

# Chemometric Calculation for Determination of Betamethasone and Neomycin Mixture in Cream Supply by Ultraviolet Spectrophotometry

Muchlisyam Bachri<sup>1\*</sup>, Julia Reveny<sup>2</sup>, Yade Metri Permata<sup>1</sup>, D. N. Panjaitan<sup>1</sup>

<sup>1</sup>Chemistry Department, Faculty of Pharmacy, Universitas Sumatera Utara, Padang Bulan, Medan, Indonesia; <sup>2</sup>Technologie Department Faculty of Pharmacy, Universitas Sumatera Utara, Padang Bulan, Medan, Indonesia

## Abstract

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**\*Correspondence:** Muchlisyam Bachri. Chemistry Department, Faculty of Pharmacy Universitas Sumatera Utara, Padang Bulan, Medan, Indonesia. E-mail: muchlisyam@usu.ac.id

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**BACKGROUND:** Chemometric can be defined as a branch of analytical chemistry using statistical principles to design and select optimal analytical procedures and experiments. The chemometric technique that applies in the design of quantitative calibration curves in the spectral analysis is very important in quality control of the component contained in the drug mixture of 2 or 3 drug components or more, especially the component that has the adjacent wavelength when the spectrum overlap.

**AIM:** The purpose of this study was to conduct research that examines betamethasone and neomycin mixture in cream with the UV spectrophotometric methods using a chemometric calculation.

**METHODS:** Chemometric calculation for determination of betamethasone and neomycin mixture in cream supply by ultraviolet spectrophotometric.

**RESULTS:** The result of betamethasone and neomycin levels were 91.35% and 97.56%, relative standard deviation (RSD) for betamethasone and neomycin 0.93%; 1.73% and recovery percentage 99.09%; 99.94%. On the multivariate calibration of PLS betamethasone and neomycin with each RMSEC value of 0.0230 and 0.3553 with the value of RMSECV 0.7187 and 0.3586 with RMSEP value 0.1558 and 0.0820. Thus, the predictive ability of the research is still acceptable and is well used for grade determination of betamethasone and neomycin content fulfil the requirement for cream preparation according to USP edition XXX.

**CONCLUSION:** Methods of UV spectrophotometric with chemometric can be used in the determination of BET and NEO levels in cream preparations and BET level is 91.35% with a range of 90-110%, and NEO level is 97.56% with a range of 94.45-98.71%. These levels have met the requirements of the levels listed in Indonesian Pharmacopoeia, 2014.

## Introduction

Betamethasone (BET) is an active semi-synthetic pharmaceutical ingredient which is a glucocorticoid. This drug contains fluorine, has a big workforce, but long-term use of fluoride-containing corticosteroid drugs may lead to a permanent capillary and arteries dilation that causes skin atrophy occurs [1]. Betamethasone (BET) can be determined by using UV Spectrophotometry on ethanol solvent at 240 nm wavelength [2].

Neomycin (NEO) is an aminoglycoside class antibiotic which is a group of antibiotics whose amino sugar is incorporated in a glycoside bond. This antibiotic has a broad spectrum and is bactericidal by

the inhibitory mechanisms of protein synthesis. This antibiotic binds to the 30S subunit of the bacterial ribosome (some also bind to 50S subunit of the ribosomal) and inhibits peptidyl-tRNA translocation from site A to site P, and causes errors in mRNA readings and results in bacteria unable to synthesise vital proteins for growth [3].

The combination of BET and NEO is produced by several drug factories. This medicinal mixture is used to treat skin diseases such as inflammatory manifestations and pruritus in severe or severe psoriasis and corticosteroid-sensitive dermatosis [3].

The ultraviolet spectrophotometric method is modified so that it can be used for mixed drug analysts having more than has a maximum absorption

with two adjacent wavelengths. Therefore, the chemometric method is performed. Chemometric can be defined as a branch of analytical chemistry that using statistical principles to design and select optimal analytical procedures and experiments. The chemometric technique that applies in the design of quantitative calibration curves in the spectral analysis is very important in quality control of the component contained in the drug mixture of 2 or 3 drug components or more, especially the component that has the adjacent wavelength when the spectrum overlap. Besides that, this technique is successfully applied to all analytical methods in spectrophotometry [4]. Spectrophotometry ratio absorbance and area under the curve are two valid modified to quantify BET and NEO in cream [5], [6].

