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Stability Indicating RP-HPLC Method for Determination of Phenylephrine Hydrochloride, Cetirizine and Nimesulide in Pharmaceutical Formulation and in Bulk Powder

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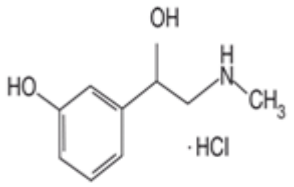
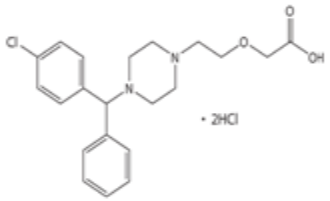
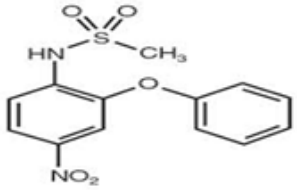
ABSTRACT

A novel stability indicating high pressure liquid chromatographic (HPLC) method was developed and validated for quantitative determination of Phenylephrine HCl (PHE), Cetirizine (CET) and Nimesulide (NIM) in bulk drug and in tablets. Isocratic, HPLC method, using a C18 reversed phase column with mobile phase 70% Methanol, 30% aqueous contained 0.05% orthophosphoric acid as mobile phase adjust pH at 3 and ambient temperature. The proposed method was investigated to separate the drug from its stress degradation products. The flow rate was 0.7ml/min, column oven temperature was ambient and detection of column effluent was performed at 225nm. All three drugs were subjected to the stress conditions of hydrolysis (acid and base), oxidation and in neutral. Stress degraded samples were analyzed by the developed procedure. The analyte was well separated from its degradants. The described method showed excellent linearity over a range of 1-5, 1-5, 20-100µg/ml for PHE, CET, NIM respectively. The R^2 of Phenylephrine hydrochloride (PHE), Cetirizine (CET) and Nimesulide (NIM) were found to be 0.999, 0.999 and 0.999 respectively. Degradation of PHE, CET and NIM were observed in acid, base and in 3% H_2O_2 conditions only. The developed method was validated with respect to the linearity, accuracy (recovery), precision, specificity and robustness. The forced degradation studies proved stability indicating power of the method.

1. INTRODUCTION

Phenylephrine (PHE) chemically is (1R)-1-(3hydroxy-phenyl) - 2-(methylamino) ethanol hydrochloride and is used as sympathomimetic (decongestants). Cetirizine (CET) provides prompt relief of itchy watery eyes, runny nose, sneezing, itching of the nose or throat due to respiratory allergies. Chemically it is ±-[2-[4- [(4-chlorophenyl) phenylmethyl]-1-piperazinyl]ethoxy]acetic acid dihydrochloride. Nimesulide (NIM) is a selective COX-2 inhibitor which provides analgesic & antipyretic effect. Chemically it is N-(4-Nitro-2-phenoxyphenyl) methanesulfonamide. Structural formula of PHE, CET and NIM (Table 1).

Table 1: Structures of Phenylephrine hydrochloride (PHE), Cetirizine (CET) and Nimesulide (NIM).

Name of Drugs	Phenylephrine Hydrochloride (PHE)	Cetirizine Hydrochloride (CET)	Nimesulide (NIM)
Structural formula			

The mixture of three drugs is recommended to relieve symptoms such as nasal and sinus congestion, allergic symptoms of the nose or throat due to upper respiratory tract allergies and sinus pain associated with headache. The multidrug mixture is also used as an adjunct with antibacterial in sinusitis, tonsillitis, and otitis media. The tablet contains a variable amount of all ingredients due to their recommended pharmacological dose; the tablet contains 5mg of Phenylephrine hydrochloride, 5mg Cetirizine and 100mg of Nimesulide. This variable amount of ingredients in such a multi-drug formulation makes the process of routine analysis difficult. Moreover, the active compounds have very different polarity and, therefore, chromatographic behavior. So far no single HPLC method is reported to determine the mentioned ingredients quantitatively in this combination. The literature reveals a number of analytical methods published for PHE, CET and NIM with some other drug combinations.

An HPLC method for Phenylephrine in combination with Chlorpheniramine Maleate has been reported[1].

