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"STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF PALONOSETRON HYDROCHLORIDE IN ITS PARENTERAL DOSAGE FORM"

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ABSTRACT

A selective and simple stability indicating reversed phase HPLC method using Naphthalethyl stationary phase C18 column (250 x 4.6mm, 5µm) was developed and validated for the quantitative determination of Palonosetron Hydrochloride in its Parenteral dosage form. Chromatographic separation (Rs > 2) was achieved with Isocratic mode-Mobile Phase: Solution-A: Solution-B (35:65) (Solution A-Buffer (Potassium dihydrogen Phosphate, Triethylamine pH 2.5), & Solution B-Buffer: ACN (50:50) of elution at a flow rate of 1 mL/min and with UV detection at 210 nm. Retention time of Palonosetron hydrochloride was found to be 7 min. This drug was subjected to Hydrolysis, oxidation, Photolysis and thermal to apply stress conditions. Linearity for Palonosetron hydrochloride was found In the Range of 28.06-84.19 microgram/milliliter (R2 = 0.999). The accuracy of the present method was evaluated at 50%, 100% and 150%. The % recoveries of Palonosetron hydrochloride was found to be in the range of 99–101%. Precision studies were carried out and the relative standard deviation (RSD) values were less than 2.

KEYWORDS: Palonosetron Hydrochloride, Parenteral dosage form, Stability Studies, HPLC, Forced Degradation.

INTRODUCTION[1-3]

Palonosetron Hydrochloride is chemically (3aS)-2-[(3S)-1-azabicyclo[2.2.2]octan-3-yl]-3a,4,5,6-tetrahydro-3H-benzo[de]isoquinolin-1-one; hydrochloride. Palonosetron Hydrochloride is used in the prevention and treatment of chemotherapy-induced nausea and vomiting (CINV). Palonosetron is a 5-HT3 antagonist, commonly known as a Setron. This drug acts by blocking Serotonin from binding to the 5-HT3 antagonist. Methods such as HPLC, LC-MS, and simultaneous UV-spectrophotometric method, HPLC-ESI are reported for estimation of Palonosetron Hydrochloride alone or in combination with other drugs.

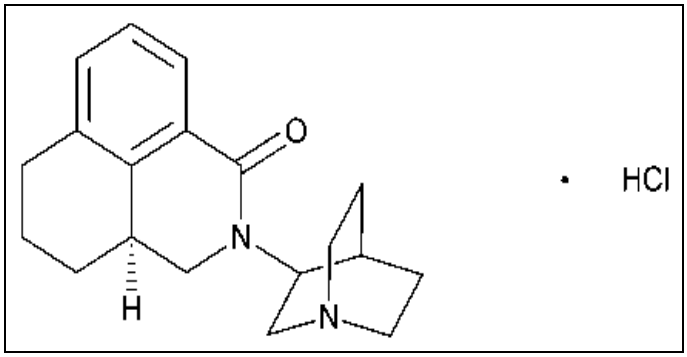

Fig. 1 Palonosetron Hydrochloride structure.

A literature search reveals that few analytical methods were reported for Stability indicating RP-HPLC method development and Validation for estimation of Palonosetron hydrochloride. Hence a Simple, Rapid, Sensitive and Accurate stability indicating RP-HPLC method was developed for determination of Palonosetron Hydrochloride in its Parenteral dosage form.

MATERIALS AND METHODS[4-17]

Material and Reagents

Acetonitrile, Methanol, Potassium di-Hydrogen Phosphate, Ortho Phosphosphoric Acid, Hydrochloric acid, Sodium hydroxide, Hydrogen peroxide (50%) were procured from Merck and Finar Reagent. Palonosetron Hydrochloride standard Gifted by ZYDUS CADILA HEALTH CARE, Moraiya, Ahmedabad.

Equipment

Chromatographic separation was performed on RP-HPLC system consist of model Shimdzu Prominence-1 LC-2030C 3D series PDA detector with 10µl loop volume. UV spectrophotometer which consists of model Shimadzu UV 1800 Spectrophotometer is also used to measure the wavelength of Palonosetron Hydrochloride.

