Research Article

Development and Validation of UV Spectrophotometric Method for Determination of Isradipine Loaded into Solid Lipid Nanoparticles

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ABSTRACT

This paper describes a simple, accurate, specific and validated method for determination of isradipine loaded into solid lipid nanoparticles. A study was carried out of all the parameters established as per ICH, to validate an analytical method for solid lipid nanoparticles i.e. linearity, range, accuracy, precision and sensitivity. A wavelength maximum of Isradipine (in Methanol: chloroform mixture) was selected at 327 nm. Method was found to be linear in the range of 5µg/mL to 30 µg/mL with a correlation coefficient (R^2) of 0.999. This sensitive method was capable to recover accurately and precisely from 80 % level to 120 % level of target concentration. Method was successfully validated as per ICH guideline. In addition, this proposed method was simple, sensitive, and easy to apply and requires relatively inexpensive instruments. The proposed method can be used for analysis of determination of isradipine loaded into solid lipid nanoparticles.

Keywords: Isradipine, UV Spectrophotometric method, entrapment efficiency, solid lipid nanoparticle.

INTRODUCTION

Isradipine is a calcium channel blocker of the dihydropyridine class. It is usually prescribed for the treatment of high blood pressure in order to reduce the risk of stroke and heart attack[1]. On a milligram-per-milligram basis, Isradipine is the most potent drug of this class. It was approved by the FDA in December 1990 for the treatment of hypertension[2]. The mechanism of action is similar to other calcium-channel antagonists, acts by inhibiting the influx of extracellular calcium across the cell membrane of myocardial and vascular smooth muscle. The serum calcium levels remain unchanged. It increases myocardial oxygen supply (secondary to coronary vasodilatation) and also decreases myocardial oxygen demand (secondary to decreased after load and lack of an increase in heart rate). These effects seem to best explain the benefit of it and other dihydropyridine in the treatment of angina. It also causes vasodilatation in coronary, skeletal, and cerebral vasculature. More recent research in animal models suggests that isradipine may have potential uses for treating Parkinson's disease [3]. The drug is commercially available in tablet dosage form (2.5 mg) with also controlled release 5 mg and 10 mg in capsule dosage form [3].

Isradipine-3-methyl5-propan-2-yl4-(2,1,3-benzoxadiazol-4-yl)-2,6-dimethyl-1,4dihydropyridine-3,5-dicarboxylateis a yellow colored crystalline powder and it is insoluble in water, but freely soluble in methanol& chloroform. Molecular weight of Isradipine is371.387 g/mol and formula is $C_{19}H_{21}N_3O_5$. Its structure is given in Figure 1[3, 4].

The main purpose of this study is to develop a simple, rapid, accurate, linear, sensitivity, robust and reproducible spectrophotometric method for the determination of isradipine loaded into solid lipid nanoparticle. The proposed UV spectrophotometric method could be widely used in routine analysis of quality control laboratories.

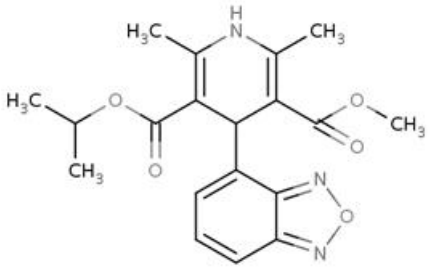

Figure 1: Structure of Isradipine

MATERIALS AND METHODS

Instrument, Chemicals, Reagents and Samples

Analysis of Isradipine was carried out on UV-Visible double beam Spectrophotometer (Shimadzu UV-1800).Isradipine reference standard was kindly gifted by Shasun Pharmaceuticals Limited, Chennai, India. Methanol (AR Grade), Chloroform ((AR Grade), was purchased from loba chemie pvt. Ltd, Mumbai, India, was used as a diluent. Dynasan 114 was gifted by Sasol, gmbh, Germany. Poloxamer 407 purchased from Sigma Aldrich. Cooling centrifuge (Remi, Mumbai, India) for centrifugation of solid dispersion to remove lipid and drug which are unentrapped. Double distilled water used in preparation of solid lipid nanoparticles.

Selection of wavelength

In order to ascertain the wavelength of maximum absorption (λmax) of the drug, solution of the drug (10µg/mL) in methanol: chloroform (7:3) was scanned using spectrophotometer within the wavelength range of 200 – 400 nm against methanol: Chloroform (7:3) as blank. The resulting spectrum is shown in figure 2 and the

absorption curve showed characteristic absorption maxima at 327 nm for Isradipine.

