# DEVELOPMENT AND VALIDATION OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR ANALYSIS OF SIBUTRAMINE HYDROCHLORIDE AND ITS IMPURITY

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**ABSTRACT**
A simple, Precise, Rapid reproducible and selective reverse phase HPLC method has been developed for the estimation of Sibutramine Hydrochloride monohydrate and its Impurity in Bulk as well as Formulation. The analyte was resolved by using Mobile phase (Sodium Dihydrogen phosphate and Acetonitrile) at the flow rate of 1.0 Ml/Min. on Isocratic HPLC system consisting of Jasco Make UV visible Detector of model UV 1575 & Jasco make HPLC pump of model PU 1580.An ODS C- 8 RP Column (4.6mm ID, 250mm L, particle size 5 Micron, at wavelength of 230 nm.

**Keywords**: Sibutramine hydrochloride, impurity*- 5-(2R)-2-aminopropyl)-2-methoxybenzene sulphonamide.

## INTRODUCTION

Sibutramine hydrochloride monohydrate is an orally administered agent for the treatment of obesity. Chemically, the active ingredient is a racemic mixture of the (+) and (-) enantiomers. (Li Ding *et al*., 2004) UV Spectroscopy and other Analytical Methods publish several Methods for its assay (Radhakrishna *et al.*, 2005). Several methods have been cited in various literatures but the prescribed method has unique advantage over it as it has not only capability of analyzing the product but also Impurity in Bulk and Formulated Dosages. This method can even be employed in routine determination of Sibutramine Hydrochloride in routine quality Control in process stage as well as in Finished stages and even for formulated dosages. Novelty of this method is its use in Impurity Profiling as prescribed method is statistically proved better choice of An Analyst. Impurity Profiling is the common name of the Analytical activities, the aim of which is the detection, identification/structure elucidation and quantitative determination of organic and inorganic impurities, as well as Residual solvents in Bulk Drugs and Formulation dosages (Sabina *et al.,* 2006). Since this is the best way to characterize quality and stability of Bulk Drugs and Formulated Dosages, this is the core activity in modern drug analysis. Due to the very rapid development of the analytical methodologies available for this purpose and the similarly rapid increase of the demands as regards the purity of the drugs, it is an important task to give a summary of the problems and various possibilities offered by modern analytical Chemistry for their solution (Valarmathi *et al.*, 2004).

## MATERIALS AND METHODS

A Jasco HPLC-1575 Series Chromatograph equipped with Intelligent Pump, UV Detector Jasco-1575 and autosampler Jasco AS 1555 was used. The column used was Stainless less steel C-18, 250x4.6mm, 5 Micron, Lichrosphere operating at room Temperature. The elution was carried out isocratically at flow rate of 1.2 ml/min. using Potassium Dihydrogen Orthophosphate buffer and Acetonitrile (50:50) and pH adjusted with 10%Ortho Phosphoric acid to 5.00 (±0.2) as Mobile phase. The detector was set at 275 nm. The responses of peak area were recorded and integrated using Browin Chromatographic Software.

***Chemicals and Reagent***

Potassium Dihydrogen Orthophosphate, Aceto Nitrile Water were used of HPLC grade purity. Ortho phosporic acid was used of Analytical Grade.

*1) Stationary Phase:* Lichrosphere C 8, with 5 Micron particle size ,Length 25 Cm with ID 4.6mm was used.

*2) Preparation of Mobile Phase:* Buffer Solution-Weigh accurately ON Sortorious make GMP model balance with 6 digit about 3.4005 gm of Potassium Dihydrogen Orthophosphate in a 500 ml of volumetric flask, add 250 to 300 ml of Milli Q water, & dissolve with the aid of sonication.

Mix 50 Parts of Buffer with 50 parts of Acetonitrile. Adjust the pH of this solution to 5.0±0.02 with 10% Orthophosphoric acid, then dilute upto the mark with water passed w through the Millipore make water purification system model Milli Q, check the pH to 5.0. (±0.2). Sonicate and Filter it through 0.45 Micron Filter paper & degas it by Sonication.

