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Development and Validation of a Stability-Indicating RP-HPLC Method for Quantification of Azelnidipine in Pharmaceutical Dosage Forms

Sagar Kalidas Dongare[1] and Ganesh S. Mhaske ,ms.seema bansode,uttekar pravin
Sagardongare2004@gmail.com ,
hariomganesh79@gmail.com,
bseema109@gmail.com
uttekar.p.s@gmail.com

[1]Department of Pharmaceutical Quality Assurance, Late Laxmibai Phadtare College of Pharmacy, Kalamb, Walchandnagar, Tal. Indapur, Dist. Pune - 413114, Maharashtra, India

Abstract

A rapid reverse-phase high-performance liquid chromatographic method was developed for the quantitative determination of azelnidipine in a pharmaceutical dosage form and evaluated using commonly applied analytical-validation characteristics. Separation was achieved on a Zodiac C18 column (250 × 4.6 mm, 5 µm) using methanol and phosphate buffer adjusted to pH 6.7 with orthophosphoric acid (50:50, v/v) as the mobile phase. The flow rate was 1.0 mL/min, the injection volume was 20 µL, UV detection was performed at 279 nm, and the total run time was 7.5 min. Azelnidipine eluted at approximately 2.26 min, while the co-formulated component eluted at approximately 5.23 min. Mean recoveries at 80%, 100%, and 120% levels were 100.27%, 99.18%, and 99.46%, respectively. Repeatability showed a relative standard deviation of 0.721%; intra-day and inter-day precision values were below 2%. The reported limit of detection and limit of quantification for azelnidipine were 0.377 and 1.143 µg/mL, respectively. The marketed formulation assay was 98.00 ± 0.41% of label claim. Stress testing indicated the highest degradation under reductive conditions (8.6%), followed by acidic conditions (5.5%). Although the dissertation states satisfactory linearity, recalculation of the tabulated calibration data produced an R^2 of approximately 0.993; therefore, the original integration data and regression model should be reconfirmed before regulatory or journal submission. Subject to this verification, the method is rapid, economical, and potentially suitable for routine quality-control analysis.

Keywords: Azelnidipine; RP-HPLC; method development; analytical validation; forced degradation; pharmaceutical dosage form

1. Introduction

Azelnidipine is a long-acting dihydropyridine calcium-channel blocker used in the management of hypertension. It reduces calcium entry through L-type channels in vascular smooth muscle, thereby promoting vasodilation and lowering peripheral vascular resistance. Reliable quantification of the active pharmaceutical ingredient in finished dosage forms is essential for assuring identity, strength, quality, and batch-to-batch consistency.

Reverse-phase HPLC is widely used for pharmaceutical assay because it offers adequate selectivity, sensitivity, precision, and compatibility with routine quality-control laboratories. During product development and stability testing, an analytical procedure should distinguish the analyte from excipients, process-related impurities, and degradation products. Forced-degradation experiments under hydrolytic, oxidative, reductive, and thermal conditions are therefore useful for evaluating method specificity and understanding degradation behavior.

The objective of the present work was to develop a simple and economical RP-HPLC procedure for azelnidipine in a pharmaceutical dosage form, optimize chromatographic conditions, and evaluate accuracy, precision, sensitivity, robustness, system suitability, assay performance, and stress-degradation behavior.

2. Materials and Methods

2.1 Chemicals and reagents

Azelnidipine reference material was reported to have been supplied by Ajanta Pharma Pvt. Ltd. Methanol, acetonitrile, potassium dihydrogen phosphate, sodium hydroxide, hydrochloric acid, hydrogen peroxide, sodium borohydride, and orthophosphoric acid were of analytical or HPLC grade as stated in the dissertation. Purified water was used for solution preparation.

2.2 Instrumentation and chromatographic conditions

Chromatographic analysis was carried out using a Hitachi HPLC system equipped with a UV-visible detector and a manual Rheodyne injector fitted with a 20 µL loop. Separation was performed on a Zodiac C18 column (250 × 4.6 mm internal diameter, 5 µm particle size). A UV-visible spectrophotometer and ultrasonic bath were used during wavelength selection and solution preparation.

