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Research Article

A STABILITY INDICATING HPLC METHOD FOR THE DETERMINATION OF SIBUTRAMINE HYDROCHLORIDE IN BULK AND COMMERCIAL FORMULATIONS

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ABSTRACT

A simple stability-indicating high performance liquid chromatographic method for the assay of sibutramine hydrochloride in the presence of its degradation products was developed using reverse phase Symmetry C-18, 150mm X 4.6mm, 5µm column, in the mobile phase phosphate buffer (pH 5.5) and acetonitrile (30:70, v/v) at flow rate 1mL/min with UV detection at 225nm. The retention time was found to be 2.618min. Validation of an analytical method was established by laboratory studies. Selectivity was validated by subjecting the stock solution of sibutramine to acidic, alkaline, oxidative and thermal degradation. The proposed method was found to be linear at concentration of 20 to 60µg/mL (R^2=0.999). The limit of detection and limit of quantification was 0.04µg/mL and 0.12µg/mL respectively and the method was found to be specific. Method precision and precision of the system was found to be within the limits of the acceptance criteria. Relative standard deviation for precision of the method and precision of the system was found to be 1.4% and 0.4% respectively. The percentage recovery ranges from 99-101%. The results indicate that there is no interference from excipients for the proposed method, thus making the method simpler, less time consuming and suitable for routine quantitative estimation of sibutramine hydrochloride capsule formulation. As the method could effectively separate the drug from its degradation products, it can be employed as a stability-indicating one.

Keywords: Sibutramine, Stability-indicating HPLC, Stress degradation, Formulations.

INTRODUCTION

The stability-indicating method is defined as validated quantitative analytical method that can detect the change with time in the chemical, physical or microbiological properties of the drug substance and the drug product, that are specific so that the content of active ingredient, degradation can be accurately measured without interference[1]. Stability testing provides information about degradation mechanisms, potential degradation products, possible degradation pathways of the drug as well as interaction between the drug and the excipients in drug product[2]. Sibutramine hydrochloride monohydrate is an orally administered agent for the treatment of obesity. Sibutramine is a centrally acting serotonin reuptake inhibitor[3]. Chemically, it is a racemic mixture of the (+) and (-) enantiomers of cyclobutanemethan amine, 1-(4-chlorophenyl)-N,N-dimethyl-α-(2-methylpropyl)-,hydrochloride, monohydrate[4] (Fig. 1). Sibutramine produces its therapeutic effects by norepinephrine, serotonin and dopamine reuptake inhibition. Sibutramine and its major pharmacologically active metabolites

(M_1 and M_2) do not act via release of monoamines[5].

Literature survey revealed that a few analytical methods have been reported for the estimation of sibutramine in pharmaceutical dosage forms and in biological fluids by spectrophotometry[6-8], HPLC[9-13], LC-MS[14-21], GC-MS[22], Chemiluminescence[23] and X-ray analysis[24] techniques. The aim of the present study was to establish a simple, reliable, sensitive stability-indicating HPLC method with UV detection for the determination of sibutramine in both bulk drug and in capsule dosage form.

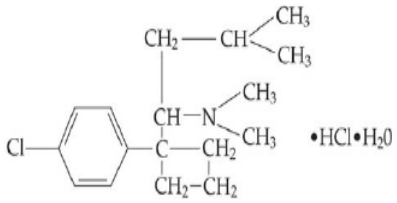

Fig. 1: Chemical structure of sibutramine hydrochloride monohydrate

EXPERIMENTAL

Chemicals and reagents

Pure sample of sibutramine hydrochloride monohydrate was generously supplied by Nosch Labs Pvt Ltd, Hyderabad, India. Sibutramine hydrochloride capsules was purchased from local market. HPLC grade water obtained from Milli-Q water purification system was used throughout the study. HPLC grade acetonitrile and potassium dihydrogen orthophosphate was purchased from Merck Specialities Pvt Ltd, Mumbai, India.

