WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 14, 558-574. Research Article ISSN 2277–7105

VALIDATION OF STABILITY INDICATING HPLC METHOD FOR THE ASSAY OF FELODIPINE (API).

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Article Received on
15 Sep. 2017,
Revised on 05 October 2017,
Accepted on 25 October 2017
DOI: 10.20959/wjpr201714-9927

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ABSTRACT

Three impurities were identified from Felodipine Active Pharmaceutical Ingredient (API). The method for analysis for felopdine is chosen high performance liquid chromatography (HPLC) and was validated according to ICH guidelines. The Retention time of standard solution is 12.952 mins and Retention time of sample solution is 13.028 mins. System precision of its the RSD is 0.05% Method precision the RSD is 0.30%. Overall RSD is 0.46% in Ruggedness. The test method is validated for Specificity, Precision and Ruggedness and found to be meeting the predetermined acceptance criteria. The validated method is Specific, Precise and Rugged for Assay of Felodipine API .Hence this method can be introduced into routine use for the assay of Felodipine API. The parameters such as HPLC, Specificity, Precision, Ruggedness, System Suitability and Linearity were analyzed.

KEYWORDS: HPLC, Specificity, Precision, Ruggedness, System Suitability and Linearity.

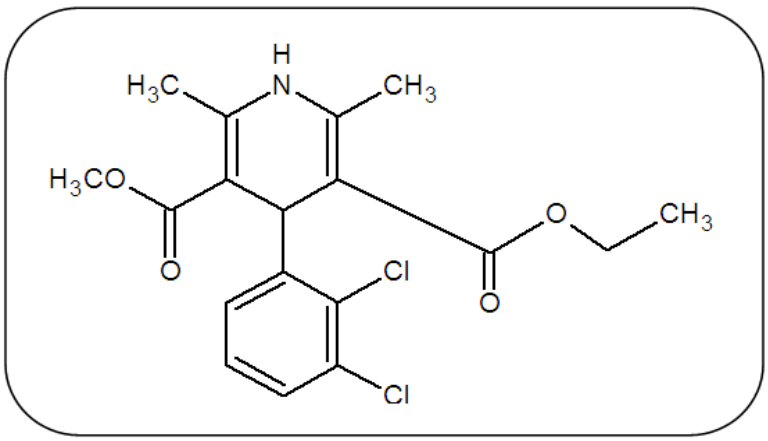
INTRODUCTION

Felodipine is a calcium antagonist (calcium channel blocker) of a dihydropyridine derivative. Felodipine is a slightly yellowish, crystalline powder. It is insoluble in water and is freely soluble in dichloromethane and ethanol. These potential advantages make it recommendable for the combination antihypertensive therapy to be used as initial treatment, particularly in

patients with target-organ break or sterner initial hypertension.[1-2] Calcium antagonists are vasodilatory and tend to increase plasma rennin, therefore combination with a β-blocker is theoretically sound.[3-4] Felodipine, with its intrinsically long half-life alone or together with β-blocker, is likely to produce superior ischemia reduction in clinical practice when patients frequently forget to take medication or take doses irregularly.[4-5]

In the European Union such the requirements are common and as regards the acceptable content of impurities (relative substances), the guidelines Q3A (R2)[6] and Q3B (R2)[7] for active substances and drug products respectively were adapted. Relative substances in drug substances and drug products, according to the mentioned guidelines, these are divided into: degradation products, unreacted raw materials, intermediates and process impurities originated from raw materials and finally by- products. According to the Rules Governing Medicinal Products in the European Community[8], validation is the action of proving, in accordance with the principles of good manufacturing practice that any procedure, process, equipment, material, activity or system actually leads to the expected results. Reproducible and accurate analytical results are a prerequisite throughout pharmaceutical development and manufacturing.[9, 10] Achieving these depends on the use of valid and robust methods. Critical factors that should be evaluated include; accuracy (as evidenced by selectivity, specificity and lack of bias), precision, recovery, linearity and system suitability.[11] The validation of cleaning methods is an important element of both qualification and process validation for drug substance and drug product manufacture.[12-13] The objective is to minimize the possibility of significant cross- contamination. Present study is undertaken knowing importance of analytical method of validation.

Structure



Chemical name: (±)-Ethyl methyl 4-(2, 3-dichlorophenyl)-1, 4-dihydro-2, 6-dimethyl-3, 5-pyridine dicarboxylate. **Molecular formula:** $C_{18}H_{19}Cl_2NO_4$; **Molecular weight:** 384.26.

A stability indicating HPLC method has been developed for the determination of percentage assay of Felodipine in Felodipine API. This report is intended for the validation of stability indicating HPLC method for the Assay of Felodipine in Felodipine API.

MATERIAL AND METHODS

Following equipments were used for the validation studies.

HPLC Systems - AR/VAL/HPLC-30, 31; Waters 2695 separation module; Waters 2996 PDA Waters Empower Software; Balance (AR/LAB-II/BALN-11); Columns: C18/AR/369, C18/AR/363.

***Felodipine standard*:** Use the standard as such and use 99.06 % potency on as is basis for calculations. This standard kept in tightly closed container. The Felodipine was available from Supriya Lifescience ltd, Mumbai.

***Reagents* :** Tertiary butyl alcohol (AR grade); Perchloric acid (AR grade); Monobasic sodium phosphate (AR grade); Phosphoric acid (AR grade); Acetonitrile (HPLC grade); Methanol (HPLC grade); Ceric sulfate (AR grade); Sodium hydroxide (AR grade); Methylene chloride (AR grade); Water (Milli Q or equivalent).

Methodology followed in analytical method validation

***Preparation of Buffer*:** Dissolve 6.9 g of monobasic sodium phosphate in 400 mL of water in a 1-liter volumetric flask. Add 8.0 mL of 1 M phosphoric acid, dilute with water to volume and mix.

