

## AUTOMATED WEIGHING OPTION: PROVING THE ACCURACY OF AUTOMATED SAMPLE PREP

Camilla Liscio, Anatune Ltd., Girton, Cambridgeshire (UK).

### INTRODUCTION

One of the most attractive advantages of using automated sample preparation is its capability to provide consistent accuracy and precision to the analytical method.

What makes this possible?

Automation delegates tasks to a robot. This is obviously not only valuable from a health & safety and time perspective, but it allows the methodical control of all experimental variables involved such as timing, temperatures, and speeds (e.g. in mixing, centrifuging and liquid handling). In fact, these are the many aspects of sample preparation which are subject to variability and uncertainty, and can lead to inaccurate and imprecise results. The consistency in performing the analytical workflow increases the robustness and accuracy and minimises analytical variability.

There is, though an additional and very powerful way to add even more accuracy and precision to your automated sample preparation: a gravimetric approach.

Gravimetric sample preparation involves the weighing of solids and liquids involved in the analytical workflow into a vial on a balance. Having access to gravimetric data for your sample preparation not only boosts accuracy and minimises out-of-specifications errors, but it can provide very useful insights on the preparation steps, offering a greater understanding of what caused the error in the first place.

The GERSTEL Multipurpose Sampler (MPS) provides this integrated weighing option. Vials are placed in the balance by the MPS then liquid samples, standards, reagents or diluents are added, weighed and registered separately. Results are automatically transferred to pre-defined Microsoft Excel tables for convenient processing. Each sample is reported in a separate line, each addition in a separate column.

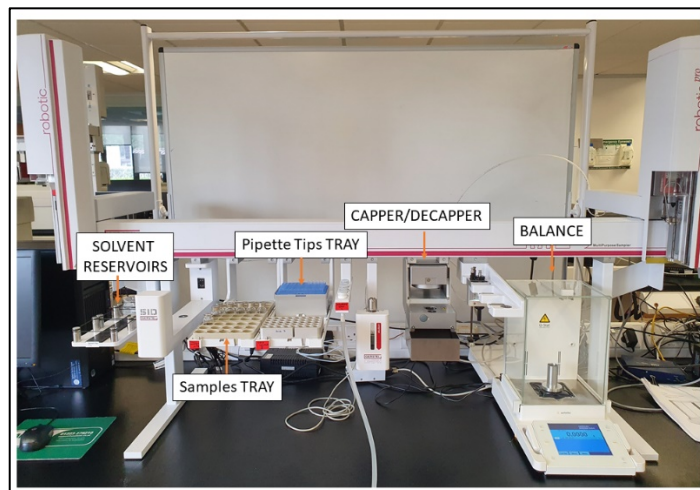
This application note will showcase the capability of the weighing option proving performances in terms of accuracy and precision for automated sample preparation, focusing specifically on liquid handling.

### INSTRUMENTATION

A GERSTEL Dual Head 1.6 m MPS Robotic was configured with the following modules:

- Automated Balance Sartorius Cubis I (4 decimal places)
- Capper/Decapper module
- Solvent reservoirs 180 mL
- Trays for 10 mL
- Pipette Tool (1 mL tips)
- Large Liquid Tool (10 mL syringe)
- Prep Syringe Module PSM (2.5 mL Syringe)

Figure 1 shows the described configuration for the evaluation of the weighing option.



**Figure 1:** GERSTEL MultiPurpose Sampler (MPS) equipped with integrated Balance option

### Experiment 1: Weighing Repeatability

#### MATERIALS AND METHODS:

Twelve 10 mL vials were accurately weighed before and after the addition of 1 mL water using the MPS PSM Tool with a 2.5 mL syringe. Five consecutive weighings were recorded for each sample for both the empty vial and after the addition of solvent.

#### RESULTS & DISCUSSION:

Average Relative Standard Deviations (RSD%) for five repeated weighings of twelve 10 mL vials, both empty and after addition of 1 mL of water, were both 0.001% (weighing on four decimal places). Average ( $n=12$ ) volume (water density 0.997 g/mL) for the added 1 mL of water was  $0.9992 \pm 0.0003$  (RSD% 0.03%) which gave an average liquid handling accuracy of 99.9%.

### Experiment 2: Liquid Handling of Different Solvents

#### MATERIALS AND METHODS:

Vials ( $n=2$ ) were weighed empty and then weighed again after the addition of 1 mL Water, MeOH and DCM respectively, using the MPS PSM Tool with a 2.5 mL syringe and 900  $\mu$ L using the pipette tool equipped with 1mL tips in combination with the Capper/Decapper module. All vials (pierced when using the syringe and unpierced when using the pipette tool) were left at room temperature and then reweighed after 1 hour to check for potential evaporation of the solvent.

