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RESEARCH ARTICLE

Rp-Hplc Method for the Simultaneous Estimation of Phenylephrine Hydrochloride, Guaiphenesin, Bromhexine Hydrochloride and Cetirizine Hydrochloride in Pharmaceutical Liquid Formulation

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ABSTRACT

A simple, rapid, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Phenylephrine HCl, Guaiphenesin, Bromhexine HCl and Cetirizine Hydrochloride, in combined dosage form. A Qualisil, C18 column, 250 mm x 4.6 mm, 5µm in isocratic mode with mobile phase containing 0.05M KH_2PO_4-1.0% HCl Buffer: ACN (62: 38) pH was adjusted to 2.5 by TEA. The flow rate 1.0ml/min and effluents were monitored at 254nm. The retention time of the Phenylephrine HCl, Guaiphenesine, Bromhexine HCl and Cetirizine Hydrochloride was found to 2.78, 3.88, 6.90 and 8.07 mins. respectively. The different analytical parameters such as accuracy, precision, robustness, limit of detection and limit of quantification were determined according to the International Conference on Harmonization (ICH) Q2R1 guidelines. The detector response was linear in the range of 0.2-1.0 µg/ml, 2-10 µg/ml, 0.16-0.8 µg/ml and 0.1-0.5 µg/ml, for Phenylephrine HCl, Guaiphenesine, Bromhexine HCl and Cetirizine Hydrochloride respectively. The proposed method was successfully applied for the simultaneous estimation of all drugs in pharmaceutical dosage forms.

KEYWORDS

RP-HPLC, Cetirizine Hydrochloride, Bromhexine Hydrochloride, Phenylephrine Hydrochloride, Guaiphenesin, Validation, Cough formulation and method development

INTRODUCTION

The liquid formulation cold and cough is a combination of mucolytic, expectorant, anti-allergic and nasal decongestant. It relieves multiple symptoms of congestion, runny nose, itching and sneezing and at the same time loosens viscid mucus. It is indicated in cough associated with nasal congestion as in respiratory tract infections/allergies.

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An extensive literature survey revealed UV, HPLC[15], HPTLC[16] and colorimetric determination for the estimation of Bromhexine HCl (B-HCl)[17, 19], Guaiphenesin (GPN)[17, 18,20,23], Cetirizine HCl (C-HCl) and phenylephrine HCl (P-HCl) either individually or in combination with other drugs.

But there is no method which describes the RP-HPLC simultaneous determination of P-HCl, GPN, B-HCl and C-HCl. The objective of this investigation was to develop simple, précis, accurate and economical procedures for simultaneous estimation of P-HCl, GPN, B-HCl and C-HCl from the pharmaceutical preparation.

Drug Profiles [1-14]

Guaiphenesin[15,16] is an expectorant which increases bronchial secretions and thereby reduces viscosity of tenacious sputum.

Guaiphenesin is (RS)-3-(2-methoxyphenoxy)-1, 2- propanediol having molecular weight 198.22 and formula as $C_{10}H_{14}O_4$. It is white or almost white, crystalline powder; odourless or with a slight characteristic odour. It is Soluble in ethanol (95%) and in chloroform; sparingly soluble in water; slightly soluble in ether.

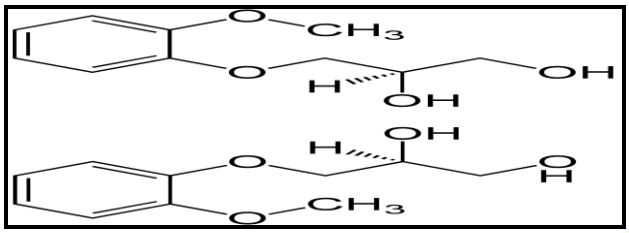

Figure 1: Chemical Structure of Guaiphenesin

Cetirizine Hydrochloride (C-HCl) is a third generation antihistamine that provides relief in allergic conditions. It reduces allergic vasodilation and nasal mucosal congestion commonly seen in upper respiratory infections and allergies. It is an orally active and selective H_1-receptor antagonist. The chemical name is (±) - [2- [4- [(4-chlorophenyl) phenylmethyl] -1-piperazinyl] ethoxy]acetic acid, diHydrochloride. Cetirizine Hydrochloride is a racemic compound with an empirical formula of $C_{21}H_{25}ClN_2O_3$•2HCl. The molecular weight is 461.82 and the chemical structure is shown below:

