METROLOGICAL EVALUATION OF A SYRINGE PUMP FOR PRECISION LIQUID DISPENSING

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Abstract: The aim of this paper is to present a metrological evaluation of an automated syringe pump. The syringe pump was developed to handle automatically micro volumes. Consists of a step motor responsible for moving the plunger of the syringe, a precision syringe, power electronics to drive the motor and a microcontroller to control the stepper motor. In order to control the system a user interface was implemented with the microcontroller.

The evaluation of this system was done in two steps: the first was to evaluation of the positioning characteristics of the actuator. The second one was the evaluation of the volumes handled. For that it was used the international standard ISO 4787 as the basis for the calibration methodology.

Key words: Automated syringe pump, metrological evaluation, uncertantity analysis.

1. INTRODUCTION

A common demand for industrial and biotechnological laboratories is liquid handling systems. These systems should handle small quantities of liquids and should be accurate and repeatable. The liquid can be reagents, diluents or any other substance required in a chemical reaction. In general the chemical reactions used in laboratory tests are very sensitive to changes in the volume of the substances involved and therefore it is necessary to quantify the deviations inherent in handling pre-defined volumes.

Usually automated liquid handlers are composed of a mechanical part responsible for the movement, and another part responsible for serving as a reservoir for liquids. The mechanical part consists of an engine and systems to control the movement and positioning. This control can be done in open loop in the case of using a stepper motor or using closed-loop for DC motor coupled to an encoder. In both options you also need some kind of power electronics to drive motors and a micro controller to implement the control loop for the movement.

As the electrical motor provides a precise rotation motion on its axis it is necessary that this movement is converted into linear motion. This conversion can be done through a number of mechanical devices.

To handle the liquids is usually used a precise syringe. This syringe has a plunger to vary its internal volume and move the liquids. It is because of the use of syringe / plunger that makes need to have a mechanism to move linearly.

In the problem studied in this paper we have two fundamental types of quantities that are directly related. These quantities are the linear displacement of the plunger and the amount of liquid that enters or leaves the syringe. This division will be made to facilitate the analysis of the uncertainties of the two processes. The uncertainties that can be evaluated in this calibration are the errors inherent in the construction of mechanical components such as gears and their clearances, pulleys, belts, motors, firmware of engine control, power electronics for activate the motor and the volumetric deviation of the syringe.

The mechanical system will be evaluated together with the controlling firmware and electronic motor drive, having as observed magnitude for evaluate these components the linear displacement. In order to do the evaluation of the repeatability and positioning accuracy for the entire operational range the system was tested. Should also be evaluated the referencing function where the system moves to a known position called HOME. The observation of the zeroing is important because the error founded in this procedure will be carried out for all other systems.

After a precise evaluation of the postioning system (step motor, power electronics, microcontroller and its firmware) by a tridimensional metrological machine, the transfer's volumes were measured. The measurements in this stage were the mass of bustling liquid. Once the liquid density applied is known, it is possible to say which was the volume transferred.

After these two steps will be possible a cross-check of the measurements making it possible to say the volume of liquid that will be moving into or out of the syringe if the plunger has a certain linear displacement.

2. SYSTEM TO BE CALIBRATED

The system to be calibrated is a syringe pump that contains: a mechanical device with step motor, linear axis, electronic power, optical sensor, and also a microcontroller. The mechanical device consists of a metal frame to give rigidity to the system, a precision syringe, a stepper motor to move the plunger of the syringe. The control system consists of a power electronic circuit to drive the motor and a microcontroller which controls the system and the communication.

The motor applied in the system is a linear stepper motor. It was chosen because its provide linear motion to output and to be compact. To drive current in the motor was used a CI Allegro a3984. This IC is a MICROSTEPPING driver to bipolar stepper motors in the modes full step, half step and microstepping. The control logic of the bridges responsible for reversing the polarity of the motor coils and the current control is all done in the IC. To move the step motor it is necessary only to send a pulse and set a pin for direction.

