INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com

ISSN 2230 – 8407

Research Article

VALIDATION AND SIMULTANEOUS ESTIMATION OF BETAMETHASONE VALERATE AND NEOMYCIN SULFATE CREAM BY ABSORBANCE RATIO SPECTROPHOTOMETRY METHODS

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Article Received on: 29/10/18 Approved for publication: 02/01/19

DOI: 10.7897/2230-8407.100240

ABSTRACT

Absorbance ratio is a method that has been developed in the simultaneous determination of UV spectrophotometry. There are no research reports on this method on simultaneous estimation of betamethasone valerate and neomycin sulfate mixture in cream preparations. Therefore, this study aims to develop and validate the absorbance ratio method for the simultaneous estimation of betamethasone valerate and neomycin sulfate mixture in cream preparations with 70% ethanol as a solvent and at the intersection of wavelength 245 nm. The result showed that the regression equation for BTS is Y = 0.04039 X + 0.00349 and the regression equation for neomycin sulfate is Y = 0.00254 X + 0.00165 with validation tests were LOD was 0.645 µg/mL, LOQ was 2.15 µg/mL, accuracy was 100.07%, precision was 0.87% for betamethasone valerate and LOD was 2.215 µg/mL, LOQ was 7.385 µg/mL, accuracy was 100.68%, precision was 0.82% for neomycin sulfate. Levels of BTS was 111.1 ± 0.97% and levels for neomycin sulfate was 100.91 ± 0.37%. The proposed method has met ICH guidelines and can be used for simultaneous estimation of betamethasone valerate and neomycin sulfate in cream form.

Keywords: Betamethasone Valerate, Neomycin Sulfate, Absorbance Ratio,Validation, Spectrophotometric.

INTRODUCTION

The combination of betamethasone valerate (BTV) and neomycin sulfate (NES) in a cream form is used to treat an inflammation of an infected skin. BTV (9α-fluoro-16β-methylprednisolone) is an active semi-synthetic drug which is glucocorticoid. This drug is a strong anti-inflammatory and is an immunosuppressive agent. NES is aminoglycosides that is a group of antibiotics in which amino sugars are incorporated into glycoside bonds. This antibiotic has a broad spectrum and is bactericidal with an inhibitory mechanism in protein synthesis[1-3].

BTV can be determined by high-performance liquid chromatography (HPLC) in a glacial acetate-methanol acetic acid and UV spectrophotometry in ethanol solvents at a wavelength of 240 nm[4-5]. NES has a maximum insignificant uptake but is found in the wavelength range 230-360 nm and its potential determination is carried out microbiologically[4-6].
Kumar, K. et al. (2016) determined the concentration of BTV and NES by using spectrophotometry in derivatives with methanol and water in a ratio of 6: 4(v/v)[7].

Absorbance ratio is a method that has been developed in the simultaneous determination of UV spectrophotometry. The absorbance ratio at each of the two wavelengths is a constant value because it is free of concentration or path length[8-9].

The study aims to develop and validate the absorbance ratio method for simultaneous determination of BTV and NES combinations in a cream form.

MATERIALS AND METHODS

Instruments

UV Spectrophotometer (Shimadzu 1800) and a set of Personal Computer (PC) equipped with 2.42 UV-Probe software, Minitab®17 application

Chemicals

The raw materials of BTV and NES were obtained from Kimia Farma Plant Medan, Sumatera Utara, Indonesia. Ethanol absolute E.Merck was obtained from PT. Rudang, Medan, Indonesia. A cream form was a local product of PT Kimia Farma Plant, Medan, Indonesia.

Preparation of BTV and NES Standard Solution

The UV spectrum of 50 mg raw material BTV was dissolved in 100 ml 70% ethanol solvent to obtain standard solution which had 500 µg/mL concentration. Pipetted 5 ml of standard solution into a 50 mL volumetric flask and treated with a 70% ethanol solvent to obtain 50 µg/mL standard work solution BTV.

Weighed carefully 100 mg NES, put into a 100 mL volumetric flask, added 25 ml 70% ethanol then sonicated for 15 min. Added the solution with a 70% ethanol solvent, pipetted 80 mL into a 100 mL volumetric flask and added the solvent to the marked line to obtain a solution of 800 µg/mL standard work solution NES.