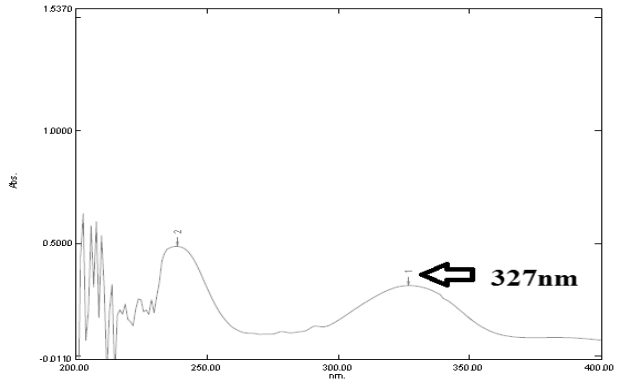

Figure 2: UV Spectrum of 10µg/mL Isradipine in Methanol: chloroform (7:3)

Standard Solution Preparation

10 mg Isradipine was accurately weighed and transferred to 100mL amber colored volumetric flask & diluted up to the mark with methanol: chloroform (7:3) to produce a stock solution of 100 µg/mL concentrations. 0.5mL of aliquot was diluted to 10mLwith methanol: chloroform (7:3) to give solution having 5 µg/mL concentrations. Similarly, solutions having concentration of 10, 15, 20, 25 and 30 µg/mL were prepared which were used for the construction of calibration curve (Tablet No 1).

Preparation of isradipine loaded solid lipid nanoparticles[5].

The SLNs were prepared High speed homogenization and ultrasoniaction method. Briefly, Dynasan 114 was melted to above its melting point and isradipine was dissolve in melted lipid. Poloxamer 407 was dissolved into double distilled water and heated to above melting point of lipid. Then transfer hot aqueous surfactant solution into drug lipid mixture and premix with high speed homogenizer (17,500 RPM for 10 min) and then sonication given for 2cycles. (1 cycle= 2min).Solid dispersion is centrifuge (8000 RPM for 10 min) to remove lipid and unentrapped drug.

Determination of Entrapment Efficiency (EE)[6].

The EE was determined by analyzing entrapped drug into solid lipid nanoparticles (SLN) in the supernatant obtained after centrifuging the SLN dispersion in high speed centrifuge at 8000 rpm for 10 min at 30°C using Remi cooling centrifuge (Mumbai, India).SLN containing supertant is dissolving into Methanol: chloroform (7:3) mixture.

$$\% \text{ Entrapment Efficiency:} = \frac{\text{Amount of entrapped drug into solid lipid nanoparticle}}{\text{Total Amount of drug}} * 100$$

Solid lipid Nanoparticle (Test) solution Preparation

SLN dispersion equivalent to 10 mg of Isradipine was weighed and transferred to 100 mL amber colored volumetric flask separately & make up to 100 mL of Methanol: chloroform mixture (7:3). 2.0mL of test solution was diluted up to 10 mL with Methanol: chloroform mixture (7:3) and the absorbance of test solution (20µg/mL) was recorded against Methanol: chloroform mixture (7:3) as a blank at 327 nm.

Method Validation[7, 8]

Validation can be defined as establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. The method was validated for several parameters like linearity, accuracy, precision, ruggedness, robustness, limit of detection (LOD), and limit of quantification (LOQ) according to ICH guidelines.

Linearity

For quantitative analysis of Isradipine, the linearity curve was plotted. Linearity range of Isradipine was established in concentration range of 5µg/mL to 30 µg/mL. The slope and intercept along with its correlation coefficient is given in Figure 3.