The microcontroller is responsible for managing the placement of the syringe plunger, sending commands to the IC Allegro and receives user commands via a serial communication.

3. LITERATURE REVIEW

3.1 Gravimetry

For the range volume in which this system will work that is up to 500 micro liters, is very common to use the gravimetric technique where it is possible to obtain the amount of volume by measuring the weight and density of the liquid. The normal procedure for doing this is the measurement of volume making two weighings of the recipient containing the liquid that we want to measure its volume. The first is to weigh the empty container and the second weighing with the amount of liquid that we want to know the volume. By equation 1 described in ISO 4787 we can calculate the liquid volume.

$$V_{20} = (I_L - I_E) \times \frac{1}{\rho_W - \rho_A} \times \left(1 - \frac{\rho_A}{\rho_B}\right) \times [1 - \gamma(t - 20)] \quad (1)$$

where,

V_{20} = volume at a temperature of 20 ° C, in ml,

I_L = result of weighing the container filled with water, in grams,

I_E = result of weighing the empty container in grams,

ρ_W = density of water at calibration temperature t in g / ml,

ρ_A = air density in g / ml (for temperatures very close to 20 ° C and standard atmospheric pressure can be used the average value of 0.0012 g / ml),

ρ_B = density of the masses that calibrating the balance (for mass in conformity to OIML 33, normally use the value of 8.0 g / ml),

γ = volumetric coefficient of thermal expansion of the material which is made the laboratory glassware in $°C^{-1}$,

t = water temperature used for calibration in ºC.

3.2 Uncertainty

The VIM (International Vocabulary of terms Fundamental and General Metrology) defines uncertainty as a parameter associated with the result of a measurement that characterizes the dispersion of values that can be primarily attributed to the measurand. This characterization of measurement uncertainty is extremely important to be able to assess how good are the measures and therefore must arise which their possible sources. To estimate the overall uncertainty, we need the contribution of each component of uncertainty, they can be estimated from the following terms:

- Standard uncertainty (u_i)
- Combined standard uncertainty (u_c)
- Expanded uncertainty (U)

3.2.1 Type A standard uncertainty

This type of uncertainty is evaluated according a valid statistical methods for data processing such as calculating the standard deviation of the observations. For that, the first step is the mean values:

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n} \quad (2)$$

The Type A standard uncertainty is related to the experimental standard deviation, which is obtained from the following expression:

$$s = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n-1}} \quad (3)$$

3.2.2 Type B standard uncertainty

The assessment of this type of uncertainty is effected by means other than statistical analysis, due to the fact that they are obtained through various observations. It is usually based on scientific judgments using all available information obtained from measurement data and previous experiences or general knowledge of the behavior of instruments like:

- Data from previous measurements;
- Specification of the manufacturer;
- Data provided from calibration certificate
- Uncertainties provided by references in manuals or other documents.
- Operating Procedures
- The effects of environmental conditions in the above information

Thus, uncertainty type B possesses some distributions more frequently associated with its components of uncertainty. One of these is the rectangular distribution, which is characterized by a probability density equal for all values within the limits "u - a" and "u + a", and zero outside them. Its deviation is given by:

$$u(x) = \frac{a}{\sqrt{3}} \quad (4)$$

The rectangular distribution is applied for cases follows:

• When there is no information about the nature of the component;
• To read digital equipment;
• For changes in volume due to temperature difference;
• For the stated accuracy of the equipment;
• Glassware is not Class A.

3.2.3 Combined standard uncertainty

The combined standard uncertainty estimates the combined uncertainty from various sources of error. If the various sources of error act independently, this number can not be obtained by simple addition of each uncertainty. Statistical aspects should be taken into account so in this case, the combined uncertainty (u_c) of the influence of various sources of uncertainty can be estimated from the standard uncertainties of each source of error by:

$$u_c = \sqrt{u_1^2 + u_2^2 + \cdots + u_n^2} \quad (5)$$

The above equation is valid only if all variables evaluated in this study are independent, that is the case in this work.

