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Stability Indicating RP-HPLC Method Development and Validation for Dutasteride and Silodosin Combination of Drugs in Pharmaceutical Dosage Forms

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ABSTRACT

This research work outlines the advancement & verification of a HPLC technique for the parallel quantification of Dutesteroid & Silodosin using an Inertsil C18 column with a MP composed of phosphate buffer &ACN. The chromatographic conditions were optimized for a detection at 235 nm, with a flow speed of 1 ml per min and an volume injected of 20 µl. Key validation parameters including specificity, linearity, precision, ID precision,accuracy,LOD, LOQ, and robustness were assessed, demonstrating that all values met established limits. The technique exhibited linearity over the conc. extent 20 µg/ml to 100 µg/ml, yielding high correlation coefficients ($R^2 > 0.999$). Precision studies showed low %RSD values, indicating technique reliability. Degradation studies revealed minimal degradation under different stress conditions, supporting the method's robustness. Overall, the verified HPLC technique is suitable for regular quantitative analysis of Dutesteroid & Silodosin in pharmaceutical formulations.

Keywords: Benserazide, Levodopa, LOD, HPLC, Precision

ARTICLE INFO

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CONTENTS

1. Introduction

In the dynamic field of pharmaceutical sciences, ensuring the stability, efficacy, and safety of drug formulations is paramount. Stability-indicating methods are essential tools for assessing the stability of pharmaceutical compounds under various conditions, thus ensuring their therapeutic effectiveness throughout their shelf life. High-Performance Liquid Chromatography (HPLC) is widely regarded as one of the most powerful and reliable techniques for stability testing due to its high resolution, sensitivity, and specificity. Reverse Phase HPLC (RP-HPLC) is particularly favored for its capability to separate compounds of varying polarities. This study aims to develop and validate a stability-indicating RP-HPLC method for the simultaneous estimation of Dutasteride and Silodosin in pharmaceutical dosage forms, following the stringent guidelines set by the International Council for Harmonisation (ICH). Dutasteride, a 5-alpha-reductase inhibitor, is commonly used in the treatment of benign prostatic hyperplasia (BPH). It works by inhibiting the conversion of testosterone to dihydrotestosterone (DHT), thereby reducing prostate size and alleviating associated symptoms. Silodosin, an alpha-1 adrenoceptor antagonist, is also used in the management of BPH. It selectively blocks alpha-1 receptors in the prostate and bladder neck, resulting in relaxation of smooth muscles and improvement in urine flow. The combination of Dutasteride and Silodosin offers a synergistic approach to the management of BPH, making their simultaneous analysis in pharmaceutical formulations essential for quality control and therapeutic efficacy.

The development of an RP-HPLC method for the concurrent analysis of Dutasteride and Silodosin involves several critical steps. The first step is the optimization of chromatographic conditions, including the selection of the mobile phase, column type, flow rate, and detection wavelength. The mobile phase must be carefully chosen to achieve optimal separation and resolution of the drug compounds. Commonly used mobile phase components include mixtures of water, methanol, and acetonitrile, often with the addition of buffer solutions to maintain pH stability. The choice of column, typically a C18 column, is crucial as it directly impacts the separation efficiency and resolution.

Once the chromatographic conditions are optimized, the method must undergo rigorous validation according to ICH guidelines. Validation ensures that the analytical method is reliable, reproducible, and suitable for its intended purpose. The key parameters assessed during validation include specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), and robustness. Specificity tests the method's ability to accurately identify and quantify Dutasteride and Silodosin in the presence of other components, such as excipients and potential degradation products. Linearity evaluates the method’s ability to produce results that are directly proportional to the concentration of the analytes over a specified range.

Accuracy and precision are critical attributes for ensuring the reliability of the analytical method. Accuracy is determined by comparing the test results with those obtained using a reference method or known standards, while precision is assessed by measuring the consistency of results from multiple analyses of the same sample. The LOD and LOQ are essential for determining the method’s sensitivity, indicating the smallest amount of the analyte that can be reliably detected and quantified. Robustness testing evaluates the method's reliability under varying conditions, such as changes in pH, flow rate, and temperature, ensuring that the method remains consistent and accurate under different operational scenarios.

