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Development of Validated RP-HPLC Method for Determination of Sibutramine Applying QbD Approach

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Abstract:- Sibutramine, {1-[1-(4-chlorophenyl) cyclobutyl]-3-methylbutyl} dethylamine is potent serotonin and norepinephrine inhibitor. Pharmacologically it acts as antihypertensive and anti-obesity agent via its secondary (M1) and primary (M2) amine metabolites. In present work an attempt has been done to develop a simple, rapid, precise and accurate isocratic reversed-phase HPLC method for the determination of sibutramine. As a novelty along with validation of developed method as per ICH guidelines, a QbD approach has been applied. As critical quality attributes related to analytical method have been identified and optimized more robustness and accurate method HPLC method has been developed. Slection of column, composition of mobile phase and flow rate were identified as critical quality attributes where by selection of KYA TECH HIQ Sil C18 (4.6 mm × 250 mm) 5µm column, Acetonitrile: water in composition 80:20 v/v as mobile phase with flow rate 1mL/min was done. The λ_{max} was detected as 239 nm. The linearity range of the proposed method was found to be in the range of 10–50 µg/ml (*r* = 0.999). The limits of detection was 0.081 µg/ml and the limits of quantitation were 0.172 µg/ml respectively.

Keywords:- *Sibutramine, Quality by Design (QbD), Analytical Method Development and Validation. RP-HPLC.*

I. INTRODUCTION

Chemically Sibutramine, {1-[1-(4-chlorophenyl) cyclobutyl]-3-methylbutyl} dethylamine is potent serotonin and norepinephrine inhibitor**. It** shows pharmacological action by its primary (M2) and secondary (M1) amine metabolites and it acts as antihypertensive and anti-obesity agent. Sibutramine generated its therapeutic properties inhibiting of serotonin (5-hydroxytryptamine, 5-HT), norepinephrine (NE), and to small level, dopamine reuptake at the neuronal synapse. Inhibition of these reuptake neurotransmitters, sibutramine inspires a sense of satiety and reduction in appetite, thereby decrease in food consumption. Documentation records from animal readings also mention that sibutramine also rises energy expenditure through thermogenic special effects in both fed and basal states, but this has been not established in human sibutramine and its most important active metabolites (M1 and M2) don't release of monoamines. [1-3]

In present work by using QbD approach a simple, linear, precise, accurate and reproducible indicating RP-HPLC method have been developed and validated as per ICH guidelines for estimation of Sibutramine . While applying QbD approach to HPLC method, the critical quality attributes were identified as composition of selection of column, mobile phase, flow rate and retention time. For analysis of Sibutramine absorbance maxima was found to be 239 nm and Beer's range was found to 10-50µg/ml.[4-6]

Due to application of QbD approach the developed HPLC method was validated as per ICH guidelines and hence can be implemented in r routine quality control analysis of sibutramine. [7-9]

II. MATERIAL AND METHOD

- *Instruments*

Under application of QbD approach to RP- HPLC method development for determination of retention factor as well as composition of mobile phase, use of various columns like C8, Silica and KYA TECH HIQ Sil C18 (4.6 mm × 250 mm) 5µm column was done for sibutramine. From results it was seen that proper retention with better peak symmetry was obtained by using KYA TECH HIQ Sil C18 (4.6 mm × 250 mm) 5µm column. Further for confirmation of peek symmetry and purity during method development the use of photodiode array detector MD 2010 was done and the solutions were scanned between range of 200 -400. The output signal was integrated using Jasco LC- Net II/ADC Software.

- *Material*

A sample of sibutramine was obtained as gift sample from stride pharmaceuticals, Bangalore, India. HPLC graded Acetonitrile was purchased from Loba chemicals Mumbai.

- *Mobile Phase*

In applying QbD approach one of the critical quality attribute which may affect final result was found to be composition of mobile phase. Various combinations of Acetonitrile: Water like 100:0, 90:10, 80:20, 70: 30 60:40 in

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%V/V were used. Results were noted and it was found that at composition of 80:20 of Acetonitrile: Water the peak symmetry and retention time were found to be satisfactory.

