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R E S E A E C H A R T I C L E

RP-HPLC Method Development and Validation for Simultaneous Estimation of Bupropion and Naltrexone in Bulk and Pharmaceutical Dosage Forms

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A B S T R A C T

Thus the proposed stability indicating RP-HPLC technique for the synchronic determination of Naltraxone and Bupropione in bulk and pharmaceutical dosage form was accurate, precise, linear, reliable, simple, economic and robust. The method can be used for routine analysis of marketed product of Naltraxone and Bupropione in combined tablet formulation. Water 2695 series with PDA Detector and C18 (Hypersil BDS, 250X4.6mm, 5), injection of 10 l was injected and eluted with the mobile phase [Potassium dihydrogen phosphate buffer (pH 4.0 was adjusted with OPA): Acetonitrile) (45:55 v/v)] which was pumped at flow rate of 1.0ml at 230nm. The peak of Naltraxone and Bupropione were found well separated at 2.4676min and 3.805min respectively. The developed method was validated for various parameters as per ICH Guidelines like among the analytical techniques available in estimation and quantification RP- HPLC method is an emerging technique reliable in vast areas of research that incited the author to undertake method development and validation as per ICH guidelines for the same. Stability indicates for new RP-HPLC technique for synchronic estimation of Naltraxone and Bupropione in bulk and pharmaceutical dosage forms. RP-HPLC method was developed with Water2695 series with PDA detector and C18 (Hypersil BDS, 250X4.6mm, 5) column, injection of 10 ml was injected and eluted with the mobile phase of [Potassium di hydrogen phosphate buffer (pH 4.0 was adjusted with Ortho phosphoric acid): Acetonitrile) (45: 55v/v)] which was pumped at flow rate of 1.0ml at 230nm.

Keywords: Bupropion, naltraxone, RP-HPLC, validation, Methanol.

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Naltrexone is a medication primarily used in the management of alcohol dependence and opioid dependence. Naltrexone is a pure opioid antagonist and works by blocking the activity of opioids. Naltrexone and its active metabolite 6β-naltrexol are competitive antagonists at the μ- opioid receptor (MOR), the κ-opioid receptor (KOR) to a lesser extent, and to a far lesser and possibly insignificant extent, at the δ-opioid receptor (DOR). The Ki affinity values of naltrexone at the MOR, KOR, and DOR have been reported as 0.0825 nM, 0.509 nM, and 8.02 nM, respectively, demonstrating a MOR/KOR binding ratio of 6.17 and a MOR/DOR binding ratio of 97.2. The blockade of opioid receptors is the basis behind naltrexone's action in the management of opioid dependence—it reversibly blocks or attenuates the effects of opioids. Its mechanism of action in alcohol dependence is not fully understood, but as an opioid receptor antagonist is likely to be due to the modulation of the dopaminergic mesolimbic pathway (one of the primary centers for risk-reward analysis in the brain, and a tertiary "pleasure center") which is hypothesized to be a major center of the reward associated with addiction that all major drugs of abuse are believed to activate.[citation needed] Mechanism of action may be antagonism to endogenous opioids such as tetrahydropapaveroline, whose production is augmented in the presence of alcohol. Bupropion is known to affect several different biological targets. It often is described as a norepinephrine-dopamine reuptake inhibitor (NDRI), and is also a nicotinic antagonist. However, bupropion does not appear to have significant dopaminergic actions in humans under normal clinical circumstances. Chemically, bupropion belongs to the class of aminoketones and is similar in structure to stimulants such as cathinone and amfepramone, and to phenethylamines in general. Bupropion was first made by Nariman Mehta and patented by Burroughs Wellcome in 1969, which later became part of what is now GlaxoSmithKline. It was first approved for medical use in the United States in 1989. It was originally called by the generic name amfebutamone, before being renamed in 2000.

2. Materials and Methods

Ortho phosphoric acid, Hydrochloric acid, Hydrogen peroxide, Potassium dihydrogen orthophosphate.