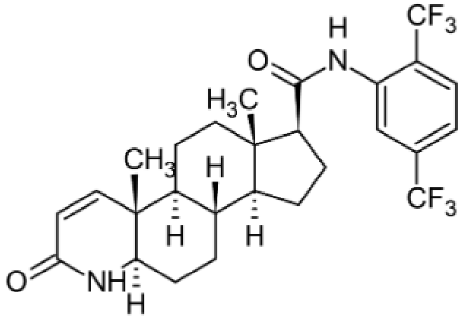
The stability-indicating aspect of the method involves subjecting the drug formulations to various stress conditions, such as heat, light, oxidation, and hydrolysis, to evaluate their stability and identify potential degradation products. This is crucial for understanding the degradation pathways and ensuring the drugs' stability over their shelf life. The ability to detect and quantify degradation products alongside the active pharmaceutical ingredients (APIs) is a key feature of a stability-indicating method, contributing to the overall assessment of the drug's quality and safety.

The successful development and validation of a stability-indicating RP-HPLC method for the simultaneous estimation of Dutasteride and Silodosin have significant implications for pharmaceutical quality control. It ensures that these critical drugs meet the required standards of efficacy, safety, and quality, thereby enhancing patient outcomes and contributing to public health. Furthermore, this validated method can be applied in routine quality control, stability testing, and during the manufacturing process to ensure the consistent quality of pharmaceutical products.

In conclusion, the development and validation of a stability-indicating RP-HPLC method for the simultaneous estimation of Dutasteride and Silodosin represent a crucial step in pharmaceutical analysis. By following a systematic approach to method development and adhering to rigorous validation protocols as per ICH guidelines, this study aims to establish a robust, reliable, and accurate analytical method. This method will not only ensure compliance with regulatory standards but also contribute to the overall improvement of pharmaceutical analytical practices, thereby supporting the continued development of safe and effective treatments for benign prostatic hyperplasia.

Drug Profile

Drug Profile for Dutasteride



Dutasteride

Structure: Dutasteride Structure

IUPAC Name: (5α,17β)-N-{2,5-bis (trifluoromethyl) phenyl}-3-oxo-4-azaandrost-1-ene-17-carboxamide

Molecular Formula: C[27]H[30]F[6]N[2]O[2]

Molecular Weight: 528.53 g/mol

Melting Point: 242-250°C

pKa : 8.5

Category: 5-alpha-reductase inhibitor

Solubility: Slightly soluble in ethanol and methanol; practically insoluble in water.

Description: Dutasteride is a synthetic 4-azasteroid compound that inhibits both type 1 and type 2 isoforms of steroid 5 alpha-reductase, used primarily to treat benign prostatic hyperplasia (BPH) in men with an enlarged prostate[1].

Mechanism of Action: Dutasteride works by inhibiting the conversion of testosterone to dihydrotestosterone (DHT), a hormone that causes the enlargement of the prostate[2].

Pharmacodynamics: Dutasteride reduces serum DHT levels by more than 90%, leading to a reduction in prostate size and improvement in urinary flow[2].

Pharmacokinetics:

- **Absorption:** Oral bioavailability is approximately 60%.
- **Distribution:** Volume of distribution is about 300-500 L.
- **Metabolism:** Metabolized in the liver by CYP3A4 and CYP3A5.
- **Route of Elimination:** Primarily excreted in feces.
- **Protein Binding:** Over 99% bound to plasma proteins.
- **Half-Life:** Approximately 5 weeks[2].

