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Precision and Accuracy of Methanol and Acetonitrile Dispenses Using the Thermo Scientific Matrix® Hydra® DT

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Key Words

- Thermo Scientific Matrix Hydra DT
- Thermo Scientific Matrix D.A.R.T.s Tips
- Methanol Dispense
- Acetonitrile Dispense
- Cross-Contamination

Abstract

The Thermo Scientific Matrix Hydra DT is a compact, liquid handler capable of automating pipetting tasks, including plate stamping, reformatting, sample transfers and serial dilutions. The instrument's small footprint makes it ideal for use in standard-size biological hoods and in laboratories where bench top space is limited. In this experiment the Matrix Hydra DT, equipped with 100 µl Thermo Scientific Matrix D.A.R.T.s® (Disposable Automated Research Tips) tips, is used to aspirate and dispense methanol and acetonitrile solutions. These solutions were mixed with tartrazine, which is used to verify the precision (coefficient of variance or CV) and accuracy of each dispense. The results indicated that the Matrix Hydra DT can dispense different volumes of methanol at a CV of less than 15.6% with less than 11% error and acetonitrile at a CV of less than 6.6% with less than 18% error. However, in order to obtain accurate and precise measurements, alterations to the aspirate settings of the Matrix Hydra DT were necessary. These alterations improved the methanol dispense precision to a CV of less than 8.3% with less than 7% error.

Introduction

The Matrix Hydra DT, a compact, bench top automated liquid handler, was used to perform the following experiments to determine the precision and accuracy of reagent additions using methanol and acetonitrile solutions. While the Matrix Hydra DT efficiently automates reagent additions, the instrument's wide application compatibility allows laboratories to streamline other routine tasks such as PCR set up, plate to plate transfers and plate reformatting. The Matrix Hydra DT can work as a standalone instrument using on-board programming, or can be linked to a PC and directed by the intuitive ControlMate software. Matrix D.A.R.T.s tips, used with the Matrix Hydra DT, eliminate possibilities of cross-contamination and liquid carryover.

The Matrix Hydra DT is calibrated using a tartrazine dye solution. In this study, we use methanol and acetonitrile to determine the performance of the Matrix Hydra DT. The reagents in this experiment were prepared as would be used by our customer for a specific scientific application. The reagents used in these tests have different properties in terms of viscosity, density, evaporation rates, etc., which affects the precision and accuracy of dispenses. In this experiment, we discuss the necessity to alter "Aspirate" settings in the menu of the Matrix Hydra DT in order to obtain precise and accurate dispenses.

Figure 1: The Matrix Hydra DT is shown equipped with a single position state. Three-position states are also available.

Materials:

1. Distilled Water
2. Tartrazine Solution (Yellow 5)
3. Methanol
4. Acetonitrile
5. Thermo Scientific Matrix Hydra DT, 0.5-100 µl (Item no. 1096-DT-100)
6. Thermo Scientific Matrix Disposable Automation Reservoir, 96-Channel, 125 ml (Item no. 1064-05-8)
7. Thermo Scientific Matrix 96-Well Polystyrene Microplate, Clear, Flat Bottom (Item no. 4915)
8. Plate Shaker
9. Centrifuge
10. Tecan Genios Reader
11. 100 µl Thermo Scientific Matrix D.A.R.T.s Tips (Item no. 5526)
12. Thermo Scientific Matrix WellMate® (Item no. 201-10001)
13. Single Channel Thermo Scientific Matrix Electronic Pipettes (Item nos. 1022 & 1024)

Methods:

1. The Matrix Hydra DT was calibrated and programmed as follows:
   - Aspirate Screen:
     - Prime is turned "ON"
     - Aspirate Volume = 1.0 µl
     - Speed = 5
   - Dispense Screen:
     - Dispense Volume = 1.0 µl
     - Speed = 5
2. The following solutions were made:
   - Distilled Water and Tartrazine:
     - For 1 µl dispenses: 98% distilled water, 2% dye
     - For 100 µl dispenses: 99.9% distilled water, 0.1% dye
   - Methanol and Tartrazine:
     - For 1 µl dispenses: 98% methanol, 2% dye
     - For 100 µl dispenses: 99.9% methanol, 0.1% dye
   - Acetonitrile and Tartrazine:
     - For 1 µl dispenses: 49% acetonitrile, 50% distilled water, 1% dye
     - For 100 µl dispenses: 94.9% acetonitrile, 5% distilled water, 0.1% dye
3. Using the Matrix WellMate, 96-well plates were filled with 250 µl of distilled water for 1 µl dispenses, and 150 µl for 100 µl dispenses
4. Disposable automation reservoirs were filled with respective solutions
5. Using the Matrix Hydra DT on-board programming, 1 µl or 100 µl was aspirated of each solution and dispensed into a fresh 96-well microplate
6. All of the plates were shaken for 5 min. on a plate shaker and then spun down at 1750 rpm for one min.
7. The plates were scanned using a Tecan Genios Reader set to 412 nm at 5 flashes
8. In order to determine the actual amount of reagent pipetted, a three point standard curve was generated for each solution made
   - Designated amounts of each reagent (0.5, 1.0 and 1.5 µl for 1.0 µl dispenses and 95, 100 and 105 µl for 100 µl dispenses) was pipetted in triplicate into a 96-well plate
   - The plate was shaken for 5 min. and spun down at 1750 rpm for one min.
   - The plate was read on the Tecan reader set to 412 nm at 5 flashes
   - The measurements for each volume were averaged and then plotted, displaying the equation of the trend line using Microsoft® Excel
   - The equation generated was used to calculate the actual volume pipetted in all dispenses using the specific reagent by correlating the average absorbance to the volume
9. A tip change was performed between uses of each solution
10. Each solution at each volume was tested in triplicate
11. For the 1 µl methanol dispense, the aspirate settings were later changed to:
    - Aspirate Screen:
      - Prime is turned "ON"
      - Aspirate Volume = 2.0 µl
      - Speed = 5
      - Air Gap = 1.0 µl
12. The dispense settings remained the same, and aspirate/dispense cycles using the altered settings were also tested in triplicate

Results

Dispenses performed in this experiment were wet, dispensed into 250 µl distilled water for 1 µl dispenses and into 150 µl distilled water for 100 µl dispenses. Also, dispenses were neat; samples were aspirated from the reservoir and dispensed directly to the 96-well plate. The addition of tartrazine dye to methanol and acetonitrile was used to determine the CV and actual volumes dispensed for each experiment.

	Set Dispense Volume (µl)	Actual Volume Dispensed (µl)	%CV
Water	1	1.0997	4.5100
	100	101.7837	0.5321
Methanol	1	1.1118	15.6073
	100	111.0910	0.7702
Acetonitrile	1	1.1800	6.6423
	100	111.6197	0.6359

Table 1: Precision and Accuracy of Triplicate Runs of Methanol and Acetonitrile Dispenses with the Matrix Hydra DT

1 µl Air Gap Before Aspirate	Volume Aspirated	Actual Volume Dispensed	%CV
No	1 µl	1.1118	15.6073
Yes	2 µl	1.0733	8.3200

Table 2: Effect of Changing Aspirate Settings on Precision and Accuracy of 1 µl Methanol Dispense

Conclusion

The purpose of this procedure was to determine the precision and accuracy at which the Matrix Hydra DT can dispense methanol and acetonitrile at 1 µl and 100 µl volumes (minimum and maximum range of the Matrix Hydra DT equipped with a 96-channel, 100 µl pipetting head fitted with 100 µl Matrix D.A.R.T.s tips). In order to achieve the desired accuracy and precision, we changed the aspirate settings to include an air gap and to aspirate a larger volume than it dispensed. These changes positively influenced the results of the 1 µl dispense and would be easily integrated into any protocol where limited reagents are not an obstacle. If a further increase in precision and accuracy is desired, or limited reagents are a factor, the instrument can be calibrated with a specific reagent. The Matrix Hydra DT can precisely and accurately dispense a wide range of laboratory reagents and buffers. However, the chemical nature of these reagents may require the use of different settings to optimize performance and achieve results of very high accuracy.

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