A Validated UV- Visible Spectrophotometric Method for Simultaneous Estimation of Zolpidem Tartrate and Sibutramine Hydrochloride in Pharmaceutical Dosage form

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Abstract **- The proposed methods are simple, sensitive, rapid, reproducible, with good precision and accuracy needs no heating, colour development is instantaneous, color developed is stable for more than 24hours. Further, the controlling of experimental conditions is minimum. Various Spectrophotometric methods are available in the literature for estimation of drugs by ion association complex method. No method is reported in the literature for estimation of the selected drugs by using proposed dyes. The selectivity and sensitivity of the spectrophotometric methods depends only on the nature of chemical reactions involved in color development and not on the sophistications of the experiment. UV and Visible spectrophotometric methods are highly versatile, sensitive. Hence it is recommended to use proposed dyes for the estimation of the selected drugs by spectrophotometry. These methods can be used for routine determination of ZLPT and SBT in bulk samples and in pharmaceutical formulations.**

Index Terms **- UV-Visible Spectrocopic Method; Zolpidem Tartrate; Sibutramine HCL;0.1 N HCl.**

INTRODUCTION

Drugs and pharmaceuticals play a very significant role in the present days for the prevention, control and curing of different kinds of human diseases. It is a common observation and the practical truth that a single drug of a particular composition is marketed in various brand names by different manufactures. The possibility of minor changes in chemical composition and standard of the drug will have a profound effect on the physiological and biological activities of the patient. It is very much painful in the present day's scientist in general and to the analytical pharmaceutical chemist in particular to note in about entry of the spurious and substandard drugs into market, which definitely will have an adverse effect on the human beings at large. It is with this challenge in mind, a thorough investigations had done to evaluate the purity of the various drugs released into the market. An extensive survey of the chemical and biochemical literature to know whether the reports involving simple experimental techniques such as the Spectro photometric techniques are available for ascertaining the assay and purity of the drugs.

Various instrumental techniques (HPLC, GC, Fluorimetry, NMR, IR, UV and Visible Spectro photometric techniques) are available in the literature for the assay of drugs. These methods are either expensive or do not give reproducible results. Usually, Spectrophotometric technique is simple and less expensive. The selectivity and sensitivity of the Spectrophotometric methods depend only on the nature of chemical reactions involved in color development and not on the sophistication of experiment.

1) Zolpidem Tartrate:

Zolpidem Tartrate is a prescription medication used for the short-term treatment of insomnia, as well as some brain disorders. It is a short-acting nonbenzodiazepine hypnotic that potentiates gamma-aminobutyric acid (GABA), aninhibitory neurotransmitter, by binding to gamma-aminobutyric acid (GABAa) receptors at the same location as

benzodiazepines. It works quickly (usually within 15 minutes) and has a short half-life (2-3 hours).

Molecular structure:

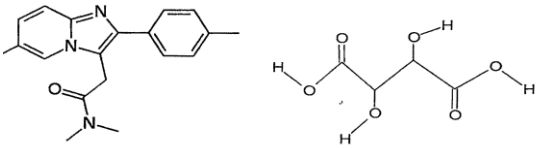

Fig.no .1. Molecular structure of Zolpidem Tartrate

N,N,6-Trimethyl-2-(4-methylphenyl)-irnidazo[1,2-a]pyridine- 3-acetamide. Zolpidem Tartrate is Yellowish white colored amorphous solid which is Soluble in Methanol, Ethanol, Acetonitrile. It is available under the trade name of Zolfresh - 5 mg, 10 mg.

2) Sibutramine HCL:

Sibutramine HCL, usually available as Sibutramine hydrochloride monohydrate, is an orally administered agent for the treatment of obesity, as an appetite suppressant. It is also under review by the FDA and the. European Medicines Agency. It is a centrally acting serotonin-norepinephrine reuptake inhibitor structurally related to amphetamines, although its mechanism of action is distinct.

Molecular structure:

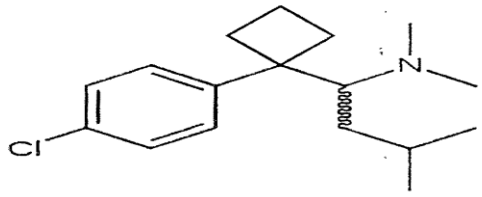

Fig.no .2. Molecular structure of Sibutramine HCL

Sibutramine HCL (±)-dimethyl-l-[l-(4- chlorophenyl) cyclobutyl]-N,N,3-trimethylbutan-1-amine.

Sibutramine HCLWhite color amorphous solid which is Soluble in Water, Methanol, Ethanol, Acetonitrile. It is available under the trade name of Obsesave - 10 mg, 15 mg

PREPARATION OF REAGENTS

All the chemicals and reagents used are of analytical grade and solutions are prepared in distilled water.

(i) TP OO solution (0.5% w/v)
(ii) BCG solution (0.5% w/v)
(iii) WFB solution (0.2% w/v)
(iv) EBT solution (O.5% w/v)
(v) HC1 solution (0.1N)
(vi) Buffer solution pH 3.5 (potassium acid phthalate – HCL)
(vii) Buffer solution (pH 1.5)
(viii) DDQ (0,1% w/v)

Preparation of standard Zolpidem Tartrate (ZLPT) drug stock solution:

The stock solution (lmg/ml^{-1}) of Zolpidem Tartrate (ZLPT) is prepared by dissolving 100 mg of drug in 100 ml of methanol. A portion of this stock solution is diluted stepwise with the methanol to obtain the working standard ZLPT drug solutions of concentrations 50 µg/ml^{-1} and 100 µg/ml^{-1}.

Preparation of standard Sibutramine HC1 (SBT) drug stock solution:

The stock solution (lmg/ml^{-1}) of Sibutramine HC1 (SBT) is prepared by dissolving 100 mg of drug in 100 ml of distilled water. A portion of this stock solution is diluted stepwise with the distilled water to obtain the working standard SBT drug solutions of concentrations 50 µg/ml^{-1} and 100 µg/ml^{-1}.

ION ASSOCIATION COMPLEX METHOD

Ion association complex is a special form of molecular complex resulting from two components extractable into organic solvents from aqueous phase at specific pH. In this ion association complex, one component is a chromogen (dye) possessing charge (cationic or anionic in nature) and so insoluble in organic solvents. The other is drug solution possessing opposite charge (anionic or' cationic in nature) to that of chromogen.

Procedure:

The drug solution (dissolved in suitable solvent), when treated with different dye solutions (TPOO, WFB, BCG, EBT) at specific pH or in acidic medium gives different colored solutions due to the formation of ion association complex. The intensity of the colored ion association complex is directly proportional to the concentration of drug solution present in the mixture. The formed colored ion association complex is extracted with chloroform using separating funnel from mixture. After systematic and detailed study of the various parameters, the proposed procedure is recommended for the determination of different drugs in bulk samples and pharmaceutical formulations. The ion association complexes can however usually be detected readily because of differences in physical

properties like color, absorption spectra, solubility in various solvents, stability of colored species etc.

METHOD-1:

Procedure;

In method-1 the drug solution (dissolved in suitable solvent), possessing secondary or tertiary aromatic amine is treated with TPOO (Tropaeoline-OO) dye in acidic medium (0.1M HC1). The resultant solution is extracted with chloroform, the formed ion association complex is present in Greenish yellow color and it is extractable in chloroform layer. The color of ion association complex is stable for more than 24 hours. The intensity of the colored ion association complex increases with increase in the concentration of drug solution. The absorbance of the extractable ion association complex is measured at the wavelength of maximum absorbance for each drug against the reagent blank (prepared in a similar manner, devoid of drug solution). After systematic study of various parameters, the amount of drug is determined from the calibration curve made between the absorbance and amount of drug (concentration of the drug).

Assay of Zolpidem Tartrate (ZLPT) by method-1:

1.0 ml of ZLPT drug solution (100µg/ml) is transferred into a separating funnel. To this drug solution, 1.0 ml of TPOO dye and 2.0 ml of 0.1 M HC1 are added. The final volume is adjusted to 8 ml with distilled water. Again 10 ml of chloroform is added and the reaction mixture is shaken gently for 5 min and allowed to stand for 5 min, so as to separate aqueous and chloroform layer. The chloroform layer is separated out. This chloroform extractable solution possesses greenish yellow color, for that absorbance is scanned in the wavelength range of 350 to 500 nm against reagent blank (prepared in similar manner, devoid of drug solution). The absorption spectrum is given in figure .3

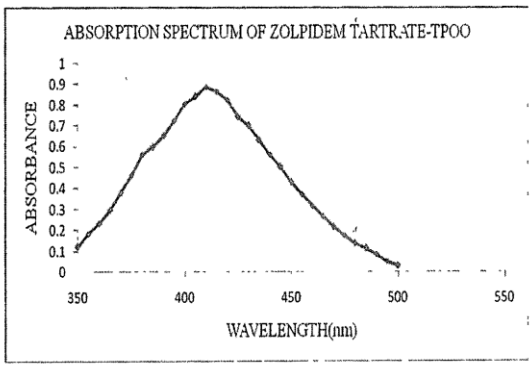

Fig.no .3.Absorption spectrum of Zolpidem Tartrate - TPOO

From the figure: 3. It is clear that the ZLPT drug solution treated with TPOO dye solution has maximum absorbance at 410 nm. Hence all further studies are made at 410 nm.

