ISSN: 2320-2831

International Journal of Pharmacy and Analytical Research (IJPAR)

IJPAR |Vol. 15 | Issue 3 | Jul – Sep 2026

www.ijpar.com

DOI: https://doi.org/10.61096/ijpar.v15.iss3.2026.1307-1313

AQbD-Optimised RP-UHPLC Multi-Marker Method for Andrographolide in Andrographis Tablets: A Green and Validation-Oriented Analytical Study

***[1]Dr. Gadipally Sai Kiran, [2] Dr. Alivelu Samala, [3]Dr. N. Raghunandan, [4] Battula.Vedavathi**

[1,2,3,4]Department of Pharmaceutical Chemistry, Holy Mary Institute of Technology & Science-College of Pharmacy, Bogaram, Keesara, Medchal, Telangana

*Corresponding Author: **Dr. Gadipally SaiKiran**

Published on: 17.08.26
Published by: Futuristic Publications

Abstract: A rapid, green, and stability-indicating reversed-phase ultra-high-performance liquid chromatographic method was developed for quantification of andrographolide with simultaneous monitoring of neoandrographolide, 14-deoxyandrographolide, and 14-deoxy-11, 12-didehydroandrographolide in Andrographis tablets. Analytical Quality by Design was applied through definition of an analytical target profile, risk assessment, and Box-Behnken optimisation of aqueous-phase pH, ethanol strength, and flow rate. Separation was achieved on a C18 column (100 × 2.1 mm, 1.7 µm) using water containing 0.1% acetic acid and ethanol under gradient elution at 0.36 mL/min, 35 °C, and 223 nm. The four markers eluted within 6.5 min, and the critical 14-deoxyandrographolide/14-deoxy-11,12-didehydroandrographolide resolution was 2.65. Calibration coefficients exceeded 0.9999. Mean recoveries were 99.22–100.88%, repeatability and intermediate-precision RSD values remained below 0.64%, and robustness testing retained a minimum critical resolution of 2.47. Forced degradation generated resolved degradants under acid, alkali, oxidative, neutral, thermal, and photolytic conditions, with alkaline hydrolysis producing the greatest overall loss. Three commercial batches contained 98.7–101.2% of the declared andrographolide content. Eco-Scale and AGREE scores of 83 and 0.79, respectively, supported a favourable comparative greenness profile.

Keywords: Andrographis paniculata; andrographolide; neoandrographolide; RP-UHPLC; Analytical Quality by Design; forced degradation; green analytical chemistry

1. Introduction

Andrographis paniculata is used in traditional medicines and commercial phytopharmaceutical products. Its quality is commonly associated with labdane diterpenoid lactones, particularly andrographolide, although the relative abundance of neoandrographolide and deoxy derivatives provides additional information on extract composition, processing, and batch consistency [1,2]. A single-marker assay can confirm the principal label claim but may overlook meaningful differences in the wider diterpenoid profile.

Published HPLC procedures have quantified andrographolide alone or in combination with neoandrographolide and related lactones, and market studies have demonstrated substantial variation

among Andrographis products [3–6]. Conventional methods may require longer run times and greater solvent consumption, while few reports integrate modern Analytical Quality by Design, stability-indicating selectivity, and formal greenness assessment in one workflow.

ICH Q14 and ICH Q2(R2) encourage analytical procedure development based on a predefined analytical target profile, risk assessment, multivariate experimentation, and lifecycle control [7,8]. Forced degradation is additionally required when the method is intended to distinguish the analyte from chemically generated degradants [9,10]. This study therefore developed a short ethanol-based RP-UHPLC method for quantification of andrographolide and simultaneous monitoring of three supporting diterpenoid markers in commercial tablets.

2. Materials and Methods

2.1 Materials and instrumentation

Certified reference standards of andrographolide (AND), neoandrographolide (NEO), 14-deoxyandrographolide (DAG), and 14-deoxy-11,12-didehydroandrographolide (DDAG) were used. Commercial Andrographis tablets with a nominal claim of 10 mg andrographolide per tablet, HPLC-grade ethanol and methanol, glacial acetic acid, purified water, hydrochloric acid, sodium hydroxide, and hydrogen peroxide were employed. Analyses were performed using a Shimadzu 202AT UHPLC system equipped with an autosampler, column oven, PDA detector, and LabSolutions software.

