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Pharmacokinetic evaluation of newly developed isradipine sustained release formulation

Selvadurai Muralidharan[1]*, Khu bao ling [1], Dhanyasri [1], Geevetha Kaur [1], Kong wen kiat [1], Kumaraswamy Santhi [2], Subramani Parasuraman[3] and Jayarajkumar [2]

*Corresponding author:

Selvadurai Muralidharan

[1] Chemistry Unit, Faculty of Pharmacy, AIMST University, Malaysia.

[2] Technology Unit, Faculty of Pharmacy, AIMST University, Malaysia.

[3]Pharmacology Unit, Faculty of Pharmacy, AIMST University, Malaysia.

Abstract

A specific and efficient method using High Performance Liquid Chromatography (HPLC) has been developed to validate the pharmacokinetics of sustained-release formulation containing Isradipine. The objective of the present study is to develop and validate PK of sustained release formulation containing Isradipine. The plasma samples of Isradipine were extracted using the protein precipitation technique (PPT). The detection wavelength of Isradipine, which was 325nm, was determined using UV spectrophotometer. Reversed phase Thermos c_{18} column was used for separation. 10mM ammonium acetate buffer (pH 4) and acetonitrile at a ratio of 20:80% v/v was used as the mobile phase with the flow rate of 1.0 ml/min. The linearity achieved in this method was in the range of 10-120 ng/ml. HPLC method provides extremely precise results and is an excellent and efficient method compared to others. The development of a sustained release formulation offers advantages such as prolonged blood levels of the drug and improved patient compliance. The formulated sustained release tablets containing Isradipine is capable of exhibiting sustained release properties, stable and feasible for industrial scale production. Thus they are capable of reducing the dose intake, minimize the blood level oscillations, dose related adverse effects, cost and ultimately improve the patient compliance in the hypertension.

Keywords: Isradipine, Sustained release formulation, bioavailability studies.

Introduction

Isradipine is 3,5-Pyridinedicarboxylic acid, 4-(4-benzofurazanyl)-1,4-dihydro-2,6-dimethyl-,methyl 1-methylethyl ester which is a dihydropyridine calcium channel blocker. Its pharmacodynamic effects include dilating effects in arterioles which reduce systemic resistance and lower blood pressure, with a small increase in resting heart rate. [1, 2]

Only a limited number of analytical and bio-analytical methods have been reported for the quantification of Isradipine in various matrices. In this research, we have formulated sustained-release tablet of Isradipine. A drug delivery system (DDS) is defined as a formulation or a device that enables the introduction of a therapeutic substance in the body and improves its efficacy and safety by controlling the rate, time, and place of release of drugs in the body. [3]

Sustained release, sustained action, prolong action, controlled release, extended action, depot are terms used to identify drug delivery systems that are designed to achieve prolong therapeutic effect by continuously releasing medication over an extended period of time after administration of single dose. In the case of orally administer this period is measured in hours while in the case of injectables this period varies from days to months.[4] Sustain release with the introduction of extended release matrix tablet have proved to be an effective tool to control the release of drug without involving the complex production procedures. By the sustained release method therapeutically effective concentration can be achieved in the systemic circulation over an extended period of time, thus achieving better compliance of patients. Numerous sustain release oral dosage forms such as membrane controlled system, matrices with water soluble/insoluble polymers or waxes and osmotic systems have been developed, intense research has recently focused on the designation of SR systems for poorly water soluble drugs. However generating such a system requires certain consideration of which the half life and the pharmacological action of the drug form an essential part.[5] It is desirable that the duration of drug action becomes more a design property of a rate controlled dosage form and less or not at all a property of the drug molecule's inherent kinetic properties. Thus, optimal design of a sustained/ controlled release system necessitates a thorough understanding of the pharmacokinetics and pharmacodynamics of the drug. When the drug is administered in a conventional dosage form, it results in a fluctuation of drug concentration at the site of action (peak and valley pattern) and therefore in systemic circulation and tissue compartment.[6] The advantages of sustained-release formulation include reduced dose frequency and improved compliance. It also has a reduced side-effect profile especially those related to rapid rise in peak serum concentration and local irritation due to a slow

release or targeted nature of delivery, resulting in some cases in reduced local irritation and a steady rise in serum levels. [7, 8]
Bioavailability is one of the essential tools in pharmacokinetics, as bioavailability must be considered when calculating dosages for non-intravenous routes of administration.[9, 10] Bioavailability studies are designed to determine either an absolute bioavailability (relative to an IV formulation) or relative bioavailability (with an alternate reference dosage form with good absorption characteristics).[11] They can be used to compare different routes of administration, for example oral versus IV or IP versus IM. Bioequivalence studies are designed to compare drug products. The objective is to determine if these products are bioequivalent. The dosage forms should be similar, especially the route of administration.[12] The purpose of bioequivalence studies is to reduce toxicological studies and full-scale clinical trials to prove that the product is of good quality, safe and effective. Bioequivalence studies are typically performed after minor changes of a marketed product or by manufacturers of generic drugs. [13]
Chromatography is a technique by which a mixture sample is separated into components.[14] High-performance liquid chromatography (HPLC), which is used in this research, is also referred to as high-pressure liquid chromatography. It is a technique in analytic chemistry used to separate the components in a mixture, to identify each component, and to quantify each component.[15] HPLC relies on pumps to pass a pressurized liquid and a sample mixture through a column filled with a sorbent, leading to the separation of the sample components.[16] This gives HPLC superior resolving power when separating mixtures, which is why it is a popular chromatographic technique. [17] Reversed phase HPLC is the most commonly used form of HPLC. Reverse phase is the choice for the majority of samples, but if acidic or basic analytes are present then reverse phase ion suppression (for weak acids or bases) or reverse phase ion pairing (for strong acids or bases) should be used. [18-21]

Materials And Methods

Reagents and Chemicals Used

Isradipine, Methanol (Merck), Acetonitrile (QReC), Tricholoroacetic acid (Bendosen), Ammonium acetate (Bendosen), Ortho-phosphoric acid (QReC), Microcrystalline cellulose (Merck), Hydroxypropyl methylcellulose (R&M), Talc (Unilab Chemical), Magnesium stearate (R&M), Sodium citrate (Bendosen), Xylene (BDH Prolabo), Sodium chloride (Bendosen)

Development of In vitro Dissolution Methods

The release characteristics of test and reference formulations of Isradipine were determined using USP XXIII dissolution apparatus (type II, paddle) at 50 rpm. The dissolution medium used phosphate buffers pH 6.8 maintained at 37±0.5˚C. Dissolution tests were performed on six tablets. Five ml of the samples were withdrawn at 0.0, 1.0, 3.0, 5.0, 7.0, 7.5, 8.0, 8.5, 9, 10, 14.0, 18.0 and 24.0 h time intervals. Equal quantity of the dissolution medium was replaced to the dissolution jar after each sampling. Percentage drug release and cumulative release at various time intervals were calculated and compared.

