Ethanol Solutions for MVS® Volume Verifications

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ARTEL

Introduction

Ethanol is used in pharmaceutical and other life science laboratories for innumerable experimental protocols. Ethanol is often combined and/or used as an additive in aqueous-based solutions and these solutions are used for many types of experiments. For purposes of this application note, the term *ethanol solution* is employed to refer to an aqueous sample solution with less than 50% ethanol by volume. Aliquots and target volumes of ethanol solutions are usually transferred to the sample holder or assay test plate using a pipettor, such as a multichannel handheld pipette or an automated liquid handler. In many cases, accurate transfer of the target volumes is vital to the integrity of the assay. More importantly, many laboratories do not have an easy and rapid way to verify the accuracy and precision of the pipetting equipment, especially for complex reagents such as ethanol solutions. Described herein is a method to employ the Artel Multichannel Verification System (MVS®) to measure the volume of ethanol solution transferred from a pipettor under test into a microtiter plate. Much of the information in this document has been explained in detail in reference 1.

Requirements

(1) MVS, with Data Manager software 2.0 or higher
(2) Training on MVS operation
(3) MVS Diluent, Stock, and Baseline Solutions
(4) MVS Calibrator plate
(5) Ethanol solution with known density value
(6) 96-well or 384-well microtiter plates
(7) Pipettor or liquid handler
(8) Analytical balance
(9) Sample container, preferably amber bottle with cap
(10) Transfer pipettes or small funnels

More specifically, this document describes the proof-of-concept methodology employed to verify 8-µL target volumes of a ~50% ethanol solution (v/v) in a standard 96-well plate using the MVS. Additionally, this application note serves as a guide for preparing custom ethanol solutions for MVS verification at specific target volumes. It should be pointed out that ethanol solutions can be measured and quantified with the MVS between *approximately* 0.4 – 49.9 µL in a standard 96-well plate or 0.1 to 9.9 µL in a standard 384-well plate.

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Materials & Methods

All purchased materials were used as received. Ethanol (ACS reagent, absolute; 0.7892 g/mL) was acquired from Sigma-Aldrich. The MVS and all MVS-related consumables were from Artel (Westbrook, ME). A Sartorius R160D analytical balance was employed for all gravimetric determinations. All solutions were allowed to equilibrate to room temperature prior to being used. All solution components were transferred to the analytical balance with disposable transfer pipettes and subsequently weighed on the analytical balance. Following each component addition, the balance was allowed to reach equilibrium, the information was collected and the balance was tared before the next component was added. In all cases, a clean, amber glass bottle and cap were used to prepare and store the ethanol solutions. Once all components were added, the bottle was mixed by inversion at least 20 times.

Proof-of-concept Testing

To verify the 8-µL transfer of a ~50% ethanol solution (v/v) in a 96-well plate, four steps were performed: (a) an initial 54% ethanol solution (v/v) was prepared; (b) this ethanol solution was gravimetrically-combined, and therefore slightly diluted, with MVS Stock Solution to prepare the ethanol (alternative) test solution; (c) the weight and density of the ethanol solution and weight of the MVS Stock Solution were incorporated into the MVS software; and (d) the prepared ethanol test solution was dispensed with the liquid handler followed by volume verification and analysis using the MVS. These four steps are summarized in **Figure 1** and are described in more detail below.

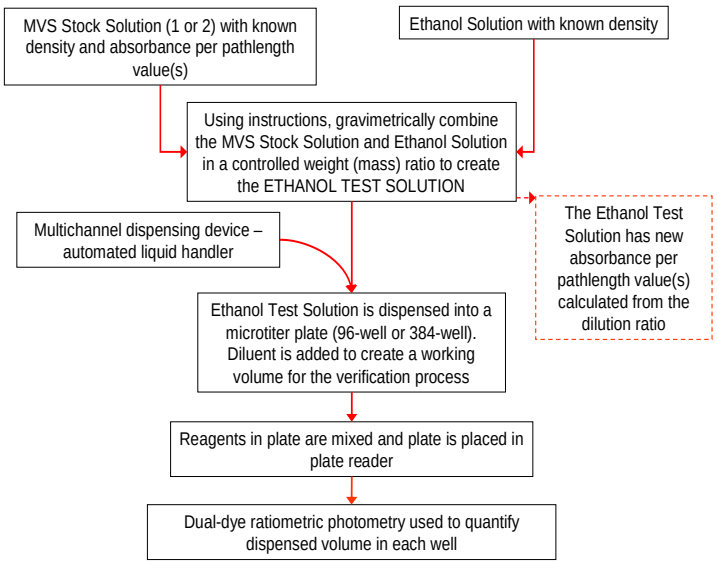

Figure 1. Overview of the method used to prepare and verify target volumes of ethanol solutions using the MVS.

