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Original Article

SYNTHESIS, CHARACTERIZATION AND QUANTITATION OF REGIOISOMERIC IMPURITY IN NIMODIPINE BULK AND FORMULATION

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ABSTRACT

Objective: The present research work was directed towards the synthesis characterization and quantitation of regioisomeric impurity of Nimodipine i.e. diethyl 1, 4-dihydro-2,6-dimethyl pyridine dicarboxylate in bulk and tablet formulation, by UV,IR,NMR and GC-MS techniques and a RP-HPLC method was developed as per ICH Q2B guidelines for quantitation of 1, 4-Dihydro-2, 6-Dimethyl-4-(p-nitro phenyl) pyridine-3,5 dicarboxylate (NI) from bulk and formulation.

Methods: The synthesis of NI was carried out by Hantzch pyridine synthesis, by using p-nitrobenzaldehyde, ethylacetoacetate, in presence of ammonia and methanol as a catalyst. The percentage yield was found to be 89.29%. Recrystallization and purification of NI was done. The preliminary evaluation was done on laboratory scale via melting point, elemental analysis and TLC.

Results: The melting point of impurity was found to be 156-158^{0}C. The TLC of impurity was carried by using Chloroform: Methanol (9:1) and the R_f was found to be 0.79. The confirmation of structure of NI was carried out by using sophisticated techniques i.e., FT-IR, NMR (^{13}C and ^{1}H), GC-MS etc. The RP-HPLC method was developed to quantify the NI in Nimodipine bulk and formulation as per ICH Q2B guidelines. The method validation was done as per ICH guidelines.

Conclusion: The validated optimized method was found to be linear, précised, robust, rugged and accurate. Finally NI was quantified from bulk Nimodipine and its marketed tablet formulation. It was concluded that the amount of NI, present in tablet was found to be 0.1% and in the bulk 0.067% respectively. Thus it was revealed that the NI was found to be within the limit laid down ICH guidelines (Not more than 0.1 %).

Keywords: NI, IR, NMR, GCMS, RP-HPLC, Validation.

INTRODUCTION

Nimodipine is known as Isopropyl 2-methoxy ethyl 1,4- Dihydro 2, 6-dimethyl-4-(3-nitrophenyl) pyridine -3, 5-dicarboxylate, chemically [1]. It is a calcium channel antagonist of the 1, 4-dihydropyridine class and has been widely used for the treatment of hypertension, arrhythmias and angina pectoris, etc. During the manufacturing process of an active pharmaceutical substance or product, some intermediates are formed. These intermediates may affect the safety and efficacy issues of the pharmaceutical products. [2, 3] The current research is directed towards the impurity profiling of drugs and marketed formulations. [4] Now days the topic gains the most importance as compared to the purity of drugs and formulations.[3] Qualification of the impurities is the process of acquiring and evaluating data that establishes biological safety of an individual impurity; thus, revealing the need and scope of impurity profiling of drugs in pharmaceutical research.[5,6] As per ICH, Impurity profiling is a group of analytical activities, having aim of isolation, structure elucidation, identification and quantitative determination of organic and inorganic impurities and residual solvents in bulk drugs & pharmaceutical formulations.[7,8] Impurity is any component of the drug substance or product that is not the chemical entity of it, or is any substance coexisting with the original drug, like starting materials or intermediates or that is formed, due to any side reactions[2].Impurities are unwanted chemical that remains within the formulation or API in small amounts which may influence quality, safety and efficacy, thereby causing serious health hazards. [8] Identification of impurities is done by a variety of Chromatographic and spectroscopic techniques, alone or in combination with another technique. There are various methods for detecting and characterizing impurities with TLC or HPTLC or HPLC etc. The pharmacopoeias, such as the Indian Pharmacopoeia (IP), British Pharmacopoeia (BP) and the United States Pharmacopoeia (USP), are slowly incorporating limits to allowable levels of impurities present in the APIs or formulations.[3,5,9] Also, the ICH has published guidelines on impurities in new drug substances [ICH, Q3A], products [ICH, Q3B], and residual solvents [ICH, Q3C].[3,4,7,10] According to ICH guidelines on impurities in new drug products, identification of impurities less than 0.1% level is not considered to be necessary, unless potential impurities are expected to be unusually toxic or potent.[10] According to ICH, the maximum daily dose qualification threshold is considered as follows;≤ 2g/day 0.1% or 1 mg per day intake (whichever is lower) ≥ 2g/day 0.05% [5,6,7].It assures identity, strength, purity, efficacy, safety and quality of drug substances and products.[2] Stereochemistry related impurities includes regioisomeric impurities. These are having similar chemical structure but different spatial orientation and are stereo isomers in which isomers differ in the arrangement of substituent on a rigid structure. [11, 12]

