ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Stability Indicating RP- High-Performance Liquid Chromatography Determination of Tegaserod Maleate in bulk and solid dosage formulations

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ABSTRACT

A simple, fast, precise and accurate rapid reverse phase high-performance liquid chromatography (HPLC) method was developed for the quantitative determination of tegaserod maleate in bulk & solid dosage formulations. The drug was chromatographed on (SUPLEX PKB-100) column. Eluents were monitored at a wavelength 277 nm using a mixture of acetonitrile and 0.05 M potassium phosphate buffer pH 3.0, consisting of 0.005 M 1-Octanesulphonic acid sodium salt, methanol in the ratio of 50:22:28. A linear response (r = 0.9999) was observed in the range of 25μg/ml-75μgmL^{-1}. The proposed method is sufficiently selective to distinguish the parent drug and the degradation products after hydrolysis, photolysis, or chemical oxidation and from excipients .The method showed good recoveries (average 99.45%) and the relative standard deviation ≤ 1.0%. The developed method can be used for routine quality control analysis of tegaserod maleate in solid dosage as well as bulk formulations.

Keywords: Tegaserod Maleate, bowel syndrome drugs, acetonitrile, HPLC

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ISSN: 0975-8585

INTRODUCTION

Tegaserod maleate (Fig.1) is chemically known as 2-[(5-Methoxy-1H-indole-3-yl) methylene]-N-pentylhydrazinecaroximidamide [1, 2] widely used in the treatment of irritable bowel syndrome. Literature survey reveals that the drug is not found official in any pharmacopoeia. So far very few liquid chromatography procedures have been described for the determination of tegaserod in microsomal incubation samples by using mass spectra as a detector (HPLC/MS).

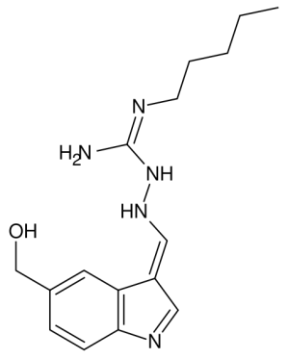


Figure1. Chemical structure of tegaserod
(M F of Tegaserod maleate: $C_{16}H_{23}N_5OC_4H_4O_4$)

EXPERIMENTAL

Chemicals and Reagents:

Pure material of tegaserod maleate, pharmaceutical-containing Tegaserod maleate were claimed to contain 6.0 mg of the drug. Acetonitrile, methanol was HPLC grade (Merck), potassium dihydrogen phosphate was AR grade, and 1-octane sulphonic acid sodium salt and Milli Q water filtered through 0.45u filter were used.

Apparatus and Chromatographic Conditions:

The HPLC apparatus consisted of E. Merck Hitachi system equipped with a model L-7100 pump, an automatic sample injection device L-7200, a variable wave length UV-Visible detector L-7400 controlled by interface module with HSM soft ware. A Supelco C18 column 25cm $\times$ 4.6mm ID5-μm particle size was selected. The flow rate of the mobile phase was 1 mL/min, and the HPLC system was operated at room temperature (25 ± 2^0C).

Preparation of Mobile Phase:

A mobile phase was composed by, potassium phosphate 0.5 M (containing 0.005 M 1-octane sulphonic acid sodium salt), Methanol and Acetonitrile in the ratio 50:22:28. The pH of

the potassium dihydrogen phosphate was adjusted to pH 3.0 using 20% orthophosphoric acid. The mobile phase was degassed prior to use.

Preparation of Standard Solution:

The stock solution of Tegaserod maleate reference standard (125μg/ml) was prepared in mobile phase because the drug is freely soluble in the mobile phase. The working standard solution (25μg/ml to 75μg/mL) was obtained by diluting the stock solution in the mobile phase. The standard stock solution for the recovery and assay (500μg/mL) was prepared using mobile phase. The working standard solution for assay level (50μg/mL) was obtained by diluting the stock solution using mobile phase (Table-2).