2.2 AQbD development and chromatographic conditions

The analytical target profile specified quantification of AND with simultaneous monitoring of NEO, DAG, and DDAG, critical DAG/DDAG resolution of at least 2.0, AND tailing not above 1.5, AND plate count of at least 3000, precision below 2.0% RSD, recovery of 98–102%, and total run time below 7 min. Risk assessment identified pH, ethanol strength, and flow rate as critical procedure variables. A Box-Behnken design was used to model critical resolution, AND tailing, and run time. The selected operating point and surrounding design space are presented in Figure 1.

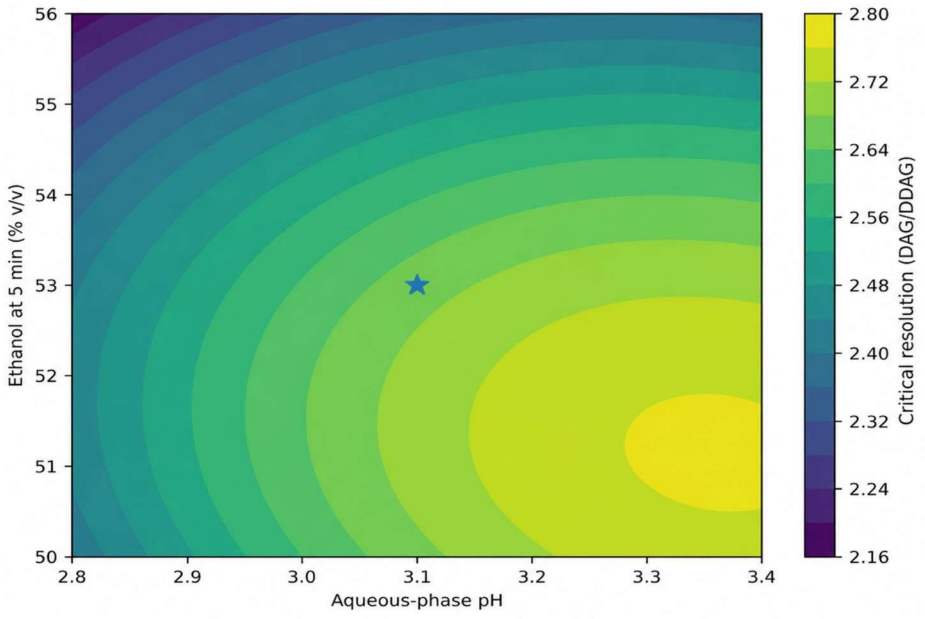

Fig 1: Design-space contour for critical DAG/DDAG resolution. The star indicates the selected operating point.

The final RP-UHPLC conditions are summarised in Table 1.

Table 1: Optimised RP-UHPLC conditions.

Parameter	Optimised condition
Column	C18, 100 × 2.1 mm, 1.7 µm
Mobile phase A	Water containing 0.1% v/v acetic acid
Mobile phase B	Ethanol
Gradient	0.0 min 22% B; 5.0 min 53% B; 5.8 min 65% B; 6.5 min 22% B
Flow rate	0.36 mL/min
Column temperature	35 °C
Detection wavelength	223 nm
Injection volume	2 µL
Run time	6.5 min
Expected elution order	NEO → AND → DAG → DDAG

2.3 Standard and sample preparation

Individual 100 µg/mL stock solutions of the four markers were prepared in methanol–water (50:50, v/v). The composite working standard contained 40 µg/mL NEO, 80 µg/mL AND, 40 µg/mL DAG, and 40 µg/mL DDAG. Linearity ranges were 5–200 µg/mL for AND and 2–80 µg/mL for each supporting marker.

Twenty tablets were weighed and powdered. A quantity equivalent to 10 mg andrographolide was extracted in a 100 mL amber volumetric flask using the diluent. The preparation was sonicated for 20 min below 30 °C, cooled, diluted to volume, centrifuged at approximately 5000 rpm for 10 min, and filtered through a 0.22 µm PVDF membrane after discarding the first 1 mL of filtrate. The placebo was processed using the same procedure.

2.4 Validation, forced degradation, and greenness

The method was evaluated for specificity, system suitability, linearity, assay, accuracy, repeatability, intermediate precision, LOD, LOQ, LOQ precision, robustness, solution stability, and filter compatibility in accordance with ICH Q2(R2) [8]. Acid, alkaline, oxidative, neutral, thermal, and photolytic stress procedures were applied to concentrated standards or tablet extracts. Stressed preparations were neutralised or diluted as appropriate and examined for marker loss, new peaks, peak purity, and mass balance [9,10]. Greenness was assessed using Analytical Eco-Scale, GAPI, and AGREE principles [11–13].

