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RESEARCH ARTICLE

Development and validation of stability indicating RP-HPLC method for the determination of Ondansetron in pure and table dosage form

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ABSTRACT

Ondansetron is a potent and highly selective serotonin 5-ht3-receptorantagonist which has demonstrated important antiemetic activity and goodtolerability in the prevention of chemotherapy-induced nausea and vomiting.the wavelength for estimation of ondansetron was found to be 250 nm.to prepare the buffer by using dissolve 1 ml of formic acid (opa) in1000 ml of water. Adjusted the ph to 4.25 using triethyl amine and the solution is filtered and sonicated for 5 min. The %rsd for intra-day precision and inter-dayprecision for ondansetron was found to be 1.59% and 0.39%, respectively (limit%rsd <2.0%). The %assay of ondansetron was found to be 99.5%. The hplcmethod is more economical for analysis of bulk drugs and pharmaceutical formulations.

Keywords: Retention time, Liniarity, Degradation, Spiked, Robustaness, Ruggedness

INTRODUCTION

Analytical chemistry[1] is often described as the area of chemistry responsible for characterizing the composition of matter, both qualitatively and quantitatively. Analytical methods are classified according to the property of the analyze measured. Pharmaceutical analysis[2] is the branch of chemistry involved in separating, identifying, and determining the relative amounts of the components making up a sample of matter. It is mainly involved in the qualitative identification or detection of compounds and quantitative measurements of the substances present in bulk and pharmaceutical preparation.

Types of analysis

Qualitative analysis

Qualitative analysis[3] seeks to establish the presence of a given element or compound in a sample.

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Quantitative analysis

Quantitative analysis[4] seeks to establish the amount of a given element or compound in a sample.

Introduction to chromatography

The term chromatography[5] (Greek kromatos–colour&graphos-written means color writing) Mikhail Tswett (1906) – invented the chromatography. The IUPAC has defined chromatography as "a method used primarily for the separation of component of a sample, in which the components are distributed between two phases, one of which is stationary while the other moves." The stationary phase may be a solid or liquid supported on a solid or a gel and may be packed in a column, spread as a layer or distributed as a film. The mobile phase may be gaseous or liquid.

Types of chromatographic methods:[6]

Based on modes of chromatography

- Normal phase chromatography
- Reverse phase chromatography

Based on principle of separation

- Partition chromatography
- Adsorption chromatography
- Ion-exchange chromatography
- Size exclusion chromatography
- Affinity chromatography
- Chiral phase chromatography

Base on elution technique

- Isocratic separation
- Gradient separation

Based on the scale of operation

- Analytical high performance liquid chromatography (HPLC)
- Preparative HPLC.

HPLC

HPLC[7] is also known as high pressure liquid chromatography**.** HPLC is a process, which separates mixture containing two or more components under high pressure. In this the stationary phase is packed in a column, one end of which is attached to a source of pressurized liquid mobile phase. HPLC is the fastest growing analytical technique for the analysis of drugs. Its simplicity, high specificity, and wide range of sensitivity make it ideal for the analysis of many drugs in both dosage forms and biological fluids. It is essential form of column chromatography in which the stationary phase is consist of small particles (3–50 μm) packings contained in a column with a small pores (2–5 mm) one end of which is attached to a source of pressurized liquid eluent (mobile phase). The three forms of high performance liquid chromatography most often used are ion-exchange, partition, and adsorption.

Advantages of HPLC

The advantages of HPLC are as follows:

- Specific, sensitive, and precise method for analysis of different complicated samples
- Speed of analysis
- Analysis of many polar substances, metabolic products, and thermolabile as well non-volatile substances
- Purification of synthetic or natural products
- Assay of active ingredients, impurities, and degradation products
- In pharmacodynamics and pharmacokinetic studies.

Instrumentation of HPLC:[8]

Chromatographic parameters

Retention time (t_R)

This is the time of emergence of the peak maximum of the component after injection. This is the sum of the times the component spends in the mobile phase (t_M) and in the stationary phase [Figures 1, 2, 3].

Adjusted retention time

It is the time the component spends in the stationary phase and is given by t_R'.

$t_R' = t_R' - t_M$

The value of t_M is obtained by measuring the time to elute an unretained substance,
for example, Air or methane.

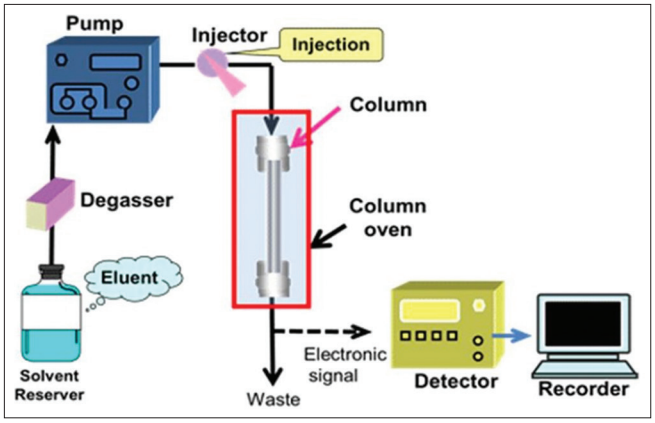

Figure 1: Instrumentation of HPLC

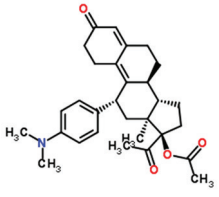

Figure 2: Chemical structure of ondenstron

It is the ratio of the time the component spends in the stationary phase to the time in the mobile phase.
$K = t_R\text{-}t_M/t_M$

