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Characterization and Validation of Impurities in Pharmaceutical Bulk Drug by HPLC Methods

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Abstract: *Three impurities were identified by HPLC methods. These impurities have process related or batch impurities. The identified impurities were found by two different chromatograms isolated by HPLC method. These impurities were not more than 0.3% and unspecified impurities are not more than 0.1%. These impurities were identified by using HPLC system; AR/VAL/HPLC-30, 31, Columns: C-18/AR/363, C-18/AR/369; Vacuum Oven (AR/LAB-I/VACO-01).Humidity Dessicator (AR/LAB-II/HDCR-01). Linearity range for Felodipine is 0.9990. LOD for Felodipine 0.003 & LOQ0.011, for Impurity-A, 0.003 &0.011, Impurity -B, 0.002 &0.005 and Impurity –C, 0.003 & 0.011. % RSD Values for Felodipine, impurities ABC are 4.30,5.87, 4.43 and 9.51. We have also calculated some parameters for validation such as identification, specificity, linearity, precision and system suitability.*

Keywords: *HPLC method, Validation, linearity, LOD, LOQ and force degradation method.*

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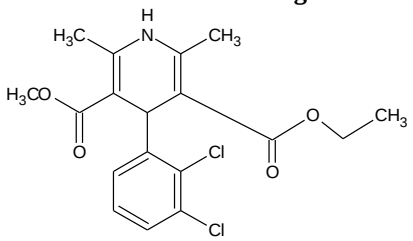
I. Introduction

Felodipine (drug) is a calcium antagonist (calcium channel blocker) and is a dihydropyridine derivative with a racemic mixture. Buccal delivery of drugs provides an attractive alternative to the oral route of drug administration particularly in overcoming the problem related with the later technique of dosing. Issues like initially pass digestion and drug degradation in the vicious gastrointestinal condition can be dodged by regulating sedate by means of buccal route [1] . Moreover, the oral cavity is effortlessly open for self medicine and can be instantly ended if there should arise an occurrence of poisonous quality just by expelling the dose shape from buccal hole. It is additionally conceivable to control drugs to patients who can't be dosed orally by means of this route [2-3] . The most essential objective in mucoadhesion comprises of drug focusing on, controlled and supported discharging, expanding of habitation time and bioavailability and diminishing of antagonistic effect [4-5]. Felodipine is practically totally retained from the gastrointestinal tract after oral measurements in any case, experiences broad initially pass digestion, with a bioavailability of around 15%. It is widely used in the gut and the liver furthermore, is discharged altogether as metabolites, around 70% of a measurement being discharged in pee and leftover portion in faces [6-7]. Keeping in mind the end goal to beat such first pass digestion and poor bioavailability, the drug is chosen as reasonable possibility for bio glue buccal drug conveyance.

II. Material and Methods

Structure of drug



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

A HPLC method was developed for determination of percentage related substances of Felodipine API. This report is intended for validation of an HPLC method for related substances of Felodipine in Felodipine API.

Impurities will be quantified as known Impurities – Impurity- A, Impurity -B and Impurity-C. All other peaks will be considered as unknown impurities[8].

Instruments and Reagents: Following equipments were used for the validation studies.
HPLC SYSTEM - AR/VAL/HPLC-30, 31 ;Waters 2695 separation module ; Waters 2996 PDA; Waters 2487 dual absorbance detector; Empower software ;pH Meter (AR/LABII/PHMT-02,03) ;Balance (AR/LABII/ BALN-05,08,AR/VAL/ BALN-11) ;Columns: C18/AR/363, C18/AR/369 ;Vacuum Oven (AR/LAB-I/VACO-01); Humidity Dessicator (AR/LAB-II/HDCR-01).*Standard:* Use the standard as such and use % potency on as is basis for calculations. Keep the container tightly closed. *Impurity- A standard*: Use the standard as such and use % potency on as is basis for calculations. Keep the container tightly closed. *Impurity- B standard*: Use the standard as such and use % potency on as is basis for calculations. Keep the container tightly closed. *Impurity-C standard*: Use the standard as such and use % potency on as is basis for calculations. Keep the container tightly closed. *Standard Potency is* 99.6: Impurity- A Standard, Potency-97.95%: Impurity -B Standard ; Potency-99.6% : Impurity -C Standard; potency-99.4%.**(Figure-4,5,6)***Reagents:* $(CH_3)_3$-C-OH (AR grade); $HClO_4$ (AR grade) :Na_3PO_4 (AR grade) CH_3CN (HPLC grade) : CH_3OH (HPLC grade) ;Ceric sulfate (AR grade) NaOH (AR grade) CH_3Cl (AR grade) ; Water (Milli Q or equivalent).*Preparation of Buffer***:** Dissolve 6.9 gms 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.

Procedure: Inject the Blank and sample solution (40 µL) into the chromatograph. Record the chromatograms, and measure the area counts for the impurity peaks. Disregard the peak due to blank.

Order of Elution:

Sr. No.	Name	RRT
1.	Impurity B	About 0.70
2.	Impurity A	About 0.82
3.	Felodipine	-
4.	Impurity C	About 1.40

RRTs (Relative Retention Times) are with respect to Felodipine Peak.

Calculations: Calculate the percentage of each impurity by the formula:

$$\% \text{ Impurity} = \frac{AT}{AS} \times \frac{\text{Std. wt. (mg)}}{100\text{ mL}} \times \frac{5\text{ mL}}{100\text{mL}} \times \frac{5\text{ mL}}{50\text{ mL}} \times \frac{100\text{ mL}}{\text{Spl. Wt. (mg)}} \times \frac{P}{100} \times 100$$

Where,
AT = Area count of impurity peak in the chromatogram of sample solution.
AS = Average area count of drug peak in the chromatogram of diluted standard solution.
P = % Potency of drug working standard on as is basis.

III. Result and Discussions

Specificity: The ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Analytical techniques that can measure the analyte response in the presence of all potential sample components should be used for specificity validation. It is not always possible to demonstrate that a single analytical procedure is specific for a particular analyte. In this case a combination of two or more analytical procedures is recommended to achieve the necessary level of discrimination. A frequently used technique in pharmaceutical laboratories is high performance liquid chromatography and to some extent gas chromatography. In specificity of developed HPLC technique for determination of drug in bulk drug and pharmaceutical research was investigated by chromatographic study. A typical HPLC chromatogram of drug standard preparation and in test sample. The HPLC chromatograms recorded for mixture of inactive ingredients revealed no peaks within retention time around 10.5 minutes, and peak purity was 99.99%. The peak purity index show that Felodipine in is clearly separated from reply of any interfering peak(s)[9]. **(Table-2,3)**

Blank Interference: Injected Blank in an HPLC using the Chromatographic system described in the Methodology by using a photodiode array detector. *Acceptance criteria***:** Blank should not show any peak at the retention time of drug Peak. *Conclusion***:** No interference was observed from Blank at the retention time of Felodipine and known impurity peaks. **(Figure-1)**

Identification : Prepared a representative Standard solution and Sample solution of drug API as per the Methodology. Injected System Suitability solution, Standard Solution, Sample Solutions, individual impurities solutions and Spiked Sample Solution as above in HPLC using the Chromatographic system described in the Methodology by using a photodiode array detector. *Acceptance Criteria***:** Results should be comparable with respect to Retention time. Peak purity should pass for drug and known impurities in control sample and spiked sample. Peak purity passes for Felodipine and known impurities in control sample and spiked sample. Hence the method is Selective [10]. **(Figure-2,3 & Table-1)**

LOD/LOQ: The limit of quantitation (LOQ) of an individual analytical procedure as lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices and is used particularly for determination of impurities or degradation products. The quantitation limit is generally determined by analysis of samples with known concentrations of analyte and by establishing minimum level at which analyte can be quantified with acceptable accuracy and precision.. If the required precision of the method at the limit of quantitation has been specified, 5 or 6 samples with decreasing amounts of the analyte are injected six times. The amounts range from the known LOD as determined above to 20 times the LOD. the detection limit of an individual analytical procedure as the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The limit of detection (LOD) is the point at which a measured value is larger than the uncertainty associated with it. It is lowest concentration of analyte in a sample that can be detected but not necessarily quantified. The limit of detection is frequently confused with sensitivity of method. The sensitivity of an analytical method is capability of method to discriminate small differences in concentration or mass of test analyte. In practical terms, sensitivity is the slope of the calibration curve that is obtained by plotting response against analyte concentration or mass. Based on determination of Prediction linearity, six replicate injections were made for LOD &LOQ[11]. **(Table-4 ,5)**

Linearity: linearity of an analytical procedure as its ability to obtain test results that are directly proportional to concentration of analyte in sample. Linearity may be demonstrated directly on test substance or by separately weighing synthetic mixtures of test product components. Linearity is determined by a series of five to six injections of five or more standards whose concentrations span 80–120 percent of expected concentration range. The response should be directly proportional to the concentrations of the analytes or proportional by means of a well-defined mathematical calculation. A linear regression equation applied to results should have an intercept not significantly different from zero. If a significant nonzero intercept is obtained, it should be demonstrated that, this has no effect on the accuracy of method [12]**. (Figure- & Table-)**

Experiment**:** A series of Standard preparations (minimum of five preparations) of drug and impurity standards were prepared over a range of the LOQ to 150% of specification limits (taken as 0.1% of Impurity A, 1% of Impurity B and 1% of impurity C).*Acceptance criteria***:** 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 drug tablets is linear.

