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Research Article

Development and validation of UV spectrophotometric method for estimation of regioisomeric impurity in Felodipine bulk and formulation

Akash A. Vadangale*[1], Pawar S.S.[2], Pande V.V.[3], Arote S.R.[4] and Musmade Deepak S.[2]

[1]*Department of Quality Assurance Techniques, S.R.E.S, Sanjivani College of Pharmaceutical Education and Research, Kopargaon, Maharashtra, India, 423601.*
[2]*Department of Pharmaceutical Chemistry, S.R.E.S, Sanjivani College of Pharmaceutical Education and Research, Kopargaon, Maharashtra, India, 423601.*
[3]*Department of Pharmaceutics, S.R.E.S, Sanjivani College of Pharmaceutical Education and Research, Kopargaon, Maharashtra, India, 423601.*
[4]*Department of Pharmacology, S.R.E.S, Sanjivani College of Pharmaceutical Education and Research, Kopargaon, Maharashtra, India, 423601.*

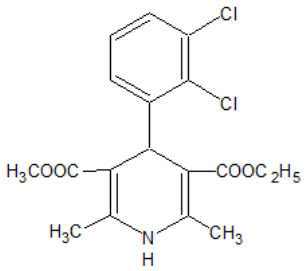
Abstract

The process related impurity of Felodipine i.e: Diethyl-4 (2-chlorophenyl) 2,6 dimethyl-1,4-dihydro pyridine 3,5 dicarboxylate was synthesized**,** characterized and quantified in bulk and formulation. The synthesis of intermediate was carried out by Hantzch process using O-chlorobenzaldehyde, ethylacetoacetate, in presence of ammonia and methanol as a catalyst. The percentage yield was found to be 78 %. Recrystallization and purification of FI was done. The preliminary evaluation was done on laboratory scale via melting point, elemental analysis and TLC. The melting point of impurity was found to be 136-140 ^{0}C. The TLC of impurity was carried by using Benzene: Methanol (6:1) and the R_f was found to be 0.64.The regioisomeric impurity was synthesized, purified, and characterized by IR, NMR and UV method was developed for quantification of synthesized impurity. The method was validated as per ICH Q2B guidelines. The UV method was found to be linear, precise, accurate, robust and rugged. Finally Diethyl-4 (2-chlorophenyl) 2,6 dimethyl-1,4-dihydro pyridine 3,5 dicarboxylate impurity was quantified from Felodipine bulk and its marketed tablet formulation.
Keywords: FI, Spectrophotometric analysis, R_f.

1. Introduction

Felodipine is Ethyl methyl 4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate. It is a calcium channel antagonist of the 1, 4-dihydropyridine class and has been widely used for the treatment of hypertension, Arrhythmia and angina pectoris, etc. Its empirical formula is $C_{18}H_{19}Cl_2NO_4$ and having the molecular weight 384.26 gm.[1]

Figure 1: Chemical structure of Felodipine



* Correspondence Info
Mr. Akash A. Vadangale
Department of Quality Assurance Techniques,
S.R.E.S, Sanjivani College of Pharmaceutical
Education and Research, Kopargaon, Maharashtra, India, 423601.
E-mail: Vadangale.akash@gmail.com

According to ICH impurity profiling of a drug substance is, "A description of the identified and unidentified impurities present in a new drug substance" or 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[2,3]. An organic impurity within the manufacturing process along with a good control strategy is an integral part of the quality control of drug substance.

2. Materials and Methods

O-chlorobenzaldehyde (AR), Ethylacetoacetate (AR), Ammonia (AR), Methanol (AR) were purchased from Merck Chemicals, India.

2.1. Instruments

2.1.1 UV-Visible Spectrophotometer

The UV spectra were recorded by using UV-Vis Spectrophotometer (UV-1650 PC) SHIMADZU INC.

2.1.2 FT-IR

The IR spectra were recorded by using Fourier Transform Infrared Spectrophotometer Model No. 8400S SHIMADZU by KBr press pellet technique.

