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DEVELOPED AND VALIDATED FOR THE ESTIMATION OF BUPROPION AND DEXTROMETHORPHAN IN A FIXED DOSE COMBINATION OF TABLET

Short Title: HPLC method development and validation

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Abstract
Objective: The aim of this study was to develop a simple, accurate, precise method for the estimation of bupropion and dextromethorphan in a fixed dose combination of tablet and robust high performance liquid chromatography (HPLC) for assay analysis for such a fixed combination.
Materials and Methods: Chromatographic analysis was performed and separations were achieved on a Denali C18 150 × 4.6 mm, 5 micron using mobile phase composition of orthophosphoric acid and acetonitrile in the ratio of 600:400 (v/v), the flow rate of 1.0 mL/min, injection volume is 10 µL and run time 6 minutes in isocratic elution. UV detection was carried out at a wavelength of 221 nm. The temperature was maintained at 30°C. Well-resolved peaks were observed with high number of theoretical plates, lower tailing factor and reproducible relative retention time. The method was validated and all the validation parameters were found to be within the acceptance limits.
Results: A simple, accurate, precise method has been developed for the estimation of bupropion and dextromethorphan in a fixed dose combination of tablet. The optimized method included the following parameters: Column temperature of 30°C, 40% acetonitrile as the mobile phase and flow rate of 1.0 mL/min. Retention times were 2.25 min and 3.12 min for bupropion and dextromethorphan, respectively. The method was found to be linear in the range of 17.5-105 µg/mL (for R2 <0.999) and 7.5-45 µg/mL for (R2 >0.999) for bupropion and dextromethorphan, respectively. Both APIs were dissolved more than 90% within 5 min.
Keywords: Bupropion, dextromethorphan, stress degradation, RP-HPLC method development and validation.

Introduction

AUVELITY is a combination of dextromethorphan hydrobromide, an uncompetitive NMDA receptor antagonist and sigma-1 receptor agonist, and bupropion hydrochloride, an aminoketone and CYP450 2D6 inhibitor[1].
Dextromethorphan is an uncompetitive antagonist of the NMDA receptor (an ionotropic glutamate receptor) and a sigma-1 receptor agonist. The mechanism of dextromethorphan in the treatment of MDD is unclear. The mechanism of action of bupropion in the treatment of MDD is also unclear; however, it may be related to noradrenergic and/or dopaminergic mechanisms. Bupropion increases plasma levels of dextromethorphan by competitively inhibiting cytochrome P450 2D6, which catalyses a major biotransformation pathway for dextromethorphan. Bupropion is a relatively weak inhibitor of the neuronal reuptake of norepinephrine and dopamine and does not inhibit monoamine oxidase or the reuptake of serotonin [1].
Bupropion hydrochloride: Bupropion hydrochloride is an antidepressant of the aminoketone class. It is chemically unrelated to tricyclic, tetracyclic, selective serotonin reuptake inhibitor, or other known antidepressant agents. Its structure closely resembles that of diethylpropion; it is related to phenylethylamines and also acts as a nicotinic acetylcholine receptor antagonist[2-6].
Dextromethorphan hydrobromide: Dextromethorphan hydrobromide is an oral non-narcotic antitussive cure widely used in practical medicine. It was very well absorbed by the digestive system and it does not bind to plasma proteins. A combination of pseudophedrine hydrochloride, chlorpheniramine maleate, and paracetamol is used in pharmaceutical preparations for reducing symptoms which are usually associated with the common cold[7-10].

Individual HPLC methods reported for each drug were inappropriate for their simultaneous determination because of interferences due to corresponding chromatographic peaks. However, these procedures require the use of more than one column, mobile phase or flow rate, which can be time-consuming and uneconomical. Recently, a method has been reported for the simultaneous estimation of bupropion and dextromethorphan. But the chromatogram revealed that the bupropion peak was eluted in the void volume where the interference was observed with the blank peak, and the placebo chromatogram was not recorded to identify the interference at bupropion and dextromethorphan. The reported method showed degradation was more than 10% for bupropion and dextromethorphan in acid, base, and peroxide, but degradation chromatograms were not shown[11]. The main aim of this method is to determine and validate the bupropion and dextromethorphan in a fixed combination of tablet dosage forms based on International Conference on Harmonization guidelines [12]. This method was developed to use as a reproducible procedure for the quantitative analysis of drug samples. The designed method can be considered advisable to develop a precise, accurate, and simple RP-HPLC method.

The chemical name for bupropion is (±)-1-(3chlorophenyl)-2-[(1,1-dimethylethyl) amino]-1-propranone hydrochloride. The molecular formula is $C_{13}H_{18}ClNO•HCl$ and the molecular weight is 276.2 grams per mole. The chemical structure of bupropion is shown in Figure 1.

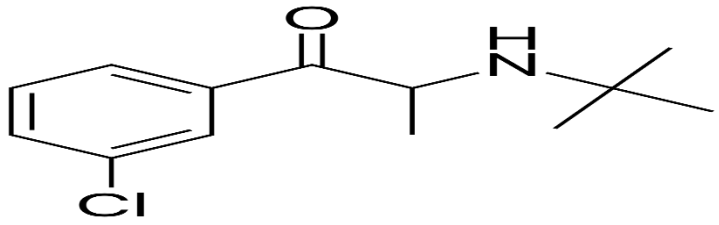

Figure 1: Chemical structures of Bupropion

The chemical name for dextromethorphan is (9S,13S,14S)-3-Methoxy-17-methylmorphinan hydrobromide. The molecular formula is $C_{18}H_{26}BrNO$ and the molecular weight is 352.3 grams per mole. The chemical structure of dextromethorphan is shown in Figure 2.