Instrumentation

Waters HPLC system equipped with a 2695 binary pump and a 2487 dual absorbance detector having Waters Empower2 software and Rheodyne injector with 20µl fixed loop. All samples were filtered through 0.45µm membrane filter. Mobile phase and sample/standard preparations were degassed using a sonicator.

Chromatographic conditions

Reverse phase high performance liquid chromatographic method was developed on a Symmetry C-18, 150mm X 4.6mm, 5µm column, using mobile phase containing phosphate buffer (pH 5.5 adjusted with Orthophosphoric acid) and acetonitrile (30:70, v/v) at ambient temperature. The elution was carried out isocratically at flow rate of 1mL/min. The UV detector was set at 225nm.

Preparation of standard solution

Accurately weigh and transfer 10mg of sibutramine working standard into a 10mL volumetric flask, add about 7mL of mobile phase as diluent, sonicate to dissolve it completely and make volume upto the mark with the same solvent. Further pipette 1mL of the above stock solution into a 10mL volumetric flask and dilute upto the mark with diluent. Mix well and filter through 0.45µm filter. Further pipette 4mL of the above stock solution into a 10mL volumetric flask and dilute upto the mark with diluent. Mix well and filter through 0.45µm filter.

Preparation of sample solution

Accurately transfer the contents of 20 capsules of sibutramine, ground into a fine powder and calculate the average weight. Weigh and transfer the sample equivalent to 10mg of sample into a 10mL volumetric flask, add about 7mL of mobile phase as diluent, sonicate to dissolve it completely and make volume upto the mark with the same solvent. Further pipette 1mL of the above stock solution into a 10mL volumetric flask and dilute upto the mark with diluent. Mix well and filter through 0.45µm filter. Further pipette 4mL of the above stock solution into a 10mL volumetric flask and dilute upto the mark with diluent. Mix well and filter through 0.45µm filter. All experiments were conducted in triplicate.

Degradation studies

Standard sibutramine hydrochloride at a concentration of 40mg/mL was used in all the degradation studies. The standard sibutramine were subjected to stress conditions in 0.1M HCl and 0.1M NaOH, at the temperature 60°C at different time intervals, after completion of the degradation processes, the solutions were neutralized and diluted with mobile phase. For the peroxide degradation studies, standard sibutramine of 40mg/mL was dissolved in 3% hydrogen peroxide and subjected to stress condition at the temperature 60°C at different time intervals. Thermal degradation was performed by exposing solid standard drug to dry heat at 105°C for 48hrs. These degraded solutions were withdrawn periodically and subjected to analysis after suitable dilution with mobile phase to get 40µg/mL. 20µL of this

degraded solution were injected using the same chromatographic conditions.

Method validation

The analytical method validation was carried out as per ICH method validation guidelines. The validation parameters addressed were accuracy, precision (intra-day and inter-day), specificity, linearity, limit of detection, limit of quantitation, robustness and stability of sibutramine in mobile phase. Standard plots were constructed for sibutramine in the range of 20 to 60µg/mL. Accuracy was determined by fortifying the mixture of pre-analysed standard of three unknown concentrations of the drug with the marketed samples.

RESULTS AND DISCUSSION

Development and optimization of the stability-indicating HPLC method

An isocratic method validation was found necessary to optimize the separation of major degradation products formed under various stress conditions. The best resolution was achieved with initial run of phosphate buffer (pH 5.5) and acetonitrile in the ration of 60:40, v/v at a flow rate of 1mL/min, the retention time was observed at 3.20min. The mobile phase ratio was changed to 30:70, v/v at the same flow rate, a retention time 2.618min was obtained. The method worked well with the mixture of degradation solutions and was even applicable to capsule formulation. The typical chromatogram of sibutramine standard was shown in Fig. 2.

Degradation behaviour

HPLC studies on the combination under different stress conditions indicated the following degradation behavior (Table 1).