Preparation of Maximum Absorption Spectrum of BTV

Pipetted 5.5 mL of the BTV standard work solution, transferred it into a 25 mL volumetric flask, added 70% ethanol solvent up to the marked line, then BTV was scanned between 200-400 nm and wavelength 240 nm was selected for the study.

Preparation of Maximum Absorption Spectrum of NES

Pipetted 5.5 mL of the NES standard work solution, transferred it into a 25 mL volumetric flask. The volume was added using 70% ethanol solvent up to the marked line. The NES solution was scanned between 200-400 nm and wavelength 260 nm was selected for the study.

Determination of the Intersection of Absorption for BTV and NES

The intersection of absorption for BTV and NES was known by overlapping the absorption curve of 11µg/mL BTV and 170µg/mL NES and measured at wavelengths between 200 - 400 nm. The wavelength where the intersection of the two curves is the absorption point of both solutions[7-9].

METHOD VALIDATION[10-13]

The absorbance ratio technique used in this study were all validated based on validation methods required by ICH guidelines[11].

Linearity

Aliquots of BTV and NES standard solutions each pipetted carefully, each transferred into two groups of 50 mL measuring flask and added ethanol solvent to obtain the concentration range of 5.6–20.5µg/mL for BTV and 85–340µg/mL for NES[12-13].

Accuracy and Precision

The determination of precision is based on the relative standard deviation (RSD) value with the standard deviation requirement is relatively less than 2%. The determination of accuracy is done with the addition of a standard solution[10-11].

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

LOD is obtained from the lowest amount that can be detected while LOQ is the lowest limit that can be measured or quantized from the studied drug with acceptable reliability[10-11].

Preparation of Calibration Curves of BTV and NES

Different aliquots of the BTV's and NES' standard work solutions were transferred into 25 ml volumetric flask. The solutions were added with 70% ethanol, so that the final concentrations for BTS were 5.5 µg / mL; 11µg / mL; 15.5µg / mL; 20.5 µg/mL and the final concentrations for NES were 85µg/mL; 170µg/mL; 255µg/mL and 340 µg/mL. The absorption spectrum of each solution was recorded at the wavelength 240 nm for BTV and 260 nm for NES at maximum absorption spectrum and wavelength 245 nm for isoabsorption point. Analysis of the relationship between concentration and absorbance values is obtained by linear regression equation y = ax +b[12].

Determination of Absorption Index Value in the BTV and NES Combination by Absorbance Ratio Method.

The intersection absorption wavelength of the BTV and NES mixture was obtained. It was used to determine the value of the absorbance ratio by calculating the concentration of each component with the following mathematical equations[7]:

$$Ca = \frac{Qm - Qy}{Qx - Qy} \; x \; \frac{A}{a1}$$

$$Cb = \frac{Qm - Qx}{Qy - Qx} \; x \; \frac{A}{b1}$$

Simultaneous Determination of BTV and NES in a Cream Form

Weighed 10 cream tubes and homogenized them in a mortar. Carefully weighed the cream equivalent to 5 mg of NES and calculated the equivalence of BTV contained therein. Put the weighed cream into a 50 mL volumetric flask, dissolved the cream with 5 mL 96% ethanol then dissolved it with 70% ethanol solvent and homogenized them with sonicator for 15 minutes. Added 3.5 mL of NES' standard solution up to the marked line, filtered, and pipetted as much as 10 mL. Put into a 25 mL volumetric flask, then filled with 70% ethanol solvent up to the marked line to obtain a test solution containing BTV and NES and measured at the point of intersection absorption[7-9].

RESULTS

Maximum Absorption Spectrum

Determination of maximum absorption spectrum was done at 200-400 nm wavelengths for BTV at a concentration of 11 µg/ml, while for NES at a concentration of 170 µg/ml. The measurement of absorption spectrum raw material mixture was based on the sample concentration ratio where the concentration of NES and BTV was 5: 1, consisting of 5 mg NES and 1 mg BTV, while maximum absorption was at a concentration of 170 µg / ml NES. and BTV at 11 µg / ml. Therefore, the NES concentration was added for maximum absorption which fulfilled the Lambert-Beer law, which is a concentration of 170 µg/ml NES.