Preparation of Standard and Sample Isradipine Solutions

Standard Stock Solution of Isradipine

10 mg of Isradipine working standard was accurately weighed and transferred into a 10 ml volumetric flask and dissolved in methanol - water mixture (1:1) and made up to the volume with the same solvent to produce a 1mg/ml of isradipine. The stock solution was stored in refrigerator at –20 ± 20 C until analysis.The stock solution was diluted to suitable concentrations for spiking plasma to obtain calibration curve (CC) standards and quality control (QC) samples.

Calibration Curve Standards and Quality Control Samples

Calibration standards for control plasma were prepared by spiking this stock solution to obtain the concentration levels of 10, 20, 40, 60, 80 and 120 ng/ml in human plasma. Quality control samples were prepared as bulk, at a concentration of 10 ng/ml (LQC), 60 ng/ml (MQC) and 120 ng/ml (HQC).These samples were stored below -50 C until use.

Plasma Samples

Calibration standards, validation QC samples and healthy volunteer plasma samples were prepared by adding 0.5 ml plasma to 2.0ml centrifuge tube and 0.5 ml of precipitating agent (10% w/v tricholoroacetic acid) was added and then vortex for 2 min. The resulting solution was centrifuged at 4000 rpm for 7 min. The supernatant layer was separated and estimated by HPLC.

Chromatographic Conditions

Reversed phase HPLC method was chosen for Isradipine. The standard solutions of Isradipine were scanned from 200–400 nm and the UV spectra obtained were recorded. From the UV spectra, the detection wavelength selected was 325 nm for Isradipine respectively. 10mM ammonium acetate buffer (pH 4) and acetonitrile at a ratio of 20:80% v/v was used as the mobile phase with the flow rate of 1.0 ml/min. Reversed phase Thermos c18 column was used as the stationary phase.

Validation of HPLC methods

Validation parameters tested were, selectivity/specificity, sensitivity, linearity, precision, accuracy, stabilities, recovery, ruggedness and robustness.

Selectivity/ Specificity

In order to prove that the method chosen was specific and selective the following two sets of samples (blank samples and

samples containing analyte and internal standard) were processed and injected into the HPLC using the extraction procedure.

Sensitivity

The lower limit of quantification for Isradipine 10 ng/ml.

Linearity

The calibration curve was plotted using response factor and concentration of the standard solutions. Linearity was established using four calibration curves over the range of (10 to 120 ng/ml for Isradipine) using the weighted least square regression analysis.

Precision and Accuracy

The precision and accuracy of the method was determined by analyzing two batches each consisting of one set of calibration curve with six replicates of quality control samples at four concentration levels [Quality Control samples at Low (QCL), Middle (QCM) and High(QCH)].

Stability Studies

Various stability studies were carried out. Room temperature stock solution stability, refrigerated stock solution stability, freeze thaw stability, bench top stability, short term stability and long term stability were determined. The short term stability of stock dilutions of analyte was evaluated at room temperature. Aqueous stock dilution of the analyte was prepared. One portion of the stock dilution was placed in the refrigerator between 2-8 C, while the other portion was placed at room temperature for 24 h. Stock dilution stored at room temperature (stability samples) was compared with refrigerated stock dilutions considered as 'comparison samples'. Six replicate injections of the above solutions were made. The long term stability of the stock solution when stored in the refrigerator for a given period of time was determined. Stock solutions of the analyte was prepared and stored in the refrigerator between 2 - 8 C for 7 days (stability stock). The stock solution stabilities of the analyte was determined with a comparison stock solution, which was prepared freshly. Five replicate injections of the above solutions were made. The response of comparison samples were corrected by multiplying with correction factor to nullify the difference between the measured weights or the dilutions made. The freeze thaw stability test was done to ensure that the analyte was stable in the biological matrix even after multiple freeze-thaw cycles. The bench top stability study was carried out using six quality control samples each at Quality Control sample at Low concentration (QCL) and Quality Control sample at High concentration (QCH) levels were stored at room temperature for 3 and 6 h. In long-term stability study, six samples of each quality control samples at low and high concentrations were stored below -50 C in the deep freezer. The stability of the analyte was evaluated by comparing each of the back calculated concentrations of stability Quality Control sample (QCs) with the mean concentrations of the respective QCs analyzed in the first accepted precision and accuracy batch . To evaluate the stability of the samples in the autosampler after processing for the anticipated run time, six sets of quality control samples each at low and high concentrations were placed in the auto sampler for 24 h and 48 h.

Recovery

To determine recovery of this method, six replicates of aqueous quality control samples (un extracted) with concentrations close to spiked Quality Control sample at Low concentration (QCL), Quality Control sample at Middle concentration (QCM) and Quality Control sample at High concentration (QCH) concentration (extracted) were prepared. These un-extracted samples were injected along with precision and accuracy batch.

Ruggedness

Ruggedness of the method was studied by changing the experimental conditions such as operators, instruments, source of reagents, solvents and column of similar type. Chromatographic parameters such as retention time, asymmetric factor, capacity factor and selectivity factor were evaluated.

Robustness

Robustness of the method was studied by injecting the standard solutions with slight variations in the optimized conditions namely, ± 1% in the ratio of acetonitrile in the mobile phase, ± 0.5 units in the pH of the buffer, ± 0.5 ml volume of the triethylamine in aqueous phase and ± 0.1 ml of the flow rate.

