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Development and Validation of a Stability Indicating RP-HPLC Method for Determination of Ondansetron in Orally Disintegrating Films

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ABSTRACT

A simple, precise, rapid and accurate stability indicating reversed-phase high performance liquid chromatography (RP-HPLC) method is developed for the estimation of ondansetron in orally disintegrating films. The separation was achieved by using a Waters 2695 HPLC System consisting of analytical column Unisphere-C8 (5µm; 150x4.6mm) and wavelength detector- Waters 2489 UV is used for analysis. The mobile phase consisting of A- phosphate buffer (pH 5.4): acetonitrile in the ratio of 72:28 (v/v) is used. The flow rate is 1.0 mlm^{-1} and the effluents are monitored at 247 nm. The retention time is about 5.0 m. The detector response is linear in the concentration range of 40.0-120.0 μgml^{-1}. The respective linear regression equation being y = 30503 + 65831. The percentage assay of ondansetron is 100.6%. The method is validated as per ICH guideline by determining its specificity, accuracy, precision, linearity & range, ruggedness, robustness and system suitability. The results of the study show that the proposed method is simple, rapid, precise and accurate, which is useful for the routine determination of ondansetron in its orally disintegrating films.

KEY WORDS: Ondansetron, RP-HPLC, Validation, System suitability tests.

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INTRODUCTION

Ondansetron (**Fig.1**) is 4H–Carbazol-4-one, 1, 2, 3, 9-tetrahydro-9-methyl-3[(2-methyl-1H-imidazol-1-yl) methyl]-(±) - (m.f. $C_{18}H_{19}N_3O$; m.w. 293.36) [1, 2].

Ondansetron is a serotonin 5-HT_3 receptor antagonist used mainly as an antiemetic to treat nausea and vomiting following chemotherapy. Its effects are thought to be on both peripheral and central nerves. Ondansetron reduces the activity of the vagus nerve, which activates the vomiting center in the medulla oblongata, and also blocks serotonin receptors in the chemoreceptor trigger zone. It has little effect on vomiting caused by motion sickness, and does not have any effect on dopamine receptors or muscarinic receptors.

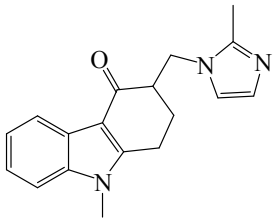

Figure-1: Chemical structure of ondansetron

Literature survey revealed spectrophotometric methods and HPLC methods in conventional dosage form for estimation of ondansetron individually as well as in combination are available [3,4,5,6,7]. An attempt has been made to develop a new stability indicating RP-HPLC method for its estimation in orally disintegrating films with good accuracy and precision [8, 9, 10, 11]. The method is validated according to the ICH guidelines Q2 (R1)[12]

EXPERIMENTAL

INSTRUMENTATION

Waters 2695 HPLC System, consisting of degasser, quaternary pump, column oven, and variable wavelength detector Waters 2489 UV IS used for analysis. The analytical column Unisphere C-8 (5µm; 150x4.6mm) is used. The waters empowers software ran on HP computer operated with Windows XP professional used for this method.

REAGENTS AND CHEMICALS

Acetonitrile used was of HPLC grade from E. Merck, India. HPLC grade water was obtained using millipore water purification system. Working standard of Ondansetron with potency of 97.50 % (on as is basis) was obtained from Dr. Reddy Laboratories Limited. Other chemicals were analytical grade of above 99% purity. All volumetric-glassware were pre-calibrated by the manufacturer

(Borosil) and were of grade A. Orally disintegrating films containing Ondansetron has been developed in the laboratories.

CHROMATOGRAPHIC CONDITIONS

The analysis was carried out with UV detection at 247 nm using a 10 l injection volume. Assay was performed using a C-8 reversed-phase column eluted with buffer and acetonitrile (72:28, %v/v) at a flow rate of 1.0 mlm^{-1}. Chromatography was carried out at ambient temperature. The solvents were mixed, filtered through a membrane filter of 0.45 micron pore and degassed in ultrasonic bath prior to use.

STANDARD SOLUTION PREPARATION

STANDARD STOCK SOLUTION

Standard stock solutions of 400 μgml^{-1} of ondansetron were prepared in diluent (Methanol).

WORKING STANDARD SOLUTION

Transferred 5 ml of standard stock solution to a 25 ml volumetric flask. Diluted up to the volume with methanol and mixed. It was filtered through a .22 µ membrane filter.

