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NEW HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF DOLASETRON IN PHARMACEUTICAL DOSAGE FORMS

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Abstract:
A simple, precise, rapid and accurate reverse phase HPLC method was developed for the estimation of dolasetron in capsule dosage form. Waters Spherisorb® 4.6x250mm i.d; column of 5µm particle size, with mobile phase consisting 0.01M ammonium acetate in water :methanol :tetra hydro furan in the ratio 700:240:60 was used. The flow rate was 1.0 ml/min and the effluents were monitored at 295 nm. The retention time was 11.57 min. The detector response was linear in the concentration of 20-60µg/ml. The respective linear regression equation being Y=3000181x+356238.2. The limit of detection and limit of quantification was 0.5µg/ml and 0.15µg/ml respectively. The assay of dolasetron in bulk was found to be 99.85%. From the recovery studies it was found that about 191.10 % on average of dolasetron was recovered which indicates high accuracy of the method. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of dolasetron in bulk drug and in its pharmaceutical dosage form.

Keywords: *Dolasetron, RP-HPLC, system suitability,linearity, recovery studies, LOD, LOQ.*

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INTRODUCTION:

Dolasetron is a 5-HT3 antagonist used in the prophylaxis treatment for women with predominant irritable bowel syndrome (IBS) [1,2]. IBS is a gastrointestinal disorder characterized by abdominal pain, distressed bowel function, and abdominal distension dolasetron was included in the United States Pharmacopeia (USP) prioritized list of chemical medicine monographs in 2013 [3].Dolasetron is chemically (1s,3R,5r,7S)-10-oxo-8-azatricyclo[5.3.1.0^{3,8}]undecan-5-yl 1H-indole-3-carboxylate. The molecular formula is $C_{19}H_{20}N_2O_3$ and molecular weight 324.38 g/mol.

Literature survey reveals few UV spectrophotometric [5,6], LC-MS[7,8] and HPLC [9,10] chromatographic methods for the estimation of dolasetron in pharmaceutical dosage forms. The availability of an HPLC method with high sensitivity and selectivity will be very useful for the determination of dolasetron in pharmaceutical formulations. The aim of the study was to develop a simple, precise and accurate reversed-phase HPLC method for the estimation of dolasetron in bulk drug samples and in pharmaceutical dosage form.

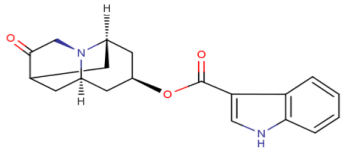

Fig1: Structure of Dolasetron

EXPERIMENTAL:

Materials and Methods:

Dolasetron was obtained as a gift sample from Hetero Drugs Ltd Hyderabad. Ammonium acetate, tetra hydro furan and methanol used were of HPLC grade (Qualigens). Commercially available dolasetron capsules (Anzemet® 50 mg)[4] were procured from local market.

Instrument:

Quantitative HPLC was performed on a Waters Alliance 2695 Separations Module is a high performance liquid chromatographic system with variable wave length PDA-Detector and powered with Empower-2 Software. The column used was Waters Spherisorb® 5µm CN, 4.6x250mm (250x4.6 mm i.d; particle size 5 µm).

HPLC Conditions:

The mobile phase comprises of 0.01M ammonium acetate in water methanol and tetra hydro furan in the ratio 700:240:60, diluent used was water : acetonitrile in the ratio 50:50 (v/v).

The contents were filtered through a 0.45µm membrane filter, pumped from the respective solvent reservoirs to the column at a flow rate of 1.0 ml/min. The run time was set at 20.0 min and the column temperature was 45^{0}C. Prior to the injection of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The eluents were monitored at 295 nm.

Preparation of Standard Stock solution:

A standard stock solution of the drug was prepared by dissolving 50 mg of dolasetron in 100 ml volumetric flask containing 50 ml of water, sonicated for about 15 min and then made up to 100 ml with water to get approximately 500µg/mL.

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Working Standard solution:

5ml of the primary standard stock solution of 500µg/mL was taken in 50 ml volumetric flask and thereafter made up to 50 ml with mobile phase to get a concentration of 50µg/ml.

