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A Validated Specific Reverse Phase Liquid Chromatographic Method for the estimation of Sibutramine Hydrochloride Monohydrate in bulk drug and capsule dosage forms

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Abstract: A simple, specific, accurate and precise reverse-phase high performance liquid chromatographic method was developed and validated for the estimation of sibutramine hydrochloride monohydrate in capsule dosage forms. A Phenyl Hypersil BDS C-18, 5 µm column having 250 x 4.6 mm i.d. in isocratic mode, with mobile phase containing Acetonitrile: 50 mM Ammonium dihydrogen phosphate (65:35, v/v; pH 6) was used. The flow rate was maintained at 1.0 mL/min and effluent was monitored at 225 nm. The retention time of sibutramine hydrochloride monohydrate was 6.18 min. The developed method was validated as per ICH guideline Q2B. Limit of detection and limit of quantification for estimation of sibutramine hydrochloride monohydrate were found to be 0.09 µg/mL and 0.26 µg/mL, respectively. Recoveries of sibutramine hydrochloride monohydrate in capsule formulation were found to be in the range of 99.87-100.45 %. Proposed method was successfully applied for the quantitative determination of sibutramine hydrochloride monohydrate in capsule dosage forms. It can also be used for analysis of samples during stability testing.
Key words: Sibutramine hydrochloride monohydrate, RP-HPLC, Validation, Stability testing.

1. Introduction

Sibutramine hydrochloride monohydrate (SBHM) is chemically, a racemic mixture of the (+) and (-) enantiomers of cyclobutanemethanamine, 1-(4-chlorophenyl)-N, N-dimethyl-α-(2-methyl propyl)-, hydrochloride, monohydrate and has an empirical formula of $C_{17}H_{29}C_{l2}NO$[1]. SBHM is an orally administered agent for the treatment of obesity. It is a centrally acting serotonin-norepinephrine reuptake inhibitor. Sibutramine is a derivative of the amphetamine precursor, β- phenethylamine, and blocks presynaptic nerve terminal reuptake of norepinephrine, serotonin, and dopamine[2]. In humans, sibutramine is rapidly metabolized, to desmethyl metabolites, M1 (mono-desmethyl sibutramine) and M2 (di-desmethyl sibutramine). It is also indicated for the management of obesity, including weight loss and maintenance of weight loss, and it should be used in conjunction with a reduced calorie diet[3]. No pharmacokinetic data for the parent drug sibutramine has been reported in the literature. It has been reported that sibutramine exerts its pharmacological actions predominantly via its secondary amine M1 and primary amine M2 metabolites, although sibutramine is also pharmacologically active[4, 5].

Based on the literature review, it was found that a number of studies involving method for estimation of SBHM have been carried out. Thus, a number of analytical methods such IR[6], GC-MS[7], LC/ESI-MS/MS[8], LC-MS[9], LC–ESI–MS[10], colorimetry[11], HPLC-MS-MS[12] etc. have been developed but no method have been developed so far for estimation of sibutramine hydrochloride monohydrate in bulk drug and capsule dosage forms by RP-HPLC method with UV detection.

A successful attempt has been made to estimate SBHM by RP-HPLC analysis. Proposed study involves development of RP-HPLC method using simple mobile phase containing acetonitrile and buffer for quantitative estimation of sibutramine hydrochloride monohydrate in capsule dosage form, which is sensitive and requires shorter analysis time. The developed method was validated as per ICH guidelines[13, 14]. As per ICH guideline Q1A (R2), drug was subjected to all stress conditions such as hydrolysis (acidic, alkaline), oxidation

(30% H2O2v/v), photolysis (As per ICH guideline Q1B), thermal degradation study[15, 16].

