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METHOD DEVELOPMENT AND VALIDATION OF NALTREXONE HCl ANDBUPROPION HCl IN BULK AND PHARMACEUTICAL FORMULATION (CONTRAVE TABLET) BY VISIBLE SPECTROSCOPY (COLORIMETRIC) USING SIMULTANEOUS EQUATION

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ABSTRACT

An accurate, Precise, selective and sensitive economical visible Spectrophotometric-method has been developed and validated for the simultaneous estimation of Naltrexone HCl and Bupropion HClin bulk and tablet(CONTRAVE) dosage form. According to USP standard percentage purity for Naltrexone HCl & Bupropion HCl was found to ≤90-110 and 98-102% respectively. The label claim for Contrave tablet was found to be 8/90mg ie; 8mg of Naltrexone HCl& 90mg of Bupropion HCl(1:11.25 ratio). The method is based on oxidation followed by coupling of 3-methyl-2-benzothiazolinone hydrazine (MBTH) in presence of ferric chloride (catalyst) and Hydrochloric acid to form green colored chromogen. The solvent is used as drug is freely soluble in ethanol and make up with distilled water. The results of analysis have been validated statistically in accordance to ICH guidelines. Linearity was observed in the concentration range of 1-6µg/ml & 11.25-67.5µg/ml for Naltrexone HCl and Bupropion HCl (Pure Drug) respectively and Recovery studies were carried out to confirm the accuracy of the method. Experimental assay shows that the percentage purity of marketed formulation (Contrave tablet) was found to be 107.81% and 98.54% within the range for Naltrexone HCl and Bupropion HCl respectively and calculated by using Simultaneous Equation Method.

INTRODUCTION

Orexigen Therapeutics developed a proprietary fixed combination of Naltrexone HCl and Bupropion HCl (Contrave 8/90mg) single tri-layer tablet. It contains 8mg of Naltrexone and 90mg of Bupropion respectively [1-5]. The Naltrexone /Bupropion combination known as Contrave is designed to initiate weight loss and sustain it over a longer period of time by switching off natural compensatory mechanisms involved in the typical weight loss plateau stage. NAL is chemically (5α)-17-(cyclopropylmethyl)-4,5-epoxy-3,14-dihydroxymorphinan-6-onhydrochloride and it is used in the treatment of alcoholism and an opioid antagonist.BUP is chemically (±) 2-(tert-butylamino)-3'-chloropropiophenone hydro chloride is an minoketone derivative. It is a second generation antidepressant and a selective inhibitor of the neuronal reuptake of catecholamines (noradrenalin and dopamine) with minimal effect on the reuptake of serotonin and no inhibitory effect on monoamine oxidase [6-9].

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Mechanism of Action

Contrave Tablet (8/90mg) (Naltrexone HCl & Bupropion HCl)

Bupropion and Naltrexone are centrally active drugs that have shown potential efficacy – alone and in combination- for the treatment of obesity. Bupropion has greater efficacy as monotherapy. Naltrexone potentiates the effects of bupropion; thus, this synergistic combination has the potential for additional weight loss compared to monotherapy. Current Phase III trials will yield further safety and efficacy information regarding these drugs in combination [10].

Literature survey reveals that no visible spectroscopy study was reported on the development and validation of Naltrexone HCl and Bupropion HCl (contrave tablet) in bulk and pharmaceutical formulation by visible spectroscopy using Simultaneous Equation Method [11-14].

My aim was to develop an accurate, precise, selective, sensitive and economical, Visible Spectroscopy method for the determination of Naltrexone HCl and Bupropion HCl in bulk and pharmaceutical formulation using simultaneous Equation method [15].

EXPERIMENTAL METHODS

Materials and Instruments

1) A SHIMANDZU model PHARMASPEC-1800 UV-Visible double beam spectrophotometer with 1cm matched quartz cell was used for recording spectra
2) Shimadzu balance A Y 220
3) Cuvettes – quartz cells
4) Digital Ultrasonic Cleaner (sonicator)

DRUG SAMPLES

Pure Drug

Naltrexone HCl was received as gift samples from Glenmark Pharmaceuticals , Mumbai and Bupropion HClwas received as gift samples from Glenmark Pharmaceuticals, Mumbai.

