Application Note

intelligent sample preparation
(w) www.eprep.com.au
(e) info@eprep.com.au

Analyical Accuracy and Precision| Application Note 2019

ePrep Sample Preparation Workstation

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INTRODUCTION

This White Paper is intended to provide an indication of the level of performance which the ePrep is capable.

The ePrep (Figure 1) is a liquid handling Sample Preparation Workstation designed for analytical laboratories and has the functionality to carry out a wide range of functions in sample preparation. Its modular deck and advanced but simple to use control software allow it to be efficient for both small sample batches as well as larger batch sizes typical of chromatographic analysis.

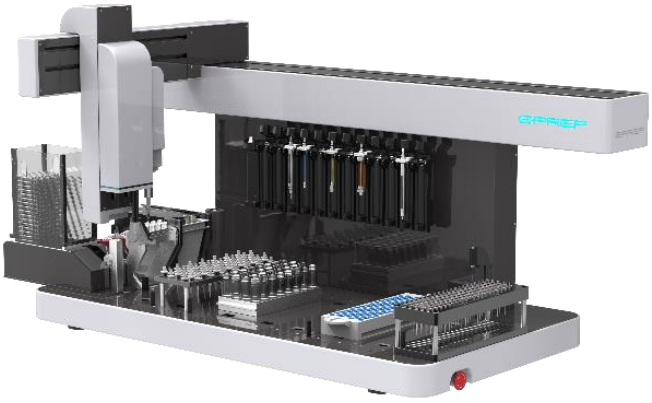


Figure 1. ePrep Sample Preparation Workstation

ePrep's robotic operation makes sample preparation processes easier, faster, and reproducible; providing an accurate, repeatable and reliable sample preparation especially for complex Workflows.

Figure 2 provides an illustration of precision and accuracy. The goal of the analytical analysis is consistent and reliable precision and accuracy.

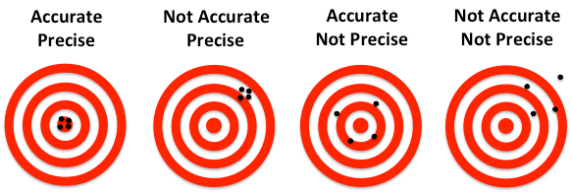


Figure 2. Precision and Accuracy Explained

Because analytical grade syringes are used in the ePrep, unsurpassed accuracy and reproducibility can be achieved. They can also allow micro volumes, high pressure dispensing and the ability to handle aqueous, organic and volatile samples.

ePrep uses high-resolution motors to drive the syringe plunger. The linear precision of these motors is in the order of 20 microns which translates to around 0.03% of the syringe volume. Digital feedback is also used to ensure control movements are correct every time. In addition, the mechanical design has minimised backlash effects in couplings and drive screws with software features further minimising any backlash contribution.

ePrep REPRODUCIBILITY

Dispensing of Volatile Solvents

System precision was determined by dispensing a range of liquids with different densities, surface tensions and volumes. Volumes are measured gravimetrically on a five figure analytical balance (Shimadzu AP225WD). The performance on the ePrep system is compared with a handheld pipette system.

The volume from the gravimetric measurement was determined using temperature corrected density tables.

Dispensing 20µL volumes of volatile solvents acetone, hexane/toluene and chloroform with the ePrep shows excellent accuracy measured against the Set Volume (Table 1). %RSD for all solvents shows dispensing mechanism to be highly repeatable. When compared to the dispensing of the same liquids using a calibrated air displacement pipette (Table 2), the ePrep system is far more reliable when dealing with a range of solvents.

EPREP	***Hexane: Toluene (1:1)***	***Chloroform***	***Acetone***	***Water***
Set Volume	20µL	20µL	20µL	20µL
Avg Dispense Volume	19.85	19.60	19.95	20.05
SD	0.05	0.04	0.07	0.03
%RSD	0.24	0.22	0.36	0.15

Table 1. ePrep with 100µL syringe measured at 20% of the total syringe volume. Triplicate samples were analysed gravimetrically

PIPETTE SYSTEM	***Hexane: Toluene (1:1)***	***Chloroform***	***Acetone***	***Water***
Set Volume	20µL	20µL	20µL	20µL
Avg Dispense Volume	19.85	18.83	19.59	20.46
SD	0.45	0.80	0.55	0.35
%RSD	2.28	4.25	2.83	1.71

