

The Benefits of Gravimetric Sample Preparation

Improving the Efficiency and Quality of Analytical Workflows

Dr. Joanne Ratcliff and Dr. Jan Prochnow, Mettler-Toledo AG

Analytical sample and standard preparation in a pharmaceutical or QA/QC laboratory is typically a manual procedure that requires that solids be weighed and diluted to the mark volumetrically. A minimum of 50 ml of solution is usually prepared for analytical measurements, even though the amount required for each injection is merely a fraction of this. More than 99.9% of a prepared solution is typically disposed of as waste. Automation of this workflow provides the opportunity to reduce the impact of human variability and uncontrolled environmental factors. Adding the liquid gravimetrically improves precision and reduces solvent and substance consumption by more than 75%.



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Traditional sample preparation technique

The traditional protocol for preparing an analytical sample or standard is to weigh out the specified amount of substance manually on an analytical balance. The substance is then transferred to a volumetric flask and diluted to the mark by filling with the appropriate amount of solvent. 50 ml or 100 ml volumetric flasks are typically used because the larger the flask, the lower the relative error – even though the required amount for today’s analytical methods is only a fraction of this. Modern HPLC or UHPLC (ultra high-performance liquid chromatography) instruments typically require 10–20 microliters per injection. Hence, more than 99.9% of prepared solutions are disposed of without ever being used. The reason for this excess is a combination of two factors: limitations in the minimum weight of powder that can be dosed on a specific analytical balance, and constraints in liquid dosing using volumetric glassware.

Measurement Uncertainty and Minimum Weight

An analytical balance has a specific minimum weight according to USP regulations. This means that there is a lower limit on the amount of solid that can be weighed in order to ensure that the required accuracy is achieved. The relative measurement uncertainty of any balance is a hyperbolic function of the weight on the balance (Fig. 1). There is a point where the uncertainty of the measurement becomes too high to have sufficient confidence in the accuracy of the weighed amount. This point is referred to as minimum sample weight or minimum weight.

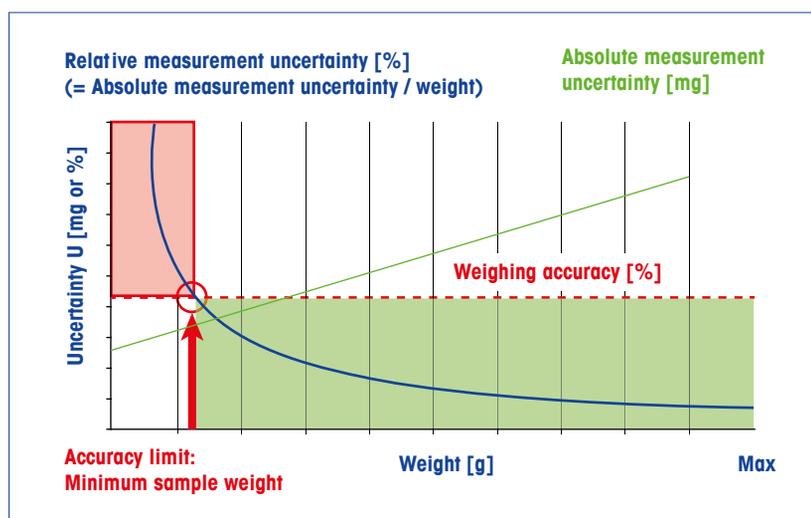


Fig. 1 Relative measurement uncertainty decreases hyperbolically with net weight. Accuracy limit is the Minimum Weight

This minimum weight is determined by the repeatability of the measurement but it varies due to changing environmental conditions, such as vibrations or draft, and the skill of the operator. Therefore, it is recommended that a safety factor of typically 2 or 3 is applied, depending on how stable the conditions or critical the applications. The safety factor is a multiplier. For example, a minimum weight of 10 mg with an applied safety factor of 2, means that 20 mg is the smallest amount that should be weighed to meet the accuracy requirements.

Constraints of Volumetric Flasks

Being constrained to Class A volumetric glassware may force an analyst to use more substance than necessary because they are limited to the specific intervals of the flasks. In most cases the amount of substance weighed is rounded up to match the volumetric flask size available. If the diluent is weighed instead, there is no size limitation and the minimum amount of solvent can be delivered gravimetrically to achieve the desired concentration.