3.2.4 Expanded uncertainty

The value obtained by equation 5 represents a range of values around the mean value, within which, with a probability defined statistically, one expects to find the measurement error. Typically corresponds to a probability of 68% and has a normal distribution.

In engineering it is common to work with confidence levels of 95%. To achieve approximately 95% should be multiplied by a numerical coefficient called the coverage factor, calculating the called, expanded uncertainty (U).

$$U = k \times u_c \quad (6)$$

The number of effective degrees of freedom (υ_{ef}) through the Welch-Satterthwaite equation:

$$\upsilon_{ef} = \frac{u_c^4}{\sum_{i=1}^{n} \frac{u_i^4}{\upsilon_i}} \quad (7)$$

where:
u_c is the combined uncertainty;
u_i is the standard uncertainty associated with the i-ésima source of uncertainty;
υ_i is the number of degrees of freedom associated with i-ésima source of uncertainty;
n is the total number of sources of uncertainties analyzed.

The value of "k" for a confidence level of 95% can then be obtained. So finally the expanded uncertainty can be calculated by:

$$U_{95} = K_{95} \times u_c \quad (8)$$

4. METHODOLOGY

4.1 Evaluation of positioning

The measurements were made in a three dimensional measuring machine Mitutoyo M BR-443 located in the metrology laboratory at PUC Minas.

The measurements were made with the pump attached firmly to the measuring table by using screw and a function of the machine to measure the distance between a point and a plan. The plan chosen was the motor plate (detail of figure 01) and the point is the end of the spindle (detail B of figure 01).

To prevent that irregularities of flatness of plate interfere in the measures were scored three points needed to identify the plan and one point in the shaft end and then all measurements were made using these as reference points.

The measurements were made in two parts, the first having the direction of movement starting in the home point (maximum displacement) to the point of measurement and the second block with the reverse, ie, starting at zero and going to the point of measurement. In both cases it was followed the following sequence of operations:

- Sent the order to movement to the starting point (home or zero point),
- Sent the order to movement to the measuring point ,
- Making the measurement,
- Repeat the operation.

This sequence was repeated ten times for each measurement point. The measurement points were 1100, 1000, 800, 600, 400, 200, 100 and 0 steps of the motor.

To evaluate the position after the home function were chosen 5 points (0, 250, 500, 750 and 1000 steps of the motor) as starting point. Initially the order was sent for movement to the starting point and then the order was sent to home function. Again ten repetitions were made for each starting point.

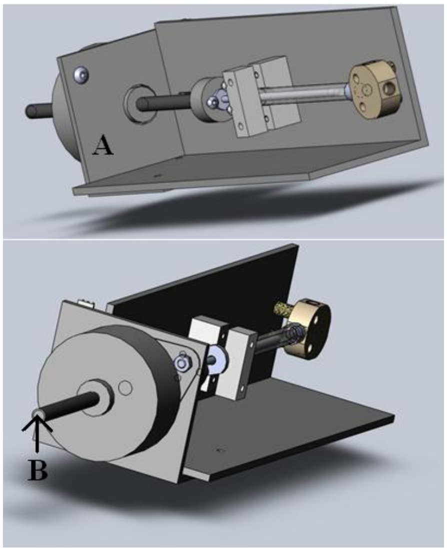

Figure 01: Location of the points of displacement measures at the pump.

4.2 Volumetric assessment

The measurements were made using a balance Gehaka BG 400. It was made two sets of measures for evaluation. The first set was done with the volumetric flask containing a random volume of water and the second was made with the recipient.

The procedure for measurements with the volumetric flask was their initial weight after the pump draws in the amount of liquid to the test the volumetric flask was weighed again. The difference between two masses of the amount of liquid sucked by the pump and using the equation 1 for calculated the volume.