HPLC METHOD DEVELOPMENT:

Buffer Preparation(0.01N KH_2PO_4):Accurately weighed 1.36 gm of potassium di hydrogen phosphate in 1000ml of volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water and pH was adjusted near four with dilute Ortho phosphoric acid.

Mobile phase: KH2PO4 (pH-was adjusted with ortho phosphoric acid) and Acetonitrile taken in the ratio (45:55 v/v).

Standard preparation: Accurately weighed 1.36gm of potassium dihydrogen phosphate in 100ml of volumetric flask add 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water and pH was adjusted to 4 with dilute Ortho phosphoric acid.

Diluents: Acetonitrile: Water (50: 50 v/v).

Chromatographic Conditions:

- Column name: BDS 250mm
- Flow rate: 1ml
- Condition: Isocratic
- Sample temperature:30˚C
- Column temperature: 30˚C
- Injection volume: 10 ml
- Run time: 20 min
- Wave length: 230nm

Method validation:

Specificity: Specificity shall be established by demonstrating that the procedure is unaffected by the presence of interference at the retention time of the Bupropione and Naltraxone with respect to mobile phase, diluents, placebo and degradants.

System suitability: To verify the analytical system is working properly and can give accurate and precise results, the system suitability parameters are to be set. Inject separately 10 micro litres each of the following solutions into the HPLC.

Precision: Perform the method precision on the drug product. Prepare a sufficient quantity of composite blend for the set of analysis. Prepare six assay preparations as per test method and inject near a HPLC system. Calculate the % assay, %RSD, (coefficient of variation) and confidence interval.

Accuracy: Recovery study shall be performed in the concentration range of 50% to 150% of the target concentration of test. Minimum three concentration levels are recommended perform the accuracy study on drug product by varying the sample qualities with respect to spike levels.

Linearity: Linearity shall be established by demonstrating that the area response obtained is directly proportional to the concentration of standard solution. Demonstrate the linearity in the range of 25% to 150% of the target assay concentration.

Robustness: The robustness of the method decided by creating slight changes within the chromatographically conditions. The robustness of the proposed HPLC method assessed for retention time, theoretical plates and tailing factor.

Limit of Detection & Limit of Quantification: The limit of detection (LOD) and limit of quantification (LOQ) were calculated in keeping with ICH recommendations wherever the approach supported the signal-to-noise. Recording signals obtained with known low concentrations of analyte was compare among the signals of blank samples. A signal-noise ratio 3:1 and 10:1 is considered for calculating LOD and LOQ respectively.

3. Results and Discussions

System suitability parameters all are passed in this trail so these chromatographic conditions are choose for optimization..Run time is reduced from 10min to 6min.

Precision:

Table 1: Results of Method Precision

S.No	Sample weight (mg)		PEAK AREA	
	Naltraxone	Bupropione	Naltraxone	Bupropione
1	8	90	2270553	993413
2	8	90	2278100	993859
3	8	90	2282356	998213
4	8	90	2283157	998930
5	8	90	2285957	999663
Average			2280028.2	996815.8
Std. Dev			6001.7	2952.0
% RSD			0.3	0.3

It was concluded that the method is precise as the % RSD is not more than 2% and assay result is between 98% to 102% of labeled amount of drug substance. Accuracy values proved good recovery and method was accurate. Correlation coefficient obtained is 0.998, 0.999 for Naltraxone and Bupropione respectively. Hence the method is linear. it is conceded that the method is robust for variations that all system suitability values meet the acceptance criteria set forth in the test method.