Cetirizine has been reported for analytical methods such as HPLC for estimations in formulations and plasma,[2-3] spectrophotometry,[4] Capillary zone electrophoresis [5] a stability indicating assay method is also reported [6-7]. Nimesulide the other analyte of multidrug combination has been quantified by analytical methods such as HPTLC[8], UV spectrophotometry[9], differential pulse voltammetric[10], FT-NIR spectroscopy[11] and HPLC[12]. Stability indicating HPLC method for determination of Nimesulide in bulk and in tablet dosage form[13]. Stability indicating HPLC method for determination of Labetalol HCL in pharmaceutical formulation[14].

To the best of our knowledge, the methods described in the literature do not cover the analysis of three analytes PHE, CET and NIM in a pharmaceutical mixture in the form of tablet formulation. Therefore, the main objective of this work was to develop a single separation method for quantifying these three analytes which are present in variable concentrations in tablet dosage form.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Working standards of pharmaceutical grade Phenylephrine hydrochloride, Cetirizine and Nimesulide were obtained as generous gifts from Cure Medicines (I) PVT. LTD (Pune, Maharashtra, India). They were used without further purification. Fixed dose combination tablet Nimucet cold (INTAS Pharmaceuticals Ltd) containing 5mg Phenylephrine hydrochloride, 5mg Cetirizine and 100mg Nimesulide was purchased from local market. All the chemicals were of HPLC grade, purchased from Merck Chemicals, India. Used water was double distilled and filtered through 0.45 micron filter.

2.2. Instrumentation

The HPLC system consisted of Youngling (S.K) Gradient System UV Detector and processed by making use of Autochro-3000 software. The chromatographic separations were carried out on a reverse phase Primesil-C18 column (4.6 x 250 mm i. d., particle size 5 µm).

2.3. Preparation of standard stock and sample solution

2.3.1. Preparation of standard stock solution

Preliminarily sample preparation was done in a Methanol taking accurately weighed quantity of 5mg of PHE, 5mg of CET and 100mg of NIM transferred to 50ml volumetric flasks separately to give stock solutions of 100µgm/ml of PHE, 100µgm/ml of CET and 2000µgm/ml of NIM.

2.3.2. Preparation of mixed standard solution

A mixed standard solution was prepared from these stock solutions by transferring 0.1ml of each of the stock solution to a 10ml volumetric flask and diluting with a mobile phase to get a solution of 1, 1 and 20µgm/ml of PHE, CET and NIM respectively.

2.3.3. Preparation of sample solution of tablet

For preparation of sample solution of pharmaceutical mixture, 10 tablets (Nimucet Cold Tab, INTAS Pharmaceuticals) were weighed and powdered finely. Tablet powder equivalent to 5mg of PHE, 5mg of CET and 100mg of NIM was transferred to a 50ml volumetric flask and dissolved in Methanol up to the mark. The solution was ultrasonicated for 15 min and filtered through 0.45µ membrane filter. The solutions were further diluted to obtain resultant concentration 1, 1 and 20µgm/ml of PHE, CET and NIM respectively. The resultant mixture was subjected to HPLC analysis in developed chromatographic conditions (Table 6 and fig. 1).

Table 6: Results for assay of marketed formulation

Commercial formulation	Ingredients	Labeled amount (mg)	Amount found (mg)	Found %
Nimucet cold tab	PHE	5	5.20	104.00
	CET	5	5.08	101.60
	NIM	100	98.81	98.81