2. Experimental

2.1. Chemicals and reagents

Pure samples of SBHM (Potency 99.54%) were obtained from Zydus Cadila Healthcare Ltd., (Ahmadabad, India). Acetonitrile (HPLC grade) was procured from E. Merck (India). HPLC grade water was obtained by double distillation and purification through Milli-Q water purification system, ammonium dihydrogen phosphate and other chemicals of analytical reagent grade was procured from Qualigens. Capsule A (Reductil, 10 mg, Abbott lab, Brazil) and capsule B (Reductil, 15 mg, Abbott lab, Brazil) were obtained from Zydus Cadila Healthcare Ltd., (Ahmadabad, India).

2.2. Instrumentation

Shimadzu HPLC system equipped with LC-2010HT with SPD-10Avp UV and PDA detector having Class-VP software and rheodye injector with 20 µl fixed loop. Sartorius weighing balance, BP211D was used for experimental purpose. All samples were filtered through 0.45 µm membrane filter.

2.3 Chromatographic condition

Reverse phase liquid chromatographic method was developed on a Phenyl Hypersil C-18, 250 x 4.6 mm, 5 µm column, using mobile phase containing acetonitrile: 50 mM ammonium dihydrogen phosphate buffer pH 6.0 by sodium hydroxide; (65:35, v/v) at ambient temperature. Solvent mixture of methanol and Mill-Q water (65:35, v/v) as diluting agents for sample preparation. Initially the method was developed for SBHM pure sample then it was extended to stress samples. The standard and all stress samples were prepared in diluting solution. The flow rate was kept at 1.0 mL/min, column temperature 30°C and detection was carried out at 225 nm. The sample was injected using 20 µl fixed loop, and the total run time was 8 min throughout analysis.

2.3. Preparation of standard Solution

2.3.1 Standard Solution

Accurately weighed 50 mg of SBHM working standard (Potency 99.54%) was transferred into 100 mL volumetric flask, dissolved and volume was made up to the mark with diluting solution. The final stock solution contained 500µg/mL of SBHM. 10 mL of SBHM stock solutions was transferred to a 100 mL clean volumetric flask and the volume was made up with diluting agent and mix well. The solution was then filtered through 0.45 µ nylon filter. 20 µL of final solution (50 µg/mL) was injected into the HPLC system and chromatograms were recorded.

2.3.2 Diluting solution

Solvent mixture of methanol and Mill-Q water (65:35, v/v) was used as diluting solution.

2.4. Stress testing

2.4.1 Acid and base-induced degradation

The bulk drug was subjected to hydrolysis by refluxing the sample solution in hydrochloric acid (1N, 5N), sodium hydroxide (1N, 5N) for different time interval and temperature. The samples from all hydrolysis studies were withdrawn and neutralized at an interval of each 1 hr. Sample was analyzed with respect to unstressed sample as per proposed assay method.

2.4.2 Hydrogen peroxide-induced degradation

The method described above except that 30% hydrogen peroxide (v/v) solution was added in place of HCl/NaOH and kept for different time interval and temperature. The stressed sample analyzed with respect to unstressed sample.

2.4.3 Thermal degradation

The sample solution was subjected to thermal degradation by keeping at 100°C for 24 hr and 48hr. followed by analysis with respect to unstressed sample.

2.4.4 Photolytic degradation

As per ICH guideline Q1B, photostability test was performed. Photostability test was performed as per solid state photostability study in which solid state pure drug was sufficiently spread on petri plates (1 mm thick layer) and exposed to photo degradation test at 2600 lux hour for 7 days. At the same time controls of all samples were also exposed to same conditions in photostability chamber.

All stressed sample were withdrawn periodically and analyzed by developed HPLC method. The % residual drug to be remained was calculated from the standard calibration curve. Using the peak purity test, the peak purity was checked at every stage of above mentioned studies.

2.5. Validation of method

Validation of the developed method was done according to ICH Q2B guideline 1996.

