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Use Your Autosampler to Make Calibration Standards

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analytical solutions to analytical problems

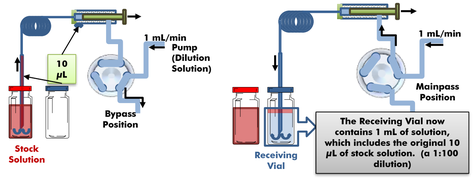
INTRODUCTION AND GENERAL SETUP

- **We describe a feature of many autosamplers that produces dilutions and mixtures.**
  - **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 Required**
  - **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 place column inlet tubing into waste container**
  - **Program pump to deliver desired dilution solution**
  - **Set flow to 0.5 - 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?

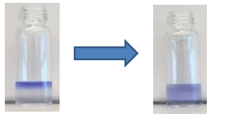
Step 1: Draw Stock Solution
Draw desired volume of stock solution using "Draw" command

Step 2: Dilution
Use "Valve" or "Main In" command to switch injection valve to Mainpass into the receiving vial for a specified time.



Important

Step 3:
Mix/agitate the receiving vial thoroughly.



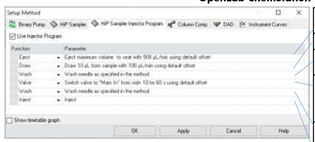
Receiving Vial After Procedure

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

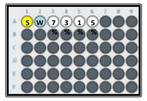


Needed to reset sampling syringe to home position.
This is the volume of stock solution.
Wash options will vary depending on your model.
This example uses a position relative to the sample. Depending on your model, you may have to specify a relative position for tray, row, and column.
Wash options will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

- **Other Method Settings**
  - **Pump**
    - **Flow: 1 mL/min**
    - **Stop Time: 0.1 min.**
  - **Injector**
    - **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 (S)**
    - **Wash vial in P1-A2 (W)**
    - **7% in P1-A3**
    - **3% in P1-A4**
    - **1% in P1-A5**
    - **5% in P1-A6**



Results

Compare Replicate Preparations

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

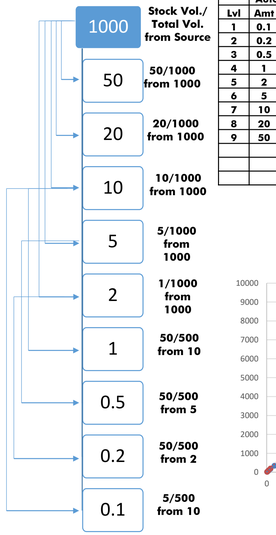
Compare Preparation Levels

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

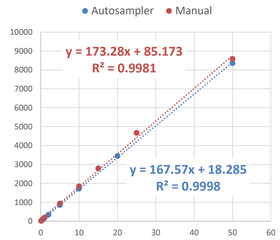
Compare with manually prepared standard

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

CASE STUDY 2: DNPH DERIVATIVE

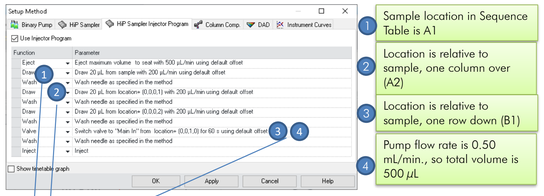


	Autosampler (Program)			Manual (Serial)		
Lvl	Amt	Area	RF	Amt	Area	RF
1	0.1	17.231	172.31	0.1	22.258	222.58
2	0.2	34.431	172.16	0.5	103.12	206.24
3	0.5	85.098	170.20	1	196.42	196.42
4	1	171.45	171.45	5	933.34	186.67
5	2	343.7	171.85	10	1845.2	184.52
6	5	859.47	171.89	15	2792.7	186.18
7	10	1724.8	172.48	25	4672.4	186.90
8	20	3449.2	172.46	50	8587.4	171.75
9	50	8359.5	167.19			
		Avg	171.3		Avg	192.7
		SD	1.71		SD	15.6
		RSD	1.00		RSD	8.11

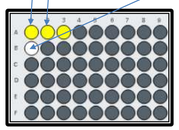


CASE STUDY 3: MAKING MIXTURES

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

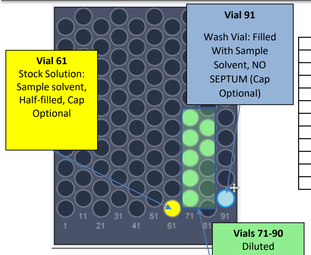


1. Sample location in Sequence Table is A1
2. Location is relative to sample, one column over (A2)
3. Location is relative to sample, one row down (B1)
4. Pump flow rate is 0.50 mL/min., so total volume is 500 µL



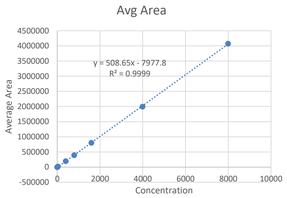
The Sample+ option allows you to specify a position relative to the Sample location. You specify the change for Tray, Plate, Row, and Column.

CASE STUDY 4: WIDE RANGE CURVE FOR GC



Level	Diluton	Conc	Avg Area	RF
1	10000	0.7992	368.600047	461.2
2	500	15.984	7275.203026	455.2
3	200	39.96	18492.79934	462.8
4	20	399.6	195377.5766	488.9
5	10	799.2	393765.4735	492.7
6	5	1598.4	803435.2665	502.6
7	2	3996	1993227.733	498.8
8	Stock	7992	4073564.138	509.7
			Avg	484.0
			SD	21.1
			RSD	4.4

Destination	Source	Concentration	Dilution Level
61		10,000 (Stock)	
71*	61	5,000	2X
72	61	2,000	5X
73	61	1,000	10X
74	61	500	20X
75	61	50	200X
84	74	20	500X
85	75	1	10,000X



***Note: This dilution requires use of a vial insert that can hold a minimum of 100 uL.**

SUMMARY

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.

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).