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Stress degradation studies on Sibutramine HCl and development of a validated stability-Indicating HPLC assay for bulk drug and Pharmaceutical dosage form

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ABSTRACT

A stability indicating HPLC assay method was developed for the quantitative determination of Sibutramine HCl in bulk and pharmaceutical dosage form. It involved a 250 mm x 4.6 mm i.d 5 µm Phenomenex C-18 column. The mobile phase consisted of phosphate buffer with pH adjusted to 5 with ortho-phosphoric acid and acetonitrile in the ratio of (60:40, v/v) and was pumped at a constant flow rate of 1 mL/min. Measurements were made at a wavelength of 222 nm. The retention time of Sibutramine HCl was found to be 4.52 min. The calibration curve was linear over the range of 2.5-12.5 µg/mL (correlation coefficient (r^2) = 0.9990). The limit of detection (S/N = 3) was 12.12 ng/mL and the limit of quantitation (S/N = 10) was found to be 36.46 ng/mL. Sibutramine was subjected to different stress conditions prescribed as per ICH guidelines such as acid hydrolysis, base hydrolysis, oxidation with hydrogen peroxide and photolysis. Degradation was not observed in all the tests performed. The evaluation of stress samples was calculated against a reference standard. The method developed was validated with respect to linearity, accuracy and precision.

Keywords:liquid chromatography; method validation; Stability indicating assay; Sibutramine

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INTRODUCTION

Stress studies of the drugs as per International conference on Harmonization (ICH) Guidelines must be carried out to indicate its stability characteristics, to identify the degraded products and to check the suitability of the analytical procedure involved. The analytical test procedures should be stability indicating and it should be fully validated. Hence, the objective of this work was to study the stress degraded products and to determine the intrinsic stability of the Sibutramine HCl. Sibutramine is chemically known as 1-1(4-chlorophenyl)-N, N-dimethyl–α (2-methylpropyl) hydrochloride monohydrate[1]. (Figure 1).

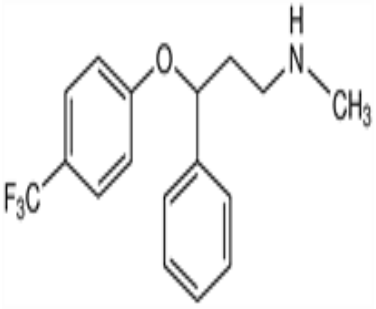


Figure 1: Structure of Sibutramine HCl

It is used in the management of obesity by increasing the amount of the two neurotransmitters namely serotonin and nor-epinephrine[2]. It inhibits the re-uptake of these neurotransmitters by the nerve cells and alters the balance of neurotransmitters within the nerve cells. Unlike other diet drugs (fenfluramine, dexfenfluramine) which increase the release of serotonin and nor-epinephrine from the cells, the action of sibutramine is similar to anti-depressants which inhibit the reuptake of serotonin [3-4]. UV-Visible spectroscopy [5] and few chromatographic methods have been reported for the estimation of Sibutramine HCl in pharmaceutical formulation [6-12]. Stability-indicating assay method for sibutramine HCl was not reported so far and hence the present study was undertaken to develop a stability indicating HPLC method for Sibutramine HCl in bulk drug and pharmaceutical dosage forms.

MATERIALS AND METHODS

Chemicals

Samples and standards of Sibutramine HCl were supplied by M/s Abbott Ltd, Mumbai, India. Commercially available 10 mg Sibutramine HCl capsules were purchased. The HPLC grade acetonitrile, analytical reagent grade sodium hydroxide, sodium bisulphate and hydrochloric acid, hydrogen peroxide was purchased from Merck, Germany. HPLC grade water was prepared by using Millipore MilliQ plus water purification system.

