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Synthesis, Characterization and HPLC Analysis of Streptomycin with its Derivatives

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Abstract A new streptomycin derivative was synthesized and identified using UV-Vis, FTIR, and HNMR; the streptomycin first derivative has a moderate inhibition *Escherichia coli* compared to the standard medication, which did not give any inhibition to this type of bacteria. A sensitive, accurate RP-HPLC method was developed to separate these streptomycin derivatives from the standard streptomycin drug and applied for the drug analysis in pharmaceuticals at optimal separation conditions using the Nucludar C18-DB column. The results showed good separation between the standard streptomycin drug and its prepared derivatives; the first, second, and third derivatives have t_R of 2.102, 6.19, and 7.695 min, respectively, while the standard streptomycin appeared at t_R of 5.352 min. This method was applied for the separation and estimation of streptomycin in ABBOTT and TROGE pharmaceuticals via standard graph, and the results proved that this method is accurate and the obtained concentration values match what was proven by the drug manufacturer.

Key Words Streptomycin, RP-HPLC, 2-hydroxynaphthaldehyde, β-naphthol, phenol, Biological activity

1. Introduction

Streptomycin is an aminoglycoside antibiotic medication, initially isolated from the bacterium *Streptomyces griseus*. It is used to treat various bacterial infections, including tuberculosis, brucellosis, and tularemia, and exhibits active properties against numerous Gram-negative bacteria.

Streptomycin appears as white crystals with a melting point of 12 °C (54 °F). Its molecular weight is 581.580 g·mol^{-1}, and its molecular formula is $C_{21}H_{39}N_7O_{12}$. The IUPAC name for Streptomycin is 2-[4-[3-[4,5-dihydroxy-6-(hydroxymethyl)-3-methylamino-oxan-2-yl]oxy-4-formyl-4-hydroxy-5-methyl-oxolan-2-yl]oxy-3-guanidino-2,5,6-trihydroxy-cyclohexyl]guanidine see Figure 1.

Various methods have been employed for estimating Streptomycin, including High-Performance Liquid Chromatography (HPLC) [1], [2], Liquid Chromatography/Mass Spectrometry (LC/MS) [3], [4], Spectrophotometry [5], [6], Cloud Point Extraction [7], Enzyme-Linked Immunosorbent Assay (ELISA) [8], Chemiluminescence [9], Fluorescence Polarization Immunoassay (FPIA) [10], and using a Modified Screen-Printed Electrode [11].

In this present study, Streptomycin and its three synthetic derivatives can be monitored directly by a simple, fast and cheap development RP-HPLC procedure for estimation of Streptomycin in pure form and pharmaceuticals.

2. Experimental

Chemicals and Solvents

Streptomycin, used as a pure standard, was sourced from Samara Drug Industries (SDI), Iraq. Various pharmaceutical formulations, including tablets, capsules, and vials, were utilized, all of which were available in the Iraqi market. For the procedures, de-ionized water and acetonitrile of HPLC grade from BDH were employed.

Equipment and Chromatographic RP-HPLC Conditions

HPLC LC-10A Shimadzu, Japan was used in this study set with a Shimadzu LC-10A double delivery pump, with Nucludar C18-DB column $(3\mu mx50x4.6mmI.D)$, aliquot of 20 μL of Streptomycin solution was manually injected via an isocratic mobile phase, %80 H_2O with 0.1% acetic acid + %20 Acetonitrile v/v, pH=4.5 at flow rate 1.1 ml.min^{-1} at 260 nm wavelength using a UV-Vis 10 A- SPD detector at 45°C column temperature. UV-VIS Spectrophotometer 1700 Shimadzu-Japan, FT-IR 8400 Shimadzu- Japan and H-NMR,