Another method of drug analysis for the assay was UV spectrophotometry combined with the multivariate calibration of PLS. This method can determine the level of multicomponent compounds that have overlapping problems on UV spectra [7]. Various studies have been published that used chemometric among them are Moxifloxacin and Metronidazole [8], and Simultaneous Determination of Two Anti-Hypertensive Drugs [9].

Based on the above, it's an interesting idea to conduct research that examines BET and NEO mixture in cream with the UV spectrophotometric methods using a chemometric calculation.

## Material and Methods

The apparatus in this research were UV Spectrophotometer (Shimadzu 1800) and a set of Personal Computer (PC) equipped with 2.42 UV-Probe software, Minitab® 17 application, 1 cm cuvette, glass tools (Oberoi), analytical balance (Boeco Germany). The raw material of betamethasone valerate, neomycin sulfate from Kimia Farma Plan Medan, Sumatera Utara, Indonesia, and ethanol absolute (Merck). The sample is Betason N Cream contains BET 1 mg, and NEO 5 mg is a local product by PT Kimia Farma Plan Medan Indonesia.

The procedure started by making a standard spectrum. Thoroughly weighed 50 mg raw material of BET, put into a 100 mL volumetric flask, filled the volume with 70% ethanol solvent up to the mark line to obtain of 500 µg/mL (standard solution I)), then pipetted 5 ml of standard solution I into a 50 mL volumetric flask and treated with a 70% ethanol solvent up to a mark line (50 µg/mL) (standard solution II). Weighed carefully 100 mg NEO, put into a 100 mL volumetric flask, then added 23 ml distilled water and 96% 25 ml ethanol then sonification for 15 min, sufficient the volume with 96% ethanol solvent up to t mark line to obtain solution with 1000 µg/mL

concentration (standard solution I), then pipetted 80 mL of standard solution I into a 100 mL volumetric flask and added solvent to the mark line to obtain a solution of 800 µg/mL (standard solution II). Pipetted each 3.5 mL; 6.0 mL; 8.5 mL and 11 mL of standard solution II of BET into a 25 mL volumetric flask, then the volume added by using a 70% ethanol solvent up to the mark line to obtain concentrations of BET solution of 5.5 µg/mL; 10.5 µg/mL; 11.5 µg/mL and 15.5 µg/mL respectively. Then made the absorption spectrum of BET. Pipetted each 3.0 mL; 5.5 mL; 8.0 mL and 11.0 mL standard solution II of NEO into a 25 mL volumetric flask, then the volume added by using a 96% ethanol solvent up to the mark line to obtain a concentration of neomycin solution of 85 µg/mL; 170 µg/mL; 255 µg/mL and 340 µg/mL respectively. Then made the absorption spectrum of NEO.

Pipetted 5.5 mL each of standard solution II of BET and NEO was introduced into a 25 mL volumetric flask, then its volume was added using 70% ethanol solvent up to a marked line with a concentration of 11 µg/mL, then measured its uptake at 200-400 nm wavelength.

Maximum absorption spectrum of BET and NEO mixture made by pipetted a 0.8 mL standard solution II of BET and 0.9 mL standard solution II of NEO into a 10 mL volumetric flask, then the volume added by using an ethanol solvent up to the mark line to obtain a solution containing a mixture of BET and NEO with a concentration of 4 µg/mL and 8.7 µg/mL respectively. Then made the absorption spectrum of BET and NEO mixture.

Method validation of this method done based on validation methods performed by Harmita (2004) includes linearity, accuracy, precision, the limit of detection and limit of quantification (LOD & LOQ) [10].

The calculation of PLS Multivariate Calibration Model done by grouping the concentration data and the absorbance of the calibration solution that was presented in the Microsoft Excel software paperwork were transferred into Minitab 17 working papers with the copy-paste function. Processed statistical data of partial least square (PLS) is selected by using the Stat option on the work panel of Minitab 17. then selected regression partial least square. The model of PLS of BET was performed using; response column is filled with a choice of variable concentration of BET and model column is selected the absorbance variable at 200-400 wavelength with 3 nm interval. Acquired the calculated value and the true value of the multivariate calibration model of PLS of BET [11], then transferred back into Microsoft Excel software working paper with copy-paste function to obtained R<sup>2</sup> and RMSEC. The same treatment for the NEO calibration process.

Cross-Validation Leave-one-out did by transferred data from Microsoft Excel software working paper by using copy-paste function into Minitab 16 working paper. Selected calibration model

of PLS by pressing the stat option, then selected regression partial least square. Enter the BET concentration variable into response and absorbance variable into the model. Then press the options button, which further specifies the leave sequential processes one at a time.