Parameters Fixation:

In developing the proposed method a systematic study of the effects of various relevant parameters in the method concerned are concentrations of reagents, temperature, time required for the reaction, stability of reagent for the colored species are undertaken by varying one parameter at a time and controlling all other Parameters to get maximum color development and minimum blank color, reproducibility and reasonable period of stability of final colored species formed/

(A) Effect of Concentration of Acid (0.1 M HCI) on Ion Association Complex:

In a series of separating funnels containing 1.0 ml of ZLPT drug solution (l00µg/mi), 1.0 ml of TPOO dye and varying amounts of 0.1 M HCI are added. The contents are made up to 8 ml with distilled water. To this 10 ml of chloroform is added, the reaction mixture is shaken gently for 5 min and allowed to stand for 5 min, so as to separate aqueous and chloroform layer. The absorbance of resultant solutions are measured at 410 nm and data is presented in the table.1

Table 1.Effect of concentration of 0.1 M HC1 on the absorbance of complex:

S. No	Volume of 0.1 M HC1	Absorbance at 410nm
1	0.5 ml	0.743
2	1.0 ml	0.924
3	1.5 ml	0.989
4	2.0 ml	0.962
5	2.5 ml	0.960

The data in the table:1. Indicates that 1.5 ml of 0.1 M HC1 is necessary for achieving maximum absorbance and hence maintained throughout the experimental studies.

(B) Effect of Concentration of TPOO Dye on Ion Association Complex:

In a series of separating funnels containing 1.0 ml of ZLPT drug solution (l00ug/ml), 1.5 ml of 0.1M HC1 and varying amounts of TPOO dye are added. The contents are made up to 8 ml with distilled water. To

this 10 ml of chloroform is added, the reaction mixture is shaken gently for 5min and allowed stand for 5min, so as to separate aqueous and chloroform layers. The absorbance of resultant solutions is measured at 410 nm and data is presented in the table:2.

Table 2.Effect of concentration of TPOO dye on the absorbance of complex:

S.No	Volume of TPOO dye	Absorbance at 410nm
1	0.5 ml	0.778
2	1.0 ml	0.802
3	1.5 ml	0.761
4	2.0 ml	0.748
5	2.5 ml	0.716

The data in the table:2. Indicates that 1.0 ml Of TPOO dye is necessary for achieving maximum absorbance and hence maintained throughout the experimental studies.

(C) Assay Procedure for Zolpidem Tartrate (ZLPT) Drug:

Aliquots of the standard ZLPT drug solution ranging from 0.5 to 2.5 ml are transferred in to a series of separating funnels. To each funnel 1.0 ml of TPOO dye and 1.5 ml of 0.1 M HC1 are added. The contents are made up to 8 ml with distilled water. To this 10 ml of chloroform is added, the reaction mixture in each separating funnel is shaken gently for 5 min and allowed to stand for 5 min. so as to separate aqueous and chloroform layers. The chloroform layer is separated out and absorbance 'is measured at 410 nm against the reagent blank (prepared in a similar manner, devoid of drug solution).

The drug calibration graph is constructed by plotting absorbance values against the concentration of ZLPT drug solution. The calibration curve is found to be linear over the concentration range of 50 -250 µg/ml for ZLPT drug. The amount of ZLPT present in the sample is estimated from the calibration graph. The data and results are presented in the table:3 and figure.4

Table 3.Effect of concentration of Drug on the absorbance of complex:

S.No	Concentration of Drug	Absorbance at 410nm
1	50 µg/ml	0.344
2	100 µg/ml	0.672
3	150 µg/ml	0.943
4	200 µg/ml	1.286
5	250 µg/ml	1.573

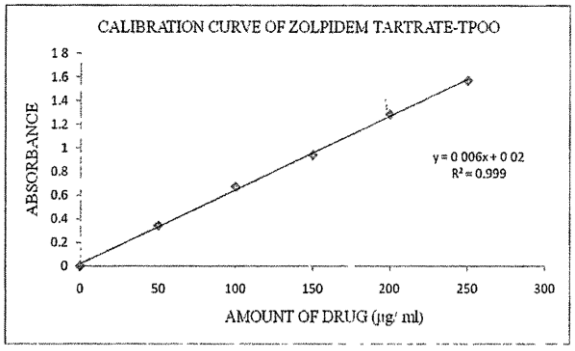

Fig.no .4. Calibration curve of Zolpidem Tartrate - TPOO

Assay of Zolpidem Tartrate (ZLPT) in Pharmaceutical Formulations:

For analysis of tablet formulation, 20 tablets of ZLPT are weighed accurately and finely powdered. An accurately weighed portion of powdered sample, equivalent to 50 mg of ZLPT was taken in a 50 ml volumetric flask containing 25 ml of methanol, sonicated for 20 min. The resultant solution is'filtered through whatsmann filter paper no: 41 in to another 50 ml volumetric flask. The filter paper was washed several times with methanol. The washings were added to the filtrate and the final volume was made up to the mark with methanol. 5 ml filtrate of the sample solution was diluted to 10ml with methanol and treated as per the procedure of Calibration curve. Amount of the drug present in the sample was computed from respective calibration curve.

Assay of Sibutramine Hc1 (SBT) By Method-1:

1.0 ml of SBT drug solution (l00µg/ml) is transferred into a separating funnel. To this drug solution, 1.0 ml of TPOO dye and 2.0 ml of 0.1 M HC1 are added. The final volume is adjusted to 8 ml with distilled water. Again 10 ml of chloroform is added and the reaction mixture is shaken gently for 5 min and allowed to stand for 5 min, so as to separate aqueous and chloroform layer. The chloroform layer is separated out. This chloroform extractable solution possesses greenish yellow color, for that absorbance is scanned in the wavelength range of 350 to 500 nm against reagent blank (prepared in similar manner, devoid of drug solution).The absorption, spectrum is given in the figure:5.

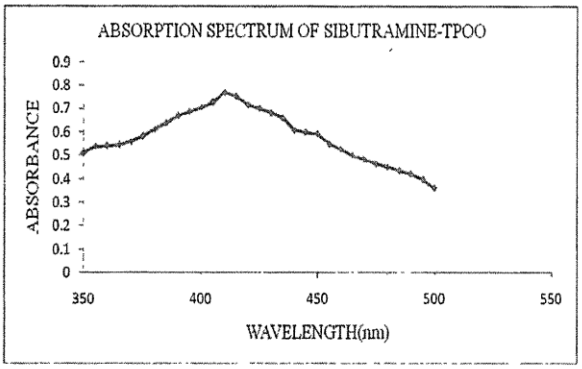

Fig.no .5. Absorption Spectrum of Sibutramine - TPOO

From the figure: 5. It is clear that the SBT drug solution treated with TPOO dye solution has maximum absorbance at 410 nm. Hence all further studies are made at 410 nm.

(A) Effect of Concentration of Acid (0.1M HCI) on Ion Association Complex:

In a series of separating funnels containing 1.0 ml of SBT drug solution (l00µg/ml), 1.0 ml of TPOO dye and varying amounts of 0.1 M HCI are added. The contents are made up to 8 ml with distilled water. To this 10 ml of chloroform is added, the reaction mixture is shaken gently for 5 min and allowed to stand for 5 min, so as to separate aqueous and chloroform layer. The absorbance of resultant solutions is measured at 410 nm and data is presented in the table:4.

Table 4.Effect of concentration of 0.1 M HCI on the absorbance of complex:

S.No	Volume of 0.1 M HCI	Absorbance at 410nm
1	0.5 ml	0.392
2	1.0 ml	0.621
3	1.5 ml	0.791
4	2.0 ml	0.823
5	2.5 ml	0.816

The data in the table:4. Indicates that 2.0 ml of 0.1 M HCI is necessary for achieving maximum absorbance and hence maintained throughout the experimental studies.

(B) Effect of Concentration of TPOO Dve on Ion Association Complex:

In a series of separating funnels containing 1.0 ml of SBT drug solution (l00µg/ml), 2.0 ml of 0.1M HC1 and varying amounts of TPOO dye are added. The contents are made up to 8 ml with distilled water. To this 10 ml of chloroform is added, the reaction mixture is shaken gently for 5min and allowed stand for 5min, so as to separate aqueous and chloroform layers. The absorbance of resultant solutions is measured at 410 nm and data is presented in the table:5.

