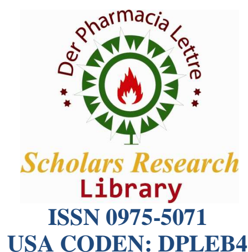
Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (17):26-31
(http://scholarsresearchlibrary.com/archive.html)



ISSN 0975-5071
USA CODEN: DPLEB4

Colorimetric method for the determination of Paromomycin Sulfate in Bulk and Dosage forms using Ascorbic acid

Mohamed E. Adam, Shaza W. Shantier*, Reem S., Magdi A. Mohamed and Elrasheed A. Gadkariem

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Khartoum, P.O. Box 1996, Khartoum, Sudan

---

ABSTRACT

A simple and accurate colorimetric method was developed for the estimation of paromomycin sulphate (PM) in bulk and pharmaceutical formulations. The method was based on generation of colored complex from reaction between PM and ascorbic acid having analytically useful maxima at 390 and 530 nm. The factors such as concentration of ascorbic acid and heating time affecting the color development were optimized and incorporated in the procedure. Regression analysis of Beer's plot showed good correlation (r=0.999) in a concentration range of 40-200μg/mL. The limits of detection and quanification at 390 and 530nm were 3.95μg/mL, 5.95μg/mL and 13.17μg/mL, 19.83μg/mL, respectively. The average recovery for the commercial preparation (Paromomycin Sulfate injections, 1g/ 2 mL) was 102.23±3.37%; n=3, which reflected no interference by the injection excipients. Based on the molar ratio method, the reaction stoichiometry was found to be 1:1.

Key words: Paromomycin**,** Ascorbic acid**,** Spectrophotometric

---

INTRODUCTION

Paromomycin sulfate (Figure 1), is a broad spectrum antibiotic produced by streptomyces riomosus var. It is used to treat intestinal infection such as cryptosporidiosis and amebiasis and other disease like lishmaniasis. Paromomycin topical cream with or without gentamicin is an effective treatment for ulcerative cutaneous leishmaniasis [1].

Many HPLC methods were reported for determination of Paromomycin alone and in combination with other amnioglycosides (2-4). Ascorbic acid has been frequently utilized as an analytical reagent in pharmaceutical analysis. It was used for the colorimetric determination of penicilins, cephalosporins having α-aminoacyl functions and aminoglycosides (5-10). Based on these reports, simple, sensitive and accurate colorimetric method was developed for the determination of PM in bulk and injection forms using ascorbic acid.

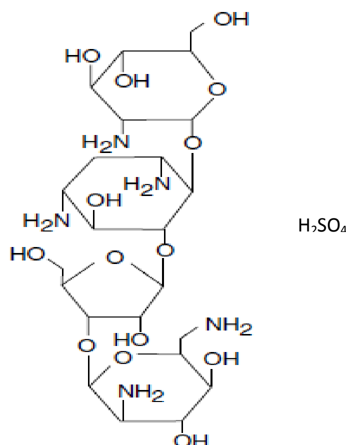


Figure 1: Chemical structure of Paromomycin sulphate

MATERIALS AND METHODS

Paromomycin sulfate standard was kindly provided by the Central Lab, Sudan. Paromomycin sulfate Injection (1g/2ml), Gland pharm Ltd, India.

Ascorbic acid, British drug house, England. Dimethylsulfoxide (DMSO) s.d Fine chem. Limited, India. Dimethyl Formamide (DMF), Merck K.G.A, Germany.

Spectrophotometric studies were carried out on Shimadzu UV-1800ENG240V, Koyoto, Japan.

Preparation of stock solutions

Standard stock solution

0.01g of paromomycin sulfate standard was accurately weighed and dissolved in 10ml distilled water. The solution was transferred into 25ml volumetric flask and the volume was completed to mark with distilled water (solution A; 4000µg/ml).

Sample stock solution

0.2ml of the sample injection was diluted to 10ml with distilled water. 1ml of the resultant solution was further diluted to 25ml using distilled water (solution B; 4000µg/ml).

