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Simple and Rapid Method on High Performance Liquid Chromatography (HPLC) for Estimation of Streptomycin Sulphate

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Abstract: A new simple, rapid and accurate reverse phase high Performance liquid chromatography assay has been developed for the estimation of Streptomycin. The estimation of streptomycin was done by Hitachi D-2000 Elite HPLC system manager using gradient pump system. From two pumps (L-2130) A containing mobile phase Methanol was adjusted on 40% flow while the pump B containing Mobile phase buffer (Orthophosphoric acid + Triethylamine) on 60%. The flow rate was 1.0ml/min; the separation was done by using C-18 column and detected by UV-Visible Detector at 240 nm. The retention time was within 2.62-2.63 minutes. Standard curves were linear over the concentration range of 0.01 to 2 µg/L. The extraction recovery of streptomycin was withen 96 and 103%. The proposed method was found to be rapid accurate, repeatability and consistent. It was successfully applied for the analysis of the Streptomycin Sulphate. This was rapid from before reported method.

Key words: HPLC · Streptomycin Sulphate · Optimization · Method validity

INTRODUCTION

Streptomycin sulfate (STR), di[*O*-2-deoxy-2-methylamino-*a*-*L*-glucopyranosyl-(1→2)-*O*-5-deoxy-3-*C*formyl-*a*-*L*-lyxofuranosyl-(1→4)-*N*,*N*′-diamidino-*D*streptamine] trisulfate. [CAS: 3810-74-0] is a substance produced by the growth of certain strains of *Streptomyces griseus* or obtained by other means. Its molecular formula is$(C_{21}H_{39}N_7O_{12})2\bullet 3H_2SO_4$ and relative molecular mass 1457.100 It is marketed as a sulfate salt of STR which is more soluble than the free base. [1] STR is one of the most widely used aminolglycoside antibiotics. It is used to treat infections in humans, veterinary medicine, as well as in plant agriculture. Which must be determined and all impurities must meet specified limits before a manufactured lot is used clinically. The current United States Pharmacopeia (USP 30, NF 25) compendial method for streptomycin sulfate measures streptomycin A as the primary antibiotic [2,3].

Its one of the first antibiotics to be discovered. It was used for a range of bacterial infections but has been largely replaced owing to the discovery of more effective antibiotics. In recent years, its popularity has increased once more because of its use in combination with other drugs for the treatment of tuberculosis. STR is bacteriocidal to both Gram-positive and Gram-negative. [4].

A comprehensive review of analytic data for STR has been published. [5] it is poorly and irregularly absorbed form the gastrointestinal tract. It is readily absorbed after intramuscular administration and widely distributed throughout the body. About one-third of STR in the circulation is bound to plasma proteins. About 30-90% of a dose is excreted unchanged in the urine in 24 hours. [6] The onset of ototoxic side effects of aminoglycosides is delayed and gradual. Thus, some investigators have postulated a metabolic transformation or activation to produce a molecular species derived from the antibiotic which may show greater toxicity than the drug itself, or alternatively these derivatives may act in combination with the antibiotic, producing the ototoxic effect [7]. Studies with kanamycin [8], gentamicin [9,10] and streptomycin (STR) [11] have been performed, but the resulting metabolites have not been identified and characterized.

Several papers have been published proposing HPLC methods for the identification [12] and control of the impurities [13] in STR using UV detection [14,15] mass spectrometric [16] and pulsed amperometric detection (PAD) [17].

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MATERIALS AND METHODS

Chemicals and Reagents: Streptomycin Sulphate (99% purity, Batch: 052648) was supplied by MP. Biomedicals, LLC Germany. Methanol, Acetonitril, ortho phosphoric acid and diethyl-amin of HPLC grade. (sigma aldrich Germany). Monopotassium phosphate and citric acid, Extra pure (Scharlau Spain) Deionized water and Solvents of LC grade was obtained by filtering through 0.45µm filter membarane and degass for 20 minutes by ultra sonic cleaner.