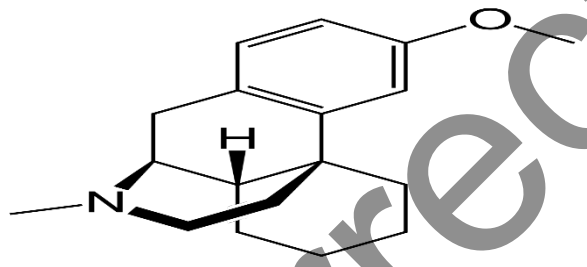

Figure 2: Chemical structures of Dextromethorphan

Material and Methods

Chemicals, Reagents and Instruments:

Bupropion, dextromethorphan, orthophosphoric acid (H_3PO_4), acetonitrile and Milli-Q water. Denali C18 150 × 4.6 mm, 5 micron column, HPLC instrument equipped with UV-VIS spectrophotometer & PDA detector.

Chromatographic Conditions:

Flow rate: 1.0 mL, Injection volume: 10 µL, Detector: 221 nm, column temperature: 30°C, Column: Denali C18 150 × 4.6 mm, 5 micron and Run time: 6 minutes.

Mobile Phase and Solutions Preparation:

Preparation of Buffer:

1 mL of orthophosphoric acid solution was diluted to 1000 mL with Milli-Q water.

Preparation of Mobile phase:

Mix 600 mL of buffer and 400 mL of acetonitrile and sonicate to degas.

Preparation of Diluent:

Mix 500 mL of water and 500 mL of acetonitrile and sonicate to degas.

Standard Preparation:

As much as 35 mg of bupropion and 15 mg of dextromethorphan were accurately weighed and transferred according to working standards into a 50 mL clean, dry volumetric flask; 10 mL of diluent was added and sonicated for 10 minutes, the final volume was made up to the mark with diluent (700 µg/mL bupropion and 300 µg/mL of dextromethorphan).

From the above stock solution, 1 mL was taken into a 10 mL volumetric flask and made up to the mark with diluent (70 µg/mL bupropion and 30 µg/mL of dextromethorphan).

Sample Preparation:

An equivalent weight of 70 mg of bupropion fixed dose combination tablet powder was accurately weighed and transferred into a 100 mL volumetric flask; 75 mL of diluent was added and sonicated for 25 minutes, further, the volume was made up to the mark with diluent and filtered by Milli-Q filter (700 µg/mL bupropion and 300 µg/mL of dextromethorphan), then 1 mL of filtered sample stock solution was transferred to a 10 mL volumetric flask and made up to the mark with diluent (70 µg/mL bupropion and 30 µg/mL of dextromethorphan).

Degradation studies:

Oxidation:

From stock solution of 700 µg/mL bupropion and 300 µg/mL of dextromethorphan, 1 mL was pipetted and 1 mL of 20% hydrogen peroxide (H_2O_2) was added separately. The resultant solution was kept for 60 min at 30 °C. For the HPLC study, the resultant solution was diluted to obtain 70 µg/mL of bupropion and 30 µg/mL of dextromethorphan, then 10 µL injection volume was injected into the system and the chromatogram was recorded to assess the stability of the sample.

Acid Degradation Studies:

From stock solution of 700 µg/mL bupropion and 300 µg/mL of dextromethorphan, 1 mL was pipetted and 1 mL of 2 N hydrochloric acid was added separately. The resultant solution was refluxed for 30 min at 60 °C. Next, the acid neutralized with an equivalent volume of sodium hydroxide solution. For the HPLC study, the resultant solution was diluted to obtain 70 µg/mL of bupropion and 30 µg/mL of dextromethorphan, then 10 µL injection volume was injected into the system and the chromatogram was recorded to assess the stability of the sample.

Alkali Degradation Studies:

From stock solution of 700 µg/mL bupropion and 300 µg/mL of dextromethorphan, 1 mL was pipetted and 1 mL of 2 N sodium hydroxide solution was added separately. The resultant solution was refluxed for 30 min at 60 °C. Next, the base neutralized with an equivalent volume of hydrochloric acid solution. For the HPLC study, the resultant solution was diluted to obtain 70 µg/mL of bupropion and 30 µg/mL of dextromethorphan, then 10 µL injection volume was injected into the system and the chromatogram was recorded to assess the stability of the sample.

Thermal Degradation Studies:

The solution was exposed to heat at 105 °C for 6 hours, then 1 mL of the stock exposed solution of 700 µg/mL bupropion and 300 µg/mL of dextromethorphan was pipetted. For the HPLC study, the resultant solution was diluted to obtain 70 µg/mL of bupropion and 30 µg/mL of dextromethorphan, then 10 µL injection volume was injected into the system and the chromatogram was recorded to assess the stability of the sample.

Photostability studies:

The solution was exposed to UV light at 1.2 million lux hours and 200 watt hour /m^2 for four days, then 1 mL of stock exposed solution of 700 µg/mL bupropion and 300 µg/mL of dextromethorphan was pipetted. For the HPLC study, the resultant solution was diluted to obtain 70 µg/mL of bupropion and 30 µg/mL of dextromethorphan, then 10 µL injection volume was injected into the system and the chromatogram was recorded to assess the stability of the sample.

