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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF DEXTROMETHORPHAN HBR AND TRIPROLIDINE

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ABSTRACT

High-performance liquid chromatographic (HPLC) and UV spectrophotometric methods were developed and validated for the quantitative determination of Dextromethorphan HBr and Triprolidine. A simple, rapid, sensitive, accurate, precise isocratic elution mode analytical method was developed and validated for the simultaneous estimation of Dextromethorphan HBr and Triprolidine. Hypersil BDS C18 (250 mm × 4.6 mm, 5 µm) was used as a stationary phase. The mobile phase used was [MeOH: Phosphate Buffer (35:65)] pH 4 adjusted using 1% orthophosphoric acid at the flow rate of 1 mL/min, Injection volume was 20 µL and the UV detector 2000 was used at 240 nm with methanol as diluent. The linearity of the Proposed method was investigated in the range of 80-120µg/ml (r2 = 0.999) for Dextromethorphan HBr and 5-15µg/ml (R^2 = 0.999) for Triprolidine. The % Accuracy was found to be in the range of 100.06-101.75% and 99.99-101.95% for Dextromethorphan HBr and Triprolidine respectively. LOD was found to be 2.788µg/ml and 0.330µg/ml for Dextromethorphan HBr and Triprolidine respectively. LOQ was found to be 8.448µg/ml and 1.001µg/ml for Dextromethorphan HBr and Triprolidine respectively. The results revealed that the developed method is suitable for the routine analysis for determination of Dextromethorphan HBr and Triprolidine in Syrup dosage form.

KEYWORDS: Dextromethorphan HBr, Triprolidine RP-HPLC, ICH Q2 (R1).

INTRODUCTION

Dextromethorphan Hydrobromide represents the hydrobromide salt variation of dextromethorphan. Dextromethorphan is a synthetic compound, a methylated dextrorotary counterpart to levorphanol Pub Chem (2005). Levorphanol shares a connection with codeine and is a non-opioid derivative of morphine. Dextromethorphan serves as an antitussive agent and lacks analgesic or addictive qualities. It has the ability to cross the blood-brain barrier and activate sigma opioid receptors within the cough control centre of the central nervous system, effectively suppressing the cough reflex(Prommer, 2014).

Dextromethorphan functions as a drug resembling opioid by binding to and acting as an antagonist to the NMDA glutamatergic receptor. Furthermore, it acts as an agonist on the opioid sigma 1 and sigma 2 receptors, functions as an antagonist for the alpha3/beta4 nicotinic receptor, and targets the serotonin reuptake pump.

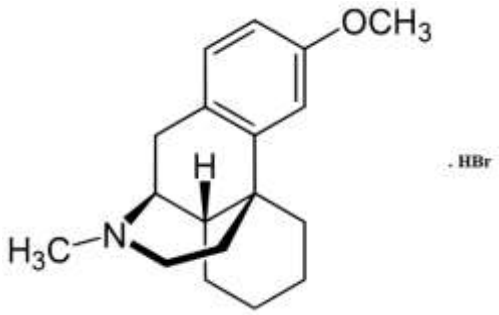
Triprolidine, on the other hand, attaches to the histamine H1 receptor, preventing the action of naturally occurring histamine(Paton & Webster, 1985). This blockade leads to temporary relief from the negative symptoms caused by histamine(Mann, Crowe, & Tietze, 1989).

Literature survey revealed the interest in the determination of Dextromethorphan HBr and Triprolidine in pharmaceutical formulations either alone or in combination with other act with other active principles (Pandey, Alam, & Mishra, 2021). The simultaneous analysis of the three components has been performed by a few methods only, including UV spectrophotometry and HPLC (Siddiqui et al., 2020).

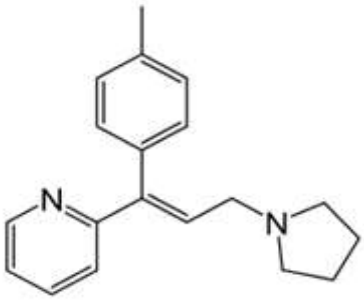
Various methods are reported for the analysis of individual drug and in combination with other drugs but no UV and HPLC method reported for these drugs in combined dosage form. Therefore, it was thought worthwhile to develop UV and RP-HPLC Method for the simultaneous estimation of Dextromethorphan HBr and Triprolidine in their Syrup dosage form.

