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Research Article

Development and Validation of an RP-HPLC Method for Quantification of Levodopa in Methanolic Extract of *Vicia faba* Seeds

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ABSTRACT

Vicia faba seeds are known to contain Levodopa (L-DOPA), a therapeutic precursor used in Parkinson's disease. This study reports the development and validation of a simple, rapid, and sensitive RP-HPLC method for quantitative estimation of L-DOPA in methanolic extracts of *Vicia faba*. Separation was achieved on a C18 column using methanol:0.5% acetic acid (70:30, v/v) at 1.2 mL/min with detection at 284 nm. The method exhibited excellent linearity (10–500 µg/mL, $r^2 = 0.9999$), high accuracy (98.57%–99.46%), and good precision (%RSD < 2.6%). Sensitivity parameters were strong with LOD 0.690 µg/mL and LOQ 2.092 µg/mL. System suitability parameters met USP criteria, confirming robust chromatographic performance. The method is practical for routine QC, herbal standardization, and phytopharmaceutical development involving *Vicia faba*.

INTRODUCTION

Herbal medicines continue to play a significant role in modern healthcare, serving as sources of pharmacologically active compounds [1,2]. Levodopa (L-DOPA) is an essential precursor for dopamine synthesis and remains the first-line treatment for Parkinson's disease [3].

While *Mucuna pruriens* has been extensively studied for its high L-DOPA content, *Vicia faba* (broad bean) also contains physiologically

relevant concentrations of L-DOPA and is consumed traditionally for neurological benefits [4–6]. Accurate quantification of L-DOPA in *Vicia faba* is crucial for herbal standardization, QC testing, and phytopharmaceutical applications [7].

HPLC is considered the most reliable tool for L-DOPA analysis due to its sensitivity, precision, and specificity [8]. However, existing methods largely target *Mucuna pruriens* and often involve long run times, complex buffers, or incomplete validation [9–11]. Very few reports present a

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rapid, fully validated RP-HPLC method specifically optimized for *Vicia faba* seed extracts.

Gap

A simple, cost-effective, rapid, and fully ICH-validated RP-HPLC method exclusively for *Vicia faba* seed extract is not adequately reported in literature.

Objective

To develop and validate an RP-HPLC method for estimation of Levodopa in methanolic extract of *Vicia faba* seeds as per ICH Q2(R1) guidelines.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Levodopa standard (Torrent Pharmaceuticals, India), HPLC-grade methanol, acetic acid, 0.1 M HCl, and Milli-Q water were used.

2.2 Plant Material

Vicia faba seeds were collected locally (Gurugram, Haryana) and authenticated by a qualified botanist, and a laboratory sample was preserved for reference.

2.3 Extraction Procedure

50 g seed powder was Soxhlet-extracted with methanol for 9 h. Filtrate was concentrated under reduced pressure at 40–45°C and stored at 4°C.

2.4 Standard Preparation

100 mg Levodopa was dissolved in 0.1 M HCl and diluted to 100 mL (1 mg/mL stock). Working

standards (10–500 µg/mL) were prepared by serial dilutions.

2.5 Sample Preparation

350 mg extract was mixed with 25 mL 0.1 M HCl, sonicated (60 min), filtered, and diluted to 100 mL. Final solution was filtered (0.45 µm).

2.6 Chromatographic Conditions

Waters Alliance e2695 HPLC with UV detector; Eurosphere C18 (250 × 4.6 mm, 5 µm).

- Mobile phase: Methanol : 0.5% acetic acid (70:30, v/v)
- Flow rate: 1.2 mL/min
- λ_{max} : 284 nm
- Injection volume: 20 µL
- Column temp: 30°C
- Run time: 5 min

2.7 Method Validation (ICH Q2 R1)

Validation included λ_{max} determination, linearity, accuracy, precision, LOD/LOQ, and system suitability.

3. RESULTS

3.1 UV Absorption Maximum (λ_{max})

Levodopa exhibited λ_{max} at 284 nm.