Formulation

Contrave (8/90mg) tablet (Naltrexone HCl-8mg + Bupropion HCL-90mg) were procured from market (India Mart-Mumbai)

REAGENTS

All the chemicals and reagents used were of analytical grade:

- Deionised Water
- Ethanol
- MBTH reagent
- Ferric Chloride
- Hydrochloric acid

MECHANISM OF REACTION

The method is based on the diazotization of C=O group and coupling with MBTH reagent to form a green colored complex [16].

Reagent

This method followed by the coupling of Naltrexone HCl and Bupropion HCl (Fig2 &Fig3)with the MBTH reagent in presence of ferric chloride and HCL to form green colored complex , by the reaction of NH (amine) group present in the MBTH reagent with the C=O functional group of Naltrexone HCl and Bupropion HCL, by eliminating one water (H_2O) molecule [17, 18].

METHOD DEVELOPMENT AND OPTIMIZATION

Selection of Solvent

Selection of suitable solvent is influenced by the Wavelength expected to be studied.

Ethanol – Drug is freely soluble in ethanol.

Distilled Water – Distilled Water as solvent for dilution.

Preparation of Naltrexone HCl and Bupropion HCl (standard Stock solution) in combined Form

5mg of Naltrexone and 56.2mg of Bupropion was accurately weighed and transferred to 50 ml volumetric flask, dissolved in 30ml ethanol and finally make up with 20ml of distilled water to give a standard solution of 100µg/ml & 1125µg/ml of Naltrexone and Bupropion respectively (according to 1:11.25 ratio of Tablet dosage form).

Selection of Reagent

MBTH used as Reagent.

Ferric Chloride solution as Catalyst.

Preparation of analytical solutions

Preparation of MBTH reagent solution (0.5%)

0.5% (0.5gm) of MBTH reagent was accurately weighed and it dissolved in distilled water in 50ml volumetric flask and volume was made up to50ml with distilled water [19].

Preparation of Ferric chloride solution (1%)

1% (1gm) of Ferric chloride solution was accurately weighed and dissolved it in Hydrochloric acid(0.2% in 100ml Distilled Water) in 50ml volumetric flask and volume was made up to 50ml with Hydrochloric acid.

Optimization of reaction condition

The conditions in which reagent reacts with Naltrexone and Bupropion were investigated. Both the drugs were reacts with the MBTH reagent only at the cool condition at 4-8°c for 15 minutes. All reaction conditions were optimized at cool temperature [20].

Optimization of reagent concentration

Optimization of concentration of Hydrochloric acid, MBTH reagent, Ferric chloride required to achieve

maximum sensitivity of the developed complex was ascertained by adding different volumes has shown below:

From the above result, I optimized the condition for Maximum Absorbance of stable green colour complex was found at 1.5ml of 1% Ferric chloride solution in 0.2% Hydrochloric acid & 1.5ml of 0.5% MBTH reagent solution.

Simultaneous Equation Method:

The sample containing two absorbing species Naltrexone and Bupropion (X &Y) each of which absorbs at the λ_{max} of the other. So the absorbance of each drugs were measured at both wavelengths λ_1&λ_2 respectively. The both the drugs are determined by simultaneous method (Vierodt's method).

$C_x = (A_2ay_1\text{-}A_1ay_2/ax_2ay_1\text{-}ax_1ay_2)$

$C_y = (A_1ax_2\text{-}A_2ax_1/ax_2\,ay_1\text{-}ax_1ay_2)$

C_x= Concentration of Naltrexone HCl

C_y = Concentration of Bupropion HCL

ax_1& ax_2 = absorptivity of Naltrexone HCl

ay_1 & ay_2 = absorptivity of Bupropion HCl

By using this formula Percentage purity of both drugs Naltrexone and Bupropion can be estimated…

RESULTS AND DISCUSSION

Selection of Wavelength

Preparation of Naltrexone HCl (standard stock solution- 100 µg/ml)

5mg of naltrexone (pure drug) was accurately weighed and transferred to 50 ml volumetric flask and solubilize the drug by using ethanol and the volume was made up to 50ml with distilled water.(stock solution)From the above stock solution pipetted out 1µg/ml (0.1ml) and transferred to 10ml volumetric flask. Then added 1.5ml of ferric chloride solution (1%) and 1.5ml of MBTH reagent (0.5%) solution and kept the solution at cool temperature at 4-8°C for 15 minutes.After that diluted up to the mark with distilled water to 10ml volumetric flask&green colored complex were formedPrepared the blank by adding 1.5ml MBTH (0.5%) & 1.5ml ferric chloride (1%) and made up with distilled water.Scanned in the visible range from 400-800nm.From the spectra of drug, wavelength of naltrexone was selected for further studies.

