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RP-HPLC AND SPECTROPHOTOMETRIC METHODS FOR THE SIMULTANEOUS ESTIMATION OF BUPROPION HCl AND NALTREXONE HCl

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ABSTRACT: A simple, sensitive, precise, accurate and cost effective reverse phase high performance liquid chromatographic method for the simultaneous estimation of bupropion HCl and naltrexone HCl from a single formulation was developed and validated in accordance to ICH guidelines. The two drugs were eluted under isocratic mode using a 250 x 4.6 mm i.d, 0.45 µm Phenomenex Chromosil C18 column. The mobile phase was composed of a mixture of methanol, acetonitrile and water in the ratio of 60:20:20 v/v (pH adjusted to 4.8) at a flow rate of 1.0mL/min. The limit of detection and limit of quantification were found to be 0.5µg/mL and 1.7µg/mL, for both the drugs. The label claim of the combined dosage form was in good agreement with the experimental assay amounts; 99.5% and 99.7% for bupropion HCl and naltrexone HCl, respectively. This method can be successfully applied for the simultaneous estimation of bupropion HCl and naltrexone HCl combination for routine quality control analysis.

INTRODUCTION: Naltrexone is an opioid receptor antagonist used primarily in the management of alcohol and opioid dependence (**Fig. 1a**) [1]. It is marketed in generic form as its hydrochloride salt, naltrexone hydrochloride, under the trade names Revia and Depade. Once-monthly extended-release injectable formulation is also marketed under the trade name Vivitrol, in some countries including the United States. Naltrexone undergoes rapid and almost complete absorption after oral administration. Approximately 96% of the dose is absorbed from the gastrointestinal (GI) tract [2].

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Naltrexone undergoes extensive first-pass metabolism, and oral bioavailability ranges from 5% to 40%. Peak plasma levels (C_{max}) of naltrexone, as well as those of the active metabolite 6-β-naltrexol, occur within one hour after oral administration. The mean elimination half-life of naltrexone is four hours, and the mean elimination half-life of 6-β-naltrexol is 13 hours [3]. Protein binding of naltrexone is only 21%.

Bupropion is a drug primarily used as an antidepressant and smoking cessation aid (**Fig.1b**) [4]. It is taken in the form of tablets, and is marketed under the trade names of Wellbutrin and Zyban. In contrast to many other antidepressants, it does not cause weight gain or sexual dysfunction. The most important side effect is an increase in risk for epileptic seizures, which caused the drug to be withdrawn from the market for some time and then caused the recommended dosage to be reduced. Bupropion is rapidly

absorbed after oral administration [5]. Bupropion undergoes extensive hepatic metabolism via cytochrome P450 (CYP) 2B6 to hydroxybupropion as well as non–CYP-mediated metabolism to erythrohydrobupropion and threohydrobupropion. All three metabolites are active, but their activity is only 20% to 50% as potent as that of the bupropion. The time to C_{max} is approximately five hours[6]. Bupropion is 84% protein-bound.

