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Development of a 100% Dimethyl Sulfoxide (DMSO) Sample Solution for Liquid Handler Performance Verification

Dimethyl sulfoxide (DMSO) has become one of the most important solvents used for high-throughput screening (HTS) and compound management applications due to its ability to readily dissolve a wide range of compounds. It is also the solvent of choice for the new breed of acoustic droplet ejection (ADE) liquid handling instruments, developed by companies such as **Labcyte** (Sunnyvale, CA) and **EDC Biosystems** (Fremont, CA), which have revolutionized HTS and compound management.

Because DMSO has very different physical properties than water, the liquid most commonly used for calibration and performance verification, it behaves differently when handled by air-displacement pipettors or acoustic droplet ejectors, creating the need for a solvent-specific volume verification method.

Artel (Westbrook, ME) has developed and characterized a 100% DMSO-based dye solution for use with the **Artel** MVS® Multichannel Verification System. This article discusses the challenges and performance capabilities of a DMSO-based dual-dye solution developed for the MVS. The DMSO-based solution is capable of measuring dispensed volumes as small as 10 nL. A comparison of the performance of this new dye versus gravimetric measurements is presented.

Introduction

Nearly all assays performed within a microwell plate are concentration-, and therefore volume-, dependent. Ensuring that volumes transferred during the preparation of assay plates are accurate is therefore of critical importance.[1]

Research conducted by Nathaniel Hentz, Ph.D., of North Carolina State University (Raleigh) has shown that the volumes dispensed during drug screening programs can have a significant effect on the results obtained by assay screens.[2] Any error made during these deliveries affects the concentration of the drug candidate and can lead to misleading data about compound activity being generated. Data were recently presented that suggest that if two successive liquid transfers are inaccurate, critical reagent concentrations could be affected by as much as 50%.[3]

Traditionally, liquid delivery performance has been measured using water, or aqueous-based test solutions. This approach gives a clear picture of how automated liquid handlers (ALH) function when water is the test solvent. The dual-dye Ratiometric Photometry™ method used by the **Artel** MVS was initially developed to test the performance of liquid handling devices when dispensing aqueous-based MVS dyes that were made to mimic water. This absorbance-based method employs two different solutions:

1. MVS Sample Solution, which contains a known concentration of red dye (and in some sample solutions a known, fixed concentration of blue dye); and
2. MVS Diluent, which contains a known, fixed concentration of blue dye. The red and blue dyes utilized within the sample solution and diluent both have distinct absorbance maxima at two different wavelengths, 520 nm (red) and 730 nm (blue).

The MVS Sample Solutions are shown in *Figure 1*.

When using the MVS, the desired volume of red sample solution is dispensed into 96- or 384-well microtiter plates, after which diluent is added to increase the total working volume to either 200 µL (96-well plates) or 55 µL (384-well plates). Photometric measurements are then collected for both the red and blue dyes at their respective wavelengths. The system simultaneously calculates accuracy and

Figure 1 – MVS Sample Solutions.

precision with no need to prepare standard curves or solutions. Measurement results are traceable to international standards, which allows for standardization between methods, instruments, and locations.

However, it is commonly understood that this approach does not truly represent ALH performance when dispensing organic solutions. While an aqueous-based test can be used to predict a change in instrument performance, pipetting performance with the actual solvent to be used needs to be measured if assay variability is to be minimized.

An application was developed for the MVS which allowed for limited DMSO testing down to 100 nL in a 384-well microtiter plate. This application required the user to prepare a custom test solution of approximately 75–80% DMSO created using DMSO and aqueous MVS Stock 1 Sample Solution. While this capability permitted for volume verification with a DMSO solution, it did not provide for testing pipetting performance of a truly 100% DMSO-based solution.[4]

A 100% DMSO Sample Solution has been developed for use with the MVS. DMSO Range E

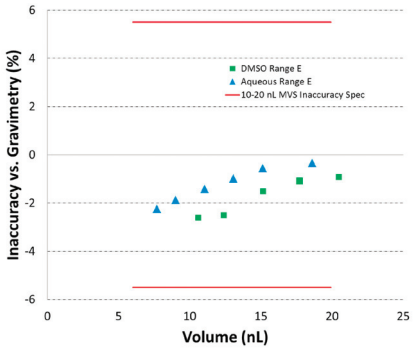

Figure 2 – MVS performance at 10–20 nL in 384-well MVS Verification Plates.

