Development and Validation of Bisacodyl Analysis Method in Tablet with Absorbance Method and Area under Curves Method in Ultraviolet Spectrophotometry

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Abstract

A precise, accurate, fast, and economical simple method has been developed and validated for the analysis of bisacodyl tablets using ultraviolet spectrophotometry with absorbance method and area under the curve. This method is carried out at a maximum absorption wavelength of 263.60 nm and the area under the curve in the range 244.40 - 297.60 nm using a 0.1 N hydrochloric acid solvent. The method of analysis is validated by linearity, detection limits, quantification limits, precision, and accuracy. The linearity of the absorbance method shows the regression equation $y = 0.02156x + 0.09258$ ($r = 0.99963$), and the linearity of the wide area method under the curve shows the regression equation $y = 0.30618x + 1.17400$ ($r = 0.99787$). Limit detection and quantification limits on the absorbance method 1.411099 and 4.276059, respectively, while the detection and quantification limits on the method of area under the curve 1.968774 and 5.9659197, respectively. Accuracy obtained by the absorbance method and the area under the curve also meet the requirements in the validation of 80 - 120%. The precision obtained by the absorbance method and the area under the curve fulfilled the validated requirement with a yield of RSD ≤ 2%. Statistical analysis was performed by paired sample t test which showed that the two methods did not significantly.

Keywords: Bisacodyl, absorbance method, area under the curve method, ultraviolet spectrophotometry

1. Introduction

Bisacodyl is a diphenylmethane derivative which is a popular contact laxation that works directly against the colon wall by reinforcing its peristaltic work that makes the stool soft. In addition to its use as a common laxative, it is also often used for surgery or x-ray examination. Bisacodyl resorption in the small intestine may reach 50% and after deacetylation in the liver is partially removed by bile and undergoes enterohepatic cycles. The remaining active metabolites are excreted through the kidney, and the unabsorbed portion is beneficial to the intestinal wall [1].

Bisacodyl belongs to diphenylmethane class with chemical name 4,4'-(pyridin-2-ylmethylene)bis(4,1-phenylene) diacetate (Figure 1). Bisacodyl contains not less than 98.0 % and not more than 101.0 % of $C_{22}H_{19}NO_4$, calculated on the dried basis [2].
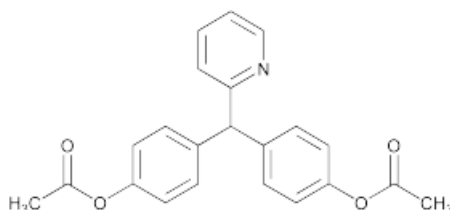

Figure 1: Structure of bisocodyl [2]

According to Pharmacopoeia Indonesia edition V [3], the determination of bisacodyl level is done by high performance liquid chromatography method. The solvent used was a mixture comprising 0.074 M sodium acetate and acetonitrile solution detected at a wavelength of 265 nm. In British Pharmacopeia the determination of bisacodyl level is done by liquid chromatography method. The solvent used acetic acid, acetonitrile, water detected by a spectrophotometer at a wavelength of 265 nm [4].

Two simple, rapid and sensitive spectrophotometric methods were proposed for determination of bisacodyl in pure and in tablet forms [5]. The method I depended on reaction of drug and tetracyanoethylene by charge transfer complex with maximum absorbance at 398 nm. Beer's law was obeyed in the range 0.05-0.3 µgml-1. Method II based on bromination-oxidation reaction using bromate-bromide mixture with thymol blue and methyl orange as reagents and measuring the absorbance of the unbleached dye at 545 nm and 510 nm. Beer's law was obeyed in the range 4-24 $µgml^{-1}$ and 4-10 $µgml^{-1}$. Under optimized conditions, the experimental conditions were optimized and Beer's law was obeyed over the applicable concentration ranges. The methods were applied successfully to the tablets containing bisacodyl. The results obtained are in good agreement with those obtained using official and reference methods.

A simple, sensitive, and accurate indirect spectrophotometric method for the determination of bisacodyl in pure form and in some of its pharmaceutical preparations has been developed [6]. The method is based on the oxidation of bisacodyl by iron (III), and subsequent complexation of iron (II) with o-phenanthroline. Forming a red-colored complex (ferroin) having the maximum absorbance at 510 nm. Beer's law is obeyed in the concentration range of 0.5-5 µg/ml. The molar absorptivity and Sandell's sensitivity were 1.55×104 $L.mol^{-1}.cm^{-1}$ and $0.0233µg.cm^{-2}$ respectively. The relative standard deviation (RSD) was less than 1.5 (n=11). The limits of detection and quantitation are 0.083 and 0.25 µg.ml-1 respectively. The method is applied successfully for determination of bisacodyl in environmental water samples and in some pharmaceutical formulations (tablets and suppositories). A statistical comparison of these results with those of official method shows good agreement and indicates no significant difference in the precision.

