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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF PROCESS RELATED IMPURITY FROM NIFEDIPINE

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Abstract - The process related impurity of Nifedipine diethyl 1, 4-dihydro-2, 6-dimethyl pyridine 3, 5 dicarboxylate in bulk and formulations was synthesized. The characterization of synthesized impurities by using FTIR, NMR and MS. The RP-HPLC method was developed according to ICH Q2B guidelines for quantitation of impurity in bulk and formulations. The method was validated as per ICH guidelines. The method was found to be linear, precise, accurate, robust and rugged. The diethyl 1,4-dihydro-2,6-dimethyl pyridine 3,5 dicarboxylate impurity was quantified from bulk Nifedipine and its marketed tablet formulations. It was revealed that the amount of impurity present in tablet batch I and II was found to be 0.26% and 0.32% respectively and the bulk was found to be negligible.

Keywords: RP-HPLC, Validation, impurity, Nifedipine

INTRODUCTION

The identification and qualification of impurities in Active Pharmaceutical Ingredient's (API's) and pharmaceutical products, is a very intensive activity performed at many levels of the drug discovery and beyond. Impurities related to starting materials, by-products, breakdown products or polymorphs. They can appear at the API production level as well as during or after formulation process. Impurities in APIs are of significant concern as they may carry activity responsible for the eventual undesirable side effects or toxicity and/or may interfere with the drug's activity. Thus monitoring impurities in API and drug product is a prerequisite to insure drug safety and quality. In Pharmaceutical World, an impurity is considered as any other organic materials, besides the drug substances, or ingredients, arises out of synthesis or unwanted chemicals that remains with Active Pharmaceutical Ingredient's (API's). The impurity may be developed either during formulation or upon aging of both API's and formulations. Presence of impurities in trace quantity in drug substance or drug product is inevitable. Therefore, their level should be controlled and monitored. They reinforce or diminish the pharmacological efficacy of the Active Pharmaceutical Ingredient's. [1] ICH defines impurities profile of a drug materials is "A description of the identified and unidentified impurities, present in a new drug substance."For Pharmaceutical products, impurities are defined as "substance in the product that are not the API itself or the excipient used to manufacture it " i.e. impurities are unwanted chemical that remains within the formulation or API in small amounts which can influence Quality, Safety and Efficacy, thereby causing serious health hazards. [2] 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. [3] Identification of impurities is done by a variety of Chromatographic and Spectroscopic techniques, either alone or in combination with other techniques.

There are different methods for detecting and characterizing impurities with TLC, HPTLC, HPLC etc. Conventional Liquid Chromatography, particularly, HPLC has been exploited widely in field of impurity profiling; the wide range of detectors, and stationary phases along with its sensitivity and cost effective separation have attributed to its varied applications. Various regulatory authorities like ICH, USFDA, Canadian Drug and Health Agency are emphasizing on the purity requirements and the identification of impurities in Active Pharmaceutical Ingredient's (API's). According to ICH guidelines on impurities in new drug products, identification of impurities below the 0.1% level is not considered to be necessary, unless potential impurities are expected to be unusually potent or toxic. 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% [4]

MATERIALS AND METHODS

Materials

The Nifedipine bulk drug was purchased from Yarrow Chemical products, Mumbai, India and two tablet formulations of different batches were purchased from local market of Nashik

Methods

HPLC Method Development and Validation

The quantitation of 1, 4-DHP from tablet formulation was carried out by HPLC method. The LC20AD Prominence Liquid Chromatograph SPD20-A Shimadzu, Japan with UV-Vis detector and C18 column with dimension on 25 x 0.6 cm was used for the method development with flow rate 1.0 ml/min at wavelength 237 nm. The methanol: Water (40:60) was used as a mobile phase, for development of chromatogram. The method was validation for synthesized compound and various parameters according to ICH guidelines (Q2B) were studied.

1. Preparation of Mobile phase

The selection of mobile phase was according to solubility. The methanol: water was selected as mobile phase in ratio of 40:60 and was filtered on membrane filter (0.45 μ) to remove degassing and was stirred for 10-15 min.