Uses:

- Treatment of benign prostatic hyperplasia (BPH)
- May be used off-label for androgenetic alopecia (male pattern baldness)

Dosage:

- Typical dose for BPH: 0.5 mg once daily

Side Effects:

- Decreased libido
- Erectile dysfunction
- Ejaculation disorders
- Breast tenderness or enlargement[1]

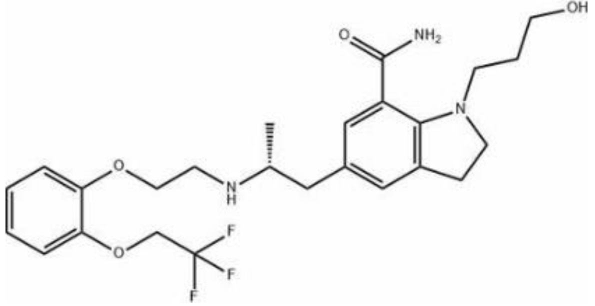
Drug Interactions:

- CYP3A4 inhibitors (e.g., ketoconazole) may increase dutasteride levels.
- Should not be used with other 5-alpha-reductase inhibitors[2].

Storage:

- Store at room temperature, between 20°C to 25°C (68°F to 77°F).
- Protect from light and moisture[2].

Drug Profile for Silodosin



Silodosin

Structure: Silodosin Structure

IUPAC NAME: 1-(3-Hydroxypropyl)-5-[(2R)-2-{2-[2-(2,2,2-trifluoroethoxy)phenoxy]ethylamino}propyl]-2,3-dihydro-1H-indole-7-carboxamide

Molecular Formula:C[25]H[32]F[3]N[3]O[4]

Molecular Weight: 495.54 g/mol

Melting Point: 105-106°C

pKa: 7.1

Category: Alpha-1 adrenergic receptor antagonist

Solubility: Slightly soluble in water; soluble in methanol and ethanol.

Description: Silodosin is an alpha-blocker used to treat the symptoms of benign prostatic hyperplasia (BPH), such as difficulty urinating, painful urination, and urinary frequency and urgency[1].

Mechanism of Action: Silodosin selectively blocks alpha-1 adrenergic receptors in the prostate and bladder neck, leading to relaxation of smooth muscle in these areas and improved urine flow[2].

Pharmacodynamics: Silodosin reduces the symptoms of BPH by relaxing the muscles in the prostate and bladder neck, which helps to improve urine flow and reduce symptoms of BPH[2].

Pharmacokinetics:

- **Absorption:** Rapidly absorbed with peak plasma concentrations reached within 2-3 hours.
- **Distribution:** Volume of distribution is approximately 49.5 L.
- **Metabolism:** Extensively metabolized in the liver, primarily by CYP3A4.
- **Route of Elimination:** Primarily excreted in feces (54.9%) and urine (33.5%).
- **Protein Binding:** Approximately 97%.
- **Half-Life:** Approximately 13 hours[2].

Uses:

- Treatment of benign prostatic hyperplasia (BPH)

Dosage:

- Typical dose for BPH: 8 mg once daily with a meal

Side Effects:

- Dizziness
- Diarrhea
- Retrograde ejaculation
- Nasal congestion
- Orthostatic hypotension[1]

Drug Interactions:

- CYP3A4 inhibitors (e.g., ketoconazole) may increase silodosin levels.
- Should not be used with other alpha-blockers[2].

Storage:

- Store at room temperature, between 20°C to 25°C (68°F to 77°F).
- Protect from light and moisture[2].

2. Materials and Methods

Chemicals used:

For the estimation of Sacubitril and Valsartan, various high-purity chemicals and reagents are employed. Trifluoroacetic acid of HPLC grade, sourced from Qualigens, is used specifically for these drugs. Formic acid, also of HPLC grade from Qualigens, is utilized for the estimation of Sacubitril and Valsartan. Additionally, water of HPLC grade from Qualigens is used for all drugs. Acetonitrile, another HPLC grade chemical from Qualigens, is also employed for all drugs. Lastly, methanol of HPLC grade from Rankem is used across all drugs.

Instruments used: For the analytical procedures, an electronic balance, model SAB224CL, manufactured by Scaletech, is utilized for precise weight measurements. Ultrasonic cleaning is carried out using an ultra-sonicator, model SE60US, from Enertech. Thermal treatments are performed using a thermal oven produced by Yamto. A pH meter, model PH-7000, from SMIS is used for accurate pH measurements. Filtration is conducted using filter paper with a pore size of 0.45 microns from Millipore. Lastly, the High-Performance Liquid Chromatography (HPLC) system employed is the Agilent 1220 Infinity, model LC(G4288C), which ensures high resolution and reliable chromatographic analysis.

