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ORIGINAL ARTICLE

Validated Spectrophotometric Method for Determination of Polymaxin-B Sulfate in Pharmaceutical Formulations

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Keywords: *Spectrophotometric, validation, PMBS complex.*

Author`s Contribution
All the authors contributed significantly to the research that resulted in the submitted manuscript.

Article info.
Received: November 09, 2017
Accepted: December 01, 2017

Funding Source: *Nil*
Conflict of Interest: *Nil*

Cite this article: *Mannan A, Khan AJ, Khan M, Abbas G. Validated Spectrophotometric Method for Determination of Polymaxin-B Sulfate in Pharmaceutical Formulations. RADS J. Pharm. Pharm. Sci. 2017;5(4):33-38.*

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ABSTRACT

Objective: The simple and selective spectrophotometric method have been proposed for the determination of PMBS, in pure form and their pharmaceutical formulation.

Method: The method is based on the formation of color complex between ferric chloride and PMBS, actually, vanillin forms imine in the presence of buffer at 60-70°C with PMBS. The imines are the good legends for transition metals. Here the green color is the color of complex of iron and PMBS, which absorbs EMR at 600 nm.

Results: Different variables and parameters affecting the reaction was studied and optimized. Beers plots were obeyed in a general concentration range 50ppm – 0.5mg/ml with co-relation co-efficient not less than 0.9991. The proposed method was successfully applied to the analysis of the cited drugs in their dosage forms.

Conclusion: The proposed method was validated according to ICH and USP guidelines with respect to specificity linearity, accuracy, precision, robustness and ruggedness.

INTRODUCTION

Polymaxin B Sulfate (PMBS) is an antibiotic primarily used for resistant gram Negative infections. It is derived from the bacterium Bacillus Polymexa. Polymaxin b is composed of a number of related compound, Such as B1-1, B2, B3 and B6. Polymaxins B1 and B2 are considered major components [1]. The related compounds are structurally identical with the exception of a variable fatty acid group on each fraction. Results from in vitro studies have shown marginal differences in Mic data when comparing the fractions. The chemical structure of PMBS is shown in Figure **1**.

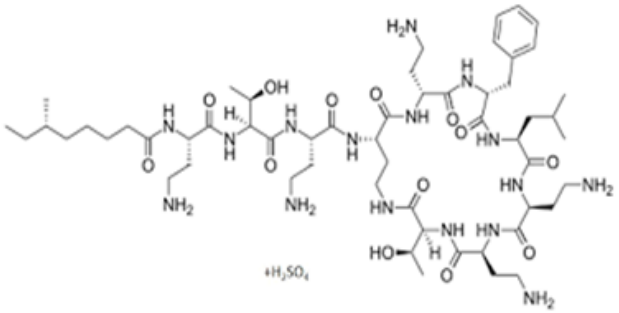

Figure 1. Polymaxin-B Sulfate.

It has a bactericidal action against the proteus and Neisseria genera. Polymaxin bind too the cell membrane and alter its structure,making it more permeable.The resulting water up take leads to cell death. Polymaxins are cationic,basic peptides that act like Detergent (Surfactant). Polymaxins are a group of non-ribosomal polypeptides (NRPS) cyclic natural products biosynthesized from bacteria belonging to The

genus Bacillus, more specifically the sub genus paenibacillus [2-5]. The bactericidal activities were compared and it was found that Polymaxin-B retained antimicrobial activity after encapsulation. At a PMBS concentration of 0.3mg/ml, both positively and negatively charged liposomal Polymaxin-B formulations and free drug, killed all cells after 1hr [6-8].

GENERAL EXPERIMENTAL

Apparatus

Spectrophotometer 1601 (Shemadzu Japan) with matched 1cm quartz cell connected to Epson Lx 300 printer was used for all measurements.

Material and reagents

All the materials were analytical grade Samples of PMBS and other reagent were generously supplied by their respective manufacturers, and were used without further purifications. Polymaxin B Sulfate (PMBS) from Taizhou Bona Chemicals Co. Ltd. China, Vanillin 99.9% assay from Merck Ferric chloride 99.0% assay from Merk and buffer pH 7 from fisher Scientific. While other all solvents were analytical grade.