Table 2: Linearity studies of Naltraxone

Levels	**Concentration (mg/ml)**	**Response (mean area)**
0% Level	0	0
20% Level	20	2285660
40% Level	40	286224
80% Level	80	2292264
100% Level	100	2301304
120% Level	120	2355896
Slope		0.84572
Correlation Coefficient		0.998

Table 3: Linearity studies of Bupropione

Levels	**Concentration (mg/ml)**	**Response (mean area)**
0%	0	0
20% Level	20	1005320
40% Level	40	1006642
80% Level	80	1007252
100% Level	100	1011606
120% Level	120	1014319
Slope		0.78652
Correlation Coefficient		0.999

Table 4: Results of LOD

Components	LOD Values	Acceptance Criteria
Naltraxone	2.95	3.0
Bupropione	2.97	

Table 5: Results of LOQ

Components	**LOQ values**	**Acceptance Criteria**
Naltraxone	9.92	10.0
Bupropione	9.96	

Table 6: Results of Robustness (Variation in flow rate)

Analyte	Flow Rate Decreased		Flow Rate Increased	
	Naltraxone	Bupropione	Naltraxone	Bupropione
Retention time	2.565	3.850	2.320	3.550
Tailing	1.20	1.1	1.2	1.13
Theoretical plates	5232.2	7421.3	4956.0	7012.3

Table 7: Results of Robustness (Variation in organic composition)

Analyte	**Organic Composition Decreased**		**Organic Composition Increased**	
	Naltraxone	Bupropione	Naltraxone	Bupropione
Retention time	2.578	3.850	2.365	3.650
Tailing	1.2	1.1	1.20	1.3
Theoretical plates	5132.0	6958.3	4936.2	5983.9

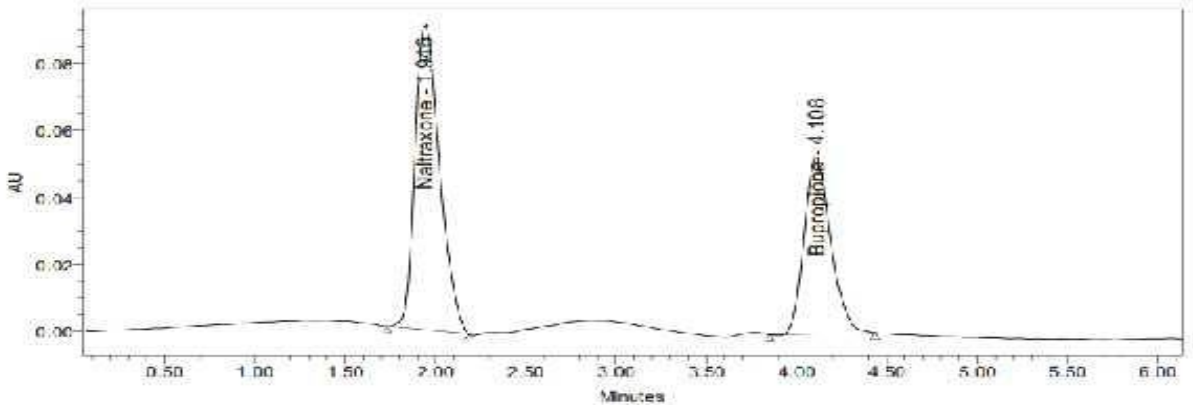

Figure 1: Trial-9 chromatogram for Bupropione and Naltraxone

Table 8: Accuracy Studies for Naltraxone

Sample No.	Spiked Level	Sample weight (mg)	Sample area	mg/ml added	mg/ml found	% Recovery	% Mean Recovery
1	50%	0.4	1182724	0.4	0.4	100.0	100.1
2	50%	0.4	1183571	0.4	0.39	99.6	
3	50%	0.4	1186318	0.4	0.42	101.0	
1	100%	0.8	2113673	0.8	0.79	99.8	99.8
2	100%	0.8	2122445	0.8	0.8	100.0	
3	100%	0.8	2129499	0.8	0.75	95.6	
1	150%	1.6	3508712	1.6	1.56	97.8	99.99
2	150%	1.6	3519906	1.6	1.6	100.0	
3	150%	1.6	3548681	1.6	1.59	99.9	

