International Journal of Biomedical and Advance Research
ISSN: 2229-3809 (Online); 2455-0558 (Print)
Journal DOI: https://doi.org/10.7439/ijbar
CODEN: IJBABN
Original Research Article

Simultaneous estimation of Cetrizine hydrochloride and Phenylephrine hydrochloride in tablet dosage form by RP-HPLC

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QR Code

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***Article History:**
Received: 20/12/2017
Revised: 26/12/2017
Accepted: 27/12/2017
DOI: https://doi.org/10.7439/ijbar.v8i12.4542

Abstract

A simple, economical, specific, accurate, precise and validated Reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the simultaneous estimation of Cetrizine hydrochloride and Phenylephrine hydrochloride in synthetic mixture and its combined dosage form. The chromatographic separation was achieved on C18 column (Intersil, 220 mm × 4.6 mm i.d., 10 µm particle size) at ambient temperature using disodium hydrogen phosphate (Na_2HPO_4) buffer; pH was adjusted to 6.50 using ortho-phosphoric acid and a mobile phase of Methanol: Acetonitrile: Disodium hydrogen phosphate (80: 4: 19, v/v) at flow rate 2.0 ml/min. Quantification was achieved with UV detector at wavelength 270 nm. The retention time was found to be 1.2 ± 0.04 min and 3.1 ± 0.06 min for cetirizine HCl and phenylephrine HCl respectively. The calibration curves were linear with correlation coefficient 0.998 and 0.999 in the range of 10-50 µg/ml for Cetrizine hydrochloride and Phenylephrine hydrochloride. The methods were validated in terms of linearity, precision, accuracy, LOD, LOQ and ruggedness according to ICH guideline.

Keywords: Phenylephrine Hydrochloride, Cetirizine hydrochloride, Reversed-phase HPLC, Disodium hydrogen phosphate, Methanol, Acetonitrile.

1. Introduction

HPLC methods are useful in the determination of drugs in pharmaceutical dosage forms and biological sample. Owing to the widespread use of HPLC in routine analysis, it is important that good HPLC methods are developed and that these are thoroughly validated [1,2]. The separation mechanism in reversed phase chromatography depends on the hydrophobic binding interaction between the solute molecule in the mobile phase and the immobilised hydrophobic ligand, i.e. the stationary phase [3]. Reversed phase mode is the most popular mode for analytical and preparative separation of compounds of interest in chemical, biological, pharmaceutical, food and biomedical sciences. In this mode, the stationary phase is nonpolar hydrophobic packing with octyl or octa decyl functional group bonded to silica gel and the mobile phase is polar solvent. In summary, separations in reversed phase chromatography depend on the reversible adsorption/ desorption of solute molecules with varying degrees of hydrophobicity to a hydrophobic stationary phase [4].

Cetirizine Hydrochloride (CTZ HCl), 2-(2-{4-[(4-chlorophenyl) (phenyl) methyl] piperazine-1-yl} ethoxy) acetic acid hydrochloride is selective Histamine H-1 antagonist used primarily as an anti-allergic agent. Phenylephrine Hydrochloride is 3 - [(1R)-1- hydroxyl -2-(methylamino) ethyl] phenol hydrochloride. It is a selective α-1 adrenergic receptor agonist used primarily as a

decongestant [5,6]. Liquid chromatography is the only available official method for the estimation of both drugs in single dosage forms [7]. CTZ HCl and PHE HCl combination is not official in any pharmacopoeia, hence no official method is available for estimation of these two drugs in combined dosage forms.

Literature survey reveals that several analytical methods have been reported for the determination of PPEH and CETH as individual and combined dosage form with each other and with other combination of other drugs such as RP-HPLC, Spectrophotometric, HPTLC, Fluorimetry and ion- pair Chromatographic method [8-17]. The aim of present work was to develop simple, sensitive, accurate, and precise methods for routine analysis. The proposed method was validated according to ICH guidelines [9].