Method of analysis

The processed standards and samples were analysed using optimised chromatographic conditions mentioned earlier and the chromatograms were recorded. The quantification of the chromatogram was performed using peak area ratios (response factor) of the drug. The calibration curves were constructed routinely during the process of pre-study validation and in-study validation.

Animal Information

Four healthy, adult, either gender of New Zealand White Rabbits, weighing 2 – 2.5 kg was obtained from Central Animal house, Universiti Sains Malaysia, Penang, Malaysia. The animals were housed in large, spacious rabbit cages at an ambient room temperature with 12-h-light/12-h-dark cycle. The animals were fed with water and normal rabbit pellet fed, green leaves vegetables *ad libitum*. The animal experiment protocol was approved by the Institutional Animal Ethical Committee of AIMST (AUHAEC 9/FOP/2013) and the study was carried out according to Animal Research Review Panel (ARRP) guidelines.

In vivo data analysis

The pharmacokinetic parameters C_{max}, t_{max}, AUC_{0-t}, AUC_{0-} , $t_{1/2}$ and k_{el} were determined using WinNonlin Standard edition version 5.1

for individual drug treatments from the observed plasma concentration-time data.

Statistical Analysis

Statistical analysis was performed using a SPSS Version 13.0. The pharmacokinetic parameters like C_{max}, T_{max}, $t_{½}$, K_{el}, AUC_{0-t} and AUC_{0-} of both formulations are presented in Mean ± S.D. One way ANOVA (Analysis of Variance) was employed in the statistical analysis of the determined parameters in this study. Statistical significance was defined at $P<0.05$.

Results And Discussion

Evaluation of Compression

The sustained release tablets were prepared by direct compression technique. The powder of nine different formulations were evaluated for angle of repose, loose bulk density (LBD) and tapped bulk density (TBD) as shown in Table 1 of Isradipine.

Table 1 Formulation prepared by direct compression method (F_1-F_9) for Isradipine

F[a]	Isradipine	MCC	HPMC	Ethyl Cellulose	Talc	Magnesium Stearate	Total (mg/tab)
F_1	5	145	20	20	5	5	200
F_2	5	155	15	15	5	5	200
F_3	5	165	10	10	5	5	200
F_4	5	145	40	-	5	5	200
F_5	5	155	30	-	5	5	200
F_6	5	165	20	-	5	5	200
F_7	5	145	-	40	5	5	200
F_8	5	155	-	30	5	5	200
F_9	5	165	-	20	5	5	200

[a] Code of formulations

Tablet Manufacture

Development of Isradipine SR tablets

The physical properties of different batches of developed tablets are given in Table 2 & 3 (Figure 1) of Isradipine respectively. All the batches showed uniform thickness. The average percentage deviation of 20 tablets of each formula was less than ± 5% and hence all formulations passed the test for uniformity of weight as per official requirements (Pharmacopoeia of India 1996). Good uniformity content was found among three different batches of tablets. Another measure of tablets strength is friability. In the present study, the percentage friability for all the formulations was below 1%, indicating that the friability is within the prescribed limits. All the tablets formulations showed acceptable pharmaco technical properties and complied with the specifications for weight variation, drug content, hardness and friability.

Table 2 Comparison of the physical properties of the matrix tablets containing Isradipine

Formulation F[a]	Hardness (kg/cm^2)[b]	Thickness (mm)[b]	Friability (%)[b]
F_1	4.9000±0.58	3.419±0.14	0.320±0.08
F_2	4.700±0.91	3.421±0.45	0.355±0.07
F_3	4.900±0.79	3.586±0.04	0.294±0.05
F_4	4.800±0.81	3.677±0.04	0.333±0.07
F_5	4.900±0.52	3.791±0.07	0.307±0.09
F_6	5.000±0.39	3. 636±0.05	0.320±0.08
F_7	4.900±0.65	3.648±0.06	0.318±0.06
F_8	4.800±0.69	3.457±0.04	0.331±0.04
F_9	5.000±0.71	3.515±0.05	0.326±0.07

[a] Code of formulations

[b] Results represents the mean of replicate determination with the standard deviation given in parenthesis

Table 3 Granule properties of the different formulations of Isradipine

Formulation F[a]	Angle of repose (θ)	LBD[b] (g/ml)	TBD[c] (g/ml)
F_1	27.54	0.342	0.385
F_2	28.17	0.317	0.374
F_3	29.13	0.308	0.391
F_4	26.97	0.319	0.375
F_5	27.36	0.347	0.347
F_6	30.70	0.331	0.327
F_7	29.13	0.301	0.391
F_8	31.74	0.347	0.393
F_9	27.69	0.350	0.374

[a]Code of formulations.
[b]Loose Bulk Density.
[c]Tapped Bulk Density

Figure 1: Photograph of Isradipine sustained release tablets

In vitro Release

All the batches have shown that as the polymer concentration increases, the drug release rate decreases for Isradipine. (Figure 2). The dissolution of the marketed IR tablets indicated that more than 80% of the drug is released within 1h, which complies with the pharmacopoeial specifications. In all the batches, we observed that as the polymer concentration increases, the drug release rate decreases. The mechanism of drug release from these formulations is clearly revealed in Figure 3-6 for Isradipine. *In vivo* studies were carried out for the optimized formulation in six healthy rabbits and the pharmacokinetic studies were carried out for the optimized formulation and compared with the marketed formulation.

Kinetics and Mechanism of Drug Release

The *in vitro* drug release profiles were plotted according to zero – order, first- order, Higuchi and Peppas equations to understand the mechanism of drug release and to compare the differences in the release profile of optimized batches of Isradipine tablets (Figure 4-7) respectively.

Pharmacokinetic Data

The order of treatment administration was randomized in two sequences (AB and BA) in blocks of two. In each dosing session, volunteers received Reference Product A (Immediate release formulations) and Test B (Sustained release formulations). A wash out period of seven days was allowed between dose administrations. Blood samples (4 ml) were collected at 0 (before drug administration) 0.0, 1.0, 3.0, 5.0, 7.0, 7.5, 8.0, 8.5, 9.0, 10.0, 14.0, 18.0 and 24.0 h post dosing. The samples were centrifuged and plasma was separated. There were no serious adverse effects observed during the entire study.

