EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 3
118-134
Year: 2019

VALIDATION OF AN ANALYTICAL METHOD FOR A COMPARATIVE PROFILES OF IN VITRO THERAPEUTIC EQUIVALENCE OF IMMEDIATE RELEASE TABLETS OF CHLORPHENIRAMINE MALEATE 4 MG

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Article Received on 13/01/2019 **Article Revised on 03/02/2019** **Article Accepted on 23/02/2019**

ABSTRACT
The present study was carried out with the purpose of validating an analytical method for the quantification of the immediate release of tablets of chlorpheniramine maleate 4 mg and the determination or the dissolved percentage of them in three buffers, using a UV-Vis spectrophotometer for the analysis of these data. The analytical results show that the method complies with the parameters of linearity, precision and accuracy. The calibration curve of each medium was carried out in a concentration range of 2-14 µg / mL, linearity was demonstrated in each medium, considering the following criteria: homoscedasticity, variance analysis, correlation coefficient and random distribution data; precision was evaluated in terms of repeatability and intermediate precision; and finally, the accuracy was evaluated by the determination of recovery percentage. Therefore, the methodology complies with the specifications established by USP 40.

KEYWORDS: Chlorpheniramine maleate, pharmaceutical equivalence, validation, analytical methods.

1. INTRODUCTION
The Chlorphenamine (IUPAC; 3- (4-chlorophenyl) -3- (pyridin-2-yl) propyl] dimethylamine), as a free base has a molecular weight of 274.78 grams / mole, has a protein binding of 72% and a half-life of 21-27 hours. This compound belongs to the class of organic compounds known as pheniramines. These are compounds containing a pheniramine moiety, which is structurally characterized by the presence of a 2-benzylpyridine linked to an dimethyl(propyl)amine to form a dimethyl [3-phenyl-3-(pyridin-2-yl)propyl]amine skeleton. Its mechanism of action is related to antagonism of the H1 receptors. It is used in allergic reactions such as antihistamine, and anti-flu formulations. It is used in both animals and humans. Therapeutically, several types of salts are used, such as hydrochloride, CAS number 56343-98-7, or maleate, CAS number 113-92-8.[1]

The Chlorphenamine is administered alone and in combination in various pharmaceutical dosage forms, such as for example syrups, oral solutions, intramuscular injections, creams, immediate-release tablets, modified-release tablets. The 4 mg immediate release tablets are a class 1 medicine (high permeability / high solubility) according to the international biopharmaceutical classification. Therefore the dissolution profiles for determining the therapeutic interchangeability between multisource tablets and the reference product.[1]

To make pharmaceutical analysis, it is necessary to validate the analytical methodology to reliably reproduce the results. Among the parameters that allow to validate an analytical procedure are precision, accuracy, linearity interval, linearity, repeatability, reproducibility, among others which depend on the pharmaceutical form, the type of test, or if the medicine has an approved monograph at the pharmacopoeical level.[1]

The validation of an analytical procedure is defined as: "The process established by laboratory research, that the performance characteristics of the procedure comply with the requirements provided for analytical applications".[1] So, the validation of an analytical method, in addition to being a normative requirement, provides and ensures that the data is reliable.

There are typical analytical characteristics that are used for validation, such as: accuracy, precision, specificity, limit of detection, limit of quantification, linearity, interval and robustness.[2]

Within the performance elements required for the validation, established by USP 40, the quantification of chlorpheniramine in solution, only the parameter of precision must be checked, and the other parameters are made depending on the specific nature of the test. According to what is established in the American Center Technical Regulations, laboratories that perform official analytical methods, should only check the linearity and accuracy of the system; however, it is also recommended to include the accuracy parameter.[3]

Below are shown the parameters made in the investigation that must met the acceptance criteria:

Accuracy

Several international guides adopt different ways to describe the definition of accuracy of an analytical procedure. For example, in USP 40, it is defined as the proximity between the test results obtained with the analytical procedure, and the true value. On the other hand, the ICH Q2 guide only defines it as "unbiasedness". ISO, is a combination of unbiasedness and precision.[2]

For the determination or evaluation of this characteristic in a drug, it can be done through a comparison between the application of the analytical procedure regarding to an analyte of known purity, such as that of a reference standard; it can also be established by a comparison of the results obtained from the procedure, with a second procedure, in which it is known that its accuracy has been checked or defined; therefore, it must be a well-characterized procedure.[2]

The ICH guide mentions that for the accuracy assessment, a minimum of three concentrations and three repeated determinations of each concentration must be used.

The acceptance criteria for this parameter are:

1. The recovery percentages obtained must be within 100% ± 4S, where S is the highest standard deviation obtained in determining the accuracy of the method or system.
2. The slope must be greater than or equal to 0.95 and the intercept must be equal to the initial concentration.
3. The relative standard deviation of the recovery percentage must be less than or equal to 3%.

Precision

It is defined as the degree of concordance of the results obtained with the individual tests, of an analytical procedure, when it is applied repeatedly to multiple samples of a sample that is homogeneous. It can be expressed by the coefficient of variation or as the deviation that are given from a series of measurements.[2]

It can be established in three levels:

- Reproducibility: It refers to performing the analytical procedure in different laboratories. It is considered when you want to standardize an analytical procedure; for example, in the inclusion of procedures in pharmacopoeias.
- Intermediate precision: Variation of the procedure within a laboratory is evaluated; either on different days, different analysts, or with different equipment from the same laboratory. It is not necessary to study these factors individually.
- Repeatability: Consists in performing the analytical procedure in the same laboratory, for a short period of time, with the same team and analyst.[2]

The Health's Ministry indicates that according to the ICH guidelines, it is recommended to make nine determinations; working with three independent samples, at three different levels of concentration that are within the specified range for the procedure.[4]

As for the acceptance criteria for repeatability and intermediate precision validation system, these indicate that relative standard deviation (RSD%) must be less than or equal to 2% relative error (ER%) should not be greater than 2% while for the validation of the method, it provides that the relative standard deviation (RSD%) must be less than or equal to 3%, in both parameters.[4][5][6]

Linearity and interval

The linearity of an analytical procedure refers to its ability to obtain test results that are directly proportional to the concentration of analyte present in the samples that are in an interval. In some cases, a mathematical transformation is required such as: square root, logarithm or reciprocal, to achieve linearity.[2] [4]

The linearity interval of an analytical procedure is the range between the lowest and highest concentration of analyte in the samples, where it has been shown that the analyte can be determined accurately and linearly.[2] [4] [5] [6]

For the determination, a minimum of 5 concentrations is recommended to establish linearity. A linear relationship must be evaluated through the range of the analytic procedure. The following should be evaluated:

- Homocedasticity
- The analysis of variance of the linear regression must show:
  - The intercept is statistically different from zero, by means of a test t with a probability level of 5%.
  - Deviation not significant regarding to the regression.

- The correlation coefficient (r) of the linear regression must be between 0.98 and 1.00; and the coefficient of determination (r^2) of each of the curves must be greater than or equal to 0.9950.

Random distribution of residues, systematic trends that are indicative of non-linearity should not be observed.[4] [5] [6]

2. MATERIALS AND METHODS

Equipment instruments

- UV-Vis Thermo Spectrophotometer Scientific Genesys.
- Ohaus Pioneer analytical balance.
- Branson 3510 ultrasonic bath.
- pHmeter Thermo Scientific Orion 5 Star.
- Fridge.

Laboratory materials

- Quartz cuvette
- Droppers
- Stirring tablet.
- Mortar and pistil.
- Wash bottle.
- Manual agitator.
- Gauged balls of: 25,100 and 500 mL.
- Beakers of different capacities.
- Test tubes of different capacities.
- Volumetric pipettes of: 1, 2, 3, 4 and 5 mL.
- Graduated pipette of 10 mL.
- Spatula.
- Foil.
- Syringes
- Naylon filters of 0.45 *u* m.
- Separator funnel.
- Paper towels.
- Filter paper.