Equipment

Shimadzu HPLC with LC-20AT prominence liquid chromatogram, Rheodyne 7725i with 20 µL loop injector, SPD-M20A Prominence-diode array detector and Sonica ultrasonic cleaner sonicator was used. The output signal was monitored and processed using lab solution software on a HCL computer. Water baths equipped with MV controller were used for the hydrolytic studies. Stability studies were carried out in a humidity chamber and photo stability studies were carried out in a photo stability chamber. Thermal stability studies were performed in a dry air oven.

Chromatographic Conditions

The phenomenex C-18, 250 x 4.6 mm i.d. with 5 µm particles chromatographic column used was a stationary phase. Phosphate buffer, pH adjusted to 5.0 using orthophosphoric acid and acetonitrile, in the ratio of 60: 40 (v/v) was as mobile phase A and B respectively. The flow rate of 1.0 mL/min was used. The column temperature was maintained at 25ºC ± 2ºC and the detection was monitored at a wavelength of 222 nm.

Preparation of Stock Solutions

A stock solution of sibutramine HCl standard and sample (5 µg/mL) was prepared by dissolving an appropriate amount in acetonitrile: buffer (40:60 v/v).

Preparation of Sample Solution

Twenty capsules were weighed and the contents were transferred into a clean and dry mortar and ground well. Then 20 mg equivalent of the drug was transferred to 100 mL volumetric flask, 70 mL of mobile phase was added and kept on a rotating shaker for 10 minutes to disperse the material completely and sonicated for 10 minutes and diluted to get the final concentration was 5 µg/mL.

Stress Studies

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities Stress testing of the drug substance can help to identify the likely degradation products, which can in turn help establish the degradation pathway and the intrinsic stability of the molecule [13]. All stress decomposition studies were performed at an initial drug concentration of 10 µg/mL.

LIQUID STATE DEGRADATION STUDY

Acid hydrolysis

Twenty five mg of the sample was transferred to a round-bottomed flask, and then 10 mL of 1M HCl was added to the above, and refluxed for 2 h in a boiling water bath. At the end of the

exposure, the solution was cooled and neutralized with 1 M NaOH and transferred into a 250 mL volumetric flask and the volume was made up with mobile phase.

Base hydrolysis

Twenty five mg of the sample was transferred to a round bottomed flask, and then 10 mL of 1M NaOH was added to the above, and refluxed for 2 h in a boiling water bath. At the end of the exposure the solution was cooled and neutralized with 1.0 M HCl and transferred into a 250 mL volumetric flask and the volume was made up with mobile phase.

Oxidation

Twenty five mg of the sample was transferred to a round-bottomed flask for an exposure of 2 h. Five to 10 mL of 10% hydrogen peroxide was added to the above, and refluxed for 2 h in a boiling water bath. At the end of the exposure, the solution was cooled and transferred into a 250 mL round bottomed flask and the volume was made up with mobile phase.

Reduction

Twenty five mg of the sample was transferred to a round-bottomed flask for an exposure of 2 h. Five to 10 mL of 10% aqueous sodium bisulphate was added to the above, and refluxed for 2 h in a boiling water bath. At the end of the exposure the solution was cooled and transferred into a 250 mL round-bottomed flask and the volume was made up with mobile phase.

Hydrolysis (Neutral solution study)

Twenty five mg of the sample was transferred to a round-bottomed flask and 5 to 10 mL of distilled water was added and the contents were refluxed for 2 h in a boiling water bath. After 2 h the solution was cooled and transferred into a 250 mL volumetric flask and the volume was made up with distilled water.

SOLID STATE DEGRADATION STUDY

Dry heat degradation studies

Two hundredmg of drug and the formulation were heated separately in an oven at 80˚C and analyzed after a time interval of 24 and 48 h respectively.