A simple, precise, accurate, rapid and economical method for determination of bisacodyl has been developed using UV spectrophotometry [7]. The determination was carried out at absorption maxima of 264 nm using methanol and 1M glacial acetic acid as solvents. The analytical method was validated for linearity, accuracy, robustness and specificity and system suitability for both the solvents namely methanol and 1 M glacial acetic acid. The method used for determination of bisacodyl using solvents (methanol and 1 M glacial acetic acid) complies with the acceptance criteria set for the aforementioned analytical parameters. Hence, the current method stands validated. The currently developed method can be used for routine quality control quantitation of bisacodyl in suppositories.

Based on the above description can be seen that there is no research on the determination of bisacodyl tablet content by ultraviolet spectrophotometry with absorbance method and the area under the curve method. Therefore, in this study we determined the bisacodyl level in tablet formulation by ultraviolet spectrophotometry with absorbance method and area under the curve method.

2. Materials and Methods

2.1 Instrument

The instrument used in this research is UV-Vis spectrophotometer (Shimadzu UV-1800), sonication apparatus (Branson 1800), pH meter, analytical scale (Precissa XB 220A), Erlenmeyer (Iwaki Pyrex), glass cup (Iwaki Pyrex) (Iwaki Pyrex), funnel (Iwaki Pyrex), micro pipette (Iwaki Pyrex), filter paper (Whatman No. 41), dropper drip, suction ball, spatula, stirrer, parchment paper, aluminum foil, and other supporting tools research.

2.2 Materials

The materials used in this research are bisacodyl raw material (PT JPN Pharma), Dulcolax® (Boehringer Ingelheim), Custodiol® (PT Pharos), hydrochloric acid (Merck), disodium hydrogen phosphate (Merck), sodium hydroxide (Merck), citric acid (Merck).

2.3 Solvent Preparation

2.3.1 Preparation of 0.1 N Hydrochloric Acid

Dilute 85 mL of concentrated hydrochloric acid with carbon dioxide-free distilled water up to 1000 mL. Pipette 100 mL of this solution, put in a 1000 mL quantity flask and add carbon dioxide-free distilled water to the limit and stir until homogeneous.

2.3.2 Preparation of 0.1 N Sodium Hydroxide

Dissolve 162 g of sodium hydroxide crystals in 150 mL carbon dioxide free water. Cool the solution to room temperature, strain through hardened filter paper. Add 54.5 mL of clear filtrate to a sealed container and dilute it with carbon dioxide-free water up to 1000 mL. Pipette 100 mL of this solution, put in a 1000 mL quantity flask and add carbon dioxide-free water to the limit and stir until homogeneous.

2.3.3 Preparation of Phosphate-buffered pH 7.2

Mix 87.0 mL of a solution of sodium dihydrogen phosphate R 7.15 % with 13.0 mL of citric acid solution R 2.1 %.

2.4 Preparation of Bisacodyl Standard Solution 1000 ppm

2.4.1 In 0.1 N Hydrochloric Acid

Create a standard solution of bisacodyl with a concentration of 1000 ppm, by carefully weighing 50 mg bisacodyl using an analytical scale, put in a 50 mL measuring flask, then add a portion of 0.1 N HCl, shake until dissolved and then be sufficient with 0.1 N HCl until boundary mark.

2.4.2 In 0.1 N Sodium Hydroxide

Create a standard bisacodyl solution of 1000 ppm, by carefully weighing 50 mg bisacodyl using an analytical scale, put in a 50 mL measuring flask, then add a portion of 0.1 N NaOH, shake until dissolved and then be sufficient with 0.1 N NaOH to the mark limit.

2.4.3 In phosphate buffer pH 7.2

Create a bisacodyl standard solution of 1000 ppm, by carefully weighing 50 mg of bisacodyl using an analytical scale, insert it into a 50 mL measuring flask, then add a partial solution of phosphate citrate pH 7.2, shake until dissolved and then supplied with a phosphate citrate pH 7.2 to the limit mark.

