VOLUME - 9, ISSUE - 12, DECEMBER - 2020 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Pharma

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF PHENYLEPHERINE HYDROCHLORIDE AND CETIRIZINE HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORM

Anil Kumar Goud.T
Prachi Kabra*
Department of Quality Assurance Nargund College of Pharmacy, Bangalore - 560 085 Karnataka, India.*Corresponding Author

Sridhar Vanga
Department of Pharmaceutical Chemistry, Vaageswari Institute of Pharmaceutical Sciences, Beside LMD Police Station, Ramakrishna Colony, Karimnagar,Telangana.-505481, India.

ABSTRACT RP-HPLC method have been developed and validated for simultaneous estimation of Phenylephrine hydrochloride and Cetirizine hydrochloride in bulk and pharmaceutical formulation.The RP-HPLC method for Phenylephrine hydrochloride and Cetirizine hydrochloride was developed with Ketotifen Fumarate as an Internal Standard using GraceSmart C18 column (250 mm × 4.6 mm, 5 m) as stationary phase and Acetonitrile:10mM Sodium Phosphate Dibasic Anhydrous buffer solution (40:60 % v/v, pH 6.2) as mobile phase. Mobile phase was maintained at a flow rate of 1.5 ml/min and detection was carried out at 220 nm. The amount of Phenylephrine and Cetirizine in marketed formulation by RP-HPLC methods was found to be in the range of 99.46 – 99.95 % and 99.24 – 100.84 %, respectively. Retention time was found to be 1.437 min, 2.373 min and 6.690 min for Ketotifen Fumarate, Phenylephrine hydrochloride and Cetirizine hydrochloride respectively. Results of assay and validation study were found to be satisfactory. The developed methods were statistically compared using One Way ANOVA with the help of Tukey-Kramer Multiple Comparison Test which suggested that there is no significant difference among the results of all the developed methods for both the drugs. So, the methods can be successfully applied for the routine analysis of Phenylephrine and Cetirizine in pharmaceutical formulation.

KEYWORDS : Phenylephrine hydrochloride, Cetirizine hydrochloride, Ketotifen fumarate, Absorption Ratio Method, Multi-Component Method, RP-HPLC.

1. INTRODUCTION

An allergy refers to an exaggerated reaction by our immune system in response to bodily contact with certain foreign substances. It is exaggerated because these foreign substances are usually seen by the body as harmless and no response occurs in non- allergic people. Allergic people's bodies recognize the foreign substance and one part of the immune system are turned on. Allergy-producing substances are called "allergens." Examples of allergens include pollens, dust mite, molds, danders and foods[1]. Recently a combination of Phenylephrine and Cetirizine came in to the market which is prescribed as an anti allergic agent.

Phenylephrine is a sympathomimetic with mainly direct effects on alpha-adrenergic receptors and weak beta-adrenergic activity. It causes vasoconstriction of the arterioles of the nasal mucosa and conjunctiva; activates the dilator muscle of the pupil to cause contraction; produces vasoconstriction of arterioles in the body and produces systemic arterial vasoconstriction. Phenylephrine is nasal decongestant its effectiveness as a decongestant stems from its vasoconstriction of nasal blood vessels, thereby decreasing blood flow to the sinusoidal vessels, leading to decreased mucosal edema [2].

Cetirizine, a human metabolite of hydroxyzine, is an antihistamine; its principal effects are mediated via selective inhibition of peripheral H_1 receptors. Cetirizine competes with histamine for binding at H_1-receptor sites on the effector cell surface, resulting in suppression of histaminic edema, flare and pruritus. The antihistaminic activity of Cetirizine has been clearly documented in a variety of animal and human models [3].

The combination of Phenylephrine and Cetirizine have additive or synergistic role in cough and anti allergic preparation, since both acts by attenuating the sign and symptoms of common cold and allergy [4].

Extensive literature survey revealed that there are very few analytical method reported for simultaneous estimation of Phenylephrine and Cetirizine. Hence, investigation of new chromatographic method (using Internal Standard) are in need for the quantitative estimation of these drugs in combination in pharmaceutical dosage form.

2. MATERIALS AND METHODS

Instrument and apparatus:

HPLC System,Liquid Chromatography: Shimadzu LC-20AT,UV-Visible Detector: Shimadzu SPD-20A, Analytical Column: GraceSmart C18 (250 mm × 4.6 mm, 5 μm)

Data Processor: Spinchrome CFR Software, Version 2.1.4.93,Injector: Rheodyne-7725i (Fixed Capacity Loop of 20 μl),Syringe**:** Hamilton, 25 μl, Electronic Weighing Balance (Sartorius-TE 214 S), Ultrasonicator (PCI Analytics), UV-Visible Spectrophotometer (Shimadzu-1700, Software Version-UV Prob 2.32), Digital pH Meter (Digisun Electronics-7007),Vacuum Pump (Servewell Instruments Pvt. Ltd.) with Supor 200 Membrane Filter, 0.2 μm (Pall India Pvt. Ltd.)

