



## **EXTRACTIVE SPECTROPHOTOMETRIC METHODS FOR DETERMINATION OF SIBUTRAMINE HYDROCHLORIDE USING ACIDIC TRIPHENYLMETHANE DYES**

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### **ABSTRACT**

Three simple and sensitive extractive spectrophotometric methods have been described for the assay of sibutramine hydrochloride either in pure form or in pharmaceutical formulations. The developed methods involve formation of coloured chloroform extractable ion-pair complexes of the drug with bromothymol blue (BTB), bromophenol blue (BPB) and bromocresol green (BCG) in acidic medium. The extracted complexes showed absorbance maxima at 415 nm for all three methods. Beer's law is obeyed in the concentration ranges 2.0-25, 2.0-20 and 2.5-25 µg/mL with BTB, BPB and BCG, respectively. The effects of concentration of dye, pH, and interference of excipients have been studied and optimized. The limits of detection and quantification have been determined for three methods. All the three methods have been validated as per the guidelines of ICH. The methods have been applied to the determination of drug in commercial tablets and results of analysis were validated statistically through recovery studies.

**Key words:** Sibutramine hydrochloride, Bromothymol blue, Bromophenol blue, Bromocresol green, Spectrophotometry.

### **INTRODUCTION**

Sibutramine hydrochloride monohydrate (SHM), with IUPAC name as (±)-dimethyl-1-[1-(4-chlorophenyl)cyclobutyl]-*N,N*,3-trimethylbutan-1-amine, is an effective serotonin (5-HT) and noradrenaline (NA) re-uptake inhibitor, which acts increasing both satiety and metabolism<sup>1-4</sup>. Its satietogenic effect occurs by enhancing central 5-HT and NA functions.

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The metabolic effects are based on stimulation of thermogenesis due to the activation of the  $\beta_3$ -adrenoceptors in the adipose tissue<sup>2-4</sup> and consequently, it helps to prevent compulsive eating and inhibits the sensation of hunger in obese patients and shows a considerable effect on weight loss<sup>5</sup>.

Because of its physiological significance, the quantitative determination of this drug attracted the attention of analytical chemists and almost all analytical methods have been applied to accomplish the purpose<sup>6-13</sup>. However, methods on spectrophotometric determination of this drug involving ion pair complexes with common and versatile acidic dyes *viz.*, bromothymol blue (BTB), bromophenol blue (BPB) and bromocresol green (BCG) are not reported yet. This prompted the authors to develop extractive spectrophotometric methods for the determination of sibutramine hydrochloride using the above mentioned dyes.

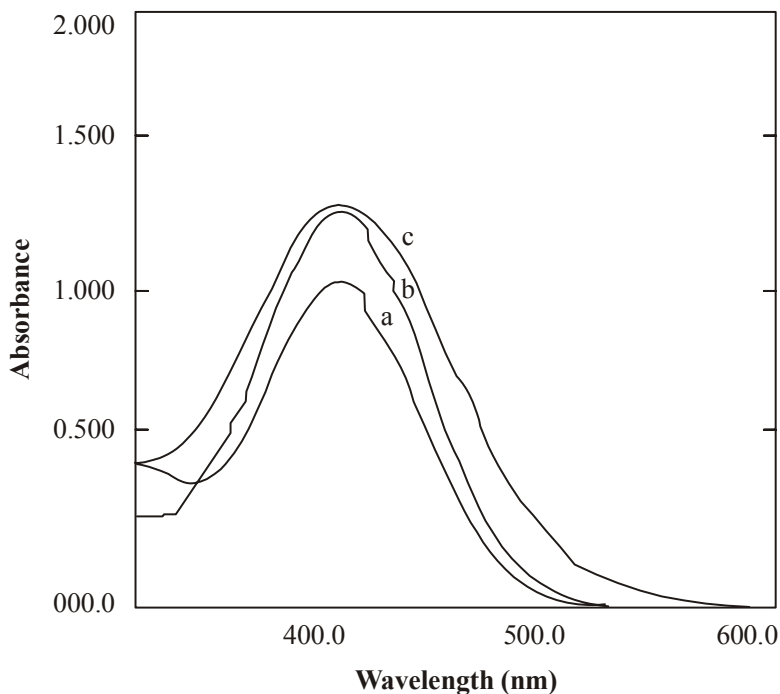
In this paper, We report three simple and sensitive extractive spectrophotometric methods for the assay of sibutramine hydrochloride. The methods are based on ion-pair complexation of drug with dyestuffs such as bromothymol blue (BTB), bromophenol blue (BPB) and bromocresol green (BCG) and subsequent extraction into chloroform and to measure the absorbance of colour complex.

