INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN:2320-2831

IJPAR |Vol.7 | Issue 4 | Oct - Dec -2018
Journal Home page: www.ijpar.com

Research article *Open Access*

Analytical method development and validation of plerixafor in its dosage form in the presence of tetra-aza-cyclotetra decane derivatives by HPLC

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ABSTRACT

A simple and selective HPLC method is described for the determination of plerixafor Chromatographic separation was achieved on a Zorbax eclipse C_{18} 250x4.6mmx5 micron using mobile phase consisting Water: Methanol: Acetonitrile in the ratio 40:35:25v/v with detection wavelength of 221 nm. Linearity was observed in the range 50-125 µg /ml For Plerixafor (r^2 =0.9964) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed method is fully validated with parameters like accuracy, precision, linearity, limit of detection, limit of quantification, robustness and ruggedness. The proposed method is stability indicating with parameters like acid base peroxide, photolytic and thermal degradation. No interference found from all three impurities of Plerixafor that is tetraazacyclotetradecane derivatives.

Keywords: Plerixafor, HPLC method, Quantification, Formulation, Related substances

INTRODUCTION

In the pharmaceutical-world, an impurity is defined as any other inorganic or organic material, or residual solvents other than the drug substances, or ingredients, arise out of synthesis or unwanted chemicals that remains with APIs. Now a days, not only purity profile but also impurity profile has become mandatory according to various regulatory authorities. Very few methods were reported for estimation of plerixafor in the presence of impurities A, B, C in the injection formulation. in the presence of impurities A, B, C in the injection formulation.

Plerixafor (PRX) belongs to the class anticancer drug.Plerixafor consists of two cyclam rings with a phenylenebis (methylene) linker [1, 2]. It inhibits CXCL12 binding to CXCR4 and subsequent downstream events including chemotaxis [3, 4]. The molecular interactions of plerixafor had been defined as a unique binding mode to CXCR4. Plerixafor rapidly mobilizes Hematopoietic Stem Cell within hours compared with the multi-day

treatment required by Granulocyte-Colony Stimulating Factor in mouse, dog and non-human primate. The mobilized cells once transplanted are capable of timely and endurable engraftment [5, 6]. Plerixafor is a hematopoietic stem cell mobilizer. It is used to stimulate the release of stem cells from the bone marrow into the blood in patients with non-Hodgkin lymphoma and multiple myeloma for the purpose of stimulating the immune system. These stem cells are then collected and used in autologous stem cell transplantation to replace blood-forming cells that were destroyed by chemotherapy [7].

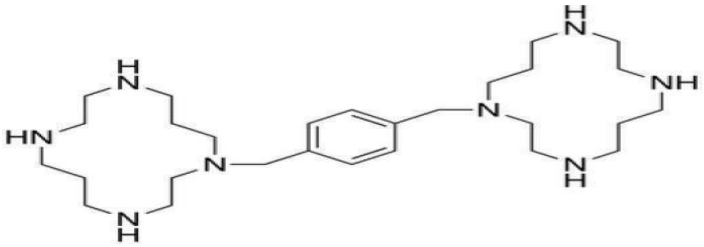


Fig 1. Structure of Plerixafor
1,1'-[1,4-phenylenebis(methylene)]-bis—1,4,8,11-tetraazacyclotetradecane

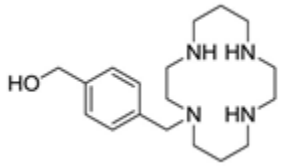


Fig 2: Impurity A
Benzenemethanol,4-(1,4,8,11-tetraazacyclotetrade-1-ylmethyl)-(4-((1,4,8,11-tetraazacyclotetradecan-1-yl)methyl)phenyl)methanol

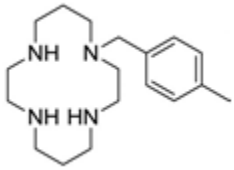


Fig 3: Impurity B
1-(4-methylbenzyl)-1,4,8,11-tetraazacyclotetradecane

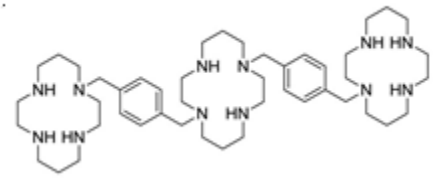


Fig 4: Impurity C
1,8-bis(4-((1,4,8,11-tetraazacyclotetradecane-1-yl)methyl)benzyl)-1,4,8,11-tetraazacyclotetradecane

REQUIREMENTS

Table 1: Equipment's

Instrument	Instrument/Column Id
HPLC	Zorbax eclipse C_{18} 250x4.6mmx5 micron column, PD detector
Analytical balance	Sartorius Practum
P^H meter	Digisun model DI-707
Sonicator	Labotec

Table 2. Chemicals

Name	Grade
Plerixafor	USP-Grade
Water	HPLC grade
Methanol	AR grade
Acetonitrile	HPLC grade
Plerixafor Brand Names	Celrixafor, Mozobil, Mozifor

METHODOLOGY [8]

Mobile Phase

Take 350ml (35%) of Methanol, 400ml of Water & 250ml of Acetonitrile were mixe-degasse & filtered-through 0.45µ-filter under vacuum-filtration.

Preparation of Standard Stock Solution

100mg of Plerixafor into 100ml-volumetric flasks & add 60ml of diluent & sonicate, make upthe volume with mobile phase. Pipette-out 0.3ml of above Plerixafor-stock into 10ml-vol flask & make up the volume.

Preparation of Sample Solution

Weight equivalent to 100mg of Plerixafer injection is taken same procedure is followed as that of Std.

Preparation of Stock A, B, C

Take 1mg of impurities A, B, C (Stds) into respective labelled vol. flask (10ml) & dilute to volume. Transfer 1 mL of this solution into a 10 mL volumetric flask & dilute to the volume.

Standard solution

To 1mL of standard stock solution**,** stock A, B, C into a 10 mL vol. flask individually & makeup the volume.

Assay method

As per the method parameters indicated in Biophore DMF of Plerixafor, Blank (diluent), Placebo, Mobile phase, Impurity A, Impurity B, Impurity C, standard and sample solutions were injected using Zorbax eclipse C_{18} 250 x 4.6mm x 5µ column to check interference with the principal peak.

RESULTS & DISCUSSION

Table 3.Optimized Chromatographic Conditions

Column	Zorbax eclipse C_{18} 250x4.6mmx5 micron
Flow rate	1.5 ml/min
Mobile Phase	Water:Methanol:ACN=40:35:25
Wavelength	221nm
Inj-volume	20 microlitre
Temperature	Ambient
Runtime	20 minutes

Table 4. Specificity check

Condition	Observation in Assay method
Diluent	No interference observed
Mobile phase	No interference observed
Placebo	No interference observed
Impurity A	No interference observed
Impurity B	No interference observed
Impurity C	No interference observed

Table 5.Linearity has been performed from 50% to 125% on working concentration.