Preparation of Mobile phase: Prepare a filtered and degassed mixture of Buffer, acetonitrile, and methanol in the ratio 40:40:20 Make adjustments if necessary.

***Preparation of Standard solution*:** Dissolve an accurately weighed quantity of about 30.0 mg of Felodipine standard to a 100 ml of volumetric flask, dissolve it and dilute with Mobile phase.

***Preparation of Resolution solution*:** Dissolve 150 mg of Felodipine in a mixture of 25 mL of tertiary butyl alcohol and 25 mL of 1 N perchloric acid, add 10 mL of 0.1 M ceric sulfate,

mix and allow standing for 15 minutes. Add 3.5 mL of 10 N sodium hydroxide and neutralize with 2 N sodium hydroxide. Shake the mixture with 25 mL of Methylene chloride in a separator. Draw off the lower layer, and evaporate it to dryness under a stream of nitrogen on a water bath. Dissolve 10 mg of the residue (Felodipine oxidation product / Impurity A) and 5 mg of Felodipine working standard in Mobile phase, dilute with Mobile phase to 100 mL and mix. Transfer 1.0 mL of the resulting solution to a 100-mL volumetric flask, dilute with Mobile phase to volume and mix.

***Preparation of Test solution*:** Transfer an accurately weighed quantity of about 30 mg of Felodipine to a 100-mL volumetric flask, dissolve in and dilute with Mobile phase to volume, and mix.

***Preparation of Impurity A Stock Solution*:** Weigh 1.5 mg of Impurity A in a 50 mL volumetric flask, add 30 mL of Mobile phase and sonicate to dissolve and make up volume with Mobile phase.

***Preparation of Impurity B Stock Solution*:** Weigh 3.0 mg of Impurity B in a 20 mL volumetric flask, add 10 mL of Mobile phase and sonicate to dissolve and make up volume with Mobile phase.

***Preparation of Impurity C Stock Solution*:** Weigh 3.0 mg of Impurity C in a 20 mL volumetric flask, add 10 mL of Mobile phase and sonicate to dissolve and make up volume with Mobile phase.

***Preparation of Felodipine Stock Solution*:** Weigh 3.0 mg of Impurity C in a 20 mL volumetric flask, add 10 mL of Mobile phase and sonicate to dissolve and make up volume with Mobile phase.

***Chromatographic system*:** Column: Waters Symmetry C18, 150 x 4.6 mm, 5 µ; Flow: 1.0 mL/minute; Detector: 254 nm; Run time: Twice the retention time of Felodipine peak.Retention time of Felodipine peak is about 14 minutes.

***Evaluation of system suitability*:** Inject resolution solution (20 µL) into the chromatograph and record the chromatograms. The resolution between Felodipine oxidation product (Impurity A) and Felodipine should not be less than 2.5. Inject standard solution (40 µL) five times into the chromatograph and record the chromatograms. Measure the area counts of

Felodipine peak. The relative standard deviation of five replicate injections should not be more than 2.0 %. The capacity factor, k', should not be less than 5.0, the column efficiency should not be less than 3000 theoretical plates &tailing factor should not be greater than 1.5.

***Procedure*:** Inject the Blank (40 µL) and sample solution (40 µL) in duplicate into the chromatograph. Record the chromatograms and measure the area counts for the Felodipine peak. Calculate the percentage of Felodipine by the formula.

Calculations

ATStd. wt. (mg)100 mL P

% Assay = ------ x ---------- x ---------- x -------- x 100

(as such)AS 100 mL Spl. Wt. (mg)100

Assay (as such)

% Assay (On dried basis) = ---------- x 100

100 - % LOD

Where as,

AT = Average area count of Felodipine peak in the chromatogram of sample solution.

AS = Average area count of Felodipine peak in the chromatogram of standard solution.

P = % Potency of Felodipine working standard on as is basis.

RESULTS AND DISCUSSION

Specificity*: Identification*: The retention time of the Felodipine peak in the chromatogram of the Sample preparation corresponds to that of the Felodipine peak in the chromatogram of the Standard preparation.[14] Retention time of Felodipine peak in standard solution is 12.952 mins. Retention time of Felodipine peak in sample solution is 13.028 mins.

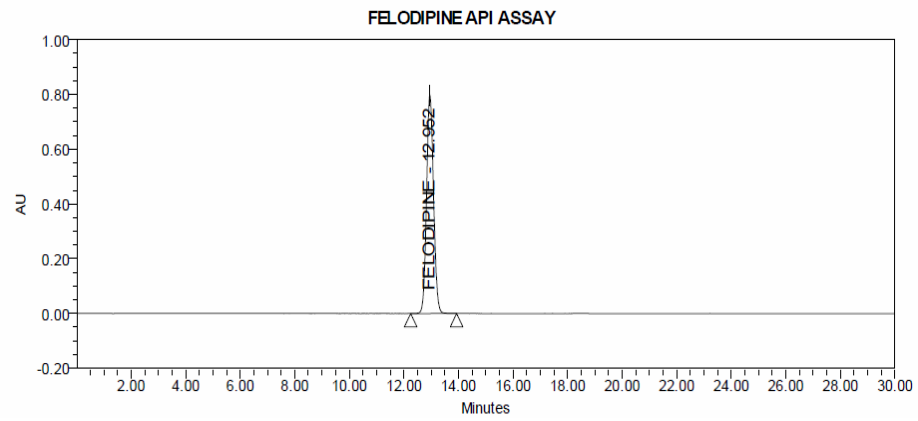
Table 1: Table for Specificity.

Sr. No.	Name	Purity Angle	Purity Threshold
1	Standard solution	0.110	1.018
2	Sample solution	0.120	1.018
3	Spiked Sample solution	0.069	1.004

The Felodipine peak is pure in Standard solution and Sample solution.