Parameter	Optimized condition
Column	Zodiac C18, 250 × 4.6 mm, 5 µm
Mobile phase	Methanol : phosphate buffer pH 6.7 adjusted with OPA (50:50, v/v)
Flow rate	1.0 mL/min
Detection wavelength	279 nm
Injection volume	20 µL
Run time	7.5 min
Mode	Isocratic

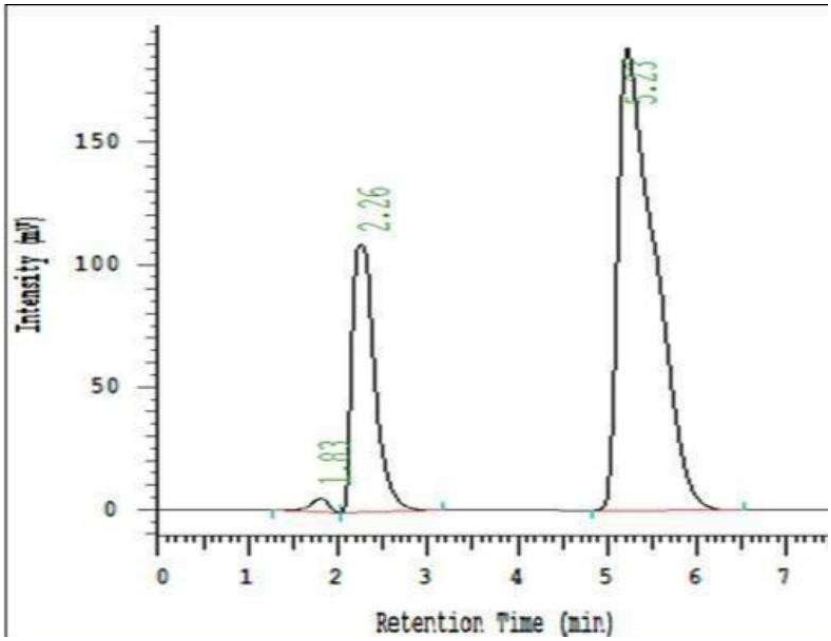

Figure 1. Optimized chromatogram showing the reported azelnidipine peak at approximately 2.26 min and the co-formulated component at approximately 5.23 min.

2.3 Preparation of standard and sample solutions

A stock solution of azelnidipine was prepared by dissolving 10 mg of reference standard in methanol and diluting to 100 mL to obtain 100 µg/mL. Working solutions were prepared by appropriate dilution with methanol or mobile phase. For formulation analysis, twenty units were weighed and powdered. A quantity equivalent to the stated label claim of azelnidipine was transferred to a volumetric flask, extracted with mobile phase by ultrasonication, filtered through a 0.45 µm membrane, and diluted to the working concentration before injection.

2.4 Calibration and validation

Calibration solutions containing 10-60 µg/mL azelnidipine were prepared from the stock solution and injected in triplicate. Method performance was assessed by recovery at 80%, 100%, and 120% spiking levels; repeatability; intra-day and inter-day precision; limits of detection and quantification; robustness following changes in flow rate; system suitability; and assay of the marketed formulation. LOD and LOQ were calculated using 3.3σ/S and 10σ/S, respectively, where σ is the standard deviation of response and S is the slope of the calibration curve.

2.5 Forced-degradation studies

For stress testing, a reported drug concentration of 1 mg/mL was exposed for 12 h to 1 N hydrochloric acid, 1 N sodium hydroxide, 5% hydrogen peroxide, 1% sodium borohydride, and dry heat at 80 °C. Acidic and alkaline samples were neutralized before dilution and chromatographic analysis. The percentage assay remaining and percentage degradation were reported for each condition.

3. Results and Discussion

3.1 Method development and chromatographic performance

Multiple aqueous-organic mobile phases containing water, methanol, and acetonitrile were screened. The final methanol-buffer system at pH 6.7 produced the best reported peak separation, resolution, and plate count within a 7.5 min run. The azelnidipine peak appeared at approximately 2.26 min. The reported resolution between the two principal peaks was 9.15, indicating ample chromatographic separation under the optimized conditions.