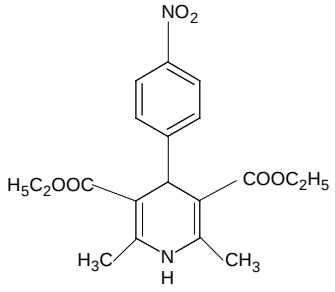

Fig. 1: 1, 4-Dihydro-2, 6- Dimethyl-4-(p- nitro phenyl) pyridine-3, 5 dicarboxylate

MATERIALS AND METHODS

Chemicals

P-nitrobenzaldehyde (AR), Ethylacetoacetate (AR), Ammonia (AR), Methanol (AR), Acetonitrile (HPLC grade), Methanol (HPLC grade), Water (HPLC grade) were purchased from Merck Chemicals, India.

Instruments

UV-Visible Spectrophotometer

The UV detection at wavelength 280 nm was selected by using UV-Vis Spectrophotometer (UV-1650 PC) SHIMADZU INC.

FT-IR

The IR spectra were recorded by using Fourier Transform Infrared Spectrophotometer Model No. 8400S SHIMADZU by KBr press pellet technique. KBr was purchased from Merck Chemicals, India and was AR Grade.

NMR

Characterization of impurities was achieved by using Varian NMR Mercury 300 MHz spectrometer, using DMSO-d_6 as a solvent and TMS as an internal reference standard for the proton experiment. All experiments were conducted at 25°C, and no shift relaxation agents were employed. The ^1H and ^{13}C NMR chemical shift values were reported on the δ scale in ppm.

GC-MS

The Q-TOF Micro mass (YA-105) spectrometer capable of recording High Resolution Mass Spectrum (HRMS) both in atomic pressure chemical ionization (APCI) and Electron spray Ionization (ESR) were used for characterization of NI.

Synthesis of NI

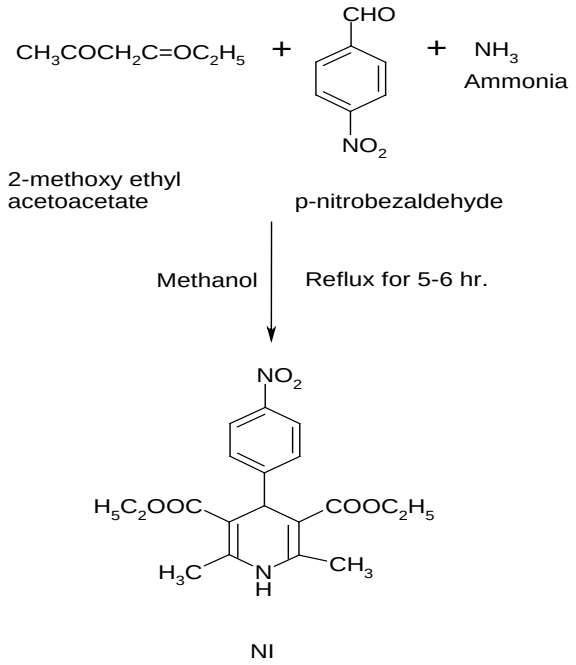

Fig. 2: Scheme for the Synthesis of NI

RP-HPLC

The HPLC method was developed by using LC20AD Prominence Liquid Chromatography SPD 20-A Schimadzu, Japan. The UV-Vis detector and C18 column with dimension on 250x 4.6 mm was used for the HPLC method development having flow rate of 1.0 ml/min at wavelength 280 nm.

The Methanol: Acetonitrile: Water in proportion of (35:38:27 v/v/v) as a mobile phase was selected for development of validated method of NI and various parameters according to ICH guidelines (Q2B) were studied. [10]

Synthesized NI Impurity

0.01 mole (1.52 gm) of p-nitrobenzaldehyde & 0.02 moles (2.60 ml) of ethylacetoacetate were added in round bottom flask. Then 5 ml of ammonia and 10 ml of methanol was added and was stir vigorously. Refluxed for 3 hrs and the solution was poured in cold water and was kept for overnight in freezer. Filtered at vacuum filter and recrystallized from Methanol.

Chromatographic Conditions

Preparation of Mobile phase

The selection of mobile phase was according to polarity and non-polarity of solvents. The methanol: acetonitrile: water was selected as mobile phase in ratio of 35:38:27(v: v: v) and was filtered on membrane filter (0.45 µ) to remove degassing and were stirred for 15-20 min.

Preparation of Stock Solution

The stock solution of 100ug/ml was prepared by dissolving 10 mg NI in 100 ml mobile phase. The dilution was prepared in various concentrations using stock solution and was dissolved in mobile phase.