Reagents and Materials:

Phenylephrine hydrochloride (Standard) , Cetirizine hydrochloride (Standard) , Ketotifen Fumarate (Standard) , Allercet-DC Tablet (PHE-10 mg, CET-10 mg; manufactured by Micro Labs Ltd), Acetonitrile (HPLC Grade), Ortho-phosphoric acid (HPLC Grade), Sodium Phosphate Dibasic Anhydrous (Analytical Grade),Water (HPLC Grade).

Preparation of Standard Stock Solution

Standard Stock Solution of PHE

25 mg of standard PHE was weighed and transferred to 25 ml volumetric flask. PHE was dissolved in 10 ml of HPLC grade water by gentle shaking and volume was made up to the mark with the same solvent to obtain final concentration of 1000 μg/ml and labeled as 'Stock PHE-A'.

From the 'Stock PHE-A' solution 2.5 ml of aliquot was pipetted out in a 25 ml volumetric flask and the volume was made up to the mark with HPLC grade water to obtain final concentration of 100 μg/ml and labeled as 'Stock PHE-B'.

Standard Stock Solution of CET

25 mg of standard CET was weighed and transferred to 25 ml volumetric flask. CET was dissolved in 10 ml of HPLC grade

water by gentle shaking and volume was made up to the mark with HPLC grade water to obtain final concentration of 1000 µg/ml and labeled as 'Stock CET -A'.

From the 'Stock CET -A' solution 2.5 ml of aliquot was pipetted out in a 25 ml volumetric flask and the volume was made up to the mark with HPLC grade water to obtain final concentration of 100 µg/ml and labeled as 'Stock CET -B.

Standard Stock Solution of KETO

10 mg of standard KETO was weighed and transferred to a 100 ml volumetric flask and dissolved it in 100 ml of HPLC grade water to obtain final concentration of 100 µg/ml of KETO and labelled as 'Stock KETO'.

Combined Standard Stock Solution of PHE and CET

25 mg of standard PHE and 25 mg of standard CET were weighed and transferred to a 25 ml volumetric flask and dissolved it in about 10 ml of HPLC grade water with 5 min of sonication. The volume was made upto the mark to obtain final concentration of 1000 µg/ml of PHE and 1000 µg/ml of CET. The solution was labeled as 'Stock PC-A'.

From the 'Stock PC -A' solution 2.5 ml of aliquot was pipetted out in a 25 ml volumetric flask and the volume was made up to the mark with water to obtain final concentration of 100 µg/ml and labeled as 'Stock PC -B'.

Identification of Separated Peak of the Drugs

For identification of peak of the drugs; the standard solutions of 100 µg/ml of PHE, CET and KETO were prepared using HPLC grade water. All these solutions were filtered through 0.2 µm Supor 200 membrane filter using syringe and injected into the Rheodyne injector (20 µl) of HPLC system and their chromatograms were recorded under the finalized chromatographic conditions as described above after getting a stable baseline and retention time was noted for each drug.

Preparation of Calibration Curve for PHE and CET

From the 'Stock PC-B' solution (PHE-100 µg/ml and CET-100 µg/ml) 0.1, 0.5, 1, and 2 ml of aliquot were pipetted out in a series of 10 ml volumetric flasks and from 'Stock PC-A' solution (PHE-1000 µg/ml and CET-1000 µg/ml) 0.5, 1, 2 and 3 ml aliquot were pipetted out in a series of 10 ml volumetric flasks. Along with this 1 ml of 'Stock KETO' solution (100 µg/ml) was added in each flask. The volume was made up to the mark with HPLC grade water to obtain the concentration of 1, 5, 10, 20, 50, 100, 200 and 300 µg/ml for both PHE and CET with 10 µg/ml of KETO (IS). The solutions were filtered through 0.2 µm Supor 200 membrane filter using syringe and injected into the Rheodyne injector (20 µl) of HPLC system and their chromatogram was recorded under the finalized chromatographic conditions as described above after getting a stable baseline. Peak area were recorded for all the peaks. Peak area ratios between PHE to KETO and CET to KETO were calculated. Calibration curves of PHE and CET were constructed by plotting the peak area ratios between PHE to KETO *vs* PHE concentration and peak area ratios between CET to KETO *vs* CET concentration, respectively.

Analysis of Tablet Formulation

Twenty tablets of PHE and CET (Allercet-DC; PHE-10 mg, CET-10 mg micro labs] were weighed and crushed to obtain fine powder. An accurately weighed tablet powder equivalent to about 25 mg of PHE (25 mg of CET) was transferred to 25 ml volumetric flask. About 10 ml of HPLC grade water was added and the solution was sonicated for 15 min. The volume was made up to the mark with the same solvent to get concentration of 1000 µg/ml of PHE and 1000 µg/ml of CET. The resulting solution was filtered through whatman filter paper No.41 and this solution was used as a 'Sample Stock'.