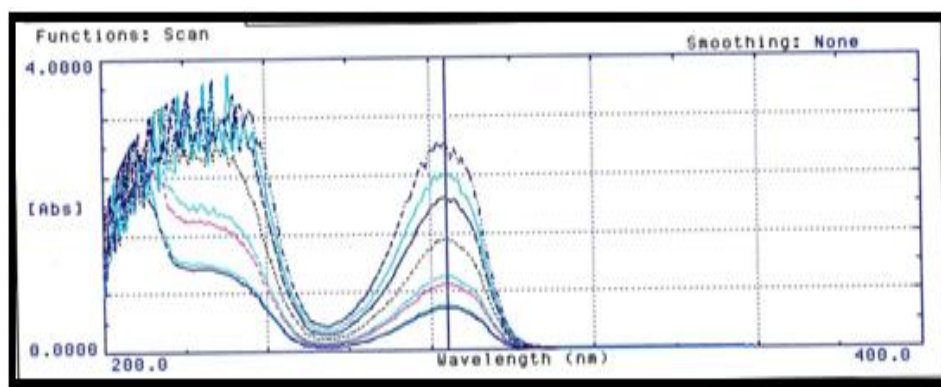


Figure 1. UV absorption scan of Levodopa showing a maximum absorbance (λ_{max}) at approximately 284 nm.

3.2 Chromatogram of Standard Levodopa

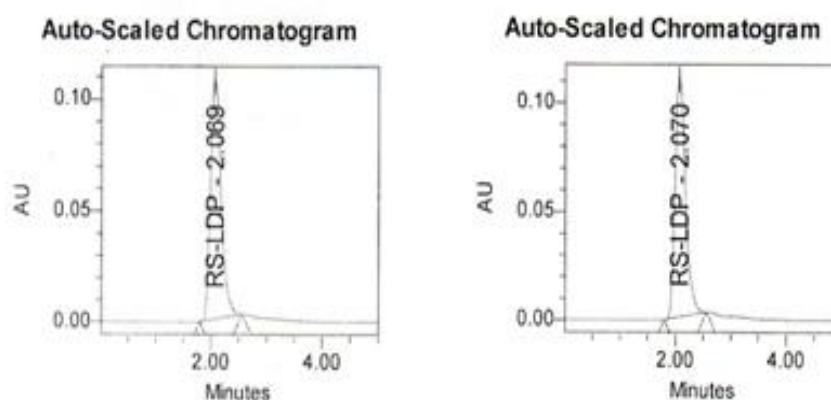


Figure 2. RP-HPLC chromatogram of Levodopa reference standard showing a sharp peak at approximately 2.06 minutes under the optimized conditions.

3.3 Chromatogram of *Vicia faba* Extract

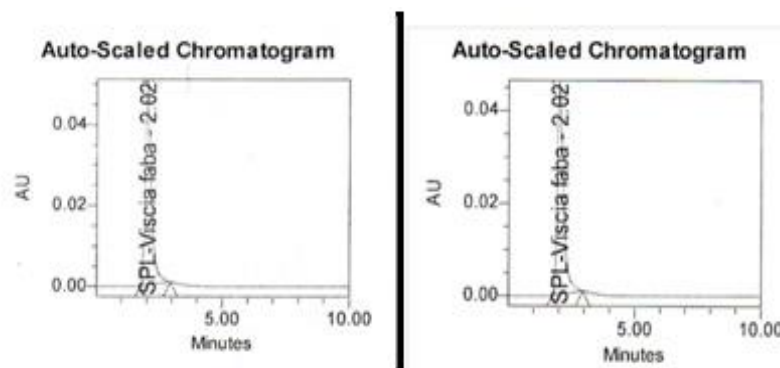


Figure 3. RP-HPLC chromatogram of methanolic extract of *Vicia faba* seeds showing the Levodopa peak at approximately 2.62 minutes.

3.4 Linearity

Linearity (10–500 µg/mL) showed $r^2 = 0.9999$.

