

RP HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF ISRADIPINE IN TABLET DOSAGE FORM

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ABSTRACT

Purpose: To develop simple and cost effective Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method for the estimation of Isradipine in bulk and tablet dosage form. Methods: The estimation was carried out on Agilent Zorbax C8 (4.6x150 mm, 5 μ), using mobile phase consisting of methanol: acetonitrile: 0.1% OPA 55:35:10. The flow rate was 1.0 ml/min column effluents were monitored at 264 nm. The proposed method has been validated as per ICH Guidelines Results: The retention time was 4.108 minute and the proposed method was linear in the concentration range of 10 to 150 μ g/ml with coefficient of correlation 0.9998. The % recoveries at 50%, 100% and 150% were found to be 99.59, 99.49 and 99.64 respectively. Conclusion: All the validation parameters were within the acceptance range. The developed method can successfully be applied for the routine estimation of the amount of Isradipine in bulk and formulations. The proposed method can be used to determine the drug content of marketed tablets.

Keywords: Reverse phase liquid chromatography, Isradipine, Validation, ICH guidelines

INTRODUCTION

Isradipine is a dihydropyridine derivative with a high specific and a low nonspecific affinity to the dihydropyridine binding site of the L-type calcium channel¹. It inhibits the inward calcium flux through 'slow' channels of cardiac and vascular tissue, thereby eliciting potent coronary, cerebral and peripheral vasodilatation. In comparison with other calcium channel blockers the drug offers the advantages of minimal cardio depressant activity, a selective action on the coronary and skeletal muscle vasculature, and a prolonged vasodilatory action².

There are few other methods reported for estimation of Isradipine includes Spectrophotometric³, Spectrofluorometric⁴, Voltammetric⁵ and HPLC^{6,7}.

MATERIALS AND METHODS

The Waters HPLC system equipped with diode array detector and auto sampler was used. Hypersil BDS C₁₈ column (4.6 mm x 150 mm i.d, 5 μ) was used for separation. The chromatographic and the integrated data were recorded using Empower 2 software.

All the reagents were of analytical grade unless stated otherwise. Milli Q water, HPLC-grade acetonitrile, methanol (Rankem, Mumbai, India), Potassium dihydrogen ortho phosphate (AR grade, S.D. Fine Chem., Mumbai, India) and ortho phosphoric acid (OPA) (AR grade, S.D. Fine Chem., Mumbai, India) were used. All solutions were filtered through 0.45 μ m membrane filters procured from Pall Pharma lab Filtration Pvt. Ltd. (Mumbai, India).

Preparation of standard and linearity solutions of Isradipine:

100 mg of Isradipine was accurately weighed and transferred to a 100 ml volumetric flask. It was dissolved in 70 ml of mobile phase and made up to the mark with volume with mobile phase and sonicated for 5 minutes (Stock solution). From this, a working standard solution of 50 μ g/ml of strength was prepared. From stock solution by taking suitable aliquots 10 μ g/ml, 25 μ g/ml, 50 μ g/ml, 75 μ g/ml, 100 μ g/ml & 150 μ g/ml were prepared.

Preparation of Sample Solution

Twenty tablets were weighed and finely powdered. An accurately weighed portion of the powder equivalent to 100 mg of Isradipine was transferred to 100 ml volumetric flask containing 70 ml of mobile phase and the contents of the flask were sonicated for 10 minutes, to ensure the complete solubility of the drug the mixture was then made up to 100ml with mobile phase. The resulting solution was thoroughly mixed and filtered through a 0.45 μ m membrane filter. From this, a solution of 50 μ g/ml of strength was prepared.

Method Validation

System Suitability

Standard solution was injected six times into system and chromatograms were recorded. %RSD (% Relative Standard Deviation) of retention time & peak area, theoretical plates and tailing factor were calculated.

Specificity

Standard solution, sample solution, blank solution and placebo solution were injected simultaneously into the system and chromatograms were recorded.

Method Precision

Assay of six different samples was carried out and %RSD was calculated.

Ruggedness (Intermediate precision)

Precision study was repeated by another analyst on another system in same lab

Accuracy

Accuracy of method was measured in terms of % recovery. Sample solutions were prepared at three different concentration levels i.e. 50%, 100% and 150%. Predetermined amount of standard was added to these solutions. % recovery was calculated by assaying the solutions.

Linearity

10 μ g/ml, 25 μ g/ml, 50 μ g/ml, 75 μ g/ml, 100 μ g/ml & 150 μ g/ml concentration solutions injected simultaneously into the system and chromatograms were recorded. Graph was plotted concentration Vs peak area, R² was noted.

RESULTS

Optimized Chromatographic Conditions

The analysis done on Agilent Zorbax C₈ column (4.6 x 150 mm, 5 μ) using mobile phase methanol: acetonitrile: 0.1% OPA (55:35:10). The other conditions are flow rate at 1.0 ml/min, column temperature 30°C, detection wave length 326 nm and injection volume 20 μ l.

