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New Analytical Method Development and Validation of Streptomycin (Anti-Biotic) Drugby RP-HPLC Method in Bulk and Pharmaceutical Dosage Form

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Abstract:
A new Reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the determination of in bulk and pharmaceutical dosage form. Chromatographic separation of Streptomycin was achieved by using Agilent Eclipse plus C_{18} (250mm ×4.6, 5µm) column. Detection was carried out at 224nm with a flow rate of 1ml/min with an injection of 20 µl was selected using mobile phase Acetonitrile and Monopotassium phosphate buffer (45:55).The standard curve was linear over a working rangeof 0.5-08 µg/ml and gave an average correlation factor 0.9987 for Streptomycin. The system was operated atambient temperature and the retention time was observed at 1.870 min for Streptomycin. The method was validated with different parameters such as Linearity, Precision, Accuracy, Robustness, Limit of detection (LOD), Limit of quantification (LOQ).The Limit of detection and Limit of quantification was found to be
0.083 µg/ml and 0.28 µg/ml of Streptomycin. The relative standard deviations of intra and inter day assay less than 2 and the method was conveniently used for routine analysis of Streptomycin in bulk and tablet dosage forms.

Keywords: Streptomycin, RP-HPLC, LOD, LOQ, Precision, Accuracy, Linearity

1. Introduction: [1]

Streptomycin is a drug of the antibacterial class, approved for the treatment of bacterial infection.

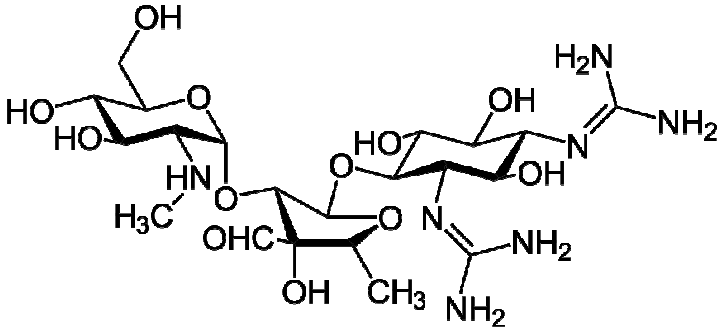


Fig 1: Structure of Streptomycin

2. Mechanism of Action:

Streptomycin functions as a protein synthesis inhibitor. It binds to the small 16S rRNA of the 30S ribosomal subunit irreversibly, interfering with the binding of formyl-methionyl-tRNA to the 30S subunit.

3. Materials and Methods

Instrumentation

The Agilent 1120 Compact LC HPLC system consisting of gradient pump (LC-10AT vp pump) (4MPa or

40barr), Standard cell, UV variable wavelength detector, rheodyne injector, and agilent syringe was used. Sonicator (EQUITRON230VAC, 50Hz), Analytical weighing balance (Shimadzu AUX 220) was used for weighing, , vacuum pump (SUPER FIT), filtration kit (TARSONS) and Nylon membrane filter (Merck Millipore) for solvents and sample filtration were used throughout the experiment. The separations were achieved on Agilent Eclipse plus C_{18} column (5µm 4.6x250mm), column length is 25 cm with UV detection at 224nm. Double beam UV-Visible spectrophotometer (SHIMADZU-UV 1700) was used for wavelength detection. The EZ Chrome Elite software-dual channel was used for evaluation, acquisition and storage of chromatographic data.

Chemicals and reagents:-

Empagliglozin is a tablet dosage form each contains 25mg of Streptomycin. HPLC grade Acetonitrile (Merck), Analytical grade monopotassium phophate buffer was used as the solvents throughout the experiment. Pharmaceutical formulation Jardiance tablet (label claim contain 25mg) was used in HPLC analysis. HPLC grade water obtained by using Direct-Q water purification system (Millipore, Milford, USA) was used in HPLC study.

Buffer Preparation:

Monopotassium phosphate Buffer solution was prepared by dissolving 0.68g of monopotassium phosphate buffer in 500ml HPLC grade water (10 mm). The pH of the resulting solution was adjusted 3 by using orthophosphoric acid.