The objective of the present work was to develop and validate a high-performance chromatographic methodology for the simultaneous determination of Dextromethorphan HBr and Triprolidine in their combined syrup formulations. Experimental design techniques as a rational, cost-effective and convenient tool to speed up the process were employed for development of the proposed method.

The novelty of the proposed method stems from its ability within a reasonable analysis time, to separate such a complex mixture without interference from the co-formulated additives and without prior sample pre-treatment. The cost effectiveness of the method in term of using inexpensive reagents and chemicals is another benefit added.



Structure of Dextromethorphan HBr



Structure of Triprolidine

MATERIALS AND METHODS

Reagent and chemicals

Dextromethorphan HBr and Triprolidine (Remus remedies) were received as gift sample. Marketed formulation (Acetified-M syrup 5 mL, containing 10 mg of Dextromethorphan HBr and 1.25mg of Triprolidine was procured from local market. HPLC grade acetonitrile and purified grade potassium di-hydrogen phosphate were purchased from Finar. All other reagents employed were of high purity analytical grade. All weighing was done on a calibrated analytical balance. Calibrated glass wares were used throughout the work. HPLC grade water were used in the UV method and RP-HPLC method respectively(Katakam, Ettaboina, & Marisetti, 2021).

METHOD DEVELOPMENT

Instrumentation

The UV method was performed on Systronic spectrophotometer (Model: UV-2206) with 1 nm spectral bandwidth. The absorption spectra of reference and test solution were carried out over the range of 200–400 nm.

The HPLC method was performed on a system equipped with a syringe (hamilton), UV-2000 detector and HPLC pump. The column used was hypersil BDS C18 column (2.5 * 0.46 cm, 5 µm). The mobile phase used was methanol: potassium dihydrogen phosphate buffer (35:65 v/v) and the final pH adjusted was 4 by using orthophosphoric acid. Injection volume was 20

µL. The flow rate was set to 1 mL/min and detection of both drugs was carried out at 240 nm by UV detector.

Standard stock solution preparation

Stock solution was prepared by dissolving Dextromethorphan HBr and Triprolidine 80mg and 10 mg respectively. that were weighed accurately and separately transferred into 100 ml volumetric flasks. Both drugs were dissolved in 25 ml of methanol to prepare standard stock solutions. After the immediate dissolution, the volume was made up to the mark with methanol. These standard stock solutions were observed to contain 800 µg/mL and 100 µg/mL respectively. Appropriate volume from this solution was further diluted to get appropriate concentration levels according to the require.

Test solution preparation

Test solutions were prepared by accurately pipetting syrup containing Dextromethorphan HBr (80 mg) and Triprolidine (10 mg) into a 100 mL volumetric flask. The ingredients were dissolved in methanol and sonicated for 10 minutes. After that, the solutions were diluted to a final volume of 100 mL, resulting in concentrations of 800 µg/mL for Dextromethorphan HBr and 100 µg/mL for Triprolidine.

Preparation of buffer

0.05 M potassium dihydrogen phosphate buffer of pH 4 was used for method development. Buffer was prepared by dissolving 6.8 g of potassium dihydrogen phosphate by diluting with HPLC grade water to 1000 ml. The pH was adjusted by ortho-phosphoric acid using pH meter (systronic). The prepared buffer was passed through 0.45 µL membrane filter and the same was used for mobile phase preparation.

Preparation of mobile phase

Mobile phase was prepared by mixing 0.05 M potassium dihydrogen phosphate buffer (pH 4) and Methanol in 35:65 (v/v) proportions. Mixture was shaken vigorously and sonicated for 30 min prior to use.

Determination of wavelength of maximum absorbance

Wavelength of maximum absorption was determined by scanning 80µg/mL and 10 µg/mL solution of Dextromethorphan HBr and Triprolidine using UV–visible double beam spectrophotometer from 200 to 400 nm using methanol as blank. Overlay spectra of both

drugs shown in figure 1.

