A New RP-HPLC Method for the Simultaneous Estimation of Naltrexone Hydrochloride and Bupropion in Its Pure and Pharmaceutical Dosage Form

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ABSTRACT

A rapid and precise high-performance liquid chromatography method has been developed for the validation of bupropion and naltrexone, in its pure form as well as in tablet dosage form. The chromatography was carried out on X-terra C18 (4.6x150mm, 5μ) column using a mixture of methanol: TEA buffer pH 4.5: Acetonitrile (65:15:20) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 212nm. The retention time of bupropion and naltrexone was 2.090, 5.289±0.02 respectively. The method produces linear responses in the concentration of 5-25mg/ml of bupropion and 45-225mg/ml of naltrexone. The method precision for the determination of assay was below 2.0% RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

INTRODUCTION

Analytical chemistry is the branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter. It is mainly involved in the qualitative identification or detection of compounds and the quantitative measurement of the substances present in bulk and pharmaceutical preparation. Measurements of physical properties of analytes such as conductivity, electrode potential, light absorption or emission, mass to charge ratio, and fluorescence, began to be used for quantitative analysis of a variety of inorganic and biochemical analytes. (1)

Highly efficient chromatographic and electrophoretic techniques began to replace distillation, extraction, and precipitation for the separation of components of complex mixtures prior to their qualitative or quantitative determination. These newer methods for separating and determining chemical species are known collectively as instrumental methods of analysis. Most of the instrumental methods fit into one of the three following categories viz spectroscopy, electrochemistry and chromatography. (2)

HPLC is a type of liquid chromatography that employs a liquid mobile phase and a very finely divided stationary phase. The rate of distribution of drugs between Stationary and mobile phase is controlled by a diffusion process. If diffusion is, minimized faster and effective separation can be achieved. For the recent study metformin and Sitagliptin was selected for estimation of the amount of analyte present in the formulation and bulk drug. (3)

The HPLC method is selected in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages like Speed many analysis can be accomplished in 20min (or) less, greater sensitivity, improved resolution, re-usable columns, ideal for the substances of low viscosity, easy sample recovery, automation, and quantification, precise and reproducible, integrator itself does calculations and suitable for preparative liquid chromatography on a much larger scale. The main objective of this study is to develop and validate new simple, sensitive, accurate and economical analytical method for the simultaneous estimation of Naltrexone and Bupropion accordance with USP and ICH guidelines. (4)

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Naltrexone is an Appetite Depressants, Narcotic Antagonists, Central Nervous System Depressants and Alcohol Antagonists available as Abernil, Adepend, Antaxon, Antaxone, Arrop, Celupan.
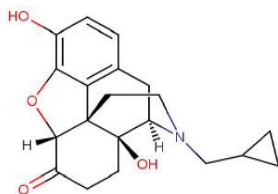
IUPAC Name: (1S,5R,13R,17S)-4-(cyclopropylmethyl)-10,17- dihydroxy-12-oxa-4 azapentacyclooctadeca-7(18),8,10-trien-14-one.

Bupropion HCl is used as a Anti-depressant, available as Bupdep
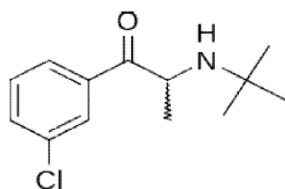
IUPAC Name**:** 2-(*tert*-butylamino)-1-(3-chlorophenyl) propan-1-one.

MATERIALS AND METHODS:

HPLC (Waters), Empower 2, 996 PDA Detector (Lab India), Digital ultrasonicator (Labman) Bupropion, Naltrexone, Water and Methanol for HPLC grade Acetonitrile (Merck)

HPLC method development (5-10)

Trails

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Bupropion and Naltrexone working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.15ml of the above Bupropion and 1.35ml of Naltrexone stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

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Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:

Initially, the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol: TEA Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Methanol: TEA Buffer: ACN in proportion 50:25:25 v/v respectively.

Optimization of Column:

The method was performed with various columns like C18 column, Symmetry, and Zodiac column. X-Terra C18 (4.6×150mm, 5μ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Preparation of Triethylamine (TEA) buffer (pH-4.5):

Dissolve 1.5ml of triethylamine in 250 ml of HPLC water and adjust the p^H 4.5. Filter and sonicate the solution by vacuum filtration and ultra-sonication.

Preparation of mobile phase:

Accurately measured 650 ml (65%) of Methanol, 150 ml of Triethylamine buffer (15%) and 200 ml of Acetonitrile (20%) were mixed and degassed in digital ultrasonicator for 10 minutes and then filtered through 0.45 μ filter under vacuum filtration.