Table 2**.** Dispensing using a conventional laboratory air displacement pipette. Triplicate sample analysed gravimetrically.

ePrep can dispense aqueous, organic and volatile samples which are not recommended with air driven pipetting systems remote syringe pumps.

ePrep ACCURACY

System Accuracy determination was carried out by gravimetric measurements using a five-figure analytical balance (Shimadzu AP225WD). The determinations were done by dispensing five replicates of 10% of the full syringe volumes and from the weights making temperature compensated conversions to volume.

Three different standard syringes were used (100µL, 1 mL and 10mL). The raw volume data was then used to determine a volume calibration factor for each syringe. The calibration factors were then applied to the second set of dispense data to determine the volume accuracy and precision that can be expected from the ePrep system when a calibrated syringe is used.

Table 3 demonstrates that extreme accuracy and reproducibility is achieved on the ePrep system, well beyond that generally required in analytical laboratories, even when the syringes are not calibrated.

	100 µL ePrep Syringe Dispense 10µL			**1mL ePrep Syringe Dispense 100µL**			**10mL ePrep Syringe Dispense 1,000µL**	
	Uncalibrated	**Calibrated**		**Uncalibrated**	**Calibrated**		**Uncalibrated**	**Calibrated**
Replicate	Actual Disp Vol (µL)	Actual Disp Vol (µL)		Actual Disp Vol (µL)	Actual Disp Vol (µL)		Actual Disp Vol (µL)	Actual Disp Vol (µL)
1	10.0803	9.9799		99.9498	100.1506		998.0924	999.3072
2	10.0502	9.9900		99.9498	100.0703		1001.2450	1002.9418
3	10.0602	9.9699		99.3173	99.6586		995.7028	996.4759
4	10.0703	10.0201		99.9096	100.1707		1000.7329	1001.2851
5	10.0301	9.9900		99.5080	99.9096		998.0522	998.2229
Average Vol (µL)	**10.0582**	**9.9900**		**99.7269**	**99.9920**		**998.7651**	**999.6466**
Volume Error (µL)	0.0582	-0.0100		-0.2730	-0.0080		-1.2350	-0.3534
% Volume Error (µL)	0.58	-0.10		-0.27	-0.01		-0.12	-0.04
RSD %	0.19	0.19		0.30	0.21		0.23	0.25

Table 3**. ePrep automated system s**yringe accuracy measurements at 10% of total volume

For the ultimate accuracy in volume from the ePrep platform, the accuracy of the syringe itself needs to be considered. Manufacturing tolerances for the full syringe volume is given as +/- 1%.

Syringe accuracy is by far the largest consideration for accuracy on the ePrep platform. The ePrep software provides for a guided syringe calibration and the application of a syringe volume correction. Calibration of the syringes further improves the accuracy of dispensed volumes as shown in Table 3.

Table 3. also further demonstrates the level of reproducibility that can be achieved with the ePrep system. Absolute volume reproducibility better than 0.3% RSD is achievable.

ePrep LINEARITY

Colorimetric 96 Well Plate Reader measurements

To demonstrate the confidence that can be achieved in preparing small volume standard calibration curves, 6 replicate series were prepared with 2 µL to 96 µL dispenses in 2 µL increments using a 100µL syringe. The resulting calibration curve looks like a single set of data but there are actually 6 replicates overlayed on each other with excellent agreement between all 6 replicates.