3. Results and Discussion

3.1. Method development

The chromatographic condition was optimized with a view to develop a stability-indicating assay method for SBHM in capsule dosage forms. Three different columns; namely, Inertsil ODS C-18 (250×4.6 mm, 5µ), Zorbax C-18 (250 × 4.6 mm, 5µ) and Phenyl Hypersil C-18 (250 × 4.6 mm, 5µ) were tried as under chromatographic conditions. The column Phenyl Hypersil C-18, gave good peak shape with response and satisfactory system suitability parameters at affordable retention time with peak purity of SBHM in presence its degradation products.

Initially mixture of methanol, acetonitrile and water has been taken; but there is no satisfactory peaks were eluted,

so later on buffer replaced in place of water. Ratio of acetonitrile and buffer was also altered to give the best separation of the peaks. Using 50 mM ammonium dihydrogen phosphate, pH 6±0.05 by sodium hydroxide and SBHM peak amongst degradation products peaks were found resolved. The final chromatographic system optimized shown in Table 1. Detection was performed at 225 nm which shown by UV scan of sample (Fig. 1). A typical UV chromatogram of SBHM by proposed method is shown in Fig. 2.

3.2 Calibration curve

The linearity of the response for SBHM assay method was determined by preparing and injecting standard solutions with concentrations of 10 - 100 µg/mL SBHM. The linear regression data for the calibration curves (Fig. 3) indicate that the response is linear over the concentration range studied with correlation coefficient, r^2 value as 0.999. The values of slope and intercept were 37950 and 13535, respectively (Table 2).

The standard deviation of y-intercept of regression line was determined and kept in following equation for the determination of detection limit and quantitation limit. Detection limit= 3.3 σ/s; quantitation limit = 10 σ/s, where σ is the standard deviation of y –intercept and s is the slope of the calibration curve.

3.2 System suitability

As per USP XXII[17], system suitability tests were carried out at before performing each of validation parameters for SBHM by the proposed HPLC method summarized in Table 3.

3.3 Analysis of stressed sample

The ICH stability guideline Q1A (R2) defines stress testing for new drug substances and drug products, to elucidate the intrinsic stability of the drug substances and drug products. The stress testing may also provide information about degradation pathways and selectivity of the applied analytical method.

Analysis of all stressed samples was performed using acetonitrile: 50mM ammonium dihydrogen phosphate buffer pH 6.0 (65:35, v/v) as the mobile phase.

It drug was found to degrade extensively on heating it in 1 N NaOH and 30% H_2O_2 for 2 h at 60°C, and 105°C/48 hr (Fig. 4, 5, 6) and while in acidic (5N HCl) and photolytic condition (2600 lux for 7 days), only 1-5 % degradation (Fig. 7, 8) was seen. The developed method was extended to marketed capsule formulation which shows identical degradation to SBHM working standard.

3.4 Validation of the method

3.4.1 Precision

Precision of the proposed method was evaluated through system precision, method precision and intermediate precision. System precision was assessed by six replicate determinations of SBHM standard solution at 100 % of target concentration. Method precision was assessed by six replicate determinations of SBHM solution at 100 % of target concentration. The method is applied repeatedly to multiple samples. Intermediate precision of the method was verified by analyzing sample of a single batch of the capsule dosage form by different analyst using different instrument and different column on different day at 100 % of target concentration (50 µg/mL), The % R.S.D. was found to be 0.004 %, 0.690 %, 1.030 % respectively, which met the acceptance criterion for the established method. The data is quoted in Table 4.

3.4.2 Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. Accuracy is performed at three levels 50%, 100% and 150% by standard addition method. Data for recovery was obtained at three different concentration of SBHM standard, is presented in Table 4.

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

LOD and LOQ were determined from standard deviation of y-intercept of regression line and slope method as per ICH guideline. For SBHM, LOD was found to be 0.01 µg/mL and LOQ was found to be 0.26 µg/mL.

3.4.4 Specificity

Separation of SBHM was found to be better with its degradation products and placebo. Better resolution was found for the drug peak from the nearest resolving peak with no interference proved that the method was found to be specific to the drug.