Reagent blank

Two ml of 0.2%w/v ascorbic acid was added to 0.5ml distilled water and the volume was completed to 10ml with DMSO.

Procedure

Construction of calibration curve

Aliquot volumes from solution A (0.1 - 0.5ml) were transferred into five stoppered glass tubes. 0.4, 0.3, 0.2, 0.1 and 0.0 ml of distilled water were added to each tube, respectively. 2ml of freshly prepared ascorbic acid (0.2% w/v) was added to each tube. The solutions were completed to10 ml with DMSO before heating in boiling water bath for 45minutes.

The absorbance of the resultant solutions was measured at 390 and 530 nm against the reagent blank. The graphs were then constructed by plotting the absorbance values versus drug concentration at each wave length.

Serial dilutions of solution B were treated as under calibration curve. The content of injection was alternatively determined by direct sample/standard comparison.

Optimization of reaction conditions

Effect of heating time

Reaction mixtures of PM standard solution (160µg/ml) and ascorbic acid were heated at time interval (15minutes). The absorbance values were then measured to determine the optimum heating time.

Effect of Ascorbic acid concentration

Two ml of freshly prepared ascorbic acid solutions (0.1%, 0.2% and 0.3% w/v) was transferred into three stoppered glass tubes containing PM standard solution (120µg/ml). The solutions were then treated as under calibration curve.

Validation of the developed method

The developed method was validated in terms of linearity, accuracy (added recovery method) and precision (repeatability and reproducibility).

Reaction stoichiometry

Serial volumes (0.1 -0.7ml) of PM standard solution ($1.5x10^{-2}$M) were transferred into seven stoppered glass tubes. 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 and 0.0 ml of distilled water were then added, respectively. 0.3ml of freshly prepared ascorbic acid solution ($1.5x10^{-2}$M) was added to each tube. The volumes were completed to 10ml with DMSO, heated for 45minutes and the absorbance values were then measured at 390nm and 530nm against blank.

The molar ratio of the reaction was obtained from a plot of concentration ration ([PM]/ [ascorbic acid]) vs absorbance values.

RESULTS AND DISCUSSION

Colorimetry is concerned with the determination of the concentration of substances by measurement of the relative absorption of light with respect to a known concentration of the substance.

The chief advantage of UV/ visible spectrophotometric methods is that it provides a simple means for determining quantities of substances. Weakly or non UV absorbing compounds can be allowed to react with a reagent to give a colored compound with higher intensity absorption depending on the presence of an active functional group such as NH_2, OH …etc

PM like other aminoglycosides is composed of 2- deoxystreptamine and teterahydropyran ring to which the amino groups are attached. PM exhibits weak UV/absorption therefore, a suitable chromogen is needed to form an absorbing chromophore that can be used for simple and sensitive determination of PM in bulk and in different pharmaceutical formulation.

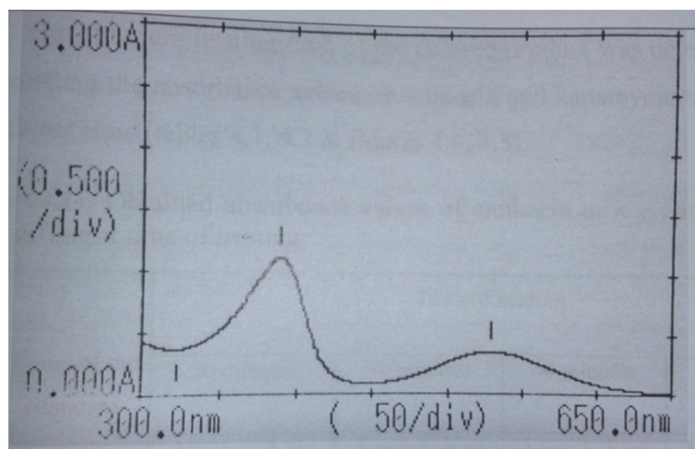


Figure 2: UV/VIS spectrum of the colored product (40µg/ml)

Ascorbic acid is reported to have a selective reaction with ammonia and primary aliphatic amines (11) and has been used for colorimetric determination of number of drugs containing primary amine (12, 13).

