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Research Article

SYNTHETIC STUDIES TOWARDS NIFEDIPINE AND ESTIMATION OF NIFEDIPINE BY HPTLC METHOD

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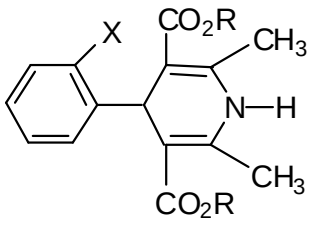
ABSTRACT

Dihydropyridines are the fastest growing class of calcium channel blockers. They act on slow L-type channels and are widely used for their property of vascular relaxation, particularly for variant angina, chronic stable angina and hypertension. They have potent antihypertensive effects. They have a greater selectivity for vascular smooth muscle than for myocardium and therefore their main effect is vasodilation. Nifedipine is a dihydropyridine calcium-channel blocker. It blocks the slow Ca channels on cell surface and prevents flow of Ca ions into the cells. It causes peripheral and coronary vasodilatation and reduction in afterload, peripheral resistance and blood pressure, but with consequent increase in blood flow and reflux coronary increase in heart rate. This leads to an increased supply in myocardial oxygen and cardiac output. They are non-rate-limiting with little or no action at the SA or AV nodes and negative inotropic activity is rarely seen at therapeutic doses. The synthesis of Nifedipine is based upon the classical Hantzsch 1, 4-dihydropyridine synthesis, i.e. the reaction of 1mole 2-nitrobenzaldehyde with 2moles methyl acetoacetate and 1mole concentrated aqueous ammonia in refluxing methanol which leads to the formation of Nifedipine. The estimation of Nifedipine was done by HPTLC method. HPTLC is becoming a routine analytical technique due to its advantages of low operating cost, high sample throughput and need for minimum sample clean up. The present analytical method is accurate, simple, precise, specific and reproducible for the estimation of Nifedipine in bulk drug and capsule dosage form.

Keywords: Nifedipine, Antihypertensive activity, Synthesis, Estimation, HPTLC method.

INTRODUCTION

Dihydropyridine chemistry began in 1882 when Hantzsch[1] first reported them as stable intermediates in the pyridine synthesis that bears his name. Several groups of investigators independently discovered that dihydropyridines[2-4] from the Hantzsch reaction are potent vasodilator hypotensives[5]. Nifedipine, **1**, is used as an antianginal and coronary dilator[6]. It dilates only resistance vessels and its tachycardia can be prevented by concurrent use of a β-blocker. The related compound, **2**, is being clinically evaluated in hypertension[7]. It also lowers blood pressure by selectively dilating resistance vessels and causes tachycardia that may be prevented by propranolol.



1 X = NO_2, R = CH_3
2 X = CF_3, R = CH_2CH_3

Dihydropyridines are the fastest growing class of calcium channel blockers and are widely for their property of vascular relaxation, particularly for variant angina, chronic stable angina and hypertension[8]. For many years, it was considered that the DHP calcium antagonist lowers blood pressure predominantly by blocking calcium influx at the level of L-type calcium channels. This is supported by the recent studies[9] that dihydropyridines also cause the release of nitric oxide from the vascular endothelium leading to vasodilation[9].

They have potent anti-hypertensive effects. The calcium channel blocking agents, typified by the dihydropyridine Nifedipine were approved initially for use in cases of atypical, nonexercise- induced angina. Clinical investigations have shown these agents to be useful for a variety of additional indications, including all types of angina as well as hypertension[10]. In search for orally active drugs for the treatment of coronary insufficency, F.Bosset synthesised in 1966 a compound designated Bay a 1040 which, following its introduction as Nifedipine (Adalat) in 1975 has since become one of the major cardiovascular drugs[11]

The structure-activity of Nifedipine shows that an orthosubstituted aryl is critical in 4 position, as is the unsubstituted nitrogen; 2,6-dimethyl is important and carbalkoxy groups are preferred to others electro withdrawing groups in the 3,5- position. The aromatic pyridine derivatives have little activity and dihydropyridines are rapidly metabolized by dehydrogenation to the pyridines.

The synthesis and characterization of Nifedipine have been reported previously[12, 13] but the main objective of the present work is to optimize the yield and the effects of parameters such as molar proportion of the reactants and time.

