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ANALYTICAL METHOD DEVELOPMENT AND ITS VALIDATION FOR ESTIMATION OF KANAMYCIN SULPHATE BY UV-VISIBLE SPECTROPHOTOMETRY AS BULK AND IN DOSAGE FORM

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Abstract: A simple, economic, sensitive, precise and accurate UV spectrophotometric method has been developed for determination of Kanamycin Sulphate as bulk and in dosage form. The quantitative determination of the Kanamycin Sulphate was carried out using the absorbance values measured at 570 nm respectively. The method was validated as per ICH guidelines. Statistical analysis of the result has been carried out revealing high accuracy and good precision. The proposed method was successfully applied to the determination of Kanamycin Sulphate as bulk and pharmaceutical formulations without any interference from common excipients. Calibration curve of Kanamycin Sulphate was linear in concentration range of 2-22 µg/ml with correlation coefficient value of 0.9996. The slope of Kanamycin Sulphate was found to be 0.0161. The results of analysis validated statistically and by recovery studies. The Developed method was found to be sensitive, specific, accurate, precise and cost effective quality control tool for the routine analysis of Kanamycin Sulphate as bulk and in dosage form.

Keywords: Kanamycin Sulphate, UV Spectrophotometry, Validation.

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INTRODUCTION

Kanamycin (acid sulphate) is derived from streptomyces kanamyceticus, consisting of three components designated a, b and c. The clinically active form is a mixture of kanamycins a and b. Kanamycin has actions and spectrum similar to neomycin, but less ototoxic. Used in gram -ve speticaemia, with monitoring of blood levels, particularly in renal failure. Kanamycin (acid sulphate) is most often applied topically.

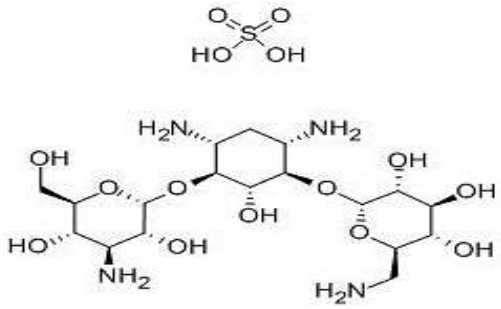

Fig. 1: Chemical structure of Kanamycin Sulphate

Kanamycin is rapidly absorbed after intramuscular injection. Peak serum levels are generally reached within approximately one hour and $T_{1/2}$ = 2.5h. Poor oral and topical absorption except with severe skin damage. Aminoglycosides like kanamycin "irreversibly" bind to specific 30S-subunit proteins and 16S rRNA. Specifically Kanamycin binds to four nucleotides of 16S rRNA and a single amino acid of protein S12. This interferes with decoding site in the vicinity of nucleotide 1400 in 16S rRNA of 30S subunit. This region interacts with the wobble base in the anticodon of tRNA. This leads to interference with the initiation complex, misreading of mRNA so incorrect amino acids are inserted into the polypeptide leading to nonfunctional or toxic peptides and the breakup of polysomes into nonfunctional monosomes. It is a used to treat Bacterial Infection, Peritonitis and Tuberculosis. Adverse effects are skin damage and eye irritation.[1-8]

2. EXPERIMENTAL

2.1 Chemicals and Reagents

Kanamycin sulphate was obtained as gift sample from Macleods Pharmaceuticals Limited, Mumbai (India). A commercial tablet formulation was purchased from the local market. Distilled water of in house production was used.

3. MATERIALS AND METHODS

3.1 Instrument

A double beam UV-VIS Spectrophotometer (CE7400, Cecil, United Kingdom) Spectral bandwidth of 1 nm and wavelength accuracy of ±0.5 nm with a pair of 10 mm matched quartz cells. All weights were taken on Digital electronic balance Precisa, Switzerland.

3.2 Preparation of Standard Stock Solution of Kanamycin

10 mg of Kanamycin sulphate and 200 mg ninhydrin was approximately weighed and transferred to 100 ml volumetric flask. The drug was dissolved in about 50 ml of distilled water and volume was made up to 100 ml with help of distilled water to obtain stock solution of drug concentration of 100 µg/ml.

3.3 Determination of λ_{max} of Kanamycin Sulphate

10 ml of the standard stock solution was diluted to 100 ml using the distilled water to obtain the final working solution of concentration of 10 µg/ml. The final solution was scanned in the range of 400 to 800 nm against distilled water as a blank. The UV-Visible spectrum of Kanamycin sulphate is shown in **Fig. 2**.

3.4 Preparation of calibration curve for Kanamycin Sulphate

0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, and 2.2 ml solutions were pipette out individually from the stock solution and transferred in a series of seven, 10 ml volumetric flasks. The volume in each flask was made up to 10 ml with solvent (distilled water) to obtain final solution in the concentration range of 2 to 22µg/ml. Then the absorbance (A)of all the solutions were measured at λ_{max} of drug, i.e. 570 nm, against distilled water as a blank. The results of calibration curve data for Kanamycin sulphate are shown in **Table 1** and the calibration curve is shown in **Fig. 3.**

3.5 Estimation of Kanamycin sulphate injection

Injection; label claimed injection contains Kanamycin sulphate 1000 mg.