For the measurements with the recipient was first made a set of measures to determine their dry weight. To calculate the volume handled by the pump, is placed in the becker initially dry, the volume determined for the test and after is made to your weighing. Again the equation 1 is used to calculate the volume.

Before to begin the testing, was made a pre loading of the system to remove air of tubing. Testing began with the pump at zero position (zero volume in the syringe) and then water was sucked from the volumetric baloon and then poured into the becker. The points evaluated were stipulated by amount of steps data by the step motor and were 100, 200, 400, 600, 800, 1000 and 1100 steps, remembering that the maximum course is 1200 steps.

The values used in the equation were:

ρ_W = 0,996 g/ml

ρ_A = 0,0012 g/ml

ρ_B = 8,0 g/ml

γ = 0×10^{-6} ºC^{-1}

$t_{água}$ = 22ºC

$t_{ambiente}$ = 20ºC

5. RESULTS

5.1.Balance of uncertainties

5.1.1 Positioning

To take stock the uncertainty should first get up the quantities that influence the measures.

In this system the following quantities were observed that could influence measures of positioning:

- Algorithm for motor control;
- Electronic Power;
- Referencing (home);
- Clearances of mechanical assembly ;
- Assembly rigidity (positioning / attachment on the machine table of measurement);
- Three-dimensional measuring machine;
- Temperature.

The exact identification of the influence of each of these quantities is difficult and how the influence of all of them combined in this uncertainty calculated by the test data of positioning is not made greater efforts to determine the individual influence quantities.

To calculate the expanded uncertainty was first calculating the type A uncertainty. This uncertainty is given by the standard deviation of measures made and can be observed in Tables 6, 7 and 8. Here only one complete table presented with the full balance of uncertainty, calculations to arrive at the values will be displayed (only for one measuring point).

Observing the values we have the largest standard deviation for the home function and to positioning is 0.0046 and 0.0048 mm respectively. The experimental standard deviation of these values taking into account that were made 10 measurements can be calculated by equation 3 will respectively:

$$R_e = \frac{0,0046}{\sqrt{10}} = 0,0015\ mm \quad (9)$$

$$R_e = \frac{0,0048}{\sqrt{10}} = 0,0015\ mm \quad (10)$$

The uncertainty of type B presented in these measures is the uncertainty of the measurements machine due to its resolution and this information is provided by the manufacturer. We consider that this uncertainty has a rectangular distribution and by equation 4 have the value:

$$u = \frac{0,0005}{\sqrt{3}} = 0,0003mm \quad (11)$$

The combined uncertainty of positioning measures can be calculated by equation 5:

$$u_c = \sqrt{0,0015^2 + 0,0015^2 + 0,0003^2} = 0,0021mm \quad (12)$$

The number of degrees of freedom is calculated by equation 7:

$$v_{ef} = \frac{0,0021^4}{\frac{0,0015^4}{9} + \frac{0,0015^4}{9} + 0} = 18,6514 \quad (13)$$

The expanded uncertainty of measurements of positioning can be calculated by equation 8 and the factor of expansion for the number of degrees of freedom calculated earlier, is 2.13.

$$U = 2,13 \times 0,0021 = 0,0045\ mm. \quad (14)$$

Below is a table with the values of the expanded uncertainties for all measured points.

Table 01: Expanded uncertainty of all points measured of the positioning of the initial tests of the zero position.

Position (steps)	Expanded uncertainty (mm)
100	0,0041
200	0,0043
400	0,0038
600	0,0045
800	0,0042
1000	0,0044
1100	0,0043

Table 02: Expanded uncertainty of all points measured of the positioning of the initial tests of the home position.

Position (steps)	Expanded uncertainty (mm)
0	0,0037
100	0,0042
200	0,0038
400	0,0037
600	0,0039
800	0,0038
1000	0,0040
1100	0,0038

5.1.2 Uncertainties in mass measurements

The magnitudes of influence observed of these measures were:

• Water purity;
• Uncertainty of the balance;
• Hydraulic connections.