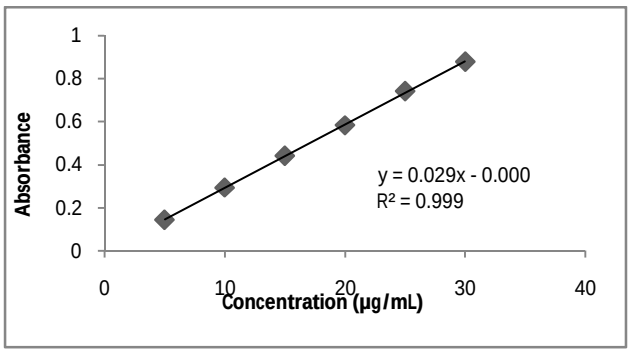

Figure 3: Linearity curve of Isradipine (5-30 µg/mL)

Specificity and selectivity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. The spectra obtained from SLN (test) solution and standard solution containing an equivalent concentration of Isradipine concludes that there was no any interference from excipients. Therefore it could be concluded that developed method is highly selective and specific. Interference of methanol: chloroform and excipients was not found on drug peak.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision of the method was determined in terms of repeatability and intraday and inter-day precision. The amounts of Isradipine was found by the number of replicates (n=3) performed by repeatability (intraday) and intermediate precision (inter-day) and reported as percent relative standard deviation (% RSD). For this 20 µg/mL concentration solutions were measured

three times in day and same was measured in next three days. The percent relative standard deviation was calculated (Table 2.1 and 2.2).

Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability of the method was determined by analyzing six samples of same concentration of drug. Chromatographs were recorded, and the area of each chromatograph was measured. The results are shown in Table 3.

Accuracy / Recovery

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. To ensure accuracy of the method, recovery studies were performed by standard addition method at 80%, 100%, and 120% level to preanalyzed samples and subsequent solutions were reanalyzed. At each level, three determinations were performed. The absorbance was measured at wavelength maxima and the amount of drug recovered from the formulation was calculated. Each level was repeated three times (n = 3). From the amount of drug found, percentage recovery was calculated (Table 4). Recovery study was carried out at three levels 80%, 100% and 120% for the formulation concentration of 20 µg/mL.

LOD & LOQ

LOD (k = 3.3) and LOQ (k = 10) of the method were established according to ICH definitions. In this study LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations-

$$LOD = 3.3\partial/s \qquad LOQ = 10\,\partial$$

Where ∂ is the standard deviation of the intercept and S is the slope of the calibrations curve. LOD and LOQ of method are 0.1115 and 0.3375 respectively.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. To determine the robustness of the method, the experimental conditions were deliberately altered and assay was evaluated. The effect of detection wavelength was studied at ±2 nm. For changes of conditions, the sample was assayed in triplicate. The results are shown in Table 5.

Stability

Solution was kept at refrigerate temperature for one day to evaluate the stability of Isradipine in standard solution and test solution. For standard solution, similarity factor was calculated from initial to one day and was found within the acceptance criteria of 0.98 to 1.02. A formula for similarity factor is mentioned below and found to be 0.98-0.99.

$$\text{Similarity factor} = \frac{\text{Absorbance of initial standard solution}}{\text{Absorbance of bench top stable standard solution}}$$

For test solution, % assay of Isradipine SLN solution were estimated against freshly prepared standard solution. The difference in % assay of SLN preparations from initial to one day was found to be within the limits(difference in % assay value from initial should be not more than 3.0%)(Table 6).

RESULTS AND DISCUSSION

The UV spectrum of isradipine in Methanol: chloroform has maximum absorption (λmax) at 327 nm. The absorbance of excipients in SLN solution did not interfere with isradipine. As a result, 327nm wavelength was selected for quantitative analysis and validation. The drug obeyed Beer–Lambert's law in the concentration range of 5–30 µg/mL with regression 0.9998 at 327nm. The overall % recovery was found to be 100.80% at 327nm, which reflect that the method is specific and selective. The developed method was found to be precise as the %RSD values for intraday and interday precision were found to be less than 2%. The developed method was found to be precise, specific, linear and accurate during method validation. Bench top stability (up to 1 day) of standard and test preparation were established by keeping the preparation at refrigerate temperature and the preparations were found to be stable. The results are summarized in table 7.

Table 1: Linearity of Isradipine (5-30 µg/ mL) (λ max.: 327 nm)

Conc. (µg/mL)	Mean Response ± S.D. (n = 6)	% R.S.D.
5	0.145±0.0008	0.5605
10	0.296±0.0007	0.2553
15	0.450±00760	1.7189
20	0.594±0.0089	1.5246
25	0.749±0.0077	1.0492
30	0.881±0.0087	0.9978
	Linearity Equation	y = 0.0294x - 0.0004
	Correlation Coefficient	0.9998
	Slope	0.0294
	Intercept	0.0004