Table 5.Effect of concentration of TPOO dye on the absorbance of complex:

S.No	Concentration of Drug	Absorbance at 410nm
1	50 µg/ml	0.784
2	100 µg/ml	0.842
3	150 µg/ml	0.838
4	200 µg/ml	0.835
5	250 µg/ml	0.828

The data in the table:5. Indicates that 1.0 ml Of TPOO dye is necessary for achieving maximum absorbance and hence maintained throughout the experimental studies.

(C) Assay Procedure for Sibutramine HC1 (SBT) Drug:

Aliquots of the standard SBT drug solution ranging from 0.5 to 2.5 ml are transferred into a series of separating funnels. To each funnel 1ml of TPOO dye and 2.0 ml of 0.1 M HC1 are added. The contents are made up to 8 ml with distilled water. To this10 ml of chloroform is added, the reaction mixture in each separating funnel is shaken gently for 5 min and allowed to stand for 5 min, so as to separate aqueous and chloroform layers. The chloroform layer is separated out and absorbance is measured at 410 nm against the reagent blank (prepared in a similar manner, devoid of drug solution).

The drug calibration graph is constructed by plotting absorbance values against the concentration of SBT drug solution. The calibration curve is found to be linear over the concentration range of 50 - 250 pg/ml for SBT drug. The amount of SBT present in the sample is estimated from the calibration graph. The data and results are presented in the table:6. and figure: 6

Table 6.Effect of concentration of Drug on the absorbance of complex:

S.No	Concentration of Drug	Absorbance at 410nm
1	50 µg/ml	0.411
2	100 µg/ml	0.757
3	150 µg/ml	1.096
4	200 µg/ml	1.458
5	250 µg/ml	1.798

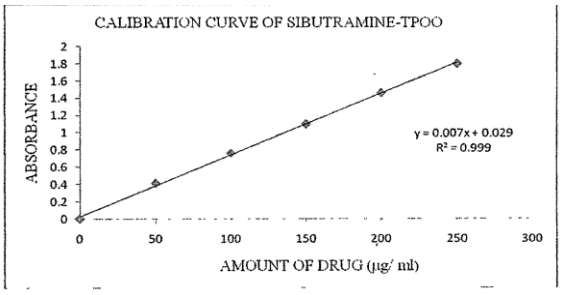

Fig.no .6. Calibration curve of Sibutramine – TPOO

Assay of Sibutramine HCL (SBT) in Pharmaceutical Formulations:

For analysis of tablet formulation, 20 tablets of SBT are weighed accurately and finely powdered. An accurately weighed portion of powdered sample, equivalent to 50 mg of SBT was taken in a 50 ml volumetric flask containing 25 ml of methanol, sonicated for 20 min. The resultant solution is filtered through whatmann filter paper no: 41 in to another 50 ml volumetric flask. The filter paper was washed several times with methanol. The washings were added to the filtrate and the final volume was
made up to the mark with methanol. 5 ml filtrate of the sample solution was diluted to 10 ml with methanol and treated as per the procedure of calibration curve. Amount of the drug present in the sample was computed from respective calibration curve.

Method-2

Procedure:

In Method-2 the drug solution (dissolved in suitable solvent), possessing secondary or tertiary aromatic amine is treated with BCG (Bromo Cresol Green) dye at 3.5 pH. The resultant solution is extracted with chloroform, the formed ion association complex is present in yellow color and it is extractable in chloroform layer. The color of ion association complex is stable for more than 24 hours. The intensity of the colored ion association complex increases with increase in the concentration of drug solution.

The absorbance of the extractable ion association complex is measured at the wavelength of maximum absorbance for each drug against the reagent blank (prepared in a similar manner, devoid of drug solution). After systematic study of various parameters, the amount of drug is determined from the calibration curve made between the absorbance and amount of drug (concentration of the drug).

Assay of zolpidem tartrate (zlpt) by method-2:

The method M-2 is based on the reaction of ZLPT drug as a tertiary aromatic amine with BCG (Bromo Cresol Green) dye at 3.5 pH. The formed ion association complex is present in yellow color and it is extracted with chloroform. The chloroform extractable layer is used to determine ZLPT drug spectrophotometrically.

Spectrum of Zolpidem Tartrate (ZLPT) - BCG Complex:

1.0 ml of ZLPT drug solution (l00µg/ml) is transferred in to a separating funnel. To this drug solution 1.0 ml of BCG dye and 2.0 ml of 3.5 pH (Buffer Solution) are added. The final volume is adjusted to 8 ml with distilled water. Again 10 ml of chloroform is added and the reaction mixture is shaken 'gently for 5 min and allowed to stand for 5 min, so as to separate aqueous and chloroform layer. The chloroform layer is separated out. This chloroform extractable solution possesses yellow color, for that absorbance is scanned in the wave length range of 350 to 500 nm against reagent blank (prepared in similar manner, devoid of drug solution). The absorption spectrum is given in the figure:7

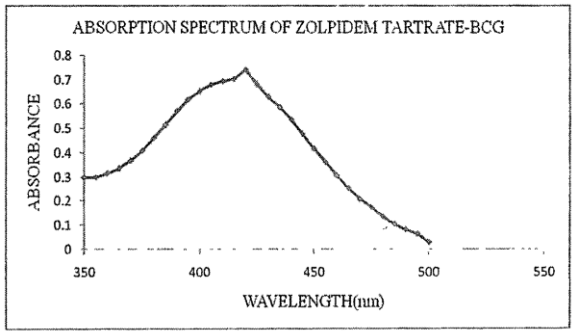

Fig.no .7. Absorption spectrum of Zolpidem Tartrate - BCG

From the figure:7 It is clear that the ZLPT drug solution treated with BCG dye solution has maximum absorbance at 420 nm. Hence all further studies are made at 420 nm.

(A) Effect of Concentration of Buffer Solution on Ion Association Complex:

In a series of separating funnels containing 1.0 ml of ZLPT drug solution (l00µg/ml), 1.0 ml of BCG dye and varying amounts of 3.5 pH (Buffer Solution) are added. The contents are made up to 8 ml with distilled

water. To this 10 ml of chloroform is added, the reaction mixture is shaken gently for 5 min and allowed to stand for 5 min, so as to separate aqueous and chloroform layer. The absorbance of resultant solutions are measured at 420 nm and data is presented in the table:7.

Table 7.Effect of concentration of buffer solution on the absorbance of complex:

S.No	Volume of Buffer solution	Absorbance at 420nm
1	0.5 ml	0.795
2	1.0 ml	0.968
3	1.5 ml	0.960
4	2.0 ml	0.962
5	2.5 ml	0.963

The data in the table:7 Indicates that 1.0 ml of 3.5 pH is necessary for achieving maximum absorbance and hence maintained throughout the experimental studies.

(B) Effect of Concentration of BCG Dve on Ion Association Complex:

In a series of separating funnels containing 1.0 ml of ZLPT drug solution (l00µg/ml),1.5 ml of 3.5 pH (Buffer Solution) and varying amounts of BCG dye are added. The contents are made up to 8 ml with distilled water. To this 10 ml of chloroform is added, the reaction mixture is shaken gently for 5min and allowed stand for 5min, so as to separate aqueous and chloroform layers. The absorbance of resultant solutions is measured at 420 nm and data is presented in the table:8.

Table 8.Effect of concentration of BCG dye on the absorbance of complex:

S.no	Volume of BCG dye	Absorbance at 420nm
1	0.5 ml	0.827
2	1.0 ml	0.946
3	1.5 ml	0.939
4	2.0 ml	0.942
5	2.5 ml	0.942

The data in the table: 8.Indicates that 1.0 ml of BCG dye is necessary for achieving maximum absorbance and hence maintained throughout the experimental studies.

(C) Assay Procedure for Zolpidem Tartrate (ZLPT) Drug:

Aliquots of the standard ZLPT drug solution ranging from 0.5 to 2.5 ml are transferred into a series of separating funnels. To each funnel 1.0 ml of BCG dye and 1.0 ml of 3.5 pH (Buffer Solution) are added. The contents are made up to 8 ml with distilled water. To this 10 ml of chloroform is added, the reaction mixture in each separating funnel is shaken gently for 5 min and allowed to stand for 5 min, so as to separate aqueous and chloroform layers. The chloroform layer is separated out and absorbance is measured at 420 nm against the reagent blank (prepared in a similar manner, devoid of drug solution).