Reactives

- Hydrochloric acid, lot K-48348717640, EMD Millipore Corporation.
- Sodium hydroxide, lot 2505-06, Químicos Arvi SA
- Monobasic potassium phosphate, lot X28C01. Laboratorios Quimar
- Sodium acetate trihydrate, lot Q-ASTO91116, Laboratorios Quimar.
- Glacial acetic acid, lot Q-CDH640816, Laboratorios Quimar.
- Distilled water.

Drugs

- Chlorpheniramine maleate, reference standard 98.5% purity. Merck.
- Chloro- trimeton ® tablets, reference product, lot 27445, Schering Plow.
- Chlorpheniramine tablets generic, lot 188049, Raven

Preparation of mediums of dissolution

0.2M hydrochloric acid buffer solution at pH 1.20 ± 0.05

Procedure

1. Dilute 400 mL of the 2M hydrochloric acid solution with a sufficient amount of distilled water, to obtain 4 L of 0.2 M hydrochloric acid.
2. Stir and mix the previous solution and adjust the pH to 1.20 ± 0.05, if necessary, with 2M sodium hydroxide or 2M hydrochloric acid.

Phosphate buffer solution at pH 6.80 ± 0.05

Procedure

1. Weigh 27.22 g of potassium phosphate monobasic and dissolve in 1 L of distilled water.
2. Add to the mixture, 448 mL of 0.2 M sodium hydroxide.
3. Add enough distilled water to obtain a volume of 4 L.
4. Mix and adjust the pH to 6.80 ± 0.05, either with 2M sodium hydroxide or 2M hydrochloric acid.

Acetates buffer at pH 4.50 ± 0.05

Procedure

1. Weigh 11.96 g of sodium acetate trihydrate and place it in a 4 L beaker.
2. Add 56 mL of acetic acid (prepare in beaker, 6.76 mL of glacial acetic acid and add distilled water to obtain 56 mL).
3. Add the previous mix to the beaker.
4. Add enough beaker of distilled water to the beaker to prepare 4 L; then mix.
5. Adjust the pH if necessary to 4.50 ± 0.05, with 2N acetic acid or 2M sodium hydroxide solution.

Preparation of the calibration curve pattern Chlorpheniramine

1. Weigh exactly 25 mg of standard chlorpheniramine and place it in a 500 mL graduated balloon.
2. Add 100 mL of diluent (either the buffer solution of hydrochloric acid at pH 1.20 ± 0.05, the phosphate buffer solution at pH 6.80 ± 0.05 or the acetate buffer at pH 4.50 ± 0.05).
3. Place the balloon in an ultrasonic bath for 5 minutes and then allow to cool to room temperature.
4. Add diluent up to the capacity mark and mix. A stock solution of 50 µg / mL of standard chlorpheniramine will be obtained.
5. Cover the graduated ball with aluminum foil and store it in the refrigerator.
6. The following aliquots will be taken, from the stock solution of 50 µg / mL: 1, 2, 3, 4, 5,6 and 7 mL. Place each aliquot in a 25 mL graduated balloon.

7. Bring the capacity mark with the diluent and mix. Concentrations of: 2, 4, 6, 8, 10, 12 and 14 µg / mL will be obtained.

3. RESULTS AND DISCUSSION

Validation of the analytical method

Validation of the system in Hydrochloric acid

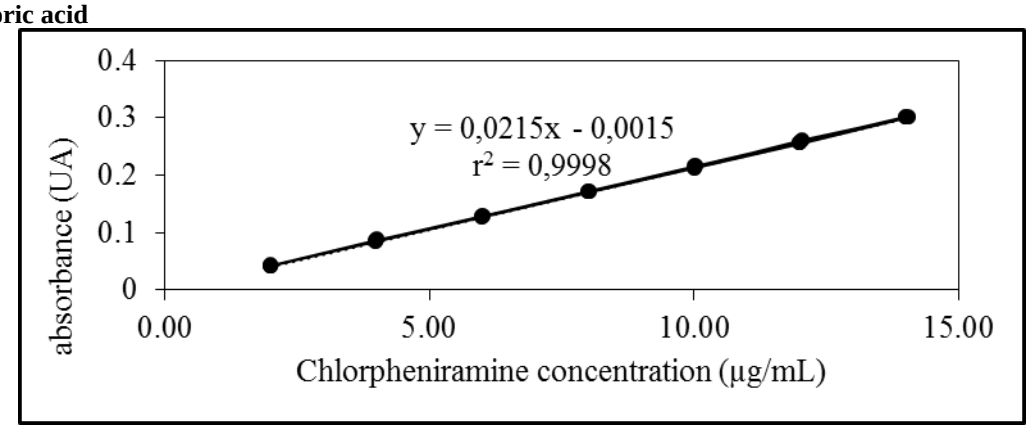

Figure 1: Calibration curve for the evaluation of the linearity of the Chlorpheniramine in HCl system.

In the previous figure, a linear behavior was observed within the concentration range used from 2 to 14 µg / mL, from the preparation of the 3 calibration curves of each of the mother solutions, and with this the linearity of the system was statistically analyzed.

Through the Hartley test an F_{max} (28.00) lower than the $_{critical}$ F (333.00) was obtained, this shows that there is no significant difference between the variances, which translates into homoscedasticity; this allows to apply the analysis of variance of the linear regression, through Microsoft Office Excel 2016, and which is shown in table 1.

Table 1: Results obtained with the variance's analysis of the linear regression.

Parameter	Value obtained
Slope (m)	0,0215
Intercept (b)	0.0015
Correlation coefficient (r)	0.9997
Coefficient of determination (r^2)	0.9999
Typical error of the slope	0.000068713
Typical intercept error	0,000615866

Source: experimental data.

To the variance analysis of the linear regression (table 1), a student T test was performed; with a confidence level of 95%, it was established that statistically, the intercept is different to zero and that the slope is statistically equal to one. At the same time, Fisher's F test, with a confidence level of 95%, indicates that there is no deviation that is statistically significant with respect to the linear regression. It is also true that the correlation coefficient is between 0.98 and 1.00; and that the coefficient of determination is greater than 0.9950.

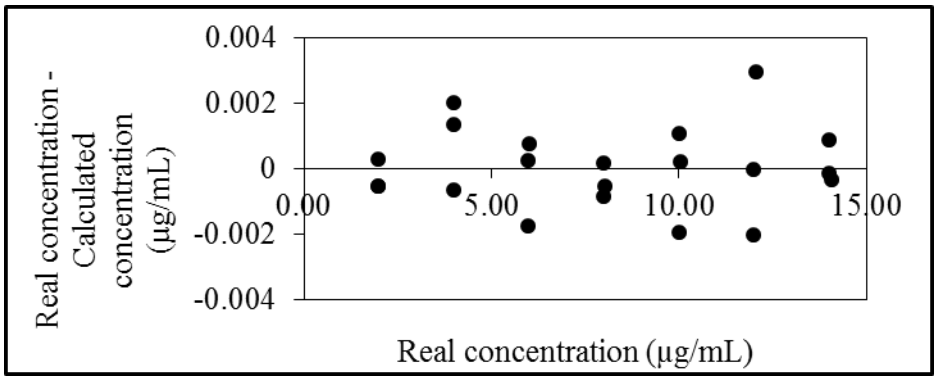

Figure 2: Analysis of system residuals in HCl.

Source: self-obtained.

