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DEVELOPMENT OF A NEW CHROMATOGRAPHIC METHOD FOR ESTIMATION OF DOLASETRON IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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ABSTRACT

Background: A simple, accurate and precise HPLC method for simultaneous determination of Dolasetron in pure and tablet dosage form has been developed. **Aim:** Development Of A New Chromatographic Method For Estimation Of Dolasetron In Bulk And Pharmaceutical Dosage Form By Rp-Hplc. **Materials and Methods:** HPLC of Waters (Model: Alliance 2695) with Phenomenex Luna C18 (4.6 mm I.D. × 250 mm, 5 µm) column was used for chromatographic separation. It contains waters injector and PDA Detector (Deuterium). Mobile phase consists of Acetonitrile: Water (50:50% v/v)) and flow rate adjusted was 1ml/min. Wavelength selected for detection was 285nm and injection volume was 10 µl. **Results and discussion:** By using the developed method, retention time of Dolasetron was found to be 3.008 respectively. The method has been validated for linearity, accuracy and precision. Linearity of Dolasetron were in the range 10–50µg/ml respectively. The percentage recoveries obtained for Dolasetron were found to be in range of 99.6 LOD and LOQ were found to be 1.16µg/ml and 3.5µg/ml for Dolasetron. **Conclusion:** A rapid and precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validated of Dolasetron, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Symmetry C18 (4.6 x 250mm, 5µm) column using a mixture of Acetonitrile and Water (50:50% v/v) as the mobile phase at a flow rate of 0.8ml/min, the detection was carried out at 285nm. The retention time of the Dolasetron was 3.0 ±0.02min. The method produce linear responses in the concentration

range of 10-50ppm of Dolasetron. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

KEYWORDS: Dolasetron; PDA Detection; RP-HPLC; Validation; Tablet dosage forms.

INTRODUCTION

Dolasetron: azatricyclo[5.3.1.03,8]undec-5-yl 1H-indole-3-carboxylate. For the prevention of nausea and vomiting associated with emetogenic cancer chemotherapy, including initial and repeat courses of chemotherapy.

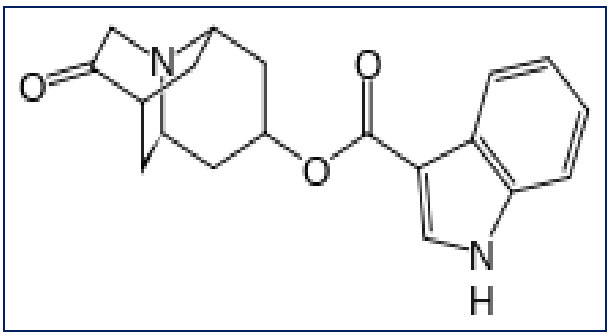

Fig 1: Chemical Structure of Dolasetron.

MATERIALS AND METHODS

Instrument used was an UV-Visible double beam spectrophotometer, SHIMADZU (model UV-1800, software – UV probe, version 2.42) with a pair of 1 cm matched quartz cells. All weighing was done on Sartorius electronic analytical balance.

Instrumentation and Chromatographic conditions[16,27]

The analysis was performed by using Chromosil C-18 column, 250 X 4.6mm internal diameter with 5 micron particle size column and UV detector set at 286.9 nm, in conjunction with a mobile phase of Acetonitrile and Water in the ratio of 60:40 v/v (pH 5 adjusted with OPA) at a flow rate of 0.8 ml/min. The retention time of Dolasetron was found to be 2.136 and 5.485 minute. The 10µl of sample solution was injected into the system.

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Dolasetron working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.3ml of the above Dolasetron stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Water, Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Acetonitrile and water in proportion 50:50 v/v respectively.

Optimization of Column

The method was performed with various columns like C18 column, X- bridge column, Xterra. Phenomenex Luna C18 (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatogram)

Column	: Symmetry C18 (4.6×250mm) 5µ
Column temperature	: Ambient
Wavelength	: 285nm
Mobile phase ratio	: Acetonitrile: Water(50:50 v/v)
Flow rate	: 0.8ml/min
Injection volume	: 10µl
Run time	: 6minutes

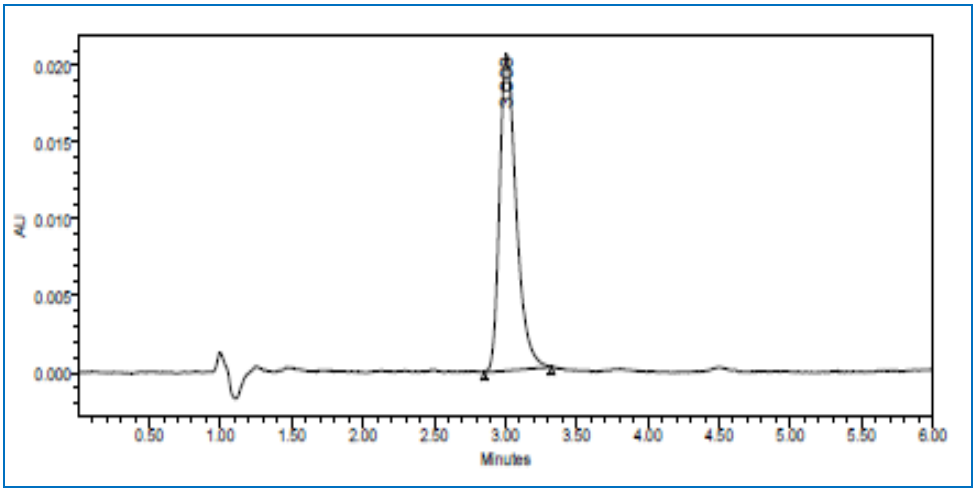

Fig 2: Typical chromatogram of mixture of Standard solution.

