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Research Article

Comparative Assessment of Analytical Methods of Orally Disintegrated Tablet of Ondansetron

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ABSTRACT

Ondansetron ODT, most widely used anti-emetic drug, in this regard, the Research and Validation of reliable analytical method for determination. The main aim of present Research work to Validate a method for estimation of Ondansetron ODT by using High Performance Liquid Chromatography.

For Chromatographic determination, mixture of Potassium phosphate:acetonitrile(52:48) was used as mobile phase, the column having length of 4.6 mm × 25 cm and containing packing L7 which is maintained at 45 ± 1 ˚C temperature. The injection volume was 10 µl and the flow rate was 1.5 mL/min. The assay method was validated for analytical parameters such as linearity, accuracy, precision, system suitability, robustness and range.

From the results it is concluded that, HPLC is less time consuming and cost reducing and the results obtained are more accurate, precise and reproducible as compared to UV.

Key-words: Ondansetron ODT, Validation, anti-emetic, HPLC etc.

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INTRODUCTION

The objective of current study is comparison of assay method of Ondansetron from Ondansetron orally disintegrating tablet by different Pharmacopoeias such as Indian Pharmacopoeia and United State Pharmacopoeia. The goal of this comparative assessment is to evaluate which method is suitable.

In this work we have performed the assay of the tablet according to two standard Pharmacopoeial books (IP and USP) and after that we have performed validation according to ICH guidelines. The assay method was validated for analytical parameters such as linearity, accuracy, precision, system suitability, robustness and range. According to IP and USP, the assay of Ondansetron Orally Disintegrating tablet was performed by HPLC.

In this method, the column used for experiment purpose, was packed with porous silica bonded to nitrile groups.C25 column contains stationary phase which is stable and it can tolerate pressure limit more than 6000 psi, for this reason mobile phase runs in uniform way and it provide uniform flow throughout the column. C25 column contains smaller particle size due to which plate increases resulting in higher efficiency. Octadecyl silane is used to increase the polarity of the stationary phase to give sharp and intense peak.

From the results it is concluded that in IP and USP method, HPLC is less time consuming and cost reducing, and the results obtained are more accurate, precise and reproducible as compared to UV.

According to IP and USP method, a mixture of Potassium phosphate:acetonitrile(52:48) was used as mobile phase, the column having length of 4.6 mm × 25 cm and containing packing L7 which is maintained at 45 ± 1 ˚C temperature. The injection volume was 10 µl and the flow rate was 1.5 mL/min.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY :[3,4,5]

Although there is spectacular advancement in the instrumental methods of analysis, the success or failure of such method largely depends upon how pure is the sample for such analysis. This is because a light impurity in analyte will lead to erroneous results. So large number of separation methods were discovered to isolate analytical species before any instrumental method is resorted. Such separation methods included not only chromatographic methods but the non-chromatographic techniques like solvent extraction, ring oven; zone refining, froth flotation, dialysis, reversed osmosis and precipitation methods. However, chromatographic methods have become most popular because of the simplicity and cost of analysis.

IUPAC has defined chromatography as: "Methods used primarily for the separation of the components of a sample, in which the components are distributed between two phases, one of which is stationary while other moves. The stationary phase may be a solid or a 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".

Some of the advantages are

- Speed (analysis can be accomplished in 20 minutes or less),
- Greater sensitivity (various detectors can be employed),
- Precise and reproducible,
- Calculations are done by integrator itself,
- Improved resolution,
- Reusable columns (expensive columns but can be used for many analysis),

Basic Terminologies in HPLC:

Baseline is any part of the chromatogram where only mobile phase is emerging from the column.

Injection point is that point in time where the sample is placed on the column.

Peak maximum is the highest point of the peak.

Dead point is the position of the peak-maximum of an unretained solute.

Dead time (t_o) is the time elapsed between the injection point and the dead point.

Dead volume (V_o) is the volume of mobile phase passed through the column 0 between the injection point and the dead point. Thus, $V_o = Qt_o$ where Q is the flow rate in ml/min and t_o is dead time.

Retention time (RT) is the time elapsed between the injection point and the peak maximum. Each solute has a characteristic retention time.

Retention Vol (VR) is the volume of mobile phase passed through the column between the injection point and the peak maximum. Thus, VR = QRt where Q is the flow rate in ml/min and RT is retention time.