Neutral Degradation Studies:

From the stock solution of 700 µg/mL bupropion and 300 µg/mL of dextromethorphan, 1 mL was pipetted and 1 mL of water was added separately. The solution was refluxed for six hours at 60 °C. For the HPLC study, the resultant solution was diluted to obtain 70 µg/mL of bupropion and 30 µg/mL of dextromethorphan, then 10 µL injection volume was injected into the system and the chromatogram was recorded to assess the stability of the sample.

Results and Discussion:

With the progress of International Conference on Harmonization (ICH) guidelines, the determination of a stability-indicating method has developed to be more clear and obligatory. The guidelines are necessary for handling of forced degradation studies under different conditions, like acid, base, photolytic, oxidation, heat and neutral. Hence the necessity of separation of several components through the study of stability samples, HPLC has gained a reputation in stability studies due to its specificity, sensitivity and high-resolution capacity. The work planned in this research was subjected to the study of the chromatographic actions of the samples of stress degradation of bupropion and dextromethorphan in the tablet dosage formulation. To the best of our knowledge, and motivated us to develop an RP-HPLC-PDA stability indicating test where the degradation products were resolved from the integral drugs.

Method Development

Initially, the analytical method was developed by using orthophosphoric acid solution and acetonitrile in the ratio of 1:1, but the resultant chromatogram observed closed eluted peaks. The resultant chromatogram is revealed in Figure 3.

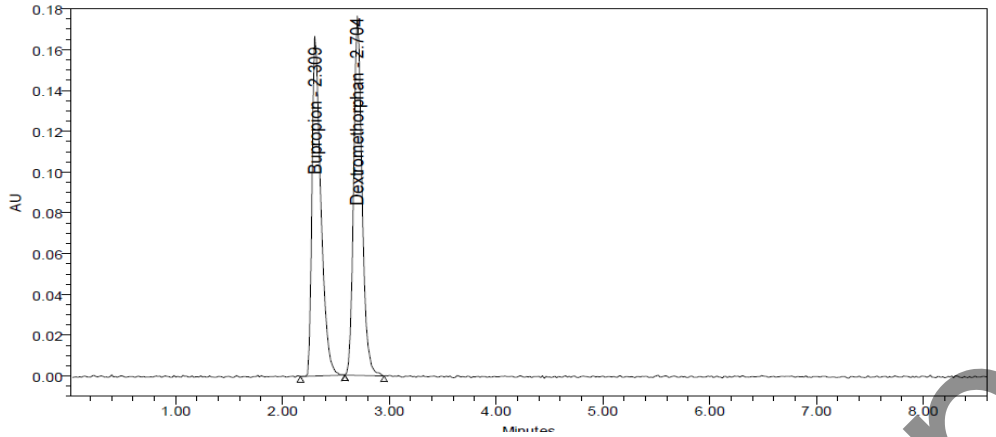

Figure 3: Standard chromatogram of Bupropion and Dextromethorphan.

Finally, the method was optimized with critical quality attributes like standard preparation, sampling preparation, column, detector, resolution of peaks and instrument. The optimized standard solution containing 70 µg/mL of bupropion and 30 µg/mL of dextromethorphan was used to validate the parameters. The resultant chromatograms are shown in Figures 4 & 5.

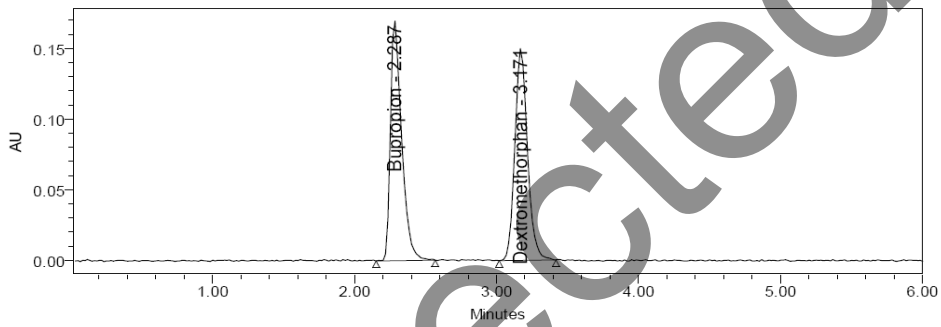

Figure 4: Standard chromatogram of Bupropion and Dextromethorphan.

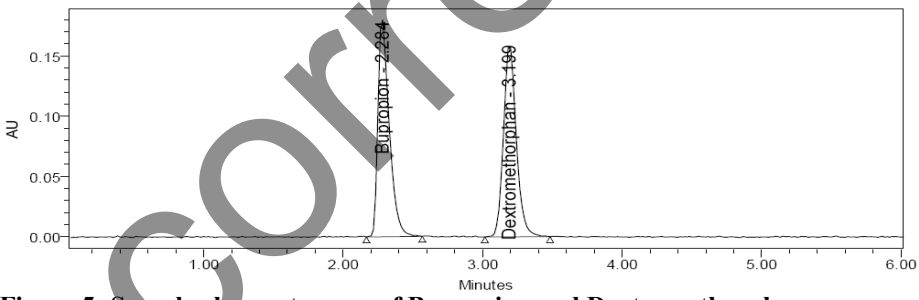

Figure 5: Sample chromatogram of Bupropion and Dextromethorphan.