2. Experiment work

2.1 Instrument and apparatus

Perkin HPLC instrument quipped with series 200 UV/ Visible detector, series 200 pump, series 200 vacuum degasser, chromatography interface 600 series link and with a universal loop injector (Rheodyne 7725*i*) of an injection capacity of 20 µL. Separation was carried out on a reverse phase C18 column (Intersil, 220 × 4.6 mm column length), under reversed phase partition chromatographic conditions and Particle size of packing was 10 µm. The equipment was controlled by a PC workstation. The output signal was monitored and integrated by LC Solutions software for the analysis. Analytical balance (Mettler Toledo), digital pH meter (Eutech instruments pH tutor) was used during analysis.

2.2 Chemicals and reagents

Working standards of phenylephrine hydrochloride (potency = 99.45%) and cetirizine hydrochloride (potency = 99.12%) were obtained as a gift samples from Cipla Ltd. Mumbai. The solution of 0.1N NaOH was prepared in double distilled water as per IP 1996 procedure. AR grade Methanol was procured from S.D. Fine Chemical Ltd., Mumbai, India. HPLC grade Methanol, Acetonitrile and Ortho-phosphoric acid were purchased from Finar Chemicals Ltd, Ahmedabad, India. Disodium hydrogen phosphate (Na2HPO4) was also purchase form Finar Chemicals Ltd, Ahmedabad, India. Whatman filter paper no.1 was purchase from Whatman International Ltd., England and HPLC grade water from RFCL limited, New Delhi, India. The commercial combined dose tablet formulation containing Phenylephrine hydrochloride and Cetirizine hydrochloride in the ratio1:1, Allercet-DC manufactured by Micro Labs Ltd, was purchased from local market.

2.3 Preparation of standard stock solution

Standard stock solution (100µg/ml) of CTZ and PHE were prepared by transferring 10mg of CTZ and PHE in 100 ml volumetric flasks separately, dissolved in diluent (methanol) with sonication for 10 minutes and diluted up to mark with diluents (methanol). From this standard stock solution, mixed standard solutions were prepared with mobile phase. The aliquot portion of the filtrate was further diluted with mobile phase to get final concentration of 50µg/ml for both the drugs.

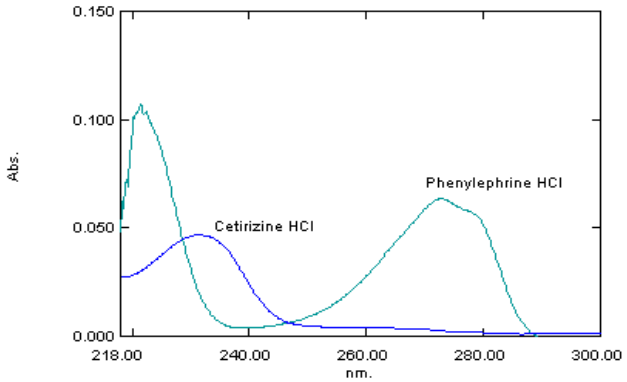
2.4 Preparation of buffer (10 mM Na_2HPO_4)

Accurately weighed quantity of 3.58 gm disodium hydrogen phosphate (Na_2HPO_4) was transferred in 1000 ml beaker, dissolved in 200.0 ml HPLC grade water and sonicated for about 10 min and diluted up to the mark with HPLC grade water. It was filtered through 0.45 µm membrane filter. Buffer pH was adjusted to 6.50 using ortho-phosphoric acid.

2.5 Selection of detecting wavelength

The suitable wavelength for detection was selected from overlain spectrum of CTZ HCl and PHE HCl. The standard solution of CET and PHE were injected under the chromatographic condition described above. Detection was carried out at different wavelength best response was achieved at 270 nm with UV/ Visible detector. So both drugs were detected at this analytical wavelength.

Figure 1: Overlay spectra of PHE (10µg/ml) and CTZ (10µg/ml)



2.6 Selection of chromatographic parameters

The following chromatographic conditions were maintained throughout the method development.