3.4.5 Stability of analytical solution

Solution stability was checked up to 15 hr. which shows no significant difference from the initial response. Thus, the solution is stable upto 15 hr.

3.4.6 Robustness

Robustness of the method was determined by analyzing standard solution at normal operating condition and also by changing some operating analytical condition such as flow rate, column oven temperature, detection wavelength, mobile phase ratio and buffer pH. System suitability test was applied at each of above mentioned condition and results of test are shown in Table 4 which indicates robustness of method.

3.5 Analysis of marketed formulations

The developed method was applied to the analysis of SBHM in capsule dosage form marketed as Capsule formulation A and B (Reductil, Label claim 10 and 15 mg strength, Abbott lab, Brazil). The results of analysis are given in Table 5.

The contents of marketed tablet dosage form were found to be in the range of 100±2% with RSD less than 2% which indicate suitability for routine analysis of SBHM in capsule dosage form.

3.6 Stability indicating property

The values of assay, degradation (%), peak purity index with stress condition are given in Table 6. The stressed

condition samples are evaluated relative to unstressed sample with respect to assay and degradation (%). The degradation (%) of SBHM indicate susceptibility of drug to alkali, peroxide, thermal condition. The peak purity index is a measure of spectral heterogeneity of a peak based on the comparison of spectra over the entire peak. The non-ideal effects are quantified and provided as a value of peak purity index. When the peak is pure, the peak purity index is greater than 0.990 as seen in Table 6.

Conclusion

Proposed study describes new RP-HPLC method using simple mobile phase for the estimation of SBHM in capsule dosage formulations. The method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in formulation. Proposed method proved to be convenient and effective for the determination of SBHM during stability testing of the bulk as well as pharmaceutical capsule dosage form. Moreover, the lower solvent consumption along with the short analytical run time of 8 min leads to cost effective chromatographic method.

Table 1: Optimized chromatographic condition of SBHM for the proposed method

Conditions	Results
Mobile Phase	Acetonitrile: Buffer –65:35, Isocratic, Buffer: - 50mM $NH_4H_2PO_4$, pH 6.0 by Sodium hydroxide
Diluting agent	Methanol: Water – 65:35
Column	PHENYL HYPERSIL C-18 (250 × 4.6 mm), 5µm particle size
Column Oven	30°C
Flow rate	1.0 ml/min
Detector	UV at 225 nm
Injection Volume	10 µl
Run time	8 minutes

Table 2: Regression analysis of the Calibration Curves for the proposed method

Parameters	Values
Calibration range(µg/ml)	10-100
Regression equation	y = 37950 x+13535
Slope(b)	37950
Intercept(a)	13535
Correlation coefficient(r^2)	0.999
Standard deviation of intercept	992.602

Table 3: System suitability parameter for SBHM by proposed method

Parameters	Values
Theoretical plate	4000
Tailing factor	1.2
Asymmetry factor	1.2-1.3
Capacity factor	600

Table 4: Summary of validation parameters

Validation Parameter	Result
Specificity	No interference was found to be w.r.t. excipients, impurities
Linearity range (µg/ml)	10-100
Target concentration (µg/ml)	50
Precision (n=6)	
System precision	0.004*
Method precision	0.690
Intermediate	1.030
Accuracy (% recovery)	99.87-100.45
LOD (µg/ml)	0.1
LOQ (µg/ml)	0.26
Solution stability	Up to 15 hr.
Robustness	Robust#

***%RSD,#As per system suitability test**

Table 5: Assay result of capsule formulation using proposed method

Brand name	Label claim (mg)	Mean ± SD	% RSD
Capsule A	10	97.78 ± 0.546	0.558
Capsule B	15	99.81 ± 0.110	0.110

Table 6: Stress study data for SBHM pure sample

Conditions	Assay degradation (%)	Peak purity
Control sample	-	0.999
Acid-5N HCl, 5 ml	4.83	0.999
Alkali-1 N NaOH, 3 ml for 2 h at 60°C	25.60	0.999
Peroxide-30% H_2O_2, 4 ml for 2 h at 60°C	24.26	0.999
Thermal deg.-105°C/48 hr	11.04	0.998
Photolytic deg, (2600 lux for 7 days)	1.01	0.999