Method Validation

The method was validated as per ICH guidelines. The different validation parameters were performed as following: linearity, precision, accuracy, specificity, and limit of detection, limit of quantitation, robustness, degradation studies and the stability indicating capability.

System Suitability Test:

System suitability was evaluated with freshly prepared standard solutions. Five replicate injections of standard solution were injected in HPLC system, the obtained areas, retention time, tailing factor, theoretical plates and % RSD were calculated. System suitability results were tabulated in tables 1 & 2. % RSD values were within the limit of not more than 2%.

Table 1: System Suitability Results for Bupropion

Injections	Retention	Area	USP Plate	USP Tailing

	Time(min)		Count	factor
1	2.265	939582	3409	1.63
2	2.284	941657	3650	1.50
3	2.287	944326	3791	1.47
4	2.290	946091	3827	1.46
5	2.291	948170	3946	1.47
Mean	2.283	943965	3724.6	1.506
Std. Dev.	0.0106	3423.1	205.6	0.0709
% RSD	0.5	0.4	5.5	4.7

Table 2: System Suitability Results for Dextromethorphan

Injections	Retention Time(min)	Area	USP Plate Count	USP Tailing factor
1	3.138	763039	6037	1.14
2	3.142	765036	6148	1.16
3	3.150	763327	6085	1.16
4	3.165	767346	6141	1.15
5	3.171	755921	6194	1.15
Mean	3.153	762934	6121	1.152
Std. Dev.	0.0143	4278.8	60.848	0.0084
% RSD	0.5	0.6	1.0	0.7

Specificity

Specificity test was carried out on a freshly prepared blank and placebo of bupropion and dextromethorphan tablet. The resultant chromatograms indicated no interference was observed from blank and placebo at retention time of bupropion and dextromethorphan in the optimized method conditions. The resultant chromatograms are depicted in Figures 6 & 7.

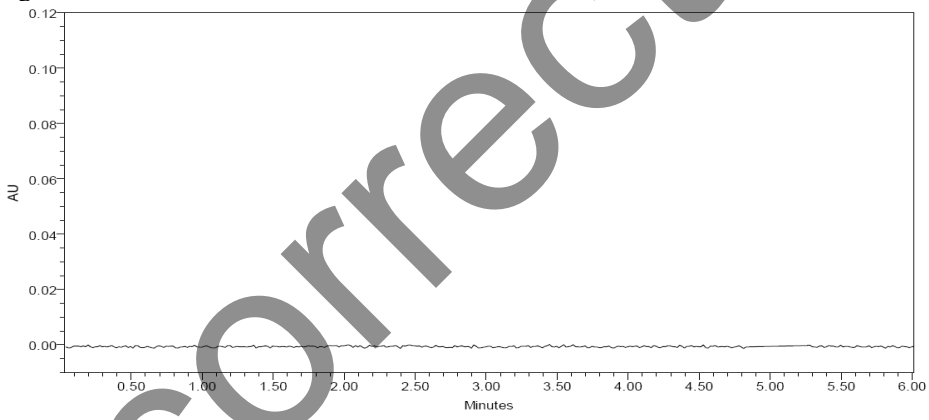

Figure 6: Blank chromatogram of Bupropion and Dextromethorphan.

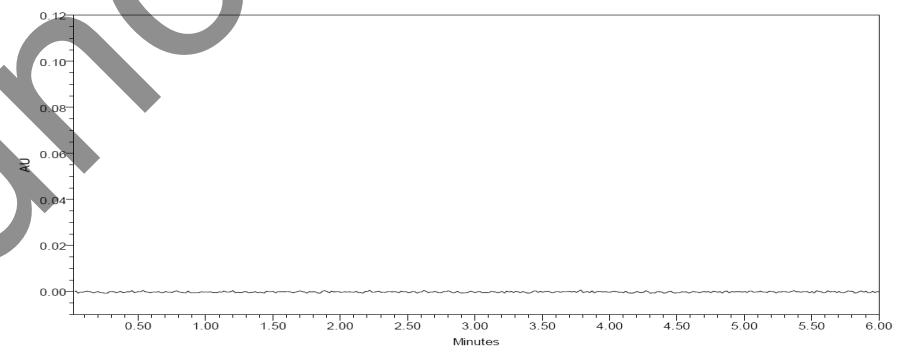

Figure 7: Placebo chromatogram of Bupropion and Dextromethorphan.

Linearity:

The linearity parameter was evaluated with standard drug solutions by preparing six different concentrations. Linearity levels were 25%, 50%, 75%, 100%, 125%, and 150% concentrations and all six linearity solutions were injected into the HPLC system, and the correlation coefficient values against drug concentrations versus peak areas were calculated. Results were obtained in table 3 and related graphs are depicted in Figures 8 & 9. Correlation coefficient values were within the limit of 0.999.