Preparation of Sample Solution (Formulation)

The sample solution of Nimodipine formulation was prepared as 100ug/ml stock solution for quantification of NI in Nimodipine formulation. The dilution was prepared in various concentrations using sample stock and was dissolved in mobile phase for quantification of NI in Nimodipine formulation.

RESULTS AND DISCUSSION

Physicochemical Properties:

Table 1: Physicochemical Properties of NI

Molecular Formula	Molecular Weight	M.P.^{0}C	Rf Value	% Yield
$C_{19}H_{22}N_2O_6$	374 gm	156-158 ^{0}C	0.79	89.29%

UV Spectrum [13]

The λmax of NI in methanol was found to be 280 nm (1) n-π* transitions. Another peak appears at 239 nm (2) π-π* transitions.

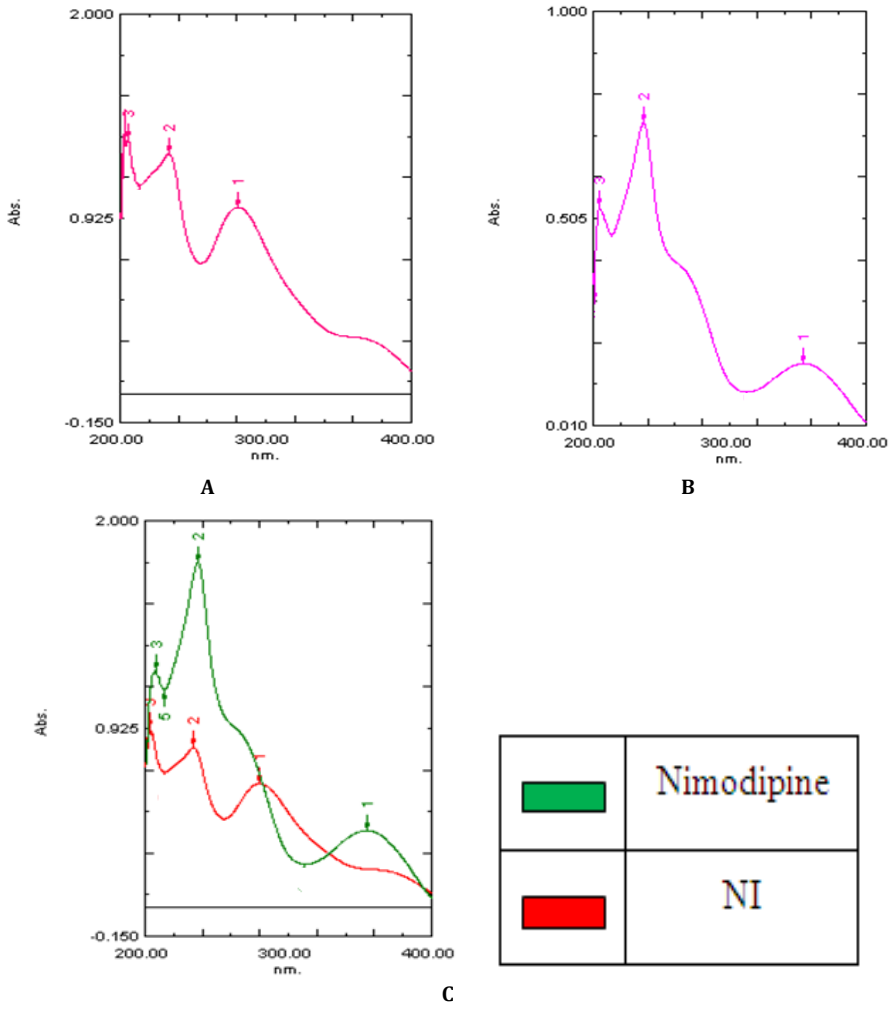

Fig. 3: UV Spectrum of A-NI and B-Nimodipine C-Overlay of NI and Nimodipine

IR Data [13, 14]

The major functional groups are primary amine, nitro and carbonyl groups. Obtained peaks in IR spectrum are as follows.

IR (KBr) cm^{-1}**:** 3400-3200 (NH- Stretch), 3150-2900(C-H Stretch), 1704(C=O Stretch), 1600-1475(C=C Stretch), 1550-1482(N-O Stretch), 1450-1375(CH_3 Bend), 1360-1320(NO_2 stretch), 900-700(Oop), 840(Substitution at para position to benzene ring).