Estimation of the Selected Drugs in Rabbit Plasma

Optimization of Chromatographic Conditions

The standard solutions were analyzed using the initial chromatographic conditions. To improve the resolution or symmetry of the peaks or to study the effect of the other chromatographic conditions, the chromatographic variables like pH of the mobile phase, the nature of stationary phase,
the composition of the mobile phase and flow rate were optimized (Figure 7).

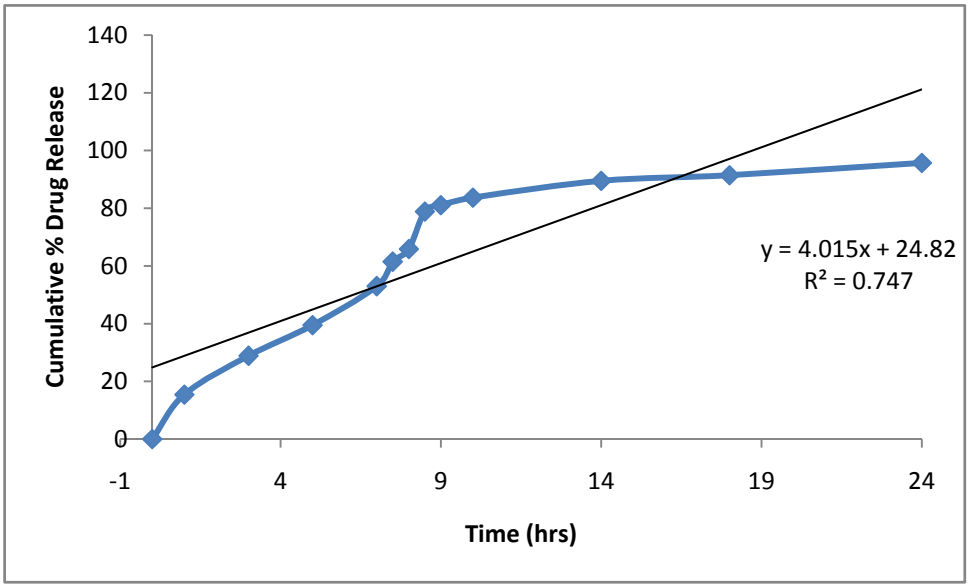

Figure 3: Zero order chart of optimized Isradipine formulation (F5)

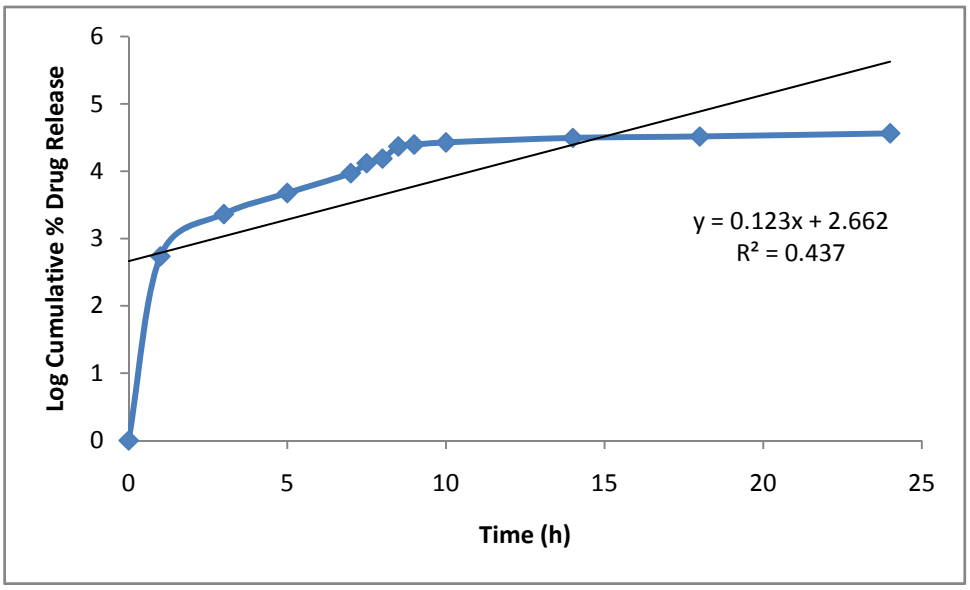

Figure 4: First order chart of optimized Isradipine formulation (F5)

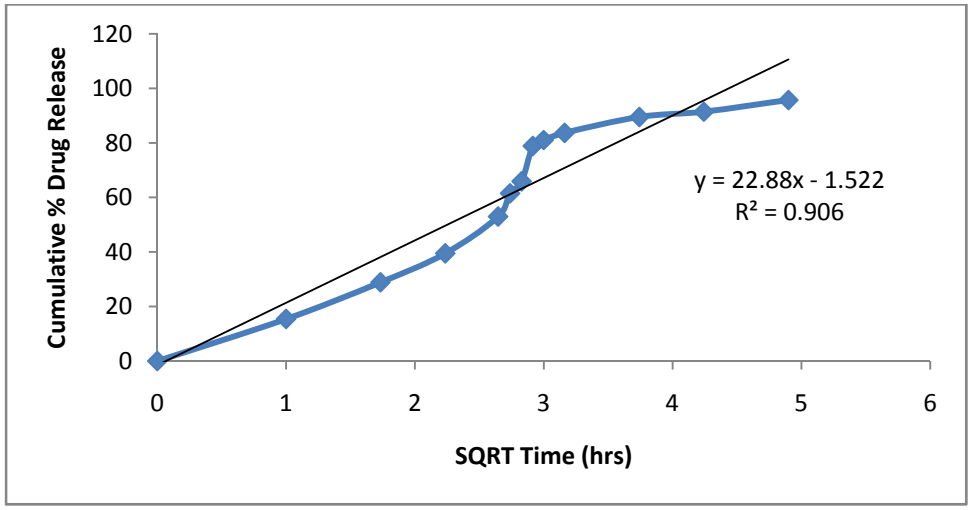

Figure 5: Higuchi chart of optimized Isradipine formulation (F5)