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Bupropion and 10mg of Naltrexone working standard into a 10ml of clean dry volumetric flasks add about 7mL of diluents and sonicate to dissolve it completely and make the volume up to the mark with the same solvent. (Stock solution). Further pipette 0.15ml of the above Bupropion and 1.35ml of Naltrexone stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

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Preparation of Sample Solution:

Take the average weight of Tablet and crush in a mortar by using a pestle and weight 10 mg equivalent weight of Bupropion and Naltrexone sample into a 10mL clean dry volumetric flask and add about 7mL of diluent and sonicate to dissolve it completely and make the volume up to the mark with the same solvent. Further pipette 1.35ml of Sample stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

VALIDATION OF METHOD

Precision & Repeatability (11-14)

Preparation of Bupropion and Naltrexone Product Solution for Precision:

Accurately weigh and transfer 10 mg of Bupropion and 10mg of Naltrexone working standard into a 10ml of clean dry volumetric flasks add about 7ml of diluents and sonicate to dissolve it completely and make the volume up to the mark with the same solvent. (Stock solution). Further pipette 0.15ml of the above Bupropion and 1.35ml of Naltrexone stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent. The standard solution was injected five times and measured the area for all five injections in HPLC. The % RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate Precision:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Accuracy:

Three replicate injections of individual concentrations was injected for (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculated the amount found and the amount added for bupropion and naltrexone and calculated the individual recovery and mean recovery values.

Robustness:

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

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For the preparation of Standard solution:

Accurately weigh and transfer 10 mg of Bupropion and 10mg of Naltrexone working standard into a 10 ml of clean dry volumetric flasks add about 7 ml of diluents and sonicate to dissolve it completely and make the volume up to the mark with the same solvent (Stock solution). Further pipette 0.15ml of the above Bupropion and 1.35ml of Naltrexone stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Effect of Variation of flow conditions:

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are the same. 10µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of mobile phase organic composition:

The sample was analyzed by variation of mobile phase i.e. Methanol: TEA Buffer: Acetonitrile was taken in the ratio and 70:5:25, 60:30:10 instead (65:15:20), remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

Trials

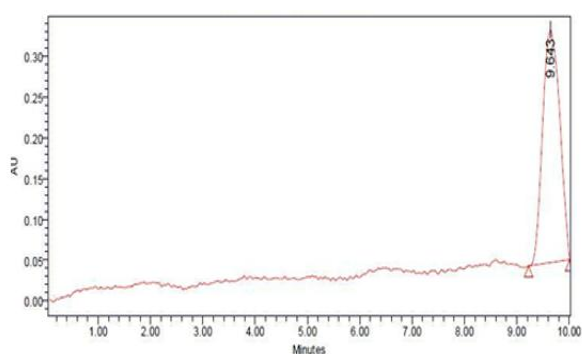**Figure 1: Chromatogram for trail 1**
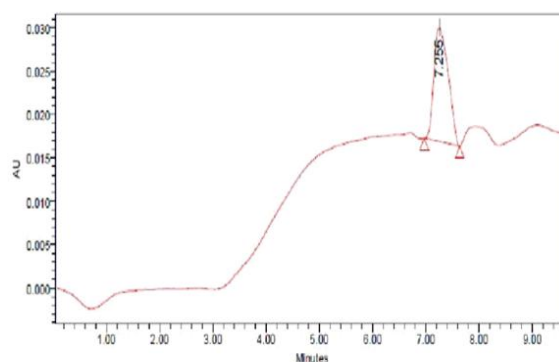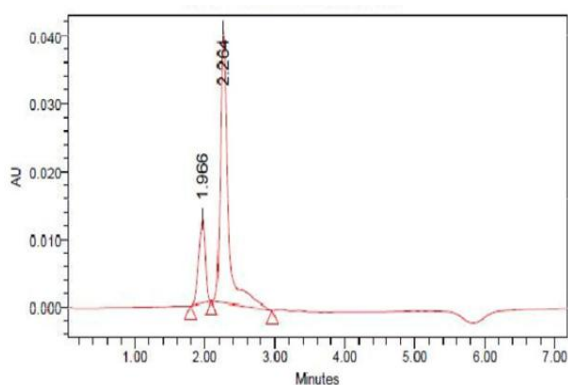
Figure 2: Chromatogram for trail 2