From the above 'Sample Stock' solution 0.5 ml of the aliquot was pipetted out and transferred to a 10 ml volumetric flask along with this 1 ml of 'Stock KETO' solution (100 µg/ml) (IS) was added. The volume was made up to the mark with HPLC grade water to obtain a solution with final concentration of 50 µg/ml of PHE, 50 µg/ml of CET and 10 µg/ml of KETO.

Similarly, from the standard 'Stock PC-A' (1000 µg/ml of PHE, 1000 µg/ml of CET) solution 0.5 ml of aliquot was pipetted out in a 10 ml volumetric flask along with 1 ml of standard 'Stock KETO' solution (100 µg/ml). The volume was made up to the mark with HPLC grade water to obtain a solution with final concentration of 50 µg/ml of PHE, 50 µg/ml of CET and 10 µg/ml of KETO.

Both solutions (Standard and Sample) were filtered through 0.2 µm Supor 200 membrane filter using syringe and injected into the Rheodyne injector (20 µl) of HPLC system and their chromatograms were recorded under the finalized chromatographic conditions as described above after getting a stable baseline. (Figure No. 5.3.17). Peak areas were recorded for all the peaks. The amount of PHE and CET present in the tablets were calculated using single point analysis by following equation.

$$C_1 = \frac{R_1 C_2}{R_2}$$

Where, C_1 and C_2 = Concentration of Sample and Standard Solution, Respectively

R_1 = Peak Area Ratio of Drug to Internal Standard of Sample Solution

R_2 = Peak Area Ratio of Drug to Internal Standard of Standard Solution

Method Validation:

Linearity and Range

The concentration ranges 1-300 µg/ml for PHE and 1-300 µg/ml for CET were prepared and analyzed. The range of analytical method was decided from the interval between the upper and lower level of calibration curves by plotting the log curve .

Accuracy

To study the accuracy, 20 tablets were weighed and powdered. Analysis of the same was carried out as shown in section 4.3.1.11. Recovery studies were carried out by standard addition method by adding the known amount of PHE and CET (reference standard) to the pre analyzed sample at three different concentration levels i.e. 80%, 100% and 120% of assay concentration and percent recoveries were calculated.

From the 'Sample Stock' solution (PHE-1000 µg/ml and CET-1000 µg/ml) 0.5 ml of the aliquot was pipetted out and transferred to three different 10 ml volumetric flasks separately along with 0.4, 0.5, 0.6 ml of aliquot from the 'Stock PC-A' solution (PHE-1000 µg/ml and CET-1000 µg/ml) and 1 ml of aliquot from 'Stock KETO' solution (100 µg/ml) (IS). The volume was made upto the mark with HPLC grade water. All these solutions were filtered through 0.2 µm Supor 200 membrane filter using syringe and injected into the Rheodyne injector (20 µl) of HPLC system and their chromatograms were recorded under the finalized chromatographic conditions as described above after getting a stable baseline. (Figure No. 5.3.18) Peak areas were recorded for all the peaks. Peak area ratios between PHE to KETO and CET to KETO were calculated. From the above data percentage Recoveries were calculated for HPLC method.

Precision

The precision of an analytical method was studied by performing intermediate precision and repeatability.

Intermediate Precision

Intra-day Precision

Intra-day precision was determined by analyzing the combined standard solutions of PHE (10, 20, 50 µg/ml) and CET (10, 20, 50 µg/ml) with KETO (10 µg/ml) at three different time intervals on the same day.

Inter-day Precision

Inter-day precision was determined by analyzing the combined standard solutions of PHE (10, 20, 50 µg/ml) and CET (10, 20, 50 µg/ml) with KETO (10 µg/ml) on three consecutive days.

Variation by Different Analyst

Sample solutions of PHE (50 µg/ml) and CET (50 µg/ml) with KETO (10 µg/ml) were prepared in triplicate and analyzed by analyst 1 and analyst 2, separately. The values obtained were evaluated using F-test and t-test to verify their reproducibility.

Repeatability

Combined standard solutions of PHE (50 µg/ml) and CET (50 µg/ml) with KETO (10 µg/ml) were prepared and analyzed six time on the same day.

Linearity and Range

The concentration ranges 1-300 µg/ml for PHE and 1-300 µg/ml for CET were prepared and analyzed. The range of analytical method was decided from the interval between the upper and lower level of calibration curves by plotting the log curve as shown in Figure No.5.3.14 for PHE and 5.3.16 for CET.

