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ORIGINAL ARTICLE

Stability Indicating Method Development and Validation for the Simultaneous Estimation of Palonosetron and Netupitant by RP-UPLC

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ABSTRACT
A simple, accurate and precise method was developed for the simultaneous estimation of the Netupitant (NTPT) and Palonosetron (PLSN) in Tablet dosage form by RP-UPLC technique. Retention times of NTPT and PLSN were found to be 1.086 min and 1.842 min respectively. Excellent chromatographic efficiency parameters were obtained with the mobile phase composition of 0.01N KH_2PO_4 buffer (4.0 pH) and acetonitrile in the ratio of 20:80%v/v pumped through an Kromasil C18 (100 x 2.1 mm, 1.8µ) reverse phase column, at a flow rate of 0.3 ml/min. Repeatability of the method was determined in the form of %RSD and findings were 0.9 and 1.0 for NTPT and PLSN respectively. LOD, LOQ values obtained from regression equations of NTPT and PLSN were 2.174, 6.587µg/ml and 0.02, 0.05 µg/ml respectively. Two analytes were subjected for acid, peroxide, photolytic, alkali, neutral and thermal degradation studies and the results shown that the percentage of degradation was found between 0.85% and 6.50%. Retention times and total run time of two drugs were decreased and the developed method was simple and economical. So, the developed method can be adopted in industries as a regular quality control test for the quantification of NTPT and PLSN
***Keywords**: Palonosetron, Netupitant, RPUPLC, Robustness, Linearity.*

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INTRODUCTION

Cancer chemotherapy induced nausea and vomiting (CINV) is a common adverse effect of most cancer drug regimens. If this condition is not controlled, it can affect quality of life and contribute to the overall survival of cancer patients' greater importance should therefore be given to antiemetic prophylaxis in the treatment of cancer. This has led to the development of new antiemetics that have substantially changed the current scenario for the prevention of CINV.

With a better understanding of the neuropharmacology of CINV and the development of new agents targeting different receptors involved in the CINV process, multi-agent antiemetic prophylactic combinations are now recommended for the highly emetogenic chemotherapy environment. Unfortunately, due to their apparent complexity, adherence to the antiemetic combinations recommended by the antiemetic guidelines has been very minimal. Several antiemetic drug classes are available on the market in different formulations (*i.e.*, tablets, IV and IM), offering a wide range of options for doctors and patients in various contexts. Alternative drug formulations can help to meet the unaddressed needs of patients and prescribers by promoting greater patient adherence to prescribed drug treatments.

Akynzeo® (Helsinn Therapeutics Inc., USA) for injection is an antiemetic combination containing 235 mg of fosnetupitant (FOS) and 0.25 mg of palonosetron (PAL). It is a freeze-dried powder in a vial and is reconstituted in 50 mL of 5% dextrose injection USP or 0.9% sodium chloride injection USP. Before the

start of chemotherapy, a patient is given a vial of reconstituted Akynzeo® as a 30minute intravenous infusion (Akynzeo® prescribing information, 2020). Figure 1 shows the chemical structures of FOS and PAL[1,2].

Netupitant is an antiemitic drug approved by the FDA in October 2014 for use in combination with palonosetron for the prevention of acute and delayed vomiting and nausea associated with cancer chemotherapy including highly emetogenic chemotherapy. Netupitant is a neurokinin 1 receptor antagonist. The combination drug is marketed by Eisai inc. And helsinn therapeutics (u.s.) Inc. Under the brand akynzeo. Palonosetron (inn, trade name aloxi) is an antagonist of 5-ht3 receptors that is indicated for the prevention and treatment of chemotherapy induced nausea and vomiting (cinv). it is the most effective of the 5-ht3 antagonists in controlling delayed cinv nausea and vomiting that appear more than 24 hours after the first dose of a course of chemotherapy and is the only drug of its class approved for this use by the US food and drug administration. As of 2008, it is the most recent 5- ht3 antagonist to enter clinical use[3,4].

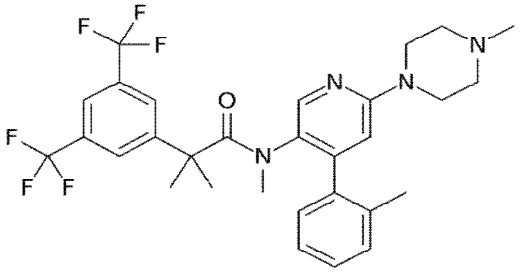

Fig. 1: Chemical structure of NTPT.