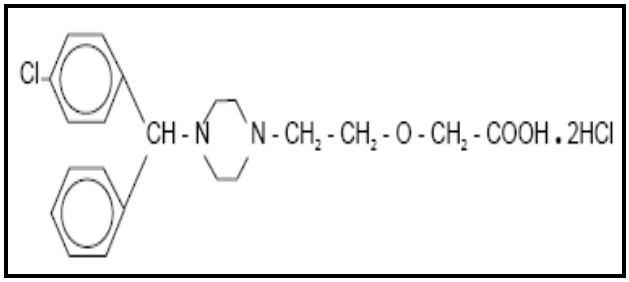

Figure 2: Chemical Structure of Cetirizine Hydrochloride

C-HCl is a white, crystalline powder and is freely soluble in water, practically insoluble in acetone and in methylene chloride.

Phenylephrine Hydrochloride[15,16] is a sympathomimetic vasoconstrictor that has been used as a nasal decongestant for many years. It constricts the blood vessels in the nasal mucus membranes and allows the air passages to open up. It is also called as (1R)-1-(3-Hydroxyphenyl)-2-(methylamino) ethanol Hydrochloride; having molecular weight and molecular formula as $C_9H_{13}NO_2$, HCl, 203.7 respectively. It is Alpha-adrenoceptor agonist.

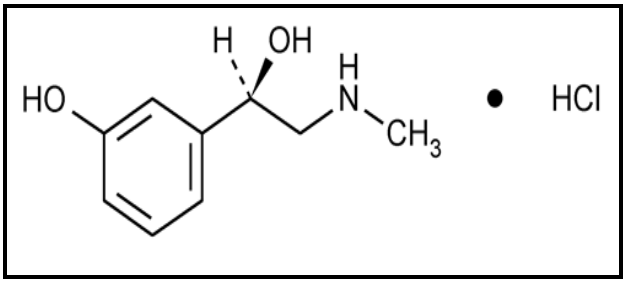

Figure 3: Chemical Structure of Phenylephrine Hydrochloride

White or almost white, crystalline powder. Which is freely soluble in water and in ethanol (96 per cent), having a melting point of 143 °C.

Bromhexine[15,16] is a mucolytic agent used in the treatment of respiratory disorders associated with viscid or excessive mucous. It alter the structure of mucus to decrease its viscosity and therefore facilitate its removal by ciliary action or expression.

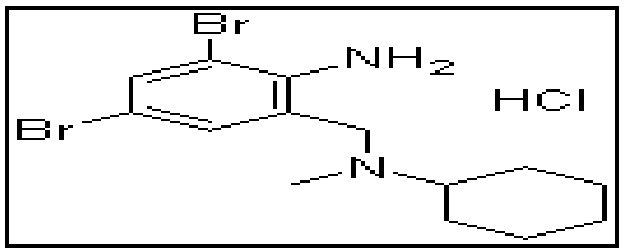

Figure 4: Chemical Structure of Bromhexine Hydrochloride

B-HCl is white or almost white, crystalline powder; odourless or almost odourless. B.HCl is 2-amino-3, 5-dibromobenzyl- (cyclohexyl) methylamine Hydrochloride. The molecular formula and weight is $C_{14}H_{20}Br_2N_2$, HCl and 412.59 respectively. It is sparingly soluble in ethanol (95%) and in methanol; slightly soluble in chloroform; practically insoluble in water.

MATERIALS AND METHOD

Guaiphenesine (GPN), Cetirizine Hydrochloride (C-HCl), Phenylephrine Hydrochloride (P-HCl) and Bromhexine Hydrochloride (B-HCl) were of USP grade. The entire reagents used were of analytical grade. Water was deionized and double distilled. The potassium dihydrogen phosphate, Triethylamine, Hydrochloric acid and phosphoric acid was of high purity HPLC grade purchased from Merck".

HPLC Instrument and Chromatographic Conditions

The method was developed on a Schimadzu LC-2010 $_{CHT}$ consisting of a quaternary pump and UV-spectrophotometric detector and system controlling module as LC solutions with an auto sampler. Chromatographic conditions were carried out in a stainless steel Qualisil C-18 column (250mm x 4.6 mm, 5µ), with 0.05M KH2PO4-1.0% HCl Buffer: ACN (62: 38 v/v) having a pH of 2.5 with TEA and the flow rate was 1.0ml/min.