Limit of Detection and Limit of Quantitation

Detection limit and Quantitation limit were determined based on the standard deviation of y-intercepts of six calibration curves and average slope of six calibration curves.[5-12]

3. RESULTS

High Performance Liquid Chromatographic Method-Reversed Phase HPLC Method

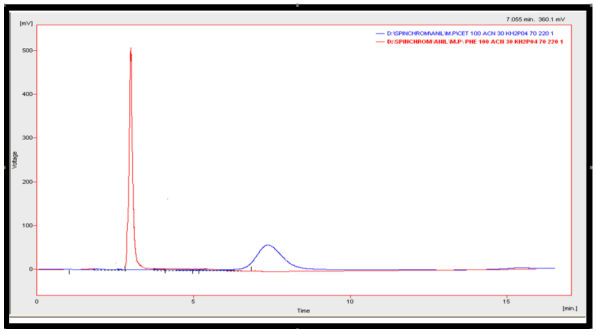

Figure No: 5.3.2. Overlain Chromatogram of PHE (100 µg/ml) and CET (100 µg/ml) in Acetonitrile: 10 mM KH_2PO_4 Buffer Solution (30:70 % v/v, pH 4.71) at a Flow Rate of 1 ml/min, at 220 nm Using C18 Column.

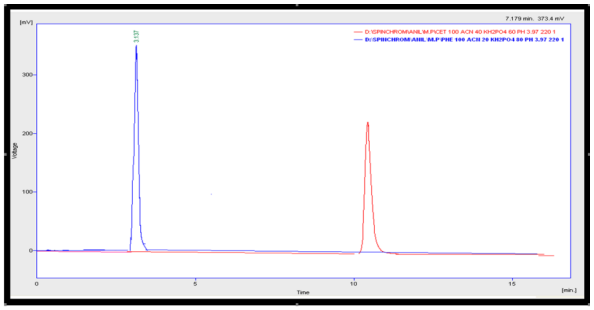

Figure No: 5.3.3. Overlain Chromatogram of PHE (100 µg/ml) and CET (100 µg/ml) in Acetonitrile: 10 mM KH_2PO_4 Buffer Solution (20:80 % v/v, pH 3.97) at a Flow Rate of 1 ml/min, at 220 nm using C18 column.

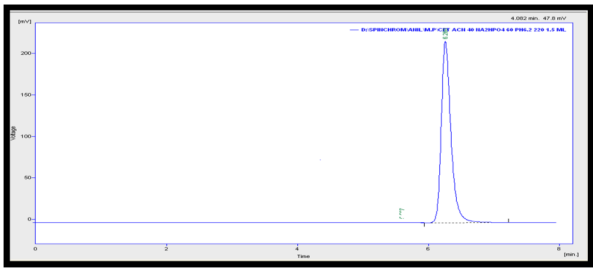

Figure No: 5.3.10. Chromatogram of CET (100 µg/ml) in Acetonitrile:10 mM Na_2HPO_4 Buffer Solution (40:60 % v/v, pH 6.2) at a Flow Rate of 1.5 ml/min, at 220 nm Using C18 Column.

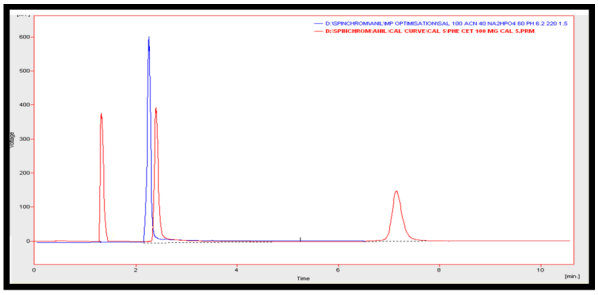

Figure No: 5.3.7. Overlain Chromatogram of KETO (100µg/ml),SAL (100 µg/ml), PHE (100 µg/ml) and CET (100 µg/ml) in Acetonitrile : 10 mM Na_2HPO_4 Buffer Solution (40:60 % v/v, pH 6.2) at a Flow Rate of 1.5 ml/min, at 220 nm Using C18 Column.

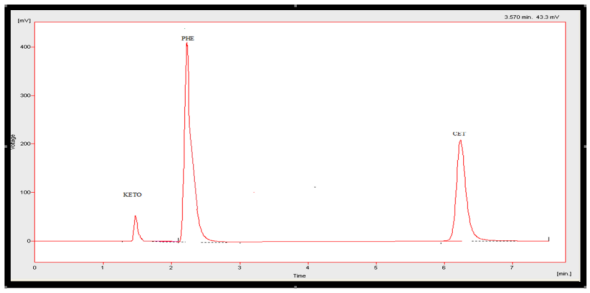

Figure No: 5.3.11. Chromatogram of KETO (10 µg/ml), PHE (100 µg/ml) and CET (100 µg/ml) in Acetonitrile:10mM Na_2HPO_4 Buffer Solution (40:60 % v/v, pH 6.2) at a Flow Rate of 1.5 ml/min, at 220 nm using C18 Column.

