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Research Article

Simultaneous estimation of phenylephrine HCl, chlorpheniramine maleate and dextromethorphan HBr in a pharmaceutical (syrup) formulations by RP-HPLC using PDA detector

S. Ashutosh Kumar *[1], Manidipa Debnath[1], D. Vimala

[1] Department of Pharmaceutical Analysis and Quality Assurance, A.K.R.G College of Pharmacy, Nallajerla, West Godavari, 534112, A.P India

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Abstract

The present study aimed to develop and validate the simultaneous estimation of phenylephrine HCl, chlorpheniramine maleate and dextromethorphan HBrin tablet dosage forms. A gradient reversed phase high-performance liquid chromatographic (RP-HPLC) method with ultraviolet detection at 220 nm has been developed for the simulataneous determination of phenylephrine HCl, chlorpheniramine maleate and dextromethorphan HBrin pharmaceutical dosage forms (Syrup). Good chromatographic separation was achieved by using a stainless steel analytical column, the Hypersil BDS C_8 column(4.6 X 250 mm; 5 µm). The system was operated at 25 ± 2°C using a mobile phase consisted of HPLC grade water (composed of TEA and 1-octane sulfonic acid sodium salt) (pH adjusted to 3.2 using orthophosphoric acid) and acetonitrile, mixed at gradient mode, manitained flow rate at 1.0 mL/minute. The slope, intercept, and correlation coefficient were found to be y = 34306x - 11042 (r^2= 0.999) for phenylephrine HCl, y = 35874x - 13101 (r^2= 0.999) for chlorpheniramine maleate anddextromethorphan HBry = 25516x - 26579 (r^2 = 0.999), respectively. The proposed method was validated for its specificity, linearity, accuracy, and precision. The method was found to be suitable for the quality control of phenylephrine HCl, chlorpheniramine maleate and dextromethorphan HBr simultaneously in a bulk drug samples as well as in a formulations.

Keywords: Phenylephrine HCl, chlorpheniramine maleate, dextromethorphan HBr, gradient separation, RP-HPLC

INTRODUCTION:

PhenylephrineHCl (Figure 1) is chemically, (R) – 3[1-m-hydroxy-2-(methyl amino) methyl] benzyl alcohol hydrochloride used as decongestant. Oral phenylephrine is extensively metabolized by MAO enzyme in the gastrointestinal tract and liver. So compared to orally taken pseudoephedrine it has a reduced and variable bioavailability of only up to 38%. It is a direct selective alpha adrenergic receptor agonist; it does not cause release of endogenous noradrenalin, as pseudoephedrine does. It has low side effects like CNS stimulation, irritability, insomnia, anxiety and restlessness[drugbank.ca/drugs).

Chlorpheniramine maleate (Figure 2) is chemically, (RS)-3-(4-chlorophenyl)-3-(pyrid-2-yl) propyldimethylamine hydrogenmaleate. It is an antihistamine drug that is widely used inpharmaceutical preparations for symptomatic relief of common coldand allergic diseases. It inhibits the effects of histamine on capillarypermeability and bronchial smooth muscles. It is a firstgeneration alkylamine antihistamine used in the prevention of thesymptoms of allergic conditions such as rhinitis and urticaria[rxlist.com).

***Corresponding Author:** *S. Ashutosh Kumar*, Department of Pharmaceutical Analysis and Quality Assurance, A.K.R.G College of Pharmacy, Nallajerla, West Godavari, 534112, A.P, India.
***E-mail**: ashu_mpharm2007@gmail.com*
Mobile No: +919640060617