~Level	Concentration (mg/mL)	Average Peak Area
50	0.2010	2606411
75	0.3015	3907659
100	0.4020	5194537
125	0.5025	6385743

PRECISION STUDY

Table 6.System precision has been performed by injecting same solution

Inj.No.	Retention time of Plerixafor	Peak area of Plerixafor	Tailing Factor
1	14.176	4877849	1.84
2	14.175	4868191	1.85
3	14.182	4856044	1.82
4	14.189	4880706	1.85
5	14.189	4869394	1.85
Avg.	14.188	4869288	1.8
% RSD	0.10	0.2	

Table 7. Method Precision (Repeatability)

Method precision was performed for 6 different preparations

Method Precision (Repeatability)			
Solution ID	**Conc. mg/mL**	**Peak area**	**% of Plerixafor**
Preparation 1	0.40	5347337	101.2
Preparation 2	0.40	5359411	101.4
Preparation 3	0.40	5382425	101.8
Preparation 4	0.40	5389279	102.0
Preparation 5	0.40	5396820	102.1
Preparation 6	0.40	5393126	102.0
Average			101.8
Std Deviation			0.38
% of RSD			0.39

RELATED SUBSTANCES

As per the method parameters indicated in the Biophore DMF of Plerixafor, Blank (diluent), Placebo, Mobile phase, Impurity A, Impurity B, Impurity C, reference standard and sample solutions were injected using Zorbax eclipse C_{18} 250 x 4.6mm x 5µ column and flow rate of 1.5mL/minute to check interference with principal peak. Chromatograms were presented in Annexure-I.

Table 8. Specificity check

Condition	Observation in RS Method
Diluent	No interference observed

Mobile phase	No interference observed
Placebo	No interference observed
Impurity A	No interference observed
Impurity B	No interference observed
Impurity C	No interference observed

Table 9. Linearity has been performed from LOQ to 200%

Level	Concentration (mg/mL)	Average Peak Area
12.5 (LOQ)	0.0005	4666
25	0.0010	6295
50	0.0020	20710
75	0.0030	27965
100	0.004	37279
125	0.0050	44922
150	0.0060	56825
200	0.0080	74165

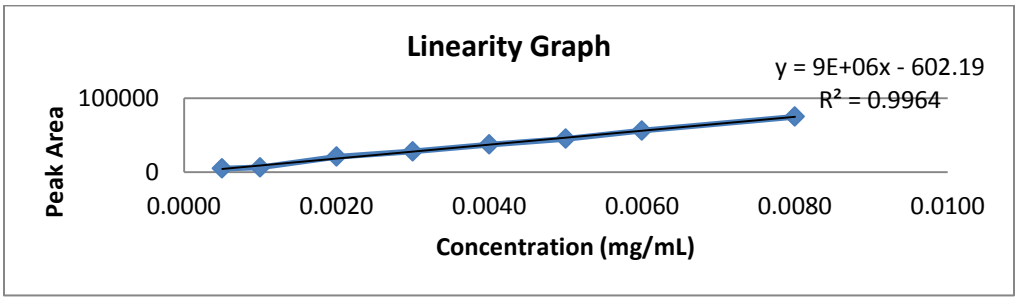


Fig 5: Linearity

Table 10. LOD & LOQ Solution

Parameters	Concentration (mg/mL)	% on working concentration	S/N
LOD	0.0002	0.01	2.9
LOQ	0.0005	0.025	9.1

Stress testing

Plerixafor Injection sample was subjected to forcible conditions (Acid, base, and peroxide) and injected into assay and related substances methods to establish the stability indicating capability of the method. Chromatograms were presented in Annexure-I.

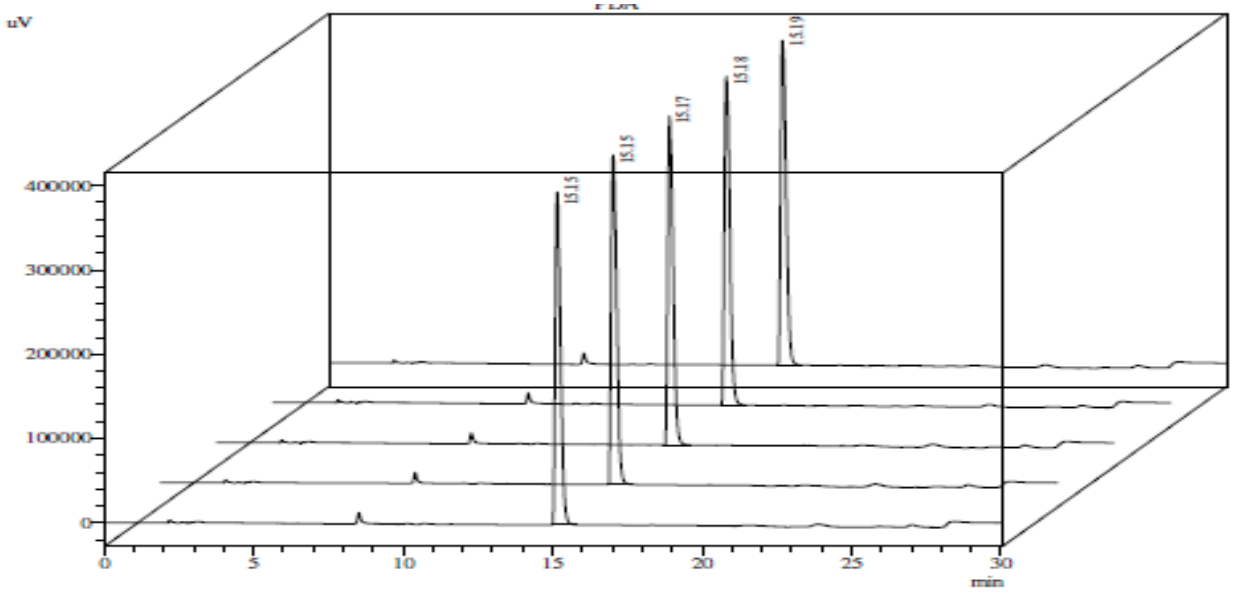
Table 11. Forced degradation study

Condition	Time period	% Assay	Peak purity Index	Peak purity threshold	% Total Impurities	Mass balance
1N Acid at 60°C	72hrs	99.7	1.00000	0.99994	0.20	100.0
2N NaOH at 60°C	72hrs	97.1	1.00000	0.99994	1.80	98.9
3% Peroxide at 60°C	72hrs	97.8	1.00000	0.99994	3.00	100.8

Table 12. Summary.

Parameters	Acceptance criteria	Result
Specificity	Mobile phase, Placebo and diluent should not show any interference with the main peak.	No interference observed
Linearity (50% to 125%) Assay	Correlation coefficient, R, NLT 0.99	0.999
Linearity (LOQ to 200%) RS	Correlation coefficient, R, NLT 0.99	0.996
System precision	Tailing factor : NMT 2.0	1.8
	The % RSD for area : NMT 2.0	0.2
Method precision	% RSD for area: NMT 2.0%	0.39
LOD	S/N ratio around 3	2.9
LOQ	S/N ratio around 10	9.1

Based on the chromatographic profile obtained from the study, assay and related substance method is specific, linear and precise and can be used to analyze Plerixafor and its degradation products in Plerixafor injection.

