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Research

Development Of A New Chromatographic Method For Estimation Of Dolasetron In Bulk And Pharmaceutical Dosage Form By Rp-Hplc

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Abstract

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, RP-HPLC, validation.

INTRODUCTION

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components.[1]

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP, and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also quality is important in every product or service, but it is vital in medicines as it involves life.

There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these conditions standard and analytical procedures for these drugs may not be available in Pharmacopoeias. In instrumental analysis, a physical property of the substance is measured to determine its chemical composition. Pharmaceutical analysis comprises those procedures necessary to determine the identity, strength, quality and purity of substances of therapeutic importance.[2]

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs. In brief the reasons for the development of newer methods of drugs analysis are:

1. The drug or drug combination may not be official in any pharmacopoeias.
2. A proper analytical procedure for the drug may not be available in the literature due to Patent regulations.
3. Analytical methods for a drug in combination with other drugs may not be available.
4. Analytical methods for the quantitation of the drug in biological fluids may not be available.
5. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable.[1,2]

Different methods of analysis

The following techniques are available for separation and analysis of components of interest.

Spectral methods

The spectral techniques are used to measure electromagnetic radiation which is either absorbed or emitted by the sample. E.g. UV-Visible spectroscopy, IR spectroscopy, NMR, ESR spectroscopy, Flame photometry, Fluorimetry.[2]

Electro analytical methods

Electro analytical methods involved in the measurement of current voltage or resistanceas a property of concentration of the component in solution mixture.

E.g. Potentiometry, Conductometry, Amperometry.[2]

Chromatographic methods

Chromatography is a technique in which chemicals in solutions travel down columns or over surface by means of liquids or gases and are separated from each other due to their molecular characteristics.

E.g. Paper chromatography, thin layer chromatography (TLC), High performance thin layer chromatography (HPTLC), High performance liquid chromatography (HPLC), Gas chromatography (GC).[2]

Miscellaneous Techniques

Mass Spectrometry, Thermal Analysis.

Hyphenated Techniques

GC-MS (Gas Chromatography – Mass Spectrometry), LC-MS (Liquid Chromatography – Mass Spectrometry), ICP-MS (Inductivity Coupled Plasma- Mass Spectrometry), GC-IR (Gas Chromatography – Infrared Spectroscopy), MS-MS (Mass Spectrometry – Mass Spectrometry).

MATERIALS AND METHODS

Dolasetron from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK). Acetonitrile for HPLC from Merck, Phosphate buffer from Sura labs.

HPLC method development

Trails

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 Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

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

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

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 diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

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

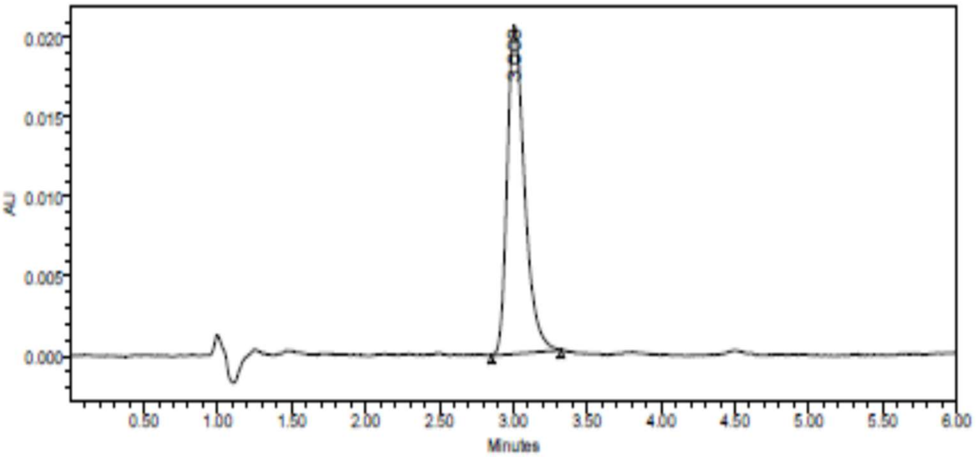


Fig 1: Optimized Chromatogram (Standard)

Table 1: Optimized Chromatogram (Standard)

S.no	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Dolasetron	3.008	627611	41541	1.1	9917

Optimized Chromatogram (Sample)

