APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

Author:

Adam J. Patkin, Ph.D.

PerkinElmer, Inc.
Shelton, CT

Calibration Dilution Workflow for Automated Standard Preparation on the TurboMatrix MultiPrep+

Introduction

Preparation of calibration standards for Gas Chromatography (GC) and Gas Chromatography/ Mass Spectrometry (GC/MS) analysis can be expensive and time-consuming, especially when it needs to be done on a daily basis for routine analyses. This expense and time is due to:

- Purchase of the required volumetric and transfer glassware
- Cleaning of the glassware to prevent carry-over
- Cost of purchase and disposal of high-purity solvents and analytical standards
- Skilled technician or scientist time to carefully prepare the standards

Automation of calibration standard preparation with the PerkinElmer TurboMatrix MultiPrep+ robotic autosampler:

- Eliminates the volumetric and transfer glassware, replacing it with syringes and disposable vials
- Eliminates labor, solvent, and waste disposal costs for cleaning this glassware
- Eliminates potential for cross contamination from reuse of volumetric and transfer glassware
- Can reduce the volume of solvent required from tens or hundreds of mL per calibration level to 0.6 mL or less, along with associated waste disposal costs of unused material
- Reduces the human time and skill requirements to filling vials with solvent and standards, and adding empty vials to receive new dilution standards

In addition, the MultiPrep+ automation software can queue the preparation of a set of calibration standards to occur immediately before they are injected along with analytical samples, or it can operate in a stand-alone mode.

PerkinElmer®
For the Better

The Sample Manager software of the MultiPrep+ provides several methods of standards preparation. This Application Note describes the "Calibration Dilution" workflow operating in a stand-alone mode. This dilution workflow can prepare a series of dilution standards, with an optional internal standard, from a single standard stock solution vial. All prepared standards and the optional internal standard are on the same 2 mL vial rack.

Workflow Overview

A user-specified Sample Manager method defines which dilutions are to be prepared, how many replicate sets of dilutions, and if an internal standard is to be spiked into the vial. The method also defines all syringes, washing, mixing, temperatures, etc.

Calibration Dilution uses a 10 µL and a 1000 µL syringe to prepare up to three replicate sets of selected dilutions of 1:2, 1:5, 1:10, 1:20, 1:50, 1:100, 1:200, 1:500, and 1:1000. The final volume of each dilution level vial is user selectable as either 0.5 mL or 1 mL. Undiluted stock solution, solvent blank, and solvent with internal standard vials may also be prepared.

The vial positions are fixed in the tray, as shown in Table 1 and Figure 1. If there is an internal standard it is placed in vial #54. Any dilution ratio can be selected individually for preparation.

Table 1. Vial Positions in Sample Tray.

Vial (3 Replicates)	Dilution Ratio
1, 19, 37	1:1 (Undiluted Stock Solution)
2, 20, 38	1:2 Dilution
3, 21, 39	1:5 Dilution
4, 22, 40	1:10 Dilution
5, 23, 41	1:20 Dilution
6, 24, 42	1:50 Dilution
7, 25, 43	1:100 Dilution
8, 26, 44	1:200 Dilution
9, 27, 45	1:500 Dilution
10, 28, 46	1:1000 Dilution
11, 29, 45	Solvent with 10 µL internal standard
12, 30, 48	Solvent Without Internal Standard 0.5 mL Always Prepared to Save Solvent
54	Internal Standard

Blue: Vial positions for dilution replicate 1, Positions 1-12
Yellow: Vial positions for dilution replicate 2, Positions 19-30
Purple: Vial positions for dilution replicate 3, Positions 37-48
Orange: Internal Standard, Position 54

Figure 1. Sample Tray Layout.

The four 10 mL vial Standard Wash station contains the standard stock solution in vial #4. Vials #1, #2, and #3 contain the dilution solvent used to prepare the replicate dilution sets 1, 2, and 3.

The basic sample preparation workflow is:

1. Transfer required volume of diluent with the 1000 µL syringe to vials #1 to #12
2. Optionally add 10 µL of the internal standard to vials #1 to #11
3. Transfer stock solution with either the 1000 µL or the 10 µL syringe (depending on the volume to transfer) to vials #1 to #10
4. Vortex mix vials #1 to #12
5. Repeat steps 1-4 for the vials of replicates 2 and 3 if selected

If running in an on-line configuration, a GC injection sample list can be scheduled to occur immediately after the completion of standards preparation for unattended operation.

Automated Calibration Dilution is best for lower-volatility solvents and analytes because of the needle holes put into the vial septa. For example, methanol or isooctane solvent with compounds of higher boiling point may be quantitatively stable for several hours to days; long enough to prepare and inject. It is not recommended for high-volatility solvents (e.g. diethyl ether, methylene chloride, carbon disulfide) or analytes which have boiling points at or below room temperature.

The standards are usually prepared in a 2 mL vial tray. A 10/20 mL vial tray can also be used, but since only 0.5 or 1.0 mL standards can be prepared, this is of limited utility. Also, internal standards are not possible with the 10/20 mL tray.