Photolysis:

Photo degradation studies were carried according to option 2 of QIB in ICH guidelines [14]. Samples were illuminated at 1.2 million lux h and integrated near the ultraviolet energy of not less than 200 watt/hm^2. Samples were withdrawn at appropriate time and subjected to HPLC analysis after suitable dilution (10 µg/mL)

METHOD VALIDATION

Precision

The precision of the assayed method was evaluated by carrying out six independent assays of Sibutramine HCl at three different concentrations viz. 5 µg/mL, 10 µg/mL and 15 µg/mL. Percentage RSD was calculated for all the samples and standard.

Linearity

Linearity test solutionswere prepared from the stock solution in 5 concentration levels from 2.5,5, 7.5, 10 and 12.5 µg/mL. The slope, % intercept and correlation coefficient were calculated.

Accuracy

The accuracy of the method was evaluated in triplicate in different concentration levels i.e. 8,10 and 12 µg/mL in bulk sample and the percentage of recoveries were calculated.

Specificity and Selectivity

The specificity and selectivity of the method was established through the study of peak purity and purity profile of all other peaks.

Solution Stability

The solution stability of Sibutramine HCl was carried by leaving the test solution in a tightly capped volumetric flask at room temperature for 48 h. The same sample solution was assayed for 24 h interval up to the study period against freshly prepared solution of Sibutramine HCl. % RSD of assay of Sibutramine HCl was calculated for subject period during solution stability.

RESULTS AND DISCUSSION

Method development and optimization

A stability indicating HPLC method was developed as per ICH guidelines[13-14]for the determination of Sibutramine HCl and its Pharmaceutical dosage form as there are no reports about the stability indicating method for Sibutramine HCl. The chromatographic conditions like detection of wavelength, pH and the composition of mobile phase, nature of stationary phase, peak modifiers or additives, flow rate, etc., were optimized for the best possible separation of the drug present in the dosage form.

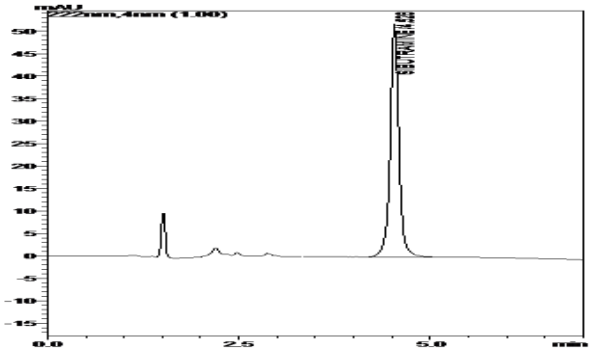


Figure. 2. Chromatogram of Sibutramine HCl in bulk form

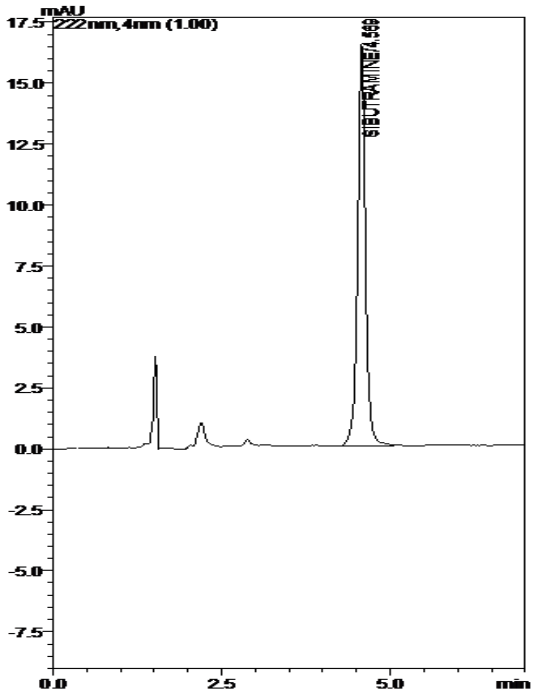


Figure. 3. Chromatogram of Sibutramine HCl in formulation

METHOD VALIDATION.