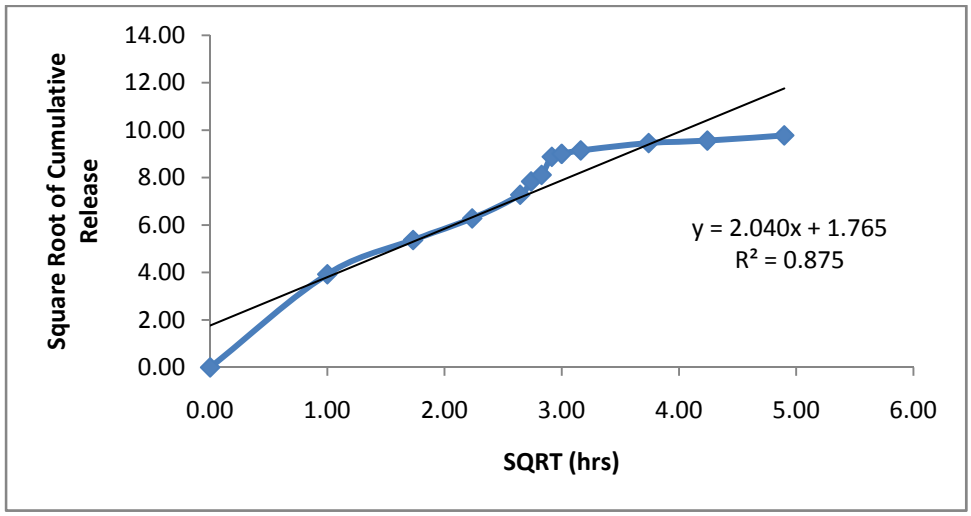

Figure 6: Peppas chart of optimized Isradipine (F_5)

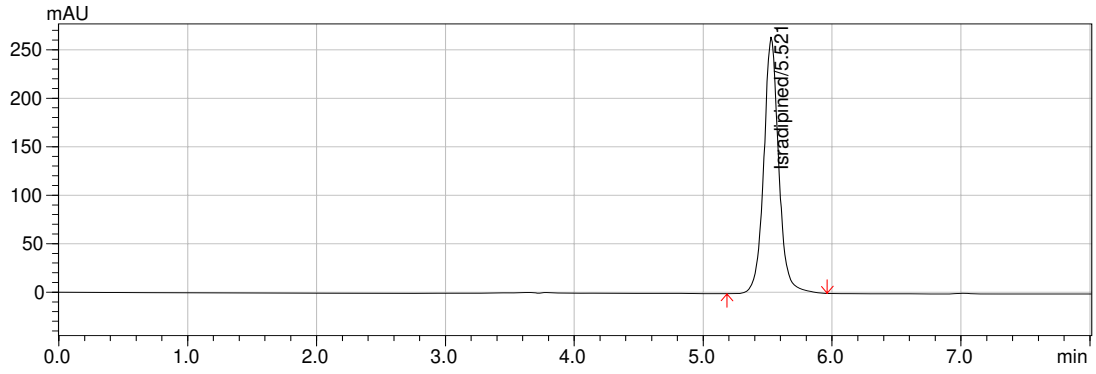

Figure 7: Typical chromatogram of standard solution of Isradipine

Specificity

HPLC-UV analysis of the blank human plasma samples showed the separations of Isradipine, no interference with either of these were observed. Hence the specificity of the method was established by comparison with human plasma (control). Representative chromatograms of extracted blank plasma indicating no interference in the blank plasma and in drug-free human plasma at the retention time of 5.5 for the drug Isradipine (Figure 8).

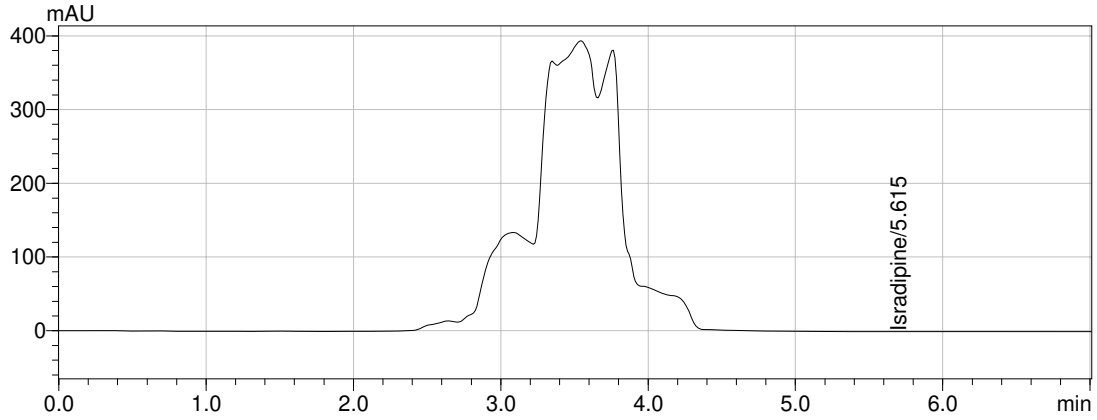

Figure 8: Typical chromatogram of blank rabbit plasma

Sensitivity

The Lower limit of quantification for Isradipine 10 ng/ml.

Linearity

The linearity range for Isradipine was found to be 10, 20, 40, 60, 80 and 120 ng/ml. The results are given in Table 4 and is shown in Figure 9 with correlation coefficient (r^2) was greater than 0.99.

Table 4 Concentrations-response linearity data for Isradipine

	Nominal Concentration (ng/ml)					
	10	**20**	**40**	**60**	**80**	**120**
1	9.652	19.234	39.0124	58.6321	78.3021	119.6348
2	9.524	19.537	39.8521	59.1430	79.1247	118.9346
3	8.996	18.9938	38.5247	59.0347	78.9624	119.5623
4	9.014	19.024	19.6350	58.6314	79.3428	119.6831
Mean	9.2965	19.1972	34.25605	58.8603	78.933	119.4537
S.D (±)	0.340707	0.250458	9.762771	0.267585	0.448547	0.349609
C.V (%)	3.664891	1.304661	28.49941	0.45461	0.568263	0.292674
% Nominal	92.965	95.986	85.64013	98.1005	98.66625	99.54475
N	4	4	4	4	4	4