Retention volume (V_R)
This is the volume of carrier gas required to elute one half of the compound from the column by the peak maximum and is given by:
$V_R = t_R \times f$

Adjusted retention volume (V'_R)
This allows for the gas hold up volume of the column which is due to the interstitial volume of the column and the volume of the injector and detector systems, it is given by:
$V'_R = t'_R \times f$

Relative retention volume
Retention volumes for compounds are expressed relative to the retention volume of a standard compound on the same column under the same conditions of a standard compound examined. Therefore, this ratio is given by:
V_N (Sample)/V_N (Standard) = t'_R (Sample)/t'_R (Standard)
Relative retention volumes can therefore be represented by ratios of the distances on the recorder chart and are the same as relative retention times.

Height equivalent to a theoretical plate
The column is considered as being made up of a large number of parallel layers or theoretical plates, and when the mobile phase passes down the column the components of a mixture on the column distribute themselves between the stationary and mobile phases in accordance with their partition that equilibrium is established within each plate. The equilibrium, however, is dynamic and the components move down the column at a definite rate depending on the rate of movement of the mobile phase. A column may be considered as being made up of a large number of the oretical plates where distribution of sample between liquid and gas phase occurs. The number of theoretical plates (n) in a column is given by the relationship.
$n = 16(t_R/W)^2 = 5.54\ (t_R/W_{1/2})$

Where, W = peak width, that is, the segment of the peak base formed by projecting the straight sides of the peak to the base line.
$W_{1/2}$ = peak width at half height.

Drug profile: ondansetron

Ondansetron[9] is a competitive serotonin Type 3 receptor antagonist. It is effective in the treatment of nausea and vomiting caused by cytotoxic chemotherapy drugs, including cisplatin, and has reported anxiolytic and neuroleptic properties

- IUPAC name: 9-methyl-3-[(2-methyl-1*H*-imidazol-1-yl)methyl]-2,3,4,9-tetrahydro-1*H*-carbazol-4-one
- Molecular formula: $C_{18}H_{19}N_3O$
- Molecular weight: 293.363 g/mol.

CHEMICAL STRUCTURE

Mechanism of action

Ondansetron is a selective antagonist of the serotonin receptor subtype, 5-HT_3. Cytotoxic chemotherapy and radiotherapy are associated with the release of serotonin (5-HT) from enterochromaffin cells of the small intestine, presumably initiating a vomiting reflex through stimulation of 5-HT_3 receptors located on vagal afferents. Ondansetron may block the initiation of this reflex. Activation of vagal afferents may also cause a central release of serotonin from the chemoreceptor trigger zone of the area postrema, located on the floor of the fourth ventricle.

Pharmacodynamics

Ondansetron is a highly specific and selective serotonin 5-HT_3 receptor antagonist, not shown to have activity at other known serotonin receptors and with low affinity for dopamine receptors. The serotonin 5-HT_3 receptors are located on the nerve terminals of the vagus in the periphery, and centrally in the chemoreceptor trigger zone of the area postrema. The temporal relationship between the emetogenic action of emetogenic drugs and the release of serotonin, as well as the efficacy of antiemetic agents, suggests that chemotherapeutic

agents release serotonin from the enterochromaffin cells of the small intestine by causing degenerative changes in the gastrointestinal (GI) tract.

Pharmacokinetics

After oral administration, Ondansetron achieves elimination half-life of 32 h. The Cmax and area under the curve are dose-dependent. The oral bioavailability of Ondansetron is about nearly 100%.

Absorption

Ondansetron is absorbed from the GI tract and undergoes some limited first-pass metabolism. Mean bioavailability in healthy subjects, following administration of a single 8-mg tablet, was recorded as being approximately 56–60%.

Protein binding

The plasma protein binding associated with Ondansetron was documented as approximately 73%.

Metabolism

The plasma protein binding associated with Ondansetron was documented as approximately 73%. The major urinary metabolites are glucuronide conjugates (45%), sulfate conjugates (20%) and hydroxylation products.

Excretion

Following oral or IV administration, Ondansetron is extensively metabolized and excreted in the urine and feces.

Indication and dosage

Prevention of nausea and vomiting associated with initial and repeat courses of emetogenic cancer chemotherapy, including high-dose cisplatin. [Figures 4-8]

Contraindications

- Known to have hypersensitivity (e.g., anaphylaxis) to Ondansetron or any of the components of the formulation
- Receiving concomitant Apomorphine due to the risk of profound hypotension and loss of consciousness.