HPLC Development:

Choosing λmax:

Spectrum of UV with 10µg/ml Dutesteroid & Silodosinin MP ratio was noted by examining in the scale of 200 to 400nm and the isobestic λmax of both the drugs obtained at 235 nm.

Optimization of Column:

INERTSIL (250 x 4.6 mm, 5µm) is find out optimum as it produce excellent shape of peak & R_S at 1.0ml per min flow speed.

Optimized Chromatographic Conditions

Instrument used : RP-HPLC with Auto Sampler and PDA detector
Column : INERTSIL (250 x 4.6 mm, 5µm)
MP ratio : (30:70) Phosphate buffer (0.05M) pH 4.6: ACN
Λmax : 235nanometer
Flow speed : 1ml per min
Volume injected : 20µl
Temperature : Ambient

Buffer & Mobile Phase Making:

Phosphate buffer pH 4.6 Preparation: By adding 6.4g Phosphate in 1L HPLC grade water. Adjust this solution to pH 4.6 by using acid / base based on the PH of the resulted solution.

Mobile phase Making:

Mix a 300 ml Phosphate buffer (30%), ACN 700ml (70%)&remove gases in ultra-sonication water bath for few min. Filter by vacuum filtration instrument using 0.45µ filter paper.

Diluent: Phosphate buffer: Acetonitrile (30:70) ratio.

ASSAY:

Standard Solution Preparation:

Precisely measure & poured 2.5mg of Dutesteroid& 20mg Silodosin standard into a 25 ml VF add Diluents & to dissolve fully sonicate& fill up till the margin. Additional take out 0.3ml from solutions into a 10ml VF & fill up till the margin using diluents. (3ppm Dutesteroid & 24ppm Silodosin).

Sample Solution Preparation:

Precisely measure & poured equal to 25mg of Dutesteroid & Silodosin equal wt of the sample into a 25ml VF add few ml of dilutant& to fully dissolve sonicate fully get volume up till the margin. Additional take out 0.3ml from solutions into a 10ml VF & fill up till the margin using diluent. (3ppm Dutesteroid and 24ppm Silodosin).

Procedure: Inject 10 µL of the std, sample into the HPLC system &note down the areas for the Dutesteroid and Silodosinpeaks.

Formula: Calculation: (For Dutesteroid and Silodosin)

$$\% \, Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Averageweight}{LabelClaim} * \frac{P}{100} * 100$$

Acceptance limitations of System Suitability:

1) Tailing factor should be < 2.
2) Theoretical Plates should be > 2000.

Forced Degradation Studies Precisely measure & poured 10mg of Dutesteroid & Silodosin standard into a 10 ml VF add Diluents and to fully dissolve sonicate& get volume up till the margin using the solvent.

Additional take out 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Acid degradation:

Take out 3 ml of Dutesteroid & Silodosin of the above stock soln into a 10ml V/Fpour 3 ml of 0.1N Hcl & sonicated for 10mins and kept it in darkness for 12 hours then refluxed under heat at 60°Cin a heating mantle for 1 hrs. Neutralized the sample soln using 0.1N NaOH &make up to the mark with diluents. The Sample was vaccum filtration through 0.44µm& injected into HPLC system.

Base Degradation:

Take out 3 ml of Dutesteroid &Silodosin of the above stock soln into a 10ml V/F pour 3 ml of 0.1N NAOH& sonicated for 10mins and kept it in darkness for 12 hours then refluxed under heat at 60°C in a heating mantle for 1 hrs. Neutralized the sample soln using 0.1N Hcl& make up to the mark with diluents. The Sample was vaccum filtration through 0.44µm & injected into HPLC system.

Thermal Degradation:

Take out 3 ml of Dutesteroid &Silodosin of the above stock soln into a 10ml V/F& put in oven under heat at 105°Cfor 12 hrs&make up to the mark with diluents. The Sample was vaccum filtration through 0.44µm & injected into HPLC system.