Figure 2 shows a random distribution of the residues. No systematic trends are observed, which are indicative of non-linearity. With the confirmation of all the acceptance criteria set forth above, the linearity of the system in HCl is confirmed.

Accuracy of the system

The precision of the system was established in two levels: repeatability and intermediate precision.

Table 2: Repeatability of the system in HCl.

Concentration (µg / mL)	Sample No.	Measurement ABS	% Relative error	Average ABS	Standard Deviation	% Relative standard deviation
4	1	0.085	-0,3195	0,084	0.03	0.67
4	2	0,084	0.8426	0,084	0.03	0.67
4	3	0,084	0.8426	0,084	0.03	0.67
8	1	0.171	-0.1369	0.171	0.03	0.34
8	2	0.171	-0.1369	0.171	0.03	0.34
8	3	0,170	0.4441	0.171	0.03	0.34
12	1	0.257	-0.0761	0.257	0.03	0.22
12	2	0.257	-0.0761	0.257	0.03	0.22
12	3	0,258	-0.4634	0.257	0.03	0.22

Source: experimental data

The following criteria are met: that the percentage of relative standard deviation (DSR%) is less than or equal to 2%, and that the relative error (ER%) is not greater than 2%.

Table 3: Intermediate accuracy of the system in HCl.

Concentration (µg / mL)	Day	Measurement ABS	% Relative error	Average ABS	Standard Deviation	% Relative standard deviation
8	1	0,172	0,5453	0.171	0.05	0.62
8	1	0,173	-0.0379	0.171	0.05	0.62
8	1	0.171	1,1286	0.171	0.05	0.62
8	1	0,170	1,712	0.171	0.05	0.62
8	1	0.171	1,1286	0.171	0.05	0.62
8	1	0,172	0,5453	0.171	0.05	0.62
8	2	0.171	1,1286	0,170	0.05	0.62
8	2	0,172	0,5453	0,170	0.05	0.62
8	2	0,170	1,712	0,170	0.05	0.62
8	2	0.171	1,1286	0,170	0.05	0.62
8	2	0,172	0,5453	0,170	0.05	0.62
8	2	0,173	-0.0379	0,170	0.05	0.62

Source: experimental data.

The evaluation of the intermediate precision was carried out during 2 consecutive days and with 2 different analysts.

The acceptance criterion is met, which establishes that the relative standard deviation (DSR%) is less than or equal to 2%, and that the relative error (ER%) is not greater tha.

Phosphate

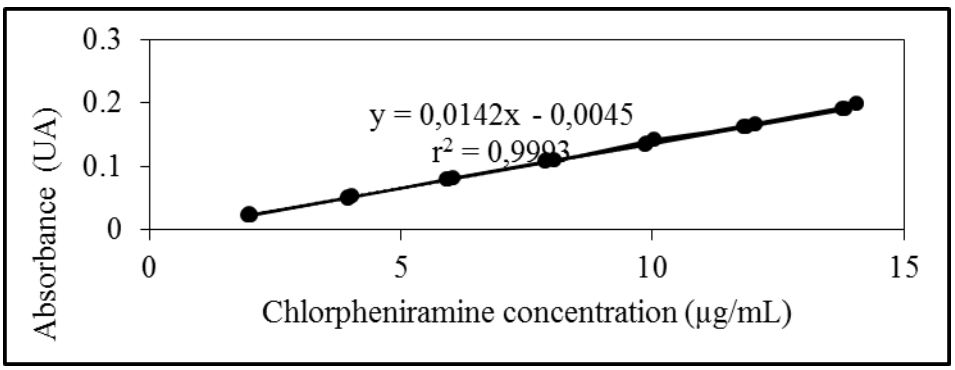

Figure 3: Calibration curve for the evaluation of the linearity of the Chlorpheniramine in Phosphate system.

In the previous figure, a linear behavior was observed within the concentration range from 2 to 14 µg / mL, from the preparation of the 3 calibration curves of each of the mother solutions, and with this the linearity of the system was statistically analyzed.

Through the Hartley test an F_{max} (22.33) lower than the $_{critical}$ F (333.00) was obtained, this shows that there is no significant difference between the variances, which translates into homoscedasticity; this allows to apply the analysis of variance of the linear regression, through Microsoft Office Excel 2016, and which is shown in table 4.

Table 4: Results obtained with the analysis of variance of the linear regression.

Parameter	Value obtained
Slope (m)	0,0142
Intercept (b)	0.0045
Correlation coefficient (r)	0.9992
Coefficient of determination (r^2)	0.9999
Typical error of the slope	0.00008698
Typical intercept error	0,0007726

Source: Experimental data.

To the variance analysis of the linear regression (table 4), a student T test was performed; with a confidence level of 95%, it was established that statistically, the intercept is different to zero and that the slope is statistically equal to one. At the same time, Fisher's F test, with a confidence level of 95%, indicates that there is no deviation that is statistically significant with respect to the linear regression.

It is also true that the correlation coefficient is between 0.98 and 1.00; and that the coefficient of determination is greater than 0.9950.

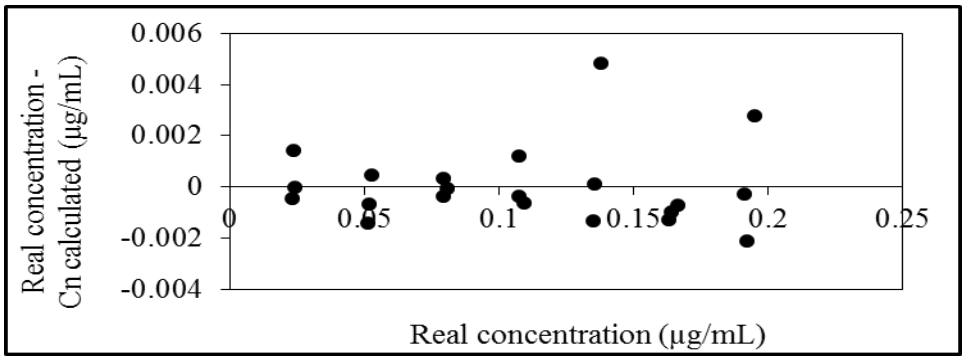

Figure 4: Analysis of system residuals in phosphate.

In Figure 4 a random distribution of residues is observed. No systematic trends are observed, which are indicative of non-linearity.

With the confirmation of all the acceptance criteria discussed above, the linearity of the phosphate system is confirmed.

Precision of the system in phosphate

The accuracy of the system was established in two levels: repeatability and intermediate precision.

Table 5: Repeatability of the system in phosphate.

Concentration (µg / mL)	Sample No.	Measurement ABS	% Relative error	Average ABS	Standard Deviation	% Relative standard deviation
4	1	0.051	1,2398	0.051	0.00	0.00
4	2	0.051	1,2398	0.051	0.00	0.00
4	3	0.051	1,2398	0.051	0.00	0.00
8	1	0.108	-0.1717	0.107	0.04	0,52
8	2	0.107	0,7179	0.107	0.04	0,52
8	3	0.107	0,7179	0.107	0.04	0,52
12	1	0.162	1,138	0.164	0.12	1.03
12	2	0.165	-0.6442	0.164	0.12	1.03
12	3	0.165	-0.6442	0.164	0.12	1.03

Source: experimental data.

The following criteria are met: that the percentage of relative standard deviation (DSR%) is less than or equal to 2%, and that the relative error (ER%) is not greater than 2%.

Table 6: Intermediate precision of the system in phosphate.