System Suitability : The resolution between drug oxidation product (Impurity A) and drug peaks in resolution solution was recorded as per method everyday. Also % RSD for six replicate injections, capacity factor, USP Tailing and USP Tangent was recorded. The resolution between drug oxidation product (Impurity A) and drug should not be less than 2.5[14]. **(Figure- 8 & Table-7)**

Diluted standard solution: The relative standard deviation of six replicate injections should not be more than 5.0 %. The capacity factor, k', should not be less than 5.0, the column efficiency should not be less than 6000 theoretical plates and the tailing factor should not be greater than 1.5. System suitability complies for all parameters.(**Figure- 9**)

Forced Degradation Studies: Forced degradation studies are undertaken to degrade active drug deliberately. These studies are used to evaluate an analytical method's ability to measure an active ingredient and its degradation products without interference. Samples or drug product (spiked placebo) and drug substance are exposed to acid, base, oxidizing agent, reducing agent and water. The degraded samples were then analyzed using method to determine if there are interferences with the active. Thus, stability-indicating property was evaluated. **(Table-8,9)**

i) Acid Degradation (5N HCl) :Transferred an accurately weighed quantity of about 30 mg of Felodipine to a 100-mL volumetric flask, added 50 ml of mobile phase and sonicated to dissolve. Added 5 mL of 5N HCl and heated at 70°C for 3 hours. Added 5 mL of 5N NaOH to neutralize the solution. Cooled to room temperature and diluted to volume with Mobile phase and mixed. **(Figure-10)**

ii)Base Degradation(2N NaOH):Transferred an accurately weighed quantity of about 30 mg of Felodipine to a 100-mL volumetric flask, added 50 ml of mobile phase and sonicated to dissolve. Added 5 mL of 2N NaOH and heated at 70°C for 3 hours. Added 5 mL of 2N HCl to neutralize the solution. Cooled to room temperature and diluted to volume with Mobile phase and mixed. **(Figure-11)**

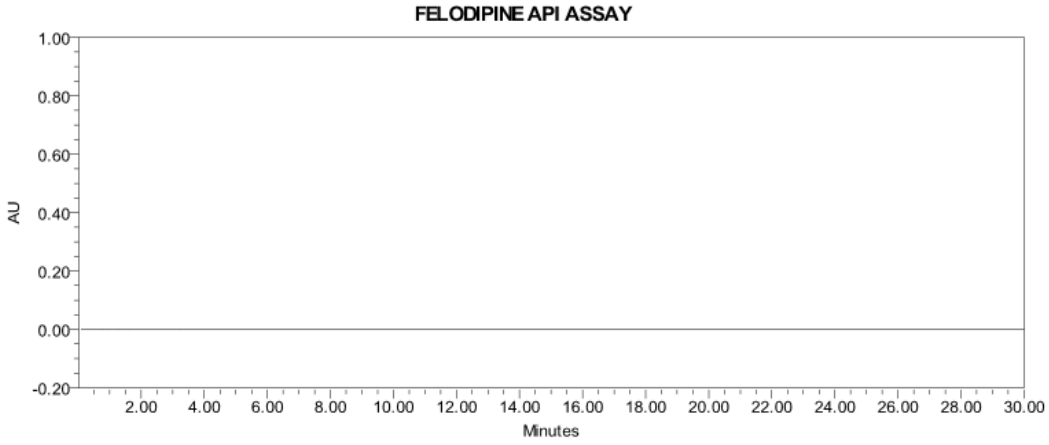
iii)Peroxide Degradation (30% H_2O_2) :Transferred an accurately weighed quantity of about 30 mg of Felodipine to a 100-mL volumetric flask, added 50 ml of mobile phase and sonicated to dissolve. Added 5 mL of 30% H_2O_2 and heated at 70°C for 3 hours. Cooled to room temperature and diluted to volume with Mobile phase and mixed. **(Figure- 12)**

iv) Thermal Degradation (105°C/72 hours) : To study the effect of temperature, approximately 50 mg diacerein was exposed at 105°C for 72 hours. It was then dissolved in 10 ml of methanol and the volume was adjusted to 50 ml with the mobile phase. The above solution was further diluted with the mobile phase, to give a solution of final concentration equivalent to 10 µg/ml. **(Figure-13)**

***v)Humidity Degradation (25°C/92%RH for 72 hours)* :** Thermal degradation test should be performed on active pharmaceutical ingredient and doses form with or without humidity. Solid drug substance, placebo and drug product samples should be exposed to heat with and without humidity. Take extra precaution while applying heat stress to liquid samples, if you are going to prepare sample by diluting further due to heat sample may loss water and concentration of drug substance in solution may change. Information on rate of degradation of primary and secondary degradation products can be understand by generating multiple time points results. In the event of stress conditions that produce insignificant degradation or not degrade due to stability of molecule, we should apply total energy equivalent to energy applied by accelerated conditions to show sufficient scientific effort to achieve degradation.Sample was exposed at 25°C/92%RH humidity condition for at least 72 hrs. & analyzed. **(Figure-14-)**

vi)Photolytic Degradation (Light) : This is very important degradation step for light sensitive molecules, but irrespective to light sensitivity, we should evaluated all molecules to identify any unacceptable change due to light exposure. Drug sample should be exposed to UV-VIS light providing an overall exposure of not less than 1.2 million lux hours and an integrated near ultraviolet energy of not less than 200 watt hours/square meter. It is preferable to expose same samples to cool white fluorescent and near ultraviolet lamp. *Acceptance criteria:* Felodipine peak should be homogeneous and there should be no co-eluting peaks. Peak purity for analyte peak should pass. The peak purity data of Felodipine peak in every degradation sample shows that the Felodipine peak and all known impurity peaks are homogeneous and there are no co-eluting peaks indicating that the method is stability indicating and specific. **(Figure-15)**

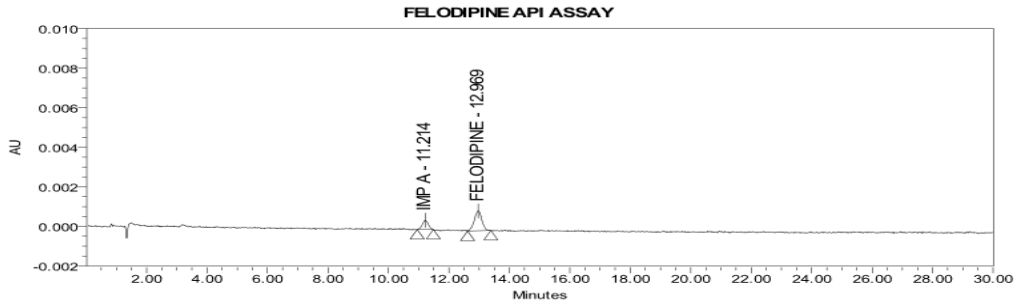
IV. Tables and Figures



Peak Results

	Sample	RT	Area	% Area	USP Resolution	USP Tailing	USP Plate
1	Blank	11.214					
2	Blank	12.969					

Figure-1: Chromatogram Blank



Peak Results

	Sample	RT	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
1	Resolution	11.214	6375	26.96		1.0	14566
2	Resolution	12.969	17270	73.04	4.19	0.9	16615

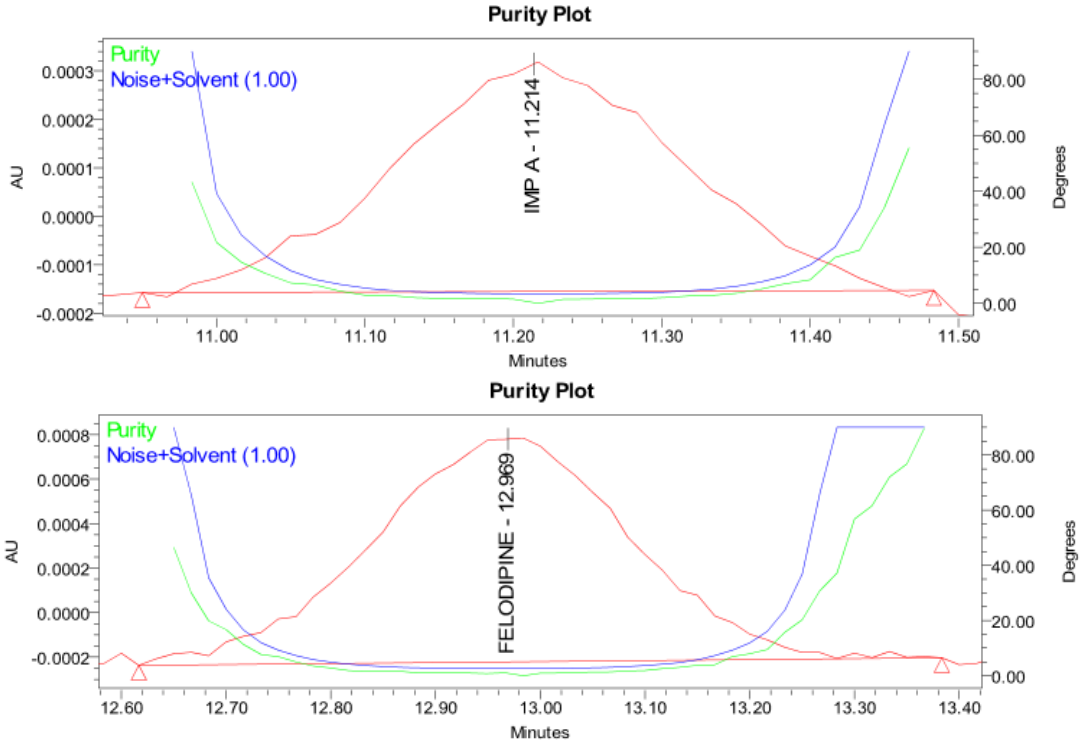


Figure-2: Resolution solution

Table-1: Identification by Retention time

Name	Retention time (min)
Felodipine	15.257
Impurity A	12.491
Impurity B	10.657
Impurity C	22.098

Peak Results

Sample	RT	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
Standerd-1	12.952	14172238			1.1	12172

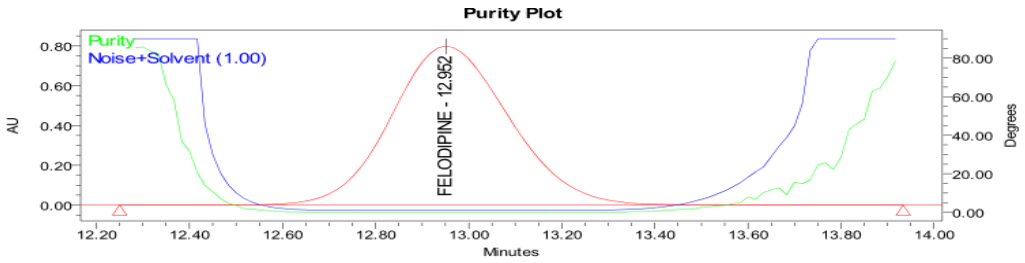
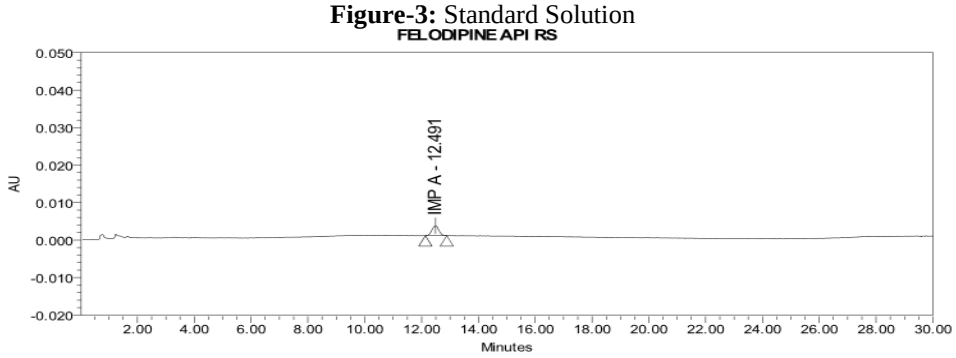