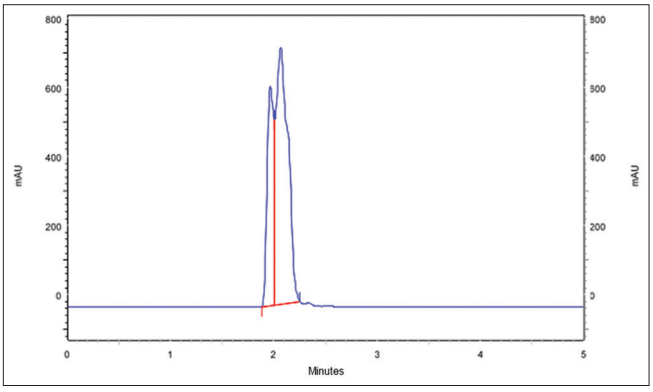

Figure 3: Trail chromatogram 2
Observation: Peak was splitted with different ranges further trail carried

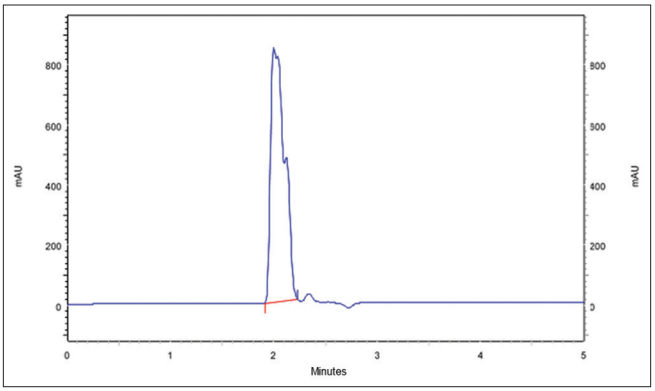

Figure 4: Trial chromatogram 3
Observation: Peak was splitted. Further trails was carried

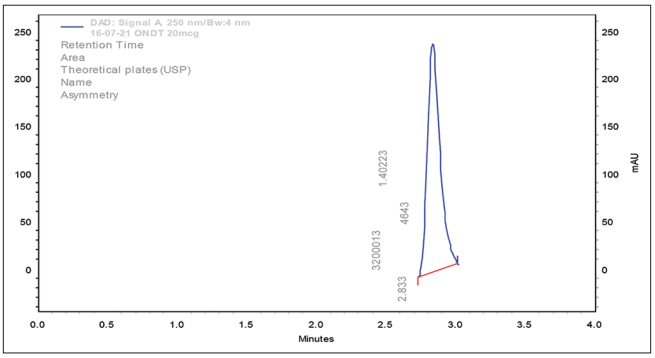

Figure 5: Optimized chromatogram
Observation: Peak shape of ondansetron was eluted with in 5 minutes of time

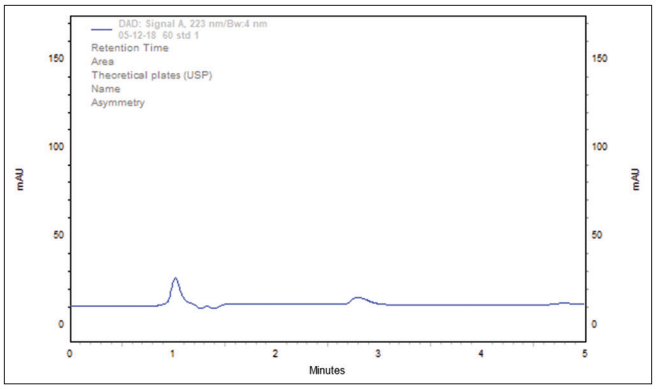

Figure 6: Blank chromatogram

EXPERIMENTAL METHODOLOGY

Instrumentation

Agilent Technologies 1260 Infinity Binary HPLC [Figure 9] was used for chromatographic studies; Shimadzu ultraviolet (UV) 1800 Double Beam UV-Visible Spectrophotometer was used for spectral studies. Shimadzu BL220H Digital Weighing Balance was used for weighing the materials.

Drugs and formulations

The reference samples of Ondansetron (API) were purchased from Yarrow Chemical Ltd., Mumbai, India. The commercial formulations (Empero ODT tablets containing 4 mg of Ondansetron) were procured from the local market.

Chemicals and solvents

Methanol (HPLC grade), acetonitrile (HPLC grade), formic acid, and triethyl amine were purchased from Merck (India) Ltd., Mumbai, India. Freshly prepared triple distilled water was used throughout the experiment.

Selection of mobile phase

The solubility of Ondansetron was carried out in a variety of polar and non-polar solvents as per Indian Pharmacopoeia standards. Based on the solubility of the compounds, finally 0.1% formic acid and acetonitrile in the ratio of 50:50 v/v were selected as the mobile phase for the drug due to its positive results.

Detection of wave length

The spectrum of diluted solutions contains 10 µg/mL of Ondansetron in mobile phase that was recorded separately on UV spectrophotometer and the solutions were scanned between 200 and 400 nm using mobile phase as blank. The peaks of maximum absorbance wavelengths were observed. The overlain spectrum was observed and the wavelength was found to be 250 nm for Ondansetron.

Method development

A mixture of formic acid and methanol in the ratio of 50:50 V/V and then the solution is filtered and sonicated for 5 min.

Trial 1: Chromatographic conditions

Mobile Phase	Formic acid (pH 3): Methanol (50:50 V/V)
Column	Zorbax, C18 (250 mm×4.6 mm, 5 µm)
Flow Rate	1.0 mL/min
Wavelength	223 nm
Temperature	Ambient
Volume	20 µL
Run time	5 min

Trial 2: Chromatographic conditions

Mobile Phase	Formic acid: Methanol (30:70V/V)
Column	Zorbax, C18 (250 mm×4.6 mm, 5 µm)
Flow Rate	1.0 mL/min
Wavelength	223 nm
Temperature	Ambient
Volume	20 µL
Run time	5 min

Trial 3: Chromatographic conditions

Mobile Phase	Formic acid (p^H 3): ACN (50:50 v/v)
Column	Phenoxneome, C18 (150 mm×4.6 mm, 5 µm)
Flow Rate	1.0 mL/min
Wavelength	250 nm
Temperature	Ambient
Volume	20 µL
Run time	5 min

Buffer preparation

Dissolve 1 mL of formic acid (OPA) in 1000 mL of water. Adjusted the pH to 4.25 using triethyl

amine and the solution is filtered and sonicated for 5 min.