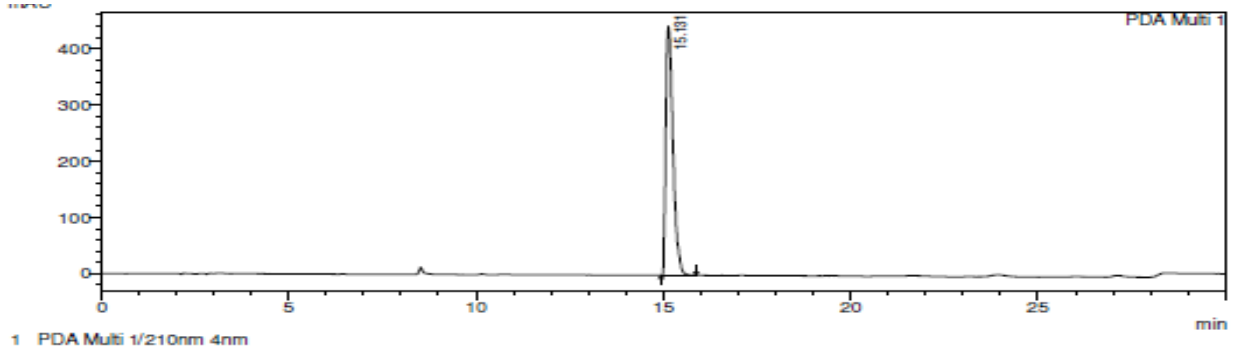


<< PDA >>

ID#1 Compound Name: PR

Vial#	Sample Name	Sample ID	Title	Ret. Time	Area	Theoretical Plate#	Tailing Factor
2	APR	Standard Solution	23062017_APR_019.lcd	15.19	4894084	30902.028	1.613
2	APR	Standard Solution	23062017_APR_020.lcd	15.18	4882804	31581.812	1.627
2	APR	Standard Solution	23062017_APR_021.lcd	15.17	4877111	31622.862	1.627
2	APR	Standard Solution	23062017_APR_022.lcd	15.15	4873326	31568.309	1.629
2	APR	Standard Solution	23062017_APR_023.lcd	15.15	4880847	32070.808	1.635
			Average	15.17	4881634	31549.164	1.626
			%RSD	0.11	0.16	1.32	0.51

Fig 6: Standard – Chromatogram

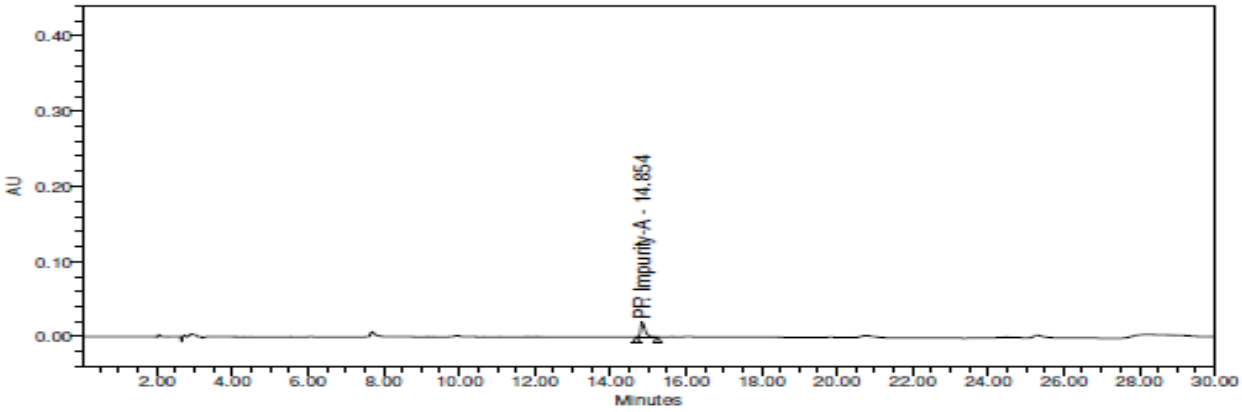


PeakTable

PDA Ch1 210nm 4nm

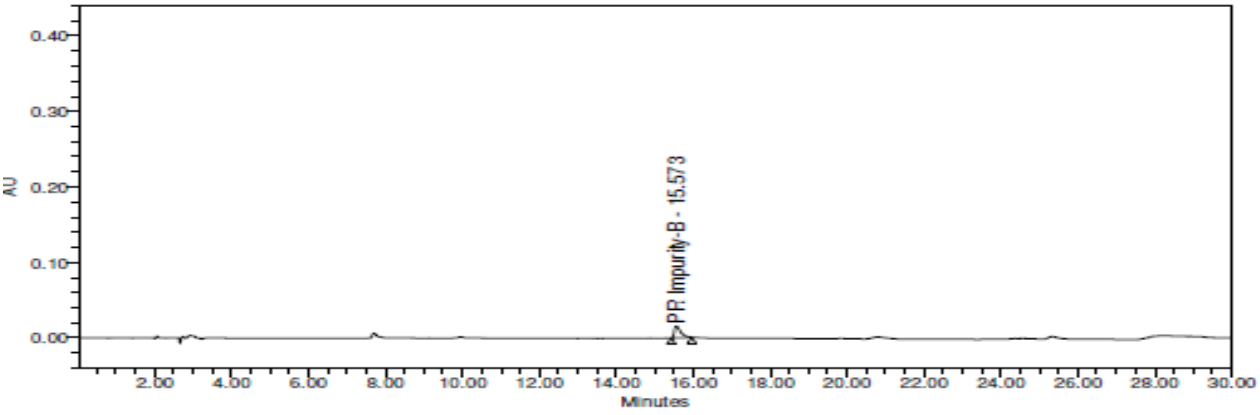
Peak#	Name	Ret. Time	Area	Area %	Peak Purity Index	Single Point Threshold
1	PR	15.131	5945548	100.00	1.00000	0.99994
Total			5945548	100.00		