Nifedipine, chemically dimethyl 1,4-dihydro-2, 6-dimethyl-4- (2-nitrophenyl) pyridine-3, 5-dicarboxylate is an anti-anginal vasodilator. The drug is official IP[14], BP[15] and USP[16]. The IP and BP describe titrimetric assay procedure and USP describes hplc assay procedure for the bulk drug. The assay procedure of capsules dosage form are done in IP (UV method), BP (HPLC method) and USP (HPLC) method. Other reported methods involve HPLC[17-22], GC[23, 24], UV[25-29], GC-MS[30, 31], PMR[32], HPTLC[33], NIR[34], VIS[35], Colorimetric[36], Voltammetric[37-39] etc.

The present method here a simple HPTLC method for the estimation of Nifedipine which is very easy to perform, rapid, convient, high sensitivity, selectivity and precision.

MATERIALS AND METHOD

2-Nitrobenzaldehyde (min.99%) and methanol AR were purchased from Spectrochem Pvt. Ltd. Methyl acetoacetate (min.98%) was purchased from Sisco Research Laboratories Pvt. Ltd. Ammonia solution (min.25%) GR was purchased from Merck.

Nifedipine reference standard (>98% pure) was obtained from Sigma-Aldrich.Capsules dosage form Depin10 and Nicardia10 were manufactured by Zydus Cadila and J.B.Chemicals & Pharmaceuticals Ltd.

SYNTHESIS OF NIFEDIPINE

2-Nitrobenzaldehyde (20g, 0.132mole), methyl acetoacetate (36cc, 38.73g, 0.333mole) were taken in a 250cc round bottomed flask and dissolved in 34cc methanol. 16cc 25% ammonia was added. The mixture was refluxed in a 250cc heating mantle for 5hours, cooled to room temperature and left for overnight in the refrigerator. The precipitated yellow solid was filtered through Buchner funnel and washed with 50ml cold methanol. The solid so obtained was recrystallised from methanol

EXPERIMENTAL

Melting points were recorded in open glass capillaries and are uncorrected.AR grade solvents were used for TLC. The purity of the compound was checked by TLC using precoated Merck TLC plate of silica gel $60F_{254}$(layer thickness 0.2mm) under radiation at 254nm with cyclohexane-ethyl **acetate (**1:4) mixture as an eluent.

IR spectrum was recorded as KBr pellet using Perkin Elmer FT-IR Spectrum1 infrared spectrophotometer in the range 4000-400cm-. UV spectra were recorded in methanol, 0.1N NaOH and 0.1N HCl using

Perkin Elmer UV Lamda 20 ultraviolet spectrophotometer in the range 200-400nm.

Assay of laboratory prepared Nifedipine as per IP[14]

Nifedipine (130mg) was dissolved in a mixture of 25ml of 2-methyl-2-propanol and 25ml of 1M perchloric acid. This mixture was then titrated with 0.1M ceric ammonium sulphate, using 0.1ml of ferroin solution as indicator until the pink colour was discharged.

ESTIMATION OF NIFEDIPINE IN CAPSULE DOSAGE FORM BY HPTLC METHOD

Preparation of standard stock solution:

An accurately weighed quantity of 50mg of Nifedipine reference standard was dissolved in 100ml of methanol to give a standard stock solution **(solution1)** of concentration 500μg/ml. 2ml of this standard stock solution was further diluted to 100ml with methanol to give a working standard solution **(solution2)** of concentration 10μg/ml of Nifedipine reference standard.

Linearity and Calibration

Varying quantities of working standard solution **(solution2)** was diluted to 10ml with methanol to give solution of concentrations 2.0, 2.5, 3.0, 3.5 and 4.0μg/ml of Nifedipine RS. 5μl of these prepared solution of five concentrations were spotted (1 spot each) in the chromatoplate previously washed with mobile phase using an automatic application device. The chromatoplate was then developed in a presaturated twin trough chamber containing mobile phase. After deveplopment the plate was scanned at 283nm and peak areas and height were measured.

Analysis of the marketed formulation

Preparation of sample solution

20 capsules were weighed accurately and ground to a fine powder. A quantity of powder equivalent to average weight of the capsule was weighed and dissolved in methanol and volume was made up to 100ml with methanol. The solution was filtered through whatman filter no.4 and then 0.45 nylon filter, 10ml of filtrate was diluted to 100ml with methanol and this sample solution was used for estimation.

Assay: 5μl of standard solution of Nifedipine RS (3.0μg/ml) and the prepared sample solution were spotted as sharp bands on the chromatoplate using Linomat5 spotting. The chromatoplate was then developed in a presaturated twin trough chamber containing mobile phase. After development, the bands of the drug were scanned at 283nm using a densitometer. The peak area values of standard and sample were used to calculate the amount of Nifedipine present per capsules

METHOD VALIDATION

The analytical method was validated according to ICH guidelines[40].

