Agilent 7696A Sample Prep WorkBench: How to Automate Preparation of a Sample Set by Serial Dilution for Measurement of Flame Ionization Detector Performance

Application Note

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Introduction

A challenge that arises more often than the analyst might like, is the need to prepare a set of samples by serial dilution. Serial dilution starts with a single sample of known concentration. It is then used to prepare a set of dilutions, each usually differing from the previous one, by a constant factor. Each sample is made from the previous one in the series. This task may be driven by the need to calibrate an instrument with specific analytes or measure such things as detector performance: linearity, sensitivity and minimum detectable level (MDL). If the samples are not stable over time, they may need to be prepared weekly or even daily. To minimize errors in manual preparations or reduce the frequency of tiresome dilutions, the user will often prepare larger volumes of sample than needed, which leads to unnecessary waste and expense.

The Agilent 7696A Sample Prep WorkBench provides a solution to this problem by automating the serial dilution process precisely so that small volumes of sample can be routinely prepared when needed over as large a concentration range as desired. The preparative method for serial dilution starts with a measured volume of solvent in an empty vial followed by a measured volume of sample. After mixing, this step is repeated using a new vial of solvent and an aliquot from the last dilution. For example, measuring the performance of a flame ionization detector (FID) requires a set of samples, each diluted by a factor of ten from the previous sample. The starting sample is a normal hydrocarbon such as n-tridecane (C_{13}). Each dilution consists of 90% solvent and 10% previous sample (v:v). A set of seven or eight samples, as prepared in this application, are required to demonstrate the normal seven orders of magnitude of FID linearity. As described below, eight sets of test samples were prepared over a two week period. Three were prepared manually and five with the Agilent 7696 Sample Prep Workbench at a total volume per sample of either 1 mL or 0.5 mL. Repeatability over all sets was excellent whether measured by sample weight in each set or by FID performance.

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Experimental

The Agilent 7696A Sample Prep WorkBench was used to prepare a set of eight samples, each diluted by a factor of ten from the previous sample. Two sequences were used so that samples could be weighed after each addition. The first used a method that added a fixed amount of solvent to each vial. The second started with a manually-prepared 10% solution of C_{13} in solvent, then added enough solution to the next vial to make a tenfold less concentrated solution. After mixing, an aliquot of the freshly made sample was used to make the next dilution in the series until the eight sample set was complete. The empty vials were tared, and then weighed after each sequence to measure reproducibility of transfers across the series. The same preparations were also done manually for comparison.

Hardware Configuration

The Agilent 7696A Sample Prep WorkBench was equipped with two Agilent 7693A Automated Liquid Samplers. The back injector contained an enhanced syringe carriage containing a 500-µL syringe (p/n G4513-60561). The front injector used a standard syringe carriage containing a 100-µL syringe (p/n 5183-2042). The back injector was used for solvent delivery to each of the empty vials (first sequence) and the front injector was used for sample transfer from one sample to the next (second sequence).

Sample Preparation

Two protocols were used that differed only in the volume of the prepared dilution. The first used 900 µL solvent + 100 µL sample and the second used half these amounts: 450 µL solvent + 50 µL sample.

A single Agilent 7696A Sample Prep WorkBench resource layout was used for both sequences:

Resource Layout:

Vial Range	Name	Type	Usage
2-9	MT vial	Empty container	1 use/vial
12-19	Solvent	Chemical resource	1 use/vial

The single sample required was a solution of 10% C_{13} in isooctane. It was prepared by adding 100 µL C_{13} to a 1 mL volumetric and diluting to mark.*

The first sequence prepared the 1 mL sample (900 µL + 100 µL) by adding 900 µL solvent to an empty vial (see Appendix for syringe parameters). The sequence specified vials 2 through 9.

* I started with the 10% C_{13} instead of 100% C_{13} to avoid any volume shrinkage that might occur when mixing two neat compounds by volume.

The second sequence specified sample dilutions according to the following steps. (see Appendix for syringe parameters):

Step	Function
1	Add 100 µL of Sample (Front) to vial #2
2	Mix vial #2 at 1500 RPM for 0 min 5 sec
3	Add 100 µL of vial #2 to vial #3
4	Mix vial #3 at 1500 RPM for 0 min 5 sec
5	Add 100 µL of vial #3 to vial #4
6	Mix vial #4 at 1500 RPM for 0 min 5 sec
7	Add 100 µL of vial #4 to vial #5
8	Mix vial #5 at 1500 RPM for 0 min 5 sec
9	Add 100 µL of vial #5 to vial #6
10	Mix vial #6 at 1500 RPM for 0 min 5 sec
11	Add 100 µL of vial #6 to vial #7
12	Mix vial #7 at 1500 RPM for 0 min 5 sec
13	Add 100 µL of vial #7 to vial #8
14	Mix vial #8 at 1500 RPM for 0 min 5 sec
15	Add 100 µL of vial #8 to vial #9
16	Mix vial #9 at 1500 RPM for 0 min 5 sec

Results

Over a period of two weeks, eight serial dilution runs were made: Three manual (two at 1 mL and one at 0.5 mL); five with the Agilent 7696A Sample Prep WorkBench (three at 1 mL and two at 0.5 mL).