Chromatographic Conditions: The HPLC (Hitachi D-2000 Elite system manager) equipped with two pumps L-2130, autoinjector/autosampler L-2200 syring loading sample injector valve's fitted with 10µl sample loop of 200 vails and UV-VIS detector L-2420. The Chromatographic separation was achieved using Column oven L-2300 and column intersil ODS-3 C18 (GL Sciences Inc. Tokyo Japan 5µm, 250×4.6 mm). filterting assembly (Model Rocker-300 Thaiwan) and ultrasonic cleaner Ceia (Model CP-104 Italy) were used for solvents filtration and degassing.

Method Validation: Method validation parameters studied were limit of detection (LOQ), limit of quantification (LOD), linearity, repeatability and accuracy.

The LOQ was defined as the lowest concentration that could be determined with acceptable accuracy and precision. The LOD was determined by diluting solutions of known concentration until the response was three times the noise while LOQ was defined as the lowest concentration that could be determined with acceptable accuracy and precision. The LOQ was calculated on the basis of minimal accepted value of S/N 10. The Linearity was determined by calibration curves. For the construction of calibration curve, six calibration standard solutions were prepared and each standard solution was injected once. The repeatability was estimated by assaying six replicate samples on day-1 and day-2. The Accuracy was evaluated by the recovery determination.

Preparation of Solution

Solvents Preparation: (Orthophosphoric acid + Triethylamine). 100ml of 1M of Orthophosphoric acid was dissolved in 1000ml distilled water and and the P^H was adjust to 3.0±0.1 with Triethylamine.(Monopotassium Phosphate + citric acid) 0.075 M monopotassium

Phosphate was well mixed with 1.0 M citric acid. Methanol and Acetonitirl used as directly. All the solvents were filtered through 0.45µm filter membarane and degass for 20 minuts by ultrasonic cleaner.

Standard Preparation: A standard stock solution of streptomycin (100mg/100mL) was prepared by dissolving the drug in 40ml methanol and 60ml Buffer. The concentration of standard was 1mg/ml solution was further diluted with mobile phase in same ratio to 0.1, 0.2, 0.3, 0.5, 1.0 and 2.0µg/ml. All the solutions were stored at at room 4°C and were brought to room temperature before use.

Sample Preparation: The sample solution was prepared and analyzed in the same manner as a standard solution.

Optimaization of Mobile Phase Composition: The Mobile phase composition was optimized on the basis of ChP method. The aqueous phase were composed of Buffers (Orthophosphoric acid + Triethylamine) and (monopotassium Phosphate + citric acid) while the organic phase was selected from methanol, acetonitrile and their Mixture in different proportions. Many efforts were made on the adjustment of the ratios of the components of mobile phases. The best separation and recovery were made by using 40% methanol with 60% buffer. (Orthophosphoric acid + Triethylamine).

Chromatography: The optimized mobile phase was a methanol and Buffer. Pump A was adjusted at flow rate 40% for Methanol while pump B at 60% flow rate for Buffer. The flow rate was set at 1 ml/min. The injection volume was 10µL for samples and standerdes, which is injected to the column by autosampler. The separation was achieved using Column oven L-2300 at 40ºC and column intersil ODS-3 C18 (GL Sciences Inc. Tokyo Japan 5um, 250×4.6 mm) and the detection wavelength was set at 240 nm. The UV absorbance of the effluent was scanned by UV Spectrophotometer (optima S-3000 Kyoto, Japan) over the range of 200-400nm and was obtained by measuring the absorption of 0.1µg/ml solution, prepared from stock solution. This showed a maximum absorbance on 240nm.

RESULT AND DISCUSSION

LOD and LOQ: Limit of detection (LOD) and limit of quantification (LOQ) of the assay method were determined. Results showed that the detection limit of the tested drugs was 0.03 µg/ml (Figure 1). For measurement, consideration was given only when the first condition was satisfied for ascertaining the presence of target compounds i.e. Streptomycin with a signal/noise ratio of 3 (S/N = 3). The LOQ calculated was 0.1 µg/ml (Figure 2).

