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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF NALTREXONE HYDROCHLORIDE AND BUPROPION HYDROCHLORIDE IN ORAL DOSAGE FORM (TABLETS) BY RP-HPLC TECHNIQUE

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ABSTRACT

A simple, rapid, accurate, precise specific and sensitive reverse phase high performance liquid chromatographic method has developed and validated for the simultaneous estimation of Naltrexone hydrochloride (NTX) and Bupropion hydrochloride (BUP) in tablet dosage form. The chromatographic separation was performed using phenomenex C_{18} Column Inertsil ODS having dimensions of 4.6x250mm having particle size of 5µm, with mobile phase consisting of Buffer and Acetonitrile (60:40% v/v), flow rate was adjusted to 1.0ml/min and detection wavelength at 224 nm. The proposed method has been validated for linearity, range, precision, accuracy and robustness were within the acceptance limit according to the ICH Q2B guidelines. The retention times of Naltrexone Hydrochloride and Bupropion Hydrochloride were 2.5 mins and 4.6 mins respectively. The linearity was performed in the concentration in the range of 6.25µg/ml to 18.75µg/ml and 37.5µg/ml to 112.5µg/ml and with a correlation coefficient of 0.998 and 0.999 respectively. % RSD for system precision was found to be 0.504 and 0.922,% RSD for repeatability 0.429 and 0.350, % RSD for intermediate precision was 0.937 and 0.0503 respectively. The % percentage purity of Naltrexone Hydrochloride and Bupropion Hydrochloride was found to be 99.63% and 99.20% respectively. The method was found to be robust even by change in the mobile phase ±5% in less flow condition.

Keywords: - Naltrexone hydrochloride, Bupropion hydrochloride, RP-HPLC, Method development, Validation

INTRODUCTION

Naltrexone Hydrochloride is chemically 17-(cyclopropylmethyl)-4,- 5α-epoxy-3, 14 Dihyroxymorphinan-6-one,its chemical formula is $C_{20}H_{23}NO_4HCl$, Molecular weight is 341.401, pKa value is 7.39. It is white to pale cream colored powder, soluble in water. Its melting point is 274-276˚C. It belongs to category opioid antagonist. The drug is available in tablet form (50mg) and is official in any pharmacopoeia Literature survey reveals that few spectrophotometric methods[2-3] has been reported for the estimation of Naltrexone Hydrochloride. The sructure of Naltrexone Hydrochloride is shown in Fig.1.

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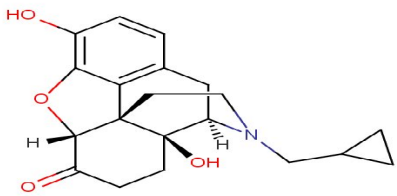


Fig.1. Chemical structure of Naltrexone Hydrochloride

Bupropion, chemically (±)-2-(tert-butylamino)-1-(3-chlorophenyl)-propan-1-one, previously known as amfebutanone is an atypical antidepressant and also used as an smoking cessation aid. It acts as a nor-epinephrine and dopamine reuptake inhibitor as well as α3, β4 receptor antagonist its chemical formula is $C_{13}H_{18}CINO$, Molecular weight is, 377.86, pKa value is18.29. It is available in white to pale yellow colored. It is freely soluble in water. Melting point is 233-234˚C, belongs to category antidepressant. The drug is available in tablet form

(100mg, 150mg and 450mg) and is official in any pharmacopoeia.Literature survey reveals that few spectrophotometric methods[4] , HPLC methods[5] Spectrophotometric[6] has been reported for the estimation of Bupropion Hydrochloride. The sructure of Bupropion Hydrochloride is shown in Fig.2.

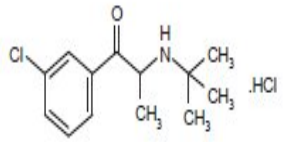


Fig.2. Chemical structure of Bupropion Hydrochloride

MATERIALS AND METHODS

Instrumental and analytical conditions:

Reagents and chemicals:

The pharmaceutical drug sample Naltrexone Hydrochloride and Bupropion Hydrochloride were obtained Glaxo Smith Kline's pharmaceutical Pvt.Ltd and Elisa pharmaceutical India Ltd. respectively. All the chemicals used of HPLC grade. Phosphate buffer and acetonitrile was purchased from Thermo Fischer Scientific India Pvt. Ltd. Milli Q Water was used in the buffer preparation.