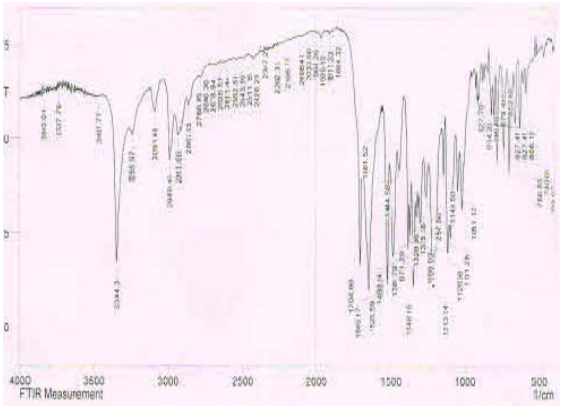

Fig. 4: IR Spectrum of NI

NMR Data [14, 15]

^{1}H NMR (DMSO)

δ=9 (s,1H,NH of 1,4-dihydropyridine), 8.108 (s,6H,CH_3 of 1,4-dihydropyridine), 7.44 (q,4H,CH_2 proton of ester), 5.01 (t,6H,,CH_3 proton of ester), 4.01 (s,1H attached to1,4-dihydropyridine ring), 2.301 (d,2H, CH attached to nitrobenzene ring), 1.142 (d,2H,CH attached to nitrobenzene ring). ^{1}H NMR Spectrum of NI was recorded and is shown below.

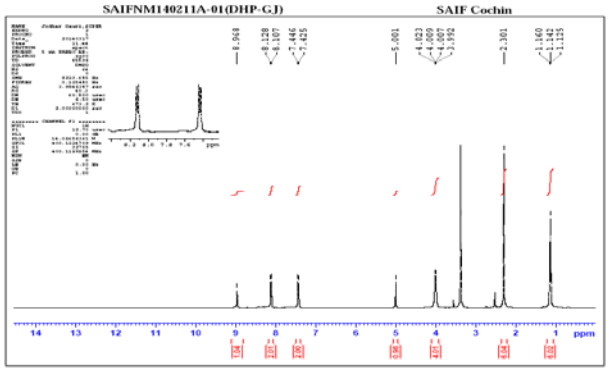

Fig. 5: ^{1}H NMR Spectrum of NI

^{13}C NMR (DMSO)

δ=14.062,(2C, CH3 Carbon attached to CH_2),59.169(2C,CH2 Carbon attached to CH_3),166.5(2C, Carbonyl carbon attached to 1,4-dihydropyridine ring), 18.188(2C,CH3 Carbon attached to 1,4-dihydropyridine ring),146.265(2C, CH_2=CH_2 of 1,4- dihydropyridine ring),100.838(2C, CH_2=CH_2 of 1,4- dihydropyridine ring),40(1C, Carbon attached to 1,4- dihydropyridine),155.48(1C, Carbon attached to nitrobenzene ring),128.566(2C,CH Carbon attached to meta position of p-nitrobenzene ring),123.208 (2C, CH Carbon attached to ortho position of p-nitrobenzene ring),145.796(1C, Carbon attached to nitrobenzene ring).

^{13}C NMR Spectrum of NI was recorded and is shown below.

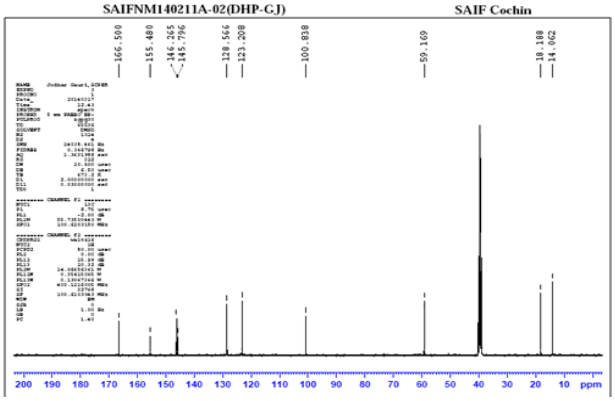

Fig. 6: ^{13}C NMR Spectrum of NI.

GC-MS Data [11, 12]