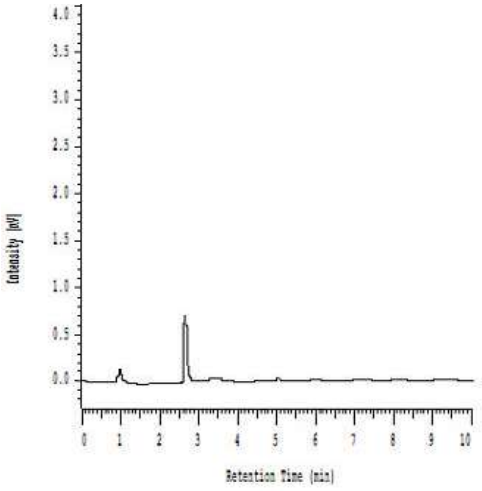


Fig. 1: LOD for 0.03 µg/ml
*Peak of 10µl Injection of STR
**Conc. 0.03µl/ml

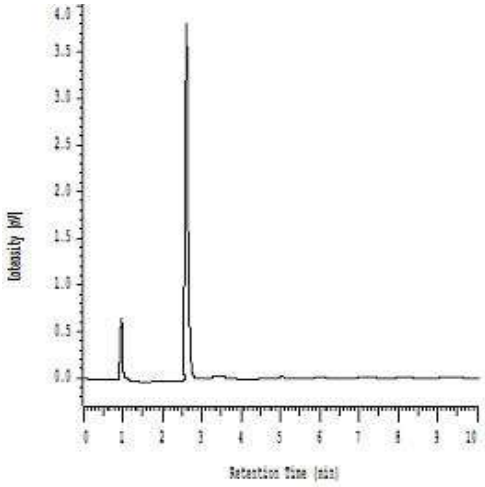


Fig. 2: LOQ for 1.0 µg/ml
*Peak of 10µl Injection of STR
**Conc. 0.03µl/ml

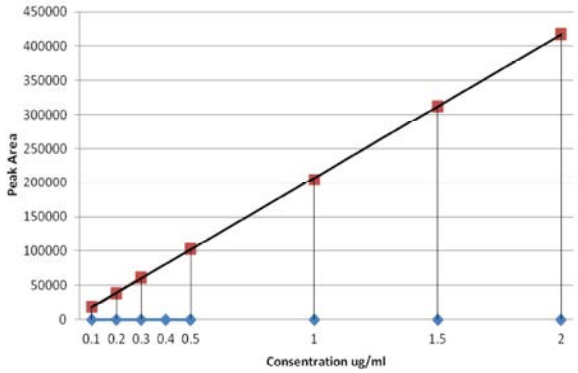
So, LOQ was started from this concentration. The LOQ was calculated on the basis of minimal accepted value of S/N 10.

Linearity: The standard solution is further diluted to 0.1, 0.2, 0.3, 0.5, 1.0 and 2.0µg/ml. Intra-day and inter-day precision was determined by injecting 10 ul six standard solutions. (n = 6). The mean of the recorded peak area of inter day and intra day is taken for calibration curve. (Table 1) The peak areas which were automatically measured by an integrator of HPLC instrument. The calibration curve obtained by plotting peak area against concentration in (Graph 1) which showed linearity in

Table 1: Peak Areas of Streptomycin Sulphate

		Peak Areas		
S.No	Concentration µg/ml	Inter Day	Intra Day-2	Mean
1	0.1	18600	18650	18625
2	0.2	38789	38562	38675
3	0.3	61217	61385	61301
4	0.5	102590	103040	102815
5	1.0	204232	205123	204677
6	2.0	417986	418826	4184406

Each 10µl Injection of Streptomycin Sulphate
Peak Area (automatically measured by an integrator of HPLC instrument)



Graph 1: Linear Curve of STR
Linear Curve of Streptomycin Sulphate Standered for 10µl Injection

accordance to Beer's law over this range and the linearity equation was y = 20997x-2837 and the regression coefficient r^2 were in the range from 0.9995-0.9999 (n=6).