System Suitability

A system suitability experiment was performed before determination of Nifedipine

Limit of detection (LOD) and Limit of quantitation (LOQ)

The limit of detection (LOD) and the limit of quantitation (LOQ) were evaluated by applying different dilutions of the standard solution of Nifedipine along with the blank (methanol).

Stability of solute during plate development

The decomposition of the analyte during spotting or development was confirmed by two-dimensional chromatography using the same mobile phase in both directions.

In situ stability after TLC development

The standard solution of Nifedipine of concentrations 60μg/ml, sample solution of capsule having concentration 60μg/ml were applied to the TLC plates. After development the plates were scanned and was stored in the dark by covering with black paper and rescanned after 24hrs.

Instrumental Precision

Precision of the instrument was checked by repeated scanning of the same spot

(500ng/spot) of standard Nifedipine solution

Repeatability

Repeatability of the method was checked by analysing a standard solution of Nifedipine after application 5μg/ml on a TLC plate for 6 times

Specificity

The specificity was also confirmed by overlaying the spectra of standard Nifedipine with the spectra of sample recorded on TLC scanner in UV range

Accuracy

To study the accuracy of the method, recovery experiment was performed by the standard addition method to determine if there are positive or negative interferences from excipients present in the formulations. The recovery of the added standard was studies at three different levels, each being analyzed in a manner similar to as describe for assay. Each set of additions was repeated 6times and percentage recovery was calculated.

Robustness

The robustness of the method was established by changing in mobile phase composition, temperature and relative humidity. Robustness of the method was done at concentration 100ng/spot.

RESULT

The results obtained after synthesis of Nifedipine with different molar concentration of reactants and time are summarized in Table1. The maximum yield obtained was recorded as 60%(w/w). The compound was identified by comparison of analytical data (M.P., IR and UV) with those of authentic sample (Sigma-Aldrich). The purity of the compound was monitored by TLC. Analysis was done according to IP[12] method and the results were given in **Table2.** The maximum purity obtained was recorded as 100.67%(w/w). It should be emphasized that the entire manufacturing process was protected as much possible from light.

Table 1

Experiment	2-Nitro benzaldehyde (g)	Methyl alcohol (ml)	Methyl acetoacetate (ml)	Ammonia (ml)	Time (hr)	Yield* (g)	%Yield
1	20	34	34	16	5.0	31	59.51
2	20	34	34	16	5.5	30	57.59
3	20	34	34	16	6.0	31	59.51
4	20	34	35	16	5.0	32	60.35
5	20	34	35	16	5.5	31	58.46
6	20	34	35	16	6.0	31	58.46
7	**20**	**34**	**36**	**16**	**5.0**	**35**	**64.86**
8	20	34	36	16	5.5	32	59.30
9	20	34	36	16	6.0	31	57.45
10	20	34	37	16	5.0	32	58.30
11	20	34	37	16	5.5	32	58.30
12	20	34	37	16	6.0	30	54.66
13	20	34	38	16	5.0	32	57.33
14	20	34	38	16	5.5	31	55.54
15	20	34	38	16	6.0	29	51.96

Yield of isolated pure product

Melting point: 172^{0}C (Lit[16, 20] mp 172-174^{0}C)

The **purity** of the compound was monitored by TLC with different solvent system and is but a mobile phase comprising of ethyl acetate: cyclohexane (4:1) which gives good separation of Nifedipine (R_f=0.5). Well-defined spots were obtained when the chamber was saturated with mobile phase for 1hour

The **UV spectra** showed absorption maxima at 235.89nm and 236.46nm in methanolic and ethanolic solution.

The **IR spectral** assignments of standard and laboratory prepared Nifedipine are obtained

The assay results were given in **Table2.** The maximum purity obtained was recorded as 100.67%(w/w)

Table 2

Experiment No.	Assay* (%w/w) of Nifedipine	IP limit
1	100.67	
2	100.14	
3	99.35	
4	98.91	
5	99.58	
6	100.07	
7	100.07	
8	99.18	98.0% to 102%(w/w)
9	99.21	
10	99.77	
11	99.73	

*Average of three readings

DISCUSSION

The proposed HPTLC method was precise, accurate and selective. The method was rapid, sensitive, reproducible and economical. It does not suffer any positive or negative interference due to common excipient present in pharmaceutical preparations and can be conveniently used for the analysis of Nifedipine in bulk drug and dosage forms. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase, unlike HPLC, thus lowering analysis time and cost per analysis.

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