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HPLC Method Development and Validation for the Simultaneous Estimation of Benserazide and Levodopa

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ABSTRACT
This research work outlines the advancement & verification of a HPLC technique for the parallel quantification of Benserazide& Levodopa. The chromatographic conditions were optimized, & SST parameters were evaluated, confirming compliance with established acceptance limits. The assay results showed a % assay of 99.55% for Benserazide& 101.32% for Levodopa. The technique exhibited linearity over the conc. extent of 10–50 µg per ml for both compounds, with R^2 of 0.9998 respectively. Precision & ID precision studies indicated low %RSD values, confirming the method's reliability. Robustness testing revealed that variations in flow speed & MP composition. Limits of detection & quantification were determined, with LOD values of 0.05 ppm for Benserazide& 0.07 ppm for Levodopa. Overall, the verified HPLC technique is suitable for regular QA of Benserazide and Levodopa in formulations.

Keywords: Benserazide, Levodopa, LOD, HPLC, Precision

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

In the landscape of pharmaceutical sciences, ensuring the accuracy and reliability of drug analysis is paramount for maintaining the quality and safety of pharmaceutical products. High-Performance Liquid Chromatography (HPLC) has emerged as a leading analytical technique due to its high resolution, sensitivity, and specificity. Among the various forms of HPLC, Reverse Phase HPLC (RP-HPLC) is particularly favored for its versatility in separating compounds with different polarities. This study focuses on the development and validation of an RP-HPLC method for the simultaneous estimation of Benserazide and Levodopa in pharmaceutical dosage forms, following the stringent guidelines set by the International Council for Harmonisation (ICH). Benserazide and Levodopa are critical components in the treatment of Parkinson's disease. Levodopa is a precursor to dopamine, a neurotransmitter that is deficient in the brains of individuals with Parkinson's disease. By crossing the blood-brain barrier and converting to dopamine, Levodopa helps alleviate the motor symptoms associated with the disease. However, when administered alone, Levodopa is

rapidly decarboxylated to dopamine in peripheral tissues, reducing its availability to the brain and causing side effects. Benserazide, a peripheral decarboxylase inhibitor, is co-administered with Levodopa to inhibit this peripheral conversion, thereby increasing the amount of Levodopa that reaches the brain and enhancing its therapeutic efficacy. The simultaneous estimation of these drugs in pharmaceutical formulations is crucial for ensuring their optimal dosage and effectiveness.

The development of an RP-HPLC method for the concurrent analysis of Benserazide and Levodopa 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. Common 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 also 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 Benserazide and Levodopa 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 successful development and validation of an RP-HPLC method for the simultaneous estimation of Benserazide and Levodopa hold 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.

Drug Profile of Benserazide

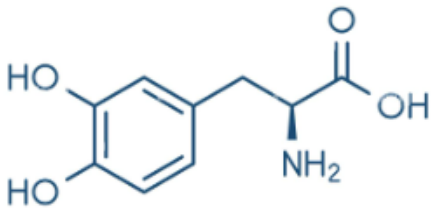

Fig.1

Basic Information

- **IUPAC Name**: (S)-2-amino-3-(3,4-dihydroxyphenyl) propanoic acid
- **Molecular Formula**: $C_9H_{11}NO_4$
- **Molecular Weight**: 197.19 g/mol
- **Category**: Central nervous system agent, Antiparkinsonian agent

Physical Properties

- **Melting Point**: 275-280°C (decomposes)
- **pKa**: 2.32 (carboxyl group), 9.74 (amino group)

Solubility

- **Solubility**: Soluble in water; slightly soluble in ethanol; insoluble in chloroform and ether.

Drug Profile of Benserazide

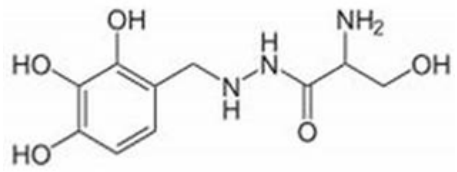

Fig.2

Basic Information

- **IUPAC Name**: (RS)-2-Amino-3-hydroxy-N'-(2,3,4-trihydroxybenzyl)propanehydrazide
- **Molecular Formula**: $C_{10}H_{15}N_3O_5$
- **Molecular Weight**: 257.25 g/mol
- **Category**: Peripheral decarboxylase inhibitor

Physical Properties

- **Melting Point**: 234-238°C
- **pKa**: Not well-documented

Solubility

- **Solubility**: Soluble in water, ethanol, and methanol.

2. Materials and Methods

Table 1: Chemicals used

For the analytical estimation, several high-purity chemicals and reagents are employed. Trifluoroacetic acid and acetic acid, both of HPLC grade, are sourced from Qualigens. Water and acetonitrile, also of HPLC grade, are provided by Qualigens. Additionally, methanol of HPLC grade is supplied by Rankem. These reagents ensure the high

accuracy and precision necessary for the simultaneous estimation of the target compounds.

