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VISIBLE SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF TOBRAMYCIN IN PHARMACEUTICAL FORMULATIONS THROUGH SCHIFF'S BASE FORMATION

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ABSTRACT

Three simple spectrophotometic methods for the analysis of Tobramycin in pure form or in pharmaceutical formulations have been developed based on the reaction of the drug with aromatic aldehydes, Vanillin, paradimethyl amino cinnamaldehyde (PDAC) and paradimethyl amino benzaldehyde (PDAB) in acidic medium producing coloured Schiff's bases having λ_{max} at 570 nm, 420 nm and 415, nm respectively. Good agreement with Beer's law was found in the range of 40– 160 µg/mL for Method A, 50–200 µg/mL Method B and 80–400 µg/mL for Method C. The methods are simple, precise and accurate with excellent recovery of 98–102%. The results obtained are reproducible with coefficient of variation of less than 1.0%.

Key words : Tobramycin, Pharmaceutical formulations, Schiff's base, Spectrophotometry

INTRODUCTION

Tobramycin (TM) is a simple aminoglycoside antibiotic with an extended spectrum of activity against gram negative and aerobic bacilli[1]. It is official in Indian pharmacopoeia[2] which recommends a microbiological assay for TM estimation. Chemically, it is 0,3– amino–3–deoxy–D–glucopyranosyl [1→6–diamino–2,3, 6–tridexoy–D–ribo–hexoglucopyranosyl]–2–deoxy streptamine[3]. Literature survey revealed the presence of two HPLC[4,5] methods a conductometric method[6], a liquid chromatographic method[7], a turbidimetric method[8] and two visible spectrophotometric procedures[9,10] for estimation of TM in pharmaceutical formulations. The reported first spectrophotometirc method is based on the oxidative coupling of the drug with 3–methyl–2–benzothiazolinone hydrazine hydrochloride (MBTH). The second reported method is based on the oxidation of the drug with ferric chloride and potassium ferricyanide. Both the methods are based on only single analogy and suffer from low sensitivity.

The analytically useful functional groups of TM namely vic–amino diol, aliphatic primary amino group and secondary hydroxyl group have not been fully exploited. Hence attention was focused on developing simple spectrophotometric methods exploiting the varied functional

groups of the drug. In the present paper, authors present three spectrophotometric methods using aromatic aldehydes namely vanillin, PDAC and PDAB. It is well known that aldehydes form coloured condensation schiff's bases with primary amines. This famous reaction has been used as a basis for development of these simple methods of visible spectrophotometry for the estimation of TM in pharmaceutical formulations.

EXPERIMENTAL

Instruments

a) An Elico SL 171 spectrophotometer with 1cm matched quartz cells for spectral measurements

b) An Elico L –120 digital pH meter for pH measurements

Chemicals and Reagents

All the chemicals used were of analytical grade and all the solutions were prepared in pure methanol. Freshly prepared solutions were always used.

a) Vanillin (Qualigens, 0.2%) – 300 mg in 100 mL chloroform.

b) Paradimethylamino cinnamaldehyde (PDAC) (BDH, 0.4%) – 400 mg in 100 mL chloroform.

c) Paradimethylamino benzaldehyde (PDAB) (BDH, 0.3%) – 300 mg in 100 mL chloroform.

Concentrated sulfuric acid and methanol were obtained from Qualigens and used as such. Pure bulk sample of TM was obtained from Aristo Pharmaceuticals, Manideep. Commercial injections of TM were procured from local market.

Preparation of standard drug solution : A standard drug solution of TM containing 1 mg/mL was prepared by dissolving 100 mg of pure drug in 100 mL of methanol. From this, working standard solutions are prepared by suitable dilutions with methanol. (Method A–400 µg/mL, Method B–500 µg/mL and Method C– 800 µg/mL).

Preparation of sample drug solution : Three brands of commercial injections were analysed by the proposed methods. In each method, injection solution equivalent to 100 mg of TM was successively extracted with 25 mL portions of chloroform 3 times and combined filtrate was evaporated to dryness. The residue was then dissolved in 100 mL methanol to get 1 mg/mL solution and this solution was suitably diluted with methanol as given under the assay procedure for bulk samples.

Assay procedure : Method A comprises of transferring into a series of 10 mL volumetric flasks, aliquots of TM (1.0 – 4.0 mL, 400 µg/mL) were added followed by 2 mL of vanillin in 3 mL concentrated sulfuric acid and total volume in each volumetric flask was brought up to 7 mL with methanol and placed in a hot water bath for 15 min. Then the flasks were cooled, the

volume made up to 10 mL with methanol and after 10 min, absorbance was measured at 570 nm against a reagent black. The concentration of TM present is deduced from the calibration graph.