Trial 4: Chromatographic conditions (Optimized conditions)

Mobile Phase	Formic acid: ACN (50:50, V/V)
Column	Phenomenex, C18 (150mm×4.6mm, 5µm)
Flow Rate	0.6 mL/min
Wavelength	250 nm
Temperature	Ambient
Volume	20 µL
Run time	5 min

Preparation of standard stock solution

About 10 mg of Ondansetron is accurately weighed and transferred into a 10 mL (1000 µg/mL) clean dry volumetric flask containing mobile phase. The solution was sonicated for 5 min and the drug was dissolved completely. The volume was made up to the mark with a further quantity of the mobile phase to get a stock concentration of Ondansetron. Further pipette 1.0 mL of the above stock solution into a 10 mL volumetric flask (100 µg/mL)and the volume was made up to the mark with the mobile phase.

Preparation of sample solution

Twenty tablets were weighed and finely powdered. An accurately weighed portion of powder sample equivalent to 10 mg of Ondansetron is transferred into a 10 mL (1000 µg/mL) clean dry volumetric flask containing mobile phase. The solution was filtered and sonicated for 5 min. The volume was made up to the mark with a further quantity of the mobile phase to get a stock concentration of Ondansetron. Transfer mL of the above solution to 10 mL volumetric flask and diluted up to mark with diluents. Further pipette 1 mL of the above stock solution into a 10 mL volumetric flask and the volume was made up to the mark with the mobile phase.

Optimized method

This method is based on the absorbance of Ondansetron which is selected based on the results of linearity and reproducibility.

Preparation of calibration curve

Working standard solutions were prepared for the Ondansetron from the standard solution of 1000 µg/mL. Different aliquots were taken from standard stock solution and diluted with water: ACN separately to prepare 5 µg/mL, 10 µg/mL, 15 µg/mL, 20 µg/mL, and 25 µg/mL solutions, respectively. The results are furnished in Table 1. Then, the construction of calibration curve was plotted by taking the above prepared solutions of different concentrations ranging from 5 to 25 µg/mL [Table 2].

Validation

System suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters such as theoretical plates, resolution, and asymmetric factor were evaluated.

Linearity

Linearity was performed by preparing standard solution of Ondansetron at different concentration

Table 1: Preparation of standard solution of Ondansetron

S. No.	Stock solution	Amount of solvent	Conc. (µg/mL)
1	0 mL	10 mL	0
2	0.5 mL	9.5 mL	5
3	1 mL	9 mL	10
4	1.5 mL	8.5 mL	15
5	2 mL	8 mL	20
6	2.5 mL	7.5 mL	25

Table 2: Results for system suitability of Ondansetron

Injection	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	2.91	1,872,079	4,652	1.35
2	2.97	1,865,632	4,235	1.3
3	2.89	1,798,961	4675	1.32
4	2.94	1,893,625	4,642	1.31
5	2.91	1,893,215	4,823	1.36
6	2.86	1,892,561	4,612	1.34
7	2.91	1,849,563	4,125	1.32
8	2.92	1,892,145	4,632	1.31
9	2.97	1,885,612	4,528	1.34
10	2.91	1,862,457	4,926	1.31
Mean	2.91	1,870,585	-	-
%RSD	1.1619	1.5819	-	-

levels, that is, 5–25 µg/mL. The absorbance was measured at 250 nm. Each measurement was carried out in triplicate. Linearity was proven by regression analysis by the least square method. The straight line in the calibration curve [Figure 10] obeyed linearity in the concentration range of 5–25 µg/mL for Ondansetron. The correlation and linearity results are presented in Table 3.

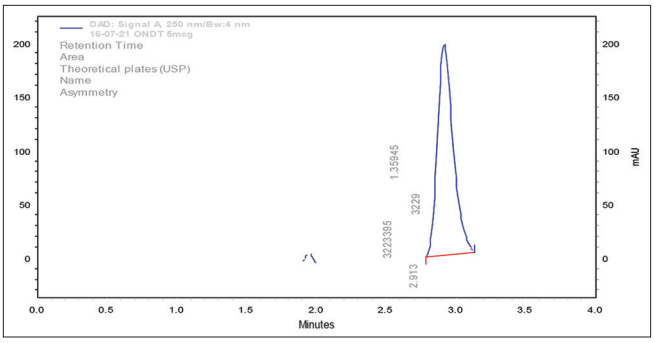

Figure 7: Standard chromatogram of ONDT linearity 5 µg/mL

Table 3: Linearity results of Ondansetron

S. No.	Concentration (µg/mL)	Peak area
1	0	0
2	5	923,395
3	10	1,872,079
4	15	2,628,643
5	20	3,620,013
6	25	4,409,482
Slope		17,653
Intercept		35,567
Regression Equation (y)		y=17653×+35567
Correlation Coefficient		0.999

Table 4: Intra- and inter-day precision results for Ondansetron

S. No.	Intra-day Time (Hours)	Peak area	Inter-day Days	Peak area
1	0	1,872,079	1	1,872,079
2	3	1,812,352	2	1,863,254
3	6	1,832,496	3	1,854,923
4	9	1,895,614	4	1,861,529
5	12	1,862,315	5	1,854,168
6	15	1,864,291	6	1,869,245
Mean		1,856,524	Mean	1,862,533
SD		29639.0231	SD	7288.21
%RSD		1.5964794	%RSD	0.3913

Precision

Precision is the degree of repeatability of an analytical method under normal operational conditions. The intermediate precision of the method was confirmed by intra-day and inter-day analysis. The concentration used for the precision studies is 10 µg/mL.

