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Research Article

DEVELOPMENT AND VALIDATION OF ISRADIPINE IN BULK AND IN ITS PHARMACEUTICAL FORMULATION BY RP-HPLC METHOD

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ABSTRACT:

A simple, sensitive and selective RP-HPLC method for the estimation of Isradipine in pharmaceutical formulation was developed and validate in the present work. Chromatographic separation of drug is performed with kromasil c18 column having internal diameter 100mmX4.6 mm and 5µm diameter and the mobile phase consisting of a mixture of water , methanol and tetrahydrofuran(50:40:10, v/v/v), Isocratic elution at a flow rate of 1.4 ml/min with UV detection at 330nm at 25˚c is used in this method. The proposed RP-HPLC method is successfully applied for the determination of isradipine in pharmaceutical dosage form. The validation studies are carried out and it's fulfilling ICH requirements.The method is found to be specific, linear, precise (including both intra- and inter- day precision), accurate and robust. The proposed method was successfully applied for the quantitative determination of Isradipine in tablet formulation.

Key Words: Rp-Hplc Method; Isradipine; development and validation; Tablet dosage form.

INTRODUCTION

Isradipine is chemically 3-methyl 5-propan-2-yl 4-(2,1,3-benzoxadiazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate[1-4], and it belongs to the dihydropyridine (DHP) class of calcium channel blockers (CCBs), the most widely used class of CCBs. the chemical formula for isradipine is $C_{19}H_{21}N_3O_5$. It is soluble in water, the chemical structure for isradipine is following on figure-1, literature survey revealed that numerous methods have been reported for estimation of isradipine in pharmaceutical formulations has been reported. The main objective of the work was to develop[5-7] simple, fast, inexpensive, sensitive and accurate methods which could be applied to analyse Isradipine in pure form and in pharmaceutical dosage form.

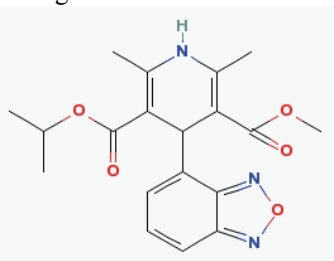

Fig.01 Chemical Structure of isradipine

MATERIALS AND METHODS

Pharmaceutical formulation

Tablets contained isradipine labelled to contain 10 mg of isradipine per tablet

Reagents and Materials:

Methanol HPLC grade, HPLC grade water was obtained by double distillation and purification through mille-Q water purification system.

Preparation of Standard solution:

Standard stock solution of Isradipine was prepared using methanol to get 1 mg/ml, further dilution was made by diluting 1 ml to 10 ml with mobile phase to obtain 100 µg/ml. For the construction of calibration graph, the aliquots of stock solution of Isradipine (1-6 ml of 100 µg/ml) was diluted with mobile phase to get 10-60µg/ml. The solutions were injected and chromatograms were recorded. Beer's law obeyed in the concentration range of 10-60 µg/ml.

Preparation of Sample solution:

For analysis of tablet formulation, the tablet powder equivalent to 100 mg of Isradipine was taken and made up to 100 ml with methanol the solution was sonicated for 10 min and filtered through Whatmann filter paper No.41. From clear solution, further dilutions were made by diluting 10 ml to 100 ml with mobile phase to obtain 100 µg/ml. 3.0 ml of test solution (30 µg/ml) was taken in six 10 ml volumetric flasks and made up to mark with mobile phase. The test solutions (10 µg/ml) were injected and chromatograms were recorded. For recovery studies, to the preanalysed formulation, solutions of raw material containing different concentrations were added and the amount of drug recovered was calculated. To each 3.0 ml of preanalysed formulation solution (30 µg/ml) added 1, 2, 3ml of raw material stock solution 100 µg/ml into 10 ml volumetric flasks and made up with mobile phase. The procedure was repeated as per the analysis of formulation. The amount of drug recovered was calculated by using slope and intercept values from the calibration graph.

Finally the method was validated as per ICH guide lines for precision, accuracy, specificity, linearity, reproducibility, LOD and LOQ.

Chromatographic Conditions

Prepare a mixture of 550 mL of water, 350 mL of methanol and 100 ml of tetra hydrofuran mix well and sonicate to degassed and the solvents were pumped from the solvent reservoir in the ratio of 50:40:10,v/v/v into the column. The flow rate of mobile phase was maintained at 1.4ml/min and detection wavelength was set at 330nm with a run time of 20min. The volume of injection loop was 25µl prior to injection of the drug solution the column was equilibrated for at least 25min with the mobile phase flowing through the system. The column and the HPLC system were kept in ambient temperature.