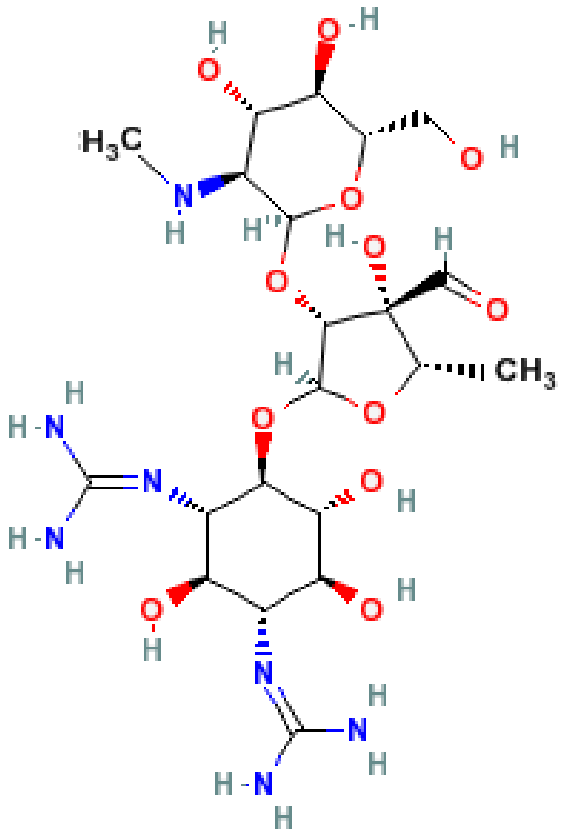

Figure 1: Chemical structure of Streptomycin.

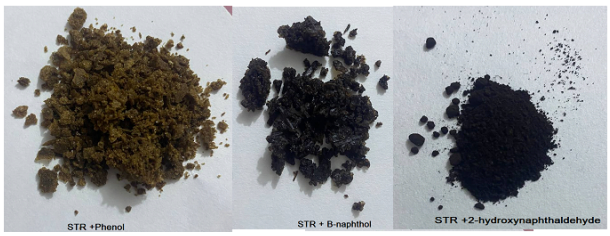

Figure 2: The three Streptomycin derivatives that were prepared are in the form of precipitates

Bruker, 500 MHz was used to characterize the synthetic derivatives.

Synthesis and Characterization of Streptomycin AZO Derivatives

Streptomycin first, second and third derivatives are prepared from the reaction of streptomycin through phenol, β-naphthol also 2-hydroxynaphtaldehyde with the presence of H_3PO_4, $NaNO_2$ or HNO_3 using water as a solvent, Figure 2.

A 1.034 g (1.78 mmol) of streptomycin was dissolved in 8mL of 85% phosphoric acid H_3PO_4 by heating and stirring then cooled using an ice bath to 0°C, a 4 mL of concentrated HNO_3 was added also 0.13 g (1.87 mmol) of $NaNO_2$ in 2mL D.W with strong stirring for 10 minutes and keep the temperature below 5°C. A solutions of phenol, β-naphthol and 2-hydroxynaphthaldehyde was prepared through dissolving 0.17, 0.25 and 0.3g respectively in 0.5 mL D.W with stirring, a few drops of of these solution was added. The mixture was transferred into 100 mL cold water, the formed precipitate was filtered and washing several times by cold water for and re-crystallized with ethanol, the purity was confirmed using TLC method, Figure 3.

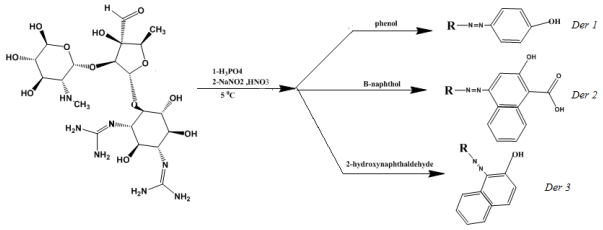

Figure 3: The proposed synthetic equations for the three Streptomycin derivatives

Standard Calibration Curve

Standard Streptomycin dissolved in appropriate solvent and filtered with $2.5\mu m$ disposable filters. A standard 100 $\mu g.ml^{-1}$ streptomycin and a series of 0.1 -10 $\mu g.mL^{-1}$ concentrations was prepared by the appropriate dilution using the appropriate solution 14g.L^{-1} Na_2SO_4 +1.5 g.L^{-1} sodium 1-octanesulfonate + 50 ml.L^{-1} acetonitrile +50 ml.L^{-1} phosphate buffer as a solvent. A $20\mu L$ of these prepared solutions was injected and RP-HPLC analysis at the finest conditions and the peak area of each concentration was calculated. A standard graph for Streptomycin was prepared in 0.1-10 $\mu g.mL^{-1}$ range and used to determine the unknown concentration using the method of least squares [12].

