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Isocratic-Reverse Phase Liquid Chromatographic method for the quantification of isradipine by UV detection in tablets

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ABSTRACT

A simple isocratic RP-HPLC method was developed and subsequently validated for the analysis of Isradipine in pharmaceutical dosage forms. Separation was achieved by using kromasil C_{18} (100 x 4.6mm, 5µm) column with flow rate of 1.7ml/minute and Analytes were monitored by UV detection at 326 nm, using a mixture of mobile phase containing 500 ml of water, 400ml of methanol and 100ml of tetra hydro furan mix well and sonicate to degas it. The retention time for Isradipine was found to be 8.95 minutes. Calibrate curves for Isradipine was linear over the concentration range 50-400 µg/ml with correlation coefficient 0.999. The percentage estimations of the Isradipine in market formulations by RP-HPLC were found in between 99.61-99.86%. The developed method was validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values. The recovery of the drug by standard addition method was found in range of 99.99-101% LOD and LOQ were 0.003µg/ml and 0.01µg/ml respectively. Thus the proposed method was found to be accurate, precise, reproducible and specific and can be successfully applied for simultaneous quantification of Isradipine in pharmaceutical dosage forms for future.

Key words: Isradipine, Isocratic, Reverse phase high performance liquid chromatography,

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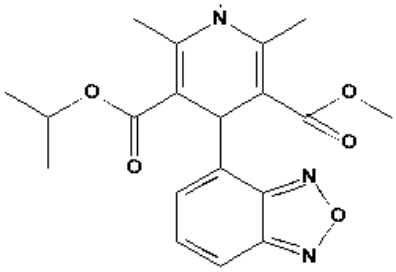
INTRODUCTION

Isradipine is 4-(4-benzofurazanyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinecarboxylic, methyl, 1-methylethyl ester which is a cardiovascular drug[14] belongs to the dihydropyridine (DHP) class of calcium channel blockers (CCBs), the most widely used class of CCBs. Isradipine binds to calcium channels with high affinity and specificity and inhibits calcium flux into cardiac and arterial smooth muscle cells. It exhibits greater selectivity towards arterial smooth muscle cells owing to alternative splicing of the alpha-1 subunit of the channel and increased prevalence of inactive channels in smooth muscle cells. Isradipine may be used to treat mild to moderate essential hypertension.

Fig: 1 Structure of Isradipine



Reviewing the literature revealed that all the reported methods for the determination of Isradipine in dosage forms and biological fluids rely on the use of chromatographic techniques such as Thin layer chromatography (TLC) [3,4], GC [5-9] and HPLC [4,17]. El-Jammal et al[10,11] studied the cyclic voltammetry of calcium antagonists dihydropyridines (including Isradipine) in aqueous medium. Although chromatographic methods offer a high degree of specificity, yet, sample cleanup and the instrumentation limitations preclude their use in routine clinical studies. The proposed method was developed as an alternative substitute to the chromatographic methods, and the results obtained were promising. The presence of the reducible furazan ring structure in the molecular formula of Isradipine initiated the present study. Compared with the reported chromatographic methods, which require lengthy extraction and clean-up procedures, the proposed method does not require a prior extraction step. Just dilution of the urine with the buffer solution eliminates its potential interference. The plasma, however, needs precipitation of the proteins; dilution of the centrifugate with the buffer solution eliminated its interference.

MATERIALS AND METHODS

The references sample of Isradipine was obtained from Axis clinical pvt.Ltd, Hyderabad, India.

CHEMICALS AND REAGENTS

Methanol and Tetra Hydro furan of HPLC grade were purchased from Merck (India) Ltd. Mumbai, India. Purified water was prepared by using 0.45 Millipore Milli-Q water purification systems.

Preparation of Mobile phase:

Prepare a mixture of 500 ml of water, 400ml of methanol and 100ml of tetra hydro furan mix well and sonicate to degas it.

Preparation of Isradipine Standard Stock solution

Weigh and transfer about 25mg of working standard into 100ml amber coloured volumetric flask and add 10ml of acetonitrile and sonicate the flasks with intermediate shaking for 2 mins. Take out the flask and make upto the volume with diluent.