Thus, the developed method is based on reaction of PM with ascorbic acid in presence of DMSO to produce a pink-purple colored complex with λ_{max} 390 and 530nm (Figure 2)

Optimization of reaction conditions
The different experimental factors affecting the color development, intensity and stability were studied.

Heating was found necessary for oxidation of ascorbic acid and hence the formation of a colored complex. The maximum colored was developed after heating for 60 minutes; however more consistent results were obtained when heating in a boiling water bath for 45minutes which was selected as a fixed time of heating (Table 1).

Table1. Effect of heating time on color intensity

λ_{max}	Heating time (minutes) / Absorbance values (160µg/ml)			
	15	30	45	60
390nm	0.345	0.900	1.128	1.199
530nm	0.115	0.266	0.367	0.401

For faster and intense color development, DMSO was used as solvent. As reported by Pesez and Bartos (14), the sample should contain no more than 1%v/v water in the final volume for high sensitivity and color stability.

2ml of 0.2%w/v ascorbic acid was chosen as the optimum concentration for maximum color development and stability (Figure 3).

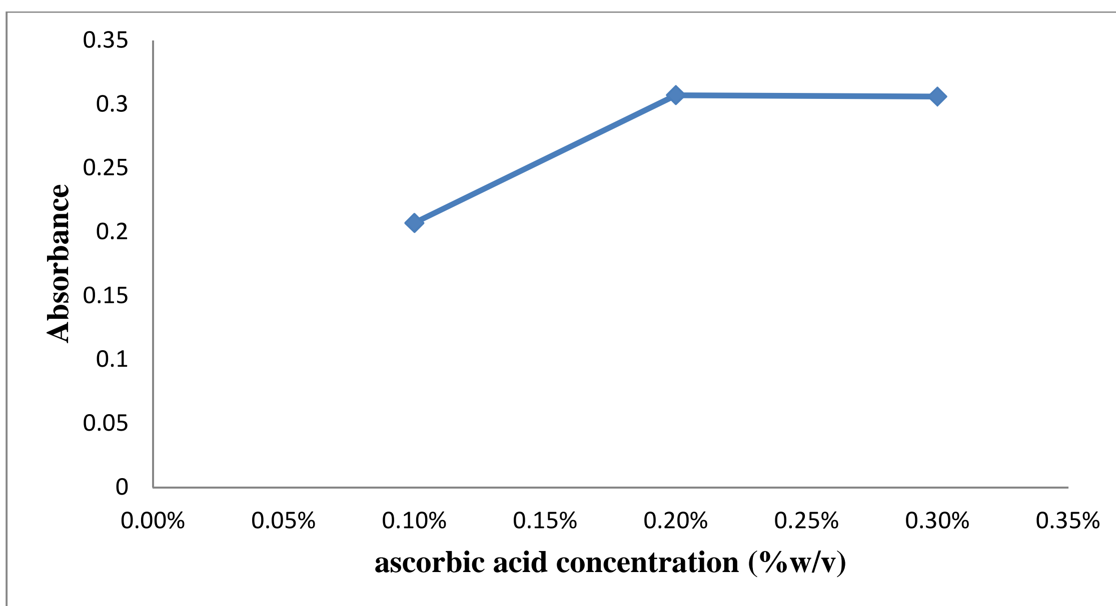


Figure 3: Effect of ascorbic acid concentration on color intensity

The sequence of reagents addition as described in the method was found essential for color development and stability (not less than 24 hours).

Method validation
Linearity
The constructed calibration curves obeyed Beer's law over the concentration range 40 - 200µg/ml with good correlation coefficient. The linearity data was calculated at 95% confidence limit and summarized in Table 2.

