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THREE-WAVELENGTH SPECTROPHOTOMETRIC METHOD VALIDATION FOR DETERMINATION OF PREDNISONE TABLET CONTAINING COLORING DYES

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INTRODUCTION

Asthma is a chronic inflammatory disease that ranks fifth out of the top ten causes of morbidity[1]. The drug therapy commonly used for asthma is corticosteroid, for example is prednisone[2]. The determination of the drug in the pharmaceutical dosage form is very important to ensure its safety and efficacy. Assay method of prednisone in tablet according to Farmakope Indonesia is using High Performance Liquid Chromatography (HPLC)[3], which has good accuracy and precision, but high cost and took a longer analysis time. These aspects are making it less suitable for quality control on a regular basis. UV-Vis spectrophotometry method could be used as the alternative assay method to determine prednisone in tablet, as prednisone has chromophore and auxochrome groups[4]. UV-Vis spectrophotometry method has shorter analysis times and lower operating costs while still providing a high precision[5]. Spectrophotometric method UV-Vis has been used in previous studies of prednisone and proven to give good results, for example in the dissolution test[6,7]. Singh and Verma[8] conducted research to determine prednisone in tablets based on the color occurred after prednisone reacted with $FeCl_3$ and $K_4Fe(CN)_6$, forming a blueish green solution. The solution then measured by spectrophotometer giving the analytical wavelength at 780 nm.

The addition of coloring agents in pharmaceutical preparations intended to identify products in production and distribution, distinguishing the same dose of the drug, preventing product counterfeiting and stability indicators[9]. However, coloring agent or dye in the formulation could affect the analysis process, such as background interfering in the analytical instrument measurement. In Indonesia, usually we can find prednisone tablet in green color. Dyes used in this study is a mix of light green and tartrazine resulting in a light green color. These dyes also have a cluster of chromophore and auxochrome to provide absorbance in the UV-Vis spectrum region. Observing the prednisone solution containing 0.1% light green and tartrazine solution at the maximum wavelength of prednisone (238 nm) will give absorbance of the solution 5% higher.

To overcome this problem, the analysis of the spectrophotometry is applied by multi-wavelength method. Dual- or three-wavelength spectrophotometry methods are very useful to eliminate the absorbance influence of interfering components.

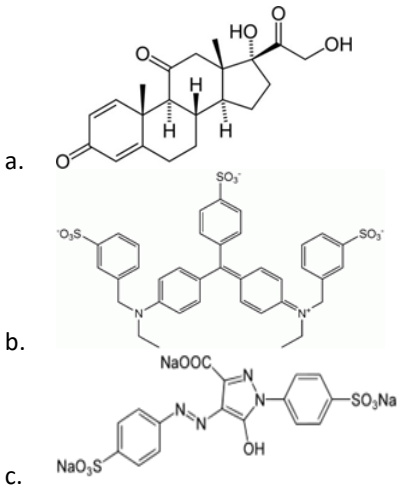

Figure 1. Chemical Structure of : a. Prednisone, b. Light green dye, c. Tartrazine

The aim of this research was validating three-wavelength spectrophotometry method to determine prednisone in tablet, containing coloring dyes. The method validation parameters defined in this research are the selectivity, linearity, accuracy and precision[3,10,11].

METHOD

Chemical

Pure prednisone, light green, and tartrazine (Zhejiang Xianju Pharmaceutical co.ltd.), methanol AR (Merck) was used for solvent.

Apparatus

Analytical instrument spectrophotometer Hitachi UH-5300 with 1 cm cuvette, analytical balance, micropipette, ultrasonic Branson 3510, laboratory glassware.