Preparation of Standard Stock Solution (224.5ppm)

Weigh accurately and transfer of about 22.5 mg of Palonosetron Hydrochoride standard into 100ml volumetric flask. Add about 70ml of diluent and sonicate to dissolve. Make volume up to the mark with diluents and mix.

Preparation of working standard solution (56.12ppm)

Pipette and transfer 5 ml of standard stock solution into 20 ml volumetric flask. Make volume uo to the mark with Diluent and mix.

AS Such Sample (50ppm)

As Such Sample is taken for method development.

As such Placebo

As Such placebo Sample is taken.

Each ml contains-Glacial Acetic Acid, Sodium Acetate Anhydrous, Mannitol, Edetate Disodium Dehydrate, Water for injection, HCl or NaOH for adjust pH.

Chromatographic Conditions[18,19]

The Cosmosil ∏ Nap packed Column C18 (250mm*4.6mm), 5µm was used as the stationary phase. Elution was Isocratic by Solution A: Solution B (35:65) %v/v where Soluion A- Buffer: 0.025 M Pottasium dihydrogen Phosphate pH adjusted by OPA+ Triethylamine pH of Buffer: 2.5±0.05 and Soluion B: Buffer: Acetonitrile (50:50) %v/v. It was filtered through 0.45µ (micron) membrane filter and degassed. The mobile phase was pumped at 1.0 ml/min. The eluents were monitored at 210 nm. The injection volumes of sample and standard were 10µl.Total run time was 15 min. Column temperature was 35 ºC. Chromatograms are shown in figure: 2.

Validation of develop method[20]

System Suitability

It was demonstrated by making six replicate injections of Standard solution prepared as per the test method. The peak area of Palonosetron Hydrochloride was recorded. The theoretical plates and tailing factor were evaluated for the Palonosetron Hydrochloride peak. The values of system suitability results obtained are shown in Table 2.

Acceptance criteria

% RSD of Area of five replicate standard injections should not be more than 2.0.

Theoretical Plates for the analyte peak should not be less than 2000.

Tailing factor for the analyte peak should not be more than 2.0.

Linearity

The linearity for Palonosetron Hydrochloride is established over the range of 50% -150% of target concentration. These solutions were injected into the HPLC system and area response of the same was recorded. A plot of concentration Vs Y-intercept of the plot were evaluated. The observations were shown in Table 3.

Table 1: Linearity Preparation.

Linearity level	Volume of linearity std stock solution (224.5ppm) to be taken (ml)	Dilute to volume with diluents	Final concentration (µg/ml)
50%	2.5	20	28.06
80%	4	20	44.90
100%	5	20	56.12
120%	6	20	67.35
150%	7.5	20	84.19

Acceptance Criteria

The correlation coefficient should be 0.999.

Curve was shown in Figure 3.

Method Precision

It was demonstrated by making six replicate injections of Standard solution prepared as per the test method, Injected in Chromatographic system & determined the %Assay of these samples. Evaluate the precision of the method by computing the % RSD.

Precision considered at three levels: Repeatability, Intermediate (Intraday) Precision and Reproducibility (Interday) Precision.

Intraday precision

Solutions containing 80%, 100% & 120% Level of Palonosetron Hydrochloride was analysed for three times on same day and % RSD was calculated.

Interday precision

Solutions containing 80%, 100% & 120% Level of Palonosetron Hydrochloride was analysed for three times on three different successive days and % RSD was calculated.

Repeatability

Method precision of experiment was performed by preparing the standard solutions of Palonosetron Hydrochloride (56.12µg/ml) for six times and analysed as per proposed method and % RSD was calculated.

Acceptance criteria

% RSD of peak area should not be more than 2.0%.

Data shown in the Table 4,5,6.

Accuracy

The accuracy of the test sample was demonstrated by preparing recovery samples at the level of 50%, 100% & 150% of target concentration. The above samples were chromatograph and the % recovery for the amount added was estimated. Each solution was injected in triplicate and the % recovery was calculated by measuring the Assay. The observations were shown in Table 7.

Acceptance Criteria

The % Recovery at each level should be 98% to 120% of added concentration.