The drug calibration graph is constructed by plotting absorbance values against the concentration of ZLPT drug solution. The calibration curve is found to be linear over the concentration range of 50 - 250 pg/ml for ZLPT drug. The amount of ZLPT present in the sample is estimated from the calibration graph. The data and results are presented in the table:9. and figure: 8

Table 9.Effect of concentration of Drug on the absorbance of complex:

S.No	Concentration of Drug	Absorbance at 410nm
1	50 µg/ml	0.399
2	100 µg/ml	0.805
3	150 µg/ml	1.155
4	200 µg/ml	1.524
5	250 µg/ml	1.889

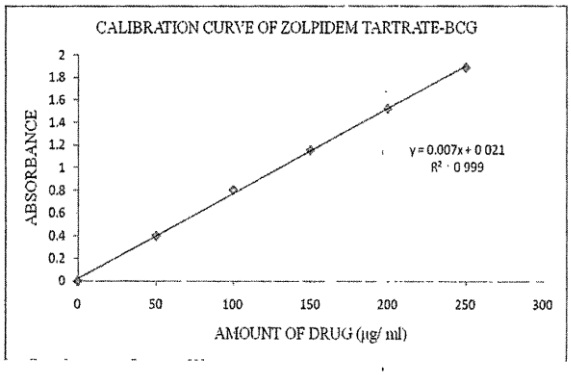

Fig.no .8. Calibration curve of Zolpidem Tartrate - BCG

Assay of Zolpidem Tartrate (ZLPT) in Pharmaceutical Formulations:

For analysis of tablet formulation, 20 tablets of ZLPT are weighed accurately and finely powdered. An accurately weighed portion of powdered sample, equivalent to 50 mg of ZLPT was taken in a 50 ml volumetric flask containing 25 ml of methanol, sonicated for 20 min. The resultant solution is filtered through whatmann filter paper no: 41 in to another 50

ml volumetric flask. The filter paper was washed several times with methanol. The washings were added to the filtrate and the final volume was made up to the mark with methanol. 5 ml filtrate of the sample solution was diluted to 10 ml with methanol and treated as per the procedure of calibration curve. Amount of the drug present in the sample was computed from respective calibration curve.

ASSAY OF SIBUTRAMINE HCI (SBT) BY METHOD-2:

The Method-2 is based on the reaction of SBT drug as a tertiary aromatic amine with BCG (Bromo Cresol Green) dye at 3.5 pH. The formed ion association complex is present in yellow color and it is extracted with chloroform. The chloroform extractable layer is used to determine SBT drug spectrophotometrically.

Spectrum of Sibutramine HCI (SBT) - BCG Complex:

1.0 ml of SBT drug solution (l00µg/ml) is transferred in to a separating funnel. To this drug solution 1.0 ml of BCG dye and 2.0 ml of 3.5 pH (Buffer Solution) are added. The final volume is adjusted to 8 ml with distilled water. Again 10 ml of chloroform is added and the reaction mixture is shaken gently for 5 min and allowed to stand for 5 min, so as to separate aqueous and chloroform layer. The chloroform layer is separated out. This chloroform extractable solution possesses yellow color, for that absorbance is scanned in the wave length range of 350 to 500 nm against reagent blank (prepared in similar manner, devoid of drug solution).The absorption spectrum is given in the figure: 9.

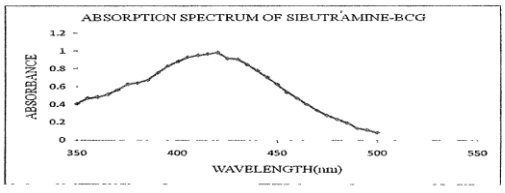

Fig.no .9. Absorption Spectrum of Sibutramine -BCG

From the figure:9. It is dear that the SBT drug solution treated with BCG dye solution has maximum absorbance at 420 nm. Hence all further studies are made at 420 nm.

(A) Effect of Concentration of Buffer Solution on Ion Association Complex:

In a series of separating funnels containing 1.0 ml of SBT drug solution (100µg/ml), 1.0 ml of BCG dye and varying amounts of 3.5 pH (Buffer Solution) are added. The contents are made up to 8 ml with distilled water. To this 10 ml of chloroform is added, the reaction mixture is shaken gently for 5 min and allowed to stand for 5 min, so as to separate aqueous and chloroform layer. The absorbance of resultant solutions are measured at 420 nm and data is presented in the table:10.

Table 10.Effect of concentration of buffer solution on the absorbance of complex:

S.No	Volume of Buffer solution	Absorbance at 420nm
1	0.5 ml	0.782
2	1.0 ml	0.947
3	1.5 ml	0.942
4	2.0 ml	0.943
5	2.5 ml	0.938

The data in the table: 10. Indicates that 1.0 ml of 3.5 pH is necessary for achieving maximum absorbance and hence maintained throughout the experimental studies.

(B) Effect of Concentration of BCG Dve on Ion Association Complex:

In a series of separating funnels containing 1.0 ml of SBT drug solution (100µg/ml), 1.5 ml of 3.5 pH (Buffer Solution) and varying amounts of BCG dye are added. The contents are made up to 8 ml with distilled water. To this 10 ml of chloroform is added, the reaction mixture is shaken gently for 5min and allowed stand for 5min, so as to separate aqueous and chloroform layers. The absorbance of resultant solutions are measured at 420 nm and data is presented in the table:11.

Table 11.Effect of concentration of BCG dye on the absorbance of complex:

S.No	Volume of BCG Dye	Absorbance at 420nm
1	0.5 ml	0.722
2	1.0 ml	0.931
3	1.5 ml	0.928
4	2.0 ml	0.925
5	2.5 ml	0.925

The data in the table:11. Indicates that 1.0 ml Of BCG dye is necessary for achieving maximum absorbance and hence maintained throughout the experimental studies.

(C) Assay Procedure for Sibutramine HCL (SBT) Drug:

Aliquots of the standard SBT drug solution ranging from 0.5 to 2.5 ml are transferred into a series of separating funnels. To each funnel 1.0 ml of BCG dye and 1.0 ml of 3.5 pH (Buffer Solution) are added. The contents are made up to 8 ml with distilled water. To this 10 ml of chloroform is added, the reaction mixture in each separating funnel is shaken gently for 5 min and allowed to stand for 5 min, so as to separate aqueous and chloroform layers. The chloroform layer is separated out and absorbance is measured at 420 nm against the reagent blank (prepared in a similar manner, devoid of drug solution).

The drug calibration graph is constructed by plotting absorbance values against the concentration of SBT drug solution. The calibration curve is found to be linear over the concentration range of 50 – 250 µg/ml for SBT drug. The amount of SBT present in the sample is estimated from the calibration graph. The data and results are presented in the table:12. and figure: 10.

Table 12.Effect of concentration of Drug on the absorbance of complex:

S.No	Concentration of Drug	Absorbance at 410nm
1	50 µg/ml	0.395
2	100 µg/ml	0.732
3	150 µg/ml	1.089
4	200 µg/ml	1.409
5	250 µg/ml	1.743

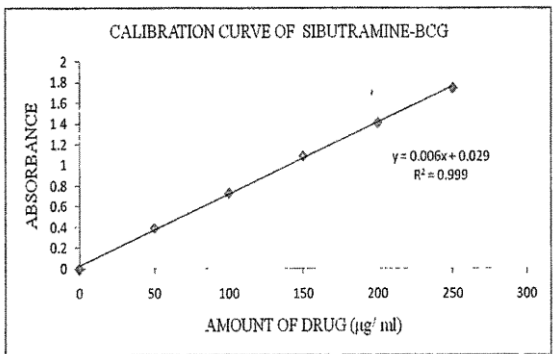

Fig.no .10. Calibration curve of Sibutramine –BCG

Assay of Sibutramine HC1 (SBT) in Pharmaceutical Formulations:

For analysis of tablet formulation, 20 tablets of SBT are weighed accurately and finely powdered. An accurately weighed portion of powdered sample, equivalent to 50 mg of SBT was taken in a 50 ml volumetric flask containing 25 ml of methanol, sonicated for 20 min. The resultant solution is filtered through whatmann filter paper no: 41 in to another 50 ml volumetric flask. The filter paper was washed several times with methanol. The washings were added to the filtrate and the final volume was made up to the mark with methanol. 5 ml filtrate of the sample solution was diluted to 10 ml with methanol and treated as per the procedure of calibration curve. Amount of the drug present in the sample was computed from respective calibration curve.

METHOD-3

Procedure:

In Method-3 the drug solution (dissolved in suitable solvent), possessing secondary or tertiary aromatic amine is treated with WFB (Wool Fast Blue) dye at 1.5 pH. The resultant solution is extracted with chloroform, the formed ion association complex is present in blue color and it is extractable in chloroform layer. The color of ion association complex is stable for more than 24 hours. The intensity of the colored ion association complex increases with increase in the concentration of drug solution. The absorbance of the extractable ion association complex is measured at the wavelength of maximum absorbance for each drug against the reagent blank. The amount of drug is determined from the calibration curve made between the absorbance and amount of drug (concentration of the drug).

ASSAY OF ZOLPIDEM TARTRATE (ZLPT) BY METHOD-3:

The Method-3 is based on the reaction of ZLPT drug as a tertiary aromatic amine with WFB (Wool Fast Blue) dye at 1.5 pH. The formed ion association complex is present in blue color and it is extracted with chloroform. The chloroform extractable layer is, used to determine ZLPT drug spectrophotometrically.