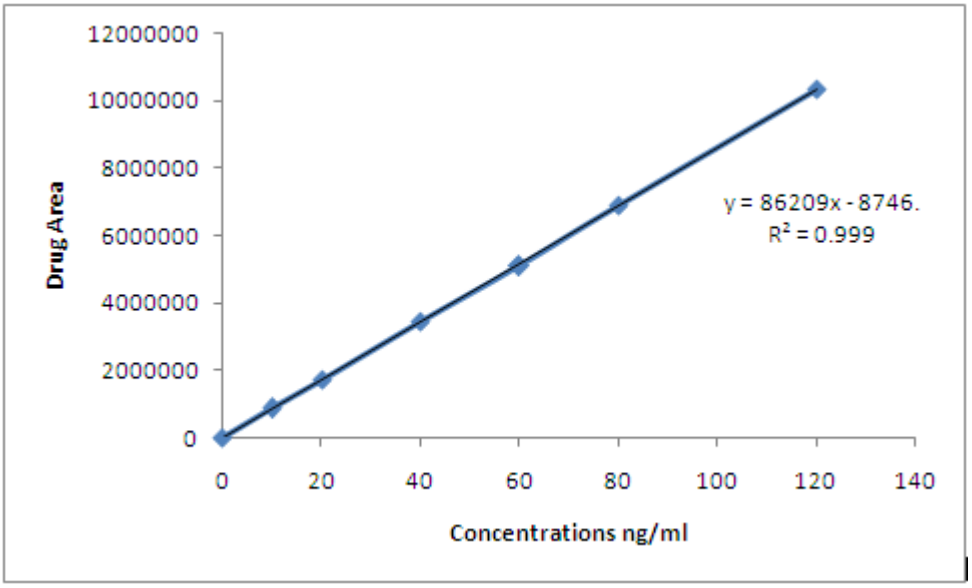

Figure 9: Calibration curve of Isradipine

Precision and Accuracy

The precision of the assay was measured by the percent coefficient of variation over the concentration range of LOQ, low, middle and high quality control sample of Isradipine during the course of validation. The accuracy of the assay was defined as the absolute value of the ratio of the calculated mean values of the LOQ, low, middle and high quality control samples to their respective nominal values, expressed as percent. The results are given in Table 5a – 5c.

Table 5a Within Batch Precision and Accuracy for Isradipine

	Nominal Concentration (µg/ml)		
	LQC	MQC	HQC
	10	40	120
1	8.9451	39.6248	119.3267
2	9.0534	39.5201	118.942
3	9.5368	38.9751	119.365
4	9.0147	39.0548	118.2341
5	8.9957	39.884	119.0318
Mean	9.10914	39.41176	118.9799
S.D (±)	0.242228	0.386726	0.455225
C.V (%)	2.65917	0.981244	0.382607
% Nominal	91.0914	98.5294	99.14993

Table 5b Between Batch / Inter day Precision and Accuracy for Isradipine

	Nominal Concentration (µg/ml)		
	LQC	MQC	HQC
	10	40	120
1	9.6524	39.5248	119.6384
2	8.9967	38.0358	119.3057
3	9.4217	39.2547	119.8637
4	8.9936	38.9932	119.8527
5	9.3542	39.7542	118.9075
Mean	9.28372	39.11254	119.5136
S.D (±)	0.285693	0.666241	0.407343
C.V (%)	3.07735	1.703394	0.340834
% Nominal	92.8372	97.78135	99.59467
N	5	5	5

Table 5c Intra Day Precision and Accuracy for Isradipine

	Nominal Concentration (µg/ml)		
	LQC	MQC	HQC
	10	40	120
1	9.6325	39.5241	119.6358
2	8.9421	38.5972	118.4207
3	9.5274	38.9524	118.4562
4	9.0657	39.0547	119.0358
5	8.9427	39.5204	118.6395
Mean	9.22208	39.12976	118.8376
S.D (±)	0.332627	0.396489	0.508655
C.V (%)	3.606849	1.013267	0.428026
% Nominal	92.2208	97.8244	99.03133
N	5	5	5

Stabilities

The stability studies of plasma samples spiked with selected drugs were subjected to three freeze-thaw cycles, short term stability at room temperature for 3 h and long term stability at – 70ºC over four weeks. In addition, stability of standard solutions was performed at room temperature for 6 h and freeze condition for four weeks. The mean concentrations of the stability samples were compared to the theoretical concentrations. The results indicate that selected drugs in plasma samples can be stored for a month without degradation in frozen state. The results of short term storage at room temperature stability and freeze-thaw cycles indicate no degradation of selected drugs in plasma as well as in sample solution and hence plasma samples could be handled without special precautions. The results are given in Table 6a -6f.

Table 6a Stock Stability of Isradipine

S.No	Drug area	Drug area	Drug area
	0 h	3 h	6 h
1	7563217	7421359	7512019
2	7452186	7521019	7563208
3	7632958	7796302	7653074
4	7523252	7541286	7520318
5	7693254	7563027	7426581
Mean	7572973	7568599	7535040
S.D	93838.39	138355.7	82515.94
C.V(%)	1.239122	1.828022	1.095096

Table 6b 20 C Stability of Isradipine in plasma

	Nominal Concentration (µg/ml)	
	LQC	HQC
	8.9651	119.6327
	9.0214	118.0215
	8.5329	118.5723
	9.3041	119.0367
	8.9935	118.5321
Mean	8.9634	118.7591
S.D	0.276442	0.606365
C.V(%)	3.084114	0.510584
% Nominal	89.634	98.96588
N	5	5

Table 6c Short Term Room Temperature Stability of Isradipine

	Concentration (µg/ml)	
	LQC	HQC
	9.6325	119.6327
	8.9752	119.032
	8.8821	118.9637
	9.0752	119.0328
	8.9964	119.8763
	9.11228	119.3075
Mean	0.298833	0.417985
S.D	3.279459	0.350343
C.V (%)	91.1228	99.42292
% Nominal	9.6325	119.6327
N	5	5

Table 6d Auto sampler Stability of Isradipine in plasma

	Nominal Concentration (µg/ml)	
	LQC	HQC
	8.9652	119.6387
	9.0214	119.8534
	8.7568	118.9365
	9.1024	118.9652
	9.6527	119.307
Mean	9.0997	119.3402
S.D	0.334521	0.405332
C.V (%)	3.676179	0.339645
% Nominal	90.997	99.45013
N	5	5

