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Method Development and Validation of Cetirizine hydrochloride, Phenylephrine hydrochloride and Nimesulide by UV and HPLC

Vilas Arsul*[1], Ashok Kagane[1], Supriya Wagh[2]

ABSTRACT

Phenylephrine HCL, Cetirizine HCL and Nimesulide, one of the most commonly prescribed Nasal decongestant, Antihistamine and Non-steroidal anti-inflammatory respectively, In this regard, the research and development of reliable analytical methods for self-determination. It is freely soluble in water, methanol and acetonitrile. Insoluble in acetone.

The main aim of present Research work is to develop and validate a method for estimation of Cetirizine HCL, Phenylephrine HCL & Nimesulide by the using of UV visible spectrophotometry and High Performance Liquid Chromatography.

Double beam UV –visible spectrophotometer with 10 mm matched quartz cell (Shimadzu -1700) one is sample and other is reference were use for quantitative estimation of Phenylephrine HCL, Cetirizine HCL and Nimesulide. The various trials were carried out by varying parameters.

RP-HPLC method for estimation of pharmaceutical dosage form was developed successfully.

Chromatographic separation was performed on Phenomenex Gemini C18 stainless steel column with dimension 250 × 4.6 mm, 5 µm particle size. The mobile phase consisting of a acetonitrile : water (ph3) 60:40 v/v) was delivered at rate of 1 ml/min. The detection was made at 229 nm. Mobile phase was filtered through a 0.45 µ membrane filter paper and degassed before analysis. Separation was performed at ambient temperature.

Key-words: Method development, Cetirizine HCL, Phenylephrine HCL, Nimesulide, UV Spectroscopy, HPLC etc.

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INTRODUCTION:

Modern analytical chemistry generally requires precise analytical measurements at very low concentrations, with a variety of instruments. Frequently, high-resolution separations have to be achieved with selective chromatographic methods prior to analytical determinations. Therefore, the knowledge of instrumentation used in chemical analysis today is of paramount importance to assure future progress in various fields of scientific endeavour. The optimal usage of instrumentation with more meaningful data generation that can be interpreted reliably is possible only with the improved knowledge of the principles of the instrumentations used for measurements well as those utilized to achieve various separations.

HPLC is an recently used techniques for quantitative and qualitative analysis. The HPLC is an method for analysis is very precise, accurate, and specific, HPLC techniques required very less time for analysis and it required very minor quantity of sample hence recently it is use in pharmaceutical or other industry. The HPLC techniques are precise for analysis of Phenylephrine HCL, Cetirizine HCL and Nimesulide at parameter as per method hence it is selected for analysis.

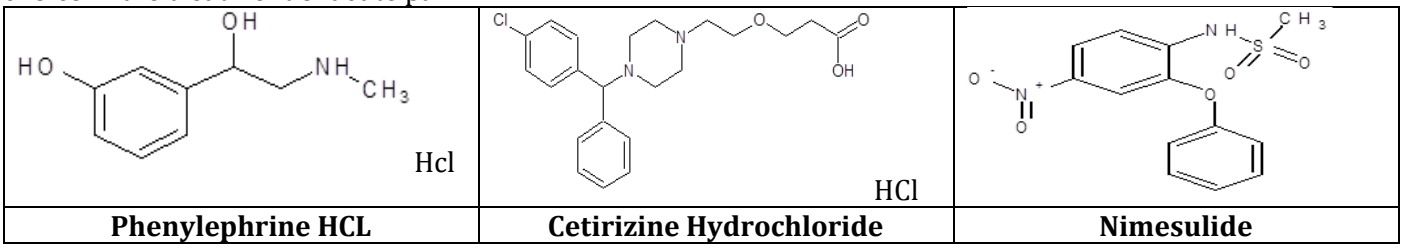
Phenylephrine HCL, Cetirizine HCL and Nimesulide, one of the most commonly prescribed Nasal decongestant, Antihistamine and Non-steroidal anti-inflammatory respectively, In this regard, the research and development of reliable analytical methods for self-determination. It is freely soluble in water, methanol and acetonitrile. Insoluble in acetone.

The main aim of present Research work is to develop and validate a method for estimation of Cetirizine HCL, Phenylephrine HCL & Nimesulide.