Concentration (µg / mL)	Day	Measurement ABS	% Relative error	Average ABS	Standard deviation	% Relative standard deviation
8	**1**	**0.11**	**-0.9668**	**0.11**	**0.05**	**0.57**
8	**1**	**0,111**	**-1,8728**	**0.11**	**0.05**	**0.57**
8	**1**	**0.109**	**-0.0607**	**0.11**	**0.05**	**0.57**
8	**1**	**0.11**	**-0.9668**	**0.11**	**0.05**	**0.57**
8	**1**	**0.11**	**-0.9668**	**0.11**	**0.05**	**0.57**
8	**1**	**0.11**	**-0.9668**	**0.11**	**0.05**	**0.57**
8	**2**	**0.11**	**-0.9668**	**0.1102**	**0.05**	**0.67**
8	**2**	**0.11**	**-0.9668**	**0.1102**	**0.05**	**0.67**
8	**2**	**0,111**	**-1,8728**	**0.1102**	**0.05**	**0.67**
8	**2**	**0.11**	**-0.9668**	**0.1102**	**0.05**	**0.67**
8	**2**	**0,111**	**-1,8728**	**0.1102**	**0.05**	**0.67**
8	**2**	**0.109**	**-0.0607**	**0.1102**	**0.05**	**0.67**

Source: experimental data.

The evaluation of the intermediate precision was carried out during 2 consecutive days and with 2 different analysts.

The acceptance criterion is met, which establishes that the relative standard deviation (DSR%) is less than or equal to 2%, and that the relative error (ER%) is not greater than 2%.

Acetate

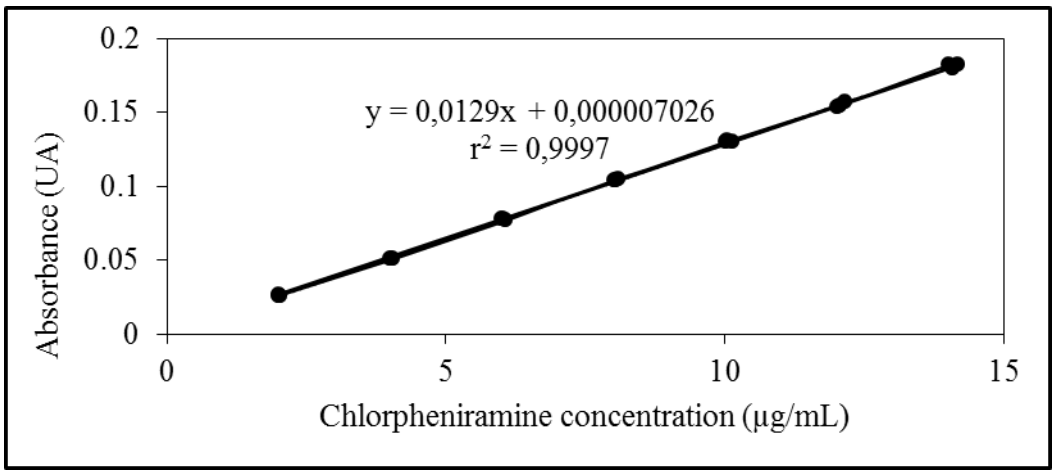

Figure 5: Calibration curve for the evaluation of the linearity of the system of Chlorpheniramine in Acetate.

In the previous figure, a linear behavior was observed within the concentration range used from 2 to 14 µg / mL, from the preparation of the 3 calibration curves of each of the mother solutions, and with this the linearity of the system was statistically analyzed. Through the Hartley test an F_{max} (3.00) lower than the Fcrítico was obtained (333.00). This shows that there is no significant difference between the variances, which translates into homoscedasticity; this allows to apply the analysis of variance of the linear regression, through of Microsoft Office Excel 2016, and which is shown in table 7.

Table 7: Results obtained with the analysis of variance of the linear regression.

Parameter	Value obtained
Slope (m)	0.0129
Intercept (b)	0.000007026
Correlation coefficient (r)	0.9997
Coefficient of determination (r^2)	0.9997
Typical error of the slope	0.00005014
Typical intercept error	0,00045127

Source: experimental data.

To the variance analysis of the linear regression (table 7), a T student test was performed; with a confidence level of 95%, it was established that statistically, the intercept is equal to zero and that the slope is statistically equal to one. At the same time, Fisher's F test, with a confidence level of 95%, indicates that there is no deviation that is statistically significant with respect to the linear regression.

It is also true that the correlation coefficient is between 0.98 and 1.00; and that the coefficient of determination is greater than 0.9950.

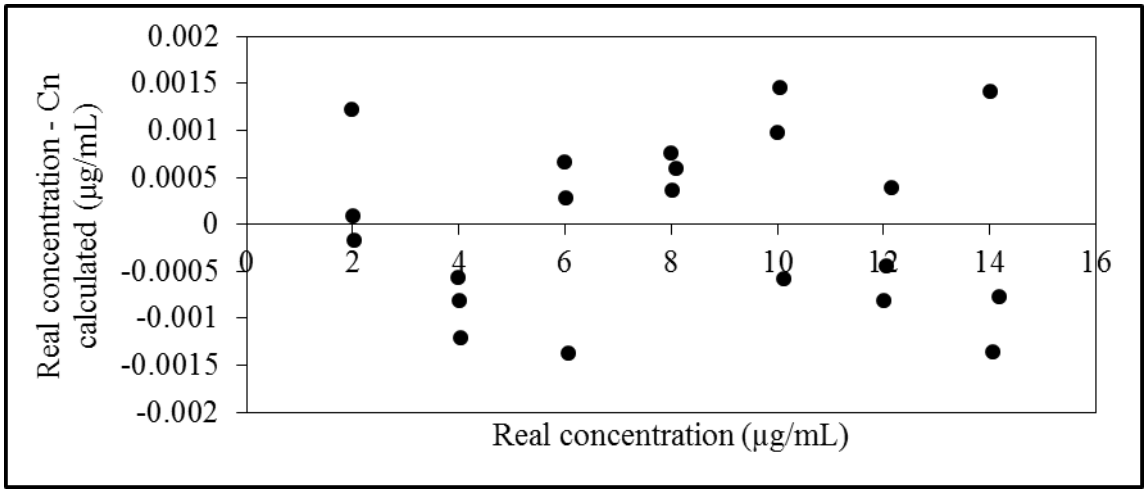

Figure 6: Analysis of system residuals in acetate.

Source: Self-obtained.

Figure 6 shows a random distribution of the residues. No systematic trends are observed, which are indicative of non-linearity.

With the confirmation of all the acceptance criteria stated above, the linearity of the system in acetate is confirmed.

Accuracy of the system in acetate

The precision of the system was established in two levels: repeatability and intermediate precision.

Table 8: Repeatability of the system in acetate.

Concentration (µg / mL)	Sample No.	Measurement ABS	% Relative error	Average ABS	Standard deviation	% Relative standard deviation
4	1	0.053	-1,4742	0,0523	0.04	1.12
4	2	0.052	0.4389	0,0523	0.04	1.12
4	3	0.052	0.4389	0,0523	0.04	1.12
8	1	0.105	-0.4766	0.1047	0.04	0.55
8	2	0.104	0.4799	0.1047	0.04	0.55
8	3	0.105	-0.4766	0.1047	0.04	0.55
12	1	0.156	0.4935	0,1563	0.04	0.37
12	2	0.157	-0.1441	0,1563	0.04	0.37
12	3	0.156	0.4935	0,1563	0.04	0.37

Source: experimental data.

The following criteria are met: that the percentage of relative standard deviation (DSR%) is less than or equal to 2%, and that the relative error (ER%) is not greater than 2%.

Table 9: Intermediate precision of the system in acetate.