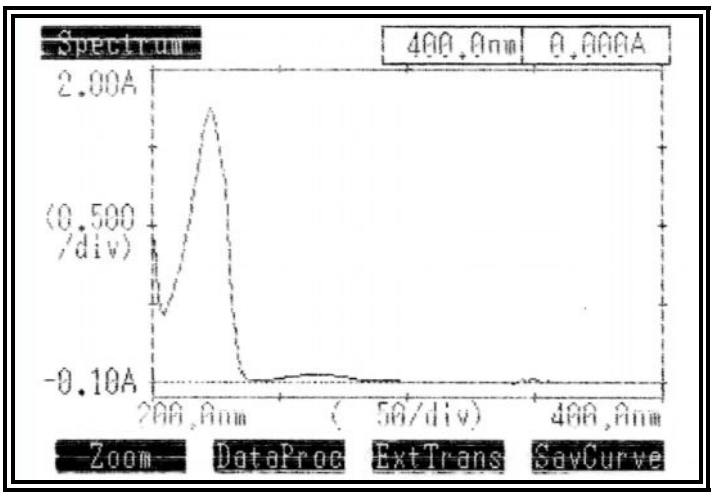

Fig. 1: UV scan of SBHM in range of 200 to 400 nm

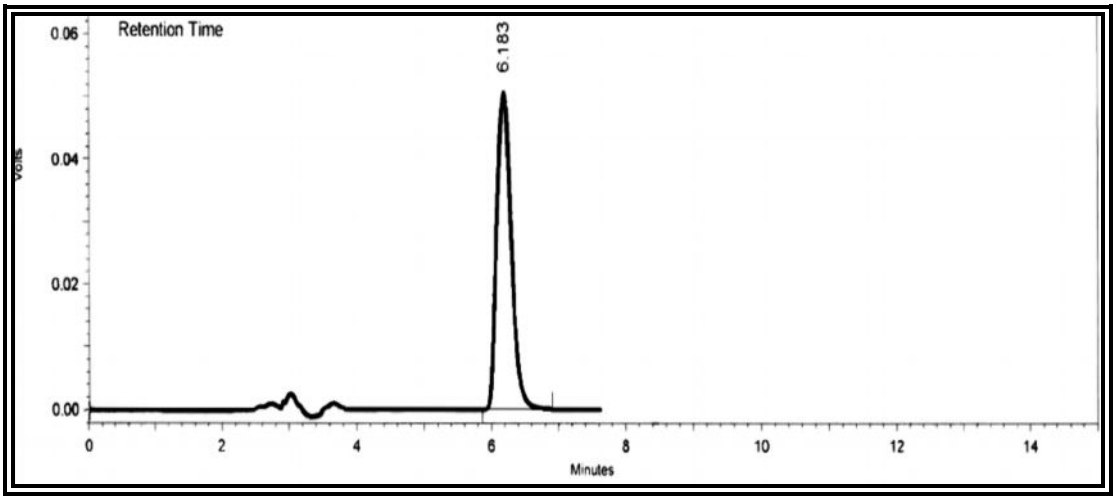

Fig. 2: RP-HPLC chromatogram of SABHM (RT 6.18 min)