Method Accuracy and Precision

Three different concentrations 2, 4 and 8 $\mu g.ml^{-1}$ of streptomycin within the limits of the calibration curves was selecting with three repetitions for each one. A $20\mu L$ of Streptomycin solutions was injected using manual injection and the peak area was measured and recorded. The SD, %RE and %RSD was calculated for the precision and accuracy determination.

Application on Pharmaceutical Preparations

A solutions of TROGE and ABBOTT pharmaceutical preparations containing a concentration of 20 $\mu g.ml^{-1}$ of streptomycin was prepared and analysis at optimal RP-HPLC via 14g.L^{-1} Na_2SO_4 +1.5 g.L^{-1} sodium 1-octanesulfonate + 50 ml.L^{-1} acetonitrile +50 ml.L^{-1} Phosphate buffer as a mobile phase solvent at 2 ml.min^{-1} flow rate and pH = 4.5 at 210 nm using UV detector, where $20\mu L$ of streptomycin solution was injected.

3. Results and Discussion

Streptomycin Derivatives Characterization

These Streptomycin first, second and third derivatives were identified by UV-Vis, FT-IR and ^{1}HNMR.

UV-Vis Spectroscopy

The absorbance spectra of the Streptomycin azo derivatives with a concentration of 100 $\mu g.ml^{-1}$in 80:20% H_2O: MeOH were determined using UV-Vis spectrophotometer in a 200-800 nm wavelength range. The first, second and third streptomycin derivatives gave the highest absorbance at λ_{max} 320, 401and 318 plus 401.5 nm respectively.

FT-IR Spectra

The FT-IR spectra of streptomycin Der-1, Der-2 and Der-3 azo derivative showed a wide band at 3541, 3405 and 3417 cm^{-1} which is owing to O-H stretching bond and one absorption band at 3093-2785, 3055-2862 and 2978 cm^{-1} suitable to the aromatic C-H bond stretching frequency, and an absorption band due to NH_2 stretching bond at 3389-3297, 3271-3224 and 3224-3214cm^{-1} ,one absorption band at 1635, 1627 and 1643 cm^{-1} due to the CH=N stretching frequency. and stretching frequency of the NH bond at 3150, 3178 and 3109 cm^{-1}.

The frequency of stretching C=O bond show at 1697, 1627 and 1666 cm^{-1} and one beam is due to the stretching of N=N bond at 1550, 1597 and 1581 cm^{-1} and an absorption beam due to the aromatic C=C stretching bond at 1465, 1465 and 1450 cm^{-1}.

^{1}H-NMR Spectra

The ^{1}H-NMR analysis of the three streptomycin Der-1, Der-2 and Der-3 Azo derivative showed a multiple signal between 8-7.5, 8-7.3 and 8- 7.4 ppm due to CH arom in the aromatic rings. A separate signal at 9.1 ppm due to 2H of the NH_2 group, also single signal at 8.8 ppm due to 1H of the NH group. A single signal at 5.5, 5.1 and 5.5 ppm owing to 3H of the NH-CH_3 group also lone signal between 2.3-1-4 , 2.4-1-2 and 2.3-1-4 ppm due to 3H of the CH_3 and a distinct signal at 9.6 , 9.9 and 9.6 ppm refers to CH for the aldehyde group and a lone signal at 11, 10 and 11 ppm refers to 1H for the OH group.

Biological Activity of Synthesized Streptomycin Derivatives

The antibacterial activity of 50 and 100 μg/mL concentrations of prepared streptomycin derivatives were examined against four types of medical importance bacteria; *Staphylococcus aureus* and *Staphylococcus epidermidis* as a Gram-positive also *Pseudomonas aeruginosa* and *Escherichia coli* as a Gram-negative; that cause various diseases other than their competence to resist therapeutic chemicals also antibiotics.

The results showed that the first streptomycin derivative Der-1 have a moderately inhibition *Escherichia coli* compared to the standard antibiotic medication which didn't give any inhibition to this type of bacteria, while other derivatives does not have the ability to inhibit these types of bacteria.

