Pipetting Comparison of DMSO Versus Aqueous Solutions as Measured Using Dual-Dye Technology

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Abstract

DMSO (dimethyl sulfoxide) has become one of the most important solvents used throughout pharmaceutical and life science laboratories. This is particularly true for high-throughput screening (HTS) and compound management applications due to the ability of DMSO to readily dissolve a wide range of compounds, and allow them to be stored in a frozen state without compromising the integrity of the compounds.

While DMSO has been widely used for some time, liquid delivery performance has traditionally been measured using water, or aqueous-based test solutions. The dual-dye photometric method used in the Artel MVS® was initially developed to test the performance of liquid handling devices when dispensing aqueous-based MVS dyes. Though this approach clearly demonstrates how automated liquid handlers (ALH) function when water is the test solvent, it is commonly understood that this approach does not represent performance when dispensing organic solutions. DMSO, in particular, has very different physical properties than water, which causes DMSO solutions to behave very differently when handled by common air-displacement pipettors, as well as increasingly used acoustic-based devices. These well-known handling differences enforce the need for volume verification using the specific solvent being dispensed.

Due to the need for volume verification using an organic-based test solution, Artel has continued to develop and characterize a new line of 100% DMSO Sample Solutions. These DMSO-based solutions allow for volume verification up to 10 µL in 96-well microtiter plates and 2.5 µL in 384-well microtiter plates.

This poster will examine the differences in liquid delivery by automated liquid handlers dispensing both aqueous and DMSO-based test solutions. For the study discussed herein, an ALH was first optimized to dispense aqueous test solutions, after which these optimized aqueous method(s) were used to dispense identical target volumes of both DMSO- and aqueous-based MVS Sample Solutions into microtiter plates, which were then measured with the MVS to determine actual delivered volume. The ALH was then optimized to dispense DMSO-based test solutions, after which the same testing was repeated.

Introduction

The Artel MVS utilizes a dual-dye, dual-wavelength volume measurement process. 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.

Though the MVS was originally developed for use with aqueous-based test solutions, the data presented herein indicates that optimization with aqueous solutions may not represent ALH performance when dispensing organic solutions like DMSO. In order to minimize assay variability, it may be necessary to optimize liquid handlers using the actual solvent that is dispensed. Artel's product line of 100% DMSO Sample Solutions originated with DMSO Range E, which allows for testing of ultralow volumes (down to 10 nL in 384-well microtiter plates). The product line has now expanded to include DMSO Range C and D Sample Solutions, which enable testing up to 10 µL in a 96-well microtiter plate and 2.5 µL in a 384-well microtiter plate with the MVS. DMSO Sample Solutions were developed to be compatible with all existing MVS materials, including Diluent and the characterized microplates also known as MVS Verification Plates. As with all MVS Sample Solutions, results are traceable to international standards.

Materials

- Artel MVS
- Range C, D, and E Sample Solutions (aqueous and DMSO)
- Baseline Solution, Diluent, and Verification Plates
- Automated Liquid Handler, associated tips and reservoirs
- WellMate dispenser (Thermo Scientific, Hudson, NH)

Methods

For this study the default liquid class for water was used to transfer all aqueous MVS solutions. For DMSO solutions, the default DMSO liquid class was used. A "universal" pipetting technique was utilized for both liquid classes, where only the calibration factors were adjusted for optimization. Individual liquid class variables (air gaps, aspirate rates, etc.) were left untouched. To begin, the ALH was optimized for dispense of aqueous solutions using the method defined in References 1 and 2, which uses a linear regression to determine the scaling factor and offset volume over a given volume range. The liquid handler was optimized over multiple volume ranges (0.25 – 0.75 µL, 1 – 1.75 µL, and 2 – 10 µL). The theoretical dispensed volume was determined using the following linear equation:

$$V_{TD} = (V_T * S) + V_{offset} \qquad \text{eq 1}$$

$V_{TD} = Theoretical\ Dispensed\ Volume$
$V_T = Target\ Volume$
$S = Scaling\ Factor$
$V_{offset} = Offset\ Volume$

The scaling factor and offset volume are the slope and y-intercept values, respectively, when plotting the theoretical dispensed volume (y) versus MVS measured volume (x).