Table 2: Precision

2.1. Intraday Precision:

Conc. (µg/mL)	Absorbance	Conc. (µg/mL)	Mean absorbance ± S.D.	Mean Conc. (µg/mL) ± S.D.	% R.S.D.
10	0.295	10.0381	0.295±0.0005	10.04±0.0196	0.195
	0.295	10.0381			
	0.296	10.0721			
20	0.589	20.0421	0.591±0.0020	20.11±0.0680	0.338
	0.591	20.0112			
	0.593	20.1783			
30	0.884	30.0803	0.886±0.0017	30.14±0.0589	0.195
	0.887	30.1823			
	0.887	30.1823			

2.2. Interday Precision

Conc. (µg/mL)	Absorbance	Conc. (µg/mL)	Mean absorbance ± S.D.	Mean Conc. (µg/mL) ± S.D.	% R.S.D.
10	0.294	10.0041	0.29433±0.0005	10.01±0.0196	0.1961
	0.294	10.0040			
	0.296	10.0301			
20	0.586	19.9401	0.585±0.0045	19.90±0.155	0.7833
	0.580	19.7385			
	0.589	20.0421			
30	0.873	29.7062	0.874±0.0095	29.74±0.3246	1.9104
	0.865	29.4300			
	0.884	30.0801			

Table 3: Repeatability

No.	Conc. (µg/mL)	Absorbance	Conc. (µg/mL)
1	20	0.584	19.87
2		0.586	19.94
3		0.580	19.73
4		0.589	20.04
5		0.591	20.11
6		0.593	20.17
Mean		0.5871	19.9798
S.D.		0.0047	0.1630
% R.S.D.		0.8161	0.8161

Table 4: Accuracy (Recovery Study)

Level of Recovery	Sample Conc. (µg/mL)	Std. Added (µg/mL)	Total amount (µg/mL)	Absorbance	Amount Recovered (µg/mL)	% Recovery	Mean % Recovery
80	10	8	18	0.525	18.86	99.24	100.38
	10	8	18	0.532	18.10	100.57	
	10	8	18	0.536	18.23	101.32	
100	10	10	20	0.593	20.17	100.89	100.89
	10	10	20	0.595	20.24	101.23	
	10	10	20	0.590	20.07	100.38	
120	10	12	22	0.356	12.11	100.94	100.51
	10	12	22	0.362	12.31	100.64	
	10	12	22	0.360	12.12	100.94	

Table 5: Robustness

Conc. (µg/mL)	Absorbance		Conc. µg/mL		% Assay	
	325 nm	329 nm	325 nm	329 nm	325 nm	329 nm
20	0.591	0.592	20.11	20.14	100.55	100.60
	0.589	0.590	20.04	20.07	100.31	100.38
	0.590	0.591	20.07	20.04	100.38	100.31
Average	0.590	0.591	20.07	20.11	100.38	100.55
S.D.	0.00080	0.00081	0.0277	0.0278	0.138	0.139
% R.S.D.	0.1383	0.1381	0.1383	0.1381	0.1381	0.1383

Table 6: Test (SLN) Solution Stability

Conc. (µg/mL)	Initial Absorbance	Initial Conc. (µg/mL)	% Assay	Abs. after 24 hr.	Conc. After 24 hr. (µg/mL)	% Assay after 24 hr.	Difference in % Assay
20	0.584	19.93	99.54	0.590	20.11	100.56	1.02
	0.590	20.11	100.56	0.598	20.38	101.09	1.36
	0.591	19.82	99.03	0.599	20.46	102.04	3.06

Table 7: Summary of Validation Parameters

Parameter	Isradipine loaded Solid lipid nanoparticle
λ max	327 nm
Linearity	5- 30 µg/mL
Equation	y = 0.0294x - 0.0004
R^2	0.9998
LOD	0.1115 µg/mL
LOQ	0.3378 µg/mL
Repeatability (%RSD, n = 6)	0.8161
Intraday Precision (%RSD, n = 3)	0.2431
Interday Precision (%RSD, n = 3)	0.6903
% Recovery	100.08
Standard solution stability (Similarity Factor)	0.99
Test (SLN) solution stability (Assay value difference from initial)	1.80

CONCLUSION

The developed UV spectrophotometric method for the determination of isradipine loaded into Solid lipid nanoparticle has the advantage of being fast and applicable over a wide concentration range with high precision and accuracy. The method was validated as per the ICH guidelines. The results of the validation were found to be satisfactory and therefore this method can be applied successfully to analyze drug formulations (SLN).

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