The mobile phase was filtered through 0.45 µm Millipore membrane filter and degassed. The detection performed as 254nm using deuterium lamp.

Preparation of Standard Solution

Standard Stock Solution for Guaiphenesin

An accurately weighed GPN (25.0mg) was transferred to 25ml of volumetric flask, dissolved in 10ml of the mobile phase and diluted up to the mark with mobile phase. (Solution A) Transferred an accurately measured 10ml of the stock solution to 25ml volumetric flask and diluted up to the mark with mobile phase. Filter the solution with 0.2µ nylon membrane filter and allow to inject 20µl.

Standard Stock Solution for Cetirizine Hydrochloride

An accurately weighed C-HCl (25.0mg) was transferred to 50ml of volumetric flask, dissolved in 15ml of the mobile phase and diluted up to the mark with mobile phase. (Solution B) Transferred an accurately measured 5ml of the stock solution to 50ml volumetric flask and diluted up to the mark with mobile phase.

Standard Stock Solution for Phenylephrine Hydrochloride

An accurately weighed P-HCl (25.0mg) was transferred to 25ml of volumetric flask, dissolved in 15ml of the mobile phase and diluted up to the mark with mobile phase. (Solution C) Transferred an accurately measured 5ml of the stock solution to 50ml volumetric flask and diluted up to the mark with mobile phase.

Standard Stock Solution for Bromhexine Hydrochloride

An accurately weighed B-HCl (20.0mg) was transferred to 25ml of volumetric flask, dissolved in 5ml of the mobile phase and diluted up to the mark with mobile phase. (Solution D) Transferred an accurately measured 5ml of the stock solution to 50ml volumetric flask and diluted upto the mark with mobile phase.

Combine Standard

Transferred an accurately weighed 50mg quantity of guaiphenesin and measured volumes of Solution B, C and D: 5ml, each respectively to 50ml volumetric flask and diluted up to the mark with the mobile phase. (Figure 5)

Sample Solution

For the determination of the content of P-HCl, GPN, B-HCl and C-HCl; the formulations equivalent to 50mg, 2.5mg, 5.0mg and 4.0mg was transferred to a 50ml volumetric flask and the content was allowed to dissolve in 30 ml of the mobile phase and make up the volume to 50 ml volumetric flask with the mobile phase (Figure 6).

Preparation of KH_2PO_4-HCl Buffer

Transfer an accurately measured quantity of potassium dihydrogen orthophosphate to 1000ml volumetric flask, dissolved the content in 600ml of distilled water, add 10ml of the hydrochloric acid and 5ml of phosphoric acid to

the flask and make up the volume to the mark with distilled water.

Preparation of Mobile Phase

The two components of the mobile phase was mixed in the ratio of (62: 38% v/v) and the pH maintained to 2.5 with triethylamine. Then the final mobile phase was filtered through a 0.45µm nylon membrane filter and sonicated for 12min.

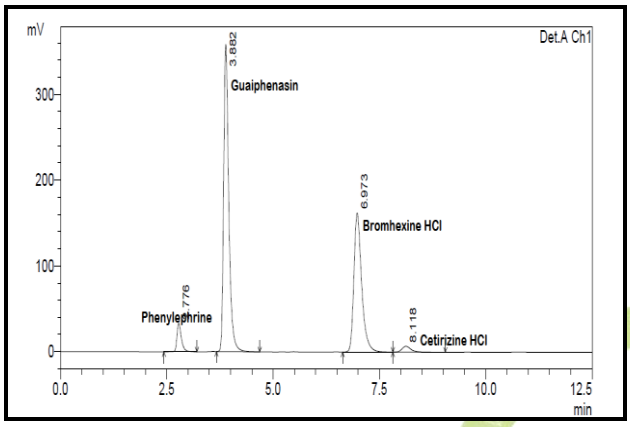

Figure-5: Shows the HPLC Graphs of P-HCl, GPN, B-HCl and C-HCl in Combine Standard

