

## APPLICABILITY OF A COLORIMETRIC METHOD FOR EVALUATING STREPTOMYCIN SULPHATE LOADED SOLID LIPID NANOPARTICLES

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Received: 06 Nov 2011, Revised and Accepted: 17 Dec 2012

### ABSTRACT

Streptomycin (STR), an antitubercular drug belongs to the class of aminoglycosides. Latter are purported to be deficient of a characteristic chromophore or fluorophore moiety which is essential for spectrophotometric analysis. Most of the studies thus report on other time consuming and highly sophisticated techniques including gas chromatography, high performance liquid chromatography (HPLC), and liquid chromatography with mass spectrometric detection (LC-MS) and immunoassays.

The present study reports on a simple and reproducible colorimetric method of analysis of streptomycin and its application to evaluation of streptomycin sulfate loaded solid lipid nanoparticles (STRS-SLNs). The method is based on the nucleophilic substitution reaction between STR and sodium nitroprusside (SNP), resulting in the formation of a red-colored product in an alkaline medium. The adduct is measured at a maximum absorption wavelength at 495 nm in visible light. The method was found to be linear in the range of 4.0 - 256.0  $\mu\text{gml}^{-1}$  for drug samples. When observed under TEM, SLNs were found to be spherical in shape. The size of the nanoparticles observed under TEM was in the range of 57.0 $\pm$ 6 nm thus confirming the small size of the prepared nanoparticles. The total drug content and entrapment efficiency of STRS-SLNs was estimated to be 89.0% $\pm$ 1.2% and 60.0%  $\pm$  2.1% (n=6) respectively. Further the method was also evaluated for Determining STRS in spiked human plasma samples with mean recovery of 89.1% $\pm$ 4.7.

**Keywords:** Solid lipid nanoparticles, Streptomycin sulphate, UV spectrophotometry, Plasma

### INTRODUCTION

Streptomycin (STR) is a water-soluble aminoglycoside derived from *Streptomyces griseus*. It is marketed as the sulphate salt. Streptomycin sulphate (STRS) is a bactericidal antibiotic. It acts by interfering with normal protein synthesis. Streptomycin has been shown to be active against most strains of the following organisms both in vitro and in clinical infections. Some of the commonest being *Brucella* (brucellosis), *Escherichia coli*, *Aerobacter aerogenes*, *Klebsiella pneumoniae*, and *Enterococcus faecalis* (in urinary tract infections), *Haemophilus ducreyi* (chancroid), *Haemophilus influenzae* (in respiratory, endocardial, and meningeal infections-concomitantly with another antibacterial agent), *Klebsiella pneumoniae* (pneumonia), *Mycobacterium tuberculosis*, *Pasteurella pestis*, *Streptococcus viridans*, and *Enterococcus faecalis* (in endocardial infections-concomitantly with penicillin)<sup>1</sup>. Streptomycin is administered by parenteral route i.e, intramuscular route (i.m) due to its unreliable oral absorption. In view of this, a controlled release formulation i.e streptomycin sulphate loaded solid lipid nanoparticles; (STRS-SLNs) of streptomycin was developed and characterised so as to improve its absorption<sup>2</sup>.

Quantification of aminoglycoside antibiotics using standard detection methods of refractive index and ultraviolet or visible light absorption is unsatisfactory, as the extinction coefficient for antibiotics is not optimum for accurate and sensitive detection<sup>3</sup>. Since streptomycin does not possess a strong UV-absorbing chromophore group, the state-of-the-art analytical method for their determination at trace levels is liquid chromatography (LC) with post-column derivatization and fluorescence detection<sup>4</sup>.

Currently, there is no analytical method reported or available for the determination of streptomycin from its developed drug delivery system. Although there exists database on the determination of streptomycin using various sophisticated techniques,<sup>5</sup> however scarce data is available on detection of any of its developed delivery system.

The present investigation reports on an analytical method for the detection of streptomycin from its developed solid lipid nanoparticles. The UV spectroscopic method described herein presents a simple, sensitive, and rapid technique of analysis which can be used in a laboratory with a high throughput of samples<sup>6</sup>.