Table 9: Accuracy Studies For Bupropione

Sample No.	Spiked Level	Sample weight (mg)	Sample area	mg/ml added	mg/ml found	% Recovery	% Mean Recovery
1	50%	5	521676	5	4.9	99.8	100.0
2	50%	5	52199	5	5	100.0	
3	50%	5	523030	5	4.7	99.56	
1	100%	10	977779	10	9.8	99.67	101.0
2	100%	10	979030	10	10	100.0	
3	100%	10	91150	10	9.9	99.9	
1	150%	15	1570240	15	14.5	98.6	99.89
2	150%	15	1578806	15	14.8	99.5	
3	150%	15	1580909	15	15	100.0	

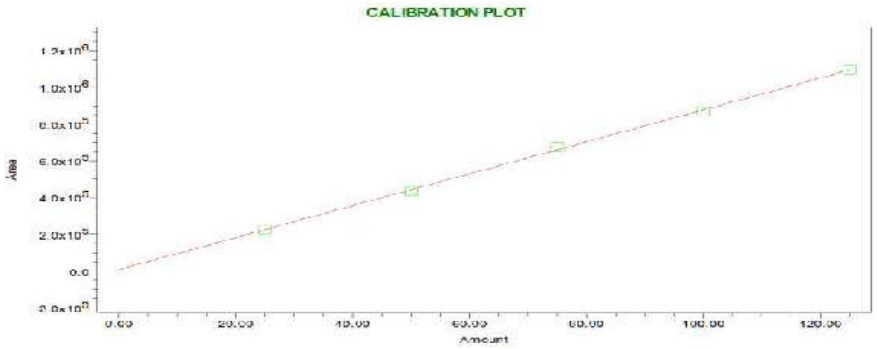

Figure 2: Linearity plot of Naltraxone

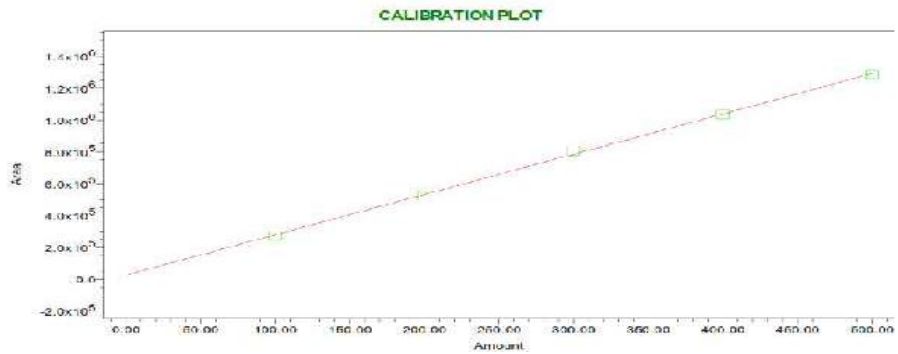

Figure 3: Linearity plot of Bupropione

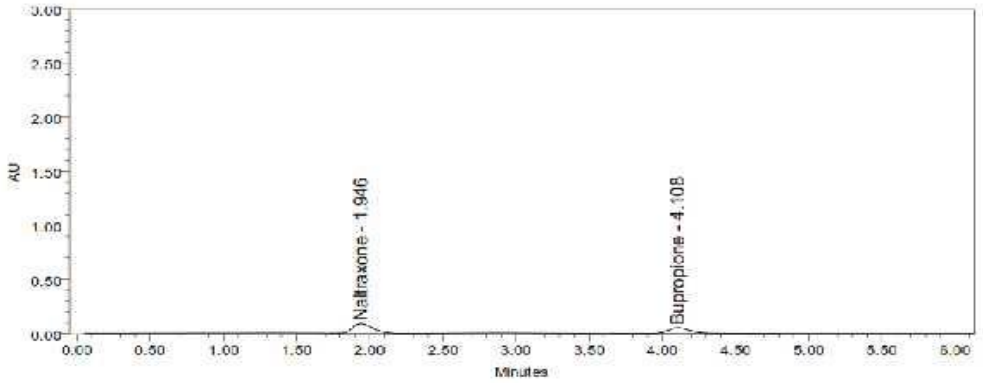

Figure 4: chromatogram of Bupropione & Naltraxone showing LOD

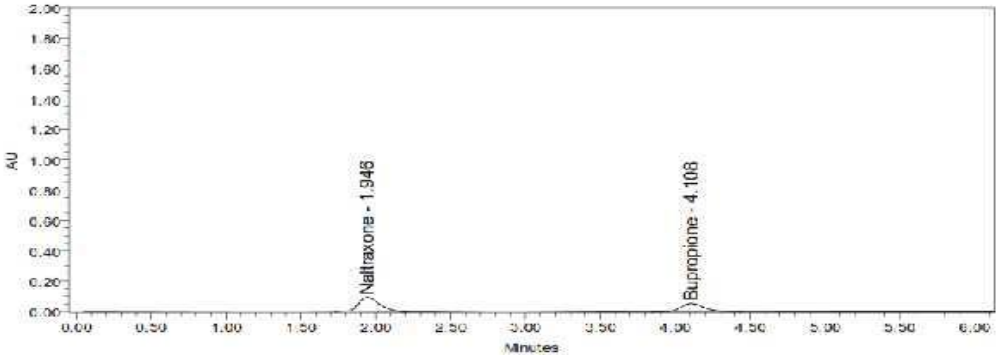

Figure 5: chromatogram of Bupropione & Naltraxone showing LOQ

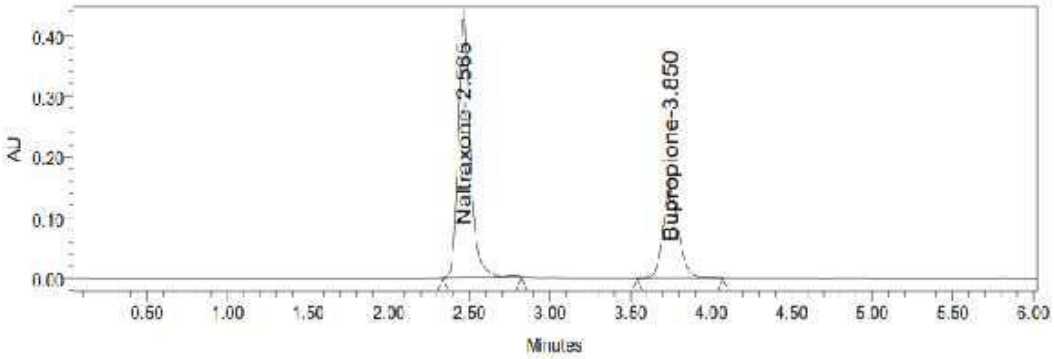

