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High performance thin layer chromatographic determination of nifedipine from bulk drug and from pharmaceuticals

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Abstract

A stability indicating high performance thin layer chromatographic (HPTLC) method for quantification of nifedipine, as bulk drug and from solid oral dosage forms has been developed. The extraction solvent was methanol and the mobile phase was chloroform:ethyl acetate:cyclohexane (19:2:2, v/v/v). The calibration curve of nifedipine in methanol was linear in the concentration range of 180–720 ng. The mean values of correlation coefficient, slope and intercept were 0.995 ± 1.02, 1.467 ± 0.56 and 184.16 ± 2.15. The limit of detection for nifedipine was 20 ng and limit of quantification was 40 ng. No interference was found from photodecomposition products. The percent recovery of nifedipine using the described procedure was 99.08 ± 1.51. The coefficient of variation for within day and between day analysis was 0.60 and 0.84% for 480 ng and 0.47 and 0.25% for 720 ng nifedipine concentration. The method was utilized to monitor concentration of nifedipine from sustained release marketed solid oral dosage forms as also from the developed sustained release liquid filled and multi unit hard gelatin capsules.

Keywords: Nifedipine; Degradation; High performance thin layer chromatographic

1. Introduction

Nifedipine, (3,5-pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-dimethyl ester, a calcium channel blocker is one of the most widely used coronary vasodilators [1–3]. There are several spectroscopic [4] and chromatographic methods reported for assay of nifedipine and its related compounds in pharmaceuticals [5–10].

High performance thin layer chromatography (HPTLC), the part of instrumental planar chromatography is regarded as a reliable, fast and accurate method for quantitative drug analysis which facilitates accurate sample application and scanning in situ. Several samples can be run simultaneously using a small quantity of mobile phase, hence minimizing analysis time and cost per analysis.

The present paper describes a simple, rapid, precise, specific and stability indicating HPTLC method for quantification of nifedipine, a light sensitive drug, as bulk drug and solid oral dosage forms.

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2. Experimental

2.1. Chemicals and reagents

Nifedipine USP grade, USP nifedipine nitrophenylpyridine analog reference standard and USP nifedipine nitrosophenylpyridine analog reference standard were supplied by Novartis Ltd., (India). Analytical grade solvents were purchased from Ranbaxy fine chemicals (India).

2.2. Preparation of standard curve

A stock solution of nifedipine (1 mg/ml) was prepared in methanol. Standard solution of 60 μg/ml was used for the analysis.

2.3. Instrumentation

The samples were spotted on HPTLC aluminum plates (10 cm × 10 cm) precoated with silica gel 60_{F254} (layer thickness 0.2 mm) (Merck) using Camag Linomat IV model. The samples were streaked in the form of narrow bands of length 3 mm, 10 mm from the bottom edge, 10 mm from margin, 5 mm apart at a constant rate of 15 s/μl using a nitrogen aspirator. The migration distance was 8 cm with migration time of 15 min. Densitometric analysis of separated components was carried out using Camag TLC scanner II (Camag, Switzerland) in the absorbance mode at 238 nm. Scanning speed was kept at 1 mm/s. Integration of chromatograms was performed using the Camag TLC scanner/integrator system (Perkin Elmer, USA).

2.4. Selection of mobile phase

Various solvent systems reported in the literature for TLC analysis of nifedipine were tried [11]. The selection of solvent system was based on the separation of degradation products from the pure drug. Appropriately modified solvent system of chloroform:ethyl acetate:cyclohexane (19:2:2, v/v/v) was selected.

2.5. Standard curve of nifedipine in methanol

Appropriate volumes of standard solution (60 μg/ml) were spotted to obtain nifedipine in the concentration range of 180–720 ng ($n = 3$)

2.6. Accuracy and precision of the assay

The accuracy of the assay was tested at 180, 420 and 720 ng level of nifedipine. The concentration of nifedipine in extracted samples ($n = 6$) at each level was compared with the theoretical concentration.

The intra-day precision was evaluated by analyzing samples repeatedly at concentration of 480 and 720 ng of nifedipine (per spot) ($n = 3$). The inter-day precision was similarly evaluated by repeated analysis of samples at concentration of 480 and 720 ng of nifedipine (per spot) once a day for 6 days.

2.7. Preparation of samples

2.7.1. Marketed tablets (Searle India Ltd., Mumbai)

The drug was extracted from the tablets (label claim 20 mg/tablet) as follows.

Ten tablets were crushed to a fine powder and mixed well. Powder equivalent to 20 mg of drug was accurately weighed and dissolved in methanol and shaken thoroughly. The contents were diluted to 100 ml and then centrifuged for 10 min. A 3 ml volume of supernatant was diluted to 10 ml with methanol and used for analysis.

2.7.2. Multi unit controlled release capsules (in-house developed, UDCT, Mumbai)

Capsule contents (label claim 20 mg nifedipine/capsule) were emptied in a volumetric flask and dissolved in methanol with the help of magnetic stirrer. The rest of the procedure was same as mentioned under marketed tablets.

2.7.3. Liquid filled controlled release hard gelatin capsules (in-house developed, UDCT, Mumbai)

The contents of the capsule (label claim 20 mg nifedipine/capsule) was dispersed in methanol with the help of magnetic stirrer. The rest of the

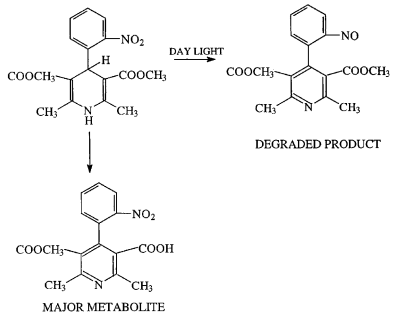

Fig. 1. Degradation of nifedipine by light.

procedure was same as mentioned under marketed tablets.

