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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF ONDONSETRAN USING REVERSE PHASE HPLC METHOD &IT'S APPLICATION TO DIFFERENT PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A new simple, accurate, precise and reproducible RP-HPLC method has been developed for the estimation of Ondonsetran in it's bulk & different pharmaceutical dosage forms using inertsil ODS C18 column (250 x 4.6 mm, 5 µm) in isocratic mode. The mobile phase consists of 0.1MKH2PO4:ACN: MeOH (30:40:30)v\v pH6.0 . The detection was carried out at 216 nm for Ondonsetran. The method was linear over the concentration range 48-112 µg/ml. The validation of method was carried out utilizing ICH-guidelines.

Keywords: Ondonsetran, reverse phase HPLC, validation.

INTRODUCTION

Ondonsetran, Chemically it is (±) 1, 2, 3, 9-tetrahydro-9-methyl-3-[(2-methyl-lH-imidazol-l-yl) methyl]-4H-carbazol-4-one, monohydrochloride, dehydrate (Fig 1). is a serotonin 5-HT_3 receptor antagonist used to prevent nausea and vomiting caused by cancer chemotherapy, radiation therapy, and surgery.It has little effect on vomiting caused by motion sickness, and does not have any effect on dopamine receptors or muscarinic receptors.It is on the World Health Organization's List of Essential Medicines, a list of the most important medication needed in a basic health system.

Ondansetron is a selective serotonin 5-HT_3 receptor antagonist. The antiemetic activity of the drug is brought about through the inhibition of 5-HT_3 receptors present both centrally (medullary chemoreceptor zone) and peripherally (GI tract). This inhibition of 5-HT_3 receptors in turn inhibits the visceral afferent stimulation of the vomiting center, likely indirectly at the level of the area postrema, as well as through direct inhibition of serotonin activity within the area postrema and the chemoreceptor trigger zone[1].

According to literature survey few spectrophotometric [1-6], HPLC [7-11] methods have been reported for the determination of Ondonsetran in single and in combination with other drugs. Analytical methods are reported for the determination of Ondonsetran in it's different dosage forms like tablet, suspension, injection by spectrophotometric and HPLC have been reported.. However very few HPLC methods were reported for the estimation of ondonsetran &it's application to different dosage forms like tablet, suspension, injection.

The aim of present work was to develop and validate as per ICH guidelines [18-19], a sensitive HPLC method that can be applied for the estimation of Ondonsetran in different dosage forms.

MATERIALS

Ondonsetran was received gratis from Hetero drugs, Hyderabad and was used as received. HPLC grade acetonitrile was purchased from SD Fine Chem Pvt. Ltd. (Mumbai, Maharashtra). Ultra-pure water was obtained from ELGA (Bucks, UK) water purification

unit. All other chemicals were of analytical reagent grade.

EXPERIMENTAL WORK

Chromatographic conditions: The HPLC system (AGILENT 1200 series) consisted of quaternary gradient system (600 Controller), in-line degasser, UV Detector and manual sampler (Waters, model 717 plus). Data was processed using Spin chrome software.

Isocratic elution of the mobile phase 0.1 M Dipotassium Phosphate buffer (pH 6) acetonitrile and Methanol in the ratio of 30:40:30 v/v with the flow rate of 1 ml/min. Separation was performed on a Waters C_{18} (250 x 4.6 mm i.d, 5 µ particle size) analytical column and a pre-column to protect the analytical column from strongly bonded material. Integration of the detector output was performed using the spin chrome software to determine the peak area. The contents of the mobile phase were filtered through a 0.45 µm membrane filter and degassed by sonication before use. Mobile phase was used as diluents.

The flow rate of the mobile phase was optimized to 1 ml/min which yields a column back pressure of 110–112 kg/cm. The run time was set at 7 min and a column temperature was maintained at 35°C. The volume of injection was 10 µl, prior to injection of the analyte, the column was equilibrated for 30–40 min with the mobile phase. The eluent was detected at 216 nm. The developed method was validated in terms of specificiy, linearity, accuracy, limit of detection (LOD), limit of quantification(LOQ), intra-day and inter-day precision and robustness for the assay of Ondonsetran in it's different dosage forms as per ICH guidelines.