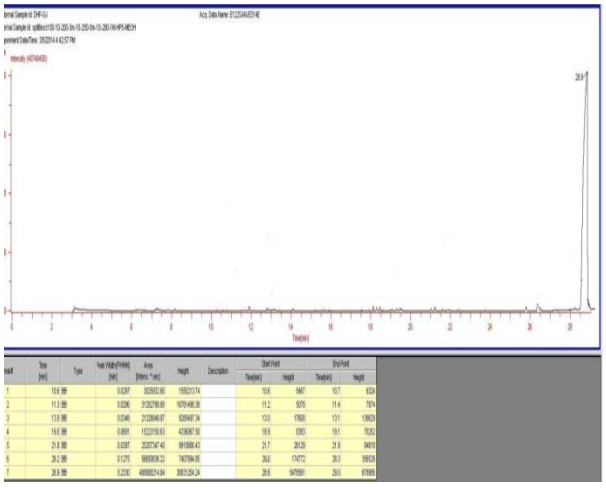

Fig. 9: GC of NI

Gas Chromatogram of NI shows a single peak at 28.9 min.which indicates purity of synthesized NI. Mass spectrum at 28.9 min was recorded and is given below. Peak appear at 374 indicates presence of molecular ion peak. Major base peak at 252 shows 100% abundance. Peak at 252 appear due to elimination of C_6H_4-NO_2. GC Chromatogram of NI showed a single peak at 28.9 min.

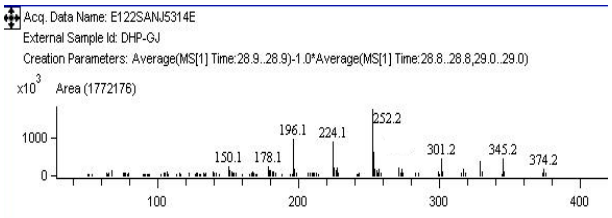

Fig. 8: Mass Spectrum of NI

Mass Fragmentation Pattern

Mass fragmentation pattern for NI is decided from mass spectrum obtained at retention time 28.9 minutes and is shown below.

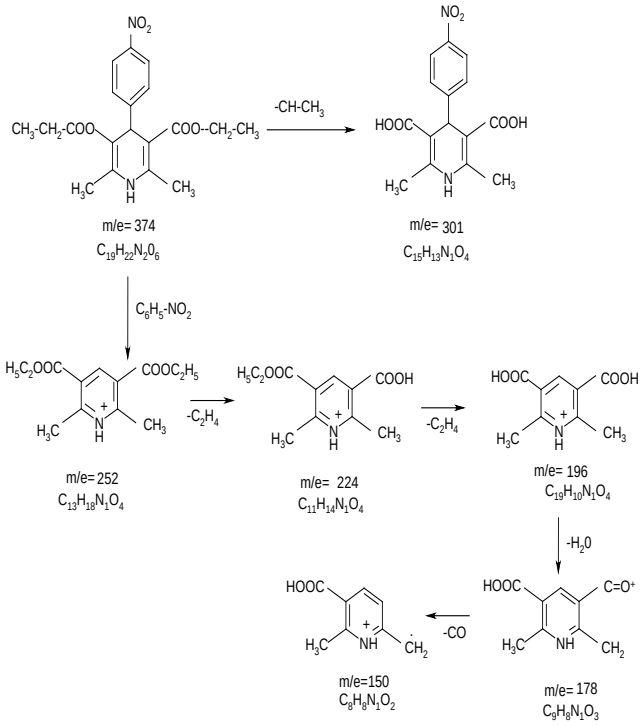

Fig. 7: Mass Fragmentation Pattern of NI

HPLC Method Development [16]

Validation experiment was performed to demonstrate system suitability, linearity, precision, accuracy study, ruggedness and robustness as per ICH Q2B guidelines.

System Suitability Parameters

The area of respective concentrations, theoretical plates, number of theoretical plates per cm, Tailing factor and the peak symmetry was recorded.

Linearity

Dilution of standard impurity in the range of 400-1400 ng/ml were prepared by taking suitable aliquots of working standard solution in different 10 ml volumetric flasks and diluting up to the mark with mobile phase. 20 µl was injected from it each time on column at flow rate of 1 ml/min. The standard from elute was monitored at 280 nm and corresponding chromatogram were obtained from these chromatograms peak area were calculated. A plot of peak area over concentration was constructed. Regression of the plot was computed by least square regression method.

Precision

Precision of analytical method was studied by multiple injections of homogenous samples. 6 replicate of 800 ng solution were prepared and injected for precision at the same flow rate of 1ml/min. The intra-day, inter-day and intermediate precision were used to study the variability of the method. S.D. and % R.S.D. were calculated for both.

Accuracy

Accuracy of the method was studied using the method of standard addition. Standard impurity solutions were added to the unknown bulk and tablet formulation of Nimodipine. The percent recovery was determined at three different levels (50%, 100% and 150%). Impurity content was determined and the percent recovery was calculated.

Robustness

Robustness was studied by changing parameters like change in flow rate. The S.D. and % R.S.D. between the change parameter were calculated.

Ruggedness

Ruggedness was studied was carried out by using different analysts. The S.D. and % R.S.D. were calculated.