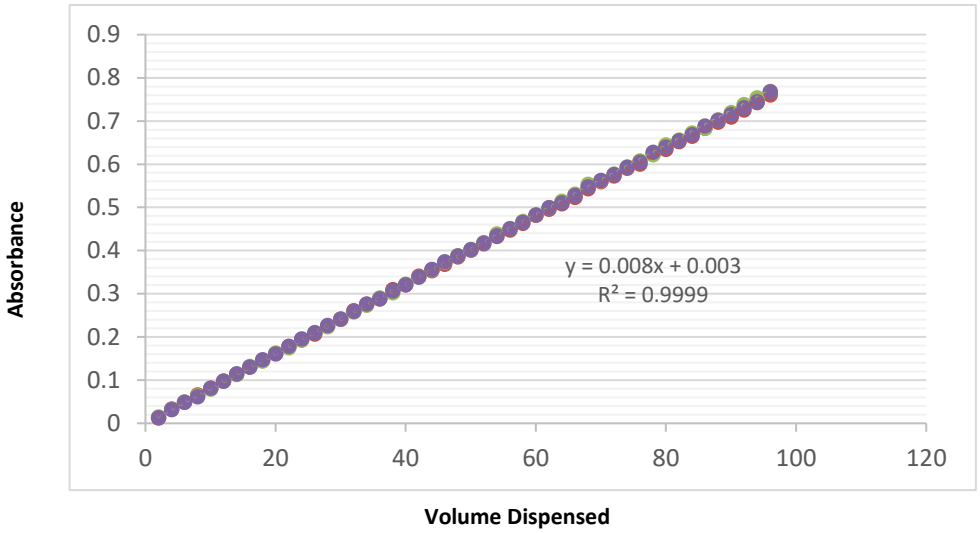
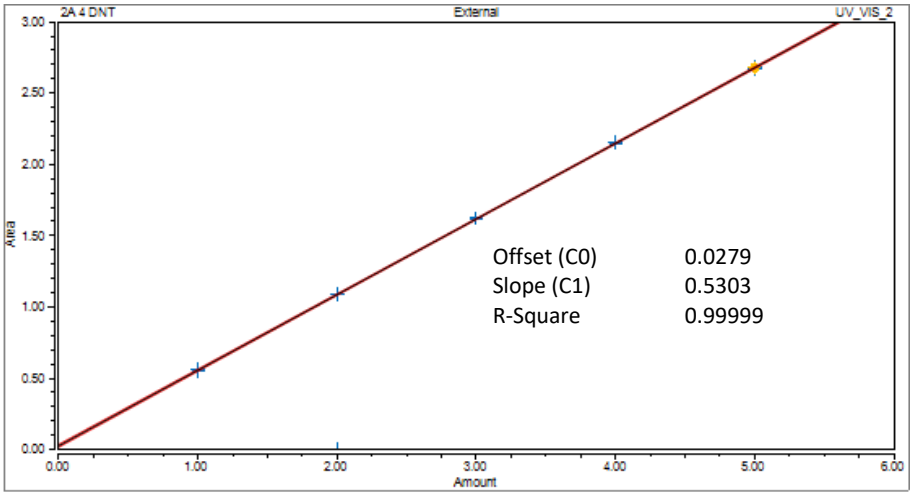


Figure 3: Linearity of dispense volume 2µL increments from 2µL to 96µL. Six replicate calibration curves overlaid.

HPLC-UV measurements

Generally, chromatographic analytical systems do not achieve the level of precision that the ePrep system achieves. Accurate Calibration curve standards were prepared using ePrep and measured with an HPLC-UV detector dispensing of 2-amino 4, 6-Nitrotoluene using the 1mL syringe. Linearity R^2 = 0.99999 (Figure 4).



Calibration Details	2A 4 DNT		
Calibration Type	Lin, WithOffset	Offset (C0)	0.0279
Evaluation Type	Area	Slope (C1)	0.5303
Number of Calibration Points	17	Curve (C2)	0
Number of disabled Calibration Points	0	R-Square	1

Figure 4. Calibration curve of 2 amino 4, 6 nitrotoluene. HPLC-UV, performed in triplicate

ePrep CARRY OVER CONSIDERATION

ePrep features an in-deck Wash Station that is accessed in Workflows to automatically prime and wash syringes to manage carryover and safe waste disposal. Aside from the in-deck wash station specific vials/tubes can be set as wash source or waste containers to allow customisation of wash parameters dependant on sample needs. The ePrep also allows the use of different syringes for different parts of a Workflow. The carry-over achieved depends on the syringe type, sample characteristics and user set parameters in the software.

Where zero potential for cross-contamination is required, the option of disposable single-use syringes (Syngles) is being provided. Check with ePrep for availability

Theoretical Syringe Wash Values at default ePrep Wash Parameters

Table 4 outlines the theoretical wash (dilution only) factors for a 10000ppm solution with 100µL, 1mL and 10mL syringes.

ePrep default wash parameters have been selected to optimise speed and near-zero-carryover wash for most "non-sticky" compounds at moderate concentrations, however for high concentration or known sticky samples wash parameters may need to be adjusted to increase effectiveness.