Acidic degradation

The standard drug sibutramine at 60°C in 0.1M HCl was found to be degrade 5.99% at 1 hour, the degradation product was appeared at retention 2.640min was identified (Fig. 3).

Alkaline degradation

The sibutramine drug was found to be degrade in alkaline hydrolysis of 0.1M NaOH at 60°C and degraded to an extent of 9.99% at 1hour, the degradation product was appeared at retention 2.644min was identified (Fig. 4).

Oxidative degradation

The drug sibutramine was found to be labile to hydrogen peroxide at 60°C and 4.16% of the drug was decomposed at 1hour, the degradation product was appeared at retention 2.750min was identified (Fig. 5).

Thermal degradation

In the thermal degradation, more degradation was seen on subjecting the drug to dry heat 105°C for 48hrs and 11.34% of the drug was decomposed, the degradation product was appeared at retention 2.642min was identified (Fig. 6).

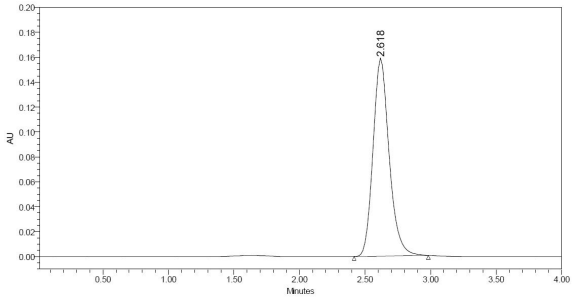

Fig. 2: Typical chromatogram of sibutramine hydrochloride monohydrate

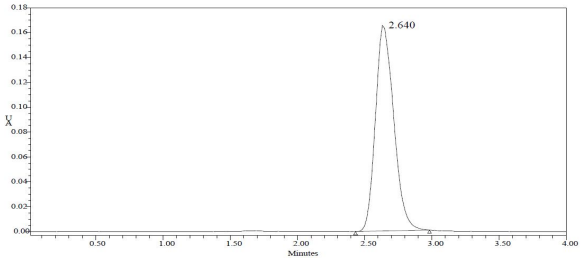

Fig. 3: Typical HPLC chromatogram recorded during acidic degradation

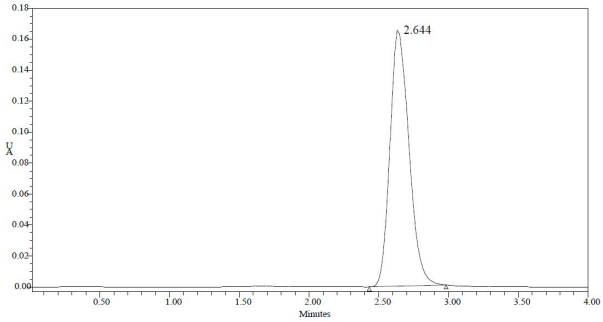

Fig. 4: Typical HPLC chromatogram recorded during alkaline degradation

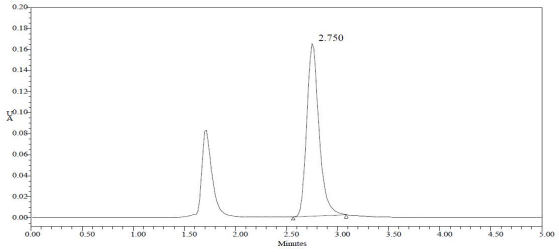

Fig. 5: Typical HPLC chromatogram recorded during oxidative degradation

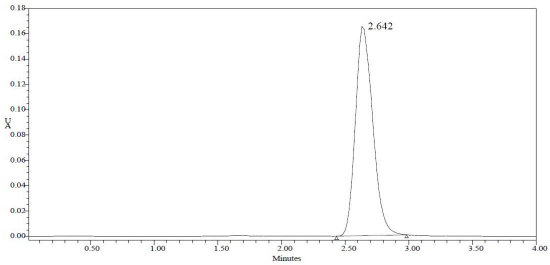

Fig. 6: Typical HPLC chromatogram recorded during thermal degradation

Validation of method

System suitability

A system suitability test was performed to evaluate the chromatographic parameters (number of theoretical plates, tailing of the peak) before the validation runs. The results of system suitability parameters were given in Table 2.