Figure-3: Standard Solution



Peak Results

Sample	RT	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
Impurity-A	12.491	44564	100.0		1.0	11044

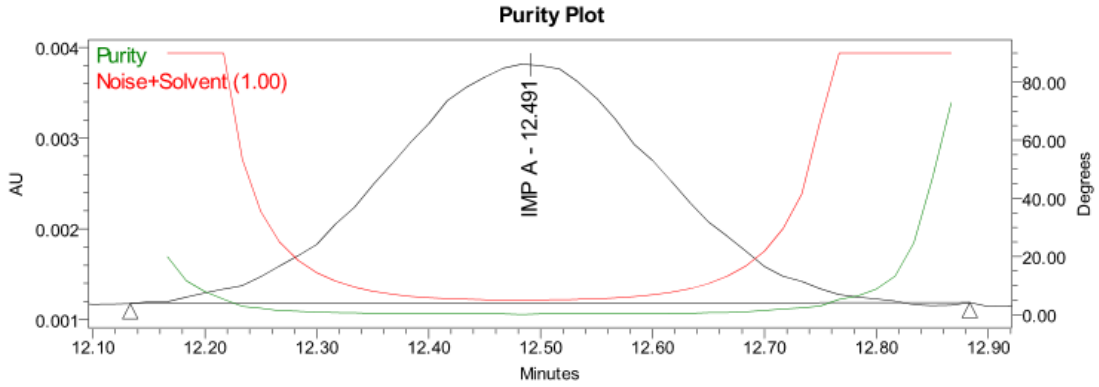
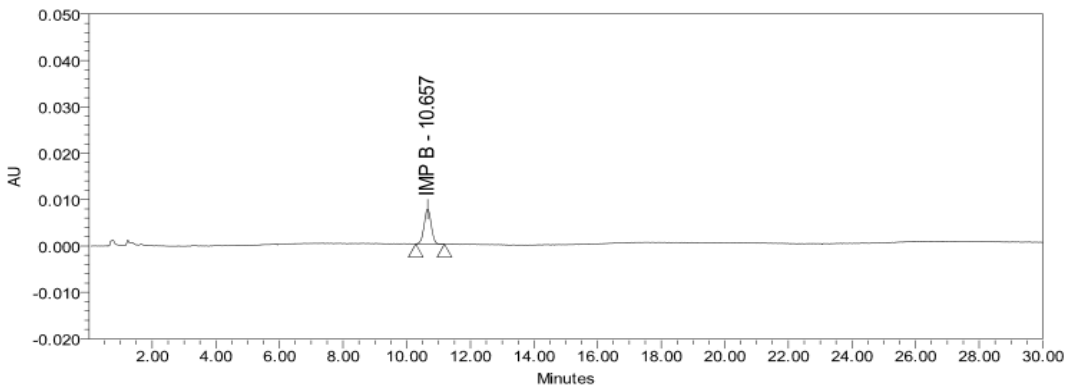


Figure -4: Chromatogram of Impurity- A



Peak Results

Sample	RT	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
Impurity-B	10.657	117010	100.0		1.0	10689

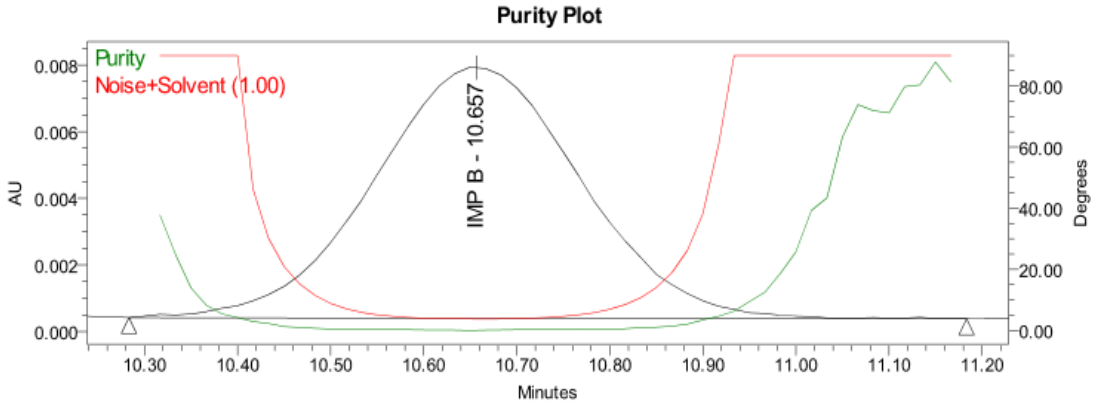
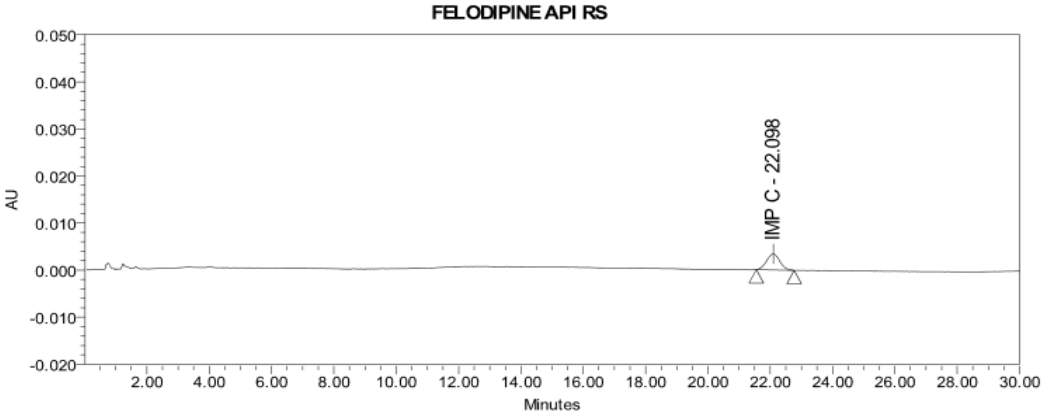


Figure-5: Chromatogram of Impurity- B



Peak Results

Sample	RT	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
Impurity-C	20.098	101473			1.0	13645

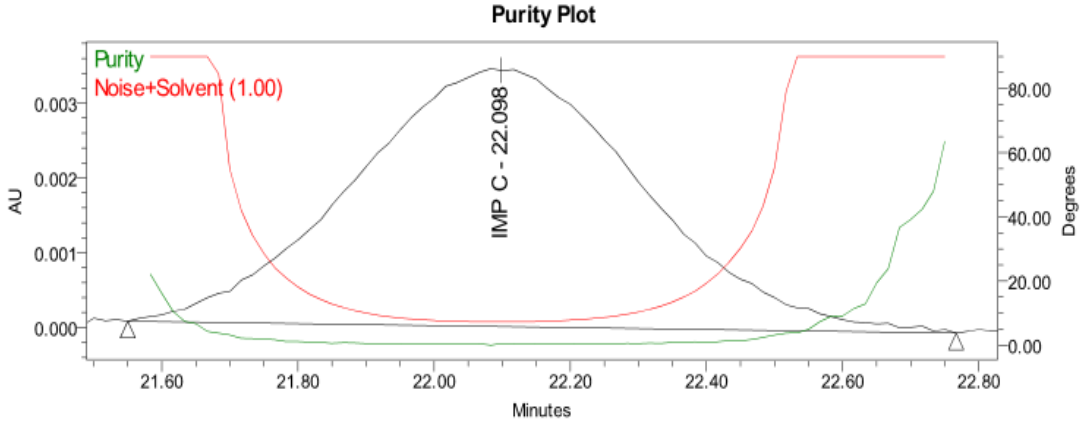
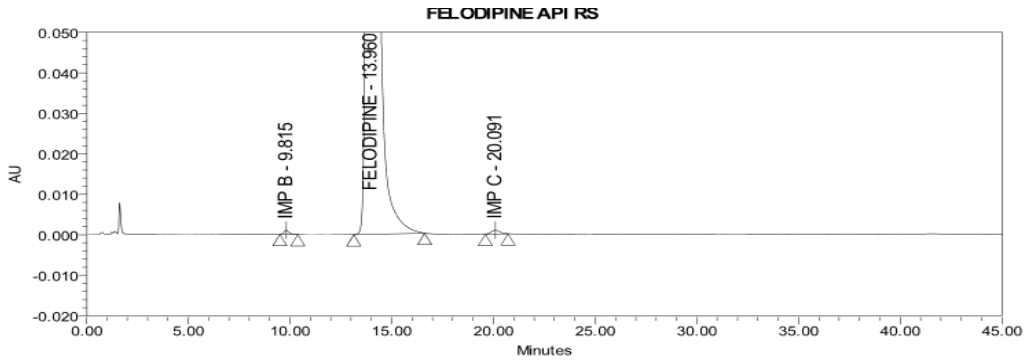


Figure-6 : Chromatogram of Impurity- C

Table -2: Peak purity of standard and Control sample

Sample	Felodipine	
	Purity angle	Purity Threshold
Standard - Felodipine	0.672	6.762
Sample solution	0.036	1.039



Peak Results

Sample	RT	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
Impurity-B	9.815	18966	0.13		1.4	7952
Felodipine	13.960	15096437	99.68	7.15	1.5	7285
Impurity-C	20.091	29002	0.19	8.58	1.1	9840