Preparation of Sample solution:

Anzemet® Injection (dolasetron mesylate injection) is a clear, colorless, non-pyrogenic, sterile solution for intravenous administration. Each milliliter of Anzemet ® Injection (dolasetron mesylate injection) contains 20 mg of dolasetron mesylate and 38.2 mg mannitol, USP, with an acetate buffer in water for injection. The pH of the resulting solution is 3.2 to 3.8. Anzemet Injection (dolasetron mesylate injection) multidose vials contain a clear, colorless, nonpyrogenic, sterile solution for intravenous administration. Each Anzemet multidose vial contains 25 mL (500 mg) dolasetron mesylate. Each milliliter contains 20 mg dolasetron mesylate, 29 mg mannitol, USP, and 5 mg phenol, USP, with an acetate buffer in water for injection. The pH of the resulting solution is 3.2 to 3.7. Five vials of Anzemet multidose vial contains 25 mL (500 mg) dolasetron mesylate were collected and then mixed thoroughly. A sample of the blended sterils liquid, equivalent to 50 mg of the active ingredient was mixed with 70 ml of mobile phase in 100 ml volumetric flask. The mixture was allowed to stand for 1 hr with intermittent sonication for complete solubility of the drug, and then filtered through a 0.45 µm membrane filter, followed by addition of mobile phase up 100 ml to obtain a stock solution of 500µg/mL. The resultant solution was further diluted by taking 5 ml of the stock solution with 50 ml of mobile phase to get the concentration of 50µg/mL.

RESULTS AND DISCUSSION:

Validation for the method was carried out as per ICH Q_2(R1) guidelines. The validation parameters such as system suitability, linearity, recovery studies, robustness, detection limit, quantitation limit were studied.

System Suitability:

The system suitability tests were carried out on freshly prepared standard stock solution of dolasetron. The system was suitable for use, the tailing factors for dolasetron were 1.85 and USP theoretical plates were found to be significantly high around 7245.

Table 1: Performance & Detection Characteristics of HPLC method

Parameter	Results of the proposed HPLC method	
	Dolasetron Standard solution	Dolasetron Sample (Anzemet®-60 mg I.V.Injections) Solution
Retention time (min)	11.570	11.823
Theoretical plates (n)	7245	7430
Plates per meter (N)	28980	29720
HETP	$3.45065x10^{-5}$	$3.36473x10^{-5}$
Peak asymmetry (T)	1.85	1.92
Linearity range (µg/mL)	20-60	
Limit of Detection (µg/mL)	0.05	
Limit of Quantification (µg/mL)	0.15	

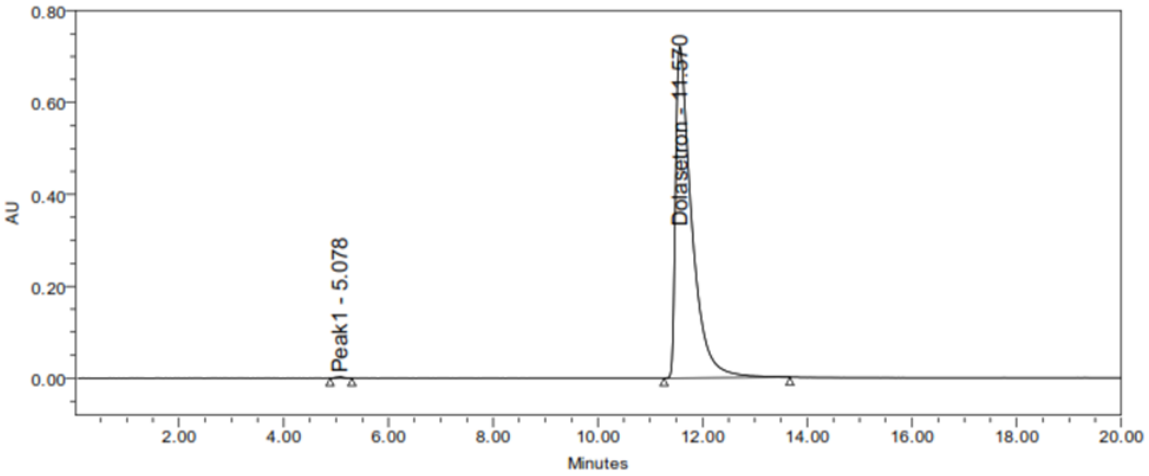

Fig 2: Typical System suitability Chromatogram of Dolasetron Working standard solution.