Table 2: Instruments used:

For the analytical procedures, an electronic balance, model SAB2032, manufactured by Scaletech, is used for precise weight measurements. Ultrasonic cleaning is conducted using an ultra-sonicator, model SE60US, from Labman Scientific India. Thermal treatments are performed in a thermal oven, model i-THERM A17782, from Dwaraka Scientific. pH measurements are accurately obtained using a pH meter, model ORION STAR A111, from Thermo Scientific. Filtration tasks utilize filter paper with a pore size of 0.45 microns, supplied by Millipore. Finally, the High-Performance Liquid Chromatography (HPLC) system employed is the Waters 2690 Separation Module, which ensures high resolution and reliable chromatographic analysis, and is manufactured by Waters.

Method Development:

Choosing λmax:

Spectrum of UV with 10µg/ml Benserazide & levodopa in MP ratio was noted by examining in the scale of 200 to 400nm and the isobestic λmax of both the drugs obtained at 220 nm.

Optimization of Column:

DIKMA SPURSIL C18-EP (3.0 x 150mm, 3µm) is find out optimum as it produce excellent shape of peak & R_S at 1.0 ml per min flow speed.

Optimized Chromatographic Conditions:

Instrument used : RP-HPLC with Auto Sampler and PDA detector

Temperature : Ambient

Column : Dikma Spursil C18 (3.0x150mm, 3µm)

Mobile phase :(50:50) KH_2PO_4PH 3.5: Acetonitrile

Flow speed : 0.9 ml per min

λmax : 220 nanometers

volume injected : 10 µl

Time duration : 10 min.

Buffer & Mobile Phase Making:

Phosphate buffer pH 3.5 Preparation:

By adding 6.8gm of KH2PO4 in 1L HPLC grade water. Adjust this solution to pH 3.5 by using sodium hydroxide soln.

Mobile phase making:

Mix a 500 ml Buffer (50%) ,ACN 500ml (50%) & remove gases in ultra-sonication water bath for few min. Filter by vacuum filtration instrument using 0.45µ filter paper.

Diluent:KH2PO4 buffer: Acetonitrile (50:50) ratio.

System Suitability: Tailing factor for Benserazide & levodopa in Std solution shouldn 't>2.0. For Standard solution Theoretical plates for the Benserazide and levodopa peaks shouldn 't<2000

ASSAY:

Procedure: Inject 10 µL of the std, sample into the HPLC system & note down the areas for the Benserazide & levodopa peaks.

Formula: Calculation: (For Benserazide and levodopa)

