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"DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEIOUS ESTIMATION OF BUPROPION AND DEXTROMETHORPHAN IN SYNTHETIC MIXTURE"

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ABSTRACT

It is rapid, simple, sensitive and accurate RP-HPLC method was Analytical method development for the estimation of Bupropion and Dextromethorphan. A reversed-phase high performance liquid chromatography method is developed and validated for the determination of both drugs. With the help of RP- HPLC it gives us to good resolution and better separation for the both drugs. The separation was conducted by using Cybersil C18 column (250mm x 4.6mm x 5µm) with mobile phase consisting Phosphate buffer: Methanol (30:70 v/v) pH :4 of buffer. The mobile phase was delivered at flow rate of 1.0 ml/min. The eluent was monitored at wavelength 217 nm and found a sharp and symmetrical peak of Dextromethorphan and Bupropion were found to be 4.51 min and 6.42 min respectively. The method was validated for linearity, accuracy, precision, system suitability. The method was found to be linear over the concentration range for the drugs 5-10 µg/ml with coefficient R2 for DEX 0.9979 and BUP 0.9981. Therefore, proposed method can be successfully used for routine analysis of Bupropion and Dextromethorphan in bulk as well as synthetic mixture.

Keywords: Bupropion, Dextromethorphan, RP-HPLC, Stationary phase, Mobile phase, chromatography.

1. Introduction:

- Bupropion is chemically 2-(tert-butyl amino)-1-(3-chlorophenyl) propan-1-one [(1)]. Bupropion is a weak dopamine and norepinephrine reuptake inhibitor that is used to alleviate the symptoms of depression. Bupropion is extensively metabolized in humans. Three metabolites are active: hydroxy bupropion, which is formed via hydroxylation of the tert-butyl group of bupropion, and the amino-alcohol isomers, threohydrobupropion and erythrohydrobupropion, which are formed via reduction of the carbonyl group. In vitro findings suggest that CYP2B6 is the principal isoenzyme involved in the formation of hydroxy bupropion [(2)].
- Dextromethorphan is chemically 4-methoxy-17-methyl-17-azatetracyclo [7.5.3.01,10.02,7] heptadeca-2(7),3,5-triene [(3)]. Dextromethorphan, is in the morphinan class of medications with sedative, dissociative, and stimulant properties (at lower doses) [(4)]. Dextromethorphan and its major metabolite, dextromethorphan, also block the NMDA receptor at high doses, which produces. Dextromethorphan is an agonist of NMDA and sigma-1 receptors. It is also an antagonist of α3/β4 nicotinic receptors. However, the mechanism by which dextromethorphan's receptor agonism and antagonism translates to a clinical effect is not well understood [(5)].
- Bupropion is officially in United States pharmacopeia. USP describes HPLC method for its estimation. Various methods like UV, HPLC, Stability indicating RP-HPLC, LC-MS [(7-25)] method for determination of Bupropion is reported in literature for estimation of BUP in pharmaceutical formulation.
- Dextromethorphan is officially in United States pharmacopeia and Indian pharmacopeia. IP and USP describes HPLC method for its estimation. Various methods like UV, HPLC, Stability indicating RP-HPLC, Stability indicating RP-HPTLC, UHPLC-MS [(26-45)] method for determination of Dextromethorphan is reported in literature for estimation of DEX in pharmaceutical formulation.

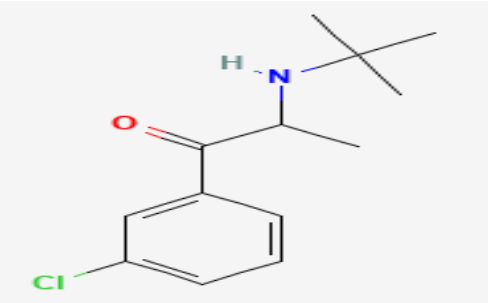

Figure 1: Chemical Structure of Bupropion [(2)]

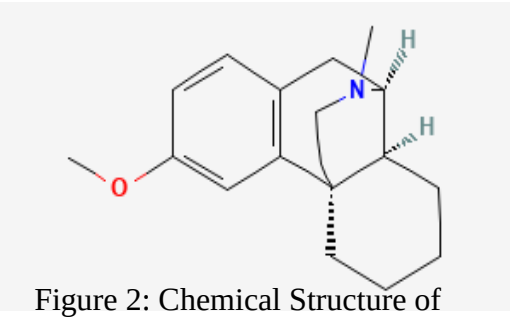

Figure 2: Chemical Structure of Dextromethorphan [(3)]

2. MATERIALS AND METHODS

- Sample of **Bupropion (BUP)** procured from Pure chem Pvt Ltd, Ankleshwar, Gujarat. **Dextromethorphan (DEX)** as procured from Alpha Chemika, Gujarat.