Spectrum of Zolpidem Tartrate (ZLPT) - WFB Complex:

1.0 ml of ZLPT drug solution (100µ|g/ml) is transferred into a separating funnel. To this drug solution, 1.0 ml of WFB dye and 2.0 ml of 1.5 pH (Buffer Solution) are added. The final volume is adjusted to 8 ml with distilled water. Again 10 ml of chloroform is added and the reaction mixture is shaken gently for 5 min and allowed to stand for 5 min, so as

to separate aqueous and chloroforin layer. The chloroform layer is separated out. This chloroform extractable solution possesses blue color, for that absorbance is scanned in the wavelength range of 500 to 650 nm against reagent blank (prepared in similar manner, devoid of drug soiution).The absorption spectrum is given in the figure:11

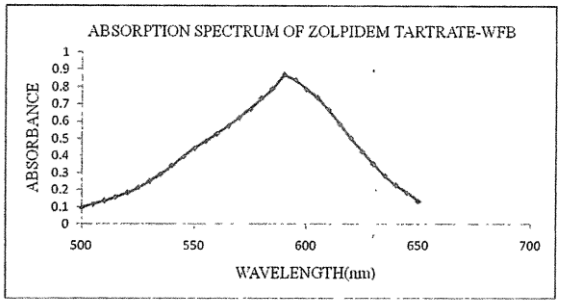

Fig.no .11. Absorption spectrum of Zolpidem Tartrate -WFB

From the figure: 11. It is clear that the ZLPT drug solution treated with WFB dye solution has maximum absorbance at 590 nm. Hence all further studies are made at 590 nm.

(A) Effect of Concentration of Buffer Solution on Ion Association Complex:

In a series of separating funnels containing 1.0 ml of ZLPT drug solution (l00ug/ml), 1.0ml of WFB dye and varying amounts of 1.5 pH (Buffer Solution) are added. The contents are made up to 8 ml with distilled water. To this 10 ml of chloroform is added, the reaction mixture is shaken gently for 5 min and allowed to stand for 5 min, so as to separate aqueous and chloroform layer. The absorbance of resultant solutions is measured at 590 nm and data is presented in the table:13.

Table 13.Effect of concentration of Buffer Solution on the Absorbance of complex:

S.No	Volume of Buffer solution	Absorbance at 590nm
1	0.5 ml	0.404
2	1.0 ml	0.496
3	1.5 ml	0.669
4	2.0 ml	0.847
5	2.5 ml	0.838

The data in the table:13. Indicates that 2.0 ml of 1.5 pH (Buffer Solution) is necessary for achieving maximum absorbance and hence maintained throughout the experimental studies.

(B) Effect of Concentration of WFB Dye on Ion Association Complex:

In a series of separating funnels containing 1.0 ml of ZLPT drug solution (l00µg/ml), 2.0 ml of 1.5 pH (Buffer Solution) and varying amounts of WFB dye are added. The contents are made up to 8 ml with distilled water. To this 10 ml of chloroform is added, the reaction mixture is shaken gently for 5min and allowed stand for 5min, so as to separate aqueous and chloroform layers. The absorbance of resultant solutions is measured at 590nm and data is presented in the table:14.

Table 14.Effect of concentration of WFB dye on the absorbance of complex:

S.No	Volume of WFB Dye	Absorbance at 590nm
1	0.5 ml	0.806
2	1.0 ml	0.898
3	1.5 ml	0.891
4	2.0 ml	0.895
5	2.5 ml	0.893

The data in the table:14. Indicates that 1.0 ml Of WFB dye is necessary for achieving maximum absorbance and hence maintained throughout the experimental studies.

(C) Assay Procedure for Zolpidem Tartrate (ZLPT) Drug:

Aliquots of the standard ZLPT drug solution ranging from 0.5 to 2.5 ml are transferred in to a series of separating funnels. To each funnel 1.0 ml of WFB dye and 2.0 ml of 1.5 pH (Buffer Solution) are added. The contents are made up to 8 ml with distilled water. To this 10 ml of chloroform is added, the reaction mixture in each separating funnel is shaken gently for 5 min and allowed to stand for 5 min, so as to separate aqueous and chloroform layers. The chloroform layer is separated out and absorbance is measured at 590 nm against the reagent blank (prepared in a similar manner, devoid of drug solution).

The drug calibration graph is constructed by plotting absorbance values against the concentration of ZLPT drug solution. The calibration curve is found to be linear over the concentration range of 50 - 250 pg/ml for ZLPT drug. The amount of ZLPT present in the sample is estimated from the calibration graph. The data and results are presented in the table:15 and figure:12.

Table 15.Effect of concentration of Drug on the absorbance of complex:

S.No	Concentration of Drug	Absorbance at 590nm
1	50 µg/ml	0.402
2	100 µg/ml	0.788
3	150 µg/ml	1.165
4	200 µg/ml	1.559
5	250 µg/ml	1.996

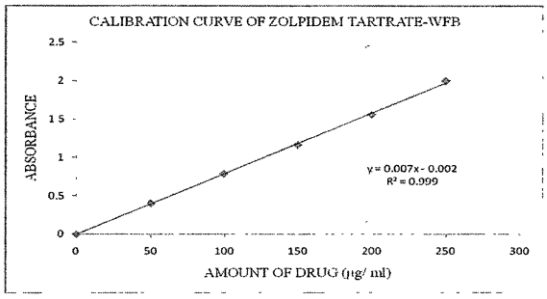

Fig.no .12. Calibration curve of Zolpidem Tartrate – WFB

Assay of Zolpidem Tartrate (ZLPT) in Pharmaceutical Formulations:

For analysis of tablet formulation, 20 tablets of ZLPT are weighed accurately and finely powdered. An accurately weighed portion of powdered sample, equivalent to 50 mg of ZLPT was taken in a 50 ml volumetric flask containing 25 ml of methanol, sonicated for 20 min. The resultant solution is filtered through whatmann filter paper no: 41 in to another 50 ml volumetric flask. The filter paper was washed several times with methanol. The washings were added to the filtrate and the final volume was made up to the mark with methanol. 5 ml filtrate of the sample solution was diluted to 10 ml with methanol and treated as per the procedure of calibration curve. Amount of the drug present in the sample was computed from respective calibration curve.

Assay of sibutramine HC1 (SBT) by method-3:

The Method-3 is based on the reaction of SBT drug as a tertiary aromatic amine with WFB (Wool Fast Blue) dye at 1.5 pH. The formed ion association complex is present in blue color and it is extracted with chloroform. The chloroform extractable layer is used to determine SBT drug spectrophotometrically.

Spectrum of Sibutramine HCI (SBT) - WFB Complex:

1.0 ml of SBT drug solution (l00µg/ml) is transferred in to a separating funnel. To this drug solution 1.0 ml of WFB dye and 2.0 ml of 1.5 pH (Buffer Solution) are added. The final volume is adjusted to 8 ml with distilled water. Again 10 ml of chloroform is added and the reaction mixture is shaken gently for 5 min and allowed to stand for 5 min, so as to separate aqueous and chloroform layer. The chloroform layer is separated out. This chloroform extractable solution possesses blue color, for that absorbance is scanned in the wavelength range of 500 to 650 nm against reagent blank (prepared in similar manner, devoid of drug solution).The absorption spectrum is given in the figure: 13.

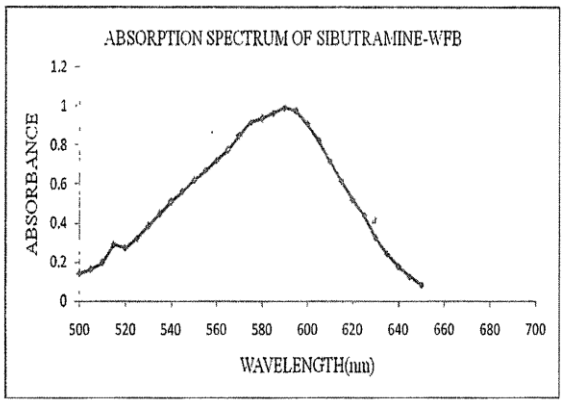

Fig.no .13. Absorption Spectrum of Sibutramine – WFB

From the figure:13. It is clear that the SBT drug solution treated with WFB dye solution has maximum absorbance at 590 nm. Hence all further studies are made at 590 nm.

(A) Effect of Concentration of Buffer Solution on Ion Association Complex:

In a series of separating funnels containing 1.0 ml of SBT drug solution (l00µg/ml), 1.0ml of WFB dye and varying amounts of 1.5 pH (Buffer Solution) are added. The contents are made up to 8 ml with distilled water. To this 10 ml of chloroform is added, the reaction mixture is shaken gently for 5 min and allowed to stand for 5 min, so as to separate aqueous and chloroform layer. The absorbance of resultant solutions is measured at 590 nm and data is presented in the table:16

Table 16.Effect of concentration of Buffer Solution on the Absorbance of complex:

S.No	Volume of Buffer solution	Absorbance at 590nm
1	0.5 ml	0.694
2	1.0 ml	0.847
3	1.5 ml	0.963
4	2.0 ml	0.948
5	2.5 ml	0.932

The data in the table:16. Indicates that 1.5 ml of 1.5 pH (Buffer Solution) is necessary for achieving maximum absorbance and hence maintained throughout the experimental studies.