Chromatographic condition:-

Mobile phase consisting Monopotassium phosphate buffer and Acetonitrile was used in the ratio of (55:45) ata flow rate of 1ml/min. Wavelength was observed at 224nm and the retention time was observed at 1.870min with an injection of 20µl by maintaining ambient temperature conditions.

Preparation of Stock solution for HPLC estimation of Streptomycin

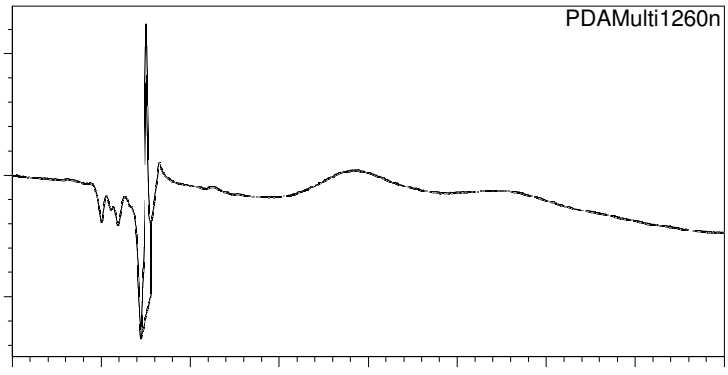
A tablet is powdered which contain 25 mg of active ingredient is transferred into 250 ml of volumetric flask and is dissolved in 25ml of acetonitrile and monopotassium phosphate buffer(45:55) volume were made up to the mark with same solvent. This gave the concentration of 1 mg/ml and sonicated for 2min.

Preparation of standard solution

METHOD VALIDATION AND DEVELOPMENT

The developed method was validated according to ICH guidelines with respect to accuracy, precision, linearity, specificity, and robustness, limit of detection (LOD), limit of quantification (LOQ), ruggedness and system suitability

From stock solution different concentration range of 0.5-08 µg/ml was prepared and sonicated for 2.25min.



.Figure 2- A typical chromatogram for Streptomycin

Linearity:-

The procedure within a given range to obtain test result which are directly proportional concentration (amount)

of analyte in the sample. The working standard were prepared, aliquots of different concentration such as 0.5µg/ml, 1µg/ml, 2µg/ml, 4µg/ml, 6 µg/ml and 8 µg/ml with acetonitrile and buffer mixture. Six dilutions of each concentration were prepared separately. From above dilution, 20µl concentration were injected to the HPLC system and their chromatogram was observed. Peak areas were recorded for all the chromatograms anda standard calibration curve of peak area against concentration was plotted.

Concentration(µg/ml)	Area
0.5	2155328
1	2838642
2	3825289
4	6087845
6	8654239
8	10196552

Table No.1: **Linearity data for Streptomycin**

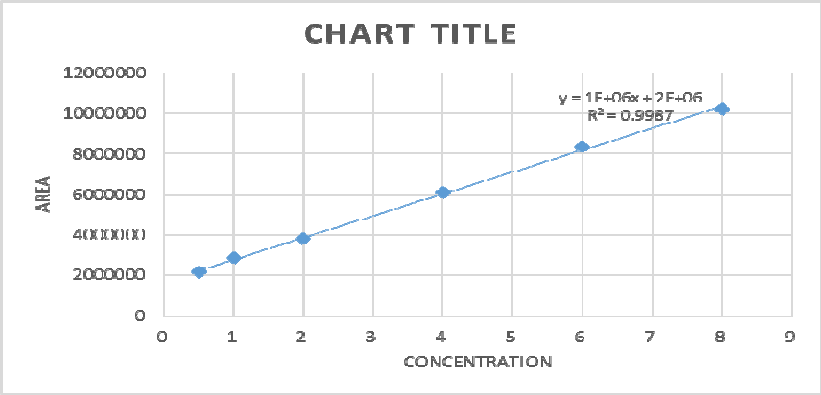


Figure 4- linearity graph of Streptomycin

Precision:-

The precision of an analytical procedure express the degree of agreement among the individual test results when the method is applied repeatedly to multiple sampling of a homogenous sample under the prescribed conditions.

The precision of the assay was determined in terms of intra and inter day variation in the peak area for a set of drug solution 2µg/ml, assayed six times on the same day and on different 2 days.

