

MICROMAN® E: Improve HPLC Analytics with Proper Pipetting of Difficult Liquids

APPLICATION NOTE AN0994

APPLICATION BENEFITS

HPLC typically requires the use of volatile solvents unsuitable for air displacement pipetting. Volatile solvents evaporate quickly leading to leaks and decreased accuracy. Effective sample preparation for HPLC requires an appropriate tool for accurate and precise dispensing.

SOLUTIONS

Comparisons between an air displacement pipette with tips and a positive displacement pipette, MICROMAN® E, with capillary pistons, demonstrate significant improvements in accuracy and precision when MICROMAN E is used for transfer of volatile solvents. Thus, MICROMAN E, in combination with capillary pistons, is capable of pipetting volatile liquids as accurately and precisely as water.

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INTRODUCTION

Sample preparation and pre-analysis steps for HPLC are critical for overall accuracy and reliability. These steps typically introduce greater variability than the measurement itself. HPLC protocols often require the transfer of microliter volumes of volatile solvents. MICROMAN® E, a positive displacement pipette, is ideal for accurate transfer of volatile solvents.

Dispensing systems in the laboratory can function either by employing the air displacement principle, or they constitute a positive displacement system. In an air displacement pipette, an air cushion separates the liquid in the plastic tip from the piston inside the pipette. The behavior of the air cushion is influenced by the physical properties of the liquid (density, viscosity, surface tension, and volatility). Interaction of the air cushion with volatile solvents is problematic with air displacement pipetting.

The properties of volatile solvents affect the elasticity of the air cushion. Evaporation is a continuous phenomenon and its properties are due to the balance between the liquid and gas states of a solution. Liquids such as acetone, hexane, and methanol evaporate quickly, which expands the column of air inside the pipette, leading to leaks. Drops are visible on the rim of the tip and may fall on the bench, which can cause risk to users. This is particularly concerning when working with hazardous, volatile liquids, such as organic solvents, which are flammable, toxic, carcinogenic, and/or uncolored on the bench.

Evaporation of acids and other corrosive reagents in the air cushion can reach and damage the pipette shaft, seal, and piston.

With positive displacement pipettes, the liquid does not come into contact with the pipette. There is no air cushion; therefore, the physical properties of the liquid have very little influence on the volume of the liquid to be aspirated or dispensed.

This application note demonstrates the advantages of using a positive displacement pipette, Gilson's MICROMAN® E with capillary pistons, to pipette volatile liquids during the sample preparation for HPLC as compared to using air displacement pipettes and tips.

MATERIALS & METHODS

For each test, 100 microliters of ISO 3696 grade 3 distilled water or acetone (Sigma) were dispensed and the volume determined gravimetrically. For air displacement (standard pipette), 100 microliter tips were used. For positive displacement, the Gilson MICROMAN® E (M100E) with a nominal volume of 100 µL and capillary pistons (CP10) were used. Ten replicates were conducted for each test.

Four different air displacement pipetting modes were tested (forward, forward saturated, reverse, and reverse saturated) with a standard pipette and the forward mode in positive displacement with MICROMAN E.

The systematic error (inaccuracy) and random error (imprecision) of each pipette were determined according to ISO 8655 standards.

Each test was performed in ten replicates and the average volume was determined by gravimetric measurements.

Laboratory technicians were experienced and formally trained in all pipetting methods used in this study.

Principle and Pipetting Mode Description

The forward mode is the regular aspirate and dispense mode (Figures 1 and 2). In general, the precision of the forward mode relies on precise draining by air pressure (air displacement pipette) or internal wiping of the pipette barrel (positive displacement pipette).

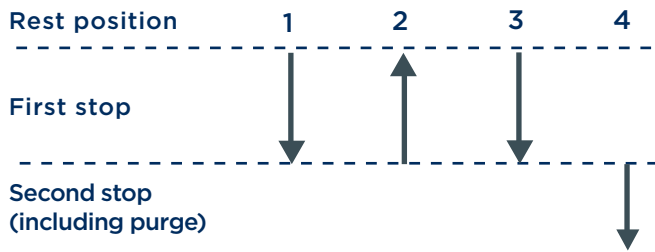


Figure 1

Forward Mode Pipetting

1. Preparation: Hold the instrument in a nearly vertical position. Depress the plunger smoothly to the first stop position.
2. Aspiration: Immerse the pipette tip in the liquid. Allow the plunger to move up smoothly to the rest position. Wait one second so that all the liquid has time to move up into the tip.

3. Distribution: Place the pipette tip at an angle (10° to 45°) against the inside wall of the receiving vessel. Depress the plunger smoothly to the first stop position.
4. Purge: Wait one second, and then depress the plunger to the second stop position. This “blow-out” stroke removes any remaining sample from the tip. Remove pipette tip end from sidewall by sliding it up the wall.