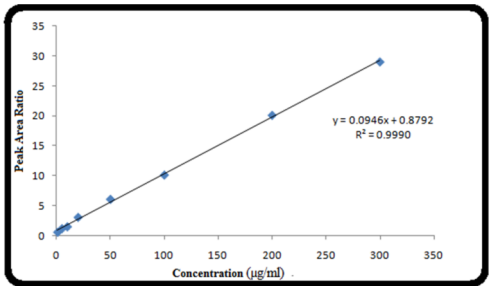

Figure No: 5.3.13. Calibration Curve of PHE of RP-HPLC Method

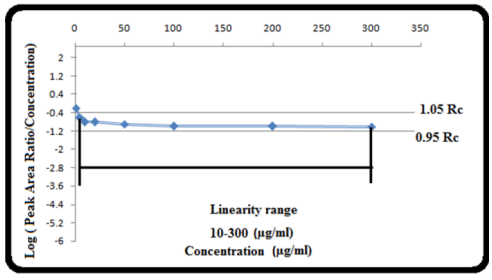

Figure No: 5.3.14. Graph for Linearity Study of PHE

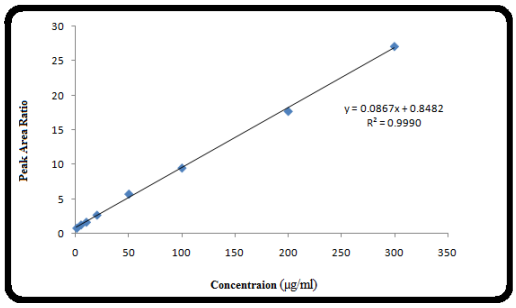

Figure No: 5.3.15. Calibration Curve of CET of RP-HPLC Method

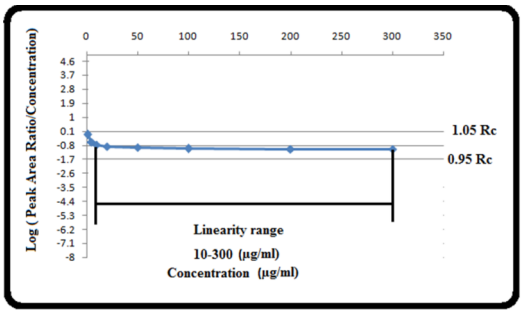

Figure No: 5.3.16. Graph for Linearity Study of CET

Table No: 5.3.4. Results of Calibration Curve of PHE

Conc. (µg/ml)	Peak Area Ratio							±SD	%RSD
	I.	II.	III.	IV.	V.	VI.	Mean		
1	0.621	0.624	0.619	0.635	0.642	0.623	0.627	0.0090	1.4493
5	1.211	1.265	1.220	1.215	1.219	1.243	1.228	0.0208	1.6977
10	1.547	1.578	1.523	1.582	1.511	1.539	1.546	0.0287	1.8562
20	3.121	3.109	3.179	3.089	3.052	3.119	3.111	0.0419	1.3475
50	6.111	6.109	6.150	6.166	6.021	6.140	6.116	0.0516	0.8441
100	10.200	10.320	10.190	10.110	10.010	10.150	10.163	0.1030	1.0142
200	20.110	20.050	20.190	20.170	20.010	20.120	20.108	0.0688	0.3422
300	28.990	29.110	29.010	28.860	29.050	28.950	28.995	0.0857	0.2956

Table No: 5.3.5. Linear Regression Analysis of Calibration Curves for PHE

Parameters	PHE at 220 nm
Linearity Range (µg/ml)	5-300
Slope	0.0946
Intercept	0.8972
Correlation Coefficient (r^2)	0.9990
SD of Y-intercept	0.0280
Average of Slope	0.0940
LOD (µg/ml)	0.983
LOQ (µg/ml)	2.979

Table No: 5.3.7. Linear Regression Analysis of Calibration Curves for CET

Parameters	CET at 220 nm
Linearity Range (µg/ml)	1-300
Slope	0.0867
Intercept	0.8482
Correlation Coefficient (r^2)	0.9990
SD of Y-intercept	0.0576
Average of Slope	0.0863
LOD (µg/ml)	2.2025
LOQ (µg/ml)	6.6743

Table No: 5.3.8. Results of Chromatogram of Sample Solution (Allercet – DC)

Analyte	Retention Time (min)	Area (mV.s)	Tailing Factor	Theoretical Plates	Resolution (R)
KETO	1.437	162.470	1.583	3176	-
PHE	2.373	1747.145	1.500	4494	7.711
CET	6.690	1361.960	1.436	9685	20.933

Table No: 5.3.9. Assay Results of Tablet Formulation by RP-HPLC Method

Sr. No.	Amount Present (mg/tab)		Amount Found (mg/tab)		% Assay	
	PHE	CET	PHE	CET	PHE	CET
1	10	10	9.95	9.92	99.52	99.29
2	10	10	9.96	10.02	99.61	100.28
3	10	10	9.99	10.05	99.95	100.59
4	10	10	9.97	9.92	99.72	99.24
5	10	10	9.94	10.01	99.46	100.19
6	10	10	9.95	10.08	99.59	100.84
Mean			9.96	10.07	**99.64**	**100.07**
± SD			0.0177	0.6690	0.1774	0.6690
%RSD			0.1780	0.6685	0.1780	0.6685