2.8. Recovery study

The recovery studies were carried out by addition of 480 ng of the standard nifedipine solution to preanalysed tablet solution samples and the mixtures were reanalysed by the proposed method ($n = 3$)

2.9. Degradation of nifedipine

Light activated degradation of nifedipine has been reported [12] (Fig. 1). Nifedipine was degraded in the solution by the following procedure.

The standard solution (60 μg/ml) was exposed to the diffused sunlight (Natural) for 2 h. A 8 μl volume of this solution was spotted on the plate. Simultaneously USP nifedipine nitrophenylpyridine analog reference standard, USP nifedipine nitrosophenylpyridine analog reference standard were spotted separately on the same plate.

3. Results and discussion

R_f value of 0.3 was obtained using the solvent system chloroform:ethyl acetate:cyclohexane (19:2:2, v/v/v). All standard curves were linear over the range 180–720 ng. The standard curve is depicted in Fig. 2. The mean values ($\pm$%CV) of coefficient of correlation, slope and intercept were 0.99 ± 1.02, 1.46 ± 0.56, 184.16 ± 2.15. The limit of detection for nifedipine was 20 ng. The limit of quantification was 40 ng.

The results in Table 1 revealed excellent accuracy and high precision of the assay method. The low coefficient of variation was indicative of acceptable inter-day and intra-day precision of the assay. The recovery of nifedipine was 99.08% and is reported in Table 2.

There was no interference from the common excipients present in the tablets, liquid filled and multi-unit capsules. The drug content in the various dosage forms of nifedipine is depicted in Table 3.

A stability indicating reversed phase HPLC assay for nifedipine has been reported by Pietta et

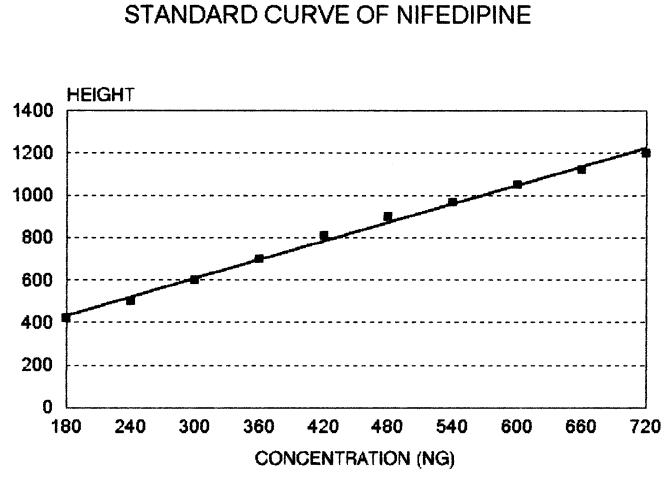

Fig. 2. Standard curve of nifedipine.

Table 1
Accuracy and precision of the assay

Concentration spotted (ng/spot)	Concentration found (%)	%CV
Accuracy		
180	100.92	0.33
420	98.08	0.06
720	99.86	0.06
Intra-day precision		
480	99.18	0.60
720	99.23	0.47
Inter-day variation		
480	98.46	0.84
720	100.06	0.25

Table 2
Recovery study of the assay

Spot no.	Amount found	Percent recovery ± S.D.
1	20.22	
2	19.74	99.08 ± 1.51
3	19.49	

al. [12]. Wherein photodegraded product of nifedipine (nitroso derivative) being more polar, eluted earlier than nifedipine. Analogously, our HPTLC method revealed an additional spot with R_f value of 0.5, which was well separated from the spot of nifedipine (R_f 0.3). Based on the reports of Pietta et al, this additional spot was assumed to be nitroso pyridine analog of nifedipine which was obtained by the photodecomposition of nifedipine after exposure to light. This was confirmed by comparison with the R_f value of USP nifedipine nitrophenylpyridine analog reference standard which also gave R_f value of 0.5. The USP nifedipine nitrosophenylpyridine analog reference standard also shows the same R_f value as USP nifedipine nitrophenylpyridine analog reference standard which is well separated from the pure drug.

The developed analytical method could therefore be considered a stability indicating method for analysis of nifedipine. A single spot at R_f value of 0.3 was obtained in the drug sample extracted from tablets. It could therefore be suggested that no degradation of nifedipine occurred in the marketed samples. As the nifedipine metabolite (USP nifedipine nitrophenylpyridine analog reference standard) is separated from nifedipine this method could also be used for the estimation of nifedipine in biological samples after appropriate optimization.

4. Conclusion

The proposed method is simple, rapid, sensitive and precise. It could be used as a stability indicating assay technique for analysis of nifedipine as bulk drug and pharmaceutical dosage forms. It could also be extended to study the degradation kinetics of nifedipine and for its estimation in plasma and other biological fluids.

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Table 3
Analysis of nifedipine from dosage forms ($n = 10$)

Formulation	Labelled claim (mg)	Amount found (mg ± %CV)	Average drug content (%)
Marketed tablets	20	19.80 ± 1.38	99.00
Multi unit controlled release capsules	20	19.97 ± 1.29	99.85
Liquid filled controlled release hard gelatin capsules	20	20.06 ± 1.44	100.31

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