APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

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Serial 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 serial dilution standard preparation with the PerkinElmer TurboMatrix MultiPrep+ robotic autosampler:

- Eliminates the volumetric and transfer glassware, replacing it with syringes and disposable vials
- Eliminates 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 1.6 mL or less
- 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.

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The Sample Manager software of the MultiPrep+ provides several methods of standards preparation. This Application Note describes the "Serial Dilution" workflow. This dilution workflow can prepare a series of standards in 2 mL vials from three to eight orders-of-magnitude dilution. This wide dynamic range can be very useful for the dilution of high concentration commercial stock standards, preparing working standards, and for determining appropriate concentration working ranges for GC and GC/MS analysis. Once the working range has been determined, the "Calibration Dilution" workflow, described elsewhere, may be useful because it gives more calibration points across a typical GC or GC/MS working range.

Workflow Overview

A user-specified Sample Manager method defines how many serial 10:1 dilutions are to be prepared and how many replicate sets of dilutions. The method also defines all syringes, washing, mixing, temperatures, etc.

Calibration Dilution uses a 100 µL and a 1000 µL syringe to prepare up to three replicate sets of the specified number of dilution steps. The final volume of each dilution level is 1 mL. The vial positions are fixed in the tray, as shown in Table 1 and Figure 1.

Table 1. Vial Positions in Sample Tray.

Vial (3 Replicates)	Dilution Ratio
1, 10, 19	1 : 10 Dilution
2, 11, 20	1 : 100 Dilution
3, 12, 21	1 : 1000 Dilution
4, 13, 22	1 : 10,000 Dilution
5, 14, 23	1 : 100,000 Dilution
6, 15, 24	1 : 1,000,000 Dilution
7, 16, 25	1 : 10,000,000 Dilution
8, 17, 26	1 : 100,000,000 Dilution
54	Neat Sample

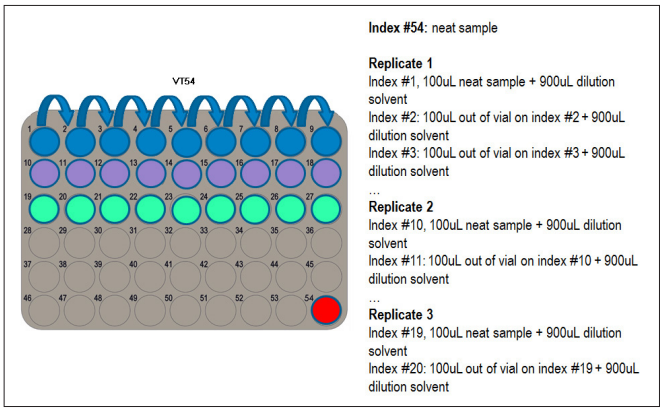

Figure 1. Sample Tray Layout.

The workflow is to first use the 1000 µL syringe to add 900 µL of dilution solvent to all the vials. Next, the 100 µL syringe is used to take 100 µL out of the neat stock sample in vial #54 and transfer it to vial #1. Vial #1 is now homogenized, optionally by a high-speed vortex mixer, and if not, by the syringe sample pumps with sample from vial #1 prior to the next step. Now, 100 µL of the 10:1 dilution is transferred to vial #2 from vial #1. Vial #2 is now mixed. This continues for the specified number of dilutions. If two or three sets of dilution standards have been specified, they occur next.

The four 10 mL vial Standard Wash station contains 10 mL of dilution solvent in each of vials #1, #2, and #3, for the dilution solvent used to prepare replicate dilution sets 1, 2, and 3.

A GC injection sample list can be scheduled to occur immediately after the completion of standards preparation, for unattended operation.

Automated Serial 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.

Cost Savings for Solvents and Standards

Table 2 shows the total quantity of diluent solvent and stock standard solution required to prepare a single complete set of standards and three replicate standard sets. It assumes that all eight dilution levels are prepared, and that the diluent solvent is the same as the syringe wash solvent. The total includes all syringe diluent cleaning washes, and priming rinses with stock solution and standards between dilution levels. Each replicate set starts with a new diluent vial.

Table 2. Required Solvent and Standard Volumes.