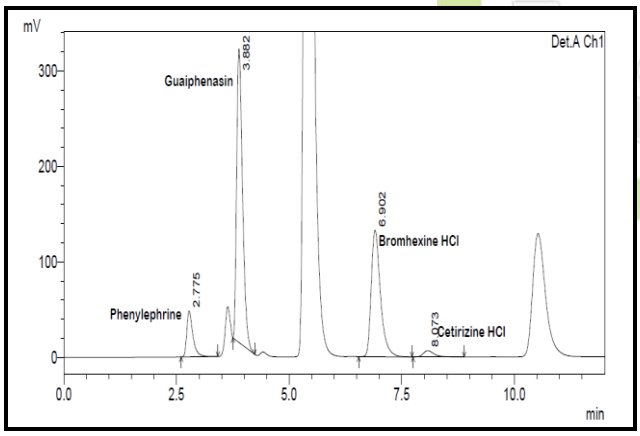

Figure-6: Shows the HPLC Graphs of P-HCl, GPN, B-HCl and C-HCl in Pharmaceutical Cough Preparation

Validation of the Method[2]

The method validation parameters such as linearity, precision, accuracy, limit of detection and limit of quantification, robustness, specificity and solution stability etc, were ascertained according to the International conference on Harmonization (ICH) guidelines.

Linearity and Range

The calibration curves were plotted over a concentration range of 0.2-1.0 µg/ml, 2-10 µg/ml, 0.16-0.8 µg/ml and 0.1-0.5 µg/ml for P-HCl, GPN, B-HCl, and C-HCl 0.24 to 60µg/ml for each P-HCl, GPN, B-HCl and C-HCl. Aliquots of mix standard solutions for P-HCl, GPN, B-HCl and C-HCl were transferred into a series of 10ml volumetric flasks and the volume was made up to the mark with mobile phase. An aliquot (20µl) of each solution was injected under the operating chromatographic conditions as described above and responses were recorded. Calibration curves were constructed by plotting the peak areas versus the concentration, and the regression equations were calculated, the curves of P-HCl, GPN, B-HCl and C-HCl are shown in [figure 7(a), 7(b), 7(c), 1(d)].