Preparation of Isradipine sample solution

Transfer 5 tablets into a 200ml amber coloured volumetric flask and add 20ml of acetonitrile and sonicate the flasks with intermediate shaking for 15mins.take out the flask add 100ml of diluent and sonicate the flasks with intermediate shaking for 30 mins and sonication bath temperature to be maintained below 25ºc throughout the sonication. Make upto the volume with diluent. Filter a portion of sampler solution through 0.45µm nylon membrane filter.

FIG: 2 A TYPICAL CHROMATOGRAM OF ISRADIPINE BLANK

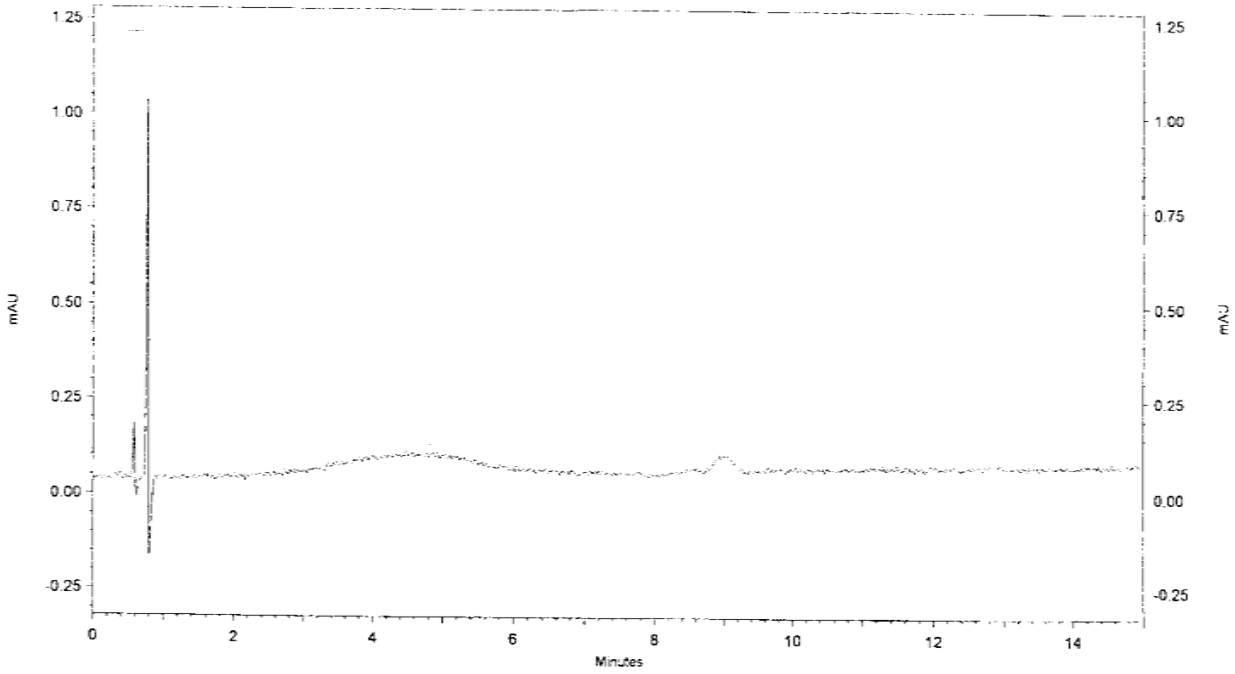


