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DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR SIMULTANEOUSESTIMATION OF DEXTROMETHORPHAN HBR AND BUPROPION HCL

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Abstract: Dextromethorphan (DXM) and Bupropion (BUP) is a combination medication used for the treatment of major depressive disorder. A simple, sensitive and accurate HPTLC method has been developed and validated for estimation of Dextromethorphan hydrobromide and Bupropion hydrochloride in bulk and pharmaceutical dosage form as per ICH guidelines. The proposed method successfully achieved effective chromatographic separation at wave length of 215 nm using Chloroform: Methanol (9: 1 v/v) as the mobile phase which gave good resolution and acceptable peak parameters. The method gives excellent linearity in the concentration ranges from 50-300 ng/band for DXM and 100-600 ng/band for BUP. The method was found to give compact spots for the drugs with Rf values of DXM 0.28 ± 0.14 and BUP 0.75 ± 0.11. The limit of detection and limit of quantification for Dextromethorphan hydrobromide was found to be 3.213 ng/ band and 9.736 ng/band and for Bupropion hydrochloride 6.432 ng/band and 19.490 ng/band respectively. The method was validated with reference to linearity, accuracy, precision, specificity and robustness and found to be within limit according to ICH guidelines.

Key words: Dextromethorphan HBR, Bupropion HCL, HPTLC, Method Validation.

I. INTRODUCTION

Major Depressive disorder is a leading cause of disability worldwide. According to World Health Organisation (WHO) it affects an estimated 264 million people of all ages globally. In 2020, the prevalence of depression was estimated to be around 4.4% of the global population, with higher rates among women than men. Major depressive disorder is a mental health condition marked by persistent feelings of sadness, lack of interest in activities, suicidal thoughts and physical symptoms such as fatigue and changes in appetite or sleep patterns. The various medications used for this condition includes Antidepressant medications, such as selective Serotonin Reuptake Inhibitors (SSRIs), Serotonin-Norepinephrine Reuptake Inhibitors (SNRIs), or Tricyclic Antidepressants (TCAs), can help alleviate symptoms of depression[1].

In the year 2022, an oral fixed dose combination of Dextromethorphan Hydrobromide and Bupropion HCl was approved in the USA for the treatment of Major Depressive Disorder and is available under the brand name Auvelity[2].Dextromethorphan hydrobromide, which is morphinan-structured with a 3-methoxy-17-methyl (9, 13, 14)-hydrobromide component, is a medication utilized for its antitussive properties (i.e., cough suppression). It is used for pain relief and in certain mental conditions. Its mechanism involves increasing the threshold for coughing by acting on the cough center[3].Bupropion hydrochloride (BUP), represented by the chemical structure of (±)-2-(tert-butylamino)-3′-chloropropiophenone hydrochloride, is an aminoketone derivative with a pKa of 7.94. As a second generation antidepressant, it differs from typical tricyclic antidepressants in terms of its neurochemical properties. Research suggests that Bupropion hydrochloride selectively inhibits the neuronal reuptake of catecholamines (namely, noradrenalin and dopamine), while having minimal impact on the reuptake of indolamines (such as serotonin) and no inhibitory effect on monoamine oxidase. BUP in sustained release form is used in smoking cessation as the first licensed non-nicotine pharmacological therapy. The structures of Dextromethorphan HBr and Bupropion HCl are shown in the Fig. 1.

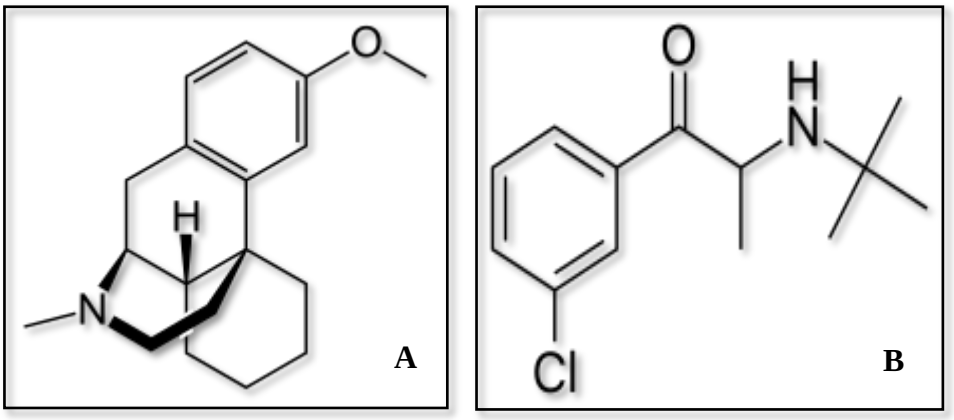

Figure 1: Chemical Structure of A. Dextromethorphan HBr[5] B. Bupropion HCl[6]

