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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF BECLOMETHASONE DIPROPIONATE, PHENYLEPHRINE HYDROCHLORIDE AND LIGNOCAINE HYDROCHLORIDE IN CREAM

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ABSTRACT

A simple, rapid, economical, precise and accurate RP-HPLC method for simultaneous estimation of PHE, LIG and BEC in their combined dosage form has been developed. A reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of PHE, LIG and BEC in their combined dosage form has been developed. The separation was achieved by Inertsil ODS C_{18} column (250 x 4.6 mm,5µm) column and Water (pH 4.0): Methanol (70:30) as mobile phase, at a flow rate of 1 ml/min and the effluent was monitored at 240 nm using PDA detector. Retention time of PHE, LIG and BEC were found to be 4.653min, 5.603 min and 11.083 min, respectively. The method was validated in terms of linearity, precision, accuracy and specificity, limit of detection and limit of quantization. Linearity of the PHE, LIG and BEC were in range of 2 – 6 µg/ml, 50 – 150 µg/ml and 0.5 – 1.5 µg/ml respectively. The percentage assay of three the drugs were found to be 98.135%, 96.931% and 98.625% for PHE, LIG and BEC respectively from the cream formulation. Repeatability, Intra-day and Inter-day Precision obtained for PHE, LIG and BEC in the range of 0.167 – 0.532, 0.125 – 0.406 and 0.683 – 1.541% RSD for intra-day and 0.607 – 1.321, 0.579 – 1.034 and 0.554 – 0.902% RSD for inter day and 0.869, 0.778 and 0.924% RSD for repeatability. The method was found to be precise, accurate and specific during the study. The proposed method enables rapid quantification and simultaneous analysis of three drugs from commercial formulations without any excipients interference. The method can be used for routine analysis of marketed products of PHE, LIG and BEC in combined cream formulation.

KEYWORDS: Reverse Phase High Performance Liquid Chromatography Method, Infra-Red Spectroscopy Method, Lignocaine HCl, Phenylephrine HCl, Beclomethasone Dipropionate.

INTRODUCTION

Lignocaine HCl is designated chemically as 2-(diethylamino)-N-(2,6-dimethylphenyl) acetamide (Figure 1) is a compound of the Carboxylic acids and derivatives class and a local anestheticand cardiac depressant is used as an antiarrhythmic agent.[1] It acts by inhibiting the ionic fluxes required for the initiation and conduction of impulses thereby effecting local anesthetic action and it alters signal conduction in neurons by blocking the fast voltage gated sodium channels in the neuronal cell membrane that are responsible for signal propagation. With sufficient blockage the membrane of the postsynaptic neuron will not depolarize and will thus fail to transmit an action potential. Various analytical methods have been reported for the estimation of Lignocaine HCl as alone as well as in combination with other drugs. They include spectrophotometric methods and TLC[2], HPLC[3], LC/MS/MS[4], stability indicating UPLC[5] and HPLC methods with Diclofenac Diethylamine[6], Miconazole Nitrate[7], Oxycodone[8], Hydrocortisone acetate.[9]

Beclomethasone Dipropionate is designated chemically as 9α-chloro-11ß-hydroxy-16ß-methyl-3, 20-dioxopregna-1,4-diene-17,21-diyldipionate (Figure 2) is a compound of the potent glucocorticoid steroid class and is used to treat anti-rhinitis and prophylaxis of asthma1. It acts by inhibiting leukocyte infiltration at the site of inflammation, interference in the function of mediators of inflammatory response, suppression of humoral immune responses, and reduction in edema or scar tissue. Various analytical methods have been reported for the estimation of Beclomethasone Dipropionate as alone as well as in combination with other drugs. They include spectrophotometric methods[10], HPLC[11], MS[12], LC/MS/MS[13] and HPLC[14] methods with Salicylic acid[15], Salbutamol Sulphate[16] and Levosalbutamol Sulphate.[17]

Phenylephrine HCl is designated chemically as Benzenemethanol,3-hydrox-[(methylamino)methyl]-hydrochloride (R)-m-Hydroxy-[(methylamino)methyl]benzyl alcohol hydrochloride (Figure 3) is a compound of the vasoconstrictor agents class and is used to treat nasal congestion, but may also be useful in treating hypotension and shock, hypotension during spinal anaesthesia, prolongation of spinal anaesthesia, paroxysmal supraventricular

tachycardia, symptomatic relief of external or internal hemorrhoids, and to increase blood pressure as an aid in the diagnosis of heart murmurs. It acts α_1-adrenergic receptors subtypes1. Various analytical methods have been reported for the estimation of Phenylephrine HCl as alone as well as in combination with other drugs. They include spectrophotometric methods[18], stability indicating HPLC methods with Ciprofloxacin[19] and HPLC Paracetamol[20] and Ebastine.[21]