Peak height (h) is the distance between the peak maximum and the baseline geometrically produced beneath the peak.

EXPERIMENTAL WORK

APPARATUS AND INSTUMENTS:

Table No.1: Instruments Used

Sr. no.	Instruments	Specification
1	HPLC System	Shimadzu HPLC System
2	Pump	LC-20 AT Prominence Liquid Chromatography
3	Column	4.6 mm X 25 cm column packed with porous silica bonded with nitrile groups.
4	Detector	SPD-M20A PDA Detector
5	Data Processor	LC solution
6	Degasser	DGU-20 A5 Prominence Degasser

REAGENTS AND MATERIALS:

Active Pharmaceutical Ingredients (API)

Table No.2: Name of API and Suppliers:

Sr. no.	Name of API	Supplier
1	Ondansetron	Symed Lab.Limited,Jinnaram Mandal,Medak(Dist) A.P

Marketed Tablet Formulation:

Table No.3: Marketed Formulation

Sr. no.	Brand Name	Strength	Name of company	Batch no.	Mfg. Date	Exp. Date
1	Ondem-MD 4	Ondansetron-4 mg	Alkem labs.Ltd.	3131320	JULY 2013	JUNE 2015

ASSAY OF ONDANSETRON ODT AS PER USP:

DILUENT: 0.01 N Hydrochloric acid

PREPARATION OF PHOSPHATE BUFFER:

Dissolved about 2.72 gm of monobasic potassium phosphate in 1000 mL of water.It is adjusted with 1 N sodium hydroxide or 0.5 N sodium hydroxide to a PH of 5.4.

PREPARATION OF MOBILE PHASE:

Filtered and degassed mixture of phosphate buffer and Acetonitrile (52:48) is prepared.

ONDANSETRON RELATED COMPOUND A SOLUTION:

Amount of USP Ondansetron related compound A RS is dissolved in diluent, and diluted stepwise with diluent to obtain a solution having a known concentration of about 0.14 mg per mL.

CONCENTRATED ASSAY PREPARATION:

Transfer 10 tablets to an appropriate volumetric flask so that the final concentration is about 400 µg of Ondansetron per ml.Add Diluent to fill about 60% of flask capacity. Shake by mechanical means for about 5

mins,and dilute with diluent to volume.Filter a portion of this solution through 0.45-µm polypropylene membrane, discarding the first 5 ml of the filtrate.

SYSTEM SUITABILITY SOLUTION:
Transferred 8 mL of Ondansetron related compound A solution and 8 mL of the standard preparation to a 50 mL volumetric flask. It is diluted with diluent to volume and mixed.

STANDARD PREPARATION:
Accurately weighed quantity of USP Ondansetron RS is dissolved in diluent,and diluted quantitatively, andstepwise, with diluent to obtain a solution having a known concentration of about 40 mcg per mL.

ASSAY PREPARATION:
Transferred 5 mL of concentrated assay preparation to a 50 mL volumetric flask. Diluted with diluent to volume and mixed.

CHROMATOGRAPHIC SYSTEM:
The liquid chromatography is equipped with a 216-nm detector and a 4.6 mm*25 cm column that contains packing L10.The flow rate is about 1.5 mL per minute. Chromatograph the System suitability solution, and record the peak responses as directed for procedure. The relative retention about 1.1 for Ondansetron related compound A, and 1 for Ondansetron, the resolution R,between Ondansetron related compound A and ondansetron is not less than 1.5,and the tailing factor is NMT 2.0;for the ondansetron peak. Chromatograph the standard preparation and record the peak responses, as directed for procedure. The relative standard deviation for replicate injection is NMT 2.0%.

PROCEDURE:
Separately injected equal volumes (about 10µl) of the standard preparation and the assay preparation into the chromatograph, recorded the chromatograms and measured the responses for the Ondansetron peaks. Calculated the quantity in mg, of Ondansetron in the portion of tablets taken by the formula.

$$(10V)C(r_u/r_s)$$

In which V is the volume used to prepare the concentrated assay preparation, in mg per mL, of USP Ondansetron RS in the Standard preparation, and r and r are the peak responses obtained from the assay preparation and the Standard preparation respectively.

