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Automated Calibration Standard Preparation using the MAESTRO Software Calibration Standard Wizard

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Keywords

Sample Preparation, High Throughput Lab Automation, LC-MS/MS, Method Calibration.

Abstract

Preparation of calibration standards and quality control samples are critical tasks in chemical analysis. The accurate and precise transfer of liquid standards and solutions is critical for ensuring the validity of analytical results. Automating the accurate transfer of liquid standards and solutions helps to improve the quality of the analytical procedure while freeing the analyst from performing tedious and repetitive manual tasks.

GERSTEL MAESTRO software provides unsurpassed flexibility for sample preparation, method development and analytical efficiency. The software operates all GERSTEL modules and system solutions used with the MultiPurpose Sampler (MPS) in a simple and efficient manner. MAESTRO can be operated as stand-alone software or integrated into a variety of laboratory chromatography data systems. The method used to prepare calibration standards can be quickly setup using the Calibration Standard Wizard available in the MAESTRO software.

Introduction

The Calibration Standard Wizard available with the MAESTRO software enables users to automate the creation of Prep Sequences that will prepare calibration curves based on a few pieces of information such as the required concentrations of the calibration curve standards to be prepared, the required final volume of each standard, whether to add an internal standard to each calibration standard, and where the necessary solutions are located on the GERSTEL MultiPurpose (MPS) robotic autosampler. The Calibration Standard Wizard will automatically generate the Prep Sequence, selecting the correct syringe size to use from those available on the MPS, and setting up the appropriate commands to move and mix the vials, if selected.

In this study, replicate calibration curves were generated using the Calibration Standard Wizard and the MPS robotic sampler to prepare the associated standards followed by injection to an LC-MS/MS system. Calibration curves were prepared for a variety of analyte classes including synthetic cathinones, hormones, and pesticides. Accuracy and precision of the calibration curves were subsequently evaluated.

GERSTEL AppNote 224

Experimental

Materials

All stock solutions for the synthetic cathinone compounds were purchased from Cerilliant. An intermediate analyte stock solution containing the synthetic cathinone compounds monitored was prepared in acetonitrile at a concentration of 10.0 µg/mL. Deuterated analogues, D_3-mephedrone and D_8-methylenedioxypyrovalerone (MDPV) were purchased from Cerilliant. An internal standard stock solution containing the deuterated internal standards was prepared in methanol at a concentration of 10.0 µg/mL.

A reference standard containing testosterone, androstenedione, 17β-estradiol, 17α-ethynylestradiol, estrone, and equilin was purchased from Restek®. An intermediate stock solution containing all hormone compounds monitored was prepared in methanol at a concentration of 10.0 µg/mL. Deuterated internal standards D_3-testosterone and D_5-17β-estradiol were purchased from Cerilliant. An internal standard stock solution containing the deuterated internal standards was prepared in methanol at a concentration of 10.0 µg/mL.

Pesticide stock solutions were obtained from Restek. An intermediate stock solution containing all pesticides monitored was prepared by combining the appropriate volumes of each pesticide stock solution to give a final concentration of 2.0 µg/mL.

Sample Preparation

Synthetic Cathinone Calibration Curves

Stock and working internal standard solutions were prepared in 2 mL vials and placed onto the MPS. A diluent solution consisting of (90:10) 0.1% formic acid in water:acetonitrile was placed into a solvent reservoir on the MPS. An agitator option was configured with the system for use in mixing the calibration standard samples. Corresponding vials for any necessary intermediate stocks were placed into their respective positions on the MPS.

Hormone Calibration Curves

Stock and working internal standard solutions were prepared in 2 mL vials and placed onto the MPS. A diluent solution consisting of 100% methanol was placed into a solvent reservoir on the MPS. An agitator option was configured with the system for use in mixing the calibration standard samples. Corresponding vials for any necessary intermediate stocks were placed into their respective positions on the MPS.