(a) Preparation of a ~54% ethanol solution (v/v). For proof-of-concept testing, the initial ethanol solution was simply a combination of deionized water and ethanol. In many cases, the user will have the ethanol solution readily available. A 54.3% ethanol solution (v/v), or 48.4% ethanol by mass, was prepared by combining 18.8037 g ethanol with 20.0765 g deionized water (38.8802 g total). Note, the density value of pure ethanol is approximately 0.7892 g/mL and this value was used to calculate the amount of ethanol in the prepared ethanol solution. See **Figure 2** for the preparation details for the initial ethanol solution. In order for a target volume of this solution to be verified with the MVS' photometric technology, gravimetric combination with an MVS Stock Solution must be achieved, *i.e.*, MVS dye must be added to the ethanol solution to enable photometric measurement of target volumes.

For preparing other ethanol solutions at varying concentrations, or to obtain the approximate density values for solutions with approximately 1% – 56% ethanol (v/v), please refer to **Table 1** at the end of this document.

(1) DI water added =	20.0765	g
(2) pure ethanol added =	18.8037	g
total weight of solution = (1 + 2) =	38.8802	g
% ethanol (g/g) = [(2/(1+2))*100] =	48.3632	%
(3) density of water =	1.0	g/mL
(4) density of ethanol =	0.7892	g/mL
(5) vol water = (1/3) =	20.0765	mL
(6) vol ethanol = (2/4)=	23.8263	mL
(7) total volume = (5+6) =	43.9028	mL
(8) % ethanol (v/v) = [(6/(5 + 6))*100] =	54.2705	%

Figure 2. Details for the preparation of the initial 54.3% ethanol solution (v/v).

(b) Preparation of the ethanol test solution. Using easy-to-follow instructions[3], the prepared 54% ethanol solution (v/v) was combined with MVS Stock Solution 1 to create the ethanol "alternative" test solution. The process is summarized in **Figure 3. The term *alternative* is used in the MVS Data Manager software to describe a custom test solution that is chemically different from the commercially-available, aqueous-based MVS Sample Solutions.** For purposes of this application note, the custom alternative test solution prepared with ethanol is simply referred to as the *ethanol test solution.*

This ethanol test solution is then dispensed into a microtiter plate for volume verification and analysis with the MVS. For preparing other ethanol test solutions with varying amounts of ethanol, or to obtain information on the approximate amount of MVS Stock Solution 1 or 2 to add to the ethanol solution, please refer to **Table 2** at the end of this document.

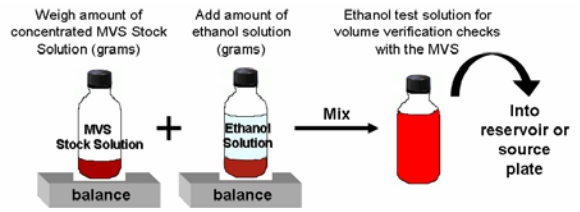

Figure 3. Overview of the preparation of the ethanol test solution from the ethanol solution and the MVS Stock Solution.

(c) Enter necessary information into MVS Data Manager software. The density value of the prepared 54.3% ethanol solution (v/v) is approximately 0.9183 g/mL[2]. In the case presented here, all of the ethanol solution (38.8802 g) was combined with 4.8085 g MVS Stock Solution 1 in an amber glass bottle to prepare the ethanol test solution. The weight and density values of the ethanol solution and the weight of MVS Stock Solution 1 were entered into the MVS Data Manager software. The dye concentration in the final, ethanol test solution is calculated based on the dilution ratio of the MVS Stock Solution 1 to ethanol solution. In the example discussed here, as shown in **Figure 4**, the resulting ethanol test solution is comprised of 10.1% of MVS Stock Solution 1 (v/v) and 89.9% of the initial 54% ethanol solution (v/v).