A few methods have been developed for the estimation of Bupropion individually and in combination[7-13]. Similarly, HPLC method has been developed for estimation of Dextromethorphan HBr[14]. But till date, No analytical HPTLC method has been reported for simultaneous estimation of Dextromethorphan HBr with Bupropion HCl in combination. In this study, HPTLC method for the simultaneous estimation of Dextromethorphan HBr and Bupropion HCl has been developed and further validated.

II. MATERIALS AND METHODS

2.1 Materials

Pure sample of bupropion HCL (Mw: 276.20 g/mol) was obtained as a gift sample from Lupin Pharmaceuticals and Pure sample of Dextromethorphan HBR (Mw: 239.74 g/mol) was obtained from Swapnroop Research PVT LTD and the marketed formulation was (Auvelity). Hydroxy Propyl Methyl Cellulose (HPMC) was obtained from Clariant Chemicals India Ltd. Polyvinyl Pyrollidone (PVP-K30) was obtained from Lab Chemie Industries, Mumbai, India. Avicel, aerosil and magnesium stearate were purchased from Research Lab Fine Chemical Industries, Mumbai, India.

2.2 Instrumentation and Software

In the present investigation, a CAMAG HPTLC (High-Performance Thin-Layer Chromatography) system manufactured in Muttenz, Switzerland was employed. The experimental setup comprised a CAMAG Linomat V semi-automated sample applicator, CAMAG TLC Scanner III, Hamilton syringe (100 µL; Bonaduz, Switzerland), CAMAG Win CATS software V-1.4.2 and UV-Visible Double beam spectrophotometer (Jasco Model V-730) furnished with a single Monochromator. The evaluation of the data was carried out utilizing Microsoft Excel software, which involved linear regression analysis to determine the quantification of extracts and validation parameters.

2.3 Experimental Method Development

2.3.1 Preparation of Standard Stock Solution

Standard stock solution of Dextromethorphan HBr and Bupropion HCl were prepared separately by dissolving 100 mg of drug in 10 ml of methanol to get concentration of 1000µg/ml. From the respective standard stock solution, working standard solution was prepared containing 100 µg/ml of each Dextromethorphan HBr and of Bupropion HCl, separately in methanol.

2.3.2 Preparation of sample solution for tablet formulation analysis

Contents of twenty tablets each containing 45 mg of Dextromethorphan HBr and 105 mg of Bupropion HCl were weighed and powdered. Powder equivalent to 10 mg of Dextromethorphan HBr (23.333 mg of Bupropion HCl) was transferred to 10 ml volumetric flask and was diluted with methanol and volume made to 10 ml (1000 µg/ml of Dextromethorphan HBr and 2333 µg/ml of Bupropion HCl) with methanol. Solution was filtered and further dilutions were made with mobile phase to get the final concentration of 50 µg/ml of Dextromethorphan HBr and 116.65 µg/ml of Bupropion HCl. 2 µl volume was applied on TLC plate and developed under optimized conditions.

2.3.3 Chromatographic Conditions

The samples were spotted in the form of bands of width of 6 mm with space between bands of 8 mm, with a 100 µl sample syringe (Hamilton, Bonaduz, Switzerland) on precoated silicagel aluminum plate 60 F 254 (10 ×10) with 250 µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland).Chloroform: Methanol (9: 1 v/v)was used as mobile phase. The slit dimensions5 mm × 0.45 mm and scanning speed of 20 mm/sec was employed. The linear ascending development was carried out in 10 cm × 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) using as mobile phase. The optimized chamber saturation time for mobile phase was 15 min. The length of chromatogram run was 8 cm and development time was approximately 15 min. TLC plates were dried in a current of air with the help of a hair drier. Densitometry scanning was performed on CAMAG thin layer chromatography scannerat 210 nm for all developments operated by WINCATS software version 1.4.2. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to400 nm.