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Figure 3: Chromatogram for trail 3   **Figure 4: Chromatogram for trial 4**
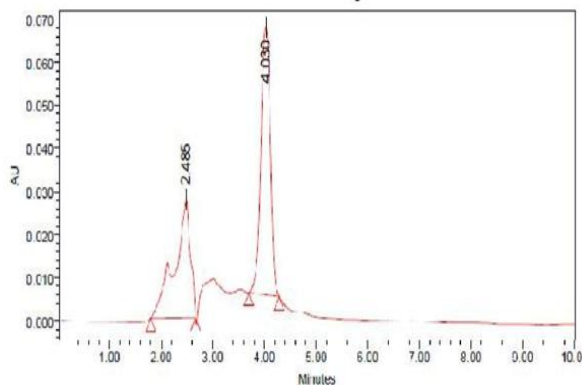
Table 1: Trial results

Trial	Peak Name	Rt	Area	Height	USP Tailing	USP Plate count
1	Naltrexone	9.643	43562	2341	7.1	134
2	Bupropion	7.255	45635	2534	5.6	595
	Bupropion	7.255	45635	2534	5.6	595
3	Bupropion	1.966	24536	1256		1292
	Naltrexone	2.264	53443	2134	3.1	677
4	Bupropion	2.48	243567	64546	2.1	1140
	Naltrexone	4.030	2964774	198276	2.5	1345

Optimized Chromatograms of Standard and sample"
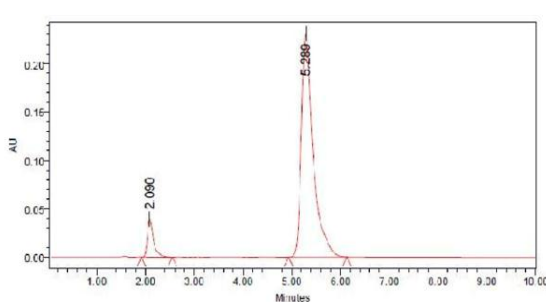
Figure 5: Optimized Chromatogram   **Figure 6: Optimized Chromatogram (Sample)**
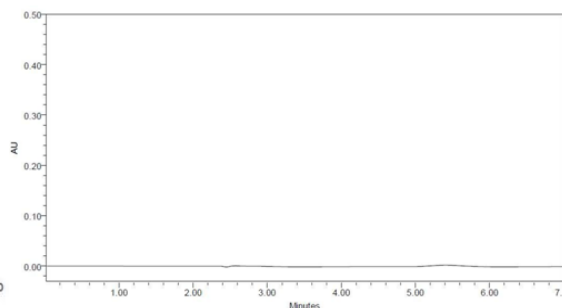
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Table 2: Peak results for optimized

Peak Name	Rt	Area	Height	USP Resolution	USP Tailing	USP Plate count
Bupropion	2.090	372126	39690		1.70	5587
Naltrexone	5.289	3864998	231194	9.80	1.77	5698

Validation:

System suitability:
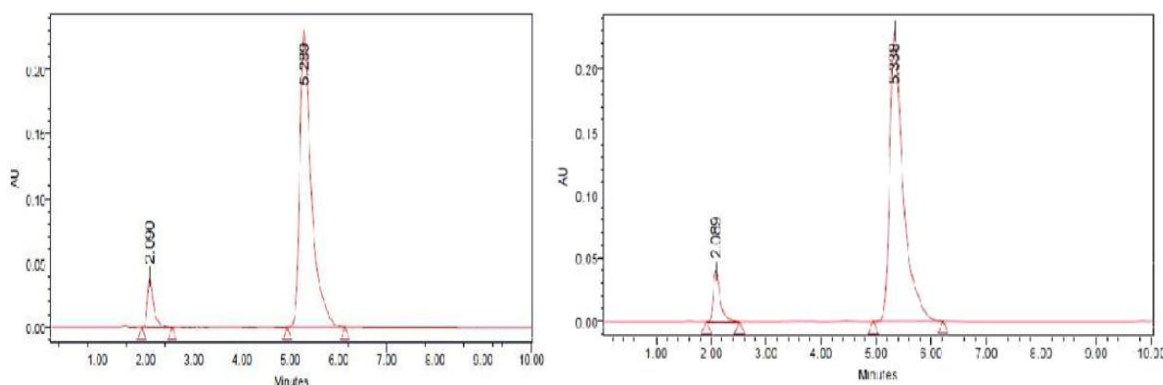
Figure 7: Chromatogram of standard injection -1   Figure 8: Chromatogram of standard injection 2
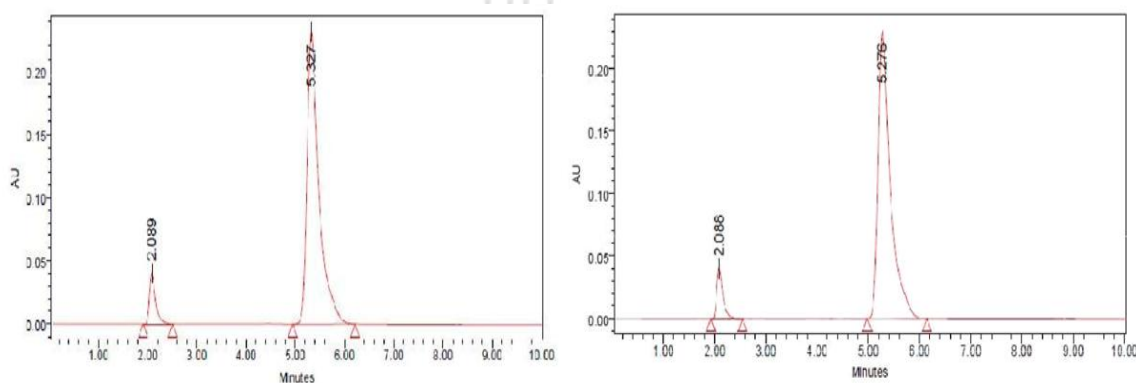
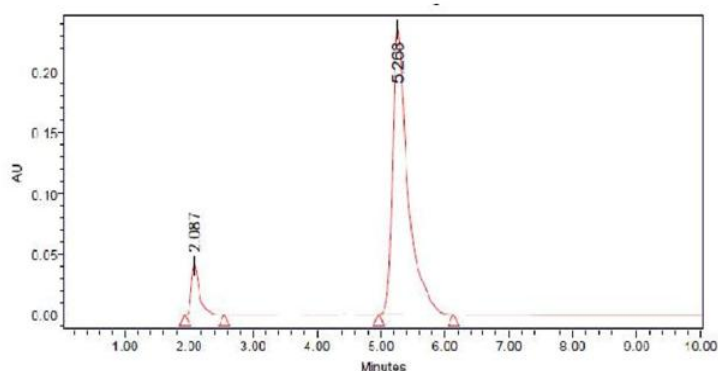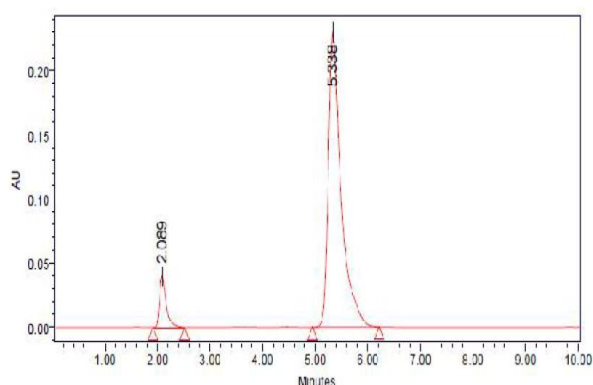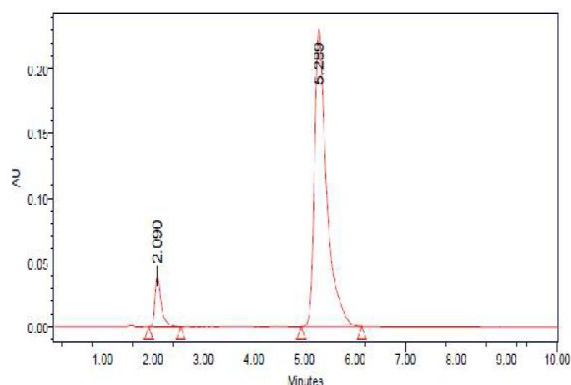**Figure 9: Chromatogram of standard injection -3   Figure 10: Chromatogram of standard injection-4**

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Figure 11: Chromatogram of standard injection-5

Table 3: Results of system suitability

Peak Name	Rt	Area	Height	USP Tailing	USP Plate count	
Bupropion	2.090	342126	39690	5463	1.42	
Bupropion	2.090	342426	39690	5576	1.42	
Bupropion	2.089	342564	39690	5576	1.42	
Bupropion	2.089	347976	39690	5098	1.44	
Bupropion	2.085	352914	40963	5674	1.47	
Mean		345601.2				
SD		4756.58				
% RSD		1.3				
Naltrexone	5.289	3864998	231194	5786	1.46	9.80
Naltrexone	5.289	3864998	232184	5908	1.47	9.81
Naltrexone	5.338	3881443	231044	5487	1.48	9.81
Naltrexone	5.327	3896952	231969	5032	1.40	9.83
Naltrexone	5.262	3900103	233541	5389	1.43	9.82
Mean		3881699				
SD		16802.33				
% RSD		0.4				

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Specificity:

Assay of Standards

Figure 12: Chromatogram standard injection -1   **Figure 13: Chromatogram standard injection -2**
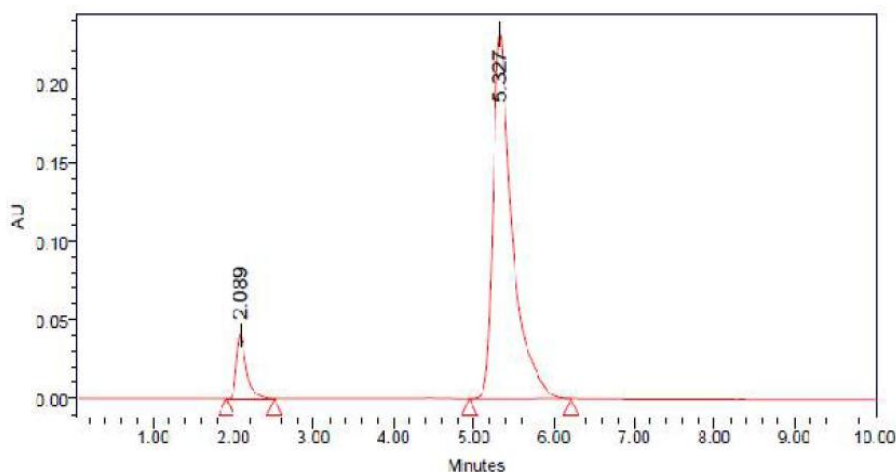
Figure 14: Chromatogram standard injection -3

Table 4: Peak results for assay standard

Name of the drug	Rt	Area	Height	USP Resolution	USP Tailing	USP Plate count	Injection
Bupropion	2.090	348126	39690		1.70	5587	1
Naltrexone	5.289	3864998	231194	9.80	1.77	5628	1
Bupropion	2.089	352564	39990		1.66	5571	2
Naltrexone	5.338	3881443	231044	9.93	1.83	5688	2
Bupropion	2.089	357976	40396		1.68	5530	3
	5.327	3896952	231969	9.91	1.86	5712	3

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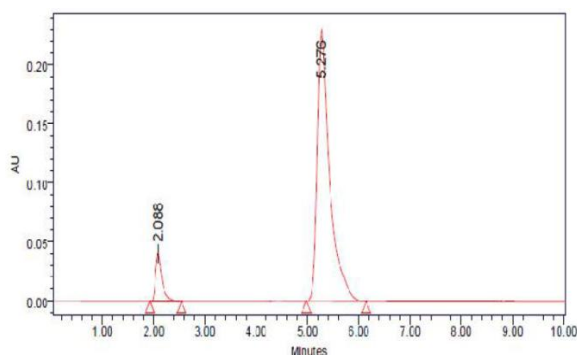
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Assay of Samples
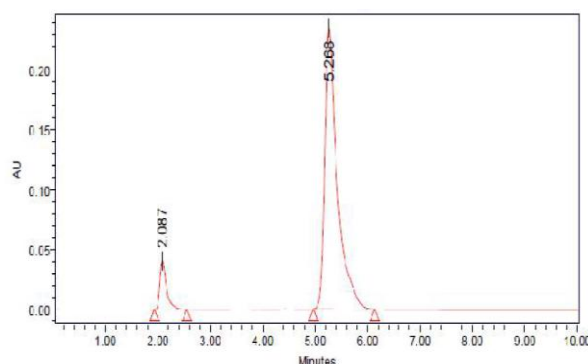

Figure 15: Chromatogram sample injection -1   **Figure 16: Chromatogram**
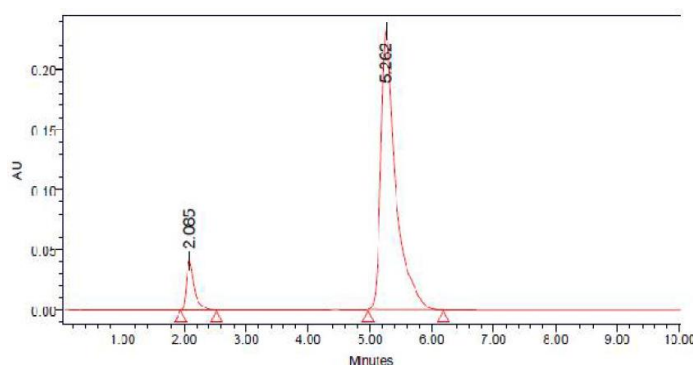
sample injection -2

Figure 17: Chromatogram sample injection -3

Table 5: Peak results for Assay sample

Name of the drug	Rt	Area	Height	USP Resolution	USP Tailing	USP Plate count	Injection
Bupropion	2.088	352290	40269		1.69	5516	1
Naltrexone	5.276	3883794	231354	9.75	1.89	5677	1
Bupropion	2.087	356547	41157		1.72	5557	2
Naltrexone	5.268	3896493	234961	9.82	1.91	5804	2
Bupropion	2.085	358914	40963		1.75	5489	3
Naltrexone	5.262	3900103	233541	9.78	1.95	5790	3