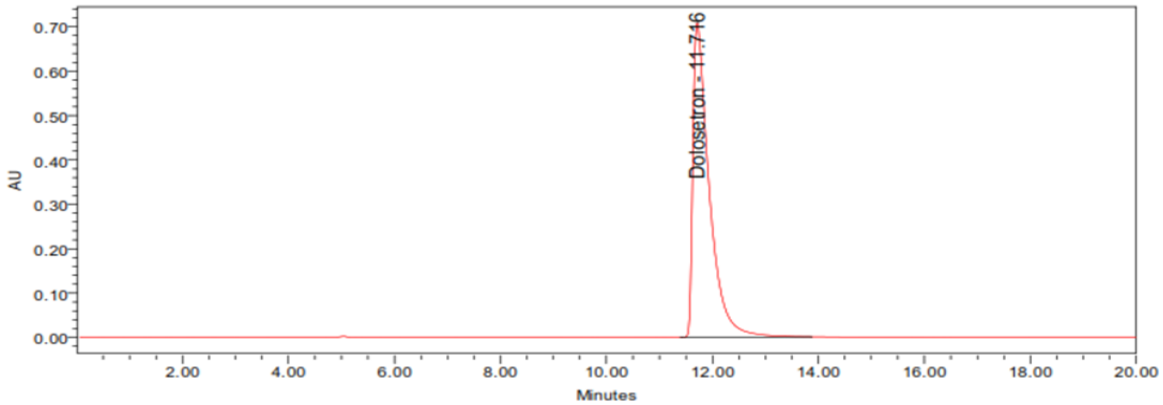

Fig 3: Typical Chromatogram of Dolasetron Working standard solution.

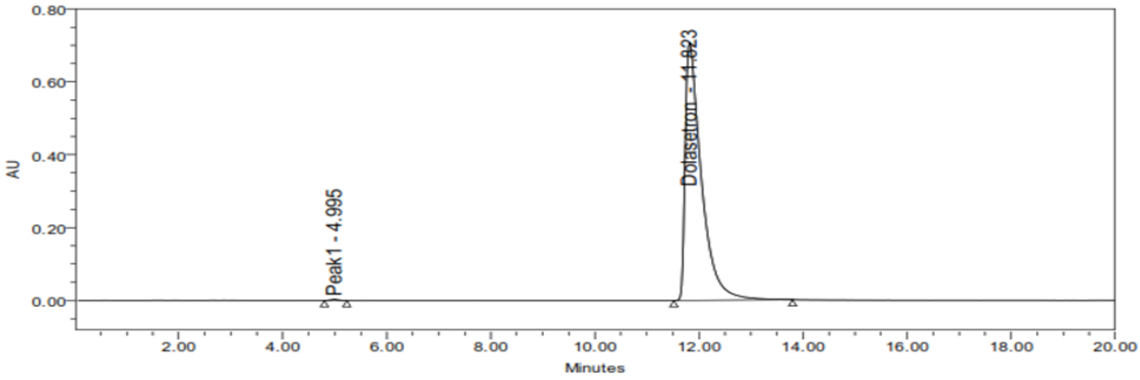

Fig 4: Typical Chromatogram of Dolasetron Working sample (Anzemet®-500 mg I.V.Injections) solution

Precision:
The precision of the method was ascertained separately from the peak area obtained by actual determination of 6 replicas of a fixed amount of drug and formulation. The HPLC systems was set up the described Chromatographic conditions, mentioned as above and follow the system to equilibrate, and then injected the 50 µg/ml concentration of dolasetron standard 6 times and recorded the response (peak area). The proposed method was extended to the pharmaceutical dosage forms by injecting the 50 µg/ml of dolasetron sample with the formulated sample from (Anzemet®-500mg, Sanofi Aventis, I.V.Injections) contains dolasetron of same concentration 6 times and recorded the response (peak area). The percent relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated and presented in Table 2.