	100µL Syringe	1mL Syringe	10mL Syringe
Default ePrep Syringe Wash Parameters			
Default Wash Volume (µL)	*30*	*100*	*800*
Default Wash Cycles	*4*	*3*	*2*
Default Wash Aspiration	*60*	*70*	*200*
Default Wash Dispense	*250*	*400*	*500*
Theoretical Dilution Concentration after each wash using default parameters (ppm)			
Initial @ 10000ppm	10,000	10,000	10,000
Wash #1	1,400	420	126
Wash #2	196.00	17.64	1.59
Wash #3	27.440	0.741	0.020
Wash #4	3.842	0.031	0.000
Wash #5	0.538	0.001	0.000
Wash #6	0.075	0.000	0.000
Wash #7	0.011	0.000	0.000

Table 4. ePrep Wash Default Parameters and Theoretical Syringe/Needle Concentration on Wash (Dilution Only)

Chromatographically Tested Wash Parameters

Caffeine was selected as a reference 'non-sticky' compound with Chlorohexidine as a sticky compound to test chromatographically the wash parameters of ePrep.

A 10,000 ppm solution containing caffeine and chlorohexidine was cycled 3 times to 30% of the syringe volume. Then each syringe was washed using it's default ePrep wash parameters and each wash was collected for analysis by UHPLC-UV.

Table 5 shows that with the caffeine there is almost no carry over after the standard wash cycles within the EPREP default settings (100µL syringe – 4 cycles, 1000µL syringe – 3 cycles, 10,000µL syringe - 2 cycles). Chlorohexidine shows higher carryover. A minimum of 6 syringe wash cycles reduced the carry over to undetectable levels.

Caffeine Conc after wash (ppm)

	100 µL Syringe	**1mL Syringe**	**10mL Syringe**
Wash 1	1287	596	160
Wash 2	264	60.9	22
Wash 3	38	11.7	0.2
Wash 4	3.7	0.00	0.00
Wash 5	0.00	0.00	0.00
Wash 6	0.00	0.00	0.00
Wash 7	0.00	0.00	0.00
Wash 8	0.00	0.00	0.00
Wash 9	0.00	0.00	0.00
Wash 10	0.00	0.00	0.00

Table 5. Washing effects with caffeine (Initial conc. 10,000ppm)

The default wash settings are very effective in clearing non-sticky compounds from the syringes. However, more sticky compounds can adsorb to syringe and needle surfaces with the default wash conditions being insufficient/inefficient (ultra-pure water) for its elimination. Difficult to clean compounds may require the use of different wash solvents. Table 6 shows the results obtained by using MeOH as washing eluent, the results highlight that the increased organic component is suspected to releases compound which had been adsorbed onto the surface on the syringe.

Chlorhexidine Conc after wash (ppm)
100µL Syringe

	MeOH wash	**Water wash**
Wash 1	154.60	179.83
Wash 2	15.86	24.65
Wash 3	10.52	5.00
Wash 4	5.24	1.93
Wash 5	4.44	1.29
Wash 6	1.10	0.86
Wash 7	0.91	0.00
Wash 8	0.00	0.00
Wash 9	0.00	0.00
Wash 10	0.00	0.00

Table 6. usage of a MeOH wash vs water based wash (Initial Conc. Chlorohexidine 1000ppm)

CONCLUSION

The ePrep Sample Preparation Workstation is a cost-effective robotic system for the preparation of standards and samples for chromatographic analysis in an Analytical Laboratory.

ePrep can achieve high levels of accuracy, precision and reproducibility out-of-the-box. With some small adjustments to Workflow parameters, unprecedented performance can be consistently achieved.

REFERENCE PUBLICATIONS

1. A Review on Step-by-Step Analytical Method Validation, www.iosrphr.org Volume 5, Issue 10 (October 2015), PP. 07-19
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3. Analytical procedures and Method Validation 2000, FDA
4. Validation of Analytical Methods Agilent, Agilent Methods 5990-5140EN