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Research Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING UV SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF SIBUTRAMINE HYDROCHLORIDE MONOHYDRATE IN BULK AND CAPSULE DOSAGE FORM

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ABSTRACT

A simple, sensitive, reproducible and cost effective stability indicating UV Spectrophotometric method has been developed for quantitative determination of Sibutramine Hydrochloride Monohydrate in bulk and pharmaceutical formulations. The UV spectrum was scanned between 200 to 400 nm and 223 nm was selected as maximum wavelength for absorption. Beer's law was obeyed in the concentration range of 5-25 µg/ml. Good accuracy (99.78-100.10%), precision (%RSD 0.1766-0.3679) were found, the method was successfully applied to the pharmaceutical dosage form containing the above-mentioned drug without any interference by the excipients. The limit of detection and limit of quantification was found to be 0.13 µg/ml & 0.42 µg/ml respectively. Results of the analysis were validated as per ICH guidelines. Forced degradation studies includes the effect of temperature, oxidation, photolysis and susceptibility to hydrolysis across a wide range of pH values, were carried out according to the ICH requirements which can be used for the routine and quality control analysis of SHM in raw material and pharmaceutical formulations.

Keywords: Sibutramine Hydrochloride Monohydrate, Spectrometry, Stability indicating, Validation.

INTRODUCTION

During the pharmaceutical development of a new drug, it is necessary to select as soon as possible the formulation with the best stability characteristics. Regulations regarding stability testing for registration application are provided by current International Commission for Harmonization (ICH), which emphasizes the stress testing conditions with the aim of assessing the effect of severe conditions on the drug in practice, the effects of pH and temperature changes on drug stability are often used in such studies. The results of such studies are of vital importance in the estimation of a drug product shelf life during early stages of its pharmaceutical development. The results may also serve as guides for better drug design, drug formulation and drug analysis [1].

Sibutramine hydrochloride monohydrate (SHM), chemically identified as a racemic mixture of enantiomers (+) and (-) of {N -[1 -(4 - chloro- phenyl)- cyclobutyl]- 3- methyl- butyl}-N- Ndimethyl-amine (Fig. 1), is an effective serotonin (5-HT) and noradrenaline (NA) re-uptake inhibitor which acts increasing both satiety and metabolism 1-4. Its satietogenic effect occurs by enhancing central 5-HT and NA functions. The metabolic effects are based on stimulation of thermogenesis due to the activation of the β3-adrenoceptores in the adipose tissue [2, 3], SHM is a white and crystalline powder, molecular weight 334.3 g mol– 1, melting point 191.0-192.0 °C, soluble in methanol and water (2.9 mg L– 1 at pH 5.2) [4, 5]. It is commercially available as pharmaceutical dosage forms in capsules.

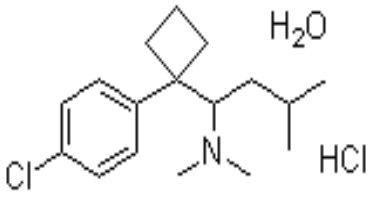

Fig. 1: Chemical structure of SHM

The United States Pharmacopeia has not yet incorporated a monograph for SHM [6], but some methods have been described for the substance determination. The high performance liquid chromatography (HPLC) has been used for the enantiomers separation and for SHM quantification in capsules. The preparation of the pure enantiomer and its determination by single crystal X-ray analysis has been also established. In addition, the quantification of Sibutramine and its active metabolites in biological fluids by liquid chromatography-electrospray ionization tandem mass spectrophotometry have been reported [2, 7, 8]. The goal of this paper is to propose an alternative, low cost, easy handling method to determinate SHM in capsules by UV-VIS spectrophotometry. Also, the complete analytical validation was performed according to the literature [9-11].

Among the various methods available for the determination of drugs, spectrophotometry continues to be very popular, because of their simplicity, specificity and low cost.

In the present study a simple, sensitive, accurate and reproducible analytical method with better detection range for estimation of SHM in pure form and in its pharmaceutical dosage forms was developed and validated. Based on forced degradation studies, the method was also tested for its stability indicating ability according to the ICH requirements which can be used for the routine and quality control analysis of SHM in bulk and pharmaceutical formulations.

MATERIALS AND METHODS

Sibutramine hydrochloride monohydrate was obtained as a gift sample from Hetero Drugs Hyderabad. All solvents and other chemicals used were of analytical reagent grade purchased from Research lab, Mumbai. A Labindia UV/VIS double beam spectrophotometer (model 3000+) with 1 cm matched quartz cells was used for all spectral measurements. Double distilled water used throughout the experiment.