Table 3: Linearity concentration table

Linearity level	Bupropion		Dextromethorphan	
	Concentration (µg/mL)	Peak area	Concentration(µg/mL)	Peak area
25 %	17.5	233642	7.5	189050
50 %	35.0	477356	15.0	386094
75 %	52.5	715373	22.5	573548
100 %	70.0	946633	30.0	787591
125%	87.5	1178712	37.5	965859
150 %	105.0	1413042	45.0	1145652
Correlation coefficient	0.999		0.999	

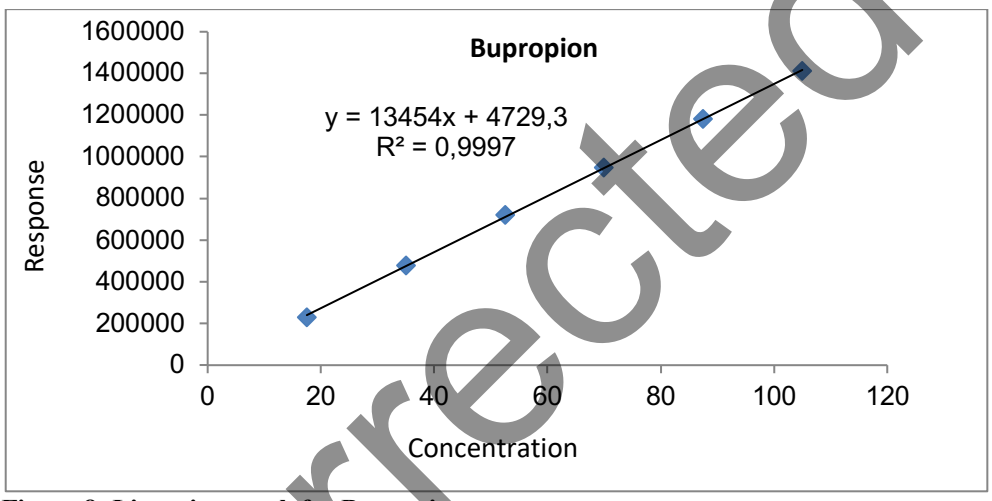

Figure 8: Linearity graph for Bupropion.

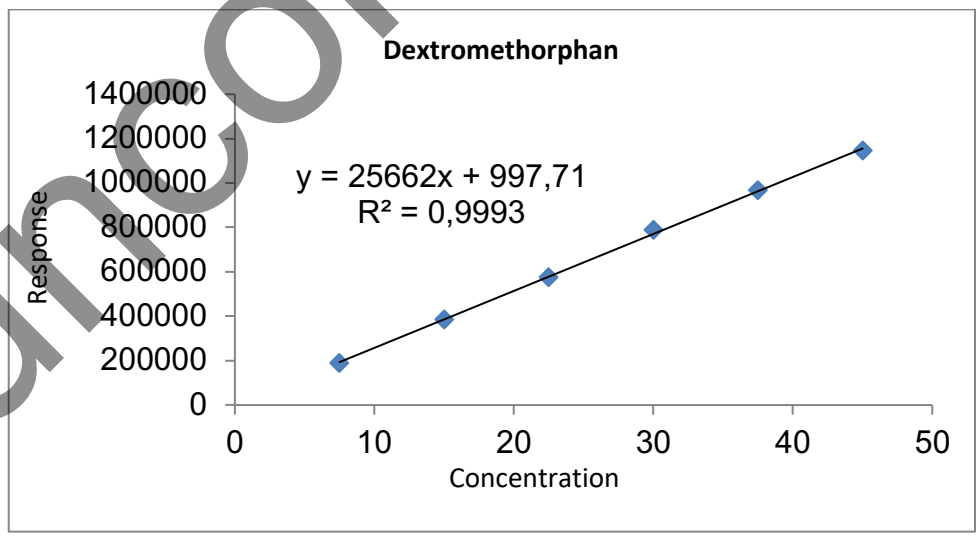

Figure 9: Linearity graph for Dextromethorphan.

Precision:
Precision was performed by preparing the six replicate sample preparations from a homogeneous sample. Six replicate solutions were carried out as per the test procedure mentioned in the materials and method section. % of RSD results were calculated for areas and %assay. The obtained results are tabulated in table 4. Precision results were found satisfactory and % RSD values were below 2 %.

Table 4: Precision Results

S. No	Bupropion		Dextromethorphan	
	Area	%Assay	Area	%Assay
1.	946549	100.08	763992	99.96
2.	946327	100.06	769671	100.70
3.	947135	100.14	766504	100.29
4.	942561	99.66	769536	100.68
5.	950484	100.50	763402	99.88
6.	949639	100.41	767899	100.47
Mean	947116	100.14	766834	100.33
S.D	2807.2	0.30	2699.9	0.35
%RSD	0.3	0.3	0.4	0.4

Intermediate Precision:
Intermediate precision was performed by preparing the six replicate sample preparations from a homogeneous sample using the different analysts, columns, and laboratory. Six replicate solutions were carried out as per the test procedure mentioned in the materials and method section. % of RSD results were calculated for areas and %assay. The obtained results are tabulated in table 5. Intermediate precision results were found satisfactory and % RSD values were below 2 %.