Pesticide Calibration Curves

Stock standard solution was prepared in 2 mL vials and placed onto the MPS. A diluent solution consisting of 100% acetonitrile was placed into a solvent reservoir on the MPS. An agitator option was configured with the system for use in mixing the calibration standard samples. Corresponding vials for any necessary intermediate stock solutions were placed into their respective positions on the MPS.

Instrumentation

To generate the synthetic cathinone compound calibration curves, all automated Prep Sequences were performed using a MPS robotic^{PRO} sampler as shown in figure 1. All analyses were performed using an Agilent 1260 HPLC with an Agilent Poroshell 120 EC-C18 column, (3.0 x 50 mm, 2.7 µm) and an Agilent Ultivo Triple Quadrupole Mass Spectrometer with Jet stream electrospray source. Sample injections were made using the GERSTEL LC-MS Tool into a 6 port (0.25 mm) Cheminert C2V injection valve outfitted with a 2 µL stainless steel sample loop.

For the hormone compound calibration curves, all automated Prep Sequences were performed using a MPS robotic^{PRO} sampler as shown in figure 1. All analyses were performed using an Agilent 1260 HPLC with a Phenomenex Gemini C18 column, (2.0 x 150 mm, 3 µm) and an Agilent Ultivo Triple Quadrupole Mass Spectrometer with Jet Stream Electrospray source. Sample injections were made using the GERSTEL LC-MS Tool into a 6 port (0.25 mm) Cheminert C2V injection valve outfitted with a 10 µL stainless steel sample loop.

Figure 1: GERSTEL MPS robotic^{PRO} sampler.

GERSTEL AppNote 224

For the pesticide compound calibration curves, all automated PrepSequences were performed using a GERSTEL MPS robotic^{PRO} sampler equipped with a 100 µL and a 1000 µL syringe. All subsequent analyses were performed using an Agilent 1260 HPLC with an Agilent Eclipse Plus C18 RRHD column, (2.1 x 50 mm, 1.8 µm) and an Agilent Ultivo Triple Quadrupole Mass Spectrometer with Jet stream electrospray source. Sample injections were made using a GERSTEL robotic^{PRO} sampler with the LC-MS Tool into a 6 port (0.25 mm) Cheminert C2V injection valve outfitted with a 2 µL stainless steel sample loop.

The individual LC and mass spectrometer method parameters used for analysis of the prepared calibration standards can be found in the corresponding application notes for synthetic cathinones[1], hormone compounds[2], and pesticides[3], respectively.

Results & Discussion

MAESTRO software's Calibration Standard Wizard enables users to automate the creation of Prep Sequences that will prepare calibration curves based on a few pieces of information such as the required concentrations of the calibration curve standards, how much of each standard is required, whether to add an internal standard, and where the necessary solutions are located on the MPS robotic sampler. The Calibration Standard Wizard will automatically generate the Prep Sequence, selecting the correct syringe size to use based on what is available on the MPS robotic sampler, and setting up the appropriate commands to move and mix the vials, if selected.

The Calibration Standard Wizard's data input screen allows the user to enter calibration standard concentration and volume values as well as other pertinent information about location of solutions, concentrations of stocks, and how the samples are to be mixed during the process. The user can create a maximum of 15 calibration standards and can choose between concentration units of ppm (µg/mL), ppb (µg/L) or (ng/mL), or ppt (ng/L).

As shown in figure 2, an example Calibration Standard Wizard method was created to prepare calibration curve solutions with concentrations of 1000, 500, 100, 50, 10, 5, and 1.0 ng/mL, respectively, from a 10.0 µg/mL stock solution. The stock solution was positioned in vial position 1 of a VT54 tray for 2 mL vials (Rack 11). The internal standard was positioned in vial position 54 of a different VT54 tray for 2 mL vials (Rack 1). The MPS was equipped with both a 1000 µL and a 100 µL syringe. Dilution solvent was in a 100 mL solvent reservoir (SolvRes1). An agitator option configured for 2 mL vials was selected as the mixing device. Rinsing of the syringes was configured to take place using 100 mL solvent reservoirs (Wash Vial 1 and Wash Vial 2). Intermediates were selected to be created into 2 mL vials held in a VT40 tray for 2 mL vials (StackFront). The final calibration standards were selected to be created in positions 1 through 7 of a VT40 tray for 2 mL vials (StackRear), respectively, with a final volume of 1 mL each. Once completed, the Calibration Standard Wizard allows the user to save the dilution strategy for future use.