Column	:	C_{18} Intersil, 220 × 4.6 mm column length
Particle size of packing	:	10 µm
Mobile Phase	:	Methanol: Acetonitrile disodium hydrogen phosphate p^H was adjusted to 6.50 with ortho phosphoric acid
Detection wavelength	:	270 nm
Flow rate	:	2 ml/min.
Temperature	:	Ambient
Sample size	:	20 µl

2.7 Preparation of calibration curves

The aliquot portions standard stock solutions of CTZ HCl (50µg/ml) and PHE HCl (50µg/ml) were further diluted with mobile phase to get the series of concentrations ranging from 10-50µg/ml for both the drugs. The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. Then each dilution of both the drugs was injected and peak areas recorded. The graphs plotted as concentration of drug Vs peak areas and were shown in figure 2 and 3.

Figure 2: Standard calibration curve for CTZ HCl

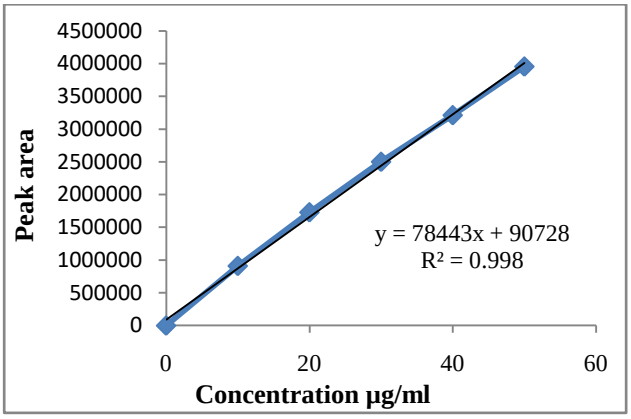
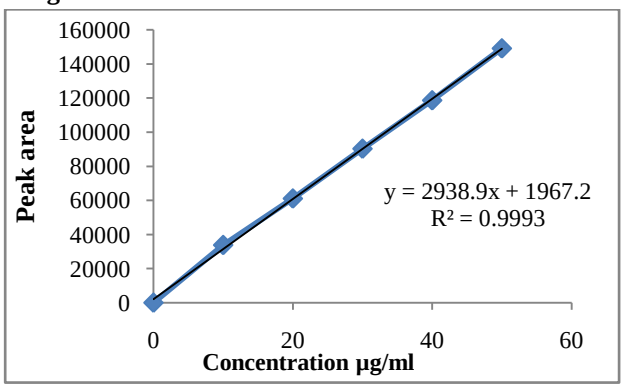


Figure 3: Standard calibration curve for PHE HCl



2.8 Application of proposed method to standard laboratory mixture

The laboratory mixture was prepared in the ratio of 50:50 % w/w for CTZ HCl and PHE HCl respectively.

2.8.1 Standard solution

Accurately weighed quantities equivalent to 10 mg of both the drugs were dissolved in methanol in 100 ml volumetric flask. Volume was made up to the mark with methanol. The solution was filtered through Whatman filter paper No.1. The aliquot portion of the filtrate was further diluted with mobile phase to get final concentration of 50µg/ml for both the drugs.

2.8.2 Samples Preparation

Five different laboratory mixtures (10-50µg/ml) of CTZ HCl and PHE HCl were prepared by same procedure as for laboratory mixture standard so as to get the final concentration 50 µg/ml for both the drugs. The mobile phase was allowed to equilibrate with stationary phase until steady base line was obtained. Then laboratory mixture was injected and chromatogram and peak areas were recorded (Figure 4). The amount of each drug in laboratory mixture was calculated using following formula;

$$\% \text{ Estimation} = \frac{A_t}{A_s} \times \frac{D_S}{Dt} \times \frac{W_S}{W_t} \times 100$$

Where,

A_t = Area for sample solution
D_s = Dilution factor for standard
W_s = Weight of standard (mg)
A_S = Area for standard solution
D_t = Dilution factor for sample
W_t = Weight of sample (mg)