# Sets of 8 Dilutions	Solvent (mL)	Standard (mL)
1	11.42	0.12
3	34.26	0.36

Each dilution level omitted saves 1.12 mL of solvent.

Smaller volumes of the stock solution can be optionally used with 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.)

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
- 1000 µL (57 mm needle, 23 Gauge) syringe and D8/57 tool (N6496004)
- 100 µL (57 mm needle, 23S Gauge) syringe and D7/57 tool (N6496002)
- 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)
- Large Wash station (N6496024), replacing Standard Wash for dilution solvent washing
- Solvent Module (N6496020), replacing Standard Wash for dilution solvent
- 57 mm, 23 Gauge needles (required for bottom sensing vials)
- 10 µL (85 mm needle, 23S Gauge) syringe and D7/85 tool (N6496003) for GC injection

Pre-Run Checklist

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

Stock Standards Preparation

Pesticide grade isooctane was used as the diluent and cleaning solvent, and analytical standard grade *n*-decane, *n*-undecane, *n*-dodecane, *n*-tridecane, and *n*-tetradecane as the analytes. The stock solution was prepared gravimetrically at 1.92, 2.34, 1.73, 2.16, and 1.86 mg/mL, respectively. The naphthalene internal standard was prepared at 1.01 mg/mL.

Methods

The TurboMatrix Sample Manager Calibration Dilution method is shown in Table 3. The GC injection method is shown in Table 4. Most parameters were used at their default values.

The PerkinElmer Clarus 690 GC used a 1 µL injection split 100:1 into a capillary split/splitless injector at 250 °C onto a PerkinElmer Elite™ 5MS, 30 m x 0.25 mm ID x 0.25 µm column. Carrier gas was 1.5 mL/min helium (99.999 + % purity). The oven program was 90 °C for 1.5 min, then ramp to 157 °C at 10 °C/min. The split flow was reduced to 10:1 at 2 min to reduce gas consumption. The Clarus SQ 8T mass spectrometer operated under UltraTune conditions with a 250 °C transferline and ion source, monitoring *m/z* 57 for the alkanes.

Table 3. Sample Manager Serial Dilution Method.

Configuration			
100 µL Sample Syringe	P/N N6556086	1000 µL Solvent Syringe	P/N N6556089
Dilution Vial Rack	Rack 1	Dilution Solvent Station	Standard Wash 1
Wash Station	Large Wash 1	Vortex Mixer	Vortex Mixer 1
Dilute			
Dilution Steps	8	Repetitions	1
Sample Fill Rate 100 µL Syringe	25 µL/s	Solvent Fill Rate 1000 µL Syringe	50 µL/s
Rinsing			
Stock Solution Rinse Cycles	2	Dilution Sample Rinse Cycles	2
Wash 100 µL Syringe After Each Sample	On	Wash Cycles for 100 µL syringe	2
Wash Cycles for 1000 µL Syringe	2	Rinse Volume for 100 µL Syringe	10 µL
Rinse Volume for 1000 µL Syringe	20 µL		
Mixing			
Mixing Speed	1200 rpm	Mixing Time	6 s
Advanced			
Bottom Sense Stock Solution	Off	Height From Bottom of Stock Solution	0.5 mm
Syringe Overfill	5 %	Wash Vial Depth	44 mm
Waste Port Depth - Solvent	12 mm	Sample Vial Penetration Speed	50 mm/s
Fast Expel for 1000 µL Syringe	50 µL	Target Vial Depth	10 mm
Dilution Solvent Station Depth	44 mm	Solvent Vial Depth	44 mm
Stock Solution Penetration Depth	30 mm	Sample Pumps	5
Sample Vial Penetration Depth	30 mm		

Table 4. Sample Manager Injection Method.