The proposed method was validated for system suitability, linearity, LOD, LOQ, inter- and intraday accuracies and precisions, robustness, ruggedness, selectivity, and recovery of sibutramine HCl by RP-HPLC according to the ICH guidelines.

System Suitability

System-suitability tests were performed where retention time (Rt), tailing factor (*T*), theoretical plates (*N*), HETP, LOD, LOQ and capacity factor (*k*) were evaluated for five replicate injections of Sibutramine HCl. The results presented in Table 1 are within the acceptable limits.

Table 1. System suitability parameters

Parameters.	Sibutramine HCl
Retention time (min)	4.52
Number of theoretical plates	7028
HETP	21.34
Tailing factor	1.07
Capacity factor	2.008
LOD (ng/mL)	12.12
LOQ (ng/mL)	36.46

Linearity

Linear calibration plot for this method was obtained over the calibration ranges tested i.e. 2.5-12.5 µg/mL and the correlation coefficient obtained was greater than 0.9990 (Figure 4). The results showed that an excellent correlation existed between the peak area and concentration of

the analyte. The slope and y-intercept of the calibration curve were 30763 and 33012 respectively.

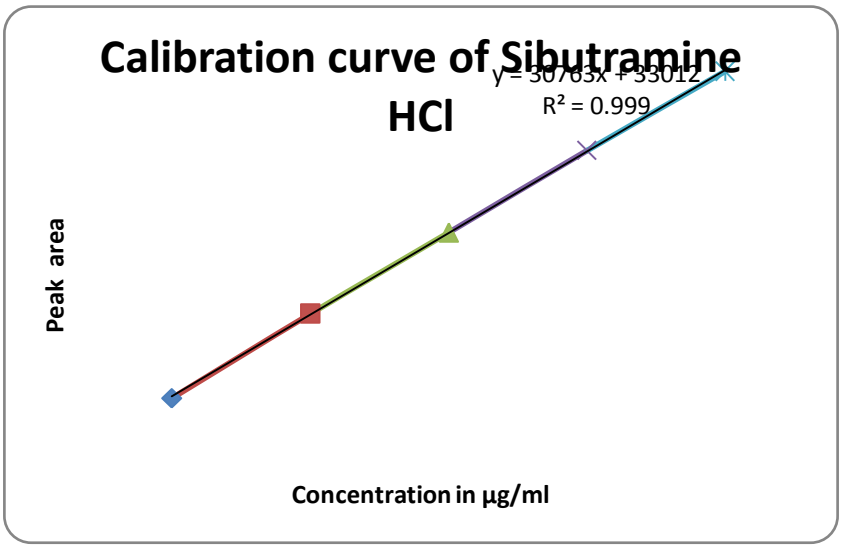


Figure 4. Calibration curve of Sibutramine HCl

Accuracy and precision.

The accuracy of the assay method was evaluated (n=5) at three concentration level. The % RSD value (less than 2) and the assay results of the pharmaceutical dosage forms (n=5) showed 97.80, 97.93 and 98.07% recovery of Sibutramine HCl, which clearly implicates that there is no interference due to the residual solvents, impurities, mobile phase and diluents in the estimation of Sibutramine HCl in bulk form and in formulation (Figure. 2 & 3) by the proposed validated RP-HPLC method.

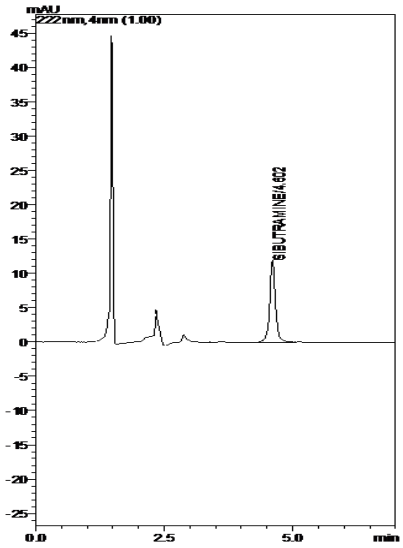
The percentage RSD values for the precision study were 0.019% (system precision) and 0.0024% (method precision), confirming good precision of the method. All the above facts confirm that the developed validated HPLC method is specific and selective for the estimation of Sibutramine HCl in bulk form and in formulation.

