Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2015, 7 (4):284-290
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Development and validation of UV spectrophotometric method for estimation of process related impurity in felodipine bulk and formulation

Vaibhav M. Thorat*[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*
[2]*Department of Pharmaceutical chemistry, S.R.E.S, Sanjivani College of Pharmaceutical Education and Research, Kopargaon, Maharashtra, India*
[3]*Department of Pharmaceutics, S.R.E.S, Sanjivani College of Pharmaceutical Education and Research, Kopargaon, Maharashtra, India*
[4]*Department of Pharmacology, S.R.E.S, Sanjivani College of Pharmaceutical Education and Research, Kopargaon, Maharashtra, India*

ABSTRACT

*This research is directed towards synthesis and characterization of process related impurity of Felodipine i.e.diethyl 4-(4-chlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate **(FI)** in bulk and tablet formulation by UV, IR and NMR techniques and its quantitationby UV spectrophotometric method development. The synthesis of **(FI)** was carried out by Hantzch process using p-chlorobenzaldehyde, ethylacetoacetate in presence of ammonia and methanol as catalyst. The preliminary evaluation was done on laboratory scale viz. melting point, TLC and elemental analysis. The regression coefficient was found to be 0.999 and Relative Standard Deviations were below 2%. The method was validated as per ICH guidelines and was found to be linear, precise, accurate, robust and rugged.*

Keywords: Felodipine, Hantzch process, Impurity, Spectrophotometric analysis

---

INTRODUCTION

Felodipine chemically is Ethyl methyl 4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylatewith molecular formula $C_{18}H_{19}Cl_2NO_4$ and molecular weight 284.3 [1]. Felodipine is under class of Calcium Channel Blocker used in the treatment of myocardial infraction, heart failure [2, 3].Felodipine decreases arterial smooth muscle contractility and subsequent vasoconstriction by inhibiting the influx of calcium ions through voltage-gated L-type calcium channels [4].

UV Spectrophotometric method was developed using methanol as solvent. The developed method was optimized and validated as per guidelines of International Conference on Harmonization (ICH) According to ICH guidelines on impurities in new drug product, when the impurity is less than 0.1% level it is not considered to be necessary, unless impurities found to be toxic or potent [5,6].

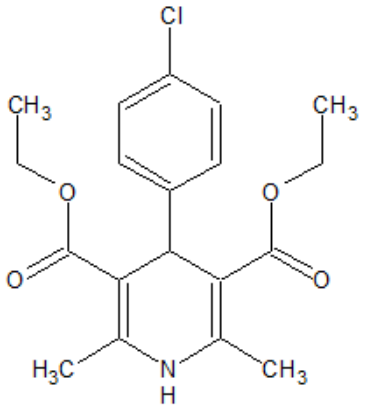


Figure 1: Chemical structure of FI

MATERIALS AND METHODS

Chemicals

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

Synthesis of Felodipine Impurity

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

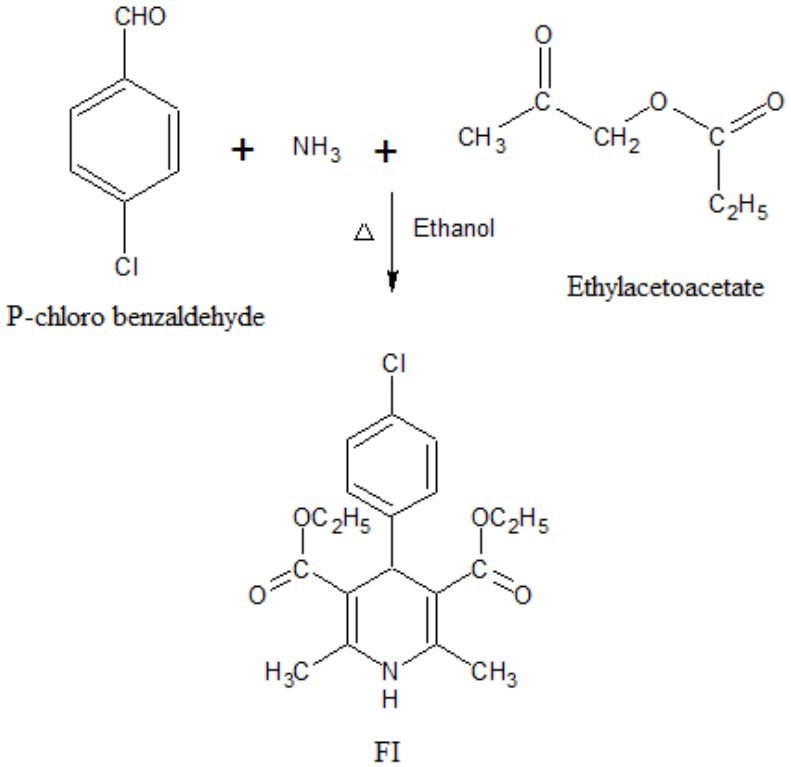


Figure 2: Synthesis of FI

FT-IR

The IR spectrum was recorded using KBr press pellet technique by using Fourier Transform Infrared Spectrophotometer Model No. 8400S SHIMADZU INC.