Equipment:

A Waters e2695 gradient system with Empower-2 software and 2489 UV/Vis detector is the most sensitive and versatile dual wave length absorbance detector was used. It was manufactured by the company Waters, Alliance, Japan. Intelligent LC pump with sampler programmed at 20µL capacity per injection was used.

Chromatographic conditions:

The column used was Inertsil ODS C18 column (250mm×4.6mm, 5µm particle size) was used for analytical separation. The mobile phase consists of an aqueous solution of mixed phosphate buffer (pH 3 adjusted with ortho-phosphoric acid and acetonitrile in the ratio of (60:40%v/v). The flow was adjusted to 1ml/min. The instrument was operated at an ambient temperature. The injection volume was 20µL. The UV detection was achieved at 224 nm which is the isobestic point shown in Fig.3.

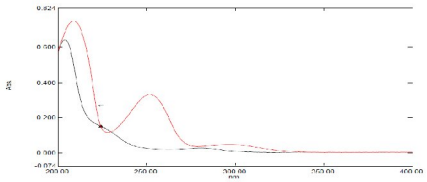


Fig.3.UV spectra showing Isobestic point of Naltrexone hydrochloride and Bupropion Hydrochloride.

Wavelength	A bsorbance	Description
224 nm	0.151	NTX & BUP

Preparation of analytical solutions:

Preparation of mixed phosphate buffer solution:

Accurately weighed 3.275 g of 0.02M KH_2PO_4 and 0.69 g of 0.03 M KH_2PO_4 dissolved in 100 ml of water filter, sonicate and adjust it with pH 3 using orthophosphoric acid and sonicate it and filter it.

Preparation of mobile phase:

Mix a mixture of phosphate buffer pH 3 adjusted with orthophosphoric acid 60% and acetonitrile 40% and degassed in ultrasonic water bath for 5 min. Filter through 0.22µ filter under vaccum filtration.

Diluent preparation: Mobile phase was used as the diluent.

Preparation of the individual Naltrexone Hydrochloride standard preparation:

25mg of NTX working standard was accurately weighed & transferred in to a 100 ml clean dry volumetric flask and add about 10 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution) Further pipette out 1 ml of Naltrexone Hydrochloride from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of individual Bupropion Hydrochloride standard preparation

75mg of BUP working standard was accurately weighed & transferred into a 100ml clean dry volumetric flask and add about 10 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1 ml of Bupropion Hydrochloride from the above stock solution into a 10 ml volumetric flask & was diluted up to the mark with diluent.

Preparation of sample solution

265mg of Naltrexone Hydrochloride & Bupropion Hydrochloride tablet powder was weighed accurately & transferred into a 100ml clean dry volumetric flask & add about 50 ml of diluent & sonicate to dissolve it completely & make volume up to the mark with the same solvent (stock solution). Further pipette out 10 ml of Naltrexone Hydrochloride & Bupropion Hydrochloride from the above stock solution into a 100 ml volumetric flask & was diluted up to the mark with diluent.

Method Development and Validation of HPLC:

The suggested analytical method was validated according to ICH guidelines (Q2B) [13] with respect to certain parameters such as specificity, linearity, accuracy, precision, robustness and system suitability.

Specificity:

The specificity was carried out to determine whether there are any interference of any impurities (presence of components may be unexpected to present) in retention time of analytical peak. Forced

degradation studies are carried out by using 0.1M HCl, 0.1M NaOH, heat and UV light.

Linearity:

Linearity of an analytical procedure as its ability (within a given range) to obtain test results that are directly proportional to the concentration (amount) of analyte in the sample. The linearity of the method was established by a spiking a series of sample mixtures of Naltrexone Hydrochloride and Bupropion Hydrochloride the solutions of six different concentration levels 6.25-112.5µg/ml are injected in to the HPLC system. Calibration curve was constructed for the standard solutions by plotting their response ratios (ratios of the peak area of the analytes) against their respective concentrations linear regression was applied and slope-a, intercept-b, correlation coefficient-R^2 and standard error were determined.

Accuracy:

Accuracy was determined in terms of percentage recovery the accuracy study was performed for 80%, 100% and 120 % for Naltrexone Hydrochloride and Bupropion Hydrochloride. Standard and sample solutions are injected in to HPLC system in triplicate and percentage recoveries of Naltrexone Hydrochloride and Bupropion Hydrochloride were calculated. The area of each level was used for calculation of % recovery.