Intra-day and inter-day precision

To study the intra-day and inter-day precision, the analysis of drugs was repeated for 6 times in the same day and different days. Six replicate mixed standard solution of Ondansetron was measured with the same concentration and the relative standard deviation (%RSD) was calculated. The %RSD was found to be 1.084% and 0.906% which are well within the acceptable criteria of not more than 2.0. Results of intra-day and inter-day precision are given in Table 4.

Accuracy

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed drug sample solution. Percent recovery was calculated by comparing the peak area before and after the addition of the standard drug. The standard addition method was performed at three concentration levels in triplicate at 50%, 100%, and 150%.

50% spiked

0.5 mL was pipette out from standard 100 µg/mL solution and transferred to 10 mL volumetric flask and labeled as 50% spiked. 1 mL was pipette out from working sample solution and transferred to the same volumetric flask and labeled as 50% spiked and diluted to 10 mL with mobile phase.

100% spiked

1 mL was pipette out from standard 100 µg/mL solution and transferred to 10 mL volumetric flask and labeled as 100% spiked. 1 mL was pipette out

from working sample solution and transferred to the same volumetric flask and labeled as 100% spiked and diluted to 10 mL with mobile phase.

150% spiked

1.5 mL was pipette out from standard 100 µg/mL solution and transferred to 10 mL volumetric flask and labeled as 150% spiked. 1mL was pipette out from sample stock solution and transferred to the same volumetric flask and labeled as 150% spiked and diluted to 10 mL with mobile phase. The results are furnished in Table 5.

Specificity

The wave length was specific for Ondansetron according to its structure. A study conducted to establish specificity of the proposed method involved injecting blank and placebo using the chromatographic conditions defined for the proposed method. It was found that there is no interference due to excipients used in the tablet formulation and also found good correlation between the absorbance of standard and sample.

Robustness

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate and wavelength [Table 6]. System suitability parameters were compared with that of method precision.

Acceptance criteria

The system suitability should pass as per the test method at variable conditions.

Ruggedness

Ruggedness of the method was confirmed by the analysis of samples that was done by different analysts. Samples of Ondansetron at 10 µg/mL

Table 5: Results for accuracy of Ondansetron

Recovery/Spike level at about [%]	Amount of ONDT added (ppm)	Peak area	Conc. found (µg/mL)	% Recovery	% Mean recovery
50	5	2,796,982	4.94	98.81	99.49
50	5	2,821,594	5.07	101.4	
50	5	2,792,158	4.91	98.28	
100	10	3,789,621	10.17	101.76	100.44
100	10	3,745,892	9.942	99.42	
100	10	3,759,741	10.01	100.16	
150	15	4,491,326	14.87	99.16	99.58
150	15	4,499,892	14.92	99.49	
150	15	4,516,238	15.01	100.11	

Table 6: Robustness results of Ondansetron

S. No.	Parameter	Optimized	Used	Rt (min)	Peak area	%RSD
1	Flow rate	0.8 mL/min	0.4 mL/min	3.12	1,872,563	0.52
			0.6 mL/min	2.91	1,889,265	0.78
			0.8 mL/min	2.82	1,883,652	0.59
2	Wavelength	250 nm	248 nm	2.95	1,879,523	0.91
			250 nm	2.91	1,872,564	0.42
			252 nm	2.93	1,865,921	0.38
3	Mobile mobile phaseEphas	ACN: buffer (50:50)	48:52	2.98	1,892,354	0.54
			50:50	2.91	1,821,564	0.72
			52:48	2.87	1,885,421	0.56

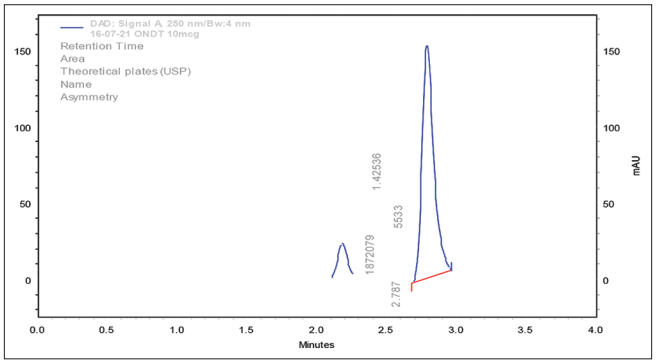

Figure 8: Standard chromatogram of ONDT linearity 10 µg/mL

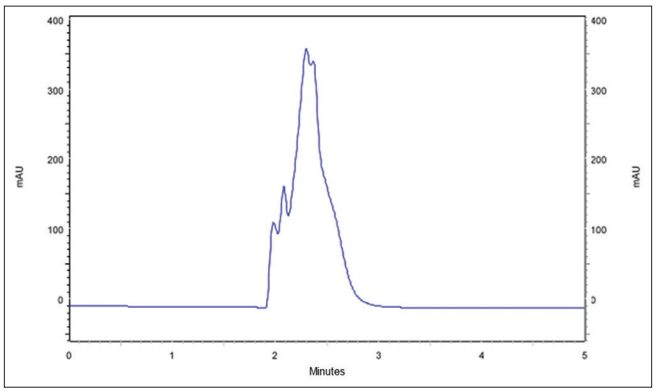

Figure 9: Trail chromatogram 1
Observation: Peak eluted with bad shape. Further trial was carried