ASSAY OF ONDANSETRONAS PER IP:
PREPARATION OF MOBILE PHASE:
A mixture of 52 volumes of 0.272% w/v solution of monobasic potassium phosphate adjusted to PH 5.4 with 1 M sodium hydroxide and 48 volumes of Acetonitrile. The flow rate of mobile phase was 1.5 ml/min.

PREPARATION OF TEST SOLUTION:
Weigh and powder 20 tablets. Disperse a quantity of powder containing about 40 mg of Ondansetron with 100 ml 0.01 M Hydrochloric acid.

PREPARATION OF REFERENCE SOLUTION:
A 0.004 % w/v solution of Ondansetron RS in 0.01 M Hydrochloric acid.

ASSAY PROCEDURE:
Inject reference solution and test solution. The relative retention time with reference to Ondansetron for Ondansetron impurity A is about 1.1.The tailing factor is NMT 2.0 for Ondansetron peak. Calculate the content of**$C_{18}H_{19}N_3O$**in the tablets.

PREPARATION OF STOCK SOLUTION OF ONDANSETRON IP AND USP:

Dissolved about 40 mg of Ondansetron sample in 100ml of diluents. This solution was of 400ppm.

Preparation of solution:

Preparation of 20.0 µg/ml solution: (For 50%)

Quantity of 0.5 ml was taken from above stock solution and volume made upto 10 ml by diluent to obtain the solution having final concentration 20.0 µg/ml.

Preparation of 30.0 µg/ml solution: (For 75%)

Quantity of 0.75 ml was taken from above stock solution and volumemade upto 10 ml by diluent to obtain thesolution having final concentration 0.75 mcg/ml.

Preparation of 40.0 µg/ml solution: (For 100%)

Quantity of 1.0 ml was taken from above stock solution and volumemade upto 10 ml by diluent to obtain thesolution having final concentration 40.0 mcg/ml.

Preparation of 50.0 µg/ml solution: (For 125%)

Quantity of 1.25 ml was taken from above stock solution and volume made upto 10 ml by diluent to obtain thesolution having final concentration 50.0 mcg/ml.

Preparation of 60.0 µg/ml solution: (For 150%)

Quantity of 1.5 ml was taken from above stock solution and volume made upto 10 ml by diluent to obtain thesolution having final concentration 60.0 mcg/ml.

VALIDATION OFANALYTICAL METHOD OF ONDANSETRON IP AND USP:

Following Method validation Parameters were studied for Ondansetron ODT IP and USP Method-

1) Linearity:

Linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.

Linearity of the method was studied by preparing concentration of drugs in linear range. For the drug, appropriate dilutions of standard stock solutions were analysed as per the developed method. Calibration curve was plotted in the concentration range of 20-60 µg/ml for Ondansetron. The acceptance criteria of linearity should not be less than 0.999.

2) Accuracy:

Accuracy of an analytical procedure is the closeness of agreement between the conventional true value or an accepted reference value and the value found.

In order to ensure the suitability and reliability of proposed method, recovery studies were carried out. The accuracy of the method was performed by conducting the recovery studies (50,100% and 150%) of pure drugs from marketed formulation, by standard addition method.

3) Precision:

Method Precision is a multiple measurements of a sample by the same analyst. A minimum of 6 determinations at the test concentration (6 times of a single batch).For Assay %RSD should be less than 2%. The System precision indicates the performance of HPLC System, as system suitability test. In this, multiple injections (NLT 5) of a homogeneous Sample (Standard Solution).

4) Range:

The range of an analytical method is the interval from the upper to the lower level that has been demonstrates to be determined with precision, accuracy and linearity.

FORMULA FOR CALCULATION:

1) %RSD (Relative Standard Deviation):

$$\% \text{ RSD} = \frac{\text{Std.dev} \times 100}{\text{Mean}}$$

2) Slope:

$$Y = mX + C$$

Where, Y = peak area, m = slope

X = concentration (µg/ml) and C = intercept

3) **Standard Deviation:** $\sigma = \sqrt{\frac{\sum(X-X)^2}{N-1}}$

4) **Mean:**

$$M = \frac{\text{Sum of all observation}}{\text{Total number of observation}}$$

5) **% Assay:** $= \frac{\text{std.abs.}}{\text{sample abs.}} \times \frac{\text{sample weight}}{\text{std weight}} \times \frac{\text{avg.weight}}{\text{label claim}} \times \text{potency}$

RESULT

Validation of Ondansetron ODT was performed by using HPLC. Assays were also performed as per IP-2010 and USP. Assay results of Ondansetron ODT were same as per IP-2010 and USP. % purity of drug found was 95.19%.Validation of Ondansetron ODT was done for different parameters like accuracy, precision, linearity, robustness etc. For linearity data, graph is plotted (concentration Vs Area). Linearity data shows as concentration increases it gives more peak area.