However an extensive literature search didn't reveal any estimation method for both the drugs in their combined dosage form. Therefore, attempt was made to develop and validate simple, precise, and accurate, stability indicating RP-HPLC method for simultaneous determination of both the drugs in their combined dosage form. The parent guideline on drug analytical method validation FDA.[22]

MATERIALS AND METHODS

Reagents and Chemicals

Lignocaine HCl, Beclomethasone Dipropionate and Phenylephrine HCl were obtained as gift samples from Saga laboratory, Changodar. Lignocaine HCl, Beclomethasone Dipropionate and Phenylephrine HCl combined dosage form cream were purchased from local market. HPLC grade Acetonitrile, Water and methanol of analytical grade were obtained from SD Fine Chem Ltd.

Instruments and Chromatographic Conditions

Young lin HPLC system was used for method development, degradation studies and validation. Data acquisition was performed on YL 9100 HPLC software. The separation were achieved on Inertsil ODS C_{18} (250 × 4.6 mm, 5µm) column. The column was maintained at room temperature and the eluent was monitored at 240nm using PDA detector. The mixture of Water (pH 4.0): Methanol in proportion of 70:30 %v/v at a flow rate of 1.0 ml/min was used as a mobile phase. The injection volume was 20µl.

Preparation of Standard Solutions of PHE, LIG and BEC

Accurately weighed and transferred 100mg of LIG, 10 mg of BEC and 40 mg of PHE into two different 100ml volumetric flasks and volume was made up to the mark with methanol and used as standard stock solution (1000µg/ml, 10µg/mL and 40µg/mL for LIG, BEC and PHE respectively.) From the prepared stock solutions 1 ml of LIG, BEC and PHE stock solution was taken and transferred to 10 mL volumetric flask and volume was made up to the

mark by mobile phase to get working standard solution comprising 100 µg/mL LIG, 1 µg/mL BEC and 4 µg/mL PHE.

Preparation of Sample Solutions of PHE, LIG and BEC

Cream equivalent to 4mg PHE, 100 mg LIG and 1 mg BEC was taken into a 100 ml volumetric flask. 60 ml methanol was added, Heat this solution in water bath at low temperature for 30 minutes, cool this solution. Volume was made up with methanol up to 100 ml. (PHE-40 µg/mL, LIG- 1000 µg/mL and BEC-10 µg/mL). This solution was filtered with whattman filter paper no-1. 1ml from sample stock solution was taken into a 10 ml volumetric flask and volume was made up with mobile phase. (PHE-4 µg/mL, LIG-100 µg/ml and BEC-1 µg/mL). The resulted test solution was then analyzed for assay determination.

Preparation of working sample (PHE 4 µg/mL, LIG100 µg/mL and BEC 1 µg/mL)

1ml from sample stock solution was taken into a 10 ml volumetric flask and volume was made up with mobile phase. (PHE-4 µg/mL, LIG-100 µg/ml and BEC-1 µg/mL).

System suitability parameters

System suitability tests were performed to verify that the resolution and repeatability of the system were adequate for the analysis intended. The parameters monitored for system suitability includes retention time, theoretical plate number, peak area, tailing factor and resolution. The repeatability of these parameters was checked by injecting three times the test solution of PHE-4 µg/mL, LIG-100 µg/ml and BEC-1 µg/mL. The results shown in Table 1 were within acceptable limits.

Method Validation

1) Specificity

Specificity of method can be termed as absence of any interference at retention times of samples. Specificity was performed by injecting blank and standard preparations. Chromatograms were recorded and retention times from sample and standard preparations were compared for identification of analytes.

2) Linearity and Range

A series of standard solutions 50-150 µg/ml of LIG, 0.5-1.5µg/ml of BEC and 2-6 µg/ml of PHE were prepared. An aliquot of 20µl of each solution was injected 3 times for each

standard solutions and peak area was observed. Plot of average peak area versus the concentration is plotted and from this the correlation coefficient and regression equation were generated. The calibration data of LIG, BEC and PHE is given in Table 2, while Figure 5A, Figure 5B and Figure 5C represents linearity graphs of three drugs respectively.