## EXPERIMENTAL

### Materials and methods

Sibutramine hydrochloride is procured from Samed Labs Limited, Hyderabad as a gift sample. The dyestuffs *viz.*, BTB, BPB and BCG (AR grade) supplied by SD Fine Chemicals Ltd. Mumbai, are used without any further purification. The dyestuffs were used as 0.025% solutions in doubly distilled water. Sodium acetate-hydrochloric acid buffers<sup>14</sup> of pH 2.5, 2.8 and 3.5 were prepared by mixing 50 mL of 1.0M sodium acetate solution with 50.50, 49.50 or 46.25 mL, respectively, of 1.0 M HCl solution and diluted to 250 mL with doubly distilled water. The pH of each solution was adjusted to an appropriate value with the aid of a pH meter. Chloroform (HPLC grade) supplied by SD Fine Chemicals Ltd. Mumbai is used throughout the work. Stock solutions were prepared for all the dyes and drugs (25 mg/100 mL).

The spectra (Fig. 1) of ion-pair complexes have been recorded on Shimadzu 140 double beam spectrophotometer, Thermo Nicolet 1000 and also on ELICO 159 UV-Visible single beam spectrophotometer using quartz cells of 10 mm path length. An Elico model Li-120 pH meter was used for pH measurement.



**Fig. 1: Absorption spectra of sibutramine hydrochloride-dye complex extracted into 10 mL chloroform**

- (a) Drug =  $20 \mu\text{g mL}^{-1}$  + 5 mL of 0.025% BTB + 5 mL of pH 2.8 buffer;  
(b) Drug =  $17.5 \mu\text{g mL}^{-1}$  + 5 mL of 0.025% BPB + 5 mL of pH 2.5 buffer;  
(c) Drug =  $17.5 \mu\text{g mL}^{-1}$  + 5 mL of 0.025% BCG + 5 mL of pH 3.5 buffer

### Calibration curve

Different aliquots of drug solution were transferred into 125 mL separating funnel. To this, 5 mL of buffer (pH 2.5, 2.8 and 3.5), 5 mL of dye were added and total volume was made up to 20 mL with water. 10 mL of chloroform was added and the contents were shaken for 5 min. The two layers were allowed to separate for 5 min. The organic layer was separated and absorbance of yellow colored solution which is stable atleast for 3 hrs is measured at 415 nm against blank similarly prepared. The same procedure of analysis is followed either for assay of pure drug or for dosage form. The calibration graphs (Fig. 2) are linear over the concentration ranges within the permissible range. The optical characteristics and statistical data for the regression equation of the proposed methods are presented in Table 1.

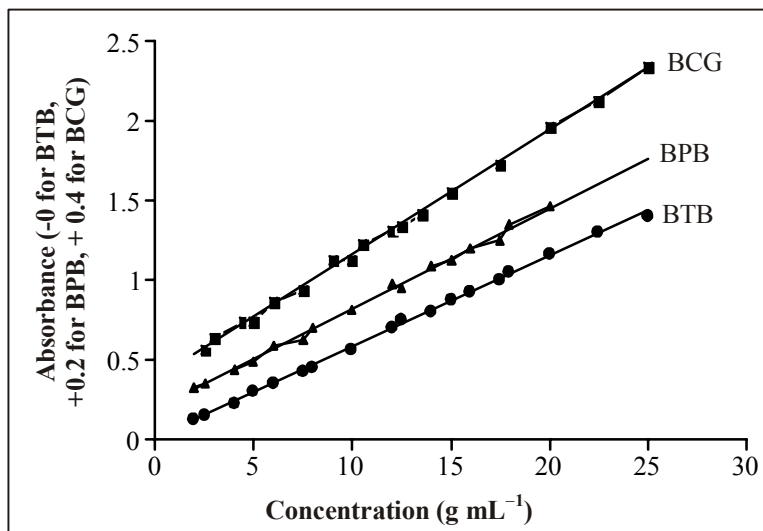


Fig. 2: Calibration graphs for drug-BTB, BPB and BCG ion-pair complexes

### Procedure for the assay of pure drug

Five different solutions of pure drug in the range of calibration curve were selected and the recovery experiments were performed. The recoveries and their relative standard deviations are tabulated in Table 2.