2. Preparation of Stock solution standard

The stock solution was prepared according to the standard procedure viz., 10 mg of synthesized compound was accurately weighed on analytical balance and using mobile phase it was dissolved to make volume up to 100 ml stock solution.

The sample was prepared in the ppm in the range of 1-6 ppm in concentrations respectively for the method validation by HPLC.

3. Preparation of sample solution (formulation)

Stock solution of 2 different batches of Nifedipine marketed formulation of 100 ppm in 100 ml volumetric flask was prepared. Dissolve 10 mg of test sample in 100 ml diluents. 1ml of this stock was diluted to 10 ml to prepare 10 ppm stock solution. For the tablet formulation 20 tablets from each 2 tablet batch were crushed respectively. The powder of this formulation equivalent to 10 mg of the drug was used to prepare the stock solution. Further dilute to 1 ppm, 2 ppm, and so on, were prepared by taking 0.1 ml, 0.2 ml and so on of standard test solution and diluting it to 10 ml. Validation experiment was performed to demonstrate system suitability, linearity, precision, accuracy study, ruggedness and robustness as per ICH guidelines.

1. System Suitability Parameters:

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

2. Linearity

Dilution of standard impurity in the range of 1-6 μg/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 into the column at flow rate of 1 ml/min. The standard from elute was monitored at 237 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.

3. Precision

Precision of analytical method was studied by multiple injections of homogenous samples. 6 replicate of 4 ppm solution were prepared and injected for precision at the same flow rate of 1ml/min. The intra-day and inter-day precision was used to study the variability of the method. SD and RSD were calculated for both.

4. Accuracy

Accuracy of the method was studied using the method of standard addition. Standard impurity solutions were added to the unknown tablet formulation of Nifedipine. The percent recovery was determined at three different levels (50%, 75% and 100%). Impurity content was determined and the percent recovery was calculated.

5. Robustness

Robustness was studied by changing parameters like change in flow rate. The SD and RSD between the change parameter were calculated.

6. Ruggedness

Ruggedness was studied was carried out by using different analysts. The SD and RSD were calculated.

7. LOD and LOQ

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

LOD= 3.3xSD/Slope

LOQ= 10xSD/Slope

5.5 Quantitation of Impurity

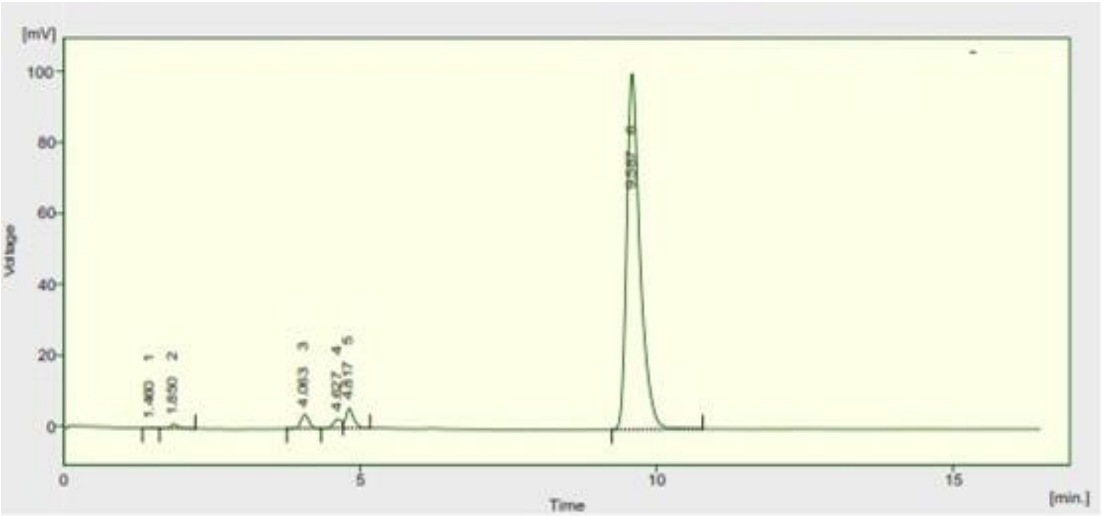
The total amount of impurity present in Nifedipine formulations was calculated for the synthesized compound and the result was compared to ICH limit for impurities in new drug substance is 0.1%.