***Acceptance criteria*:** Blank and Placebo should not show any peak at the retention time of Felodipine Peak. No interference was observed from Blank and Placebo at the retention time of Felodipine peak. Also, The Felodipine peak is pure in Standard solution and Sample solution. Therefore, the HPLC method for the determination of Felodipine in Felodipine API

is specific.



Peak Results

Sample Name	RT	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
Standard-1	11.214					
Standard-1	12.952	14172238	100.0		1.1	12172

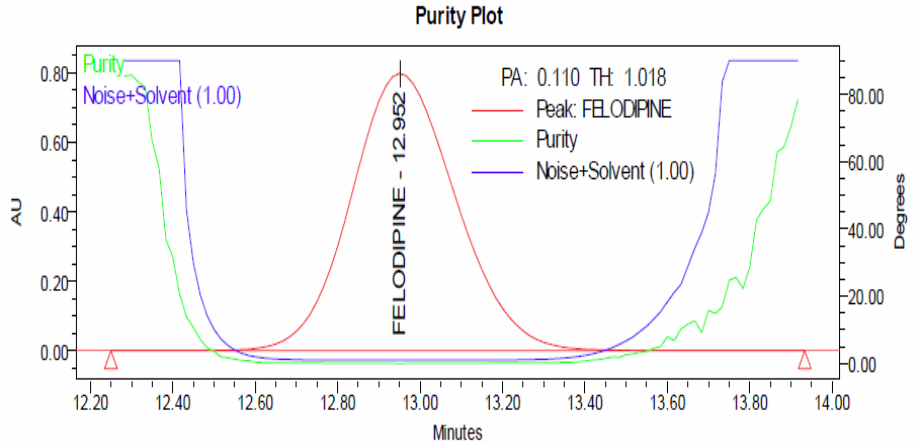
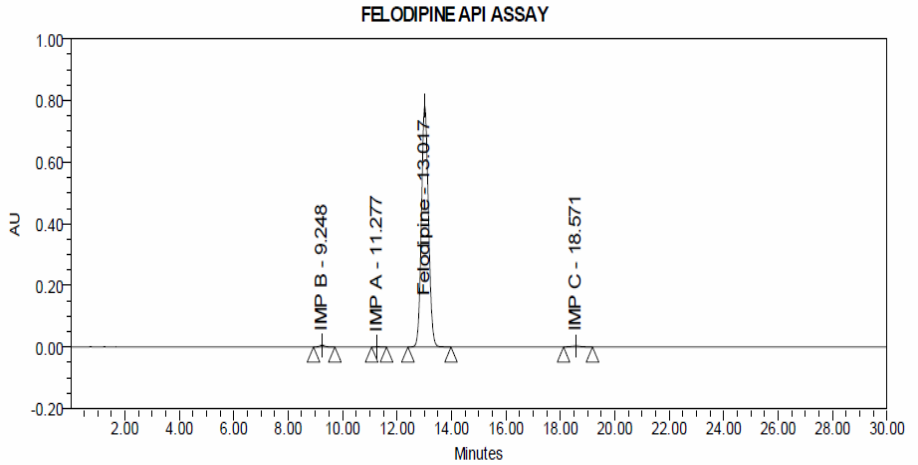
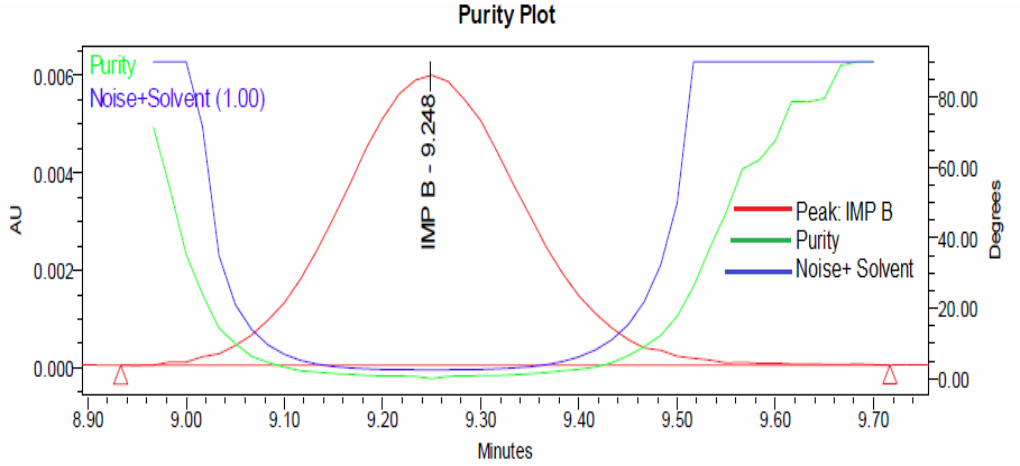


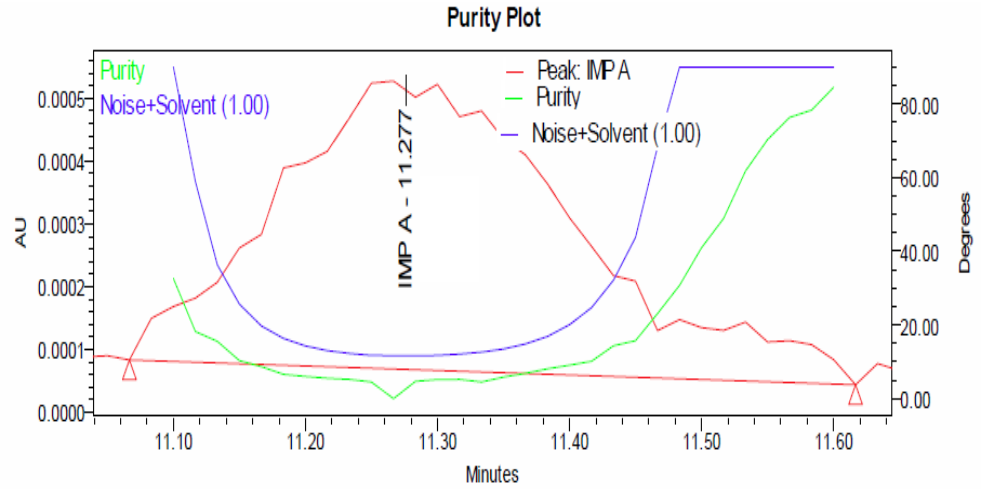
Fig 1: Standard solution.