Then performed the same treatment for the NEO validation process. Acquired the actual values and the calculated values, as well as press values from the internal validation stage and then transferred to Microsoft Excel software working papers with the copy-paste function. The accuracy and precision of the calibration model are shown by R<sup>2</sup> and RMSECV values [11].

Determination of BET and NEO levels in cream done used 10 tubes of sample cream, then removed its contents and homogenised. Weighed the cream and recorded the results of weighing. Weighed carefully an amount of the cream that equivalent to 1 mg BET (weighing is done as much as 6 repetitions) and calculated the equivalence of NEO that contained therein. Incorporated the cream that had been weighed into a 50 mL volumetric flask, dissolved with 96% ethanol solvent and homogenised with sonication for 15 minutes. Then added 0.94 ml standard solution II NEO and added 70% ethanol up to the marked line. Filtered, and disposed of the first filtrate as much as 10 ml then accommodated the next filtrate. Pipetted 5 mL and put into a 25 mL volumetric flask. Measured the uptake obtained, calculated level with multivariate PLS calibration method [12].

Calculation of sample concentration with a coefficient of each model for BET compound and NEO compound did according to the formula of [11], [12]:

$$X = t1p1 + t2p2 + \dots + tpsps + \epsilon$$

Description:

X = Concentrations of calculated samples

ts = Coefficient of the calibration model

ps = Absorbance of each sample size

ε = Correction of possible errors in the PLS calibration model

Calculated levels by using the formula (Rochman, 2007):

$$Level\left(\frac{mg}{ml}\right) = (Ct\left(\mu\frac{g}{ml}\right) \times Fp) \times \frac{X(\text{total mg in cream})}{X(mg)}$$

Description:

Ct = The sample concentration is predicted by the model

Fp = Dilution Factor

X = Average weight of the total weight of the cream sample

X = Weight of sample weighing (mg)

## Results

Determination of maximum absorption spectrum was done at 200-400 nm wavelengths. Figure 1 shows the maximum absorption spectra of BET concentrations of 11 µg/ml and NEO170 µg/ml.

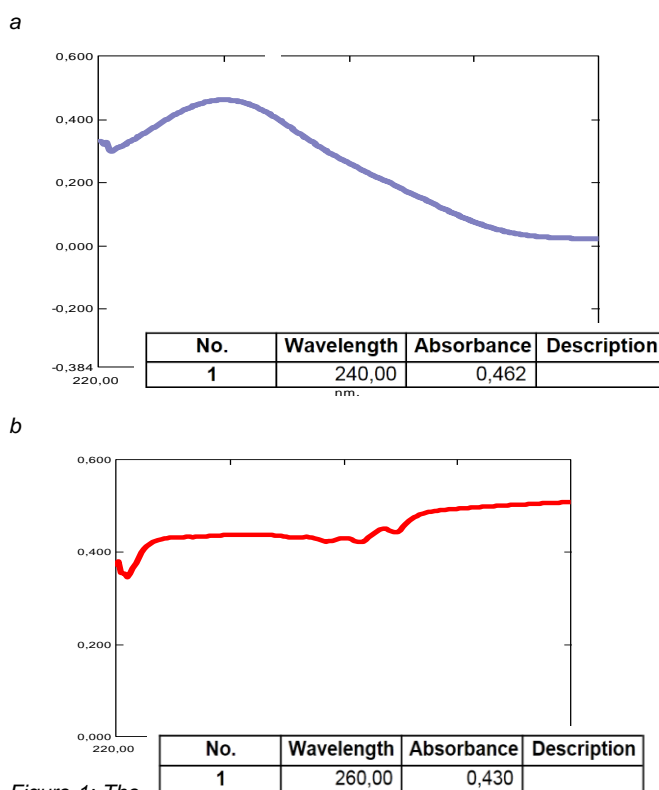


Figure 1: The maximum absorption spectrum of A) BET (11 µg/ml), B) NEO (170 µg/ml)

Figure 2 describes the overlapping spectrum of BET and NEO.