Table No: 5.3.10. Results of Accuracy Study for RP-HPLC Method

% Level of Recovery	Sr. No.	Amount of Standard Drug Added (µg/ml)		Total Amount Found (µg/ml)		Total Amount Recovered (µg/ml)		% Recovery	
		PHE	CET	PHE	CET	PHE	CET	PHE	CET
80 %	1	40	40	89.75	89.78	39.94	40.04	99.87	100.11
	2	40	40	90.33	90.21	40.52	40.47	101.30	101.19
	3	40	40	89.91	89.59	40.10	39.86	100.26	99.65
100 %	1	50	50	100.19	99.54	50.37	49.80	100.75	99.60
	2	50	50	99.66	98.42	49.85	48.69	99.71	97.38
	3	50	50	99.99	99.04	50.18	49.30	100.37	98.61
120 %	1	60	60	110.10	109.17	60.28	59.43	100.48	99.05
	2	60	60	109.94	109.35	60.13	59.62	100.22	99.36
	3	60	60	110.77	108.98	60.96	59.25	101.61	98.75

Table No: 5.3.11. Statistical Validation Data for Accuracy Study

Level of % Recovery	Mean* (% Recovery)		±SD		% RSD	
	PHE	CET	PHE	CET	PHE	CET
80 %	100.47	100.32	0.7414	0.7921	0.7378	0.7896
100 %	100.28	98.53	0.5277	1.1137	0.5262	1.1303
120 %	100.77	99.05	0.7392	0.3087	0.7336	0.3116

*Mean of 3 Estimations

Table No: 5.3.12. Results of Intra-day Precision of PHE

Concentration (µg/ml)	Peak Area Ratio at Following Time (hr)			Mean	±SD	% RSD
	0	2	4			
10	1.5441	1.5462	1.5483	1.5462	0.0021	0.1358
20	3.1013	3.1134	3.1243	3.1130	0.0115	0.3695
50	6.1019	6.1132	6.1254	6.1135	0.0117	0.1922

Table No: 5.3.13. Results of Intra-day Precision of CET

Concentration (µg/ml)	Peak Area Ratio at Following Time (hr)			Mean	±SD	% RSD
	0	2	4			
10	1.632	1.636	1.638	1.6353	0.0030	0.1868
20	2.651	2.652	2.661	2.6546	0.0055	0.2074
50	5.691	5.701	5.709	5.7003	0.0090	0.1582

Table No: 5.3.14. Results of Inter-day Precision of PHE

Concentration (µg/ml)	Peak Area Ratio at Following Day			Mean	±SD	% RSD
	1	2	3			
10	1.5483	1.5521	1.5641	1.5548	0.0082	0.5304
20	3.1243	3.1454	3.1576	3.1416	0.0166	0.5313
50	6.1254	6.1267	6.1812	6.1444	0.0318	0.5183

Table No: 5.3.15. Results of Inter-day Precision of CET

Concentration (µg/ml)	Peak Area Ratio at Following Day	Mean	±SD	% RSD

	1	2	3			
10	1.638	1.647	1.653	1.6460	0.0075	0.4586
20	2.673	2.683	2.695	2.6836	0.0110	0.4104
50	5.709	5.778	5.812	5.7663	0.0524	0.9101

Table No: 5.3.16. Results of Variation by Different Analyst Study for PHE (50 µg/ml)

(%Assay* ± SD)		Result of F-test	Result of t-test	Inference
Analyst 1	Analyst 2			
99.69 ± 0.0534	99.59 ± 0.0172	0.3218	0.0609	No significant difference

***Mean of 3 Estimations**

Table No: 5.3.17. Results of Variation by Different Analyst Study for CET (50 µg/ml)

(%Assay * ± SD)		Result of F-test	Result of t-test	Inference
Analyst 1	Analyst 2			
100.05 ± 0.4652	100.09 ± 0.6526	1.4027	0.0582	No significant difference

*Mean of 3 Estimations

Table No: 5.3.18. Results of Repeatability Study for PHE and CET

Sr. No.	Peak Area Ratio	
	PHE (50 µg/ml)	CET (50 µg/ml)
1	6.113	5.721
2	6.109	5.69
3	6.11	5.723
4	6.166	5.734
5	6.15	5.684
6	6.14	5.752
Mean	6.131333	5.717333
± SD	0.024147	0.026013
% RSD	0.393826	0.454982

Table No: 5.3.19. Result of Robustness Study: Variation in Flow Rate (ml/min)

Flow Rate (ml/min)	Analyte	Retention Time* (min)	Tailing Factor (T)	Theoretical Plates (N)	Resolution (R)
1.47#	KETO	1.473	1.846	3349	-
	PHE	2.453	1.176	5381	7.987
	CET	6.910	1.478	10313	21.384
1.50#	KETO	1.437	1.769	3176	-
	PHE	2.370	1.722	4481	7.711
	CET	6.690	1.487	9714	20.934
1.53#	KETO	1.417	1.769	3088	-
	PHE	2.293	1.438	4945	7.539
	CET	6.427	1.432	9946	19.689