Preparation the sample solution of the cream was carried out by the standard addition method until it reached maximum absorption. As the measured NES uptake did not meet the Lambert-Beer Law, additions of NES were carried out in order to achieve the maximum absorption[10-12].The maximum absorption spectrum of BTV and NES can be seen in figure 1 and figure 2 respectively[5].

The intersection of absorption for BTV and NES was known by overlapping the absorption curve of 11µg/mL BTV and 170µg/mL NES and measured at wavelengths between 200 - 400 nm. The intersection of absorption are obtained at wavelengths of 234 nm and 245 nm, and a simultaneous determination of absorbance ratio was carried out at 245 nm because this wavelength is between maximum wavelength 240 nm of BTV and 260 nm of NES and the figure can be seen in figure 3.

Accuracy test is expressed in the percentage recovery which is determined by the standard addition method. The tests were carried out on three sample concentrations with a specific range of 80%, 100%, and 120% calculated from the levels in the sample, which was consisted of three repetitions containing 70% analytes

and 30% raw materials. The percentage recovery of BTV and NES in the cream can be seen in table 1 and table 2.

The determination of wavelength, regression equation and the parameter of the validation test for BTV and NES can be seen in table 3, and the absorbance ratio at 245 nm for BTV and NES, and the regression equation at Y=0.04039X+ 0.00349 for BTV and Y=0.00258X+ 0.02148 and the absorption point calculation can e seen in table 4 and table 5.

According to Harmita (2004), in the additional method is the number of samples analyzed plus standard solution with the amount needed to meet the law requirements of lambert Beer and be analyzed. The difference between the two results is compared to the actual level and statistical calculations of BTV and NES in the 5 g cream formulation can be seen in table 6 and table 7.

Table 1: Percentage recovery of BTV in the cream formulation

No	Specific range (%)	Weight			Percentage Recovery
		70% (µg/ml)	30% (µg/ml)	Recovery (µg/ml)	
1	80%	2.61	1.12	3.72	99.76
2		2.61	1.12	3.75	100.51
3		2.61	1.12	3.69	99.03
4	100%	3.26	1.40	4.68	100.51
5		3.26	1.40	4.63	99.33
6		3.26	1.40	4.66	99.92
7	120%	3.91	1.68	5.56	99.53
8		3.91	1.68	5.59	100.03
9		3.91	1.68	5.00	101.99
Average of percentage recovery					100.07

Table 2: Percentage recovery of NES in the cream formulation

No	Specific range (%)	Weight			Percentage recovery
		70% (µg/ml)	30% (µg/ml)	Recovery (µg/ml)	
1	80%	57.20	24.50	81.68	100.86
2		57.20	24.50	81.68	100.27
3		57.20	24.50	81.68	100.86
4	100%	71.48	30.63	102.11	100.86
5		71.48	30.63	102.11	101.19
6		71.48	30.63	102.11	101.51
7	120%	85.77	36.76	122.53	100.47
8		85.77	36.76	122.53	98.69
9		85.77	36.76	122.53	100.47
Average of percentage recovery					100.68

Table 3: Determination of wavelength, regression equation and the parameter of the validation test

No	Parameter	Raw Materials		Absorbance Ratio	
		BTV	NES	BTV	NES
1	Wavelength (nm)	240	260	245	245
2	Lambert-Beer Law	0.2 – 0.8	0.2 – 0.8	0.2 – 0.8	0.2 – 0.8
3	Regression equation	Y=0.04026X+ 0.00146	Y=0.00254X+ 0.00165	Y=0.04039X+ 0.00349	Y=0.00258X+ 0.02148
4	Coefficient of correlation (R^2)	0.9995	0.9999	0.9995	0.9969
5	LOD (µg/ml)	0.645	2.15	0.701	2.61
6	LOQ (µg/ml)	2.15	7.385	2.50	7.601
7.	Precision	0.87%	0.82%	0.87%	0.82%
8.	Accuracy	100.72%	100.74%	100.07%	100.68%

