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Determination of cilnidipine from pharmaceutical formulation by high performance thin layer chromatographic method

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ABSTRACT

A suitable method was developed and validated for the determination of cilnidipine by HPTLC from pharmaceutical formulation using Nifedipine as an internal standard. The analyte and internal standard were resolved on silica gel $60F_{254}$ HPTLC plates (Merck) by using a two component mobile phase system comprising toluene-ethyl acetate, 6.0:4.0 (v/v) with a chamber saturation of 8mins. The plate was developed up to 8cm and air-dried. The plate then was scanned and quantified at 238nm. The linear dynamic range of cilnidipine was found to be 5µg mL^{-1} to 45µg mL^{-1}. The limit of detection and limit of quantification for cilnidipine was found to be 3µg mL^{-1} and 5µg mL^{-1} respectively. The proposed method is accurate, precise and rapid for the determination of cilnidipine.

KEYWORDS

HPTLC;
Cilnidipine;
Nifedipine.

INTRODUCTION

Cilnidipine[1] is a dihydropyridine (DHP) type of calcium channel antagonist. Unlike other calcium channel antagonists, cilnidipine blocks the influx of Ca^{2+} ions into both vascular smooth muscle at the level of L-type Ca^{2+} channels and neuronal cells at the level of N-type Ca^{2+} channels. The L-type Ca^{2+} channel blockade by cilnidipine affects predominantly vascular smooth muscle, thereby producing vasodilation of peripheral resistance vessels and coronary arteries. The blockade of N-type Ca^{2+} channels affects predominantly peripheral nerve endings of sympathetic neurons, thereby dilating blood vessels by lowering plasma catecholamine levels. Although few LC/MS/MS[2,3] and HPLC[4-6] methods are available, no HPTLC method has been published for the determination of Cilnidipine. Cilnidipine 20 mg is available in the market as tablet. The present paper describes a simple, precise and accurate HPTLC method for the determination of Cilnidipine from pharmaceutical formulation. The structure of Cilnidipine is shown in figure 1.

EXPERIMENTAL

Chemicals and materials

The formulation was obtained from market. Work

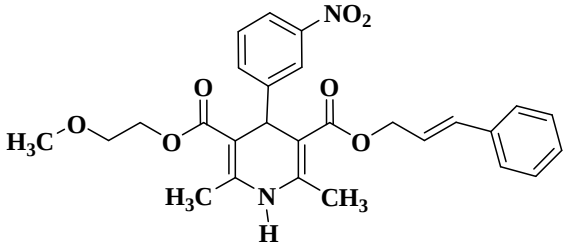

Figure 1 : Cilnidipine

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ing Standards of Cilnidipine and Nifedipine were obtained from Unique Pharmaceutical Labs (A division of J.B. Chemicals and Pharmaceuticals ltd) Thane (Maharashtra-India). Toluene and ethyl acetate used were of HPLC grade. All dilutions were performed in standard amber coloured volumetric flasks and protected from light.

Instruments

A Camag, Linomat IV sample applicator was used. Camag twin trough glass chamber (20×10cm) was used for development of plates. And Camag TLC scanner II equipped with cats 3 Version software was used for interpretation of data.

Development mobile phase and wavelength optimization

The selection of the mobile phase is of prime importance in the development of a chromatographic technique for proper elution, resolution, spot definition, symmetrical peak shapes and retention or retardation factor (R_f) reproducibility of the analytes.

In the present research work, initially a two component mobile phase system consisting of toluene (polarity=2.4) and dichloromethane (polarity=3.1) in the volume ratio of 1:1 was used and it was observed that although the spot of Cilnidipine migrated to obtain a R_f of 0.3, the spot of Nifedipine migrated marginally from the origin. Hence the polarity of the phase was increased by replacing dichloromethane with a more polar solvent- ethyl acetate (polarity = 4.4) for better migration of the two spots. With this mobile phase both the spots migrated to a good distance. The variation in the retention factors of Cilnidipine and Nifedipine with the variation in the composition of mobile phase was studied and best resolution between Cilnidipine (R_f = 0.6) and Nifedipine (R_f = 0.48) was found using 6:4 composition. A typical HPTLC chromatogram is given in figure 2.

The spots on the TLC plate were visualized in a UV chamber equipped with a UV lamp. The developed TLC plate was scanned between 200 and 400 nm using Camag TLC Scanner III .The λ_{max} for Cilnidipine and Nifedipine was found to be 240 nm and 251 nm respectively. But 238 nm which is the 'isobectic point' was chosen for further quantification. The UV scan is shown in figure 3.

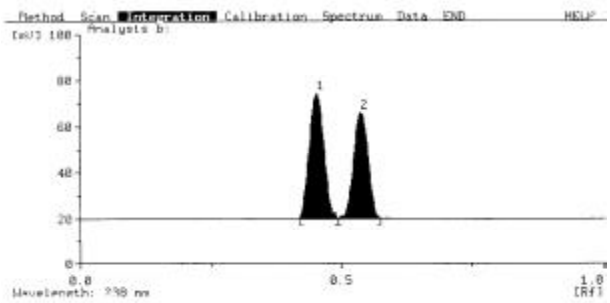

Figure 2: Typical HPTLC chromatogram. 1=Nifedipine. 2=Cilnidipine

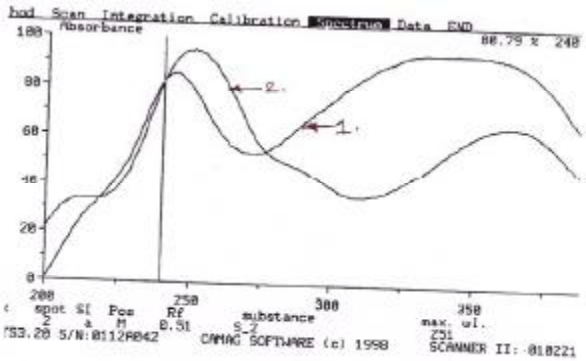

Figure 3: UV scan of (1) Cilnidipine and (2) Nifedipine

Chromatographic conditions

Application

10µL of standard were applied as bands of 8mm width using Camag Linomat IV- Applicator, and developed in Camag twin trough chamber.