The resulting ethanol test solution therefore had a final ethanol concentration of 48.79% (v/v). Note, 89.9% of a 54.27% ethanol solution is equal to 48.79% ethanol in the test solution. This 48.79% ethanol test solution (v/v) was designed and prepared to incorporate similar absorbance per pathlength values as the commercially-available aqueous MVS Sample Solutions[1]. For example, the volume range for the custom solution described herein is similar to the aqueous-based MVS Range

C Sample Solution, which covers 2.0 – 9.9 µL in a 96-well plate.

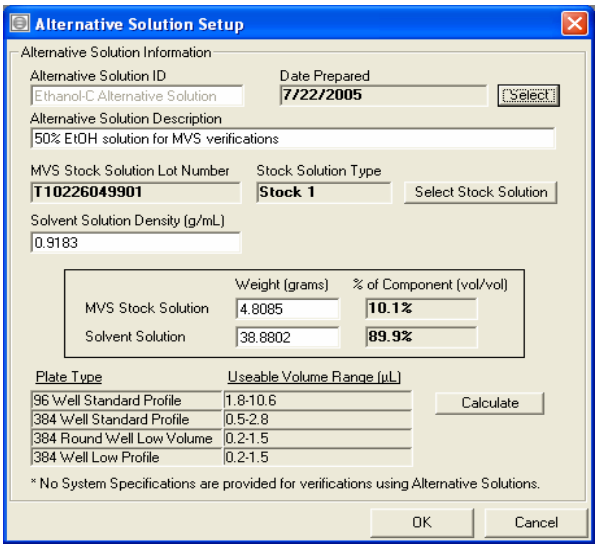

Figure 4. Graphical user interface for preparing custom test solutions in MVS Data Manager software. The weight and density values of the MVS Stock Solution 1 and ethanol solution are employed to calculate the usable volume range for various plate formats. For example, the proof-of-concept ethanol test solution shown can be dispensed from a liquid handler into a 96-well plate and the target volume can be assessed with the MVS for accuracy and precision between 1.8 – 10.6 µL or between 0.5 – 2.8 µL in a standard 384-well plate.

(d) Transfer and performance analysis of prepared ethanol test solution. After the necessary information (weights, density values) was entered into the MVS Data Manager software, 8 µL target volumes of the prepared ethanol test solution were dispensed into a microtiter plate for MVS volume verification and analysis. The proof-of-concept testing for this solution was performed with calibrated syringes. All syringes are gravimetrically-calibrated using an accredited method in a controlled test laboratory. *Because the reproducibility of the syringes has been proven, they can be used as volume transfer standards thereby removing device variability from the test procedure*. The gravimetrically-calibrated 8-µL Hamilton (Reno, NV) syringes with reproducibility adapters were used to aspirate target volume from a vial filled with the 48.79% ethanol test solution followed by a dispense into a 96-well microtiter plate. To each well containing the target volume, 192 µL of MVS Diluent solution was added to achieve a 200-µL working volume[4] in the 96-well plate. The two solutions in each well were mixed using the MVS plate shaker before the plate was placed into the MVS plate reader for measurement.

Results and Conclusions

The 48.79% ethanol test solution (v/v) was employed to monitor solution stability over time. Using the calibrated 8-µL Hamilton syringe with modified reproducibility adapter, the solutions were dispensed in replicates of eight into a 96-well Artel Verification Plate on each day of testing. The stability, or shelf-life, of the prepared test solutions, as monitored by relative inaccuracy (all values are within ± 2.2%), shows that the ethanol test solution was stable for at least four weeks. The coefficient of variation (%CV) values, or the precision data in the measurement, were within ± 0.8% (**Figure 5**). These results indicate that it may be possible for individual users to employ this methodology to assess device performance for specific or proprietary ethanol solutions for critical volume transfers.

Discussion

The proof-of-concept for preparing and verifying dispensed target volumes of the 48.79% ethanol test solution (v/v) has been shown. Although this application note does not discuss the preparation or testing of other ethanol test solutions, *i.e.*, solutions with < 50% ethanol by volume, the functionality in the MVS Data Manager software does support trial-and-error experimentation for users that wish to test their specific ethanol solutions. *Alternative test*

solutions containing > 50% ethanol (v/v) have not been evaluated by Artel.