Fig.7 Sample – Chromatogram



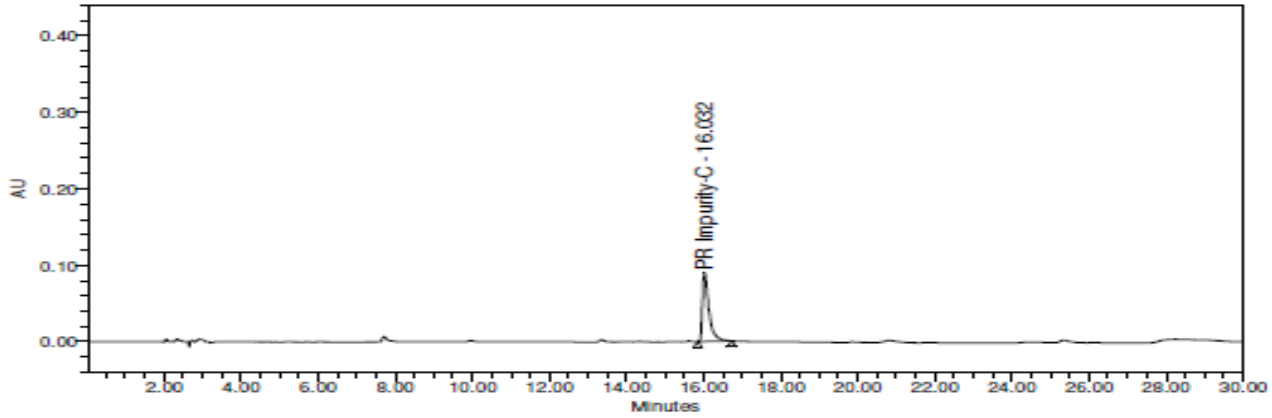
	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing
1	PR Impurity-A	14.85	151262	100.00	85662.51	1.64

Fig. 8 Impurity A – Chromatogram



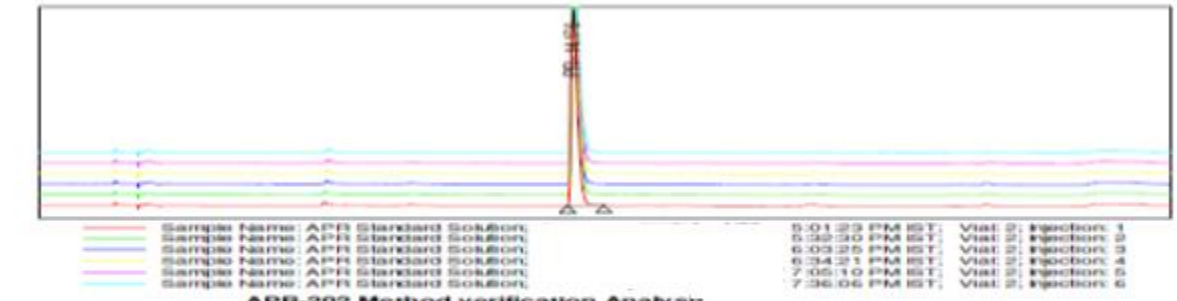
	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing
1	PR Impurity-B	15.57	178983	100.00	43287.30	1.87

Fig.9 Impurity B – Chromatogram



	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing
1	PR Impurity-C	16.03	1036043	100.00	49710.58	2.04

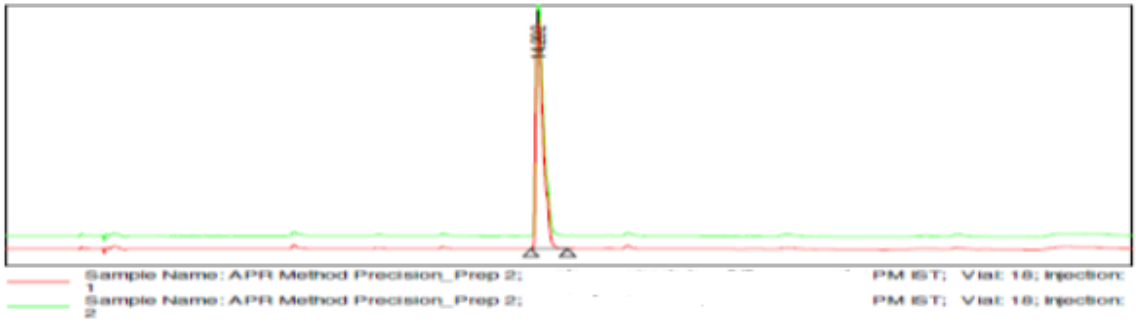
Fig.10 Impurity C – Chromatogram



APR-202 Method verification Analysis
Name : PR

	Sample Name	Vial	Inj	Name	Retention Time (min)	Area	% Area	USP Plate Count	USP Tailing
1	APR Standard Solution	2	1	PR	14.176	4977840	100.00	32414.46	1.94
2	APR Standard Solution	2	2	PR	14.175	4868191	100.00	32540.40	1.95
3	APR Standard Solution	2	3	PR	14.182	4950044	100.00	31630.91	1.92
4	APR Standard Solution	2	4	PR	14.189	4880706	100.00	31824.27	1.95
5	APR Standard Solution	2	5	PR	14.197	4869894	100.00	30488.04	1.95
6	APR Standard Solution	2	6	PR	14.213	4863649	100.00	30947.88	1.98
Mean					14.188	4885060		31593.6	1.8
Std. Dev.					0.014	9090.8			
% RSD					0.10	0.2			

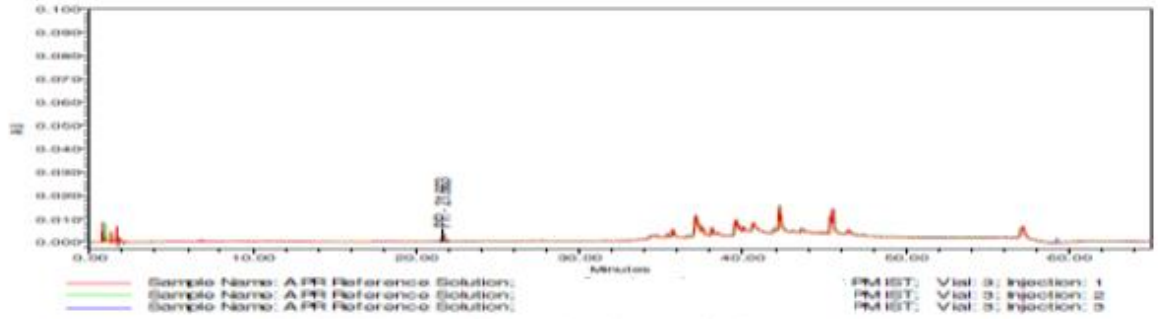
Fig.11 Assay – System Precision



APR-202 Method Precision_ Prep-2
Name :

	Sample Name	Vial	Inj	Retention Time (min)	Area	% Area	USP Plate Count	USP Tailing
1	APR Method Precision_Prep 2	18	1	14.202	5360446	100.00	27013.95	1.94
2	APR Method Precision_Prep 2	18	2	14.211	5358376	100.00	27776.80	1.93
Mean				14.207	5359411		27395.4	1.9
Std. Dev.				0.007	1464.1			
% RSD				0.05	0.0			