2.5 Determination of Maximum Bisacodyl Absorption Wavelength

Each standard solution of bisacodyl 1000 ppm with various solvents (0.1 N HCl solution, 0.1 N HCl and phosphate buffer pH 7,2) was diluted to 100 ppm by pipetting 5 mL of the standard solution into in a 50 mL measuring flask, then dilute with each solvent until the boundary mark, stirred until homogeneous. Then each 100 ppm bisacodyl standard solution was pipetted with a 1.0 mL micropipette, fed into a 10 mL measuring flask and diluted with each solvent up to the boundary marker. The solution was stirred until homogeneous so that the concentration of 10 ppm was obtained and the absorption was measured in the wavelength range 200-400 nm with ultraviolet spectrophotometry to obtain maximum absorption wavelength.

2.6 Preparation of Calibration Curve

The standard solution of bisacodyl 1000 ppm diluted to 100 ppm in the best solvent was taken with a pipette of 1 mL, 1.5 mL, 2.0 mL, 2.5 mL, and 3.0 mL respectively, inserted into a measuring flask 10 mL. The solution is diluted to the limit, stirred until homogeneous. The concentration of the solution was 10 ppm, 15 ppm, 20 ppm, and 25 ppm, 30 ppm. The absorbent and area under the curve of each solution are measured at the maximum wavelength.

2.7 Determination of Bisacodyl Levels in Tablets

Determination of bisacodyl content in tablets was done using 20 Dulcolax Tablets and 20 Custodiol Tablets. These tablets are crushed until smooth and weighed using an analytic scale. Some tablets were weighed equivalent to 50 mg of pure bisacodyl, diluted with some of the best solvent in a 50 mL measuring flask, sonicated for several minutes until dissolved and filtered using Whatmann 41 filter paper. Then the volume was supplied with the best solvent to the limit mark. This solution is piped in 5 mL, put into a 50 mL measuring flask, diluted with the best solvent to the boundary mark, the concentration of 100 ppm is obtained. This solution is pipetted 1 mL of insert into a 10 mL measuring flask, filled its volume with the best solvent to the limit marker, and homogenized to obtain a concentration of 10 ppm. This solution measured the absorbance at a wavelength of 263.60 nm and the area under the curve with ultraviolet spectrophotometry. The bisacodyl content is calculated on the basis of bisacodyl linear regression equation.

2.8 Validation of Analysis Methods

2.8.1 Linearity Test

Linearity test is done from measurement data of calibration curve, then analyzed with linear regression so that obtained correlation coefficient (r) which shows its linearity. The good linearity value is $0.99 \leq r \leq 1$ [8].

2.8.2 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection and the limits of quantification are determined from the standard curve obtained. The value of LOD = 3.3 (SD/S) and LOQ = 10 (SD/S), standard deviation (SD) is determined based on the standard deviation of residuals from the regression line equation $y = a + bx$ and S is the slope of the regression line [8].

2.8.3 Accuracy Test

The accuracy test is carried out by the recovery test by "spiking" method by adding some standard bisacodyl solution in a test solution which is known from the added standard solution concentration of 80%, 100% and 120%, each performed 3 repetitions. Then the value of recovery of the reference standard added to the test solution is calculated as expressed by percent recovery. The method qualifies if the percent recovery is within the range of 80% to 120% [8].

2.8.4 Precision Test

The precision test was performed at the repeatability level by measuring bisacodyl standard solution concentration of 10 µg/mL, 15 µg/mL and 20 µg/mL at 3 different times in one day (intraday) with repetition of 3 times each. Measurement of bisacodyl standard solution with the same concentration was performed for 3

consecutive days (interday) with repetition each 3 times. The method was precise when RSD value between 1 to 2% [8].

3. Results and Discussion

Based on the results of the experiment by testing several solvents of 0.1 N HCl, 0.1 N NaOH and phosphate citrate buffer pH 7.2, it was found that the best solvent used for bisacodyl analysis was 0.1 N HCl. When viewed from the spectrum, bisacodyl analysis with 0.1 N HCl solvent the obtained results show λ_{max} 263.60 nm where the results obtained close to the results listed on Pharmacopeia Indonesia Edition V is 264 nm. The absorbance obtained is 0.307 where the obtained result enters the range of 0.2-0.8. In addition, on the spectrum there is only one peak, 0.1 N HCl is an inorganic solvent, non-volatile, non-toxic and environmentally friendly (see Fig. 2 – 4).
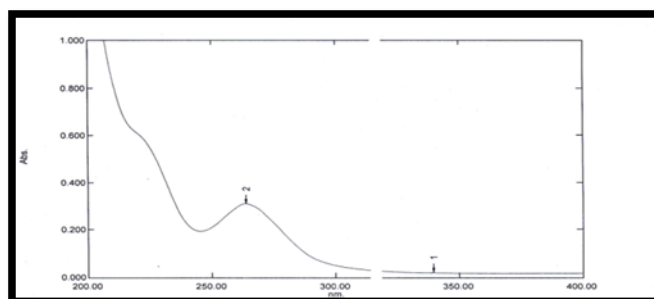