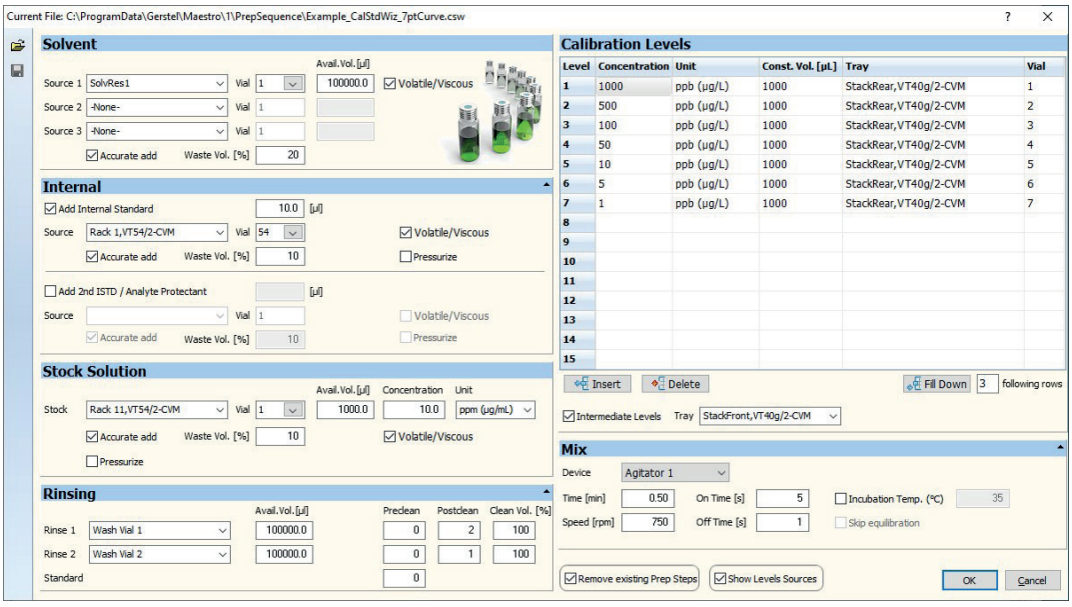

Figure 2: GERSTEL MAESTRO Calibration Standard Wizard.

GERSTEL AppNote 224

As shown in figure 3, the Calibration Standard Wizard provides a graphical representation of the strategy that will be used to prepare calibration standards allowing the user to review and confirm the process before starting it. A method check is performed to ensure that each parameter can be performed as specified.

The Calibration Standard Wizard was first evaluated by preparing standard curves for synthetic cathinones mephedrone and MDPV with their corresponding deuterated internal standards. Eight standards were prepared in triplicate using the Calibration Standard Wizard and were then analyzed using the LC-MS/MS system as described in [1]. As shown in figures 4 A-B, these standards were found to be both accurate (mephedrone average: 100%, range (92.2% - 109%, MDPV average: 100%, range (92.5% - 108%)) and precise (mephedrone average: 5.18% CV, range (0.692% - 9.76% CV, MDPV average: 5.22% CV, range (0.928% - 8.88% CV)). The linearity was good as can be seen from the R^2 values of 0.99 or better.