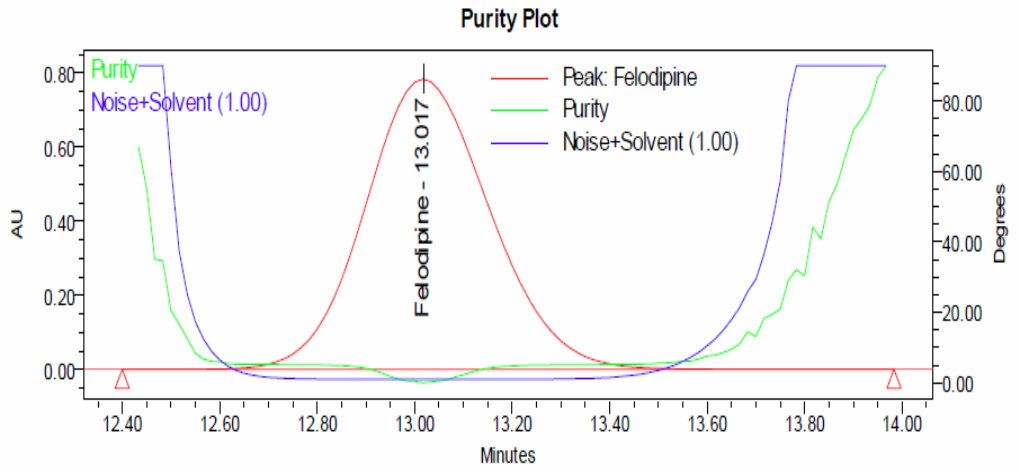


Peak Results

Sample Name	RT	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
Spiked Sample-1	9.248	78424	0.55		1.1	11189
Spiked Sample -1	11.277	7240	0.05	5.38	1.3	16782
Spiked Sample -1	13.017	14071615	98.87	3.94	1.1	12012
Spiked Sample -1	18.571	75444	0.53	9.81	1.0	12947







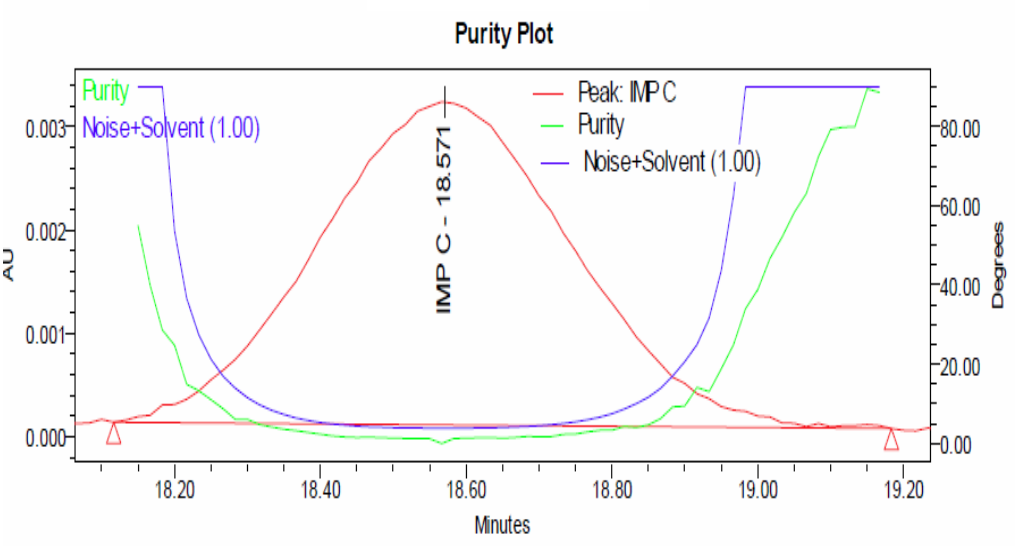
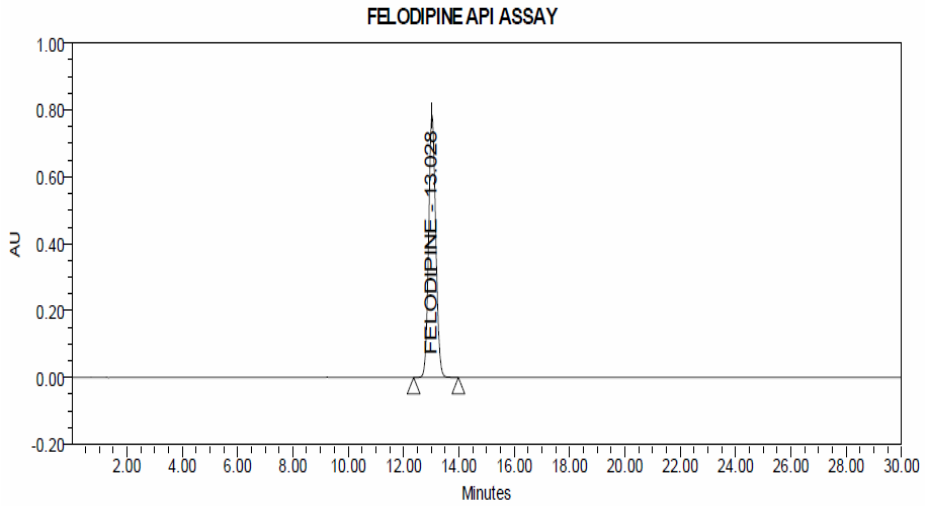


Fig 2: Spiked sample solution.