Mobile phase

Toluene: Ethyl Acetate, 6.0 :4.0 (v/v)

Saturation time

The chamber was saturated with mobile phase for 8mins.

Migration

Distance 8cm

Wavelength of detection

238nm using Camag TLC Scanner II with cats3 software

Preparation of stock solutions

Cilnidipine stock solution

Accurately weighed 20mg of Cilnidipine standard was taken in 20mL volumetric flask. This was dissolved in minimum quantity of methanol and made up to volume to get a concentration of 1000µg mL^{-1}.

Nifedipine stock solution (Internal standard solution)

Accurately weighed 20mg of Nifedipine standard was taken in 20mL volumetric flask. This was dissolved in minimum quantity of methanol and made up to volume to get a concentration of 1000μg mL^{-1}. This solution was further quantitatively diluted with methanol to obtain a 60μg mL^{-1} solution.

Standard solution

1 mL of Cilnidipine stock solution was pipetted into a 10 mL volumetric flask and diluted to volume with internal standard solution to obtain a solution having a known concentration of 20μg mL^{-1} of Cilnidipine and 60μg mL^{-1} of Nifedipine.

Linearity

Into a series of 10mL volumetric flasks, varying concentrations from 16μg mL^{-1} to 24μg mL^{-1} of Cilnidipine (80-120% of the working level) were prepared by diluting appropriate aliquots of stock solution with IS solution.. The above concentrations were applied on the chromatographic plates. The plate was developed using mobile phase comprising of toluene-ethyl in the volume ratio 6.0:4.0 in twin trough chamber to a distance of 8cm. The plate was then removed from chamber and air-dried. The plate was then scanned and quantified at 238nm.

Assay

Twenty tablets were weighed and average weight was calculated. These tablets were powdered and weight equivalent to one tablet was taken in a 100 mL volumetric flask. This was dissolved in minimum amount of methanol and diluted to volume. The solution was filtered through 0.45 micron PVDF syringe filter and 1 mL of the filtrate was diluted to 10 ml with IS solution to obtain 20μg mL^{-1} of Cilnidipine. Six such samples were prepared. 10μL of these sample solution were spotted along with standard solution on HPTLC plate precoated silica gel $60F_{254}$ under the optimized chromatographic conditions. Peak areas were recorded and the amount of Cilnidipine present in the formulation was estimated. Result of assay experiment is given in TABLE 1.

Recovery

TABLE 1: Results of assay in method precision

Sample no.	Assay (mg)	Assay (%)
1	20.06	100.29
2	20.21	101.05
3	19.86	99.30
4	19.90	99.50
5	19.81	99.04
6	20.14	100.70
Mean	20.00	99.98
SD	0.16	0.82
%RSD	0.82	0.82

TABLE 2: Results of accuracy

Level	Amount added(mg)	Amount found(mg)	% Recovery	Mean Recovery
	2.0506	2.0278	98.89	-
110%	1.9982	2.0316	101.67	98.94
	2.0207	1.9451	96.26	-
	4.0008	4.0320	100.78	-
120%	4.0111	3.9116	97.52	98.96
	4.0422	3.9844	98.57	-
	6.0100	6.2276	103.62	-
130%	6.0042	5.9430	98.98	101.88
	6.0101	6.1934	103.05	-
Mean	-	-	99.93	-
SD	-	-	2.50	-
%RSD	-	-	2.50	-

Recovery experiments were carried out to check for the presence of positive or negative interferences from excipients present in the formulation, and to study the accuracy of the method. Recovery experiment was performed by the standard addition method. Pre-analysed sample of Cilnidipine tablets 20 mg were spiked with Cilnidipine standard at three different levels 110 %, 120 % and 130 % of the labeled amount of Cilnidipine in triplicate (total 9 determinations). The results of recovery experiment are tabulated in TABLE 2.

RESULT AND DISCUSSION

The method was a normal phase HPTLC method. It makes use of a silica gel $60F_{254}$ stationary phase precoated on aluminium sheet. The mobile phase comprises toluene-ethyl acetate in the volume ratio of 6.0:4.0 which gives good separation between Cilnidipine (R_f =0.6) and Nifedipine (R_f =0.48). The linear dynamic range for Cilnidipine was found to be 5μg mL^{-1} to 45μg mL^{-1} with a coefficient of variation of 0.9990. The limit of detection and limit of quantitation for Cilnidipine was found to be 3 μg mL^{-1} and 5μg mL^{-1} respectively. The

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average recovery for Cilnidipine was found to be 99.93 %(RSD=2.5%) which shows that method is free from interference from excipients present in the formulation. The low values of standard deviation and coefficient of variation at each level of the recovery experiment indicate high precision of the method.

CONCLUSION

The high performance thin layer chromatographic method for the determination of Cilnidipine from its fixed dosage form was found to be accurate and precise. Thus, the proposed HPTLC method can be successfully applied for the routine quality control analysis of Cilnidipine from its fixed dosage form.

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