Precision:

Express the closeness of agreement between the series of measurement obtained from multiple sampling of same homogeneous sample under the prescribed conditions. Method precision was determined both in terms of repeatability (injection and analysis) and intermediate precision/Ruggedness (It shows the degree of reproducibility of test results obtained by analyzing the sample under variety of normal test conditions such as analyst, instruments). In order to determine precision, six independent sample solution preparations from a single lot of formulation Naltrexone Hydrochloride of 20µg/ml and 20µg/ml for Bupropion Hydrochloride was injected in to HPLC system, the retention time and peak area was determined and expressed as mean and %RSD calculated from the data obtained which are found to be within the specified limits.

Robustness:

Robustness of the developed method was investigated by evaluating the influence of small deliberate variations in procedure variables like flow rate (±5%) and change in wave length (±5nm). The robustness was performed for the flow rate variations from 0.9ml/min to 1.1ml/min and the method is robust only in less flow condition and even by change in the mobile phase ±5%.

System Suitability:

System suitability tests were carried out on freshly prepared standard stock solutions of Naltrexone Hydrochloride and Bupropion Hydrochloride it was calculated by injecting standards in six replicates at 6 minutes interval and the values were recorded.

RESULTS AND DISCUSSIONS

The present investigation reported is a new RP-HPLC method development and validation of simultaneous estimation of Naltrexone Hydrochloride and Bupropion Hydrochloride. The method developed was proceeding with wavelength selection. The optimized wavelength was 224 nm. In order to get the optimized RP-HPLC method various mobile phases and columns were used. From several trials final method is optimized with the following conditions: The mobile phase consists of an aqueous solution of mixed phosphate buffer (pH3 adjusted with ortho phosphoric acid) and acetonitrile in the ratio of 60:40%v/v and the column used was Thermo Scientific Inertsil ODS C18 column (250mm×4.6mm,5µm particle size). The flow rate was adjusted to 1ml/min. The instrument was operated at an ambient temperature. The UV detection was achieved at 224nm and purity analysis was performed over a wavelength range of 200-400nm. The injection volume was 20µL. The specificity of the method was to determine whether there are any interference of any impurities (the presence of components may be unexpected to present) in retention time of analytical peak. The linearity was determined as linearity regression of the claimed analyte concentration of the range 6.25-37.5µg/ml for Naltrexone Hydrochloride and 18.75-112.5µg/ml for Bupropion Hydrochloride. The calibration curve obtained by plotting peak area versus concentration and presented in **Table 1** was linear and the correlation coefficient was found to be 0.998 and 0.999 for Naltrexone Hydrochloride and Bupropion Hydrochloride respectively.The precision of the method was ascertained from determinations of peak areas of six replicates of sample solution. The %Relative Standard Deviation for system precision presented in **Table 2** was found to be 0.504and 0.922 and the % Relative Standard Deviation for method precision presented in **Table 3** was found to be 0.429 and 0.350. The accuracy study was performed in 80%, 100% and 120% .The percentage recovery was determined for Naltrexone Hydrochloride and Bupropion Hydrochloride was found to be 99.6 and 99.8% presented in **Tables 4&5.** The robustness were carried out with minor but deliberate changes in parameters i.e., detection wavelength, column temperature, and flow rate as presented in **Table 6**. Theoretical plates and tailing factor were observed and were found to be 2834.29 and 5022.67 (theoretical plates) and 1.18 and 1.38 (tailing factor). The system suitability parameters like theoretical plates (N), tailing factor (T) were calculated and were found to be more than 2000 and not more than 2 and ascertained that proposed RP-HPLC method was accurate and precise as presented in **Table 7.**Ruggedness values are mentioned in **Table 8.**

Table No. 1: Linearity results for Naltrexone Hydrochloride& Bupropion Hydrochloride

	Naltrexone Hydrochloride		Bupropion Hydrochloride	
S.No	**Concentration(µg/ml)**	**Area**	**Concentration(µg/ml)**	**Area**
1	6.25	241659	18.75	1338276
2	12.50	424675	37.50	2356096
3	18.75	641305	56.25	3576201
4	25.00	811194	75.00	4506710
5	31.25	1016933	93.75	5664898
6	37.50	1238844	112.50	6812492