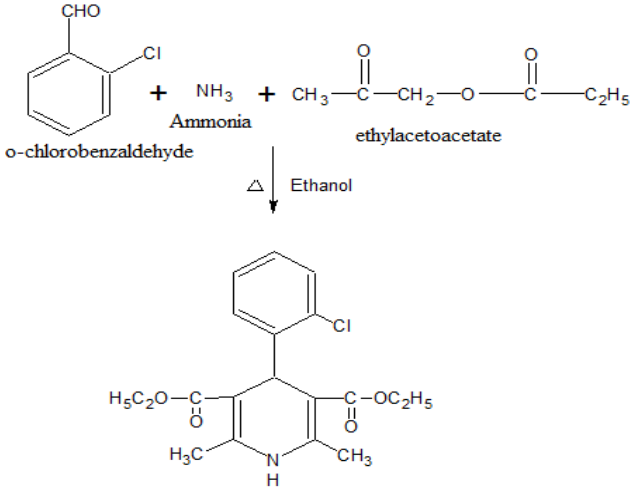
2.1.3 NMR

Characterization of impurities was achieved by using Varian NMR Mercury 300 MHz spectrometer, using $CDCl_3$ 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 ^{1}H and ^{13}C NMR chemical shift values were reported on the δ scale in ppm.

2.2. Synthesis of felodipine impurity

The synthesis of Felodipine Impurity (FI) was carried out by addition of 0.01 mole of O-chlorobenzaldehyde, 0.02moles ethylacetoacetate, 3 ml ammonia, 15 ml ethanol and was refluxed for 8 hours. Then it was cooled, poured into 150 ml ice cold water and stirred for 1 hour. Then it was filtered, dried and recrystallized twice using methanol as solvent and weighed.

Figure 2: Scheme for the Synthesis of FI.



3. Results

3.1 Physicochemical Properties

Table 1: Physicochemical Properties

Parameter	Result
Molecular Formula	**$C_{19}H_{22}ClNO_4$**
Molecular Weight	**363.30**
Melting Point	**136-140°C**
R_f Value	**0.64**
% Yield	**78%**

3.2 Thin Layer Chromatography

Mobile phase: Benzene: Methanol (6:1 v/v)

R_f Value = 0.64

Figure 3: TLC of FI

3.3 IR Data[4,5]

The major functional group are primary amine, chloro and carbonyl group are as follows:

IR (KBr) cm^{-1}: NH Stretching-3325,3225. C-H Stretching-3091,3061,2980. C=OStretching-1699. C=C Stretching-1491. CH_3 Bending-1377. C-O-C Bending-750,789. CH out of plane bending of orth-benzoid-839.06. Substitution at Ortho position of benzene ring-

3.4 NMR Data[5]

3.4.1 ^{1}H NMR ($CDCl_3$)

δ=5.8 (1H,NH of 1,4-dihydropyridine), 1.2 (6H,CH_3 of 1,4-dihydropyridine), 4.1 (4H,CH_2 proton of ester), 2.3(6H,,CH_3 proton of ester), 6.4(1H attached to1,4-dihydropyridine ring), 2.301 (2H, CH attached to chlorobenzene ring), 7.2(2H,CH attached to chlorobenzene ring).

3.4.2 ^{13}C NMR ($CDCl_3$)

δ=14.19 (2C,CH_3 Carbon attached to CH_2),50.70 (2C,CH_2 Carbon attached to CH_3), 167.72 (2C, Carbonyl carbon attached to 1,4-dihydropyridine ring), 19.23 (2C,CH_3 Carbon attached to 1,4-dihydropyridine ring), 127.20 (2C,C=C of 1,4-dihydropyridine ring), 129.15 (2C,C=C of 1,4-dihydropyridine ring), 37.38 (1C Carbon of 1,4-dihydropyridine ring), 145.59 (6 Carbon of phenyl ring).

