Development of a 100% DMSO Sample Solution for Liquid Handler Performance Verification

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Abstract

DMSO (dimethyl sulfoxide) 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 handlers, developed by companies such as Labcyte and EDC Biosystems, which have revolutionized HTS and compound management.

Research by North Carolina State University's Dr. Nathaniel Hentz has shown that the volumes dispensed during drug screening programs can have a significant effect on the results obtained by assay screens. Any error made during these deliveries affects the concentration of the drug candidate and can lead to misleading data about compound activity being generated.

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 photometric method used by the Artel MVS® Multichannel Verification System was initially developed to test the performance of liquid handling devices when dispensing aqueous-based MVS dyes that were made to mimic water.

However, it is commonly understood that this approach does not 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.

Artel has now developed and characterized a 100% DMSO-based dye solution for use with the MVS. This poster discusses the challenges and performance capabilities of a DMSO-based dual-dye solution developed for the MVS. This new DMSO-based solution is capable of measuring dispensed volumes as small as 10 nL. Comparison of this new dye with gravimetric measurements are presented in this poster.

Introduction

The Artel MVS utilizes a dual-dye, dual-wavelength volume measurement system known as Ratiometric Photometry™. 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).

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

The MVS was originally developed exclusively for use with aqueous-based test solutions. However, testing with aqueous solutions may not represent ALH performance when dispensing organic solutions such as DMSO. When evaluating pipetting performance, it may be necessary to test the liquid delivery device using the actual solvent being dispensed in order to minimize assay variability. 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.

We have now developed a 100% DMSO Sample Solution for use with the MVS. This new DMSO Range E Sample Solution is capable of measuring down to 10 nL. As with all MVS Sample Solutions, results are traceable to international standards.

Experimental: Measuring the Performance of 100% DMSO Range E Sample Solution

DMSO Range E Sample Solution was developed to be compatible with all existing MVS materials, including MVS Diluent, as well as the Artel characterized microtiter MVS Verification Plates, allowing for easy testing alongside other aqueous MVS solutions.

In order to demonstrate the performance of the 100% DMSO dye solution, a comparison was made between a gravimetrically calculated volume and an MVS measured volume. 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. The filled plates were then measured using the MVS. The two gravimetric measurements were used to calculate the volume of sample solution present in each well. This gravimetric volume was then compared to the measured MVS volume.

Results

Figure 1. MVS Performance at 10-20 nL in 384-well MVS Verification Plates.

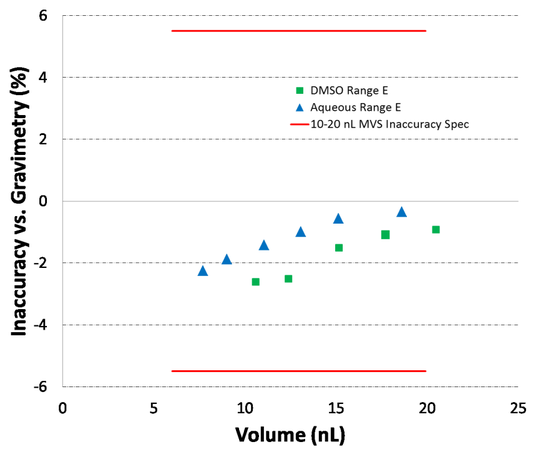

Figure 2. MVS Performance at 20-300 nL in 384-well MVS Verification Plates.

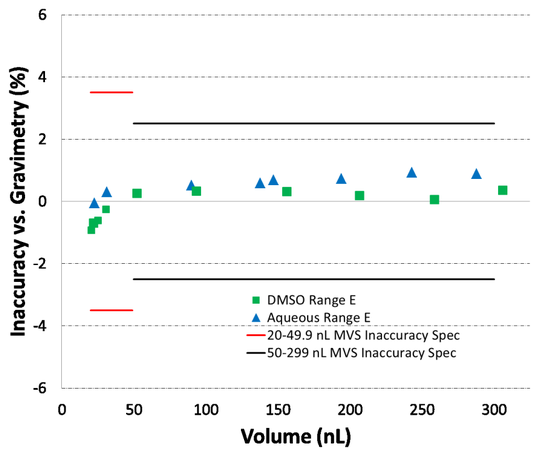

Figure 3. MVS Performance at 100-999 nL in 96-well MVS Verification Plates.

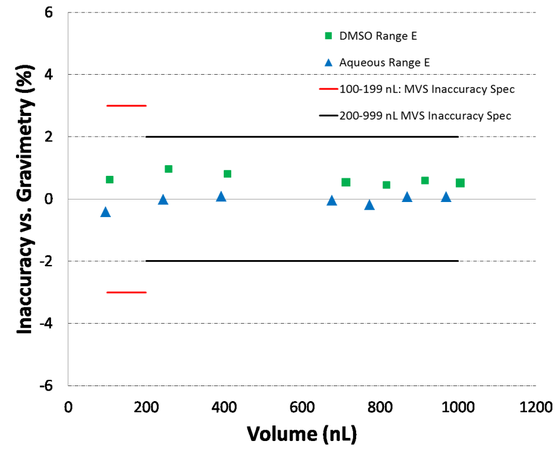

Table 1. MVS System Performance Specifications for Range E Sample Solution in 96-well Verification Plates

Volume Range	Accuracy (%)	CV (%)
0.999 µL to 0.2000 µL	2.0	0.4
0.1999 µL to 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 to 0.0500 µL	2.5	0.8
0.0499 µL to 0.0200 µL	3.5	0.8
0.0199 µL to 0.0100 µL	5.5	0.9
≤ 0.0099 µL	N/A	N/A

Conclusions

Figures 1-3 display the performance of aqueous 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 1 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.

The data herein demonstrate that 100% DMSO Range E Sample Solution adds a significant new capability to the MVS for measuring liquid delivery. Development of this solution also opens the door for higher volume range DMSO-based MVS Sample Solutions.

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