Assay of Ondansetron as per IP and USP:

Table No.4 Assay Result for Ondansetron as per IP and USP:

Formulation	Amount of Drug in Tablet (mg)		Amount of Drug taken (mg)		Amount of Drug found(mg)		%Amount of Drug found(mg)	
	ONDAN USP	ONDAN IP	ONDAN USP	ONDAN IP	ONDAN USP	ONDAN IP	ONDAN USP	ONDAN IP
Tablet	4	4	4	4	3.8	3.8	95.19%	95.19%

Validation of Ondansetron as per IP and USP:

Table No. 5 Linearity Data for Ondansetron as per IP and USP:

Sr. No.	Concentration (ppm)		Peak Area	
	USP	IP	USP	IP
1	20	50	588.240	631.548
2	30	75	937.534	909.125
3	40	100	1263.507	1260.899
4	50	125	1576.831	1540.584
5	60	150	1882.478	1888.843

Figure 1: Calibration curve of Ondansetron (USP)

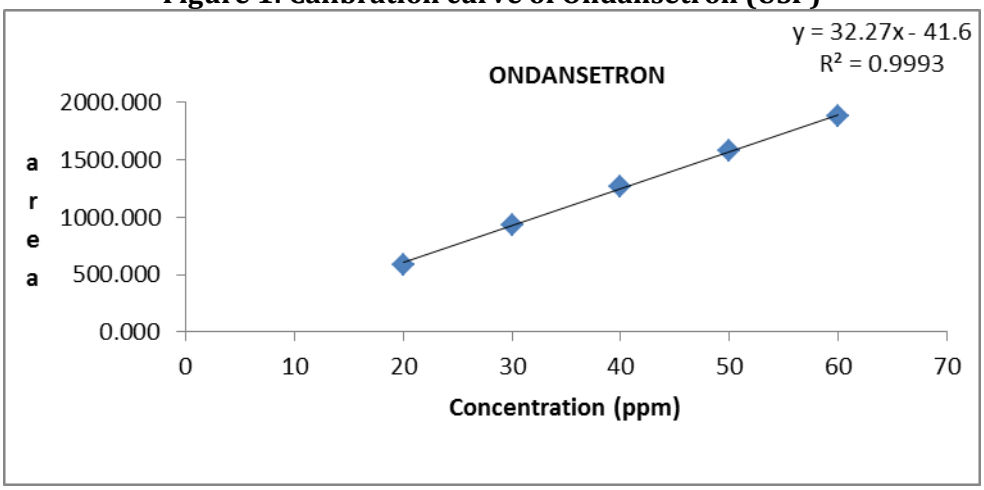
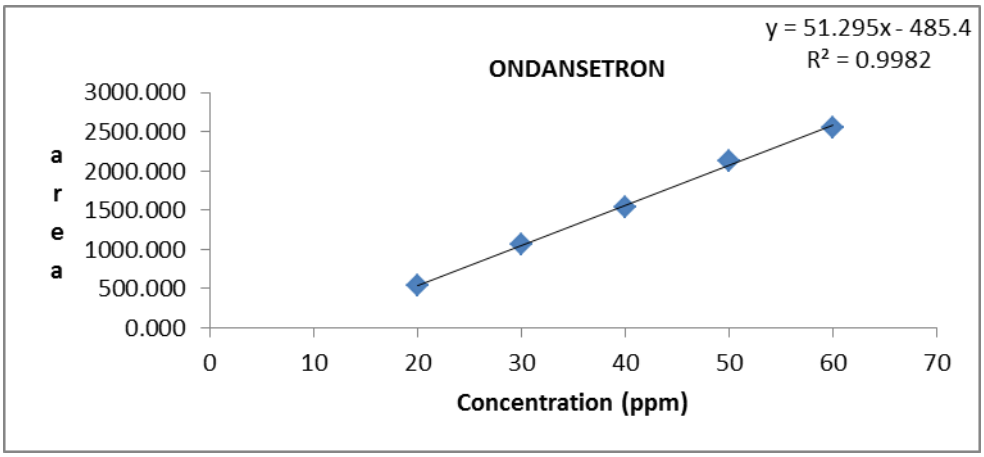