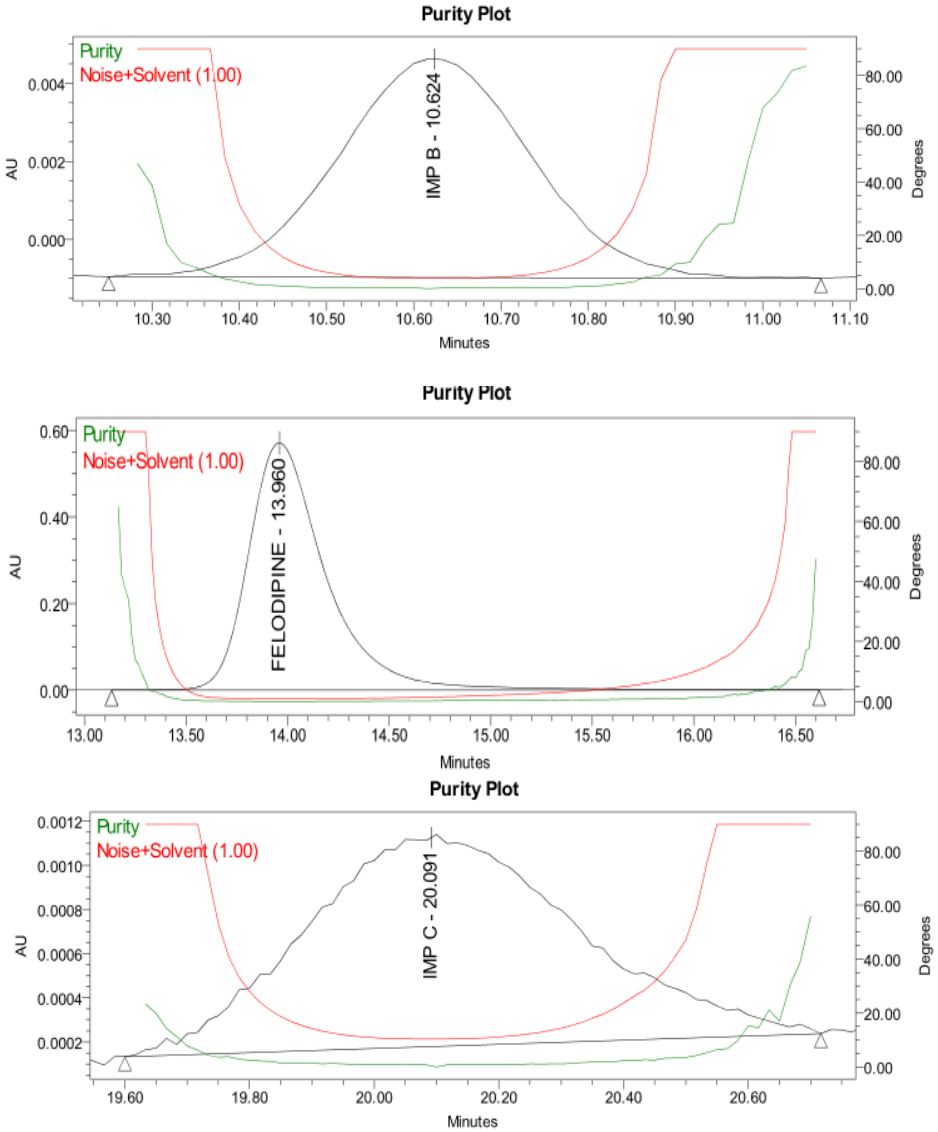
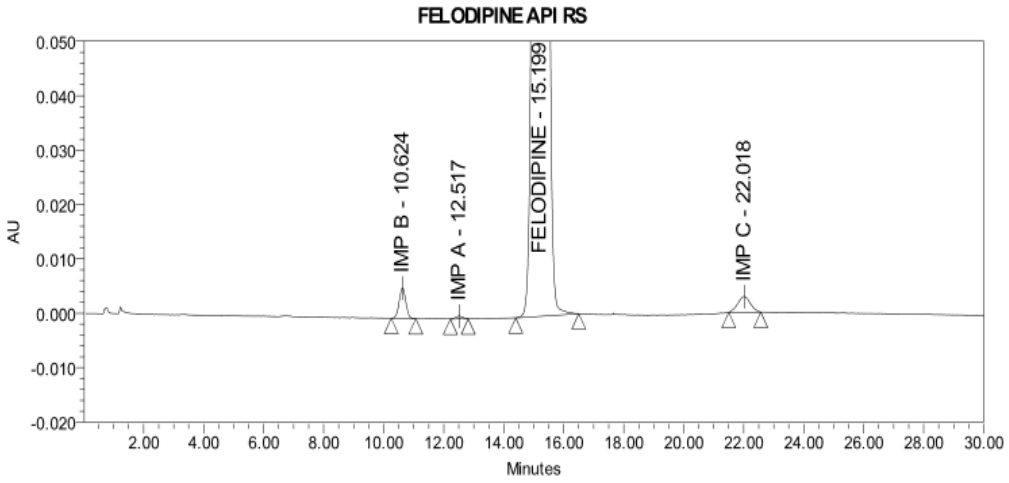


Figure-7: Sample of Felodipine API

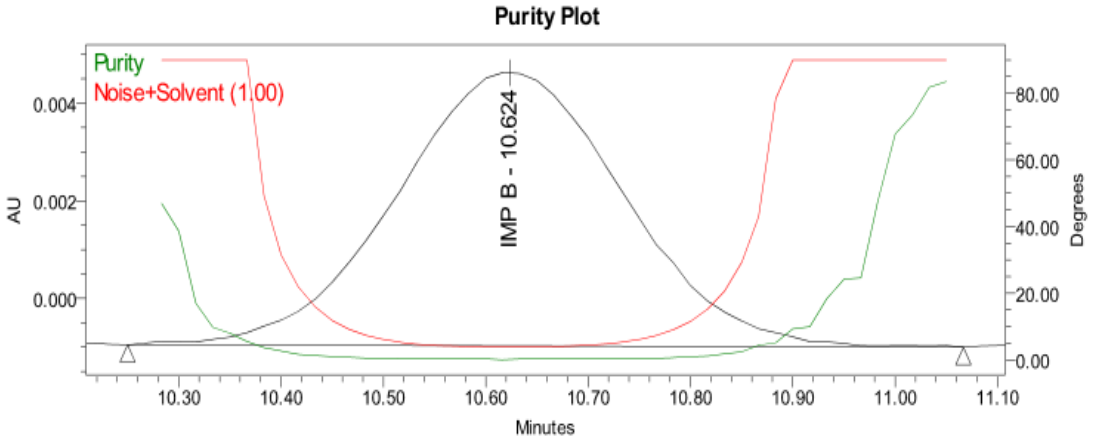
Table-3: Peak purity of spiked sample

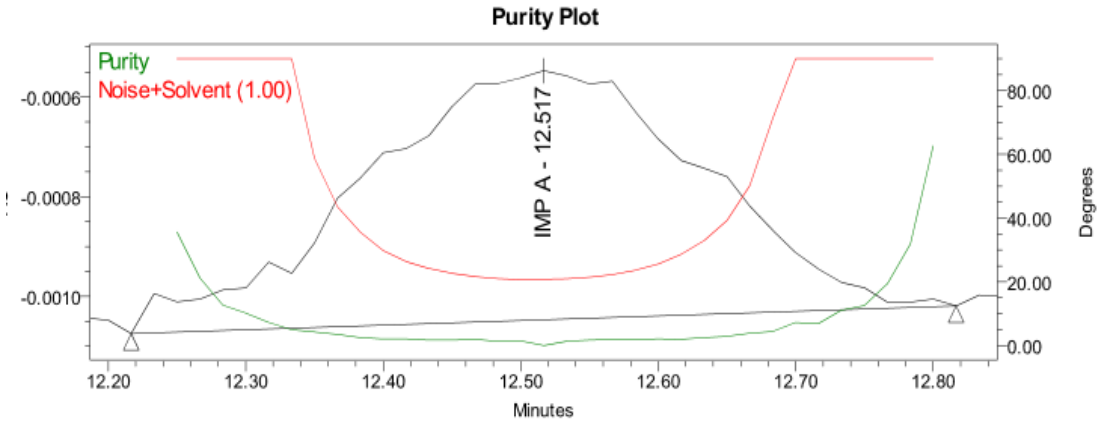
	Purity Angle	Purity Threshold
Felodipine	0.088	1.046
Impurity A	2.569	26.442
Impurity B	0.503	5.408
Impurity C	0.841	8.392



Peak Results

Sample	RT	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
Impurity-B	10.624	86687	0.55		1.0	11164
Impurity-A	12.517	8571	0.05	4.48	0.9	7645
Felodipine	15.199	15476939	98.85	5.37	1.1	11542
Impurity-C	22.018	84320	0.54	9.91	1.0	11309





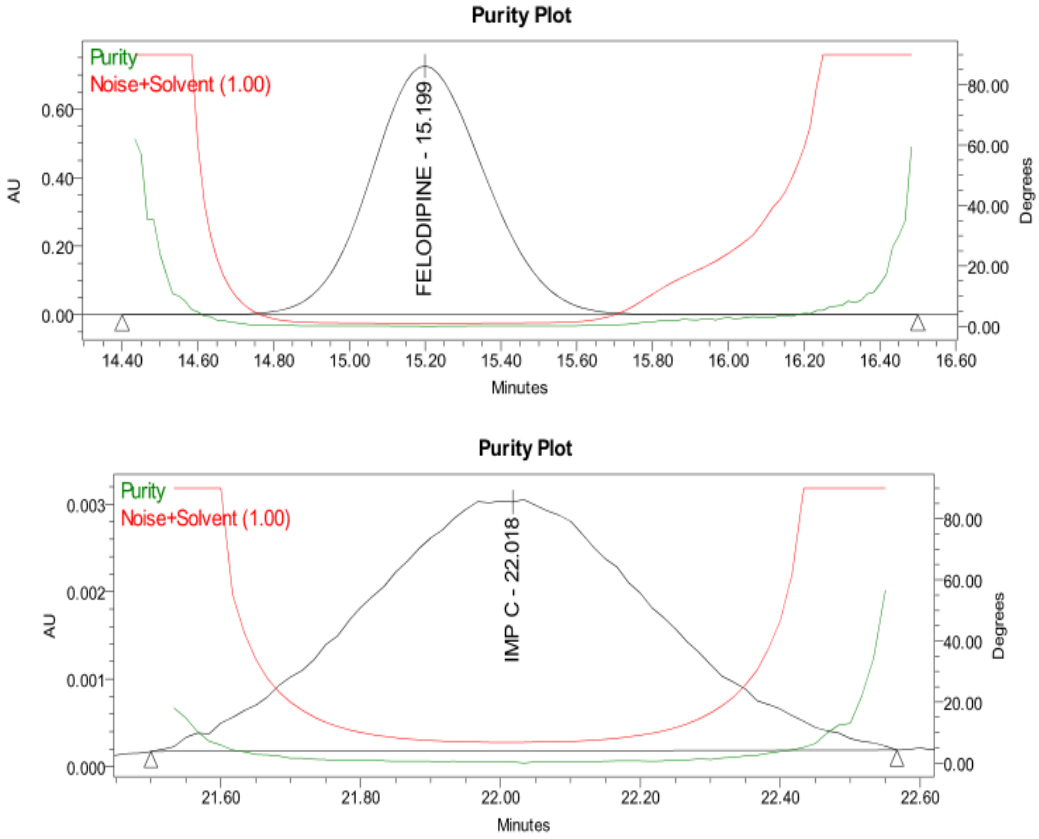


Figure-8 : Spiked Sample of Felodipine API

Table- 4 : Limit of Detection

Sr. No.	Response			
	Felodipine	Impurity- A	Impurity- B	Impurity- C
Conc.(µg/mL)	0.010	0.010	0.005	0.010
1	617	405	263	573
2	751	293	265	600
3	793	403	280	688
4	748	361	209	827
5	882	270	233	646
6	830	407	331	549
Mean	**770**	**357**	**264**	**647**
SD	**90.473**	**60.991**	**41.789**	**101.362**
%RSD	**11.75**	**17.11**	**15.86**	**15.66**