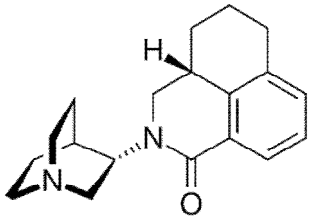

Fig. 2: Chemical structure of PLSN.

MATERIAL AND METHODS

Chemicals and Reagents

API of NTPT and PLSN were obtained from spectrum Pharma Research Solutions, Hyderabad. HPLC-grade methanol and acetonitrile were procured from Merck chemical division, Mumbai, India, Potassium dihydrogen ortho phosphate, orthophosphoric acid, sodium dihyrogen ortho phosphate and HPLC-grade water were bought from Rankem, avantor performance material India limited. Akynzeo capsules were obtained from local pharmacy.

Method development

During the method development various mobile phase compositions consisting of methanol, acetonitrile, water, phosphate buffers and different stationary phases were executed to get fine chromatographic conditions like theoretical plates, resolution, tailing and peak shape.

Optimized Chromatographic Conditions

Liquid chromatographic UPLC system of Waters equipped with PDA (photodiode array detector), auto-sampling unit and Kromasil C18 (100 x 2.1 mm, 1.8µ) reverse phase column. The mobile phase composition of 0.01N KH_2PO_4 buffer (4.0 pH) and acetonitrile in the ratio of 20:80 was pumped through a column at a flow rate of 0.3 ml/min. Column oven temperature was maintained at 30°C and the detection wavelength was processed at 274 nm. Integration of output signals were monitored and processed by waters Empower software-2.0.

Diluent

Depending up on the solubility of the drugs, diluent was optimized. Acetonitrile and water in the ratio of 50:50% v/v was used as diluent.

Preparation of Standard Stock Solutions

Exactly weighed 150mg of NTPT and 0.25mg of PLSN poured in to two 50ml volumetric flasks alone. 10ml of diluent was added and vortexed for 20 min. Flasks were made up with diluent and marked as standard stock solution 1and 2 (3000µg/ml of NTPT and 5.0 µg/ml PLSN). 1ml from each stock solution

was pipetted out and taken into a 10ml volumetric flask and made up with diluent to get 300µg/ml of NTPT and 0.5µg/ml of PLSN.

Preparation of Sample Stock Solutions

20 capsules were weighed and the average weight of each tablet was calculated. The weight equivalent to 1 capsule was transferred into a 100ml volumetric flask and 25 ml of diluent was added and sonicated for 25 min. Further the volume was made up with diluent and filtered through 0.45 µ filter (3000µg/ml of NTPT and 5.0 µg/ml PLSN). 1ml of the resultant solution was poured in to a 10ml volumetric flask and made up with diluent (300µg/ml of NTPT and 0.5µg/ml of PLSN).

Preparation of buffer

Accurately weighed 1.36gm of potassium dihyrogen ortho phosphate in a 1000ml of volumetric flask consisting about 900ml of milli-Q water and sonicate to degasify and make up the volume with water. Then 1ml of Triethylamine was added and PH adjusted to 4.0 with dilute orthophosphoric acid solution**.**

Method Validation

The developed method for NTPT and PLSN was subjected for validation for the parameters like system suitability, linearity, robustness, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy as per the guidelines of ICH[5,6].

RESULTS AND DISCUSSION

Method Development and Optimization

With different mobile phase compositions and stationary phases 3 different trials were executed and 4th trail was optimized. In all the 3 trials there was no peak resolution with extra peaks observed in trial-1 and trial-2, and poor resolution, peak shape and theoretical plates observed in trial -3. Optimized chromatographic peaks were shown in Fig. 3.

Finally, excellent chromatographic efficiency parameters were obtained with the mobile phase composition of 0.01N KH_2PO_4 buffer (4.0 pH) and acetonitrile in the ratio of 20:80%v/v pumped through an Kromasil C18 (100 x 2.1 mm, 1.8µ) reverse phase column, at a flow rate of 0.3 ml/min. Column oven temperature was maintained at 30°C and the detection wavelength was processed at 274 nm. Based on the solubility, all the dilutions were made with acetonitrile and water in the ratio of 50:50%v/v. Retention times of NTPT and PLSN were found to be 1.086 min and 1.842 min respectively. An injection volume of 1.0 µl was infused through an UPLC system to get the better performance.

