International Journal of Pharmaceutical Research and Development (IJPRD)
Platform for Pharmaceutical Researches & Ideas
www.ijprd.com

DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR ESTIMATION OF GRANISETRON IN DOSAGE FORM USING ONDANSETRON AS INTERNAL STANDARD.

Moin shakeb*1,
Chandrashekar Javali[1], Md. Karvekar[1], SomashekarP.L[1]

[1]Department of Pharmaceutical Chemistry,Government College of Pharmacy,Subbiah circle,Bengaluru-560 027, India

ABSTRACT

High performance liquid chromatography (HPLC) method has been developed for estimation of Granisetron in bulk and dosage form using Ondansetron as internal standard. Granisetron was chromatographed on a C_{18} column (250 x 4.6mn; 5µm) in a mobile phase consisting of 0.05 M potassium dihydrogen phosphate buffer (pH 6.4. adjusted with sodium hydroxide) and acetonitrile in the ratio of 70:30. The mobile phase was pumped at a flow rate of 1.0 mL/min with detection at 301 nm. The retention time for granisetron hydrochloride was 5.47 min. The detector response was linear in the concentration of 10-50 µg/mL with correlation coefficient of 0.999. The percentage recovery of Granisetron hydrochloride in dosage form was found to be 98.57%. The validation of method carried out as per ICH guidelines and the proposed method with ondansetron as internal standard(IS) found to be simple, accurate, precise and reproducible and could be used for routine quality control analysis of granisetron hydrochloride in bulk and dosage form.

Keywords *Granisetron , Ondansetron, HPLC , Dosage, Method Validation.*

Correspondence to Author

Moin shakeb

Department of Pharmaceutical Chemistry,Government College of Pharmacy,Subbiah circle,Bengaluru-560 027, India

Email: moin_shakeb@yahoo.co.in

INTRODUCTION

Granisetron is an effective and potent antiemetic drug which is used in the treatment of vomiting and nausea resulting from cancer chemotherapy and radiotherapy in adults and children. Granisetron is also effective in the management of post-operative nausea and vomiting due to the anaesthetics [1, 2]. Chemically it is *endo*-N-(9-methyl-9-azabicyclo [3.3.1] non-3-yl)-1-methyl-1H-indazole-3-carboxamide hydrochloride. Granisetron hydrochloride selectively blocks type 3 serotonin (5-HT3) receptors.

Granisetron dosage forms are not yet official in USP [3]. A review of the literature revealed that a

very few HPLC methods have been reported for estimatiom of granisetron in dosage forms using Ondansetron as internal standard.

Hence, in this present investigation an attempt has been made to develop an accurate, precise and economically viable HPLC method for the estimation of Granisetron in bulk and dosage form.

MATERIAL

Experimental :

Reagents and Materials :

Acetonitrile of HPLC grade, Potassium dihydrogen phosphate and sodium hydroxide of LR grade.Granisetron Hydrochloride was a gift sample by Cipla, kurkumbh. The commercially available Granisetron hydrochloride dosage form was procured from the local market. All chemicals and solvents of HPLC grade.

Instrumentation of HPLC :

The chromatographic separation was carried out on HPLC system (Shimadzu Co, Tokyo, Japan) with UV- Visible dual absorbance detector (PDA), phenomenix C_{18} column (250 x 4.6mn; 5µm). The mobile phase consisting of phosphate buffer (pH 6.4 adjusted with sodium hydroxide) and acetonitrile in the ratio of 70:30 v/v were filtered through 0.45µ membrane filter before use, degassed and thermolile phase was pumped into the column at a flow rate of 1.0 mL/min. The detection was monitored at 301 nm. The volume of injection loop was 20 µl.Prior to the injection of the drug solution. the column was equilibrated for at least 30 minute. with the mobile phase following through the system. The column and the HPLC system were kept in ambient temperature (25º C).

METHOD

Preparation of stock solution :

About 10 mg of Granisetron was weighed in 100 mL volumetric flask. About 50 mL of mobile phase was added, sonicated to dissolve the drug completely and the volume was made up with mobile phase. 1 mL of above solution was diluted to 10 mL with mobile phase. (10 µg/mL)

Preparation of Internal standard solution :

About 10 mg of Ondansetron was weighed in 100 mL volumetric flask. About 50 mL of mobile phase was added, sonicated to dissolve the drug completely and the volume was made up with mobile phase. 1 mL of above solution was diluted to 10 mL with mobile phase. (10 µg/mL)

Analysis of dosage form :

Accurately weighed a portion of dosage form equivalent to 1.5 mg of Granisetron in a clean and dry 50 mL volumetric flask. 30 mL of mobile phase was added, sonicated to dissolve for 5 to 10 mts and make up the volume with mobile phase and filtered through 0.45µ membrane filter. (30 µg/mL)