The intra and inter day variation in the peak ratio of the drug solution was calculated in terms of co-efficient of variation (CV) and obtained by multiplying the ratio of the standard deviation to the mean with 100(CV=SD/MEAN × 100) shown in the graph

INJECTION 15µg/ml	AREA	AVERAGE	STANDARD DEVIATION (SD)	%RSD
1	3825289	3826290	63446.53	1.65
2	3726241			
3	3835283			
4	3867213			
5	3911243			
6	3792468			

Table 2: intraday day data for precision-morning

INJECTION 15µg/ml	AREA	AVERAGE	STANDARD DEVIATION (SD)	%RSD
1	3813232	3828629	34474.44	0.90
2	3794683			
3	3824123			
4	3826262			
5	3818349			
6	3895123			

Table 3: Data for Intraday precision-afternoon

INJECTION 15µg/ml	AREA	AVERAGE	STANDARD DEVIATION (SD)	%RSD
1	3926243	3882124	52464.66	1.35
2	3926243			
3	3785243			
4	3895001			
5	3825013			
6	3816003			

Table 4: Data for Inter day precision-day 1

INJECTION 15µg/ml	AREA	AVERAGE	STANDARD DEVIATION (SD)	%RSD
1	38361232	3818480	14422.07	0.37
2	3826921			
3	3825621			
4	3795732			
5	3817371			
6	3809112			

Table 5: Data for Inter day precision-day 2

Accuracy:-

The accuracy of analytical procedure expresses the closeness of test result between the value which is accepted either as true value or reference value. The procedure for the preparation of the solution for accuracy determination at 80% (2 µg/ml), 100% (4 µg/ml) and 120% (6 µg/ml) level were prepared in the acetonitrile and Monopotassium phosphate buffer mixture.

The procedure for the preparation of the solutions for Accuracy determination at 80%, 100% and 120% level

were prepared in the acetonitrile.
For 80% Accuracy for Streptomycin:
10 mg of the pure drug was added to 08 mg of formulation
For 100% Accuracy for Streptomycin:
10mg of the pure drug is added to 10mg of formulation For 120% Accuracy for Streptomycin:
10mg of the pure drug is added to 12mg of formulation

Sl.no	Level of percentage recovery	Amount present (mg/table t)	Amount of standard drug added	Area response	Mean	SD	RSD (%)	Total amount recover	% recovery
1	80%	10	8	3823241	3846224	41449.34	0.083	2.01	100.50
				3894132					
				3821471					
2	100%	10	10	6087845	6082824	7753.82	0.13	3.99	99.75
				6073894					
				6086734					
3	120%	10	12	8654234	8694046	69870.62	0.80	6.03	100.50
				8683179					
				8774723					

Table 6: - Accuracy data for estimation of Streptomycin

Robustness:-
Robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variation and provides an indication of its repeatability.
As defined by the ICH, the robustness of an analytical procedures describes to its capability to remain unaffected by small and deliberate variation in the chromatographic conditions and found to be unaffected by small variation ±0.1ml/min in flow rate of mobile phase, and wavelength ±2nm result are shown.

Sl.no.	Parameter	Optimized	Used	Retention time(mins)
1	Flow rate	1 ml/min	0.9 ml/min	2.070
			1.1ml/min	1.697
2	Detection wavelength	224 nm	219nm	1.870
			229nm	1.867

Table No. 7:-Robustness data for estimation of Streptomycin

Limit of Detection (LOD):-
The limit of detection of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not quantified.

Limit of Quantification (LOQ):-
The limit of quantification of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected and quantitatively.
LOD and LOQ were calculated according to ICH recommendations where the approach is based on the signal-to-noise ratio. Chromatogram signals obtained with known low concentrations of analytes was compared with the signals of blank samples. A signal to noise ratio 3:1 and 10:1 was considered for calculating LOD and

LOQ respectively.

Name of drug	LOD µg/ml	LOQ µg/ml
Empagliflozin	0.083	0.28

Table 8: LOD and LOQ for estimation of Streptomycin

CONCLUSION

From the above results, method was found to be accurate, precise, linear, specific, system suitable, robust proved to be sensitive, convenient and cost effective for the estimation of Streptomycin in oral solid dosage form. The proposed method has a run time of 8mintues, which makes the method simple, cost effective and suitable for the routine analysis of Streptomycin in oral solid tablet dosage form.

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