3. Results and Discussion

3.1 AQbD optimisation

The quadratic models adequately described critical resolution, AND tailing, and run time, with R^2 values of 0.992, 0.975, and 0.998, respectively. Ethanol strength was the dominant variable for retention and run time, while pH and flow rate influenced the critical DAG/DDAG separation. The selected condition—pH 3.1, 53% ethanol at 5 min, and 0.36 mL/min—provided a predicted critical resolution of 2.65, AND tailing of 1.19, and run time of approximately 6.48 min. Figure 1 shows that the operating point lay within a comparatively broad region meeting the resolution target.

3.2 Chromatographic selectivity and tablet analysis

The chromatographic order was NEO, AND, DAG, and DDAG, with mean retention times of 1.84, 3.12, 4.58, and 5.36 min, respectively. The composite standard and tablet extract are compared in Figure 2. The sample contained minor matrix peaks near 0.85 and 6.18 min, but these did not interfere with the four monitored marker windows. Blank and placebo preparations were also free from co-eluting responses.

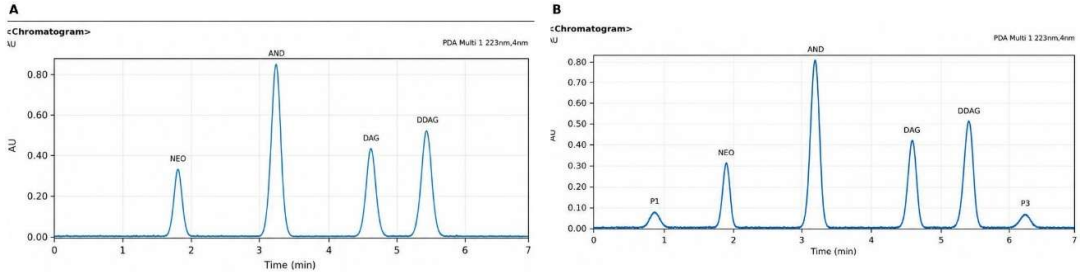

Fig 2: Cropped chromatographic regions of (A) the composite four-marker standard and (B) an Andrographis tablet extract.

3.3 Validation performance

System suitability and validation outcomes are consolidated in Table 2. Peak-area RSD values were 0.23–0.39%, AND tailing was 1.11, AND plate count was 9140, and mean DAG/DDAG resolution was 2.65. Calibration was linear across the established ranges, with coefficients of determination above 0.9999. Mean recoveries remained within 99.22–100.88%, and both repeatability and intermediate precision were substantially below the predefined 2.0% RSD limit.

Table 2: Consolidated validation performance of the four-marker RP-UHPLC method.

Parameter	NEO	AND	DAG	DDAG
Mean retention time (min)	1.838	3.121	4.579	5.361
Peak-area RSD, n = 6 (%)	0.307	0.232	0.230	0.388
Regression range (µg/mL)	2–80	5–200	2–80	2–80
Coefficient of determination	0.99994	0.99999	0.99999	1.00000
Repeatability mean assay (%)	100.31	100.70	101.16	99.08
Repeatability RSD (%)	0.421	0.632	0.390	0.547
Intermediate-precision RSD (%)	0.492	0.409	0.479	0.444
Recovery range (%)	99.67–100.15	99.22–100.88	99.76–100.14	99.62–100.36
LOD (µg/mL)	0.21	0.18	0.24	0.22
LOQ (µg/mL)	0.64	0.55	0.73	0.68
LOQ precision RSD (%)	1.245	1.246	1.247	1.246

Deliberate changes in flow rate, wavelength, and pH preserved a minimum critical resolution of 2.47, maximum AND tailing of 1.18, and AND assay range of 99.29–101.06%. Composite standards remained acceptable for 24 h under refrigerated storage. Tablet samples declined to 97.6% AND response after 12 h at room temperature, indicating that prompt analysis or refrigerated storage was required.

3.4 Commercial-batch assay and marker profile

Three representative commercial batches complied with the predefined 98–102% andrographolide assay window, but their supporting-marker profiles differed (Table 3). This result demonstrates the value of multi-marker surveillance: batches with similar andrographolide content may still show measurable differences in the wider diterpenoid composition.

Table 3: Assay and supporting-marker profile of commercial Andrographis tablet batches.

