Comparison of the measurement accuracy of automated and manual analytical pipettes

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Abstract

Analytical syringes can be used in place of pipettes for measuring and dispensing solvents and other non-aqueous liquids. In this study we compared the precision and accuracy of the Thermo Scientific eVol analytical syringe with standard pipettes and evaluated carryover effects.

Introduction

The eVol is an automated handheld analytical syringe. This can be used to dispense between 0.2 - 500 µL of liquid and achieves this with detachable syringes, which have different capacities. The barrel capacities are: (a) 0.2 - 5.0 µL, (b) 2.0 - 50 µL and (c) 20 - 500 µL.

While factory tested, the eVol is easily calibrated by the user gravimetrically.

The application areas in which the eVol can be used include:

- preparation of calibration standards
- addition of internal standards
- sample dilution

The syringe has an ergonomic design with a full colour screen and intuitive control interface.

The eVol has been demonstrated to:

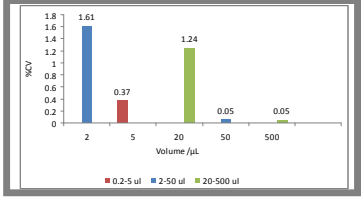
- have excellent precision compared to a conventional pipette
- be precise across its range of operation
- be capable of dispensing liquids of a range of viscosities with good precision
- be accurate by virtue of its ability to be calibrated by the user
- be easily rinsed and shows minimal carryover

Results and Discussion

Precision across the range of the eVol

Precision of the eVol was shown to be high across the working range. The greatest precision was observed at the higher range with CV's of 0.05 – 0.37% (Figure 1).

Figure 1. Precision across the range of the eVol



Comparison with a conventional pipette

When comparing the %CVs of the weighings at the upper range of the barrel, the eVol produced consistently higher precision than the manual pipette (Figure 2).

The statistical t-test on the three pairs of means for the eVol and pipette (at a significance level of 0.05) indicated that the means were statistically different. While it is not possible to statistically confirm which method of liquid transfer was most accurate, the fact that eVol had been calibrated prior to us using it did give us more confidence in this technique.

Precision of dispensing different liquids

The eVol pipette lends itself to the aspiration and dispensation of many different liquids. While DMSO was a good choice of solvent for our evaluation of the eVol's precision, we also tested the eVol precision with horse serum, water and acetonitrile by carrying out twelve replicate dispensations of 50 µL. There was a correlation between viscosity and dispensing precision, with the more viscous liquids showing the greatest precision (Figure 3).

Figure 2. Precision for the two methods of dispensing liquids

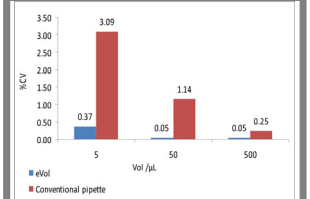
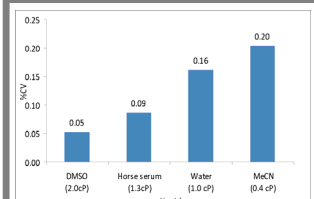


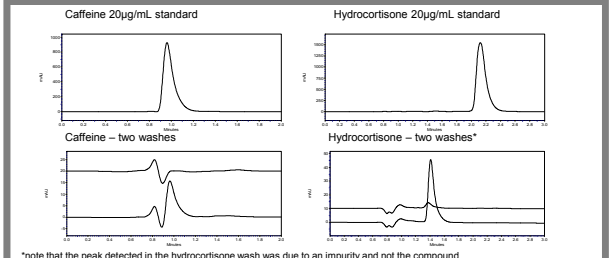
Figure 3. Precision of measuring liquids



Carryover

While a small amount (2.6%) of carryover was observed for caffeine in the first aspiration-dispensation cycle, none was observed thereafter. Hydrocortisone, which is considered a more "sticky" compound, showed no evidence of carryover. Of course, the eVol would normally be washed between dispensing one sample and aspirating another (Figure 4).

Figure 4. Chromatograms showing (a) caffeine with first two washes and (b) hydrocortisone with first two washes – both compounds at 20µg/mL in methanol / water (50:50 v/v)



*note that the peak detected in the hydrocortisone wash was due to an impurity and not the compound

Experimental Details

Equipment

Thermo Scientific eVol automated analytical syringe
Premium brand manual pipette, range 0.5 – 10 µL
Premium brand manual pipette, range 10 – 100 µL
Premium brand manual pipette, range 100 – 1000 µL
MC5 micro analytical balance (Sartorius)
Dimethyl sulfoxide (DMSO) (Fisher Scientific)
Thermo Scientific Surveyor HPLC
Thermo Scientific Hypersil GOLD 3µm 50x2.1 mm HPLC column
Chromacol screw top fixed insert vial 0.9 ml Part 09-FISV (and pre-slit septa)

All calculations were carried out using Microsoft Excel®

The micro analytical balance was evaluated in order to determine the minimum weight that could be measured on this with a measurement uncertainty of 0.1% (USP General Chapter 41). This was determined using 12 replicate injections of a 50 mg check-weight (x=49.990, s=-6.216x10^{-4}). The minimum weight was calculated as 3s/0.001 which was 1.86 mg.