Table-5 : Limit of Quantification

Sr. No.	Response			
	Felodipine	Impurity A	Impurity B	Impurity C
Conc.(µg/mL)	0.033	0.032	0.017	0.033
1	1759	732	778	1539
2	1645	722	753	1676
3	1859	824	776	1642
4	1713	811	738	1729
5	1810	788	688	1330
6	1712	825	758	1728
Mean	**1750**	**784**	**749**	**1607**
SD	**76.680**	**45.986**	**33.189**	**152.881**
%RSD	**4.38**	**5.87**	**4.43**	**9.51**

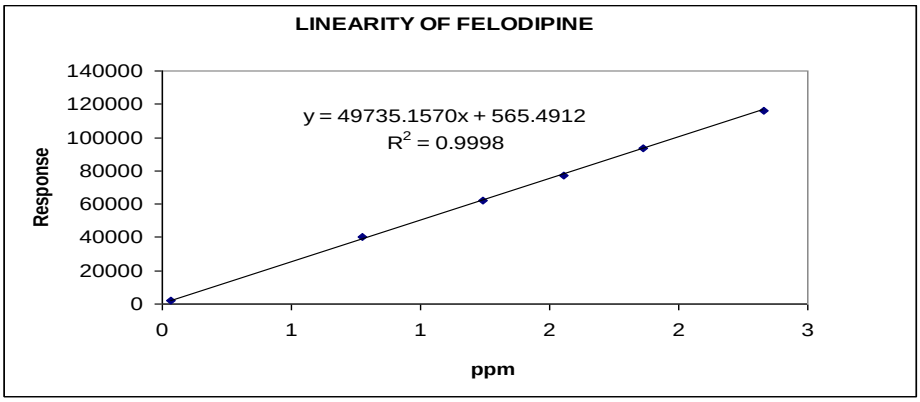
*Acceptance criteria***:** RSD for LOD: NMT 33% & RSD for LOQ: NMT 10%

***Conclusion*:**

Limit of Detection				
Conc.	Felodipine	Imp A	Imp B	Imp C
%	0.003	0.003	0.002	0.003
µg/mL	0.010	0.010	0.005	0.010
Limit of Quantification				
Conc.	Felodipine	Imp A	Imp B	Imp C
%	0.011	0.011	0.005	0.011
µg/mL	0.033	0.032	0.017	0.033

Table -6 : Table for Linearity of Felodipine

Level	Concentration (µg per ml)	Response (Area)
LOQ	0.033	1849
Lin-1	0.777	40137
Lin-2	1.243	61859
Lin-3	1.554	77512
Lin-4	1.865	93767
Lin-5	2.331	116300
	Slope	49735.2
	Intercept	**565.5**
	Correlation Coefficient	**0.99990**

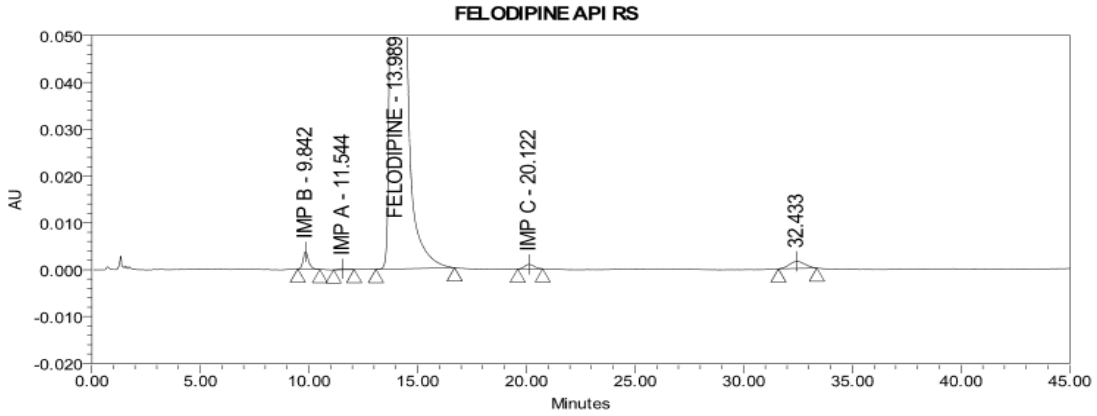


Sr. No.	Concentration (ppm)	Response
1	0.032	781
2	0.150	3320
3	0.240	5006
4	0.300	6260
5	0.360	7630
6	0.450	9576
Correlation Coefficient		0.99963

Figure-9: Linearity graph - Felodipine

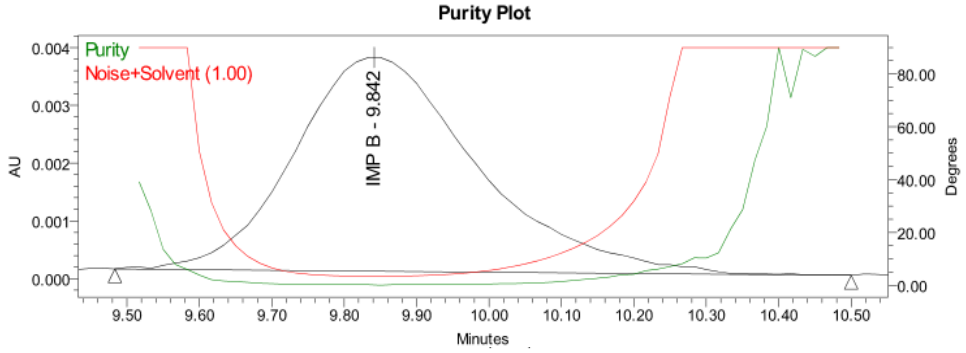
Table-7: System suitability

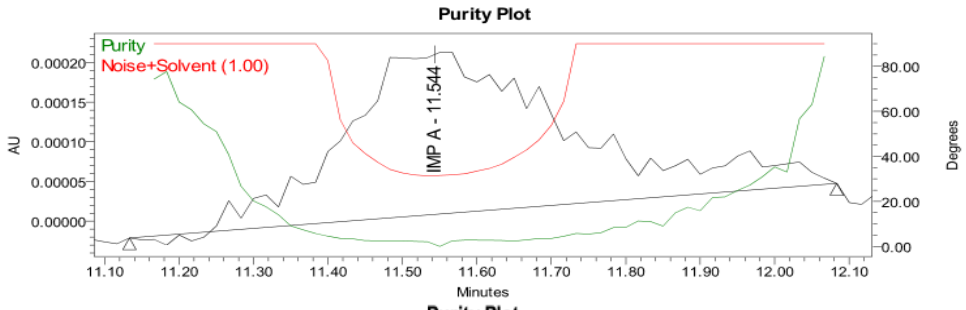
Experiment	%RSD of std	K Prime	Resolution bet^{n} Impurity- A & Felodipine	USP Tangent of Felodipine in std soln.	USP Tailing of Felodipine in std soln.
Specificity	3.25	16.4	4.53	8799	1.3
Linearity	0.32	9.67	5.29	11439	1.07
Method Precision	1.62	8.98	3.93	12960	1.0
Ruggedness	1.33	17.7	5.08	11553	1.0

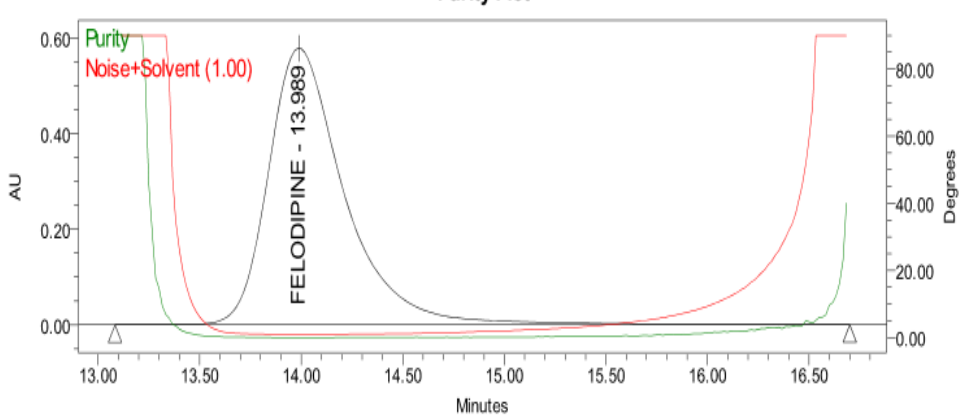


Peak Results

	Sample	RT	Area	% Area
1	Impurity-B	9.842	65092	0.13
2	Impurity-A	11.544	4339	
3	Felodipine	13.989	15170569	99.68
4	Impurity-C	20.122	30311	0.19
5		32.433	75654	0.49