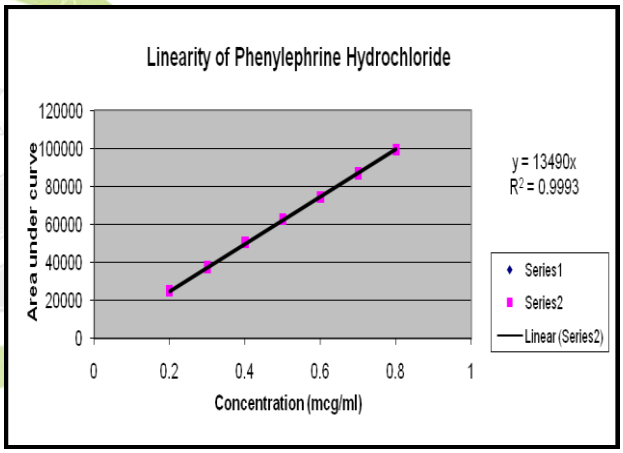

Figure 7(a): Calibration Curves of Phenylephrine HCl

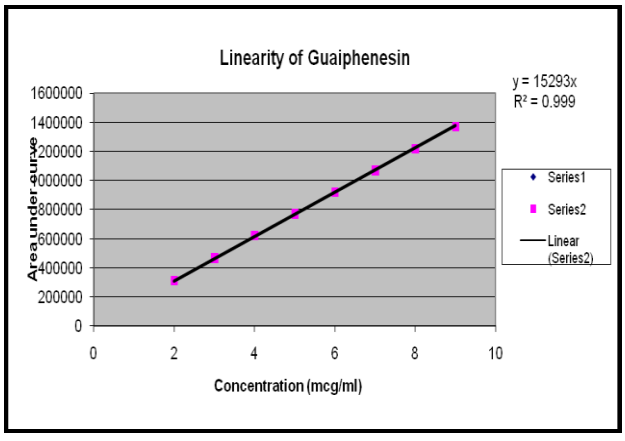

Figure 7(b): Calibration Curves of Guaiphenesin

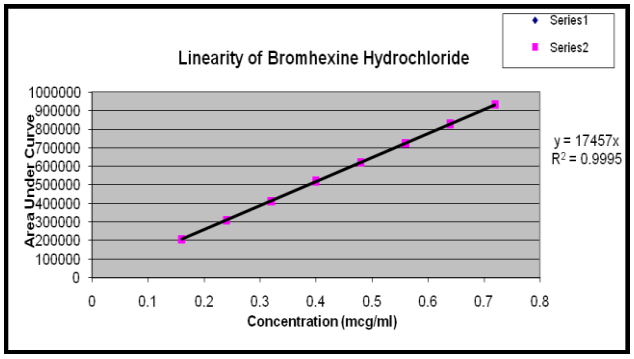

Figure 7(c): Calibration Curves of Bromhexine HCl

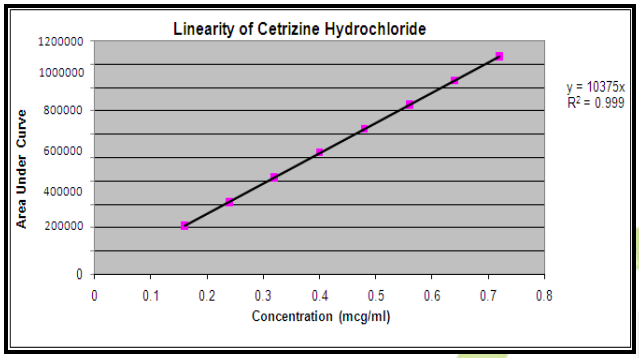

Figure 7(d): Calibration Curves of Cetirizine HCl

Method Precision (Repeatability)

The precision of the instrument was checked by repeatedly injecting (n=6) solutions of P-HCl, GPN, B-HCl and C-HCl, without changing the parameters of the proposed method. The results were reported in terms of relative standard deviation (% RSD).

Intermediate Precision

The intraday, interday precisions of the proposed method was determined by estimating the corresponding responses 3times on the same day and on three different days over a period of one week for all standard solutions of P-HCl, GPN, B-HCl and C-HCl 50mcg/ml, 250mcg/ml, 40mcg/ml and 30mcg/ml. respectively. The results were reported in terms of relative standard deviation (% RSD).

Recovery Studies

The accuracy of the proposed methods was checked by recovery study by addition of standard drug solutions to reanalyzed sample solution at three different concentration levels

Table 1: Intra-Day Precision for P-HCl, GPN, B-HCl and C-HCl

Sample*	P-HCl (mcg/ml)	GPN (mcg/ml)	B-HCl (mcg/ml)	C-HCl (mcg/ml)
Sample 1	51.295	250.089	39.875	29.998
Sample 2	51.300	250.090	40.559	30.185
Sample 3	50.828	250.055	40.615	30.265
Sample 4	50.788	250.100	40.589	29.988
Sample 5	51.200	249.989	39.870	29.978
Sample 6	50.283	249.991	39.898	30.900
AVERAGE	50.945	250.052	40.218	30.219
NOMINAL	**50mcg/ml**	**250mcg/ml**	**40mcg/ml**	**30mcg/ml**
SD	**0.399**	**0.050**	**0.406**	**0.354**
%C.V.	**0.784%**	**0.202%**	**1.011%**	**1.172%**

*sample – in house Production batch

Table 2: Inter-Day Precision for P-HCl, GPN, B-HCl and C-HCl

Sample*	P-HCl (mcg/ml)	GPN (mcg/ml)	B-HCl (mcg/ml)	C-HCl (mcg/ml)
Sample 1	50.952	249.958	39.805	29.801
Sample 2	50.591	249.989	40.560	30.185
Sample 3	49.890	250.096	39.859	30.180
Sample 4	49.907	250.102	40.551	30.102
Sample 5	49.925	249.893	39.696	30.011
Sample 6	50.071	249.909	40.016	30.001
AVERAGE	**50.222**	**249.991**	**40.081**	**30.213**
NOMINAL	**25mcg/ml**	**250mcg/ml**	**50mcg/ml**	**40mcg/ml**
SD	**0.444**	**0.090**	**0.399**	**0.416**
%C.V.	**0.885%**	**0.361%**	**0.784%**	**1.377%**

Table 3: Recovery Studies for P-HCl, GPN, B-HCl and C-HCl

Table 3.1: Recovery Studies for P-HCl

Sample*	Amount of P-HCl added	Theoretical Conc.	Actual Conc.	% Recovery
80%	80	180	179.86	99.922
80%	80	180	179.401	99.666
80%	80	180	179.993	99.996
100%	100	200	201.198	100.599
100%	100	200	200.786	100.393
100%	100	200	200.184	100.092
120%	120	220	219.809	99.913
120%	120	220	220.099	100.045
120%	120	220	220.094	100.042
Nominal	100mcg/ml			

