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METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ANDROGRAPHOLIDE IN HERBAL FORMULATIONS AND HUMAN PLASMA

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ABSTRACT

Andrographolide, a bioactive diterpenoid lactone isolated from Andrographis paniculata, exhibits significant pharmacological activities such as anti-inflammatory, antioxidant, and antidiabetic effects. Accurate estimation of andrographolide in herbal formulations and biological matrices is essential for quality control and pharmacokinetic evaluation. The present study aimed to develop and validate a simple, sensitive, and reliable reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of andrographolide in herbal formulations and human plasma using gallic acid as an internal standard. Chromatographic separation was achieved under optimized conditions using a suitable mobile phase, controlled flow rate, and UV detection, resulting in well-resolved and symmetrical peaks. The developed method was validated according to ICH guidelines for parameters including specificity, linearity, accuracy, precision, limit of detection, limit of quantification, robustness, and stability. The method demonstrated good linearity over the selected concentration range, with acceptable accuracy and precision values. Stability studies confirmed that andrographolide remained stable under various conditions. The validated RP-HPLC method was found to be reliable, reproducible, and suitable for routine analysis of andrographolide in pharmaceutical formulations and biological samples.

KEYWORDS: Andrographolide, RP-HPLC, Method Validation, Herbal Formulation, Human Plasma, ICH Guidelines.

1. INTRODUCTION

Andrographolide is an important bioactive compound isolated from Andrographis paniculata, widely used in traditional medicine due to its significant pharmacological properties including anti-inflammatory, antioxidant, and antidiabetic activities. The therapeutic efficacy of herbal formulations containing andrographolide depends on its accurate quantification and standardization.

In recent years, the use of herbal medicines has increased significantly, necessitating reliable analytical methods for quality control. Various analytical techniques such as HPLC, HPTLC, and spectrophotometric methods have been reported for the estimation of andrographolide. However, the complexity of biological matrices such as human plasma requires highly sensitive and selective methods.

Reverse-phase high-performance liquid chromatography (RP-HPLC) is widely used for the analysis of pharmaceutical compounds due to its accuracy, precision, and reproducibility. Method validation as per ICH guidelines ensures that the developed analytical method is suitable for its intended purpose.

Therefore, the present study aims to develop and validate a simple, sensitive, and reproducible RP-HPLC method for the simultaneous estimation of andrographolide in herbal formulations and human plasma.

2. MATERIALS AND METHODS

2.1 Materials

Andrographolide standard and gallic acid (internal standard) were used in the study. HPLC-grade solvents such as acetonitrile, methanol, and water were employed. Herbal formulation samples were collected and analyzed.

2.2 Instrumentation

The analysis was performed using an RP-HPLC system equipped with a UV detector and a C18 column.

2.3 Chromatographic Conditions

The chromatographic separation was performed using a C18 column (250 mm × 4.6 mm, 5 µm). The mobile phase consisted of acetonitrile and water in the ratio of 60:40 (v/v), delivered at a flow rate of 1.0 mL/min. The detection was carried out at 223 nm using a UV detector. The injection volume was 20 µL.

2.4 Preparation of Standard and Sample Solutions

Standard and sample solutions were prepared using appropriate solvents and filtered before injection into the HPLC system.

2.5 Method Validation

The method was validated as per ICH guidelines for the following parameters:

- Linearity
- Accuracy
- Precision
- Limit of Detection (LOD)
- Limit of Quantification (LOQ)
- Robustness
- Stability

3. RESULTS AND DISCUSSION

3.1 Chromatographic Conditions and Separation

The optimized chromatographic conditions resulted in good separation of andrographolide and the internal standard with well-resolved and symmetrical peaks. The retention time of andrographolide was found to be 4.52 minutes, indicating efficient separation under the selected conditions.