Preparation of standard solution: Ondonsetran was weighed (100 mg) and transferred to a separate 100 ml volumetric flask and dissolved in 50 ml of mobile phase and make up the volume upto the mark with mobile phase. Working standards of the drug was prepared from this solution.

Preparation of sample solution(Tablet): Twenty tablets (zorfan, Make: GSK) were weighed. An accurately weighed amount of the finelypowdered tablets equivalent to 4 mg of was made up to 100 mL with mobile phase. The solution was filteredfollowed by serial dilution to the required concentrations for each experiment.

Preparation of sample solution(Suspension): suspension equivalent to 2mg (oyster labs) were weighed. &made up to 100 mL with mobile phase. The solution was filtered followed by serial dilution to the required concentrations for each experiment.

Preparation of sample solution(injection): (zorfan, Make: GSK) were weighed. An accurately weighed amount of the Ondonsetran injection equivalent to 2mg was made up to 100 mL with mobile phase. The solution was filtered followed by serial dilution to the required concentrations for each experiment.

RESULTS AND DISCUSSION

Method Development: Number of mobile phase and their different proportionswere tried and finally was selected as 0.1 M Dipotassium Phosphate buffer (pH 6), acetonitrile & methanol in the ratio of 30:40:30 v/appropriate mobile phase which gave good resolution and acceptable system suitabilityparameters. The results of system suitability parameters were shown in table 2. The chromatogram of working standardsolution is shown in Fig 3. The summaries of Chromatographic conditions were given in table 1.

Method Validation:

Accuracy: Recovery assessment was obtained by using standardaddition technique which was by adding known quantitiesof pure standards at three different levels in 50%, 100%and 150% to the pre analysed sample formulation. Fromthe amount of drug found, amount of drug recoveredand percentage recovery were calculated which sense toconformation that the proposed method was accurate. The results were tabulated in Table 3.

Precision: The intraday and interday precision of the proposed methodwas determined by analyzing mixed standard solution of Ondonsetran at100% concentration 3 times on the same dayand on 3 different days. The results shown in table 4 were reported in terms of relative standard deviation.

Linearity: Calibration graphs were constructed by plotting peak area vs concentration of OND and the regression equationswere calculated. The calibration graphs were plotted over 5different linear concentrations in the range of 48-112µg/ml for OND in Tablets, suspension,injection. Aliquots (10 ml) of each solution were injected under the operating chromatographic condition describedabove [Number of replicates (n =5)]. The linearity graphs were shown in fig 4 & 5,6

Limit of detection (LOD) and limit of quantitation(LOQ): The limit of detection (LOD) and limit of quantitation (LOQ) of OND was

determined by calculating the signal-to-noise(S/N) ratio of 3:1 and 10:1, respectively according to InternationalConference on Harmonization guidelines. LOD values for OND tablet, suspension, injection were found to be 1.05mcg/ml. LOQ values OND tablet, suspension, injection were found to be 3.19mcg/ml respectively.

Assay of the dosage forms: The proposed validated method was successfully applied to determine ONDONSETRAN in different dosage forms. The results obtained were comparable with corresponding labeled amounts. The results were tabulated in table 5.

CONCLUSIONS

The proposed method has advantage of simplicity and convenience for the separation and quantitation of Ondansetran in different dosage forms which can be used for the assay of their dosage form. Also, the low solvent consumption and shortanalytical run time lead to environmentally friendly chromatographic procedure. The method is accurate, precise,rapid and selective for estimation of Ondonsetran & it's application to different pharmaceutical dosage forms. Hence it can be conveniently adopted for routine analysis.

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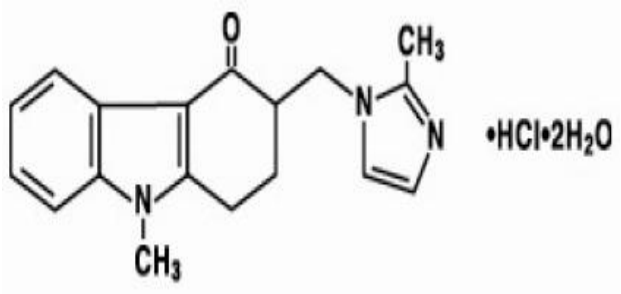

Fig 1: Chemical Structure of Ondonsetran.