Blank interference: Prepared representative Placebo solutions, Standard solution and Sample solution of Felodipine API. Injected each of the Blank, Placebo solutions, Sample solutions and Standard solution in duplicate into the HPLC using the Chromatographic system as per the Methodology utilizing a photodiode array detector.



Peak Results

Sample Name	RT	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
Sample-1	11.214					
Sample-1	13.028	14066483	100.0		1.1	12046

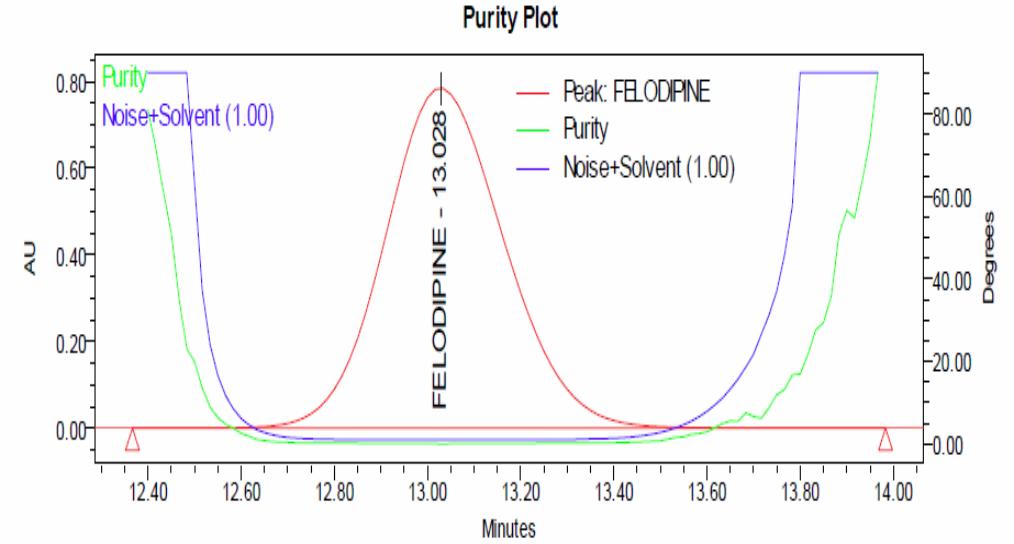


Fig 3: Sample solution.

Precision; System Precision: Five replicate injections of the Standard preparation were injected into the HPLC using the method as described under Methodology.

Table 2: Table for System Precision.

Injection	Area
1	14172238
2	14170992
3	14182519
4	14184616
5	14185838
Mean	**14179240.6**
SD	**6972.260**
%RSD	**0.05**

***Acceptance Criteria*:** RSD should not be more than 2.0%. The RSD of system precision is 0.05%. Therefore, the HPLC method for the determination of Felodipine in Felodipine API is precise.

Method Precision: Six sample solutions of Felodipine API were prepared and injected into the HPLC using the method as described under Methodology. HPLC Used: Waters Alliance, AR/VAL/HPLC-30, Column Used: C18/AR/369.

Table 3: Table for Method Precision.

Sample	% Label claim
1	99.7
2	100.1
3	99.7
4	99.4
5	99.3
6	99.9
Mean	**99.7**
SD	**0.299**
%RSD	**0.30**

***Acceptance Criteria*:** RSD should not be more than 2.0%. The RSD of method precision is 0.30%. Therefore, the HPLC method for the determination of Felodipine in Felodipine API is reproducible.

Ruggedness: Sixsample preparations of the same lot (as used in 2.2) of Felodipine APIwere made by a different analyst[15], using different column on a different day and injected in duplicate into a different HPLC (other than that used in 2.2) using the method as described under Methodology, along with Standard preparation. HPLC Used: Waters Alliance, AR/VAL/HPLC-31, Column Used: C18/AR/363.

Table 4: Table for Ruggedness.

Sample	Analyst -1 % Label claim	Analyst -2 % Label claim
1	99.7	99.8
2	100.1	99.9
3	99.7	99.3
4	99.4	100.6
5	99.3	100.4
6	99.9	100.6
Mean	**99.7**	**100.1**
SD	**0.299**	**0.522**
%RSD	**0.30**	**0.52**
Overall Mean	**99.9**	
Overall SD	**0.460**	
Overall%RSD	**0.46**	

***Acceptance criteria*:** Overall RSD for twelve results should not be more than 2.0%. The RSD of intermediate precision is 0.46%. Therefore, the HPLC method for the determination of Felodipine in Felodipine API is rugged.

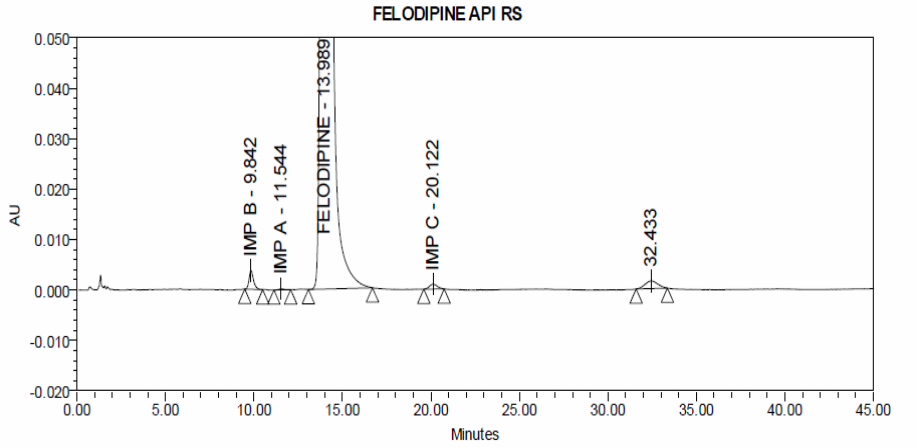
System Suitability: %RSD of five replicate injections, Retention time, USP Tailing and USP Tangent for Felodipine in Standard solution were maintained as per method on everyday.