(B) Effect of Concentration of WFB Dve on Ion Association Complex:

In a series of separating funnels containing 1.0 ml of SBT drug solution (l00µg/ml), 1.5 ml of 1.5 pH (Buffer Solution) and varying amounts of WFB dye are added. The contents are made up to 8 ml with distilled water. To this 10 ml of chloroform is added, the reaction mixture is shaken gently for 5min and allowed stand for 5min, so as to separate aqueous and chloroform layers. The absorbance of resultant solutions are measured at 590 nm and data is presented in the table:17.

Table 17.Effect of concentration of WFB dye on the absorbance of complex:

S.No	Volume of WFB Dye	Absorbance at 590nm
1	0.5 ml	0.862
2	1.0 ml	0.964
3	1.5 ml	0.959
4	2.0 ml	0.957
5	2.5 ml	0.960

The data in the table:17. Indicates that 1.0 ml Of WFB dye is necessary for achieving maximum absorbance and hence maintained throughout the experimental studies.

(C) Assay Procedure for Sibutramine HC1 (SBT) Drug:

Aliquots of the standard SBT drug solution ranging from 0.5 to 2.5 ml are transferred into a series of separating funnels. To each funnel 1.0 ml of WEB dye and 1.5ml of 1.5 pH (Buffer Solution) are added. The contents are made up to 8 ml with distilled water. To this 10 ml of chloroform is added, the reaction mixture in each separating funnel is shaken gently for 5 min and allowed to stand for 5 min, so as to separate aqueous and chloroform layers. The chloroform layer is separated out and absorbance is measured at 590 nm against the reagent blank (prepared in a similar manner, devoid of drug solution). The drug calibration graph is constructed by plotting absorbance values vs against the concentration of SBT drug solution. The calibration curve is found to be linear over the concentration range of 50 - 250 pg/ml for SBT drug. The amount of SBT present in the sample is estimated from the calibration graph. The data and results are presented in the table:18. and figure:14

Table 18.Effect of concentration of Drug on the absorbance of complex:

S.No	Concentration of Drug	Absorbance at 590nm
1	50 µg/ml	0.409
2	100 µg/ml	0.789
3	150 µg/ml	1.134
4	200 µg/ml	1.479
5	250 µg/ml	1.854

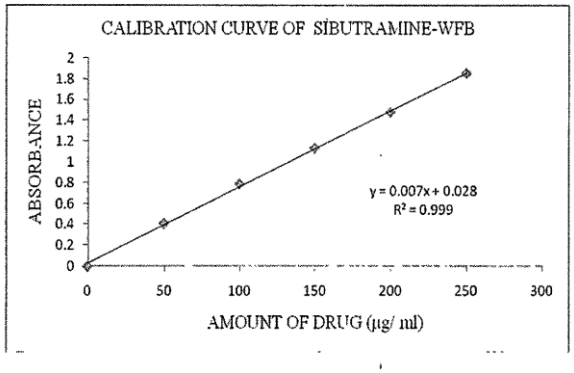

Fig.no .14. Calibration curve of Sibutramine - WFB

Assay of Sibutramine HC1 (SBT) in Pharmaceutical Formulations:

For analysis of tablet formulation, 20 tablets of SBT are weighed accurately and finely powdered. An accurately weighed portion of powdered sample, equivalent to 50 mg of SBT was taken in a 50 ml volumetric flask containing 25 ml of methanol, sonicated for 20 min. The resultant solution is filtered through whatmann filter paper no: 41 in to another 50 ml volumetric flask. The filter paper was washed several times with methanol. The washings were added to the filtrate and the final volume was made up to the mark with methanol. 5 ml filtrate of the sample solution was diluted to 10 ml with methanol and treated as per the procedure of calibration curve. Amount of the drug present in the sample was computed from respective calibration curve.

METHOD-4

Procedure;

In Method-4 the drug solution (dissolved in suitable solvent), possessing secondary or tertiary amine is treated with EBT (Eriochrom Black-T) dye at 1.5 pH. The resultant solution is extracted with chloroform, the formed ion association complex is present in purple color and it is extractable in chloroform layer.

The color of ion association complex is stable for more than 24 hours. The intensity of the colored ion association complex increases with increase in the concentration of drug solution. The absorbance of the extractable ion association complex is measured-at the wavelength of maximum absorbance for each drug against the reagent blank (prepared in a similar manner, devoid of drug solution). After systematic study of various parameters, the amount of drug is determined from the calibration curve made between the absorbance and amount of drug (concentration of the drug).

ASSAY OF ZOLPIDEM TARTRATE (ZLPT) BY METHOD-4:

The Method-4 is based on the reaction of ZLPT drug as a tertiary amine with EBT (Eriochrome Black-T) dye at 1.5 pH. The formed ion association complex is present in purple color and it is extracted with chloroform. The chloroform extractable layer is used to determine ZLPT drug spectrophotometrically.

Spectrum of Zolpidem Tartrate (ZLPT) - EBT Complex:

1.0 ml of ZLPT drug solution (l00µg/ml) is transferred into a separating funnel. To this drug solution 1.0 ml of EBT dye and 2.0 ml of 1.5 pH (Buffer Solution) are added. The final volume is adjusted to 8 ml with distilled water. Again 10 ml of chloroform is added and the reaction mixture is shaken gently for 5 min and allowed to stand for 5 min, so as to separate aqueous and chloroform layer. The chloroform layer is separated out. This chloroform extractable solution possesses purple color, for that absorption is scanned in the wavelength range of 450 to 600 nm against reagent blank (prepared in similar manner, devoid of drug solution). The absorption spectrum is given in the figure:15

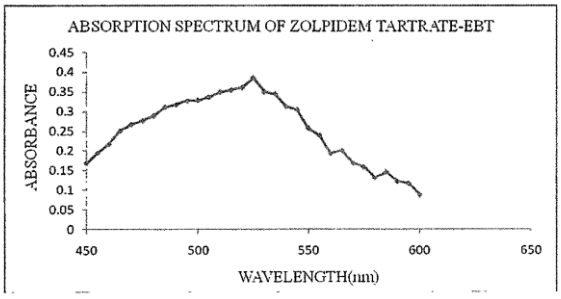

Fig.no .15. Absorption Spectrum of Zolpidem Tartrate – EBT

From the figure: 15. It is clear that the ZLPT drug solution treated with EBT dye solution has maximum absorbance at 525 nm. Hence all further studies are made at 525 nm.

(A) Effect of Concentration of Buffer Solution on Ion Association Complex:

In a series of separating funnels containing 1.0 ml of ZLPT drug solution (l00µg/ml), 1.0 ml of EBT dye and varying amounts of 1.5 pH (Buffer Solution) are added. The contents are made up to 8 ml with distilled water. To this 10 ml of chloroform is added, the reaction mixture is shaken gently for 5 min and allowed to stand for 5 min, so as to separate aqueous and chloroform layer. The absorbance of resultant solutions are measured at 525 nm and data is presented in the table:19.

Table 19.Effect of concentration of Buffer Solution on the Absorbance of complex:

S.No	Volume of Buffer solution	Absorbance at 525nm
1	0.5 ml	0.507
2	1.0 ml	0.543
3	1.5 ml	0.541
4	2.0 ml	0.539
5	2.5 ml	0.534

The data in the table:19. Indicates that 1.0 ml of 1.5 pH (Buffer Solution) is necessary for achieving maximum absorbance and hence maintained throughout the experimental studies.

(B) Effect of Concentration of EBT Dye on Ion Association Complex:

In a series of separating funnels containing 1.0 ml of ZLPT drug solution (100µg/ml). 1.0 ml of 1.5 pH (Buffer Solution) and varying amounts of EBT dye are added. The contents are made up to 8 ml with distilled water. To this 10 ml of chloroform is added, the reaction mixture is shaken gently for 5min and allowed stand for 5min, so as to separate aqueous and chloroform layers. The absorbance of resultant solutions is measured at 525 nm and data is presented in the table:20.

Table 20.Effect of concentration of EBT dye on the absorbance of complex:

S.No	Volume of EBT Dye	Absorbance at 525nm
1	0.5 ml	0.365
2	1.0 ml	0.418
3	1.5 ml	0.700
4	2.0 ml	0.692
5	2.5 ml	0.695

The data in the table: 20. Indicates that 1.5 ml Of EBT dye is necessary for achieving maximum absorbance and hence maintained throughout the experimental studies.