VALIDATION

PREPARATION OF MOBILE PHASE

Preparation of mobile phase

Accurately measured 500ml (50%) of HPLC Water and 500ml (50%) of HPLC Acetonitrile in to a 1000ml of volumetric flask and degassed in a digital ultrasonicator for 10 minutes.

Diluent Preparation

The Mobile phase was used as the diluents.

Linearity

The linearity of was obtained in the concentration ranges from 10-50.

Table 1: Linearity data of Dolasetron.

Concentration Level (%)	Concentration µg/ml	Average Peak Area
60	10	1096958
80	20	1979067
100	30	2933803
120	40	3905613
140	50	4769190

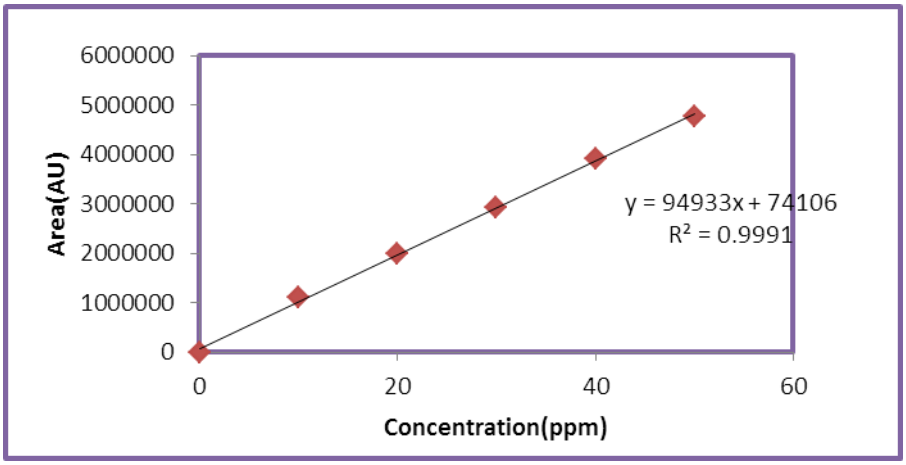

Fig. 3: Calibration graph of Dolasetron.

LINEARITY

Linearity of detector response of assay method was found by injecting seven standard solutions with concentration ranging from 10-50 µg/mL for Dolasetron respectively. The graph was plotted for concentration versus peak area. The results were shown in Table- and fig.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

REPEATABILITY

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table 2: Results of repeatability for Dolasetron.

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Dolasetron	2.942	168306	20744	7562	1.1
2	Dolasetron	2.962	168388	20788	9981	1.2
3	Dolasetron	2.963	168365	20727	6794	1.1
4	Dolasetron	2.804	162052	21841	8927	1.2
5	Dolasetron	2.865	163387	21947	7746	1.1
Mean			166099.6			
S.D			3121.629			
%RSD			1.879372			

ACCURACY

Accuracy at different concentrations (50%, 100% and 150%) were prepared and the % recovery was calculated. The accuracy results shown in below table.

Table 3: Accuracy results for Dolasetron.

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	86774	15	14.9	99.8	99.6
100%	168427	30	29.79	99.3	
150%	255311	45	44.8	99.7	

Limit of detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The LOD and LOQ values for Dolasetron 1.16 µg/ml respectively.

Quantitation limit (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined. The LOQ values for Dolasetron 3.5µg/ml.

Robustness

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Dolasetron. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Dolasetron were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor and plate count.

Table 4: Results for Robustness Dolasetron.

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.8 mL/min	168461	3.008	8846	1.12
Less Flow rate of 0.7mL/min	167261	4.608	7927	1.1
More Flow rate of 0.9mL/min	167651	3.495	6927	1.1
Less organic phase (about 5% decrease in organic phase)	168947	4.609	8826	1.2
More organic phase (about 5% Increase in organic phase)	160081	3.499	9971	1.1

SUMMARY AND CONCLUSION

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 285nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was Symmetry C18 because it was giving good peak. 35ºC temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Water: Acetonitrile (50:50% v/v) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Water: Acetonitrile was selected because of maximum extraction sonication time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery. Run time was selected to be 10 min because analyze gave peak around 3.0 and also to reduce the total run time.

Dolasetron was freely soluble in acetonitrile ethanol, methanol and sparingly soluble in water. Water: Acetonitrile (50:50% v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising.

The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

Table 5: Summary data for Dolasetron.

Parameters	Dolasetron
Retention Time (min.)	3.008
Linearity (µg/ml)	10-50µg/ml
Correlation Coefficient (r^2)	0.99
Slope	94933
Y - intercept	74106
LOD (µg/ml)	1.16µg/ml
LOQ (µg/ml)	3.5µg/ml
Repeatability (% RSD) n=6	1.879372
Intraday Precision (% RSD) n=6	0.285619
Interday Precision (% RSD) n=6	0.272518
Accuracy (%)	99.6

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