Phenylephrine hydrochloride is closely related to epinephrine. It is a useful vasoconstrictor of sustained action with little effect on the myocardium or the central nervous system. It is used by topical application in nose drops. Subcutaneous injection has been employed extensively to prevent hypotension during spinal anaesthesia and for the treatment of orthostatic hypotension.

Cetirizine is a piperazine derivative and active metabolite of hydroxyzine, is a second generation antihistaminic drug used in symptomatic treatment of seasonal allergic rhinitis and perennial allergic rhinitis as well as chronic urticaria and pruritus. CET selectively inhibits histamine H1-receptors without causing sedation due to its physicochemical properties. As an advantage compared to the first generation compounds, CET is a non-sedating derivative and it does not affect serotonergic, muscarinergic and adrenergic receptors.

Nimesulide is a non-steroidal anti-inflammatory analgesic drug having rapid analgesic action. Indicated for the treatment of acute pain, the symptomatic treatment of osteoarthritis and primary dysmenorrheal in adolescents and adults above 12 years old. These unique characteristics of Nimesulide an appealing therapeutic choice in the treatment of acute pain.



Phenylephrine HCL	Cetirizine Hydrochloride	Nimesulide

Figure 1 : Chemical Structures of Drug

MATERIALS:

Chemical: Methanol, Acetonitrile, Potassium dihydrogen phosphate, Orthophosphoric acid, Sodium Hydroxide, Hydrochloric acid

Equipment's/Instrument: HPLC Model, UV-Vis Spectrophotometer, PH Meter, Electronic Balance, Sonicator Hot Air Oven, Glassware, Melting Point Apparatus.

METHODS:

Wavelength determination

An accurately weight quantity 10 mg of PHE and CET, 200 mg of NIM were transferred to three separate 100 ml volumetric flasks containing 20 ml of 0.1N NaOH each, and volume was made up to mark with same solvent to obtain concentration 100 µg/ml of PHE and CET, 2000 µg/ml. The absorbance of the latter was recorded using UV visible spectrophotometer in range 200-400nm.

Method optimization & development of UV:

An accurately weight quantity 10 mg of PHE and CET, 200 mg of NIM were transferred to three separate 100 m l volumetric flasks containing 20 ml of 0.1N NaOH each, and volume was made up to mark with same solvent to obtain concentration 100 µg/ml of PHE and CET, 2000 µg/ml. The absorbance of the latter was recorded using different concentration of solution by UV visible spectrophotometer in range 200-400nm.

The spectrum of all three drugs were recorded and three wavelengths 234.0 nm (λ_{max} of PHE) and 231.0 nm (λ_{max} of CET) and 224.0 nm ((λ_{max} of NIM) Mean SD and %RSD were calculated.

Method validation of UV

1. Linearity

From stock solution 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 ml solutions were pipetted out and diluted up to 10ml using 0.1 N NaOH to obtain resultant solutions of 2, 4 , 6, 8, 10, 12 and 14 µg/ml. Absorbance's for each of these solutions were recorded in triplicate and calibration curve was constructed considering mean absorbance of each test solution. From the calibration curve equation of line, correlation coefficient and intercept were determined.

2. Precision

From the calibration range three QC standard decided viz. 2, 8 and 14 µg/ml as LQC , MQC and NQC respectively. The solutions for QC standards were prepared by diluting stock solution of 0.2, 0.8 and 1.4 ml solutions up to 10ml. Absorbance's of each QC standard were recorded for intraday and inter day precision in triplicates as per ICH guidelines Q2 R1.

3. Accuracy

% Accuracy was determined using observations of precision study using following formula. Limit for % accuracy is NMT 5% RSD.

4. Robustness

10µg/ml solution was selected for robustness study for the parameters like wavelength. Wavelength was subjected to minor variation of ±1 (viz.227±1). The absorbances for each of these wavelengths were recorded in triplicate. The variation should not be more than 5% RSD.