Table 6e Freeze/thaw cycle stability of Isradipine

	Concentration (µg/ml)	
	LQC	HQC
	9.8532	119.8634
	9.9754	119.0307
	8.9362	119.8639
	9.0125	118.5206
	8.9937	119.3476
	9.3542	119.3252
Mean	0.51389	0.573252
S.D (±)	5.493687	0.480411
C.V (%)	93.542	99.4377
% Nominal	9.8532	119.8634
N	5	5

Table 6f Long term stability for four weeks of Isradipine

Long Term Stability for four weeks		
	Concentration (µg/ml)	
	LQC	HQC
	9.5261	119.6325
	8.963	118.9327
	9.0247	118.2368
	9.2355	119.0325
	8.9952	119.8634
	9.1489	119.1396
Mean	0.236319	0.639563
S.D (±)	2.583037	0.536818
C.V (%)	91.489	99.28298
% Nominal	9.5261	119.6325
N	5	5

Recovery

The detailed results are presented in Table 7. The results indicate that the recovery of Isradipine was consistent at all levels (Figure 10).

Ruggedness and Robustness

No significant changes in the chromatographic parameters were observed when changing the experimental conditions (operators, instruments, source of reagents and column of similar type) and optimized conditions (pH, mobile phase ratio and flow rate).

Estimation of Selected Drugs in Plasma Samples

The calibration curve samples (CC samples), quality control samples (QC samples) and plasma sample solutions were injected with the optimized & validated chromatographic conditions and the chromatograms were recorded. The individual and mean concentration of the drugs present in the plasma samples were calculated and are presented in the Tables 8-9 (Figure 11).

In vivo Data Analysis

Pharmacokinetic parameters such as peak plasma concentration (C_{max}), time to peak concentration (t_{max}), area under the plasma concentration-time curve (AUC_{0-t} & AUC_{0-}), elimination rate constant (k_{el}) and elimination half-life ($t_{1/2}$) were calculated separately and the blood level data of selected formulations were compared. Mean plasma concentration-time profile of Isradipine was given in Table 10 and Figure 12.

Statistical Analysis

The pharmacokinetic parameters of different drug formulations of isradipine were compared statistically by one way ANOVA (analysis of variance) using SPSS version 16.0. P-value of <0.05 was considered as statistically significant. The results were expressed as the mean ± S.D. The pharmacokinetic parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of the immediate release and sustained release formulations of Isradipine was found to be significantly different by one way ANOVA.

Figure 10: Typical chromatogram of spiked rabbit plasma

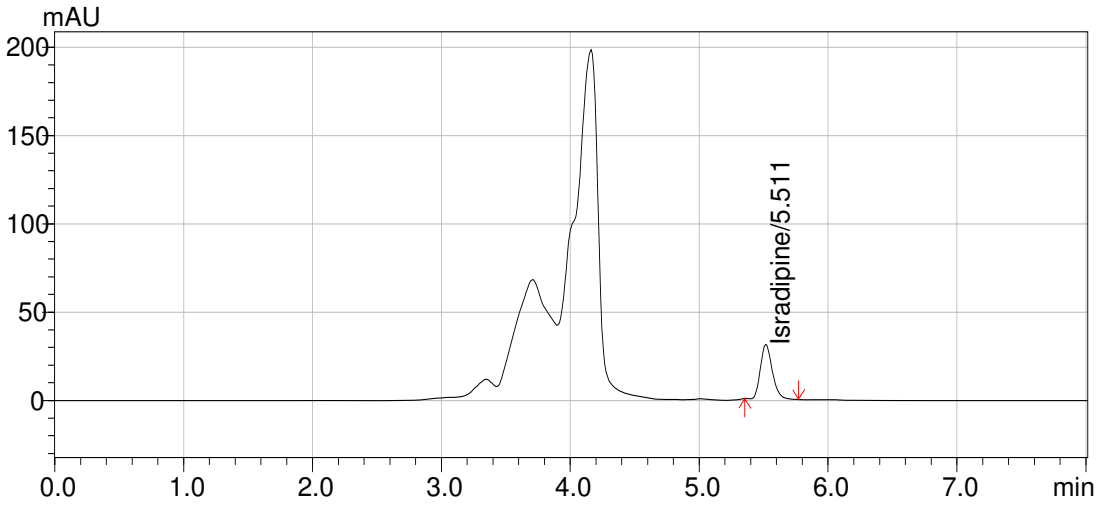

Figure 11: Typical chromatogram of Isradipine rabbit sample

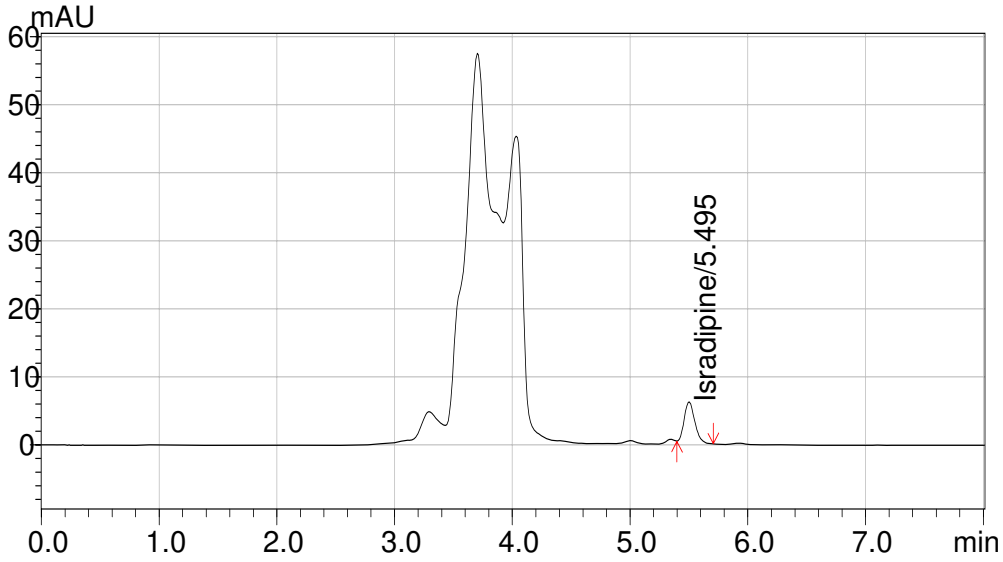

Figure 12: Mean plasma concentration-time profile of Isradipine from developed Sustained release tablets (test) and marketed immediate release tablet (Reference)

