SEKOLAH TINGGI ILMU KESEHATAN TELOGOREJO

Science and Community Pharmacy Journal
Volume 2 *Issue* 2 *Year* 2023 *Pages* 149-154
E–ISSN 2830–6139| DOI:
12.23.23456/scienceandcommunitypharmacy.cj
Web https://ojs.stikestelogorejo.ac.id/

VALIDATION OF ANALYSIS METHOD FOR DETERMINING CONTENT OF CHLORPHENIRAMINE MALATE (CTM) IN TABLET PREPARATION BY UV SPECTROPHOTOMETRY

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Abstract: In the pharmaceutical industry, quality control of a medicine is one part of Good Medicine Manufacturing Practices (CPOB) which is used to ensure that the product has quality that is appropriate for its intended use, so that the production results marketed meet the CPOB requirements. Therefore, this study aims to determine the levels of the active substance chlorpheniramine maleate (CTM) in drug samples and compare them with standards using UV spectrophotometry. The research results showed that the maximum CTM wavelength measurement obtained was 263 nm. The linear equation y = 0.01904x + 0.03139 with a correlation coefficient (R) value of 0.995. The SD obtained by sample A was 0.4483 and the % relative standard deviation (%RSD) value was 14.6%, sample B was 0.2020 and the % relative standard deviation (%RSD) value was 6.4%, sample C was 0.0542 and the % relative standard deviation (%RSD) value is 1.3%. The LOQ values obtained on samples A, B and C are not accepted as quantitation limits because they do not meet precision (CV < 20%) and accuracy (bias < ±20%). The average % recovery results obtained for samples A, B, and C were 100%. The range of CTM levels obtained was 159.307%, 145.025%, and 126.195%.

Keywords: UV Spectrophotometry, Chlorpheniramine maleat, Validation of analytical methods

INTRODUCTION

According to law, a medicine is a substance or mixture of substances to be used to determine a diagnosis, prevent, reduce, cure a disease or symptom, injury or bodily or spiritual disorder in humans or animals (Ribkah, 2018). The choice of chlorpheniramine maleate as the research object was because chloramphenicol is a drug that is widely used, especially in government health service facilities, which has the benefit of treating allergic reactions caused by various types of allergens.

In the pharmaceutical industry, quality control of a medicine is one part of Good Medicine Manufacturing Practices (CPOB) which is used to ensure that the product has quality that is appropriate for its intended use, so that the production results marketed meet the CPOB requirements. In this requirement, it is necessary to determine the level of chlorpheniramine maleate (CTM) in tablets, which according to the requirements of the Indonesian Pharmacopoeia (FI) Edition VI of 2020 is not less than 98.5% and not more than 101.10%

Determining the levels of chlorpheniramine maleate (CTM) in a preparation requires a thorough and accurate method. Therefore, validation needs to be carried out first, where this procedure is used to prove that the analytical method produces the results as expected with sufficient accuracy and accuracy.

This research uses the ultraviolet spectrophotometry method. Chlorpheniramine maleate is easily soluble in water, ethanol and chloroform but difficult to dissolve in ether. Based on this solubility, it is possible to modify the determination of chlorpheniramine maleate (CTM) levels by using an appropriate solvent.

METHODS

1. Materials

The materials used in this study include CTM tablets, namely CTM standards, 3 types of CTM samples from different production factories (Orphen, PIM, and Generic) and 0.1 N sulfuric acid solvent

2. Equipment

Equipment used in this study includes The materials that will be used in determining the levels include CTM tablets, namely CTM standards, 3 types of CTM samples from different production factories (Orphen, PIM, and Generic) and 0.1 N sulfuric acid solvent

3. Procedure

3.1. Preparation 1000 ppm CTM standar

Samples Standard chlorpheniramine maleate (ctm) powder was weighed as much as 25 mg and dissolved with 0.1 N sulfuric acid in a beaker. The solution was put into a 25 ml measuring flask until it reached the mark and was homogeneous

3.2. Determination of maximum wavelength

A standard solution of chlorpheniramine maleate (ctm) with a concentration of 15 ppm was made by pipetting 1.5 ml of the CTM stock solution and then placing it in a 10 ml volumetric flask. The solution was diluted with 0.1 N sulfuric acid to the limit mark, then shaken until homogeneous. The maximum wavelength of the solution was measured in the wavelength range between 200-400 nm.

3.3. Creating a standard curve

From the 1000 ppm stock solution a standard solution was made with a concentration series of 6; 10 ; 1.5 ; 2 and 2.5 ppm as much as 10 ml. The series solutions that have been prepared are then measured for the absorption of each concentration at the maximum wavelength obtained. The absorbance data obtained is then calculated by calculating the standard curve equation to obtain the line equation $y = a + bx$.