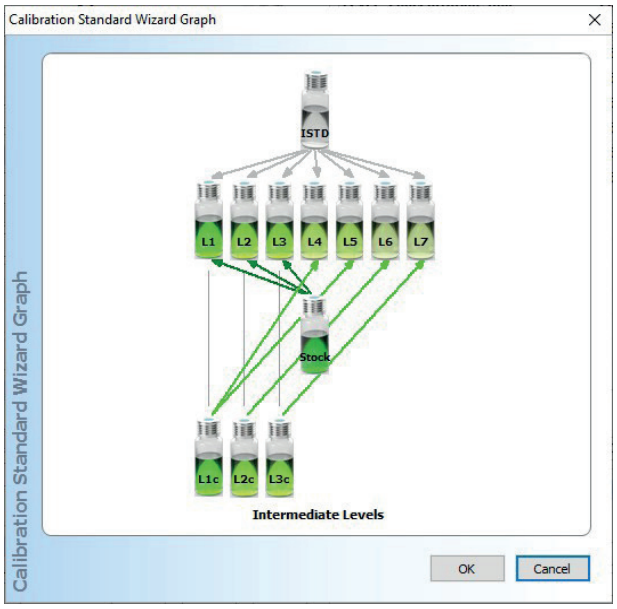

Figure 3: GERSTEL MAESTRO Calibration Standard Wizard graphical representation of the dilution and addition process.

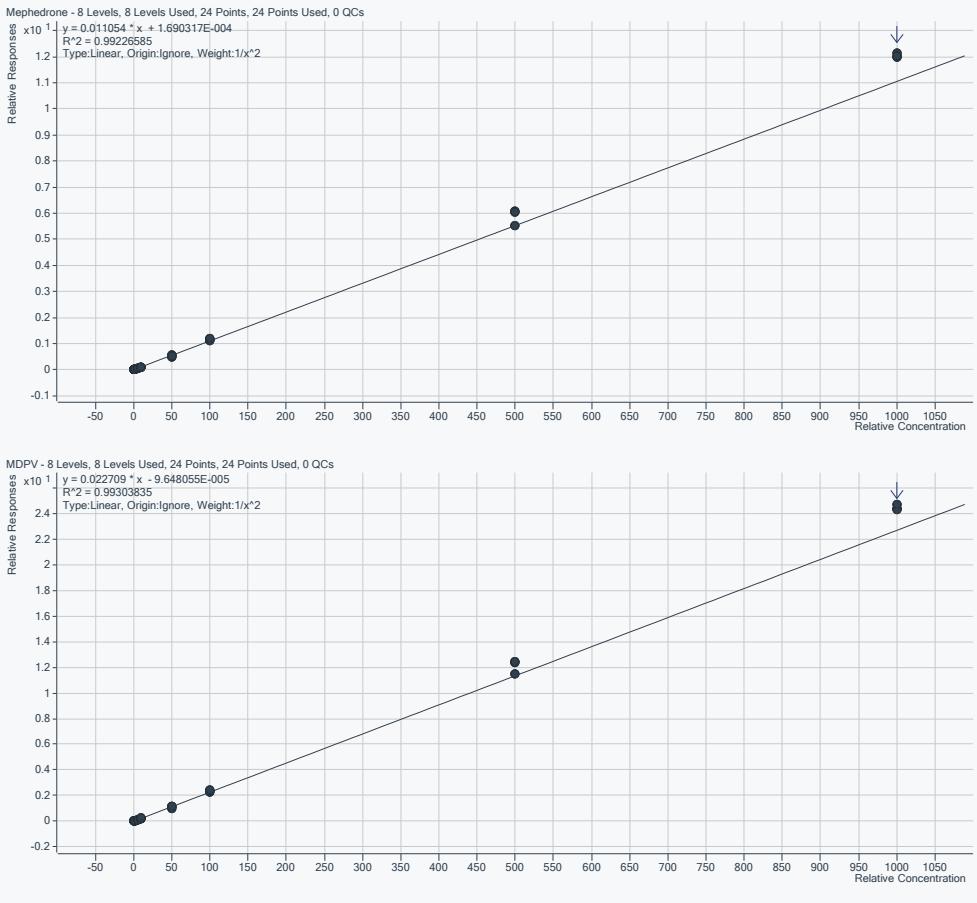

Figure 4: Representative calibration curves for mephedrone and MDPV.