2.3.4 Selection of Detection Wavelength

From the standard stock solution further dilutions were made using methanol and scanned over the range of 200 - 400 nm and the spectra was obtained. The detection wavelength was selected at the point where both the drugs showed considerable absorbance.

2.4 Method Validation

Validation of the HPTLC method was done as per ICH Q2 (R1) guidelines for parameters like linearity, accuracy, precision, robustness, LOD and LOQ[15].

2.4.1 Specificity

The specificity of the method was confirmed through peak purity profiling studies, where the purity of the peak was verified by comparing spectra at various levels, including the start, apex, and end position of the spot.

2.4.2 Linearity

From the standard stock solution (1000 µg/ml) of Dextromethorphan HBr and Bupropion HCl, solution was prepared containing 100 µg/ml of Dextromethorphan HBr and 200 µg/ml of Bupropion HCl, separately. Different volumes were applied on TLC plate to obtain linear range. Six replicates per concentration were applied. The linearity (relationship between peak area and concentration) was determined over the concentration range 50-300 ng/band for Dextromethorphan HBrand 100- 600 ng/band for Bupropion HCl.

2.4.3 LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) were determined by calculating the slope and standard deviation from the linearity graph.

2.4.4 Accuracy

Recovery studies were conducted to verify the accuracy of the method by adding standard drug to the sample at three different levels (50%, 100%, and 150%). The basic concentrations of the sample were 2 µl of 100 µg/ml of Dextromethorphan HBr and 2 µl of 1600 µg/ml of Bupropion HCl. The solutions were applied in triplicate to TLC plates to obtain densitograms, and the percent recovery, standard deviation, and % RSD were calculated.

2.4.5 Precision

The precision of the method was demonstrated by Intra-day and Inter-day variation studies. In the Intra-day studies, 3 replicates of 3 different concentrations were analyzed in a day and percentage RSD was calculated. For the inter day variation studies, 3 different concentrations were analyzed on 3 consecutive days and percentage RSD were calculated.

2.4.6 Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which wavelength, chamber saturation time and Time form application to development was altered and the effect on area was noted.

2.4.7 Assay

15 tablets with a fixed dose of DXM and BUP were prepared with accurate dosing of 45mg and 105mg per tablet, respectively in laboratory. The tablets were prepared using the wet granulation method with the API and the excipients i.e. HPMC, Avicel and PVP K-30 were carefully weighed. The wet mass was then extruded through a sieve (16 mesh) to obtain granules. The granules were dried and blended with Magnesium Stearate and Aerosil before being punched into tablets. The tablets were collected and stored for further evaluation. Sample solution was applied and area was recorded for each drug. Procedure was repeated for six times. Concentration and % purity was determined.

III. RESULTS AND DISCUSSION

The methodology section outline the plan and method that how the study is conducted. This includes Universe of the study, sample of the study, Data and Sources of Data, study's variables and analytical framework. The details are as follows.

3.1Optimization of chromatographic conditions:

After reviewing the available literature and considering the solubility of DXM and BUP in different solvents, various combinations of solvents were prepared to assess their ability to separate the two drugs effectively. The combination of Chloroform: Methanol in the ratio of 9:1v/v was found to provide well-defined and separated peaks and was selected as the mobile phase for the separation process. For further analysis, the isobestic wavelength of 215 nm was chosen as the detection wavelength.

Under the optimized chromatographic conditions 200 ng/band of DXM and 400 ng/band of BUP was applied on TLC plate and the retention factor of repeated applications was found to be0.28 ± 0.14for Dextromethorphan HBr and 0.75 ± 0.11 for Bupropion HCl. Chromatogram of Methanol blank, Dextromethorphan HBr, Bupropion HCl and Mixture are shown in Fig. 2 and its System suitability parameters were shown in Table 1.The resultant optimized chromatographic conditions are summarized in the Table 2.