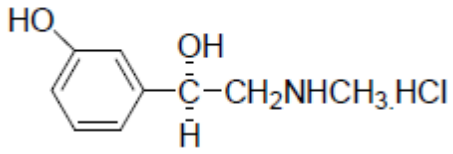

Figure 1: Chemical structure of phenylephrine

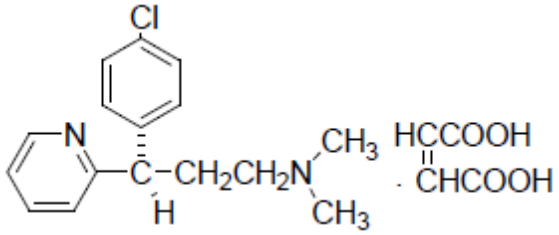

Figure 2: Chemical structure of chlorpheniramine Maleate

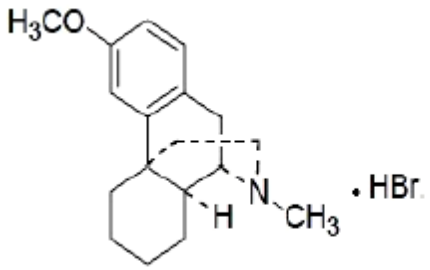

Figure 3: Chemical structure of dextromethorphan hydrobromide

Dextromethorphan hydrobromide (Figure 3) is chemically, [3-Methoxy-17-methylmorphinan hydro bromide monohydrate]1-3, is an opioid like drug acts centrally .It elevates the threshold for coughing , without inhibiting ciliary activity.Dextromethorphan hydrobromide rapidly absorbed from the gastrointestinal tract and converted into lower active metabolite (dextrorphan). The duration of action after oral administration is approximately three to eight hours for dextromethorphan hydrobromide[drugs.com).

Numerous methods have been reported for estimation of these drugs alone as well as in combination with other drugs in pharmaceutical dosage forms like capillary electrophoresis [Maria *et al.,* 2002), UV [Ivana, 2008., Sastry*et al.,* 1990., Aman*et al.,* 2002., Arun*et al.,* 2013., Joshi *et al.,* 2010., Wadher*et al.,* 2013., Khalode*et al.,* 2012., Ekram*et al.,* 2011., Michael *et al.,* 1999), HPLC [Ugo, 2006., Chawla *et al.,* 1997., Palabiyik*et al.,* 2007., Milenkova*et al.,* 2003., Useni et al., 2011) and HPTLC [Chawla *et al.,* 1997). To our best of knowledge no method had been yet reported for simultaneous estimation of these three drugs using HPLC in bulk samples and in pharmaceutical dosage forms. Therefore, the present work was aimed to develop and validate anew RP- HPLC method for simultaneous estimation of phenylephrine HCl, chlorpheniramine maleate and dextromethorphan HBrin pharmaceutical dosage forms. The presentRP-HPLC method was validated following the ICH guidelines.

EXPERIMENTAL DESIGN

***Instrumentation*:**

A Waters Alliance 2695 separation module equipped with a 2487 UV detector was employed throughout this study. Column that was employed in the method was Eclipse XDB plus C_8 column(4.6 X 150 mm; 5 µm).The sampleswere injected with an automatic injector. The 20 µL volume of sample was injected. The input and output operations of the chromatographic system were monitored by Waters Empower 2 software. The flow rate selected was 1.0 mL per min. The detection was done at 220 nm. The temperature and run time was monitored at 25 ± 2°C and 30.0 min respectively.

The ultra violet spectra of the drugs used for the investigation were taken on a Lab India UV 3000 spectrophotometer for finding out their λ_{max}values. Solubility of the compounds was enhanced by sonication on an ultra sonicator (Power Sonic 510, Hwashin Technology).

All the weighings in the experiments were done with an Afcoset electronic balance.The

Hermlemicrolitre centrifuge Z100 (model no 292 P01) was used for the centrifugation process and Remi equipment (model no- CM101DX) Cyclomixer was used.

***Reagents and materials*:**

The reference sample of phenylephrine HCl, chlorpheniramine maleate and dextromethorphan HBrwas supplied by M/s Pharma Train, Hyderabad, Telagana**.** HPLC grade water (prepared by using 0.45 Millipore Milli-Q) was procured from Standard Reagents, Hyderabad. HPLC grade acetonitrile and methanol were purchased from Merck, Mumbai. The chemicals used for preparation of buffer include tri ethyl amine, 1-Octane sulfonic acid salt (Finar Chemicals, Ahmedabad), orthophosphoricacid (Standard Reagents, Hyderabad).