(C) Assay Procedure for Zolpidem Tartrate (ZLPT) Drug:

Aliquots of the standard ZLPT drug solution ranging from 0.5 to 2.5 ml are transferred into a series of separating funnels. To each funnel 1.5 ml of EBT dye and 1.0 ml of 1.5 pH (Buffer Solution) are added. The contents are made up to 8 ml with distilled water. To this 10 ml of chloroform is added, the reaction mixture in each separating funnel is shaken gently for 5 min and allowed to stand for 5 min, so as to separate aqueous and chloroform layers. The chloroform layer is separated out and absorbance is measured at 525 nm against the reagent blank (prepared in a similar manner, devoid of drug solution).

The drug calibration graph is constructed by plotting absorbance values. against the concentration of ZLPT drug solution. The calibration curve is found to be linear over the concentration range of 50 – 250 µg/ml for ZLPT drug. The amount of ZLPT present in the sample is estimated from the calibration graph. The data and results are presented in the table:21. and figure: 16.

Table 21.Effect of concentration of Drug on the absorbance of complex:

S.No	Concentration of Drug	Absorbance at 525nm
1	50 µg/ml	0.354
2	100 µg/ml	0.683
3	150 µg/ml	0.985
4	200 µg/ml	1.315
5	250 µg/ml	1.684

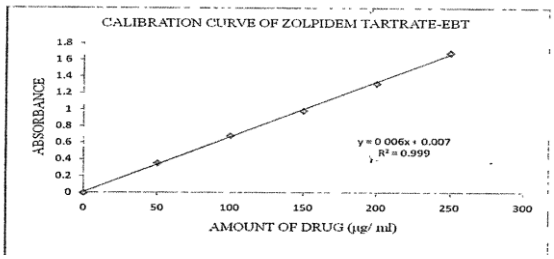

Fig.no .16. Calibration curve of Zolpidem Tartrate – EBT

Assay of Zolpidem Tartrate (ZLPT) in Pharmaceutical Formulations:

For analysis of tablet formulation, 20 tablets of ZLPT are weighed accurately and finely powdered. An accurately weighed portion of powdered sample, equivalent to 50 mg of ZLPT was taken in a 50 ml volumetric flask containing 25 mi of methanol, sonicated for 20 min. The resultant solution is filtered through whatmann filter paper no: 41 in to another 50 ml volumetric flask. The filter paper was washed several times with methanol. The washings were added to the filtrate and the final volume was made up to the mark with methanol. 5 ml filtrate of the sample solution was diluted to 10ml with methanol and treated as per the procedure of calibration curve. Amount of the drug present in the sample was computed from respective calibration curve.

ASSAY OF SIBUTRAMINE HC1 (SBT) BY METHOD-4:

The Method-4 is based on the reaction of SBT drug as a tertiary amine with EBT (Eriochrome Black-T) dye at 1.5 pH. The formed ion association complex is present in purple color and it is extracted with chloroform. The chloroform extractable layer is used to determine SBT drug spectrophotometrically.

Spectrum of Sibutramine HC1 (SBT) - EBT Complex:

1.0 ml of SBT drug solution (l00µg/ml) is transferred into a separating funnel. To this drug solution 1.0 ml of EBT dye and 2.0 ml of 1.5 pH (Buffer Solution) are added. The final volume is adjusted to 8 ml with distilled water. Again 10 ml of chloroform is added and the reaction mixture is shaken gently for 5 min and allowed to stand for 5 min, so as to separate aqueous and chloroform layer. The chloroform layer is separated out. This chloroform extractable solution possesses purple color, for that absorption is scanned in the wavelength range of 450 to 600 nm against reagent blank (prepared in similar manner, devoid of drug solution). The absorption spectrum is given in the figure:17.

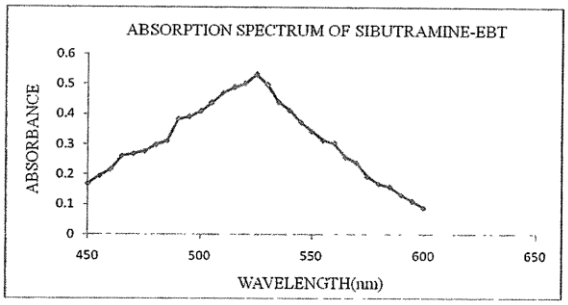

Fig.no .17. Absorption spectrum of Sibutramine – EBT

From the figure:17. It is clear that the SBT drug solution treated with EBT dye solution has maximum absorbance at 525 nm. Hence all further studies are made at 525 nm.

(A) Effect of Concentration of Buffer Solution on Ion Association Complex:

In a series of separating funnels containing 1.0 ml of SBT drug solution (100µg/m I), 1.0 ml of EBT dye and varying amounts of 1.5 pH (Buffer Solution) are added. The contents are made up to 8 ml with distilled water. To this 10 ml of chloroform is added, the reaction mixture is shaken gently for 5 min and allowed to stand for 5 min, so as to separate aqueous and chloroform layer. The absorbance of resultant solutions is measured at 525 nm and data, is presented in the table:22.

Table 22.Effect of concentration of Buffer Solution on the Absorbance of complex:

S.No	Volume of Buffer solution	Absorbance at 525nm
1	0.5 ml	0.343
2	1.0 ml	0.481
3	1.5 ml	0.493
4	2.0 ml	0.482
5	2.5 ml	0.480

The data in the table:22. Indicates that 1.5 ml of 1.5 pH (Buffer Solution) is necessary for achieving maximum absorbance and hence maintained throughout the experimental studies.

(B) Effect of Concentration of EBT Dve on Ion Association Complex:

In a series of separating funnels containing 1.0 ml of SBT drug solution (100µg/ml), 1.5 ml of 1.5 pH (Buffer Solution) and varying amounts of EBT dye are added. The contents are made up to 8 ml with distilled water. To this 10 ml of chloroform is added, the reaction mixture is shaken gently for 5min and allowed stand for 5min, so as to separate aqueous and chloroform layers. The absorbance of resultant solutions is measured at 525 nm and data is presented in the table:23.

Table 23.Effect of concentration of EBT dye on the absorbance of complex:

S.No	Volume of EBT Dye	Absorbance at 525nm
1	0.5 ml	0.488
2	1.0 ml	0.529
3	1.5 ml	0.535
4	2.0 ml	0.526
5	2.5 ml	0.518

The data in the table:23. indicates that 1.5 ml of EBT dye is necessary for achieving maximum absorbance and hence maintained throughout the experimental studies.

(C) Assay Procedure for Sibutramine HC1 (SBT) Drug:

Aliquots of the standard SBT drug solution ranging from 0.5 to 2.5 ml are transferred into a series of separating funnels. To each funnel 1.5 ml of EBT dye and 1.5ml of 1.5 pH (Buffer Solution) are added. The contents are made up to 8 ml with distilled water. To this 10 ml of chloroform is added, the reaction mixture in each separating funnel is shaken gently for 5 min and allowed to stand for 5 min, so as to separate aqueous and chloroform layers. The chloroform layer is separated out and absorbance is measured at 525 nm against the reagent blank (prepared in a similar manner, devoid of drug solution).

The drug calibration graph is constructed by plotting absorbance values against the concentration of SBT drug solution. The calibration curve is found to be linear over the concentration range of 50 - 250 µg/ml for SBT drug. The amount of SBT present in the sample is estimated from the calibration graph. The data and results are presented in the table:24. and figure: 18.

Table 24.Effect of concentration of Drug on the absorbance of complex:

S.No	Concentration of Drug	Absorbance at 525nm
1	50 µg/ml	0.287
2	100 µg/ml	0.539
3	150 µg/ml	0.785
4	200 µg/ml	1.031
5	250 µg/ml	1.289

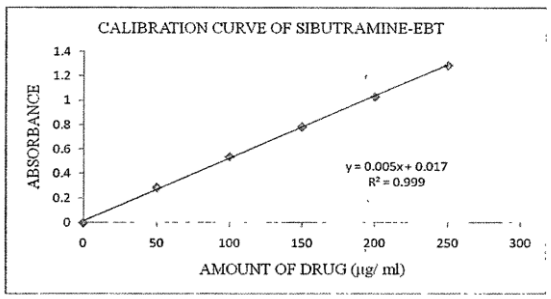

Fig.no .18. Calibration curve of Sibutramine – EBT

Assay of Sibutramine HC1 (SBT) on Pharmaceutical Formulations:

For analysis of tablet formulation, 20 tablets of SBT are weighed accurately and finely powdered. An accurately weighed portion of powdered sample, equivalent to 50 mg of SBT was taken in a 50 ml volumetric flask containing 25 ml of methanol, sonicated for 20 min. The resultant solution is filtered through whatmann filter paper no: 41 in to another 50 ml volumetric flask. The filter paper was washed several times with methanol. The washings were added to the filtrate and the final volume was made up to the mark with methanol. 5 ml filtrate of the sample solution was diluted to 10ml with methanol and treated as per the procedure of calibration curve. Amount of the drug present in the sample was computed from respective calibration curve.

