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ePrep ONE® | Application Note 2025

Automated Preparation of Calibration Standards

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INTRODUCTION

Calibration standards are critical in analytical methods, as they allow analysts to ensure that their measurements are accurate, reproducible, and traceable to known references, thereby enhancing the credibility and reliability of the analytical data.

This article discusses the challenges associated with the preparation of calibration standards and explores how automation of the liquid handling processes can be achieved using the ePrep ONE sample preparation workstation. It also demonstrates that automation can perform the necessary precision and accuracy for this crucial component of the analytical method while freeing up analysts' time for other tasks.

BACKGROUND

Standards are essential for ensuring the accuracy, precision, and reliability of the analytical results. They serve as reference points for instrument calibration and method validation, and can be made up from a single reference material or a complex mixture of multiple analytes.

Typically, calibration standard solutions are prepared at various concentration levels to cover the expected range of analyte concentrations in samples. This may also require the preparation of stock solutions before a series of dilutions are performed to create the final calibration series.

Recent shifts in the choice of analytical techniques towards more sensitive options such as the use of triple quadrupole LC(GC)-MS/MS, has resulted in a need for standards at increasingly lower concentrations. When not available "off the shelf", their preparation can involve multiple, precise dilution steps requiring careful handling of small volumes – tasks that can be more challenging for manual approaches.

For multi-component mixtures, analysts may need to combine 10s or 100s of reference materials. Depending on the method, pre-made mixtures may be commercially available, however in many cases these need to be created in-house demanding significant amounts of time and focus from laboratory staff.

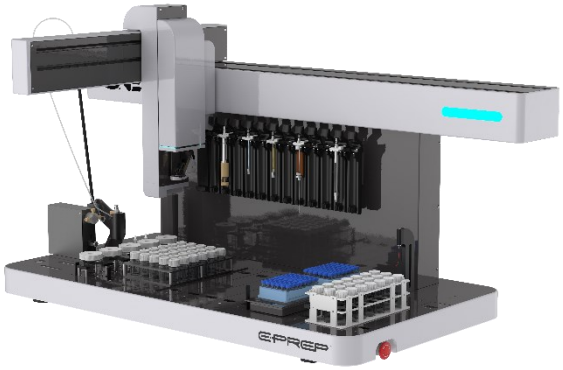


Figure 1: ePrep ONE Sample Preparation Workstation

The ePrep ONE workstation (Figure 1) was designed specifically for chromatographic methods. While calibration standards represent one application, its versatility

extends much further in sample preparation. As an offline system, it can seamlessly switch between creating stock solutions and calibrants to handling other analytical workflows as needed.

CHALLENGES WITH CALIBRATION STANDARDS

Typically, standard solutions are prepared at various concentration levels to cover the expected range of analyte concentrations in the samples. Due to limitations caused by stability, demand or cost, this may include the creation of multi-component stock solutions before a dilution series can be created. Standard solutions are typically straightforward in terms of the components that go into them. However, these elements can introduce additional complications:

Reference Materials: Certified reference materials (CRMs) are often used as calibration standards to ensure traceability and credibility of the analytes. They can be costly, and their availability can be restricted to small volumes and subject to long lead times so accuracy when handling is crucial.

Diluent: The solvents used as diluent will depend on solubility of the analytical components and should be similar to the mobile phase solvent system for liquid chromatography (LC) or need to be sufficiently volatile for gas chromatography (GC) samples. Typically, this means an organic solvent is often a necessity which can be challenging for manual pipetting processes as the volatility can affect the dispense precision.

Sample Matrix: For complex sample matrices, matrix-matched calibration standards (i.e., standards prepared in the same matrix as the samples) may be used to account for matrix effects on analyte response. As with diluents, certain complex matrices can be challenging to handle and may require the introduction of additional clean-up steps.

Once the components of the standard solutions have been addressed then it is critical to ensure that they can be prepared accurately using calibrated volumetric equipment to ensure precise concentrations. This process should also be highly repeatable as calibration standards need to be regularly recreated to ensure that the analytical method is performing as expected.