The WellMate dispenser was employed to pre-load each 96-well Verification Plate with 190-200 µL Diluent before positioning on the ALH deck. The deck was also loaded with new tip boxes and reservoirs containing the applicable aqueous solution (Range C, D, or E). The aqueous-optimized technique was then used to dispense the corresponding DMSO sample solution, keeping all parameters identical, except the DMSO liquid class. This procedure mimics the process one might use for optimization with a single solution, where a method optimized for one liquid class (in this case water) is used to dispense a different liquid class (DMSO, using the DMSO liquid class).

The ALH was next optimized to dispense DMSO solutions. This DMSO-optimized dispense technique was used to dispense a range of aqueous sample solutions, where all parameters were kept identical (again using the appropriate liquid class for the aqueous or DMSO Sample Solutions).

Results

Figure 1. ALH optimized for dispense of aqueous Sample Solutions

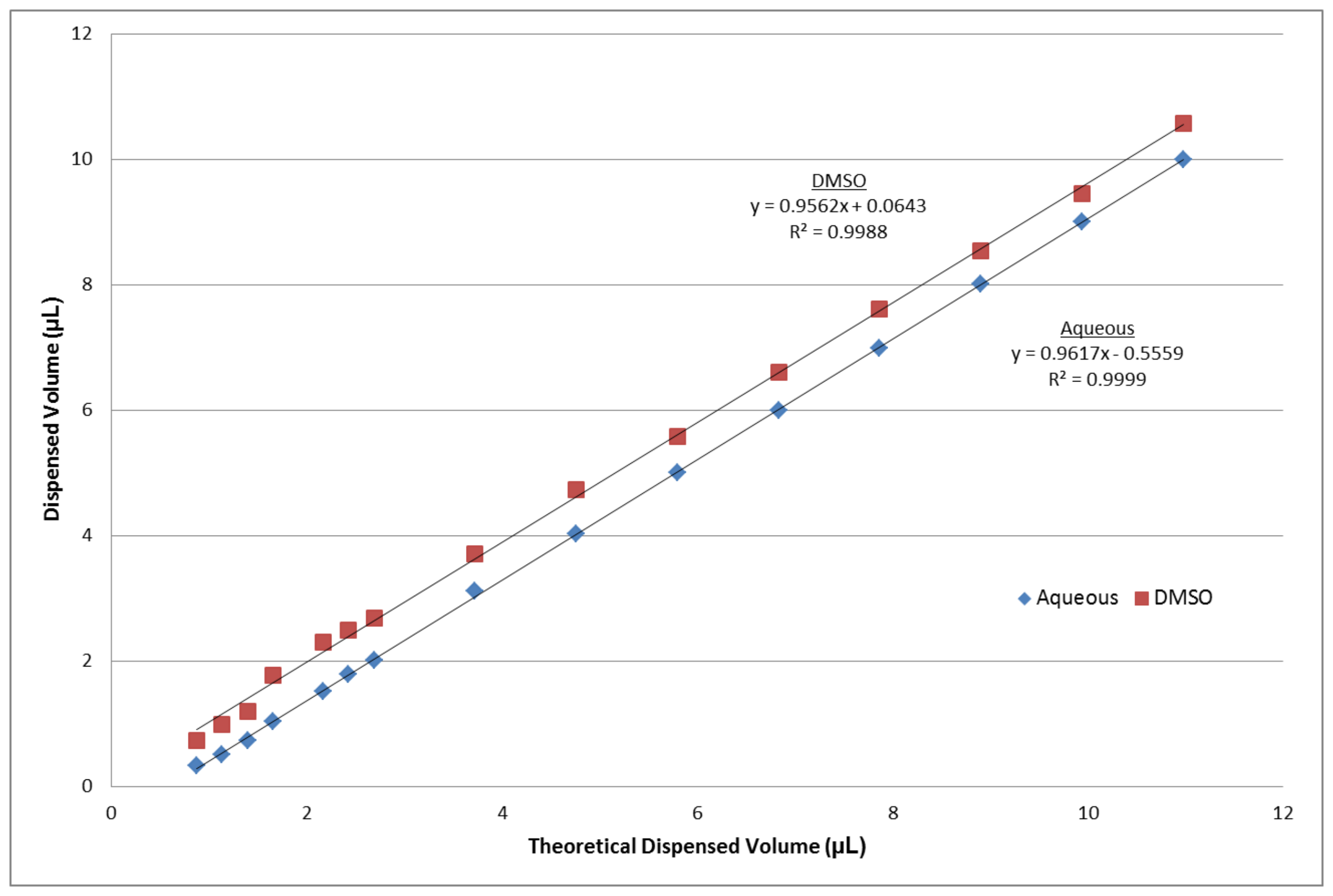


Figure 2. ALH optimized for dispense of DMSO Sample Solutions

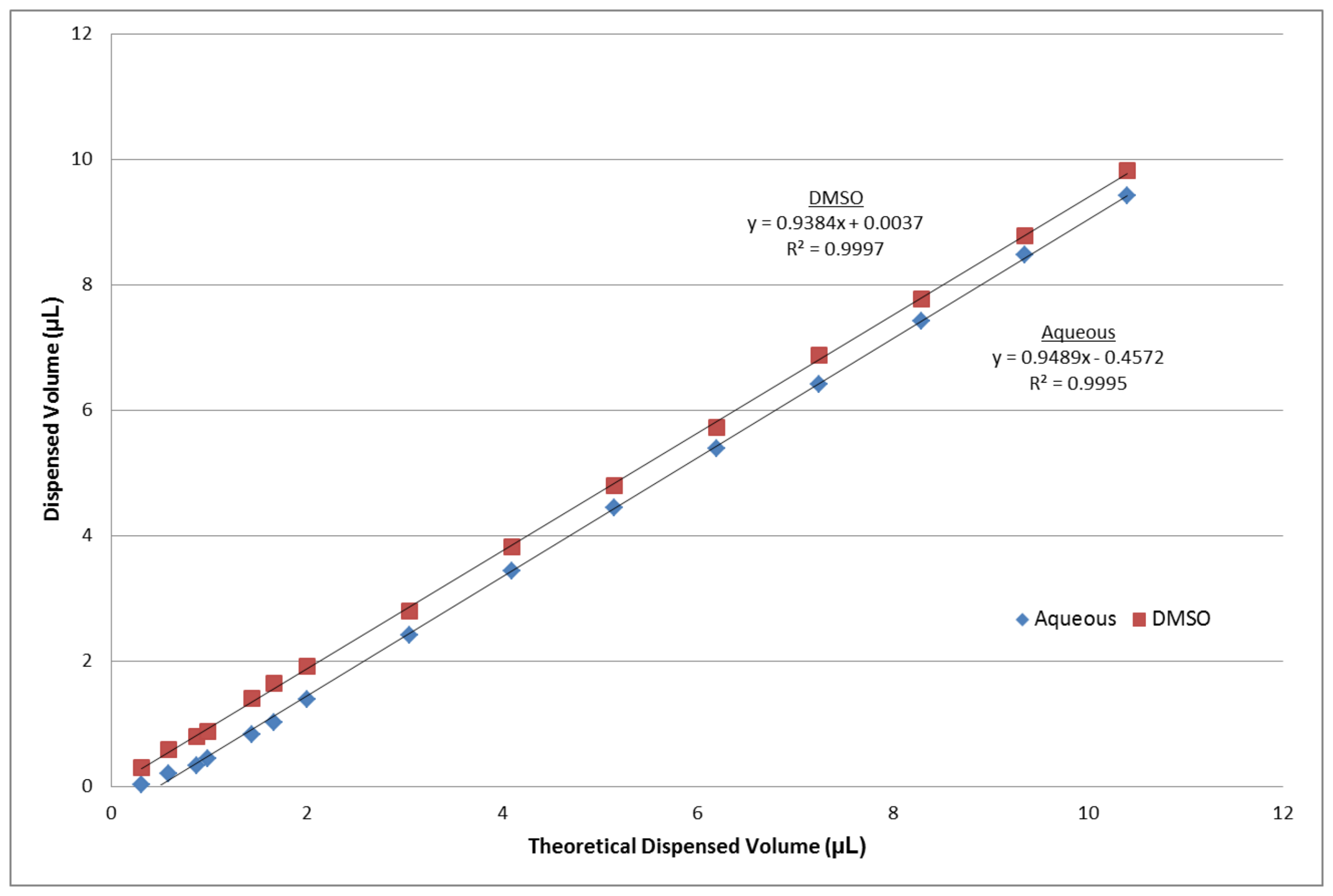


Table 1. ALH optimized for dispense of aqueous Sample Solutions

Target Volume (µL)	Theoretical Dispensed Volume (µL)	MVS Mean Volume (µL) Aqueous (Water liquid class)	MVS Mean Volume (µL) DMSO (DMSO Liquid Class)	Percent Difference
0.25	0.874	0.32726	0.73485	76.75%
0.50	1.133	0.50875	0.99443	64.62%
0.75	1.392	0.72778	1.20088	49.06%
1.00	1.651	1.03989	1.76984	51.96%
1.50	2.169	1.523	2.2918	40.31%
1.75	2.428	1.7842	2.4899	33.02%
2.00	2.687	2.0084	2.6828	28.75%
3.00	3.723	3.1081	3.7144	17.77%
4.00	4.759	4.0298	4.7294	15.97%
5.00	5.795	4.9952	5.5848	11.15%