Citation: A. Yasodha et al. Ijppr.Human, 2018; Vol. 12 (4): 454-470.
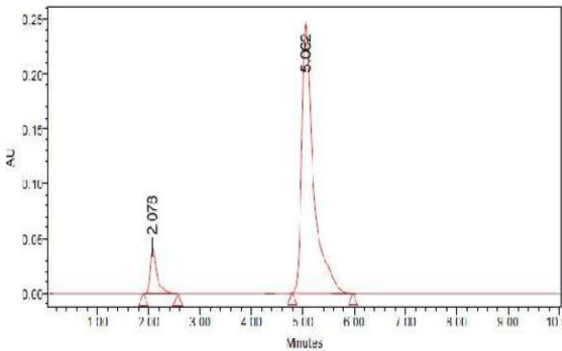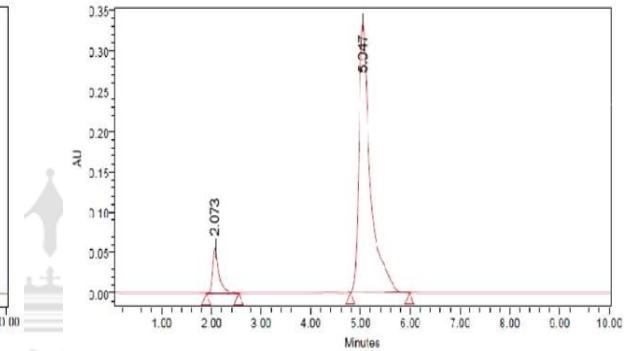
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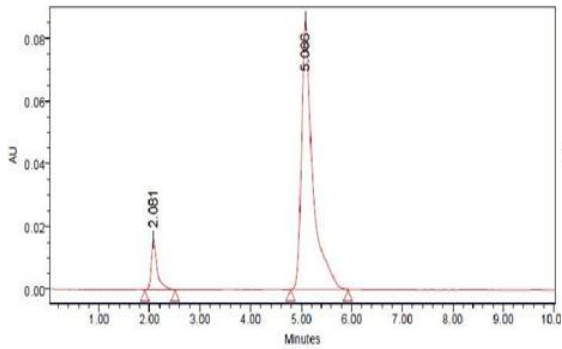
Linearity:

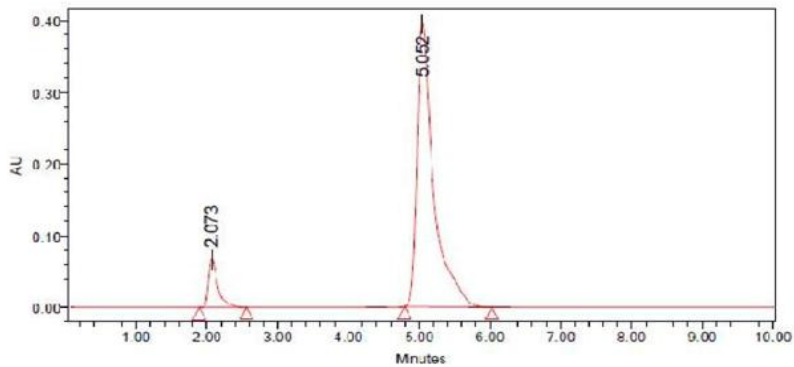**Figure 14: Linearity chromatogram at 5μg/ml**   **Figure 15: Linearity chromatogram at 10μg/ml**
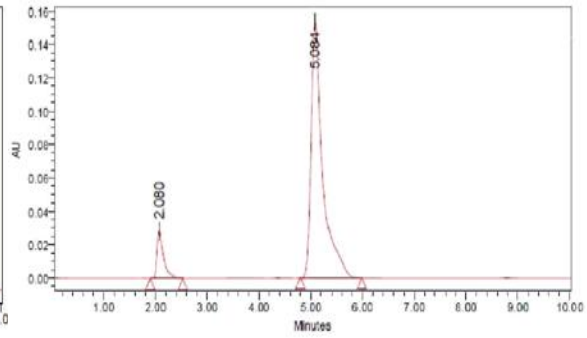
Figure 20: Linearity chromatogram at 15 μg/ml   **Figure 21: Linearity chromatogram at 20 μg/ml**