Specificity

The specificity of the test method was demonstrated by studying the interference from Mobile Phase, Diluent Blank, Placebo and different degradation pathways. Sample prepared as per procedure and injected in HPLC. The observations were shown in Table 8.

Acceptance Criteria

There should be no interference at the RT of analyte peak.

Forced Degradation Study[21]

ICH prescribed stress conditions such as acidic, basic, oxidative, thermal and photolytic stresses were carried out.

Acid degradation

Condition: 5 N HCl_0.5 ml_80°C_4 Hrs.

Sample preparation

Take the 9.0 ml of the sample into a 10ml volumetric flask. Add 0.5ml of 5N Hydrochloride acid and shake it. Keep the sample on water bath at 80ºC for 4 hrs. Cool at room temperature. Neutralize with 0.5 ml of 5N Sodium Hydroxide Solution and Shake it. Filter with 0.45µ PVDF filter.

Chromatograms are shown in figure: 4

Alkali Degradation

Condition: 5 N NaOH_0.5 ml_80°C_4 Hrs.

Sample preparation

Take the 9.0ml of the sample into a 10ml volumetric flask. Add 0.5ml of 5N Sodium Hydroxide and shake it. Keep the sample on water bath at 80ºC for 4 hrs. Cool at room temperature. Neutralize with 0.5ml of 5N Hydrochloride acid Solution and Shake it. Filter with 0.45µ PVDF filter.

Chromatograms are shown in figure: 5

Peroxide Degradation

Condition: 30% H_2O_2_0.5 ml_80°C_6 Hrs.

Sample preparation

Take the 9.5ml of the sample into a 10ml volumetric flask. Add 0.5ml of 30% Hydrogen Peroxide and shake it. Keep the sample on water bath at 80ºC for 6 hrs. Cool at room temperature. Filter with 0.45µ PVDF filter.

Chromatograms are shown in figure: 6

Photolaytic Degradation

Condition: Expose Under UV light.

Sample preparation

Take the 1 ml of the sample into a 10ml volumetric flask. Keep the sample in Photostability Chamber for Exposure up to 1.2 million lux hous under UV light.

Chromatograms are shown in figure: 7.

Thermal Degradation

Condition: Expose in Oven_100°C_2 Days.

Sample preparation

Take the 1 ml of the sample into a 10ml volumetric flask. Keep the sample in Oven for Exposure at 100°C for 2 days.

Chromatograms are shown in figure: 8.

RESULTS AND DISCUSSION

The detection wavelength was carried out in the UV range of 210 nm. Chromatographic separation was carried out using mobile phase composed Solution A: Solution B (35:65) %v/v where Soluion A- Buffer: 0.025 M Pottasium dihydrogen Phosphate pH adjusted by OPA+ Triethylamine pH of Buffer: 2.5±0.05 and Soluion B: Buffer: Acetonitrile (50:50) %v/v by using Cosmosil ∏ Nap packed Column C18 (250mm*4.6mm), 5µm as the stationary phase.

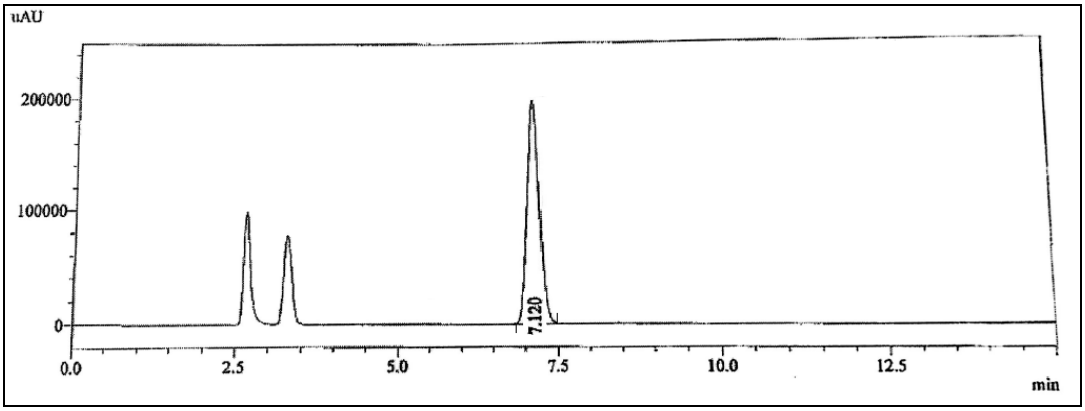

Fig. 2: Chromatogram of Palonosetron Hydrochoride Test Sample.