*% RSD was found to be less than 4 % for each drug; #Mean of 3 Estimations

Table No: 5.3.20. Result of Robustness Study: Variation in Organic Solvent Ratio in Mobile Phase

Mobile Phase (Acn:Buffer %v/v)	Analyte	Retention Time* (min)	Tailing Factor (T)	Theoretical Plates (N)	Resolution (R)
39.2:60.8#	KETO	1.460	1.846	3280	-
	PHE	2.398	2.00	6050	7.815
	CET	6.827	1.360	10659	20.978
40:60#	KETO	1.437	1.308	3310	-
	PHE	2.310	1.737	4633	7.689
	CET	6.680	1.352	11053	20.856
40.8:59.2#	KETO	1.410	1.846	3509	-
	PHE	2.290	1.875	5844	7.567
	CET	6.597	1.361	11413	20.687

*% RSD was found to be less than 4 % for each drug; #Mean of 3 Estimations

Table No: 5.3.21. System suitability Results of the Proposed Method (n=6)

Analyte	R	N	T	% RSD	
				R_t	Peak Area Ratio
KETO	-	3310	1.308	0.0814	-
PHE	7.689	4633	1.737	0.7067	0.6813
CET	20.856	11053	1.352	0.1126	0.1642
Required limits	R>2	N>2000	T<2	R.S.D. <1%	

R-Resolution factor, N-Number of theoretical plates, T-Tailing factor

4. DISCUSSION

Optimization of Chromatographic Condition

To optimize mobile phase composition, different ratios of solvents were tried. First trial was done using acetonitrile: 10Mm potassium dihydrogen ortho phosphate buffer (pH 4.71) in the ratio of 30:70 % v/v. It was observed that theoretical plates for CET peak were less than 2000. So further trial was done using acetonitrile : 10mM potassium dihydrogen ortho phosphate buffer (20:80% v/v at pH 4), acetonitrile : 10mM Sodium phosphate di-basic buffer (40:60% v/v,35:65% v/v at pH 5, 40:60 % v/v at pH 6.2), It was observed that as organic solvent content of mobile phase decreased the retention time of both the drugs increased.

Finally the mobile phase containing acetonitrile : 10 mM Sodium phosphate di-basic buffer at pH 6.2 in the ratio of 40:60 % v/v at 1.5ml/min flow rate gave satisfactory results, so this mobile phase was finalized.

Selection of analytical wavelength for detection in HPLC

The standard solutions of PHE (10 µg/ml) and CET (10 µg/ml) in HPLC grade water were scanned in the UV region of 200-400 nm and the overlain spectra were recorded. Both the drugs showed good absorbance at 220 nm, which was selected as wavelength for analysis.

Effect of pH

Considering the pKa value of PHE (8.9, 10.1) and CET (1.6, 2.9, 8.3) different pH values of the mobile phase were tried in the range of 4.0 to 6.2. Finally, the best results were obtained at pH 6.2 by using 1% ortho phosphoric acid. The choice of this pH for the mobile phase is justified by the excellent symmetry of the peaks and the adequate retention times of PHE and CET.

Effect of flow rate

The flow rate of 1.5 ml/min was selected where the column plate number (N) between the peaks was observed maximum, with the best resolution.

Internal standard

Salbutamol Sulphate (SAL) and Ketotifen fumarate (KETO) were tried as an internal standard but SAL was showing overlapping peak with PHE peak. While KETO showed sharp peak with good compatibility with the other drug and best resolution between the peaks so it was selected as an internal standard.

Assay of Marketed Formulation

Amount of drugs present in the marketed formulation (Allercet-DC) were calculated using equation mentioned in the Section No. 4.3.1.11. The mean% assay was found as 99.64 % and 100.07 % for PHE and CET respectively.

Validation Parameters

This method was validated in accordance to ICH guidelines. Percentage of recoveries of PHE and CET were found in the range from 99.71 - 101.61 % and 97.38 - 101.19 % respectively. Precision of the method was determined by % RSD found among intra-day precision, inter-day precision, repeatability. It was found to be less than 1 %. Variation of results by two

different analyst was determined by preparing and measuring the sample solutions of PHE (50 μg/ml) and CET (50 μg/ml) by Analyst 1 and Analyst 2, separately. The values obtained were evaluated using F-test and t-test to verify their precision. Calculated values for t-test were found to be 0.0609 for PHE and 0.0582 for CET, which are less than the tabulated or standard value (1.533) hence no significant difference was observed between the results of two analysts. LOD and LOQ of PHE were found to be 0.9831 and 2.9792 μg/ml, respectively. LOD and LOQ of CET were found to be 2.202 and 6.674 μg/ml, respectively.