6.00	6.831	5.9979	6.6104	9.72%
7.00	7.867	6.9907	7.6169	8.57%
8.00	8.903	8.0104	8.5415	6.42%
9.00	9.939	9.0087	9.4481	4.76%
10.00	10.975	10.0005	10.5662	5.50%

Table 2. ALH optimized for dispense of DMSO Sample Solutions

Target Volume (µL)	Theoretical Dispensed Volume (µL)	MVS Mean Volume (µL) Aqueous (Water liquid class)	MVS Mean Volume (µL) DMSO (DMSO Liquid Class)	Percent Difference
0.25	0.30175	0.02595	0.30131	168.28%
0.50	0.5885	0.20076	0.57979	97.12%
0.75	0.87525	0.3245	0.78751	83.27%
1.00	0.981	0.44789	0.88137	65.22%
1.50	1.4325	0.8237	1.4031	52.04%
1.75	1.65825	1.0197	1.6387	46.57%
2.00	1.999	1.3946	1.9146	31.43%
3.00	3.049	2.4031	2.7941	15.05%
4.00	4.099	3.4285	3.824	10.91%
5.00	5.149	4.4364	4.7968	7.81%
6.00	6.199	5.3947	5.7246	5.93%
7.00	7.249	6.414	6.8816	7.03%
8.00	8.299	7.4132	7.7761	4.78%
9.00	9.349	8.4689	8.7725	3.52%
10.00	10.399	9.4252	9.8132	4.03%