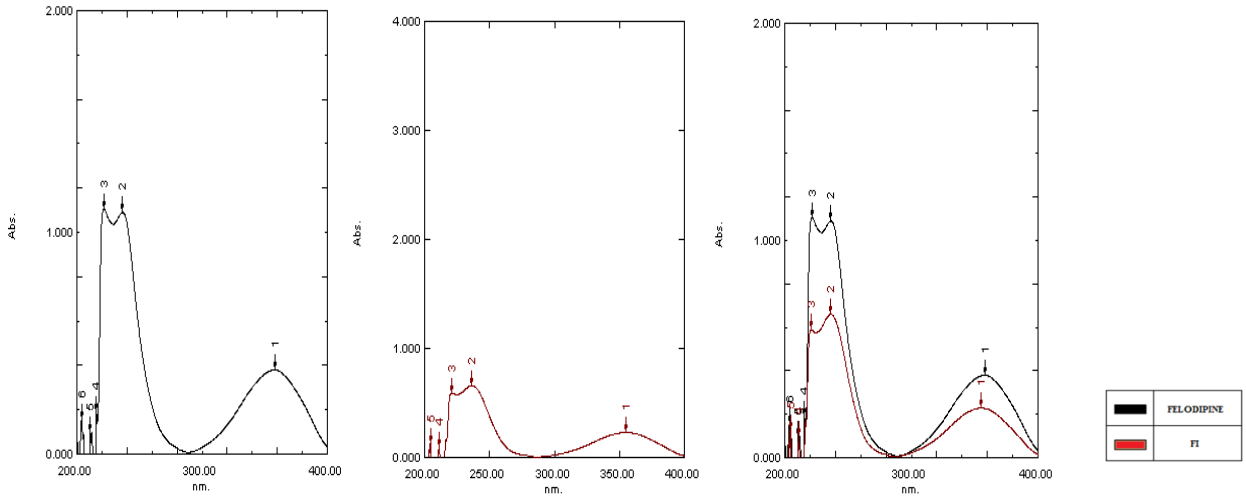
3.5 GC-MS Data[10,11]

Gas Chromatogram of FI shows a single peak at 25.45 min. which indicates purity of synthesized FI. Mass spectrum at 25.45 min was recorded. Peak appear at 365 indicates presence of molecular ion peak. Major base peak at 252 shows 100% abundance.

3.5 UV Method Development[4,9]

The λmax of FI in methanol was found to be 234 nm (1) n-π^* transitions. Another peak appears at 360 nm (2) π-π^* transitions.

Figure 4: UV Spectrum of A-Felodipine and B-FI C-Overlay of FI and Felodipine



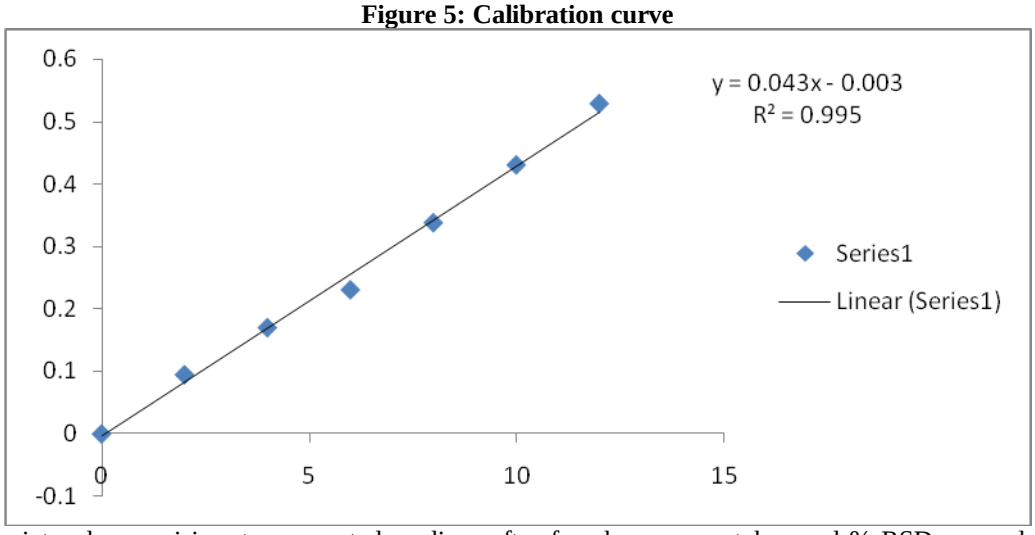
A **B** C

The aliquots of stock solution of FI (0.2, 0.4, 0.6, 0.8, 1.0) were transferred to 10 ml volumetric flask and volume was made up to 10 ml by methanol for making 2ppm, 4ppm, 6ppm, 8ppm and 10 ppm. The absorbance of solution was taken at 237 nm against methanol as a blank.

Table 2: Linearity

Sr. No	Concentration (ppm)	Absorbance
1	2	0.0946
2	4	0.1699
3	6	0.2304
4	8	0.3375
5	10	0.4298
6	12	0.5277
7	14	0.6227
8	16	0.7174
9	18	0.8110

Figure 5: Calibration curve



In intra-day precision, two repeated readings after four hours were taken and % RSD was calculated. In inter-day precision two repeated measurement were made on two consecutive days and % RSD was calculated.