A. Apparatus

HPLC manufactured by Cyber Lab having LC-100 model no. was used in this method development. Cyber-Sil, C18 column (250mm x 4.6mm, 5 µg) was used as a stationary phase. For Identification of API by using UV Visible Spectrophotometer and FT-IR. UV Visible Spectrophotometer is manufactured by Shimadzu having UV 1700 model no and FT-IR is manufactured by Agilent Technologies having Cary 630 model no.

B. Chemical and reagent

HPLC Grade Water, Methanol, Acetonitrile which is manufactured by Ranchem Ltd. AR Grade Potassium Dihydrogen Phosphate. Which also manufactured by Ranchem Ltd.

C. Preparation of Stock solution

a) Stock-A: - Bupropion (BUP)-1000 µg/ml

Accurately weighed separately and transferred about 100 mg of Bupropion (BUP) in to 100 ml volumetric flask, 50 ml methanol was added and sonicate to dissolve. Make up to the mark with diluent

- **Stock-1 – Bupropion (BUP)-100 µg/ml**

Further diluted 10 ml of Stock-A solution to 100 ml volumetric flask and volume was make up to the mark with diluent. Make up to the mark with diluent.

b) Stock-B: Dextromethorphan (DEX)-1000 µg/ml

Accurately weighed separately and transferred about 100 mg of Dextromethorphan (DEX) in to 100 ml volumetric flask, 50 ml methanol was added and sonicate to dissolve. Make up to the mark with diluent.

- **Stock-2 – Dextromethorphan (DEX)-100 µg/ml**

Further diluted 10 ml of Stock-B solution to 100 ml volumetric flask and volume was make up to the mark with diluent. Make up to the mark with diluent.

I. Preparation of Standard Stock solution of Bupropion (BUP)

Pipette out 1,2,3,4,5 and 6 ml of Bupropion (BUP) 100 µg/ml (Stock-1) in 100 ml of volumetric flask. Diluted above solution to 10 ml volumetric flask and make up to the mark with diluent to get 10,20,30,40,50 and 60 µg/ml concentration of Bupropion (BUP).

II. Preparation of Standard Stock solution of Dextromethorphan (DEX)

Pipette out 0.5,1,2,3,4 and 5 ml of Dextromethorphan (DEX) 100 µg/ml (Stock-1) in 100 ml of volumetric flask. Diluted above solution to 10 ml volumetric flask and make up to the mark with diluent to get 5,10,20,30,40 and 50 µg/ml concentration of Dextromethorphan (DEX).

III. Preparation of Standard Stock solution of Bupropion (BUP) and Dextromethorphan (DEX)

Accurately weighed and transferred about 20 mg of Bupropion (BUP) and 10mg of Dextromethorphan (DEX) in to 100 ml of volumetric flask, 50 ml of methanol was added and sonicated to dissolve. Volume was making up to the mark with diluent.

Concentration of Bupropion (BUP) is 200 µg/ml and Dextromethorphan (DEX) 100 µg/ml.

Further diluted 5 ml of above solutionto 50 ml volumetric flask and volume was make up to the mark with diluent. Concentration of Bupropion (BUP) is 20 µg/ml and Dextromethorphan (DEX) 10 µg/ml.

D. Preparation Of Buffer

Mixed: Dissolve 5.04 g disodium hydrogen phosphate and 3.01 g of potassium dihydrogen phosphate in sufficient water to produce 1000 ml. Adjust the pH 4 with glacial acetic acid. Mix well and sonicate. Filter through 0.45 µm membrane filter paper.

E. Preparation Of Mobile Phase

Prepare a mixture of phosphate buffer and Methanol in the volume ratio 30:70 % v/v. Mix well and sonicate to degas the mixture. Adjust pH 4 with glacial acetic acid.