Similarly the quantities that influence the positioning the influence of these quantities will not be evaluated separately.

The type A uncertainty is given by the standard deviation of the measurements. The values of standard deviations can be seen in Tables 9 and 10. The highest values are the measures of 0.001 g of the weighing the dry becker, and 0.0037 of the variation in weight of the volumetric balloon. O experimental standard deviation of these values considering 10 measurements that were made will be respectively:

$$R_e = \frac{0{,}001}{\sqrt{10}} = 0{,}0003\ g \qquad (15)$$

$$R_e = \frac{0{,}0037}{\sqrt{9}} = 0{,}0012\ g \qquad (16)$$

The type B uncertainty is again referring to a given uncertainty of measurement equipment, in this case the balance.

We will also consider the distribution of this uncertainty rectangular and by equation 4 have the value:

$$u = \frac{0{,}0020}{\sqrt{3}} = 0{,}0012\ g \qquad (17)$$

The combined uncertainty is:

$$u_c = \sqrt{0{,}0003^2 + 0{,}0012^2 + 0{,}0012^2} = 0{,}0017\ g \qquad (18)$$

The number of degrees of freedom is calculated by equation 7.

$$v_{ef} = \frac{0{,}0017^4}{\frac{0{,}0003^4}{9} + \frac{0{,}0012^4}{9} + 0} = 37{,}5115 \qquad (19)$$

The expanded uncertainty of measurements of positioning can be calculated by equation 9 and the expansion factor for the number of degrees of freedom is 2.08.

$$U = 2{,}08 \times 0{,}0016 = 0{,}0035 g \qquad (20)$$

Below are the values of expanded uncertainty of all measured points.

Table 03: Expanded uncertainty of all points of weighings made with the volumetric flask.

Position (steps)	Expanded uncertainty (mm)
100	0,0027
200	0,0035
400	0,0035
600	0,0032
800	0,0031
1000	0,0031
1100	0,0032

Table 04: Expanded uncertainty of all points of weighings made with the becker.

Position (steps)	Expanded uncertainty (mm)
100	0,0025
200	0,0029
400	0,0028
600	0,0028
800	0,0028
1000	0,0031
1100	0,0028

5.1.3 Uncertainties in volume measurements

For the volume calculated by equation 4 we have the influence of the set of measures (standard deviation), uncertainties of the measurements of weight, the volumetric coefficient of thermal expansion of the material it is made from the laboratory glassware ($u(\gamma)$), density of water ($u(\rho_W)$), density of air ($u(\rho_A)$) and specific mass of the weights that calibrating the balance ($u(\rho_B)$). The uncertainties of the measurements of weight are of type B and must be used the equation 4.

$$u = \frac{0{,}0034}{\sqrt{3}} = 0{,}0020 mm \qquad (21)$$

The repeatability in this case need not be calculated because will be equal to uncertainty of weighing.

To calculate the combined uncertainty of volume measurements is used the equation 6.

$$u_c = \sqrt{0{,}0020^2 + u(\gamma)^2 + u(\rho_W)^2 + u(\rho_A)^2 + u(\rho_B)^2} \qquad (22)$$

Since the values of uncertainty measures for volumetric coefficient of thermal expansion of the material is made the laboratory glassware ($u(\gamma)$), density of water ($u(\rho_W)$), density of air ($u(\rho_A)$) and the density of weights calibrated balance ($u(\rho_B)$) are not known, will be made a sensitivity analysis to identify the influence of these values on the results of volume calculations.

The analysis will be done using equation 1. It was chosen one measuring point to provide the values to be used in this equation. To see the sensitivity of the equation to a given parameter will be kept all other values fixed and varied only one parameter. The value calculated with the parameter change was compared with the reference being made to subtraction, recalculated value minus reference value.