0.45 µ membrane filters (Advanced Micro Devices Pvt. Ltd., Chandigarh, India) were used for filtration of various solvents and solutions intended for injection into the column.

***Glassware*:**

All the volumetric glassware used in the study was of Grade A quality Borosil.

Optimization of the chromatographic conditions:

Several modifications in the mobile phase were made by changing proportions of acetonitrile, methanol and water. Various modifiers were used such as chloroform, Tetrahydrofuran (THF), ethanol, Isopropyl alcohol (IPA), n-Hexane, and dichloromethane, with a 5 µ particle size column, used for separation initially. However, the best resolution of 4.969 was observed by using HPLC grade water (composed of TEA and 1-octane sulfonic acid sodium salt) (pH adjusted to 3.2 using orthophosphoric acid) and acetonitrile, mixed at different ratio, much above the desirable limit of USP resolution 2.0. The retention time obtained for phenylephrine HCl, chlorpheniramine maleate and dextromethorphan HBrare 4.069,13.608 and 14.922 min., respectively.

Preparation of buffer solution (***mobile phase*** A)**:**

The buffer solution was prepared by dissolving 1.0mL of triethylamineand 1.08 gm of 1-Octane sulfonic acid sodium salt in 900mLHPLC grade water in a 1000 mL clean and dry flask. The mixture was stirred well for complete dissolution of the salt. Further 100 mL of water was added and the pH was adjusted to 3.2 using ortho phosphoric acid.

***Preparation of mobile phase B*:**

The mobile phase B was prepared by using 500 mL of HPLC acetonitrile, degassed in ultrasonicator for 5 minutes. The resultant mobile phase B was filtered through 0.45µ membrane filter (Advanced Micro Devices Pvt. Ltd., Chandigarh, India) under vacuum.

***Diluent preparation*:**

The diluent was prepared by mixing HPLC acetonitrile and buffer solution (pH 3.2) in the ratio of 50:50 (v/v). This solution was used for diluting the drug solutions in the study.

Gradient (time %B):

0/30, 2/30, 8/40, 12/55, 20/60, 22/30, 30/30

***Preparation of standard solution*:**

About 50 mg phenylephrine HClwas weighed accurately and transferred to a 100 mL clean and dry volumetric flask. Initially, the drug was dissolved with 70 mL of diluent. The solution was sonicated for 15 min for complete dissolution of the drug. The final volume was made up with the same solvent. From the above prepared solution 1.0 mL transferred to a 10 mL clean and dry volumetric flask and it was diluted up to the mark with the same diluent. This stock solution contains 50 µg/mL of phenylephrine HCl.

Similarly, about 20 mgchlorpheniramine maleate was weighed accurately and transferred to a 100 mL clean and dry volumetric flask. Initially, the drug was dissolved with 70 mL of diluent. The solution was sonicated for 15 min for complete dissolution of the drug. The final volume was made up with the same solvent. From the above prepared solution 1.0 mL transferred to a 10 mL clean and dry volumetric flask and it was diluted up to the mark with the same diluent. This stock solution contains 20 µg/mL of chlorpheniramine maleate.

Similarly, about 100 mgdextromethorphan HBrwas weighed accurately and transferred to a 100 mL clean and dry volumetric flask. Initially,

the drug was dissolved with 70 mL of diluent. The solution was sonicated for 15 min for complete dissolution of the drug. The final volume was made up with the same solvent. From the above prepared solution 1.0 mL transferred to a 10 mL clean and dry volumetric flask and it was diluted up to the mark with the same diluent. This stock solution contains 100 µg/mL of dextromethorphan HBr.

***Preparation formulation (tablet) solution*:**

A commercial brand of syrupCorfen-DM, (manufactured by Cypress pharmacetical, Inc.) was employed for this study.Each 5 mL syrup contained 10.0 mg phenylephrineHCl, 4.0 mg chlorpheniramine maleate and 15.0 mg dextromethorphan HBr. 5 mLsyrup was accurately and the sampledrugs were extracted with small amount of diluent in a 25 mLclean and dry volumetric flask. The solution was shaken well and allowed to stand for 15 min with intermittent sonication to ensure complete solubility of drugs. The contents are made up to the mark with the diluent and filtered through a 0.45µ membrane filter.