Solution Stability and Mobile Phase Stability.

Stability of sample solution was established by reanalyzing them after 24 h and 48 h time intervals. The results from the solution stability experiments confirmed that the standard solutions of sibutramine and its formulation solutions in the mobile phase were stable at ambient temperature up to 48 h during the assay. No significant change in the chromatographic parameters was observed during the solution stability study where, the RSD (%) values for the sibutramine assay during solution stability experiments were within 1%.

Forced degradation studies

Forced degradation studies performed to identify the likely degraded products and its interference in the analysis of Sibutramine HCl. (Figure.5a-d) The standard and sample solutions of Sibutramine HCl were subjected to forced degradation with acid and base under room temperature and at elevated temperature, dry heat and oxidative procedures under room temperature and at elevated temperature as per ICH guidelines. There is no significant degradation observed in acid, alkali, oxidative and dry heat conditions and the results are shown in Table 2-7.



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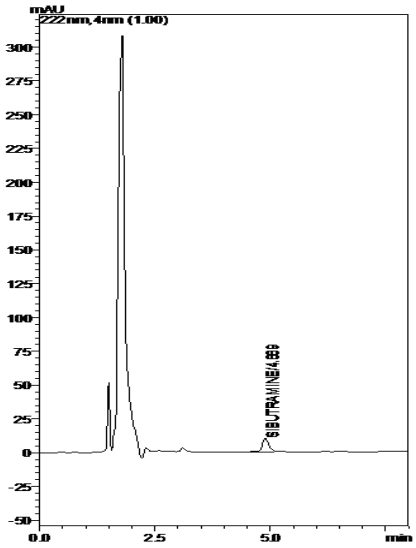

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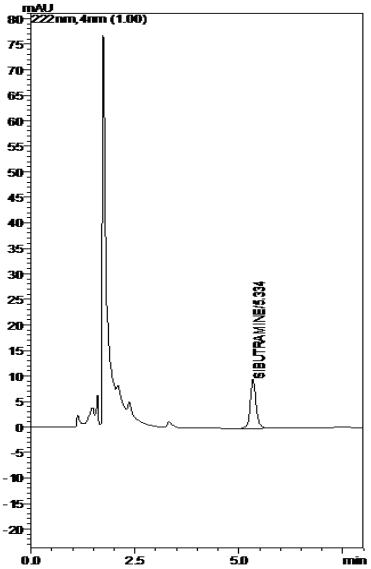

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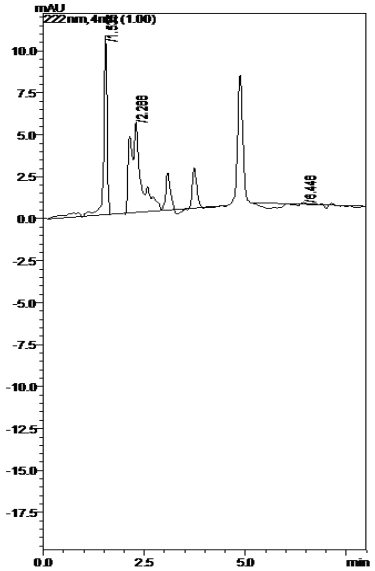