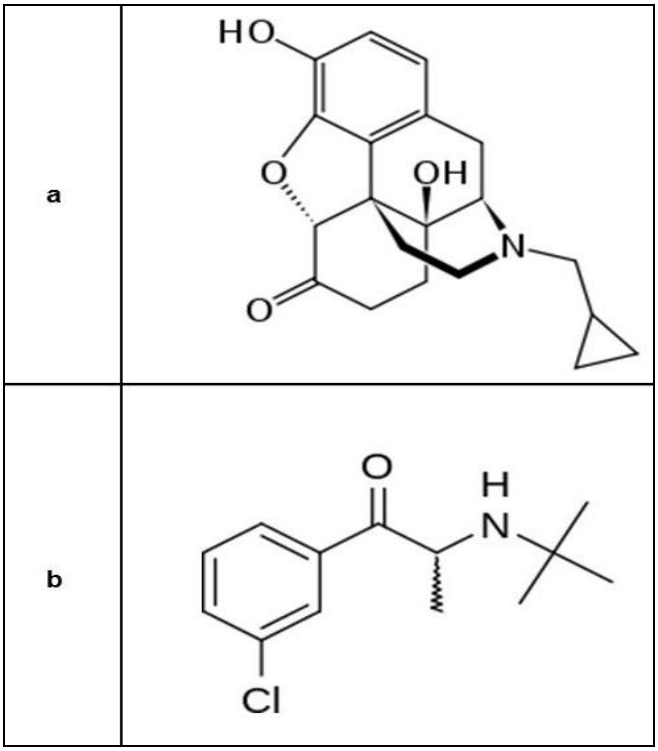

FIG.1: CHEMICAL STRUCTURE OF (a) NALTREXONE AND (b) BUPROPION

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 SR potentiates the effects of bupropion SR; thus, this synergistic combination has the potential for additional weight loss compared to monotherapy [7]. Current Phase III trials will yield further safety and efficacy information regarding these drugs in combination. The U.S. Food and Drug Administration today approved Contrave® (naltrexone hydrochloride and bupropion hydrochloride extended-release tablets) as treatment option for chronic weight management in addition to a reduced-calorie diet and physical activity [8]. Several HPLC methods have been described for the determination in biological samples [9-15], but no validated HPLC-UV/PDA methods were reported so far for the simultaneous estimation of bupropion and naltrexone HCl in combined pharmaceutical dosage forms. Hence, the main objective of the present investigation was to develop a validated RP HPLC method for the simultaneous analysis of bupropion and naltrexone HCl in bulk and dosage forms.

In the present investigation, an attempt has been made to estimate bupropion HCl and naltrexone HCl by reverse phase high performance liquid chromatography (RP HPLC). Although LC-MS/MS is a versatile tool, the development of HPLC based estimation method makes it more economical and simpler both in terms of maintenance and data interpretation. In the current study, an effort has been made to identify a common mobile phase to come up with the isocratic elution of both drugs in combination. An attempt has been made to develop and validate the RP-HPLC method to ensure the accuracy, precision, robustness and other analytical method validation parameters, as mentioned in the ICH guidelines[16, 17].

Experimental:

Chemicals and reagents: The standard drugs bupropion HCl and naltrexone HCl were obtained from Cipla Pharma Pvt. Ltd. Extended release formulation Contrave® (buproprion-90mg and naltrexone-8mg) was procured from local market. HPLC grade acetonitrile, methanol and water were purchased from Merck Ltd. All other chemicals and reagents were of analytical grade and were purchased from Fisher scientific company.

Instrumentation and chromatographic conditions:

The chromatographic separations were performed on a PEAK chromatographic system equipped with LC-P7000 isocratic pump, Rheodyne injector with 20µl fixed volume loop, variable wavelength programmable UV detector (UV7000) and a Tech comp UV-2301 double beam UV-Visible spectrophotometer to carry out spectral analysis. All the components of the system were controlled using SCL-10A VP system controller. Data acquisition and processing was done using PEAK chromatographic software (version 1.06). The detector was set at a wavelength of 254 nm.

Chromatographic separations were achieved on a reversed phase Chromosil C-18 column (250mm × 4.6mm ID; particle size 0.45µm). The mobile phase was prepared with methanol, acetonitrile and water in the ratio of 60:20:20v/v with a final pH of 4.8. The components of the mobile phase were filtered before use through 0.45µm membrane filter, degassed for 15min using ultra sonicator (1.5L). The column temperature was maintained at ambient conditions and the respective solvent reservoir was pumped to the column at the flow rate of 1mL/min. The column was equilibrated for at least 30 min with the mobile phase.

Preparation of standard drug solution:
Standard stock solution of bupropion HCl was prepared by taking a weighed quantity of 10mg in a 10mL volumetric flask containing 5ml of solvent. Standard stock solution of naltrexone HCl was prepared by taking a weighed quantity of 10mg in a 10mL volumetric flask containing 5ml solvent. From the above prepared stock solution, 1mL was taken into a 10mL volumetric flask containing 5 ml of solvent.

The contents were sonicated for about 15min and made up to final volume with the same solvent to get a working standard concentration of 1000 and 100µg/mL for bupropion and naltrexone, respectively. This stock solution of the drugs was filtered through 0.45µm membrane filter paper and stored in a refrigerator below 10^0C.

Preparation of formulation sample solution:
Ten tablets of Contrave® extended release tablets were weighed and the average weight was calculated. By using a clean pestle, the tablets were powdered uniformly. Form the tablet powder, a weighed amount of powder equivalent to 10mg of the standard bupropion HCl and 1mg of the standard naltrexone HCl was dissolved in 10ml of solvent. The contents were sonicated for 15 min and then the solution was filtered through 0.45µm membrane filters. From the above prepared stock solution, a concentration of 164µg/ml of bupropion Hcl and and 36µg/ml of naltrexone HCl were prepared by selective dilution technique.