F. Selection Of Column

Dextromethorphan (DEX) and Bupropion (BUP) are polar in nature. So, C18 analytical column were selected for HPLC method. The column was used Cybersil C18 column (250 mm × 4.6 mm, 5 µm) was used for the development of the method.

G. METHOD VALIDATION

i. Linearity and Range

Linearity was studied by preparing solutions of six different concentrations of 5, 10, 20, 30, 40 and 50 µg/ml of DEX, 10, 20, 30, 40, 50 and 60 µg/ml of BUP respectively. Each concentration was repeated six times.

Linearity was assessed in terms of slope, intercept and correlation coefficient of Dextromethorphan (DEX) and Bupropion (BUP). The calibration curves were developed by plotting absorbance versus concentrations (n = 6).

ii. Precision

Repeatability was determined by analyzing solution containg mixture of 20µg/ml for DEX and 20µg/ml for BUP is times and results are reported in terms of RSD. Intraday precision of the developed RP-HPLC method were determined by analyzing sample solutions of DEX (5, 20, 50 µg/ml) and BUP (10, 30, 60 µg/ml) at three levels covering low, medium and high concentrations of the calibration curve three times on the same day (n = 3). Interday precision was determined by analyzing sample solutions of DEX (5, 20, 50 µg/ml) and BUP (10, 30, 60 µg/ml) at three levels covering low, medium and high concentrations over a period of 3 days (n = 3). The peak areas obtained were used to calculate mean and RSD values.

iii. Accuracy

The accuracy of the method was studied by analysis of standard at three different levels, i.e., multiple level recovery studies (50%, 100% and 150%). Known amount of Dextromethorphan (DEX) (50%, 100% and 150%) and BUP (50%, 100% and 150%) were added to a pre-quantified sample solution of 20 µg/ml Dextromethorphan (DEX) and 20 µg/ml Bupropion (BUP) and the amount of Dextromethorphan (DEX) and Bupropion (BUP) were estimated by measuring the peak areas and by fitting these values to the straight-line equation of calibration curve.

iv. LOD and LOQ

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by using the following equations as per International Conference on
Harmonization (ICH) guidelines which is based on the calibration curve.

$LOD = 3.3 \times \sigma / S$

$LOQ = 10 \times \sigma / S$

Where, σ = Standard deviation of y-intercepts of regression lines

S = Slope of calibration curve.

v. Specificity

The specificity of the method was ascertained by analyzing Dextromethorphan (DEX) and Bupropion (BUP)in the presence of excipients (Lactose, micro-crystalline cellulose, Aerosil, Magnesium stearate) by preparing synthetic mixture.The results obtained of Dextromethorphan (DEX) and Bupropion (BUP) were confirmed by comparing with results of standards and calculate the % interference.

vi. Robustness

According to ICH, the robustness of the method was determined in triplicate at a concentration level of 20µg/ml for DEX and 20µg/ml BUP. The mean and RSD of peak areas were calculated. Deliberate changes in the following parameters which affects % assay of Dextromethorphan (DEX) and Bupropion (BUP) and system suitability parameters were studied.

a) Change in % organic phase of mobile phase by ± 5.0 %
b) Change in pH of buffer of mobile phase by ± 0.05 of set PH
c) Change in the flow rate of the mobile phase by ± 10 % of the original flow rate.
d) Change in detection wavelength by ± 5.0 nm

Assay of Synthetic Mixture Preparation

The synthetic mixture of Dextromethorphan (DEX) and Bupropion (BUP) was prepared in ratio of (45:105 mg). Common excipients like Hydroxypropyl methyl cellulose (HPMC) K100 (200 mg), Microcrystalline cellulose (160 mg), Magnesium stearate (120 mg), Cross Providone (80 mg), Talc (40 mg) were weighed accurately and transfer into mortar pestle along with 450 mg of DEX and 1050 mg of BUP which is equivalent to 10 tablets.