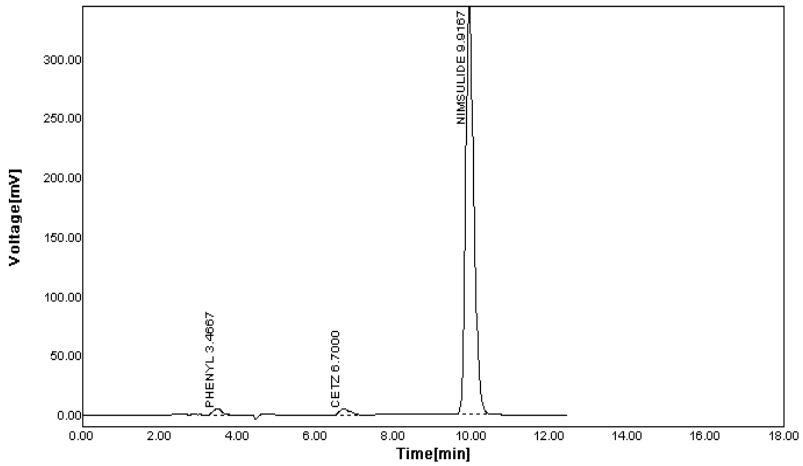


Fig. 1: Chromatogram for assay of marketed preparation

2.4. Chromatographic Conditions

The chromatographic separation was carried out on C_{18} (Primesil) column (4.6 x 250 mm id, particle size of packaging material 5µm). Mobile phase combination water and methanol in proportion of (30:70 v/v) respectively. Aqueous phase contained 0.05% orthophosphoric acid in mobile phase to adjust pH 3 at ambient temperature. Experiments concluded lack of resolution of a complex mixture of three drugs using the isocratic approach of analysis. But after many trials, method was developed in isocratic system. Flow rate of mobile phase is 0.7ml/min at ambient temperature. Chromatograms obtained on 225nm detection wavelength, and injection volume was 20µl.

Procedure for dosage forms degradation study

Acidic Degradation

10ml of methanolic stock solutions of the drug, 10ml of 1N HCl was added. The mixture was refluxed for 1 hour at 80ºC on water bath. Degraded samples were then cooled at room temperature and neutralized with 1N NaOH to pH 7. Suitable aliquot of the resultant degraded sample was withdrawn and subjected to analysis after suitable dilutions with methanol.

Alkaline Hydrolysis

10ml of methanolic stock solutions of the drug and 10ml of 0.1N NaOH was added. No need to heat the mixture. Degraded samples neutralized with 0.1N HCl to pH 7. Suitable aliquot of resultant degraded sample was withdrawn, neutralized and subjected to analysis after suitable dilutions with methanol.

Oxidative Hydrolysis

To 10ml of methanolic stock solutions of the drugs, 10ml of 3% V/V H_2O_2 was added. The mixture was refluxed for 1 hour at 80ºC temperature on water bath. Degraded sample was then cooled to room temperature. Suitable aliquot of resultant degraded sample was withdrawn, neutralized and subjected to analysis after suitable dilutions with methanol.

Neutral Conditions

Methanolic solution of the drug was refluxed for 2 hours at 80ºC temperature on water bath. Degraded samples were then cooled to room temperature. Suitable aliquot of resultant degraded sample was withdrawn and subjected to analysis.

3. RESULTS AND DISCUSSION

3.1. Method development and optimization of chromatographic conditions

The development of the method was based on the experience obtained from the HPLC method previously developed for the analysis of mixture of analytes comprising phenylephrine, paracetamol, caffeine and chlorpheniramine maleate[15] (Dewani et al., 2012) (30) by authors. Experiments previously suggest use of C-18 stationary phases of (250, 4.5 mm i.d., particle size 5µm) hence for the study a reverse phase C_{18} (Primesil) column made by core shell technology was utilized. For the separation of all the three analytes in the mixture, the composition of mobile phase was varied. Parameters such as mobile phase composition of buffer were exhaustively studied so as to achieve a reasonable degree of separation of analytes. Several binary or ternary eluants were tested using different proportions of solvent, such as acetonitrile, methanol, water and buffer at different pH conditions. Initially, isocratic mode of separation was experimented and was found insufficient to resolve the mixture with good peak characters but after many trials,

methods developed in isocratic system. Method selected so as to achieve separation of analytes with good peak characters. The mean retention time of three analytes was PHE 3.25, CET 6.73 and NIM 10.233 min respectively. Peak identification was done by injecting individual analyte in developed chromatographic conditions.

Degradation behavior of combination mixture

Acidic conditions

Acidic degradation of tablet was observed on heating methanolic solution of the drug and 1N HCL for 1 hour at 80ºC. The acidic degradation showed a peak at tR 4.3667 min. (fig. 2).