NMR
The characterization of impurity was done by using NMR. The ^{1}H and ^{13}C NMR were reported by using $CDCl_3$ as solvent.

UV
Determination of wavelength of maximum absorption
Accurately weighed 10 mg of FI was transferred to 100 ml volumetric flask and volume was made upto 100 ml with methanol. The solution was scanned from 200 – 400 nm to determine λmax.

Linearity and Range
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.

Precision
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.

LOD and LOQ
Detection limit and Quantification limit was calculated using formula
LOD= 3.3 × SD/ Slope
LOQ=10 × SD/Slope

Where, SD is calculated using values of y intercepts of regression equations.

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

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

Accuracy and Recovery
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.

RESULTS AND DISCUSSION

Physicochemical properties

Table no 1: Physicochemical properties

Molecular formula	Molecular weight	M.P °C	Rf value Benzene: Methanol (6:1 v/v)	% yield
$C_{19}H_{22}ClNO_4$	363.5	136-140	0.64	75%

Thin layer chromatography (TLC)
Rf value = 0.64

IR data[6,7,8]
The major functional groups are primary amine, chloro and carbonyl groups. Obtained peaks in IR spectrum are as follows.

IR (KBr) cm^{-1}: 3354.32 (NH- Stretch), 2958.90,3088.14 (C-H Aromatic Stretch), 2899.11 (C-H Aliphatic Stretch), 1695.49 (C=O), 1489.10 (C=C), 1375.29 (CH_3 Bend), 1174.69-1215.19 (C-O-C Stretch), 746.48-783.13 (Benzene ring Bend), 831.35 (CH out of plane bending of para-benzoid), (Substitution at para position of benzene ring)

NMR data [6,8]

^{1}H NMR ($CDCl_3$)

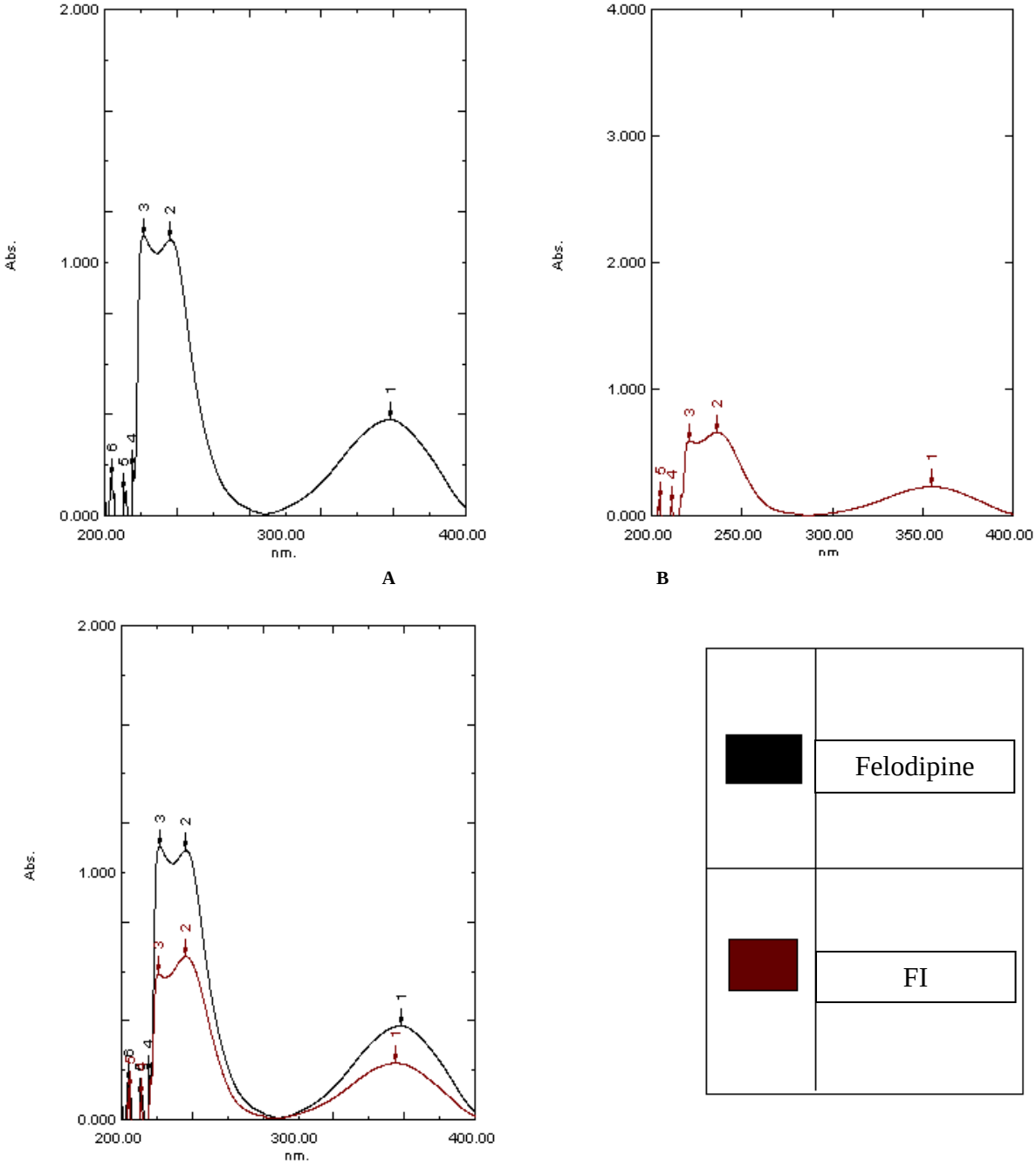
δ (ppm)= 4.945 (1H,NH of 1,4 dihydropyridine), 1.206 (6H,CH_3 of 1,4 dihydropyridine), 4.021 (4H, CH_2 proton of ester), 2.28 (6H,CH_3 proton of ester), 6.120 (1H,CH of 1,4 dihydropyridine), 7.141 (2H,CH of chlorobenzene ring), 7.207 (2H,CH of chlorobenzene ring).