Figure 22: Chromatogram for linearity at 25 μg/ml

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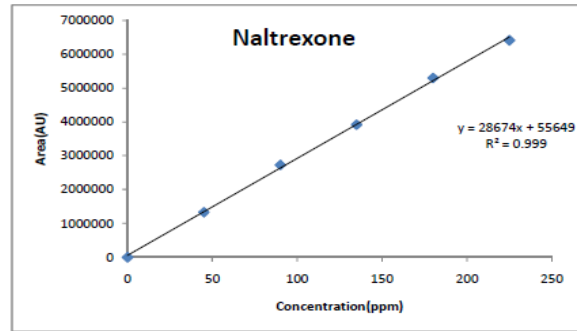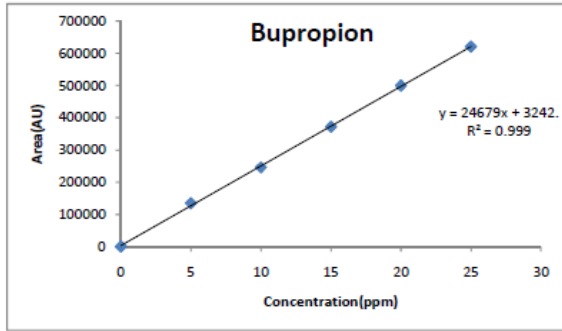**Figure 23: Calibration curve of Bupropion Figure 24: Calibration curve of Naltrexone**
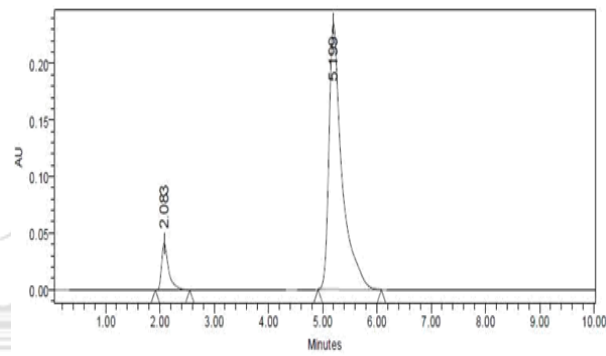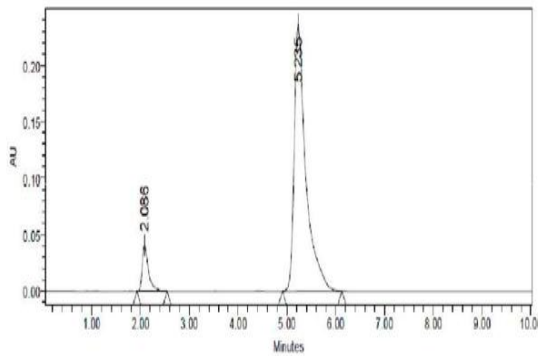
Precision and Repeatability:

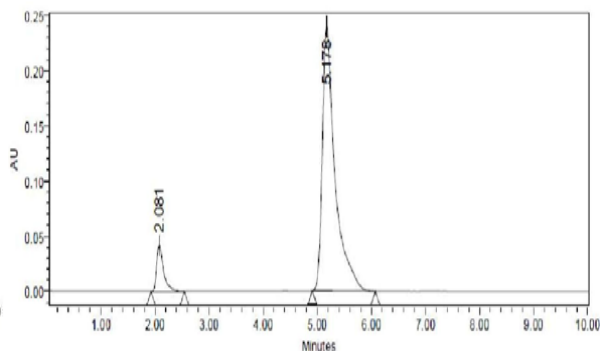**Figure 25: Precision chromatogram of injection-1   Figure 26: Precision chromatogram of injection -2**
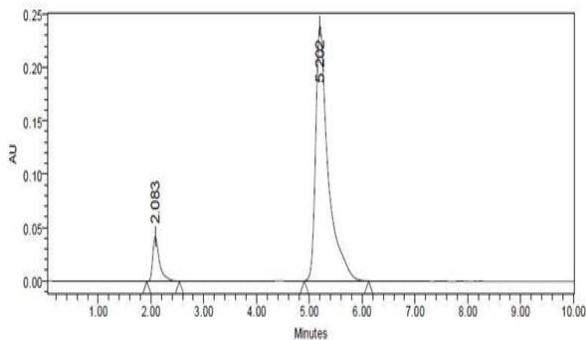
Figure 27: Precision chromatogram of injection   -3   Figure 28: Precision chromatogram of injection