From the above mixture weight accurately equivalent to 45 mg DEX and 105 mg BUP and transfer it in 10 ml volumetric flask containing few ml of Methanol and volume made up to the mark with

methanol to obtain (450 µg/ml of DEX and 1050 µg/ml of BUP) than sonicate for 15 min. The solution was filtered using Whatman filter paper No. 42 and collect the filtrate in another 10 ml volumetric flask and residue was wash with few ml amount of methanol, the filtrate and residue was combined and volume was diluted to the mark with methanol.Pipette out 1.0 ml aliquot from the above stock solution transfer it in another 10 ml volumetric flask and volume made up to the mark with methanol to obtain (45 µg/ml of DEX and 105 µg/ml of BUP) solution. Then pipette out 5.0 ml from above stock solution and volume made up to 10 ml with methanol to obtain final concentration of 22.5 µg/ml DEX and 52.5 µg/ml BUP. The volume injected was 20 µl and chromatogram was recorded with the optimized mobile phase.

3. RESULT AND DISCUSSION

A. Melting point study

The observed melting point of each mentioned drugs were similar to the standard melting point reported for respective drugs as evident from Table 1.

Table 1 Melting point study

Drugs	Reported Melting Point (°C)	Observed Melting Point (°C)
Bupropion (BUP)	233-234 °C	233-234 °C
Dextromethorphan (DEX)	122◦C -124◦C	120◦C -123◦C

B. Solubility Study

The solubility of substance fundamentally depends on the physical and chemical properties of the solute and solvent as well as temperature, pressure and the pH of the solution. The solubility profile is used for solvent selection in method development. The solubility of each drug in different solvent in shown in Table 2.

Table 2 Solubility Study

Drugs	Bupropion (BUP)	Dextromethorphan (DEX)
Water	Freely soluble	Insoluble
Ethanol, Methanol	Very Soluble	Freely Soluble
Acetonitrile	Soluble	Slightly soluble

C. Determination of Wavelength (λmax)

UV spectra of drugs in methanol depicted that the wavelength maxima of BUP and DEX were at **214 nm** and **222 nm** respectively as shown in Figure 3. For High Performance Liquid Chromatography **217 nm** was selected as detection wavelength.