Preparation of calibration curve

The calibration curve was prepared by scanning test samples ranging from 40–120 and 5-15 µg/mL at 240 nm and 260 nm for Dextromethorphan HBr and Triprolidine respectively. The calibration curve was tested by validating it with inter-day and intra-day measurements. Linearity, intra-day and inter-day measurements, accuracy and precision were determined for both. Mean of n = 5 determinations was plotted as the standard curve (figure 2 and 3).

METHOD VALIDATION

The methods were validated according to International Conference on Harmonization Q2(R1)(ICH, 2015) guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for each analyte.

Specificity / Selectivity

The ability of an analytical method to produce a response for the analyte in the presence of extraneous interference is referred to as selectivity. The method's selectivity was determined by comparing the chromatograms obtained for diluents, mobile phase, Dextromethorphan HBr and Triprolidine, sample solutions. The parameters retention time and tailing factor were obtained to indicate that the methodology used was unique (figure 4 to 9) and table 2.

Linearity

The linearity of the Dextromethorphan HBr and Triprolidine UV and HPLC technique was tested at ten different concentration levels:40,60,80,100,120 µg/mL and 5,7.5,10,12.5,15 µg/mL respectively. The method's linearity was confirmed using a least squares linear regression analysis of absorption and peak area vs. concentration data. The coefficient of determination (R^2) values better than 0.999 ($R^2 \geq 0.999$) were used as a criterion for linearity. (Shown in table 3).

Precision

Precision was determined by considering intraday (repeatability obtained by analyzing a standard solution on the same day) shown in table 4 and 5 and interday variations shown in table 6 (repeatability carried out by analyzing a standard solution on three consecutive days). The precision study was conducted by injecting 3 concentration, 3 replicates of standard solution at 40, 80,120 and 5,10,15µg/mL of Dextromethorphan HBr and Triprolidine

respectively on the same day and three days in a row.

Accuracy

The method's accuracy was assessed by performing recovery tests at three concentrations that were 80 %, 100 %, and 120 % of the target level of Dextromethorphan HBr (40 µg/mL) and Triprolidine (5 µg/mL) using the standard addition technique (shown in table 7). At each level, the trials were repeated in triplicate and % recoveries were determined. For each concentration, the % recovery and percentage relative standard deviation (%RSD), were computed.

Limit of Detection and Limit of Quantification

Several approaches for determining the detection (LOD) and quantification (LOQ) limits are described in the ICH guidelines. In this study, the LOD and the LOQ were based on the response (s) standard deviation, and the slope of the regression line (m) were calculated following equations.

LOD = 3:3*s/m

LOQ = 10*s/m

Robustness

A robustness study was conducted to assess the impacts of minor but consistent changes in chromatographic settings. Variable mobile phase flow rates (0.8 and 1.2 mL/min), ratio of mobile phase methanol: potassium dihydrogen phosphate buffer (37:63 and 33:67 v/v) and pH of the mobile phase (4.2 and 3.8). The system suitability parameters were examined after each change and the results were compared to those in the original chromatographic conditions. (Shown in table 8).

Analysis of Commercial Formulation

The test solution was created by properly measuring 1 mL of the previously produced test solution into a volumetric flask of 10 mL and filling the mark with methanol to reach a theoretical concentration of 80µg/mL and 10µg/mL of Dextromethorphan hydrobromide and Triprolidine respectively.

RESULT AND DISCUSSION

RP-HPLC and UV-method validation

RP-HPLC and UV-Spectrophotometric methods were developed for Dextromethorphan

hydro bromide and Triprolidine. Which can be conveniently employed for routine analysis in pharmaceutical dosage forms and will eliminate unnecessary tedious sample preparations. The chromatographic conditions were optimized in order to provide a good performance of the assay.