Table 3: Intra-day Precision

Sr. No	Concentration (ppm)	Absorbance	SD	%RSD
1	6	0.2834	0.0033	1.1724
2	6	0.2904		
3	6	0.2850		
4	6	0.2860		
5	6	0.2830		
6	6	0.2920		
7	6	0.2875		

Table 4: Inter-day Precision

Sr. No	Concentration (ppm)	Absorbance	SD	%RSD
1	6	0.3262	0.0026	0.77
2	6	0.3240		
3	6	0.3270		
4	6	0.3251		
5	6	0.3257		
6	6	0.3251		
6	6	0.3260		

Ruggedness was studied by changing analyst. The SD and % RSD between the changed analysts was calculated.

Table 5: Ruggedness

Sr. No	Concentration (ppm)	Absorbance 1	Absorbance 11	SD 1	SD 11	%RSD 1	%RSD 11
1	6	0.2976	0.2960	0.003645	0.001632	1.22	0.55
2	6	0.2920	0.2959				
3	6	0.2951	0.2963				
4	6	0.2960	0.2961				
5	6	0.3020	0.2961				
6	6	0.3010	0.2962				
7	6	0.2945	0.2960				

Robustness was studied by changing scanning speed. The SD and % RSD between the changed parameter was calculated.

Table 6: Robustness

Sr. No	Concentration (ppm)	Absorbance 1	Absorbance 11	SD 1	SD 11	%RSD 1	%RSD 11
1	6	0.2990	0.2945	0.001387	0.008072	0.4648	0.2736
2	6	0.2970	0.2943				
3	6	0.2983	0.2967				
4	6	0.2992	0.2953				
5	6	0.2987	0.2951				
6	6	0.2989	0.2946				
7	6	0.2985	0.2948				

To ensure the accuracy, known amounts of pure drug (50%, 100%, and 150%) were added to the sample solution and these samples were reanalysed by the proposed method and also % recovery was determined.

Table 7: Recovery

Sr. No	Drug / Formulation	Percentage recovery			Mean	SD	%RSD
		50%	100%	150%			
1	Bulk	96.45	99.98	99.66	98.69	1.95	1.97
2	Tablet	99.05	98.45	99.29	99.07	1.46	1.47

4. Discussion

4.1 Linearity and Range

The given method was obtained in range of 2-18µg/ml. The standard Calibration curve was obtained by plotting the absorbance against its concentration measured at 237 nm. The regression coefficient was found to be 0.995 and slope was found to be 0.043.

4.2 Intra-day and Inter-day Precision

The intra-day and inter-day precision study of the developed method confirmed adequate sample stability and method reliability where all the Relative Standard Deviations were below 2%.

4.3 Ruggedness

The method was performed by changing analyst and the method was found to be rugged with standard deviation 0.003645 and relative standard deviation 1.22%.

4.4 Robustness

The robustness was performed by change in scanning speed and method was robust with standard deviation 0.001387 and relative standard deviation 0.46%.

4.5 LOD and LOQ

The LOD 0.0111 and LOQ 0.06301 ensures that the method is more sensitive and selective.

4.6 Accuracy and Recovery

The results within the range 96.00 - 100.00 ensure an accurate method.

5. Conclusion

The regioisomeric impurity of Felodipine Diethyl-4 (2-chlorophenyl) 2,6 dimethyl-1,4-dihydro pyridine 3,5 dicarboxylate in bulk and formulation was synthesized, characterized and the UV method was developed according to ICH Q2B guidelines for quantitation of FI from Felodipine bulk and tablet formulation. The synthesis of FI was carried out by Hantzch pyridine synthesis. The % yield was found to be 78%. The preliminary evaluation was done on laboratory scale viz. melting point, TLC and elemental analysis. The melting point of FI was found to be 136-

140^0C. The TLC of FI was carried by using Benzene: Methanol (6:1) and the R_f was found to be 0.64.The confirmation of structure of FI was carried out by using sophisticated instruments viz, FT-IR, NMR (^{1}H and ^{13}C), A UV method was developed to identify and quantify the FI from Felodipine bulk and formulation, as per ICH Q2B guidelines. The method was found to be linear, precise, robust, rugged and accurate. Finally FI was quantified from bulk Felodipine and its marketed tablet formulation. It was observed that the amount of FI, Diethyl-4 (2-chlorophenyl) 2,6 dimethyl-1,4-dihydro pyridine 3,5 dicarboxylate present in tablet was found.

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