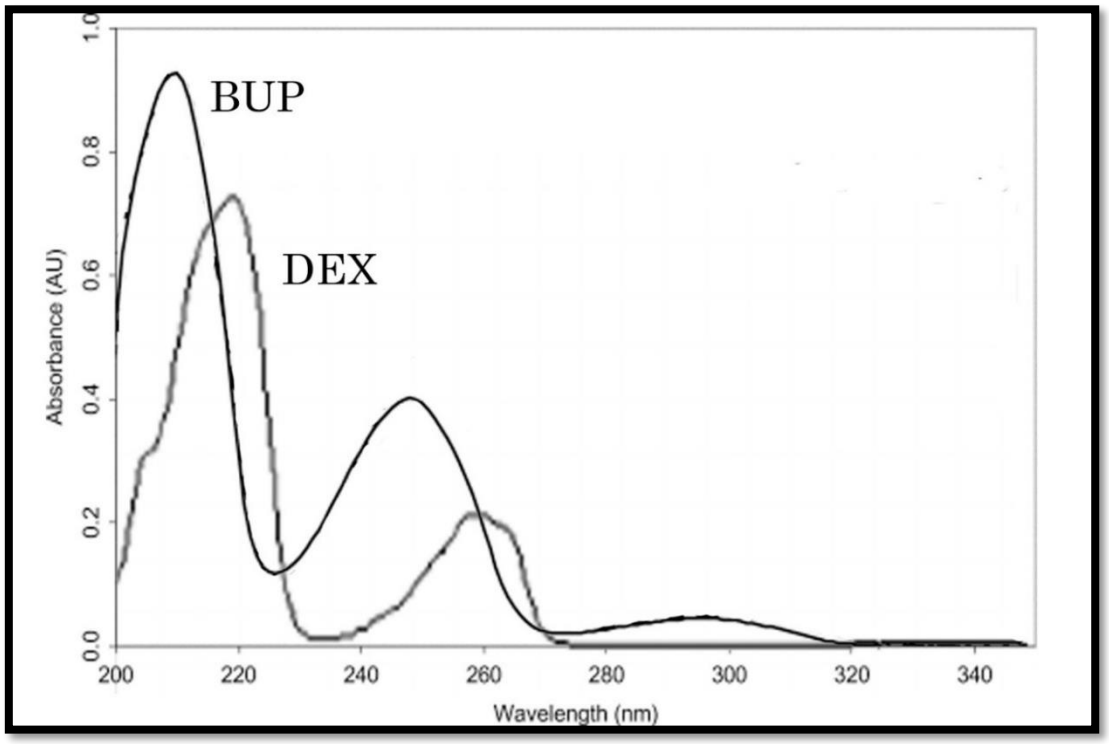

Figure 3: Overlain UV Spectrum in methanol

D. VALIDATION PARAMETERS

i. Linearity and Range

Linearity was evaluated by calculation of correlation coefficient. Responses were found to be linearity in the above concentration range with correlation coefficients of 0.9979 for DEX and 0.9981 for BUP. The results of linearity are shown in Table 3 and figure 4 or 5 for Dextromethorphan and Bupropion, respectively.

Table 3 Linearity data

Drug	Conc.	Peak Area	% RSD	Drug	Conc.	Peak Area	% RSD
DEX	5	21560.55	1.55	BUP	10	33863.65	0.93
	10	30371.53	1.55		20	55883.38	1.12
	20	48651.82	1.34		30	80543.28	0.53
	30	67231.82	0.87		40	102481.00	0.87
	40	85143.23	0.81		50	124887.77	1.68
	50	98827.33	0.76		60	142548.57	1.20