Concentration (µg / mL)	Day	Measurement ABS	% Relative error	Average ABS	Standard deviation	% Relative standard deviation
8	1	0.105	-1.5814	0.1042	0,0763	0.9439
8	1	0.103	0.3536	0.1042	0,0763	0.9439
8	1	0.104	-0.6139	0.1042	0,0763	0.9439
8	1	0.105	-1.5814	0.1042	0,0763	0.9439
8	1	0.103	0.3536	0.1042	0,0763	0.9439
8	1	0.105	-1.5814	0.1042	0,0763	0.9439
8	2	0.104	-0.6139	0.1043	0,0633	0.7826
8	2	0.105	-1.5814	0.1043	0,0633	0.7826
8	2	0.103	0.3536	0.1043	0,0633	0.7826
8	2	0.105	-1.5814	0.1043	0,0633	0.7826
8	2	0.104	-0.6139	0.1043	0,0633	0.7826
8	2	0.105	-1.5814	0.1043	0,0633	0.7826

Source: experimental data.

The evaluation of the intermediate precision was carried out during 2 consecutive days and with 2 different analysts.

The acceptance criterion is met, which establishes that the relative standard deviation (DSR%) is less than or equal to 2%, and that the relative error (ER%) is not greater than 2%.

Validation of the method in Hydrochloric acid

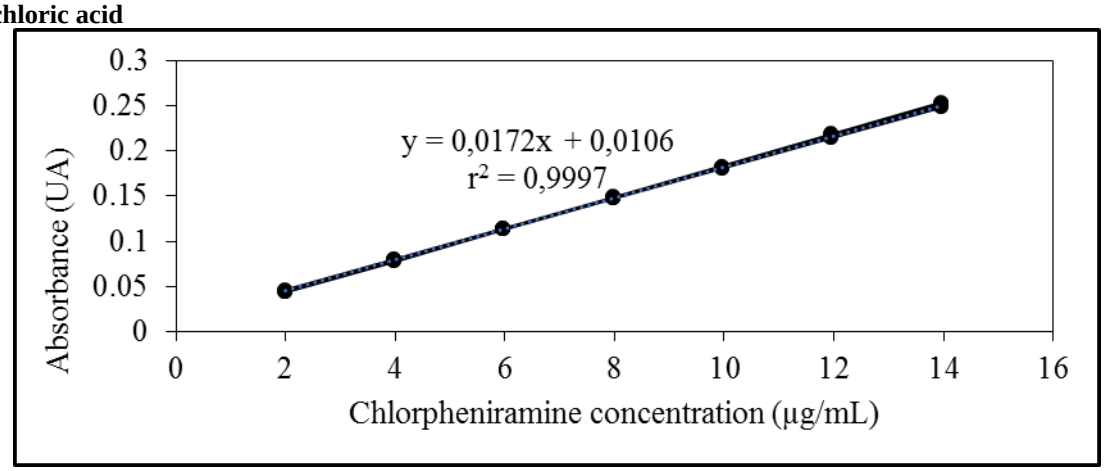

Figure 7: Calibration curve for the evaluation of the linearity of the method in Chlorpheniramine tablets of 4mg in HCl.

In the previous figure, a linear behavior of the method is observed, within the concentration range of 2 to 14 µg / mL, of the triplicate preparation of powder solutions of Chlorotronetone ® tablets of 4 mg, a which is added standard. With this, the linearity of the system was analyzed statistically.

Through the Hartley test, an Fmax (19.00) lower than the Fcrítico (333.00) was obtained, this shows that there is no significant difference between the variances, which translates into homoscedasticity; this allows to apply the analysis of variance of the linear regression, through Microsoft Office Excel 2016, and which is shown in table 10.

Table 10: Results obtained with the analysis of variance of the linear regression.

Parameter	Value obtained
Slope (m)	0.0172
Intercept (b)	0,0106
Correlation coefficient (r)	0.9997
Coefficient of determination (r^2)	0.9997
Typical error of the slope	0.00006567
Typical intercept error	0,0005856

Source: experimental data.

To the variance analysis of the linear regression (table 10), a student T test was performed; with a confidence level of 95%, it was established that statistically, the intercept is different to zero and that the slope is statistically equal to one. At the same time, Fisher's F test, with a confidence level of 95%, indicates that there is no deviation that is statistically significant with respect to the linear regression.

It is also true that the correlation coefficient is between 0.98 and 1.00; and that the coefficient of determination is greater than 0.9950.

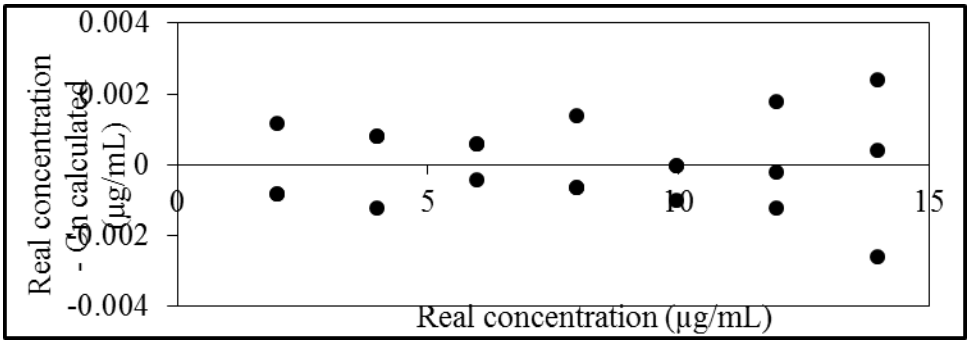

Figure 8. Analysis of residuals of the method in HCl.

Source: Self-obtained.

Figure 8 shows a random distribution of the residues. No systematic trends are observed, which are indicative of non-linearity.

With the confirmation of all the acceptance criteria stated above, the linearity of the method in HCl is confirmed.

Accuracy of the method in HCl

The accuracy of the method was determined by the triplicate preparation of three solutions of known concentration (4, 8 and 12 µg / mL) by means of the proposed method. Subsequently, these samples were enriched with the addition of standard; this in order to obtain the percentages of recovery shown below.

Table 11: Accuracy of the method by addition of standard.

Concentration (µg /mL)	Sample No.	Real total mass (µg)	Calculated total mass (µg)	Recovery percentage%
4	1	100.01	100.53	100.53
4	2	99.90	101.70	101,80
4	3	99.90	99.37	99.47
8	1	199.30	200.57	100.64
8	2	199,19	202.90	101,86
8	3	199,19	199.41	100.11
12	1	298.58	300.61	100.68
12	2	298.48	304.10	101,88
12	3	298.48	299.44	100.32
			Average	**100.81**
			Max.	**101,88**
			Min.	**99.47**
			Standard deviation	**0,0935**
			DSR	**1,8289**

Source: experimental data.

The results obtained previously, show that the percentage obtained of the analyte in the sample, is within the range that specifies the acceptance criterion, which is in this case, between 97 and 103%. Therefore, this criterion is satisfactorily fulfilled. Likewise, the relative standard deviation of the recovery percentage is below 3%, which is also in accordance with the acceptance criterion.

In the following figure, the total amount found against the added amount was plotted. It is observed that the slope obtained is greater than or equal to 0.95, and the intercept close to the initial concentration (50 µg / mL). In this way, the last acceptance criterion for the accuracy of the method by addition of standard is met.