3.2 Linearity and calibration

Concentration (µg/mL)	Mean peak area (mV·s)	SD	%RSD
0	0	0	0
10	92,164	45.38	0.0492
20	167,293	190.47	0.1138
30	306,089	119.62	0.0391
40	370,481	316.95	0.0856
50	447,930	486.02	0.1085
60	584,345	794.19	0.1359

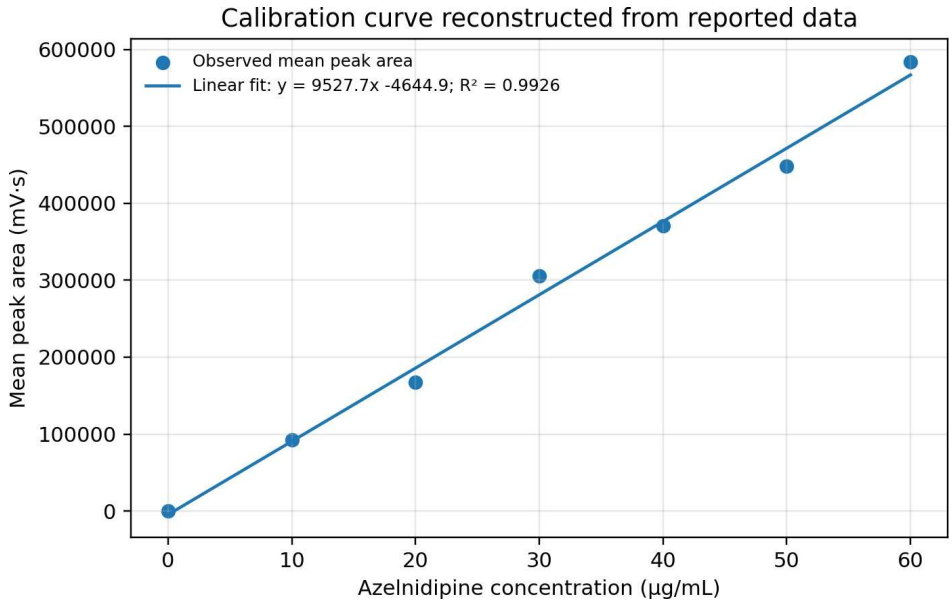

Figure 2. Calibration curve reconstructed from the tabulated mean peak areas.

The dissertation reports a linear concentration range of 10-60 μg/mL. Recalculation using the tabulated mean peak areas (including the blank) produced the regression equation y = 9527.7x -4644.9 with R^2 = 0.9926. This differs from the value of 0.999 stated elsewhere in the dissertation. Because linearity is a critical validation characteristic, the raw integration files, replicate-level data, weighting model, and any exclusion of the zero calibrator should be reviewed and the regression repeated before the method is described as fully validated.

3.3 Accuracy

Spiking level	Added standard (μg/mL)	Formulation level (μg/mL)	Mean recovery (%)	%RSD
80%	8	10	100.27	1.022
100%	10	10	99.18	0.097
120%	12	10	99.46	0.222

Mean recovery values were within 98-102%, and relative standard deviations were below 2%. These findings support acceptable trueness across the evaluated spiking levels, subject to confirmation of the underlying calculations and replicate chromatograms.

3.4 Precision

Repeatability at the working concentration produced a reported %RSD of 0.721%. Intra-day precision ranged from 0.19% to 1.03% RSD, while inter-day precision ranged from 0.15% to 0.46% RSD across the tested concentrations. All reported values were below 2%, indicating satisfactory repeatability and intermediate precision.

Nominal concentration (µg/mL)	Intra-day mean (µg/mL)	Intra-day %RSD	Inter-day mean (µg/mL)	Inter-day %RSD
10	10.03	1.03	10.41	0.46
30	30.49	0.51	30.94	0.28
100	99.14	0.19	99.19	0.15

3.5 Sensitivity, robustness, and system suitability

The reported LOD and LOQ for azelnidipine were 0.377 and 1.143 µg/mL, respectively. Flow-rate variation from 0.9 to 1.1 mL/min shifted the retention time from 2.395 to 2.085 min without reported loss of peak identity. The system suitability results were resolution 9.15, theoretical plate count 4246, injection %RSD 0.13%, and a reported tailing factor of 0.12. The unusually low tailing value should be checked against the chromatographic software output and calculation convention.

Validation characteristic	Reported result
LOD	0.377 µg/mL
LOQ	1.143 µg/mL
Repeatability	0.721% RSD
Resolution	9.15
Theoretical plates	4246
Injection precision	0.13% RSD
Retention time at 0.9 / 1.0 / 1.1 mL/min	2.395 / 2.260 / 2.085 min

3.6 Forced degradation

Stress condition	Assay remaining (%)	Degradation (%)	Mass balance (%)
Acidic, 1 N HCl, 12 h	95.2	5.5	100.7
Basic, 1 N NaOH, 12 h	96.2	4.4	100.7
Oxidative, 5% H_2O_2, 12 h	99.6	1.0	100.7
Reductive, 1% sodium borohydride, 12 h	92.1	8.6	100.7
Thermal, 80 °C, 12 h	99.3	1.3	100.7