➢ *Selection of Column*

Different varieties of columns like C8, Silica and KYA TECH HIQ Sil C18 (4.6 mm × 250 mm) 5µm column were utilized for determination of sibutramine. From results it was found that using KYA TECH HIQ Sil C18 (4.6 mm × 250 mm) 5µm column good resolution, peak symmetry, theoretical plate and retention time was obtained.

➢ *Flow Rate*

By applying QbD approach flow rate was also found to be one of the critical quality attribute, which may affect final result. Hence various flow rates like 0.8 mL/min, 1 mL/min, 1.2 mL/min. were tried. Results were noted and found that keeping 1 mL/min flow rate gives good retention time.

➢ *Stock Solution*

Standard stock solution of sibutramine 100µg/ml was prepared by accurately weighing the drug and dissolving it in 100 ml mobile phase and used for analysis.

➢ *Preparation of Standard Analytical Concentration Solutions*

Dilution of stock solution were done to get the standard final concentration in the range 10 to 50µg/ml.

- *Qbd Approach Applied in Development and Validation of RP-HPLC Method for*
- *Sibutramine*
- *System Suitability Parameter*

Table 1 Result of System Suitability Parameter

Parameter	Sibutramine
Retention time	3.50
Plate count	4565
Symmetry factor	1.306

III. VALIDATION OF DEVELOPED METHOD

➢ *Linearity Range*

With the help of serial dilution solutions of sibutramine were taken in different concentration of sibutramine was in the range of 10-50 µg/ml. The HPLC analysis of all aliquots was carried out and response (Peak area) was recorded for all the peaks. The plotting of calibration curve for sibutramine was done using peak area versus concentration.

➢ *Accuracy*

For determination of accuracy, recovery study was performed by taking different concentration of drug at three level i.e. 80%, 100%, 120%. Percentage recovery for pure drug was calculated from differences between the peak areas obtained from samples.

➢ *Precision*

For performing precision study six replicates each of standard solution of sibutramine having different concentration ranges like 30, 40 and 50µg/ml was done. The calculation of relative standard deviation was done on same day to calculate intra-day precision and on different days to calculate inter-day precision.

➢ *Robustness*

Robustness was studied for carrying out variation in wavelength, using different instrument and different analyst. The results obtained were within the acceptable limits.

➢ *LOD*

The standard deviation of the blank is depend on : Measurement of the level of analytical background response was performed by analyzing the six replicates of blank samples and calculating the standard deviation of these responses by using formula (1),

$$LOD = 3.3\ \sigma/S$$

➢ *LOQ*

The standard deviation of the blank is depend on: Measurement of the level of analytical background response was performed by analyzing the six replicates of blank samples and calculating the standard deviation of these responses by using formula (2),

$$LOQ = 10\ \sigma/S$$

➢ *Specificity*

The specificity of method was confirmed by making use of photodiode array detector MD 2010 by scanning solutions in the range of 200 – 400. The wavelength of detection was confirmed to be 239 on Jasco LC- Net II/ADC Software.

IV. RESULT AND DISCUSSION

Applying QbD approach the method validation were initially studied using C- 18 column. Mobile phase containing methanol and water was found to be not suitable, as the compound showed very close retention times and splitting of the chromatogram. Various ratio of methanol: water, acetonitrile: water were tried and it was found that Acetonitrile: water in (80:20 v/v) as mobile phase gave good resolution.

A flow rate of 1 ml/ min resulted in drug retention time within 10 minutes. The samples were scanned in range of 200 -400 wavelength by preparing sample solution of 30µg/ml and 239 nm was selected as suitable wavelength to the determination of sibutramine.

By applying QbD approach method has been validated as per ICH Q2A guidelines and carried out parameters like specificity, accuracy, precision, robustness, and linearity. The concentrations of standard stock solution was 100 µg/ml. Serial dilutions of stock solution was done to get final concentration ranging from 10µg/ml to 50µg/ml. The

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correlation coefficients was found to be more than 0.999 for the drug.