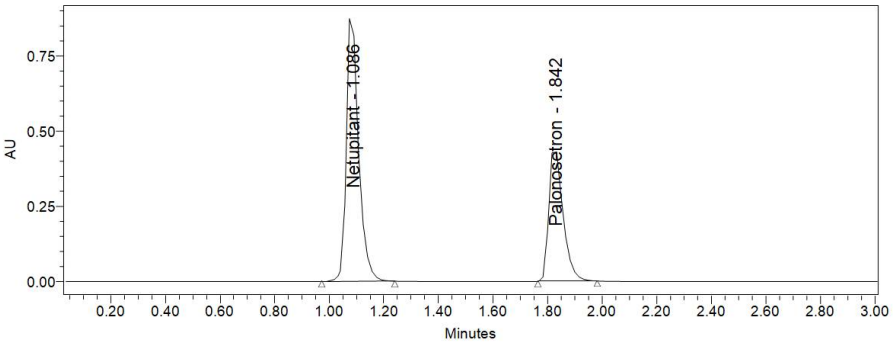

Fig. 3: Optimized chromatogram of NTPT and PLSN

Method validation

System Suitability

The system suitability variables were estimated by preparing standard solutions of NTPT and PLSN and the same were injected 6 times in to the chromatographic system [7-9]. The variables like peak tailing, resolution and USP plate count were estimated. The results were shown in Table 1.

Table. 1: System suitability parameters for NTPT and PLSN

S.No	PLSN			NTPT			
	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	USP Resolution
1	1.841	2970	1.25	1.084	6606	6606	6606
2	1.842	3002	1.25	1.086	6397	6397	6397
3	1.841	2897	1.19	1.085	5927	5927	5927
4	1.843	2827	1.19	1.084	5660	5660	5660
5	1.843	2959	1.21	1.083	5701	5701	5701
6	1.842	3149	1.17	1.086	6960	6960	6960

Specificity

Method specificity was determined by infusing the blank, placebo, standard and sample solutions in to a chromatographic system and the resulting chromatograms were evaluated for interference with the excipients, degradants and other components may expected to be present[10-13]. Blank, standard, formulation and placebo chromatograms were represented in Fig. 4.

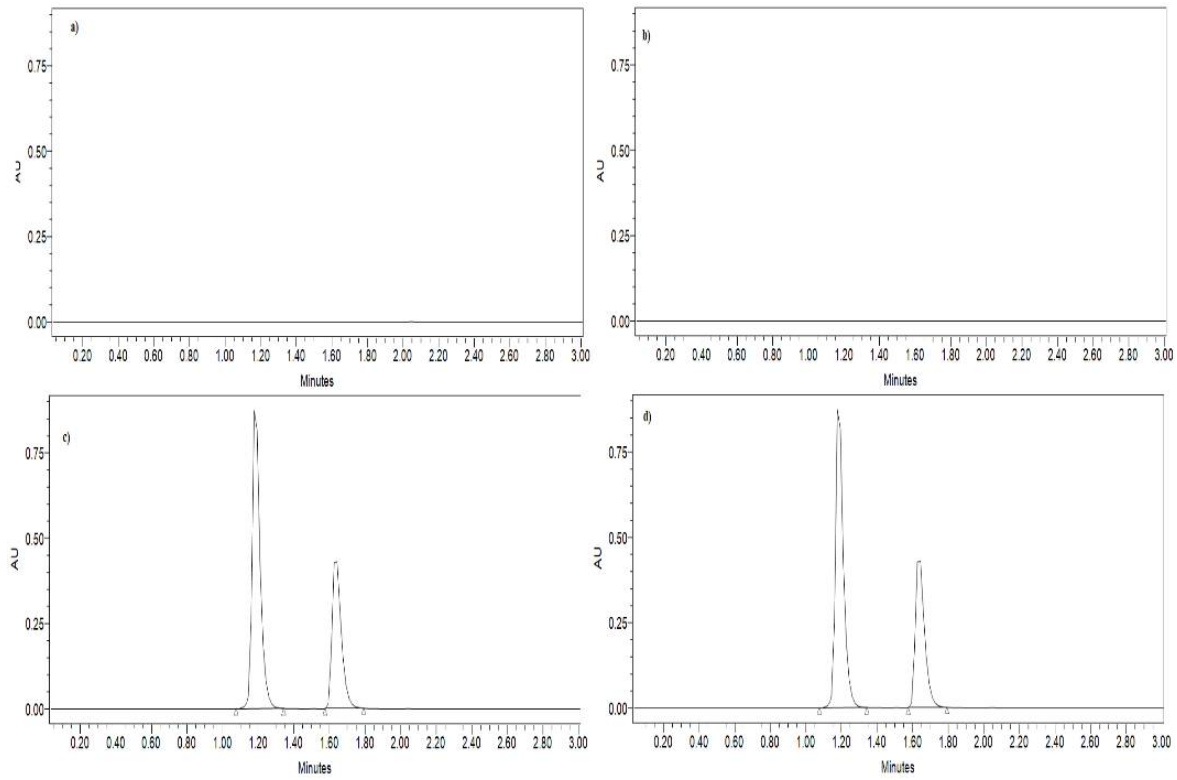

Fig. 4: Chromatograms of a) Blank, b) Placebo, c) Standard and d) Sample.