Fig.1 shows overlay spectra of both drugs of the UV-Spectrophotometric method. Which shows a peak at the wavelength of 240nm for Triprolidine and 260nm for Dextromethorphan HBr, respectively. Fig.2 depicts calibration curve for Dextromethorphan HBr at 240 nm in methanol. The points in the figure correspond to values of absorbance at five different concentrations of Dextromethorphan HBr. A regression analysis was performed which give a linear fit with r2 ≥ 0.999 for each of the analytes over their calibration ranges. The linear fit of the curve validates the given method according to ICH Q2 R1 guideline. Similarly, the calibration curves for Triprolidine are shown in the Fig.3 again it can be seen from the figure that a linear fit is obtained at different concentration. Thus, we can say that the given method follows ICH Q2 R1 guideline. Fig.4 shows Chromatogram of Dextromethorphan HBr 80 ppm and Triprolidine 10 ppm in Methanol: Phosphate Buffer (pH=4.0) (35:65%v/v) Flow rate: 1ml/min. it can be seen from the figure that the retention times (R_t) of Dextromethorphan hydro bromide and Triprolidine were 4.057 min and 7.407 min respectively. These retention times are calculated from individual chromatogram of Dextromethorphan HBr and Triprolidine. These individual chromatograms have been shown in Fig.5 and Fig.6 for Dextromethorphan HBr and Triprolidine. The chromatogram of Mobile Phase [Buffer (pH4.0): Methanol (65:35)] is shown in Fig.7. A flat line is observed without any peaks, this shows that the mobile phase has no peak interference. The developed HPLC method was accurate, precise, reproducible and very sensitive. Fig.2 and Fig 3. shows that the concentration of dextromethorphan HBr and Triprolidine lies within the range respectively and the regression coefficient of the correlation equation curve was greater than 0.999, and the method was validated by using binary mixture of both drugs with less than 2% RSD (Table 1). All the method validation parameters are well within the limits as specified in the ICH Q2 R1 guidelines as shown in Table 1. The intra- and inter-day precision (%R.S.D.) at different concentration levels was found to be less than 2% (Table 5& 6). Table 7 lists the percent recovery (content uniformity) of both drugs in the commercial formulations by HPLC and UV methods. Moreover the %R.S.D. (less variation) shows good precision of both developed methods. The calculated LOQ and LOD concentrations confirmed that the methods were sufficiently sensitive. The methods were specific as none of the excipients

interfered with the analytes of interest (Table 5). Hence, the methods were suitably employed for assaying both the drugs in commercial marketed formulation.

Table 1: Summary of validation parameters using UV Spectrophotometer.

Parameters	Dextromethorphan HBr		Triprolidine	
Wavelength (nm)	260nm	240nm	240nm	260nm
Regression equation (y = mx +c)	y= 0.0053x + 0.0803	y=0.052x+0.082	y=0.0256x + 0.0226	y=0.05x +0.0254
Correlation Coefficient (R^2)	0.998	0.995	0.996	0.0994
Intraday Precision (%RSD, n=3)	0.59-1.03	1.17-1.84	0.77-1.73	1.10-1.51
Interday Precision (% RSD, n=3)	0.64-1.55	0.57-1.28	0.59-1.13	1.53-1.88
Repeatability (% RSD, n=6)	0.60	1.20	0.76	1.45
LOD (µg/ml)	3.603	5.917	1.400	1.648
LOQ (µg/ml)	10.919	17.930	4.243	4.993
Accuracy (% RSD, n=3)	99.12-100.18		99.84-100.67	
Assay (%)	99.82		99.60	

Table 2: System suitability using RP-HPLC.

Sr. No.	System suitability parameter	Dextromethorphan HBr	Triprolidine
1.	Retention time	4.057	7.407
2.	Theoretical Plates	4674	4496
3.	Tailing Factors	1.303	1.614
4.	Resolution	9.827	
5.	Flow rate	1ml/min	

Table 3: Linearity data for Dextromethorphan HBr and Triprolidine.

Dextromethorphan HBr			Triprolidine		
Conc. (µg/ml)	**Area ± SD (n=3)**	**%RSD**	**Conc. (µg/ml)**	**Area ± SD (n=3)**	**%RSD**
40	2171.289±21.583	0.994	5	682.424±1.938	0.284
60	3205.757±32.089	1.001	7.5	1015.305±3.391	0.334
80	4383.295±25.467	0.581	10	1388.885±6.680	0.481
100	5397.235±13.709	0.254	12.5	1710.713±15.088	0.882
120	6567.476±69.024	1.051	15	2081.916±23.817	1.144

Table 4: Repeatability.