Method Validation

The described method has been validated which include parameters like System Suitability, Linearity, Accuracy, Precision, Specificity.

System Suitability

System suitability and chromatographic parameters were validated such as Theoretical plates, %RSD of Area and Tailing factor was calculated. The results are given in table 1.

Table 2: System Suitability Data for Palonosetron Hydrochloride.

Parameters	Observation	Specification
% RSD of Area	0.10	RSD < 2%
Resolution (Rs)	0.0	Rs > 2%
Theoritical Plate (N)	14496	N >2000
Tailing Factor (T)	1.21	T < 2%

Linearity

The linearity of this method was evaluated by linear regression analysis and calculated by the least square method and studied by preparing standard solutions of Palonosetron Hydrochloride at different concentration levels. The calibration curve showed in (Fig. 3) good linearity in the range of 25-75 µg/ml with a correlation coefficient (r^2) of 0.999. The results are given in table 2.

Table 3: Linearity Data of Palonosetron Hydrochloride.

Concentration(µg/ml)	Peak area ± S.D (n=3)	%RSD
28.06	1556.15±0.174	0.01
44.90	2491.91±1.541	0.06
56.12	3125.85±0.528	0.02
67.35	3722.46±1.154	0.03
84.19	4682.28±1.501	0.03

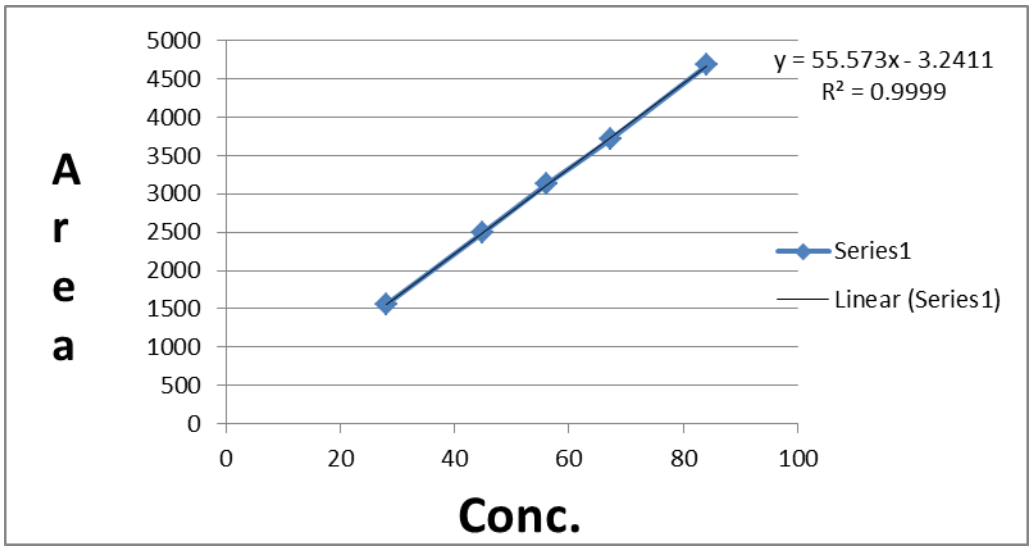

Fig. 3: Calibration curve of Palonosetron Hydrochoride.

Precision

Intraday precision

Intraday precision was performed by analyzing three different concentrations within linearity range, three times in a day (3*3 determinations).

Table 4: Intraday Precision Data for Palonosetron Hydrochloride.