Preparation of Bupropion HCl(Standard stock solution -1125µg/ml)

56.2mg of Bupropion HCl(Pure Drug) was accurately weighed and transferred to 50ml volumetric flask.From the above stock solution pipetted out 1µg/ml (0.1ml) and transferred to 10ml volumetric flask. Then added 1.5ml of ferric chloride solution (1%) and 1.5ml of MBTH reagent (0.5%) solution and kept the solution at cool temperature at 4-8□C for 15 minutes. After that diluted up to the mark with distilled water to 10ml volumetric flask &green colored complex were formed**.** Prepared the blank by adding 1.5ml MBTH (0.5%) & 1.5ml ferric chloride (1%) and made up with distilled water.Scanned in the visible range from 400-800nm .From the spectra of drug, wavelength of Bupropion HClwas selected for furtherstudies.

Overlay spectrum for both Naltrexone HCl & Bupropion HCl in Combined Form

5mg of Naltrexone +56.2mg of Bupropion was accurately weighed and transferred to 50ml of volumetric flask.Solubilized the drug solution by using ethanol and volume was made up to 5oml with distilled water.Aliquot of the standard drug solutions was pipetted into separate 10ml standard flasks for preparing six calibration standard solutions containing 1,2,3,4,5,and 6µg/ml of Naltrexone and 11.25,22.5,33.75,45,56.25,and 67.5µg/ml of Bupropion. Mix well and 1.5ml ferric chloride solution (1%), and 1.5ml of MBTH reagent (0.5%) solution was added and made up to volume with distilled water. Absorbance was measured against blank at 622nm and 658nm for Naltrexone and Bupropion respectively. Overlay spectrum of NAL & BUP are shown in fig.

Estimation of Formulation

An attempt was made to develop a sensitive and specific analytical method for analysis of Naltrexone and Bupropion in combined dosage form. 6µg/ml was selected for further studies. Absorbance spectra of the green colored drug-MBTH complex are shown in fig. with a maximum absorbance (λ_{max}) at 622nm and 658nm. Maximum absorbance of stable colour complex was found at 0.2%HCL , 1.5ml of 0.5%MBTH reagent solution, and 1.5ml of ferric chloride solution(1%).

Assay Procedure for Marketed Formulation(CONTRAVE TABLET)

20 tablets were accurately weighed and finely powdered. An accurately weighed amount of the powder equivalent to 0.005gm of Naltrexone HCl and 0.0562gm of Bupropion HCl was transferred to a 50ml standard volumetric flask. 20ml of ethanol and 10 ml of distilled water was added to the flask and mixed thoroughly. Sonicated the solution for 5min. and finally the solution were made upto the mark with distilled water and then filtered. From the above stock solution, pipetted out sample solution (6µg of Naltrexone and 67.5µg of Bupropion) and to 10ml of standard volumetric flask. 1.5ml of 0.5% of MBTH reagent and 1.5ml of ferric chloride (1%) were added to the flask, kept the solution at cool temperature for 15min (4-8°c), and the sample solution were made upto the mark with distilled water. Absorbance was measured using Ferric chloride solution (1%), MBTH (0.5%), made upto the mark with distilled water as BLANK. The absorbance of prepared sample solution was determined at 622nm for Naltrexone and 658nm for Bupropion.

Finally the concentration of the drugs and Percentage purity were calculated by using Simultaneous Equation Method.

METHOD VALIDATION

Linearity and Range

Five concentrations of the standard Naltrexone (1, 2, 3, 4, 5 and 6µg/ml) and Bupropion (11.25, 22.5, 33.75, 45, 56.25and 67.5µg/ml) were prepared and the regression coefficients were found out.

Accuracy

The accuracy of the method was determined at three percentage level 80%, 100% and 120% levels. The recovery studies were carried out three times and the percentage recovery and percentage relative standard deviation was found to be less than 2 andgiven in Table .