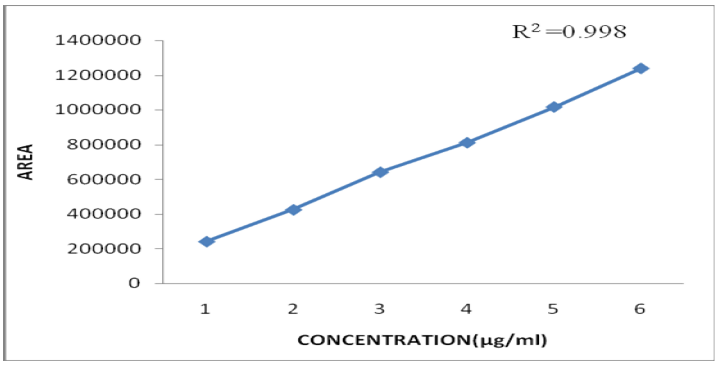


Fig .4. Linearity graph for Naltrexone Hydrochloride

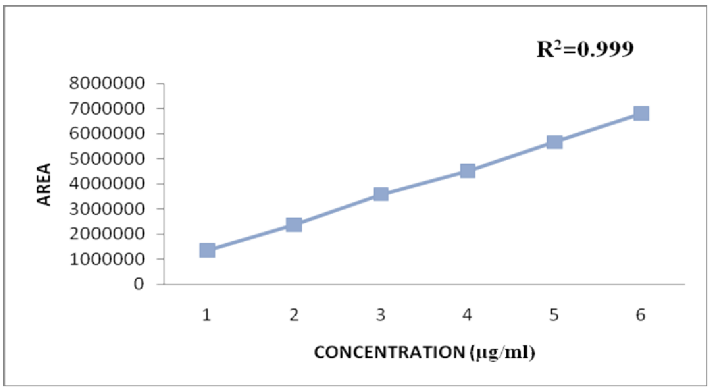


Fig.5. Linearity graph for Bupropion Hydrochloride:

Table No. 2: System Precision values for Naltrexone Hydrochloride and Bupropion Hydrochloride

Table .2.1 % RSD results for Naltrexone Hydrochloride

S.No.	**Injection**	**Peak name**	**R_t**	**Area**	**Height**
1	Injection -1	Naltrexone Hydrochloride	2.533	828174	119023
2	Injection-2	Naltrexone Hydrochloride	2.534	834710	115664
3	Injection-3	Naltrexone Hydrochloride	2.535	845370	122832
4	Injection-4	Naltrexone Hydrochloride	2.537	839258	120356
5	Injection-5	Naltrexone Hydrochloride	2.536	835962	118023
6	Injection-6	Naltrexone Hydrochloride	2.539	839965	115664
Average				835573	
Standard deviation				4208.36	
%RSD				0.504	

Table.2.2 %RSD results for Bupropion Hydrochloride

S.No.	Injection	Peak name	R_t	Area	Height
1	Injection-1	Bupropion Hydrochloride	4.654	4590330	480874
2	Injection-2	Bupropion Hydrochloride	4.657	4633377	452046
3	Injection-3	Bupropion Hydrochloride	4.657	4695188	499366
4	Injection-4	Bupropion Hydrochloride	4.652	4694235	4823636
5	Injection-5	Bupropion Hydrochloride	4.656	4689325	481874
6	Injection-6	Bupropion Hydrochloride	4.655	4682589	452046
Average				4664174	
Standard deviation				42985.13	
%RSD				0.922	

Table. No. 3.1: Method precision values of Naltrexone Hydrochloride

S.No	Injection	Peak Name	R_t	Area	Height
1	Injection-1	Naltrexone Hydrochloride	2.537	873925	121336
2	Injection-2	Naltrexone Hydrochloride	2.537	876263	115242
3	Injection-3	Naltrexone Hydrochloride	2.538	879265	115695
4	Injection-4	Naltrexone Hydrochloride	2.536	878523	120523
5	Injection-5	Naltrexone Hydrochloride	2.539	879236	118242
6	Injection-6	Naltrexone Hydrochloride	2.535	885145	118695
Average				**878726**	
Standard Deviation				**3765.94**	
%RSD				**0.429**	

Table. No. 3.2: Showing results for method precision of Bupropion Hydrochloride

S.No	Injection	Peak Name	R_t	Area	Height
1	Injection-1	Naltrexone Hydrochloride	4.66	4998044	470449
2	Injection-2	Naltrexone Hydrochloride	4.658	4983145	452745
3	Injection-3	Naltrexone Hydrochloride	4.661	4972563	4694589
4	Injection-4	Naltrexone Hydrochloride	4.678	4945623	462435
5	Injection-5	Naltrexone Hydrochloride	4.662	4982362	459475
6	Injection-6	Naltrexone Hydrochloride	4.677	4973015	4614589
Average				**4975792**	
Standard Deviation				**17439.55**	
%RSD				**0.350**	