Table 1. Optical characteristics and precision of the proposed methods

Parameter	Method A	Method B	Method C
λ_{max} (nm)	570	420	415
Beer's law limit (μg/mL)	40–160	50–200	80–400
Molar absorptivity (L mol^{-1} cm^{-1})	3.92 x 10^4	8.10 x 10^4	1.04 x 10^4
Sandell's sensitivity (mg cm^{-2} per 0.001 absorbance unit)	0.027	0.010	0.048
Regression equation (y = a + bC)* Slope (b)	1.8 x 10^{-3}	1.0 x 10^{-2}	1.52 x 10^{-2}
Intercept (a)	3.1 x 10^{-2}	4.0 x 10^{-3}	3.2 x 10^{-3}
Correlation coefficient (r)	0.9999	0.9982	0.9992
Relative standard deviation (%)**	0.301	0.781	0.402
% Range of error (confidence limits)**	0.339	0.318	0.401
0.05 level 0.01 level	0.532	0.512	0.402
% Error in bulk samples***	0.675	0.653	0.515

*Y = a + bC, where C is concentration of analyte and Y is absorbance unit, ** Average of six determinations, *** Average of three determinations.

Table 2. Assay of TM in pharmaceutical formulations

Drug	Label claim mg/injection	Amount found by proposed method (mg)			Reference Method[9]	% Recovery ** ± S.D.
		Method A	Method B	Method C		
Injection I	20	19.8	19.7	19.8	19.7	99.08 ± 0.92
Injection II	40	39.9	39.7	39.8	39.9	99.07 ± 0.93
Injection III	60	59.7	59.8	59.8	59.9	99.09 ± 0.91
Injection IV	80	79.7	79.9	79.8	79.6	99.91 ± 0.09

*Drug from different pharmaceutical companies; ** Recovery of 10 mg added to the pre–analyzed pharmaceutical dosage forms (average of 3 determinations).

In method B, aliquots of standard drug solution (1.0 – 4.0 mL, 500 µg/mL) were transferred into a series of 10 mL volumetric flasks. Then 3 mL of PDAC and 3 mL concentrated sulfuric acid were added and the total volume in each flask was brought to 7 mL with methanol and the flasks were placed in hot water bath for 10 min. The flasks were cooled to room temperature and volume in each flask was made up to 10 mL with methanol and after 15 min, absorbance was measured at 420 nm against a reagent blank. The concentration of TM present is computed from calibration curve.

In method C, aliquots of standard drug solution (1.0 – 4.0 mL, 800 µg/mL) were transferred into a series of 10 mL volumetric flasks. Then 3 mL of PDAB and 3 mL concentrated sulfuric acid were added and the total volume in each flask was brought to 7 mL with methanol and the flasks were placed in hot water bath for 10 min. The flasks were cooled to room temperature and volume in each flask was made up to 10 mL with methanol and after 15 min, absorbance was measured at 415 nm against a reagent blank. The concentration of TM present is computed from calibration curve.

RESULTS AND DISCUSSION

Optimum operating conditions used in the procedures were established adopting variation of one variable at a time (OVAT) method. The optical characteristics of the methods are presented in Table 1. The precision and accuracy of the methods was tested by measuring six replicate samples of the drug in Beer's law limits. Commercial formulations containing TM were successfully analyzed by the proposed methods. The results are presented in Table 2. As an additional check of accuracy, recovery experiments were performed by standard addition method. When pharmaceutical preparations (injections) containing TM were analyzed, the results obtained by the proposed methods were in good agreement with the labeled amounts. The recovery with the methods was found to be 99–101%.

The aromatic aldehydes have lead to numerous applications as analytical reagents. PDAB (p–dimethyl amino benzaldehyde) allows the determination of trace amounts of hydrazine[11]. Aldehydes were applied to the colorimetric determination of primary alkylamines[12] and primary aromatic amines in acidic medium. The condensation of indole derivatives in acidic medium gives the coloured product[13].

CONCLUSION

The proposed methods are applicable for assay of TM and have the advantage of a wider range. The decreasing order of sensitivity of the methods is $M_1 > M_2 > M_3$ and the increasing order of λ_{max} among the proposed methods are $M_3 > M_2 > M_1$. As the formation of coloured species differ from one another in the proposed methods depending on the chromogenic reagents, the appropriate method can be used for the assay of TM in bulk form and injections

with good precision and accuracy depending on the availability of chemicals, needs of specific situations and nature of concomitants present in the sample under analysis.

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