RESULTS AND DISCUSSION :

For HPLC method, chromatographic conditions were optimized to obtain, an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried for better separation of granisetron with Ondansetron internal standard. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity factor, run time etc). The mobile phase was consisted of phosphate buffer pH 6.4 adjusted with sodium hydroxide) and acetonitrilewith ratio of (70:30 % v/v) at 1 mL/min flow rate was quite satisfactory. Ondansetron was used as an internal standard, neutralizing the error inherent in sample injection, eliminating random errors. The optimum wavelength fixed for detection was 301 nm at which better detector response for drug and internal standard were obtained. System suitability tests are an integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. The calibration was linear for granisetron at concentration range of 10 –50 µg/mL, with regression 0.999, slope 30685.17 shown in (Fig. 1). The calibration was linear for ondansetron at concentration range of 10–50 µg/mL, with regression 0.998, respectively shown in (Fig.1). A typical chromatogram for granisetron and ondansetron (internal standard) for standard solution was shown in (Fig.1).. The average retention time for Granisetron is 5.47min and for Ondansetron (IS) was found to be 8.55 min, respectively.

Precision and accuracy were evaluated using Granisetron and Ondansetron in different concentrations, which were prepared and analyzed on same day. Day to day variability was assessed using different concentrations analyzed on different days, over a period of one week. These results show the accuracy and reproducibility of the assay. Thus, it wasconcluded that there was no significant difference on the assay, The % R.S.D. values was found to be less than 2% shows that proposed method provides acceptable intra – day and inter – day variation of granisetron and Ondansetron with good precision and accuracy reported in Table 1. The percentage recoveries were found in the range of 99.72 – 99.25 %.

Table 1. Validation parameters of determination of Granisetron, Ondansetron by HPLC.

Validation parameters	RP-HPLC	
	Granisetron	Ondansetron
Linearity and range (µg/mL)	10-50	10-50
Correlation coefficient	0.999	0.998
LOD (µg/mL)	0.02	-------
LOQ (µg/mL)	0.06	-------
Accuracy (%)	99.90	------
Precision RSD (%)	0.0091	0.154
Inter-day RSD(%)	0.0670	0.685
Inter-day RSD(%)	0.1435%	0.1398

Table 2. Results of analysis of formulation and recovery studies

Drug	Amount mg/ tablet		%Recovery
	Labeled	Found	
Granisetron	1	1.08	108.0%

CONCLUSION :

In this present study an attempt has been made to develop HPLC method for the estimation of Granisetron in bulk and tablet dosage form. The results obtained were reproducible and reliable. The linearity, precision, accuracy of the methods were evident from the statistical and analytical parameters obtained. Therefore, it is concluded that the proposed HPLC method with Ondansetron as internal standard was found to be linear, precision, specific, accurate, rugged. Hence, this method can easily and conveniently adopt for routine quality control analysis of Granisetron in pure and its dosage form.

ACKNOWLEDGEMENT :

The authors are thankful to provide necessary laboratory facility carry out for a research work in the Government College of pharmacy and drug testing laboratory, Bangalore

REFERENCES

1. Amnon CS, Igor.Radiofrequency driven skin microchanneling as a new way for electrically assisted transdermal delivery of hydrophilic drugs. J controlled release.2003;(89):311-20.
2. Turvill,j.l, Connor p, farthing M.J.G. The inhibition of cholera toxin- induced 5-HT release by the 5-HT3recepto antagonist granisetron, in the rat. British J Pharmacol.2000; 130: 464–473.
3. Wada I,Satosh M,Takeda T,Nakabayashi T,Honma T,SaitohH,Takada M and Hirano k.A rapid assay of granisetron in biological fluids from cancer patients.1998;21 :535-537.
4. Zhanga,Yea L & Stewarta JT. HPLC Determinations of Doxorubicin with Selected

Medications in 0.9% Sodium Chloride Injection USP. J Liquid Chromatogr & Related Technologies.1998;(21):15

5. Quercia RA, Zhang J, Fan C, Chow MS.Stability of granisetron Hydrochloride in an extemporaneously prepared oral liquid. .Am J Health Syst Pharm.1997;(12):1404- 1406.
6. Chung KC, Chin A, Gill MA. Stability of granisetron hydrochloride in a disposable elastomeric infusion device. Am J Health Syst Pharm.1995;(14):1541-3.
7. Bloomer JC, Baldwin SJ, Smith GJ. Characterisation of the cytochrome P450 enzymes involved in the in vitro metabolism of Granisetron.Br J clin Pharmac.1994;(38):557-66.
8. Capacio BR, Byers CE, Jackson TK, Matthews RL. An HPLC Method for the determination of Granisetron in Guinea Pig Plasma. J anal toxicol. 1993;17:151-55

*****