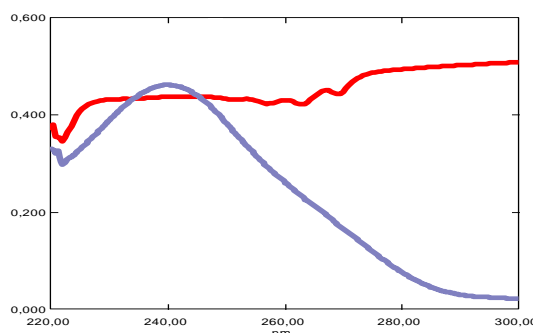


Figure 2: The overlap maximum absorption spectrum of BET (blue) and NEO (red)

The result of the determination of absorption spectrum was made to BET solution with concentration 11.13 µg/mL and NEO solution with concentration 170 µg/mL; then the absorption spectrum was made at 200-400 nm wavelength. The standard mixed absorption spectrum of BET (11.13 µg/mL) and NEO (170 µg/mL) can be seen in Figure 3.

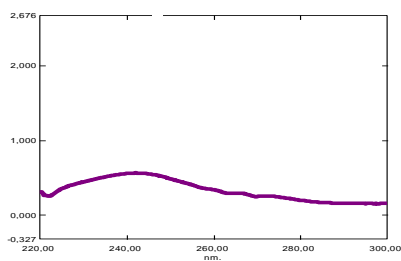


Figure 3: The standard mixed absorption spectrum of BET (11.13 µg/mL) and NEO (170 µg/mL)

Table 1 illustrated the actual value and the calculated value of each drug.

Table 1: The actual value and the calculated value using UV-PLS spectrophotometry without cross-validation

Number	Concentration			
	BET		NEO	
1	5.6	5.6001	85	84.9970
2	5.6	5.6001	85	85.0000
3	10.5	10.5089	160	159.9110
4	10.5	10.4964	160	160.0330
5	11.13	11.0873	170	170.6430
6	11.13	11.1673	170	169.2260
7	15.50	15.4942	255	255.1600
8	15.50	15.5095	255	255.0070
9	18.00	17.9695	273	273.5010
10	18.00	18.0230	273	272.8060
11	20.50	20.5306	340	339.7770
12	20.50	20.5002	340	339.9400

The curve of the relationship between the calculated values and the actual values of BET content in the calibration set can be seen in Figure 4.

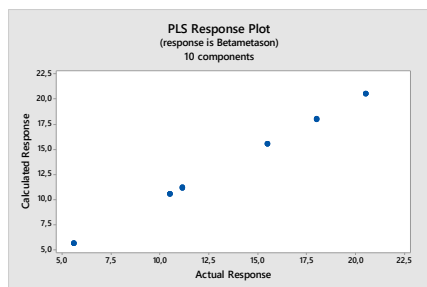


Figure 4: Curve relationship between actual BET content (actual response) and the calculated value of BET using UV-PLS spectrophotometry without cross-validation

The curve of the relationship between the calculated values and the actual values of NEO levels in the calibration set can be seen in Figure 5.

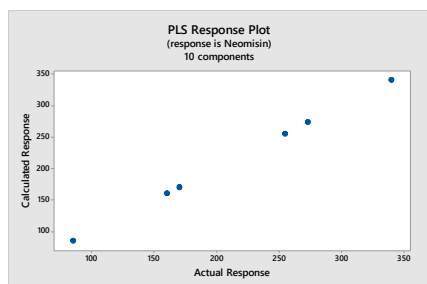


Figure 5: Curve relationship between actual NEO levels and the calculated response of NEO using UV-PLS spectrophotometry without cross-validation

Table 2 is the result of actual concentration and calculated of BET, and NEO concentration then processed using 2013 MS. Excel software to determine the value of R<sup>2</sup> and RMSEC.

Table 2: Result equations, R<sup>2</sup> and RMSEC obtained from the relationship between the actual value and calculated value using UV-PLS spectrophotometry without cross-validation

	BET	NEO
Equation	Y = 0.99937x + 0.00626	Y = 1.000002x - 0.00042
R <sup>2</sup>	0.9999	0.9999
RMSEC	0.02301	0.3554

Table 3 is the actual value (actual response) and the calculated value (calculated response) using UV-PLS spectrophotometry with cross-validation.

Table 3: The actual value (actual response) and the calculated value (calculated response) using UV-PLS spectrophotometry with cross-validation

Number	Concentration			
	BET		NEO	
1	5.6	5.1683	85	84.995
2	5.6	6.3286	85	84.999
3	10.5	10.6429	160	159.782
4	10.5	11.4842	160	160.068
5	11.13	10.5563	170	170.81
6	11.13	11.0951	170	169.346
7	15.5	14.8373	255	255.016
8	15.5	16.1939	255	254.875
9	18.0	17.7972	273	273.525
10	18.0	16.6232	273	272.665
11	20.5	20.1468	340	339.969
12	20.5	21.5863	340	339.949
R <sup>2</sup>	0.9794		0.9999	
RMSECV	0.7187		0.3582	

The profile of the relationship between calculated levels and actual levels of BET can be seen in Figure 6.