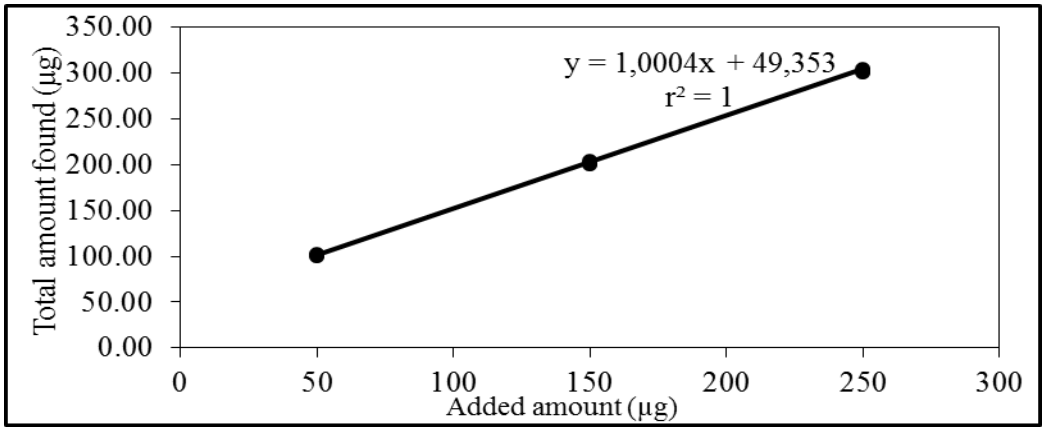

Figure 9: Correlation between the amount of chlorpheniramine found in the sample and the added amount. Accuracy of the method in HCl.

The precision of the method was evaluated in two levels: repeatability and intermediate precision. The results of each are as follows:

Table 12: Repeatability of the method in HCl.

Concentration (µg / mL)	Sample No.	Measurement ABS	% Relative error	Average ABS	Standard deviation	% Relative standard deviation
4	1	0,087	-1,2572	0,0867	0.03	0.67
4	2	0,087	-1,2572	0,0867	0.03	0.67
4	3	0.086	-0.0856	0,0867	0.03	0.67
8	1	0,173	-1.0059	0,1733	0.03	0.33
8	2	0,173	-1.0059	0,1733	0.03	0.33
8	3	0.174	-1,5917	0,1733	0.03	0.33
12	1	0.259	-0.9221	0,2593	0.03	0.22
12	2	0,260	-1,3127	0,2593	0.03	0.22
12	3	0.259	-0.9221	0,2593	0.03	0.22

Source: experimental data.

The results obtained from the relative standard deviation (DSR%), show that all of them meet the criterion of acceptance of repeatability of the method, since they are all below 3%.

Table 13: Intermediate precision of the method in HCl.

Concentration (µg / mL)	Day	Measurement ABS	% Relative error	Average ABS	Standard deviation	% Relative standard deviation
8	1	0.171	0,5459	0,1723	0.04	0.48
8	1	0,173	-0.6211	0,1723	0.04	0.48
8	1	0,172	-0.0376	0,1723	0.04	0.48
8	1	0,173	-0.6211	0,1723	0.04	0.48
8	1	0,172	-0.0376	0,1723	0.04	0.48
8	1	0,173	-0.6211	0,1723	0.04	0.48
8	2	0,172	-0.0376	0,1715	0.03	0.32
8	2	0,172	-0.0376	0,1715	0.03	0.32
8	2	0,172	-0.0376	0,1715	0.03	0.32
8	2	0.171	0,5459	0,1715	0.03	0.32
8	2	0.171	0,5459	0,1715	0.03	0.32
8	2	0.171	0,5459	0,1715	0.03	0.32

Source: experimental data.

The evaluation of the intermediate precision was carried out during 2 consecutive days and with 2 different analysts.

The results obtained from the relative standard deviation (DSR%), show that all of them meet the criterion of acceptance of intermediate precision of the method, since they are all below 3%.

Phosphate

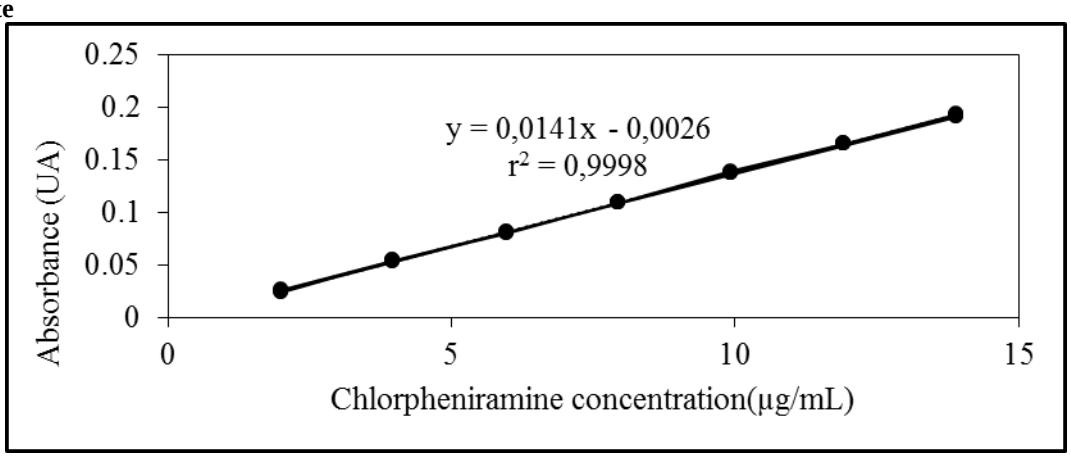

Figure 10: Calibration curve for the evaluation of the linearity of the method of Chlorpheniramine tablets of 4mg in Phosphate.

In the previous figure, a linear behavior of the method is observed, within the concentration range of 2 to 14 µg / mL, by means of the triplicate preparation of powder solutions of Chlorotronetone ® tablets of 4 mg, a which is added standard. With this, the linearity of the system was analyzed statistically.

Through the Hartley test, an F_{max} was obtained (3.00) lower than the $_{critical}$ F (333.00) this shows that there is no significant difference between the variances, which translates into homoscedasticity; this allows to apply the analysis of variance of the linear regression, through Microsoft Office Excel 2016, and which is shown in table 14.

Table 14: Results obtained with the analysis of variance of the linear regression.

Parameter	Value obtained
Slope (m)	0.0141
Intercept (b)	0.0026
Correlation coefficient (r)	0.9999
Coefficient of determination (r^2)	0.9999
Typical error of the slope	0.00004758
Typical intercept error	0,0004225

Source: experimental data.

To the variance analysis of the linear regression (table 14), a student T test was performed; with a confidence level of 95%, it was established that statistically, the intercept is different to zero and that the slope is statistically equal to one. At the same time, Fisher's F test, with a confidence level of 95%, indicates that there is no deviation that is statistically significant with respect to the linear regression.

It is also true that the correlation coefficient is between 0.98 and 1.00; and that the coefficient of determination is greater than 0.9950.

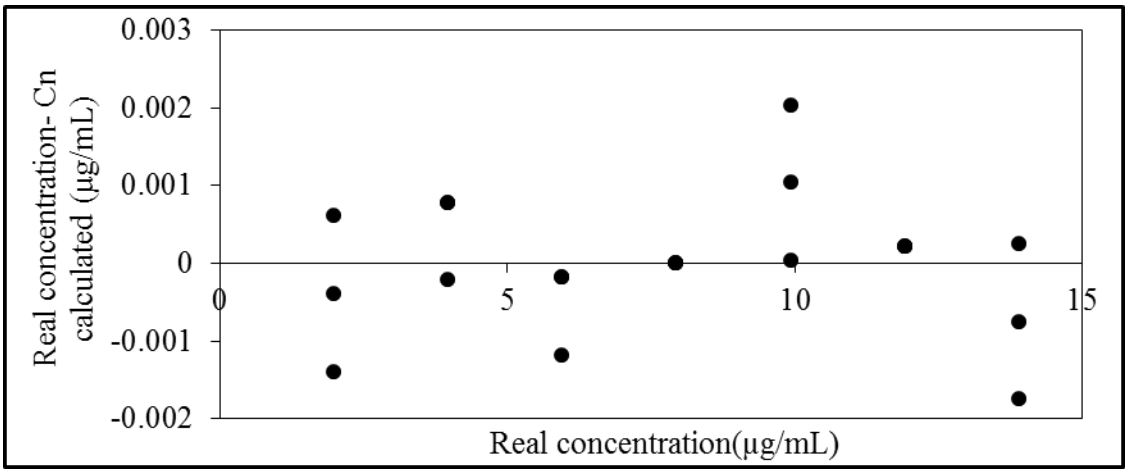

Figure 11: Residual analysis of the phosphate method.