*3) Preparation of Test Solution for the analysis of Sibutramine Hydrochloride:* Amber Coloured glassware must be used when preparing these solution .Dissolve 50 Mg of the sample in the mobile phase and dilute to 100ml

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with Mobile phase.

***Reference solution A***
Dissolve 10 mg of 5-(2R)-2-Aminopropyl)-2-methoxy-benzene sulphonamide standard, in the mobile phase and dilute to 100 ml with Mobile phase.

***Reference Solution B***
Weigh accurately 100 mg Sibutramine Hydrochloride standard, transfer in 100 ml Volumetric Flask. Add 1.0 ml of solution A in to it &dilute to 100 ml with Mobile phase.

***System suitability***
As Per USP 27 System suitability tests were carried out on freshly prepared. Reference solution B to check the various parameters. Such as efficiency, retention time, and peak tailing which was found to comply with USP requirements.

The instrumental precision as determined by six successive injections of the reference solution B give RSD below 2% of Retention Time, And area and resolution between the these two peaks more than 1.0,Column efficiency for many impurity was 1669, and for Sibutramine Hydrochloride3346 theoretical plates.

## CALCULATION OF RESULTS

***1. Sibutramine Hydrochloride Content***

$$\text{Sibutramine Hydrochloride Content} = \frac{A_{Samp.} \times W_{Std.}}{A_{Std.} \times W_{Samp.}} \times P$$

$A_{Samp.}$ = Area of Sibutramine Hydrochlode Monohydrate peak in an injection of sample.
$A_{Std.}$ = Mean Area of Sibutramine Hydrochloride Monohydrate peak in injection of analytical standard solution.
$W_{Samp.}$ = Weight of the sample taken to prepare relevant sample solution . ( in mg )
$W_{Std.}$ = Weight of Sibutramine Hydrochloride Mono-hydrate reference standard taken to prepare analytical standard solution. ( in mg )
P = Potency of Sibutramine Hydrochloride Mono-hydrate reference standard (On dried Basis).

**2. 5-(2R)-2-Aminopropyl)-2-Methoxybenzene Sulphonamide Content**

$$\text{5-(2R)-2-Aminopropyl)-2-methoxybenzene sulphonamide} = \frac{A_{Samp.} \times W_{Std.}}{A_{Std.} \times W_{Samp.}} \times P$$

Where,
$A_{Samp.}$ = Area of 5-(2R)-2-Aminopropyl)-2-methoxy-benzene sulphonamide Peak in an injection of sample.
$A_{Std.}$ = Mean Area of 5-(2R)-2-Aminopropyl)-2-methoxybenzene sulphonamide Peak in injections of analytical standard solution.
$W_{Samp.}$ = Weight of the sample taken to prepare relevant sample solution. (in mg )
$W_{Std.}$ = Weight of 5-(2R)-2-Aminopropyl)-2-methoxy-benzene sulphonamide reference standard taken to prepare analytical standard solution. (in mg)
P = Potency of 5-(2R)-2-Aminopropyl)-2-methoxy-benzene sulphonamide reference standard. (On Dried Basis)

Reference solution B is tested for Recovery and Reproducibility study and results are as follows-

***Recovery***
The table 1 shows recovery is more than 98% as well impurity.

***Reproducibility***
The data in table 2 shows reproducibility is more than 99% for the product as impurity on analysis of 3 Consecutive days in Triplicate. Assay value of each run

**Table 1**: Recovery

| Compound | Run | A | B | X | Mean | SD | CV | 95% C.L. |
|---|---|---|---|---|---|---|---|---|
| Impurity | 1 | 0.012 | 0.012017 | 1.00140947 | 0.998114 | 0.004107 | 0.411447 | 0.00754 |
| | 2 | 0.012 | 0.011993 | 0.99941821 | | | | |
| | 3 | 0.012 | 0.011922 | 0.99351302 | | | | |
| Sibutramine HCl | 1 | 0.01 | 0.009977 | 0.99773973 | 1.000989 | 0.003109 | 0.310555 | 0.005707 |
| | 2 | 0.01 | 0.010039 | 1.00393456 | | | | |
| | 3 | 0.01 | 0.010013 | 1.00129397 | | | | |

Where: A = Actual Concentration taken for analysis: B-Recovered Concentration, X = B/A; (%Recovery = X*100); Mean – Average of 3 (x) Injection; SD Standard Deviation, CV Coefficient of Variation; 95%; C.L. = Confidence Limit at 95%.