Table 5: Intermediate Precision Results

S. No	Bupropion		Dextromethorphan	
	Area	%Assay	Area	%Assay
1.	945886	100.01	761882	99.68
2.	939324	99.32	766487	100.29
3.	939052	99.29	759040	99.31
4.	945600	99.98	763144	99.85
5.	939091	99.29	759107	99.32
6.	946842	100.11	762823	99.81
Mean	942633	99.67	762081	99.71
S.D	3832.0	0.41	2800.9	0.37
%RSD	0.4	0.4	0.4	0.4

Accuracy:
The accuracy of the method was determined on three concentration levels by performing recovery studies. The recovery studies were carried out by different concentrations of both drugs added to the placebo from 50%, 100%

and 150% were evaluated. Recovery and % RSD were calculated. The obtained results were tabulated in table 6 & 7 and related graphs were depicted in Figures 10 & 11. % of recovery results were between 97 % to 103 %.

Table 6: Accuracy Results for Bupropion

S. No	Recovery level	%Assay	Average	SD	%RSD
1.	50%-1	100.2	99.9	0.7462	0.7
2.	50%-2	100.4			
3.	50%-3	99.1			
4.	100%-1	99.7	100.2	0.8907	0.9
5.	100%-2	99.6			
6.	100%-3	101.2			
7	150%-1	100.6	100.8	0.3504	0.3
8	150%-2	100.6			
9	150%-3	101.2			

Table 7: Accuracy Results for Dextromethorphan

S. No	Recovery level	%Assay	Average	SD	%RSD
1.	50%-1	100.99	99.7	1.5357	1.5
2.	50%-2	98.00			
3.	50%-3	100.09			
4.	100%-1	99.88	99.9	0.4321	0.4
5.	100%-2	100.36			
6.	100%-3	99.50			
7	150%-1	100.55	100.8	0.4211	0.4
8	150%-2	101.28			
9	150%-3	100.55			

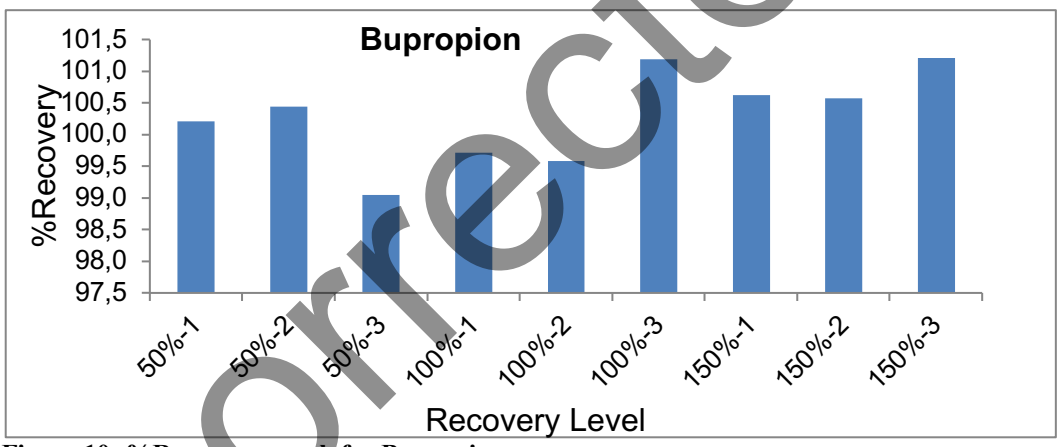

Figure 10: %Recovery graph for Bupropion.

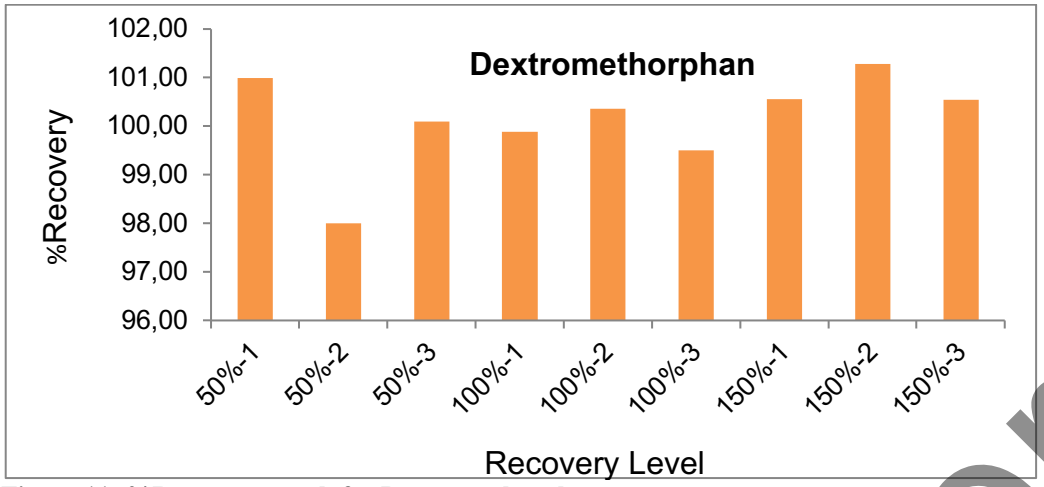

Figure 11: %Recovery graph for Dextromethorphan.

Robustness:

The robustness of the method was performed by changing the flow rate, organic and temperature. System suitability test was conducted to check the variations and the results were found satisfactory. Results were reported in the table-8.