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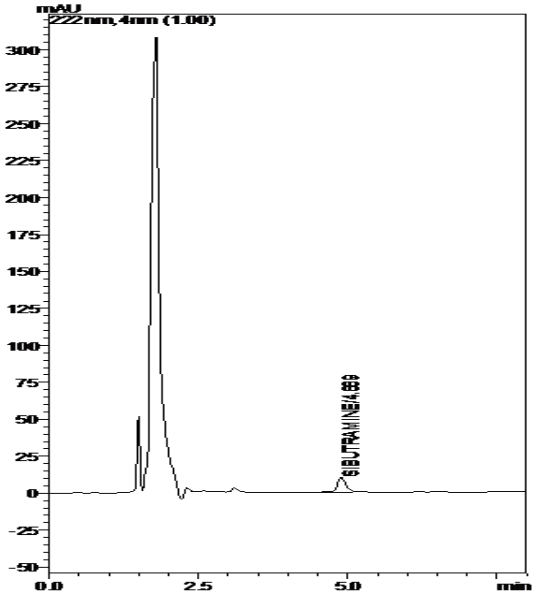

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Figure 5:(a) Acid degradation, (b) base degradation, (c) oxidative degradation, (d) dry heat degradation and (e) photolytic degradation.

Table 2: Forced Degradation data of Sibutramine HCl in Bulk form at Room Temperature

Sibutramine HCl (10 µg/ml)	Retention Time	Peak Area	Average	% Recovery
Standard Solution	4.528	334478	334435	97.93
	4.532	334392		
Base Hydrolysis	4.818	339498	339512	99.41
	4.826	339526		
Acid Hydrolysis	4.584	339812	339818	99.50
	4.589	339824		
Oxidation	5.331	320802	320806	93.94
	5.334	320810		

Table 3: Forced Degradation data of Sibutramine HCl in formulation at Room Temperature

Sibutramine HCl(10 µg/ml)	Retention Time	Peak Area	Average	% Recovery
Sample Solution	4.588	332200	332204	97.27
	4.591	332208		
Base Hydrolysis	4.888	330992	330999	96.92
	4.893	331008		
Acid Hydrolysis	4.802	336819	336812	98.62
	4.792	336805		
Oxidation	5.334	320609	320612	93.88
	5.339	320615		

Table 4: Forced Degradation data of Sibutramine HCl in Bulk form at Elevated Temperature

Sibutramine HCl(10 µg/ml)	Retention Time	Peak Area	Average	% Recovery

Standard Solution	4.548	340016	340012	99.56
	4.542	340008		
Base Hydrolysis	4.788	334602	334610	97.98
	4.794	334618		
Acid Hydrolysis	5.352	328126	328123	96.08
	5.349	328120		
Oxidation	4.349	320598	320602	93.88
	4.351	320606		

Table 5: Forced Degradation data of Sibutramine HCl in Formulation at Elevated Temperature

Sibutramine HCl(10 µg/ml)	Retention Time	Peak Area	Average	% Recovery
Sample Solution	5.337	339978	339981	99.55
	5.342	339984		
Base Hydrolysis	4.804	330498	330502	96.77
	4.809	330506		
Acid Hydrolysis	5.492	327995	328000	96.04
	5.496	328005		
Oxidation	4.787	319978	319982	93.69
	4.788	319984		

Table 6: Forced Degradation data of Sibutramine in Bulk form and Formulation under Dry Heat

Sibutramine HCl(10 µg/ml)	Retention Time	Peak Area	Average	% Recovery
Bulk form Dry Heat	4.788	336180	336183	98.44
	4.791	336186		
Formulation Dry Heat	4.886	339880	339882	99.52
	4.891	339884		

Table 7: Photolysis Degradation of Sibutramine HCl in Bulk form and its Formulation

Sibutramine HCl(10 µg/ml)	RetentionTime	Peak Area	Average Peak Area	% Recovery
Bulk form	4.548	339765	331490	97.33
	4.543	323215		
Formulation	4.172	336464	331441	97.05
	4.168	336454		

CONCLUSION

The developed RP-HPLC method proved to be simple, linear, precise, accurate and specific. The method was completely validated showing satisfactory data for all the method validation parameters that were tested. The developed method is stability-indicating and can be used for the routine analysis of the drug.

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