Figure 6: chromatogram showing less flow of 0.6ml/min

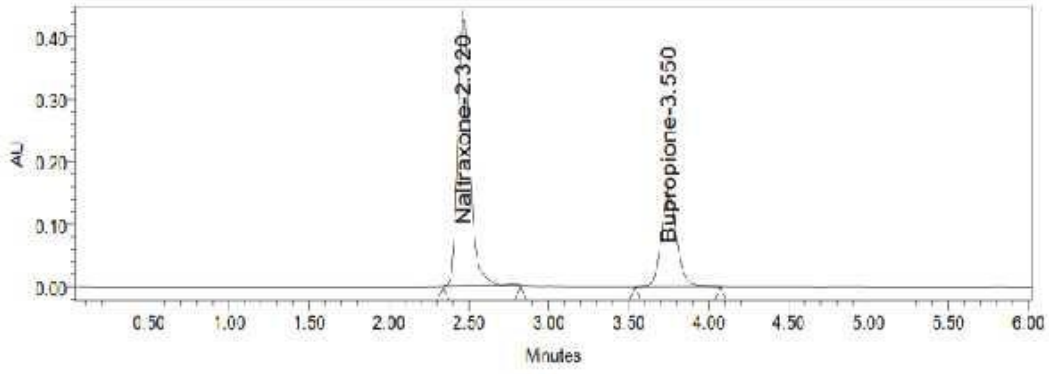

Figure 7: chromatogram showing more flow of 1.0ml/min

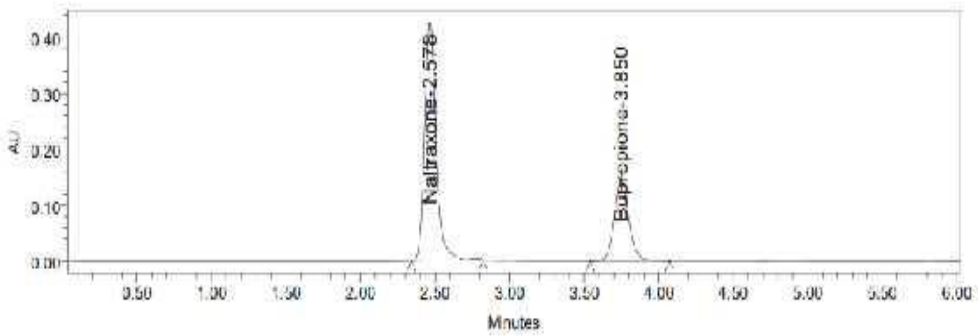

Figure 8: chromatogram showing less organic composition

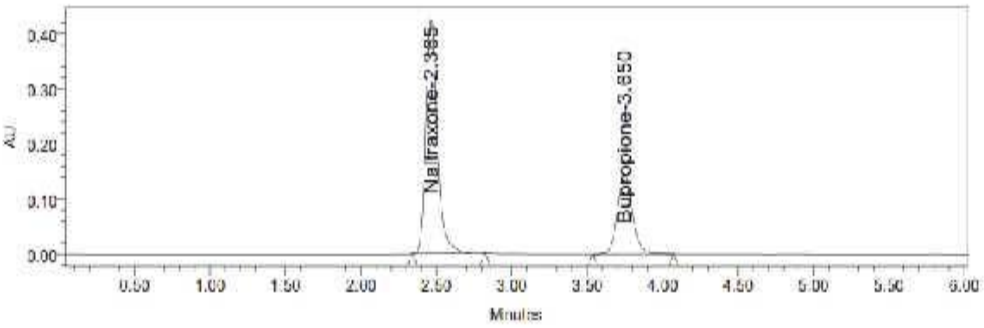

Figure 9: chromatogram showing more organic composition.

4. Conclusion

Thus the proposed stability indicating RP-HPLC technique for the synchronic determination of Naltraxone and Bupropione in bulk and pharmaceutical dosage form was accurate, precise, linear, reliable, simple, economic and robust. The method has several advantages, including simple mobile phase, rapid analysis, simple sample preparation and improved selectivity as well as sensitivity. The method can be used for routine analysis of marketed product of Naltraxone and Bupropione in combined tablet formulation. Water 2695 series with PDA Detector and C18 (Hypersil BDS, 250X4.6mm, 5µ), injection of 10µl was injected and eluted with the mobile phase [Potassium dihydrogen phosphate buffer (pH 4.0 was adjusted with OPA): Acetonitrile) (45:55 v/v)] which was pumped at flow rate of 1.0ml at 230nm. The peak of Naltraxone and Bupropione were found well separated at 2.4676min and 3.805min respectively. The developed method was validated for various parameters as per ICH Guidelines like

- System suitability
- Accuracy
- Linearity
- Specificity
- Precision
- LOD
- LOQ
- Robustness

The stability indicating analytical method validation of new RP-HPLC method was found to be satisfactory and could be used for the routine Pharmaceutical analysis of Naltraxone and Bupropione.

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