Table 1. Reproducibility for Solvent Delivery (Average of Eight Samples)

Type	Manual	Manual	Manual	7696A	7696A	7696A	7696A	7696A
Volume (mL)	0.5	1.0	1.0	0.5	1.0	1.0	1.0	0.5
Average weight (g)	*	0.6165	0.6151	0.3089	0.6176	0.6195	0.6180	0.3088
%SD	*	0.17	0.26	0.11	0.16	0.09	0.06	0.17

* Not measured.

Reproducibility for the second step was ±1 µL, for all but the last sample. Each sample except the last was used to prepare the next. The weight should not change because the same volume is added to and then removed from each sample. The average weight change regardless of whether a 1 mL or 0.5 mL preparation was involved was equivalent to ±1 µL. The volume increase of the last sample was 100 µL or 50 µL for the 1 mL and 0.5 mL volumes, respectively.

The total Agilent 7696A Sample Prep WorkBench runtime was 49 min for the 1 mL set of samples and 41 min for the 0.5 mL set. The time for the manual preparations was not measured.

Reproducibility of FID performance

The protocol used for FID linearity, sensitivity and MDL followed the ASTM protocol closely [1]. The major difference was the use of liquid samples rather than gas samples as specified by ASTM. All preparations were tested on the same FID. The linearity results (Figure 1) are essentially indistinguishable whether the samples were prepared by the Agilent 7696A Sample Prep WorkBench or manually. The average sensitivity and % SD were 26.3 and 2.4, respectively. This is very good performance for repeat runs on a single FID. The large spread in the MDL (Table 2) is caused by day-to-day variability in average detector noise in the region where C_{13} elutes. MDL is a sensitive function of noise. Table 2 and Figure 1 summarizes the results.

Table 2. FID MDL

Prep Type	Manual	Manual	Manual	7696	7696	7696	7696	7696
Volume (mL)	0.5	1.0	1.0	0.5	1.0	1.0	1.0	0.5
Sensitivity (ma-s/gC)	27.2	25.7	25.8	26.8	26.8	25.5	26.6	25.5
MDL (pgC/s)	0.96	1.14	1.66	0.92	0.68	1.31	1.23	1.15

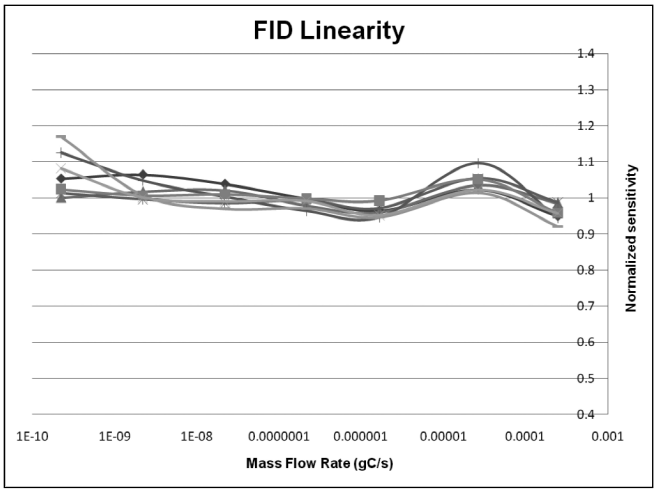

Figure 1. Linearity Plots for all eight runs overlaid.

Conclusion

The Agilent 7696A Sample Prep WorkBench simplifies the preparation of a set of samples by serial dilution. The user can prepare fresh samples only when needed at volumes no larger than necessary to satisfy the analytical requirements. The result is less boredom, less chance for operator error, less consumption of reagents, less waste disposal expense and better repeatability.

Appendix

500 µL syringe parameters:

	Tower Back	Solvent Prewash1	Solvent Prewash 2	Dispense wash	Dispense pumps	Dispense settings	Solvent postwash1	Solvent postwash2
Number pumps or washes					3			
Wash volume (µL)					50			
Draw speed (µL/min)					1250	1250		
Dispense speed (µL/min)					3000	3000		
Needle depth offset (mm)					0	0		
Viscosity delay(s)					2	2		
Turret solvent								
Air gap (% syr.vol.)						0		

100 µL syringe parameters:

	Tower Back	Solvent Prewash1	Solvent Prewash 2	Dispense wash	Dispense pumps	Dispense settings	Solvent postwash1	Solvent postwash2
Number pumps or washes		1		1	2			
Wash volume (µL)		10		20	10			
Draw speed (µL/min)		300		300	300	300		
Dispense speed (µL/min)		6000		6000	6000	6000		
Needle depth offset (mm)		0		0	0	0		
Viscosity delay(s)		2		2	2	2		
Turret solvent		A						
Air gap (% syr.vol.)						0		

Reference

1. ASTM E594-96 (2006) Standard Practice for Testing Flame Ionization Detectors used in Gas or supercritical Fluid Chromatography

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