## RESULTS & DISCUSSION:

Performances in dispensing accurate volumes of water, methanol and dichloromethane were assessed for both syringe (2.5 mL) and pipette (tool). Table 1 lists the replicate result for weights and volumes (water density 0.997 g/mL, methanol density 0.792 g/mL and dichloromethane density 1.330 g/mL) for the syringe and the pipette tool, respectively. Syringe tool accuracy in dispensing 1 mL was 100% for the water, 99.2% for the methanol and 97.3% for the dichloromethane. Pipette tool accuracy in dispensing 1mL was 97.6% for water, 89.8% for methanol and 65.5% for dichloromethane.

**Table 1:** Syringe tool and Pipette tool performances in dispensing 1 mL of solvent

Sample ID	Syringe Tool		Pipette Tool	
	Solvent [g]	Solvent [mL]	Solvent [g]	Solvent [mL]
Water 1	0.9977	1.0007	0.8760	0.8786
Water 2	0.9979	1.0009	0.8753	0.8779
Methanol 1	0.7856	0.9919	0.6440	0.8131
Methanol 2	0.7863	0.9928	0.6363	0.8034
DCM 1	1.2936	0.9726	0.7829	0.5886
DCM 2	1.2949	0.9736	0.7851	0.5903

In order to evaluate potential losses due to piercing of the vials when using the syringe tool, vials were left at room temperature for 1 hour and weighed again to assess weight losses. Table 2 summarises the weight losses for all the investigate solvents for both the syringe tool and pipette tool samples.

**Table 2:** Weight losses after storage at room temperature for 1 hour for syringe tool samples (pierced septa) and for pipette tool samples (unpierced septa)

Sample ID	Syringe	Pipette
	Weight Delta [g]	Weight Delta [g]
Water 1	0.0000	0.0001
Water 2	0.0001	-0.0001
Methanol 1	-0.0008	-0.0009
Methanol 2	-0.0004	-0.0002
DCM 1	-0.0052	-0.0046
DCM 2	-0.0043	-0.0049

Comparable results were obtained for both the syringe and the pipette tool, suggesting the piercing of the septum was not having a major impact on solvent losses. As predicted based on solvent volatility, water and methanol did not show any significant weight losses. Weight losses recorded for the dichloromethane samples were between 0.2% and 0.6%

## Experiment 3: Syringe Liquid Handling Accuracy

### MATERIALS AND METHODS:

The accuracy capability of a 2.5 mL syringe was tested across the full liquid handling range: 1/10th of the volume (0.25 mL, bottom end), middle range (1.25 mL) and top end (2.5mL). Water was used as solvent and volumes were added to 10 mL screw caps vials. The bottom end was tested with and without Accurate ADD liquid handling option. The "Accurate ADD" feature in Maestro software allows the user to avoid the transfer of air bubbles by aspiration of an additional "Waste" volume of the liquid to be transferred.

## RESULTS & DISCUSSION:

Table 3 compares accuracy dispensing performances for a 2.5 mL syringe across the available volume range using water as solvent. The bottom end of the range (0.25 mL) was evaluated with and without the Accurate Add option. As shown by the data, the Accurate Add option significantly improves performances especially when working at the bottom of the dispensing range

**Table 3:** Dispensing accuracy of a 2.5mL syringe (average and standard deviation,  $n=3$  per volume settings)

Volume	Dispensed solvent	Accuracy
0.25 mL no Accurate Add	0.2208 ± 0.0002	88.3%
0.25 mL Accurate Add	0.2504 ± 0.0001	100.1%
1.25 mL	1.2507 ± 0.0002	100.1%
2.5 mL	2.4706 ± 0.0027	98.8%

## Experiment 4: Calibration Curve Gravimetric Preparation

### MATERIALS AND METHODS:

A six-points calibration curve was prepared with the addition of Solvent (water), internal standard (IS) and standard mix stock solutions. Vials were weighed empty, and after the addition of each component. The accuracy of large volume solvent dispensation was assessed by comparing a 2.5 mL with a 10 mL syringe which allows the Accurate Add option on volume above 2.25 mL.

## RESULTS & DISCUSSION:

Table 4, 5 and 6 show the results for the preparation of a calibration curve gravimetrically. Volumes are expressed in mL. Dispensing accuracy was evaluated for each measured volume. Different syringes were used to accommodate the range of volumes to be dispensed.