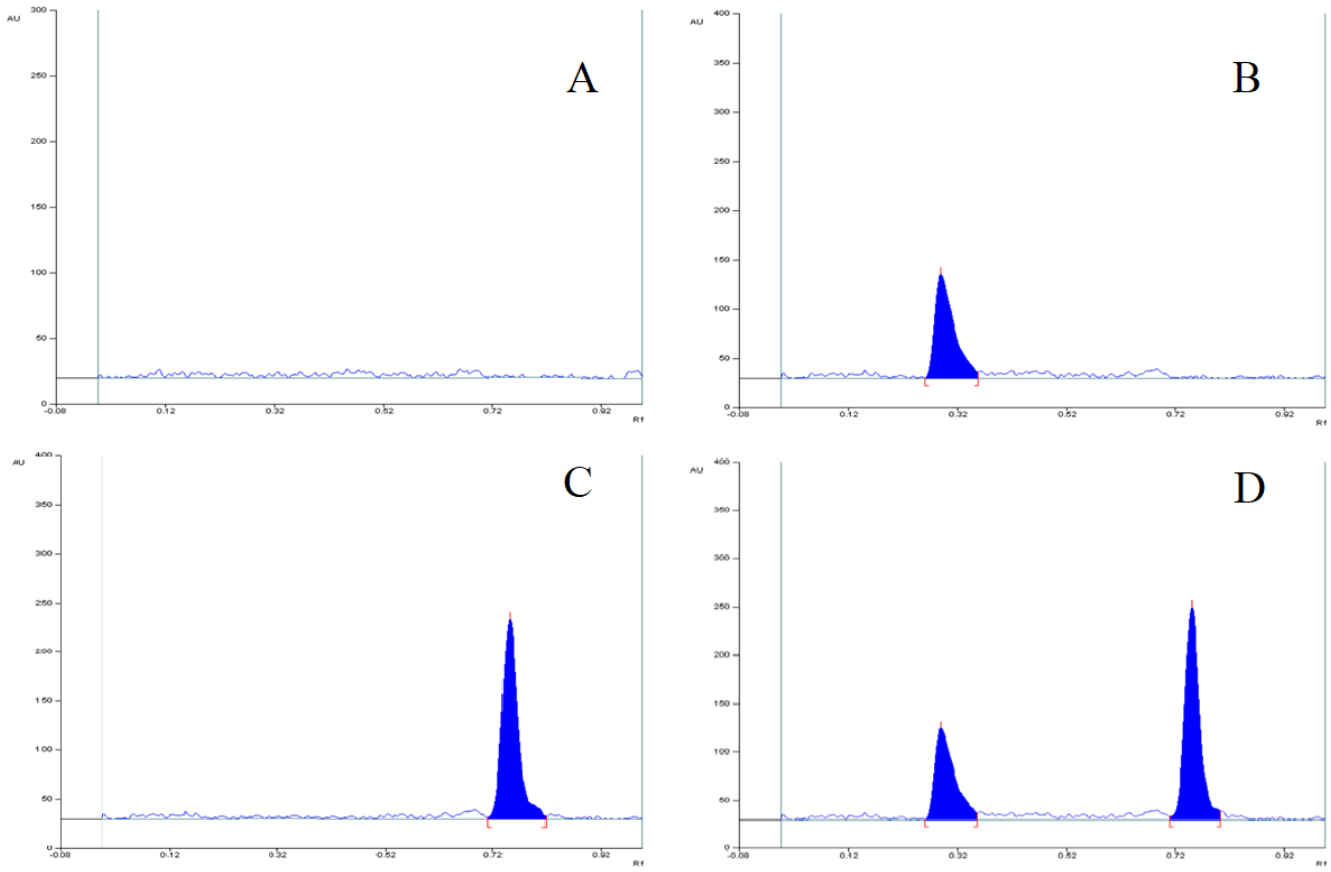

Figure 2- Chromatograms of Blank (A), Dextromethorphan HBr (B), Bupropion HCl(C) and Mixture (D).