Table 8: Robustness results

S. No	Condition	Bupropion	Dextromethorphan	Resolution between the both peaks
		%RSD	%RSD	
1.	Optimized condition	0.4	0.5	5.4
2.	Low Flow rate (0.9 mL/min)	0.6	0.8	6.1
3.	High Flow rate (1.1 mL/min)	0.3	0.7	5.9
4.	Low column Temperature (25°C)	0.7	1.0	7.2
5.	High column Temperature (35°C)	0.7	0.6	5.7
6	Low Organic volume (+4 mL)	0.8	0.8	7.5
7	High Organic volume (-4 mL)	0.4	0.7	5.2

Limit of Detection and Limit of Quantitation:

The limit of detection (LOD) is the least concentration of analyte in a sample that can be identified but not quantified. The limit of quantitation (LOQ) is defined as the least concentration of analyte in a sample that can be estimated with tolerable precision, accuracy, and reliability by a specified method under affirmed experimental conditions. The LODs were found to be 0.15 µg/mL and 0.06 µg/mL for bupropion and dextromethorphan, respectively. The LOQs were found to be 2.91 µg/mL and 0.18 µg/mL for bupropion and dextromethorphan, respectively.

Degradation Studies:

Degradation studies involving acid, base, peroxide, thermal, UV and neutral conditions were evaluated. Further, all stress degradation results were tabulated in tables 9 & 10 and the resultant chromatograms were shown in Figures 12 to 17.

Table 9: Degradation Results for Bupropion

Stress condition	% Amount	% Amount	Peak Purity

	remaining	degraded	Purity Angle	Purity Threshold
Acid	94.40	5.60	0.635	0.745
Base	95.29	4.71	0.555	0.732
oxidation	95.50	4.50	0.944	1.090
Thermal	97.60	2.40	0.725	0.917
UV	98.67	1.33	0.538	0.726
Neutral	99.60	0.40	0.552	0.753

Table 10: Degradation Results for Dextromethorphan

Stress condition	% Amount remaining	% Amount degraded	Peak Purity	
			Purity Angle	Purity Threshold
Acid	94.38	5.62	1.270	1.525
Base	95.87	4.13	1.201	1.523
oxidation	95.65	4.35	1.730	2.167
Thermal	97.58	2.42	1.915	2.304
UV	98.52	1.48	1.175	1.459
Neutral	99.01	0.99	1.187	1.498

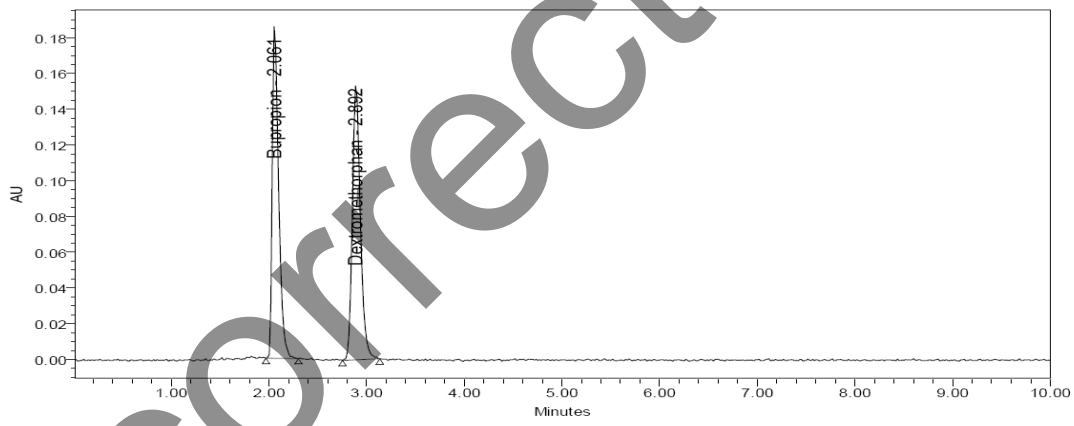

Figure 12: Acid degradation chromatogram.