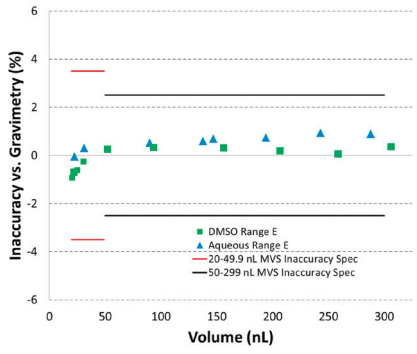

Figure 3 – MVS performance at 20–300 nL in 384-well MVS Verification Plates.

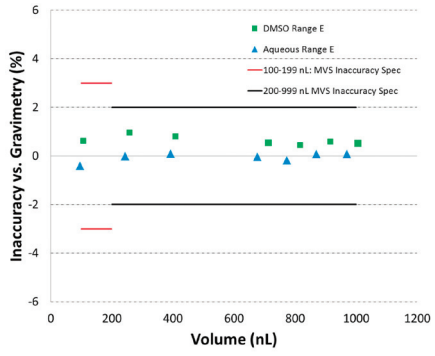

Figure 4 – MVS performance at 100–999 nL in 96-well MVS Verification Plates.

Sample Solution is capable of measuring down to 10 nL. As with all MVS Sample Solutions, results are traceable to international standards.

Evaluating the performance of 100% DMSO Range E Sample Solution

The DMSO Range E Sample Solution was developed to be compatible with all existing MVS materials, including MVS Diluent and the **Artel** characterized microwell MVS Verification Plates. Integration of this DMSO solution into MVS allows for easy comparison and standardization between various liquid handlers, no matter their location or whether they are dispensing aqueous or DMSO-based solutions.

In order to demonstrate the performance of the 100% DMSO dye solution, a comparison was made between a gravimetrically calculated volume and a volume measured using the MVS. For this experiment, representative gravimetric dilutions were prepared for specified volumes of DMSO Range E Sample Solution diluted into aqueous MVS Diluent. This test procedure was also followed for aqueous-based Range E Sample Solution to provide a performance comparison of the two solutions (DMSO vs aqueous Range E). Using a calibrated automated liquid handler, 200 µL of each prepared dilution was dispensed into every well of a 96-well Verification Plate, or 55 µL into a 384-well Verification Plate. The total volume of solution dispensed into each plate was measured gravimetrically, and the result was used to calculate the average volume dispensed into each well. The volumes dispensed into each well were then measured using the MVS. The volumes calculated from the gravimetric measurements were then compared to the volumes measured using the MVS.

Figures 2–4 display the performance of Aqueous Range E and DMSO Range E as compared to gravimetry, in both 96- and 384-well MVS Verification Plates. The data in these graphs show good agreement between gravimetric and MVS results, illustrating that the MVS performs equally well for both aqueous and DMSO Range E Sample Solutions in both plate types.

Furthermore, Figure 2 demonstrates the capability of the MVS to measure down to 10 nL in a 384-well plate. This represents an extended volume range for the MVS, which previously had a lower measurement limit of 30 nL.

Tables 1 and *2* demonstrate the MVS performance capabilities for both aqueous and DMSO Range E Sample Solutions over the volume range of 100–999 nL in 96-well plates, and the expanded volume range of 10–300 nL in 384-well plates.

Table 1 – MVS system performance specifications for Range E Sample Solution in 96-well Verification Plates

Volume range	Accuracy (%)	CV (%)
0.999 µL–0.2000 µL	2.0	0.4
0.1999 µL–0.1000 µL	3.0	0.4
≤0.0999 µL	N/A	N/A

Table 2 – MVS system performance specifications for Range E Sample Solution in 384-well Verification Plates

Volume range	Accuracy (%)	CV (%)
0.299 µL–0.0500 µL	2.5	0.8
0.0499 µL–0.0200 µL	3.5	0.8
0.0199 µL–0.0100 µL	5.5	0.9
≤0.0099 µL	N/A	N/A

Conclusion

The data herein demonstrate that 100% DMSO Range E Sample Solution adds a significant new capability to the MVS for measuring liquid handling instrument performance. In turn, this should answer the need for those scientists working in the drug discovery arena for a fast and reliable method to verify that their liquid handling instruments are accurately and precisely dispensing both water- and DMSO-based solutions.

The development of this DMSO solution has also opened the door for higher-volume-range DMSO-based MVS Sample Solutions.

References

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