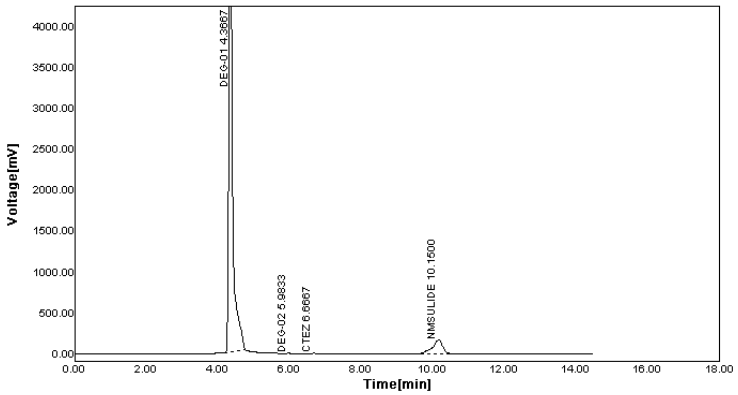


Fig. 2: Chromatogram of acidic degradation

Alkaline conditions

Alkaline degradation of tablet observed on heating methanolic solution of the drug and 0.1N NaOH for 0.5 hours at room temperature. The alkaline degradation product showed a peak at tR 4.2000 min. (fig. 3).

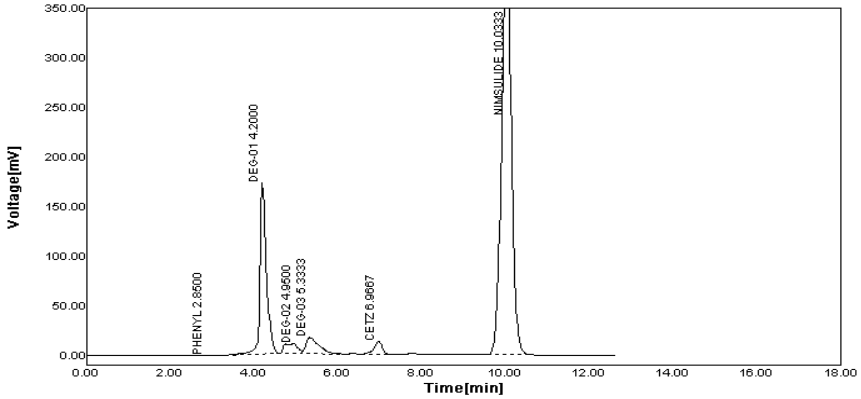


Fig. 3: Chromatogram for basic degradation

Oxidative conditions

Oxidative degradation of tablet was observed on heating methanolic solution of the drug and 3% H_2O_2 for 1 hour at 80ºC. The oxidative degradation products showed a strong peak at tR 4.6667 min. There was a strong peak due to the presence of hydrogen peroxide, well resolved from the main peak of intact drug (fig. 4).

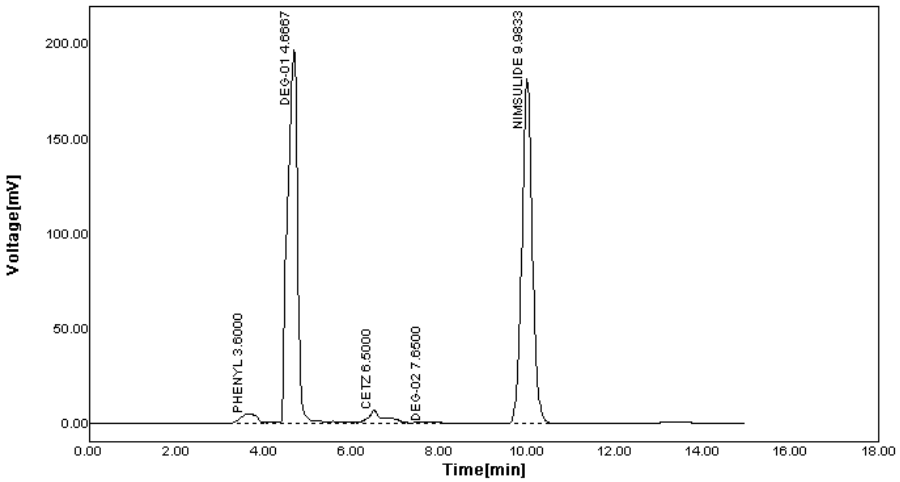


Fig. 4: Chromatogram for oxidative degradation

Neutral Conditions

No sufficient degradation of tablet observed on heating methanolic solution of the drug for 2 hours at 80ºC. It shows stability against neutral condition (Table 7 and fig. 5).