$$\%\ Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

3. Results and Discussion:

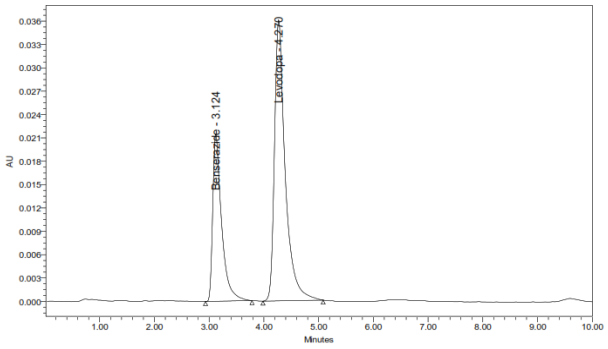

Figure 3: Standard Chromatogram for system suitability

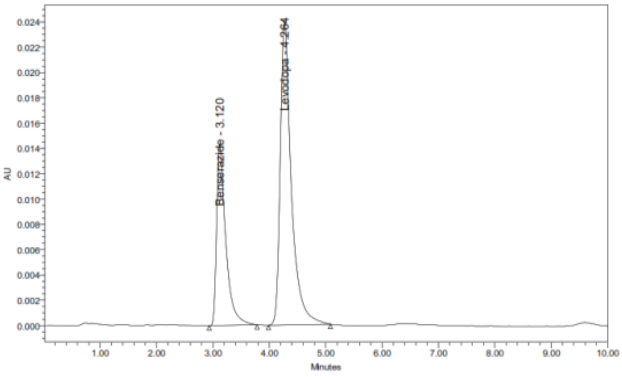

Figure 4: Standard Chromatogram

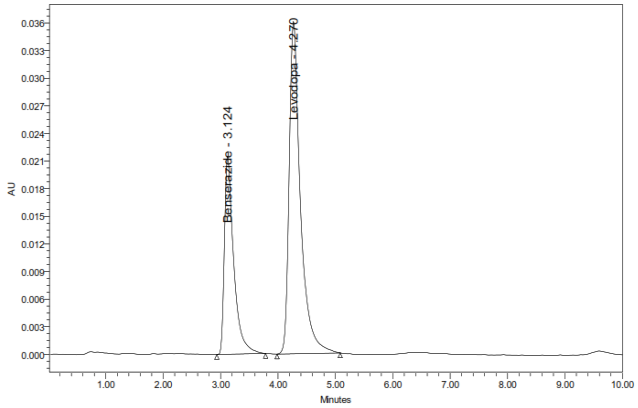

Figure 5: Sample Chromatogram

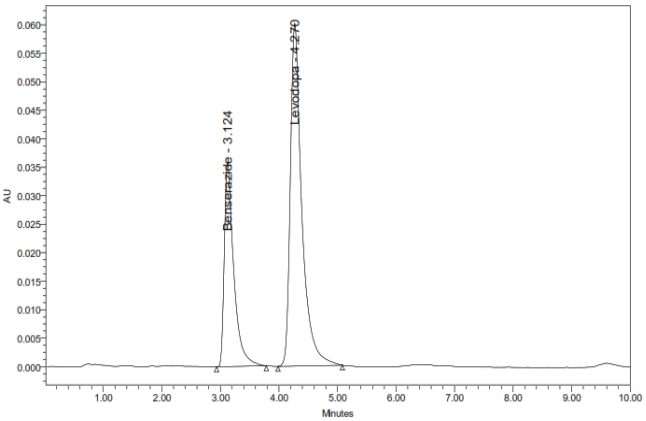

Figure 6: Linearity Chromatogram

Table 1: Areas of various conc. of levodopa

S. No	Levodopa	
	Concentration (µg per ml)	Area
1	20	943454
2	40	1886909
3	60	2830363
4	80	3773817
5	100	4817272

Table 2: Areas of various conc. of Benserazide

S. No	Benserazide	
	Concentration (µg per ml)	Area
1	5	374795
2	10	787590
3	15	1295181
4	20	1788976
5	25	2232762

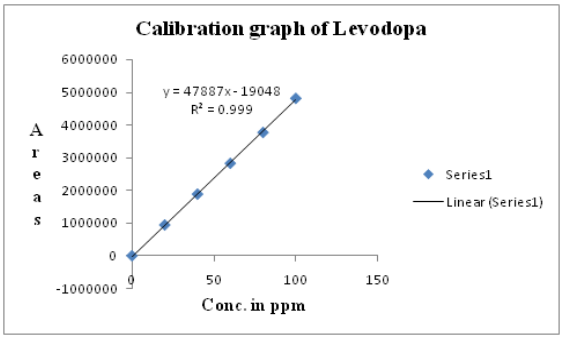

Figure 7: Calibration graph for Levodopa