Table 4: Absorbance ratio with the regression equation

Sr.No	Ca (µg/ml)	Cb (µg/ml)
1	1.60	38.84
2	1.67	38.52
3	1.72	37.98
4	1.65	38.80
5	1.63	38.85
6	1.60	38.17

Table 5: Absorbance ratio with the absorption point calculation

Sr.No	Ca (µg/ml)	Cb (µg/ml)
1	3.16	30.6
2	3.15	31.2
3	3.20	30.3
4	3.17	30.5
5	3.18	31.3
6	3.15	30.4

Table 6: Analysis of NES and BTV in the cream formulation

Replication	Level Analysis		Level Percentage	
	NES	BTV	NES	BTV
1	25.26	5.580	101.06%	111.60%
2	25.15	5.580	100.62%	111.60%
3	25.15	5.519	100.62%	110.38%
4	25.26	5.580	101.06%	111.60%
5	25.26	5.551	101.06%	111.02%
6	25.26	5.519	101.06%	110.38%

Table 7: Statistical calculations of BTV and NES in the 5 g cream formulation

No.	Drug	Mean (%)	SD (%)	Level requirements (%)
1	BTV	111.1	0.970	(90 -115)
2	NES	100.91	0.370	(90 – 135)

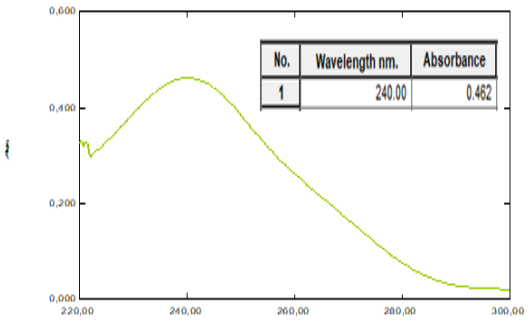

Figure 1: Maximum absorption spectrum of 11 µg/mL BTV

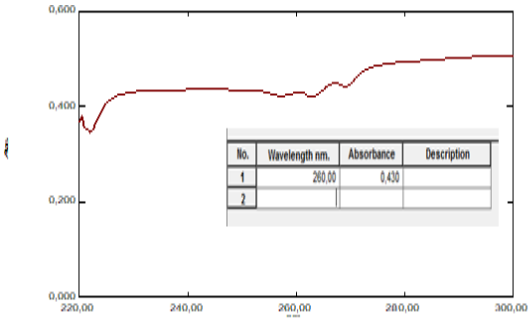

Figure 2: Maximum absorption spectrum of 170 µg/mL NES

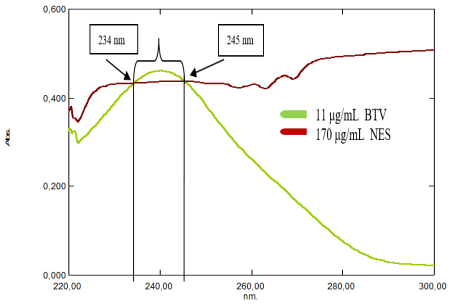

Figure 3: Intersection absorption point of BTV and NES at 234nm and 245nm

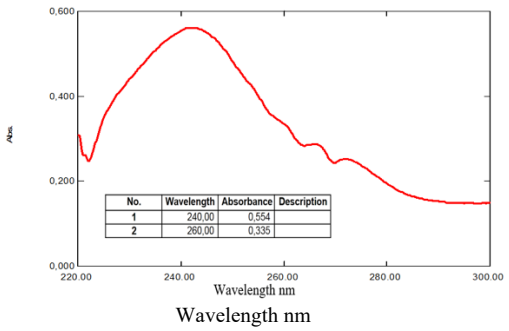

Figure 4: Absorption spectrum of BTV and NES combination at 240 nm and 260 nm

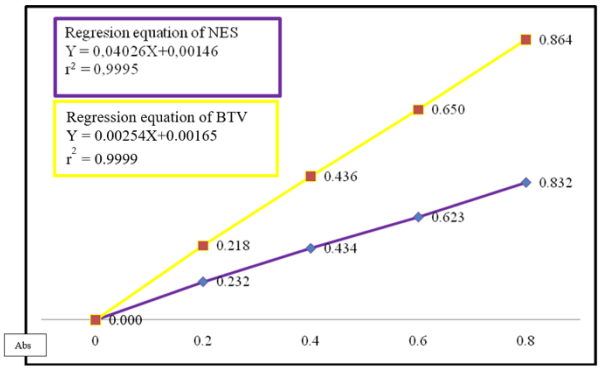

Figure 5: Calibration curve and regression equation of BTV at 240 nm and NES at 260 nm