The ePrep ONE is an analytical syringe-based system that can accurately and precisely handle a range of solvents and sample matrices. The instrument has syringes in the range of 10 uL to 10 ml, allowing for accurate handling of volumes down to 1 uL. The task-based software allows for easy optimisation of specifications such as volumes dispensed, the aspiration and dispense flow rates to easily account for differences in the physical properties of the liquids being handled.

Syringes use positive displacement to offer superior performance with a wide range of non-aqueous liquids including:

- Volatile solvents (e.g. acetone)
- Non-polar solvents (e.g. hexane/toluene)
- High-density liquids (e.g. chloroform)
- High-viscosity liquids (e.g. oils, blood)

The stainless-steel needles also remove the risk of solvent contamination from extractable & leachable components and their reusability reduces single-use plastic waste in the lab when compared to pipette based alternatives.

Given the importance of calibrants, manual preparation often requires a certain level of experience and skill, as it is important that precision and accuracy are understood and addressed by the analyst. Some sources of error in manual preparation processes can be addressed by careful and thoughtful method design, however, the performance of lab personnel is often diminished through tedious and repetitive tasks that must be done daily and at a high quality. This is where automation can offer a solution to allow highly skilled staff to be utilised where they are most needed rather than tied up with creation of standards.

Using instrumentation to create standards also offers efficiency gains e.g. the standards can be created while the user is elsewhere i.e. when key analysts need time off, work is not disrupted as the same workflow once programmed will provide the same result no matter which staff member initiates the run. In addition, the ePrep ONE also has a scheduler allowing the user to set up the method the night before and schedule the workflow to start overnight meaning standards could be created in time for the user's arrival the next morning ready to begin running on their analytical instrument.

An ePrep ONE user at the University of Technology, Sydney puts it this way:

"Mornings go like this; get standards from the refrigerator, place on deck, load method, press go. Go get a cup of coffee, return to collect my perfectly prepared 6 point calibration standards in the instrument autosampler rack ready to run."

Using automation to optimise the use of the resources available is an ideal approach that many labs are now considering for their analytical methods. Applying this to the accurate and precise preparation of calibration standards as well is vital for quantitative analysis. The ePrep ONE Sample Preparation Workstation (Figure 1) can offer improved efficiency, eliminates errors and frees vital resources for other laboratory tasks by automating the preparation workflow of standards and samples. This article outlines how automated preparation of standards in different contexts can be achieved.

APPLYING THE ePrep ONE TO CALIBRATION STANDARDS

The ePrep software is a task-based user interface. No movements need to be programmed, the groups of vials are defined and the tasks are selected. Within the ePrep software there are several tasks that allow for easy use of the system for creation of calibration standards. These include:

- **Variable dispense** – dispenses a reagent from a single input vial (or group) to a series of vials where a different volume can be dispensed to each unique output vial. The same syringe is used for the dispense but multiple aliquots can be aspirated for multiple dispenses. Ideal for making standard solutions where different final concentrations are needed.
- **Serial Dilution** – the user adds a starting dispense volume of a sample/standard from a single input vial (or vial group) to the first output vial. Subsequent dispenses to the rest of the output vials are determined by a defined increment value.

- **Serial Dispense** – designed for making up an exponentially decaying set of calibration standards. A series of vials can be preloaded with solvent using "Add Diluent". An initial solution is created by adding a defined volume of reagent/standard to the first vial, the same volume is then aspirated from the first vial to the next vial in the group. This is repeated to create a series of concentrations.
- **Make up to Volume** – the software calculates the volume needed to fill to a given total volume for each container within the output group after taking into account all of the additions made in previous tasks. Particularly useful when combined with Serial dispense or Variable dispense.

Mixing to homogenise the final solution can be done through a syringe mix process or using a vortex shaker that can be placed on the deck.