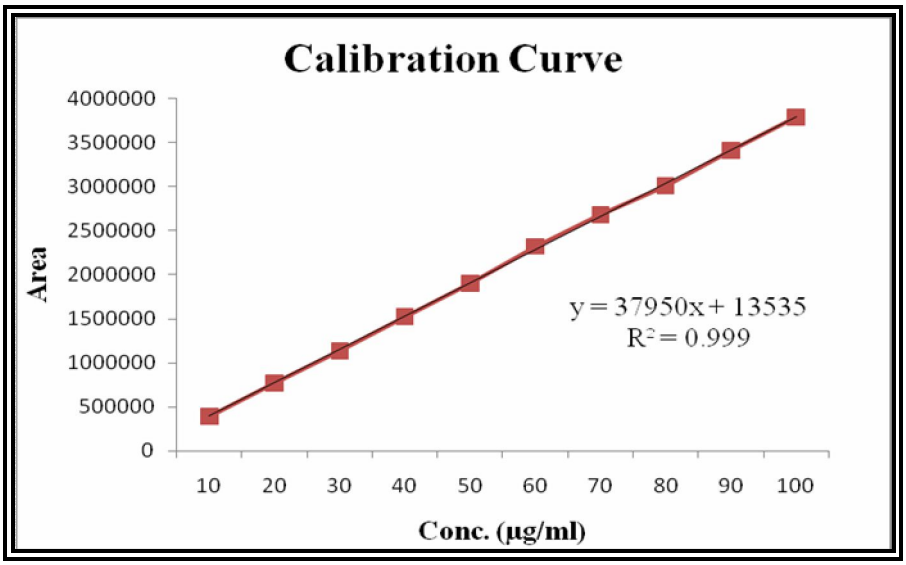

Fig. 3: Calibration curve of SBHM as per the proposed RP-HPLC method

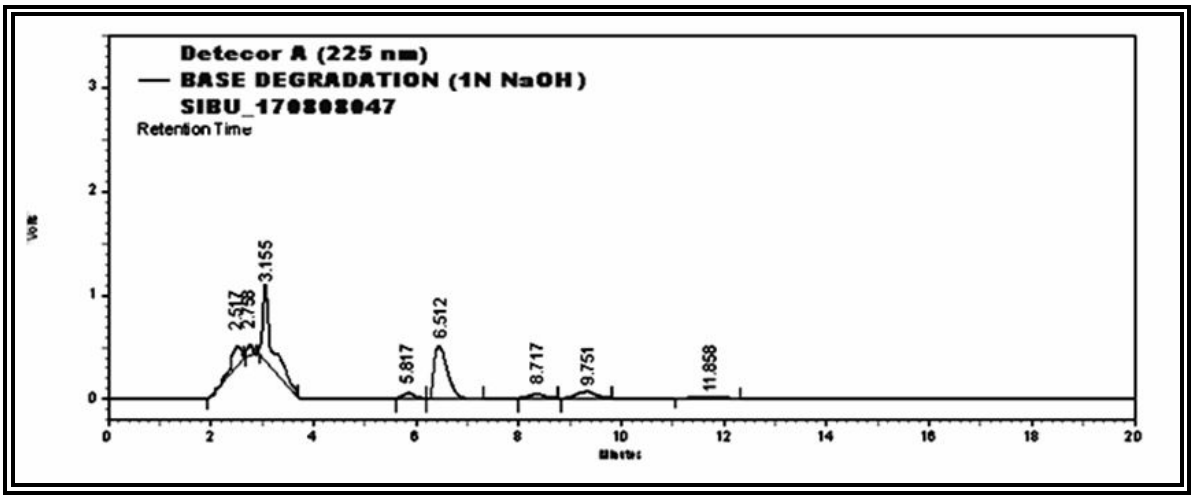

Fig. 4: HPLC chromatograms representing degradation behavior of SBHM in 1 N NaOH as per the proposed method