5.LOD and LOQ

LOD and LOQ were determined using following formulas

$$LOD = 3.3*I/(SD) \qquad LOQ = 10*I/(SD)$$

Where, I = Intercept of the graph, SD = Standard Deviation

Analysis of marketed formulation

Ten tablets weighed accurately and ground into fine powder. An accurately weighed quantity equivalent to 1 mg of CET, PHE and 20 mg of NIM was transferred to 100 ml volumetric flask containing 0.1N NaOH, sonicated for 10 min and volume was made up to the mark with same solvent and filtered through Whatmann filter paper. Aliquot portion 0.7 ml was transferred to 10 ml volumetric flask and volume was adjusted to mark with the same solvent. The sample solution was scanned over the range 400 - 200 nm, in the multicomponent mode; using three sampling wavelength 234 (λ_{max} of PHE), 231 nm (λ_{max} of CET) and 224 nm (λ_{max} of NIM). The percent label claim was calculated.

HPLC method development and validation

1. Selection of column

On the basis of reversed phase HPLC mode, stationary phase with Phenomenex Gemini C18 column (250 mm × 4.6 mm) with particle size 5 µm was selected.

2. Selection of mobile phase

The selection of mobile phase was made on the basis of literature survey. The sample solution of CET, PHE and NIM was analysed by HPLC system using different solvent components like acetonitrile and acidified water, methanol and acidified water in different concentration and pH. It was found that acetonitrile and water (pH 3) gives satisfactory result as compared to other mobile phases. Finally the optimal composition of the mobile phase determined was acetonitrile : water (pH 3) (60:40 v/v).

3. Chromatographic condition

Chromatographic separation was performed on Phenomenex Gemini C18 stainless steel column with dimension 250 × 4.6 mm, 5 µm particle size. The mobile phase consisting of a acetonitrile : water (pH 3) 60:40 v/v) was delivered at rate of 1 ml/min. The detection was made at 229 nm. Mobile phase was filtered through a

0.45 µ membrane filter paper and degassed before analysis. Separation was performed at ambient temperature. The injection volume was 20 µl.

Method development of HPLC

Preparation of stock solution

Accurately weighed 10 mg CET, 10 mg PHE and 200mg NIM was transferred into 100 ml volumetric flask. The solution was dissolved and diluted to the mark with mobile phase.

Preparation of working solution

From the standard stock solution 100 µl transferred into ephedrine tube and adjust the volume up to 1000 µl with mobile phase.

Method validation of HPLC

1. Linearity

From stock solution 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 ml were pipetted out and diluted upto 10 ml to obtain 10, 20, 30, 40, 50 and 60 µg/ml resultant solutions respectively. Calibration curve was constructed between concentration versus peak area. Results were recorded for equation of line, correlation coefficient and intercept were determined.

$$Y = mX+c$$

Where, Y- area X- Concentration m- Slope of graph c- Intercept

2. Precision

From the calibration range three QC standard decided viz. 15, 35 and 65 µg/ml as drug solution. The solutions for QC standards were prepared by diluting stock solution of 1.5, 3.5 and 6.5 ml solutions upto 10ml. Area of each QC standard were recorded for intraday and interday precision in seven replicates as per ICH guidelines Q2 R1 . Results were recorded to calculate mean, SD, %RSD.

3. % Accuracy

% Accuracy was determined using observations of precision study using following formula. Limit for % accuracy is NMT 5% RSD.

4. Robustness

10µg/ml solution was selected for robustness study for the parameters like mobile phase proportion, flow rate, wavelength. Seven replicates for parameters given in table were injected and area for each of the parameter was recorded. The variation should not be more than 5% RSD. One factor was changed at time to estimate the effect.

5. LOD and LOQ

LOD and LOQ were determined using following formulas

$$LOD = 3.3*I/(SD) \qquad LOQ = 10*I/(SD)$$

Where, I = Intercept of the graph, SD = Standard Deviation

6. System Suitability test

System suitability testing is essential for the assurance of the quality performance of the chromatographic system. A mixed working standard solution (20 µg/ml of CEFP and 10 µg/ml of SULB) was analysed by optimized chromatographic conditions as per Table.

Result and discussion:

UV method development

1. Determination of wavelength:

The spectrum of all three drugs were recorded and three wavelengths 234.0 nm (λ_{max} of PHE) and 231.0 nm (λ_{max} of CET) and 224.0 nm ((λ_{max} of NIM) were selected for estimation of drugs using multicomponent mode of instrument.