From this filtrate, suitable dilutions were made to get a concentration of 50µg/mL phenylephrine HCl, 20 µg/mLchlorpheniramine maleate and 100 µg/mLdextromethorphan HBr. Now the sample of 20 µL was injected and chromatographed. The average of the peak areas was calculated.

Method suitability: The commercial tablet formulation of amlodipine besylateand nebivolol hydrochloride namely Corfen-DM (manufactured by Cypress pharmacetical, Inc.)was analyzed by the proposed method and the results are shown in Table 1. The values were found to be in good agreement with the labeled amounts, which confirms the suitability of the method for the analysis of the drugs in pharmaceutical dosage forms.

Table 1: Recovery of phenylephrine HCl, chlorpheniramine maleate and dextromethorphan HBr from syrup Corfen-DMformulation

Label claim (mg)	Amount found (mg) (n=3)	% Amount found
Phenylephrine HCl (10.0 mg)	10.46	104.60
Chlorpheniramine maleate (4.0 mg)	3.90	97.50
Dextromethorphan HBr (15.0 mg)	15.50	103.33

RESULT AND DISCUSSION

Specificity and Selectivity: An aqueous mixture of phenylephrine HCl, chlorpheniramine maleate and dextromethorphan HBr(50, 20 and 100 µg/mL concentration respectively) was prepared and injected into the column and the retention time was checked and any interference at the retention time was checked by comparing the response in the blank. No interference was observed at the retention time for the respective drug. The method was found to be precise and specific. A typical chromatogram of phenylephrine HCl, chlorpheniramine maleate and dextromethorphan HBr standard and sample are shown in figure 3 (A & B).

***Linearity*:** In order to find out the linearity range of the proposed HPLC method, curves were constructed by plotting peak areas obtained for the analyte against their concentrations. A good linear relationship ($r^2 = 0.999$) was observed between the concentration of metoprolol tartrate andramipril hydrochlorideand their corresponding peak areas. The relevant regression equation was y = 34306x - 11042 (r^2= 0.999) for phenylephrine HCl, y = 35874x - 13101 (r^2= 0.999) for chlorpheniramine maleate anddextromethorphan HBr y = 25516x - 26579 ($r^2 = 0.999$) (where y is the peak area and x is the concentrations of phenylephrine HCl, chlorpheniramine maleate anddextromethorphan HBr (µg/mL))**.** The data are represented table 2, 3 and 4 and the calibration curves are presented in figure 4, 5 and 6.

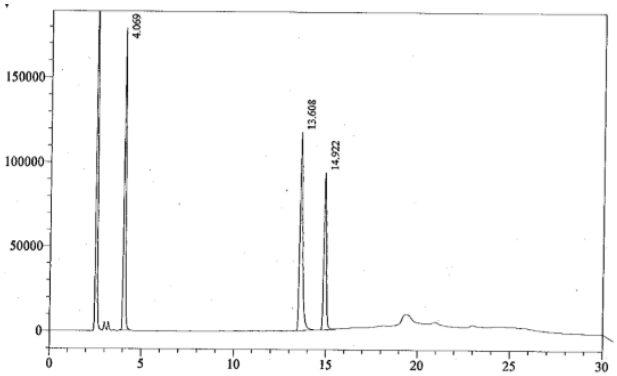

Figure 3 A: A typical chromatogram of phenylephrine HCl, chlorpheniramine maleate and dextromethorphan HBr (Standard)

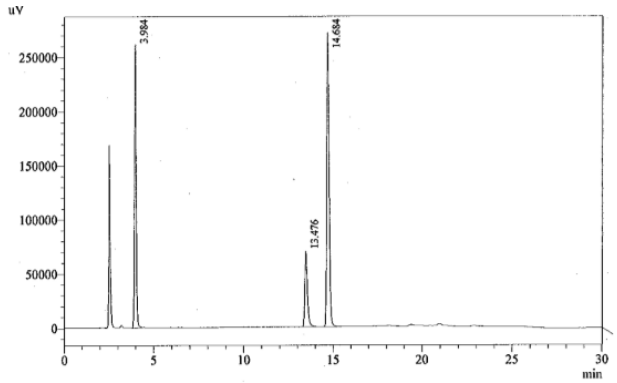

Figure 3 A: A typical chromatogram of phenylephrine HCl, chlorpheniramine maleate and dextromethorphan HBr (Sample)