Method Validation: The proposed method was subjected to validation for linearity, accuracy, precision, selectivity, sensitivity, robustness, ruggedness and system suitability parameters in accordance with International Conference on Harmonization (ICH) guidelines[18-20].

Linearity: Appropriate volume of aliquots, from naltrexone HCl and bupropion HCl standard stock solution were transferred to a 10mL volumetric flask. The volume was adjusted to the final mark with methanol to give solutions containing 10-100µg/mL for both the drugs. The prepared solutions were injected into the HPLC chromatographic system using the optimized conditions. Peak areas obtained were used to draw a calibration curve against the prepared concentration.

The best fit linear graph was obtained within the concentration range of 40100µg/mL range. The slope, Y-intercept and correlation coefficient were calculated using regression analysis. The calibration curves were linear in the studied range for both the drugs (**Fig.2**). Results of the regression line relating to the standard concentrations of drug were given in **Table 1**.

Accuracy: Accuracy was assessed by determining the recovery of the method. Standard drug was added to the pre-quantified placebo preparation at 3 different concentration levels 50%, 100% and 150%, taking into consideration percentage purity of added bulk drug samples. Each concentration was analyzed in triplicate and average recoveries were measured. The % recoveries were found to be 98.2-101.71% for naltrexone HCl and 98.9-101.4% for bupropion HCl in the proposed method. The % recoveries were found to be within the acceptance limit of 98-102% (**Table 2**). This confirms that the proposed method was found to be accurate.

TABLE 1: CHARACTERISTICS OF CALIBRATION CURVES FOR THE ESTIMATION OF DRUGS (y=ax + b, WHERE y IS PEAK AREA AND x IS CONCENTRATION, µg/mL)

Compound	Slope (a)	%RSD of a	Intercept (b)	%RSD of b	Correlation coefficient (r)
Bupropion	6466.7	1.3	10443.6	0.42	0.9995
Naltrexone	44454.3	1.6	3122.9	1.5	0.9990

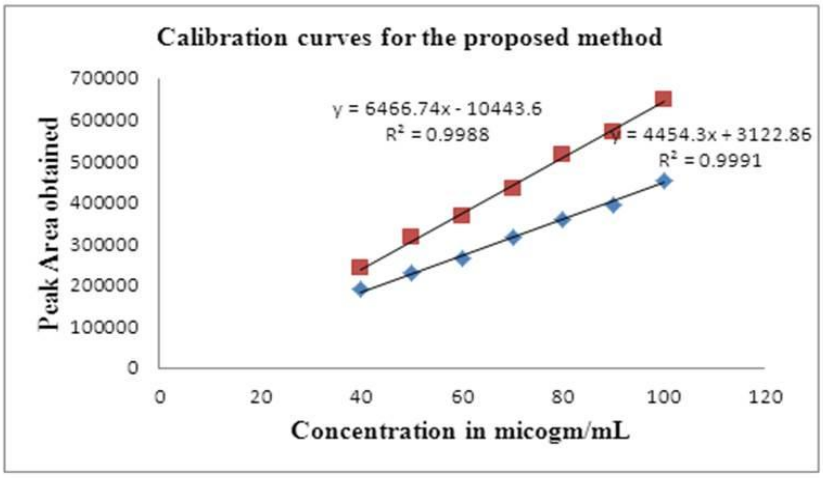

FIGURE 2: LINEARITY GRAPH FOR BUPROPION HCl AND NALTREXONE HCl

TABLE 2: ACCURACY RESULTS FOR BUPROPION HCl AND NALTREXONE HCl

% of Recovery	Concentration in µg/ml			Naltrexone HCl		Bupropion HCl	
	Target	Spiked	Final	Conc., obtained	% of Assay	Conc., obtained	% of Assay
	40	20	60	59.70	99.50	60.26	100.44
50%	40	20	60	60.55	100.92	59.49	99.16
	40	20	60	61.03	101.71	60.65	101.09
	40	40	80	78.91	98.64	79.13	98.92
100%	40	40	80	79.17	98.97	79.80	99.75
	40	40	80	78.58	98.23	81.11	101.39
	40	60	100	98.74	98.74	98.86	98.86
150%	40	60	100	98.98	98.98	99.95	99.95
	40	60	100	100.35	100.35	98.91	98.91