Preparation of standard stock solution

10 mg of SHM was accurately weighed and transferred to 100 ml volumetric flask and dissolved in about 20 ml of distilled water. The volume was made up to the mark with distilled water to give 100µg/ml stock solution.

Preparation of calibration curve for SHM

By scanning a suitable standard solution in the UV-VIS spectrophotometer in the wavelength range of 200-400 nm, the λmax of the drug was determined, shown in fig 2. Aliquots (0.5, 1, 2, 3, 4 and 5 ml) from standard solution of SHM were pipetted out in to a series of five volumetric flasks and the volume was made upto 10 ml with double distilled water. The absorbance was measured at 223 nm against reagent blank. The calibration curve was

constructed by plotting absorbance v/s concentration (µg/ ml). Correlation coefficient was also measured.

Estimation of SHM in capsule dosage form

The sample solution was obtained from the SHM capsules. Twenty capsules were weighed and the contents was removed, as completely as possible, and mixed. An accurately weighed portion of the combined contents, equivalent to about 10.0 mg of SHM, was dispersed in double distilled water using a 100.0 mL volumetric flask, to this 30 ml double distilled water was added.

The content of the flask was sonicated for 15 min and the volume was made up to mark with the same solvent and filter through Whatmann filter paper No. 40. Appropriate solutions were prepared by taking suitable aliquots and diluting them with double distilled water to give final concentration (20 µg/ml). Then the absorbance of these solutions was measured at 223 nm against blank.

Method validation

The method was validated according to ICH Q2B guidelines[9] to determine the Linearity, sensitivity, precision, and accuracy of the analyte. Linearity of the proposed method was determined by measuring the absorbance of the standard solutions in the concentration range of 5-25 µg/ml and performing least square regression analysis. In addition, the accuracy of the proposed method was checked using standard addition method and recovery studies were carried out at 80%, 100% and 120% of target concentration. The percent analytical recovery was calculated by comparing the concentration resulted with the addition of spiked samples with actual expected theoretical increase in concentration. Intra-day precision was determined by carrying out the analysis for six concentrations at two different time interval in a day. Similarly inter-day precision was determined by performing analysis on two consecutive days. LOD and LOQ of the proposed methods were calculated. Recovery of the analyte of interest from a given matrix can be used as a measure of the accuracy or the bias of the method.

Stability studies of SHM

Stability studies were performed by forced degradation study of SHM and it includes the study of effect of temperature, oxidation, photolysis and susceptibility to hydrolysis across a wide range of pH values. For acidic hydrolysis 0.1, 0.2, 1.0 N HCl, for basic hydrolysis 0.1, 0.2, 1 N NaOH, for oxidation study 0.1%, 0.2% and 1% H_2O_2 was used. For carrying out photolysis studies the drug was treated with sunlight for 3 days and thermal stress was applied by heating the drug at 60^0C for 2 hrs.

RESULTS AND DISCUSSION

The development of a simple, economic, rapid, sensitive, and accurate analytical method for the routine quantitative determination of samples will reduce unnecessary tedious sample preparations and the cost of materials and labor. The absorption spectrum of SHM in double distilled water is shown in Fig 2.

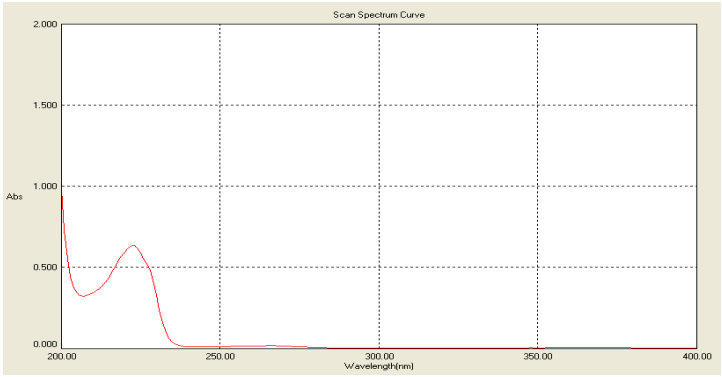

Fig. 2: UV spectrum of SHM

The λmax of the drug for analysis was determined (223 nm) by taking scans of the drug sample solutions in the entire UV region. Calibration curve data was constructed in the range of the expected concentrations of 05 to 25 µg/ml. Beer's law was obeyed over this concentration range. The regression equation was found to be Y=0.032x+0.026(Fig. 3).