Preparation of Sample Solution:

The commercially available 20 tablets of tegaserod maleate (claim 6mg/tab as tegaserod) are weighed accurately and finely powdered. The powder equivalent to 50 mg of tegaserod maleate in a 100 mL volumetric flask mixed with 90 mL of mobile and kept in a sonicator for 10 minutes, cooled to room temperature diluted up to the mark with mobile phase and mixed well, finally filtered through 0.2μ membrane filter. Take 1 mL of filtrate in 10 volumetric flasks dilute with mobile phase and mixed well to get the concentration range of 25-75μg/mL which is 50-150% (v/v).

Robustness of the method:

The robustness was evaluated by variation in the mobile phase, including pH values and composition. The stability of reference substance stock solution was also determined. While developing robustness of the method was checked by varying proportions of the mobile phase constituents, flow rate and pH and found that the results were not adversely affected by these changes.

Linearity of Detector Response:

The experiment was carried out in triplicate to ascertain accuracy and precision of the method. A graph of concentration of Tegaserod maleate v/s absorbance was plotted and co-efficient of variation for tegaserod maleate was found to be 0.999. The regression analysis of the calibration data carried out to determine the relationship between the absorbance and concentration [3, 4]. The result showed a linear relationship between concentration and the detector response.

Linearity of the method was verified by 2-6 mL of standard Tegaserod maleate standard stock solution taken in a series of 10mL calibrated volumetric flask made up to the mark with mobile phase so as to get a concentration of 25-75μg/mL which is 50-150 % (v/v) of expected assay range.

ISSN: 0975-8585

Limit of detection and Limit of Quantitation:

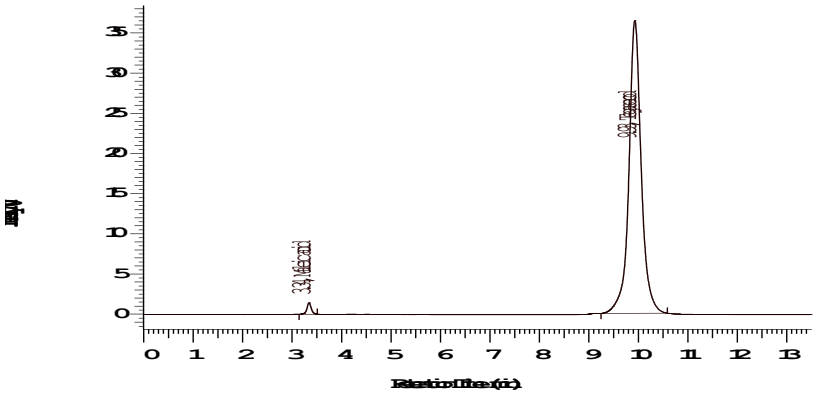
For the proposed method limit of detection and limit of quantitation for Tegaserod maleate was found to be 5-25µg/mL. The signal to noise ratio obtained for above parameters are well within the criteria of ICH guidelines indicating good quantitation capability of the method**.**

Assay:

20µL of standard and sample solutions are injected into liquid chromatograph. From the peak area of Tegaserod maleate the amount of drug in sample is calculated. The values are in Table 1.

Table 1: Assay

Experiment	Bulk drug % of assay	Fomulation (Tablets) Claim (mg)	Found (mg)	% of Assay
I	99.87	6.0	5.94	99.0
II	99.98	6.0	5.93	98.83
III	99.69	6.0	5.95	99.17
Average	99.85		5.94	99.0
SD	0.146		0.01	0.17
% of RSD	0.15		0.17	0.17



Chromatogram showing the peak of Tegaserod as standard at RT 10.0 mins
Figure.2 Chromatogram of standard (Tegaserod maleate)

Recovery:

To study the accuracy, reproducibility, and precision of the method recovery experiments were carried out in triplicate. Different known concentrations of standard at 3 different levels were added to the powdered sample and the mean recovery was found to be more than 99.45%. The values are in Table 2.

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Table 2: Recovery

Level	Amount of powdered sample taken (gms)	Amount of Standard stock solution (500µg/ml) added (in ml)	Total drug recovered (mg)	Recovery (%) (as Tegaserod)
1(110%)	1.4375	10	6.55 (109.17%)	109.17/110X100 = 99.25
11(120%)	1.4505	20	7.19(119.83%)	119.83/120X100 = 99.86
111(130%)	1.4495	30	7.74(129.0%) Mean recovery	129.0/130X100 = 99.45 99.45 %

Stability in solution

The stability of tegaserod maleate in solution containing mobile phase have been determined by keeping one sample in refrigerator and other in a tightly capped volumetric flask placed at ambient temperature under normal lighting conditions. The samples were checked for assay on two successive days of storage and compared with freshly prepared sample. The RSD values of experiments were found to be below 2.0% in both cases. This indicated that the Tegaserod maleate is stable in the solution.