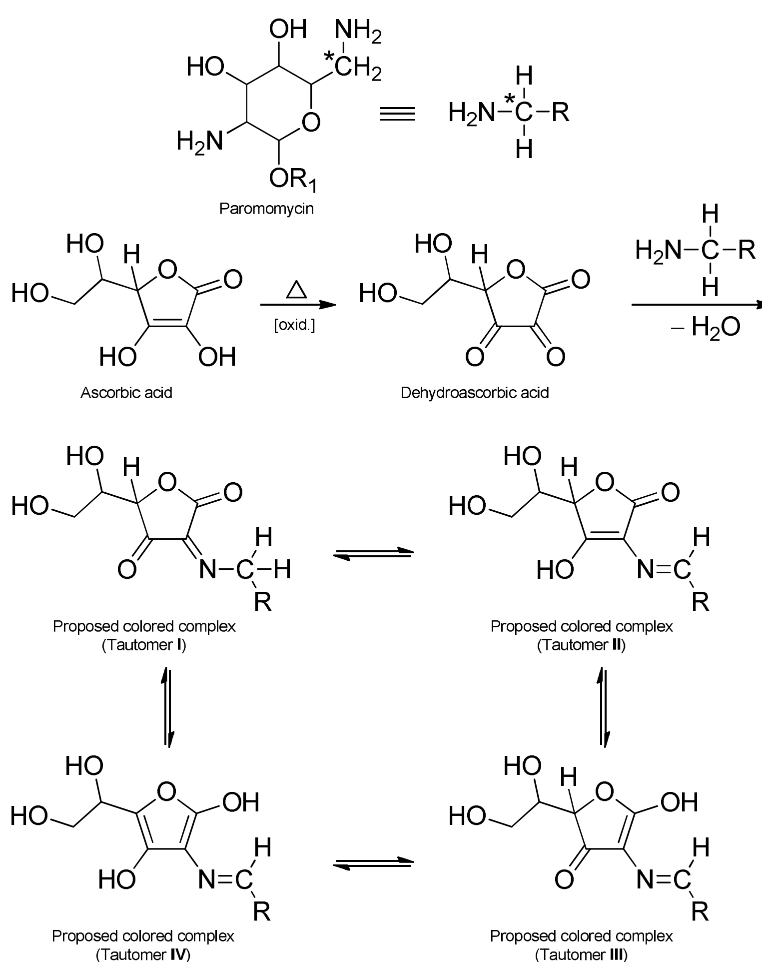
Table 2. Linearity data of the developed method

Parameter	Wavelength	
	390nm	530nm
Slope ± ts_b	0.0072±2.40 x10^{-4}	0.0025±1.27x10^{-4}
Intercept ± ts_a	0.005±0.038	-0.007±0.017
Correlation coefficient	0.9998	0.9996
Range	40 - 200µg/ml	40 - 200µg/ml
LOD	3.95µg/ml	5.95µg/ml
LOQ	13.17µg/ml	19.83µg/ml

Analytical assay

The developed method was successfully applied for the quantitation of PM injection formulation. The injection content percent was found 103.03 ±1.6 % and 101.95±1.91%; n=4 at 390nm and 530nm, respectively. Alternatively, the assay results were calculated using the following adopted formula:

$$\text{Content }\% = \frac{\text{slope of sample curve}}{\text{slope of standard curve}} \text{ x } 100$$



Scheme 1. Proposed mechanism for the color producing reaction

Accuracy and precision

The recovery percentage method was applied to evaluate the accuracy of the procedure and the freedom from interference by the injection excipients. The obtained results showed good recovery of the injection (102.10±3.32% and 102.10 ±3.37%; n=3 at 390nm and 530nm, respectively), which reflected the accuracy of the developed method.

Three concentrations of PM within the linearity range were used to evaluate the precision of the developed method. The obtained RSD% values for the within-day and between-days determination were within the range 0.00 - 1.70% and 0.56 - 1.14%; n=3. These low values (less than 2%) reflected the precision of the developed method.