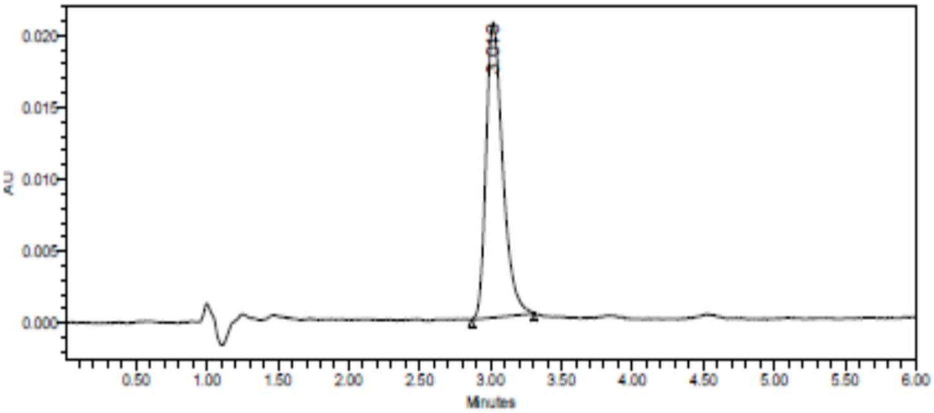


Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S.no	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Dolasetron	3.018	738718	27711	1.2	8937

Theoretical plates must be not less than 2000, Tailing factor must be not less than 0.9 and not more than 2.
It was found from above data that all the system suitability parameters for developed method were within the limit.

Assay (Standard)

Table 3: Peak results for assay standard

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Dolasetron	2.804	163305	21223	1.2	5817.1	1
2	Dolasetron	2.865	163387	20960	1.1	6144.5	2
3	Dolasetron	2.988	168830	20736	1.1	6452	3

Assay (Sample)

Table 4: Peak results for Assay sample

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Dolasetron	2.804	162052	28151	1.1	8862	1
2	Dolasetron	2.865	163387	21761	1.2	7872	2
3	Dolasetron	3.018	168406	21956	1.1	6926	3

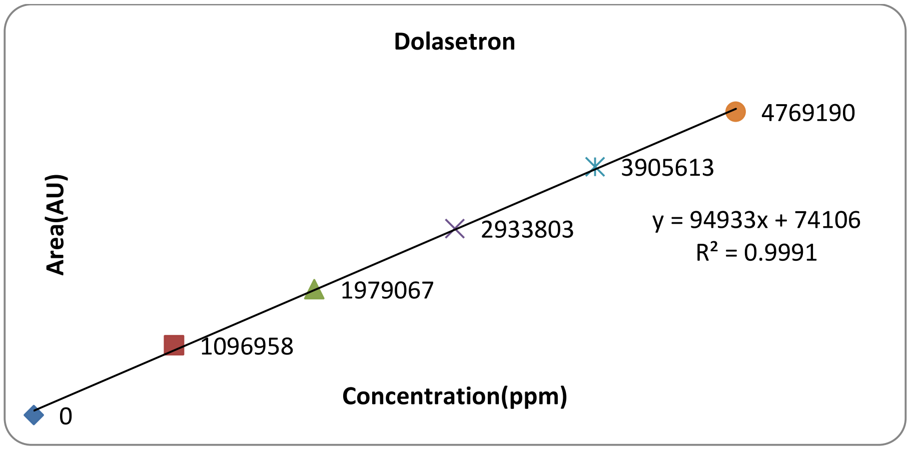
$$\%\text{ASSAY} = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

=164615/165174*10/30*30/0.124*99.8/100*1.2409/100*100
=99.5%
The % purity of Dolasetron in pharmaceutical dosage form was found to be 99.5%.

Linearity

Chromatographic data for linearity study

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



Repeatability

Table 5: 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			
Std.dev			3121.629			
%RSD			1.879372			

%RSD for sample should be NMT 2, The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision

Table 6: Results of Intermediate precision for Dolasetron

S.No	Peak Name	RT	Area (μV*sec)	Heigh (μV)	USP Plate count	USP Tailing
1	Dolasetron	2.982	167483	20736	8846	1.21
2	Dolasetron	2.988	167196	20583	9173	1.1
3	Dolasetron	3.001	167072	20647	8892	1.2
4	Dolasetron	3.008	167281	20631	9937	1.1

5	Dolasetron	3.018	167508	20545	9946	1.2
6	Dolasetron	3.018	168406	21452	8817	1.1
Mean			167491			
Std. Dev.			478.3856			
% RSD			0.285619			

%RSD of six different sample solutions should not more than 2

Table 7: Results of Intermediate precision Day 2 for Dolasetron

S.No	**Peak Name**	**RT**	**Area (µV*sec)**	**Height (µV)**	**USP Plate Count**	**USP Tailing**
1	Dolasetron	2.982	168830	20736	9371	1.1
2	Dolasetron	2.988	168066	20583	8916	1.2
3	Dolasetron	3.001	168565	20647	7917	1.1
4	Dolasetron	3.008	168387	20631	9971	1.1
5	Dolasetron	3.018	168406	20545	7977	1.2
6	Dolasetron	3.018	167508	20655	6018	1.1
Mean			168293.7			
Std. Dev.			458.6304			
% RSD			0.272518			

%RSD of six different sample solutions should not more than 2

Accuracy

Table 8: The 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	

Robustness

Table 9: Results for Robustness

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

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Dolasetron in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

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.

This method can be used for the routine determination of Dolasetron in bulk drug and in Pharmaceutical dosage forms.

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