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Calibration standards prepared using an automated Prep Sequence generated with the Calibration Standard Wizard were compared to manually prepared calibration standards. Triplicate calibration curves were prepared for each sample set using a stock containing testosterone, androstenedione, 17β-estradiol, 17α-ethynylestradiol, estrone, and equilin. The internal standards, D3-testosterone and D5-17β-estradiol were combined and used for both preparation sets. Table 1 shows a comparison of the accuracy and precision results from the manually and automatically prepared sample sets at each concentration level. As shown, standards prepared using the Calibration Standard Wizard were found to be as accurate as or better than manually prepared calibration standards. The average accuracy both for manually prepared and automatically prepared standards was 100%. Manually prepared standards resulted in an average precision of 3.69% compared with 3.22% CV for automatically prepared standards.

Table 1: Comparison of average accuracy, precision, and linearity from manually vs. automatically generated hormone calibration curve replicates.

Linearity

r^2	Androstenedione	Equilin	Estrone	17α-Ethynylestradiol	17β-Estradiol	Testosterone	Average Linearity
Manual	0.997	0.995	0.995	0.989	0.997	0.997	0.993
Wizard	0.995	0.992	0.996	0.998	0.999	0.999	0.996

Average % Accuracy

% Accuracy	Androstenedione	Equilin	Estrone	17α-Ethynylestradiol	17β-Estradiol	Testosterone	Average % Linearity
Manual	0.997	0.995	0.995	0.989	0.997	0.997	0.993
Wizard	0.995	0.992	0.996	0.998	0.999	0.999	0.996

Average Precision (% CV)

% CV	Androstenedione	Equilin	Estrone	17α-Ethynylestradiol	17β-Estradiol	Testosterone	Average Linearity (% CV)
Manual	1.94	3.87	4.46	4.46	6.21	1.66	3.69
Wizard	2.22	5.17	2.84	2.84	3.85	1.60	3.22

The robustness of standards prepared using the Calibration Standard Wizard was evaluated over a five-day period using a mixture of pesticide standards. To challenge the robustness further, no internal standard was used during the analysis of the pesticide standards. As shown in figure 5, the average accuracy of all pesticide standards prepared over a five-day period was found to be 100% (range: 86% - 106%) with an average precision of 5.35% (range: 0.788% - 11.6%).