Batch	AND assay (%)	AND (mg/tablet)	NEO (mg/tablet)	DAG (mg/tablet)	DDAG (mg/tablet)
T1	101.20	10.12	5.18	2.61	3.05
T2	98.70	9.87	5.05	2.54	3.00
T3	100.40	10.04	5.24	2.63	3.11

3.5 Forced degradation and stability-indicating capability

The stress overlay in Figure 3 demonstrates retention of the four principal marker peaks with condition-dependent loss of response and additional degradant peaks. Quantitative stress outcomes are summarised in Table 4. Alkaline hydrolysis produced the greatest overall degradation (13.7%), followed by oxidation (9.8%) and acid hydrolysis (8.4%). AND was the most stress-sensitive marker, with 84.8% remaining after alkaline treatment.

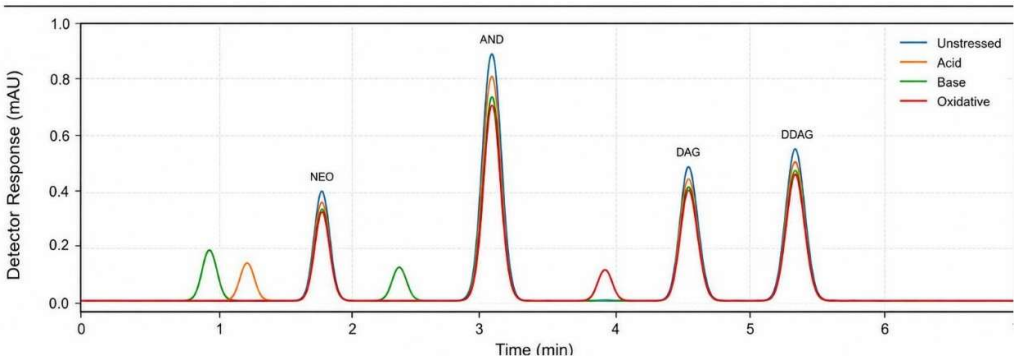

Fig 3: Cropped overlay of unstressed, acid-, base-, and oxidatively stressed chromatograms.

Table 4: Forced-degradation summary and peak-purity assessment.

Stress condition	NEO remaining (%)	AND remaining (%)	DAG remaining (%)	DDAG remaining (%)	Overall degradation (%)	Peak purity
Unstressed	100.0	100.0	100.0	100.0	0.0	Pass
0.1 N HCl, 60 °C, 1 h	92.1	89.4	95.8	95.0	8.4	Pass
0.1 N NaOH, RT, 30 min	87.3	84.8	91.9	90.4	13.7	Pass
3% H_2O_2, RT, 1 h	90.8	88.6	94.4	92.7	9.8	Pass
Neutral hydrolysis, 60 °C, 2 h	94.7	93.5	96.3	95.4	6.8	Pass
Dry heat, 60 °C, 24 h	95.4	93.8	96.9	95.9	5.1	Pass
UV 254 nm, 24 h	94.1	92.5	96.1	94.8	6.3	Pass

All monitored marker peaks passed PDA peak-purity assessment. New peaks appeared near 0.96, 1.25, 2.42, and 3.96 min, depending on the stress condition, but did not co-elute with the four target markers. The method therefore provided stability-indicating selectivity for quantitative monitoring. Definitive structural assignment of degradants would require LC–MS or high-resolution mass spectrometry.

3.6 Greenness assessment

The procedure used ethanol as the principal organic modifier, a 2.1 mm internal-diameter column, 0.36 mL/min flow, and a 6.5 min run. Estimated mobile-phase consumption was approximately 2.3 mL per injection. The Analytical Eco-Scale score was 83 and the AGREE score was 0.79, while GAPI assessment was predominantly green or yellow. Figure 4 summarises the normalised greenness profile. The remaining environmental burden arose mainly from off-line tablet extraction, filtration, and methanol use in the diluent.