3) Precision

The method was validated in terms of intra-day inter-day precision. The solution containing PHE-4 µg/mL, LIG-100 µg/ml and BEC-1 µg/mL was injected six times for repeatability study. Inter-day and Intra-day study was performed by injecting 50,100,150 µg/ml of LIG and 0.5,1,1.5µg/ml of BEC and 2,4,6µg/ml of PHE solutions three times for each aliquots. The %RSD for precision study was found less than 2% as shown in Table 3.

4) Accuracy

Accuracy was determined by calculating recovery of PHE, LIG and BEC by the standard addition method. Known amounts of standard solutions of PHE (1.6, 2 and 2.4µg/ml), LIG (40, 50 and 60µg/mL) and BEC (0.4, 0.5 and 0.6µg/ml) were added to a pre quantified test solutions of PHE (2µg/ml), LIG (50µg/ml) and BEC(0.5µg/ml). Each solution was injected in triplicate and the recovery was calculated by measuring peak areas. Results obtained are shown in Table 4.

5) LOD and LOQ

LOD and LOQ for PHE and LIG and BEC were calculated as suggested by ICH guidelines using equations LOD = 3.3 σ/s and LOQ = 10 σ/s, respectively. Where, σ is the SD of the response and S is the slope of the calibration curve.

6) Robustness

The robustness study was performed to evaluate the influence of small but deliberate variation in the chromatographic condition. The robustness was checked by making two small changes. The mobile ration was changed by ± 0.2 ml and flowrate was changed by ± 0.02 ml/min and pH was changed by ± 0.2. After each changes sample solution was injected and system suitability parameters were observed. The results were shown in Table 5.

FIGURES

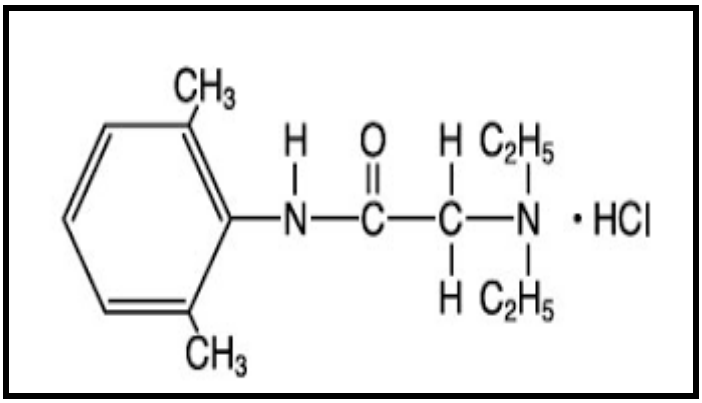

Figure 1: Chemical structure of LIG.

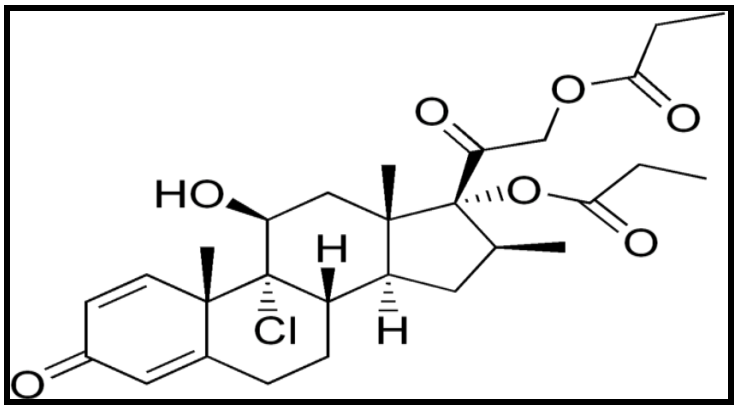

Figure 2: Chemical structure of BEC.