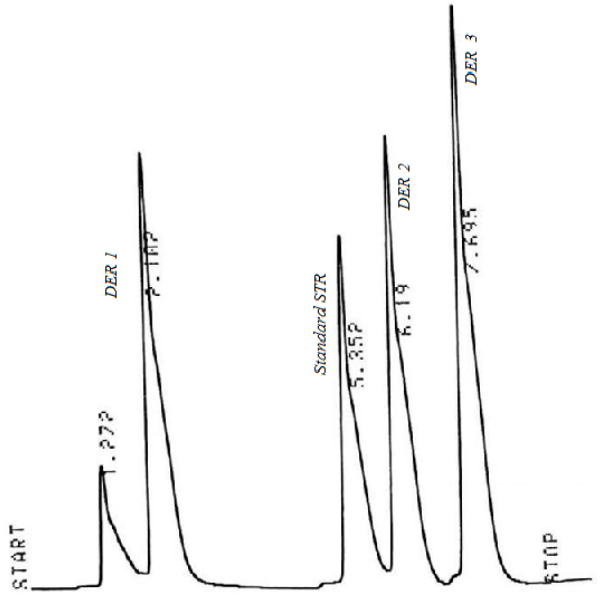

Figure 4: Chromatogram of Streptomycin and its three derivatives at optimal HPLC conditions

HPLC Separation of Streptomycin AZO Derivatives

The Streptomycin Azo derivatives were separated from its standard streptomycin at the optimum conditions. The results showed that the first derivative, the second and third derivative have t_R of 2.102, 6.19 and 7.695 min respectively while the standard appeared at t_R of 5.352 min, Figure 4.

Streptomycin Analysis

For the purpose of drug determination, a standard calibration curve was prepared. The linear equation y = b x + c, y: Detector response, b: slope=14533, c: intercept=1013.5 with Correlation Coefficient, r = 0.9993, the LOD and LOQ are 0.01 and 0.033 respectively.

1) Method Accuracy and Precision

The precision with accuracy of the developed RP-HPLC method was examined as it expressed in terms of the standard deviation, SD; relative standard deviation, RSD% and relative error. The results showed that the SD value was 0.80, RSD% ranged between 0.09 to 0.15 and percent relative error don't exceed 0.5% which proved the high accuracy and precision of this method.

2) Determination of Streptomycin in Pharmaceuticals

To estimate the active substances in the drugs, this method was applied for the separation and estimation of streptomycin in ABBOTT and TROGE pharmaceuticals via standard graph equation y= 14533 x + 1013.5 at the experimental optimal conditions; the pharmaceutical streptomycin peak for both

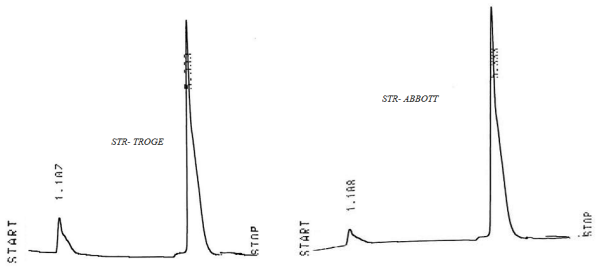

Figure 5: Chromatogram of the TROGE and ABBOTT pharmaceuticals analysis at optimal RP-HPLC conditions

TROGE and were appeared at 5.333 minutes, Figure 5, the results proved that this method is accurate and the find concentration equal what was proven via the drug manufacturer.

4. Conclusions

Streptomycin Azo derivatives were prepared and characterized by UV-Vis, FT-IR, and HNMR. These prepared streptomycin derivatives were successfully separated from each other and from the original Streptomycin using the RP-HPLC and successfully used to estimate Streptomycin in pharmaceutical samples. The biological effectiveness of these prepared streptomycin Azo derivatives was tested, and one of them demonstrated good efficiency compared with the original Streptomycin to inhibit *Escherichia coli* bacteria.

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Conflict of Interest

The authors declare no conflict of interests. All authors read and approved final version of the paper.

Authors Contribution

All authors contributed equally in this paper.

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