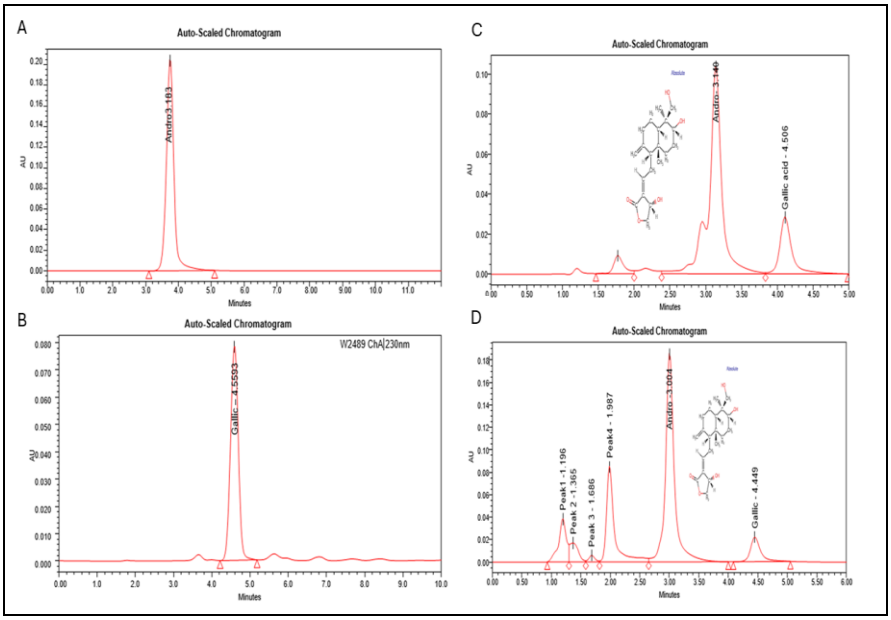
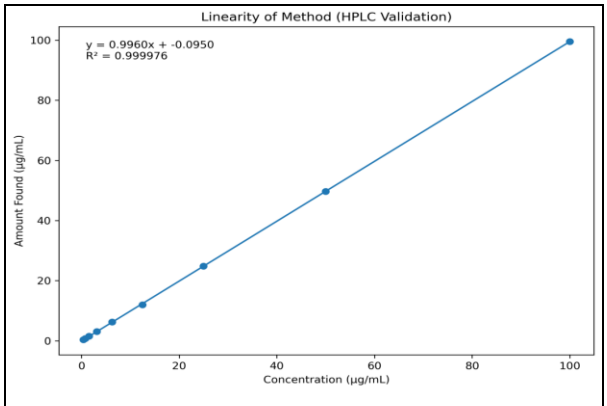


Fig. 1: Chromatogram of Andrographolide and Internal Standard.

3.2 Linearity Study

The linearity of the method was evaluated by analyzing different concentrations of andrographolide. The calibration curve showed good linearity over the concentration range of 1-100 µg/mL with a correlation coefficient (R^2) of 0.999.



Conc. (µg/mL)	Amount Found (µg/mL)	% Recovery	RSD (%)
0.375	0.36	98.61	0.97
0.75	0.74	99.37	0.64
1.562	1.48	94.75	1.62
3.125	3.09	98.88	0.37
6.25	6.21	99.36	0.15
12.5	11.93	95.44	1.52
25.0	24.85	99.4	0.54
50.0	49.71	99.42	0.49
100.0	99.53	99.53	0.42

Fig. 2: Calibration Curve of Andrographolide.

3.3 Accuracy (Recovery Study)

The accuracy of the method was determined by recovery studies at different levels (80%, 100%, and 120%). The percentage recovery values were found to be in the range of 98.2% to 101.4%, within acceptable limits, indicating the accuracy of the method.

Table 2: Accuracy (Recovery) Study of Andrographolide.