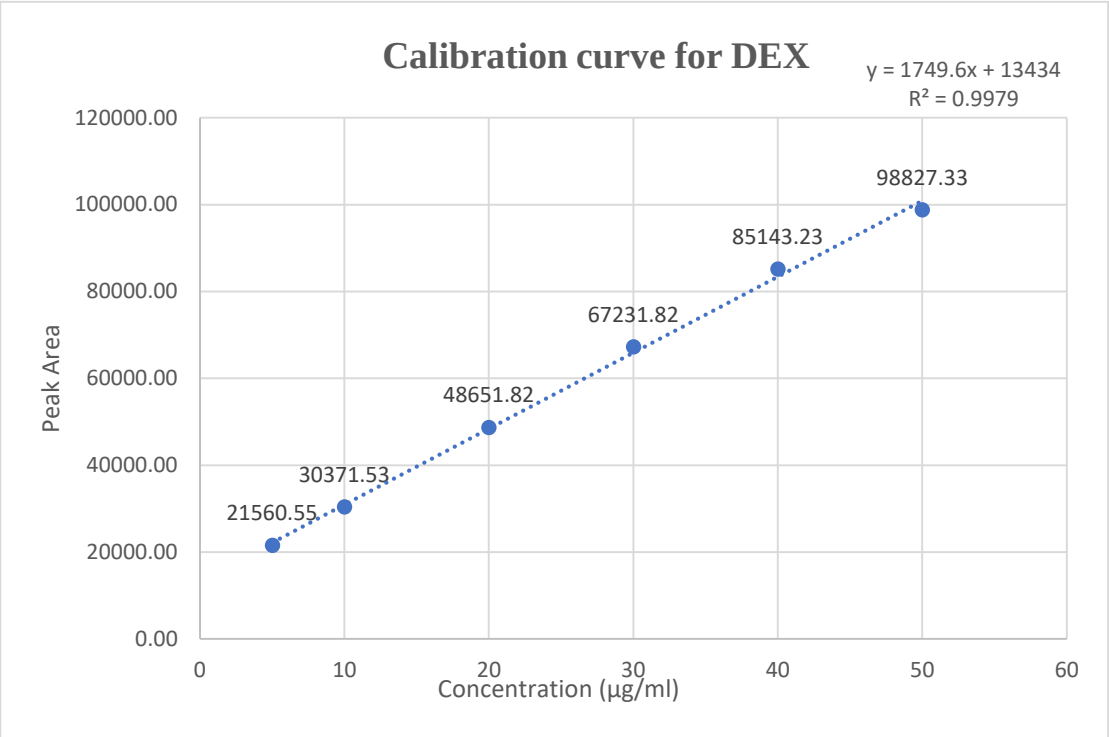

Figure 4: Calibration curve of DEX standard

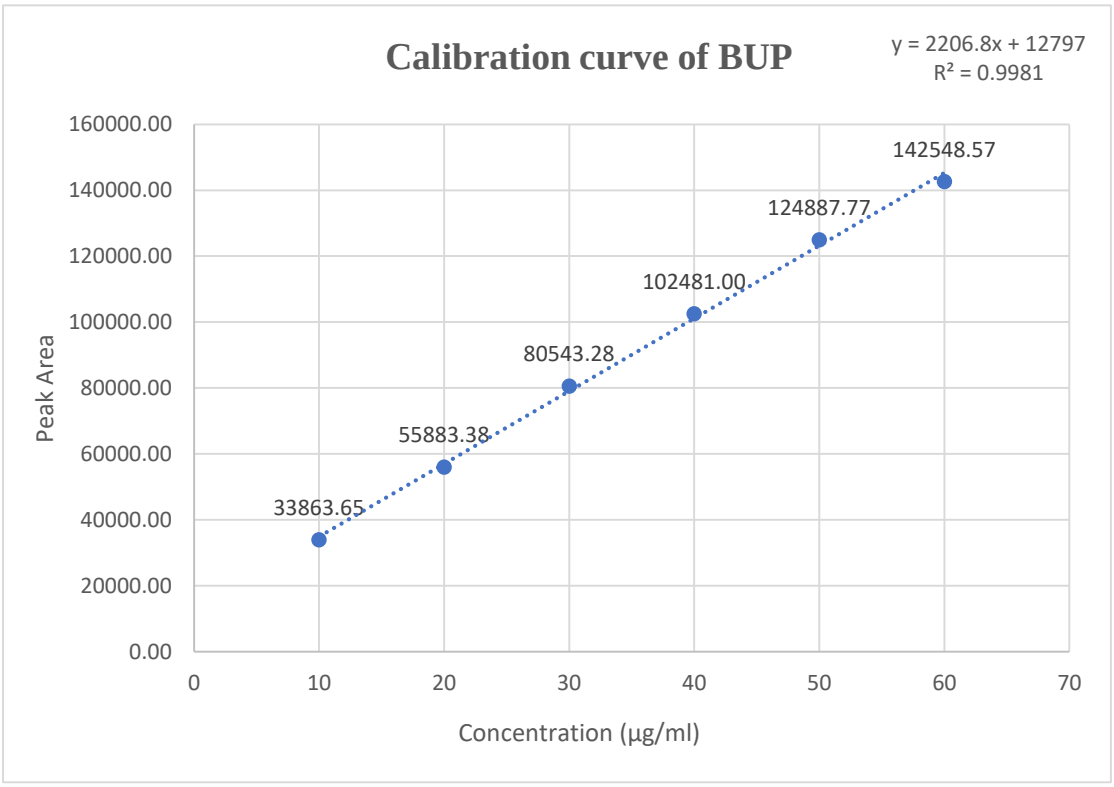

Figure 5: Calibration curve of BUP standard

ii. Pricision

a. Repeatability

In RP-HPLC method, repeatability has been carried out by Injection, repeatability. Injection repeatability was carried out by analyzing the sample solution of DEX + BUP (20 µg/ml) six times and peak area was measured and % RSD Calculated which is shown in Table 4.

Table 4. Repeatability study

Dextromethorphan (DEX)			**Bupropion (BUP)**		
Sr. No	**Conc. (µg/ml)**	**Area**	**Sr. No**	**Conc. (µg/ml)**	**Area**
1	20	48105	1	20	56398.7
2	20	47562	2	20	56174.2
3	20	48567	3	20	56575.8
4	20	48753	4	20	54749.3
5	20	46785	5	20	55738.6
6	20	47896	6	20	55863.7
Average	47944.67		**Average**	55916.72	
SD	715.55		**SD**	652.75	
% RSD	1.49		**RSD**	1.17	

b. Intraday and Interday precision

The precision of method was determined by carrying out Intraday and Interday precision. The peak areas obtained were used to calculate mean and % RSD values show in table 5.

The % RSD was found to be less than 2 % which indicate method in precise.