FIG: 3 TYPICAL CHROMATOGRAM OF ISRADIPINE PLACEBO

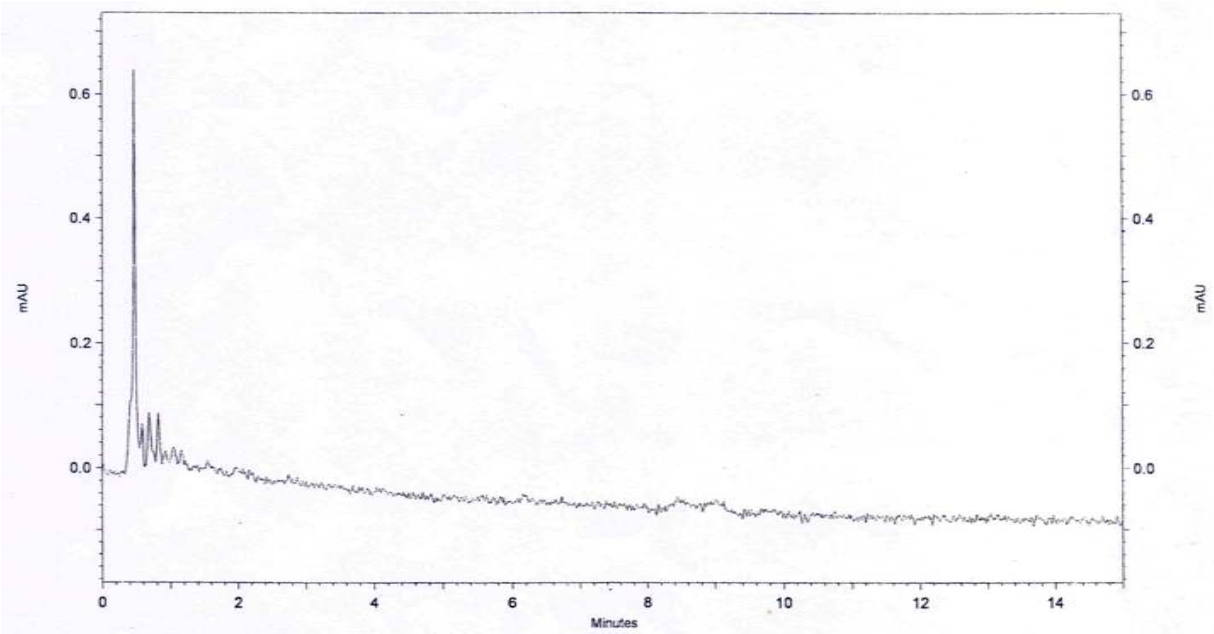


FIG: 4 A TYPICAL CHROMATOGRAM OF ISRADIPINE STANDARD

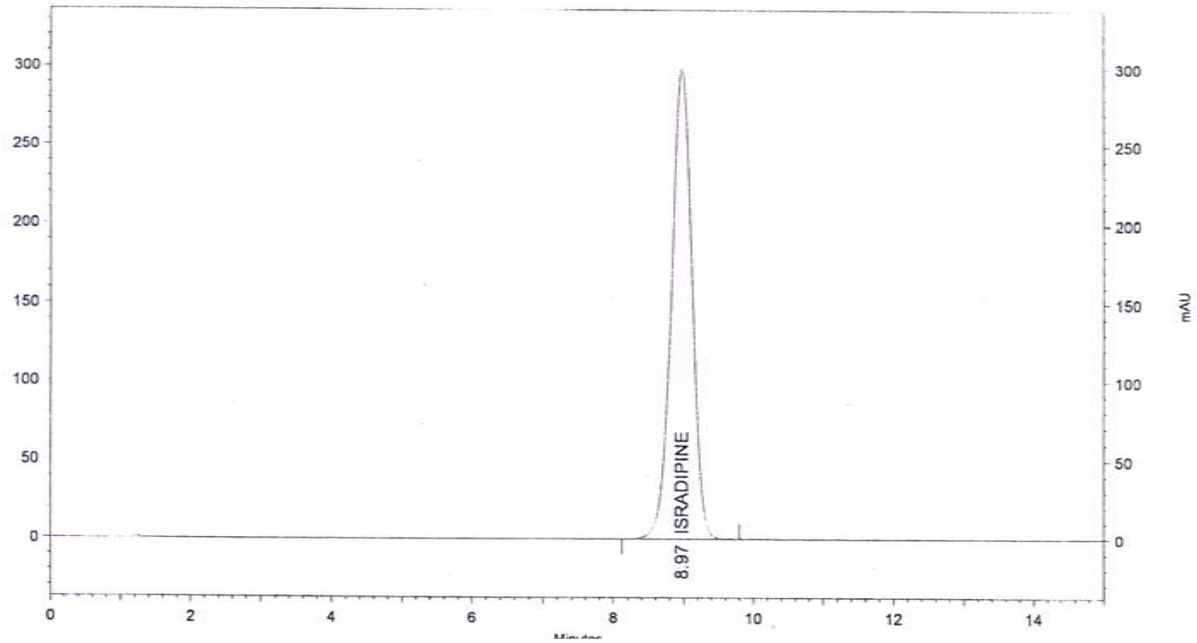
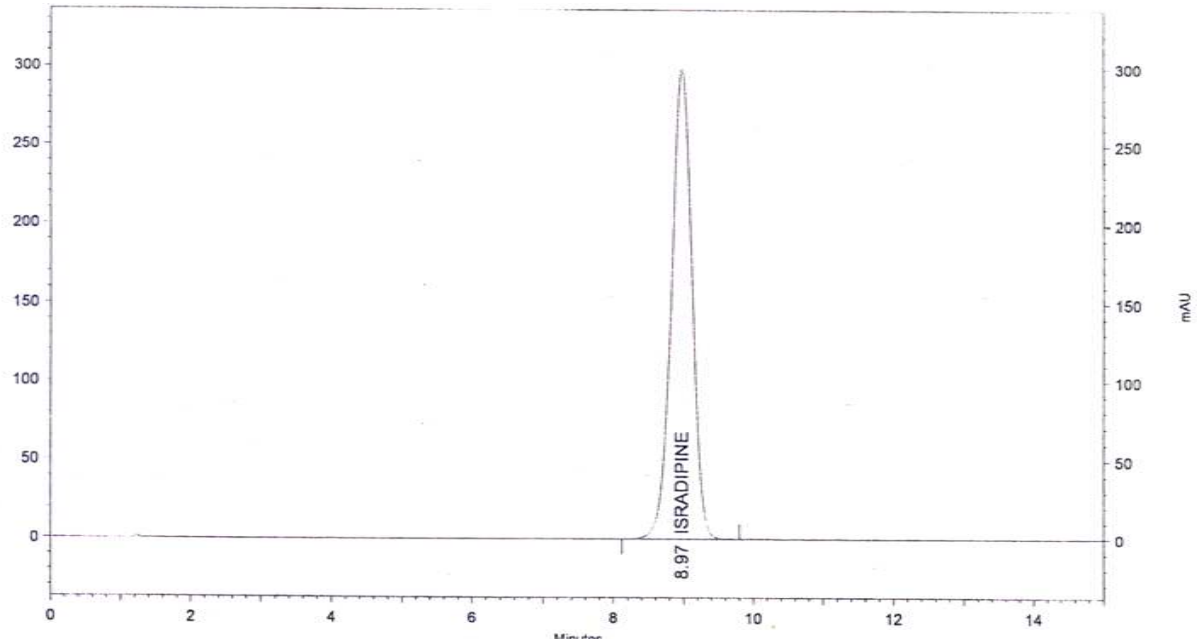


FIG:5 A TYPICAL CHROMATOGRAM OF ISRADIPINE SAMPLE



INSTRUMENTATION

2.5 Validation of the method

The developed method was validated in terms of specificity, system suitability, linearity, accuracy, limit of detection, limit of quantification, intra-day and inter-day precision and repeatability of measurement as well as repeatability of sample application.

RESULTS AND DISCUSSION

3.1 Method Development[12]

Various compositions of mobile phase with different ratios of Water, Methanol and tetra hydro furan were tried and best resolution was obtained with mobile phase consisting of water, Methanol and tetra hydro furan in the proportion of 50:40:10 % (v/v/v). A satisfactory separation and peak symmetry for Isradipine was obtained with above mentioned mobile phase Quantification was achieved with UV detection at 326 nm based on peak area. The results of the validation and system suitability results are given in Table 1.