The vial of the same batch no. was mixed properly. Weighed accurately 10.1 mg powder (equivalent to 10 mg of Kanamycin sulphate) was transferred in 100 ml volumetric flask containing small quantity of reference solvent (distilled water). The solution was filtered through whatman filter paper no. 40. Further suitable dilutions were made to obtain six replicates of 10 µg/ml solution. These solutions were analyzed and percentage recovery of Kanamycin sulphate injection was determined. Recovery data for estimation of Kanamycin sulphate in Kanamycin sulphate injection are shown in **Table 2**.

3.6 Method Validation

3.6.1 Specificity: The result obtained for the specificity study from six replicates after addition of excipients had a very negligible change in concentration from the concentration before addition of excipients. On the basis of % agreement criteria, therefore Average % agreement found to be 100.2089. Specificity study shows the good agreement with results, indicating that excipients did not interfere with the analyte.

3.6.2 Linearity: A calibration curve was constructed at optimum experimental conditions using absorbance values versus concentration in the range of 2-22 µg/ml for Kanamycin sulphate. The regression equation (y = 0.0161x+0.0033) for Kanamycin sulphate was obtained. From calibration curve data, high value of the correlation coefficient (0.9996) for Artesunate was found and the value of the intercept on ordinate, which is close to zero for each drug, shows very good linearity of the calibration graph and adherence of the method to Beer's law.

3.6.3 Precision:

3.6.3.1 Repeatability

Repeatability study showed a % R.S.D of 0.758803 for Kanamycin sulphate. Thus it is concluded that the analytical technique has a good repeatability precision as R.S.D are less than 5.3% (Specified)and less than 2% (desired). So it can be said that the proposed method is precise.

3.6.3.2 Intraday precision

For Intraday precision of the method, solution of Kanamycin sulphate were prepared at three concentration levels 8, 10, 12 (µg/ml) each in triplicate. These solutions were analyzed three times within one day.

The results of intraday precision are shown in **Table 4**.

3.6.3.3 Interday precision

For Interday precision of the method, solution of Kanamycin sulphate were prepared at three concentration levels 8, 10, 12 (µg/ml) each in triplicate. These solutions were analyzed for three consecutive days.

The results of interday precision study are shown in **Table 4**.

Intraday and Interday study were showed a % R.S.D of 0.0861367 and 1.206059 respectively for Kanamycin sulphate. So it can be said that the proposed method is precise.

3.6.4 Accuracy: An accuracy concentration for an assay method is that mean recovery should desirably be 100 ±2% at each concentration is to be range of 80-120% of the 10 µg/ml. since the % recovery 99.37 to 100.416 are within the desirable confidence interval of 98-102%. So it can be said that the proposed method is accurate.

The recovery data are shown in **Table 4**.

4. RESULTS AND DISCUSSION

According to the International Conference on Harmonization, the main objective of the validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose, and the parameters that need to be selected are the responsibility of the analyst. The solubility of Kanamycin Sulphate in distilled water, so it was used in this method. Kanamycin Sulphate in distilled water shows absorption maxima at 570 nm. The response for Kanamycin Sulphate was found to be linear in the concentration range of 2-22 µg/ml. The % mean recovery data for estimation of Kanamycin Sulphate in injection are summarized in **Table 2**. The optical characteristics of the method and regression analysis of the calibration curve are shown in **Table 3**. The results of validation parameters are shown in **Table 4**. The recovery of Kanamycin Sulphate was found to be good. Excipients used in the specificity study did not interfere with response of the drug at its analytical wavelength. Also, no significant change in response of Kanamycin Sulphate solutions was observed. Hence, the method is sensitive, specific and robust for estimation of Kanamycin Sulphate. The proposed spectrophotometric method was applied to the determination of Kanamycin Sulphate in its pharmaceutical formulations.