Wash steps automatically exist within the tasks using the in-built wash station and can be easily adjusted to increase the volume or number of cycles for more "sticky" analytes. Alternatively, these can be combined with additional wash tasks that can be directed to use other solvent sources if a stronger solvent than alcohol is required thus offering lots of solutions for preventing issues with carryover. Where a wide range of calibrant concentrations are being created, it is also possible to specify the serial number of syringes used in a task for handling of high/low concentrations to reduce the amount of washing required for individual tools to save time and solvent resources.

An example method might look like the workflow displayed in Figure 2. The purpose of this workflow is to combine 3 reference materials with a suitable volume of diluent in a single vial to create a stock solution. The corresponding calibration standards are then created with this stock solution into output vials prefilled with suitable diluent volumes.

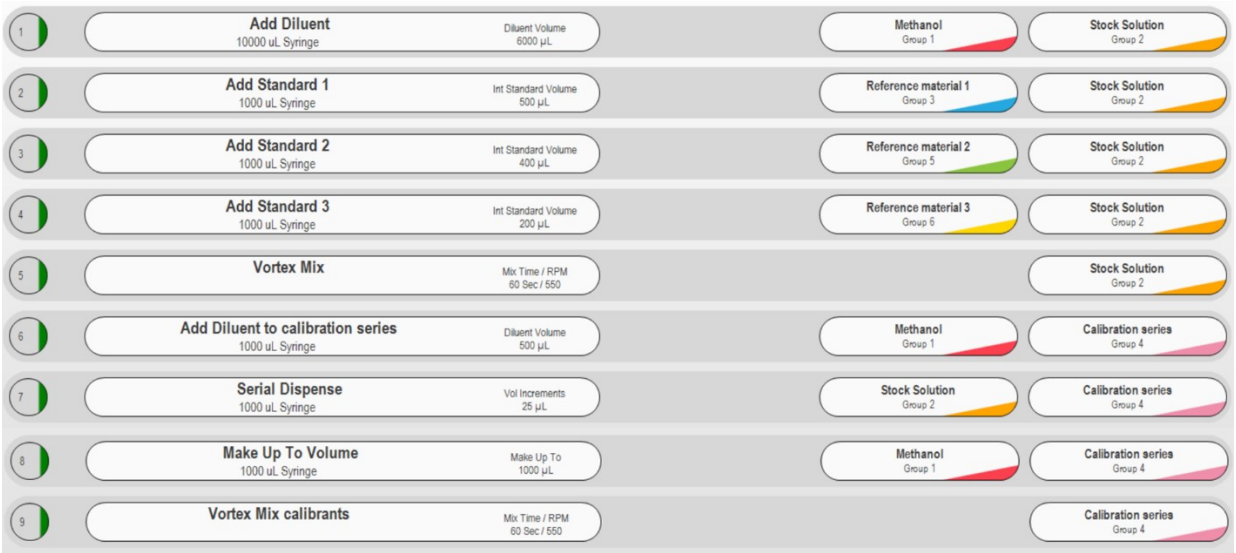


Figure 2: Upper - Workflow task list, Lower - Deck display showing vial set-up

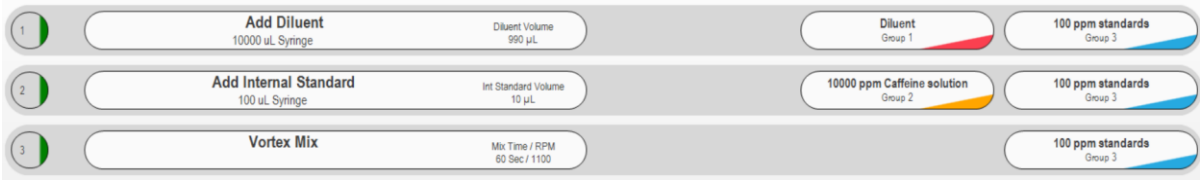
This example is a very straightforward version of how the instrument can be used to create multicomponent stock solutions from certified standards. This particular workflow takes 19 mins to create both the stock solution and 6 point calibration series using standard parameters. This could be easily expanded to a larger number of analytes, 10's of components can easily be combined to form such stock solutions. Automating the approach, optimises use of the materials available and reduces risk of errors in creating the stocks which can be particularly critical when handling reference materials as these are often costly or subject to long lead times.