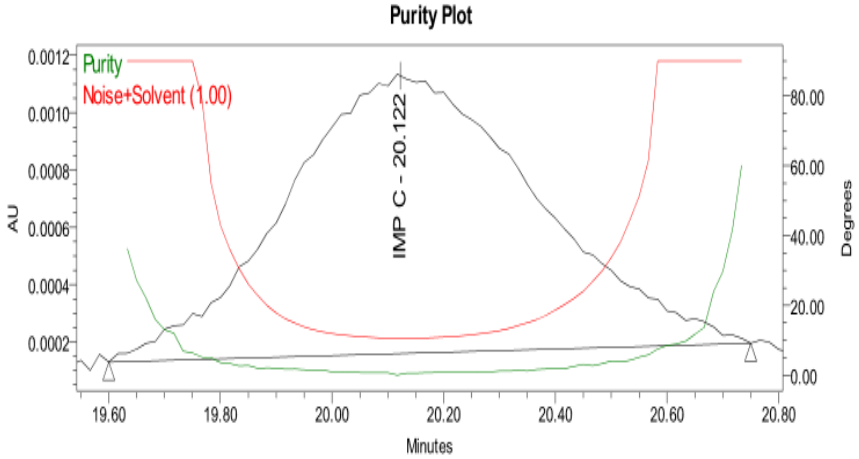
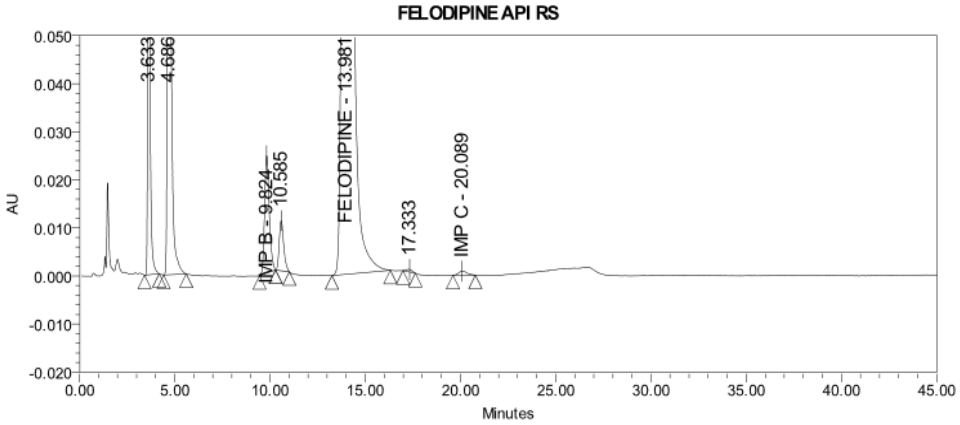
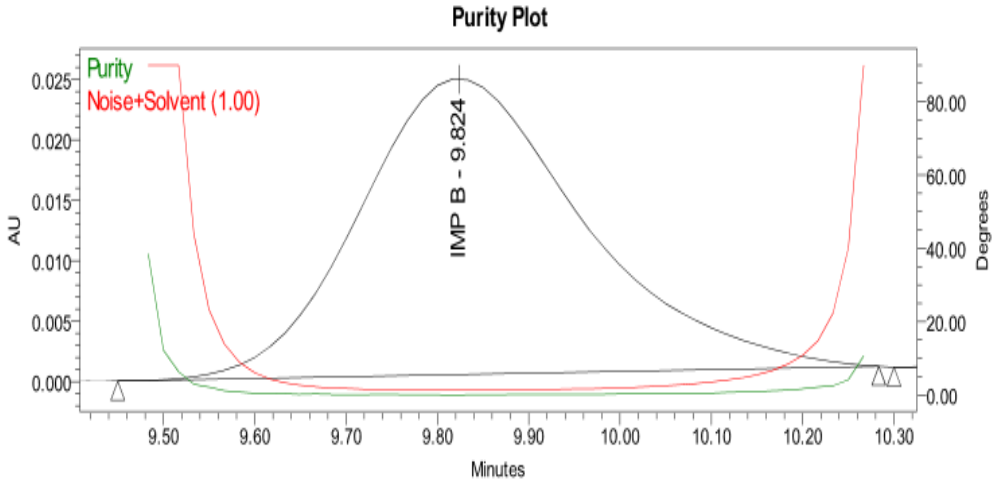


Figure -10: Acid Degradation (5N HCl)



Peak Results

	Sample	RT	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
1		3.633	752114	5.11		1.5	5895
2		4.686	2121852	14.42	4.69	1.5	6118
3	Impurity-B	9.824	417049	2.83	14.52	1.2	7862
4		10.585	170501	1.18	1.69	1.3	9547
5	Felodipine	13.981	11213773	76.22	6.13	1.4	7647
6		17.333	11047	0.08	5.35	0.9	15958
7	Impurity-C	20.089		0.18	3.96	1.2	9817



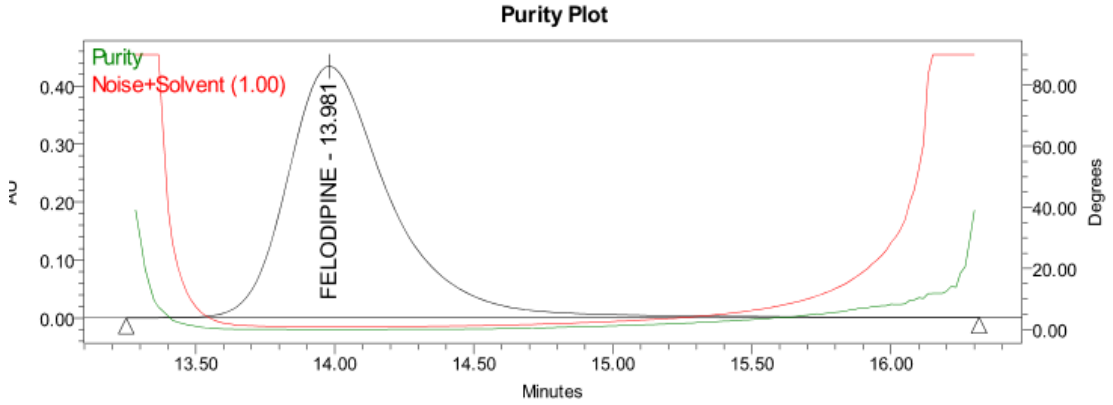
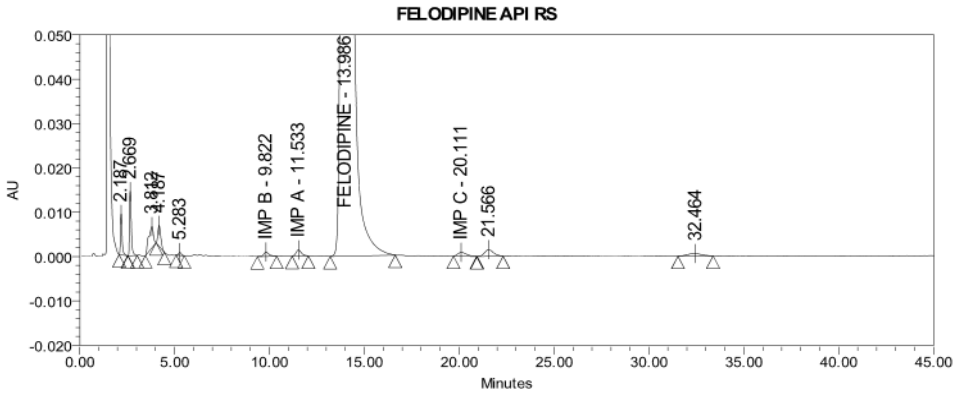
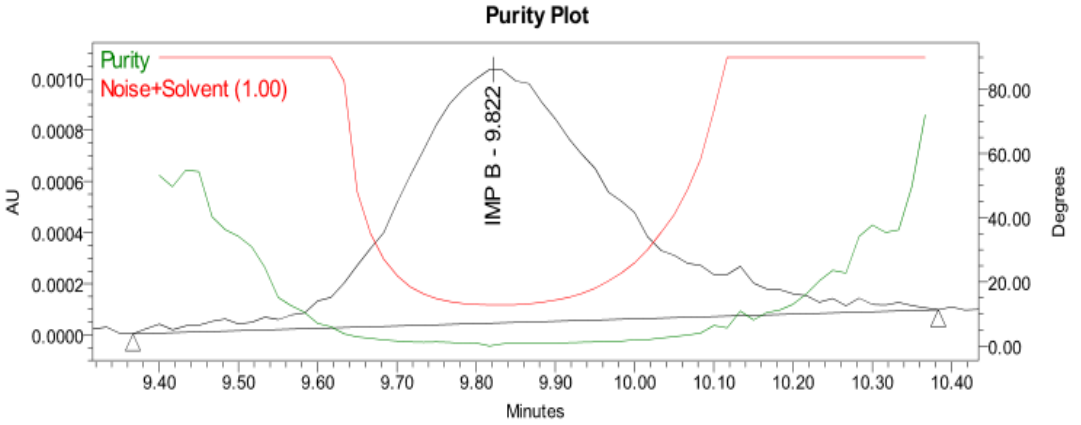


Figure-11: Base Degradation (2N NaOH)



Peak Results

	Sample	RT	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
1		2.187	54205	0.37		1.6	3380
2		2.669	95031	0.66	2.89	1.5	4047
3		3.812	84876	0.59	3.59	0.8	93
4		4.187	49340	0.34	0.99	1.4	3468
5		5.283	7023	0.05	3.80	1.1	5166
6	Impurity-B	9.822	18421	0.13	11.82	1.1	6666
7	Impurity-A	11.533	27454	0.19	3.47	1.2	7180
8	Felodipine	13.986	14023152	96.94	4.18	1.5	7401
9	Impurity-C	20.111	28609	0.20	7.97	1.4	8150
10		21.566	48538	0.34	1.63	1.2	10557
11		32.464	29341	0.20	9.10	0.9	7595