Table 1: Optical characteristics and statistical parameters for the regression equation of the proposed methods

Parameters	Extraction methods with		
	BTB	BPB	BCG
$\lambda_{\max}$ (nm)	415	415	415
Beer's law limit ( $\mu\text{g mL}^{-1}$ )	2.0-25	2.0-20	2.5-25
Molar absorptivity ( $\text{L mol}^{-1} \text{cm}^{-1}$ )	18223	20525	24324
Formation constant, K, $\text{M}^{-1}$	$1.54 \times 10^6$	$1.75 \times 10^6$	$1.84 \times 10^6$
Sandell sensitivity ( $\mu\text{g cm}^{-2}$ )	0.0175	0.0158	0.0128
Slope (specific absorptivity), b	0.057	0.0631	0.0782
Intercept (a)	0.00875	-0.0126	-0.0239
Correlation coefficient (r)	0.9993	0.998	0.999

Cont...

Parameters	Extraction methods with		
	BTB	BPB	BCG
Standard deviation of intercepts (% n = 6)	0.0048	0.0082	0.01277
Limit of detection, $\mu\text{g mL}^{-1}$	0.2779	0.4288	0.539
Limit of quantification, $\mu\text{g mL}^{-1}$	0.8337	1.2865	1.617
Regression equation	$Y = 0.057C + 0.00875$	$Y = 0.0631C - 0.0126$	$Y = 0.0782C - 0.02398$

<sup>a</sup>With respect to  $Y = bc + a$ , where  $C$  is the concentration ( $\mu\text{g mL}^{-1}$ ) and  $Y$  is absorbance

<sup>b</sup>Six replicate samples

**Table 2: Application of proposed methods for the analysis of Sibutramine hydrochloride in pure form**

Taken ( $\mu\text{g mL}^{-1}$ )	Proposed methods						Reference method <sup>6</sup>
	Found ( $\mu\text{g mL}^{-1}$ )			Recovery (%)			Recovery (%)
	BTB	BPB	BCG	BTB	BPB	BCG	
3.5	3.56	3.54	3.54	101.82	101.34	101.32	98.25
6.5	6.63	6.63	6.48	102.05	102.13	99.73	101.25
9.5	9.53	9.72	9.62	100.32	102.41	101.26	101.65
12.5	12.58	12.46	12.63	100.66	99.72	101.1	102.25
15.5	15.8	15.54	15.92	101.99	100.26	102.76	101.16
							100.6
							99.0
							103.58
							101.95
RSD (%)				0.8034	1.15	1.0638	1.6143
Mean $\pm$ SD				101.4 $\pm 0.81$	101.18 $\pm 1.16$	101.23 $\pm 1.07$	101.07 $\pm 1.63$
t-test				0.454	0.1377	0.2218	
F-test				0.249	0.508	0.4356	

### Procedure for the assay of dosage forms

Ten capsules of Slenfig-10 mg are powdered and dissolved in doubly distilled water, stirred thoroughly and filtered through a Whatman No. 42 filter paper. This solution was transferred into 100 mL standard volumetric flask and diluted with doubly distilled water as required. Different solutions of drug in the range of calibration curve were chosen and the assay was estimated using the calibration curve. The results of the recovery experiments are tabulated in Table 3.

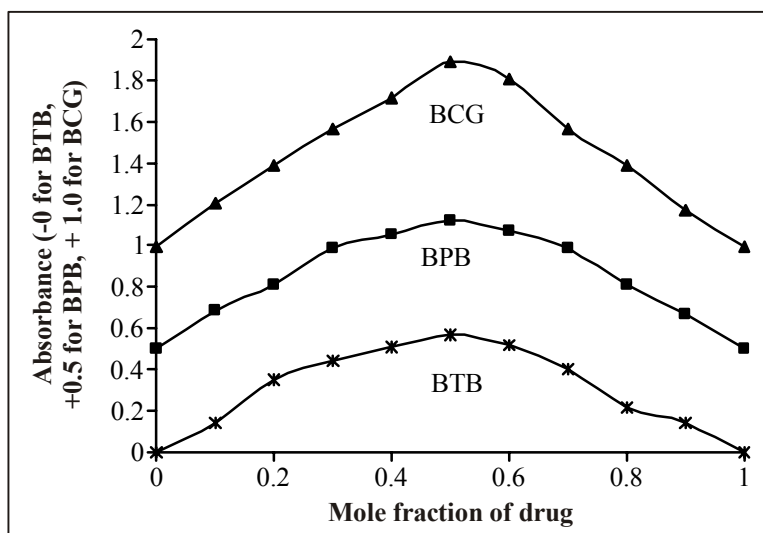
**Table 3: Application of proposed methods for the analysis of Sibutramine hydrochloride in pharmaceuticals form**