Table 2: Linearity range for phenylephrine HCl

Sr. No.	Conc. (µg/mL)	Mean Peak Area	Statistical Analysis
1	5	170506	y = 34306x -
2	25	837869.7	11042
3	50	1676576	
4	75	2605407	R^2 = 0.999
5	100	3402451	

Precision: Precision is the level of reproducibility of the results as reported between sample analyzed on the same day (intra-day) and samples run on three different days (inter-day).

To check the intra and inter-day variations of the method, solutions containing 50 µg/mL phenylephrine HCl, 20 µg/mL chlorpheniraminemaleate and 100 µg/mL dextromethorphan HBrrespectively, were subjected to the proposed HPLC method of analysis and results obtained were noted. The precision of the proposed method i.e. the intra and inter-day variations in the peak areas of the drugs solutions were calculated in terms of percent RSD and the results are presentedin table 6 and 7. A statistical evaluation revealed that the relative standard deviation of the drugatlinearity level for 6 injections was less than 2.0.

Table 3: Linearity range forchlorpheniramine maleate

Sr. No.	Conc. (µg/mL)	Mean Peak Area	Statistical Analysis
1	2	66815.3	y = 35874x
2	10	342067.2	- 13101
3	20	685114.1	R^2 = 0.999
4	30	1081370.2	
5	40	1418320.1	

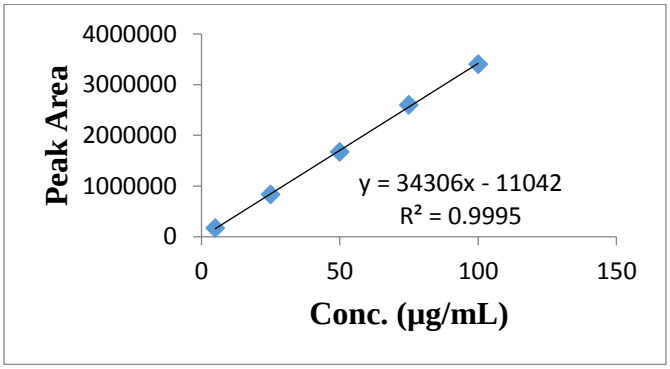

Figure 4: Calibration curve for phenylephrine HC

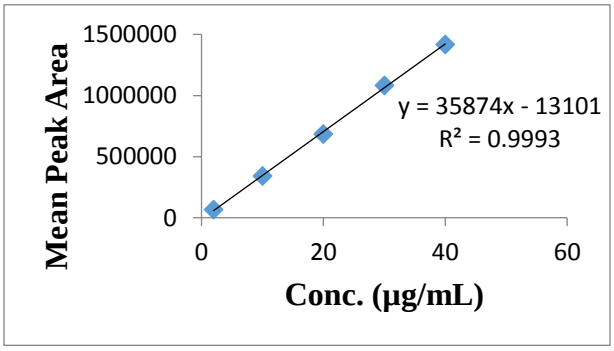

Figure 4: Calibration curve forchlorpheniramine maleate

Table 5: Linearity range for dextromethorphan HBr

Sr. No.	Conc. (µg/mL)	Mean Peak Area	Statistical Analysis
1	10	249291.6	y = 25516x - 26579 R^2 = 0.999
2	50	1233723	
3	100	2478682	
4	150	3861385	
5	200	5057272	

Table 6: Intra-day precision of the proposed method for phenylephrine HCl, chlorpheniramine maleate and dextromethorphan HBr

Injection	Peak Area Phenylephrine HCl	Peak Area Chlorpheniramine Maleate	Peak Area Dextromethorphan HBr
Mean	1700056	697959	2506760
SD	1915	1226	1732
%RSD	0.113	0.176	0.069