Dextromethorphan HBr		Triprolidine	
Conc. (µg/ml)	**Area**	**Conc. (µg/ml)**	**Area**
80	4365.939	10	1383.330
	4374.543		1386.119
	4310.806		1388.887
	4392.087		1333.751
	4370.317		1384.713

	4378.928		1387.513
Mean ± S.D	4365.437±28.227	**Mean ± S.D**	1377.386 ±21.467
%RSD	0.647	**%RSD**	1.559

Table 5: Intraday precision.

Dextromethorphan HBr			Triprolidine		
Conc. (µg/ml)	**Area Mean ± S.D. (n=3)**	**% RSD**	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% RSD**
40	2154.009 ± 17.038	0.790	5	673.923 ± 9.624	1.428
80	4353.898 ± 45.293	0.581	10	1373.856 ± 15.635	1.138
120	6517.281± 20.104	0.308	15	2057.514 ± 29.888	1.453

Table 6: Interday precision.

Dextromethorphan HBr			Triprolidine		
Conc. (µg/ml)	**Area Mean ± S.D. (n=3)**	**% RSD**	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% RSD**
40	2152.553 ± 13.781	0.640	5	674.284 ± 7.078	1.049
80	4349.532± 21.076	0.484	10	1371.217± 16.212	1.182
120	6514.320± 23.334	0.358	15	2050.547 ± 32.817	1.600

Table 7: Recovery data for Dextromethorphan HBr.

Conc Level	**Dextromethorphan HBr (40µg/ml)**				**Triprolidine (5µg/ml)**			
	Amount Added (µg/ml)	**Amount recovered (µg/ml)**	**% Recovery**	**% Mean Recovery ± SD**	**Amount Added (µg/ml)**	**Amount recovered (µg/ml)**	**% Recovery**	**% Mean Recovery ± SD**
80%	32	31.598	98.745	99.889 ± 1.026	4	3.996	99.894	100.181 ± 1.065
	32	32.233	100.728		4	3.972	99.290	
	32	32.062	100.195		4	4.054	101.361	
100%	40	39.619	99.047	99.613 ± 0.549	5	5.009	100.182	100.822 ± 0.656
	40	40.058	100.144		5	5.075	101.493	
	40	39.860	99.649		5	5.040	100.792	
120%	48	48.003	100.006	99.631 ± 0.415	6	6.078	101.301	100.814 ± 0.497
	48	47.608	99.184		6	6.018	100.307	
	48	47.856	99.701		6	6.050	100.833	

Table 8: Robustness.

Parameter	**Level**	**Dextromethorphan HBr**	**%RSD**	**Triprolidine**	**%RSD**
Flow Rate	0.8 ml/min	4501.112	0.705	1434.725	1.834
		4545.746		1395.881	
		4563.092		1445.626	
	1.2 ml/min	4230.902	0.925	1348.702	0.611
		4282.374		1356.931	
		4308.608		1365.275	
pH	3.8	4439.114	0.939	1417.948	1.090
		4501.851		1401.004	
		4519.246		1431.849	

	4.2	4151.541	0.746	1318.788	1.037
		4194.555		1302.794	
		4212.298		1329.980	
Mobile Phase	MeOH: Phosphate Buffer (33:67)	4479.887	0.502	1377.816	1.895
		4488.280		1411.576	
		4445.926		1430.417	
	MeOH: Phosphate Buffer (37:63)	4218.439	1.020	1345.997	1.235
		4273.793		1330.659	
		4304.293		1363.905	