Taken ( $\mu\text{g mL}^{-1}$ )	Proposed methods						Reference method <sup>6</sup>
	Found ( $\mu\text{g mL}^{-1}$ )			Recovery (%)			Recovery (%)
	BTB	BPB	BCG	BTB	BPB	BCG	
3	3.02	3.02	3.09	100.72	100.73	103.03	98.25
6	6.12	6.08	6.07	102.08	101.36	101.3	101.25
9	9.02	9.12	9.28	100.24	101.38	103.11	101.65
12	12.33	12.26	11.96	102.78	102.2	99.73	102.25
15	15.09	15.17	15.03	100.61	101.18	100.26	101.16
							100.6
							99.0
							103.58
							101.95
RSD (%)				1.07	0.5239	1.5318	1.6143
Mean $\pm$ SD				101.29 $\pm$ 1.08	101.37 $\pm$ 0.53	101.49 $\pm$ 1.55	101.07 $\pm$ 1.63
t-test				0.2935	0.504	0.47	
F-test				0.4438	0.1059	0.907	

## RESULTS AND DISCUSSION

Sibutramine hydrochloride forms ion-pair complexes in acidic buffer with dyestuffs such as bromothymol blue (BTB), bromophenol blue (BPB) and bromocresol green (BCG) and these complexes are quantitatively extracted into chloroform. Ion-pair complexes of drug with BTB, BPB and BCG absorbed maximally at 415 nm. The reagent blank under similar conditions showed no absorption.

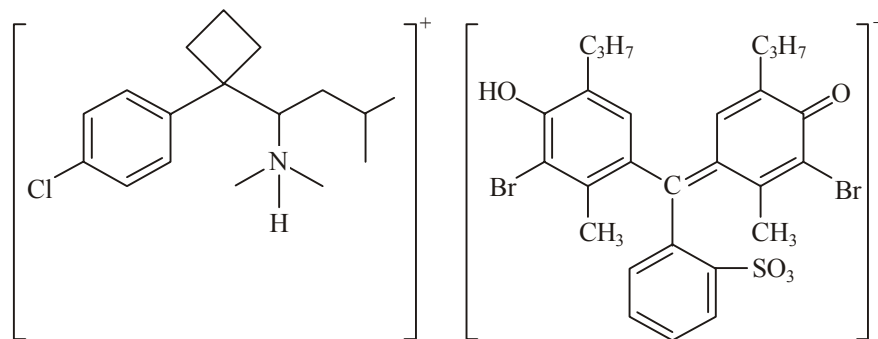
In order to establish molar ratio between sibutramine hydrochloride and dyestuffs used, the Job's method of continuous variation<sup>15</sup> has been applied. In this method, solutions of drug and dyestuff with identical molar concentrations ( $8 \times 10^{-5}M$ ) were mixed in varying volume ratios in such a way that the total volume of each mixture was the same. The absorbance of each solution was measured and plotted against the mole fraction of the drug,  $[Drug]/ [Drug] + [Dyestuff]$  (Fig. 3). This measurement showed that 1 : 1 complex was formed with each dyestuff. The formation constants<sup>16,17</sup> were also estimated and found to be  $1.54 \times 10^6$ ,  $1.75 \times 10^6$  and  $1.84 \times 10^6 K M^{-1}$  for complexes with BTB, BPB and BCG, respectively.