Selections of mixed standard solutions

Table No.1 Mixed Standards of CET, PHE and NIM

Cetirizine(µg/ml)	Phenylephrine HCl (µg/ml)	Nimesulide (µg/ml)
0.55	0.55	11
0.6	0.6	12
0.65	0.65	13
0.7	0.7	14
0.75	0.75	15

0.75	0	0
0	0.75	0
0	0	15

Available marketed formulations contain 5 mg of PHE, 5 mg of CE and 100mg of NIM. Therefore, mixed drug concentrations were selected in such a way to obtain constant ratio of 1:1:20. Eight mixed standard solutions with different concentration of PHE, CET and NIM were prepared, as shown in Table 1. All the mixed standard solutions were scanned over the range 400 - 200 nm, in the multicomponent mode; using three sampling wavelength 234 (λ_{max} of PHE), 231 nm (λ_{max} of CET) and 224 nm (λ_{max} of NIM). The spectral data from these scans was used to determine the concentration of three drugs in tablet sample solutions.

Application of proposed method for simultaneous estimation of drugs in physical mixtures

Table No. 2 Analysis of physical mixture

Drug	Amount taken(µg/ml)	Amount found(µg/ml)	% Recovery
Cetirizine HCl	14	13.38	95.57
Phenylephrine HCl	0.7	0.699	99.85
Nimesulide HCl	0.7	0.665	95

Brand Name: Nicip flu NF **Mfg. By: Cipla**
Batch No.: 652107 Avg. Wt.: 0.31457 g

Table No.3 Analysis of tablet formulation

Drug	Label claim(mg)	Amount found(mg)	% Recovery	%RSD
CET	5	4.93	98.71	1.08
PHE	5	4.88	97.68	0.78
Nimesulide	100	97.14	97.14	0.78

UV Method validation

1. Linearity

Appropriate aliquot portion of PHE, CET and NIM stock solution were transferred to separate 10 ml volumetric flasks. The volume was adjusted to the mark with same 0.1 N NaOH to obtain concentrations of 2, 4, 6, 8 and 10 µg/ml. Response of the respective solution recorded at the respective wavelength i.e. 234 nm for PHE, 231 nm for CET and 224 nm for NIM. Calibration curve was plotted, absorbance versus concentration.

Table No. 4 Absorbance data for *Phenylephrine* HCL.

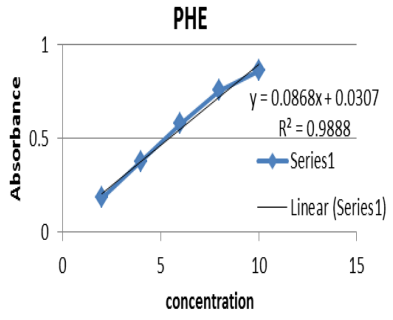
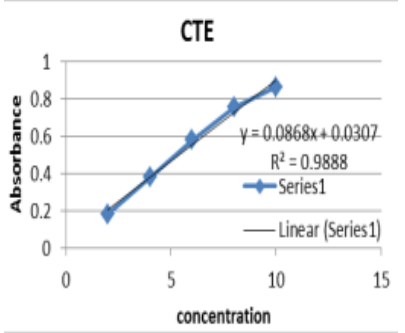
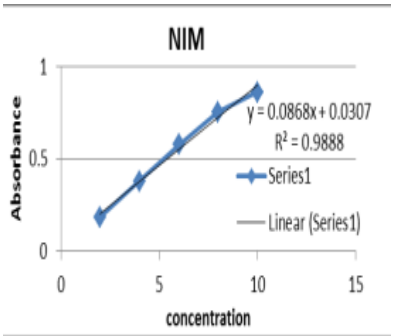
Conc (µg/ml)	*Phenylephrine* HCL Absorbance	*Cetirizine* HCL Absorbance	*Nimesulide* Absorbance
2	0.204	0.213	0.193
4	0.334	0.354	0.378
6	0.554	0.565	0.578
8	0.688	0.675	0.755
10	0.854	0.889	0.862
Calibration Curves			

Table No. 5 Linearity study of PHE, CET and NIM

Parameter	PHE	CET	NIM
Linearity range (µg/ml)	2-10	2-10	2-10
Slope	0.085	0.0083	0.086
Intercept	0.038	0.037	0.030
Correlation coefficient	0.989	0.991	0.988

From the data of Linearity study it was observed that the method is linear for Cetirizine hydrochloride, Phenylephrine hydrochloride and Nimesulide.