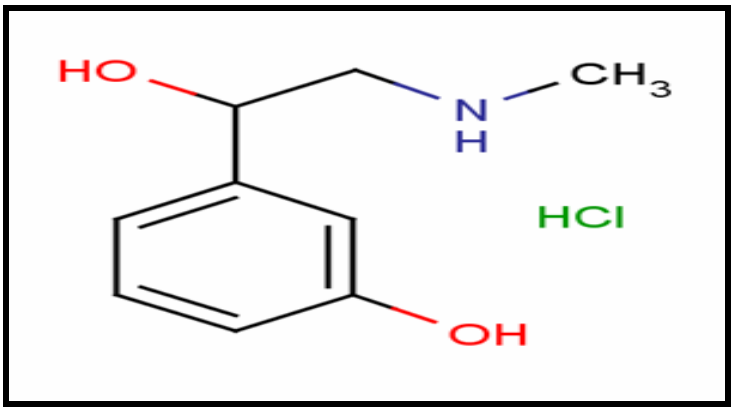

Figure 3: Chemical structure of PHE.

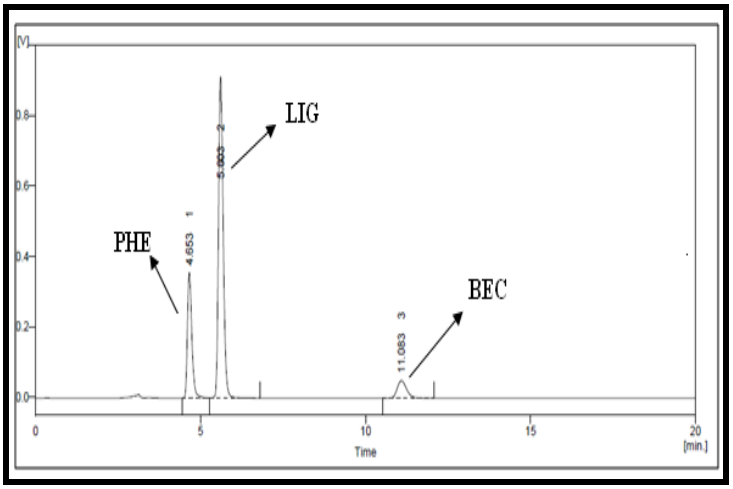

Figure 4: Optimised condition chromatogram of PHE,LIG and BEC.

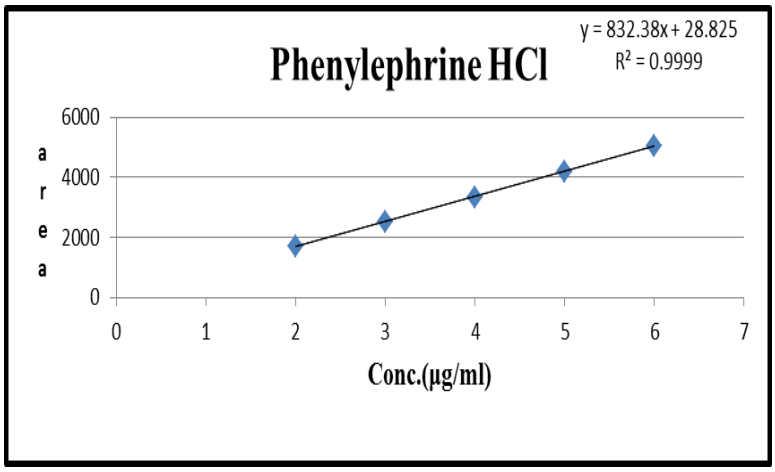

Figure 5A: Linearity graph for PHE.

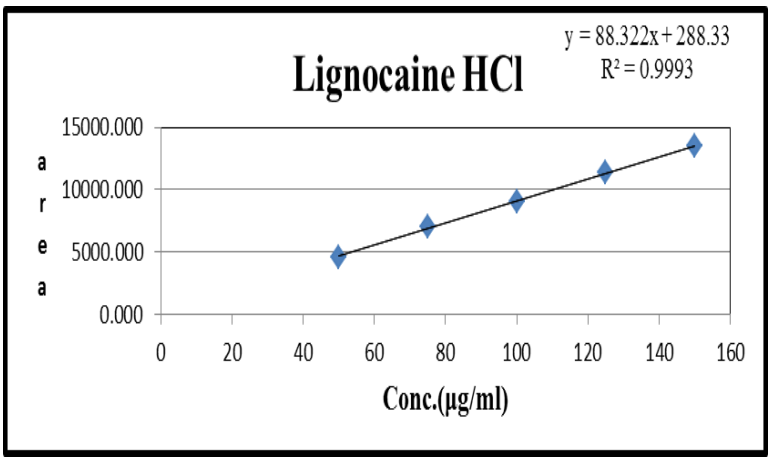

Figure 5B: Linearity graph for LIG.