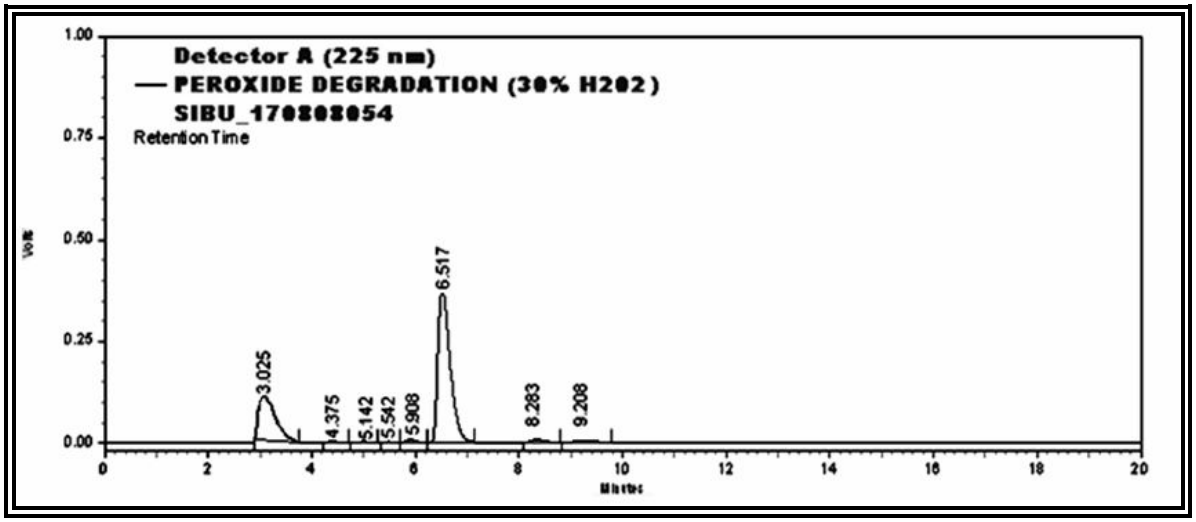

Fig. 5: HPLC chromatograms representing degradation behavior of SBHM in 30% H_2O_2 as per the proposed method

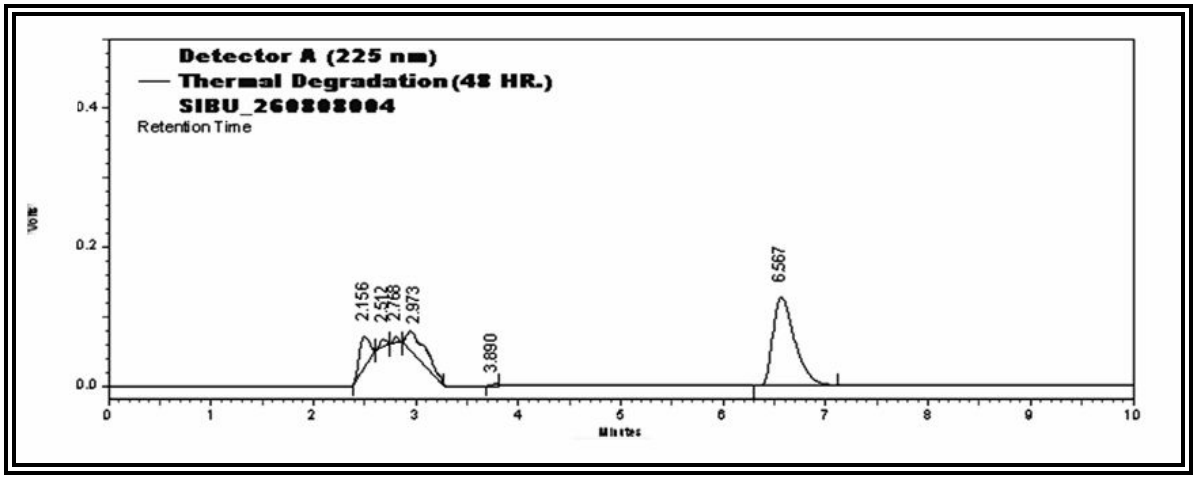

Fig. 6: HPLC chromatograms representing degradation behavior of SBHM at 105°C/48 hr as per the proposed method