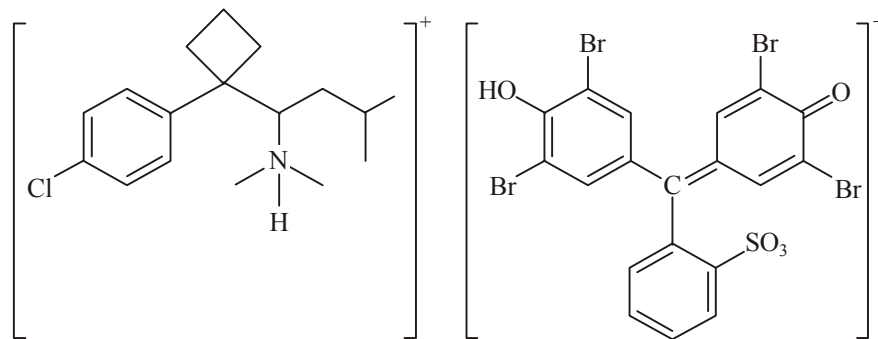
**Fig. 3: Continuous-variations study of drug-dye systems**  
**[Drug] = [Dye] =  $8 \times 10^{-5}M$**

Sibutramine hydrochloride contains tertiary amino group, which is protonated in acid medium, while sulphonic acid group is present in BTB, BPB and BCG, that is the only group undergoing dissociation in the pH range 1-5. The colour of such dyes is due to the

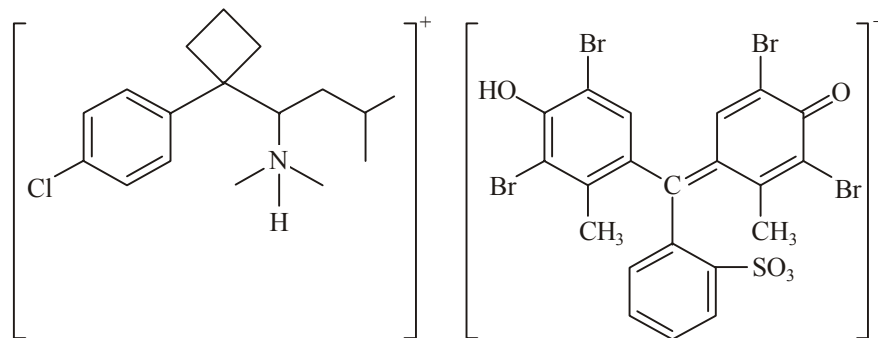
opening of lactoid ring and subsequent formation of quinoid group. It is supposed that the two tautomers are present in equilibrium but due to strong acidic nature of the sulphonic acid group, the quinoid body must predominate. Finally, the protonated Sibutramine hydrochloride forms ion-pairs with the dyestuffs which are quantitatively extracted into chloroform. The possible reaction mechanisms are proposed and given in **Scheme 1**.