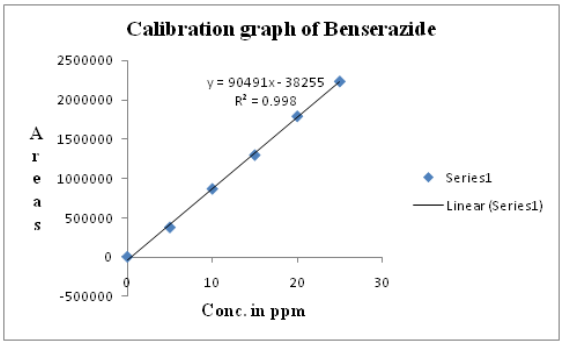

Figure 8: Calibration graph for Benserazide

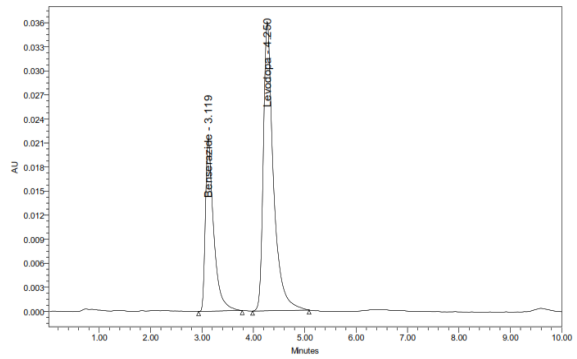

Figure 9: Precision Chromatogram

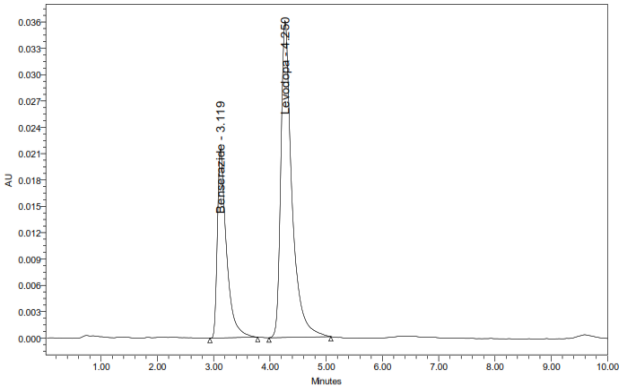

Figure 10: ID Precision -6 Chromatogram

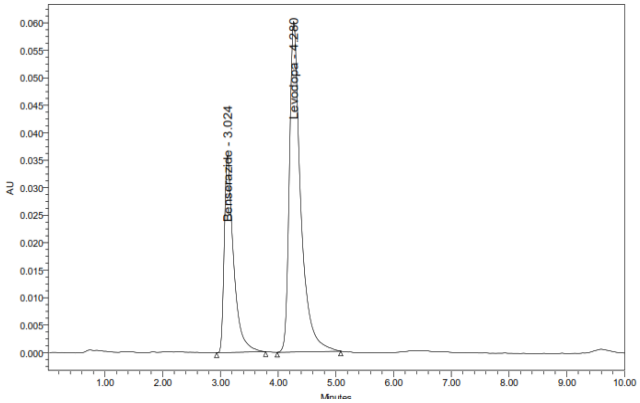

Figure 11: Accuracy 150%-3 Chromatogram

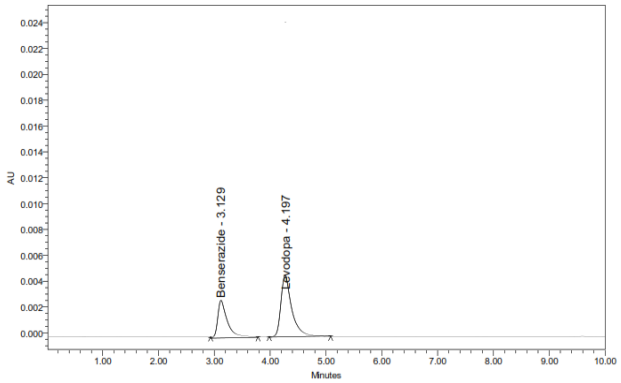

Figure 12: Benserazide& levodopa depicting LOD Chromatogram

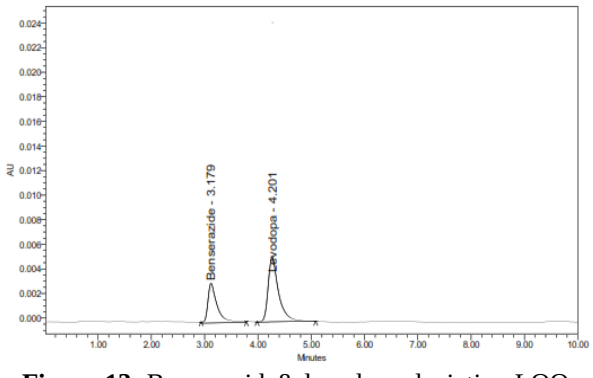

Figure 13: Benserazide& levodopa depicting LOQ Chromatogram

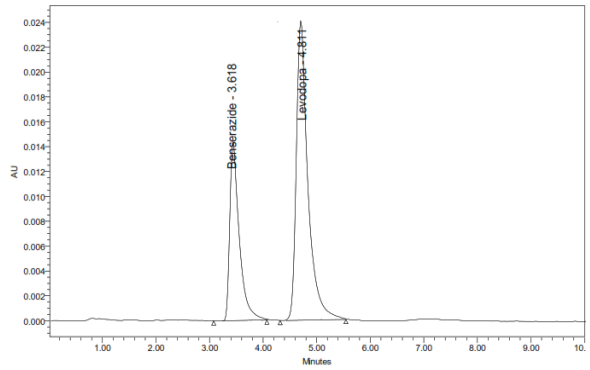

Figure 14: Robustness less flow Chromatogram

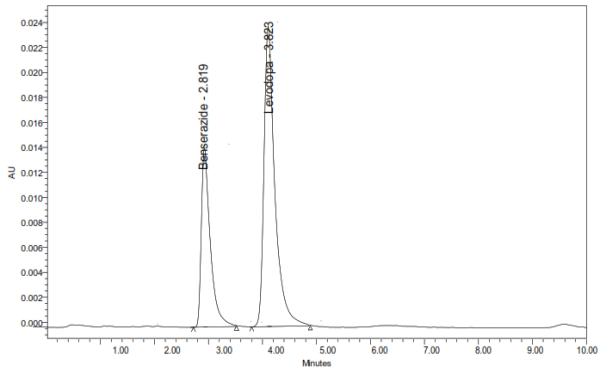

Figure 15: Robustness more flow Chromatogram

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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