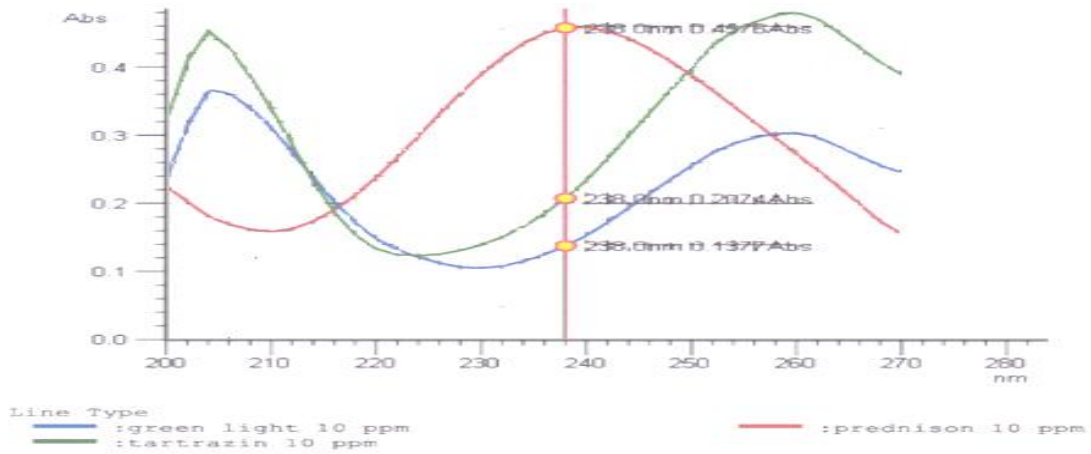

Figure 2. The overlay spectrum of prednisone, light green dye, and tartrazine.

Procedure

Standard solution

Stock solution of prednisone (1 mg mL^{-1}) was prepared by dissolving 50.0 mg of prednisone in 100.0 mL methanol.

Stock solution of light green (5 mg mL^{-1}) was prepared by dissolving 50.0 mg light green in 50.0 mL methanol. The working standard solution (10 µg mL^{-1}) was prepared by stepwise dilutions of the stock solution with methanol.

Formation of calibration curve

Aliquots containing from 5 to 17 µg mL^{-1} of prednisone were transferred into a series of 25 mL volumetric flasks. 1% light green solution and 1% tartrazine solution was added to them and the solutions were diluted to volume with methanol. The absorbance of the standard solutions was measured at λ = 234, 238 and 242 nm. These measurements were performed against the placebo prepared simultaneously.

Sample preparation for accuracy

Weighed accurately the placebo which spiked with 80%, 100% and 120% of prednisone containing in a tablet, dissolve in 5 mL methanol and transferred into 10 mL volumetric flask. The solution was ultra-sonicated for 30 minutes, diluted to volume with methanol and filtered through Whatmann filter paper No. 41. Afterward, 0.5 mL of the solution was pipetted and transferred into 25.0 mL volumetric flask, diluted with methanol to volume and shaken homogeneously.

RESULT

Based on its structure (Figure 1), prednisone has conjugated double bonds, a carbonyl group as a chromophore group, and -OH group as a group of auxochrome which can be analyzed using UV-Vis spectrophotometry. Dyes used in the tablet, in this case is a mix of light green and tartrazine, also have chromophore and auxochrome groups (Figure 1). Light green has conjugated double bonds, $-NR_2$ group, and $-SO_3$, while tartrazine has conjugated double bonds, azo group (CH_3N = NCH_3), carboxyl group (-COONa), -OH and -SO3Na. These similar chromophore of the dyes will affect the analysis result of prednisone when spectrophotometric measurement was observe at its maximum wavelength (Figure 2). To overcome the affected absorbance by the dyes, quantitative analysis was executed using three wavelengths UV-Vis spectrophotometry method. The advantage of three wavelengths spectrophotometry is the ability of the method to reduce the absorbance interfering caused by other substance in the multi components sample[8,12].

Method Validation

Selectivity

The three wavelengths was chosen by observing single standard solution of prednisone (10 µg/mL), light green (0.1 µg/mL) and tartrazine (0.1 µg/mL0, in methanol, at 200-400 nm (Figure 3). Methanl was in used as the solvent, because all the analyte were soluble in this solvent.

The pre-requisite for three-wavelength method is selection of the λ1 and λ3, such wavelength where the interfering components showed relatively same absorbance (the profile of spectra is flat) while the analyte has a significant difference of absorbance to the concentration.

According to the overlay spectra of prednisone and the mixture of dyes suggested that λ1 = 234 nm, λ2 = 238 nm (maximum wavelength of prednisone) and λ3= 242 nm were suitable to use as the analytical wavelength in this method.