Precision: The intraday precision of the proposed method was checked by carrying out six independent assays of test samples. A concentration of 60µg/mL of naltrexone HCl and bupropion HCl was prepared in six different times and the prepared solutions were analyzed using the developed method. Peak area of the resultant chromatogram was used for the determination of the precision of the method. Mean, SD and an RSD (%) value of six assays was calculated using the peak area. The % RSD was found to be 0.78 and 1.02 for naltrexone HCl and bupropion HCl, respectively. The intraday precision results were found to be within the acceptance limits of N.L.T. 2, indicating a high degree of repeatability (**Table 3**). The reproducibility of the method was carried out by estimating response of naltrexone HCl and bupropion HCl at a concentration of 60µg/mL. The solution was prepared and analyzed using the proposed method on the same day in triplicate and on three different days for same concentrations and the results were reported in terms of relative standard deviation. The % RSD was found to be 1.14 and 0.84 for naltrexone HCl and bupropion HCl, respectively. The interday results were found to be within the acceptance limits of N.L.T 2, indicating a high degree of reproducibility (**Table 3**).

TABLE 3: INTERDAY AND INTRADAY PRECISION OF THE PROPOSED METHOD

Drug	Concentration, µg/mL*	Inter-day precision		Intra-day precision	
		Mean	% RSD	Mean	% RSD
Naltrexone HCl	60	257952.8	1.14	265343.7	0.78
Bupropion HCl	60	373665.0	0.84	364241.3	1.02

*Average of 6 values

Specificity: The specificity of an analytical method may be defined as the ability to detect the analyte peak in the presence of the analyte by product, or other inactive components, such as dosage form excipient or impurities. The specificity of the method was evaluated by injecting blank solutions containing the mobile phase, which exhibits a steady zero baseline at the selected wavelengths. Also the placebo chromatogram showed no additional peaks indicating the specificity of the proposed method.

The standard solution exhibits two sharp peaks at retention times of 7.4 and 3.4 min on injecting the two standard drugs separately. This confirms that retention time of 3.4 min corresponds to that of naltrexone HCl and 7.4 min to be that of bupropion HCl in the combined standard chromatogram. This confirms that the developed method was specific. The chromatogram of blank, standard and sample were given in **Fig. 3**