Precision

Precision of the method was evaluated in terms of method precision and intermediate precision [14-16]. The method precision (repeatability) was estimated by infusing 6 standard solutions and 6 sample solutions. Intermediate precision was evaluated by infusing 6 standard solutions and 6 sample solutions on different days by different employees on different chromatographic systems [23, 25]. The peak responses of all the chromatograms were taken and standard deviation, % RSD (relative standard deviation) and percentage assay of sample solutions were calculated. The findings were represented in Table 2, and 3.

Table. 2: Repeatability results of NTPT and PLSN.

S. No	Area of NTPT	Area of PLSN
1.	1475960	19938
2.	1467092	20243
3.	1476887	20289
4.	1472173	20112
5.	1498861	19915
6.	1462369	20425
Mean	1475557	20154
SD	12665.2	202.5
%RSD	0.9	1.0

SD: standard deviation; RSD: relative standard deviation.

Table. 3: Intermediate precision results of NTPT and PLSN.

S. No	Area of NTPT	Area of PLSN
1.	1414182	19232
2.	1388187	19105
3.	1375020	18991
4.	1399247	18960
5.	1386258	19438
6.	1379109	18991
Mean	1390334	19120
SD	14352.1	185.9
%RSD	1.0	1.0

SD: standard deviation; RSD: relative standard deviation.

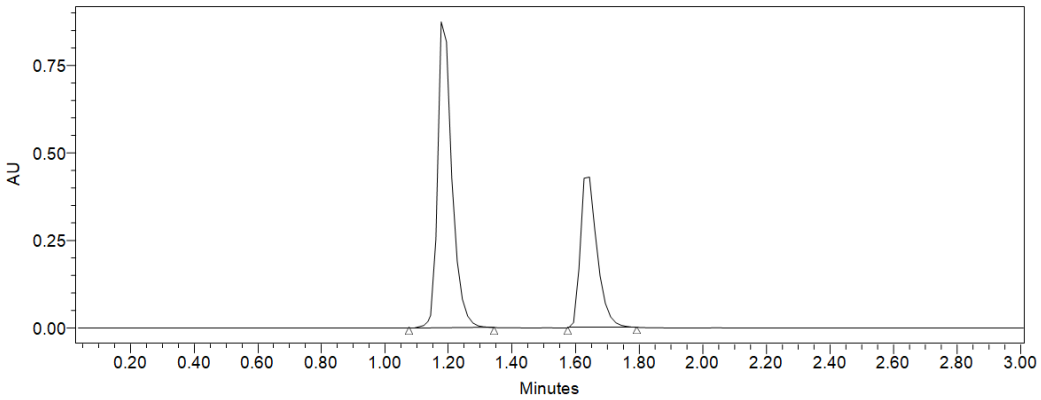

Fig. 5: Chromatogram showing standard injection.

Accuracy

Method accuracy was estimated at three variable concentrations of 50%, 100%, and 150% level by spiking the known amount of the drug analytes[17,18]. The % recovery at each level was calculated and the findings were represented in Table 6.4(Fig. 6 to 8).

Table. 4: Accuracy results of NTPT and PLSN.

	PLSN				NTPT			
% Level	Amount Spiked (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean %Recovery	Amount Spiked (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean % Recovery
50%	0.25	0.248	99.5	99.61%	150	149.856	99.90	99.98%
	0.25	0.25	100.0		150	148.957	99.30	
	0.25	0.247	99.0		150	149.591	99.73	
100%	0.5	0.497	99.5		300	299.675	99.89	
	0.5	0.495	99.1		300	299.944	99.98	
	0.5	0.507	101.4		300	298.962	99.65	
150%	0.75	0.753	100.4		450	449.892	99.98	
	0.75	0.741	98.9		450	448.685	99.71	
	0.75	0.747	99.6		450	451.107	100.25	