Typical concentrations in the pharmaceutical industry require several milliliters of solvent, which is equivalent to several grams in weight. Quantifying these amounts on an analytical balance can be done with very high accuracy due to a negligible measurement uncertainty contribution. This is because the weight of solvent required is typically several orders of magnitude higher than the minimum sample weight, and the uncertainty decreases hyperbolically with the net weight (see Figure 1).

Weighing both the sample and the solvent instead of using a volumetric flask improves reproducibility and traceability and minimizes problems associated with volumetric glassware. The vials in which the samples are prepared can be small and disposable, which eliminates concern about potential cross-contamination. Hidden costs of washing of volumetric flasks and waste disposal are also reduced.

Saving Substance and Solvent

As discussed earlier, the minimum sample weight and the safety factor depend strongly on the user and environmental influences. Automated powder dispensing with the Quantos system can reduce environmental and user variations, and thus significantly reduce both the mini-

minimum sample weight and the recommended safety factor. For automated dosing, a safety factor of 1.5 can be applied, providing conditions are stable.

For example, according to USP regulations, the XP205 analytical balance has a minimum weight of typically 21 mg and a recommended safety factor of 2. The Quantos automated dosing balance has a minimum weight 8 mg and a recommended safety factor of 1.5.

If a 1.0 mg/mL solution with a 1 g/mL density is prepared manually using a volumetric flask, 50 mg of substance and 50 mL of solvent are required. When the solution is prepared by the automated gravimetric method, 12 mg of substance and 12 mL of solvent are sufficient. A materials saving of 76% can be realized whilst remaining compliant with USP regulations. This has a significant impact on the cost of compliance.

Figure 2 illustrates how the minimum weight, the concentration, and the available flask size determine the amount of substance and solvent needed. The red and green lines

show the consumption of solvent as a function of the target concentration for the volumetric and gravimetric methods respectively. The consumption of substance is also proportional.

The red line on the graph demonstrates that with the volumetric approach only at four discrete concentrations (horizontal sections) can the minimum net sample weight of 42 mg be weighed. In all other cases, significantly more solvent is consumed because the amount of substance needs to be rounded up to the next flask available.

The green line indicates that when the diluent is weighed gravimetrically the minimum net weight of substance can be used at every target concentration (represented by a smooth curve). No rounding up to the next flask size available is necessary. When quantifying solvent by its weight rather than volume, the corresponding amount of solvent can be dispensed to match the desired concentration.