4. Determination of sample levels

Five test sample tablets that met weight uniformity were then crushed until smooth and homogeneous. The powder sample was weighed as much as 5 mg and dissolved with 0.1N sulfuric acid and placed in a 25 ml measuring flask up to the mark. 1 ml of the solution was pipetted, then diluted with sulfuric acid to a concentration of 20 ppm in 10 ml. Measure the absorption of the solution at the maximum wavelength. If the absorption of the test sample solution is still outside the absorption range of the standard solution, then the solution is diluted until the absorption falls within the range. Determination of levels was carried out by repeating 3 times.

5. Validation Of Analytical Methods

5.1 Determination of precision

In this study, precision testing was carried out by measuring the absorbance of the CTM standard solution with a concentration of 15 µg/mL at the maximum wavelength

5.2 Determination of precision (accuracy)

The standard addition method uses a recovery test on samples with standard addition with 3 replications.

5.3 Determination of linearity

Linearistic determination states the relationship between concentration and absorbance using a linear regression equation from a standard curve.

5.4 Sensitivity determination

Includes limit of detection (LOD) test and limit of quantification (LOQ) test using the linear regression line equation in the linearity test.

RESULT AND DISCUSSION

Determination of the Maximum Wavelength

Determination of the maximum wavelength is carried out because the wavelength of a compound can be different when determined under different conditions and tools. The maximum wavelength (λmax) is the wavelength at which electronic excitation occurs which provides maximum absorbance. The aim of measuring at the maximum wavelength is that the change in absorbance for each concentration unit is greatest at the maximum wavelength, so that maximum analytical sensitivity will be obtained (Gandjar and Rohman, 2007).

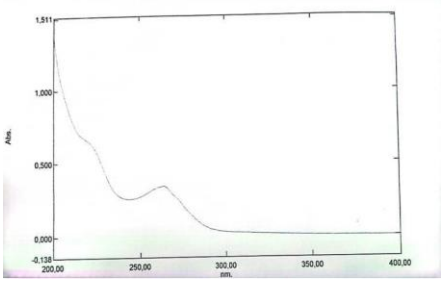

Figure 1. Maximum wavelength of CTM

Figure 1 shows the results of the maximum CTM wavelength measurement obtained which is 263 nm. This maximum wavelength indicates that the CTM absorption is in the UV region because it is in the wavelength range of 200–400 nm. Theoretically the maximum absorption for CTM is 263 nm (Anne, 2018)

Determination Linearity

Linearity shows the ability of an analytical method to obtain test results that correspond to the analyte concentration in the sample in a certain concentration range (Ermer and Miller, 2005). This can be done by making a calibration curve from a standard solution whose concentration is known. The calibration curve is a standard method that can be used to determine the concentration of an analyte based on the Lambert-Beer law. Determination of the calibration curve is carried out by analyzing the concentration of CTM standard solutions, including 6; 10 ; 15 ; 20 and 25 ppm. Solutions with a series of concentrations were measured for their respective absorbance at the maximum CTM wavelength, namely 263 nm. Measuring the absorbance of CTM standard solutions at the maximum wavelength is because in this area the largest absorption point will be obtained for each CTM standard solution.

The measurement results show that the greater the concentration of the CTM standard solution measured, the greater the absorbance obtained. This is because at higher concentrations, the concentration level of the CTM compound is also higher. In addition, the Lambert-Beer law shows that changing the concentration of a particular sample will change the absorbance at each wavelength by a constant factor (Skoog and West, 1971). Preparation of a standard calibration curve was carried out by plotting the CTM standard solution (axis x) and absorbance (y axis), then the points are connected with a straight line.

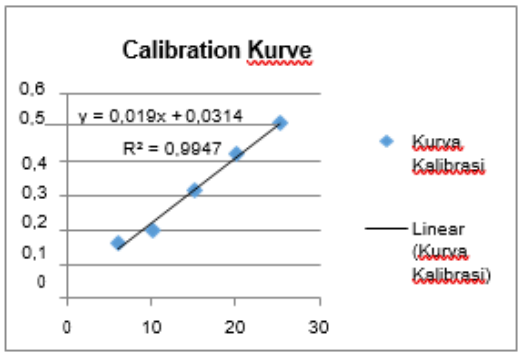

Figure 2. CTM standard curve

Based on the results of absorbance measurements of CTM solutions with various concentrations, it gives a linear equation y = 0.01904x + 0.03139 with a correlation coefficient (R) value of 0.995 and a coefficient of determination (R2) value obtained of 0.9947. The correlation coefficient value obtained is the relationship between CTM concentration and absorbance, that is, it meets the linear criteria (parameters), because a good correlation coefficient close to the value 1. The linear range values obtained indicate that the Lambert-Beer law applies in the calibration curve, so that the line equation can be used to determine the validation of the method for determining CTM levels using an ultraviolet spectrophotometer.