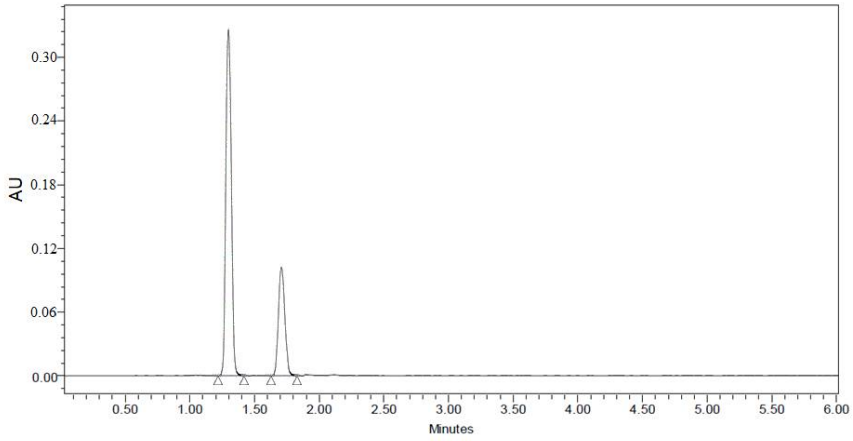

Fig. 6: Chromatogram showing accuracy 50%injection

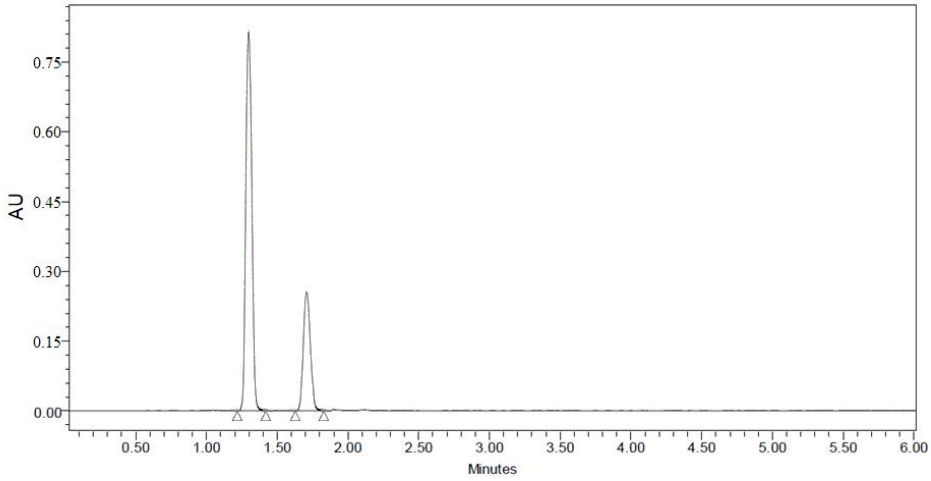

Fig.7: Chromatogram showing accuracy 100%injection

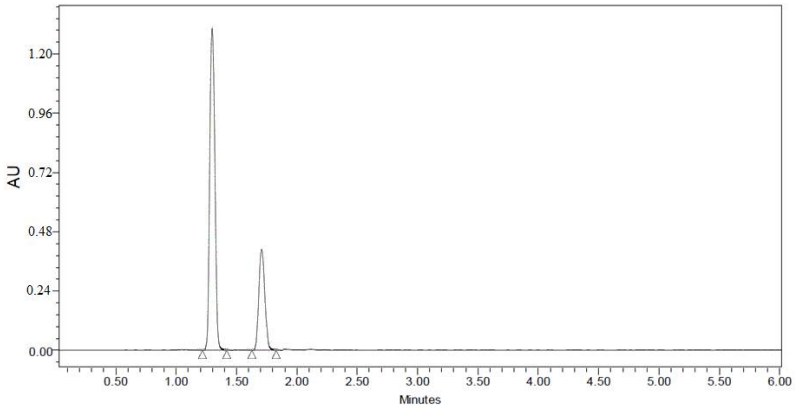

Fig. 8: Chromatogram showing accuracy 150%injection

Linearity

Linearity of the developed method was evaluated by processing 6 different concentration levels of both NTPT and PLSN over the concentration of 75 to 450 µg/ml and 0.125 to 0.75 µg/ml[15]. Each concentration level was processed in triplicates[61, 64]. The linearity plots were acquired by plotting peak response (on X-axis) versus concentration (on Y-axis). The results of the linearity were represented in Fig. 9, 10 and Table 5.