Table 5: Table for System Suitability.

	%RSD of Standard preparation	Retention time	USP Resolution	USP Tailing	USP Tangent
Method precision	0.05	12.952	4.19	1.1	12172
Ruggedness	0.10	15.297	5.08	1.1	11564

Resolution solution: The resolution between Felodipine oxidation product (Impurity A) and Felodipine should not be less than 2.5.

***Standard solution*:** The relative standard deviation of five replicate injections should not be more than 2.0%. The capacity factor, k', should not be less than 5.0, the column efficiency should not be less than 4000 theoretical plates & the tailing factor should not be greater than 1.5.



Peak Results

Sample Name	RT	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
Impurity- B	9.842	65092	0.42			
Impurity- A	11.544	4339	0.03			
Felodipine	13.989	15170569	98.86			
Impurity -C	20.122	30311	0.20			
	32.433	75654	0.49			

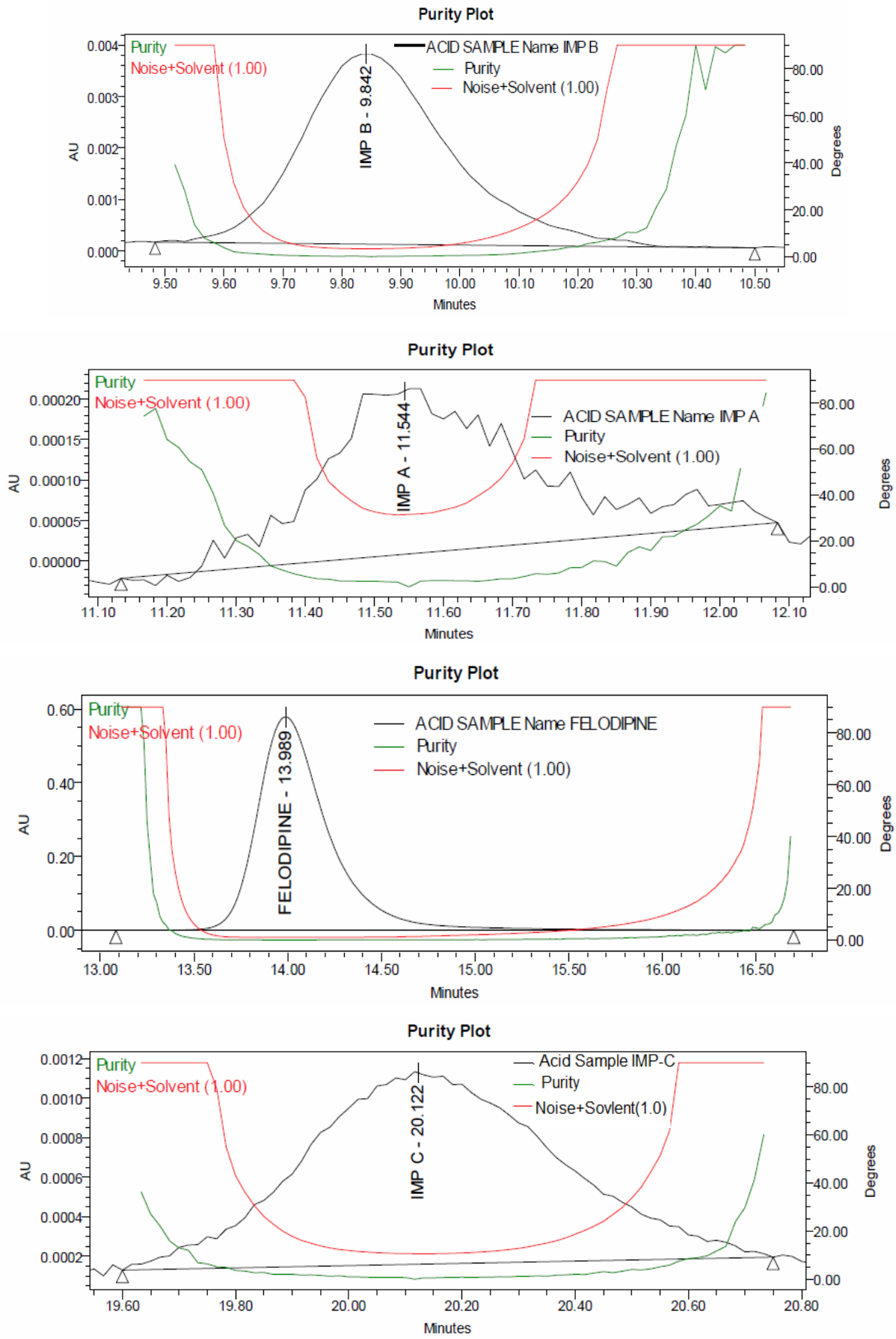


Fig. 4: Acid degradation – Sample

Linearity: A series of Standard preparations (minimum of five preparations) of Felodipine and impurity standards were prepared over a range of the LOQ to 150% of specification limit (taken as 0.1% of Impurity A, 1% of Impurity B and 1% of impurity C). Correlation coefficient should not be less than 0.99. The Correlation coefficient for Felodipine and known impurities is more than 0.99. Therefore, the HPLC method for the determination of related substances in Felodipine tablets is linear.