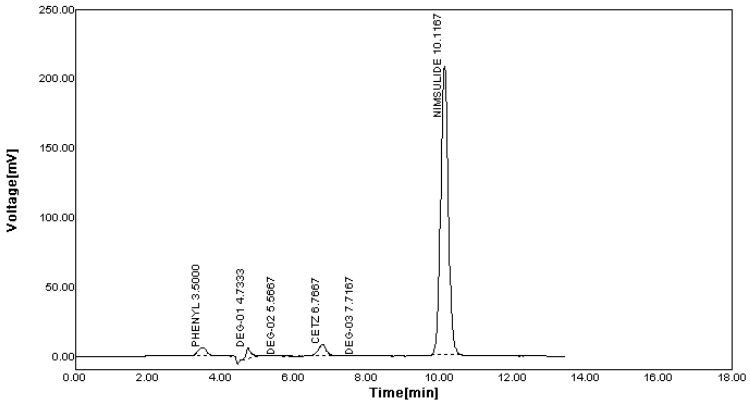


Fig. 5: Chromatogram for Neutral condition (insufficient degradation)

Table 7: Results of stability study data

Stress condition	Time in hr	Mean peak area	% recovery of analyte	Retention time of analyte
Acid hydrolysis (1 N HCL)	1 hr	37152.5859		4.3667
Base hydrolysis()	0.5 hr	1853.8484		4.2000
Oxidation (3% H_2O_2)	1 hr	3167.5762		4.6667
Neutral	2 hr	77.0688		4.7333

3.2. Method validation

3.2.1. Selectivity and linearity

Method selectivity was assessed by the peak purity test (comparison between analyte peak and auto threshold in the purity plot) using diode array detector. The analyte chromatographic peak was not found to be attributable to more than one component indicating the method to be selective[16] (Conference Harmonisation (ICH), 1995).

For linearity, an external method was used for the simultaneous determination of three ingredients. Five concentrations were chosen ranging from 20% to 100% of the target analyte concentrations in formulations. So the linearity dilution concentrations were PHE 1-5µgm/ml , CET 1-5µgm/ml, NIM 20-100µgm/ml. All the solutions were prepared by diluting in Methanol. Each concentration of standard mixture solutions was injected two times and the mean value of peak area was taken for the calibration curve. Calibration graph was obtained by plotting peak area versus concentration of standard drugs. The linear regression equations for PHE, CET and NIM were found to be y = 28.07x + 23.95, y = 33.61x + 17.48, y = 70.96x + 36.00 respectively. The regression coefficient values (R^2) were found to be 0.999, 0.999 and 0.996 respectively indicating an acceptable degree of linearity as shown in (Table 2 and fig. 6, 7, 8).

Table 2: Results of Linearity study

Parameters	Phenylephrine	Cetirizine	Nimesulide
Concentration range, µg/ml	1-5µg/ml	1-5µg/ml	20-100µg/ml
Correlation coefficient	0.999	0.999	0.999