Source: Self-obtained.

In Figure 11 a random distribution of the residues is observed. No systematic trends are observed, which are indicative of non-linearity.

With the confirmation of all the acceptance criteria discussed above, the linearity of the phosphate method is confirmed.

Accuracy of the phosphate method

It was determined by the triplicate preparation of three solutions of known concentration (4, 8 and 12 µg / mL) by means of the proposed method. Subsequently, these samples were enriched with the addition of standard; this in order to obtain the percentages of recovery shown below.

Table 15: Accuracy of the phosphate method by standard addition.

Concentration (µg /mL)	Sample No.	Real total mass (µg)	Calculated total mass (µg)	Recovery percentage%
4	1	99.68	99.93	100.25
4	2	99.66	99.93	100.27
4	3	99.62	98.13	98.50
8	1	198.97	199.09	100.06
8	2	198.95	199.09	100.07
8	3	198,91	199.09	100.09
12	1	298.26	300.05	100.60
12	2	298.24	300.05	100.61
12	3	298.20	300.05	100.62
			Average	**100.81**
			Max.	**100.62**
			Min.	**98.50**
			Standard deviation	**0,0451**
			DSR	**0.8974**

Source: experimental data.

The results obtained previously, show that the percentage obtained of the analyte in the sample, is within the range that specifies the acceptance criterion, which is in this case, between 97 and 103%. Therefore, this criterion is satisfactorily fulfilled. Likewise, the relative standard deviation of the recovery percentage is below 3%, which is also in accordance with the acceptance criterion.

In addition, the total amount found was plotted against the amount added. It is observed that the slope obtained is greater than or equal to 0.95, and the intercept close to the initial concentration (50 µg / mL). In this way, the last acceptance criterion for the accuracy of the method by addition of standard is met.

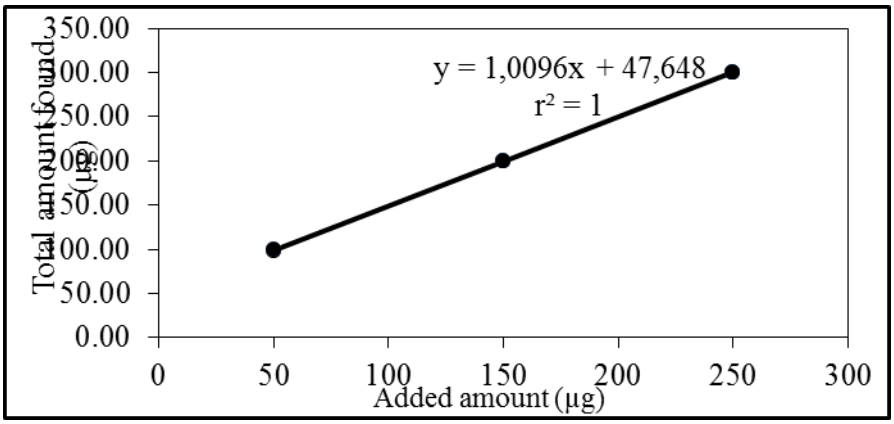

Figure 12: Correlation between the amount of chlorpheniramine found in the sample and the added amount.
Source: self-obtained.

Precision of the phosphate method

The precision of the method was evaluated in two levels: repeatability and intermediate precision. A following the results of each are as follows:

Table 16: Repeatability of the phosphate method.

Concentration (µg / mL)	Sample No.	Measurement ABS	% Relative error	Average ABS	Standard deviation	% Relative standard deviation
4	1	0.053	1,1673	0.0547	0.06	1.52
4	2	0.055	-0.8815	0.0547	0.06	1.52
4	3	0.056	-1,8192	0.0547	0.06	1.52
8	1	0,111	-1.5447	0.1107	0.02	0.21
8	2	0.11	-1,1673	0.1107	0.02	0.21
8	3	0,111	-1.5447	0.1107	0.02	0.21
12	1	0.169	-1,7777	0.168	0.02	0.14
12	2	0.168	-1.3687	0.168	0.02	0.14
12	3	0.167	-0.9821	0.168	0.02	0.14

Source: experimental data.

The results obtained from the relative standard deviation (DSR%), show that all of them meet the criterion of acceptance of repeatability of the method, since they are all below 3%.

Table 17: Intermediate precision of the phosphate method.

Concentration (µg / mL)	Day	Measurement ABS	% Relative error	Average ABS	Standard deviation	% Relative standard deviation
8	1	0.11	-0.9685	0.1102	0.05	0.67
8	1	0,111	-1,8746	0.1102	0.05	0.67
8	1	0.11	-0.9685	0.1102	0.05	0.67
8	1	0.109	-0.0624	0.1102	0.05	0.67
8	1	0,111	-1,8746	0.1102	0.05	0.67
8	1	0.11	-0.9685	0.1102	0.05	0.67
8	2	0.11	-0.9685	0.1098	0.05	0.68
8	2	0.109	-0.0624	0.1098	0.05	0.68
8	2	0.11	-0.9685	0.1098	0.05	0.68
8	2	0,111	-1,8746	0.1098	0.05	0.68
8	2	0.109	-0.0624	0.1098	0.05	0.68
8	2	0.11	-0.9685	0.1098	0.05	0.68

Source: experimental data.

The evaluation of the intermediate precision was carried out during 2 consecutive days and with 2 different analysts.

The results obtained from the relative standard deviation (DSR%), show that all of them meet the criterion of acceptance of intermediate precision of the method, since they are all below 3%.

Acetate

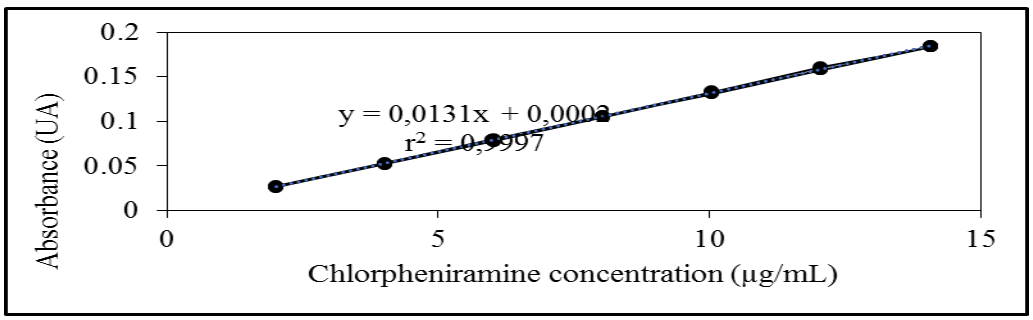

Figure 13: Calibration curve for the evaluation of the linearity of the method of Chlorpheniramine tablets of 4 mg in acetate.

In the previous figure, a linear behavior of the method is observed, within the worked concentration range of 2 to 14 µg / mL, by means of the triplicate preparation of powder solutions of Chlorotronetone ® tablets of 4 mg, a which is added standard. With this, the linearity of the system was analyzed statistically.