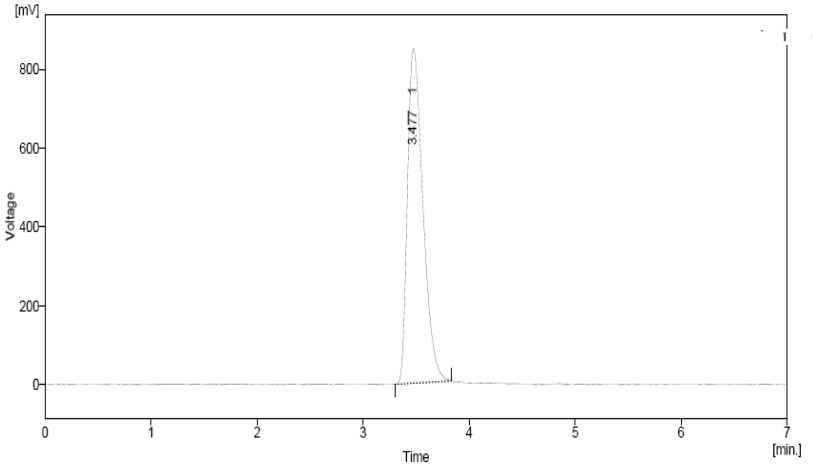

Fig.2 Typical Chromatogram of Ondonsetran tablet

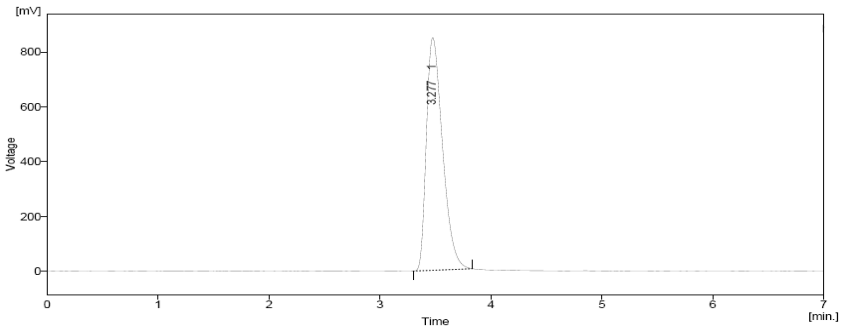

Fig.3 Typical Chromatogram of Ondonsetran injection

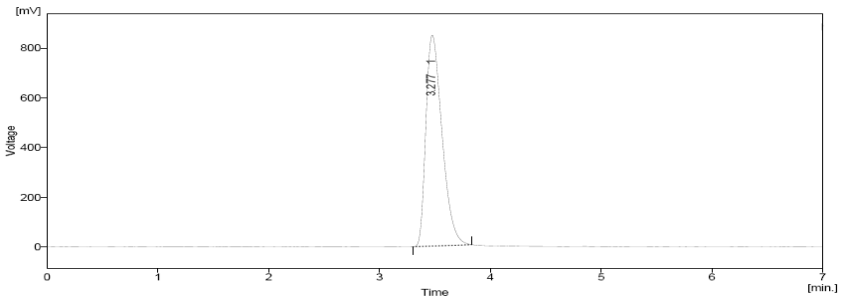

Fig.4 Typical Chromatogram of Ondonsetran suspension

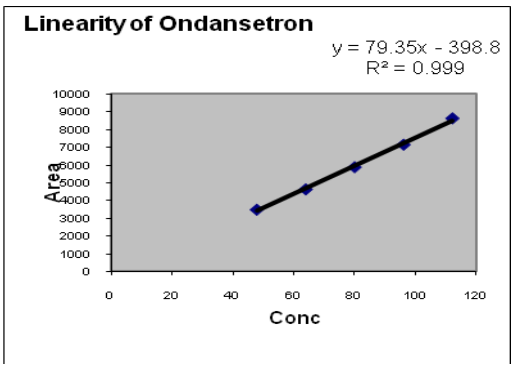

Fig 5 Linearity of Tablet

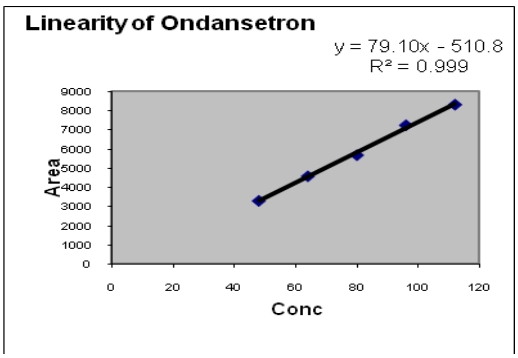

Fig 6 Linearity of Injection

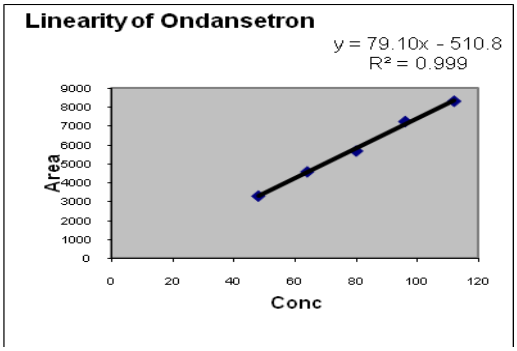

Fig 7 Linearity of Suspension

Table 1: Summary of Chromatographic conditions

S. No	Parameter	Description/Value
1.	Stationary Phase	inertsil C18 (250X4.6X5)
2	Mobile Phase	0.1 M potassium di hydrogenPhosphate buffer (pH 6) , acetonitrile& methanol in the ratio of 30:40:30 v/v
3	Flow rate	1 ml/min
4	Detection Wavelength	216 nm
5	Detector	UV detector
6	Injection	Manual sampler -Waters, model 717 plus
7	Rt's	Tablet-3.4, injection- 3.277,suspension 3.277
8	Injection volume	10 µl
9	Column Temperature	35 °C
10	Run time	7 mins
11	Diluents	Mobile Phase

Table 2: System suitability parameters

S. No	Parameter	Result		
		OND tablet	**ONDsuspension**	**ONDinjection**
1	Retention Time	3.477	3.277	3.277
2	Tailing	1.079	1.581	1.522
3	Theoretical Plates (n)	3838	3226	3026

Table 3: Results of Accuracy

S.NO	% conc at specific level	OND tablet			OND suspension			OND injection		
		Amount added (mcg\ml)	Amount found	Mean % recovery	Amount added	Amount found	Mean % recovery	Amount added	Amount found	Mean % recovery