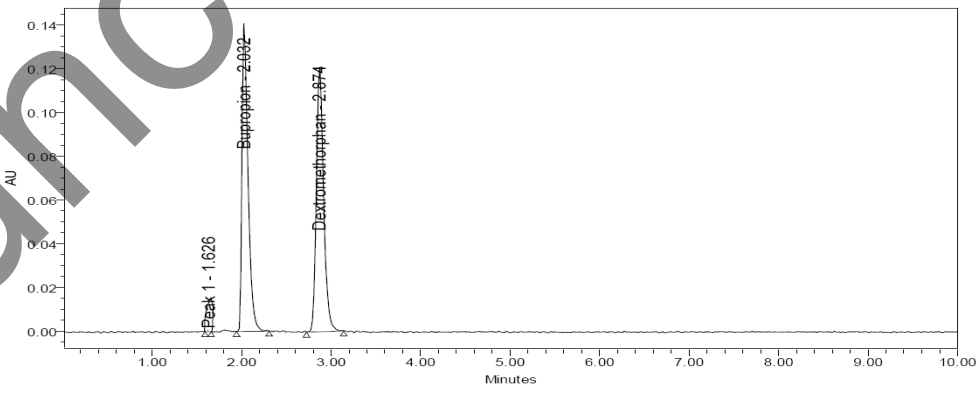

Figure 13: Base degradation chromatogram

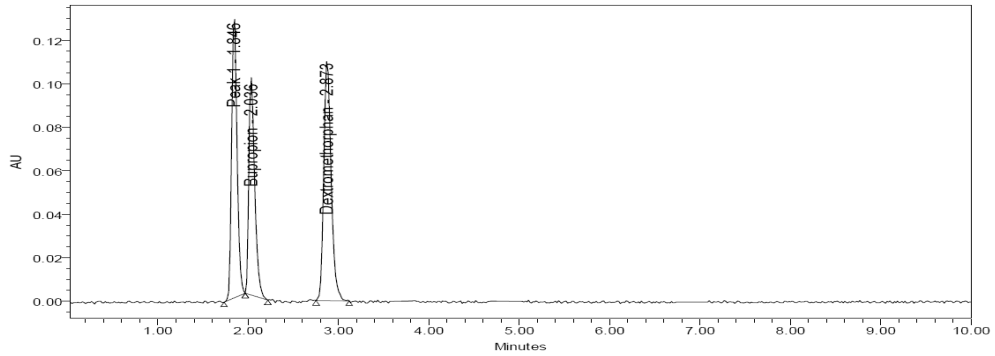

Figure 14: Oxidation degradation chromatogram

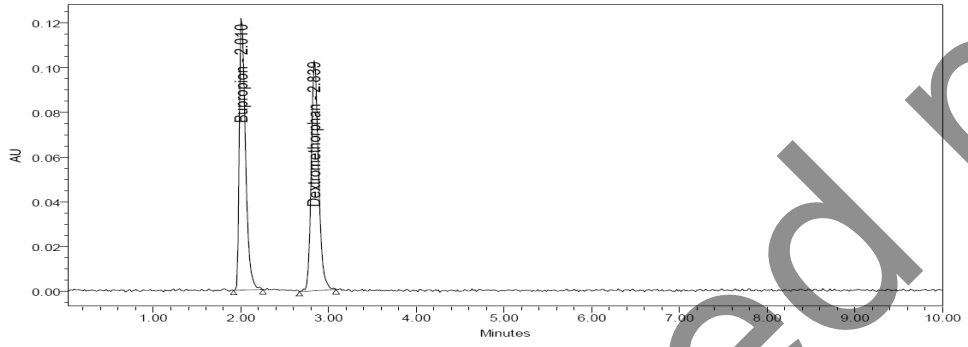

Figure 15: Thermal degradation chromatogram

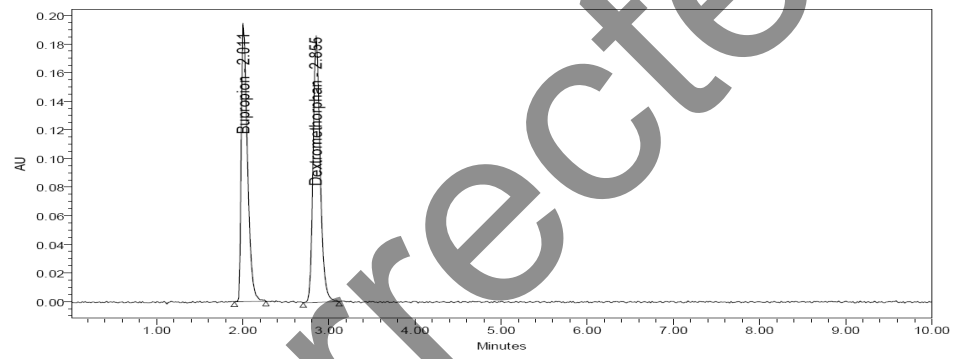

Figure 16: UV degradation chromatogram

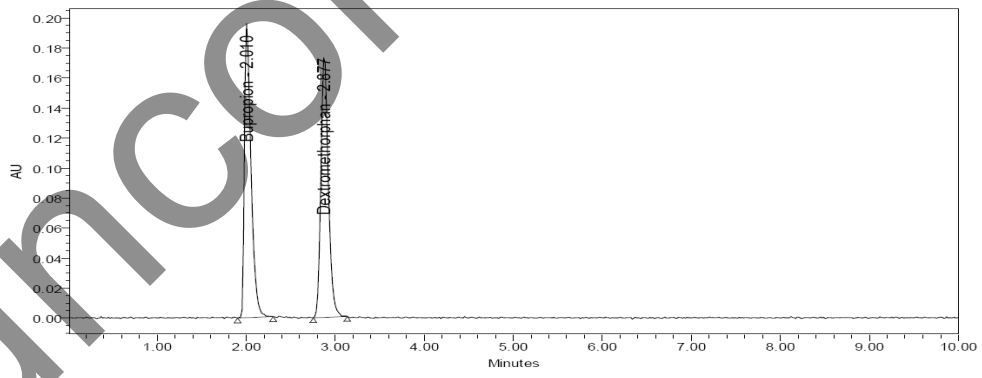

Figure 17: Water degradation chromatogram

Conclusion:

The current study describes a new and simple, reliable, economic elution RP-HPLC method for the estimation of bupropion and dextromethorphan in a fixed combination tablet dosage form. The forced degradation studies were conducted by using several degradation conditions like acidic, alkali, oxidation, thermal, UV, and neutral conditions;

the proposed method was effectively employed from the resolution of sample peaks. To our knowledge, no such detailed and stability indicating method has been reported for a fixed tablet dosage form. The developed method was completed by using a PDA as a tool for peak integrity and purity confirmation. Therefore, the proposed method can be used for the quantitation of bupropion and dextromethorphan in a fixed tablet dosage form. Finally, this method was carefully validated; as a result, it can be suggested for routine analysis testing in a quality control laboratory.

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