Forward mode with a positive displacement pipette is similar to the forward mode of air displacement pipettes and even easier as there is no need for a purge and the capillary piston can be directly ejected.

1. Preparation: Press the plunger button to the first stop. The piston moves to the appropriate position.
2. Aspiration: Immerse the capillary piston in the liquid. Release the plunger allowing it to move up to the home position. The piston moves up and the ambient pressure forces the desired volume of liquid through the orifice into the capillary.
3. Distribution: Press the plunger button to the first stop. The piston moves down and expels the liquid out of the capillary.
4. Ejection: Press the plunger all the way down to the second and last stop. Capillary and piston are ejected without hand contact.

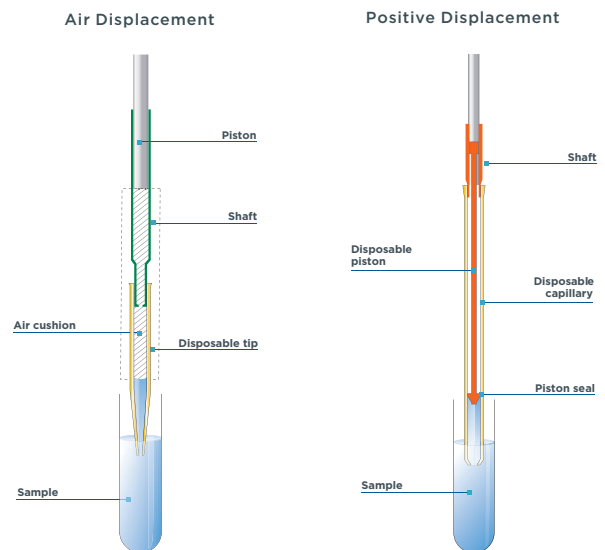


Figure 2

Two Pipetting Concepts—Air and Positive Displacement

Reverse mode pipetting: the purge stroke is used during preparation. During aspiration, an amount of liquid equal to the amount of purged air is added. This amount compensates for the liquid that remains as film inside the tip during dispensing.

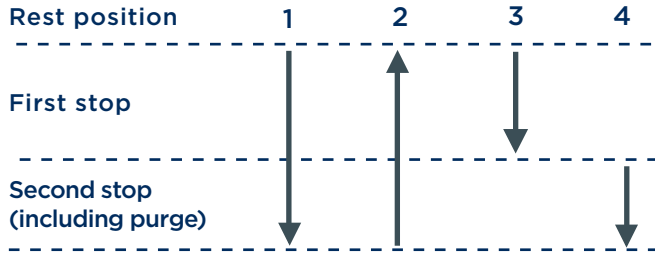


Figure 3
Reverse Mode Pipetting

1. Preparation: Hold the instrument in a nearly vertical position. Depress the plunger smoothly to the second stop position.
2. Aspiration: Immerse the pipette tip in the liquid. Allow the plunger to move up smoothly to the rest position. Wait one second so that all the liquid has time to move up into the tip.
3. Distribution: Place the pipette tip at an angle (10° to 45°) against the inside wall of the receiving vessel. Depress the plunger smoothly to the first stop position. Wait one second.
4. Complete purge: Wait one second and purge. If the pipette tip will not be re-used, depress the plunger to purge position over an appropriate waste container and eject the tip.

Saturated pipetting: the accuracy of dispensing can be improved by pre-wetting the tip by aspirating and dispensing several times with the solvent before pipetting. This allows to saturate the air cushion with solvent vapors and to improve volume delivering precision. This method can be combined with forward or reverse mode pipetting.

Calculation Description

The average volume was determined by gravimetric measurements. The systematic error or inaccuracy of a pipette can be expressed as a percentage of the nominal volume:

$$E\% = (\bar{V} - V_0) \times 100 / V_0$$

E systematic error

V₀ nominal volume

\bar{V} mean volume

A percentage of random error or imprecision was calculated using this equation:

$$RSD = \frac{SD}{\bar{V}} \times 100$$

with

$$SD = \sqrt{\frac{\sum_{i=1}^n (\bar{V} - V_i)^2}{n-1}}$$

$$\bar{V} = \frac{1}{n} \sum_{i=1}^n V_i$$

RSD random error or relative standard deviation

SD standard deviation

V_i individually measured volume

n number of measurements

\bar{V} mean volume.

RESULTS AND DISCUSSION

The results deal with the impacts of characteristics of volatile solutions and the pipetting mode used with an air displacement pipette versus a positive displacement pipette on accuracy and precision of volume gravimetric results.