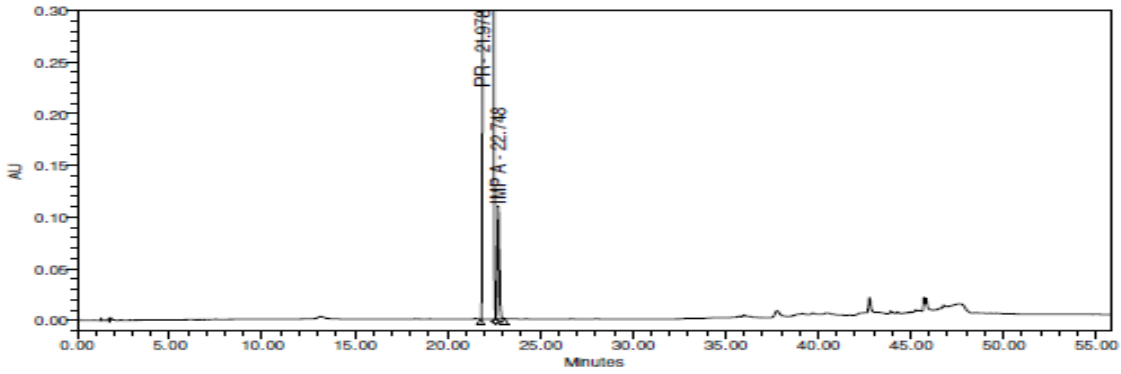
Fig.12 Assay – Method Precision



APR-202 Forced degradation analysis
Name : PR

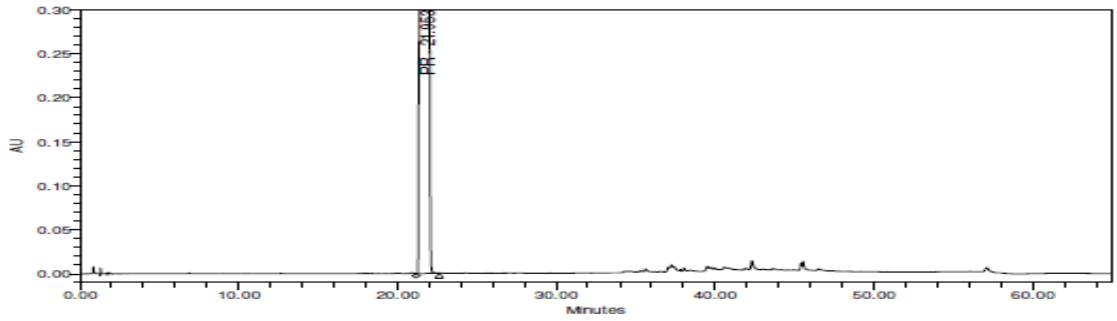
	Sample Name	Vial	Inj	Name	Retention Time (min)	Area	% Area	USP Plate Count	USP Tailing
1	APR Reference Solution	3	1	PR	21.983	27679	100.00	130379.84	1.68
2	APR Reference Solution	3	3	PR	21.978	30194	100.00	115998.34	1.17
3	APR Reference Solution	3	2	PR	21.983	27185	100.00	115951.06	1.60
Mean					21.975	28359.0		86108.8	1.5
Std. Dev.					0.010	1613.6			
% RSD					0.05	5.7			

Fig.13 Reference standard – Chromatogram (Specificity)



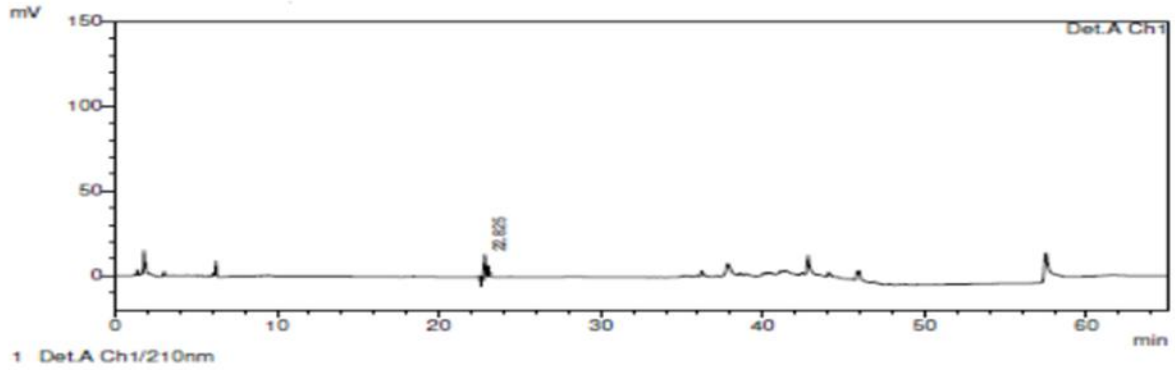
	Peak Name	RT	Area	% Area	RT Ratio	USP Resolution
1	PR	21.98	16966425	95.58		
2	IMP A	22.75	784147	4.42	1.04	1.78

Fig.14 .System suitability solution – Chromatogram



	Peak Name	RT	Area	% Area	Purity 1 Angle	Purity 1 Threshold
1	UK	20.92				
2	PR	21.95	18559108	100.00	0.077	0.259