**Table 4:** Gravimetric preparation of six-point calibration curve: Solvent

Calibration Point	Theoretical Volume	Measured Volume	Accuracy	Syringe Used
C1	4.7450	4.6957	99.0	2.5 mL
C2	4.7250	4.6724	98.9	2.5 mL
C3	4.7000	4.6473	98.9	2.5 mL
C4	4.6250	4.5718	98.9	2.5 mL
C5	4.5000	4.4471	98.8	2.5 mL
C6	4.2500	4.1969	98.8	2.5 mL

**Table 5:** Gravimetric preparation of six-point calibration curve: Internal Standard

Calibration Point	Theoretical Volume	Measured Volume	Accuracy	Syringe Used
C1	0.2500	0.2483	99.3	1 mL
C2	0.2500	0.2487	99.5	1 mL
C3	0.2500	0.2476	99.3	1 mL
C4	0.2500	0.2487	99.5	1 mL
C5	0.2500	0.2485	99.4	1 mL
C6	0.2500	0.2487	99.5	1 mL

**Table 6:** Gravimetric preparation of six-point calibration curve: Standard

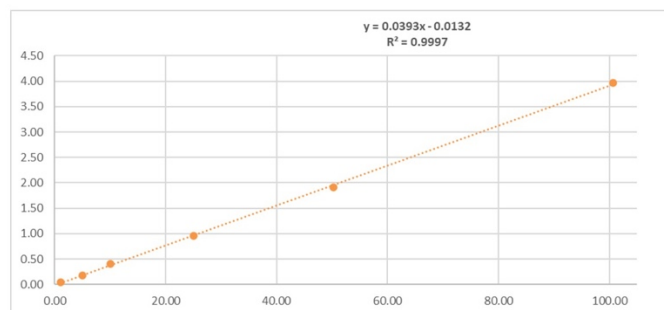
Calibration Point	Theoretical Volume	Measured Volume	Accuracy	Syringe Used
C1	0.0050	0.0050	100	10 µL
C2	0.0250	0.0247	98.7	100 µL
C3	0.0500	0.0498	99.7	100 µL
C4	0.1250	0.1236	98.9	1 mL
C5	0.2500	0.2483	99.3	1 mL
C6	0.5000	0.4973	99.5	1 mL

Furthermore, to evaluate performances in dispensing large volumes (solvent) a 2.5 mL syringe was compared to a 10 mL syringe which would allow Accurate Add. Results are summarized in Table 7.

**Table 7:** Comparison between 2.5 mL and 10 mL (with and without Accurate Add) syringes for dispensing of large volumes

Theoretical Volume [mL]	Accuracy 2.5 mL Syringe	Accuracy 10 mL syringe	Accuracy 10 mL syringe Accurate Add
4.7450	99.0	98.5	99.2
4.7250	98.9	98.5	99.3
4.7000	98.9	98.4	99.3
4.6250	98.9	98.3	99.4
4.5000	98.8	98.3	99.3
4.2500	98.8	98.2	99.4

Figure 2 shows the calibration curve obtained using the gravimetric approach. (Correlation coefficient slightly lower using volume (non-gravimetric approach) at R2 0.9996

**Figure 2:** Calibration curve prepared by gravimetric approach

## Experiment 5: Liquid handling of viscous samples

### MATERIALS AND METHODS:

900 µL and 500 µL of avocado oil were dispensed in a 10 mL screw cap vial ( $n=6$ ) using the GERSTEL Pipette Tool with 1 mL tips in combination with the Capper/Decapper module. Fill speed, wait time and air volume were optimized to limit sample dripping.

### RESULTS & DISCUSSION:

#### Experiment 5: Liquid handling of viscous samples

Accuracy in dispensing viscous samples was assessed by recording the weighing of six replicate dispenses of 500 µL and 900 µL of avocado oil. Table 8 shows the results for the two investigated volumes.

**Table 8:** Dispensing of viscous samples

Sample ID	Volume 1	Volume 2
1	0.4114	0.7234
2	0.4239	0.7073
3	0.4311	0.6805
4	0.4250	0.6798
5	0.3849	0.6888
6	0.4104	0.6221
Average	0.4145	0.6837
SD	0.0166	0.0346
RSD%	4.0	5.1

Better accuracy could be obtained when dispensing the lower volume because it allowed a larger air volume to control dripping (300 µL)

## CONCLUSIONS

This application note explored the added value of the automated weighing option to prove accuracy and precision performances of automated sample preparation. Five different experiments were run to evaluate liquid handling capabilities of the automated platform and how they can impact on the quality of the data. The automated weighing capability is a fully integrated option which can give access to even greater data accuracy thanks to the gravimetric recording of the results.