SAMPLE PREPARATION

20 strips of the product under study were weighed, cut them uniformly. A portion equivalent to the weight of 20.00mg was accurately weighed and transferred to a dry 250 ml volumetric flask and 50 ml of 0.1N HCl was added. The volumetric flask was sonicated for 15 min with intermittent shaking. Again 150ml of methanol was added and sonicated it for 15 min. Cool to room temperature and volume made up to the mark with methanol & mixed. Suitable aliquots of solution were filtered through a 0.45 m nylon filter. Each of standard (**Fig.2**) and sample preparation (**Fig.3**) were injected into the chromatograph and the responses were recorded.

METHOD VALIDATION [12]

LINEARITY & RANGE

A series of standard curves were prepared over a concentration range of 40.0 – 120.0 g/ml by diluting the standard stock solution of ondansetron (80 g/ml) in methanol (as diluent). The data from peak area versus drug concentration plots were treated by linear least square regression analysis and r^2 was found 0.9999. The standard curves were evaluated for intra-day and inter-day reproducibility. Each experiment was repeated in triplicate.

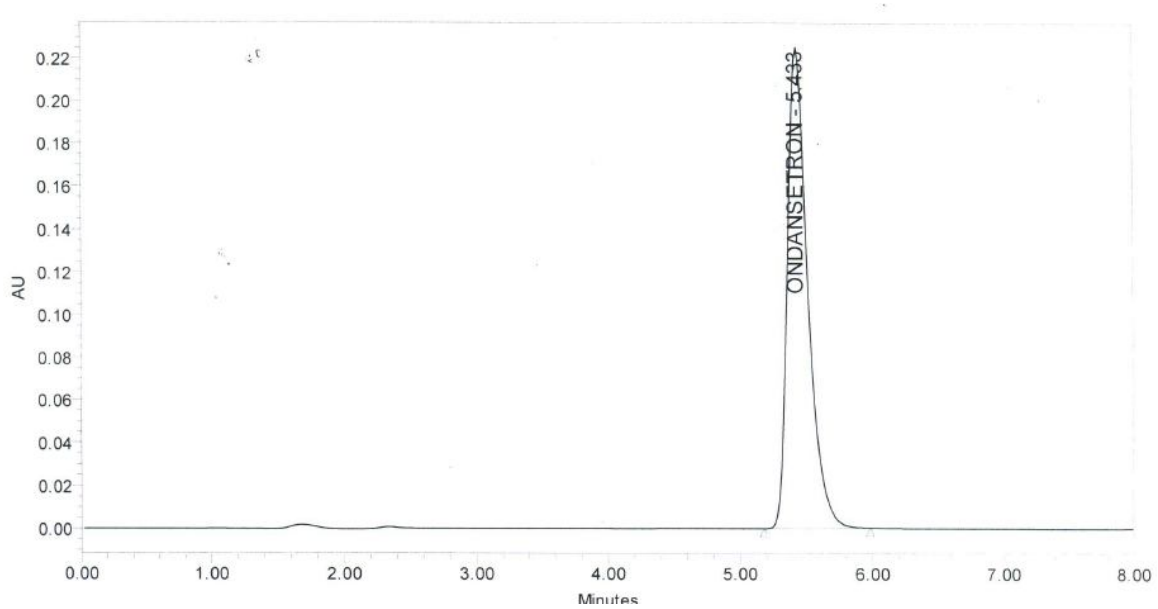

Fig.2 HPLC Chromatogram of Standard Ondansetron

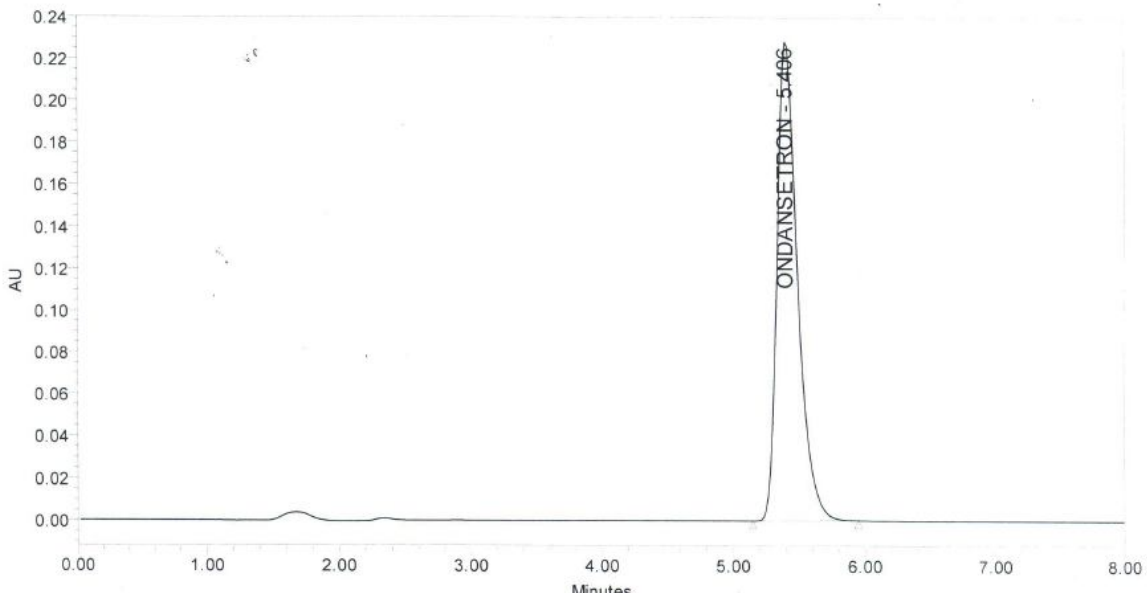

Fig. 3 HPLC Chromatogram of Ondansetron Orally Disintegrating Films Sample

PRECISION

Precision was measured in accordance with ICH recommendations. The precision study was carried out by injecting sample preparation of 80 g/ml concentration six times.

ACCURACY

Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Placebo of Ondansetron orally disintegrating film 4 mg were

spiked with ondansetron standard solution (80 gml^{-1}) so as to get three different levels (80%, 100% and 120%) and the mixtures were analyzed by the proposed method. The experiment was performed in triplicate. Recoveries (%), RSD (%) were calculated for each concentration.

RUGGEDNESS

The ruggedness of the method was demonstrated by analysis of the samples as for precision study by a second analyst. The RSD of the two sets of data indicates the ruggedness of the method.

ROBUSTNESS

The robustness of the method was determined to assess the effect of small but deliberate changes of the chromatographic conditions on the determination of Ondansetron. The different variations are in flow rates by ± 0.1 mL/min, in wavelength by ± 2 nm, in pH by ± 0.2, and in mobile phase composition by ± 2%. The concentration of the solution analyzed was 80 gml^{-1}.