To view the volume calculated was used values ranging from -10% to +10% of the value originally used in the equation. After recalculating the equation 1 with the variations of parameters was calculated using the change in recalculated value with the base value and converted into a percentage of the base. The results can be seen below.

Table 05: Sensitivity of equation 1 to its parameters.

Parameter	Variation %
Volumetric coefficient	-0,0002
Density of water	-9,1009
Density of air	0,0106
Specific mass of the weights that calibrating the balance	0,0014

All parameters analyzed has low influence on the outcome of the equation and can be neglected in the uncertainty balance, less the water density. For this parameter a variation of 10% of its value almost causes equal a variation in the value of equation 1. This shows that the uncertainty in this parameter could not be despised but as it was not possible to calculate such uncertainty will also be omitted from the uncertainty this parameter in the calculation of combined uncertainty.

Since we only have one parameter of uncertainty in this case do not need the combined uncertainty. The expanded uncertainty is of the form.

$$U = 2{,}262 \times 0{,}0020 = 0{,}0045\ mm. \quad (23)$$

The expansion factor to be used is taken from the Student table with the number of degrees of freedom calculated as the number of measurements taken minus one.

5.2 Data of the measures of Position

Table 6: Results of the measurements of the home function.

Position (steps)	Average (mm)	Standard Deviation (mm)
0	103,5347	0,0034
250	103,5334	0,0028
500	103,5312	0,0030
750	103,5299	0,0042
1000	103,528	0,0046
Average	**103,5314**	**0,0027**

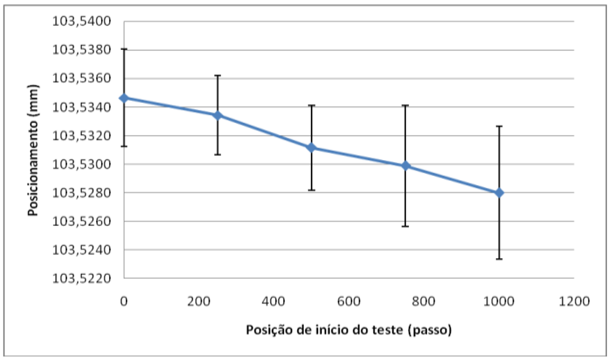

Figure 02: Graph of function after home function.

The highest and lowest standard deviation were 0.0046 mm and 0.0028 mm respectively.

The measurement results showed that the system has a linear behavior in all travel range as expected. The linear regression for the points formed by the average of each set of measurements has a value of R2 = 0.999995.

Table 7: Results of measurements of displacement starting from the zero position (measured in millimeters)..

Position (steps)	Average (mm)	Standard Deviation (mm)
1100	98,5638	0,0039
1000	93,5503	0,0042
800	83,5179	0,0031
600	73,3935	0,0048
400	63,4840	0,0040
200	53,4506	0,0044
100	48,4478	0,0043

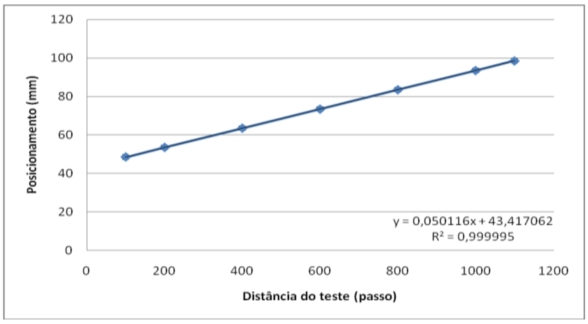

Figure 03: Graph of position starting from position 0.

Table 8: Results of the measurements of displacement starting from the home position (measured in millimeters).