Sr. No.	Concentration (ppm)	Response
1	0.033	1849
2	0.777	40137
3	1.243	61859
4	1.554	77512
5	1.865	93767
6	2.331	116300
Correlation Coefficient		**0.99990**

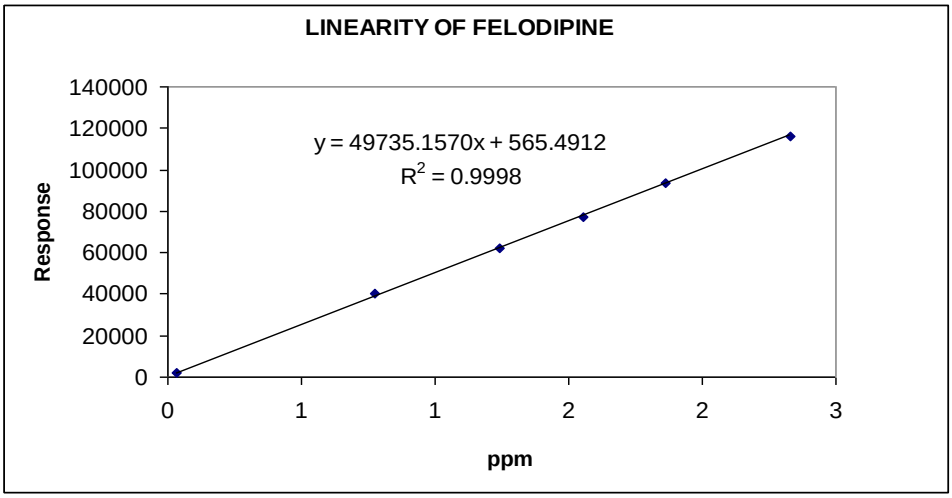


Fig 5: Linearity graph - Felodipine.

Sr. No.	Concentration (ppm)	Response
1	0.032	781
2	0.150	3320
3	0.240	5006
4	0.300	6260
5	0.360	7630
6	0.450	9576
Correlation Coefficient		**0.99963**

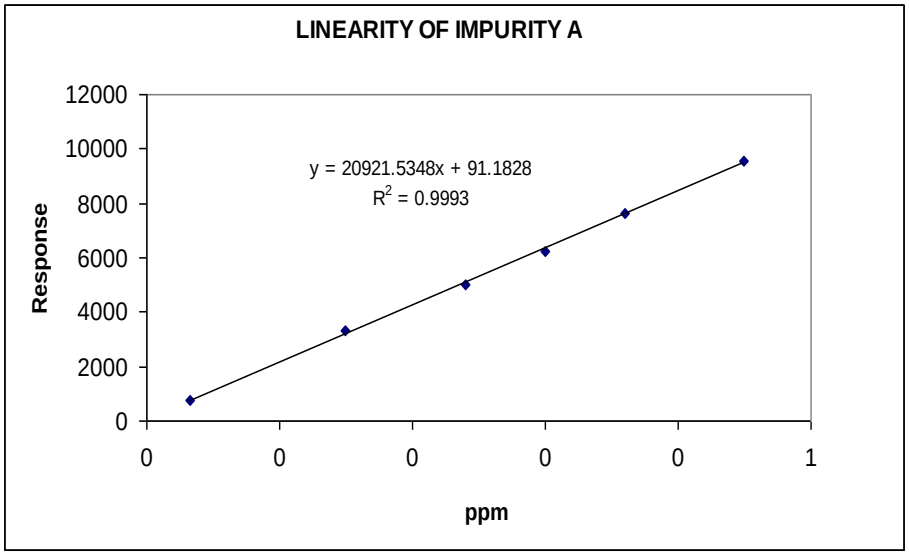


Fig. 6: Linearity graph – Impurity A.

Sr. No.	Concentration (ppm)	Response
1	0.017	724
2	1.554	81058
3	2.486	127008
4	3.108	158663
5	3.729	191265
6	4.661	238951
Correlation Coefficient		**0.99997**

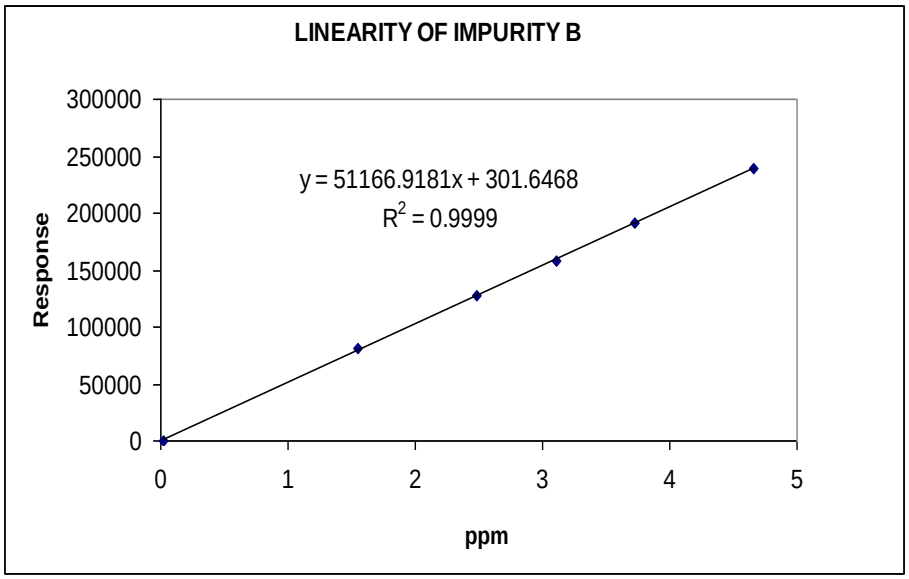


Fig. 7: Linearity graph – Impurity B.