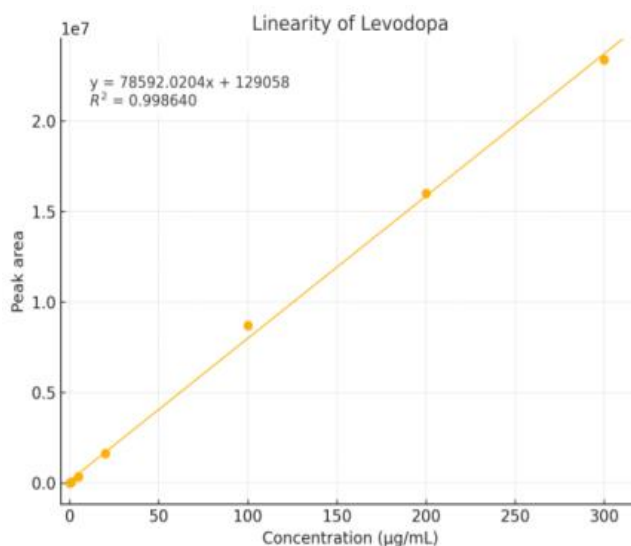


Figure 4. Calibration curve of Levodopa (10–500 µg/mL) showing excellent linearity with regression equation $y = 78592.0204x + 129058$ ($R^2 = 0.9986$).

Table 1. Linearity data of Levodopa showing the mean peak area obtained at different concentrations (10–500 µg/mL). The calibration curve demonstrated excellent linearity with regression equation $y = 45725x + 9566.505$ and correlation coefficient (r^2) = 0.9999.

Conc. (µg/mL)	Inj-1	Inj-2	Inj-3
10	113313	108112	109944
50	738942	715202	767530
100	1444560	1426635	1445756
250	3922595	3800596	4025756
500	7684255	7760000	7758283

3.5 Accuracy

Table 2. Accuracy results of Levodopa determined by recovery studies at three

concentration levels (80%, 100%, and 120%), showing percent recovery within the acceptable range of 98–102%, confirming the accuracy of the developed method.

Level	Amount (mg)	% Recovery
80%	8	98.57
100%	10	99.46
120%	12	98.89

Recoveries: 98.57% – 99.46%. Intra-day %RSD = 2.547%; Inter-day %RSD = 1.10%.

3.6 Precision

3.7 LOD/LOQ



LOD = 0.690 µg/mL; LOQ = 2.092 µg/mL.

3.8 System Suitability

Table 3. System suitability parameters for the optimized RP-HPLC method, including theoretical plates (N), tailing factor, and capacity factor, all of which comply with USP acceptance criteria, confirming robust chromatographic performance.

Parameter	Result
Theoretical Plates	6549
Tailing Factor	1.48
Capacity Factor	1.05

4. DISCUSSION

The developed RP-HPLC method showed superior chromatographic efficiency with a retention time of ~2.0–2.6 min, much shorter than many reported methods for herbal L-DOPA sources [9,10]. The methanol–acetic acid mobile phase provided sharp peak shape, consistent with literature indicating acidic modifiers improve catecholamine separation [10].

Linearity ($r^2 = 0.9999$) and wide range (10–500 µg/mL) exceeded several earlier herbal studies [9,11]. Sensitivity parameters (LOD/LOQ) were excellent compared to buffer-based or electrochemical detection methods. Accuracy (~99%) and precision (%RSD < 2.6%) confirmed reliability per ICH Q2(R1). System suitability met USP criteria, validating chromatographic performance.

Overall, this work resolves limitations of earlier methods such as long run times, complex solvents, and incomplete validation, providing the first fully optimized RP-HPLC method specifically for *Vicia faba* seed extract.

5. NOVELTY OF THE METHOD

This is among the first rapid, sensitive, and fully validated RP-HPLC methods tailored exclusively for *Vicia faba*. The short run time, simple mobile phase, and strong validation metrics enhance applicability in routine QC laboratories.

6. CONCLUSION

A rapid, sensitive, and ICH-compliant RP-HPLC method for Levodopa quantification in *Vicia faba* was successfully developed. The method's practical outcome is its suitability for routine industrial QC, herbal standardization, and phytopharmaceutical development. The approach fills a major analytical gap by providing a robust method specifically optimized for *Vicia faba*.

7. CONFLICT OF INTEREST

The authors declare no conflict of interest.

8. ACKNOWLEDGMENT

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