Cost Savings for Solvents and Standards

Table 2 shows the total quantity of diluent solvent, stock solution, and optional internal standard required to prepare a single complete set of standards and for three replicate standard sets of either 0.5 mL or 1 mL volumes of dilution standards. Each replicate starts with a new 10 mL diluent vial. The total includes all syringe diluent cleaning washes and priming rinses with stock solution and internal standard.

Table 2. Required Diluent and Internal Standard Volumes.

	1 Set of Standards		3 Replicate Sets of Standards	
Required Volume (mL)	0.5	1.0	0.5	1.0
Diluent	4.98	9.53	14.93	28.60
Stock Solution	0.94	1.88	2.82	5.65
Internal Standard	0.11	0.11	0.33	0.33

Less volume of diluent, stock, and internal standard solutions are required if only a subset of the vials is being prepared.

There are additional cost savings possible. Vial #1, the undiluted stock solution, and vial #2, the 1:2 dilution, are the major consumers of stock solution. Eliminating them from the prepared standard set will result in significant savings, with only 1.2 mL of stock standard required for all three sets. If only one set of vials is being prepared, or vials #1 and #2 are eliminated, the 10 mL vial #4 of the Standard Wash station containing the stock solution

can be replaced by a 2 mL vial by using a 10 mL to 2 mL vial adapter (P/N N6556040, and configuring #4 of the Standard Wash station for the 2 mL vial as described in the User's manual). Even smaller required volumes of the internal standard can be realized using the "bottom sensing" mode of syringe sampling, which can take three 1 µL samples from as little as 5 µL of liquid in the vial. (This mode requires a 23 Gauge liquid syringe and a conical bottom vial, and is only possible for 2 mL sample vial trays).

System Configuration

Below are recommended MultiPrep+ configurations for Serial Dilution and a typical pre-run checklist.

Minimum Recommended MultiPrep+ Configuration

The Minimum Configuration Required to Perform Calibration Dilution Is:

- Robotic tool change syringe park station
- Sample Tray holder
- 2 mL VT-54 rack
- Standard Wash station
- Large Wash station (N6496024)
- 10 µL (57 mm needle, 23S Gauge) syringe and D7/57 tool (N6496002)
- 1000 µL (57 mm needle, 23 Gauge) syringe and D8/57 tool (N6496004)
- 10 µL (85 mm needle, 23S Gauge) syringe and D7/85 tool (N6496003) for GC injection
- 2 mL vials and caps

Suggested MultiPrep+ Configuration Enhancements

For homogeneous sample mixing and lower internal standard usage:

- Vortexer (*strongly* recommended)
- Magnetic metal crimp caps for 2 mL vials (required for Vortex mixing)
- 10 mL to 2 mL vial adapter (N6556040)
- 57 mm, 23 Gauge needles (required for bottom sensing vials)
- Round-bottom 2 mL sample vials for internal standard (required for bottom sensing vials)

Pre-Run Checklist

1. Verify 10 µL sample prep syringe is mounted.
2. Verify 1000 µL sample prep syringe is mounted.
3. (Optional) Verify GC injection syringe is mounted.
4. Place stock standard with a non-magnetic cap in position #4 of Standard Wash station.
5. (Optional) Place Internal standard solution in Vial #54.
6. Replace Standard Wash vials #1-#3 (depending on number of replicates) with fresh 10 mL vials (to avoid carry-over) and dilution solvent.
7. Refill Large Wash station vials with syringe cleaning solvent. (#1 for internal standard, #2 for diluent).
8. Put new, capped sample vials in tray positions being used (Figure 1).

Experimental

Below are examples of Calibration Dilution without and with an internal standard. Samples were prepared off-line with a stand-alone MultiPrep+, and then injected into a Clarus 690 GC with an FID detector under TotalChrom control.

Stock Standards Preparation

Pesticide-grade isooctane (2,2,4-Trimethylpentane) was used as the diluent and syringe cleaning solvent. Analytical standard grade *n*-decane, *n*-undecane, *n*-tridecane, and *n*-tetradecane (2.36, 3.34, 2.16, and 2.81 mg/mL, respectively) were used as the analytes, and analytical standard grade *n*-dodecane (2.64 mg/mL) was the internal standard.

Automated Calibration Dilution Method

The Sample Manager Calibration Dilution method is shown in Table 3. For this applications note both internal and external standard calculations were made from the same internal standard calibration dilution for comparison purposes. However, if an internal standard is not required the *Internal Standard* parameter is set to "Omit" the *Solvent with Internal Standard* to "Skip" and the *Solvent without Internal Standard* to "Prepare".

Table 3. Sample Manager Internal Standard Calibration Dilution Method.