Figure 2: Calibration curve of Ondansetron (IP)



SUMMARY

The comparative study performed to validate a simple, economical, accurate, more precise and validated method for analysis of Ondansetron Orally Disintegrating tablet by performing assay as per two standard books i.e. IP and USP, which is summarized below:

For Ondansetron,as per USP and IP analysis method, the mobile phase which comprises of Potassium Phosphate: acetonitrile (52:48), with a flow rate 1.5 ml/min, the injection volume was 10 µl and the wavelength was set at 216 nm. The column used in USP method was 4.6 mm × 25 cm containing packing L10. These two standard Pharmacopoeial methods was validated as per ICH guidelines for accuracy, precision, linearity, range and robustness over a concentration range of Ondansetron and the analysis results of two Pharmacopoeial books were compared. Then standard deviation, relative standard deviation and cumulative standard deviation were calculated which shows variation in results of IP and USP and it is within the limit.

As per USP, assay by liquid chromatography requires C25 column which packing material as stationary phase and C25 column is also stable at wide pH range and this column have less particle size (4 micron) which gives an intense and better resolution peak. C25 column contains porous silica bonded to nitrile group, which is mechanically very stable and can tolerate pressure limit more than 6000 psi, due to which mobile phase runs in a uniform way and provide more uniform flow throughout the column. Stainless steel column (contains porous silica bonded to nitrile group) is used in order to increase the polarity of stationary phase to give a sharp and intense peak. C25 column contains smaller particle size due to which plate increases resulting in higher efficiency.

Simultaneous determination was also done by USP method. Acetonitrile which was used as mobile phase in combination with water having lowest absorbance especially for shorter wavelength, since the mobile phase having the lower absorbance, lower the noise. The retention time of Ondansetron was 7.3.

As per IP and USP, assay of Ondansetron was done by HPLC. HPLC is less time consuming and cost reducing method, it can give more accurate, precise, reproducible result as compared to Titration.

Also by comparing the assay and linearity results of two standards (IP and USP) I found that, same results obtained in both Standard books, because assay procedure was same in both Official books.

Table No. 6: Comparison of Assay Results of Ondansetron:

Sr. No.	Pharmacopoeia	% Assay	Coefficient correlation (R^2)
1	Indian Pharmacopoeia	95.19%	0.998
2	United State Pharmacopoeia	95.19%	0.998

Summary of Validation parameters of HPLC method of Ondansetron: As per IP and USP:

Parameters	Results	
	USP	IP
Linearity		
Concentration Range	50%-150% µg/ml	50%-150%µg/ml
Correlation coefficient (r^2)	0.998	0.998
Accuracy		
% Recovery	99.71-100.17%	99.67-99.88%
Precision (%RSD)		
Method Precision	0.87	1.07
System Precision	1.17	1.17
Robustness (%RSD)		
Flow rate (+0.2mL/min)	1.52	1.52
Flow rate (-0.2 mL/min)	1.19	1.19
Mobile phase (90%)	1.63	0.79
Mobile phase (110%)	1.59	0.36
Range (%RSD)		
50%	1.81	1.66
150%	0.51	1.17

CONCLUSION

The main goal of this work is based on to compare and selects the more suitable, accurate, precise, validated and reliable method for the determination of Ondansetron ODT which are given in standard Pharmacopoeial books like IP and USP.

Assay of Ondansetron ODT is reported in USP. The assay method of Ondansetron in IP and USP is same. This study helps to find out the problem during the analysis and solve the problem by finding alternative and

validated method. This study is designed to make a comparative and comprehensive analysis of Ondansetron Orally Disintegrating tablet.

From this comparative study, I found that we can use IP and USP both the methods for analysis purpose.

So,I concluded that IP and USP both the methods are accurate, precise and reliable for analysis purpose. If the ODT are analysed by IP and are passing the same tablets will pass as per USP and vice a versa. This will reduce the reputation of analysis of the Tablets which are analysed by IP and are to be reanalysed by USP.

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