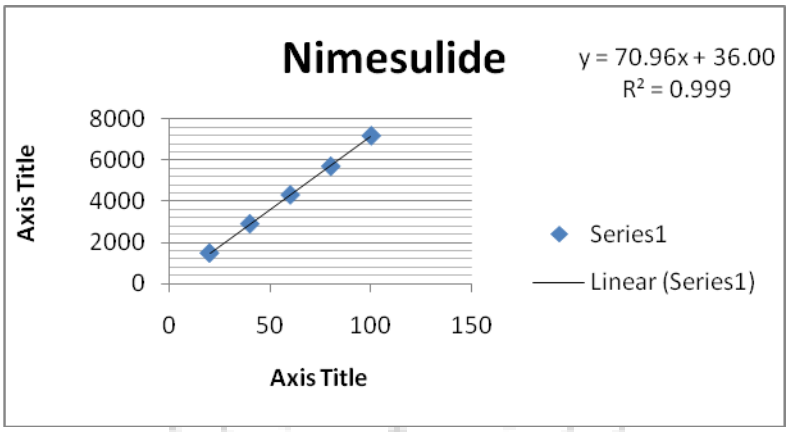


Fig. 6: Linearity graph for Nimesulide

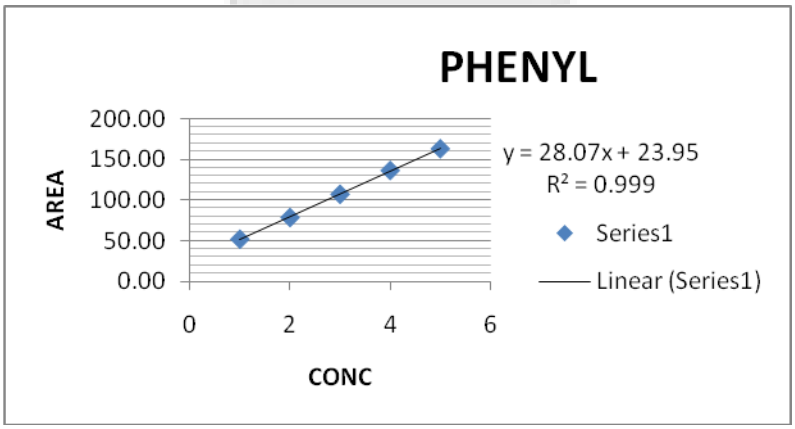


Fig. 7: Linearity graph for Phenylephrine

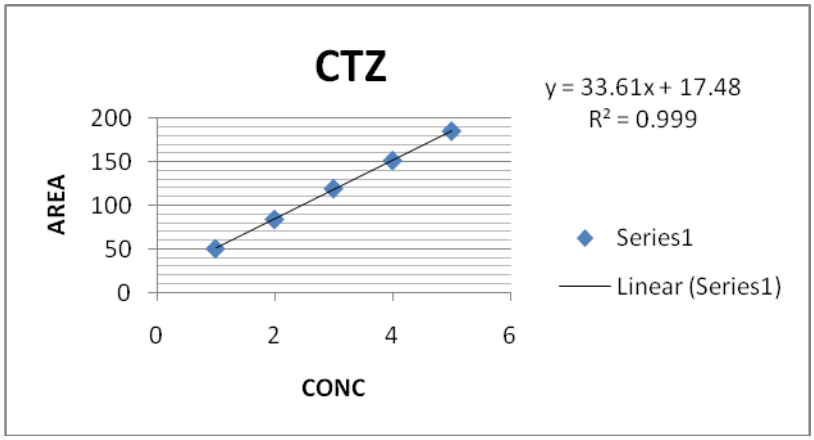


Fig. 8: Linearity graph for Cetirizine

3.2.2. Specificity

The specificity of the method was accessed from the chromatogram where complete separation of PHE, CET and NIM was achieved and against potential interferences in the presence of placebo (diluents i.e., Methanol). The peaks obtained were sharp and well separated at the baseline also excipients from formulation were not interfering with assay no interferences were detected at retention times of PHE, CET and NIM in sample solution proving the method to be specific.

3.2.3. Precision

The precision of an analytical method is the closeness of replicate results obtained from analysis of the same homogeneous sample. Precision is determined by the estimate of the relative standard deviation (RSD) values. Precision studies were carried out by carrying inter-day and intra-day studies. The precision studies were done by injecting the prepared standard solution at three concentration levels (20%, 60% and 100%) in triplicate every day up to three consecutive days for inter day studies. Intra-day studies were done by injecting the standards at three different times on the same day. %RSD values were measured the low value of RSD (%) showed that the method is precise within the acceptance limit of +2%. The intra- and inter- day variability or precision data are given. The results indicated good precision of the developed method (Table 3).

Table 3: Precision data of intra-day and inter-day assay (n= 6).