Configuration			
10 µL Syringe	P/N N6556083	1000 µL Syringe	P/N N6556089
Dilution Vial Rack	Rack 1	Standard Wash Station	Standard Wash 1
Large Volume Wash Station	Large Wash 1	Vortex Mixer	Vortex Mixer 1
Dilute			
Final Volume	1000uL	Repetitions	1
Internal Standard	Include	Sample Viscosity Delay	3 s
Sample Fill Rate 10 µL Syringe	1 µL/s	Sample Fill Rate 1000 µL Syringe	50 µL/s
Dilution Selection			
Undiluted Standard	Prepare	1:2 Dilution	Prepare
1:5 Dilution	Prepare	1:10 Dilution	Prepare
1:20 Dilution	Prepare	1:50 Dilution	Prepare
1:100 Dilution	Prepare	1:200 Dilution	Prepare
1:500 Dilution	Prepare	1:1000 Dilution	Prepare
Solvent with Internal Standard	Prepare	Solvent without Internal Standard	Skip
Rinsing			
Rinse Cycles	2	Wash Cycles	2
Rinse Volume for 10 µL Syringe	10 µL	Rinse Volume for 1000 µL Syringe	20 µL
Mixing			
Mixing Speed	1200 rpm	Mixing Time	6 s
Advanced			
Bottom Sense Internal Standard	Off	Height from Bottom of Internal Standard	0.5 mm
Bottom Sense Stock Solution	Off	Height from Bottom of Stock Solution	0.5 mm
Syringe Overfill	5 %	Sample Cleaning Viscosity Delay	1 s
Wash Vial Depth	44 mm	Waste Port Depth - Solvent	12 mm
Sample Vial Penetration Speed	50 mm/s	Fast expel for 1000 µL Syringe	20 µL
Target Vial Depth	10 mm	Solvent Vial Depth	44 mm
Stock Solution Vial Depth	30 mm	Internal Standard Vial Depth	30 mm

Analytical Conditions

The GC injection method is shown in Table 4.

Table 4. GC injection method.

Gas Chromatograph	PerkinElmer Clarus 690 GC
Injector Type	Programmable Split/Splitless
Injector Temperature	200 °C
Analytical Column	PerkinElmer Elite™ - 5HT 15 m x 0.32 mm ID x 0.1 µm
Detector	Wide-Range Flame Ionization Detector 250 °C, Air = 400 mL/min, H2 = 35 mL/min
Oven Program	70 °C for 2 min, then ramp to 220 °C at 15 °C/min
Split Flow	Splitless until 1 min, then 30 mL/min
Carrier Gas	2 mL/min Helium, 99.99+% purity

Results

Table 2 shows the internal standard calibration dilution plot, where each of the 11 calibration dilution levels shown in Table 1 (undiluted stock, 1:2, 1:5, 1:10, 1:20, 1:50, 1:100, 1:200, 1:500, 1:1000, and solvent blank with internal standard) was injected in triplicate.

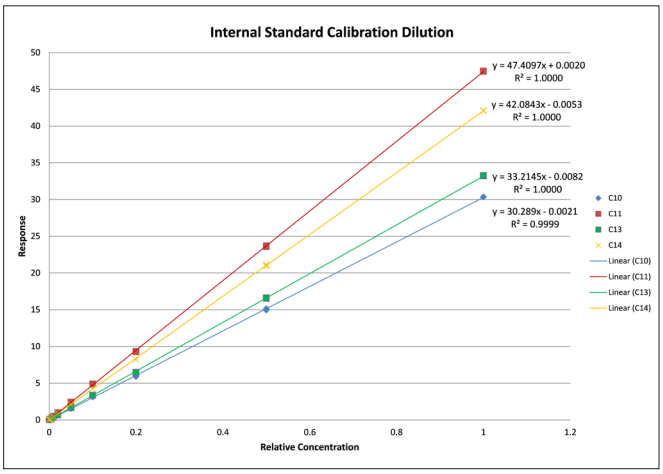

Figure 2. Internal Standard Calibration Dilution Plot.

Excellent linearity was observed over this wide dynamic range. The external standard calibration plot (not shown) was very similar.

Table 5 demonstrates that the internal standard calibration had slightly better linearity, showing slightly higher Coefficient of Determination (R^2) values, than the external standard calibration. This is expected from theory; the internal standard improves linearity by helping to compensate for injection-to-injection variations in sample volume and injector flow dynamics.

Table 5. Comparison of Internal Standard and External Standard R^2 Values.

	External Standard R^2	Internal Standard R^2	Internal – External Standard R^2
C10	0.9997	0.9999	0.0002
C11	0.9997	1.0000	0.0003
C13	0.9997	1.0000	0.0003
C14	0.9997	1.0000	0.0003

Conclusions

The Calibration Dilution workflow of the PerkinElmer TurboMatrix MultiPrep+ robotic autosampler has been demonstrated to produce user-selected calibration dilutions with excellent linearity across a 1000-fold dynamic range, using both external and internal standard calibration. This allows the preparation of fresh standards from stock solution using smaller quantities of expensive high-purity analytes and solvents than would be required by conventional manual volumetric methods. It also reduces labor costs and opportunity for human error.

Samples can be prepared on-line with direct injection into the GC using the MultiPrep, or off-line as demonstrated above.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

013990_01 PKI