2. Precision

Precision was determined by studying the repeatability and intermediate precision. Repeatability result indicate the precision under the same operating condition over the short interval of time the intermediate precision study is expressed within the laboratory variation on different days.

Precision studies (Intraday n=6)

Table No. 6 for Cetirizine HCl

conc(µg/ml)	*Morning*	*Afternoon*	*Evening*	*mean*	*SD*	*%RSD*
4	**0.233**	**0.238**	**0.24**	0.237	0.0036	1.52
6	**0.465**	**0.47**	**0.466**	0.467	0.0026	0.56
8	**0.533**	**0.539**	**0.541**	0.537	0.0041	0.77

Table No. 7 for Phenylephrine HCl.

conc(µg/ml)	*Morning*	*Afternoon*	*Evening*	*mean*	*SD*	*%RSD*
2	**0.18**	**0.178**	**0.179**	0.179	0.001	0.55
4	**0.357**	**0.345**	**0.348**	0.350	0.006	1.78
6	**0.541**	**0.544**	**0.539**	0.541	0.002	0.46

Table No. 8 for Nimesulide

conc(µg/ml)	*Morning*	*Afternoon*	*Evening*	*mean*	*SD*	*%RSD*
2	**0.174**	**0.173**	**0.171**	0.172	0.0015	0.88
4	**0.347**	**0.345**	**0.339**	0.343	0.0041	1.21
6	**0.56**	**0.555**	**0.562**	0.559	0.0036	0.64

Precision study (Interday n=6)

Table No. 9 Cetirizine HCl

DAY	*Conc.*	*Mean abs.*	*SD*	*%RSD*
1	4	0.373	0.002	0.55
2	4	0.366	0.001	0.27
3	4	0.365	0.002	0.68

Table No. 10 Phenylephrine HCl

DAY	*Conc.*	*Mean abs.*	*SD*	*%RSD*
1	4	0.361	0.021	0.69
2	4	0.365	0.002	0.68
3	4	0.366	0.003	0.98

Table No.11 for Nimesulide

Day	Conc.	Mean abs.	SD	%RSD
1	4	0.351	0.0020	0.59
2	4	0.353	0.0015	0.43
3	4	0.355	0.0005	0.16

As per % RSD for Intraday and Interday Precision study for each drug is below 2 so method is precise.

3. Accuracy

To the preanalyzed sample solutions, a known amount of standard solutions of the pure drugs were added at different level i.e. 80, 100 and 120 %. The result of recovery studies are reported in Table 12.

Table No. 12 Recovery studies (n=3)

Level of recovery	Drug	Amount of drug(tablet) taken(µg/ml)	Amount of drug added(µg/ml)	%Recovery	%RSD
80%	Cetirizine HCl	6	4.8	97.21	1.23
	Phenylephrine HCl	6	4.8	97.21	1.22
	Nimesulide	6	4.8	100.62	1.25
100%	Cetirizine HCl	6	6	96.98	1.76
	Phenylephrine HCl	6	6	97.19	1.01
	Nimesulide	6	6	97.19	1.01
120%	Cetirizine HCl	6	7.2	96.44	1.47
	Phenylephrine HCl	6	7.2	97.22	1.42
	Nimesulide	6	7.2	97.24	1.59

4 LOD and LOQ

LOD was calculated by using equation and was found to be 0.25µg/ml .similarly, LQC was calculated by using equation and was reported in below table.

Table No. 13 LOD and LOQ

Std solution	LOD(µg/ml)	LOQ(µg/ml)
CET	0.23	0.70
PHE	0.21	0.66
NIM	0.20	0.63

All factors which are consider in Accuracy and results are found within limits.

HPLC method development

1. Selection of column

On the basis of reversed phase HPLC mode, stationary phase with Phenomenex Gemini C18 column (250 mm × 4.6 mm) with particle size 5 µm was selected.

2. Selection of mobile phase

The selection of mobile phase was made on the basis of literature survey. The sample solution of CET, PHE and NIM was analysed by HPLC system using different solvent components like acetonitrile and acidified water, methanol and acidified water in different concentration and pH. It was found that acetonitrile and water (pH 3) gives satisfactory result as compared to other mobile phases. Finally the optimal composition of the mobile phase determined was acetonitrile: water (pH3) (60:40 v/v).