Configuration			
Gas Chromatograph	GC2	Syringe	P/N N6556084
Verify Barcodes	Off	Pre-injection Wash Station	Standard Wash 1
Post-injection Wash Station	Standard Wash 1	Peltier Stack 1	none
Peltier Stack 1 Temperature	20 °C	Peltier Stack 2	none
Peltier Stack 2 Temperature	20 °C		
General Syringe			
Sample Vial Depth	30 mm	Sample Pumps	6
Fill Volume	3 µL		
Pre-injection Wash			
Wash Cycles	6	Wash Solvent 1	2
Wash Solvent 2	3	Wash Solvent 3	0
Wash Solvent 4	0	Syringe Fill Volume	3 µL
Syringe Flow Rate	2 µL/s		
Sample Rinse			
Sample Rinses	1	Rinse Volume	3 µL
Delay After Pull Up	2 s	Delay After Aspirate	1 s
Sampling			
Sample Viscosity Delay	2 s	Sample Fill Rate	2 µL/s
Prep Ahead	Disabled		
Inject Sample			
Injection Mode	Normal	Injection Flow Rate	50 µL/s
Post-injection Dwell Time	0 s		
Post-injection Wash			
Wash Cycles	6	Wash Solvent 1	2
Wash Solvent 2	3	Wash Solvent 3	0
Wash Solvent 4	0	Syringe Fill Volume	3 µL
Syringe Flow Rate	5 µL/s		
Advanced			
Pre-injection Dwell Time	0 s	Height from Bottom of Sample Vial	0.5 mm
Bottom Sense Sample Vial	Off	Sample Vial Penetration Speed	50 mm/s
Waste Port Depth	10 mm	Wash Vial Depth	40 mm
Sample Cleaning Fill Rate	2 µL/s	Sample Cleaning Viscosity Delay	2 s
Air Gap	0 µL	Injection Signal Mode	PlungerDown
Injector Penetration Depth	45 mm	Injector Penetration Speed	100 mm/s

Excellent linearity was observed over a wide linear dynamic range. Examples are shown in Figure 2 and Figure 3.

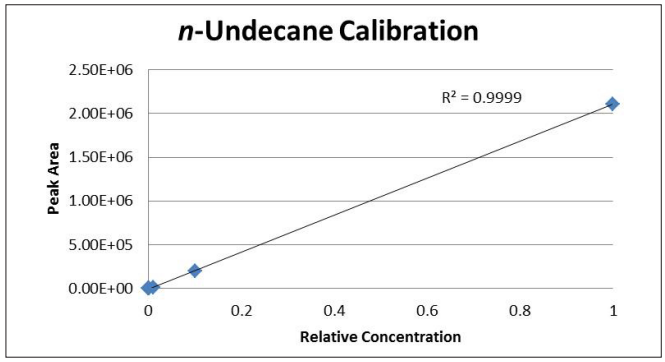

Figure 2. n-Undecane Calibration Curve.

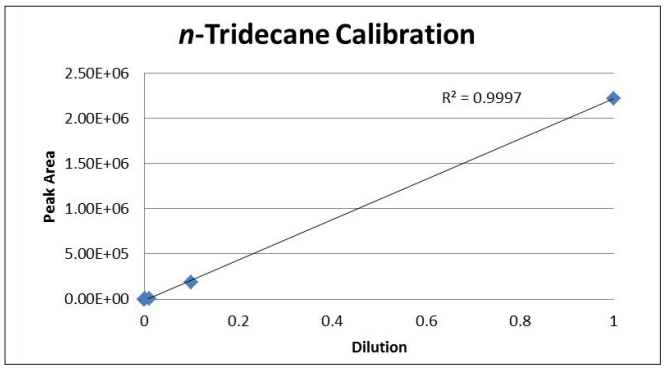

Figure 3. n-Tridecane Calibration Curve.

The mean R^2 coefficient of determination was better than 0.999 over five orders-of-magnitude dilution, limited on the low end by trace analyte contaminants in the solvent.

Table 5. Coefficients of Determination for analyte linearity.

Analyte	R^2	Decades
***n*-Decane**	0.9999	4
***n*-Undecane**	0.9999	5
***n*-Dodecane**	0.9995	5
***n*-Tridecane**	0.9997	5
***n*-Tetradecane**	0.9994	5

Conclusions

The Serial Dilution workflow of the PerkinElmer TurboMatrix MultiPrep+ robotic autosampler has been demonstrated to produce dilutions across a wide dynamic range. 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.

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