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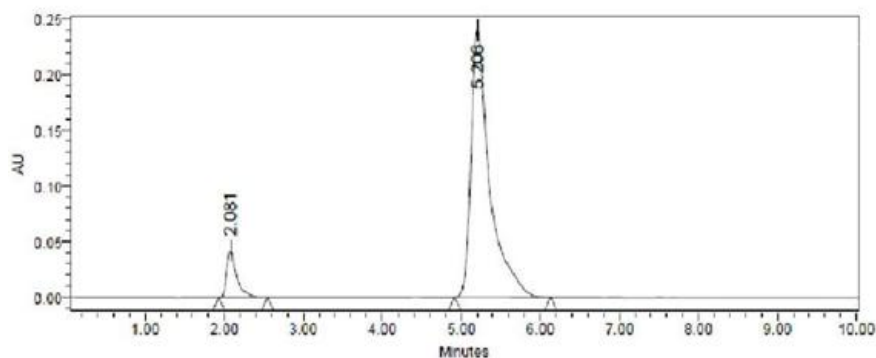
Figure 29: Chromatogram showing precision injection -5

Intermediate Precision:

Table 6: Results of Intermediate precision

Name	Rt	Area	Height	USP Plate count	USP Tailing	USP Resolution
Bupropion						
Bupropion	2.083	369246	42277	5537.8		1.6
Bupropion	2.083	370766	42708	5561.8		1.6
Bupropion	2.089	370840	42065	5489.3		1.6
Bupropion	2.083	370840	42065	5489.3		1.6
Bupropion	2.082	371041	42568	5583.2		1.8
Bupropion	2.080	371386	42211	5533.2		1.8
Mean						
Std. Dev		740.7369				
% RSD		0.19				
Naltrexone						
Naltrexone	5.178	3903548	240181	5988.3	2.0	9.8
Naltrexone	5.199	3905819	235523	5856.3	2.0	9.7
Naltrexone	5.235	3916120	238578	5930.2	2.0	9.9
Naltrexone	5.202	3916542	238814	5936.9	2.0	9.8
Naltrexone	5.206	3920943	241006	5040.0	2.0	9.5
Mean		3912594.4				
SD		7507.6				
% RSD		0.2				

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Accuracy:

Table 7: Results of accuracy

%Concentration(at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
Bupropion					
50%	192446.6	7.5	7.4	98.6	
100%	374222	15	14.8	98.66	98.7%
150%	555891.3	22.5	22.3	99.1	
Naltrexone					
50%	2001752	67.5	67.3	99.7	
100%	3927797	135	134.8	99.8	99.7%
150%	5858665	202.5	202.1	99.8	

Robustness:

Table 8: Results of robustness

Bupropion				
The parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
The actual Flow rate of 1.0 mL/min	372126	2.090	5587	1.70
Less Flow rate of 0.9 mL/min	356765	2.736	5432	1.82
More Flow rate of 1.1 mL/min	342356	1.673	5644	1.91
Less organic phase	312434	2.736	5098	1.82
More organic phase	305623	1.673	5123	1.91
Naltrexone				
The actual Flow rate of 1.0 mL/min	3864998	5.289	5698	1.77
Less Flow rate of 0.9 mL/min	3546737	6.746	5546	1.88
More Flow rate of 1.1 mL/min	3857216	4.032	5124	1.91
Less organic phase	3810347	6.746	5034	1.88
More organic phase	3875642	4.032	5612	1.91

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DISCUSSION

The maximum absorbance was found to be at 212 nm and the peak purity was excellent. Injection volume was selected to be 10μL which gave a good peak area. The column used for the study was X-Terra C_{18} because it was giving a good peak. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. The mobile phase is Methanol: TEA Buffer pH 4.5: Acetonitrile (65:15:20) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Runtime was selected to be 10 min because analyze gave peak around 2.090, 5.289 ±0.02min respectively and also to reduce the total run time. The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision were found to be accurate and well within range. The analytical method was found linearity over the range 5-25mg/ml of Bupropion and 45-225 mg/ml of Naltrexone of the target concentration. The analytical passed both robustness and ruggedness tests. In both cases, the relative standard deviation was well satisfied.

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Bupropion and Naltrexone in bulk drug and pharmaceutical dosage forms. This method was simple since diluted samples are directly used without any preliminary chemical derivatization or purification steps. Bupropion and Naltrexone were freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: TEA Buffer pH 4.5: Acetonitrile (65:15:20) was chosen as the mobile phase. The solvent system used in this method was economical. The % RSD values were within 2 and the method was found to be precise. The results expressed in Tables for the RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the spectrophotometric methods. This method can be used for the routine determination of Bupropion and Naltrexone in bulk drug and in Pharmaceutical dosage forms.

CONCLUSION

In conclusion, we can say that this is a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Bupropion and Naltrexone in bulk drug and pharmaceutical dosage forms. This method was simple since diluted samples are

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directly used without any preliminary chemical derivatization or purification steps. Bupropion and Naltrexone were freely soluble in ethanol, methanol and sparingly soluble in water.

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