LOD and LOQ

Limit of detection and limit of Quantitation of the method was calculated by formula given below

LOD= 3.3xS.D./Slope

LOQ= 10xS.D./Slope

Quantitation of NI

The total amount of impurity present in Nimodipine bulk and formulation was calculated for the NI and the result was compared to ICH limit for impurities in new drug substance and products is 0.1%.[3,17]

HPLC Chromatograms

HPLC Chromatogram of Nimodipine

HPLC Chromatogram of Nimodipine was recorded and is shown below.

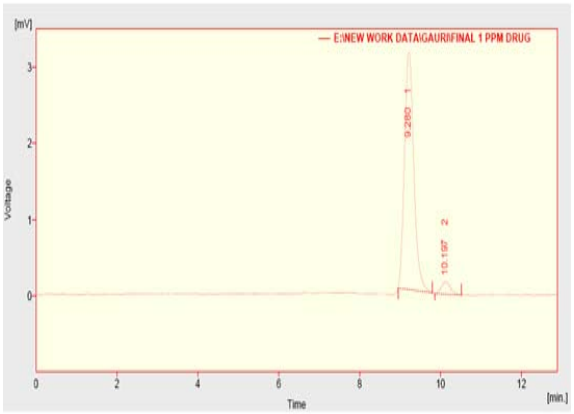

Fig. 10: HPLC Chromatogram of Nimodipine

Result Table (Uncal - E:\NEW WORK DATA\GAURI\FINAL 1 PPM DRUG)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	9.280	49.639	3.146	95.0	95.2	0.24
2	10.197	2.585	0.159	5.0	4.8	0.26
	Total	52.224	3.305	100.0	100.0	

The Retention time of Nimodipine was 9.280 min.

HPLC Chromatogram of NI

HPLC Chromatogram of NI was recorded and is shown below

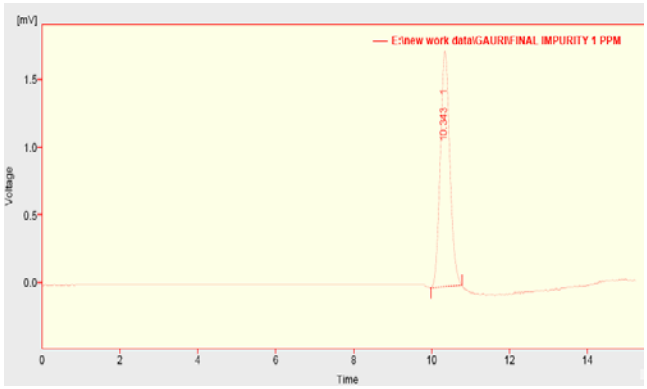

Fig. 11: HPLC Chromatogram of NI

Result Table (Uncal - E:\new work data\GAURI\FINAL IMPURITY 1 PPM)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	10.343	29.761	1.741	100.0	100.0	0.26
	Total	29.761	1.741	100.0	100.0	

The retention time of NI was 10.343 min and it shows a single peak which indicates purity of compound.

HPLC Chromatogram of Nimodipine and NI Combination

HPLC Chromatogram of Nimodipine and NI combination was recorded and is shown below.

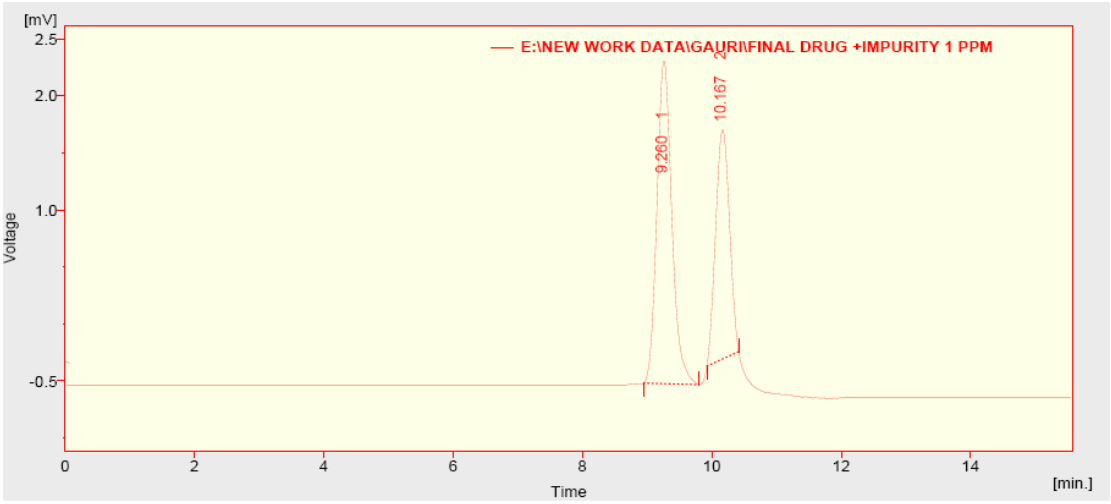

Fig. 12: HPLC Chromatogram of Nimodipine and NI Mixture

Result Table (Uncal - E:\NEW WORK DATA\GAURI\FINAL DRUG +IMPURITY 1 PPM)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	9.260	45.504	2.831	60.4	58.5	0.25
2	10.167	29.780	2.011	39.6	41.5	0.24
	Total	75.284	4.842	100.0	100.0	