### MATERIAL AND METHODS

Streptomycin sulphate was obtained from Panacea Biotec, Lalru, India, Sodium hydroxide was purchased from, Rankem, Mumbai, Sodium nitroprusside and Potassium ferricyanide from Fischer Scientific (AR), Mumbai, Soy Lecithin (Hi Media, India); Tween 80 (S.D. Fine Chemicals Ltd., India); Compritol® 888 ATO (Glyceryl Behenate, gift sample from Gattefosse, USA) were also used in the study.

All other chemicals and reagents were of analytical grade and were used without further purification.

#### Preparation of Calibration curve (CC)

Various concentrations of STRS in the concentration range of 4-256  $\mu\text{g/ml}$  were prepared and the analysis was performed with ten replication.

To 1.0 ml of varying solution range was prepared and to it 2.5 ml solution of sodium nitroprusside (SNP) reagent was added. The volume of the above solution was made upto 12.5 ml with double distilled water so as to reach the concentration range of 4-256  $\mu\text{g/ml}$ . The mixture was shaken and allowed to stand for 20 min at room temperature, and the absorbance of the solution was measured at 495 nm against a reagent blank prepared in a similar manner but

without STRS<sup>7</sup> with  $E_{1\%}^{1\text{cm}} = 30$

**SNP reagent was prepared by adding 1 g each of SNP, potassium ferricyanide and sodium hydroxide to 100 ml of water and the volume was then made up to 400 ml with distilled water.**

#### Preparation of calibration curve of Streptomycin sulphate in plasma

Calibration curve of STRS was also prepared in plasma in the concentration range of 16-256  $\mu\text{g/ml}$ . The method involved the extraction of the drug from the suitably spiked plasma (200  $\mu\text{l}$ ), samples using methanol (500  $\mu\text{l}$ ) as an extracting solvent. Post extraction, the SNP procedure of drug detection was followed and calibration curve was obtained as discussed above.

#### Validation of analytical method

**Linearity:** The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of

analytes in samples within a given range or proportional by means of well-defined mathematical transformations.

**Accuracy:** The accuracy of an analytical method describes the closeness of mean test results obtained by the method to the true value (concentration) of the analyte. Accuracy is determined by replicate analysis of samples containing known amounts of the analyte. The deviation of the mean from the true value serves as the measure of accuracy.

**Precision:** The precision of an analytical method describes the closeness of individual measures of an analyte when the procedure is applied repeatedly to multiple aliquots of a single homogeneous volume of biological matrix.

**Recovery:** The extraction efficiency was calculated by comparing the concentration of extracted plasma standards with concentration of reference standards added to blank plasma extract. The recovery study for streptomycin was accomplished at five concentration levels (16, 32, 64, 128, 256 µg/ml) in plasma.

#### Preparation of STRS-SLNs

Polysorbate 80 (45.45% w/w), soy lecithin (0.58%w/w), and water were placed together in a beaker and heated to the lipid melt temperature. Lipid (7.27%w/w) was melted at 82-85°C. Streptomycin sulfate was added to the aqueous phase, following which the hot aqueous emulsifier mix, was dropped at once into the lipid melt, under magnetic stirring to obtain a clear microemulsion. The hot microemulsion thus formed, was transferred into an equivalent amount of cold water (~2°C) under continuous mechanical stirring (5000 rpm) for 2 h. The prepared SLNs were stored in refrigerator until further analysis<sup>8,9</sup>

#### Characterization of STRS-SLNs

##### Transmission electron microscopy (TEM)

Morphology of SLNs was examined using an electronic transmission microscope (Hitachi H-100; Japan).

##### Total drug content (TDC)

Total drug content of the prepared STRS-SLNs was estimated spectrophotometrically at  $\lambda_{max}$  of 495 nm by disrupting 1 ml of the SLN dispersion using an appropriate volume of chloroform: methanol (1:1) and drying the above solution under vacuum. To the above solution 2.50 ml solution of SNP were added and volume made to 12.5 ml with distilled water. The mixture was then shaken and stood for 20 min at room temperature, and the absorbance of the solution was measured at 495 nm. The TDC was estimated by plotting the absorbance value on the prepared calibration curve

using  $E_{1\%}^{1\text{cm}}$  obtained from the calibration curve.