3. Chromatographic condition

Table No. 14 Chromatographic condition

Chromatographic Mode	Chromatographic Condition
Stationary phase	Phenomenex Gemini C18 (250 mm × 4.6 mm × 5µm)
Mobile phase	Acetonitrile : water (ph 3) (60:40)
Detection wavelength	229 nm
Flow rate	1 ml/min
Sample size	20 µl

Selection of analytical wavelength

A mixed solution of CET, PHE and NIM was injected to HPLC system. The overlay PDA spectrum of drugs was used to select the wavelength of analysis. The overlay PDA spectra shown in Figure No.2

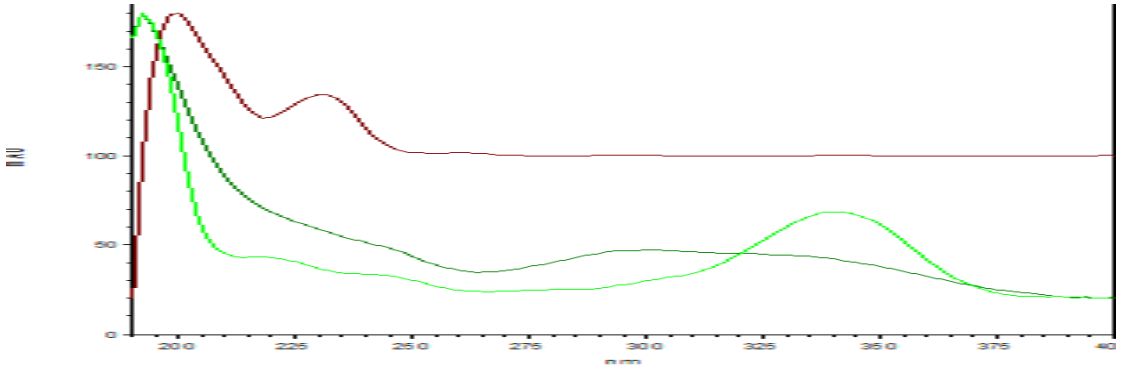


Figure No. 2: Overlay PDA spectra of PHE, CET and NIM

Table No. 15 Optimization trials

Sr. no.	Mobile phase	Retention time			Theoretical plate			Asymmetry		
		PHE	CET	NIM	PHE	CET	NIM	PHE	CET	NIM
1	Acetonitrile : water (5:95)	19.3	18.22	16.32	78845	10090	78845	0.96	0.98	0.71
2	Acetonitrile : water pH3 (60:40)	2.2	4.3	6.4	3801	1050	4933	1.3	1.6	1.3
3	Acetonitrile : water pH4 (60:40)	8.9	20.64	26.62	154	525	579	1.25	1.13	1.14
4	Acetonitrile : water pH5.5 (60:40)	6.6	2.8	6.5	250	3492	1545	1.04	1.83	1.2
5	Acetonitrile : water pH 6.2 (60:40)	6.1	2.8	5.9	49	1880	1099	1.13	1.5	1.2