Position (steps)	Average (mm)	Standard Deviation (mm)
1100	98,59386	0,0030
1000	93,5736	0,0036
800	83,43586	0,0029
600	73,43353	0,0033
400	63,49197	0,0024
200	53,37482	0,0030
100	48,37357	0,0024
0	43,35487	0,0025

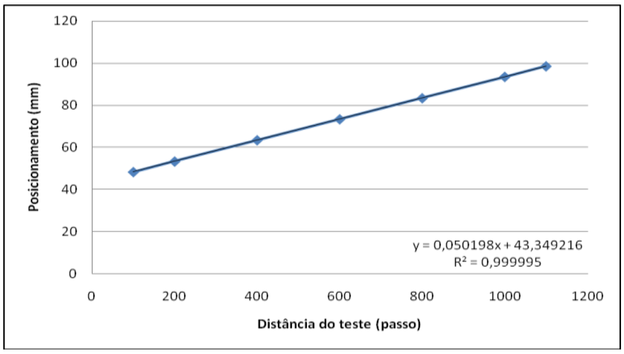

Figure 04: Graph of position starting from the home position (1200 steps).

The system presented the following characteristics: Maximum displacement: 60.1765 ± 0.0045 mm.
Relationship mm/step: 0.0501 ie a step is equal to 0.0501 ± 0.0045 mm.

5.2 Data of the measures of Volume

Table 9: Volume calculated by the weight of the becker.

Displacement (steps)	Average (ml)	Standard Deviation (ml)
100	0,0450	0,0005
200	0,0871	0,0022
400	0,1702	0,0018
600	0,2550	0,0019
800	0,3378	0,0018
1000	0,4239	0,0028
1100	0,4640	0,0018

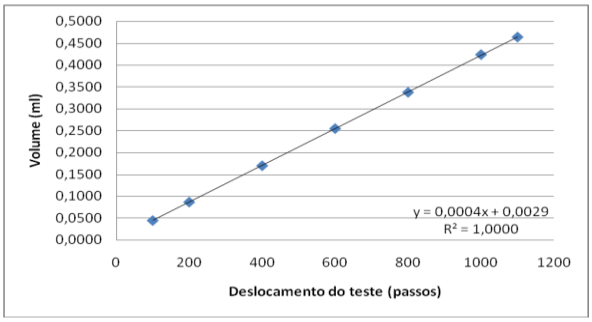

Figure 05: Graph of the volume calculated by the weight of the becker.

Table 10: Volume calculated by the weight of the volumetric flask.

Displacement (steps)	Average (ml)	Standard Deviation (ml)
100	0,0421	0,0017
200	0,0843	0,0037
400	0,1689	0,0038
600	0,2522	0,0031
800	0,3364	0,0027
1000	0,4214	0,0027
1100	0,4625	0,0031

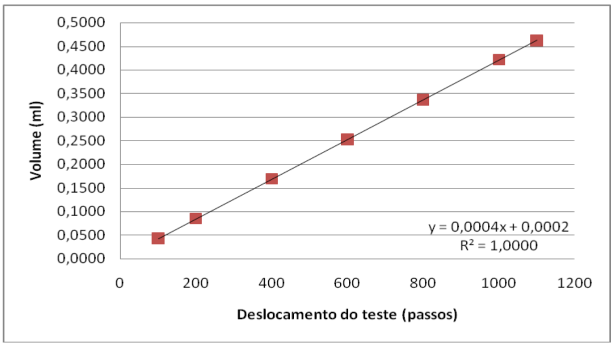

Figure 06: Graph of the volume calculated by the weight of the volumetric flask.

6. CONCLUSION

With this work was possible to develop a methodology to evaluate a syringe pump.

With this methodology it was possible to evaluate a real system and its data can be seen below.

Positioning

The system proved to be very linear as can be seen in Figure 04 and 05.

The ratio mm/steps of system is 0.0501 mm.

The uncertainty of the measurements is 0.0045 mm.

The minimum displacement is 0.0501 ± 0.0045 mm and the maximum is 60.1765 ± 0.0045 mm.

Volume

Again the system proved to be extremely linear throughout their range.

The uncertainty measures for volumes is 4.5 µl.

The maximum volume that the system can handle is 480 ± 4.5 µl.

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