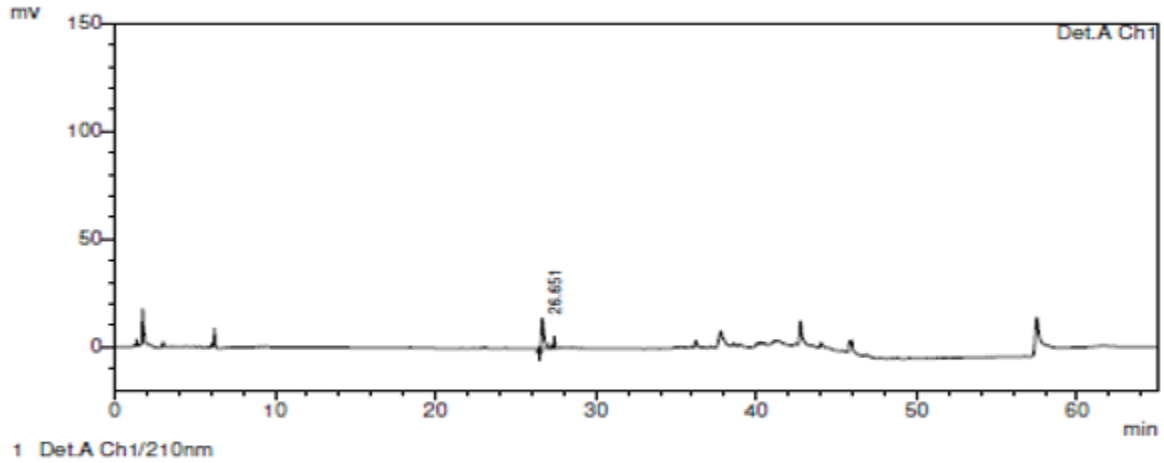
Fig.15 Sample – Chromatogram



Detector A Ch1 210nm

Peak	Name	Ret. Time	Area	Area %	RRT
1	IMP-A	22.82	102048	100.00	1.00

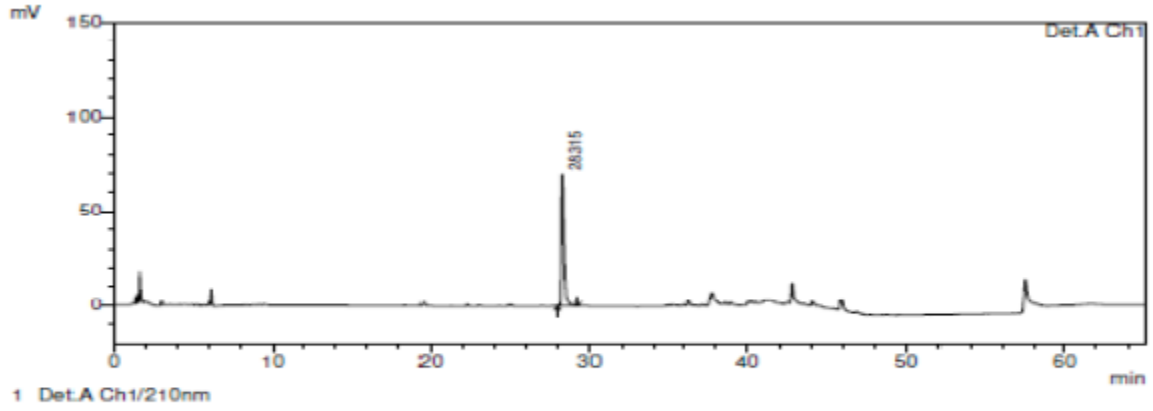
Fig.16 Impurity A - Chromatogram



Detector A Ch1 210nm

Peak	Name	Ret. Time	Area	Area %	RRT
1	IMP-B	26.65	151919	100.00	1.00

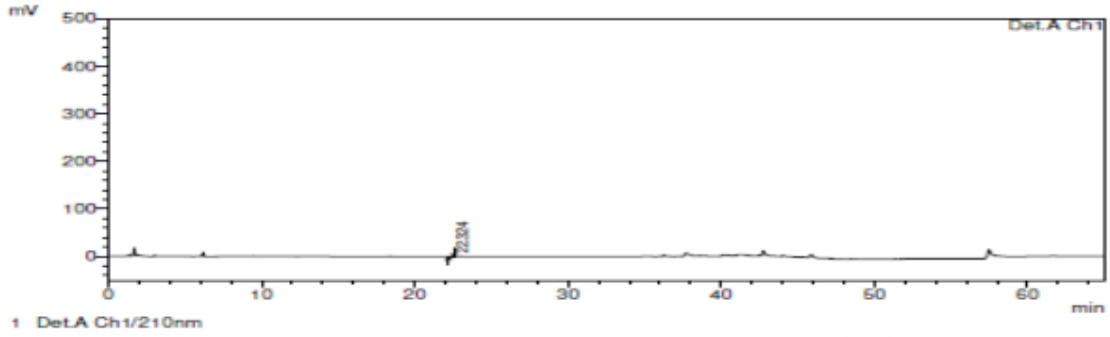
Fig: 17 Impurity B - Chromatogram



Detector A Ch1 210nm

Peak	Name	Ret. Time	Area	Area %	RRT
1	IMP-C	28.32	826819	100.00	1.00

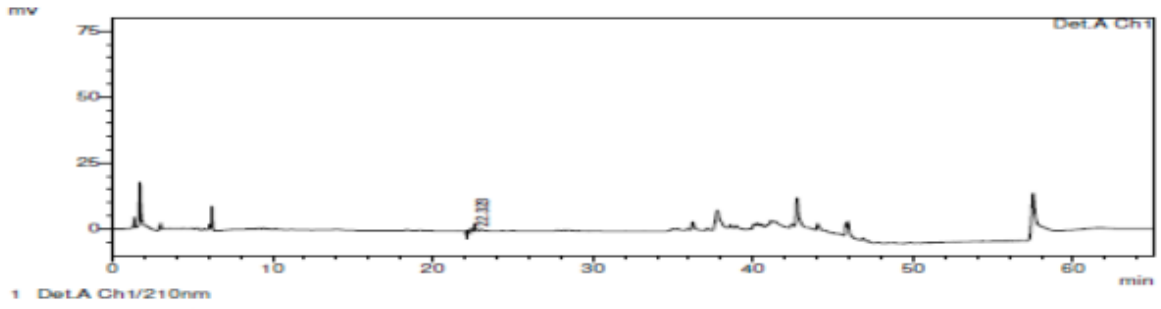
Fig: 18 Impurity C - Chromatogram



Detector A Ch1 210nm

Peak	Name	Ret. Time	Area	Area %	RRT
1	PR	22.32	2549	100.00	1.00

Fig: 19 LOD solution - chromatogram

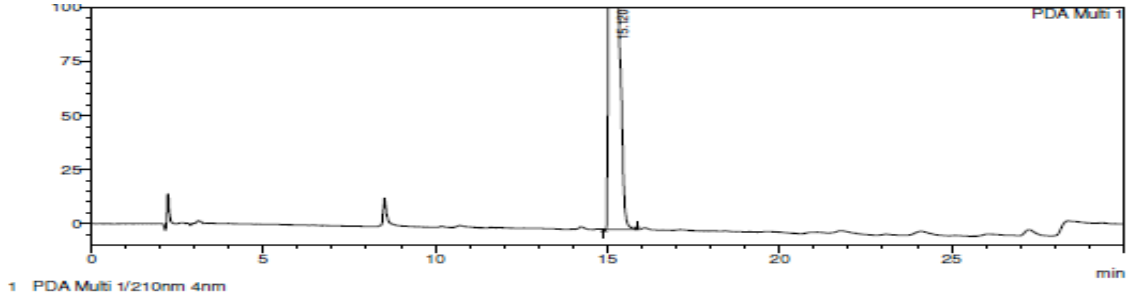


Detector A Ch1 210nm

Peak	Name	Ret. Time	Area	Area %	RRT
1	PR	22.32	4554	100.00	1.00

Fig: 20 LOQ solution- chromatogram

Acid degradation at 72Hrs Assay

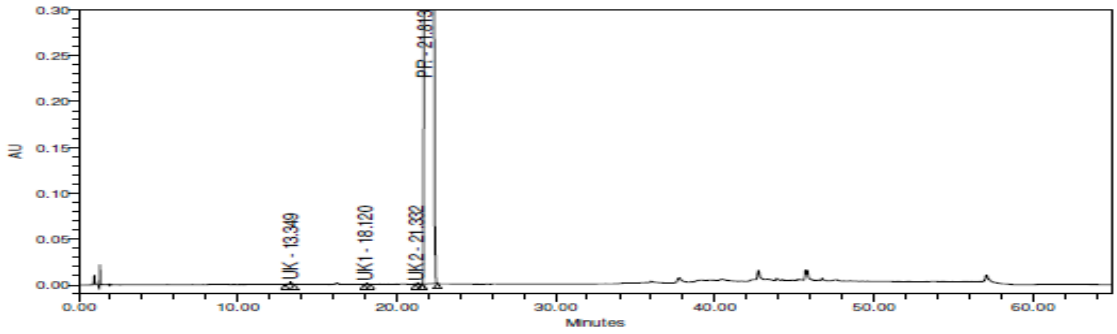


PeakTable

PDA Ch1 210nm 4nm

Peak#	Name	Ret. Time	Area	Area %	Peak Purity Index	Single Point Threshold
1	PR	15.120	5898425	100.00	1.00000	0.99994
Total			5898425	100.00		