Precision

To determine the precision of the proposed method, pure drug solutions (NAL& BUP) at a concentration within the working range were prepared and analyzed in three replicates during the same day and on three consecutive days and the results are presented in Table 3. Percentage relative standard deviation (%RSD) for intra-day was 0.519 for NAL and 0.611 for BUP and for inter-day %RSD was 0.684 for NAL and 0.522 for BUP indicating repeatability.

Robustness

To evaluate the robustness of the methods, the concentration of Ferric chloride was changed and the effect of this change on the absorbance of the sample solutions was studied. The results of this study are presented in Table 4 and indicated that the proposed method is robust.

Ruggedness

Method ruggedness was evaluated by performing the analysis following the recommended procedures by three different analysts. From the %RSD values obtained in (Table 5) it concluded that the proposed method was rugged

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ values were calculated to check the sensitivity of the method by using
following equations;

$$\text{LOD} = \frac{3.3\sigma}{S} \quad \text{LOQ} = \frac{10\sigma}{S}$$

Where □□the standard deviation and S is the slope of the curve. The values are concluded in the Table.

Table 1. Drug Profile

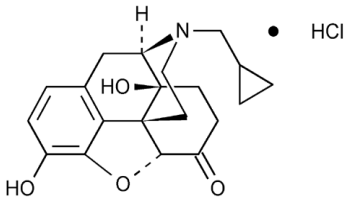
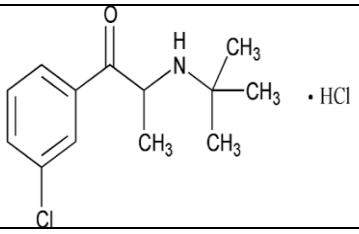
DRUG NAME	Naltrexone HCl	BUPROPION HCl
Structure		
IUPAC Name	17-(cyclopropylmethyl)-4,5αepoxy-3,14-dihydroxymorphinan-6-one	1-(3-chlorophenyl)-2-[(1,1-dimethyl-ethylamino]-1-propane,amonohydrochloride
Molecular Formula	$C_{20}H_{23}NO_4$ .HCl	$C_{13}H_{18}ClNO$ .HCl
Molecular Weight	377.87g/mol	239.74g/mol
Solubility	freely soluble in Water, soluble in Ethanol, Methanol	Freely soluble in Water, soluble in Methanol, Ethanol
Route of administration	Oral, Intramuscular	Oral, Intramuscular
Excretion	Renal	Renal
Uses	Opiate antagonist	Anti- depressant activity
Adverse Effect	Nausea, Headache, anxiety, Bone joint pain	Nausea, ,Insomnia

Table 2. Optimization Condition of Reagent Concentration

Con. Of Ferric chloride in 2% HCL	Con.of MBTH reagent
2.5% ferric chloride (2ml)	2.5% MBTH (2.5ml)
1.5% ferric chloride (1ml)	1% MBTH(2ml)
1% Ferric chloride (1.5ml)	0.5% MBTH (1.5ml)

Table 4. Reading of overlay spectrum of graph (Naltrexone HCl& Bupropion HCL)

Amount taken	Absorbance (nm)	Amount taken	Absorbance (nm)
1	0.166	11.25	0.169
2	0.251	22.5	0.255
3	0.326	33.75	0.330
4	0.397	45	0.394
5	0.459	56.25	0.451
6	0.533	67.5	0.520

Table 5. Results of Marketed Formulation by Colorimetry

Marketed Formulation	Drug	Label Claim	Estimated Amount (mg)	% Purity	% RSD
CONTRAVE (8/90mg)	NAL	8mg	8.625	107.81%	0.922
			8.698	108.36%	
			8.769	109.61%	
	BUP	90mg	88.69	98.54%	0.455
			88.99	98.87%	
			89.50	99.44	

Table 6. Linearity Reading of Naltrexone and Bupropion Spectrum

Amount taken (µg/ml)	Absorbance (nm)	Amount taken (µg/ml)	Absorbance
Naltrexone HCl at 622nm		Bupropion HCL at 658nm	
1 µg/ml	0.166	11.25µg/ml	0.169
2 µg/ml	0.251	22.5 µg/ml	0.255
3 µg/ml	0.326	33.75 µg/ml	0.330
4 µg/ml	0.397	45 µg/ml	0.394
5 µg/ml	0.459	56.25µg/ml	0.451
6 µg/ml	0.533	67.5 µg/ml	0.520