RESULT AND DISCUSSION

6. HPLC Method Validation

The ICH Q2B guidelines discuss the analytical method validation on HPLC. Currently the vast majority of process-related impurity determinations are performed by HPLC. It offered the desired sensitivity for trace level determinations with a high degree of automation. A wide variety of stationary phases and operation modes make HPLC applicable to all drug classes. The typical detection limits for process-related impurities by HPLC are 0.1% or lower and this can be routinely met in the majority of circumstances using conventional UV detectors. These methods involved the prediction of likely impurities within the synthetic process, their isolation and identification by suitable analytical techniques. The last step of the present study was to develop, validated HPLC method for detection and quantification of Diethyl 1, 4-dihydro-2, 6- dimethyl-4(o-nitrophenyl) pyridine-3, 5- dicarboxylate impurity in tablet formulations.

HPLC Chromatograph of Nifedipine



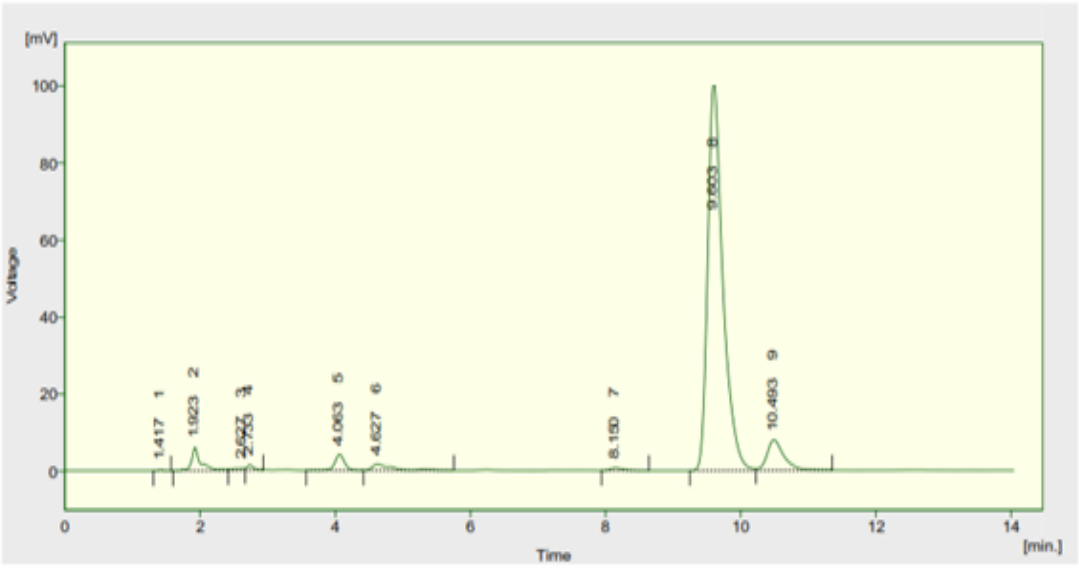
Result Table

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.460	2.424	0.289	0.1	0.3	0.13
2	1.850	11.705	1.278	0.7	1.1	0.11
3	4.063	37.267	3.919	2.2	3.4	0.15
4	4.627	25.732	2.608	1.5	2.3	0.17
5	4.817	52.839	5.630	3.1	4.9	0.14
6	9.587	1568.718	100.231	92.3	88.0	0.23
	Total	1698.684	113.955	100.0	100.0	

Figure No.01: HPLC Chromatogram of Nifedipine

The Retention time of Nifedipine was 9.5 min.