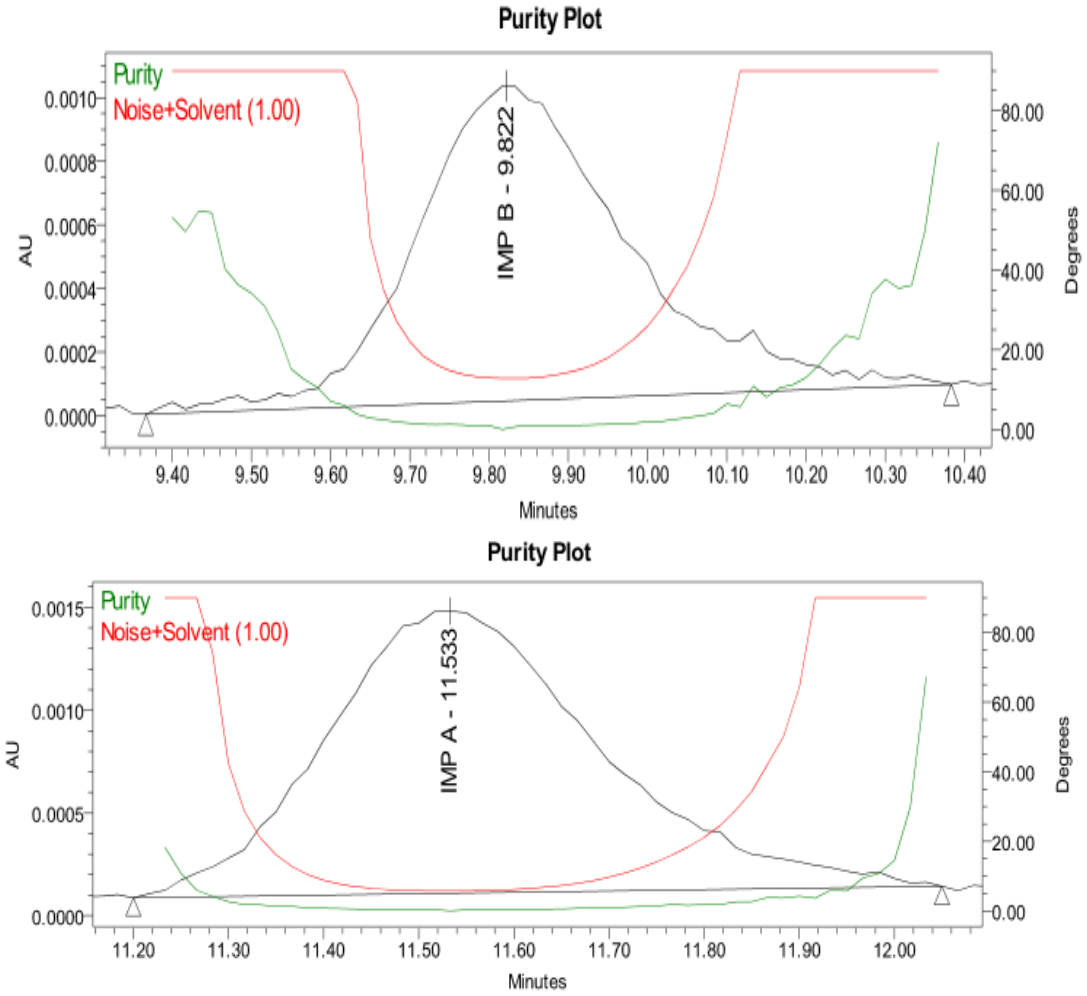
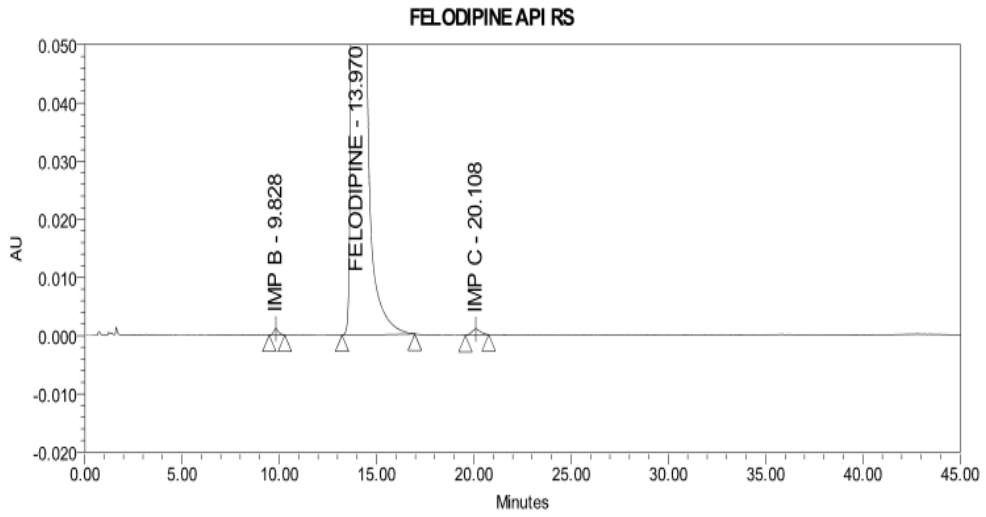


Figure-12: Peroxide Degradation (30% H_2O_2)



Peak Results

Sample	RT	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
Impurity-B	9.828	19660	0.12		1.3	6923
Felodipine	13.970	15858834	99.68	7.07	1.5	7312
Impurity-C	20.108	31617	0.20	8.05	1.1	8288

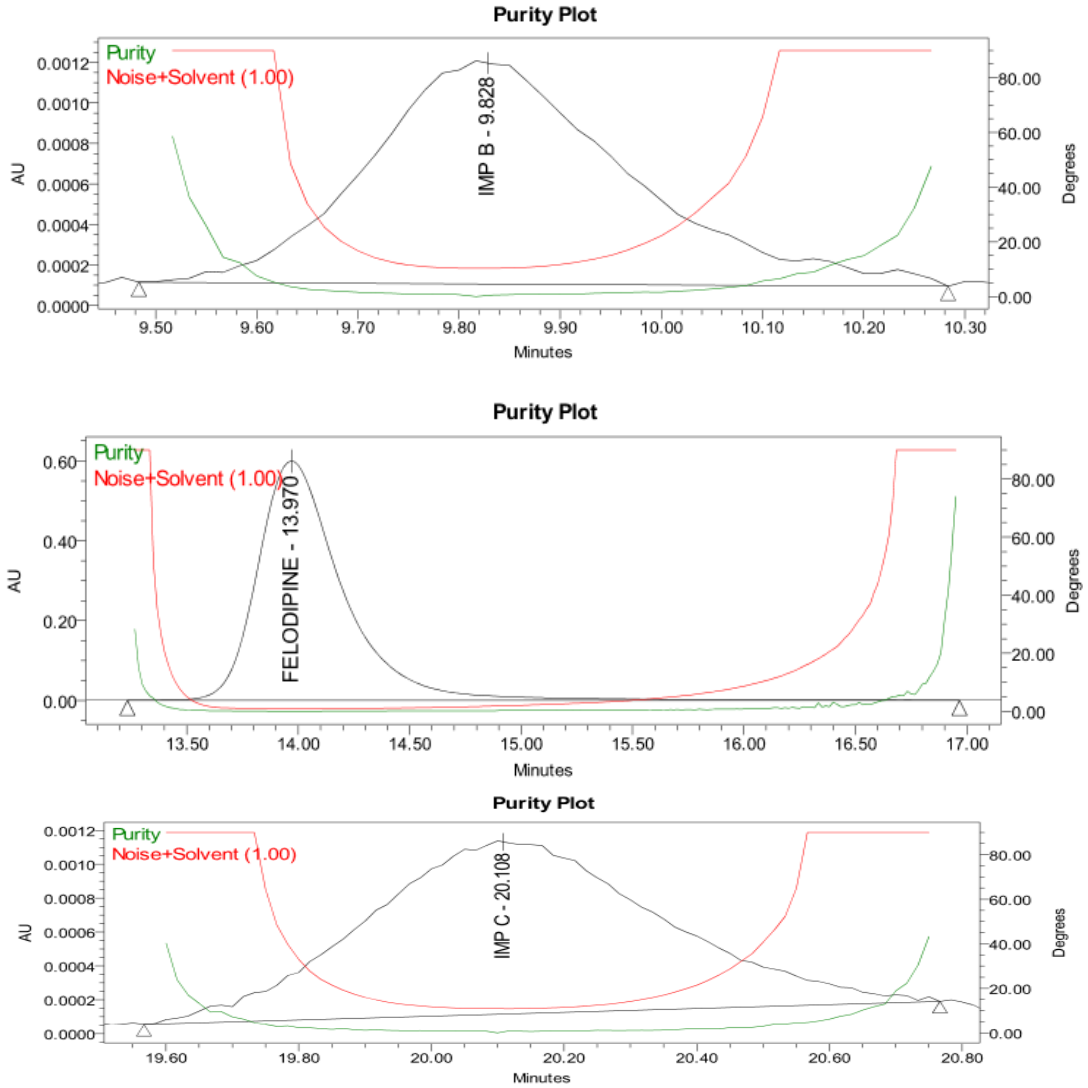
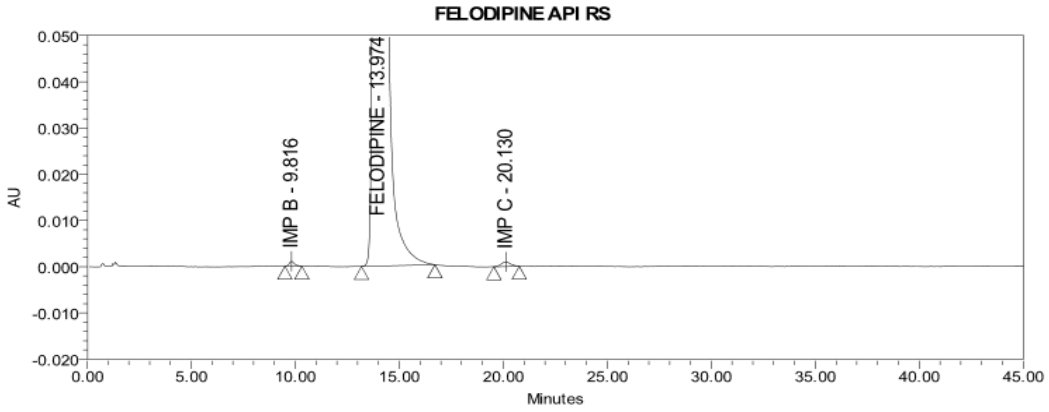


Figure-13: Thermal Degradation (105°C for 72 hours)



Peak Results

Sample	RT	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
Impurity-B	9.816	18855	0.12		1.3	7321
Felodipine	13.974	15134250	99.67	7.20	1.5	7313
Impurity-C	20.130	31370	0.21	7.63	1.1	10072