Figure 4: Typical chromatogram of standard laboratory mixture

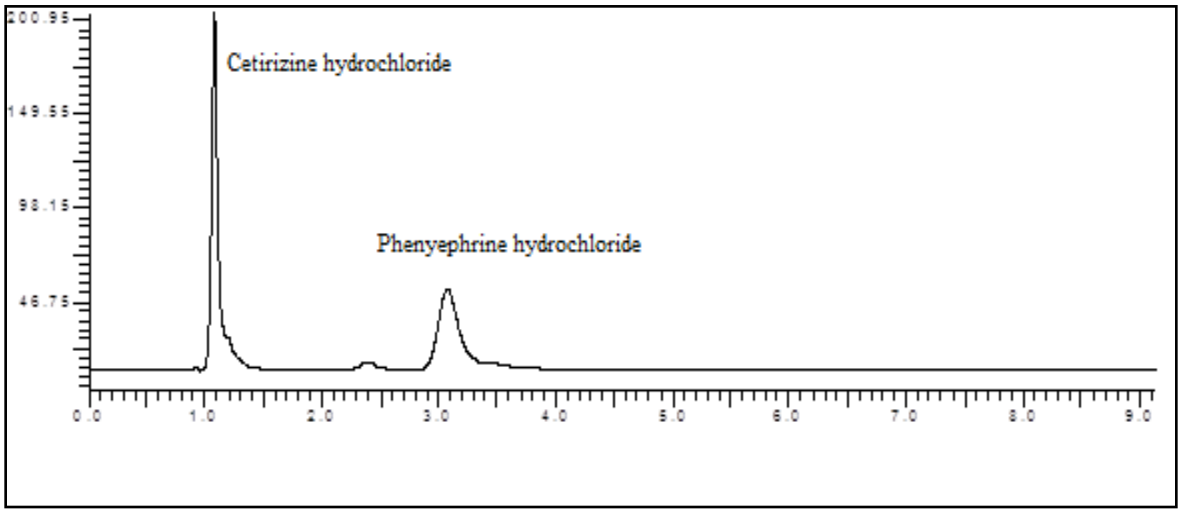


Table 2: Observations and results of standard laboratory mixture analysis

Sr. No.	Laboratory mixture	Weight of pure drug in µg/ml		Peak area		% Estimation	
		CTZ HCl	PHE HCl	CTZ HCl	PHE HCl	CTZ HCl	PHE HCl
1	Standard	50	50	384587.3	151017	99.99	100.6
2	samples	10	10	912413	158021.4	99.87	99.93
3		20	20	1690112	61041.34	98.57	101.6
4		30	30	2501234	89829.5	100.0	99.80
5		40	40	3191451	116489.4	100.6	99.4
6		50	50	381863.8	151124	100.1	100.4
					Mean	99.85	100.2
					±S.D.	0.6790	0.7727
					± R.S.D.	0.68	0.771

2.9 Application of proposed method to estimation in marketed formulation

2.9.1 Standard Solution

Accurately weighed quantities equivalent to 10 mg of CTZ HCl and 10 mg of PHE HCl were dissolved in methanol in 100.0 ml volumetric flask. Volume was made up to the mark with methanol. The solution was filtered through Whatman filter paper No.1. The aliquot portion of the filtrate was further diluted with mobile phase to get final concentration of 50µg/ml for both drugs.

2.9.2 Sample Preparation

Twenty tablets were crushed to fine powder; weighed equivalent to 10 mg of CTZ HCl was transferred to 100.0 ml volumetric flask. Then to each flask about 50.0 ml of methanol was added and sonicated for 30 min, finally volume was made to mark with methanol. The extracts were filtered through Whatman filter paper No.1 and required dilutions were made with mobile phase to get the final concentrations containing 50µg/ml for CTZ HCl and 50µg/mL for PHE HCl. The mobile phase was allowed to equilibrate with stationary phase until steady base line was obtained each (20µl) volume of standard and sample solution were injected and chromatogram and peak areas were recorded (Figure 5).