	80% Level						
Set	**Level**	**Morning**	**Noon**	**Evening**	**Mean**	**SD**	**% RSD**
1	80%	2490.2275	2465.2489	2450.2362	2468.570	20.201	0.82
2	80%	2520.5686	2483.2156	2495.2324	2499.672	19.068	0.76
3	80%	2555.3245	2550.2548	2530.2456	2545.274	13.260	0.52

	100% Level						
Set	**Level**	**Morning**	**Noon**	**Evening**	**Mean**	**SD**	**% RSD**
1	100%	3126.1489	3099.1254	3100.2521	3108.508	15.257	0.49
2	100%	3130.1378	3102.2542	3110.2215	3114.204	14.362	0.46
3	100%	3165.1245	3159.2354	3145.2531	3156.537	10.207	0.32

	120% Level						
Set	**Level**	**Morning**	**Noon**	**Evening**	**Mean**	**SD**	**% RSD**
1	120%	3773.5549	3725.2356	3706.2531	3735.014	34.700	0.93
2	120%	3720.5438	3718.1245	3700.2315	3712.966	11.095	0.30
3	120%	3745.2452	3715.2456	3705.2421	3721.910	20.817	0.56

Interday precision

Interday precision was performed by analyzing three different concentrations within linearity range, on different days.

Table 5: Inter day Precision Data for Palonosetron Hydrochloride.

80% Level						
Set	**Level**	**Day-1**	**Day-2**	**Mean**	**SD**	**% RSD**
1	80%	2490.2275	2550.2332	2520.230	42.430	1.68
2	80%	2520.5686	2575.2924	2547.931	38.696	1.52
3	80%	2555.3245	2570.2446	2562.785	10.550	0.41

100% Level						
Set	**Level**	**Day-1**	**Day-2**	**Mean**	**SD**	**% RSD**
1	100%	3126.1489	3190.2521	3158.201	45.328	1.44
2	100%	3130.1378	3202.2215	3166.180	50.971	1.61
3	100%	3165.1245	3215.2531	3190.189	35.446	1.11

120% Level						
Set	**Level**	**Day-1**	**Day-2**	**Mean**	**SD**	**% RSD**
1	120%	3773.5549	3816.2531	3794.904	30.192	0.80
2	120%	3720.5438	3800.2315	3760.388	56.348	1.50
3	120%	3745.2452	3825.2421	3785.244	56.566	1.49

Repeatability

The repeatability studies were carried out by measuring response for a single concentration (56.12µg/ml) for 6 times a day.

Table 6: Repeatability Data for Palonosetron Hydrochloride.

Sr. No.	Palonosetron Hydrochloride (56.12 µg/ml)
1	3098.80664
2	3089.11084
3	3090.05615
4	3097.22412
5	3086.77515
6	3094.42285
Mean	3092.73263
SD	4.81033054
% RSD	0.16

Accuracy

Accuracy of the method was confirmed by recovery study of Palonosetron Hydrochloride at three levels (50%, 100%, 150%) by standard addition method. The results are given in table 3.

Table 7: Accuracy Data for Palonosetron Hydrochloride.

Set	Level	Amount Added	Area	Amount Found	% Recovery	Mean % Recovery	% RSD
1	50%	0.5564	1522.837	0.5636	101.3	101.2	0.2
2	50%	0.5564	1518.694	0.5621	101.0		
3	50%	0.5564	1520.789	0.5629	101.2		

Set	Level	Amount Added	Area	Amount Found	% Recovery	Mean % Recovery	% RSD
1	100%	1.1129	3015.735	1.1162	100.3	100.3	0.3
2	100%	1.1129	3020.559	1.1180	100.5		
3	100%	1.1129	3006.238	1.1127	100.0		

Set	Level	Amount Added	Area	Amount Found	% Recovery	Mean % Recovery	% RSD
1	150%	1.6693	4482.889	1.6592	99.4	99.5	0.8
2	150%	1.6693	4456.078	1.6493	98.8		
3	150%	1.6693	4522.281	1.6738	100.3		

Specificity

Table 8: Peak Purity data of Palonosetron Hydrochloride.