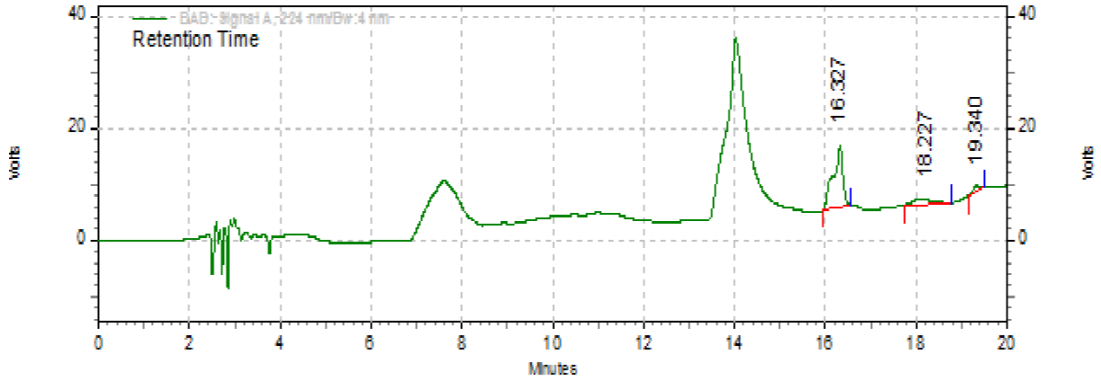


Fig no. 3 Chromatogram of trial 1

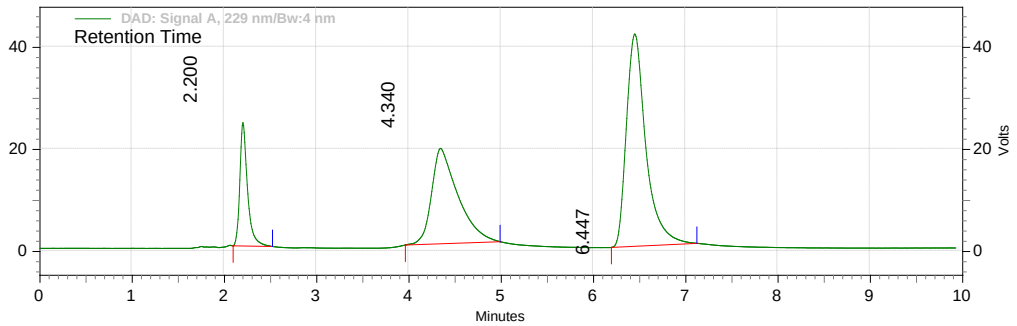


Fig no. 4 Chromatogram of trial 2

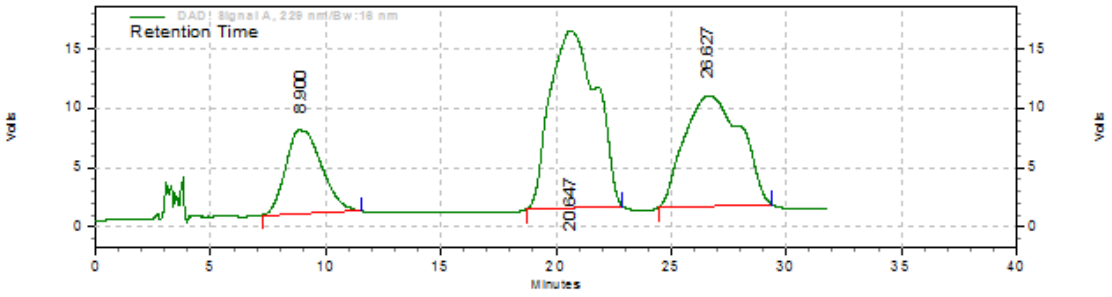


Fig no. 5 Chromatogram of trial 3

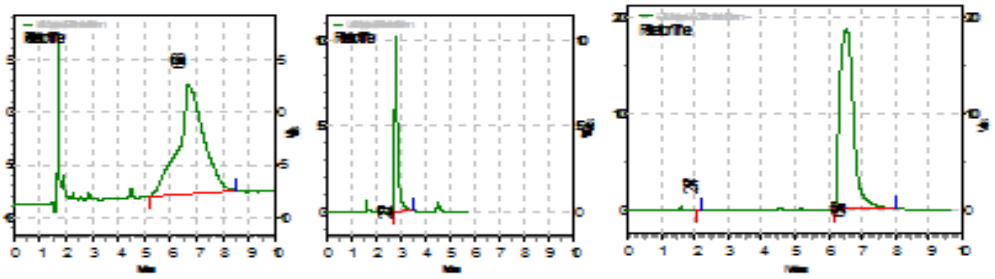


Fig no. 6 Chromatogram of trial 4

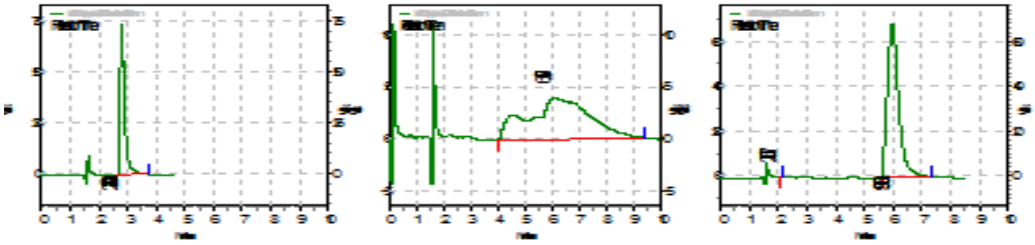


Fig no. 7 Chromatogram of trial 5

Final chromatographic condition

Chromatographic separation was performed on Phenomenex Gemini C18 stainless steel column with dimension 250 × 4.6 mm, 5 µm particle size. The mobile phase consisting of a acetonitrile : water (pH 3) 60:40 v/v) was delivered at rate of 1 ml/min. The detection was made at 229 nm. Mobile phase was filtered through a 0.45 µ membrane filter paper and degassed before analysis. Separation was performed at ambient temperature. The injection volume was 20 µl. The optimized chromatographic conditions are shown in Table No. 16.