Figure 5: Chromatograms of CTZ HCL (Rt 1.3) with PHE HCl (Rt 3.2) in marketed formulation

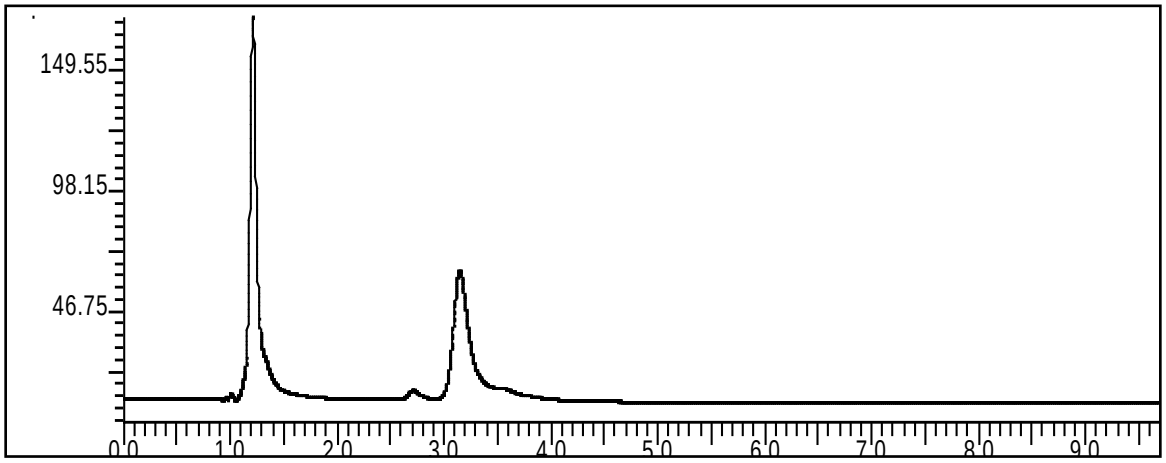


Table 3: Observation of marketed formulation analysis

Brand name: Allercet-Dc **Average weight = 173.26 mg**

Sr. No.	Weight of tablet powder (mg)	Peak area of standard		Peak area of sample	
		CTZ HCl	PHE HCl	CTZ HCl	PHE HCl
1	173.23	375834.5	150624	374231.2	150852.0
2	172.02			375345.0	151135.0
3	173.35			376016.3	153214.5
4	174.12			375324.8	152145.9
5	173.61			373462.5	157321.0

Table 4: Results of marketed formulation analysis

Brand name: Allercet-Dc **Average weight = 171.22 mg**

Sr. No	Laboratory mixture	Weight of pure drug and tablet powder (mg)		Amount estimated in average weight of tablet (µg/ml)		% Label claim	
		CTZ HCl	PHE HCl	CTZ HCl	PHE HCl	CTZ HCl	PHE HCl
1	Standard	172.20	170.32	50	50	100.04	100.2
2	Sample	171.94	171.62	10	10	99.82	100.32
3		171.32	172.34	20	20	99.99	98.92
4		170.98	172.65	30	30	100.53	100.06
5		171.03	171.25	40	40	100.21	99.98
6		170.84	171.09	50	50	100.03	99.81
					Mean	100.10	99.88
					±S.D.	0.2432	0.5029
					±R.S.D	0.2430	0.5040

2.10 System suitability parameters

System suitability test is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from five replicate injections of standard drug solution. The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. 20µl of mixed standard stock solution was injected five times separately and their system suitability parameters were recorded and shown in Table 3.