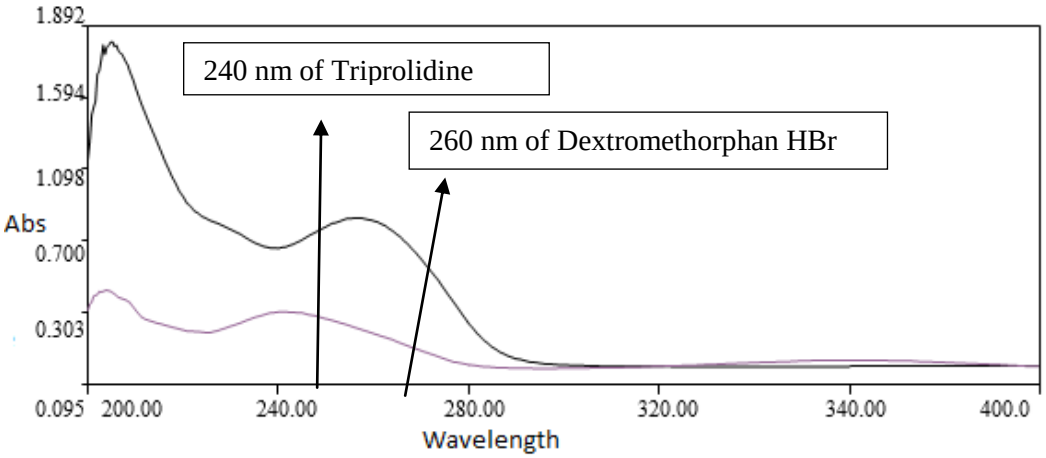


Figure 1: Overlay spectra of Dextromethorphan HBr and Triprolidine.

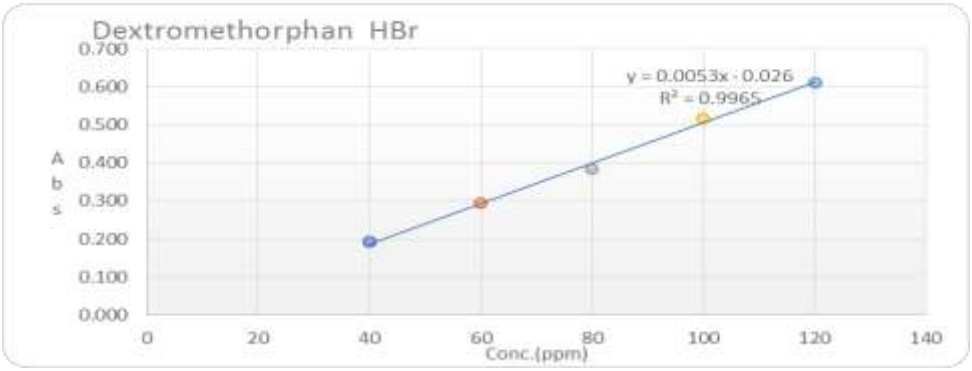


Figure 2: Calibration curve for Dextromethorphan HBr at 260 nm in methanol.

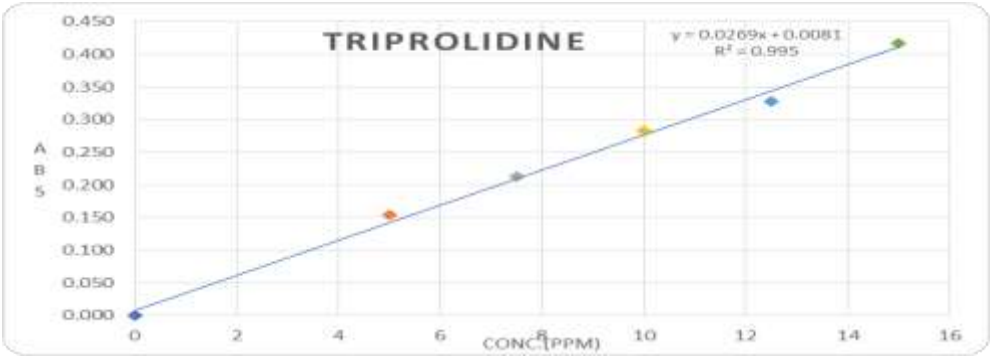


Figure 3: Calibration curve for Triprolidine at 240 nm in methanol.