Pharmaceutical formulations

The following available commercial preparations were purchased from local pharmacy and analyzed according to proposed method;

1- Ophth Neodex®Ophth Pharma
2- Polyfex®gsk
3- Trance® Innvotek
4- Bioprerd®REMINGTON PHARMA

Preparation of Reagents

Stock solution of PMBS standard

An accurately weighed 100mg of polymaxin B Sulfate RS, transferred to 100 ml volumetric flask dissolved with water and made up the volume up to the mark with same solvent. The final concentration of solution was 10,000 IU /ml.

Vanillin stock solution

An accurately weighed 100 mg of vanillin (VAN) and transferred into 100 ml volumetric flask and leveled solution up to mark with methanol the final concentration of vanillin solution was 1 mg/ml.

Ferric chloride stock solution

An accurately weighed 1gram of $FeCl_3$ and transferred into 100 ml volumetric flask and leveled solution up to mark with pure methanol. The final concentration of the solution becomes 10mg/ml.

General analytical procedure

An aliquot volume of the working standard solutions of the studied drug were accurately transferred into a 100ml beaker, added 1ml buffer and Vanillin 1ml. Incubated reaction mixture for 3-5 mints at 60-70°C. The 0.5 ml aliquot from reaction mixture at room temperature, was transferred intoa series of test tubes of size 2 x 18 mm, also added 1ml $FeCl_3$ (1%) solution in each tube which formed colored complex. The absorbance of resulting green complexwas measured against blank ($FeCl_3$) treated at 600 nm after complete mixing.

Preparation of Sample Solution

Procedure for ophthalmic solution

It was taken ophthalmic solution of Ophth Neodex® (Ophth Pharma) Trance® (Remington) and Biopred® of concentration 2000 – 5000 IU/ ml after addition of 1 ml buffer and 5 ml Vanillin it was kept for incubation at 60-70°C for 3-5 mints. Before addition of 1ml $FeCl_3$ solution (10 mg/ml) it was centrifuged. Then the general procedure was followed.

Procedure for ophthalmic ointments

An accurately weighed 5 gm of ointment containing 10000 IU/ gm in separatory funnel, added 30 ml n-Hexane to dissolve completely ointment base. Then added 25 ml of distilled water and shacked well. The concentration of

aqueous layer was 2000 IU/mg. Then the general procedure was followed.

RESULTS AND DISCUSSIONS

Absorption Spectra

The reaction between PMBS with $FeCl_3$ formed green colored complex. The absorption spectra of the green colored products were recorded at 190 – 900 nm against the corresponding blank solutions. The resulted green color complex shows absorbance at 600 nm. The absorption spectra and color of solutions have been shown as follows in Figure **2a** & **2b**.

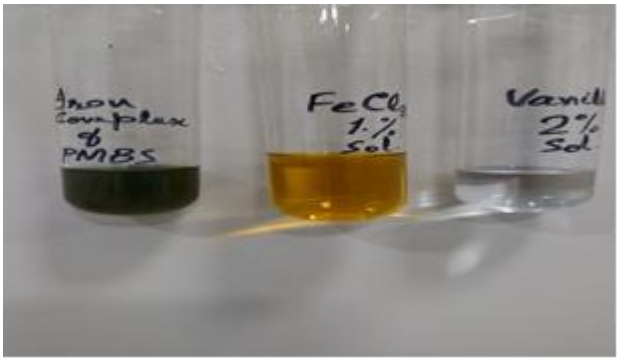

Figure 2a. PMBS complex, $FeCl_3$ & Vanillin solution.