Table 1- System suitability parameters

Name	**Rf Mean ± % RSD**	**Concentration (µg/ml)**	**Area**	**Asymmetry**
Dextromethorphan HBr	0.28 ± 0.14	200	1626.4	1.19
Bupropion HCl	0.75 ± 0.11	400	2866.9	1.06

Table 2- Chromatographic parameters

Sr. No.	**Parameter**	**Conditions used for Analysis**
1	Stationary phase	TLC aluminum plate precoated with silica gel 60 F_{254}
2.	Mobile phase	Chloroform: Methanol (9: 1 v/v)
3.	Detection Wavelength	215 nm
4.	Saturation time	15 mins

3.2Method Validation

3.2.1 Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 0.997, indicating the non interference of any other peak of degradation product or impurity.

3.2.2Linearity

The linearity was found in the range 50-300 ng/band for Dextromethorphan HBr with R^2 value of 0.9984 and in the range of 100-600 ng/band for Bupropion HCl with R^2 value of 0.9986.The results obtained are shown in Table 3for Dextromethorphan HBr and in Table 4for Bupropion HCl. The Calibration curve for Dextromethorphan HBr and Bupropion HCl are shown in Fig. 3 and Fig. 4 respectively.

Table 3- Linearity study of Dextromethorphan HBr

Replicates	Concentrations of Dextromethorphan HBr (ng/ band)					
	50	100	150	200	250	300
	Peak Area					
1	871.6	1626.4	2364.5	3161.4	4138.7	4759.2
2	860.5	1616.4	2387.9	3163.6	4096.7	4786.4
3	845.1	1592.2	2390.1	3121.7	4064.1	4718.2
4	856.2	1628.4	2377.7	3143.4	4093.6	4712.6
5	884.1	1605.1	2342.1	3150.8	4106.1	4767.1
6	852.9	1629.1	2411.1	3185.3	4095.1	4695.3
Mean	861.7	1616.3	2378.9	3154.4	4099.1	4739.8
Std.dev.	14.032	14.957	23.691	21.409	24.059	36.000
%RSD	1.628	0.925	0.996	0.679	0.587	0.760

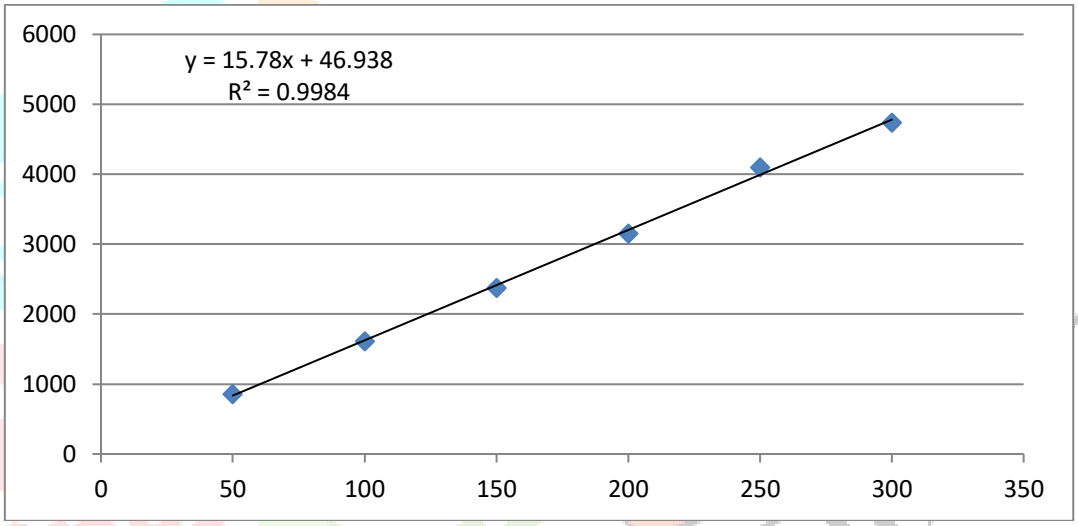

Figure 3- Calibration Curve for Dextromethorphan HBr

Table 4- Linearity study of Bupropion HCl

Replicates	Concentrations of Bupropion HCl (ng/band)					
	100	200	300	400	500	600
	Peak Area					
1	1742.8	2866.9	4102.6	5076.7	6125.1	7066.7
2	1735.3	2858.6	4039.5	5063.5	6131.3	7059.7
3	1729.4	2816.6	4031.2	5071.8	6037.7	7061.9
4	1765.4	2780.6	4050.1	5035.1	6116.5	7123.9
5	1760.5	2811.8	4039.3	4973.8	6149.2	7092.7
6	1738.4	2797.9	4136.2	4991.2	6118.8	7064.8
Mean	1745.3	2822.1	4066.5	5035.4	6113.1	7078.3
Std.dev.	14.434	34.010	42.767	43.746	38.750	25.371
%RSD	0.827	1.205	1.052	0.869	0.634	0.358