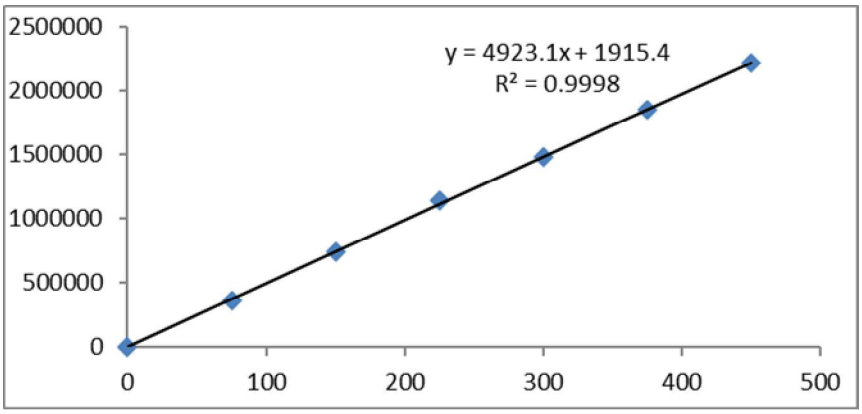

Fig. 9: Calibration curve of NTPT.

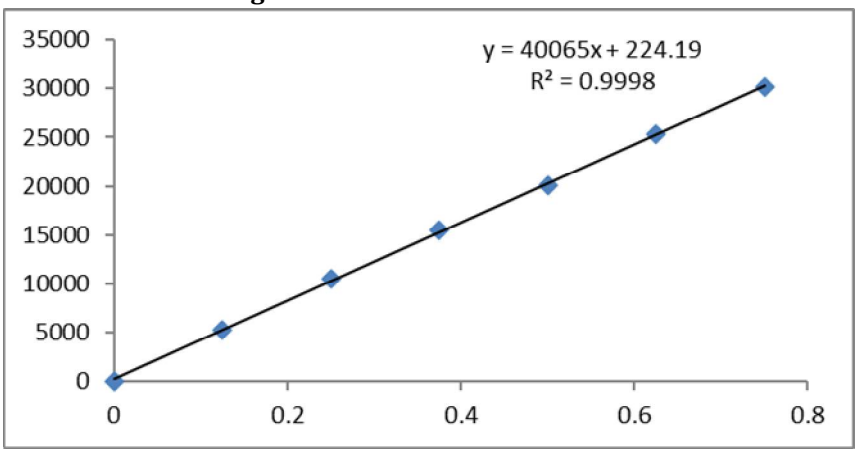

Fig. 10: Calibration curve of PLSN.

Table. 5: Linearity results for NTPT and PLSN.

NTPT		PLSN	
Conc (µg/ml)	**Peak area**	**Conc (µg/ml)**	**Peak area**
0	0	0	0
75	363525	0.125	5254
150	736887	0.25	10462
225	1134680	0.375	15451
300	1475001	0.5	20123
375	1844025	0.625	25277
450	2213099	0.75	30173

LOD and LOQ

LOD is lowest quantity of drug in a sample that can be identified but cannot be quantify exactly. LOQ is the lowest quantity of a drug in a analyte which can be quantitatively estimated with a suitable accuracy and precision[14]. The LOD and LOQ values were calculated from the linearity data by utilizing standard deviation and slope of the curve. The resulting LOD and LOQ findings were represented in Table 6, Fig. 11 and 12.

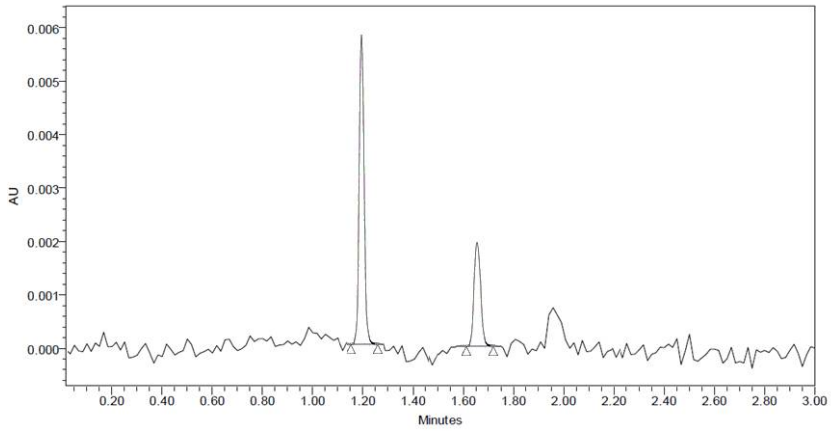

Fig. 11: LOD chromatogram of NTPT and PLSN.