Linearity:
Aliquots of standard Dolasetron stock solution were taken in different 10 ml volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of Dolasetron are in the range of 20-60 µg/ml. Each of these drug solutions (20 µL) was injected three times into the column, and the peak areas and retention times were recorded. Evaluation was performed with PDA detector at 295 nm and a Calibration graph was obtained by plotting peak area versus concentration of Dolasetron. The linearity Chromatograms presented in Figure 5.

Table 2: Precision of Standard drug with statistics

Injection No.	Name of the drug & conc. (50 µg/ml)	Retention time in min.	Peak Area
1	Dolasetron injection-1	11.597	15208163
2	Dolasetron injection-2	11.605	15159650
3	Dolasetron injection-3	11.616	15343636
4	Dolasetron injection-4	11.622	15216780
5	Dolasetron injection-5	11.630	15167117
6	Dolasetron injection-6	11.637	15220342
Mean		11.618	**15219281.3**
% RSD.		0.015	66139.4
Std. Deviation		0.13	**0.4**

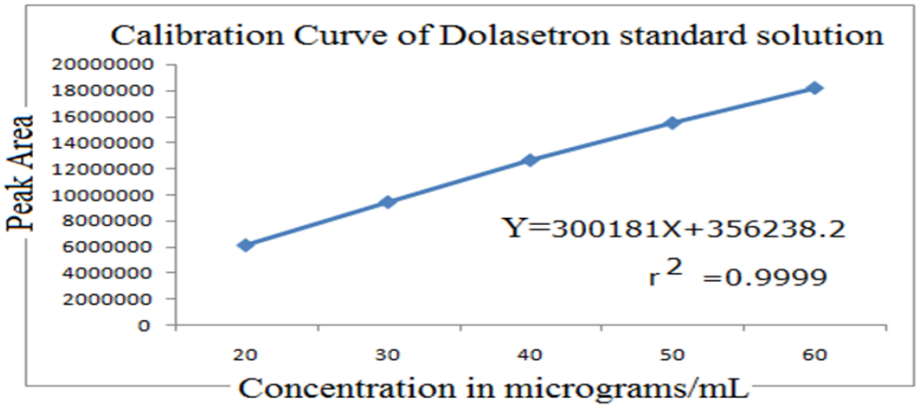

Fig 5: Standard Calibration Curve of Dolasetron.

Table 3: Optical & Regression Characteristics of HPLC method:

Parameter	Results of HPLC Method
Detection wavelength (nm)	295
Linearity range (µg/mL)	20-60
Regression Equation (y=mx + c)	Y=3000181x+356238.2
Slope (m)	3000181
Intercept (c)	356238.2
Correlation coefficient	0.9999
Relative Standard deviation*	0.1
% error in bulk samples	1.765

Assay and recovery studies:
Recovery studies were conducted by analyzing pharmaceutical formulation in the first instance for the active ingredient in the concentration of 80% of the working standard (contains 40 µg/mL of Dolasetron); 100% of the working standard solution (contains 50 µg/mL of Dolasetron) and 120% of the working standard solution (contains 60 µg/mL of Dolasetron) by the proposed method. Each concentration was injected 3 times and the peak area was recorded. Known amounts of pure drug [10% of the working standard solution contains 5 µg/mL of Dolasetron for 80% of the working standard, for 100% of the working standard, for 120% of the working standard] was then added to each 3 previously analyzed formulation and the total amount of the drug was once again determined by the proposed method (each concentration was again injected 3 times) after keeping the active ingredient concentration within the linearity limits. The Recovery data is given in Table 4.

Robustness:
A method is robust if it is unaffected by small changes in operating conditions. To determine the robustness of this method, the experimental conditions were deliberately altered at two different levels and retention time and chromatographic response were evaluated. One factor at a time was changed to study the effect. Variation of the mobile phase flow rate was varied by ±10%) and different column had no significant effect on the retention time and chromatographic response of the method, indicating that the method was robust. When the chromatographic conditions were deliberately altered, system suitability results remained within acceptance limits and selectivity for individual substance was not affected. The results of the study prove the robust nature of the method (Table 5 and Table 6).