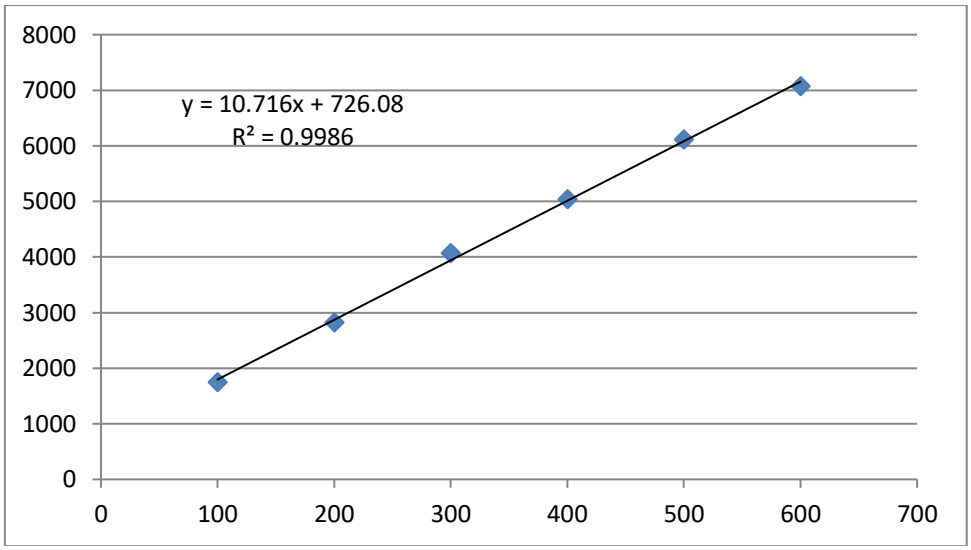

Figure 4 - Calibration Curve of Bupropion HCl

3.2.3LOD and LOQ

The limit of Detection of DXM and BUP were found to be 3.213ng/band and 6.432ng/band respectively. The Limit of Quantification of DXM and BUP was found to be9.736ng/ band and 19.490ng/band respectively.

3.2.4 Accuracy

For accuracy, the mean percentage recovery of DXM and BUP was determined at three levels: 50%, 100%, and 150%. The mean percentage recoveries of DXM were found to be 100.069 ± 0.671, 99.882 ± 1.098, and 100.168 ± 0.947, and for BUP the mean percentage recoveries were found to be 99.981 ± 0.765, 101.056 ± 0.236, and 100.707 ± 0.440, respectively. Results obtained for accuracy is shown in Table 5 and Table 6.

Table 5- Recovery studies of Dextromethorphan HBr

Level	Conc. (ng/band)		Area	Conc. (ng/band)	% Recovery	Mean % Recovery ± % RSD
	Sample	Std.				
50 %	200	100	2415.3	150.086	100.058	100.069 ± 0.671
			2399.8	149.104	99.403	
			2431.6	151.119	100.746	
100 %	200	200	3224.3	201.354	100.677	99.882 ± 1.098
			3213.6	200.676	100.338	
			3159.7	197.260	98.630	
150 %	200	300	3977.1	249.060	99.624	100.168 ± 0.947
			3976.8	249.041	99.616	
			4041.8	253.160	101.264	

Table 6- Recovery studies of Bupropion HCl

Level	Conc. (ng/band)		Area	Conc. (ng/band)	% Recovery	Mean % Recovery ± SD
	Sample	Std.				
50 %	233.3	100	4277.3	331.394	99.428	99.981 ± 0.765
			4328.2	336.144	100.853	
			4285.6	332.169	99.661	
100 %	233.3	200	5360.1	432.439	99.801	101.056 ± 0.236
			5381.8	434.464	100.269	
			5373.8	433.718	100.096	
150 %	233.3	300	6453.7	534.492	100.224	100.707 ± 0.440
			6503.5	539.140	101.095	
			6486.8	537.581	100.803	

3.2.5 Precision

The results obtained for Intraday and Inter day precision study are shown in Table 7 and Table 8 for DXM and BUP respectively. The results of precision study were reported in % RSD and were found to be less than 2.