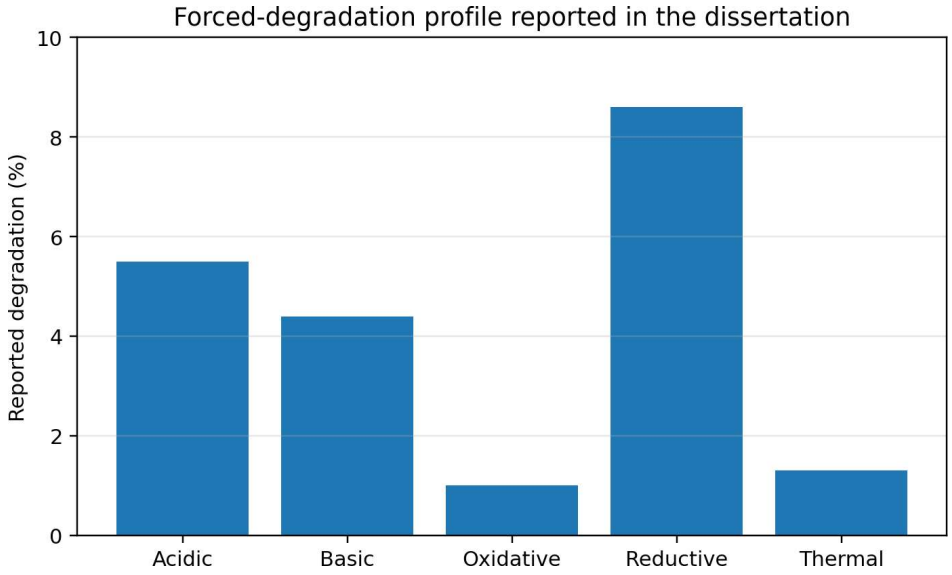

Figure 3. Reported degradation under the evaluated stress conditions.

The greatest reported degradation occurred under reductive stress, followed by acidic and alkaline hydrolysis. Oxidative and thermal degradation were limited. These data indicate differential susceptibility of azelnidipine to the tested stressors. Nevertheless, demonstration of a stability-indicating method normally also requires representative stress chromatograms, peak-purity evidence or an orthogonal selectivity assessment, and confirmation that the drug peak is free from co-eluting degradants. These items were not available in the provided dissertation extract and should be added before a strong stability-indicating claim is made.

3.7 Assay of marketed formulation

The formulation labelled to contain 3 mg azelnidipine yielded a mean measured amount of 2.94 ± 0.04 mg and an assay of 98.00 ± 0.41% (n = 6). The reported assay therefore fell within the commonly used 98-102% working acceptance range cited in the dissertation.

3.8 Study limitations and data reconciliation

The source dissertation contains internal inconsistencies that should be resolved before submission: (i) the title describes a single-analyte azelnidipine method, while several procedures and chromatograms refer to a second analyte; (ii) the marketed product name and stated 3 mg label claim require verification; (iii) the column is identified as Zodiac C18 in the optimization tables but as Aqua Sil C18 in the conclusion; (iv) azelnidipine retention time is reported as both 2.23 and 2.26 min; and (v) the calibration statistics conflict with the tabulated data. The present manuscript preserves the reported experimental values and explicitly identifies these points rather than inventing corrections.

4. Conclusion

A rapid isocratic RP-HPLC method was developed for azelnidipine using a C18 column, methanol-phosphate buffer at pH 6.7 (50:50, v/v), a flow rate of 1.0 mL/min, and UV detection at 279 nm. The method provided a short azelnidipine retention time, acceptable reported recovery and precision, low detection and quantification limits, robustness to minor flow-rate changes, and a formulation assay of 98.00%. Stress studies showed greatest degradation under reductive and acidic conditions. Before publication or regulatory application, the linearity calculation, formulation identity, column designation, chromatographic peak assignments, and stability-indicating evidence should be verified from original laboratory records. Following this reconciliation, the procedure may offer a practical and economical approach for routine quality-control analysis.

Declarations

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Conflict of interest: The authors should insert an appropriate declaration before submission.

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Ethics approval: Not applicable to the analytical laboratory study described.

Data availability: Original chromatograms, integration reports, calibration worksheets, and validation calculations should be retained by the authors and made available in accordance with the target journal policy.

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