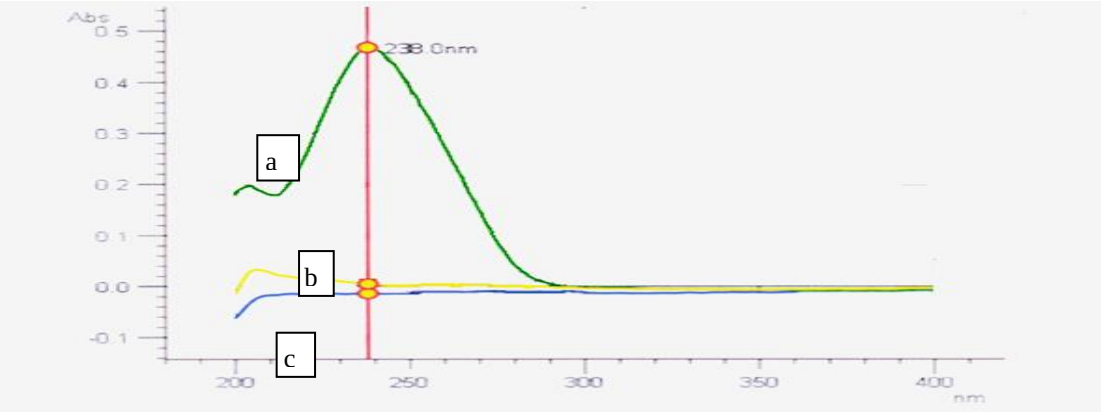

Figure 3. The overlay spectrum of : a. 10 µg/mL prednisone solution, b. 0.1 µg/mL light green solution, c. 0.1 µg/mL tartrazine solution.

Linearity

Linearity was performed through the construction of calibration curve, by plotting Δ absorbance versus concentration of the prednisone solution containing 1% light green and tartrazine (Figure 4.).

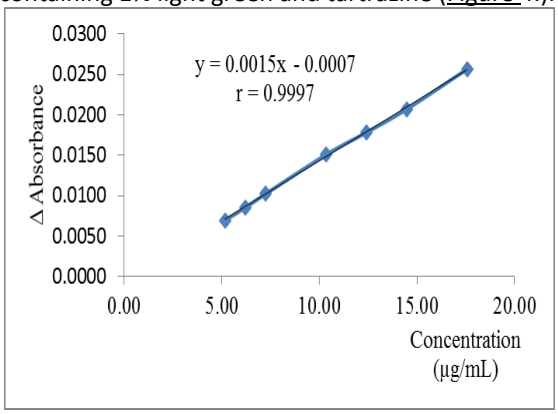

Figure 4. Calibration curve of prednisone solution containing light green and tartrazine.

Calibration curve of prednisone (Figure 4) was linier on the range of 5.0-17.0 ppm, respectively, with coefficient correlation, r, was 0.9997, Vxo was 1.18% and Xp was 0.81 µg/mL. The regression equation of the calibration curve was Y = 0.0015X – 0.0007.

Accuracy

Accuracy accomplished by spiked placebo technique to achieved percentage recovery. The percent recoveries for predisone at thre levels were found 99.53%, 9,18 nd 99.63 with 99.45 in average. The result of the recoveries study listed on the Table 1.

Table 1. % Recovery of the spiked placebo of prednisone.

Concentration of spiked placebo	% Recovery	Average	
80%	102.23		
	98.52	99.53%	
	97.83		
100%	99.83		
	99.74	99.18%	99.45%
	97.98		
120%	102.17		
	97.95	99.63%	
	98.78		

Precision

Precision were conducted for repeatability and inter mediate precision.

Table 2. Precision of the method conducted in two different days

Replicate	% Recovery	
	Day 1	Day 2
1	99,15	98,45
2	100,27	99,15
3	98,42	100,63
4	98,20	97,71
5	98,66	99,11
6	99,24	98,12
Mean	98,99	98,86
SD	0,7479	1,0301
CV	0,76%	1,04%

Based on the data (Table 2), it could be concluded that the precision was fulfill the requisite of method validation for repeatability (CV of % Recovery on day 1 was less than 2%) and intermediate precision (CV of % Recovery on day 1 and 2 are less than 2%)[13].

CONCLUSION

The three-wavelengths UV-Vis spectrophotometry method for the determination of prednisone tablet containing the coloring agent is fulfill the requirements of method validation by test of selectivity, linearity, accuracy and precision parameters. The method has prominent feature which is the simplicity, due to the needless of prior sample separation. It is also easy to be applied for routine method.

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