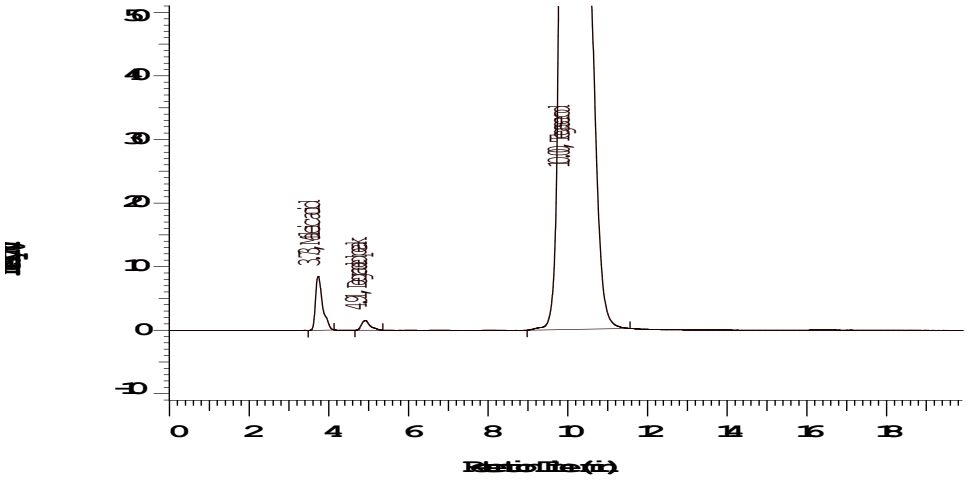
Stability study:

To check that the stability of the developed method the drug was subjected to stressed conditions like treatment with 0.5M HCl, 0.5M NaOH, 3.0% Hydrogen peroxide, at ambient temperature for one hour. The solutions prepared after subjecting to the stressed conditions [5] were analyzed by the above developed chromatographic condition but using Photo Diode Array Detector and it was observed that the drug and degradation products were well resolved.

Validation summary

System Suitability Test (SST)	Response
Theoretical plates (N) Tailing factor	9100 1.01
Linearity range Coefficient of variation(r 2)	25 to 75 µg/ml 0.9999
Accuracy Mean percentage recovery	 99.45
Limit of quantitation(LOQ) (µg/ml)	25µg/ml
Limit of detection (LOD) (µg/ml)	5µg/ml

ISSN: 0975-8585



Chromatogram showing the peaks of Tegaserod in NaOH treatment.
RT 4.91 shows degraded peak and RT 10.0 min shows peak of Tegaserod.

RESULTS AND DISCUSSION

In developed method a new generation SUPLEX PKB-100 C18, 25 cm x 4.6mm ID 5µm (Supelco) HPLC column was used. The mobile phase was a mixture of 0.05 M potassium phosphate buffer: Methanol: acetonitrile (50:22:28). System suitability study showed that the method is precise and accurate. The linearity study showed that linear regression was found to be 0.9999. Performing recovery studies has showed the reliability and reproducibility of the proposed method, and mean recovery was found to be 99.45% for Tegaserod**.** Good recoveries and low values of RSD and coefficient of variation indicated that the proposed method is precise and accurate. Further the drug was subjected to various forced degradation procedures. Analytical results show that no interferences from degradation products of the drug were observed indicating the method is specific to monitor the degradation studies of the drug.

CONCLUSION

Precise, accurate method was developed and validated for the determination of Tegaserod maleate in pharmaceutical dosage forms**.** The developed method was checked for selectivity and specificity by carrying out forced degradation studies. Analytical data of the stability studies indicates that the developed method is selective and specific for monitoring degradation studies as well as estimation of in Tegaserod maleate in pharmaceutical formulations**.**

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