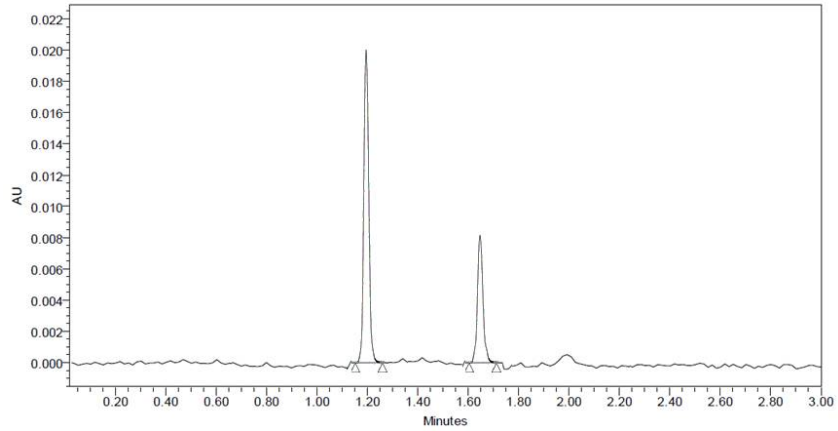

Fig. 12: LOQ chromatogram of NTPT and PLSN.

Table. 6: LOD and LOQ results for NTPT and PLSN.

Analyte	LOD (µg/ml)	LOQ (µg/ml)
NTPT	2.174	6.587
PLSN	0.02	0.05

Robustness

The method robustness was processed by introducing small variation in the optimized LC conditions [17] such as organic phase in mobile phase (±5%), flow rate (-0.27 and +0.33 ml/ min) and column temperature (±5°C). The findings were shown in the Table 7.

Table. 7: Robustness data for NTPT and PLSN

S.No.	Variation in LC conditions	% RSD for NTPT	% RSD for PLSN
1	Flow rate (-) 0.27ml/min	1.3	1.4
2	Flow rate (+) 0.33ml/min	1.1	0.9
3	Organic phase -5%	0.7	1.4
4	Organic phase + 5%	1.5	1.1
5	Temperature at 25°C	1.3	1.4
6	Temperature at 35°C	0.9	1.1

Degradation Studies

Alkali Degradation Studies

To 1 ml of each stock solution of NTPT and PLSN, 1 ml of 1N NaOH was added in to a 10 ml volumetric flask and kept at 60°C for 30 min. The resulting solution was neutralized with 1ml of 1N HCl and further, solution was made up to the mark to get 300µg/ml and 0.5µg/ml concentrations of NTPT and PLSN respectively [6,7]. From that 1.0 µl of solution was infused in to an UPLC system and the resultant chromatograms were analysed for the stability of analytes. The findings were represented in Table 8 and Fig. 13.

Table. 8: Degradation data of NTPT and PLSN.

Type of degradation	NTPT			PLSN		
	Area	%Recovered	% Degraded	Area	%Recovered	% Degraded
Acid	1401393	94.78	5.22	18916	93.67	6.33
Alkali	1425724	96.43	3.57	19453	96.33	3.67
Peroxide	1409975	95.36	4.64	18881	93.50	6.50
Thermal	1440170	97.41	2.59	19875	98.42	1.58
UV light	1461415	98.84	1.16	19897	98.53	1.47
Neutral	1465976	99.15	0.85	20027	98.53	1.47