Table 4: Recovery Peak areas of Dolasetron by Accuracy studies

S.No	Recovery at 80% dilution level Peak areas		Recovery at 100% dilution level Peak areas		Recovery at 120% dilution level Peak areas	
	Standard	Spiked	Standard	Spiked	Standard	Spiked
1	12105542	13995406	15166296	16982253	18393278	19937765
2	12062082	13993842	15149180	16975559	18204944	20143657
3	12063724	13994795	15192077	16994139	18223729	20046329
Avg	12077116.0	13994681.0	15169184.3	16983983.7	18273983.7	20042583.7
SD	24631.3	788.2	21593.9	9410.1	103738.0	102997.1
%RSD	0.2	0.0	0.1	0.1	0.6	0.5
% Recovery	104.5		98.08		99.41	

Table 5: Robustness study of Dolasetron Standard solution at 100 % level (50 µg/mL)

Parameter	Peak areas of Dolasetron in Flow increase study	Peak areas of Dolasetron in Flow decrease study	Peak areas of Dolasetron in Variable column Study
Injection-1	14069361	17028319	15322433
Injection-2	13979535	17091603	15357329
Injection-3	13951021	17129794	15365196
Mean	13999972.3	17083238.7	15348319.3
% RSD	61760.4	51252.0	22760.7
Std. Dev	0.4	0.3	0.1

Table 6: Robustness study of Anzemet®-60 mg I.V.Injections solution at 100 % level (50 µg/mL):

Parameter	Peak areas of Dolasetron in Flow increase study	Peak areas of Dolasetron in Flow decrease study	Peak areas of Dolasetron in Variable column Study
Injection-1	13970242	17016395	15274637
Injection-2	14009808	17138352	15282147
Injection-3	13985154	17099070	15320559
Mean	13988401.3	17084605.7	15292447.7
% RSD	19981.9	62251.8	24633.0
Std. Dev	0.1	0.4	0.2

Limit of Detection [LOD] and Limit of Quantification [LOQ]:

The detection limit of the method was investigated by injecting standard solutions Dolasetron into the HPLC column. By using the signal-to-noise method the peak-to-peak noise around the analyte retention time is measured, and subsequently, the concentration of the analyte that would yield a signal equal to certain value of noise to signal ratio is estimated. A signal-to-noise ratio (S/N) of 3 is generally accepted for estimating LOD and signal-to-noise ratio of 10 is used for estimating LOQ. This method is commonly applied to analytical methods that exhibit baseline noise. Chromatograms illustrating the LOD are shown in figure 2.10. The limit of detection (LOD) and limit of quantification (LOQ) for Dolasetron were found to be 0.05µg/ml and 0.15 µg/ml respectively.

CONCLUSIONS:

The author has developed a sensitive, accurate and precise HPLC for the estimation of Dolasetron in bulk drug and in I.V.Injection dosage form. From the typical chromatogram of Dolasetron , it was found that the retention time was 11.570 min. The contents of the mobile phase were Buffer: Acetonitrile 15: 85 (v/v). Solvent-A (Buffer) is 3.48 gms of Di Potassium hydrogen ortho-phosphate (0.03M) in 1000 ml of water and by adjusting the pH to 2.5 with dilute orthophosphoric acid and Solvent-B is acetonitrile in a isocratic mode of separation was used to resolute the Dolasetron at a flow rate of 1.0 ml/min and eluents were monitored at 284 nm, was found to be most suitable to obtain a peak well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (r2=0.9999) was observed between the concentration range of 100-300 µg/mL. The assay of Dolasetron in bulk was found to be 99.85%. From the recovery studies it was found that about 191.10 % on average of Dolasetron was recovered which indicates high accuracy of the method.

The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the I.V.Injections. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of bulk and sterile powder for injection dosage form of Dolasetron within a short analysis time. It can be seen from the results presented that the proposed procedure has good precision and accuracy. Results of the analysis of pharmaceutical formulations revealed that proposed methods are suitable for their analysis with virtually no interference of the usual additives present in the pharmaceutical formulations.

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