	Palonosetron Hydrochloride
Standard	1
Test	0.990

Forced Degradation

Acid Degradation

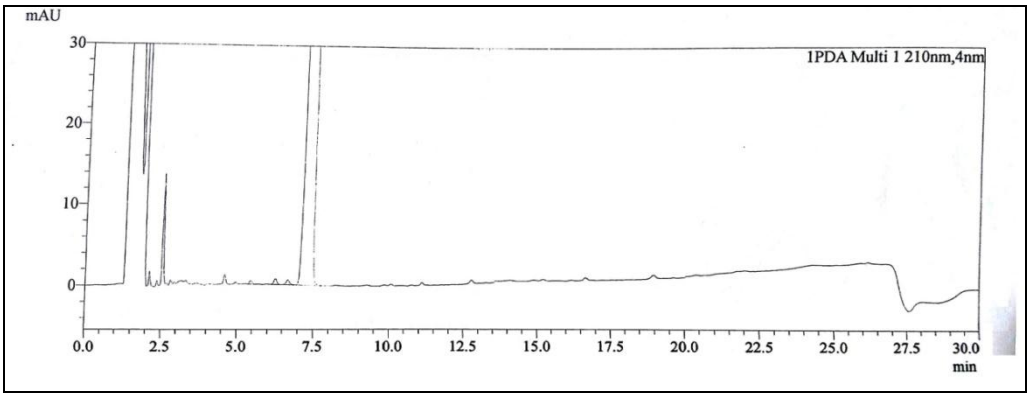

Fig. 4: Chromatograph of Acid Degradation.

Base Degradation

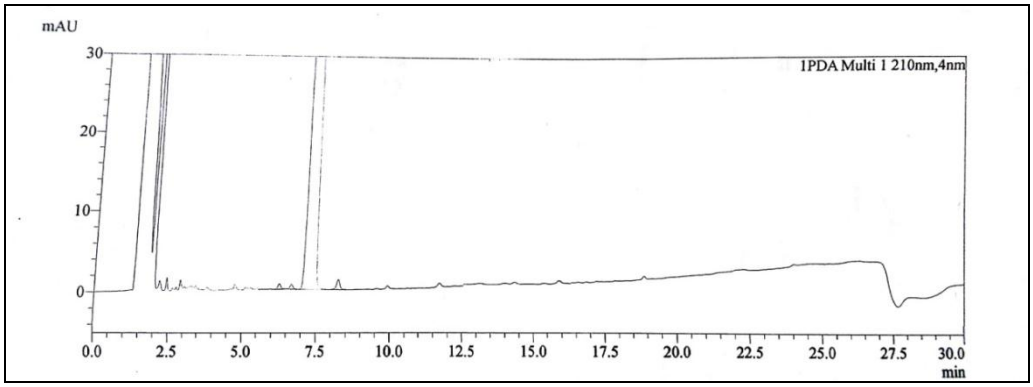

Fig. 5: Chromatograph of Base Degradation.

Peroxide Degradation

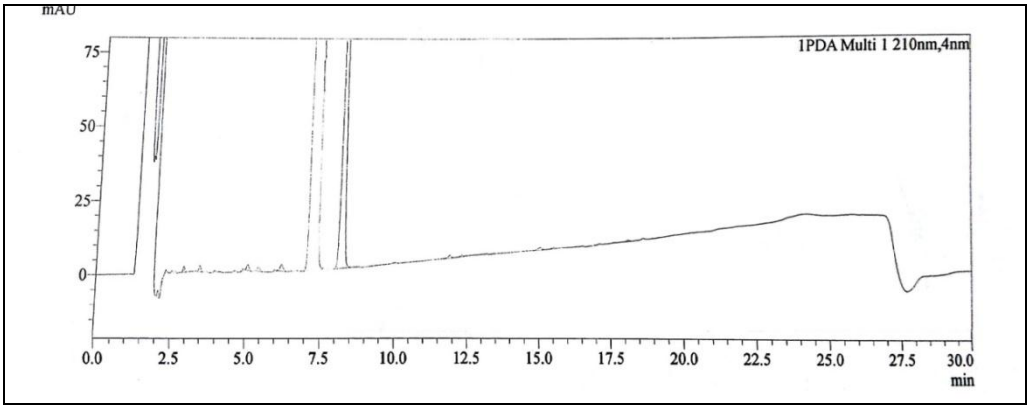

Fig. 6: Chromatograph of Peroxide Degradation.