Peroxide Degradation:

Take out 3 ml of Dutesteroid &Silodosin of the above stock soln into a 10ml V/F pour 3 ml of 3% H2O2&sonicated for 10mins and kept it in darkness for 12 hours then refluxed under heat at 60°C in a heating mantle for 1 hrs. The Sample was vaccum filtration through 0.44µm &injected into HPLC system.

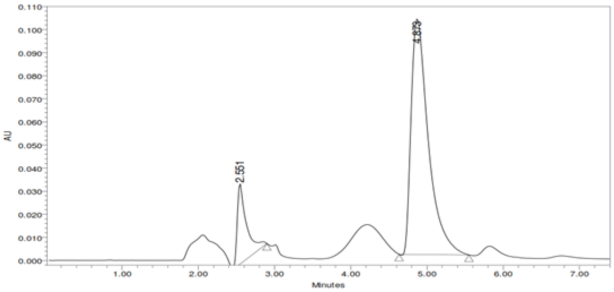
Photo Degradation:

Take out 3 ml of Dutesteroid &Silodosin of the above stock soln into a 10ml V/F pour about &sonicated for 10mins and kept it in darkness for 12 hours. The Sample was vaccum filtration through 0.44µm & injected into HPLC system.

3. Results and Discussion

Trial-1:

Column : Agilent(250×4.6mm, 5µm)
MPratio : H2O: MEOH(40:60)
λmax: 235nanometer
Flowspeed: 1ml per min
Volume injected: 10µl
Columntemperature : Ambient
Sample temperature : Ambient



Optimized conditions of Chromatographic:

Instrument used: HPLC having with Auto Sampler and PDA detector
Column : Inertsil (250 x 4.6mm, 5µm)
MP ratio: Phosphate buffer (0.05M) pH 4.6: ACN (30:70)
λmax : 235nanometer
Flow speed : 1ml per min
Volume injected : 20µl
Column temperature : Ambient