Table: 1 Specificity and System Suitability

S.No	RT	Peak Area	Plate count	Tailing
1	8.95	5963790	4331	1.0
2	8.92	5964825	4365	1.2
3	8.97	5964589	4359	1.3
4	8.92	5963871	4290	1.2
5	8.95	5963521	4385	1.1
6	8.95	5963785	4321	1.2
7	8.96	5964897	4359	1.2
8	8.96	5963589	4289	1.0
9	8.97	5963791	4356	1.1
10	8.95	5964895	4325	1.3
MEAN	8.95	5964155	4338	1.16
SD	0.017638	571.6456	-	-
%RSD	0.197076	0.009585	-	-

3.2 Method Validation

The proposed method has been validated[13] for the determination of Isradipine in bulk dosage form using following parameters.

3.2.1 Specificity

The peak purity of Isradipine was assessed by comparing the retention time (RT) of standard Isradipine. Good correlation was also found between the retention time of standards and sample of Isradipine.

3.2.2 Precision

Precision study was performed to find out intra-day and inter-day variations in the estimation of Isradipine of different concentrations, with the proposed method. Percentage relative standard deviation of all the parameters is less than 2% which indicates that the proposed method is repeatable. Results are shown in Table 2

Table: 2 Summary of Intraday and Interday precision

Conc. (µg/ml)	Inter day			Intra day		
	Amt Found (µg/ml)*	±SD	%RSD	Amt Found (µg/ml)*	±SD	%RSD
125	124.993	0.01527	0.01222	124.986	0.00577	0.00462
250	250.006	0.01527	0.00611	249.986	0.00577	0.00231
500	499.986	0.00577	0.00115	499.976	0.00577	0.00115

**N=3*

3.2.3 Accuracy

This was carried out to check the recovery of the drugs at three different levels in the formulations i.e. multilevel recovery study. The pre analyzed samples were spiked with standard Isradipine and the mixtures were analyzed by proposed method. The experiment was repeated for five times (n=3). Results of recovery studies are shown in Table 3.

Table 3 Accuracy of Isradipine

Amount added (µg)	Amount found (µg)	% Recovery	Statistical Analysis of % recovery	Conc. % of Spiked level
125	124.99	99.992	Mean	99.994
125	125.01	100.008	SD	0.0122
125	124.98	99.984	%RSD	0.0122
250	250.02	100.008	Mean	99.998
250	249.99	99.996	SD	0.0083
250	249.98	99.992	%RSD	0.0083
375	375.01	100.002	Mean	99.998
375	374.99	99.997	SD	0.0040
375	374.98	99.994	%RSD	0.0040

3.2.4 Linearity

Linearity was studied by preparing standard solutions at different concentration levels. The linearity range for Isradipine was found to be 50-400 µg/ml. The regression equation for Isradipine was found to be y = 23855x + 1739 with coefficients of correlation (R^2) = 0.999.Results and Calibration curve were given the table 4 and Fig2 respectively