Table No.4.Accuracy results for Naltrexone Hydrochloride

%Concentration (at specific level)	Average area	Amount added(mg)	Amount found(mg)	%Recovery	Mean recovery
80%	691384	20	19.718	98.59%	99.96%
100%	876441	25	24.996	99.98%	
120%	1054548	30	30.000	100.25%	

Table No.5.Accuracy results for Bupropion Hydrochloride

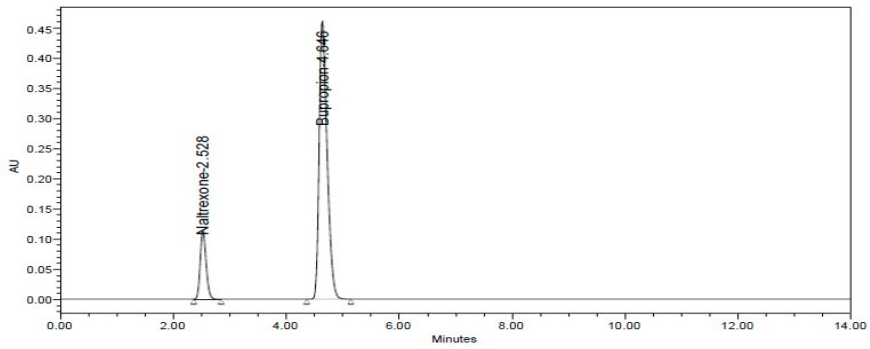
%Concentration (at specification level)	Average area	Amount add(mg)	Amount found (mg)	%Recovery	Mean recovery
80%	3967849	60	59.677	99.46%	99.8%
100%	4983928	75	74.960	99.95%	
120%	5983919	90	90.0	100.00%	

Table.No.6 Robustness results of Naltrexone Hydrochloride & Bupropion Hydrochloride

S.No	Parameter	Naltrexone Hydrochloride		Bupropion Hydrochloride	
		Rt	Area	Rt	Area
1.	Standard	2.528	811194	4.646	4506710
2.	Robustness-Flow-1	2.265	795621	4.176	4420293
3.	Robustness-Flow-2	2.8886	1040536	5.286	5785191
4.	Robustness-oven-Temp-1	2.562	927076	4.782	5133396
5.	Robustness-oven-Temp-2	2.527	931118	4.539	5177609

Table No.7: System suitability Parameters for Naltrexone Hydrochloride and Bupropion Hydrochloride

Parameter	Naltrexone Hydrochloride	Bupropion Hydrochloride
R_t	2.528	4.646
Tailing factor	1.18	1.38
Resolution		9.11
Plate count	2834.29	5022.67
Assay value	99.63	99.20



	Peak Name	RT	Area	Height	% Area	USP Resolution	USP Tailing	USP Plate Count
1	Naltrexone	2.528	811194	113452	15.254		1.18	2834.29
2	Bupropion	4.646	4506710	461057	84.746	9.11	1.38	5022.67

Fig.No.6 Standard chromatogram of Naltrexone Hydrochloride & Bupropion Hydrochloride

Ruggedness:

Table No. 8. Showing ruggedness charts 1 and 2

S.No	Naltrexone		Bupropion	
	RT	Area	RT	Area
1	2.536	8782583	4.668	48948623
2	2.535	8792582	4.667	48978771
3	2.534	8782681	4.666	48988871
4	2.533	8782789	4.665	48998971
5	2.532	8782879	4.664	48988888
6	2.531	8782978	4.663	48998198
Avg	2.534	8784415	4.666	48983720
Std	0.0019	4003.3	0.0019	18717.58
RSD	0.074	0.046	0.040	0.038

S.No	Naltrexone		Bupropion	
	RT	Area	RT	Area
1	2.536	8782583	4.668	48948623
2	2.535	8792582	4.667	48978771
3	2.534	8782681	4.666	48988871
4	2.533	8782789	4.665	48998971
5	2.532	8782879	4.664	48988888
6	2.531	8782978	4.663	48998198
Avg	2.534	8784415	4.666	48983720
Std D	0.0019	4003.2739	0.0019	18717.5774
RSD	0.074	0.046	0.040	0.038

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of Naltrexone Hydrochloride and Bupropion Hydrochloride in pharmaceutical dosage form. The method was validated for parameters like specificity, linearity, accuracy, precision, robustness and system suitability values were found to be within limits. The method has significant advantages, in terms of shorter analysis time, selectivity, and accuracy than previously reported. The validation study indicates that method can be considered suitable for carrying out quality control and routine determination of Naltrexone Hydrochloride and Bupropion Hydrochloride pharmaceutical dosage form.

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