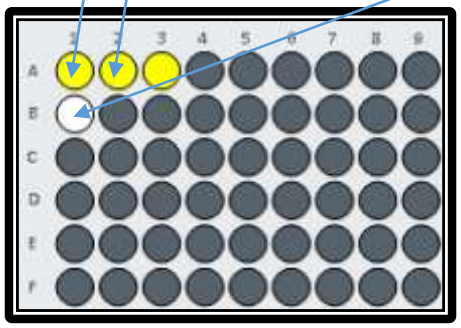
Better linearity and more consistent response factors (RF) for the autosampler method.

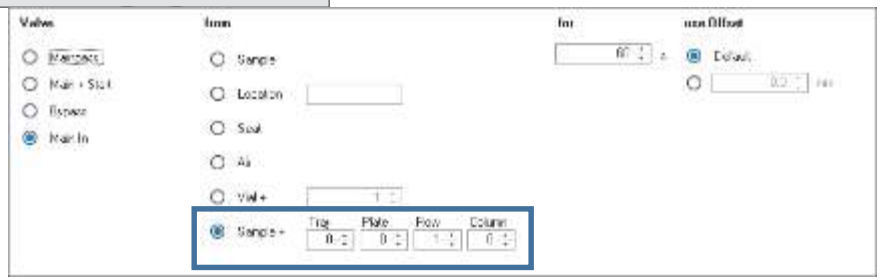
Case Study 3: Making Mixtures

Prepare a mixture of three components, each diluted 1:25 from three individual stock solutions.



1. Sample location in Sequence Table is P1-A1
2. Location is relative to sample, one column over (P1-A2)
3. Location is relative to sample, one row down (P1-B1)
4. Pump flow rate is 0.50 mL/min., so total volume is 500 µL





- The Sample+ option allows you to specify a position relative to the Sample location. You specify the change for Tray, Plate, Row, and Column.

Conclusion:
You can make individual stock solutions 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
- 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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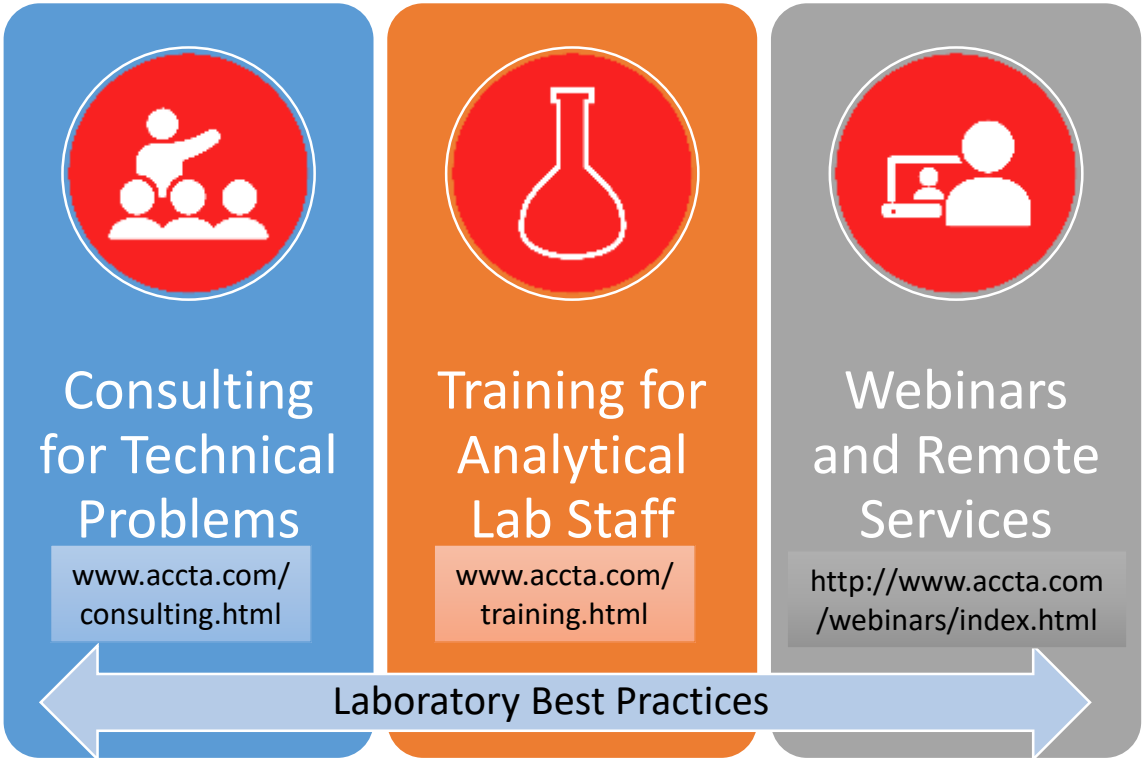

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