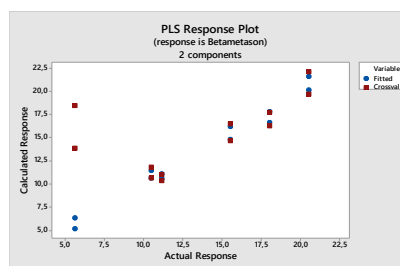


Figure 6: Relationship curve between actual response BET content and the calculated response value of BET using PLS UV spectrophotometry with cross-validation

The profile of the relationship between calculated levels and actual levels of NEO can be seen in Figure 7.

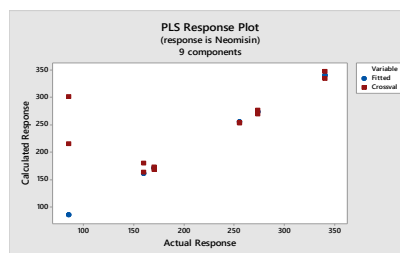


Figure 7: Curve relationship between actual response NEO levels and calculated response values NEO using UV-PLS spectrophotometry with cross-validation

External validation was performed by setting the concentration of 12 validation solutions. Table 4 is the actual value is the value made in the process of determining the concentration of the solution, while the calculated value is the value obtained from the determination of the concentration of the solution with the coefficient of calibration model that has passed the internal validation process.

**Table 4: Evaluation of actual values and calculated values of PLS calibration results from 12 validation solutions set containing BET and NEO.**

Number	Concentration			
	BET		NEO	
1	7.6	7.628	85	85.001
2	7.6	7.5628	85	85
3	7.6	7.6707	160	160.002
4	7.6	7.6280	160	160.003
5	12.8	12.8601	255	255.002
6	12.8	12.6758	255	255.001
7	12.8	12.2413	85	84.799
8	12.8	13.4187	85	85.201
9	18.0	17.9022	160	159.999
10	18	18.2079	160	160.001
11	18	17.7024	255	254.999
12	18	18.1055	255	255.002
equation	Y = 1.0008x - 0.00132		Y = 0.9999x + 0.00008	
R <sup>2</sup>	0.9960		0.9999	
RMSEP	0.15585		0.08207	

Table 5 shows the result of the validation method based on calibration data of BET and NEO obtained LOD, LOQ, accuracy and precision for BET and NEO.

**Table 5: Calculation Result of LOD, LOQ, Accuracy and Precision**

Number	Content	LOD	LOQ	Accuracy	Precision
1	BET	0.6481	2.1604	100.07%	0.2505
2	NEO	11.2184	37.3949	100.68%	0.7112

Determination of the sample content of BET and NEO was done by the spectrophotometric method of PLS. Table 6 and 7 shown the determination of BET and NEO prediction level in cream using UV-PLS Spectrophotometric method. For the concentration of BET and NEO in the sample to be in the range of calibration curve, each sample can be done standard addition method, and can also be diluted first with certain dilution factor. Pipetted 45 mL the standard solution II of NEO for standard addition method employed.

**Table 6: Determination of BET Prediction Level in Pharmaceutical Form of Cream Using UV-PLS Spectrophotometric Method**

Evaluation of BET	Sample					
	1	2	3	4	5	6
Calculated	1.8471	1.8286	1.8178	1.8075	1.8028	1.8057
Weighing	47954.6	47909.4	47871.5	47954.4	47909.3	47871.6
Dilution	5	5	5	5	5	5
Average	4791.2	4791.2	4791.2	4791.2	4791.2	4791.2
Weight (tube)						
Etiquette (mg/5g)	1	1	1	1	1	1
mg/5g Cream	0.9235	0.9143	0.9356	0.9037	0.9014	0.9028
Average						
Content	0.9136 = 91.36%					
SD	0.0137					
RSD	0.2505%					

This is done because the absorption of NEO in the sample is very small to be within the measurement area 0.2-0.6 absorption range so can be in the absorbance range under Lambert-Beer law, so it needs to be done addition of NEO standard solution II to the sample solution.