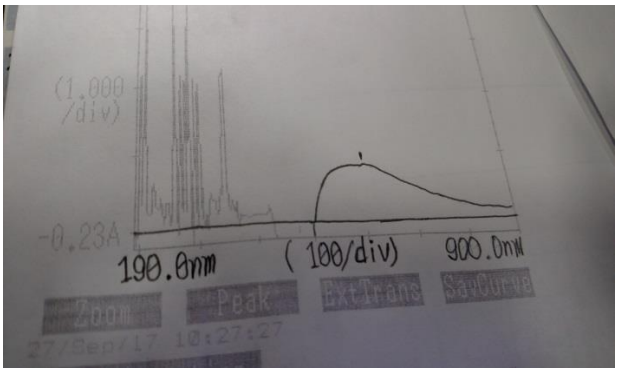

Figure 2b. Absorption spectra of PMBS & Iron complex.

Optimization of Variables

Effect of Vanillin Concentration

The effect of vanillin concentration on the intensity of the color development at the selected wave length and constant PMBS concentration and other reagents were critically examined using different volumes of vanillin solution (20 mg/ml). It was found that the maximum absorbance obtained at 2.5 ml vanilline (Figure **3**).

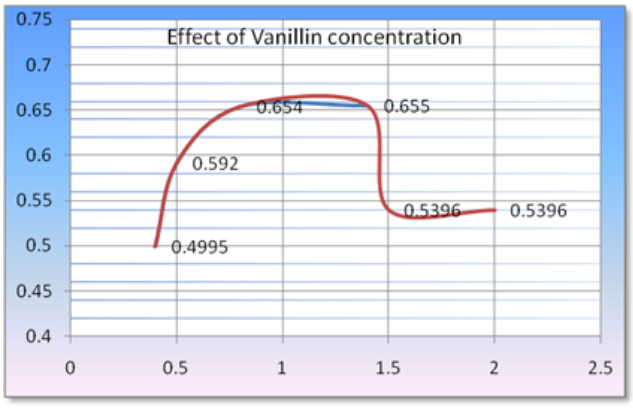

Figure 3. Effect of vanillin concentration on the absorbance of colored complexin terms of volume of Vanillin (1mg/ml).

Effect of Buffer Volume (pH-7)

The reaction mixture was treated with different volumes of buffer pH-7 to know the effect of volume opf buffer for completion of reaction. It was found that the maximum absorbance was obtained at 1.5 ml (Figure **4**).

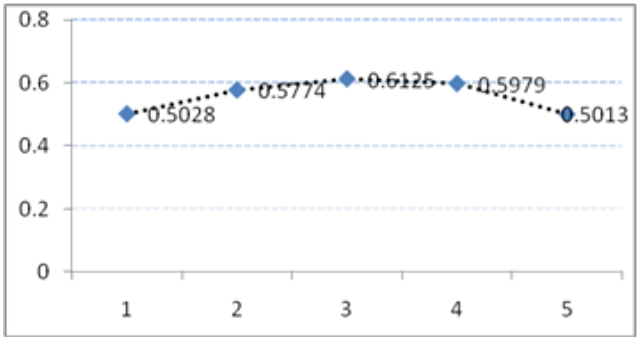

Figure 4. Effect of Buffer volume on the absorbance of formed complex of PMBS with $FeCl_3$.

Effect of Ferric Chloride (10 mg/ml)

The effect of $FeCl_3$ concentration was critically studied at the selected wave length, constant concentrations of PMBS, Vanillin and buffer at constant conditions, by using different volumes of $FeCl_3$ (10mg/ml) solution. The maximum

absorbance was found upon using 3.0 ml volume (Figure **5**).

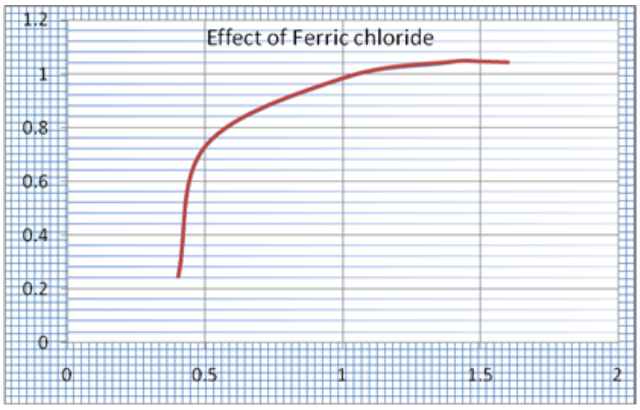

Figure 5. Effect of $FeCl_3$ volume (10mg/ml) solution on the absorbance of color complex of PMBS.