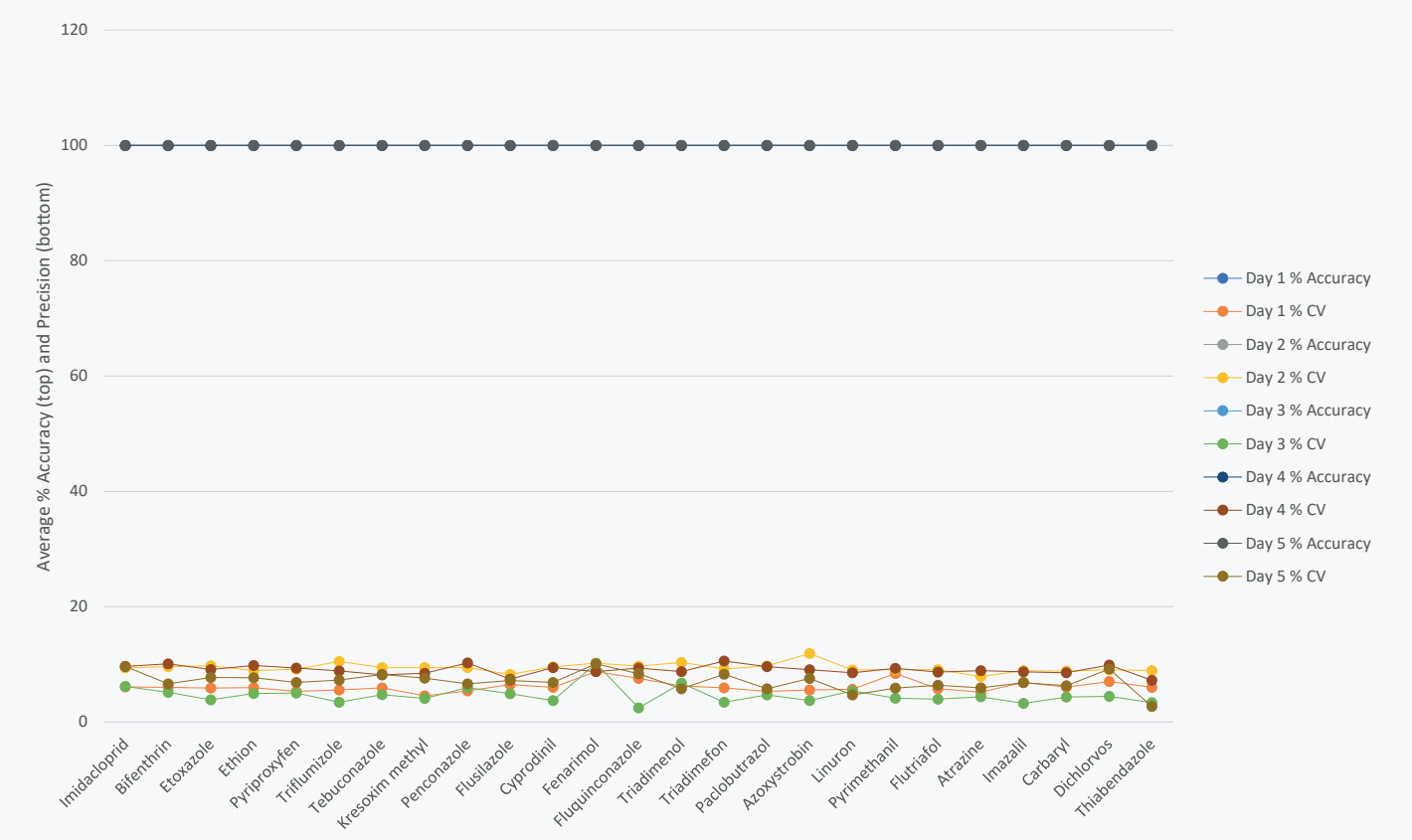

Figure 5: Average % accuracy (top) and precision (bottom) data for each pesticide calibration standard prepared using the Calibration Standard Wizard over a 5-day period. All 5 days resulted in 100% average accuracy.

Conclusions

As a result of this study, we were able to show:

- The Calibration Standard Wizard allows methods for the preparation of multi-level calibration curves to be generated within minutes.
- The GERSTEL MPS robotic^{PRO} sampler prepares calibration standards with great accuracy and precision with R^2 values of 0.99 or better for all calibration standards monitored.
- When compared to manually prepared calibration standards, standards prepared using the automated system were found to be as accurate and precise, if not better for the hormone compounds tested.
- Standard preparation robustness was proven as standards prepared and monitored over a 5-day period were shown to be both accurate and precise.

References

[1] GERSTEL Application Note No. 215, Automated Hydrolysis, Extraction and Analysis of Synthetic Cathinones in Urine using a Robotic Autosampler and LC-MS/MS Platform, **2020**.

[2] GERSTEL Application Note No. 220, Determination of Hormones in Water by Back Extraction of TF-SPME-SBSE and LC-MS/MS, **2021**.

[3] GERSTEL Application Note No. 210, Automating the Preparation of Matrix Matched Calibration Standards for the Analysis of Food Contaminants by LC/MS/MS, **2020**.