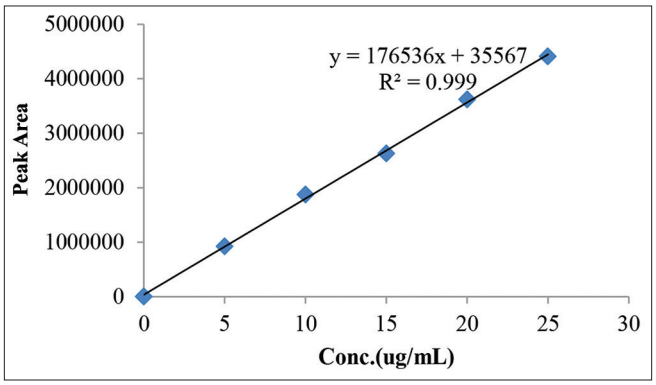

Figure 10: Calibration graph of Ondansetron

concentration were analyzed by different analysts. It was observed that there were no marked changes in absorbance, which demonstrated that the developed method was rugged in nature.

Limit of detection (LOD) and limit of quantitation (LOQ)

For this study, six replicates of the analyte at the lowest concentration were measured and quantified. Table 7 shows the values of LOD and LOQ.

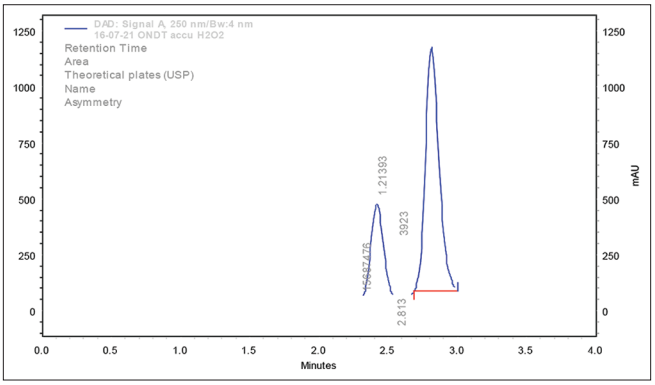

Figure 11: Chromatogram of acid degradation

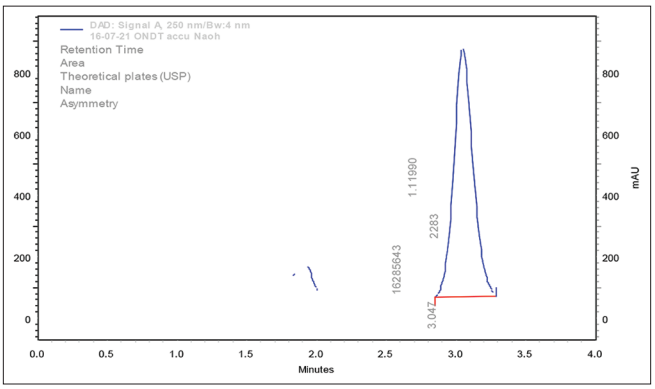

Figure 12: Chromatogram of alkaline degradation

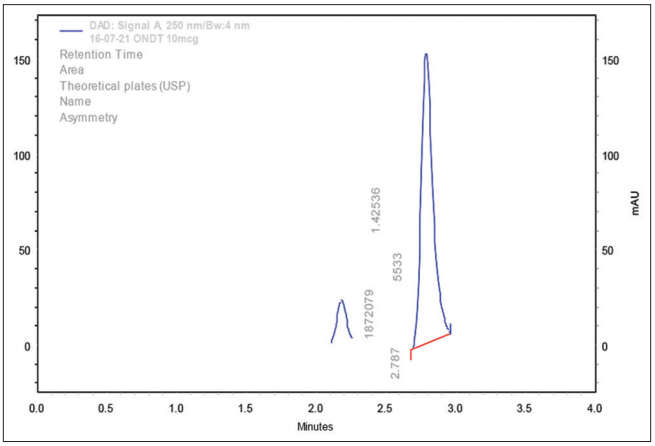

Figure 13: Chromatogram of oxidative degradation

Estimation of Ondansetron in tablet dosage forms

Commercial formulation of tablets was chosen for testing the suitability of the proposed method to estimate Ondansetron in tablet formulations. Twenty tablets were weighed and finely powdered. An accurately weighed portion of powder sample equivalent to 10 mg of Ondansetron was transferred

to a 10 mL volumetric flask. The contents of the flask were sonicated for about 10 min for complete solubility of the drug and volume made up with further quantity of mobile phase. Further pipette 0.2 mL of the above stock solution into a 10 mL volumetric flask and the volume was made up to the mark with the mobile phase.

The standard solutions and sample solutions were determined at 250 nm and the amount of the drugs present in the tablet dosage form was calculated. The results [Table 8] were compared with the label claim of Ondansetron in tablet dosage forms and yielded 98.23%.

Stability indicating studies

Degradation studies were performed and the degraded samples were injected. Degradation data of the drugs were summarized in Table 9 and the chromatograms were given in Figures 11-13. Assay of the injected samples was calculated. The forced degradation study showed that the method was highly specific.