Sr. No.	Concentration (ppm)	Response
1	0.033	1752
2	1.551	77292
3	2.481	122574
4	3.101	152891
5	3.722	184524
6	4.652	230104
Correlation Coefficient		**0.99999**

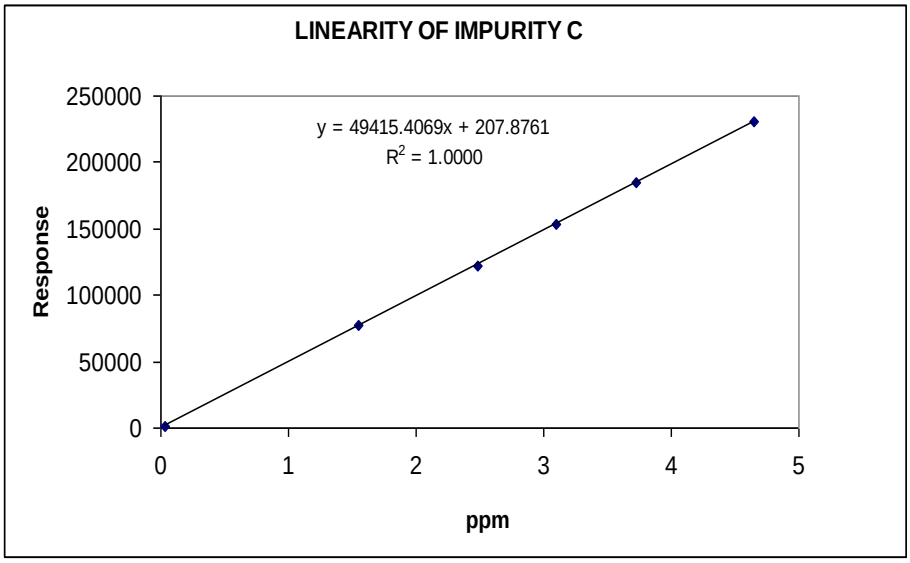


Fig 8: Linearity graph – Impurity C.

CONCLUSIONS

The Felodipine (API) sample contain Three impurities, Results should be comparable with respect to Retention time. Retention time of standard solution is 12.952 mins and Retention time of sample solution is 13.028 mins. System precision of its the RSD is 0.05% Method precision the RSD is 0.30%. Overall RSD is 0.46% IN RUGGEDNESS. The test method is validated for Specificity, Precision and Ruggedness and found to be meeting the predetermined acceptance criteria. Resolution between Felodipine oxidation product (Impurity A) and Felodipine should not be less than 2.5. The capacity factor, k', should not be less than 5.0, the column efficiency should not be less than 4000 theoretical plates and the tailing factor should not be greater than 1.5. The relative standard deviation of five replicate injections should not be more than 2.0%. The validated method is Specific, Precise and Rugged for Assay of Felodipine API. Hence this method can be introduced into routine use for the assay of Felodipine API. Correlation coefficient should not be less than 0.99. The Correlation coefficient for Felodipine and known impurities is more than 0.99. Therefore, the HPLC method for the determination of related substances in Felodipine tablets is linear.

AKNOWLEDGEMENT

The authors wish to special thanks, Satish Wagh, MD, Supriya Lifescience Ltd, Shivani Wagh, Prashant Zate, Supriya Lifescience limited, for providing necessary facilities for research work. We Thanks to PAHER, Udaipur For registration for Ph.D degree and all members who supports to this work.

REFERENCES

1. Moser, M. Current Recommendations for Initial Therapy in Hypertension: Are They Still Valid?: Introduction. American Journal of Hypertension, 1998; 11: 69S-72S.
2. Moser, M and Black, H.R. The Role of Combination Therapy in the Treatment of Hypertension. American Journal of Hypertension, 1998; 11: 73S-78S.
3. Waeber, B. Detry, J.M and Dahl of, B. Felodipine–metoprolol combination tablet: A valuable option to initiate antihypertensive therapy. American Journal of Hypertension, 1999; 12: 915-920.
4. Davies, R.F. Habibi, H and Klinke, W.P. Effect of amlodipine, atenolol and their combination on myocardial ischemia during treadmill exercise and ambulatory monitoring. Journal of the American College of Cardiology, 1995; 25: 619- 625
5. Lokhande, M.V. Rathod, N.G. Gupta, M.K., Identification and structural elucidation of process related impurities in duloxetine hydrochloride. International Journal of Chemistry and Pharmaceutical Sciences, 2013; 4(2): 34-43.
6. International Conference on Harmonization ICH Topic Q 3 A (R2) Impurities in new Drug Substances, June 2017.
7. International Conference on Harmonization ICH Topic Q 3 B (R2) Impurities in New Drug, June 2017.
8. Commission of the European Communities. The rules governing medicinal products in the European community, volume IV Good manufacturing practice for Medicinal products. Luxembourg, The commission, 1992; 162-163.
9. Commission of the European Committees. The rules governing medicinal products in the European Community, VolumeIII. Addendum note for guidance analytical validation. Luxembourg: The commission, 1990.
10. Pasteelnick, L.A. Drugs and the pharmaceutical sciences, Pharmaceutical Process Validation. Marcel Dekker, New York. 1993; 97: 411-418.
11. The Pharmaceutical Codex; principles & practice of pharmaceutics, 12th Ed, 1994; 396.

12. Pandey, V.P. Soma Sekhar, K and Shenoy, K.R.P. Analytical Method Validation And Cleaning Verification of Felodipine By HPLC Method. Pharmacology online, 2010; 2: 362-370.
13. Lokhande, M.V. Rathod, N.G and Gupta, M.K. Structural elucidation, Identification, quantization of process related impurity in Hydralazine Hydrochloride HR/AM- LC MS/MS, NMR and FTIR technique. Journal of Applied Chemistry, 2013; 6(2): 05-15.
14. Gupta, M.K and Lokhande, M.V., Structure Determination Of Impurity In Memantine Hydrochloride By Analytical Techniques . International Journal of General Medicine and Pharmacy, 2014; 3(4): 75-84.
15. Zate, P.B. Kothari, S and Lokhande, M.V. Confirmation and Quantification of Genotoxic Impurity 2-Dimethylaminoethyl chloride hydrochloride by GCMS in Chlorpheniramine /Chlorphenamine Maleate, Journal of Applied Chemistry, 2017; 10(7): 21-26.