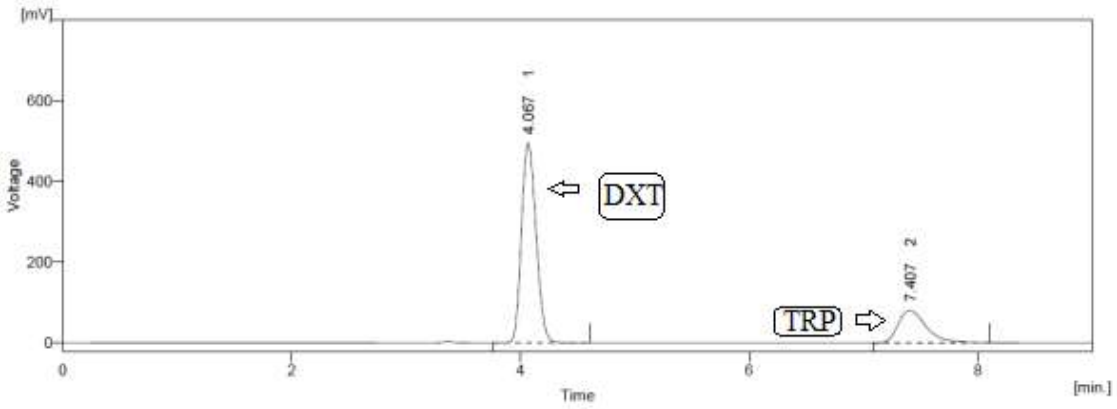


Figure 4: Chromatogram of Dextromethorphan HBr 80 ppm and Triprolidine 10 ppm in Methanol: Phosphate Buffer (pH=4.0) (35:65%v/v) Flow rate: 1ml/min.

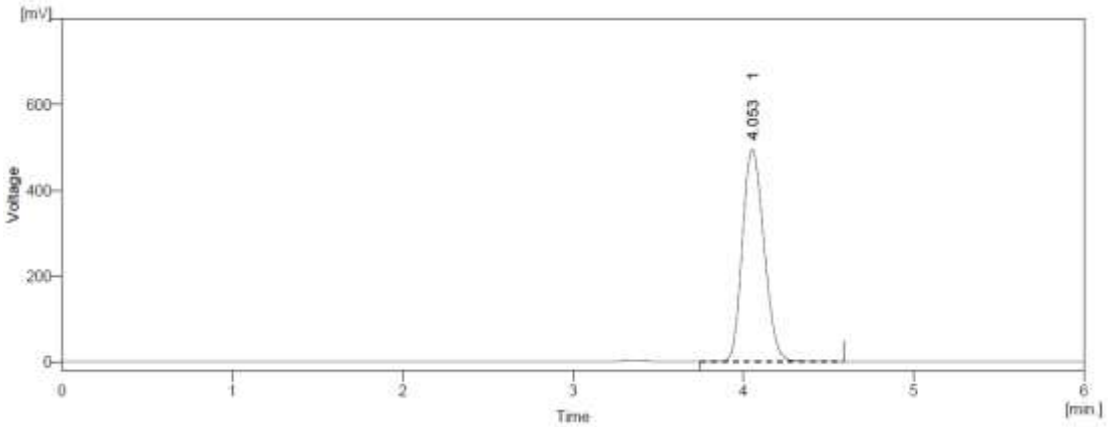


Figure 5: HPLC Chromatogram of Dextromethorphan HBr (80 µg/ml).

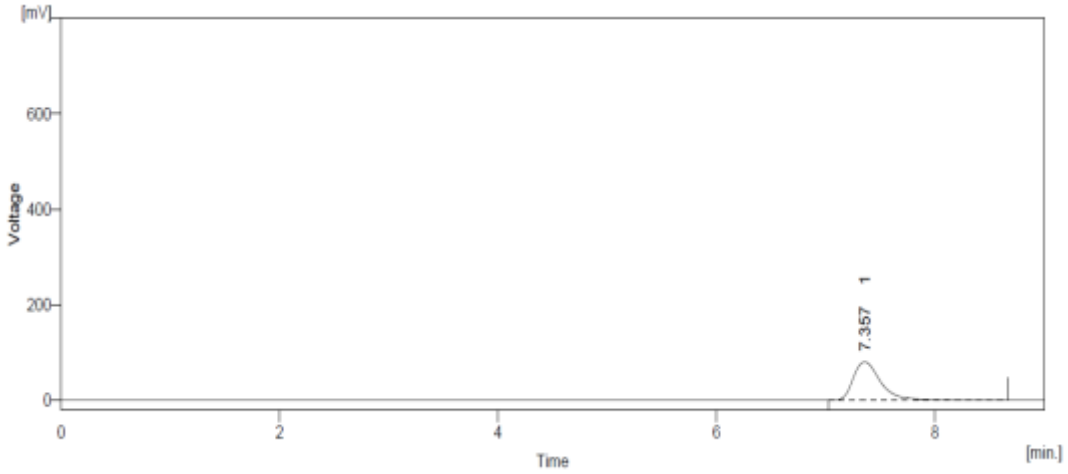


Figure 6: HPLC Chromatogram of Triprolidine (10µg/ml).