Table 5. Precision Date of DEX and BUP

Dextromethorphan (DEX)				
Conc	**Intraday precision**		**Interday precision**	
	Peak Area (Mean ± SD) n	**%RSD**	**Peak Area (Mean ± SD) n**	**%RSD**
5	21447.53 ± 250.48	1.17	21684.30 ± 359.24	1.66
20	48247.82 ± 441.80	0.92	49042.82 ± 626.83	1.28
50	99394 ± 503.32	0.51	98375.67 ± 906.93	0.92
Bupropion (BUP)				
10	33655.40 ± 249.93	0.74	33788.73 ± 591.66	1.75
30	80831.97 ± 298.11	0.37	80597.07 ± 768.96	0.95
60	142140.17 ± 678.75	0.48	141356.96 ± 1253.00	0.89

iii. Accuracy

The data shown in Table 7 indicate that the developed method is accurate. The % recovery of DEX and BUP was found to be in range of 97.97 -102.74 % and 98.60 – 101.36 %, respectively.

Table 7 Accuracy date of DEX and BUP

Level (%)	Target Conc. (µg/ml)	Spiked Conc. (µg/ml)	Total Conc. (µg/ml)	Mean Area^n	Conc. Found (µg/ml)	% Recovery
Dextromethorphan (DEX)						
0	20	0	20	49042.82	20.35	101.76
50	20	10	30	67023.65	30.63	102.10
100	20	20	40	85333.23	41.09	102.74
150	20	30	50	99142.33	48.99	97.97
Bupropion (BUP)						
0	20	0	20	46316.23	19.72	98.60
50	20	10	30	70822.83	30.83	102.75
100	20	20	40	92269.27	40.54	101.36
150	20	30	50	113853.00	50.32	100.65

iv. LOD and LOQ

The detection limits for DEX and BUP were found to be 0.74 µg/ml and 1.28 µg/ml respectively, while quantitation limits were found to be 2.45 µg/ml and 4.28 µg/ml respectively as shown in table 8 indicating sensitivity of the method.

Table 8 LOD and LOQ study

Drug	Dextromethorphan (DEX)	Bupropion (BUP)
Limit of detection (LOD)	0.74 µg/ml	1.28 µg/ml
Limit of quantification (LOQ)	2.45 µg/ml	4.28 µg/ml

v. Robustness

For robustness study, slight changes were made in detection wavelength, flow rate and mobile phase composition. The results were expressed as % RSD shown in Table 8 % RSD less than 2 indicated that the developed method was robust.

Table 9 Robustness date of DEX and BUP

Parameters	Change in condition	DEX		BUP	
		Peak Area (Mean± SD)	%RSD	Peak Area (Mean± SD)	%RSD
Flow rate Changed (1 ml/min)	0.9	44391.53	0.85	54298.7	0.57
	1.1	42721.58	0.57	55863.7	0.66
Mobile Proportion Changed Phosphate buffer: Methanol: (30:70% V/V) pH-4 of buffer	Phosphate buffer: Methanol (25:75v/v)	48652	0.66	54145.9	0.80
	Phosphate buffer: Methanol (35:65v/v)	41371.53	0.80	56198.7	1.15
Detection wavelength (269nm)	266 nm	44521.58	1.35	53863.7	0.89
	274nm	45115.51	0.78	54174.2	1.05

Analysis of synthetic mixture (assay)

The developed RP-HPLC method was successfully applied for the estimation of DEX and BUP in synthetic mixture.

The chromatogram of sample showed only drug peaks at retention time (Rt) value of 4.51 and 6.42 minute for Dextromethorphan (DEX) and Bupropion (BUP), respectively, indicating that there is no interference of the excipients present in synthetic mixture.

The content of Dextromethorphan (DEX) and Bupropion (BUP) was calculated by comparing peak areas of sample with that of the standard. The Synthetic mixtures were analyzed using proposed method which gave percentage recovery of more than 98.23 for DEX and 99.17 for BUP (Table 33).

No interference from the excipients present in the marketed tablet formulation was observed which is shown in figure 6.