HPLC chromatogram of Nifedipine and synthesized compound combination



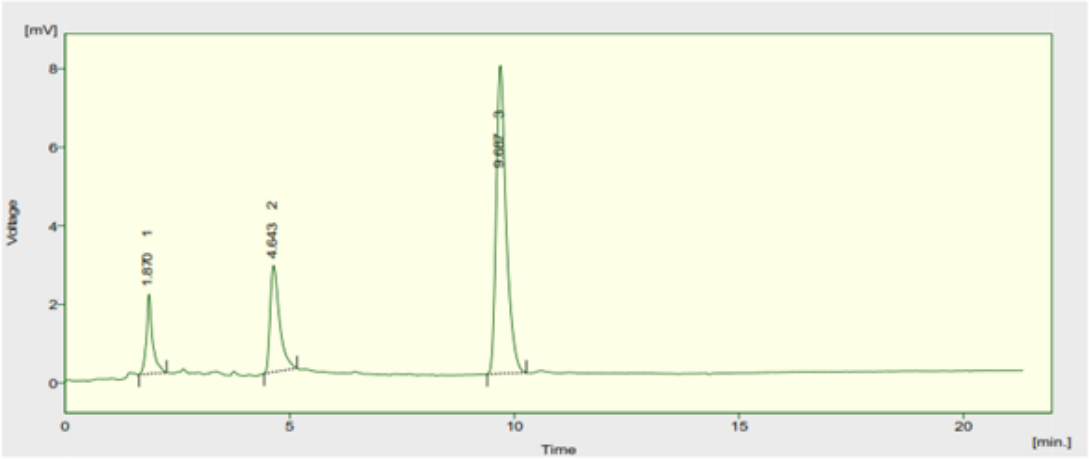
Result Table

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.417	1.559	0.259	0.1	0.2	0.09
2	1.923	56.096	6.038	3.0	4.9	0.10
3	2.627	5.744	0.488	0.3	0.4	0.20
4	2.733	8.699	1.344	0.5	1.1	0.10
5	4.063	41.275	4.124	2.2	3.3	0.15
6	4.627	31.614	1.567	1.7	1.3	0.31
7	8.150	8.444	0.642	0.5	0.5	0.20
8	9.603	1564.219	100.804	84.4	81.8	0.23
9	10.493	135.152	7.907	7.3	6.4	0.25
	Total	1852.802	123.171	100.0	100.0	

Figure No. 02: HPLC Chromatogram of Nifedipine and synthesized compound mixture.

The retention time of Nifedipine and synthesized compound in lab mixture was found at 9.60 and 10.49 min respectively.

HPCL Chromatogram of Tablet



Result Table

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.870	19.233	2.024	10.7	16.1	0.12
2	4.643	39.227	2.710	21.9	21.6	0.22
3	9.687	120.869	7.840	67.4	62.4	0.23
	Total	179.330	12.573	100.0	100.0	

Figure No.03: HPLC Chromatogram of Nifedipine Tablet

The retention time of Nifedipine tablet was found at 9.6 min.

Optimized chromatographic condition

Table No.01: Optimized chromatographic condition for RP-HPLC

Chromatographic Conditions	**SHIMADZU HPLC System**
Mobile phase	Methanol: Water(40:60)
Column	ARP-C18 (250 mm X 4.6 mm), 5µ column
Flow rate	1 ml/min
Wavelength detection	237 nm
Injection volume	20µl
Temperature	Ambient
Retention time	10.5 min
Run time	15 Min

1. Linearity

Table No.02: Result of Linearity by HPLC (Peak area vs. Conc.)

Sr. No	**Concentration(ppm)**	**Area (mill volts) at 237nm**
1.	1	122.27
2.	2	214.78
3.	3	311.07
4.	4	418.09
5.	5	512.02
6.	6	617.34