Sibutramine-bromothymol blue complex



Sibutramine-bromophenol blue complex

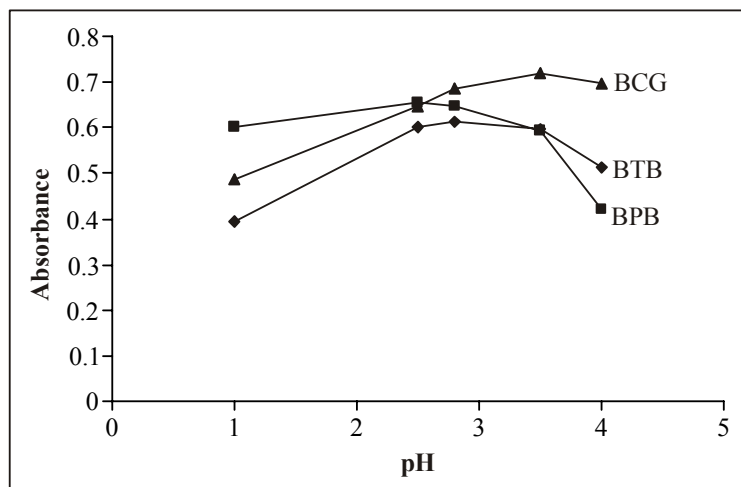


Sibutramine-bromocresol green complex

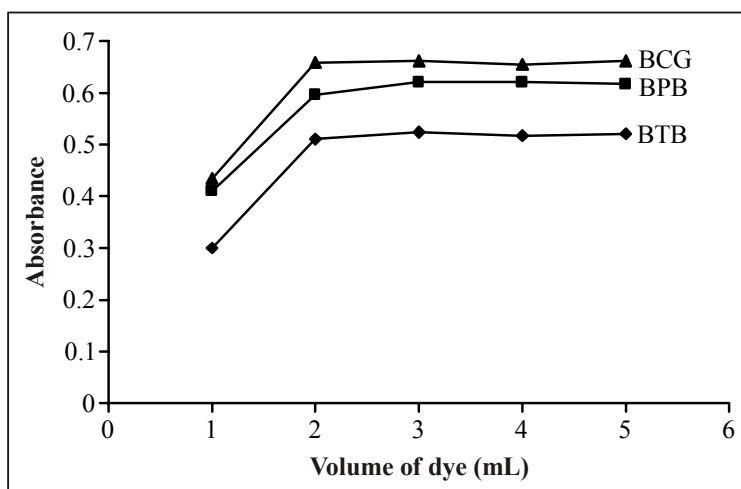
**Scheme 1: Drug-dye complex**



The influence of pH on the ion-pair formation of sibutramine hydrochloride with various dyestuffs has been studied using sodium acetate-hydrochloric acid buffer. The results are shown in Fig. 4. It is evident that absorbance of complexes with BTB, BPB and BCG was found to be constant within the pH ranges 2.2-3.3, 2.0-3.0 and 2.8-3.8, respectively. Thus, all the absorbance measurements were made at pH 2.8, 2.5 and 3.5 with BTB, BPB and BCG, respectively.



**Fig. 4: Effect of pH**  
[Drug] =  $8 \mu\text{g mL}^{-1}$ , [Dye] = 5 mL of 0.025%



**Fig. 5: Influence of the volume of 0.025% Dye**  
[Drug] =  $8 \mu\text{g mL}^{-1}$

The effect of dyestuff concentrations was also studied by adding different volumes of dyestuff to a constant amount of Sibutramine hydrochloride ( $8 \mu\text{g mL}^{-1}$ ). It is apparent from Fig. 5 that the maximum absorbance, in each case, was found with 3.0 mL of dyestuff, beyond which absorbance was constant. Thus, 5 mL of each dyestuff was used for ion-pair formation throughout the experiment.

A systematic study of the effect of foreign species present along with sibutramine hydrochloride on the determination of sibutramine hydrochloride at  $8 \mu\text{g mL}^{-1}$  levels was undertaken. This study was carried out by following the proposed procedures for a 10 mL sample system, by adding a known amount of foreign species to a sibutramine hydrochloride solution of  $8 \mu\text{g mL}^{-1}$ . Table 4 summarizes the results obtained. However, the drug content from the powdered capsules was extracted into chloroform, which completely removes any interference by the common excipients found in formulations.

**Table 4: Interference study**

Excipients ( $\mu\text{g mL}^{-1}$ )	Tolerance limit
Microcrystalline cellulose	85
Starch	165
Lactose	125
Povidone	50
Silicon dioxide	85
Titanium dioxide	30

### Validation of the proposed method

All the three proposed methods have been validated in terms of guidelines proposed by ICH<sup>18</sup> viz. selectivity, specificity, accuracy, precision, limits of calibration curve, LOD, LOQ, robustness, ruggedness and regression equation. The student t-test and variance F-test have been performed in comparison with a reference method. Table 1 summarizes the values for Beer's law limits, molar absorptivity, regression equation, correlation coefficients, relative standard deviation and recoveries. To test the reproducibility of the proposed methods, six replicate determinations of  $10 \mu\text{g mL}^{-1}$  of Sibutramine hydrochloride were made. The coefficient of variation was found to be less than 1.2% for all the procedures.

The proposed methods have been successfully applied to the determination of Sibutramine hydrochloride in pharmaceutical preparations. The performance order of the

proposed methods is BCG > BPB > BTB. The results obtained shown in Table 2 and Table 3 were compared to those obtained by a reference method<sup>6</sup> by means of *t*-test at 95% confidence level. In all cases, the average results obtained by proposed methods and reference method were statistically identical, as the difference between the average values had no significance at 95% confidence level.

The proposed methods are simple, sensitive and reproducible and can be used for routine analysis of Sibutramine hydrochloride in pure form and in formulation.

## CONCLUSION

Sibutramine hydrochloride formed ion-pair complexes with acidic dyes with 1 : 1 composition and extractable in chloroform for assay of drug. The method is validated and applied to pharmaceuticals.

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