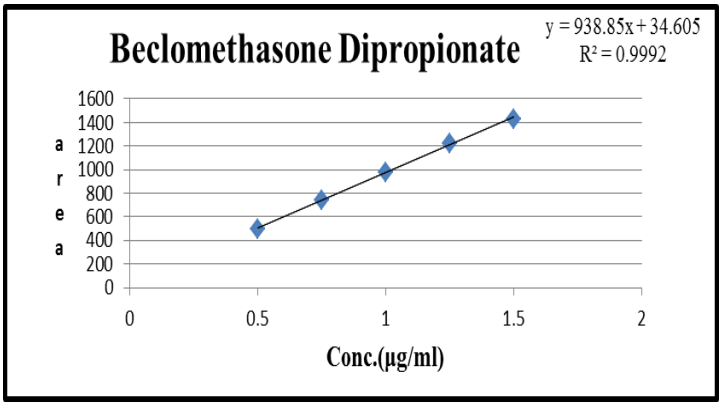

Figure 5C: Linearity graph for BEC.

TABLES

Table 1: Results for System suitability parameters.

Parameters	Data observed		
	PHE (mean ± SD)(n=3)	LIG (mean ± SD)(n=3)	BEC (mean ± SD)(n=3)
Theoretical plates per column	5879±8.00	7122±6.10	7426±7.11
Retentiontime	4.632±0.0034	5.577±0.006	11.020±0.0018
Tailing factor	1.542±0.02	1.393±0.036	1.376±0.03
Resolution	3.733±0.0245		14.040±0.0214
* = average of three determinations, SD=Standard deviation			

Table 2: Linearity study PHE, LIG and BEC.

Sr.No	Phenylephrine HCl			Lignocaine HCl			Beclomethasone Dipropionate		
	Conc. (µg/ml)	Average area *	% RSD	Conc. (µg/ml)	Average area *	% RSD	Conc. (µg/ml)	Average area *	% RSD
1	2	1695.285	0.6502	50	4602.543	0.6479	0.5	492.40167	0.6452
2	3	2532.263	1.7041	75	6873.6793	1.6623	0.75	734.23967	1.0475
3	4	3344.5663	0.3877	100	9094.8863	0.2580	1	973.135	0.9288
4	5	4192.5407	1.34877	125	11442.709	0.5659	1.25	1225.9643	0.6547
5	6	5027.0383	0.8316	150	13634.153	1.1152	1.5	1458.3183	1.6930
*= average of three determinations, RSD=Relative standard deviation									

Table 3: Precision study results for PHE, LIG and BEC.

Parameters	Conc.			% RSD		
	PHE (µg/ml)	LIG (µg/ml)	BEC(µg/ml)	PHE	LIG	BEC
Intra-day* precision	2	50	0.5	0.58	0.406	1.541
	4	100	1	0.77	0.125	0.683
	6	150	1.5	0.70	0.360	0.782
Inter-day* precision	2	50	0.5	1.321	0.849	0.759
	4	100	1	0.607	0.579	0.554
	6	150	1.5	1.144	1.034	0.902
Repeatability**	4	100	1	0.869	0.778	0.924
*= average of three determinations, **= average of six determinations						

Table:4The accuracy study of PHE,LIG and BEC.

Drug	Accuracy Level %	Amount taken (µg/ml)	Amount added (µg/ml)	Total Amount found* (mg/ml) ± S.D.	% Recovery ± SD
PHE	80%	2	1.6	1.599 ± 0.015	99.943 ± 0.971
	100%	2	2	1.978 ± 0.025	98.923 ± 1.268
	120%	2	2.4	2.397 ± 0.010	99.894 ± 0.439
LIG	80%	50	40	40.23 ± 0.208	100.578 ± 0.522
	100%	50	50	49.60 ± 0.526	99.206 ±1.504
	120%	50	60	60.082 ± 0.158	100.136 ± 0.264
BEC	80%	0.5	0.4	0.401 ± 0.004	100.288+ ± 1.013
	100%	0.5	0.5	0.499 ± 0.002	99.727 ± 0.504
	120%	0.5	0.6	0.602 ±0.001	100.308 ± 0.265
*= average of three determinations					

Table 5: Robustness study results for PHE, LIG and BEC.