1	80*	80	81.11	101.38	80	79.37	99.22	80	80.74	100.93
2	100**	96	96.08	100.09	96	96.5	99.65	96	96	96.87
3	120*	112	113.41	101.26	112	111.52	98.68	112	112.94	100.84

**Mean % Recovery of 6 replicates; **Mean % Recovery of 3 replicates*

Table 4: Results of Precision (%Assay)

Sample No	OND tablet		OND suspension		OND injection	
	Retention time	Sample area	Retention time	Sample area	Retention time	Sample area
1	3.483	5704.908	3.283	5604.908	3.283	6004.908
2	3.450	5587.778	3.250	5687.778	3.250	6087.778
3	3.473	5700.527	3.273	5600.527	3.273	6000.527
4	3.467	5510.683	3.267	5610.683	3.267	6010.683
5	3.467	5579.223	3.267	5679.223	3.267	6079.223
6	3.460	5671.643	3.260	5671.643	3.260	6071.643
	Avg Area	5625.794	Avg Area	5642.460	Avg Area	6042.460
	S.D	78.499	S.D	41.074	S.D	41.074
	%RSD	**1.40**	%RSD	**0.73**	%RSD	**0.68**

Table 5: Results of Assay in different dosage forms

Sample No.	OND TAB			OND INJ			OND SUS		
	Labeled amount (mg/tablet)	Amount Found (mg/tablet)	% Assay	Labeled amount (mg/1ml)	Amount Found (mg/1ml)	% Assay	Labeled amount (mg/5ml)	Amount Found (mg/5ml)	% Assay
1	4	3.98	99.5	2	1.996	99.8	2	1.996	99.8
2		3.96	99		1.999	99.0		1.992	99.6
3		3.99	99.75		1.992	99.64		1.96	98.0
4		4.0	100		2.0	100		2.0	100
5		3.8	95		1.996	99.8		1.996	99.8
6		3.99	99.75		1.988	99.4		1.998	99.9
Average Assay:			**98.84**	**Average Assay:**		99.60	**Average Assay:**		99.51
STD			**1.74**	**STD**		0.32	**STD**		0.68
% RSD			**1.76**	**% RSD**		0.32	**% RSD**		0.68

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