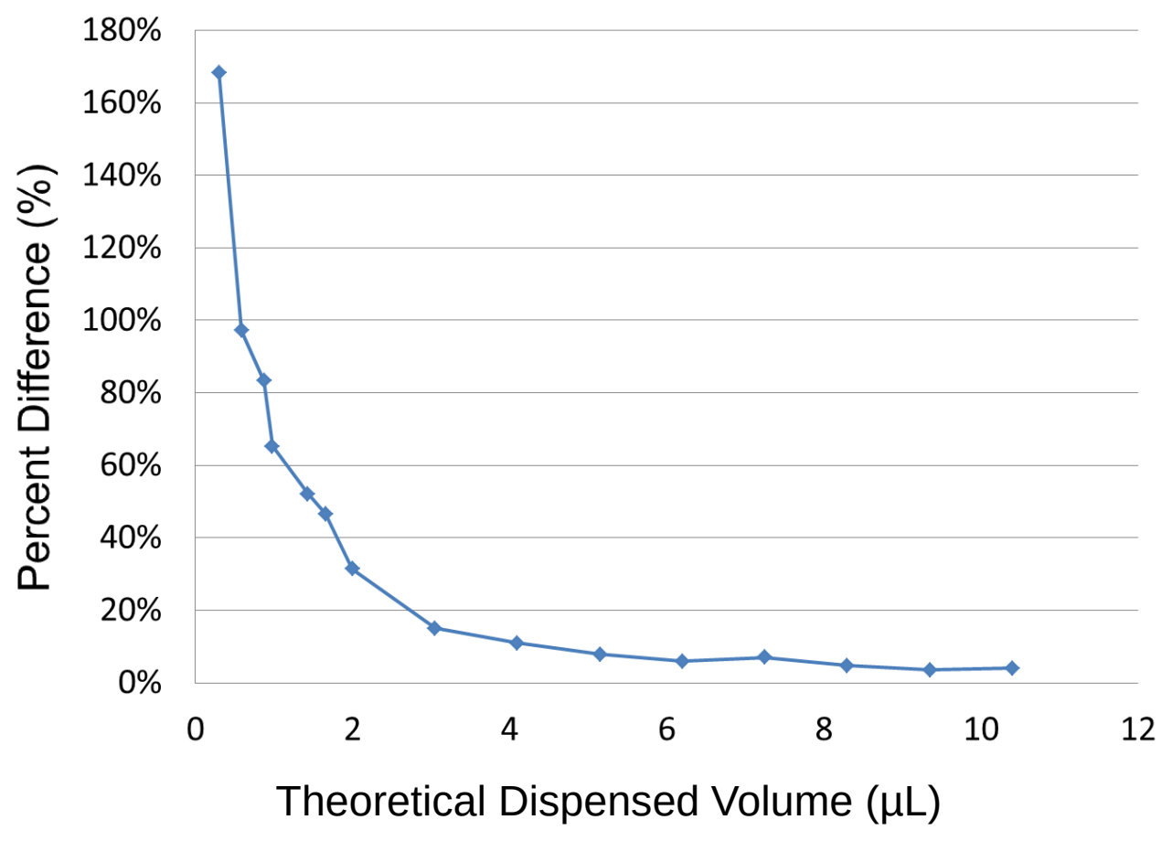
Discussion

Because DMSO has such different physical properties than water, it behaves very differently when handled by air-displacement pipettors and acoustic-droplet ejectors. Figures 1 and 2 depict the mean dispensed volume (as measured using the Artel MVS) versus the theoretical dispensed volume (Equation 1) for dispenses of both aqueous and DMSO solutions. Both charts, which show data collected after separate optimization for aqueous and DMSO dispenses, confirm that there is a clear offset between the two solution types, even when the appropriate liquid class is used for each. It should be noted that if the MVS Mean Volume were plotted versus the Target Volume, the slope and y-intercept of the optimized solution would be approximately equal to one and zero, respectively (as would be expected).

The results of this study indicate the necessity to calibrate liquid handlers using the specific solution type(s) that are normally dispensed. While calibration using a single solution type can aid in giving an overall idea of liquid handler performance, such a method may not adequately demonstrate the performance of a liquid handler when pipetting a different type of liquid such as DMSO. However, it should be noted that if there is a linear offset between two liquid classes (as is demonstrated by the data in this poster), it could be possible to calibrate with a single liquid class, provided the offset between liquid classes is known and can be accounted for. Without knowing the actual offset between the two liquid classes, it is not sufficient to calibrate with one liquid type and rely on a program's standard liquid class to ensure the proper adjustments to pipetting parameters are made.

The differences in pipetting performance for a liquid handler dispensing different types of solutions may be especially apparent in low volume dispenses, as is indicated by the data in this study. The data presented herein indicates that the difference in volume delivery between aqueous and DMSO solutions may change exponentially as the dispensed volume is decreased (as illustrated in Figure 3).
For users dispensing ultralow volumes during drug screening programs, this potential level of error (inaccuracy of greater than 100%) could have a dire effect on the results obtained by assay screens.

Figure 3. Percent Difference between aqueous and DMSO dispenses as measured using the MVS, after optimization with DMSO.



Conclusions

It is essential to measure a liquid handler's performance using the actual solution that is dispensed. Artel's 100% DMSO Sample Solutions enable users to ensure that their liquid handlers accurately and precisely dispense both aqueous- and DMSO-based solutions, using a method that is simultaneously fast and reliable.

References

1. Artel application note entitled "Optimizing Accuracy Performance on a Beckman Coulter Biomek" Doc # 12A6478 (http://www.artel-usa.com/wp-content/uploads/2013/09/12A6478_Biomek-Optimization-Application-Note.pdf).
2. Artel application note entitled "Optimizing Accuracy Performance on an Agilent Bravo Platform Using the Artel MVS®" Doc # 12A6480 (http://www.artel-usa.com/wp-content/uploads/2013/09/12A6480_Bravo-Optimization-Application-Note.pdf).

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