Fig.21 Forced Degradation - Chromatograms



	Peak Name	RT	Area	% Area	RT Ratio	Purity1 Angle	Purity1 Threshold	USP Resolution
1	UK	13.35	23679	0.12	0.61	10.632	6.925	
2	UK1	18.12	7737	0.04	0.83	10.409	7.274	19.92
3	UK2	21.33	14712	0.08	0.98	9.013	9.004	13.71
4	PR	21.81	19262166	99.76		0.086	0.256	0.99

Fig.22 Sample - Acid degradation at 72HrsRS

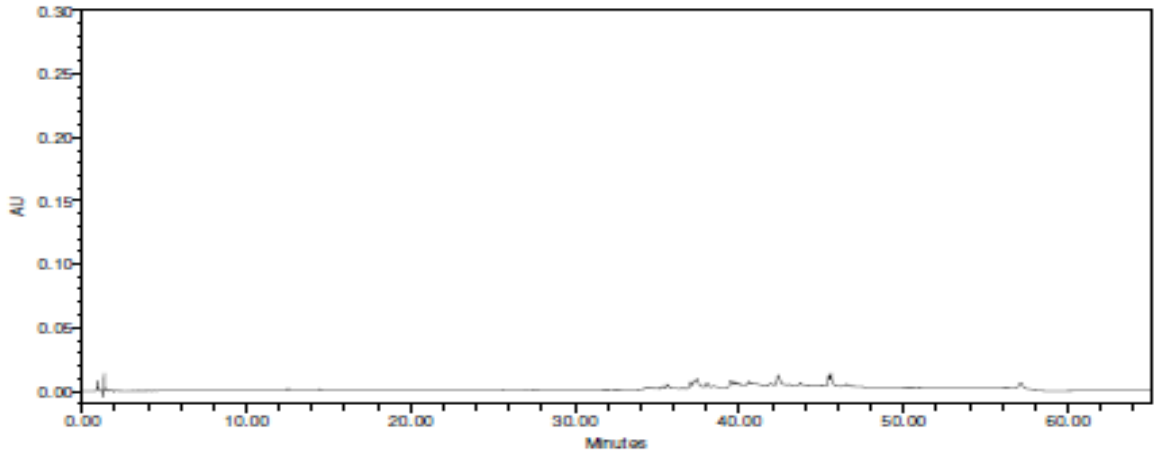
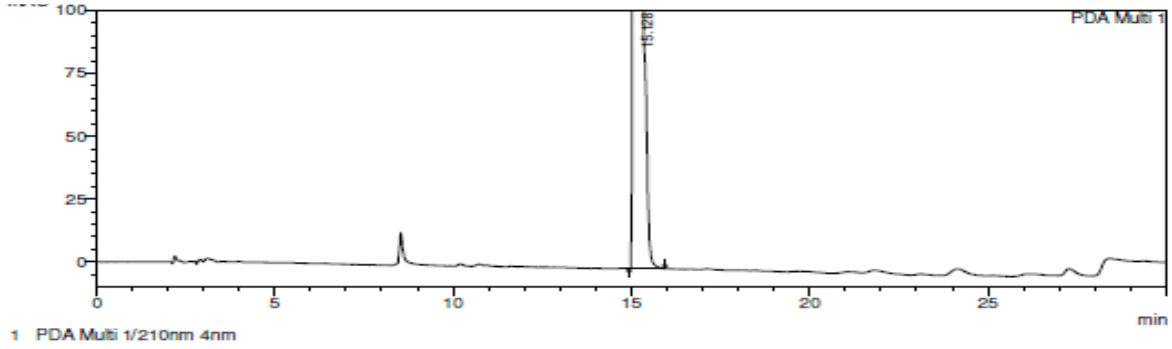


Fig.23 Sample – Acid degradation at 72Hrs RS

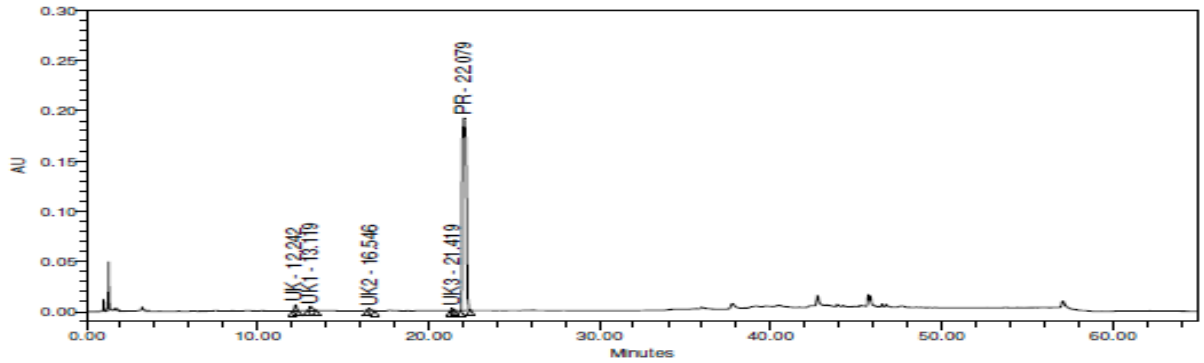


PeakTable

PDA Ch1 210nm 4nm

Peak#	Name	Ret. Time	Area	Area %	Peak Purity Index	Single Point Threshold
1	PR	15.128	5768939	100.00	1.00000	0.99994
Total			5768939	100.00		

Fig.24 Base degradation at 72Hrs Assay



	Peak Name	RT	Area	% Area	RT Ratio	Purity 1 Angle	Purity 1 Threshold	USP Resolution
1	UK	12.24	52544	1.65	0.55	1.811	2.124	
2	UK1	13.12	50895	1.60	0.59	5.414	1.742	3.26
3	UK2	16.55	21483	0.67	0.75	2.151	2.290	11.41
4	UK3	21.42	12686	0.40	0.97	4.699	5.393	20.24
5	PR	22.08	3051680	95.69		0.130	0.251	2.44