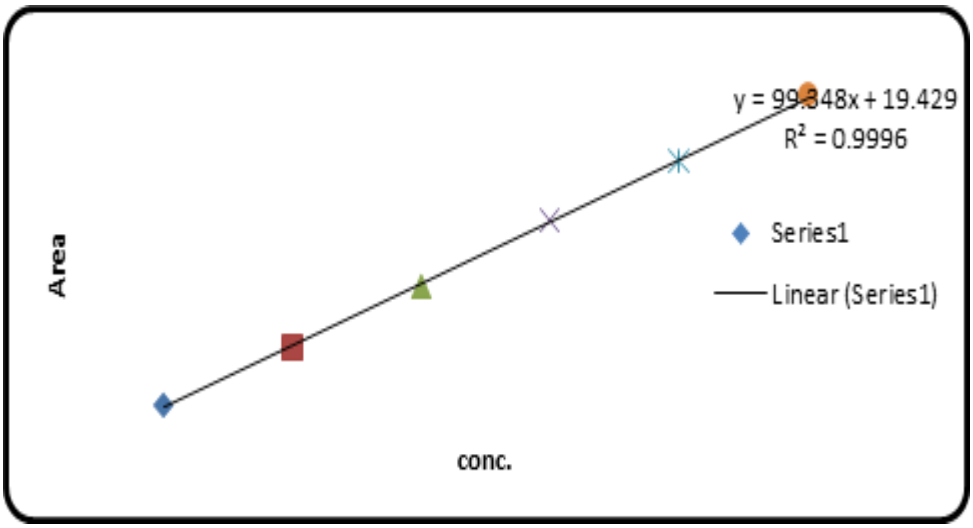


Figure No.04: Linearity of synthesized compound by HPLC

The linearity of the proposed method was estimated by regression analysis at six concentration levels in the range of 1-6 µg/ml for intermediate. The correlation coefficient (R^2) was found to be 0.999 and intercept Y= 99.34x + 19.42 was linear.

2. Precision

Table No.03: Precision by HPLC

Sr.No	Concentration(ppm)	Peak area (mV)at 237 nm	Mean	SD	% RSD
1.	4	418.29	419.15	1.331	0.317
2.	4	417.98			
3.	4	419.80			
4.	4	419.90			
5.	4	418.08			
6.	4	420.85			

The precision of the intermediate was quantified for repeated concentration of 4µg/ml in range and was reliable with their area of chromatogram as shown in above table. The Standard deviation (SD) and Relative standard deviation (RSD) was found to be 1.331 and 0.317 respectively.

a. Intraday precision after 4 hours

Table No.04: Intraday precision after 4 hours

Sr. No	Conc. (ppm)	Peak area after 4 hour at 237 nm	Mean	S.D	%RSD
1.	4	418.38	416.96	2.250	0.539
2.	4	413.51			
3	4	418.48			
4.	4	417.15			
5.	4	415.38			
6.	4	418.87			

b. Interday precision after 24 hours

Table No.05: Intraday precision after 24 hours

Sr. No	Conc. (ppm)	Peak area after 24 hour at 237 nm	Mean	S.D	%RSD
1.	4	423.12	424.42	1.760	0.414
2.	4	422.60			
3.	4	426.66			
4.	4	424.24			
5.	4	423.42			
6.	4	426.53			

The intra and inter day precision was carrying out and difference in % RSD was found not much varies and remains less than 2% indicate preciseness of method.

3. Robustness

Table No.06: Robustness study by change in flow rate

At flow rate of 0.8 ml/min

Sr. No	Conc. (ppm)	Peak area (mV) 0.8ml/min	Mean	S.D	%RSD
1.	4	786.65			

2.	4	783.58	787.45	3.122	0.395
3.	4	793.85			
4.	4	784.25			
5.	4	788.35			
6.	4	788.05			

The robustness of the Intermediate was performed for change in flow rate upto0.8 ml/min and method was robust with standard deviation 3.122 and relative standard deviation 0.395.

4. Ruggedness

Table No.07: Ruggedness study by change in analyst

Sr. No	Conc. (ppm)	Peak Area in mV		Mean		S.D		%RSD	
		Analyst I	Analyst II	I	II	I	II	I	II
1.	4	419.19	418.80	419.94	420.66	1.392	1.481	0.3314	0.3520
2.	4	418.98	420.18						
3.	4	421.90	420.36						
4.	4	419.86	421.79						
5.	4	418.39	419.93						
6.	4	421.34	422.98						

The ruggedness of the Intermediate was carried out for change in analyst and method was found to be robust.