Concentration µlg/ml	Measured mean concentration ± %RSD	
	Inter-day precision (n= 6)	Intra-day precision (n=6)
PHE		
1	1.02	0.99
3	3.06	3.01
5	5.02	5.01
CET		
1	1.00	1.01
3	2.98	2.98
5	4.98	4.98
NIM		
20	20.30	20.33
60	59.53	59.54
100	100.25	100.05

3.2.4. Accuracy

The accuracy of an analytical method expresses the closeness of agreement between the value, which is accepted reference value, and the value found. Accuracy studies were done by the standard addition method. Accuracy is expressed as % recovery of the standard spiked to previously analyzed test sample of tablet. The active ingredients were spiked in previously analyzed tablet powder sample at different concentration levels viz. 80%, 100%, and 120% each of the labeled claim and injected in developed chromatographic conditions in triplicate. The percentage recoveries were calculated from the slope and Y-intercept of the calibration curve. The recovery data for accuracy studies are shown in (Table 4).

Table 4: Recovery data for accuracy studies

Recovery level	Std added to placebo	Amount added (mg)	Mean recovery (mg)	Mean % recovery
80%	**PHE**	1.6	1.59	99.26
	CET	1.6	1.59	99.76
	NIM	32	31.65	98.9
100%	**PHE**	2	2.00	99.78
	CET	2	1.98	99.04
	NIM	40	39.78	99.70
120%	**PHE**	2.4	2.41	100.47
	CET	2.4	2.43	101.31
	NIM	48	47.97	99.95

3.2.5. System suitability parameters

System suitability tests are an integral part of the analytical method. It is used to verify the adequacy of the resolution and reproducibility of the system. For study of system suitability parameter, seven replicate injections of mixed standard (100% level of labeled claim) solution were injected and parameters such as peak area, retention time, asymmetry factor and theoretical plates of the peaks were calculated (Table 5).

Table 5: System suitability data.

Std sol.	Parameters (mean values)			
	RT(min)	**Peak area**	**Theoretical plates (TP)**	**TF**
PHE	3.2500	181.6430	3690.9	1.4812
CET	6.7333	186.9911	8661.5	1.5056
NIM	10.2333	2549.1714	12175.4	1.3659

3.2.6. Robustness studies

The robustness of a method is the ability to remain unaffected by small changes in chromatographic parameters. The experimental conditions were purposely altered and the

chromatographic resolution of PHE, CET and NIM was assessed. The chromatographic parameters included variation of flow rate. Second was deliberate change in detection wavelength and third is change in mobile phase proportion. To study the effect of flow rate on system suitability parameters ±10% change on either side of actual flow rate was made i.e., from 0.8ml/min and 0.6ml/min, while other conditions were held constant. For variation of detecting wavelength change in detecting wavelength of ±2 nm was made and system suitability parameters were recorded. For third parameter study change in actual mobile phase variation in a range ±1ml. All the robustness studies were carried using a mixed standard having resultant concentration of 5mg of PHE, 5mg of CET and 100mg of NIM. The system suitability parameters considered for deliberate changes were %RSD of peak areas, mean tailing factor and mean retention time.

4. CONCLUSION

In this study, a validated simple and reliable HPLC–DAD procedure was described for the assay of a complex multi-drug combination consisting of PHE, CET and NIM which is indicated for the treatment of allergic symptoms of the nose or throat due to upper respiratory tract allergies associated with headache. To our present knowledge, no attempts have yet been made to assay this multidrug mixture by any analytical methodology. All the three analytes (PHE, CET and NIM) were successfully resolved and quantified using a Reverse phase C_{18} (Primesil) column in a relatively short run time with the last analyte eluting at 10.2333 min the in isocratic program contributed total run time of 15 min. Reliability was guaranteed as validation experiments proved that the HPLC method is linear in the proposed working range as well as accurate, precise and specific. The good recovery percentage of tablet forms suggests that the excipients have no interference in the determination. The RSD (%) was also less than 2 showing a high degree of precision of the method. Stability study also shows good results. Formulation shows degradation in acid, alkali, oxidative reagent and in neutral condition no sufficient degradation. The proposed method was also found to be robust with respect to flow rate, change in mobile phase proportion and detecting wavelength. Hence, it can be recommended for the routine quality control of the studied drugs either in bulk form or in their combination formulated in some other dosage form.

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