Table 7: Intra-day precision of the proposed method for phenylephrine HCl, chlorpheniramine maleate and dextromethorphan HBr (on six consecutive days n = 6)

Days	Phenylephrine HCl	Chlorpheniramine Maleate	Dextromethorphan HBr
Mean	1669859	686918	2466503
SD	0.099	0.147	0.083
%RSD	1655	1008	2044

***Accuracy*:** Accuracy is expressed as the closeness of the results obtained from standard samples to that of the actual known amounts. To determine the accuracy of the proposed method, recovery studies were carried out by analyzing recovery amount (80.0, 100.0, 120.0 % of phenylephrine HCl, chlorpheniramine maleate and dextromethorphan HBr) of pure drugs at linearity level (50.0 µg/mL of phenylephrine HCl, 20.0 µg/mL of chlorpheniramine maleate and 100.0 µg/mL of dextromethorphan HBr) was added. Then each dilution was injected thrice (n=3). The percent

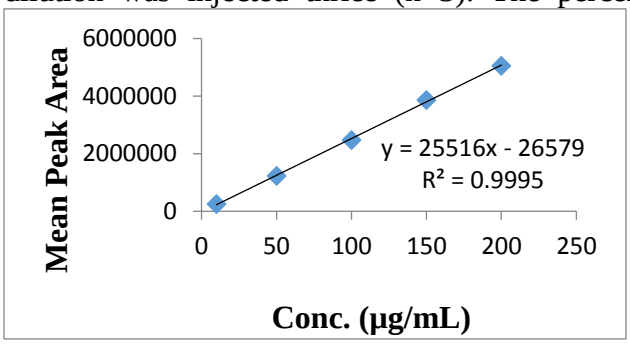

Figure 5: Calibration curve for dextromethorphan HBr

recoveries of the drugs were calculated. The results are shown in table 8, 9 and 10.

Limit of detection (LOD) and Limit of quantitation (LOQ): LOD is defined as the smallest level of analyte that gives a measurable response. LOD is based on S/N ratio (signal/noise) typically for HPLC methods. Six replicates of the analyte were measured. The LOQ is the lowest concentration that can be quantified reliably with a specified level of accuracy and precision. It is the lowest concentration at which the precision expressed by relative standard deviation (RSD) is less than 2 % and accuracy expressed by relative

Table 8: Accuracy data of the proposed method for phenylephrine HCl (4 mg), chlorpheniramine maleate (1.6 mg) and dextromethorphan HBr 8mg at 80% level

Drug	Amount Found (mg)	% Recovery	% Recovery
Phenyl	4.16	104.02	**Mean**- 103.98
	4.16	104.0	**SD**- 0.052
	4.16	103.9	**%RSD**- 0.051
Chlorph	1.69	105.7	**Mean**- 105.6
	1.69	105.8	**SD**- 0.053
	1.68	105.1	**%RSD**-0.052
Dextromet	8.40	104.9	**Mean**- 104.88
	8.39	104.9	**SD**- 0.053
	8.38	104.2	**%RSD**- 0.052

Table 9: Accuracy data of the proposed method for phenylephrine HCl (5 mg), chlorpheniramine maleate (2 mg) and dextromethorphan HBr(10 mg) at 100% level

Drug	Amount Found (mg)	% Recovery	% Recovery
Phenylephrine HCl	4.94	98.76	**Mean**- 98.77
	4.94	98.73	**SD**- 0.042
	4.94	98.82	**%RSD**- 0.042
Chlorpheniramine Maleate	2.05	102.62	**Mean**- 102.41
	2.04	102.02	**SD**-0.341
	2.05	102.60	**%RSD**- 0.333
Dextromethorphan HBr	10.16	101.64	**Mean**- 101.69
	10.17	101.68	**SD**- 0.052
	10.17	101.75	**%RSD**- 0.051

Table 10: Accuracy data of the proposed method for phenylephrine HCl (6mg)chlorpheniramine maleate (2.4 mg) and dextromethorphan HBr (12 mg) at 120% level