For robustness study, the effect of change in the pH (±2%) of mobile phase,organic phase ratio (±2%) and flow rate (±2%) on the retention time, asymmetry factor, theoretical plates and resolution were studied. Combined standard solutions of PHE (50 μg/ml), CET(50 μg/ml) and KETO (10 μg/ml) were prepared and analyzed at different pH (6.08, 6.20, 6.32) of the mobile phase, at different organic phase ratio (39.2:60.8, 40:60, 40.8:59.2 %v/v) and at different flow rate (1.47, 1.5, 1.53 ml/min). The method was found to be pH sensitive. As pH decreased PHE peak got splitted and retention time of CET was increased. Percentage RSD of retention time was found to be less than 4 %, when flow rate and organic phase changed.

Linearity Study

PHE and CET were found to be linear in the concentration range of 5-300 μg/ml and 10-300 μg/ml, respectively.

5.CONCLUSION

RP-HPLC method for estimation of Phenylephrine and Cetirizine was developed with Ketotifen as an internal standard. The methods were validated according to ICH guidelines. Results of assay and validation study were found to be satisfactory. The developed method was statistically compared using One Way ANOVA with the help of Turkey-Kramer Multiple Comparison Test. So, this method can be successfully applied for the routine analysis of Phenylephrine and Cetirizine in pharmaceutical formulation.

REFERENCES

1. http://en.wikipedia.org/wiki/Allergy(access date June 10, 2012).
2. http://www.drugs.com/mmx/Phenylephrine (access date June 15, 2012).
3. http://www.drugbank.ca/drugs/ Cetirizine (access date Nov 24, 2011).
4. http://en.wikipedia.org/wiki.answer.com/Q/Can you take cetirizine and phenylephrine together (access date June 10, 2012).
5. Soni LK, Narsinghani T, Saxena C. Development and validation of UV-Spectrophotometric assay protocol for simultaneous estimation of Ebastine and Phenylephrine Hydrochloride in tablet dosage form using simultaneous equation method. Int J ChemTech Res. 2011Oct-Dec; 3(4): 1918-25. [http:// sphinxsai.com/Vol.3No.4/chem/pdf/CT=31(1918-1925)OD11.pdf]. (access date JUNE 18, 2012).
6. Theia'a N, Al-Sabha. Spectrophotometric Assay of Phenylephrine Hydrochloride Using 4-Aminoantipyrine and Copper (II). Pak J Anal. Environ Chem. 2010; 11(1):1-7 [http://core.kmi.open.ac.uk/display/1024630]. (Access date JUNE 17, 2012).
7. Savić I, Nikolić G, Banković V. Development and validation of spectrophotometric method for Phenylephrine hydrochloride estimation in nasal drops formulations. Maced J Chem Chem Eng. 2008 Aug; 27(2):149-[56 Shttp://www.docstoc.com/docs/56442663]. (Access date JUNE 19, 2012).
8. Cieri UR. Determination of Phenylephrine hydrochloride, Chlorpheniramine maleate and Methscopolamine nitrate in tablets or capsules by liquid chromatography with two UV absorbance detectors in series.U.S. Food and Drug Administration [http:// www.ncbi.nlm.nih.gov/ pubmed/16512228]. (Access date JUNE 14, 2012).
9. Marin A, Garcia E, Garcia A, Barbas C. Validation of a HPLC quantification of Acetaminophen, Phenylephrine and Chlorpheniramine in pharmaceutical formulations: capsules and sachets. J Pharmaceut Biomed Anal.2002; 29:70114. [http://dspace.ceu.es/bitstream/10637/762/1/p%20701_14.pdf]. (Access date JUNE 14, 2012).
10. Hudecová T, Hatrík S, Zimová N, Havránek E. Validation of the HPLC method in the determination of Dioxopromethazine and Phenylephrine in eye drops. Ceska Slov Farm.2002 Mar; 51(2):91-5 [http:// www.ncbi.nlm.nih.gov/ pubmed/ 11928283]. (Access date JUNE 13, 2012)
11. Rawool ND, Venkatchalam A, Singh KH. Development and validation of a rapid RP-HPLC method for the simultaneous estimation of Cetirizine and Pseudoephedrine in pharmaceutical dosage forms. Int J Curr Pharm Res. 2013;5(1):54-60. [Available from http:// www.ijcpr.org/ Issues/ Vol5Issue1/ 642. pdf, access date Feb 28, 2013]
12. Padmavathi N, Niranjan MS. Development and validation of HPLC method for simultaneous estimation of Cetirizine dihydrochloride with Aceclofenac. IntJ Pharm Res Dev. 2012;4(3):268-73. [Available from http:// www.ijprd.com/ DEVELOPMENT % 20AND % 20 VALIDATION % 20 OF % 20 HPLC % 20 METHOD % 20 FOR % 20 SIMULTANEOUS % 20 ESTIMATION % 20 OF % 20 CETRIZINE % 20 DIHYDROCHLORIDE % 20WITH % 20 ACECLOFENAC. pdfaccess date Feb 28, 2013]