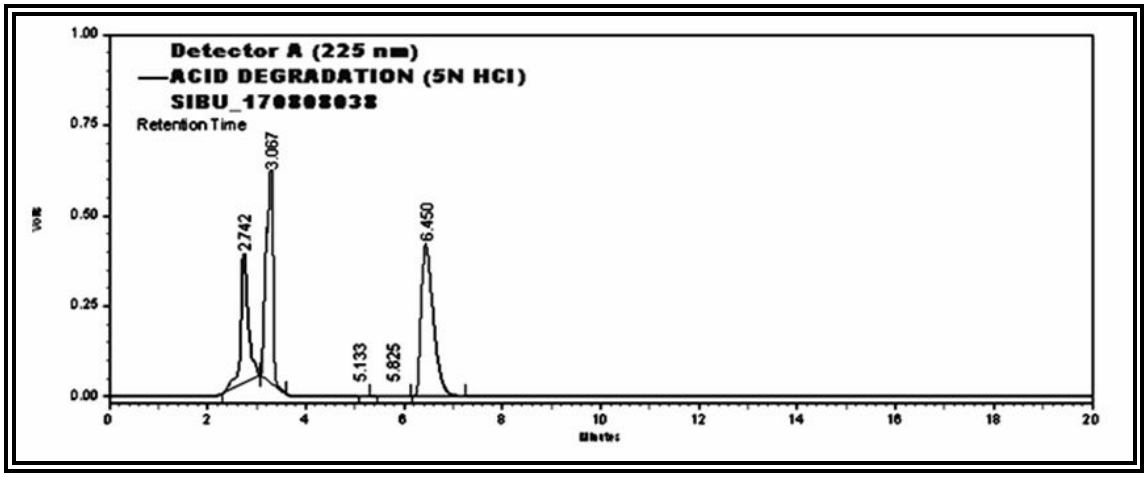

Fig. 7: HPLC chromatograms representing degradation behavior of SBHM in 5N HCl as per the proposed method

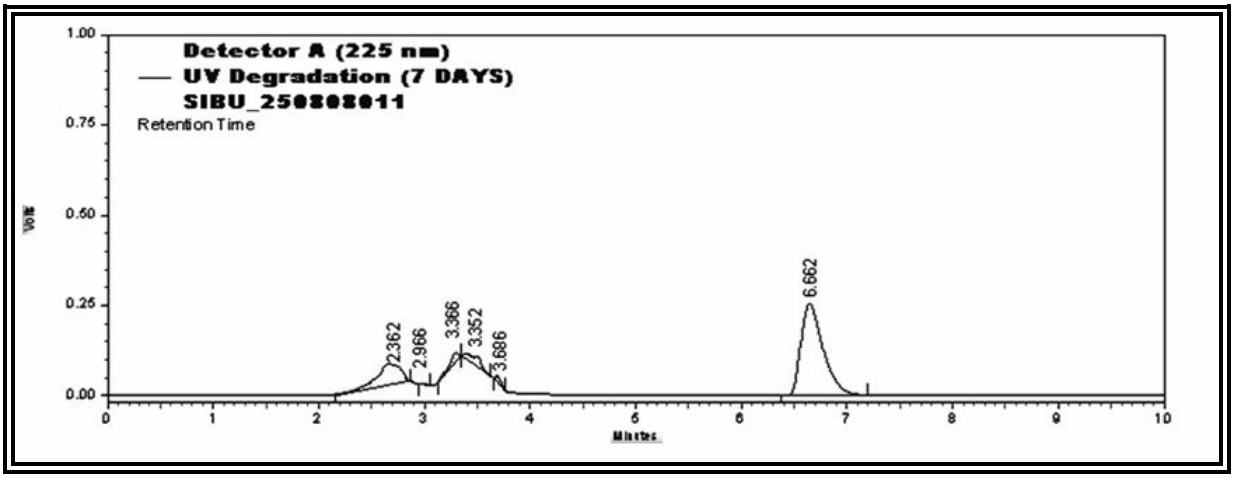

Fig. 8: HPLC chromatograms representing degradation behavior of SBHM in photo condition as per the proposed method

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