Fig: 4 Calibration curve for Isradipine

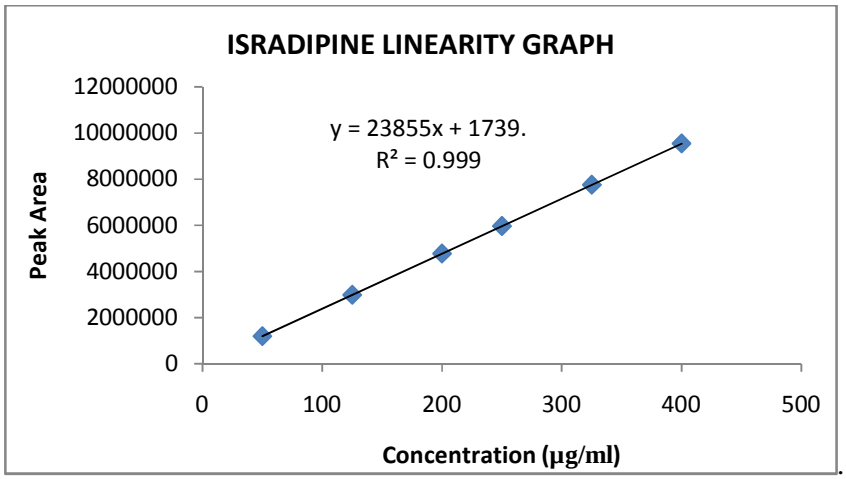


Table 4 Linearity of Isradipine

Linearity Conc.	Peak Area	Average Area	SD	%RSD
50	1192918	1192905	67.968	0.0809
	1192965			
	1192831			
125	2982413	2982262	169.920	0.0450
	2982295			
	2982078			
200	4771324	4771618	271.872	0.0271
	4771860			
	4771671			
250	5964155	5964523	339.841	0.0087
	5964589			
	5964825			
325	7754273	7753880	441.793	0.0042
	7753966			
	7753402			
400	9543343	9543195	490.087	0.0051
	9542649			
	9543595			

3.2.5 Limit of Detection and the Limit of Quantification

Calibrated curves were constructed in the lower concentration range 50-400 µg/ml for Isradipine standard deviation of y-intercept of regression line for both the drugs was determined and substituted in the following equation LOD and LOQ for the both drugs were determined by using

$$LOD=3.3 \times \sigma/s$$
$$LOQ=10 \times \sigma/s$$

Where σ is standard deviation of y- intercept of regression line and S is the slope of the calibration curve. The limit of detection and the limit of quantification of the drugs were calculated as in the text. The LOD and LOQ were found to be 0.003µg/ml and 0.01µg/ml, respectively for Isradipine.

3.2.6 Ruggedness

To demonstrate the stability of both standard and sample drug solution during analysis, both the solution were analyzed after a period of 8 hrs at room temperature for both solutions. The assay of Isradipine in standard and test solution was estimated against freshly prepared standard each time. The difference in % assay of standard and test solution from intial to 24 hrs was calculated and the result are given in the table 5, from the above study, it is established that the standard and sample solutions are stable for a period of 24 hrs at room temperature (25±2ºC).

Table 5 Solution stability

Time (hrs)	Assay	Difference
Initial (0)	101.01	0
12	100.06	0.95
18	99.99	0.07
24	99.98	0.01

3.2.7 Robustness

The robustness of the method was determined as per USP guidelines under a variety of conditions including changing the composition of organic phase ± 2%, detection wavelength by ± 2nm, change in flow rate by ± 10% or ±0.1ml, pH of buffer by ± 0.05. No marked changes were observed in the system suitability parameters and peak area. The results obtained by deliberately variation in method parameters and data are summarized below table.6

Table: 6 Robustness Studies of Isradipine

Parameter		%RSD of peak area	Theoretical Plates	Asymmetry
Flow rate ± 10% (1.7ml/min)	1.9 ml	0.0242	5962871	1.2
	1.5 ml	0.0341	5963521	1.1
Organic phase variation ± 2% H_2o: MeoH:THF (500:400 :100)	H_2o: MeoH:THF 460:420:120	0.0581	5963785	1.2
	H_2o: MeoH:THF 540:380:80	0.0141	5964897	1.3
Temperature variation ± 5ºc	30ºc	0.0025	5963589	1.2
	20 ºc	0.0158	5965750	1.2
Column variation	Agilent Zorbax Eclipse XDB C_{18}	0.0043	5963823	1.1
	X-terra RP -8	0.0086	5968658	1.2

3.2.8 The Assay

The assay value for the marketed formulation was found to be within the limits as listed in Table5. The low %RSD value indicated the suitability of the method for routine analysis of Isradipine in pharmaceutical dosage forms.

Table: 1 Assay of PRESCAL (Isradipine) tablets-2.5mg

Drug	Labeled Amount (µg/ml)	Amount taken(µg/ml) Mean(± S.D)	% Label Claim	%RSD
2.5 mg (Prescal)	2.5	2.4903±0.01050	99.612	0.4217
	5.0	4.993±0.01527	99.866	0.3059
	7.5	7.4866±0.0057	99.822	0.0771

CONCLUSION

The proposed method is simple, sensitive, precise, reproducible and accurate and hence can be used in routine for the simultaneous determination of Isradipine in bulk as well as in pharmaceutical preparations.

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