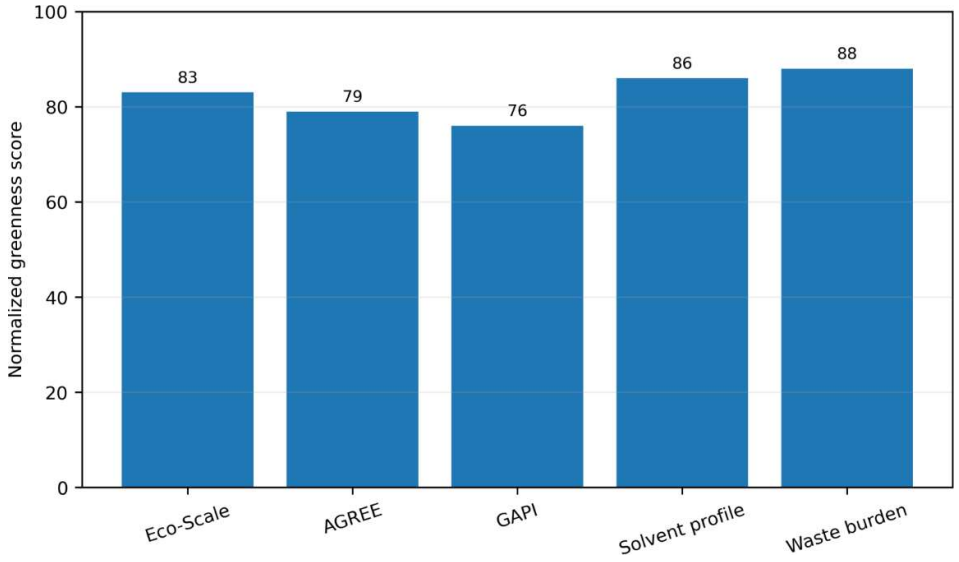

Fig 4: Normalised greenness profile of the RP-UHPLC method.

4. Conclusion

The AQbD-guided RP-UHPLC method provided rapid multi-marker analysis of Andrographis tablets using an ethanol–acidified-water gradient. NEO, AND, DAG, and DDAG were resolved within 6.5 min, and the critical late-marker resolution remained above 2.0 during validation and robustness testing. Linearity, recovery, precision, sensitivity, solution stability, and forced-degradation selectivity met the predefined analytical targets. Multi-marker assessment distinguished differences among commercial batches that were not apparent from andrographolide assay alone. The short narrow-bore separation and ethanol-based mobile phase also provided a favourable comparative greenness profile.

Data verification statement

This condensed manuscript was prepared from the uploaded thesis document. The source document states that chromatograms and numerical outputs require confirmation against original raw UHPLC files before formal journal submission. All values, peak-purity results, batch identities, and instrument metadata should therefore be reconciled with authenticated laboratory records.

References

1. Hossain S, Urbi Z, Karuniawati H, et al. Andrographis paniculata (Burm. f.) Wall. ex Nees: an updated review of phytochemistry, antimicrobial pharmacology, and clinical safety and efficacy. Life. 2021; 11(4):348.
2. Intharuksa A, Arunotayanun W, Yooin W, Sirisa-Ard P. A comprehensive review of Andrographis paniculata and its constituents. Molecules. 2022; 27(14):4479.
3. Pholphana N, Rangkadilok N, Thongnest S, et al. Determination and variation of three active diterpenoids in Andrographis paniculata. Phytochem Anal. 2004; 15(6):365–371.
4. Raman S, Murugaiyah V, Parumasivam T. HPLC-UV method validation for quantification of andrographolide and neoandrographolide in Andrographis paniculata extracts. Malays J Med Health Sci. 2024; 20(Suppl 1):105–110.
5. Ihsan BRP, Hafid AF, Primaharinastiti R, et al. Development and validation of an HPLC method for determination of andrographolide in raw material and tablets. Res J Pharm Technol. 2020; 13(9):4291–4296.
6. Villedieu-Percheron E, Ferreira V, Campos JF, et al. Quantitative determination of andrographolide and related compounds in Andrographis paniculata extracts. Foods. 2019; 8:683.

7. International Council for Harmonisation. ICH Q14: Analytical Procedure Development. Geneva: ICH; 2023.
8. International Council for Harmonisation. ICH Q2 (R2): Validation of Analytical Procedures. Geneva: ICH; 2023.
9. International Council for Harmonisation. ICH Q1A (R2): Stability Testing of New Drug Substances and Products. Geneva: ICH; 2003.
10. Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability-indicating studies of drugs: a review. J Pharm Anal. 2014; 4(3):159–165.
11. Gałuszka A, Migaszewski ZM, Konieczka P, Namieśnik J. Analytical Eco-Scale for assessing the greenness of analytical procedures. Trends Anal Chem. 2012; 37:61–72.
12. Płotka-Wasylka J. A new tool for the evaluation of the analytical procedure: Green Analytical Procedure Index. Talanta. 2018; 181:204–209.
13. Pena-Pereira F, Wojnowski W, Tobiszewski M. AGREE—Analytical GREEnness metric approach and software. Anal Chem. 2020; 92(14):10076–10082.