POINT STANDARD CHALLENGE

An even simpler example for how automation can overcome these problems is a challenge completed on the ePrep ONE versus the manual processes of a pharmaceutical laboratory. The goal was to create 10 replicates of a simple, single component 100 ppm caffeine standard from a pre-made 10000 ppm stock solution.

The manual challenge was taken on by an Experienced Analyst and a Trainee whilst an ePrep user wrote and ran the workflow for the process. Analytical precision was analysed by isocratic HPLC method.

The workflow and deck setup on the ePrep was per Figure 3.



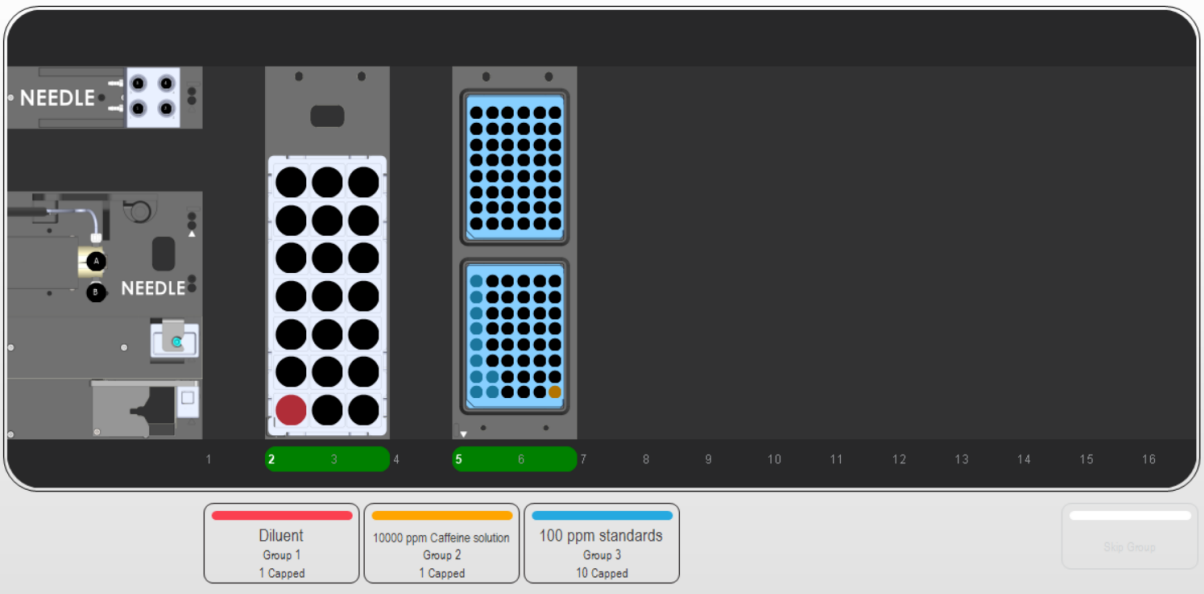


Figure 3: Upper - Workflow task list for creation of 10 x Caffeine standards, Lower - Deck display showing vial set-up

System	Hitachi Chromaster Plus
Detector	DAD
Column	Merck Purospher STAR RP18e column (55 x 4.0 mm, 3 µm)
Flow rate	1.0 mL/min
Mobile phase	80% Water 20% Acetonitrile, isocratic, pump mixed
Run time	1.5 min
Column temperature	40 °C
Injection Volume	10 µL

Table 1: Parameters for isocratic HPLC analysis of Caffeine standard (100 ppm)

	ePrep ONE	Expert	Trainee
Time	18 mins	60 mins	90 mins
Solvent Volume	10 x 1 mL	10 x 100 mL	10 x 100 mL
Precision	0.2% RSD	0.53% RSD	4.57% RSD

Table 2: Results Time taken, Solvent volume used and Precision for ePrep ONE and 2 user experiences.