➢ *Determination of Linearity and Range*

Table 2 Result of Linearity Study for Sibutramine

SR.NO	CONCENTRATION (µ/ml)	AREA
1	10	46068
2	20	91739
3	30	141767
4	40	185000
5	50	220218

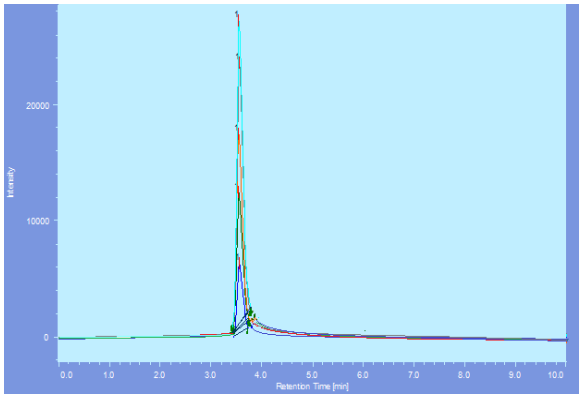

Fig 1 Overlay Chromatogram for Sibutramine

➢ *Result of Accuracy:*

Accuracy and precision of the method was determined by performing the recovery experiment. Recovery study was performed at three levels, in which sample stock solutions were spiked with standard drug solution containing 30 µg/ml, 40 µg/ml, and 50 µg/ml. Three replicate samples of each concentration level were prepared and the % recovery at each level (n = 3), and mean % recovery (n=9) were determined (Table 4). The mean recovery was 0.6371% by calculating %RSD. Performing precision study six replicates each of standard solution of sibutramine having different concentration ranges like 30, 40 and 50µg/ml was done. The calculation of relative standard deviation was done on same day to calculate intra-day precision and on different days to calculate inter-day precision (Table 5). Robustness was studied for carrying out variation in wavelength, using different instrument and different analyst. The results obtained were within the acceptable limits (Table 6). Specificity of method was confirmed by making use of photodiode array detector MD 2010 by scanning solutions in the range of 200 – 400. The wavelength of detection was confirmed to be 239 on Jasco LC- Net II/ADC Software

➢ *Accuracy Study*

Table 3 Result of Accuracy for Sibutramine

Sr. No	% Drug Solution	Conc. Estimated (%)			% RSD
1	80	95.2	96	95.6	0.5917
2	100	100.3	99.6	99.8	0.4952
3	120	102.3	101.25	102.5	0.8246

➢ *Precision Study*

Table 4 Result of Precision Study for Sibutramine Day-1 Morning

Conc. (µg/ml)	Conc. Estimated (%)	% RSD
30	100.25	0.9219
40	102.43	1.7953
50	105.54	1.3512

Table 5 Result of Precision Study for Sibutramine Evening

Conc. (µg/ml)	Conc. Estimated (%)	% RSD
30	102.4	1.51
40	100.04	1.20
50	101.9	1.45

Table 6 Result of Accuracy for Sibutramine Day-2 Morning

Conc. (µg/ml)	Conc. Estimated (%)	% RSD
30	102.48	1.83
40	102.76	1.44
50	98.48	1.38

Table 7 Result of Precision Study for Sibutramine Evening

Conc. (µg/ml)	Conc. Estimated (%)	% RSD
30	101.96	1.38
40	100.31	1.16

50	101.81	0.86

- *Robustness Study*

Table 8 Result of Robustness Study for Sibutramine

Sr. No.	Wavelength	Conc. Estimated (%)	% RSD
1	209	99.8	1.10
2	210	101.2	0.92
3	211	102.7	1.45

- *LOD and LOQ study*

Table 9 Result of LOD and LOQ for Sibutramine

Limit if Detection (µg/ml)	0.000102
Limit of Quantitation (µg/ml)	0.003118

V. CONCLUSION

From the statistic and results obtained it can be concluded that our aim to apply QbD approach has led to development of more robustness, simple, accurate, precise and reproducible HPLC method. It can be applied for routine qualitative analysis of sibutramine.

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- *Conflicts of Interest*

The authors declare no conflicts of interest.

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