^{13}C NMR ($CDCl_3$)

δ (ppm)=14.16 (2C,CH_3 Carbon attached to CH_2), 50.94 (2C,CH_2 Carbon attached to CH_3), 167.93 (2C, Carbonyl carbon attached to 1,4-dihydropyridine ring), 19.34 (2C,CH_3 Carbon attached to 1,4-dihydropyridine ring), 127.91 (2C,C=C of 1,4-dihydropyridine ring), 129.29 (2C,C=C of 1,4-dihydropyridine ring), 38.94 (1C, Carbon of 1,4-dihydropyridine ring), 144.30 (6 Carbon of phenyl ring).

UV method development

UV Spectrum [7, 9, 10]



A **B**

C

Figure 3: UV spectrum of A- Felodipine, B- FI, C- Overlay of Felodipine and FI

Table no 2: Linearity

Sr. no	Concentration (ppm)	Absorbance
1	2	0.1191
2	4	0.1725
3	6	0.2726
4	8	0.3848
5	10	0.5078
6	12	0.5931
7	14	0.6787
8	16	0.7850
9	18	0.8973

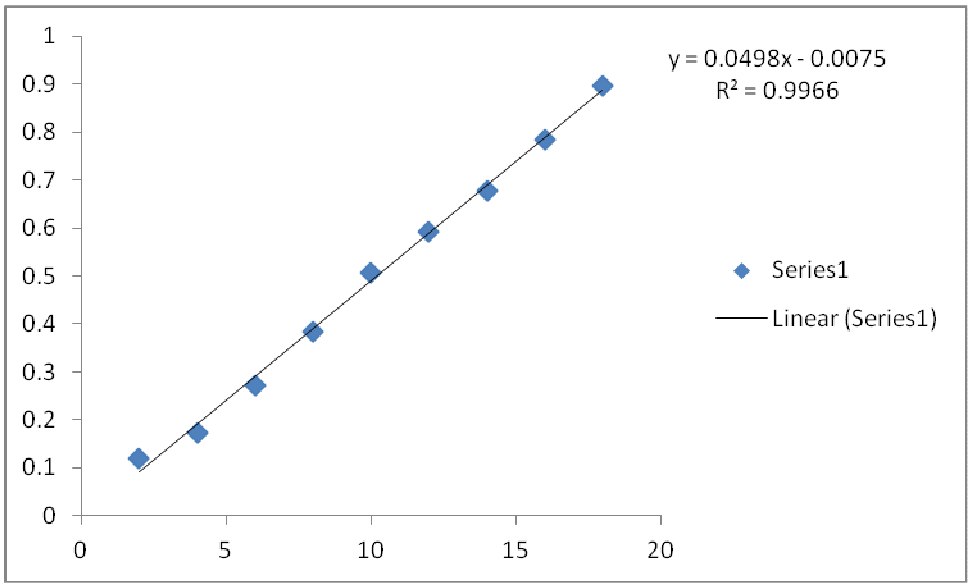


Figure 4: Calibration curve

Table no 3: Intra-day Precision

Sr. no	Concentration (ppm)	Absorbance	SD	%RSD
1	6	0.2969	0.0065	2.0
2	6	0.2992		
3	6	0.3077		
4	6	0.2922		
5	6	0.3070		
6	6	0.2913		
7	6	0.3032		