Table 7. Accuracy studies of Naltrexone and Bupropion

Drug	Theoritical % Target level	Amount added (µg/ml)	Amount recovered (mg)	% Recovery * mean	% RSD
Naltrexone(8mg)	80	4	8.694	108.67%	0.525
	100	6	8.610	107.62%	
	120	7	8.650	108.12%	
Bupropion(90mg)	80	45	89.88	99.86%	0.577
	100	67.5	89.90	99.88%	
	120	78.75	88.99	98.87%	

Table 8. Precision studies of Naltrexone and Bupropion

Drug	Amount (µg/ml)	Intra - day		Inter – day	
		% Content	%RSD	%Content	%RSD
Naltrexone (8mg)	6	106.87%	0.519	106.37%	0.684
		107.5%		107.48%	
		107.9%		106.23%	
Bupropion (90mg)	67.5	99.8%	0.611	98.77%	0.522
		100.11%		98.87%	
		100.98%		99.72%	

Table 9. Robustness results

Drug	Amount taken (µg/ml)	Parameter altered (concentration of Ferric Chloride in %)	Amount Found (mg)	% Content	% RSD
	6	0.9%	8.650	108.12%	0.949

Naltrexone (8mg)			8.627	107.83%	
			8.768	109.6%	
		1.1%	8.669	108.36%	0.060
			8.660	108.25%	
			8.668	108.35%	
Bupropion (90mg)	67.5	0.9%	91.54	101.66%	0.020
			91.50	101.69%	
			91.49	101.65%	
		1.1%	89.10	99%	0.298
			89.50	99.44%	
			88.99	98.87%	

Table 10. Ruggedness results

Drug	Analyst	Amount taken (µg/ml)	Amount Found (mg)	% Content	% RSD
Naltrexone (8mg)	Analyst I	6	8.625	107.81%	0.942
	Analyst II		8.790	109.87%	
	Analyst III		8.650	108.12%	
Bupropion (90mg)	Analyst I	67.5	88.69	98.54%	0.153
	Analyst II		89.45	99.38%	
	Analyst III		88.50	98.33%	

Table 11. LOD and LOQ results

Naltrexone (8mg)		Bupropion (90mg)	
LOD (µg/ml)	LOQ (µg/ml)	LOD (µg/ml)	LOQ (µg/ml)

Table 12. Analytical Data

Parameter	Naltrexone HCl	Bupropion HCL
Detection Wavelength	622nm	658nm
Beer's law Limit	1-6µg/ml	11.25- 67.5µg/ml
Regression equation	Y=0.072x + 0.102	Y=0.006x + 0.112
Correlation coefficient	0.9978	0.9952
Slope	0.072x	0.006x
LOD	0.3239	5.475
LOQ	0.9818	16.5916