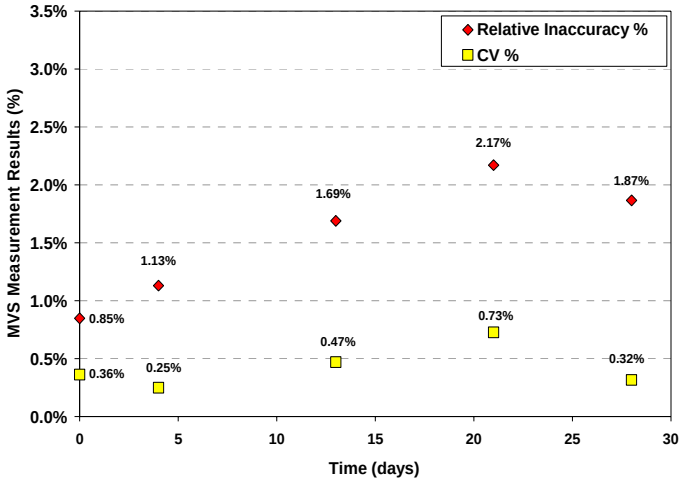

Figure 5. Using an 8-µL calibrated syringe, the 48.79% ethanol test solution was verified on five different days over a 28-day period. Based on the relative inaccuracy values, the shelf-life of the solution may be at least 4 weeks.

Table 1 (below) can be used as a guide for preparing ethanol solutions when ethanol portions are < 50% by mass (or < 56% by volume). **Table 2** (below) can be used as a guide for adding the appropriate amount and type of MVS Stock Solution to the ethanol solution to prepare an appropriate ethanol test solution that covers a specific volume range in either 96-well or 384-well standard profile plates. For example, preparing an ethanol test solution that covers 5 µL in a standard 96-well plate is different than preparing an ethanol test solution that covers 5 µL in a standard 384-well plate.

Please note, the final ethanol test solution will have a lower concentration of ethanol as compared to the initial ethanol solution because of the addition of the aqueous-based MVS Stock Solution. For instance, a 50% ethanol solution by mass (or ~56% ethanol by volume), may be diluted to approximately: (a) ~ 36% ethanol by mass for target volumes between 0.4 – 4 µL in 96-well plates; (b) ~44% ethanol by mass for target volumes between 2 – 11 µL in 96-well plates; or (c) ~ 38% ethanol by mass for target volumes between 9 – 49 µL in 96-well plates. These values are from column *l* in **Tables 1** and **2**.

References

(1) Albert, K.J.; Bradshaw, J.T.; Knaide, T.R.; Rogers, A.L. Verifying Liquid Handler Performance for Complex or Non-Aqueous Reagents: A New Approach. *J. Assoc. Lab. Autom.*, **2006**, *11*, 172-180.

(2) Handbook of Chemistry and Physics, 78th Edition, CRC Press, New York, David R. Lide, Editor-in-Chief, 1997-1998.

(3) Protocols and information for determining the proper amount of MVS Stock Solution to add to the corresponding amount of Solvent (or starter) Solution are detailed in multiple places, including the MVS User Guide, the MVS Help menu (MVS Data Manager software 2.0 and higher), as well as the Alternative Solution Helper software program found as part of MVS Data Manager 2.2 and higher. Additionally, Artel Technical Support can be contacted with any questions between 8 am – 5 pm EST (888.406.3463 x109) or via email at technical.support@artel-usa.com.

(4) Bradshaw, J. T.; Knaide, T.; Rogers, A.; Curtis, R. H. Multichannel Verification System (MVS): A Dual-Dye Ratiometric Photometry System for Performance Verification of Multichannel Liquid Delivery Devices. *J. Assoc. Lab. Autom.*, **2005**, *10*, 35-42.

Table 1. Protocol to prepare 50 g of ethanol solution using only ethanol & DI water