Results

The outcome summarised in Table 2 demonstrates the variability between the manual processes with analysts of different experience levels as well as the opportunities for improvement offered by automation.

Time

The time for the ePrep includes the time taken for the ePrep user to develop the workflow and to run it. The overall run time for the workflow outlined in Figure 3 is just under 10 minutes. An hour was needed by the Experienced Analyst in order to create the 10 replicants accurately. In an ordinary environment, it is not uncommon to expect that the more senior staff member to have other demands on their time that could cause distractions that will result in an increased risk of error. As such, the task may then fall to less experienced staff members who may take longer to complete it.

Volume

The manual method used here makes up each standard to 100 mL due to glassware availability at the time of the challenge. The typical manual process would be completed in smaller volumetric flasks i.e. 5 or 10 mL. This still uses volumes much larger than the 1 mL used per standard on ePrep ONE due to difficulties with accuracy and precision of the manual process. The scaling of the method to use smaller volumes would not typically be considered compatible with the manual process, however it not only offers the benefit of reduced solvent usage, it also allows the replicates to be made up in autosampler vials in the autosampler rack ready for moving directly to the analytical instrument. This removes additional transfer steps that would be needed in the manual process and the requirement for additional intermediate containers.

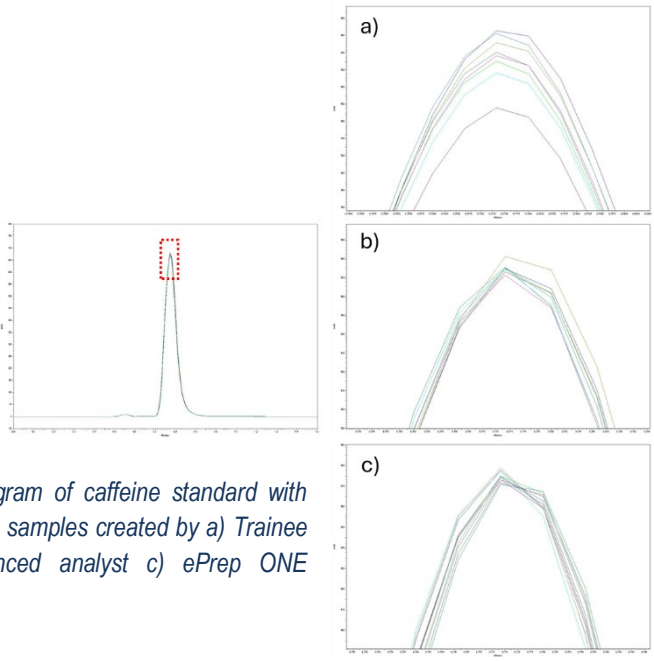


Figure 4: Chromatogram of caffeine standard with peaks of 10 replicate samples created by a) Trainee analyst b) Experienced analyst c) ePrep ONE workstation

Precision

The precision values from the chromatography in Table 2 and chromatograms in Figure 4 demonstrate clear differences between analysts experience which is to be anticipated however it was also found that the ePrep ONE to also offer an improvement in precision. The advantage of automation is that once created, the workflow can be reused without modification or easily transferred between instruments and company sites allowing for a consistent level of performance to be achieved regardless of staff availability or location.

Overall, the challenge demonstrates that automation of this process on the ePrep ONE can offer multiple improvements for the creation of a commonly used caffeine standard.

STANDARDS FOR CANNABIS RESIDUAL SOLVENTS

Another application area where the ePrep ONE has been successful used for creation of calibration standards has been for residual solvents analysis in cannabis samples. Residual solvents can be the most technically complex workflow as working with volatile gases can be extremely difficult and can lead to failed quality control samples and poor calibration curves. Experts estimate that up to 40% of samples are prepared incorrectly leading to serious errors in test results.