Fig 1. Mechanism of Action of Naltrexone and Bupropion Tablet

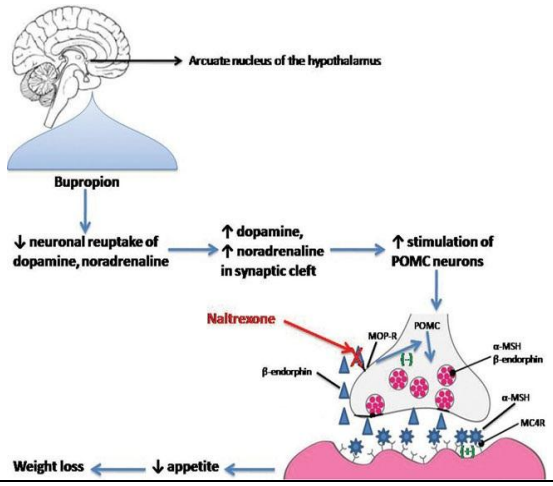


Fig 2. Reaction of Naltrexone with MBTH

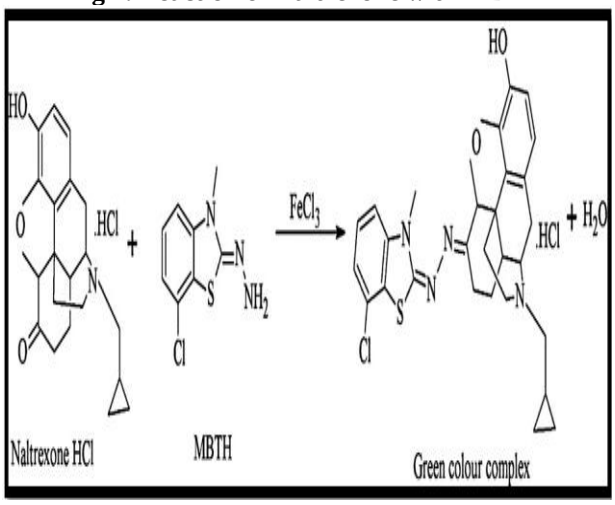


Fig 3. Reaction of Bupropion with MBTH Reagent

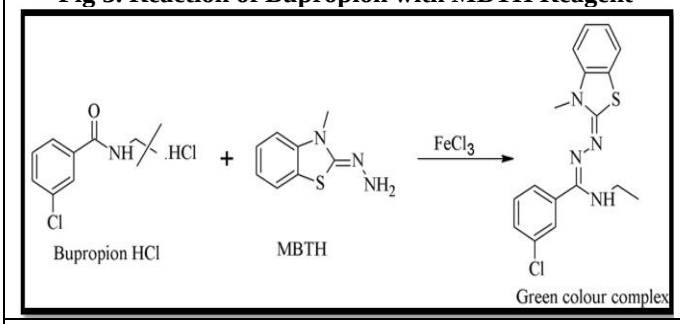


Fig 4. Visible spectrum of Blank for Naltrexone HCl

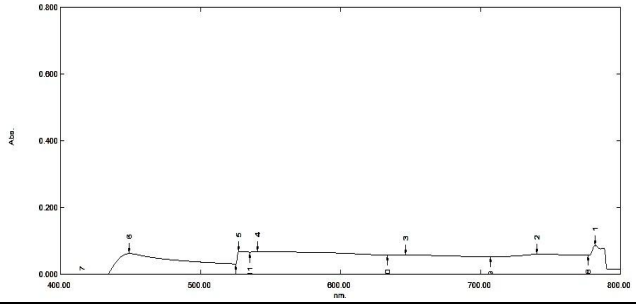


Fig 5. Visible Spectrum of Naltrexone HCl showing λ_{max} at 622nm

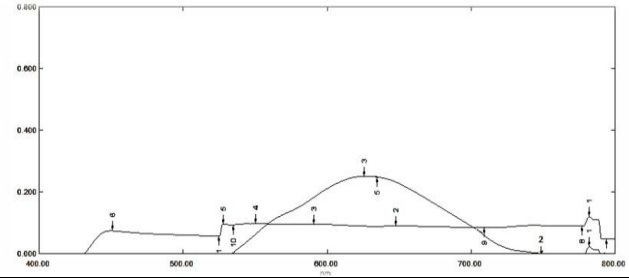


Fig 6. Visible spectrum of Blank for Bupropion HCL

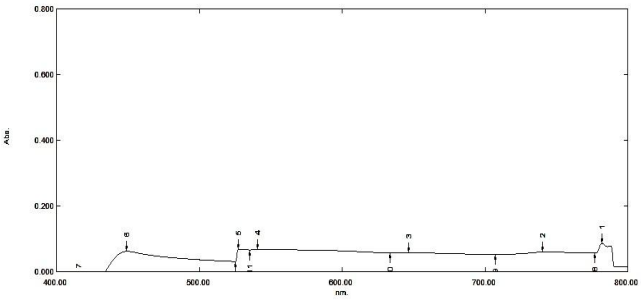


Fig 7. Visible spectrum of Bupropion HCl showing λ_{max} nm 658nm

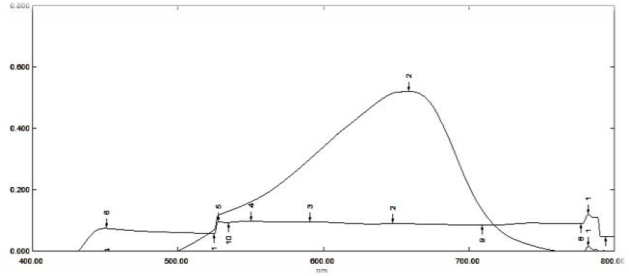


Fig 8. Naltrexone HCl at 622nmBupropion HClat 658nm

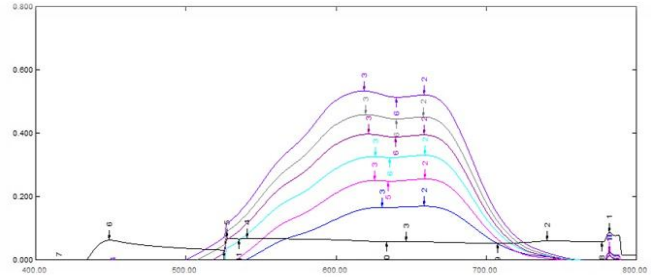


Fig 9. Visible spectrum of contrave tablet (naltrexone Hcl at622nm &bupropion Hcl at 658nm