During calibration of pipettes in forward mode, with grade 3 (ISO 3696) distilled water the systematic and random errors for pipetting 100 µL of water were similar for the standard pipette (0.23% and 0.06% respectively) compared to MICROMAN® E (-0.12% and 0.14%, Figure 4).

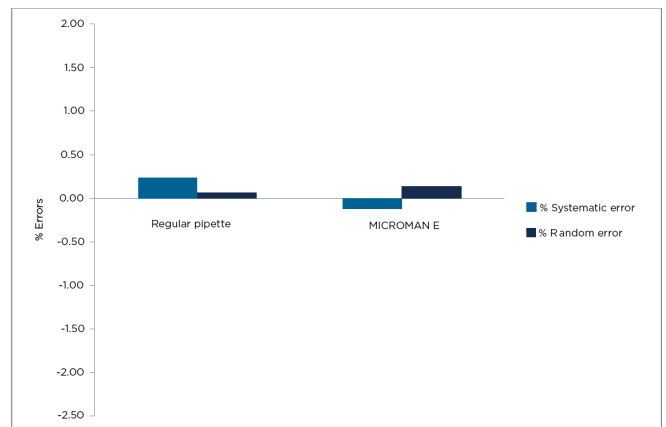


Figure 4
Systematic and random errors of water pipetting with MICROMAN® E versus standard pipette. Measurements were based on the average of ten gravimetric measurements per sample.

With a volatile liquid, acetone, the systematic and random errors of the air displacement system (-2.03% and 1.45%, respectively) are higher than the one of the positive displacement system (0.01% and 0.39%, respectively), Figure 5).

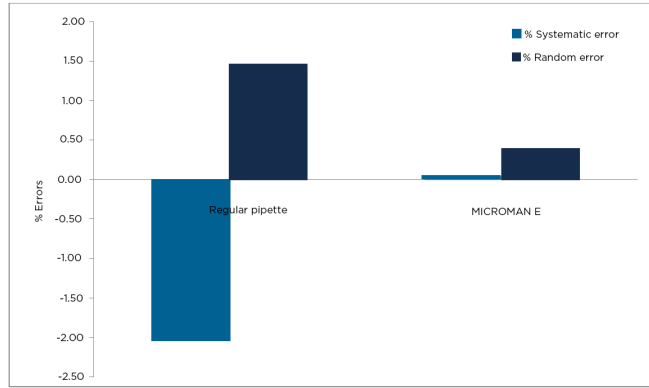


Figure 5

The MICROMAN® E outperforms standard pipettes for transfer of acetone. Measurements were based on the average of ten gravimetric measurements per sample.

The standard pipette does not meet the volumetric specifications recommended by ISO 8655 when used for volatile solvents whereas the values obtained with MICROMAN® E meet specifications.

In Figure 6, the only alternative pipetting to improve accuracy with a standard pipette is the reverse saturated pipetting mode (-1.16%) but stay out of the volumetric specification and it is far from the accuracy level of MICROMAN® E (0.01%), whose results are the best and within the volumetric specification with water.

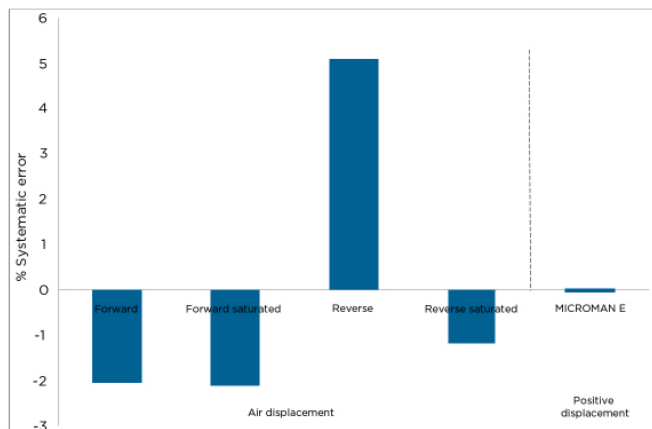


Figure 6

Systematic errors are significantly lower when using the MICROMAN® E to pipette acetone compared to four modes of air displacement pipetting. Measurements were based on the average of ten gravimetric measurements per sample.

CONCLUSIONS

Positive displacement pipettes, such as MICROMAN® E can optimize the sample preparation for HPLC. Volatile liquids are aliquoted and dispensed accurately without leaks thanks to the direct contact of the piston against the capillary; MICROMAN® E and capillary pistons are the strongest barrier against vapors and leaks due to volatile liquids. In addition, to improved accuracy, MICROMAN E also prevents corrosion of pipettes and prevents leaks of hazardous liquids.

REFERENCES

1. Gilson SAS, 2014, Nadège BELHADJ, Achieve Precise Pipetting of Problem Liquids with MICROMAN® E
2. Gilson SAS, 2015, Guide to Pipetting