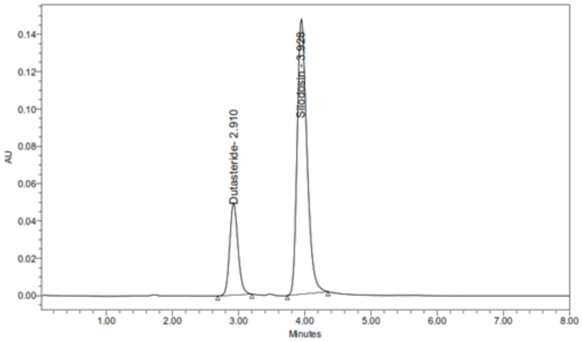


Figure 7: Chromatographic conditions

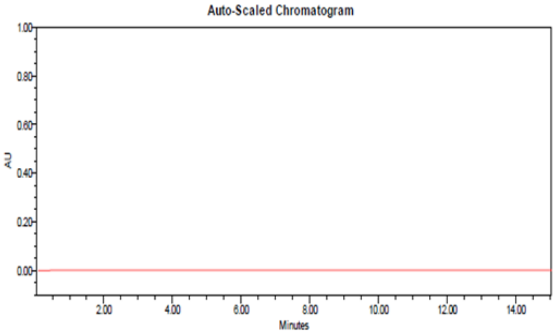


Figure 8: Blank Chromatogram

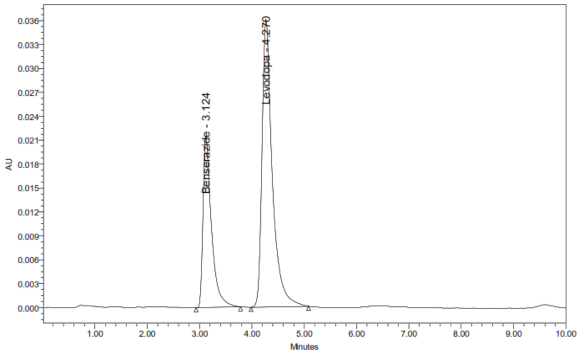


Figure 5: Sample Chromatogram

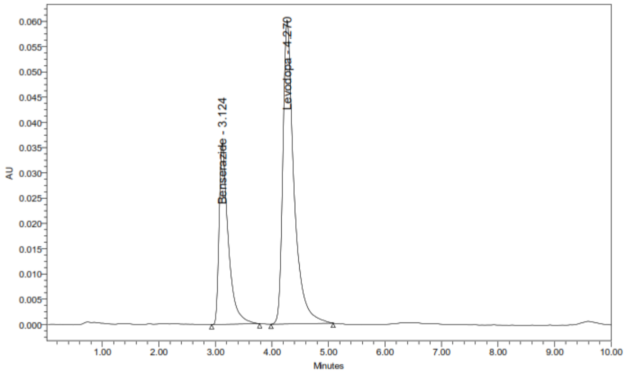


Figure 6: Linearity Chromatogram

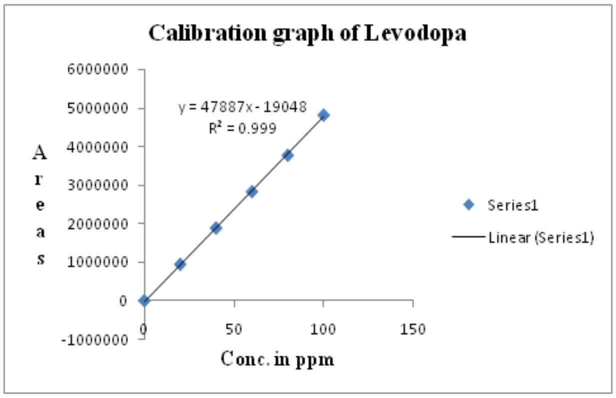


Figure 7: Calibration graph for Levodopa

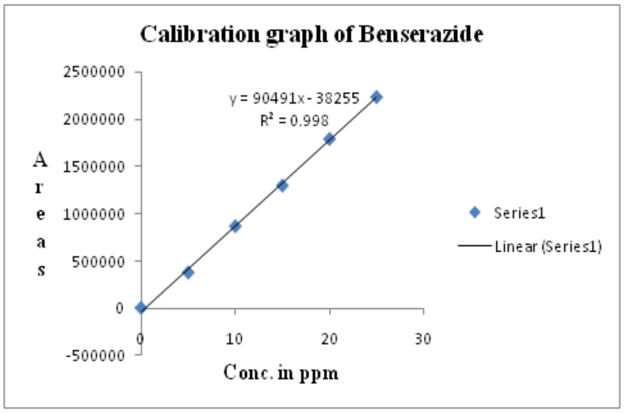


Figure 8: Calibration graph for Benserazide

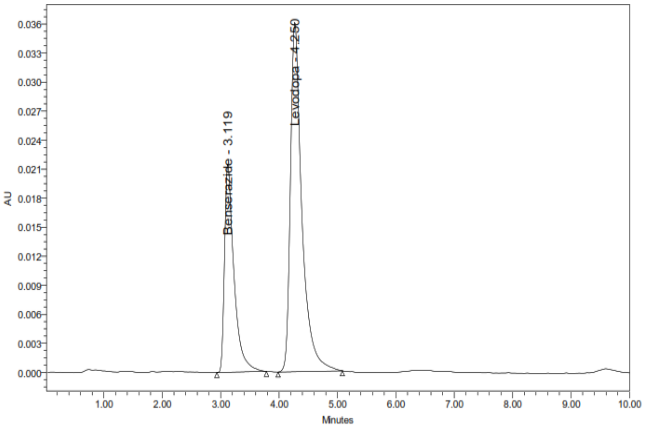


Figure 9: Precision Chromatogram

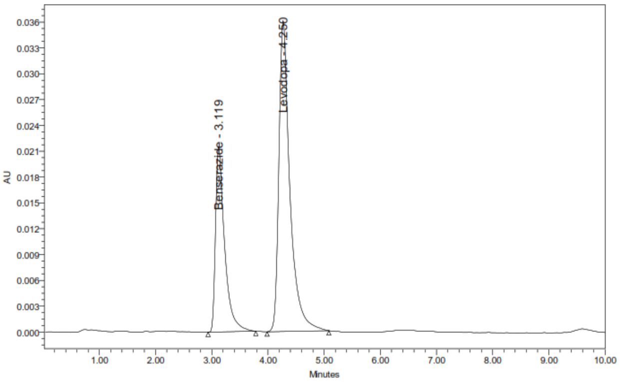


Figure 10: ID Precision -6 Chromatogram

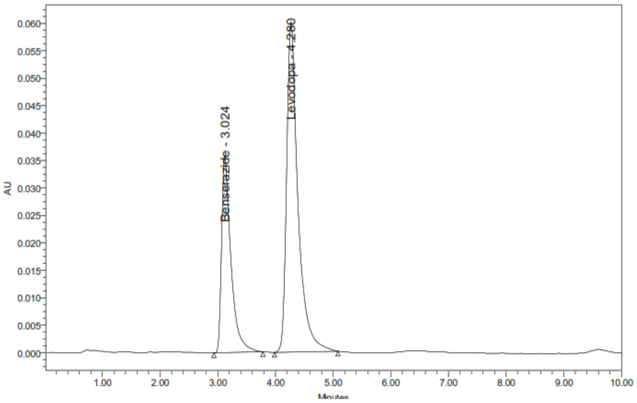


Figure 11: Accuracy 150%-3 Chromatogram

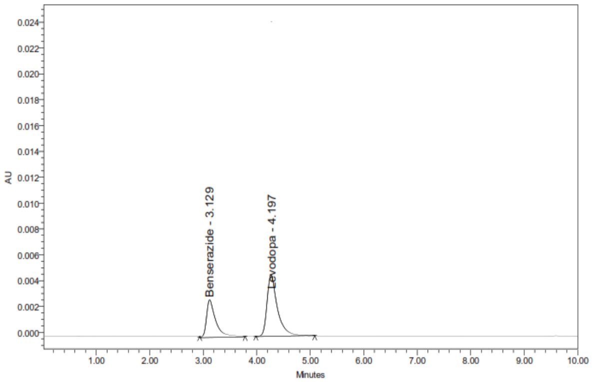


Figure 12: Benserazide& levodopa depicting LOD Chromatogram

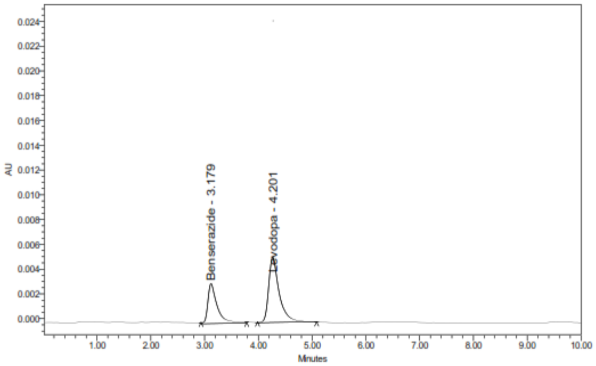


Figure 13: Benserazide& levodopa depicting LOQ Chromatogram

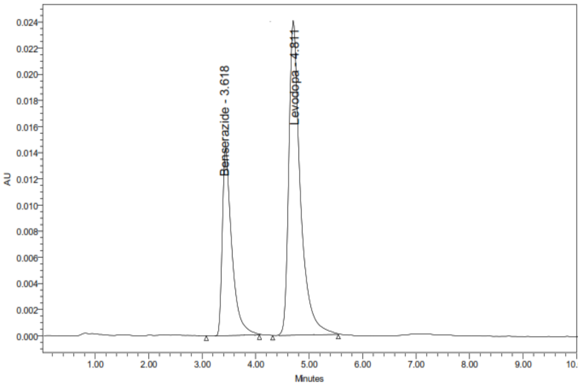


Figure 14: Robustness less flow Chromatogram

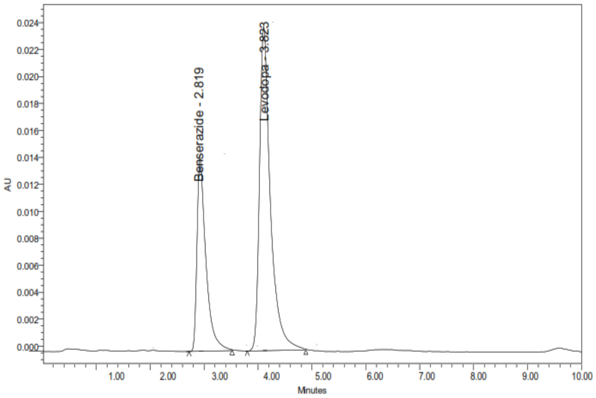


Figure 15: Robustness more flow Chromatogram

Table 3: Outcomes of Precision for Benserazide and levodopa

Injection	Benserazide Area	Levodopa Area
1 Injection	1220068	2834747
2 Injection	1206192	2834747
3 Injection	1295181	2830363
4 Injection	1205321	2834747
5 Injection	1210736	2834747
6 Injection	1206028	2834747
Avg	1209087.667	2834016.333