Table 5: Regression analysis of calibration curves for CTZ HCl and PHE HCl and system suitability parameters

Sr. No.	Parameters	CTZ HCl	PHE HCl
1	Detection wavelength	270 nm	
2	Linearity range (ug/ml)	10-50ug/ml	10-50ug/ml
3	Slope	78450	2940.7
4	Intercept	90698	1977.3
5	Correlation coefficient	0.998	0.9993
6	Retention time ±S.D.	1.2±0.04	3.1±0.06
7	Theoretical plates ±S.D.	3681±0.03	3501±0.05
8	Resolution	3.5	
9	Tailing factor	0.9507±0.072	0.915±0.05

S.D. Standard deviation of 3 determinations

2.11 Validation of developed methods

The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantification, intra-day and inter-day and repeatability of measurement as well as repeatability of sample application [18].

a) Accuracy

The accuracy of an analytical method is the closeness of test results obtained by the method to the true value. Accuracy of proposed method was ascertained on the basis of recovery study performed by standard addition method. The results of recovery studies and statistical data are recorded in Table 6.

Table 6: Observations, results and statistical data for recovery studies

Sr. No	Weight of tablet powder (mg)	Peak area of standard		Amount of pure drug added ug/ml		Peak area of sample		% recovery	
		CTZ HCl	PHE HCl	CTZ HCl	PHE HCl	CTZ HCl	PHE HCl	CTZ HCl	PHE HCl
1	173.42	373156	150684	8.1	8.0	373541	150732	100.26	100.18
2	174.31			10.3	10.2	374003	151032	99.69	100.00
3	174.25			12.1	12.1	373149	150642	100.29	99.69
							Mean	100.08	99.95
							±S.D.	0.3380	0.2478
							±R.S.D	0.338	0.248

*Mean of three estimations

b) Precision

Precision of an analytical method is the degree of agreement among individual test results. It was ascertained by replicate estimation of marketed formulation (six times) and expressed as the S.D. and R.S.D. of the series of measurements. The results were shown in Table 4.

c) Ruggedness

The study of ruggedness was carried out under three different conditions i.e. Days, Time and Analyst.

1) Different days

i) Interday study

The interday study was performed by applying the proposed method on same sample of tablet on different days. The percent label claim was calculated using same formula as in analysis of tablet. The results were shown in Table 7.

Table 7: Results of Interday study

Day	Wt. of tablet powder taken (mg)	Wt. of std (mg)		Standard Peak area		Sample peak area		%Label claim*	
		CTZ HCl	PHE HCl	CTZ HCl	PHE HCl	CTZ HCl	PHE HCl	CTZ HCl	PHE HCl
Day1	172.2	10.1	10.1	371543	150355.0	371510	150382.2	99.99	100.0
Day2	171.8	10.5	10.0	371645	151303.3	371805	151198.6	100.04	99.93
Day3	171.7	10.2	10.3	371124	150454.8	371128	150379.7	100.00	99.95
							Mean	100.01	99.96
							±S.D.	0.0264	0.0416
							R.S.D	0.026	0.042

ii) Intraday study

The intraday study was performed by applying the proposed method on same sample of tablet on same day at two hours interval. The percent label claim was calculated using same formula as in analysis of tablet. The results are shown in Table 8.

Table 8: Result of Intraday study

Time (hr.)	Wt. of tablet powder taken (mg)	Wt. of std (mg)		Standard Peak area		Sample peak area		%Label claim*	
		CTZ HCl	PHE HCl	CTZ HCl	PHE HCl	CTZ HCl	PHE HCl	CTZ HCl	PHE HCl
0	173.3	10.1	10.3	374321.4	150514.88	375213	151322.1	100.2	100.5
2	173.2	11.2	10.1	373582.6	151893.12	374642	150186.5	100.2	98.87
4	173.6	10.2	10.5	371578.0	150568.13	373784	150893.8	100.5	100.2
							Mean	100.3	99.87
							± S.D.	0.195	0.880
							%R.S.D.	0.0019	0.0088
							C.V.	0.1931	0.8813

*Mean of three estimations

iii) Different analyst

The sample and standard solutions were prepared by different analysts and analysis was done by proposed method. The percent label claim was calculated using same formula as in analysis of tablet. The results were shown in Table 9.