The retention time of Nimodipine and NI in laboratory mixture was found at 9.260 min and 10.167 min respectively.

HPLC Chromatogram of Tablet

HPLC Chromatogram of Nimodipine tablet was recorded and is shown below.

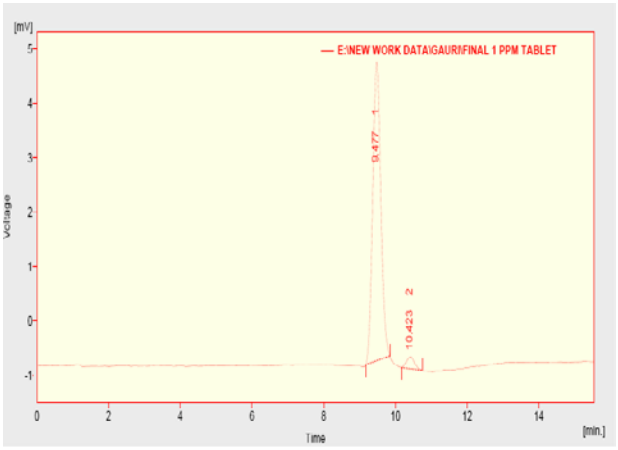

Fig. 13: HPLC Chromatogram of Nimodipine Tablet

Result Table (Uncal - E:\NEW WORK DATA\GAURI\FINAL 1 PPM TABLET)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	9.477	85.925	5.476	96.2	96.1	0.25
2	10.423	3.368	0.220	3.8	3.9	0.24
	Total	89.293	5.696	100.0	100.0	

The retention time of Nimodipine and NI in tablet was found at 9.477 min. and 10.423 min. respectively.

HPLC Chromatogram of Tablet and NI Mixture

HPLC Chromatogram of Nimodipine tablet and NI was recorded and is shown below.

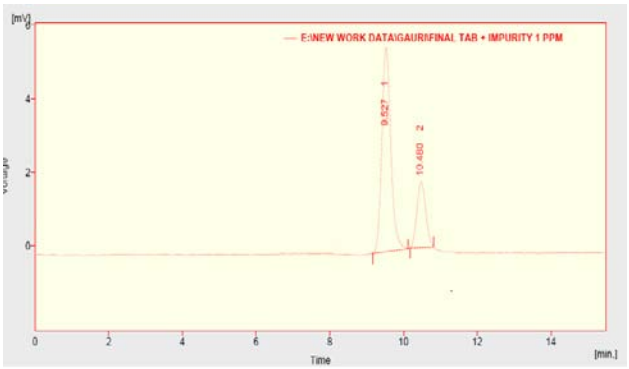

Fig. 14: HPLC Chromatogram of Tablet and NI Mixture

Result Table (Uncal - E:\NEW WORK DATA\GAURI\FINAL TAB + IMPURITY 1 PPM)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	9.527	93.794	5.541	75.9	75.6	0.26
2	10.480	29.792	1.787	24.1	24.4	0.27
	Total	123.586	7.328	100.0	100.0	

The retention time of Nimodipine in Tablet and NI in laboratory mixture was found at 9.527 and 10.480 min respectively.

a. Linearity

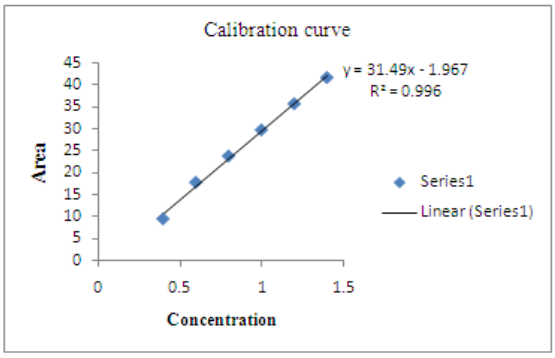

Fig. 15: Calibration Curve of NI

Table 2: Linearity Data of NI

S. No.	Parameter	Observation
1.	Linearity Range	400-1400 ng/ml
2.	Slope	31.49
3.	Intercept	-1.967
4.	Correlation Coefficient	0.9962
5.	LOD	7.545 ng/ml
6.	LOQ	22.86 ng/ml