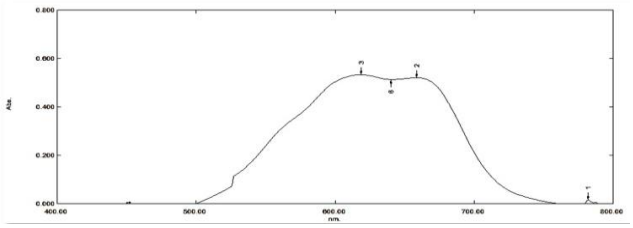


Fig 10. Calibration curve of Naltrexone HCl at 622nm

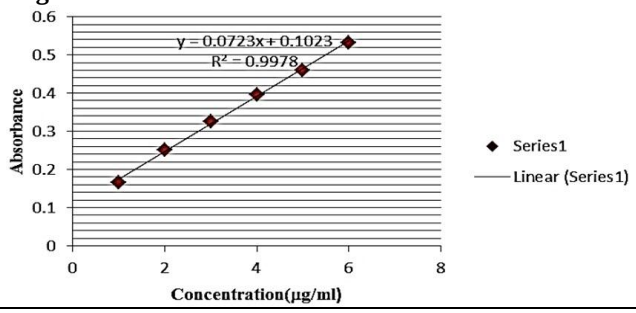
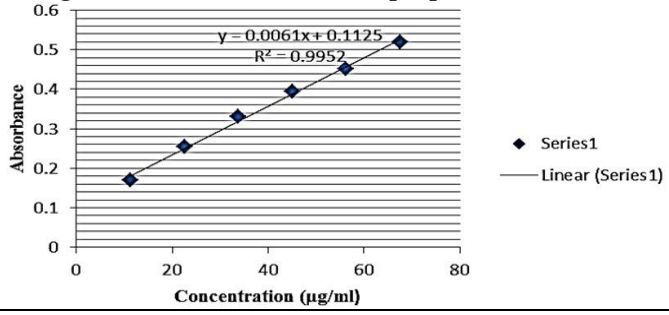


Fig 11. Calibration curve of Bupropion HClat 658nm



SUMMARY AND CONCLUSION

Literature survey reveals no Visible Spectrophotometric method was developed for the Simultaneous estimation for the analysis of Naltrexone HCl and Bupropion HCl in bulk and pharmaceutical formulation (CONTRAVE TABLET) in combined dosage form. No other methods are reported for the simultaneous estimation of these drugs in bulk drug and formulation. Hence an attempt was made to develop validated simple, accurate and precise methods for the determination of Naltrexone and Bupropion and as follows, Visible Spectrophotometric Method

Visible Spectrophotometric Metho Development And Validation

For the determination of Naltrexone and Bupropion in combined dosage form by Visible Spectrophotometric 0.5% 3–Methyl-2-benzothiazolinone hydrazine hydrochloride hydrate reagent was used to produce green colored complex. The reaction catalysts used were 1% Ferric Chloride Solution made up with 2% Hydrochloric Acid. The developed green colored complex showed maximum absorbance at 622nm and 658nm for Naltrexone and Bupropion respectively.

Linearity was found in the concentration ranges of 1-6µg/ml for Naltrexone and 11.25 - 67.5µg/ml for Bupropion. Slope, intercept and correlation coefficient values were found to be 0.001, 0.653 and 0.99 for Naltrexone and 0.047, 0.13 and 0.981 for Bupropion respectively. The developed colour was stable about 10min at room temperature. Low percentage relative standard deviation values show that the developed method is precise, robust and rugged. The recovery studies were carried out at 80, 100 and 120% levels. The method was successfully used for the simultaneous estimation of Naltrexone and Bupropion in Bulk and Pharmaceutical Formulation.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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