Acid degradation studies

To 1 mL of stock solution of Ondansetron, 1 mL of 2N hydrochloric acid was added and refluxed for 30 min at 60°C. The resultant solution was diluted to obtain 10 µg/mL solution and 20 µL solution were injected into the system and the chromatograms were recorded to assess the stability of sample [Figures 14-17]

Alkaline degradation studies

To 1 mL of stock solution of Ondansetron, 1 mL of 2N sodium hydroxide was added and refluxed for 30 min at 60°C. There sultant solution was diluted to obtain 10 µg/mL solution and 20 µL solution were injected into the system and the chromatograms were recorded to assess the stability of sample.[10-21]

Oxidative degradation studies

To 1 mL of stock solution of Ondansetron, 1 mL of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min

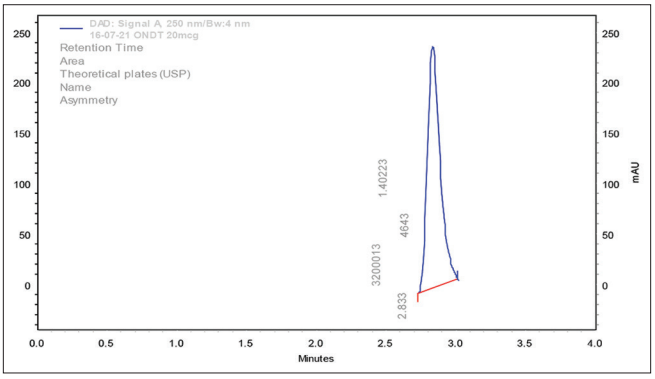

Figure 14: Standard chromatogram of ONDT linearity 15 µg/mL

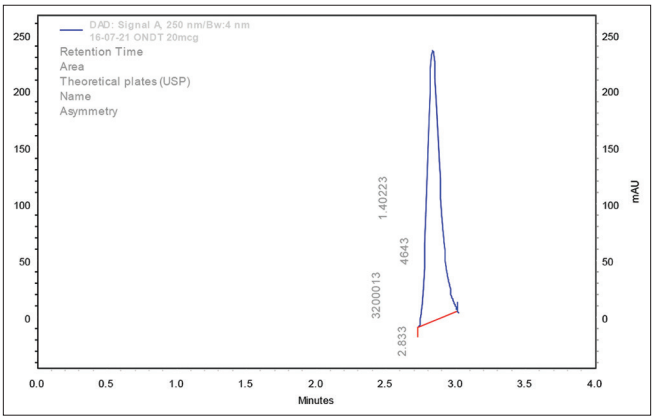

Figure 15: Standard chromatogram of ONDT linearity 20 µg/mL

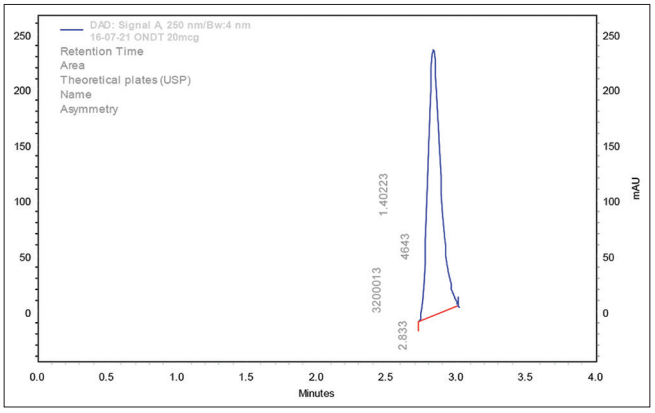

Figure 16: Standard chromatogram of ONDT linearity 25 µg/mL

at 60°C. There sultant solution was diluted to obtain 10 µg/mL solution and 20 µL solution were injected into the system and the chromatograms were recorded to assess the stability of sample.

Hydrolytic degradation studies

Stress testing under hydrolytic conditions was studied by refluxing the standard Ondansetron