Table 25.Optical Characteristics, Precision and Accuracy of Proposed Methods

DATA	METHOD-1 (TPOO)		METHOD-2 (BCG)	
	ZLPT	SBT	ZLPT	SBT
λ max	410	410	420	420
Beer's law limits (µg/ml)	50-250	50-250	50-250	50-250
Molar absorptivity (lit. mole $^{-1}$. Cm^{-1})	6.266	7.306	7.700	7.260
Sandell's sensitivity (µg/cc/0.001.absorbance)	0.159	0.136	0.129	0.137
Regression Equation (y = bc+a)	0.006X+0.02	0.007X+0.029	0.007X+0.021	0.006X+0.029
Slope (b)	0.006	0.007	0.007	0.006
Intercept (a)	0.02	0.029	0.021	0.029
Correlation Coefficient (r^2)	0.999	0.999	0.999	0.999

Table 26. Optical Characteristics, Precision and Accuracy of Proposed Methods

DATA	METHOD-3 (WFB)		METHOD-4 (EBT)	
	ZLPT	SBT	ZLPT	SBT
λ max	590	590	525	525
Beer's law limits (µg/ml)	50-250	50-250	50-250	50-250
Molar absorptivity (lit. mole $^{-1}$. Cm^{-1})	7.766	7.560	6.566	5.233
Sandell's sensitivity (µg/cc/0.001.absorbance)	0.128	0.132	0.152	0.191
Regression Equation (y = bc+a)	0.007X-0.002	0.007X+0.028	0.006X+0.007	0.005X+0.017
Slope (b)	0.007	0.007	0.006	0.005
Intercept (a)	-0.002	0.028	0.007	0.017
Correlation Coefficient (r^2)	0.999	0.999	0.999	0.999

Assay and Recovery of Drugs by Method-1(TPOO) in Dosage forms

Data	ZLPT**				SBT***			
	Tablet 1	Tablet 2	Tablet 3	Tablet 4	Tablet 1	Tablet 2	Tablet 3	Tablet 4
Labeled Amount (mg)	10	10	10	10	15	15	15	15
Found by Reference Method (mg)	9.963±0.045	9.957±0.034	9.945±0.037	9.924±0.041	14.943±0.021	14.952±0.028	14.937±0.038	14.951±0.033
Amount Found by Proposed Method (mg)±S.D.*	9.969±0.035	9.964±0.028	9.967±0.037	9.918±0.032	14.973±0.033	14.933±0.031	14.945±0.037	14.961±0.034
Relative Standard Deviation (%)	0 .353	0.292	0.373	0.332	0.226	0.213	0.251	0.232
t	0.519	0.434	0.419	1.421	0.643	0.603	0.580	0.294
F	1.633	1.734	1.454	1.854	0.698	0.794	0.571	0.666
% Recovery	99.69	99.64	99.67	99.18	99.82	99.55	99.63	99.74
Standard Error of Mean	0.014	0.011	0.015	0.013	0.013	0.012	0.015	0.014

*Average ± Standard deviation of six determinations that t- and f- values refer to comparison of the proposed methods with the reference method. Theoretical values at 95% confidence limits.
UV Spectrophotometric method (λmax 366nm), * UV Spectrophotometric method (λmax 220nm).

Assay and Recovery of Drugs by Method-2 (BCG) in Dosage forms

Data	ZLPT**				SBT***			
	Tablet 1	Tablet 2	Tablet 3	Tablet 4	Tablet 1	Tablet 2	Tablet 3	Tablet 4
Labeled Amount (mg)	10	10	10	10	15	15	15	15
Found by Reference Method (mg)	9.937±0.041	9.921±0.028	9.946±0.044	9.928±0.038	14.931±0.033	14.908±0.029	14.925±0.041	14.938±0.043
Amount Found by Proposed Method (mg)±S.D.*	9.944±0.044	9.913±0.023	9.961±0.039	9.925±0.051	14.952±0.041	14.916±0.039	14.934±0.044	14.950±0.049
Relative Standard Deviation (%)	0 .452	0.239	0.398	0.516	0.278	0.265	0.297	0.330
t	0.472	0.452	0.809	0.689	0.437	0.482	0.425	0.293
F	0.997	1.417	1.295	0.766	0.463	0.512	0.407	0.328
% Recovery	99.44	99.13	99.61	99.25	99.68	99.44	99.56	99.66
Standard Error of Mean	0.018	0.009	0.016	0.020	0.017	0.016	0.018	0.020

*Average ± Standard deviation of six determinations that t- and f- values refer to comparison of the proposed methods with the reference method. Theoretical values at 95% confidence limits.
UV Spectrophotometric method (λmax 366nm), * UV Spectrophotometric method (λmax 220nm).

Assay and Recovery of Drugs by Method-3 (WFB) in Dosage forms

Data	ZLPT**				SBT***			
	Tablet 1	Tablet 2	Tablet 3	Tablet 4	Tablet 1	Tablet 2	Tablet 3	Tablet 4
Labeled Amount (mg)	10	10	10	10	15	15	15	15
Found by Reference Method (mg)	9.926±0.028	9.926±0.041	9.917±0.038	9.939±0.036	14.927±0.042	14.941±0.039	14.924±0.034	14.947±0.044
Amount Found by Proposed Method (mg)±S.D.*	9.947±0.041	9.922±0.037	9.928±0.027	9.949±0.037	14.931±0.036	14.943±0.044	14.928±0.032	14.964±0.046
Relative Standard Deviation (%)	0 .420	0.380	0.272	0.373	0.245	0.296	0.218	0.313
t	0.806	0.691	1.582	0.733	0.760	0.619	0.453	0.676
F	1.158	1.411	1.855	1.468	0.874	0.597	1.106	0.537
% Recovery	99.47	99.22	99.28	99.49	99.54	99.62	99.52	99.76
Standard Error of Mean	0.017	0.015	0.011	0.015	0.014	0.018	0.013	0.019

*Average ± Standard deviation of six determinations that t- and f- values refer to comparison of the proposed methods with the reference method. Theoretical values at 95% confidence limits.
UV Spectrophotometric method (λmax 366nm), * UV Spectrophotometric method (λmax 220nm).

Assay and Recovery of Drugs by Method-4 (EBT) in Dosage forms

Data	ZLPT**				SBT***			
	Tablet 1	Tablet 2	Tablet 3	Tablet 4	Tablet 1	Tablet 2	Tablet 3	Tablet 4
Labeled Amount (mg)	10	10	10	10	15	15	15	15
Found by Reference Method (mg)	9.942±0.041	9.963±0.029	9.937±0.042	9.935±0.033	14.918±0.043	14.947±0.028	14.938±0.044	14.931±0.028
Amount Found by Proposed Method (mg)±S.D.*	9.959±0.039	9.972±0.036	9.945±0.050	9.921±0.038	14.921±0.044	14.950±0.052	14.952±0.031	14.941±0.025
Relative Standard Deviation (%)	0 .400	0.368	0.504	0.382	0.300	0.353	0.208	0.168
t	0.136	0.372	0.637	0.644	0.549	0.285	0.264	0.407
F	1.266	1.492	0.800	1.377	0.586	0.421	1.205	1.527
% Recovery	99.59	99.72	99.45	99.21	99.47	99.66	99.68	99.60
Standard Error of Mean	0.016	0.014	0.020	0.015	0.018	0.021	0.012	0.010

*Average ± Standard deviation of six determinations that t- and f- values refer to comparison of the proposed methods with the reference method. Theoretical values at 95% confidence limits
UV Spectrophotometric method (λmax 366nm), * UV Spectrophotometric method (λmax 220nm).

CONCLUSION

Since Zolpidem Tartrate (ZLPT) and Sibutramine HC1 (SBT) are relatively new drags and the analytical methods available for their assay are very limited, it is worth while to develop some methods for their assay. As part of the present investigations, four methods have been developed for the purpose of assay of ZLPT and SBT. The calibration curves are linear over the large of 50-250 µg/ml for ZLPT and SBT. The values of standard deviation are low, indicates high accuracy and reproducibility of the methods.

It can be seen that from the results presented above that the proposed methods have reasonable sensitivity. Statistical analysis of the results shows that the proposed procedures have good precision and accuracy. Results of the analysis of pharmaceutical formulations reveal that the proposed methods are suitable for their analysis with virtually no interference of the usual additives present in them.

The order of sensitivity among the four methods is Method-1 > Method-4 > Method-3 > Method-2. Beer's law limit (lOOgg/ml) of the proposed methods are better than many of the reported Spectrophotometric methods. All the proposed methods are simple sensitive and reliable with good precision and accuracy. These methods can be used for routine determination of ZLPT and SBT in bulk samples and in pharmaceutical formulations.

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