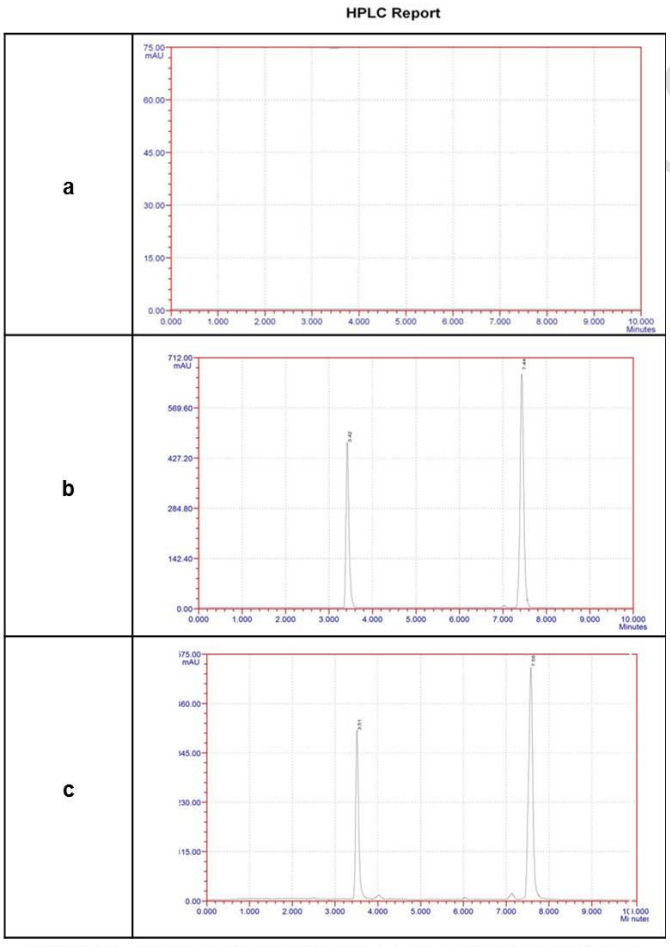

FIGURE 3: CHROMATOGRAM OF (a) BLANK, (b) STANDARD AND (c) FIXED DOSE FORMULATION OF BUPROPION AND NALTREXONE HCl

Sensitivity of the method: The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions. LOD and LOQ were estimated from signal to noise ratio. LOD is the lowest concentration resulting in a peak area of three times the baseline noise and the equation is LOD = 3.3 x SD/S. LOQ is the lowest concentration that provide signal to noise ratio more than 10 and the equation is LOQ = 10 x SD/S, where 'SD' is the average standard deviation and 'S' is the slope of the line. The LOD and LOQ were found to be 0.5µg/ml and 1.7µg/ml, respectively, for both the drugs. This confirms that the proposed method can detect the drug up to a minimum concentration of 0.5µg/ml. These results indicated that the developed method was sensitive (**Table 4**).

TABLE 4: LOD AND LOQ RESULTS FOR BUPROPION HCl AND NALTREXONE HCl

Parameter	Bupropion HCl	Naltrexone HCl
LOD	0.5 µg/ml	0.5 µg/ml
LOQ	1.75 µg/ml	1.75 µg/ml

Ruggedness: Ruggedness of the method was generally determined between two different labs, different analyst, different instrument and columns. Here ruggedness was performed by two different analysts under same environmental conditions. Standard solutions of naltrexone HCl and bupropion HCl at a concentration of 60µg/mL were prepared six times by different analysts and analyzed in the same experimental conditions. The % RSD of the resultant peak area obtained was calculated. The %RSD was found to be 0.71 for Naltrexone HCl and 0.58 for Bupropion HCl. The results of ruggedness were found to be very low than the acceptable limit (**Table 5**). This confirms that in the proposed method, no remarkable changes were observed in the peak area, with change in analysts.

TABLE 5: RUGGEDNESS RESULTS OF THE PROPOSED HPLC METHOD

Drug concentration*	Isoniazid, 60 µg/ml		Rifampicin, 60µg/ml	
Analyst	1	2	1	2
Mean peak area	261015.7	263967.3	362715.0	364156.3
% RSD	0.64	0.78	0.56	0.60

* Average of 6 values

Robustness: Robustness was performed by deliberately changing the chromatographic conditions. The important parameter to be studied was the resolution factor between two peaks. Robustness of the method was carried out by deliberately made small variation in the wavelength of the detector, pH of mobile phase and organic phase ratio by using 60µg/mL of both the drugs. The prepared solution of 60µg/mL was injected using the changed conditions. The peak area obtained was compared with the calibration values and % RSD in the results due to the change in conditions was calculated. The % RSD was found to be in the range of 0.6-1.5% for naltrexone HCl and 0.1-1.2% for bupropion HCl. The results of robustness were found to be within the acceptance limits of N.L.T. 2 (**Table 6**). This confirms that the small change in the developed conditions doesn't influence the results and % RSD was found to be very less. Hence the developed method was found to be robust.

TABLE 6: ROBUSTNESS RESULTS OF THE PROPOSED HPLC METHOD

S.No	Condition	Naltrexone HCl		Bupropion HCl	
		Mean area*	% RSD	Mean area*	% RSD
1	Standard	264514	……	364586	……
2	◙MP change-1 M:A:W (65:15:20 v/v)	262839	0.63	364970	0.10
3	MP change-2 M:A:W (55:25:20 v/v)	268558	1.52	365391	0.22
4	◘WL change-1 (252)	261300	1.21	360261	1.18
	WL change-2 (256)	266077	0.59	364819	0.06
5	pH change-1 (4.6)	261429	1.10	361749	0.77
6	pH change-2 (5.0)	260922	1.30	365674	0.29

*Average of 3 values
◙MP- Mobile phase
◘WL- Wavelength

System suitability: System suitability was defined as tests to measure the method that can generate result of acceptable accuracy and precision. The system suitability was carried out after the method development and validation have been completed. The system suitability was assessed by six replicate analyses of the drugs at concentrations of 60µg/ml for both the drug. Parameters measured for system suitability were %RSD of retention time, area, plate number (n), tailing factor and resolution of samples. The results confirm that the method obeys system suitability criteria (**Table 7**).