##### Entrapment efficiency (EE)

For determining the entrapment efficiency, SLN dispersion was ultracentrifuged at 90,000 rpm (1 lac g) for 2 h at 4°C. The clear

supernatant was decanted. The pellet of STRS-SLNs, was then washed with water to remove any untrapped drug, and re-centrifuged. The two supernatants were combined and its absorbance was recorded. The pellet obtained from the above was dissolved in suitable quantity of methanol: chloroform (1:1) and the above solution was evaporated to dryness. 2.5 ml solution of SNP were added and then diluted to 12.5 ml with distilled water. The mixture was then shaken and stood for 20 min at room temperature, and the absorbance of the solution was measured at 495 nm.

Amount of drug in the pellet gave a direct measure of the extent of drug entrapped, while the concentration of drug in the supernatant indicates untrapped free drug. Latter was used to obtain the mass balance of the total drug content. Absorbance value obtained for blank SLNs treated in a similar manner was used as the control value to compensate for any interference of the ingredients.

$$\% EE = \frac{\text{Total drug entrapped} \times 100}{\text{Total drug incorporated (TDC)}}$$

## RESULTS AND DISCUSSION

### Optimization of the parameters

#### Effect of Amount of SNP

In order to study the effect of amount of SNP on the determination of STR, the amount ranging from 0.20 mL to 3.00 mL was submitted to the proposed procedure. It was found that the absorbance of the product increased with the increase in the amount of SNP. When the amount of SNP was 2.50mL, the absorbance reached the maximum and remained stable. Therefore, 2.50mL of SNP was selected as the amount to use for optimum conditions in the later experiments.

#### Effect of Standing Time

The absorbance was measured after the solution was standing for a different number of minutes at room temperature; the STR reacts immediately with SNP at room temperature. Subsequently, the absorbance begins to increase and becomes constant after 20 min. Moreover, the absorbance can remain constant for at least 50min. Based on the preceding discussions, the condition of room temperature and 20 min of standing time were chosen as optimum.

#### Sensitivity, Limit of Detection

The calibration graph was linear over the concentration range 4-256µg/ml. Regression analysis indicated a linear relationship between absorbance and concentration.  $r^2=0.999$ . The LOD is 2 µg/ml and LOQ was found to be 4µg/ml. These small values indicate the high precision of the proposed method.

#### Standard Plot

A linear correlation  $r^2= 0.999$  obtained between absorbance and concentration values in the range of 4-256 µg/ml indicated the results to be in agreement with the Beer-Lambert law (Figure 1).

The  $E_{1\%}^{1\text{cm}}$  as obtained from the curve was estimated to be 30.

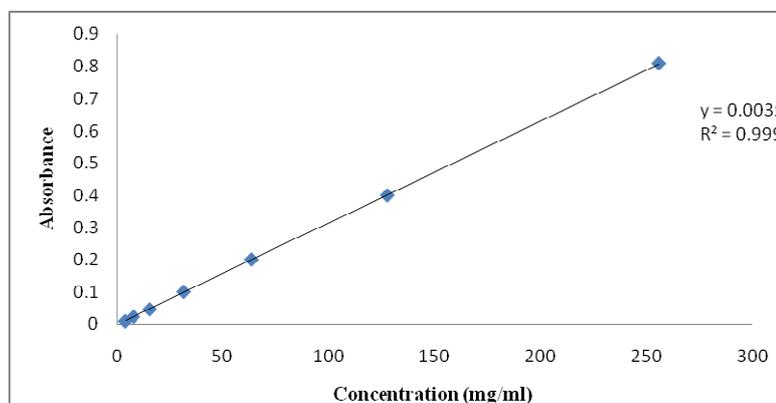


Fig. 1: Standard plot of free STRS

### Validation of spectrophotometric method of analysis for STRS

The lower limit of quantization for the developed method was found to be 2 µg/ml. The linearity of the method was determined by a weighted least-squares regression analysis of a five point standard curve. The accuracy of STRS in water was found to be in the range of 90-105 % (Table 1) and the precision was found to be < 1.06% (Table 2).

### Standard plot in plasma

Standard plot of STRS in human plasma was found to be linear over the concentration range of 16 µg/ml - 256 µg/ml with a linear correlation coefficient ( $r^2= 0.998$ ).