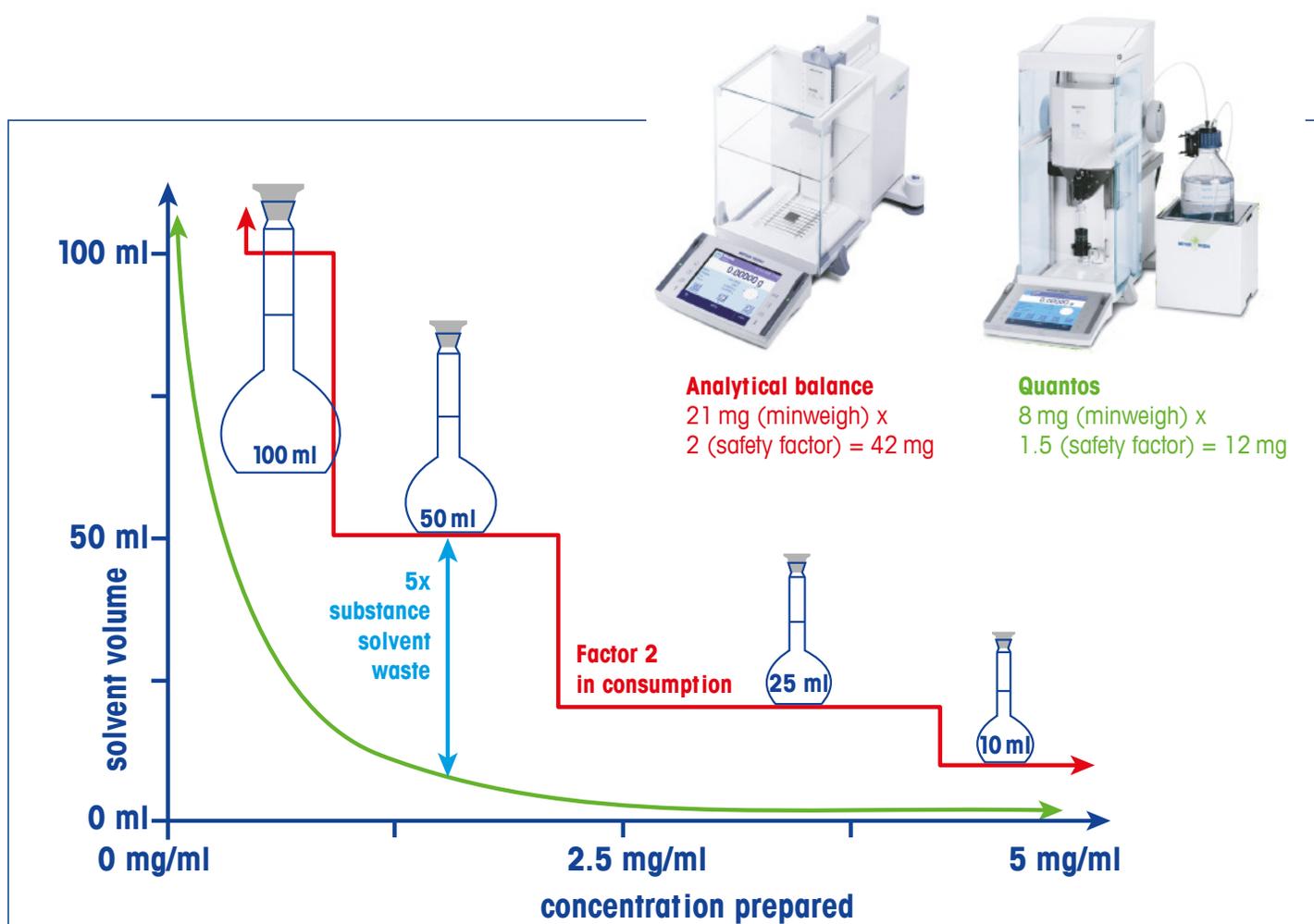


Fig. 2 Solvent consumption as a function of the desired concentration: volumetric method (red line); gravimetric method (green line)



Joanne Ratcliff is a Product Manager at Mettler-Toledo for the Quantos Automated Dosing Solutions Business Unit for 13 years. Her key areas of expertise are automated weighing, powder dispensing and sample preparation. She has a degree in Chemistry with Analytical Science and a Ph.D. in Physical Organic Chemistry using Analytical Methods from the University of Swansea in Wales.



Jan Prochnow, Ph.D. in Physics, is Head of Product Management and Business Development, Quantos Business Unit at Mettler-Toledo in Greifensee, Switzerland.

Reproducibility of Sample Concentrations

Automated gravimetric powder and liquid dispensing produces very reproducible concentrations. To demonstrate this, nine solutions of an active pharmaceutical ingredient (API) were prepared individually, by automated and manual methods. The solutions were analyzed by HPLC to measure the reproducibility.

Nine solutions with a target concentration of 0.603 mg/g were prepared. Ten milligrams of an API was dispensed automatically into nine 20 mL brown glass vials. Automation allowed 10 mg to be dispensed with accuracy to within an RSD of only 0.89%. Then, the diluent – an 80:20 mixture of acetonitrile and water – was added gravimetrically based on the exact weight of the API dispensed into each vial. The RSD of the concentrations achieved was 0.001%.

Next, a 2 μ L sample was injected into the HPLC system. The peak areas for the nine individually automatically prepared samples varied with an RSD of 0.19%; the peak areas for the individually manually prepared samples varied with an RSD of 0.60%. To highlight the reproducibility of the sample preparation procedure and eliminate the variability contribution of the HPLC injector, 10 repeat injections of the same solution were also analysed. When the same sample was injected 10 times, the peak areas varied with an RSD of 0.21%.

When sample preparation is automated by weighing the substance and the solvent, the results are extremely reproducible. The variability in the prepared solutions is insignificant because it is lower than the variability of the analytical instrumentation itself.

Conclusion

The use of volumetric flasks and manual powder weighing results in excessive use of solvent and substance. Until now, 99.9% of prepared solutions are disposed of without being used.

Automated gravimetric sample and standard preparation reduces the minimum weight of the balance and eliminates the need for volumetric flasks, therefore significantly reducing the amount of solvent and substance consumed. Therefore the cost of compliance is significantly reduced. Concentrations can be determined with very high accuracy which makes the process more reproducible and improves the quality of analytical results.

- Joanne.Ratcliff@mt.com
- jan.prochnow@mt.com

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