Fig.25 Sample - Base degradation at 72Hrs RS

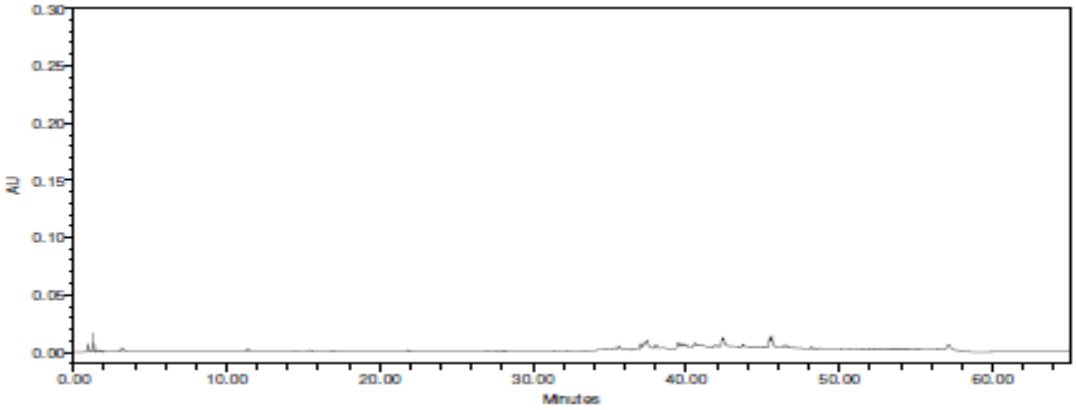
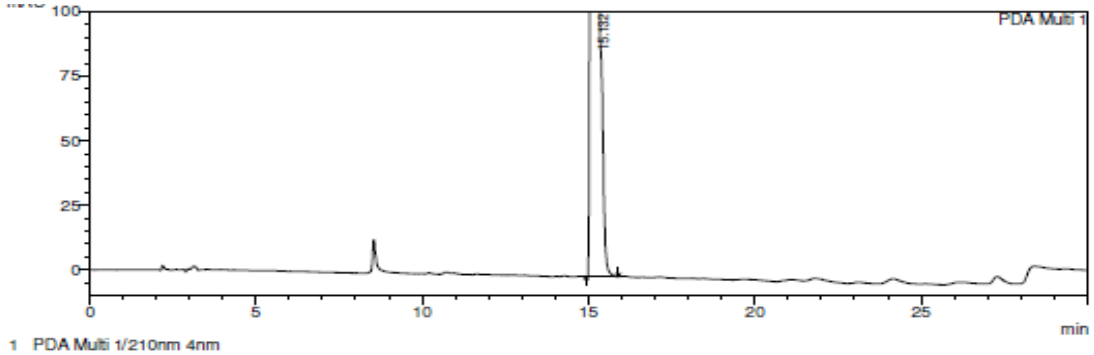


Fig.26 Placebo - Base degradation at 72Hrs RS

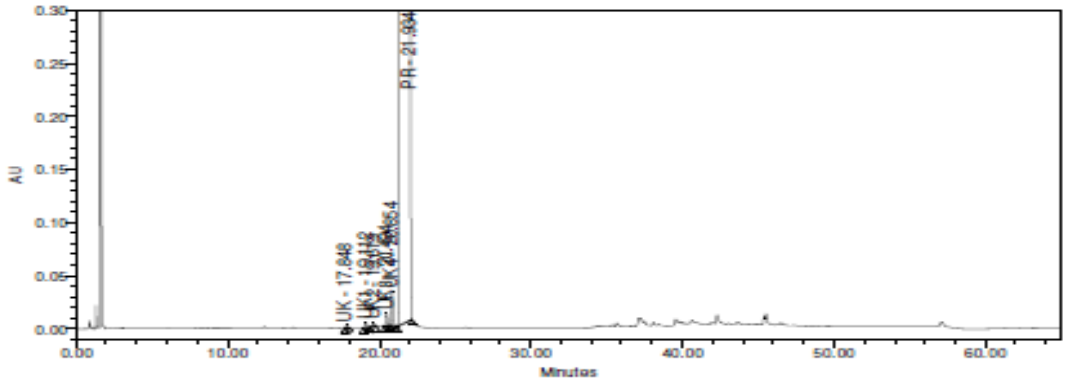


PeakTable

PDA Ch1 210nm 4nm

Peak#	Name	Ret. Time	Area	Area %	Peak Purity Index	Single Point Threshold
1	PR	15.132	5789868	100.00	1.00000	0.99994
Total			5789868	100.00		

Fig.27 Sample - Peroxide degradation at 72Hrs RS



	Peak Name	RT	Area	% Area	RT Ratio	Purity 1 Angle	Purity 1 Threshold	USP Resolution
1	UK	17.85	19104	0.10	0.81	5.455	3.585	
2	UK1	19.11	41955	0.22	0.87	2.599	2.653	6.55
3	UK2	19.62	29096	0.15	0.89	7.228	6.549	2.29
4	UK3	20.49	101735	0.53	0.93	1.108	1.303	3.70
5	UK4	20.85	246144	1.29	0.95	0.674	0.764	1.63
6	PR	21.93	18656795	97.71		0.994	0.264	2.10

Fig.28 Peroxide degradation at 72Hrs Assay

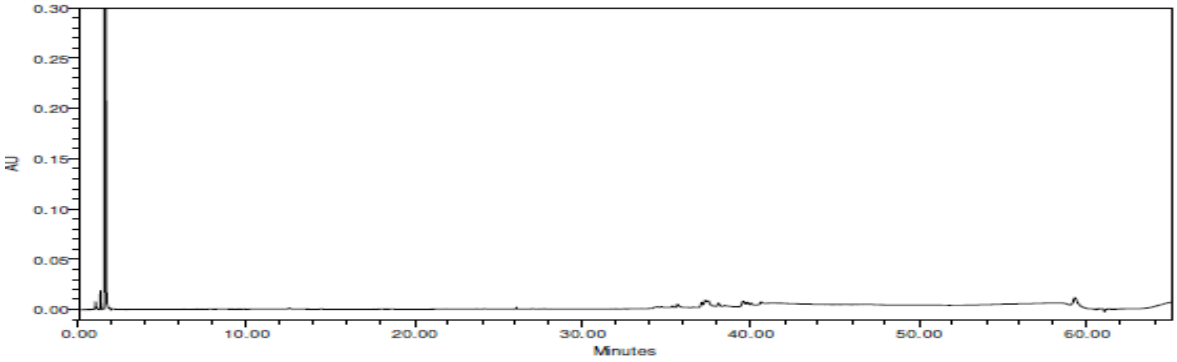


Fig.29 Placebo - Peroxide degradation at 72Hrs RS

Table: 13 Summary

Parameters	Acceptance Criteria	Result
Specificity	Mobile phase, placebo and diluents should not show any interference with the main peak.	No interference observes
Linearity (50% to 125%) Assay	R^2, NLT 0.99	0.999
Linearity (LOQ to 200%) RS	R^2, NLT 0.99	0.996
System precision	Tailing factor:NMT 2.0	1.8
	The % RSD for area:NMT 2.0	0.2
Method precision	%RSD for area:NMT 2.0%	0.39
LOD	S/N ratio around 3	2.9

CONCLUSION

Based on the chromatographic profile obtained from the study, assay and related susbstance method is specific, linear and precise and can be used to analyze Plerixafor and its degradation products in Plerixafor injection.

Acknowledgement

The authors gratefully acknowledge Enal drugs Pvt Limited, Hyderabad, Telangana, India for providing necessary facilities to carry out this work.

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