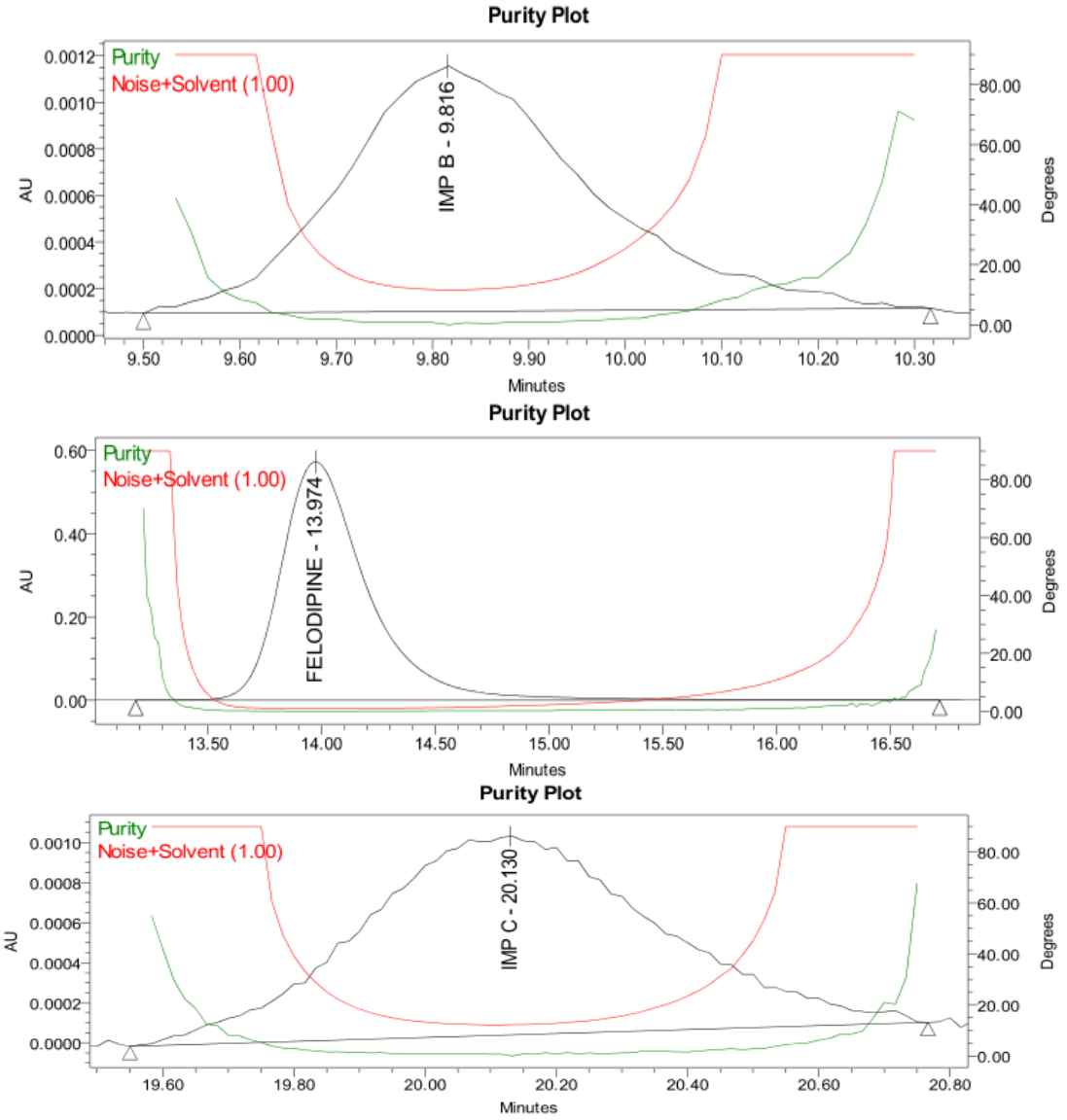
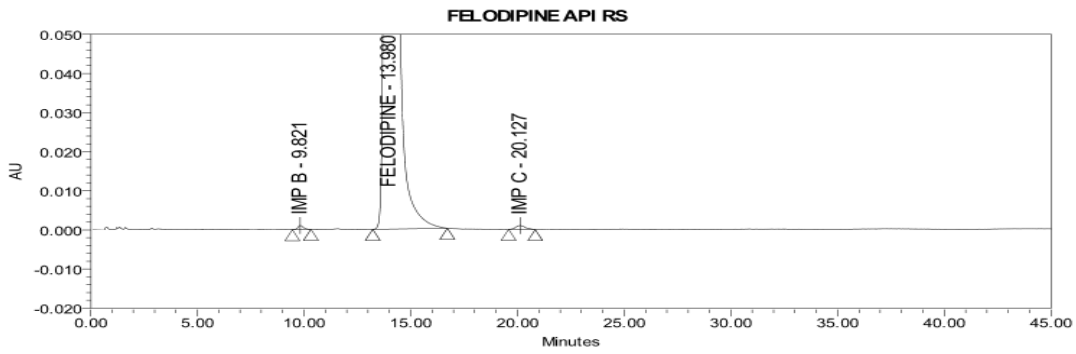


Figure-14 : Humidity Degradation ((25°C/92%RH for 72 hours))



Peak Results

Sample	RT	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
Impurity-B	9.821	19820	0.13		1.2	7898
Felodipine	13980	15282519	99.67	7.12	1.5	7306
Impurity-C	20.127	31520	0.21	8.38	1.2	13570

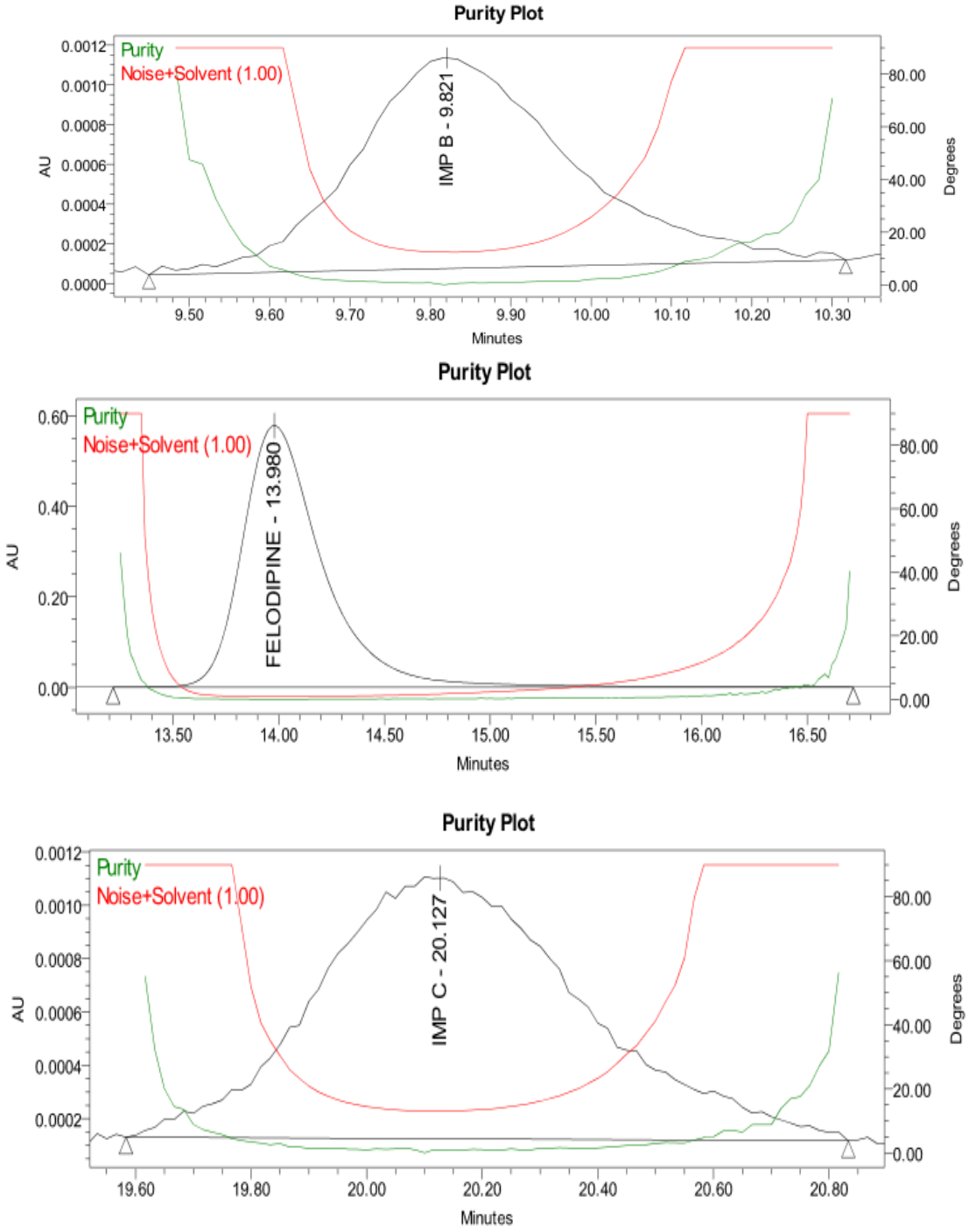


Figure-15: Photolytic Degradation (Light)

Table-8: Forced Degradation Studies

Experiment	Degradation Condition	Purity Angle	Purity Threshold
Control	--	0.036	1.039
Acid Degradation	5N HCl – 70°C/3 hrs	0.035	1.041
Base Degradation	2N NaOH – 70°C/3 hrs	0.041	1.054
Peroxide Degradation	30% H_2O_2 – 70°C /3 hours	0.110	1.054
Thermal Degradation	105°C – 72 hours	0.041	1.043
Humidity Degradation	25°C/92%RH – 72 hours	0.034	1.048
Photolytic Degradation	1.2 million lux hours	0.037	1.051

Table-9: Impurities in Forced Degradation Studies

Experiment	Degradation Condition	% Impurity A	% Impurity B	% Impurity C	% Single max. unknown	% Total
Control	--	--	0.118	0.181	--	0.30
Acid Degradation	5N HCl – 70°C/3 hrs.	0.027	0.405	0.189	0.471	1.90
Base Degradation	2N NaOH – 70°C/3 hrs.	0.992	2.427	0.155	12.349	20.37
Peroxide Degradation	30% H_2O_2 – 70°C/3 hrs.	0.171	0.115	0.178	0.592	2.76
Thermal Degradation	105°C – 72 hours	--	0.122	0.197	--	0.32
Humidity Degradation	25°C/92%RH – 72 hours	--	0.118	0.196	--	0.31
Photolytic Degradation	1.2 million lux hours	--	0.124	0.197	--	0.32

V. Conclusion

The HPLC method for the determination of Related Substances of Felodipine in Felodipine API. Three impurities were identified. The Mean Recovery for known Impurities is within limits. Therefore, the HPLC Method for the determination of Related Substances of Felodipine in Felodipine API was accurate. The correlation coefficient for Felodipine, Impurity -A, Impurity-B and Impurity-C was more than 0.99. Therefore, the HPLC Method for the determination of related Substances of Felodipine in Felodipine API was Linear. Correlation coefficient should not be less than 0.99. Overall RSD for twelve results should not be more than 10.0% Identification Results should be comparable with respect to Retention time. Peak purity should pass for Felodipine and known impurities in control sample and spiked sample. Blank should not show any peak at the retention time of Felodipine peak, and any of the Impurity peaks Felodipine peak should be homogeneous and there should be no co-eluting peaks. Peak purity passes for Felodipine and known impurity peaks. No interference observed. Peak purity for analyte peak should pass. The peak purity passes for Felodipine peak. The resolution between Felodipine oxidation product (Impurity A) and Felodipine should not be less than 2.5. Diluted standard solution, the relative standard deviation of six replicate injections should not be more than 5.0 %. The capacity factor, k', should not be less than 5.0, the column efficiency should not be less than 6000 theoretical plates and the tailing factor should not be greater than 1.5. The test method is validated for Specificity, LOQ, Linearity and range, Precision and Ruggedness and found to be meeting the predetermined acceptance criteria. The validated method is Specific, Linear, Precise and Rugged for Related substances of Felodipine in Felodipine API. Hence this method can be introduced into routine use for the related substances of Felodipine in Felodipine API.

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