**Table 7: Determination of Predicted NEO Level in Cream of Pharmaceutical Preparation Using the UV- Spectrophotometric Method with PLS calculation**

Evaluation of NEO	Sample					
	1	2	3	4	5	6
Calculated	9.876	9.793	9.75	9.713	9.704	9.695
Weighing	47954.6	47909.4	47871.5	47954.4	47909.3	47871.6
Dilution	5	5	5	5	5	5
Average	4791.2	4791.2	4791.2	4791.2	4791.2	4791.2
Weight (tube)						
Etiquette (mg/5g)	5	5	5	5	5	5
mg/5g Cream	4.938	4.8965	4.875	4.8565	4.852	4.8475
Average						
Content	4.8776 = 97.56%					
SD	0.0347					
RSD	0.7112%					

## Discussion

Determination of maximum absorption spectrum was done at 200-400 nm wavelengths. Measurements of BET at a concentration of 11 µg/ml, while for NEO at a concentration of 170 µg/ml. Based on the research results, obtains the maximum wavelength of BET at 240 nm and NEO at 260 nm. Based on Figure 1 and 2 it can be seen that the overlap of BET absorption and NEO absorption has a coincide absorption, it's mean that BET and NEO cannot be determined by using the classical spectrophotometric method, then the determination of this mixture content is used spectrophotometric method with chemometric calculation. Because of the concentration of BET in the sample was too low, so that the resulting absorbance was too low. Therefore, standard addition should be done. The absorption spectrum of 11.13 µg/mL BET and 170 µg/mL NEO (Figure 3). The calibration set consists of the true value and the calculated value (Table 1). The calibration sample is modelled by a multivariate partial least square calibration [12]. Modeling is conducted with wavelength 200-400 nm with 3 nm interval. This election aims to obtain optimum model performance even though the PLS method of computerisation can cover the entire spectrum [11], [12].

The predictive capabilities of the multivariate calibration model are validated by two processes: internal validation and external validation. Internal validation aims to address the overfitting problems that often occur in the modelling process, whereas external validation aims to determine the prediction capability of the entire multivariate PLS calibration model [12]. The relationship between the

true value and the validated predicted value or cross-validation with the leave-one-out technique with the PLS method at 200-400 nm wavelength (Table 3). Internal validation results can be seen in Table 5.

Based on Table 3, it can be seen that the coefficient of determination ( $R^2$ ) generated  $R > 0.9$  and low RMSECV value (close to zero) then the model's ability to predict to be better. Cross-validation can also determine the number of optimum components that characterise the data [13]. Based on this cross-validation, each of BET and NEO has 10 components. Based on Table 4, it can be seen that describes the prediction capabilities of the PLS calibration model obtained by each cross-validation phase. Acceptable predictive ability on external validation is seen with the  $R^2$  values obtained by betamethasone is 0.9959641 and neomycin is 0.99998 and RMSEP of betamethasone is 0.1558495 and neomycin is 0.082071. According to the parameters generated from the cross-validation test with *leave-one-out* technique and external validation that has been done, it can be concluded that this model is still well used for the determination of the sample content of pharmaceutical preparation with the value of  $R^2 > 0.9$ , RMSEP and RMSECV are small.

Table 5 shows that the LOD is the lowest analytical concentration in a sample that can still be detected. LOQ is defined as the lowest concentrations of analytes in a sample that can still meet thorough and thorough criteria. According to Harmita (2004), the precision value for analytes with part per million is not more than 2%. From the results obtained shows that the spectrophotometric with chemometric calculation is a reliable method [10].

Based on Figure 6 and Figure 7, it's been seen that all sample measurements are above are within the range of LOD and LOQ. The results of percentage recovery of accuracy the predetermined requirements. If the average yield is in the range of 80-120% [14], then the percentage of recovery according to Harmita (2004) the relative standard deviation value of part per million (ppm) is not more than 2% [10]. The profile of the relationship between calculated levels and actual levels of BET and NEO can be seen in Figure 6, and 7 indicates good of accuracy. Based on the results of the determination of the sample rate obtained RSD value for BET and NEO respectively 0.25% and 0.71%, so that both drug compounds have good precision. BET levels 91.35% with a range of 90-110% and NEO levels 97.56% with a range of NEO levels of 94.45-98.71%. The levels are compliant with the levels shown on USP XXX [15].

In conclusions, it can be concluded that based on the research, the methods of UV spectrophotometric with chemometric can be used in the determination or quantitative analysis of BET and NEO levels in cream preparations. BET level is

91.35% with a range of 90-110%, and NEO level is 97.56% with a range of 94.45-98.71%. These levels have met the requirements of the levels listed in Indonesian Pharmacopoeia V [1].

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