Recovery and Precision of Streptomycin: The current method is valid and accurate. The Accuracy was evaluated by the recovery determination. Our results showed the amount obtained by this method were between 96% and 103 %. (Table 2) the absolute recoveries of streptomycin Sulphate were determined in triplicate by direct comparison of peak area form standard versus sample (Figure 3-4). The data was analyzed statistically by calculating average mean RSD by using formula. [RSD = (S.D./mean of the recoveries) × 100%].

$$\text{Sample potency µg/ml} = \frac{\text{Peak area of Sample}}{\text{Peak area of Standerd}} \times \frac{\text{conc. of Standerd}}{\text{conc. of sample}} \times \text{Standerd potency}$$

Specificity: The specificity of the method was ascertained by analyzing standard drug and sample.

Table 2: Recovery and Precision of Streptomycin Sulphate

	Recovery					
S.No	Conc. (µg/ml)	Conc. Day 1 (µg/ml)	Conc. Day 1 (µg/ml)	Mean (µg/ml)	Recovery (%)	Precision RSD%
1	0.1	0.0967	0.0959	0.0963	96.3	0.21
2	0.2	0.1900	0.1980	0.1940	97.0	0.59
3	0.3	0.3030	0.3060	0.3040	101.3	0.19
4	0.5	0.4960	0.4990	0.4970	99.4	0.08
5	1.0	1.0160	1.0480	1.0300	103.0	0.007
6	2.0	2.0810	2.0610	2.0700	103.5	0.006

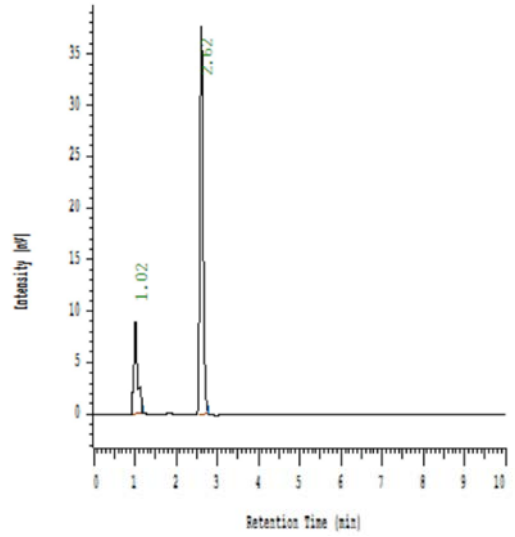


Fig. 3: Standered Peak for 1 µg/ml
*Peak of 10µl Injection of STR Standerd
**Conc. 1.0µl/ml

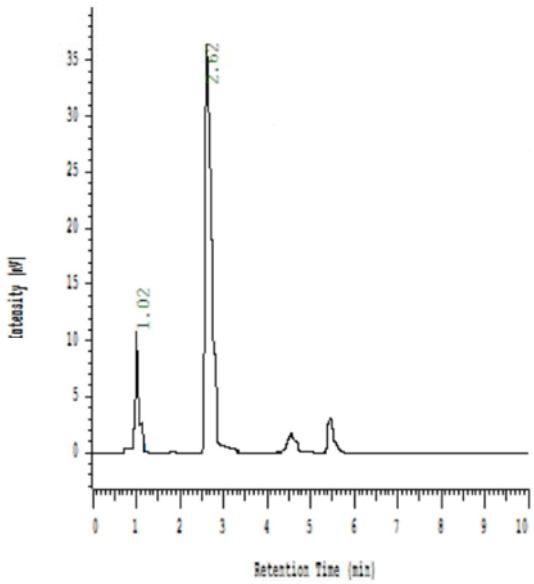


Fig. 4: Sample Peak for 1µg/ml
*Peak of 10µl Injection of STR Sample
**Conc. 1.0µl/ml

The retention time (RT) of streptomycin confirmed by comparing the RT with that of the standard, which was within 2.62-2.63 minutes. The presence of other ingredients in the formulation did not cause any interface with the streptomycin peak so specific for streptomycin sulphate.

CONCLUSION

The aim of this study was to develop a selective and sensitive HPLC method for the rapid detection of streptomycin sulfate. Various methods are available for determination but having some disadvantages of being time consuming with poor recoveries and reproducibility. While the proposed method was found to be rapid accurate, repeatability and consistent. It was successfully applied for the analysis of the drug in marketed formulation and could be effectively used for the routine analysis of formulation containing the drug without any alteration in the chromatography conditions. This was rapid from before reported method.

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