This workflow automates all liquid steps including spiking of laboratory control sample (LCS), extraction of samples and LCS, and preparation of intermediate working solutions, calibration solutions, independent calibration verification (ICV), and continuing calibration verifications (CCVs). On the ePrep ONE, this takes 116 mins for automated sample preparation of a California Authority (CA) compliance batch (1 lab air blank, 1 solvent blank, 8 calibrants, 1 ICV, 4 CCVs, 1 LCS and 20 samples.)

The calibration curve was made using a two-part residual solvent commercial reference standard in triacetin (CPI Z-G34-115300-02/ CPI Z-G34-115300-03).

Calibrant concentrations for:

1) CA category I solvents range from 0.156ppm to 10.0ppm
2) CA category II solvents range from 15.6ppm to 1000ppm. The analysis was completed by Headspace GC-MS/MS. Linearity (r^2) for all compounds was > 0.998 and all quality control samples (ICV, CCVs, LCS) pass the +/- 30% requirement in line with CA regulatory authority.

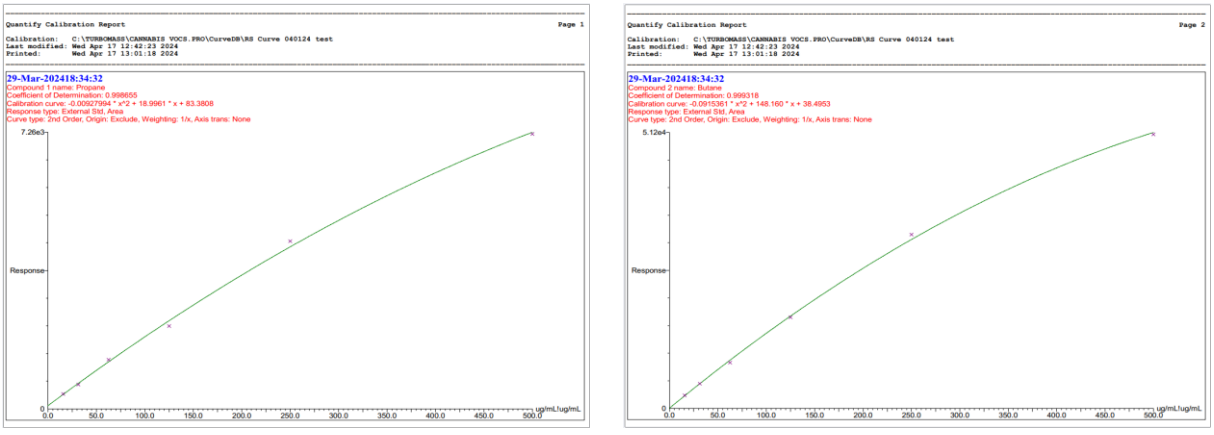


Figure 5: Left – Propane calibration curve, Right – Butane calibration curve

This method demonstrates the ability of ePrep ONE to meet the critical requirements of residual solvents preparation. This includes precise and reproducible preparation of the intermediates from the viscous stocks, prevention of carryover, and prevention of loss of analytes through the use of septa and caps during the preparation. This article focusses on

the standard aspect of the work. If more detail on the wider workflow is of interest please refer to application note 98-35039.

CONCLUSION

This application note has discussed how the ePrep ONE sample preparation work system can be applied to creating calibration standards. Key advantages of automating manual processes on the ePrep ONE include:

- Improved precision and accuracy
- Minimise reruns
- Reductions in solvent & resource usage
- More efficient use of laboratory staff

These benefits can help labs with their own goals such as increasing throughput, optimising costs and improving method sustainability.

Calibration standards represent only one area of the chromatography lab where the ePrep ONE workstation can make an impact. The system can be readily reconfigured for preparing sample batches, or integrated workflows combining both samples and standards can be developed. To explore additional applications or learn more about implementation, please contact us or review our other application notes.

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