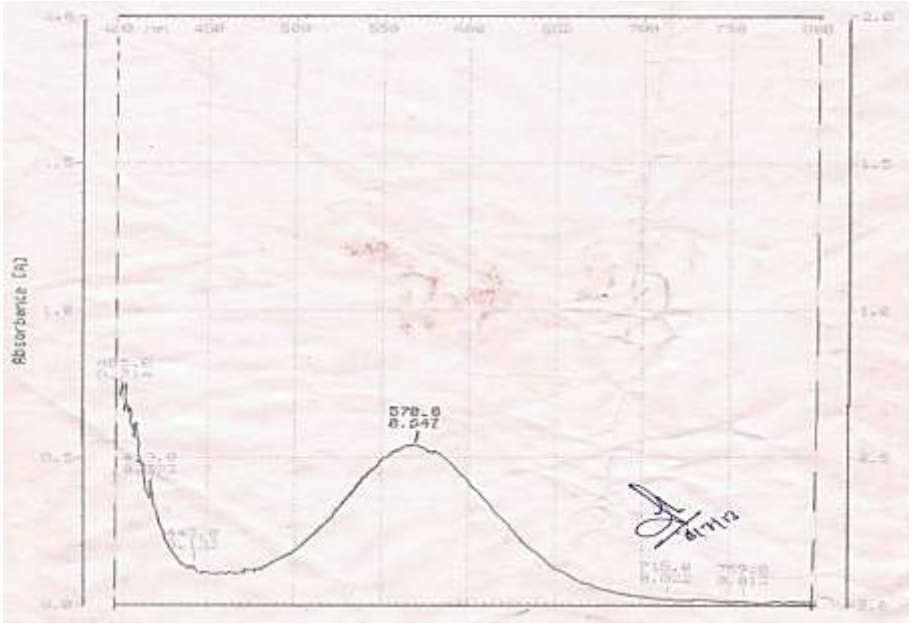

Fig. 2: UV spectrum of Kanamycin Sulphate

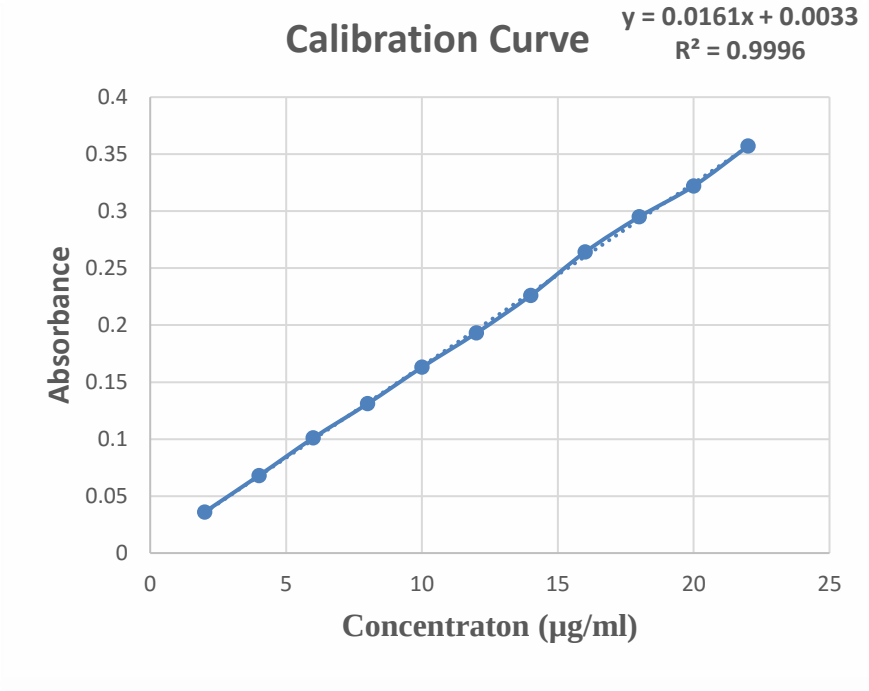

Fig. 3: Calibration curve of Kanamycin sulphate at 570 nm

Table 1: Calibration curve data for Kanamycin sulphate

S. No.	Conc. (µg/ml)	Absorbance (A)*
1.	2	0.036
2.	4	0.068
3.	6	0.101
4.	8	0.131
5.	10	0.161
6.	12	0.193
7.	14	0.226
8.	16	0.264
9.	18	0.295
10.	20	0.322
11.	22	0.357

Table 2: Assay result of Kanamycin sulphate in injection

Label claim	Amount found (mg/inj.)	Standard deviation	% Mean Recovery
Kanamycin sulphate - 1000 mg	996.06	1.452224	99.60

Table 3: Beer's law data and Regression characteristic of Kanamycin sulphate

S. No.	Parameters	Values
1.	Beer's law limit	2-22 (μg/ml)
2.	Molar absorptivity	938.0826 (lit. gm^{-1} cm^{-1})
3.	Regression equation (Y = a + bc)	0.0161x+0.0033
4.	Correlation coefficient (r^2)	0.9996
5.	Slope (b)	0.0161
6.	Intercept (a)	0.0033

Table 4: Validation parameters of Kanamycin sulphate

Validation parameters		Values
Specificity		% Agreement 100.2089
Precision(% RSD)	Repeatability	0.758803 %
	Intraday	0.861367 %
	Interday	1.206059%
Range	Working Range	2.031 to 22μg/ml
	Linearity Range	2μg/ml to 22μg/ml
	Target Concentration	10μg/ml
	Target Range	8 μg/ml, 10μg/ml, 12 μg/ml
Accuracy (% recovery)		98.66 to 101.8 %
LOD (μg/ml)		0.211691
LOQ (μg/ml)		0.641488
Percent mean recovery for Tablets		99.6066 %

5. CONCLUSION

The method was validated and found to be sensitive, specific, economic, accurate and precise. Hence, the method can be used successfully for routine analysis of pharmaceutical dosage form of Kanamycin Sulphate.

6. ACKNOWLEDGMENT

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