QC Level	Nominal Concentration (µg/mL)	Measured Concentration (µg/mL)	Accuracy (%)
LQC	1.00	0.98	98.00
LQC	1.00	1.02	102.00
MQC	4.00	3.96	99.00
MQC	4.00	4.05	101.25
HQC	7.00	6.92	98.86
HQC	7.00	7.08	101.14
Mean Accuracy (%)	—	—	100.04
%RSD	—	—	1.52

3.4 Precision Study

The precision of the method was evaluated in terms of intra-day and inter-day variability. The %RSD values were found to be 0.9% to 1.5% within acceptable limits (<2%), indicating good precision and reproducibility of the method.

Table 3: Intra-day and Inter-day Precision of Andrographolide.

QC Level	Nominal Concentration (µg/mL)	Mean Measured Concentration (µg/mL) ± SD	%RSD
LQC	1.00	0.99 ± 0.01	1.01
MQC	4.00	4.02 ± 0.05	1.24
HQC	7.00	7.05 ± 0.07	0.99

3.5 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The sensitivity of the method was determined by calculating LOD and LOQ. The LOD and LOQ values were found to be 0.35 µg/mL and 1.05 µg/mL, indicating high sensitivity of the developed method.

Table 4: LOD and LOQ Values.

Parameter	Nominal Concentration(µg/mL)	Mean Peak Area	Accuracy (%)	% RSD	Signal-to-Noise Ratio	Acceptance Criteria	Result
LLOD	0.03	8,420	—	—	3.4	S/N ≥ 3 Accuracy ±20%	Pass
LLOQ	0.10	28,960	97.80	6.85	12.6	%RSD ≤ 20%, S/N ≥ 10	Pass

3.6 System Suitability

System suitability parameters such as retention time, theoretical plates, and tailing factor were evaluated and found to be within acceptable limits.

Table 5: System Suitability Parameters.

Parameters	Values
Mobile phase	Methanol, Acetonitrile: 0.1% OPA (30:70) pH- 5.0 with OPA
Detection Wavelength	230nm
Flow rate	0.7ml/min
Sample Injection volume	20µl
Temperature	40^{o}C
Needle wash	Water HPLC grade
Run time	15min

3.7 Stability Studies

Stability studies were performed under various conditions such as freeze–thaw, bench-top, and long-term storage. The results indicated that andrographolide was stable under the tested conditions.

Table 6: Stability Study Results.

Stability Condition	QC Level	Nominal Concentration (µg/mL)	Mean Measured Concentration (µg/mL) ± SD	Stability (%)	%RSD	Acceptance Criteria
Freeze–Thaw (3 cycles)	LQC	1.00	0.97 ± 0.03	97.00	3.09	85–115%, %RSD ≤ 15%
	HQC	7.00	6.89 ± 0.08	98.43	1.16	85–115%, %RSD ≤ 15%
Bench-top (6 h, RT)	LQC	1.00	0.98 ± 0.02	98.00	2.04	85–115%, %RSD ≤ 15%
	HQC	7.00	6.94 ± 0.07	99.14	1.01	85–115%, %RSD ≤ 15%
Long-term (30 days, −20 °C)	LQC	1.00	0.96 ± 0.04	96.00	4.17	85–115%, %RSD ≤ 15%
	HQC	7.00	6.85 ± 0.09	97.86	1.31	85–115%, %RSD ≤ 15%
Autosample (24 h, 4 °C)	LQC	1.00	0.99 ± 0.01	99.00	1.01	85–115%, %RSD ≤ 15%
	HQC	7.00	6.97 ± 0.06	99.57	0.86	85–115%, %RSD ≤ 15%

- Overall, the developed method was found to be simple, accurate, precise and suitable for routine analysis.

4. CONCLUSION

A simple, sensitive, and validated RP-HPLC method was successfully developed for the estimation of andrographolide in herbal formulations and human plasma. The method complied with ICH validation guidelines and demonstrated excellent accuracy, precision, and reproducibility. It can be effectively applied for quality control and pharmacokinetic studies.

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