### Recovery

The extraction recovery of STRS in spiked plasma was determined at all levels of the calibration curve. The absolute recoveries of STRS from plasma were found to (83-95 %) as shown in (Table 3). Recovery of the STRS in presence of blank SLNs was determined in

same manner as determined for the formulation and found in the range of 97-102% this showed that the other components do not interfere with the determination of streptomycin in the proposed method.

### Characterization of STRS-SLNs

When observed under TEM, SLNs were found to be spherical in shape (Fig. 2). The size of the nanoparticles observed under TEM was in the range of 57±6 nm thus confirming the small size of the prepared nanoparticles.

### Total drug content

Total drug content of streptomycin loaded nanoparticles was estimated to be 89.0%±1.2% (n=6)

### Entrapment efficiency

The entrapment efficiency of streptomycin loaded nanoparticles was estimated to be 60.0% ± 2.1% (n=6)

**Table 1: Accuracy in measuring a known concentration of STRS (n=10)**

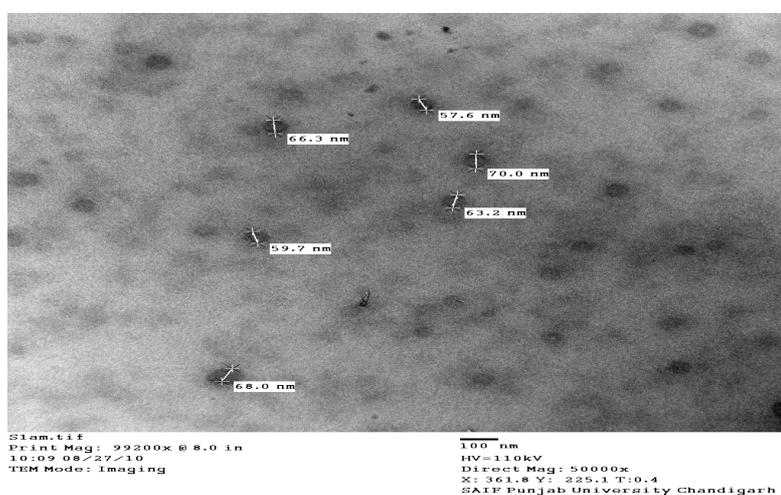
S. No.	Conc. (µg/ml)	Abs.	Nominal Conc. (µg/ml)	% Accuracy	Mean
1	8	0.024 ± 0.0001	7.2	90	
2	16	0.047 ± 0.0005	16	100	100.2
3	32	0.101 ± 0.0001	33.6	105	
4	64	0.201 ± 0.0002	66.5	103	
5	128	0.402 ± 0.0002	133	103	

**Table 2: Precision in measuring a known concentration of STRS**

S. No	Abs.	Mean	S.D	% CV
1	0.047			
2	0.046	0.046	± 0.0005	1.08
3	0.047			
4	0.047			
5	0.046			
6	0.047			
7	0.046			
8	0.046			
9	0.046			
10	0.047			

**Table 3: Accuracy and recovery data of STRS in plasma obtained from UV Spectrophotometry (n=3)**

Nominal conc. (µg/ml)	Observed conc. (µg/ml)	Accuracy %	Recovery %	Mean	±SD
16	15.2 ± 0.05	95.0	95		
32	29.2 ± 0.03	91.2	86	89.1	4.7
64	60.6± 0.04	94.6	91		
128	121.0 ± 0.04	94.5	90.9		
256	251.0 ± 0.03	98.0	83		



**Fig. 2: TEM micrograph of STRS-SLNs**

**CONCLUSION**

A new method for the determination of STR and STRS loaded solid lipid nanoparticles was established by spectrophotometry, using SNP as a chromogenic reagent. The proposed method is simple, rapid, precise, and economical. The major advantage in comparison with other methods previously described is that STR can be determined directly in visible light so that potential interference can be avoided. Furthermore, the low cost of SNP and the ease of carrying out the spectrophotometer make the proposed procedures very suitable for the analysis of STR for solid lipid nanoparticles. The method was also applied for its potential in quantification of plasma samples.

**ACKNOWLEDGEMENT**

The research grant provided by Department of Biotechnology, New Delhi, and Gift Samples provided by Panacea Biotec Ltd, Lalru is highly acknowledged.

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