Determination of Precision

Accuracy or precision is a measure that indicates the degree of agreement between individual test results, measured through the spread of individual results from the average if the procedure is applied repeatedly to samples taken from a homogeneous mixture. Accuracy can be expressed as repeatability or reproducibility. Repeatability is the accuracy of a method if it is carried out repeatedly by the same analyst under the same conditions and within a short time interval. Reproducibility is the accuracy of the method if carried out under different conditions. Precision values are calculated using standard deviation (SD) to produce Relative Standard Deviation (RSD) or Coeficient Variation (CV). Accurate criteria are given if the method provides a %RSD value ≤2%. The smaller the standard deviation value obtained, the smaller the coefficient of variation value (Riyadi, 2009).

Table 1. Data from CTM Precision Test Results

Replication	Sample A	Sample B	Sample C
1	0,586	0,635	0,516
2	0,739	0,534	0,524
3	0,591	0,574	0,525
$\sum(\mathbf{x}-\bar{\mathbf{x}})^2$	0,40196	0,16323	0,00589
SD	0,4483	0,2020	0,0542
%RSD	14,6%	6,4%	1,3%

Based on the data in Table 1, it shows that the standard deviation value obtained by sample A was 0.4483 and the % relative standard deviation (%RSD) value was 14.6%, sample B was 0.2020 and the value % relative standard deviation (%RSD) is 6.4%, sample C is 0.0542 and the % relative standard deviation (%RSD) value is 1.3%. The results obtained indicate that the test method used in validating the method for determining CTM levels in drug C samples using UV Visible spectrophotometry meets the acceptable %RSD value requirements. But samples A and B do not meet the value requirements %RSD received. The

precision value can provide information that this method cannot be used as a permanent method used in the laboratory.

Determination of Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Limit of Detection (LOD) is the smallest amount or concentration of analyte in a sample that can be detected, but does not need to be measured according to the actual value. Limit of Quantitation (LOQ) or limit of quantitation is the smallest amount of analyte in a sample that can be determined quantitatively with a good level of precision and precision. Quantitation limits are quantitative test parameters for low analyte concentrations in complex matrices and are used to determine the presence of impurities or product degradation.

Based on Table 2, it can be seen that the LOQ values obtained for samples A, B and C are not accepted as quantitation limits because they do not meet precision (CV < 20%) and accuracy (bias < ± 20%). Meanwhile, if the LOD value at this concentration is measured, it cannot provide accurate analysis.

Table 2. LOD and LOQ Value Data

Parameter	Sample A	Sample B	Sample C
LOD (mg/L)	70,6355	31,8277	8,5399
LOQ (mg/L)	235,4516	106,0924	28,4663

Determination of Accuracy

The accuracy of an analytical method is the closeness of the test result value obtained by the procedure to the actual value. Accuracy is a measure of the precision of analytical procedures (Rohman, 2007). Accuracy is expressed as the percent recovery (%Recovery) of the added analyte.

Table 3. Accuracy Test Data

Replication	Sample A	Sample B	Sample C
1	91.63%	109,96%	98,45%
2	116,98%	92,04%	100,89%
3	91,50%	98,00%	100,65%
Mean	100%	100%	100%

Table 3 shows that the average % recovery results obtained for samples A, B, and C were 100%. According to Harmita (2004), the acceptable range of percent (%) analyte recovery values is 90-110%. This range is flexible depending on the condition of the analyte being examined based on the number of samples and laboratory conditions. The %recovery obtained is within the acceptable range, namely 90-110%, so it can be said that this method has good accuracy.

Determination of CTM Levels

Determination of CTM levels is carried out by measuring the test sample solution which is suspected to contain CTM at a maximum wavelength of 263 nm with repetition 3 times/sample. Determination of these levels aims to guarantee the quality and safety of a medicinal product.

Table 4. Data on CTM Level Ranges

Parameter	Sample A	Sample B	Sample C
mg/tablet	6,3 mg	5,8 mg	4,03 mg
Rate range	159,307%	145,025%	126,195%

The data in Table 4 shows that the recovery value of mg/tablet in tablet drug samples with 3 different trademarks is 6.3 mg, 5.8 mg, and 4.03 mg. According to Werner, et al (2010) the CTM level in tablet preparations is 4 mg. The average level of paracetamol obtained was more than the level that should be present in the tablet preparation of the drug. Meanwhile, the range of CTM levels obtained was 159.307%, 145.025% and 126.195%. According to the requirements of the Indonesian Pharmacopoeia (FI) Edition VI 2020, the level of CTM active substance in a tablet is not less than 98.5% and not more than 101.10%. The results obtained from this test show a discrepancy between the test results and the established standards. Factors that influence inconsistency in test results are when taking/pipetting the solution, inaccuracy in weighing which causes less accurate results and the lack of a shaking factor before the sample solution to be measured also affects the results obtained in the test, because the solution must be truly homogeneous in order to obtain good results. maximum in testing.

CONCLUSION

Based on the results of the research that has been carried out, it can be concluded as the CTM levels obtained in drug tablet samples did not comply with established standards, the UV spectrophotometry method used in the research does not meet the parameters set in the validation test except for determining accuracy, so this method can be applied for analysis of determining chlorpheniramine maleate levels in a laboratory. More observations, furthermore, related to the development of methods for analysis drugs.

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