5. Accuracy

Table No.08: Recovery study by HPLC

Sr.No.	Drug / Formulation	Percentage recovery			Mean	S.D.	%RSD
		50%	75%	100%			
1.	Tablet I	99.22	101.30	103.79	101.43	2.288	2.255
2.	Tablet II	99.25	101.68	103.13	101.30	1.969	1.934

Accuracy study was performed by the recovery method. The results demonstrate that the percentage recovery in tablet was more than bulk due to the presence of impurity in the tablet. Percentage recovery was found to be more at higher concentration level a compare to lower concentration level.

6. Limit of detection

$$LOD = \frac{3.3 \times \text{Standard deviation}}{\text{Slope}}$$

$$LOD = \frac{3.3 \times 1.3313}{98.44}$$

LOD = 0.4462

7. Limit of quantitation

$$LOQ = \frac{10 \times \text{Standard deviation}}{\text{Slope}}$$

$$LOQ = \frac{10 \times 1.3313}{98.44}$$

LOQ = 0.1352

The LOD by HPLC was 446.2 ng and that of LOQ 135.2 ng the method is more sensitive and selective.

CONCLUSION

The plethora subscribed in this research was directed towards the synthesis, characterization and quantification of diethyl 1,4- Dihydro-2,6-dimethyl pyridine 3,5 –dicarboxylate impurity in Nifedipine and its marketed formulations by Reverse Phase High Performance Liquid Chromatography method. The synthesis of a process related impurity of Nifedipine was successfully carried out by Hantzsch pyridine synthesis. The impurity was purified by column chromatography. Characterization was done by I.R, ^{1}H-NMR, ^{13}C-NMR and GC-MS. Based on the spectral data, the structure of impurity was characterized as diethyl 1,4- Dihydro-2,6-dimethyl pyridine 3,5 –dicarboxylate. An efficient isocratic RP-HPLC was developed and validated according to ICH guidelines with respect to specificity, accuracy, linearity and precision. The validated RP-HPLC method was used for detection and quantitation of diethyl 1,4-Dihydro-2,6-dimethyl pyridine 3,5 –dicarboxylate, a process related impurity of Nifedipine , from Nifedipine bulk and tablet formulations. The above method was found to be specific, accurate, precise, rugged and robust and can be used for routine analysis.

REFERENCES

[1]. Ingale SJ, Sahu CM, Paliwal RT, Vaidya S, Singhai AK. Advance approaches for the impurity profiling of pharmaceutical drugs: A review.*International Journal of Pharmacy and Life Sciences* 2011; 2(7): 955-962.

[2]. Rao NR, Nagaraju V. An Overview of the recent trends in development of HPLC methods for the determination of impurities in drugs. *Journal of Pharmaceutical and Biomedical Analysis* 2003; 33: 335-337.

[3]. Shah RS, Patel MA, Naik MV, Pradhan PK, Upadhyay UM. Recent Approaches of Impurity profiling in Pharmaceutical Analysis: A Review. *International Journal of Pharmaceutical sciences and Research* 2012; 3(10): 3603-3617

[4]. Bari SB, Kadam BR, Jaiswal YS, Shirkhekar AA. Impurity Profile: Significance in Active Pharmaceutical Ingredients. *Eurasian Journal of Analytical Chemistry* 2007; 2(1): 32-53.

[5]. ICH Q3A, Impurities Testing Guideline: Impurities in New Drug Substances,TheEuropean Agency for the Evaluation of Medicinal Products, 1995, 1-11.

[6]. ICH Q3B (R2), Impurities in New Drug Products, European Medicines Agency, 2006, 3-14.

[7]. Krishnaiah VSR, Bhaskar P, Jayaram B, Rama B, Raju V, Mohan RPM. Development and Validation of a Reversed-Phase HPLC method for the Analysis of Nimodipine in Pharmaceutical Dosage forms. *Asian Journal of Chemistry* 2003; 15(3): 1302-1306.