Linearity

Five-point calibration curves were obtained in a concentration range from 20 to 60µg/mL for sibutramine, three independent determinations were performed at each concentration. The response for the drug was linear and the calibration equation was y=34971.68x+5670.6 with R^2 = 0.999.

Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection and limit of quantitation for sibutramine was found to be 0.04µg/mL and 0.12µg/mL respectively.

Accuracy

The percentage recovery ranges from 99-101%. The results show that there is no interference from excipients for the proposed method, thus making the method simple, less time consuming and suitable for routine quantitative estimation of sibutramine capsule formulation. The recovery study data is given in Table 3.

Precision

Method precision and precision of the system was found to be within the limits of acceptance criteria. For the analytical method and system precision the relative standard deviation was found to be 1.4% and 0.4% respectively. The data for intra- and inter- day precision is given in Table 4.

Ruggedness

The ruggedness was established by determining sibutramine using the same chromatographic system and the same column by two analysts on a different day. The assay result indicated that the method was capable with high precision. Additionally, good separations were always achieved which suggested that the method was selective for all components under the test.

Solution stability

The stability of solution under study was established by keeping the solution at room temperature for 24hrs. The result showed no significant change in concentration and thus confirms the stability of the drug in the solvent used for the analysis.

Analysis of marketed products

The validated method was applied for the analysis of sibutramine hydrochloride capsules of strength 10mg from two different manufacturers. In both cases assay obtained is more than 99% and no interference of impurity peak observed in sibutramine peak. The results of analysis are given in Table 5.

CONCLUSION

This study presents a simple and validated stability-indicating HPLC method for estimation of sibutramine in the presence of degradation products. The developed method is specific, accurate, precise and robust. All the degradation products formed during forced decomposition studies were well separated from the analyte peak demonstrating that the developed method was specific and stability-indicating**.** The method could be applied with success even to the analysis of marketed products sibutramine capsule formulation, as no interference was observed due to excipients or other components present.

Table 1: Degradation studies of sibutramine

Stress conditions	Degradation time	Area of peak	% Degradation	% of active drug present after degradation
Standard Drug	-	1424568	-	-
Acidic	1 hour	1339142	5.99	94
Alkaline	1 hour	1282142	9.99	90
Oxidative	1 hour	1365278	4.16	95.8
Thermal	48 hours	1262894	11.34	88.7

Table 2: Summary of validation parameters

System suitability	Results
Theoretical plates (N)	2191
Linearity range (µg/mL)	20-60
Retention time (min)	2.618
Tailing factor	1.2
Correlation coefficient	0.999
LOD (µg/mL)	0.04
LOQ (µg/mL)	0.12

Table 3: Recovery study data of sibutramine

Concentration (at specification level)	Concentration of sibutramine (µg/mL)	Peak area	Amount added (mg)	Amount found (mg)	% Recovery	% Mean recovery
80%	32	1114284	8.2	8.16	99.6	99.9
100%	40	1419773	10.3	10.40	101	
120%	48	1649248	12.2	12.08	99	

Table 4: Intra- and inter-day precision of sibutramine

Concentration (µg/ml)	Intra-day precision		Inter-day precision	
	SD	%RSD	SD	%RSD
40	19127	1.4	5347	0.4

Table 5: Assay result of capsule formulation using proposed method

Formulation	Labelled strength (mg)	Amount found (mg)	%Assay	%RSD
Formulation-1	10	10.07	100.7	0.314
Formulation-2	10	9.98	99.8	0.425

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