Photolaytic Degradation

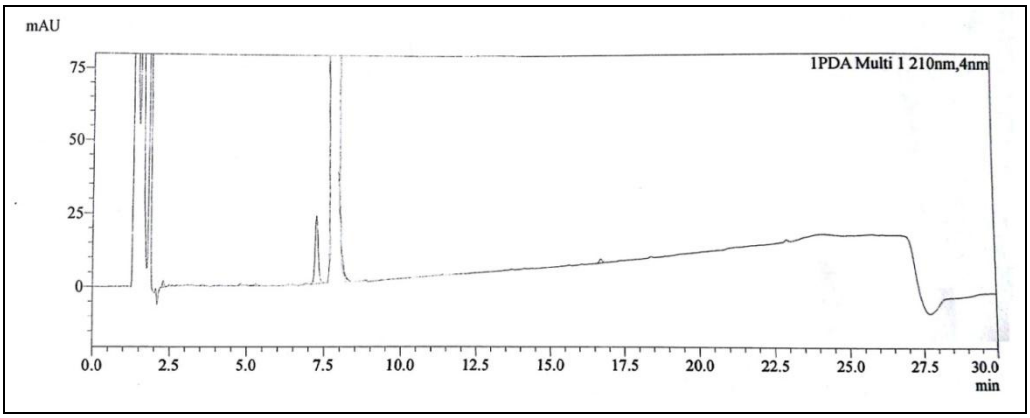

Fig. 7: Chromatograph of Photolaytic Degradation.

Thermal Degradation

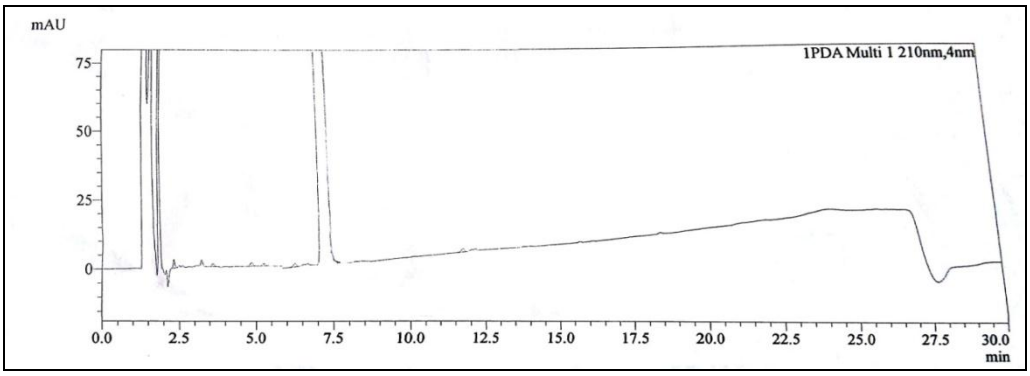

Fig. 8: Chromatograph of Thermal Degradation.

Palonosetron Hydrochloride undergoes significant degradation in acid, base, peroxide, thermal and UV. Comparatively, more degradation was found with Peroxide Degradation. Forced Degradation Summary is given in Table 9.

Table 9: Forced Degradation Summary.

Condition	Area	%Assay	% Degradation	Peak Purity
As Such	2585921	98.83%	-	-
Acid Degradation	2333265	89.27%	9.7%	0.999995
Base Degradation	2332723	89.25%	9.8%	0.999996
Peroxide Degradation	2271128	86.89%	12.1%	0.999996
Photolaytic Degradation	2599634	100.53%	-	0.999996
Thermal Degradation	2531257	97.88%	0.95%	0.999995

Hence, a method of the analysis of Palonosetron Hydrochloride in Parenteral dosage form shows that the degradation product doesn't interfere with the analytical determination.

Hence the proposed analytical method is also useful for the determination of Palonosetron Hydrochloride stability in a sample of the pharmaceutical dosage form.

CONCLUSION

Stability indicating RP-HPLC methods have been developed and validated for the determination of Palonosetron Hydrochloride in Parenteral dosage form. The methods are found to be specific as there was no interference of any co-eluting impurities after stress degradation. The proposed method is found to be Simple, Accurate, Precise and Robust. Hence it can be used successfully for the routine analysis of Palonosetron Hydrochloride in Pharmaceutical dosage forms and for analysis of stability samples obtained during accelerated stability study.

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