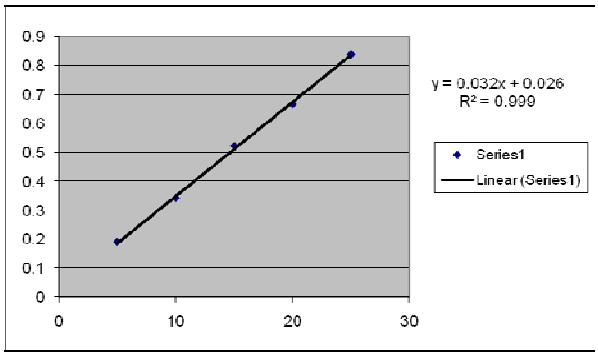

Fig. 3: Calibration curve of SHM at 223 nm

The correlation coefficient (r) of the standard curve was found to be 0.9990. The summary of analytical parameters and calibration curve data are presented in Table 1 and Table 2 respectively.

Table 1: Optical characteristics of the proposed method

Parameters	Result
Measured wavelength (λmax)	223 nm
Beers law limit (µg/ml)	5-25
Regression equation (y = m x + c)	Y=0.032x+0.026
Slope	0.032
Intercept	0.026
Correlation coefficient (r)	0.9990
LOD µg/ml	0.13
LOQ µg/ml	0.42

Table 2: Calibration curve data for SHM

Sr. No.	Conc. (µg/ml)	Absorbance
1	5	0.191
2	10	0.342
3	15	0.522
4	20	0.666
5	25	0.838

Performing replicate analyses of the standard solutions was used to assess the accuracy and precision of the proposed methods (Table 3 and 4). The LOD and LOQ were found to be 0.13µg/ml and 0.42 µg/ml respectively.

To study the accuracy of the proposed method and to check the interference from excipients used in dosage forms, recovery experiments were carried out by the standard addition method. The mean recovery was found to be 99.78-100.10. The proposed methods can be successfully applied for assay in tablet dosage forms without any interference (Table 3).

Table 3: Result of recovery studies

Level of % recovery	% Mean* recovery	S.D	% RSD
80	100.10	1.6683	1.6667
100	99.95	0.7705	0.7709
120	99.78	0.7223	0.7239

* Mean of three determinations at each

To determine the precision of the method SHM solutions at concentration 30 µg/ml. Intra-day precision was determined by carrying out the analysis for six concentrations at two different time interval in a day. Similarly inter-day precision was determined by performing analysis on two consecutive days. The method was found to be precise since % RSD values for interday precision were found to be 0.1755, 0.2809 respectively and for intraday precision it was 0.1755, 0.3679 respectively. Results are shown in Table 4.

Table 4: Statistical validation for interday and intraday precision

Parameters	Concentrations (µg/ml)	
	20	20
Intraday*		
% Mean±S.D	99.37±0.1755	98.85±0.3637
%RSD	0.1766	0.3679
Interday*		
% Mean±S.D	99.37±0.1755	100.08±0.2809
%RSD	0.1766	0.2807

*Denotes average of six determinations S.D= Standard Deviation

%RSD = %Relative Standard Deviation

The application of this procedure is explained in the experimental section. The obtained results demonstrate the validity and accuracy of the proposed method for the determination of SHM in tablets. The stability studies indicates that appreciable changes were observed by treating the drug with thermal stress, oxidation, acid and basic hydrolysis, however drug was stable in sun light stress condition. The results are shown in Table 5.

Table 5: Result of forced degradation study of SHM

Sr. No.	Conditions applied	Conc. taken(µg/ml)	Conc. Found (µg/ml)	Observation
1	Acidic Hydrolysis			
	0.1 N HCl	20 µg/ml	23.24	Degraded
	0.2 N HCl	20 µg/ml	23.42	Degraded
	1 N HCl	20 µg/ml	23.56	Degraded
2	Basic Hydrolysis			
	0.1 N NaOH	20 µg/ml	Change in λmax	Degraded
	0.2 N NaOH	20 µg/ml	Change in λmax	Degraded
	1 N NaOH	20 µg/ml	Change in λmax	Degraded
3	H_2O_2 (Oxidation)			
	0.1%	20 µg/ml	5.15	Degraded
	0.2%	20 µg/ml	7.2	Degraded
	1%	20 µg/ml	10.72	Degraded
4	Sunlight-Treatment			
	Day 1	20 µg/ml	19.98	Stable
	Day 2	20 µg/ml	20	Stable
	Day 3	20 µg/ml	20	Stable
5	Thermal Stress (60^0 C, 2 hrs)	20 µg/ml	14.11	Degraded

CONCLUSION

These results reveal that the developed method was simple, economic, rapid, accurate and precise and consequently, can be applied to the determination of SHM tablet in pharmaceuticals without any interference from the excipients. Based on forced degradation studies according to the ICH requirements, this method can be used for the routine and quality control analysis of SHM in raw material and pharmaceutical formulations.

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