Standard Deviation	5726.21441	1789.760505
%RSD	0.47	0.06

Table 4: ID precision outcomes for Benserazide and levodopa

Injection	Benserazide Area	Levodopa Area
1 Injection	1220068	2834747
2 Injection	1206192	2734747
3 Injection	1295181	2830363
4 Injection	1205321	2834747
5 Injection	1210736	2834747
6 Injection	1206028	2834747
Avg	1209087.667	2817349.667
Standard Deviation	5726.21441	40504.85456
%RSD	0.47	1.43

Table 5: Accuracy data for levodopa

%Conc. (at specification Level)	Areas*	Added Amount (mg)	Found Amount (mg)	% Recovery	Avg Recovery
50%	1287761	7.5	7.2	96	98.23
100%	2830363	15	14.78	99.6	
150%	3440574	22.5	22.3	99.1	

Table 6: Accuracy data for Benserazide

%Conc. (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	647590	7.5	7.2	96	98.23
100%	1295181	15	14.78	99.6	
150%	1942771	22.5	22.3	99.1	

Table 7: LOD Results

Drug name	noise of Baseline (µV)	obtained Signal (µV)	Signal /Noise ratio	Conc. In PPM
Benserazide	75	220	2.93	0.05µg/ml
Levodopa	75	223	2.97	0.07µg/ml

8: LOQ Results

Drug name	noise of Baseline (µV)	obtained Signal (µV)	Signal /Noise ratio	Conc. In PPM
Benserazide	75	745	9.93	0.1 µg/ml
Levodopa	75	747	9.96	0.2µg/ml

Table 9: Outcomes of difference in flow speeds for Benserazide and levodopa

S. No	Flow Rate (ml per min)	SST outcomes of Levodopa	
		Plate Count	Tailing
1	0.7	2691	1.12
2	0.9	3338	1.16
3	1.1	2974	1.20

4. Conclusion

The validation of the chromatographic technique for analyzing Benserazide& Levodopa demonstrated that the technique meets all specified acceptance limits. The SST tests confirmed that the R's between the two drugs was greater than 2, the theoretical plates were above 2000, & the tailing factors were within the acceptable limit of 2, ensuring optimal performance of the technique. The %assay results showed that Benserazide& Levodopa were within 99.55% &99.6% of their label claims, respectively. The technique's linearity was established with R^2 of 0.9997 for Levodopa & 0.9989 for Benserazide, showing good linear response over the tested concentration ranges. Precision & ID precision assessments revealed %RSD values well within the acceptable limit of 2%, demonstrating the method's reproducibility & ruggedness. Accuracy was confirmed with recoveries ranging from 96% to 99.6% for both drugs, meeting the required 98-102% recovery extent. LOD& LOQ were established with satisfactory signal-to-noise ratios, & robustness studies indicated that differences in flow speed& MP ratio did not significantly affect the technique's performance. The results ensure that the technique provides reliable & consistent data for both Benserazide and Levodopa, making it a dependable tool for regular analysis.

Conflict of Interest:

The authors declare no conflict of interest

5. References

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