SYSTEM SUITABILITY TESTS

The chromatographic systems used for analyses must pass the system suitability limits before sample analysis can commence. The injection repeatability, tailing factor (T), theoretical plate number (N) and % RSD (% relative standard deviation) for the principal peak were the parameters tested on a 80 g/mL sample of Ondansetron to assist the accuracy and precision of the developed HPLC system.

SPECIFICITY

The specificity of the method was determined by purity angle and purity threshold of standard and test solution using photo diode array detector.

RESULTS AND DISCUSSION

Ondansetron, a weak acid, is sparingly soluble in water. The final decision on mobile phase composition and flow rate was made on the basis of peak shape, peak area, tailing factor, baseline drift, ease of preparation, use of readily available cost-effective solvents and time required for analysis. Initial trial experiments were conducted, with a view to select a suitable solvent system for the accurate estimation of the drug. These included methanol–water, acetonitrile-water, methanol-buffer, acetonitrile: buffer, methanol–acetonitrile-water and acetonitrile–methanol in different ratio. Flow rates between 0.5 and 1.2ml/min were studied. A mobile phase system comprising of buffer-acetonitrile (72:28 % v/v) was found to be optimum and a flow rate of 1.0 mlm^{-1} gave an optimal

peak shape and was selected. The methanol was used for the extraction of the drug from the formulation containing excipients. No internal standard was used because no extraction or separation step was involved. The solvents were mixed, filtered through a membrane filter of 0.45 micron pore and degassed in ultrasonic bath prior to use. Using a reversed-phase C8 column, the retention times for ondansetron was observed to be 5.33 & 5.406 min. Total run time was kept 8.0 min. The maximum absorption of ondansetron was detected at 247 nm and this wavelength was chosen for the analysis. The developed method was linear showing the coefficient of correlation of 0.9999. % RSD of accuracy study for three levels (80, 100 and 120 %) showed below 2.0% and precision was found to be 0.54. The method was also found to be robust as there was no significant change in the peak area, peak shape and retention time of ondansetron. The system suitability tests performed verified the resolution, column efficiency and repeatability of the chromatographic system.