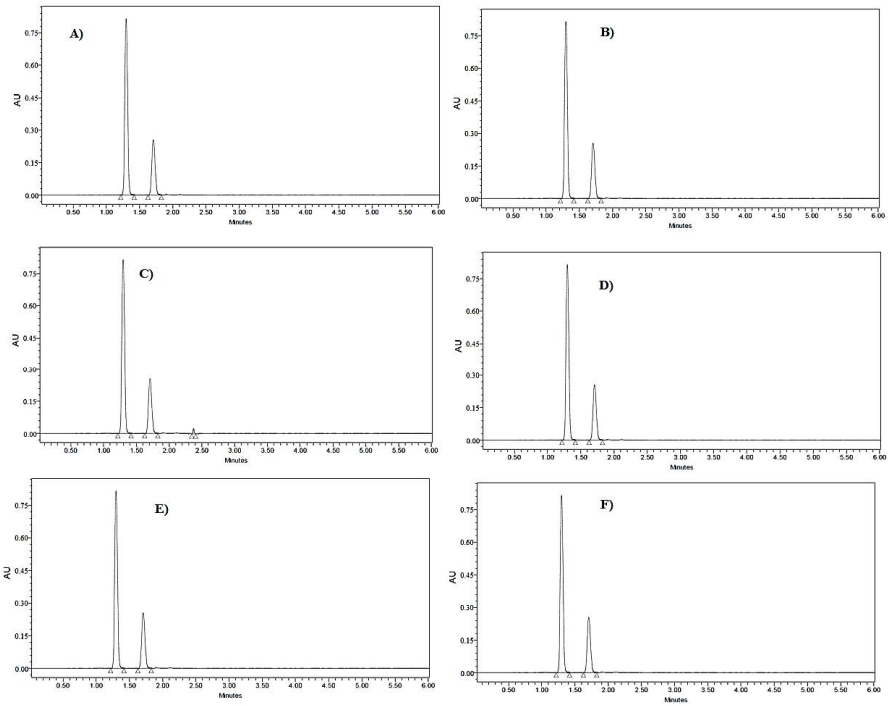

Fig. 13: Chromatogram for A)alkali B) photo C) acid D) neutral E) oxidation F) dry heat degradation study.

Photolytic Stability Study

For the photolytic stability study, NTPT 3000µg/ml and PLSN 5.0µg/ml solutions were exposed to UV-light by placing the solutions in UV cabinet for 1day or 200 Watt hours/m^2 in photo stability chamber. The resulting solutions were combined in a 10ml volumetric flask and made up to the mark with diluent to get 30µg/ml and 0.5µg/ml concentrations of NTPT and PLSN respectively. From that 1.0 µl of solution was infused in to an UPLC system and the resultant chromatograms were analysed for the stability of analytes. The findings were represented in Table 8 and Fig. 13.

Acid Degradation Studies

To 1 ml of each stock solution of NTPT and PLSN, 1 ml of 1N Hydrochloric acid was added in to a 10 ml volumetric flask and refluxed at 60°C for 30 min. The resulting solution was neutralized with 1ml of 1N NaOH and further the resulting solution was made up to the mark to get 30µg/ml and 0.5µg/ml concentrations of NTPT and PLSN respectively. From that 1.0 µl of solution was infused in to an UPLC system and the resultant chromatograms were analysed for the stability of analytes. The findings were represented in Table 8 and Fig. 13.

Neutral Degradation Studies

To 1 ml of each stock solution of NTPT and PLSN, 5 ml of water was added in to a 10 ml volumetric flask and kept for refluxing at 60°C for 6 h. Further, the resulting solution was made up to the mark to get 30µg/ml and 0.5µg/ml concentrations of NTPT and PLSN respectively. From that 1.0 µl of solution was infused in to an UPLC system and the resultant chromatograms were analysed for the stability of analytes. The findings were represented in Table 8 and Fig. 13.

Oxidation

To 1 ml of each stock solution of NTPT and PLSN, 1 ml of 20% hydrogen peroxide (H_2O_2) were added in to a 10 ml volumetric flask and kept at 60°C for 30 min. Further, the resulting solution was made up to the mark to get 30µg/ml and 0.5µg/ml concentrations of NTPT and PLSN respectively. From that 1.0 µl of solution was infused in to an UPLC system and the resultant chromatograms were analysed for the stability of analytes. The findings were represented in Table 8 and Fig. 13.

Dry Heat Degradation Studies

To a 10 ml volumetric flask add 1ml each stock solution of NTPT and PLSN and monitored at 105°C for 6 h in an hot air oven to perform the dry heat stability study. Further, the resulting solution was made up to the mark to get 30µg/ml and 0.5µg/ml concentrations of NTPT and PLSN respectively. From that 1.0 µl of solution was infused in to an UPLC system and the resultant chromatograms were analysed for the stability of analytes. The findings were represented in Table 8 and Fig. 13.

CONCLUSION

A simple, accurate and precise method was developed for the simultaneous estimation of the NTPT and PLSN in Tablet dosage form by RP-UPLC technique. Retention times of NTPT and PLSN were found to be 1.086 min and 1.842 min respectively. Excellent chromatographic efficiency parameters were obtained with the mobile phase composition of 0.01N KH_2PO_4 buffer (4.0 pH) and acetonitrile in the ratio of 20:80%v/v pumped through an Kromasil C18 (100 x 2.1 mm, 1.8µ) reverse phase column, at a flow rate of 0.3 ml/min. Repeatability of the method was determined in the form of %RSD and findings were 0.9 and 1.0 for NTPT and PLSN respectively. LOD, LOQ values obtained from regression equations of NTPT and PLSN were 2.174, 6.587µg/ml and 0.02, 0.05 µg/ml respectively. Two analytes were subjected for acid, peroxide, photolytic, alkali, neutral and thermal degradation studies and the results shown that the percentage of degradation was found between 0.85% and 6.50%. Retention times and total run time of two drugs were decreased and the developed method was simple and economical. So, the developed method can be adopted in industries as a regular quality control test for the quantification of NTPT and PLSN.

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