Parameter	Change Level	Area		
		PHE	LIG	BEC
Mobile phase composition (±2.0 ml)	58:42	3253.847	8834.928	948.165
	60:40#	3236.593	8817.686	942.673
	62:38	3251.715	8656.624	952.938
	Mean±SD	3247.385 ± 9.407	8836.413±19.511	947.925±5.137
	% RSD	0.290	0.221	0.542
pH (±0.2)	4.8	3209.521	8714.713	935.286
	5.0#	3236.593	8817.686	942.673
	5.2	3185.228	8688.650	930.334
	Mean ± SD	3210.447 ±25.695	8740.350± 68.231	936.098 ± 6.209
	% RSD	0.800	0.781	0.663
Flow rate(±0.02 ml/min)	0.98	3266.44	8868.934	951.669
	1.0#	3236.593	8817.686	942.673
	1.02	3276.869	8922.178	959.265
	Mean±SD	3259.967±20.904	8869.599±52.249	951.202±8.306
	% RSD	0.641	0.589	0.873
#= actual parameter as control standard				

Table 6: Analysis of marketed formulation by proposed method,

Cream	Anovate cream								
	Phenylephrine HCl (0.1% w/w)			Lignocaine HCl (2.5% w/w)			Beclomethasone Dipropionate (0.025% w/w)		
Labeled amount(mg)	1 mg			25 mg			0.25 mg		
Amount found	0.9773	0.9942	0.9724	24.11	24.54	24.03	0.2423	0.2459	0.2443
Mean ± SD	0.9813±0.0114			24.2329±0.2766			0.2442±0.0018		
%RSD	1.162			1.142			0.749		
% Assay	97.73	99.42	97.24	96.47	98.19	96.12	96.56	98.39	97.73
Mean ± SD	98.135±1.1409			96.931±1.106			98.625±1.0300		
%RSD	1.162			1.141			1.0444		

RESULT AND DISCUSSION

System suitability study

The detection was carried out in the UV region at 240nm. The different composition of mobile phase was testing and the composition giving retention time of 4.632 min for PHE, 5.577 min LIG and 11.020 min for BEC with good resolution and theoretical plates was selected, that optimized mobile phase was Water (pH 4.0): Methanol (70:30 v/v). A chromatogram of the mixture in optimized conditions is shown Figure 4 and the system suitability parameters are shown in Table 1.

Method Validation

A) Specificity

The method was found to be specific as there was no interference observed in any of the parameters under observation.

B) Linearity and Range

The linearity of PHE, LIG and BEC were found between 2-6 µg/ml, 50-150 µg/ml and 0.5-1.5 µg/ml respectively. The results are shown in Table 2.

C) Precision

The %RSD for repeatability study for PHE, LIG and BEC were found 0.869, 0.778 and 0.924 respectively. The Inter-day and Intra-day study also show %RSD value for PHE, LIG and BEC within the acceptable limit. Results for precision study are shown in Table 3.

D) Accuracy

Accuracy of the method was confirmed by recovery study at three levels (50%, 100% and 150%) of standard addition. Percentage recovery for PHE was found to be 98.923-99.943%,

LIG was found to be 99.206-100.578, while for BEC it was found to be 99.727-100.308% as shown in Table 4.

E) LOD and LOQ

The LOD was found to be 0.036 µg/ml for PHE, 1.402 µg/ml for LIG and 0.016 µg/ml for BEC, while the LOQ was found to be 0.110 µg/ml for PHE, 4.250 µg/ml for LIG and 0.049 µg/ml for BEC.

F) Robustness

The typical variations studied under this parameter were mobile phase composition, pH and Flowrate. Overall %RSD was found to be less than 2% for all the variations which indicates that the proposed method is robust. Robustness data are shown in Table 5.

G) Analysis of marketed formulation by proposed method

Applicability of the proposed method was tested by analyzing the commercially available marketed formulation. The percentage of PHE, LIG and BEC were found to be 98.135% for PHE, 96.931% for LIG and 98.625% for BEC. Results as % Assay are shown in Table 6.

CONCLUSION

From the above discussion it can be concluded that the proposed method is specific, precise and accurate. Results are in good agreement with label claim which indicates there is no interference of exicipients. Therefore the proposed method can be used for routine analysis of Phenylephrine HCl, Lignocaine HCl and Beclomethasone Dipropionate in combined cream formulation.

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