b. Repeatability

Table 3: Results of Repeatability Studies

S. No.	Parameter	S.D.	%R.S.D.
1.	Precision	0.07141	0.2999
2.	Intraday Precision (After 4 Hrs.)	0.1306	0.5445
3.	Interday Precision (After 24 Hrs.)	0.2711	1.0577
4.	Intermediate Precision	0.0908	0.2891
5.	Robustness (At flow rate 0.8 ml/min)	0.9028	1.6559
6.	Ruggedness		
	Analyst I	0.1394	0.5812
	Analyst II	0.2556	1.0107

c. Accuracy

Table 4: Results of Recovery of NI

S. No.	Drug / Formulation	Amount of Drug (ng/ml)	Amount of Impurity Added (ng/ml)	Amount Recovered (ng/ml)
		2000	1000	950
1.	Bulk	2000	2000	1930
		2000	3000	2910
		2000	1000	960
2.	Tablet	2000	2000	1940
		2000	3000	2950

Table 5: Results of Accuracy Studies

S. No.	Drug / Formulation	Percentage Recovery			Mean	S.D.	%R.S.D.
		50%	100%	150%			
1.	Bulk	95.98	96.70	97.23	96.63	0.6274	0.6493
2.	Tablet	95.50	97.32	98.25	97.36	0.8756	0.8993

d. System Suitability Parameters

Table 6: System Suitability Parameters

S. No.	Property	Values	Official Limits
1.	Retention time(t_R)	10.343 min	-
2.	Theoretical Plates(N)	8744	N ≥ 2000
3.	Resolution(R)	2.280	R ≥ 2
4.	Tailing Factor(T)	0.98	T ≤ 2

e. Summary of Retention time and Asymmetry

Table 7: Summary of Retention Time and Asymmetry

S. No.	Compound	Retention Time (Min.)	Asymmetry
1.	Nimodipine	9.280	1.152
2.	NI	10.343	1.155
3.	Nimodipine Tablet	9.477	1.063

f. Quantitation of NI

Table 8: Quantitation of NI in Bulk and Tablet

S. No.	Bulk/Formulation	Quantitation of NI
1.	Bulk Nimodipine	**0.0672%**
2.	Nimodipine Tablet	**0.1%**

Thin Layer Chromatography (TLC)

The Mobile phase Chloroform: Methanol (9:1 v/v)

R_f Value = 0.79

(Iodinated) (Plane)

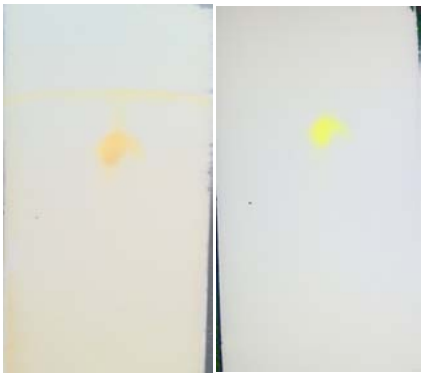

Fig. 16: TLC of NI

CONCLUSION

The regioisomeric impurity of Nimodipine diethyl 1, 4-dihydro-2, 6-dimethyl pyridine 3, 5 dicarboxylate in bulk and formulation was synthesized, characterized and the RP-HPLC method was developed according to ICH Q2B guidelines for quantitation of NI from Nimodipine bulk and tablet formulation. The synthesis of NI was carried out by Hantzch pyridine synthesis. The % yield was found to be 89.29%. The preliminary evaluation was done on laboratory scale viz. melting point, TLC and elemental analysis. The melting point of NI was found to be 156-158^{0}C. The TLC of NI was carried by using Chloroform and Methanol (9:1) and the R_f was found to be 0.79.The confirmation of structure of NI was carried out by using sophisticated instruments viz, FT-IR, NMR (^{1}H and ^{13}C), GC-MS.A RP-HPLC method was developed to identify and quantify the NI from Nimodipine bulk and formulation, as per ICH Q2B guidelines. The method was found to be linear, precise, robust, rugged and accurate. Finally NI was quantified from bulk Nimodipine and its marketed tablet formulation. It was observed that the amount of NI, diethyl 1, 4-dihydro-2, 6-dimethyl-4(p-nitro phenyl) pyridine-3, 5-dicarboxylate present in tablet was found to be 0.1 % and in bulk drug, it was found to be 0.0672 %. Thus it was found that the impurity was found to be within the limit laid down as per ICH

guidelines (not more than 0.1 %). Thus impurity profiling can act as a quality Control tool.

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