System Suitability

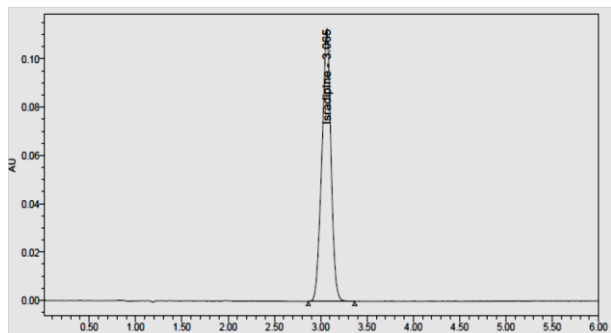
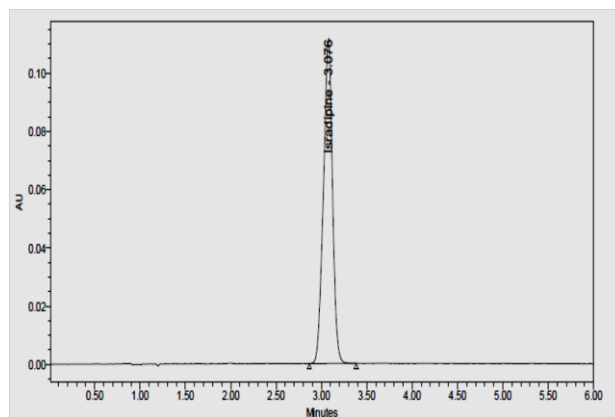
System suitability results were well under limit.

Table 1: Results of System suitability (n=6)

% RSD of Retention time	0.24
% RSD of Peak Area	0.27
Tailing Factor	0.95
Theoretical Plates	4265

Specificity

The chromatograms of sample and standard were identical to each other. The blank and placebo injections were identical, there was not any interference of excipients.

**Fig 1: Standard Chromatogram of Isradipine****Fig 1: Sample Chromatogram of Isradipine****Precision**

The % RSD values found to be 0.85 for Isradipine for six different samples.

Intermediate Precision

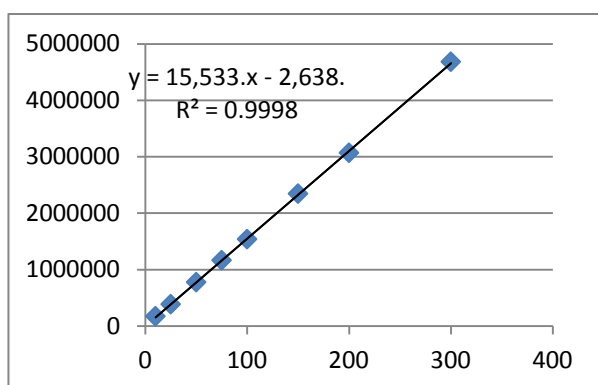
The % RSD Value was found to be

Table 2: Results of Precision and Intermediate Precision

Sample no	1	2	3	4	5	6	Mean	%RSD
Precision	99.561	99.942	99.853	100.098	99.579	100.336	99.895	0.300
Int.med. precision	99.75	99.34	99.14	99.93	99.64	100.14	99.656	0.371

Linearity

R² values found to be as 0.9998 and regression equation $y = 15533.x - 2638$.

**Accuracy**

The mean % recovery values were found to be in normal range. Results were shown in table

Table 3: Results of Accuracy (% Recovery studies)

% Concentration	Amount added ($\mu\text{g/ml}$)	Amount found ($\mu\text{g/ml}$)	% Recovery
50	5.00	4.975	99.50
100	10.00	9.949	99.49
150	15.00	14.946	99.64

DISCUSSION**Method Development**

The selection of column and mobile phase was done trial error method. The drug is insoluble in water, soluble in methanol and acetonitrile. Initially 0.1% ortho phosphoric acid (OPA) was tried with methanol isradipine eluted in more than 12 min and peak shape was not good. Acetonitrile was added to the mobile phase in different ratios. The mobile phase containing 55:35:10 methanol:

acetonitrile: 0.1% OPA elutes isradipine in less than 4 min with good peak symmetric properties. Kromasil C8 (4.6x150 mm, 5 μ), Zodiac C8 (4.6x150 mm, 5 μ) and Agilent Zorbax C8 (4.6x150 mm, 5 μ) columns were tried, elution with good peak symmetry was observed with Agilent Zorbax C8 (4.6x150 mm, 5 μ). 0.5 mL/min, 1.0 mL/min and 1.0 mL/min flow rate were tried, 1.0 mL/min found to be suitable. The column temperature kept at 30°C. Injection volume kept at 20 μL . On observation 3D spectra of sample in diode array detector response at 326 nm, it has shown good response, hence 326 nm selected as the detection wavelength.

Method Validation

% Relative standard deviation (%RSD) of retention times and peak areas were less than 1, average of tailing factor <2 and theoretical plates >4000 hence method passes system suitability tests.. The standard and sample chromatograms were identical to each other, there was no interference of excipients in analysis of drugs that proves method is specific. The average amount of drugs found in six samples were 99.89% When analysis was performed by second analyst on second system the results were well under limit, that proves method precision. The method was linear in the range of 10 - 150 $\mu\text{g/ml}$. The mean % recoveries at 50%, 100% and 150% concentration level were found to be 99.59, 99.49 and 99.64 respectively.

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