Table 3.2: Recovery Studies for GPN

Sample*	Amount of P-HCl added	Theoretical Conc.	Actual Conc.	% Recovery
80%	320	720	720.991	100.138
80%	320	720	721.829	100.254
80%	320	720	721.901	100.264
100%	400	800	801.111	100.138
100%	400	800	804.421	100.552
100%	400	800	803.333	100.416
120%	480	880	881.99	100.226
120%	480	880	882.285	100.256
120%	480	880	879.088	99.896
Nominal	400mcg/ml			

Table 3.3: Recovery Studies for B-HCl

Sample*	Amount of P-HCl added	Theoretical Conc.	Actual Conc.	% Recovery
80%	80	180	179.86	100.013
80%	80	180	179.401	99.374
80%	80	180	179.993	100.005
100%	100	200	201.198	99.987
100%	100	200	200.786	100.618
100%	100	200	200.184	100.05
120%	120	220	219.809	99.977
120%	120	220	220.099	100.511
120%	120	220	220.094	98.318
Nominal	80 mcg/ml			

Table 3.4: Recovery Studies for C-HCl

Sample*	Amount of P-HCl added	Theoretical Conc.	Actual Conc.	% Recovery
80%	40	90	91.611	101.79
80%	40	90	89.99	99.988
80%	40	90	90.022	100.024
100%	50	100	101.89	101.89
100%	50	100	100.17	100.172
100%	50	100	99.79	99.79
120%	60	110	110.233	100.211
120%	60	110	110.02	100.018
120%	60	110	110.018	101.016
Nominal	50 mcg/ml			

Table 4: Robustness Studies for P-HCl, GPN, B-HCl and C-HCl

Factor	Level	Retention Time			
		P-HCl	GPN	B-HCl	C-HCl
Flow Rate ml/min					
0.9	-0.1	2.866	4.011	7.055	8.101
1.0	0	**2.770**	**3.882**	**6.908**	**8.068**
1.1	+0.1	2.699	3.799	6.900	7.997
pH of the Mobile Phase					
2.4	-0.1	2.783	3.898	6.922	8.102
2.5	0	**2.770**	**3.882**	**6.908**	**8.068**
2.6	+0.1	2.834	4.008	6.930	8.656
% Acetonitrile in the Mobile Phase					
36	-2	2.749	3.855	6.888	8.041
38	0	**2.770**	**3.882**	**6.908**	**8.068**
40	+2	2.789	3.995	6.950	8.200

80%, 100% and 120% of the entire API) within the range of linearity for the drugs. Each being analyzed in a manner similar to as described for assay and the recovery of added standard was calculated. From the data obtained, added recoveries of standard drugs were found to be accurate.

Limits of Detection (LOD) and Limits of Quantification (LOQ)

The limit of detection and the limit of quantification of the drugs were derived by calculating the signal to noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ). The limit of detection and limit of quantification of the developed method were determined by injecting progressively low concentration of the standard solutions using the developed method. The LOD of P-HCl, GPN, B-HCl and C-HCl was found to be 30 µg/ml, 100 µg/ml, 40µg/ml and 20.0 µg/ml respectively. The LOQ is the smaller concentration of the analyte response that can be quantified accurately the LOQ was 100 µg/ml, 250 µg/ml, 70µg/ml and 50.0 µg/ml respectively.

Robustness

The robustness was studied by analyzing the same samples of P-HCl, GPN, B-HCl and C-HCl by deliberate variations in the method parameters. The change in the response of P-HCl, GPN, B-HCl and C-HCl were noticed. Robustness of the method was studied by changing composition of mobile phase by ±2% of the organic solvent, flow rate by ±0.1ml/min and pH by ±0.1nm. The results were reported (Table-3.0).

Specificity

Specificity of the method was established by observing the interferences of the common excipients used for pharmaceutical dosage form with the principal peaks. Further the recovery study confirms that there was no interferences from sample placebo with the actives; hence it showed that the developed analytical method was specific for the estimation of P-HCl, GPN, B-HCl and C-HCl.

CONCLUSION

The proposed validated high performance liquid chromatographic method can be used for routine quality control analysis of Phenylephrine Hydrochloride, Guaiphenesin, Bromhexine Hydrochloride and Cetirizine Hydrochloride in pharmaceutical dosage forms.

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