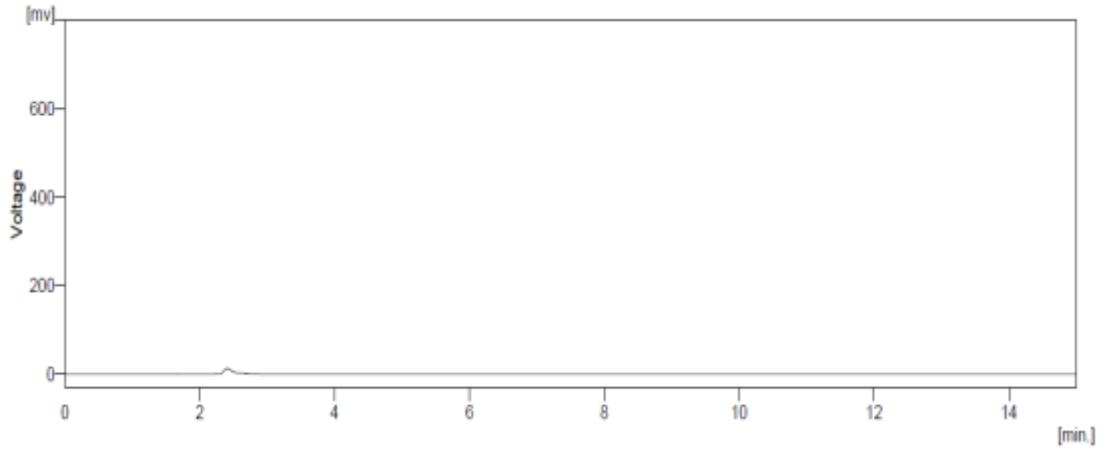


Figure 7: Chromatogram of Mobile Phase [Buffer (pH4.0): Methanol (65:35)].

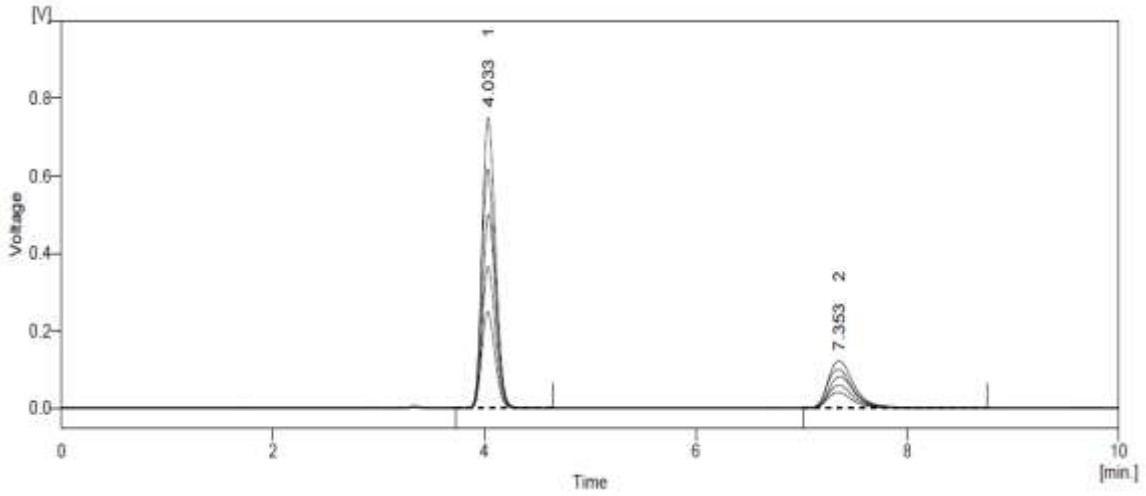


Figure 8: Overlay chromatogram.

CONCLUSION

From all results it was concluded that the developed RP-HPLC method is simple, sensitive, accurate, precise, and selective. Percentage recovery shows that the method is free from interference of excipients used in the formulation. The results obtained on the validation parameters have been met with ICH guidelines and requirements. The method was found to have suitable application in routine laboratory analysis.

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