Calibration Curve

Appropriate aliquots of standard Isradipine stock solution were taken in different volumetric flasks and resultant

solution was diluted up to the mark with mobile phase to obtain final concentration of 10, 20, 30,40,50,60 and 70µg/ml of Isradipine. These solutions were injected into chromatographic system, chromatograms were obtained and peak area ratio was determined for each concentration of drug solution. Calibration curve of Isradipine was constructed by plotting peak area ratio versus applied concentration of Isradipine and regression equation was computed. Similarly the sample solution was chromatographed and concentration of Isradipine in tablet sample was found out using regression equation.

Method Validation

The method was validated for accuracy, precision, linearity, specificity, limit of detection, limit of quantitation and robustness by following procedures.

Accuracy

The accuracy of the method was determined by calculating recovery of Isradipine by the method of standard addition. Known amount of Isradipine was added to a pre quantified sample solution and the amounts of Isradipine as estimated by measuring the peak area ratios and by fitting these values to the straight line equation of calibration curve (Table 1). The recovery studies were carried out three times over the specified concentration range and amount of Isradipine was estimated by measuring the peak area ratios by fitting these values to the straight line equation of calibration curve. From the above determination, percentage recovery and standard deviation of percentage recovery were calculated.

Precision

The intra-day precision study of Isradipine was carried out by estimating the correspondence responses six times on the same day with 100µg/ml concentration and inter-day precision study of Isradipine was carried out by estimating the correspondence responses six times next day with 100µg/ml concentration.

Linearity and range

The linearity of the method was determined at six concentration levels ranging from 10-70µg/ml for Isradipine.

Limit of detection and limit of quantification

Limit of detection = 10ng

Limit of quantification = 30ng

Stability

In order to demonstrate the stability of both standard and sample solutions during analysis, both the solutions were analyzed over a period of 8 hours at room temperature

Robustness

Robustness of the method was studied by changing the composition of organic phase by ±4% and the PH by ± 0.1, and also by observing the stability of the drugs for 24 hours at ambient temperature in the mobile phase

RESULTS AND DISCUSSION

Selection of the detection wavelength

The UV spectra of Isradipine in 60:40v/v mixture of buffer and Acetonitrile was scanned in the region between 200 and 400nm and shows λmax at 330nm.

Optimization of the chromatographic conditions

Proper selection of the stationary phase depends upon the nature of the sample, molecular Weight and solubility. The drug Isradipine is polar. The Mixture of phosphate buffer and acetonitrile was selected as mobile phase and the effect of composition of mobile phase on the retention time of Isradipine was thoroughly investigated. A short run time and the stability of peak asymmetry were observed in the ratio of 60:40% v/v of phosphate buffer and acetonitrile. The retention time of Isradipine was found to be 9.97min, which indicates a good baseline (Figure-3). The number of theoretical plates was found to be 4300 (USP) which indicates efficient performance of the column. The calibration curve for Isradipine was obtained by plotting the peak area ratio versus the concentration of Isradipine over the range of 10-70µg/ml, and it was found to be linear with $r^{2} =$ 0.99997. The regression equation of isradipine concentration over its peak area ratio was found to be y =100.00%, where x is the concentration of isradipine and Y is the respective peak area. The data of regression analysis of the calibration curve was shown in table. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The limit of detection and limit of quantification for Isradipine was found to be 10ng and 30ng, indicates the sensitivity of the method. The system suitability and validation parameters were given in (Table.04).The high percentage of recovery of Isradipine was found to be 100.37% indicates that the proposed method is highly accurate. The absence of additional peaks indicates no interference of the recipients used in the tablets

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REFERENCES

1. The Merck Index –An encyclopedia of chemicals, drugs and biologicals, 11th edn., Merck and company, USA,1989, p 3757

2. Martindale – The complete drug reference, 33rd edn., pharmaceutical press, London, 2002 p 732

3. Chitra K, Sujatha K, Ahmed IR, Shalini K, Priya BL and Varghese SS, Indian J.Pharm.Scie., 2005; 67 (1):98-100

4. Kuchekar BS, Randive VS, Chaudhary AP and Bhise SB, Indian.J.pharm scie., 2003; 65:184-186 Shenoy KRP, The Eastern Pharmacist, 2000; 512:135- 137

6. Yogini J, Gokul T and Sanjay S, J.Liq.Chrom.&Rel.Tech.2007; 30(8): 1115-1124

7. ICH guidelines Q2A, Text on validation of analytical procedures, 1994.

8.Snyder LR Kirkland JJ and Glach JL, Practical HPLC Method development 2nd Edition Wiley Intersciences publication ,John Wiley&sonsInc1997-709.