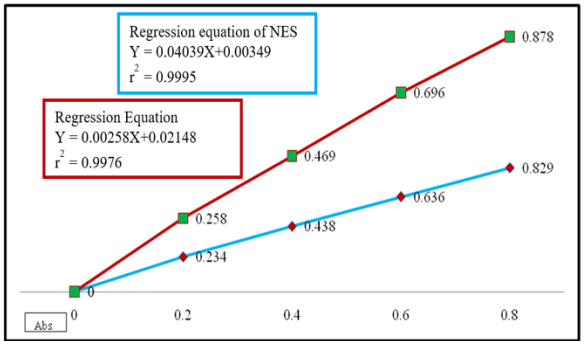

Figure 6: Calibration curve and regression equation of BTV and NES at 245 nm

DISCUSSION

Based on figure 1 and figure 2 can be seen that the qualitative absorption spectrum for each BTV and NES were obtained. The maximum absorption for BTV was at 240 nm and for NES was at 260 nm. Indonesian pharmacopoeia stated that the quantitative determination of samples could be carried out if the maximum absorption wavelength was not more than 2 nm than what was stated in the literature. It meant that 240 nm for BTV and 260 nm for NES could be used for sampling.[4]

In figure 3, the overlapping of the BTV and NES absorption curves was obtained by two absorption intersection points at 234 nm and at 245 nm. To calculate the levels of BTV and NES in the sample by the absorbance ratio method, the sample measurements were carried out at the 245 nm absorption point because at this is the point of the intersections between the BTV absorption spectrum at 240 nm and the NES absorption spectrum at 260 nm.

Erram and Typical in Kamal et al 2016 stated about the development of an intersection point method. This method could only be used for a spectrum of two components that had the same absorptivity at a point which was called an iso absorptivity point. Experimental testing could be done with this method in order to obtain measurement values at certain concentrations of two drug mixtures whose results were compared with the measurements on the absorbance spectrum of each which had the same absorptivity, so that the ratio absorbance value of each drug was obtained.[7]

Determination of Absorption Spectrum for BTV and NES in the Cream

Determination of absorption spectrum was made using a standard solution of 11 µg / mL BTV and 170 µg / mL NES and measured at a wavelength of 200-400nm and obtained maximum absorption from BTV at 240 nm and NES at 260 nm. This fulfills the requirement that maximum absorption for BTV and maximum absorption of NES is not significant at 230-360nm[2].

Validation of LOD, LOQ, Accuracy, and Precision[10-13]

The precision value for analytes was not more than 2%. The average yield was in the range of 80-120% The RSD values were both less than 2%[10].
Based on the obtained curve, a linear relationship is achieved if the values of b = 0 and r = +1 or -1 depending on the direction of the line. While the value of a indicates the sensitivity of the analysis, especially the instruments used[11-13].

The obtained results showed that the method had a good equality. That meant the absorbance ratio method for simultaneous evaluation of BTV and NES combination in the cream had fulfilled the validation requirements because it met all validation parameters[10--11].

CONCLUSION

The absorbance ratio method by ultraviolet spectrophotometry could be used to determine the levels of BTV and NES combination in a cream and met the requirements of accuracy, precision, linearity, LOD, and LOQ.

ACKNOWLEDGMENT

The authors wish to thank for Prof. Runtung., Rector of Universitas Sumatera Utara and Prof Erman Munir., Chairman of the USU Research Institutes in conducting research of USU Talenta 2018.

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Cite this article as:

Muchlisyam Bachri *et al*. Validation and simultaneous estimation of betamethasone valerate and neomycin sulfate cream by absorbance ratio spectrophotometry methods. Int. Res. J. Pharm. 2019;10(2):48-52 http://dx.doi.org/10.7897/2230-8407.100240

Source of support: Universitas Sumatera Utara, Conflict of interest: None Declared

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