(For the product and impurity) is taken in to consideration and SD & RSD is calculated.

**Table 2**: Reproducibility: Performed by analyst on 3 consecutive days in Triplicate analysis

| Day | Run | Impurity | Sibutramine HCl |
|---|---|---|---|
| 1 | 1 | 2746178.00 | 24348461.00 |
| 1 | 2 | 2752958.50 | 24454437.00 |
| 1 | 3 | 2758496.00 | 24560259.50 |
| 2 | 4 | 2739105.00 | 24351020.00 |
| 2 | 5 | 2720213.00 | 24484170.00 |
| 2 | 6 | 2744250.00 | 24476808.50 |
| 3 | 7 | 2721655.00 | 24407703.00 |
| 3 | 8 | 2722091.00 | 24396490.50 |
| 3 | 9 | 2744311.00 | 24472651.50 |
| Average | | 2738806.39 | 24439111 |
| Standard Deviation | | 13423.094 | 65207.99 |
| 2 V | | 0.98 | 0.54 |
| Limit | | 10% | 2.0% |

## RESULTS AND DISCUSSIONS

As per USP XXVII, system suitability was carried out freshly prepared reference solution B to check various parameters such as efficiency, resolution and peak tailing which found to comply with USP requirements (table 3).

***Limit of detection- and limit of quantitation***

The limit of detection (LOD) and Limit of Quantitation (LOQ) For Impurity- LOD – 0.0002% [2 ppm] of Concentration **LOQ** – 0.0005% [5 ppm] of Concentration. The optimum mobile phase of Acetonitrile and Potassium Dihydrogen Orthophosphate as mobile phase was selected because it was found to be ideally resolve peak of impurity from Sibutramine Hydrochloride. Wavelength was selected by scanning standard drug over the wide range of wavelength 200 nm to 400 nm. The analysis was performed at 275nm detector setting.

The content of an impurity in Sibutramine Hydrochloride by proposed method. The lower values of reproducibility indicate that the method is precise and accurate. The mean recoveries of Impurity were in the range of 99.3% to 100%, which shows that there is no interference from the mobile phase, which also confirm the reproducibility and reliability of the method.

## CONCLUSION

The proposed method is simple rapid and selective. Percent Relative standard Deviation was very slow, below 2.0% which indicate that method is highly precise. Short Analysis time (≤10 min.) coupled with simplicity and ease of operation warrants use of the method for analysis of Sibutramine Hydrochloride along with its impurity stated above in Bulk as well as in Formulated dosages for Assays and for said Related Substance by HPLC. Therefore, method can be useful in routine quality Control analysis in bulk.

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**Table 3**: System suitability parameter

| Compound | Run 1 | Run 2 | Run 3 | Run 4 | Run 5 | Mean | SD | RSD(%) | T.P. | R.F. | T.F. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sibutramine HCl Area (in MV) | 24043360 | 24112985 | 24063107 | 24188160 | 24101903 | 24101903 | 55891.6211 | 0.2318971 | 3346.90 | 5.752 | 1.00 |
| Retention Time (Min.) | 5.00 | 5.00 | 5.1 | 5.05 | 5.00 | 5.03 | 0.04 | 0.79% | | | |
| Impurity Area (in MV) | 2748640 | 2734322 | 2742995 | 2730302 | 2739065 | 2739064.8 | 7183.51833 | 0.26226171 | 1669.064 | - | 1.00 |
| Retention Time (in Min.) | 3.55 | 3.6 | 3.55 | 3.62 | 3.50 | 3.524 | 0.02244 | 0.6356% | | | |

SD- Standard Deviation, RSD- Relative Standard Deviation, T.P.- Theoretical Plates (NLT 1000 No.), R.F. Resolution Factor, T.F. – Tailing Factor. (Limit -Max 1.5%)

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