Table 9: Results of different analysts

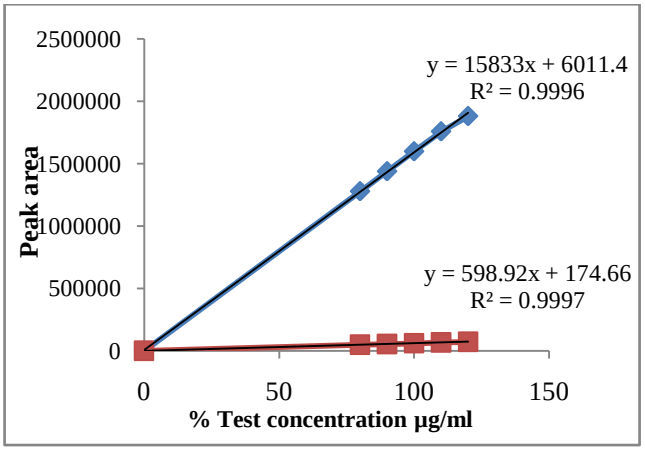
Analyst	Wt. of tablet powder taken (mg)	Wt. of std (mg)		Standard Peak area		Sample peak area		%Label claim*	
		CTZ HCl	PHE HCl	CTZ HCl	PHE HCl	CTZ HCl	PHE HCl	CTZ HCl	PHE HCl
Analyst 1	171.7	10.3	10.2	376332	15878.9	376401	15873.3	100.0	99.96
Analyst 2	171.5	10.1	10.5	376268	15793.6	376310	157990.3	100.0	100.03
Analyst3	171.5	10.3	10.1	376265	15796.3	376270	15789.6	100.0	99.95
							Mean	100.00	99.98
							±S.D.	0.0057	0.043
							R.S.D.	0.0057	0.043

***Mean of three estimations

d) Linearity and range

According to USP 80% to 120% of test concentration was taken and dilution was done appropriately, (Figure 6).

Figure 6: Plot of linearity and range for CTZ HCl and PHE HCl



e) Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were estimated from the set of 5 calibration curves used to determine method linearity.

LOD= 3.3*σ/S and LOQ= 10*σ/S

Where,
σ = the standard deviation of y-intercepts of regression lines
S = the slope of the calibration curve

3. Discussion

A validated RP-HPLC method for the simultaneous estimation of CTZ HCl and PHE HCl in tablet was developed under experimental condition described. Both the drugs were resolved using C_{18}Intersil, 220 × 4.6 mm column using Methanol: Acetonitrile: disodium hydrogen phosphate p^H was adjusted to 6.50 with ortho phosphoric acid (80:4:19 v/v/v) as mobile phase with a flow rate of 2 ml/min, UV detection was carried out at 270 nm. A critical evaluation of proposed method was performed by statistical analysis. The retention time (RT) for CTZ HCl was 1.2 min and for PHE HCl 3.1 min. Both the drugs showed linear response in concentration range of 10-50 µg/ml with correlation coefficient of 0.998 (for CTZ HCl) and 0.9993 (for PHE HCl). Results of marketed formulation analysis shows % R.S.D. value less than 2.0% indicating reproducibility of the results. % recovery ±S.D. was found to be 100.08±0.338m and 99.95±0.247 for CTZ HCl and PHE HCl which shows high degree of accuracy. Hence, the results from the validation experiments showed that the method is reliable and accurate therefore it can be successfully applied for the routine quality control analysis of Phenylepherine Hydrochloride and Cetirizine Hydrochloride in tablet dosage form.

4. Conclusion

The proposed RP-HPLC technique enable the quantitation of phenylephrine hydrochloride and cetrizine hydrochloride binary mixture with good accuracy and precision, either in laboratory prepared samples or in pharmaceutical dosage forms. The results of the analysis of pharmaceutical formulation by the proposed method were highly reproducible and reliable and it is in good agreement with the label claim of drug. The sensitivity, reproducibility, simplicity and short analysis time for the method makes it valuable in the routine analysis. The results of the recovery studies performed show the high degree of accuracy of the proposed methods. High recovery shows that the method is free from interference of excipients used in formulation.

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