Proposed reaction mechanism

The reaction between PM and ascorbic acid was found to proceed with a 1:1 ratio as determined by the molar ratio method. The reaction is proposed to take place via nucleophic addition mechanism with PM and dehydroascorbic acid being the nucleopile and the electrophile, respectively (Scheme 1). The mechanism involves the reaction of the PM's less sterically hindered amino group with the carbon of the most electrophilic carbonyl group of dehydroascorbic acid to generate an imine (tautomer I). The resultant imine undergoes tautomerism to give tautomers II, III or IV (scheme 1). Tautomer IV appears to be the most stable as it can be envisioned as a derivative of the aromatic heterocycle furan. The furan derivative, tautomer IV, absorbs UV/visible at two wave lengths, 530 and 390 nm. The low intensity absorption at 530 nm is supposed to be due to $n=\pi^*$ transition whereas the high intensity absorption at 390 nm is supposed to be due to $\pi=\pi^*$ transition. The proposed mechanism explains why one mole ascorbic acid is required to couple with one mole of PM to yield the color coupling complex.

CONCLUSION

The developed method was proved to be simple, accurate and precise for the determination of PM in bulk and dosage forms. Ascorbic acid is considered a suitable, cheap and available reagent for the analysis of PM without interference from excipients. The developed method can be used for the routine analysis of PM.

REFERENCES

[1] S. Sweetman, Martindale: The Complete Drug Reference, London, Pharmaceutical Press, **2002**, 33rd ed.
[2] J. Pick, L.L. Oison, WY. Ellis, P. Lim, *J. Pharm. Biomed. Anal.,* **1997**, 16, 1, 131-137.
[3] R. Oretel, V. Neumeister, W. Kirch*, J. chroma. A,* **2004**, 1058, 1-2, 197-201.
[4] L.L. Oison, J. Pick, WY. Ellis, P. Lim. *J. Pharm. Biomed. Anal*., **1997**, 15, 6, 783- 793
[5] E.A. Gadkariem, M.A Abounassif, A.M. Wahabi, *Talanta*, **1985**, 33, 180-181.
[6] H.A.AL- Khamees, F.S. El- Shafie, M.E. Hagga, M.E. Alawady , E.A. GadKariem, *Sci. Pharm.,* **1995**, 63, 191.
[7] N. Rahman, M. Sing, M. Nasrulhoda, *J. Braz. Chem. Soci.,* **2005**, 16, 5, 1001-1009.
[8] S.W. Shantier, E. A GadKariem , K.E Ibrahim, *Int. J. Dru. Form. Res.,* **2012**, 2, 260-272.
[9] S.W. Shantier, E. A GadKariem, K.E Ibrahim, M.E. Hagga, *Res. J. Pharm. Bio. Chem. Sci.,* **2012**, 3, 1, 566-573.
[10] H.A. ELobied, E.A.Gadkariem, K.A. AL.Rashood, H.A. Al-Khamees, F.S. El- Shafie, G.A.M. Bawazeer, *Anal. Lett*., **1999**, 32,14, 2809-2823.
[11] E.A. Gadkariem, M.M. Mutasim, K.E.Ibrahim, H.A. El-Obeid*, J. Biomed. Sci*., **2009**, 5, 267-274.
[12] F.S. El-Shafie, E.A. Gadkariem, K.A. Al-Rashood, H.A. Al-Khamees, H.A. El-Obeid, *Anal Lett*., **1996**, 29, 3, 381–393.
[13] H.A. El-Obeid, E.A. Gadkariem, K.A. Alrashood, H.A. Al-Khamees, F.S. El-Shafie, G.A.M. Bawazeer, *Anal Lett.,* **1999**, 32, 2809–2823.
[14] M. Pesez, J. Bartos . Aliphatic amine textbook of colorimetric and fluorimetric analysis of organic compounds and drugs. **1974**. p.155.