Name	Amount Found (mg)	% Recovery	% Recovery
Phenylephrine HCl	6.00	99.99	100.24
	6.02	100.3	- 0.051
	6.03	100.42	**%RSD**- 0.050
Chlorpheniramine Maleate	2.45	101.9	**Mean**- 102.57
	2.47	102.90	**SD**- 0.052
	2.47	102.90	**%RSD**- 0.051
Dextromethorphan HBr	12.18	101.54	**Mean**- 102.02
	12.27	102.23	**SD**- 0.052
	12.28	102.31	**%RSD**- 0.051

difference in the measured and true value is also less than 2 %. In other words, the analyte response is 10 times greater than the noise response. Six replicates of the analyte were analyzed and quantified.The limits of detection for phenylephrine HCl, chlorpheniramine maleate and dextromethorphan HBrobtained by the proposed method was 0.19, 0.32 and 0.58 µg/mL and limits of quantification for phenylephrine HCl, chlorpheniramine maleate and dextromethorphan HBr obtained by the proposed method was 0.62, 0.97 and 1.90µg/mL.

Table 11: Results of the robustness study for phenylephrine HCl

Parameters		***Phenylephrine HCl***		
		Retention Time	**Peak Area**	**Tailing Factor**
Standard		4.069	1696920	1.39
Flow rate	0.9	3.969	1699395	1.49
	1.1	3.969	1700193	1.38
Wavelength	215	4.078	1699827	1.50
	225	4.089	1701542	1.41
pH	3.0	4.012	1702461	1.59
	(3.4)	4.056	1700056	1.39
Mobile	28	4.098	1696920	1.38
phase B	32	4.078	1699395	1.49
Temperature	20	4.056	1701542	1.47
	30	4.078	1702461	1.38

***Robustness*:** The optimized HPLC conditions were slightly modified to evaluate the robustness of the method. Small variations were made in the mobile phase ratio and flow rate. From the results, it was indicated that the selected factors remained unaffected by small variations in these quantities as well as the method was robust even by change in flow rate ± 0.1 mL/min and change in detection wavelength ± 5nm. The results are shown in table 11, 12 and 13.

CONCLUSION

It can be concluded that the proposed RP-HPLC method developed for the quantitative determination of phenylephrine HCl, chlorpheniramine maleate and dextromethorphan HBrin bulk samples and in its formulations is simple, selective, sensitive, accurate, precise and rapid. The method was proved to be superior to

Table 12: Results of the robustness study for chlorpheniramine maleate

Parameters		*Chlorpheniramine maleate*		
		Retention Time (min.)	**Peak Area**	**Tailing Factor**
Standard		13.608	695678	1.39
Flow rate	0.9	13.780	698643	1.38
	1.1	13.678	697496	1.49
Wavelength	215	13.548	698493	1.27
	225	13.788	698423	1.49
pH	3.0	13.068	699024	1.69
	3.4	13.368	697959	1.58
Mobile	28%	13.908	695678	1.69
phase B	32%	13.218	698643	1.37
Temperature	20**°C**	13.238	697959	1.49
	30**°C**	13.348	695678	1.59

Table 13: Results of the robustness study for dextromethorphan HBr flow rate (ml/min), Wavelength (nm)

Parameters		*Dextromethorphan HBr*		
		Retention Time (min.)	**Peak Area**	**Tailing Factor**
Flow	0.5	14.922	688170	1.39
	0.9	14.129	687768	1.38
	1.1	14.078	687275	1.49
Wavelength	215	14.097	686061	1.27
	225	14.622	686679	1.49
pH	3.0	14.892	685552	1.49
	3.4	14.078	686918	1.38
Mobilephase	28%	14.789	688170	1.69
B	32%	14.546	687768	1.87
Temperature	20**°C**	14.970	686679	1.29
	30**°C**	14.912	685552	1.59

most of the reported methods. The mobile phases are simple to prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence this method can easily be adopted as an alternative method to reported ones for the routine determination of phenylephrine HCl, chlorpheniramine maleate and dextromethorphan HBrdepending upon the availability of chemicals and nature of other ingredients present in the sample. The method also finds use in clinical, biological and pharmacokinetic studies of phenylephrine HCl, chlorpheniramine maleate and dextromethorphan HBr.

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