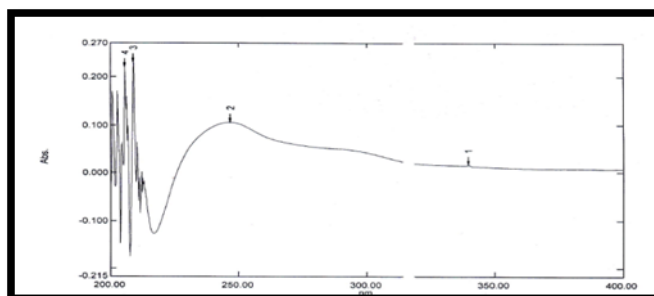Figure 2: Ultraviolet spectrum of 10 μg/mL bisacodyl solution in 0.1 N HCl

Figure 3: Ultraviolet spectrum of 10 μg/mL bisacodyl solution in 0.1 N NaOH
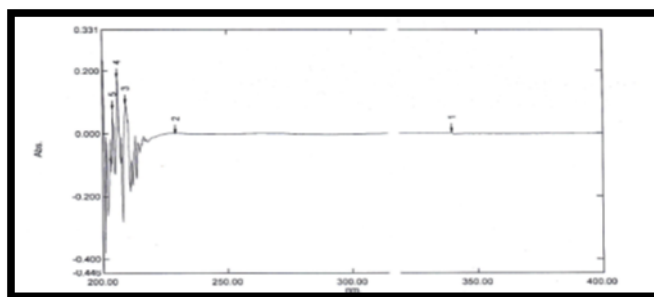

Figure 4: Ultraviolet spectrum of 10 μg/mL bisacodyl solution in phosphate citrate buffer pH 7.2

The bisacodyl calibration curve with concentrations of 10, 15, 20, 25 and 30 μg / mL is shown in Figure 5. The relationship between concentration and absorbance shows linear regression equation y = 0.02156x + 0,09258. While the bisacodyl calibration curve by looking at the relationship between the concentration with the area under the curve shows linear regression equation y = 0.30618x + 1.17400 (Figure 6).
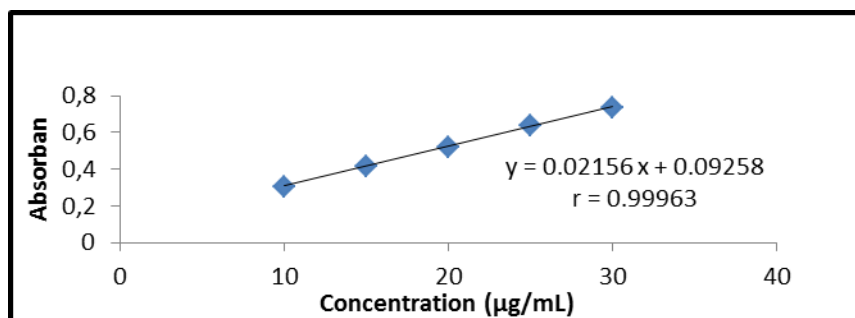

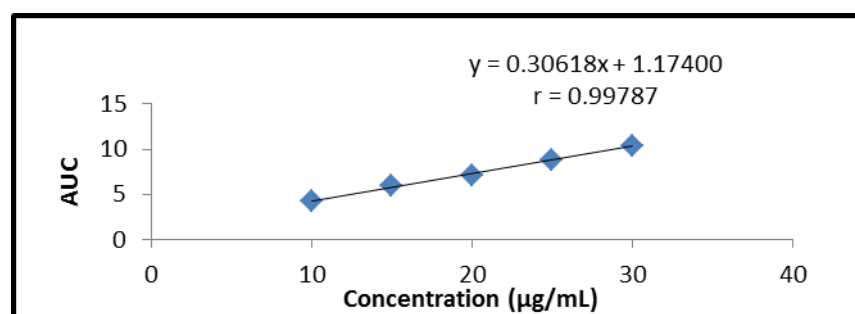Figure 5: Bisacodyl calibration curve in 0.1 N HCl with absorbance method

Figure 6: Bisacodyl calibration curve in 0.1 N HCl with area under curve method

In the determination of bisacodyl tablet sample with the trade name Dulcolax, obtained bisacodyl with absorbance method of 100.84% with standard deviation 0.009%, whereas with method of area under the curve obtained bisacodyl value equal to 96.58% with standard deviation 0.035% . Levels of bisacodyl tablets meet the requirements in accordance with Pharmacopoeia Indonesia edition V that is 90% to 110% [3].