Effect of Reaction Time

The reaction mixture was heated in water bath for different intervals of time. The absorbance was taken after each interval. It was found that the maximum absorbance was attained at between 10-15 mints (Figure **6**).

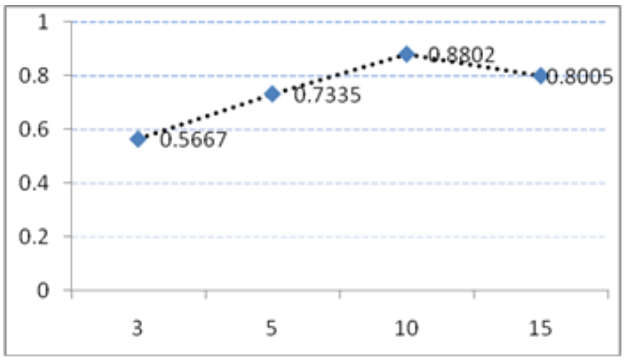

Figure 6. Effect of buffer volume on absorbance of color complex.

Effect of Temperature

The temperature plays a vital rule for enhancing rate of reaction. The rate of reaction 10 times increases by increasing one degree temperature. Therefore different tubes of reaction mixture were prepared and incubated at 40°C, 50°C, 60°C, 70°C, and 100°C. It was found that the maximum absorbance was attained at temperature between 60-70°C (Figure **7**).

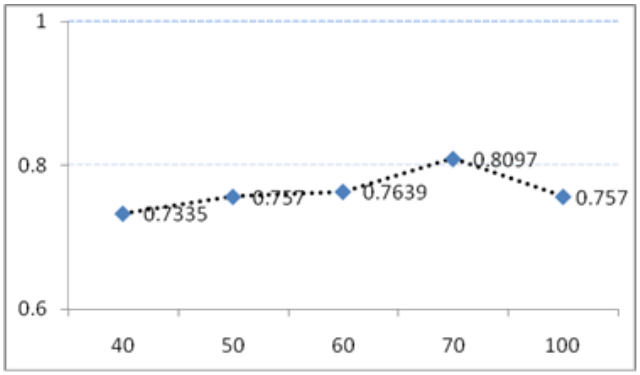

Figure 7. Temperature Effect on formation of color complex.

Stability of Complex

Stability of complex was studied after completion of incubation. It was found that the absorbance was remained stable for at least 72 hours. This indicates good stability of the formed complex.

Validation of Proposed Method

The proposed method was validated according to USP 1995 and ICH guidelines with respect to specificity Linearity, Accuracy, Precision, Robustness and Ruggedness.

Linearity

The linearity was indicated by co-relation co-efficient and regression co-efficient obtained. The correlation and regression co-efficients of the complex were in the range of 0.998 – 0.999 indicating good linearity as shown in Table **1**.

The limit of detection (LOD) and limit of quantitation LOQ for the proposed method was calculated using the following equation (USP-1995).

$$LOD = 3.3\,\partial/S \qquad LOQ = 10\,\partial/S$$

Where ∂ is the standard deviation of intercept, S is the slop of calibration curve. The results are summarized in Table **1**, Figure **8**. The calculated detection limits for studied drug was 0.1 ug/ml, while the quantitation limit for studied drug was 9.05ug/ml, indicating good sensitivity of the proposed method (Table **1**).

Table 1. Optimization parameters.