TABLE 7: SYSTEM SUITABILITY PARAMETERS OF BUPROPION HCl AND NALTREXONE HCl

Parameter*	Naltrexone HCl	Bupropion HCl	Acceptance limit
Retention time (min)	3.4	7.4	-
Area	264514.6	364586.1	-
No. of theoretical plates	7598	35105	More than 2500
Tailing factor	0.75	1.09	Less than 2
Resolution	…..	25.2	More than 2

*Average of 3 values

Formulation analysis: Formulation analysis was carried out in a marketed extended release formulation Contrave®. The prepared formulation solution was analyzed using the developed method. The resultant chromatogram confirms that the components in the formulation were retained in the same retention time as standard. Sharp peaks were observed in the chromatogram without any of the interfering peaks. Peak area of the two peaks was noted and the amounts were found to be 99.68% and 99.45% for naltrexone HCl and bupropion HCl, respectively.

The experimental results of the amount of naltrexone HCl and bupropion HCl combination tablet were in good agreement with the percentage of label claim. A good agreement of result indicates that there was no interference from any of the excipients which were normally present in the tablet. The results are shown in **Table 8** and formulation chromatogram was given in **Fig.3**.

TABLE 8: FORMULATION ANALYSIS OF BUPROPION HCl AND NALTREXONE HCl COMBINATION

Brand name	Available form	Label claim	Concentration µg/ml	Amount found µg/ml	% Assay
Contrave®	Tablet	BPPN- 90mg NTX-8 mg	BPPN-60 NTX-60	BPPX-59.81 NTX-59.67	BPPN-99.68 NTX-99.45

RESULTS AND DISCUSSION:

Method development: Due to the polar properties of bupropion HCl and naltrexone HCl, a reversed phase HPLC system was used to analyze both compounds with sufficient separation and fine peak shapes within a short analysis time. Therefore, all the experiments were carried out on a C18 column by trying various mobile phase conditions systematically. The initial trails were selected based on the solubility of the two components and the separation was achieved in the selected solvents.

To investigate the appropriate wavelength for simultaneous determination of bupropion HCl and naltrexone HCl, solutions of these compounds in the mobile phase were scanned by UV–visible spectrophotometry in the range 200–400 nm. From the overlaid UV spectra, suitable wavelength choices considered for monitoring the drugs were 254nm, 238nm and 210nm. Solutions of each substance in the mobile phase were also injected directly for HPLC analysis and the responses (peak area) were recorded at 254nm, 238nm, and 210 nm. It was observed there was no interference from the mobile phase or baseline disturbance at 254nm, in contrast with 238nm and 210nm. It was, therefore, concluded that 254nm is the most appropriate wavelength for analysis of the two substances with suitable sensitivity.

Because the compounds of interest are predominantly polar and of low molecular mass, two reversed-phase columns of different polarity, a Chromosil C18 Column; 250x4.6 mm 0.45µm id and a CN-Phenomenex (250 mm × 4.6 mm i.d., 5-µm particles) were tried. Several mobile phases containing different ratios of solvent were evaluated with the C18 column. The mobile phase conditions were optimized so the peak from the first-eluting compound did not interfere with those from the solvent, excipients, or sample components. Other criteria, viz. time required for analysis, appropriate k range ($1 < k < 10$) for eluted peaks, assay sensitivity, solvent noise, and use of the same solvent system for extraction of drug from formulation matrices during drug analysis, were also considered. After each change of mobile phase, the column was re-equilibrated by passage of at least ten column volumes of the new mobile phase. The retention times of the solutes decreased with increasing concentration of organic modifier. It was noticed that the k value was too low at the lowest concentrations of the organic modifier. In contrast, acetonitrile concentrations which were too high resulted in k values were too high ($k > 10$) resulting in excessively long runtimes.

It is well known that multiple-component mobile phases result in better separation efficiency than binary mobile phases, because with these both solvent strength and selectivity can be varied simultaneously to obtain the desired retention times. After the initial experiments, symmetric peak shape was obtained with a mobile phase of methanol: acetonitrile: water in the ratio of 60:20:20 (v/v) and this mobile phase was found to be suitable for separation of both the drugs. Usage of the optimized mobile phase resulted in a quality separation in terms of peak symmetry, optimum resolution, reasonable run time, and acceptable k values. No further improvement in peak symmetry was observed when pH of the mobile phase was changed to acidic /or basic. Increasing the flow rate reduces the total run time and retention times; and also effects the separation and resolution of the two drugs. Decreasing the flow rate of the mobile phase increases the run time and alters the peak shape. Hence an optimized mobile phase flow rate of 1.0 mL/min was found to be suitable for the separation of bupropion HCl and naltrexone HCl.