Table 7- Precision study Dextromethorphan HBr

Concentration (ng/band)	Intra-day Precision Studies			Inter-day precision study		
	Area	% Recovery	Avg % Recovery ± % RSD	Area	% Recovery	Avg % Recovery ± % RSD
100	1630.2	100.333	100.361± 0.780	1641.4	101.043	100.443± 0.525
	1618.5	99.592		1625.8	100.055	
	1643.2	101.157		1628.6	100.232	
200	3230.2	100.864	99.885± 0.982	3207.3	100.138	100.43± 0.411
	3199.4	99.888		3231.4	100.902	
	3168.3	98.902		3210.8	100.249	
300	4740.4	99.144	99.104± 0.513	4787.2	100.132	99.96± 0.534
	4761.6	99.592		4799.2	100.386	
	4713.6	98.578		4750.7	99.361	

Table 8- Precision study of Bupropion HCl

Concentration (ng/band)	Intra-day Precision Studies			Inter-day precision study		
	Area	% Recovery	Avg % Recovery ± % RSD	Area	% Recovery	Avg % Recovery ± % RSD
200	2866.1	99.852	100.272 ±0.52	2864.6	99.782	100.695 ±0.858
	2871.6	100.108		2901.4	101.499	
	2887.6	100.855		2886.5	100.803	
400	5033.7	100.495	100.237 ±0.235	4998.5	99.674	100.035 ±0.323
	5013.9	100.033		5018.4	100.138	
	5020.3	100.182		5025.1	100.294	
600	7160.5	100.075	100.001 ±0.246	7140.2	99.759	100.354 ±0.683
	7138.1	99.727		7168.5	100.199	
	7168.7	100.203		7226.6	101.103	

3.2.6 Robustness

Method robustness was assessed by varying wavelength, chamber saturation time, and time of application to development, and recording the corresponding effect on area. Results are shown in Table 9.

Table 9- Robustness Study

Drug	% RSD Found for Robustness Study (Peak Area)								
	Wavelength			Chamber Saturation Time (Min)			Time form application to development (min)		
	210	215	216	14	15	16	0	30	60
DXM	0.703	0.535	0.842	0.921	0.549	0.674	1.142	0.926	0.756
BUP	0.707	0.626	0.489	0.613	0.492	1.396	0.853	1.336	1.048

3.2.7 Assay

Assay of DXM and BUP were performed and its % purity and % RSD were determined. Results are presented in Table 10 with a representative chromatograph in Fig. 5.

Table 10- Assay results of tablet formulation

Sr. no.	Dextromethorphan HBr			Bupropion HCl		
	Peak area	Amount Recovered (ng/band)	% Recovery	Peak area	Amount Recovered (ng/band)	% Recovery
1	1618.7	99.605	99.605	3228.5	233.522	100.095
2	1628.3	100.213	100.213	3258.6	236.331	101.299
3	1631.2	100.397	100.397	3234.3	234.063	100.327
4	1618.4	99.586	99.586	3197.1	230.592	98.839
5	1624.7	99.985	99.985	3208.9	231.693	99.311
6	1629.4	100.283	100.283	3224.5	233.149	99.935
Mean	1625.12	100.011	100.011	3225.32	233.225	99.968
SD	5.51	0.349	0.349	21.28	1.986	0.851
% RSD	0.339	0.349	0.349	0.660	0.852	0.852