Table no 4: Inter-day Precision

Sr. no	Concentration (ppm)	Absorbance	SD	%RSD
1	6	0.3462	0.0026	0.77
2	6	0.3429		
3	6	0.3512		
4	6	0.3530		
5	6	0.3478		
6	6	0.346		
6	6	0.3454		

Table no 5: Ruggedness

Sr. no	Concentration (ppm)	Absorbance 1	Absorbance 11	SD 1	SD 11	%RSD 1	%RSD 11
1	6	0.2986	0.2986	0.005425	0.005808	1.84	1.99
2	6	0.2972	0.2806				
3	6	0.2854	0.2913				
4	6	0.2961	0.2886				
5	6	0.2869	0.2901				
6	6	0.2916	0.2954				
7	6	0.2979	0.2955				

Table no 6: Robustness

Sr. no	Concentration (ppm)	Absorbance 1	Absorbance 11	SD 1	SD 11	%RSD 1	%RSD 11
1	6	0.3050	0.3099	0.003714	0.0033	1.22	1.05
2	6	0.3066	0.3105				
3	6	0.2979	0.3123				
4	6	0.2991	0.3085				
5	6	0.3056	0.3175				
6	6	0.3059	0.3154				
7	6	0.3070	0.3151				

Table no 7: Recovery

Sr. no	Drug / Formulation	Percentage recovery			Mean	SD	%RSD
		50%	100%	150%			
1	Bulk	95.69	98.78	99.32	97.93	1.95	1.99
2	Tablet	96.0	97.4	99.29	97.56	1.63	1.67

Method Validation

Linearity and Range

The given method was obtained in range of 2-18 µg/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 0.999 and slope was found to be 0.0498

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%.

Ruggedness

The method was performed by changing analyst and the method was found to be rugged with standard deviation 0.005808 and relative standard deviation 1.99%.

Robustness

The robustness was performed by change in scanning speedand method was robust with standard deviation 0.0033 and relative standard deviation 1.05%.

LOD and LOQ

The LOD 0.2650and LOQ 0.8835ensures that the method is more sensitive and selective.

Accuracy and Recovery

The results within the range 96.00-99.00 ensure an accurate method.

CONCLUSION

The synthesis of a process-related impurity of Felodipine was successfully carried out by suitable synthetic procedure. Its characterization was carried out by IR, ^{1}H NMR and ^{13}C NMR. The result and statistical data states that the UV spectrophotometric method was found to be linear, precise, robust, rugged and accurate as per ICH guidelines.

Acknowledgement

Author wish to express their sincere thanks to Principal, SRES's Sanjivani College of Pharmaceutical education and research, Kopargoan for his constant encouragement and support.

REFERENCES

[1] British Pharmacopoeia, The Department of Health, British Pharmacopoeia Commission Office,volume I,**2011**, 1, 776-778.
[2] The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals, Merck Research Laboratories, Merck and Co., Inc **2001**,13, 3981-3982.
[3] K.D. Tripathi, Essential of Medical Pharmacology, Jaypee Brothers Medical Publishers (P) Ltd, New Delhi; **2008**,6, 181-182.
[4] V. Shinde, S. Gosavi, D. Musmade, S. Pawar, V. Kasture,*Indo American Journal of Pharmaceutical Research*, **2014**, 4, 2231-6876, 181-188.
[5] Validation of Analytical Procedure: text and methodology, in: International Conference on Harmonization (ICH), Q2 (R1), IFPMA, Genevia, Switzerland, **2005**.
[6] G.P. Jadhav, V.S. Kasture, S.S. Pawar, A.P. Lodha, A.R. Vadgaonkar, R.K. Ajage, S.G. Deshpande, *International Journal of Pharmacy and Pharmaceutical Sciences*, **2014**, 6, 7, 401-402
[7] D.A.Skoog., D.M.West, F.J.Holler, S.R.Crouch, Fundamentals of Analytical Chemistry, Thomson Brooks/cole, Singapore, **2004**,8 ,906-946.
[8] R. M.Silverstein, F. X.Webster, Spectrometric Identification of Organic Compounds, John Wiley and Sons publications, **2005**,6, 81-109.
[9] F. Settle, Handbook of Instrumental Techniques for Analytical Chemistry,Pearson Education, **1997**, 481-499.
[10] D. L. Pavia, G. M. Lampman, G. S. Kriz, J. R. Vyvyan, Spectroscopy, Cenage learning, New Delhi,**2007**,367-397.