Detection and chromatography:

Taking into consideration of system suitability parameters like retention time, tailing factor, number of theoretical plates, and other peak responses like capacity factor, peak asymmetry and resolution of drugs, the method was devloped. The mobile phase consisting of varying percentages of organic phases and buffers of different pH were

tested at a suitable overlay maximum absorption wavelength of 254 nm on a C18 stationary phase. Finally a mobile phase of methanol: acetonitrile: water in the ratio of 60:20:20 (v/v) on Chromosil C18 Column (250x4.6 mm 0.45µm id) at a wavelength of 254nm and flow rate of 1.0mL/min in isocratic mode was found to be suitable for the separation and estimation of naltrexone HCl and bupropion HCl. Under the optimized chromatographic conditions, naltrexone HCl and bupropion HCl eluted at 3.4min and 7.4min, respectively with a resolution of 25.2. Both the peaks in the HPLC chromatogram obey the system suitability criteria.

Method validation:

The best fit linear graph was obtained within the concentration range of 40-100µg/mL range for both the drugs with a regression equation of y=4454.3x+3122.86; R^2=0.999 for naltrexone HCl and y=6466.74x-10443.6; R^2=0.999 for bupropion HCl, respectively. Spiked recovery of 40µg/mL target was performed for both the drugs using standard addition method at three different concentration levels 50%, 100% and 150%. The % recoveries were found to be 98.2-101.71% for naltrexone HCl and 98.9-101.4% for bupropion HCl in the proposed method. The % recoveries were found to be within the acceptance limit of 98-102%. Hence the developed method was found to be accurate.

Standard solution at 60µg/mL of naltrexone HCl and bupropion HCl were used for the determination of precision, ruggedness and robustness of the method. The % RSD in three precision studies were found to be 0.78, 1.14, 0.71 for naltrexone HCl and 1.02, 0.84 and 0.58 for bupropion HCl, respectively in intraday, interday and ruggedness studies. The same concentrated solution was used for the determination of robustness of the method. On change in the mobile phase ratio, pH and wavelength of the detector, no remarkable change was observed in the peak area and % RSD was found to be less than two in all the altered conditions. Hence the developed method was found to be precise.

The applicability of the method in lowest concentration of sample was determined by studying the sensitivity. LOD and LOQ were determined by using signal to noise ratio of the analyte. The LOD was found to be 0.5µg/mL and LOQ was found to be 1.7µg/ml for both the drugs. This confirms that the proposed HPLC method can detect the drug up to a minimum concentration of 0.5µg/mL. Hence the proposed method was found to be sensitive.

Assay: The utility of the developed method for the routine analysis of naltrexone HCl and bupropion HCl in pharmaceutical formulations was determined using a markedly available combined dosage form Contrave®. In the assay study, label claim were in good agreement with the experimental amounts, 99.7% and 99.5% for naltrexone HCl and bupropion HCl, respectively.

CONCLUSION: A simple, sensitive, precise, accurate, robust and cost-effective reverse phase high performance liquid chromatographic method was developed and validated for the simultaneous estimation of bupropion HCl and naltrexone HCl from combined formulation. The mobile phase consists of a mixture of methanol, acetonitrile and water in the ratio of 60:20:20v/v (pH adjusted to 4.8). It does not use any tedious preparation of buffers which commonly block the column, and no carry over effect was observed.

In conclusion, method validation following ICH guidelines indicated that the developed method has high sensitivity, acceptable recovery, reliability, specificity and excellent efficiency with a total running time of 10 min per sample and retention time of 3.4 and 7.4 min for naltrexone HCl and bupropion HCl, respectively, which is important for large batches of samples. The method showed good linearity in the ranges of 40-100 µg/mL, with high correlation coefficient values (R^2> 0.999) for both the drugs. The LOD was found to be 0.5µg/mL and LOQ was found to be 1.7µg/ml for both the drugs. The proposed RP-HPLC method was applied successfully for the routine analysis of the tablet sample of naltrexone HCl and bupropion HCl and the results of the assay were obtained within the specification limit.

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Reviewer's recommendations:

1. Specify designation and current full address of corresponding author.
2. Check for spelling, grammar and punctuation error(s).