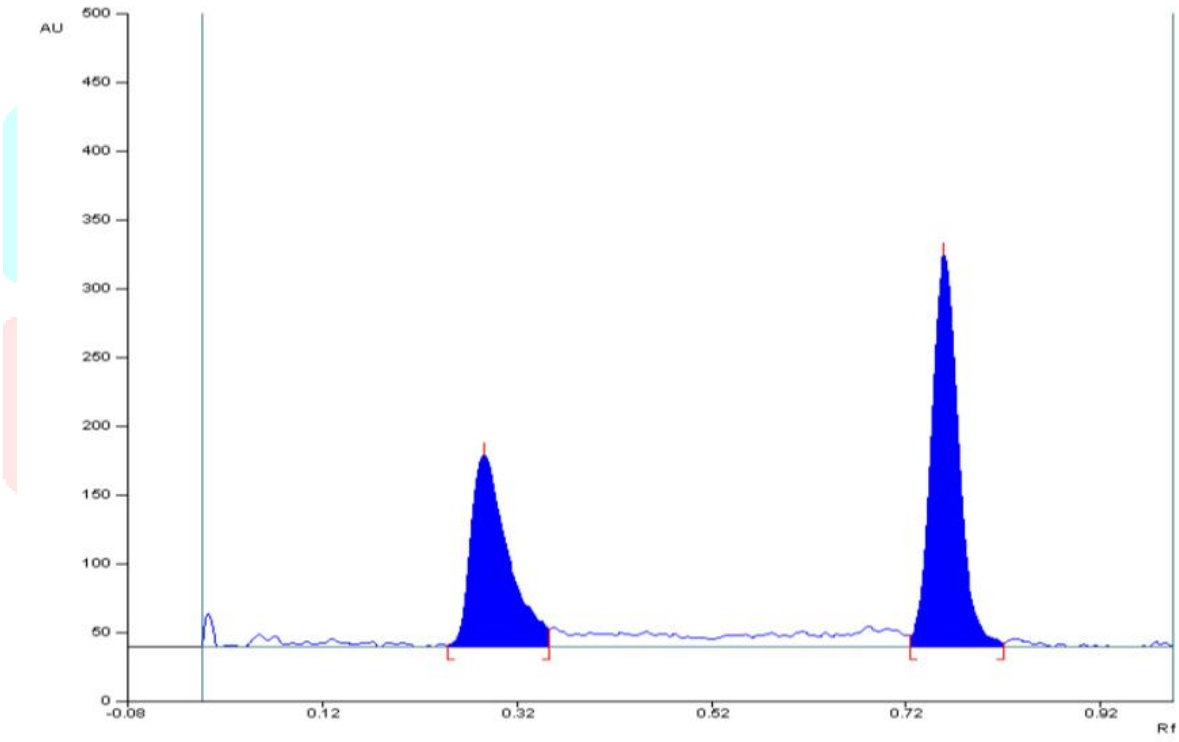

Figure 5- Densitogram of Test Solution Dextromethorphan HBr and Bupropion HCl

A summary of the validation parameters results is provided in the Table 11.

Table 11- Summary of Results of Validation Parameters

Sr. No.	Validation Parameter	Results	
		Dextromethorphan HBr	Bupropion HCl
1	Linearity	y = 15.78x +46.938 R^2 = 0.9954	y = 10.716 x + 726.08 R^2 = 0.9986
2	Range	50-300 ng/band	100 - 600 ng/band
3	Assay (Mean ± % RSD)	100.011± 0.349	99.968± 0.852
4	Precision	%RSD	%RSD
	A) Intraday precision	0.513 – 0.982 %	0.235 – 0.520 %
	B) Interday precision	0.411 – 0.534%	0.323 – 0.858 %
5	Accuracy	% Recovery	% Recovery
	50%	100.069 ± 0.671	99.981 ± 0.765
	100%	99.882 ± 1.098	101.056 ± 0.236
	150%	100.168 ± 0.947	100.707 ± 0.440
6	LOD	3.213 ng/ band	6.432 ng/band
7	LOQ	9.736 ng/band	19.490 ng/band
8	Specificity	Specific	Specific
9	Robustness	Robust	Robust

IV. CONCLUSION

A precise, accurate and reproducible HPTLC (High Performance Thin Layer Chromatography) technique has been developed and verified for simultaneous determination of Dextromethorphan HBr and Bupropion HCl in both bulk drug and tablet dosage form. The % Recovery studies demonstrate that the method is unaffected by the use of excipients. Consequently, this approach can be employed for quantitative analysis of Dextromethorphan hydrobromide and Bupropion HBr in pharmaceutical dosage forms. The technique was developed using low-cost, readily available solvents, making it an economically viable option for drug analysis.

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