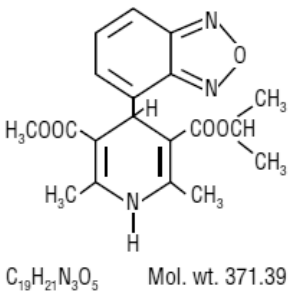

Fig.01.Chemical structure of Isradipine

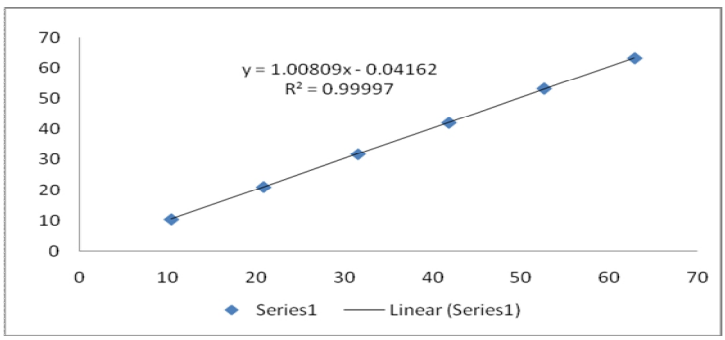

Fig.02Linearty graph of isradipine

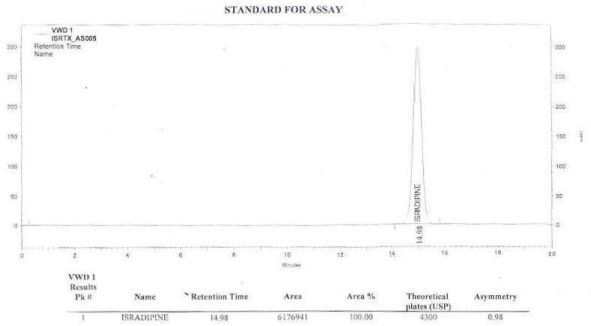

Fig.03.Typical chromatograph of Isradipien

Table .01.Linear data of Isradipine

Range	Concentration(µg/ml)	Response
10	24.9	617859
20	49.82	1235388
40	99.64	2510776
100	249.1	6176889
150	373.65	9265411
200	498.2	12353882

Table .02 Accuracy studies

Accuracy				
Amount added(mg)	Amount recovered(mg)	% Recovery	Mean %Recovery	%RSD
10.4	10.43	100.29	99.95	0.23
10.38	10.38	100		
10.31	10.3	99.9		
10.44	10.45	100.1		
10.4	10.38	99.81		
10.42	10.38	99.62		
20.79	21.03	101.15	101.01	0.25
20.77	21.01	101.16		
20.9	21.05	100.72		
31.63	31.52	99.65	100.45	0.72
31.25	31.45	100.64		
31.68	32.02	101.07		
41.58	41.99	100.99	100.6	1.11
42.41	42.13	99.34		
41.41	42.02	101.47		
52.79	53.52	101.38	101.11	0.23
52.57	53.07	100.95		
52.37	52.89	100.99		
62	62.59	100.95	100.56	0.44
62.57	63.12	100.88		
63.87	63.76	99.83		
62.95	63.23	100.44		
62.62	63.2	100.93		
63.04	63.25	100.33		

Table .03. Precision studies

Method precision	
Assay Results	
mg/unit	% of LC
9.86	98.6
9.78	97.8
9.80	98.0
9.83	98.3
9.76	97.6
9.94	99.4
9.83	98.3
0.7	0.7

Table.04. System Precision and System Suitability

System suitability parameters	Results	Acceptance criteria
% RSD for area count of five replicate injections of standard	0.01	NMT 5.0
Tailing factor	1.2	NMT 2.0
Theoretical plates	11685	NMT 3000
Check standard recovery (%)	100.37	95.0-105.0

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