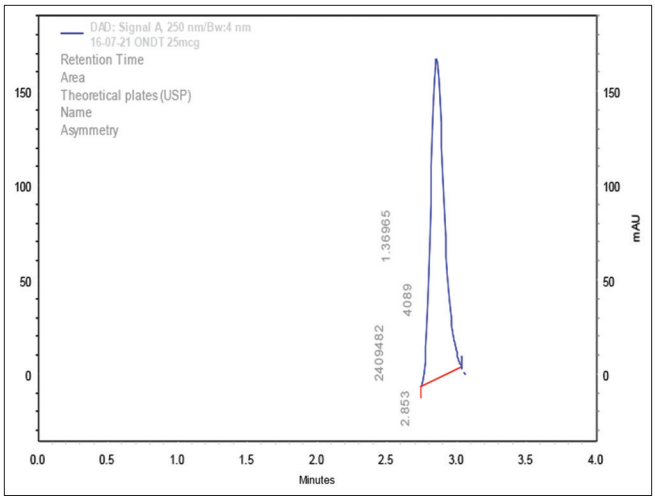

Figure 17: Sample chromatogram of Ondansetron

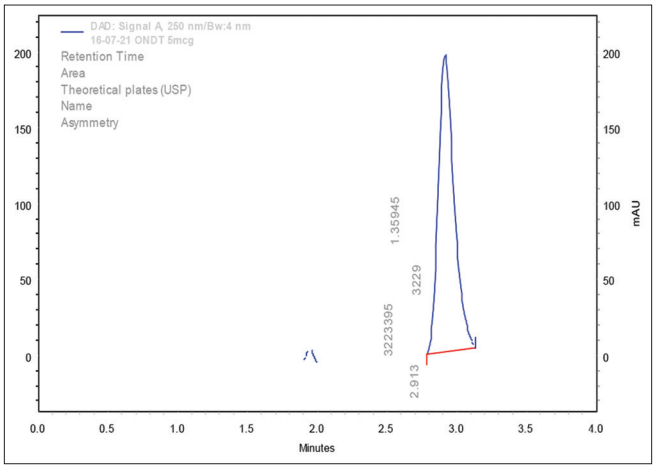

Figure 18: Chromatogram of neutral degradation

solution in water for 6 h at a temperature of 60°C. The resultant solution was diluted to obtain 10 µg/mL solution and 20 µL solution were injected into the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSION

- The present study was aimed at developing a simple, sensitive, precise, and accurate HPLC method for the estimation of Ondansetron from bulk samples and their tablet dosage forms
- Ondansetron, attempts were made using mobile phases containing solvents of varying polarity, at different concentration level with implicating PhenoxneomeC18 (150 mm × 4.6 mm, 5 µm) column as a stationary phase
- Variousmobilephasesystemssuchasacetonitrile, methanol, water, and orthophosporic acid at different concentration levels with different pH were tried. Among the different mobile phase combinations employed, best resolution with sharp well defined peaks obtained with mobile phase composed of 0.1% Formic acid (pH 4.25) acetonitrile in the ratio of 50:50 V/V
- The wavelength for estimation of Ondansetron was found to be 250 nm
- The absence of additional peaks in the absorption spectrum indicates non-interference of the commonly used excipients in the tablets and, hence, the method is specific
- The linearity was found satisfactory in the concentration range of 5–25 µg/mL for Ondansetron
- The regression equation of the linearity curve between concentrations of Ondansetron over its absorbance was found to be y = 17653× + 35667 (where y is the Peak area and x is the concentration of Ondansetron in µg/mL)
- The correlation coefficient (R^2) was found to be 0.999 for Ondansetron. The results show that an excellent correlation exists between absorbance and concentration the drug within the concentration range indicated
- Precision of the method was studied by repeated measurements of drug solution and results showed lower %RSD values. The %RSD for intra-day precision and inter-day precision for Ondansetron was found to be 1.59% and

Table 7: LOD and LOQ of Ondansetron

Parameter	Measured value (µg/mL)
Limit of detection	1.531
Limit of quantitation	4.64

Table 8: Assay results of Ondansetron formulation

Formulation		Label claim	Amount found	%Assay
Ontoron-MD	Ondansetron	4 mg	3.98 mg	99.5%

Table 9: Forced degradation data of Ondansetron

Type of Stress	Retention time (min.)	%Assay	%Amount degraded
Acid degradation	2.918	85.60	14.40
Alkali degradation	2.894	75.70	22.30
Oxidative degradation	2.867	86.37	14.63
Hydrolytic degradation	2.935	96.74	03.26

0.39%, respectively (limit %RSD <2.0%). This reveals that the method is quite precise

- The percent recoveries of the Ondansetron were studied at three different concentration levels. The percent individual recovery of both the drugs at each level was within the acceptable limits. The mean recovery of the drug is 99.49–100.44%. The high percentage of recovery indicates that the proposed method is highly accurate
- The LOD and LOQ of Ondansetron were found to be 1.53 μg/mL and 4.64 μg/mL, respectively. Obtained by the proposed method indicate that the method is sensitive
- Validated method was applied for the estimation of Ondansetron in commercial tablet dosage forms. The %assay of Ondansetron was found to be 99.5%. The assay results showed the drug contents of this product to be in accordance with the labeled claims. No interfering peaks were found in the absorption spectrum of the tablet formulation indicating that excipients used in tablet formulations did not interfere with the estimation of the Ondansetron by the proposed HPLC method. This confirms the suitability of the method for the analysis of the drug in pharmaceutical dosage forms
- HPLC studies of Ondansetron under different stress conditions indicated the following degradation behavior
- In acidic degradation, the degradation product of Ondansetron was appeared at retention time of 2.81 min and the %degradation is 14.40%. In alkaline degradation, the degradation product of Ondansetron was appeared at retention time of 3.047 min and the %degradation is 23.90%. In oxidative degradation, the degradation product of Ondansetron was appeared at retention time of 2.787 min and the %degradation is 14.63%. In hydrolytic degradation, the degradation product of Ondansetron was appeared at retention time of 2.91 min and the %degradation is 3.16%. The results of analysis are given in Table 8. The typical chromatograms of degradation behavior of Ondansetron in different stress conditions are shown in Figures 11-13,18.

SUMMARY AND CONCLUSION

In the present investigation, an attempt has been made to develop simple, rapid, sensitive, precise, and accurate HPLC method for the determination of Ondansetron in bulk sample and pharmaceutical formulations. The advantage of proposed method is its simple procedure for its sample preparation. The satisfying recoveries, low correlation coefficient, and assay results confirmed the suitability of proposed method for the routine quality control analysis for determination of Ondansetron in pharmaceutical formulations. The method was validated as per International Conference on Harmonization Guidelines and the results are within the limits. To conclude, the HPLC method is more economical for analysis of bulk drugs and pharmaceutical formulations.

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