In order to avoid cavitation, when aspirating solvents of different viscosities, all aspirations and dispensations were carried out using the minimum speed (i.e. 1).

The statistical F-test was used to compare the standard deviations of two or more precisions, and the t-test was used to test whether two means were the same at a given confidence.

Calibration

The eVol pipette was calibrated for each syringe barrel to ensure that the device was operating at its maximum accuracy. The calibration process uses repeat weighings of a liquid of known density. In our experiments, we used DMSO (density =1.1004 g/ml). DMSO was used here and in other parts of the experiment due to its high boiling point (189 °C) and relatively low evaporation rate, which produced very stable readings on the balance.

Precision across the range of the eVol

The precision of conventional pipettes was evaluated by weighing 12 replicates of DMSO.

We evaluated the precision of the eVol across its range of 0.2 – 500 µL using the three syringe barrels. 12 replicate weighings of DMSO were made at:

1. the low end of the range of a given barrel, using repeat dispensations from the same aspiration
2. the top of the range of a given barrel, using a single aspiration and dispensation

A statistical F-test was used to compare the standard deviations of the measurements and maximum capacity of each barrel [Statistics and Chemometrics for Analytical Chemistry, James N Miller and Jane C Miller, 5th Edition (2005), Pearson Education Limited].

Comparison of eVol with conventional pipettes

A statistical t-test (two tailed) was used to compare the means of the measurements at the maximum capacity of each barrel [Statistics and Chemometrics for Analytical Chemistry, James N Miller and Jane C Miller, 5th Edition (2005), Pearson Education Limited].

Precision of dispensing different liquids

12 replicate weighings of DMSO, horse serum, water and acetonitrile were made. In order to minimize the evaporation of the more volatile liquids, vials with reduced volume inserts fitted with pre-slit septa were used.

Carryover

Caffeine and hydrocortisone (both 20 µg/mL) were used to evaluate the carryover of the syringe. Both solutions were prepared in methanol/water (50:50). A 500 µL volume of the carryover solution was aspirated and dispensed, followed by three washes of the blank solvent of the same volume. Blank injections were made before and after the injection of the test compounds to ensure that there were no instrument carryover effects.

Method summary:

Column	Hypersil GOLD™ 3µm 50x2.1 mm
Mobile phase	methanol/water (40:60)
Temperature	40 °C
Flow	0.2 mL/min
Detection	UV 254 nm

Conclusion

The eVol shows higher precision than a conventional pipette. This advantage of builds upon the inherent repeatability of having an inbuilt motor drive the aspiration and dispensing cycle, rather than an the operator.

Appendix – Results and statistical treatment

Table 1. Precision across the range of the eVol

Barrel	Volume (µL)	Std Dev	Average
1	0.2	0.0062395	0.22025
1	5	0.0205042	5.4816667
2	2	0.0356153	2.2100833
2	50	0.0291562	55.383583
3	20	0.2717776	21.945167
3	500	0.2630092	551.95525

Table 2. Comparison with a conventional pipette

Volume (µL)	Standard deviation, s		Mean wt /mg
	eVol	Pipette	
5	0.020504	.	5.4816667
50	0.029156	.	55.383583
500	0.263009	.	551.95525
5	.	0.1710273	5.5421833
50	.	0.632709	56.232917
500	.	1.355652	556.21108

Table 3. Statistical t-testing of mean weights of DMSO dispensed using the eVol and the conventional pipette

	Volume dispensed /µL		
Volume	5	50	500
x1 (pipette)	5.5421833	56.232917	556.21108
x2 (eVol)	5.4816667	55.383583	551.95525
s1 (pipette)	0.1710273	0.632709	1.355652
s2 (eVol)	0.0205042	0.0291562	0.2630092
Degrees of freedom	11.316148	11.046717	11.826901
t-value	2.55	6.28	44.63
t-critical (P=0.05)	2.18	2.18	2.18
t-critical (P=0.02)	2.68	2.68	2.68
t-critical (P=0.01)	3.05	3.05	3.05

Table 4. Manual pipette dispensions

Range (µL)	Volume dispensed (µL)
0.5 - 10	5
10 - 100	50
100 - 1000	500

For additional information, please visit our Chromatography Resource Centre which can be found at: www.thermoscientific.com/crc

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