Table 1 Date of determination of DEX and BUP in synthetic mixture

Formulation	**Drug**	**Amount Taken (µg/ml)**	**Amount Found[n] (µg/ml)**	**%DEX ± SD**	**%BUP ± SD**
Synthetic mixture	DEX	22.50	22.10	98.23 ± 0.56	99.17 ± 0.67
	BUP	52.50	52.07		

n= Average of three determination

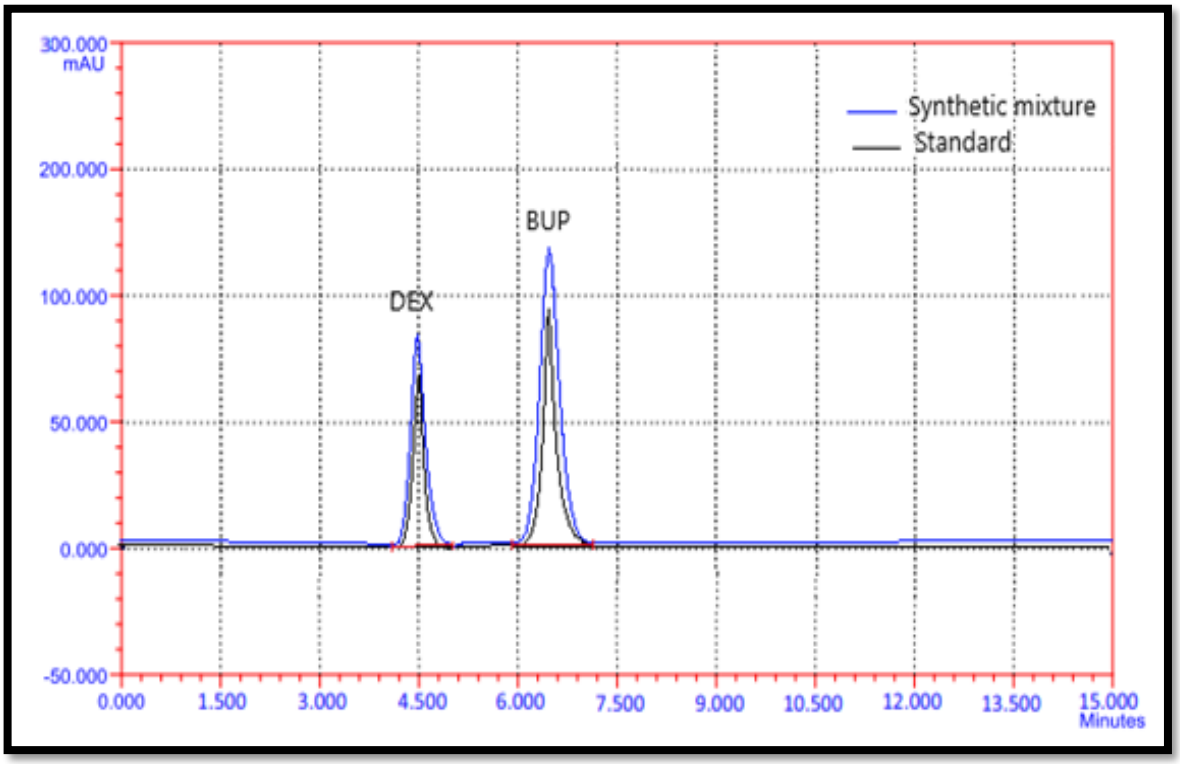

Figure 6: HPLC Chromatogram of standard solution of DEX and BUP in mix standard.

CONCLUSION

Simple and sensitive RP-HPLC were developed for simultaneous estimation of Bupropion (BUP) and Dextromethorphan (DEX) in their synthetic mixture. RP-HPLC method was developed using Cyber Lib C18 (250 x 4.6mm, 5µm) column as a stationary phase and Phosphate buffer: Methanol: (30:70% V/V) pH-4 of buffer as mobile phase. The flow rate was maintained at 1 ml/ min and detection was carried out at 217 nm where Bupropion (BUP) and Dextromethorphan (DEX) have significant absorbance. The retention times of Bupropion (BUP) and Dextromethorphan (DEX) was found to be 4.51 minute and 6.42-minute respectively. RP-HPLC method is linear in the concentration range of 5- 50 µg / ml for DEX and 10- 60 µg/ ml for BUP, with correlation coefficient found to be 0.9979 for DEX and 0.9981 for BUP. The recovery was in the range of be 97.97 % - 102.74% for DEX and 98.60 %-102.75% for BUP, respectively. The detection limits for DEX and BUP were found to be 0.73µg/ml and 1.28 µg/ml respectively, while quantitation limits were found to be 2.44 µg/ml and 4.28 µg/ml respectively. The method was found to be accurate, precise, specific, selective, repeatable, robust and reproducible.

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