S. No.	Parameters	Observations
1	λ max	600 nm
2	LOD	0.1 ug/ml
3	LOQ	9.05 ug/ml
4	Regression Eq	Y= 0.0021x + 0.0217
5	Slop (m)	0.0021
6	R	0.9998
7	R2	0.9991
8	Absorptivity	600 nm

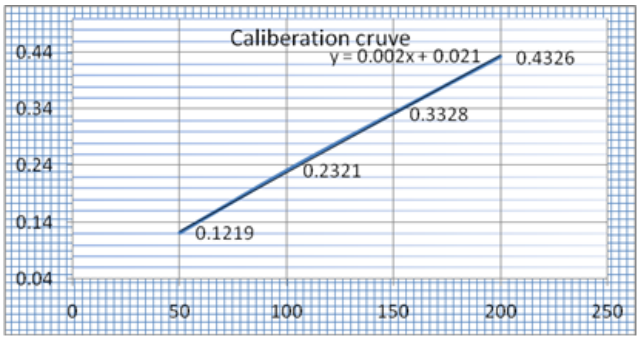

Figure 8. Linearity curve of PMBS- $FeCl_3$.

Accuracy

The accuracy was checked at 3 different concentration levels with a specified rang. Three replicate measurements were recorded at each concentration level. The results were recorded as percent recovery ± RSD as shown in Table **2**. The results obtained show the close agreement between the measurement and the true values (Table **2**).

Precision

Precision was checked at three concentration levels. Three replicate measurements were recorded at each concentration level. The results were summarized in Table **3**. The calculated % RSD was below 4% indicating excellent precision of the proposed procedure at both levels of reproducibility and repeatability Test results were tabulated in Table **3**.

Robustness

Robustness of the proposed method was checkedby varying small change in experimental variables on the analytical method while the other variables were kept unchanged and percentage recovery was calculated each time. The small variations in any of the variables did not significantly affect the results. This gave an indication for the reliability of the method during routine work (Table **4**).

Table 2. Accuracy.

S. No	Concentration of PMBS	% Recovery	Mean of Results	± SD	± RSD
1	50 PPM	98.9 %	101.5 %	±3.21	±3.17
2	100 PPM	99.9 %			
3	150 PPM	105.7 %			

Table 3. Precision.

Parameters		% Recovery of TOB	Mean value (%)	± S.D	± RSD
Intraday	1	100.48	103.45	±2.81	±2.77
	2	106.51			
	3	103.38			
Interday	1	98.24	99.9		
	2	99.7			
	3	101.89			

Table 4. Robustness.

Volume of Vanillin (ml)	Volume of Buffer (ml)	Volume of $FeCl_3$ (ml)	% Recovery	± RSD
2.5	1.5	2.5	99.76	±1.01
2.0	1.0	2.0	97.95	
3.0	2.0	3.0	99.05	

Table 5. Ruggedness.

	Temperature	% Recovery	Mean Value	± RSD
Analyst 01	15°C	**101.23**	**±0.82**	**±0.81**
	25°C	100.72		
	40°C	100.15		
Analyst 02	15°C	102.3		
	25°C	101.63		
	40°C	99.98		

Table 6. % Recovery of available.

Available Formulations	% Recovery	Mean Value	±SD	±RSD
Ophth Neodex®	99.9%	100.4	±1.26	±1.26
Polyfex® E/O	98.9%			
Trance® E/D	102.3%			
Biopred®	100.5%			

Ruggedness

The ruggedness of an analytical method is the degree of reproducibility of test obtained by the analysis of the same sample under a variety of normal test conditions such as different laboratories, different analysts, different instruments, different lots of reagents different assay temperatures, different assay times etc. Ruggedness of the proposed method was determined by changing of analysts or variation in temperature conditions. Therefore the absorbance of the complex was taken at 15°C, 25°C and at 40°C (Table **5**).

Applications to Pharmaceutical Dosage Forms

The proposed method was applied for determination of investigated drug in commercial pharmaceutical dosage forms. The obtained percent recovery values were shown in Table **6**.

CONCLUSION

This is a simple and cheap method for determination of Polymaxin B Sulfate in pure, bulk and in pharmaceutical formulations within a short time.

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