In the determination of bisacodyl level in Tablet Custodiol with absorbance method, obtained bisacodyl level of 100.38% with standard deviation 0.008%, whereas with method of area under the curve 97.62% with standard deviation 0.008%. The bisacodyl content in the Custodiol Tablets obtained meets the requirements of Pharmacopoeia Indonesia edition V that is 90-110% [3].

Linearity is determined by processing data about the relationship between concentration (x) with absorbance (y) and concentration (x) with the area under the curve (y) obtained from the calibration curve. From the linear regression equation, then obtained the value of correlation coefficient. The calibration curve between concentration and absorbance gives linear results with r = 0.99963 and the calibration curve between the concentration and the area under the curve gives a linear result with r = 0.99787. The two correlation coefficients above have a better value because they are closer to 1 in accordance with the literature which states the acceptance criterion is the correlation coefficient value (r) close to 1 ($0.995 \le r \le 1$) [8].

The detection limits and quantification limits of bisacodyl on the absorbance method showed results of 1.411099 μg/mL and 4.276059 μg mL, respectively. While the detection limits and quantification limits of bisacodyl on the area under the curve method showed the results of 1.968774 μg/mL and 5.9659197 μg/mL, respectively.

Accuracy is measured as the number of recovered analytes. The recovery was measured by the addition of standard bisacodyls of 80%, 100% and 120%, on the Dulcolax sample. Percent return of bisocodyl in succession with absorbance method was 96.31%; 96.44%; 101.82% and the average recovery rate was 98.19%. While the percent of recovery obtained by the method of area under the curve is 97.73%; 97.64%; 105.99% and the average recovery percentage is 100.45%. In this way both methods are within the allowed range of (80% to 120%) [8].

The determination of bisacodyl intraday precision in Dulcolax Tablets is done in the morning, afternoon and evening with three different concentrations. The absorbance method showed RSD at a concentration of 10 µg/mL of 0.53%, 0.99% and 1.64%, respectively; at a concentration of 15 µg/mL of 0.31%, 0.01% and 1.0%, respectively; and at a concentration of 20 µg/mL of 0.60%, 0.27% and 0.24%, respectively. While the area under the curve method shows RSD at a concentration of 10 µg/mL of 0.58%, 1.53% and 1.45%, respectively; at 15 µg/mL concentration of 1.16%, 1.19% and 0.86%, respectively; and at a concentration of 20 µg/mL of 0.77%, 0.03% and 0.08%, respectively.

Determination of bisacodyl interday precision in Dulcolax Tablets was performed for 3 consecutive days at three different concentrations. The absorbance method at the concentration of 10 µg/mL on the first, second and third day showed RSD 0.26%, 0.51% and 0.52%, respectively; at concentrations of 15 µg mL on the first, second, and third day showed RSD 0.54%, 0.64% and 0.62%, respectively; and at concentrations of 20 µg/mL on the first, second and third day showed RSD 0.83%, 0.49% and 0.13% respectively. While the method of the area under the curve at the concentration of 10 µg/mL on the first, second, and third day showed the RSD of 0.66%, 0.57% and 0.26%, respectively; at concentrations of 15 µg/mL on the first, second and third day showed RSD of 0.83%, 0.38% and 0.62%, respectively; and at concentrations of 20 µg/mL on the first, second and third days showed RSD of 1.01%, 0.05% and 0.05% respectively.

4. Conclusion

The best solvent used for bisacodyl analysis by absorbance method and the area under the curve by ultraviolet spectrophotometry is 0.1 N HCl. The absorbance method and the wide area method under the curve indicate that both methods are valid methods for bisacodyl analysis. Methods of absorbance and wide-area methods under the curve of validated ultraviolet spectrophotometry can be used for the determination of bisacodyl tablet levels. Bisacodyl levels in Dulcolax Tablets with absorbance method and area under the curve showed 100.84% and 96.63%, respectively. Levels of bisacodyl in Dulcolax Tablet and Tablet Custodiol meet the requirements of Pharmacopeia Indonesia edition V 2014 with bisacodyl levels in tablets listed between 90-110%. There was no statistically significant difference between the absorbance method and the area under the curve method for bisacodyl analysis in tablets.

Acknowledgements

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