Through the Hartley test, an F_{max} (3.00) lower than the $_{critical}$ F (333.00) was obtained. This shows that there is no significant difference between the variances, which translates into homoscedasticity; this allows applying the analysis of variance of the linear regression, through

Microsoft Office Excel 2016, and which is shown in table 18.

Table 18: Results obtained with the analysis of variance of the linear regression.

Parameter	Value obtained
Slope (m)	0,0131
Intercept (b)	0.0002
Correlation coefficient (r)	0.9999
Coefficient of determination (r^2)	0.9997
Typical error of the slope	0.00004844
Typical intercept error	0,0004354

Source: experimental data.

To the variance analysis of the linear regression (table 18), a student T test was performed; with a confidence level of 95%, it was established that statistically, the intercept is equal to zero and that the slope is statistically equal to one. At the same time, Fisher's F test, with a confidence level of 95%, indicates that there is no deviation that is statistically significant with respect to the linear regression.

It is also true that the correlation coefficient is between 0.98 and 1.00; and that the coefficient of determination is greater than 0.9950.

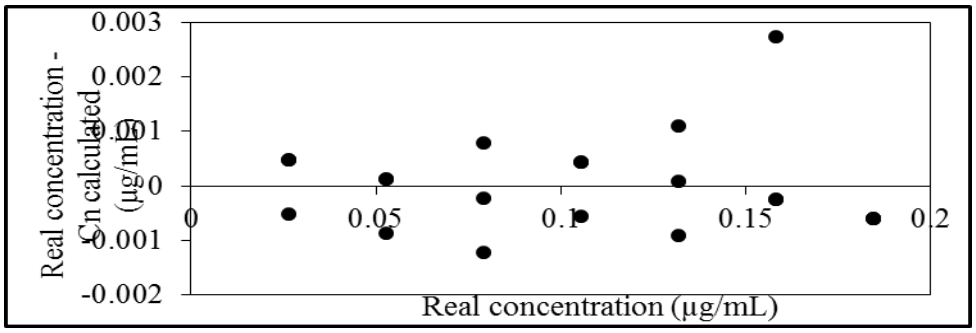

Figure 14: Residual analysis of the acetate method.

Source: Self-obtained.

Figure 14 shows a random distribution of the residues. No systematic trends are observed, which are indicative of non-linearity.

With the confirmation of all the acceptance criteria discussed above, the linearity of the acetate method is confirmed.

Accuracy of the acetate method

It was determined by the triplicate preparation of three solutions of known concentration (4, 8 and 12 µg / mL) by means of the proposed method. Subsequently, these samples were enriched with the addition of standard; this in order to obtain the percentages of recovery shown below:

Table 19: Accuracy of the method by addition of standard.

Concentration (µg / mL)	Sample No.	Real total mass (µg)	Calculated total mass (µg)	Recovery percentage%
4	1	100.73	102.37	101.63
4	2	100.71	102.37	101,65
4	3	100.71	102.37	101,65
8	1	201,60	203.92	101.15
8	2	201.58	203.92	101,16
8	3	201.58	203.92	101,16
12	1	302.46	305.47	100.99
12	2	302.44	305.47	101.00
12	3	302.44	303.55	100.37
			Average	**101,19**
			Max.	**101,65**
			Min.	**100.37**
			Standard deviation	**0,0479**
			DSR	**0.9576**

Source: experimental data.

The results obtained previously, show that the percentage obtained of the analyte in the sample, is within the range that specifies the acceptance criterion, which is in this case, between 97% and 103%. Therefore, this criterion is satisfactorily fulfilled. Likewise, the relative standard deviation of the recovery percentage is below 3%, which is also in accordance with the acceptance criterion.

In addition, in the following figure, the total amount found against the added amount was plotted. It is observed that the slope obtained is greater than or equal to 0.95, and the intercept close to the initial concentration (50 µg / ml). In this way, the last acceptance criterion for the accuracy of the method by addition of standard is met.

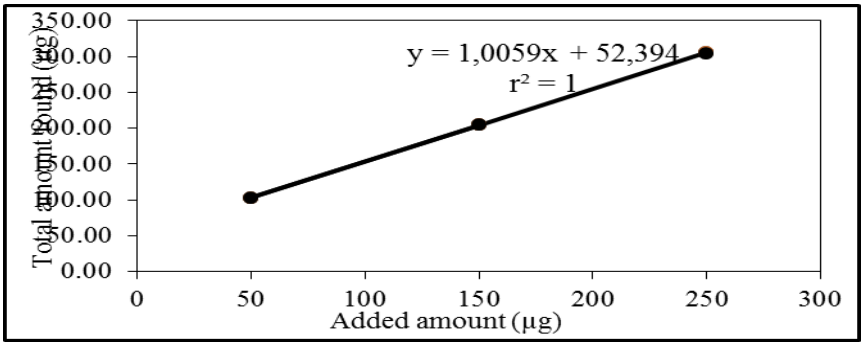

Figure 15: Correlation between the amount of chlorpheniramine found in the sample and the added amount.

Accuracy of the acetate method

The precision of the method was evaluated in two levels: repeatability and intermediate precision. The results of each are as follows:

Table 20: Repeatability of the acetate method.

Concentration (µg / mL)	Sample No.	Measurement ABS	% Relative error	Average ABS	Standard deviation	% Relative standard deviation
4	1	0.053	0.4071	0.053	0.00	0.00
4	2	0.053	0.4071	0.053	0.00	0.00
4	3	0.053	0.4071	0.053	0.00	0.00
8	1	0.105	0.8141	0.105	0.00	0.00
8	2	0.105	0.8141	0.105	0.00	0.00
8	3	0.105	0.8141	0.105	0.00	0.00
12	1	0.158	0.3166	0.158	0.00	0.00
12	2	0.158	0.3166	0.158	0.00	0.00
12	3	0.158	0.3166	0.158	0.00	0.00

Source: experimental data.

The results obtained from the relative standard deviation (DSR%), show that all of them meet the criterion of acceptance of repeatability of the method, since they are all below 3%.

Table 21: Intermediate precision of the acetate method.

Concentration (µg / mL)	Day	Measurement ABS	% Relative error	Average ABS	Standard deviation	% Relative standard deviation
8	1	0.106	-0.4859	0.1057	0.06	0.78
8	1	0.105	0.4673	0.1057	0.06	0.78
8	1	0.106	-0.4859	0.1057	0.06	0.78
8	1	0.107	-1,4389	0.1057	0.06	0.78
8	1	0.105	0.4673	0.1057	0.06	0.78
8	1	0.105	0.4673	0.1057	0.06	0.78
8	2	0.105	0.4673	0.1045	0.04	0.53
8	2	0.104	1,4204	0.1045	0.04	0.53
8	2	0.105	0.4673	0.1045	0.04	0.53
8	2	0.105	0.4673	0.1045	0.04	0.53
8	2	0.104	1,4204	0.1045	0.04	0.53
8	2	0.104	1,4204	0.1045	0.04	0.53

Source: experimental data.

The evaluation of the intermediate precision was carried out during 2 consecutive days and with 2 different analysts.

The results obtained from the relative standard deviation (DSR%) show that all of them meet the criterion of acceptance of intermediate precision of the method, since they are all below 3%.

4. CONCLUSIONS

The validation procedure carried out, verifies that the performance requirements for the analytical applications for which they were planned are met. That said, it is evident that the data obtained from the analytical method for quantifying chlorpheniramine and the determination of the percentage dissolved in the medium or dissolution, are reliable; in the evaluation of the linearity of the system, the results show linearity in the described concentration range (2-14 µ g / mL) plus the results demonstrate the accuracy and precision of the method. Of this way it is guaranteed that the obtained results can be applied for new works by other researchers. However, if you want to make any changes in the method or equipment, you must make a new extension of the validation.

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