LINEARITY

Peak area versus drug concentration was plotted to construct a standard curve for Ondansetron and linearity was shown in concentration range of 40.0 gml^{-1} to 120.0 gml^{-1}. The polynomial regression for the calibration plots showed good linear relationship with coefficient of correlation, r = 0.9999; slope = 30503 and intercept = 65831 over the concentration range studied. **Fig.4**

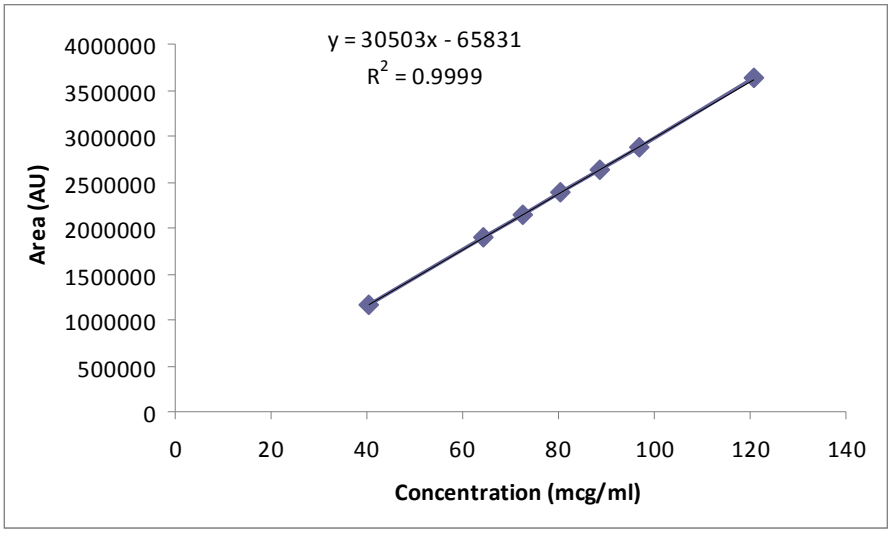

Fig. 4-Linearity graph of Ondansetron

PRECISION

The % assay for film was calculated and % RSD was found to be 0.54%.which proved that the method was precise, as depicted in **Table 1**.

Table No.1: "Precision of developed method at working level"

Sample No.	% Assay
1	99.60
2	100.70
3	100.40
4	101.10
5	101.0
6	100.80
Mean	100.6
SD	0.54
% RSD	0.54

ACCURACY

The % recovery was calculated for triplicate samples and for all levels and mean recovery was calculated. The mean recovery was well within the acceptance limit hence the method was accurate, as depicted in **Table 2**.

Table No.2: "Recovery studies of ondansetron orally disintegrating films 4 mg"

Sample No.	Amount added (mg)	Amount recovered (mg)	% Recovery
Accuracy 80 % -1	16.41	16.55	100.90
Accuracy 80 % -2	16.50	16.60	100.60
Accuracy 80 % -3	16.33	16.44	100.70
Accuracy 100 % -1	20.14	20.41	101.30
Accuracy 100 % -2	19.51	19.35	99.20
Accuracy 100 % -3	19.60	19.71	100.60
Accuracy 120 % -1	24.49	24.88	101.60
Accuracy 120 % -2	23.62	23.72	100.40
Accuracy 120 % -3	24.33	24.61	101.20
Mean	100.70		
SD	0.691		
%RSD	0.69		

RUGGEDNESS

The % assay and RSD for samples prepared by second analyst was calculated and found within limit. Then RSD of analyst 1 and analyst 2 was calculated and found within limit. This proved that the method is rugged, as depicted in **Table 3**.

Table No.3: "Ruggedness Analysis"

Analyst 1		Analyst 2	
Sample	**% Assay**	**Sample**	**% Assay**
1	99.60	1	101.00
2	100.70	2	100.90
3	100.40	3	101.30
4	101.10	4	100.90
5	101.00	5	101.70
6	100.80	6	101.20
Mean	100.60	Mean	101.20
SD	0.548	SD	0.308
% RSD	0.54	% RSD	0.30

ROBUSTNESS

The results of the analysis (% RSD ranged from 0.61 to 1.047 %) of the samples under the conditions of the above variations indicated the nature of robustness of the method.

SYSTEM SUITABILITY TESTS

The results of the system suitability tests assure the adequacy of the proposed HPLC method for routine analysis of Ondansetron. The RSD of six consecutive injections performed under the precision test (**Table 1**) was found to be 0.54% and thus shows good injection repeatability. The tailing factor (T) for Ondansetron peak was found to be 1.52, reflecting good peak symmetry. The theoretical plate number (N) was found to be 7775, thus demonstrating good column efficiency.

SPECIFICITY

The chromatograms obtained showed separation of the analyte from the excipients was complete, i.e. there was no interference from the excipients under the chromatographic conditions used for the analysis. No interference of the placebo mixtures with the peak of ondansetron was observed.

CONCLUSION

The HPLC method developed is accurate, precise, reproducible and specific. The method is linear over a wide range, economical and utilizes a mobile phase which can be easily prepared. All these factors make this method suitable for quantification of Ondansetron in bulk drug and in newly developed orally disintegrating films. The method developed was then subjected to validation as per ICH guidelines and showed that method is linear, precise, accurate and robust[12].

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