Ethanol solution ID:	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	*l*
Desired mass % of ethanol in water	**1.00**	**2.00**	**4.00**	**8.00**	**12.00**	**16.00**	**20.00**	**26.00**	**32.00**	**38.00**	**44.00**	**50.00**
contributing mass of ethanol (g)	0.50	1.00	2.00	4.00	6.00	8.00	10.00	13.00	16.00	19.00	22.00	25.00
contributing mass of DI water (g)	49.50	49.00	48.00	46.00	44.00	42.00	40.00	37.00	34.00	31.00	28.00	25.00
density of pure ethanol (g/mL)	0.789	0.789	0.789	0.789	0.789	0.789	0.789	0.789	0.789	0.789	0.789	0.789
density of DI water (g/mL)	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
theoretical contributing volume of ethanol (mL)	0.63	1.27	2.53	5.07	7.60	10.14	12.67	16.47	20.27	24.08	27.88	31.68
theoretical contributing volume of water (mL)	49.50	49.00	48.00	46.00	44.00	42.00	40.00	37.00	34.00	31.00	28.00	25.00
total theoretical volume of solution (mL)	50.13	50.27	50.53	51.07	51.60	52.14	52.67	53.47	54.27	55.08	55.88	56.68
Theoretical volume % of ethanol in water	**1.26**	**2.52**	**5.01**	**9.92**	**14.73**	**19.44**	**24.06**	**30.81**	**37.35**	**43.71**	**49.89**	**55.89**
approx. density of ethanol solution (g/mL, from CRC)	0.9963	0.9945	0.9910	0.9847	0.9792	0.9739	0.9687	0.9602	0.9504	0.9392	0.9269	0.9139

Table 2. Protocol to prepare the ethanol test solution for volume verifications by combining the appropriate amount of MVS Stock Solution (1 or 2)* to the 50 g ethanol solution

Ethanol solution ID & summary (from Table 1):	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	*l*
Ethanol solution with desired mass % of ethanol in water (as above)	**1.00**	**2.00**	**4.00**	**8.00**	**12.00**	**16.00**	**20.00**	**26.00**	**32.00**	**38.00**	**44.00**	**50.00**
Ethanol solution with theoretical volume % of ethanol in water (as above)	**1.26**	**2.52**	**5.01**	**9.92**	**14.73**	**19.44**	**24.06**	**30.81**	**37.35**	**43.71**	**49.89**	**55.89**
Total mass of ethanol solution (as above, g)	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
Approx. density of ethanol solution (as above, g/mL)	0.9963	0.9945	0.9910	0.9847	0.9792	0.9739	0.9687	0.9602	0.9504	0.9392	0.9269	0.9139
Add this much of MVS Stock 1 or 2 (grams) to cover approximate volume ranges in either standard 96-w or 384-w plates:												
Approx. mass of **MVS Stock Solution 1 (g)** to add to ethanol solution to measure ~ **0.4 - 4 uL in a standard 96-well plate** *or* ~ **0.1 - 1.1 uL in a standard 384-well plate**	16.864	16.909	16.999	17.179	17.358	17.538	17.718	17.987	18.257	18.526	18.796	19.066
Resulting mass % of ethanol in ethanol test solution for MVS verifications	0.748	1.495	2.985	5.954	8.908	11.845	14.767	19.121	23.441	27.727	31.979	36.198
Approx. mass of **MVS Stock Solution 1 (g)** to add to ethanol solution to measure ~ **2 - 11 uL in a standard 96-well plate** *or* ~ **0.5 - 2.9 uL in a standard 384-well plate**	5.496	5.511	5.540	5.598	5.657	5.716	5.774	5.862	5.950	6.038	6.126	6.213
Resulting mass % of ethanol in ethanol test solution for MVS verifications	0.901	1.801	3.601	7.194	10.780	14.359	17.929	23.272	28.597	33.906	39.198	44.473
Approx. mass of **MVS Stock Solution 2 (g)** to add to ethanol solution to measure ~ **9 - 49 uL in a standard 96-well plate** *or* ~ **2.4 - 9.9 uL in a standard 384-well plate**	13.238	13.273	13.344	13.485	13.626	13.767	13.908	14.119	14.331	14.543	14.754	14.966
Resulting mass % of ethanol in ethanol test solution for MVS verifications	0.791	1.580	3.157	6.301	9.430	12.546	15.648	20.275	24.871	29.438	33.975	38.482

*amount and type of MVS Stock Solution are dependent on volume range and plate density

An example in using these tables: verify a 5-µL transfer of a 20% ethanol solution (g/g) in a 96-well plate. Refer to column *g*. In this case, to the 50 g of 20% ethanol solution (g/g), add 5.774 g MVS Stock Solution 1. The resulting test solution will be approximately 17.9% ethanol by mass. If a higher percent of ethanol is desired in the final ethanol test solution, a higher concentration of ethanol is required in the initial ethanol solution.

For instance, to measure a 20-µL dispense of a 20% ethanol solution (g/g) in a 96-well plate, the solution in column *h* could be prepared. To the 50 g of the 26% ethanol solution (g/g), add 14.119 g of MVS Stock Solution 2 resulting in a 20.3% ethanol test solution (g/g).

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