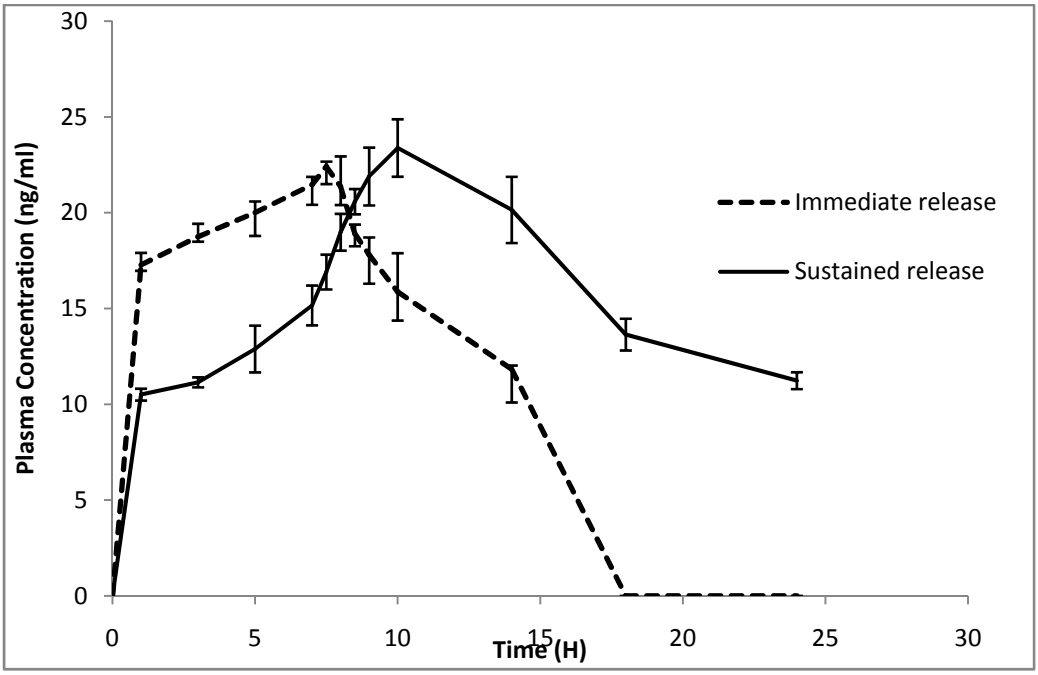

Table 7 Recovery study of Isradipine

	LQC Response		MQC Response		HQC Response	
	Extracted	Unextracted	Extracted	Unextracted	Extracted	Unextracted
1	95324	110527	145978	163254	175326	196375
2	93512	111726	143205	168935	180234	197524
3	95307	112850	139635	159938	179635	195325
4	94521	111763	148759	163290	172562	198635
5	95310	113968	147526	167851	180436	189973
Mean	94794	112166	145020	164653	177638	195566
S.D (±)	795.0432	1299.749	3656.172	3694.892	3523.746	3363.285
C.V (%)	0.838699	1.158764	2.52114	2.24404	1.98366	1.719766
N	5		5		5	
% Recovery	84.5		88.0		90.8	

Table 8 Individual plasma concentrations (µg/ml) and pharmacokinetic parameters for Isradipine immediate release product

Time	V_1	V_2	V_3	V_4	V_5	V_6
C_{max}	22.0547	23.1069	23.5628	22.7816	22.4012	22.1835
T_{max}	7.5	8	8	7.5	7.5	7.5
AUC_{0-t}	263.717075	258.6703	262.300275	260.087	263.9672	262.76255
K_{eli}	0.066070574	0.095531896	0.094115689	0.095420567	0.091893018	0.097332071
$T_{1/2}$	7.687102212	7.25566232	7.364842027	7.264127631	7.542979859	7.121467485
AUC_{0-}	528.9864507	384.244066	388.5969652	384.117912	393.4808488	376.8547219

Table 9 Individual plasma concentrations (µg/ml) and pharmacokinetic parameters for Isradipine sustained release product

Time	V_1	V_2	V_3	V_4	V_5	V_6
C_{max}	24.9598	22.4698	24.0319	24.1347	22.0317	24.1068
T_{max}	10	14	10	10	14	10
AUC_{0-t}	366.36415	369.492325	370.983875	366.885	368.043925	368.30225
K_{el}	0.056561371	0.060143556	0.058082162	0.058297412	0.061047289	0.059803453
$T_{1/2}$	12.25478046	11.52487851	11.93390806	11.88984478	11.3542664	11.5904208
AUCo-	561.9570054	568.7854948	561.056001	554.4559335	556.9581294	549.6161965

Table 10 Mean plasma concentrations (µg/ml) for Isradipine

Time (h)	Immediate release formulation		Sustained release formulation	
	Mean	S.D[a]	Mean	S.D[a]
0.0	0	0	0	0
1	17.27737	0.625	10.50568	0.31
3	18.74718	0.676	11.14482	0.26
5	20.00767	0.581	12.88667	1.22
7	21.45483	0.417	15.16033	1.04
7.5	22.40798	0.262	16.9046	0.91
8	21.36378	1.579	18.98007	0.96
8.5	18.90922	0.474	20.5741	0.66
9	17.80477	0.906	21.88982	1.51
10	15.87255	2.015	23.38023	1.50
14	11.82442	0.200	20.14647	1.73
18	0	0.000	13.63805	0.83
24	0	0.000	11.2333	0.44

Conclusion

A simple and sensitive method for the determination of Isradipine in plasma by HPLC was developed and validated. Adequate specificity, precision and accuracy of the proposed method were demonstrated over the concentration range of 10.0 – 120.0 ng/ml. The method was accurate, reproducible, specific and applicable to the evaluation of pharmacokinetic profiles of Isradipine and suitable for the pharmacokinetic study of Isradipine.

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