Table No. 16 Optimized Chromatographic Conditions

Chromatographic Mode	Chromatographic Condition
Stationary phase	Phenomenex Gemini C18 (250 mm × 4.6 mm × 5µm)
Mobile phase	Acetonitrile : water (ph 3) (60:40)
Detection wavelength	229 nm
Flow rate	1 ml/min
Sample size	20 µl

The chromatogram of CET (10 µg/ml) PHE (10 µg/ml) and NIM (200 µg/ml) with the optimized mobile phase was as depicted in Figure No. 8.

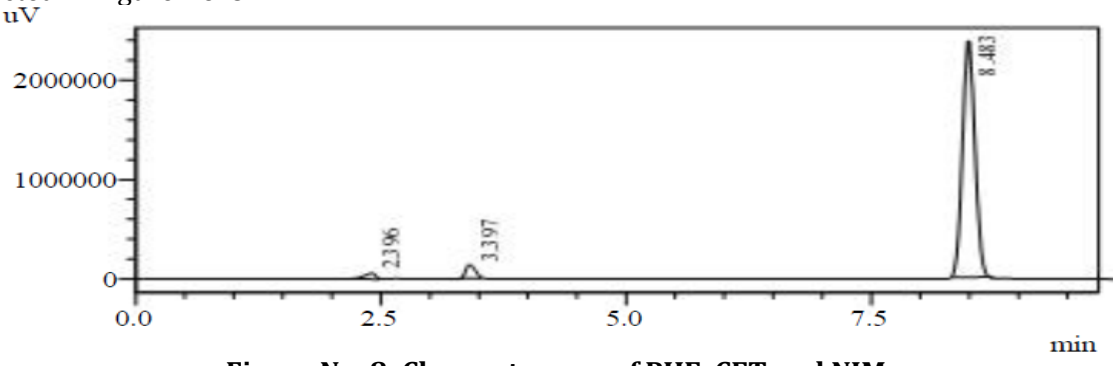


Figure No. 8: Chromatogram of PHE, CET and NIM

The retention time of PHE was obtained at 2.396 min, retention time of CET was obtained at 3.397 min and for NIM at 8.483 min.

Table no. 17 system suitability test

Drug	Peak area	Asymmetry	Retention time	Resolution	Theoretical plates
PHE	195678	0.0	2.39	0.0	2609
CET	481644	0.0	3.39	4.49	4317
NIM	4101042	1.12	8.48	21.67	16721

HPLC Method validation

1. Linearity

From the working standard solution, aliquots portion of about 1.0 – 2.0 ml was transferred into a 10 ml volumetric flasks. The solution was diluted to the mark with mobile phase to obtain 10-20 µg/ml of CET and PHE, 200-400 µg/ml of NIM.

Table No. 18 Calibration curve data & Figure 9 of Phenylephrine HCL

Conc. (µg/ml)	Peak Area
10	200561
12	214552
14	248974
16	287416
18	298745
20	324578

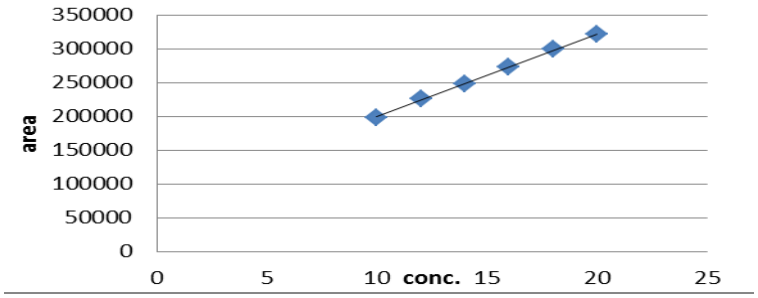
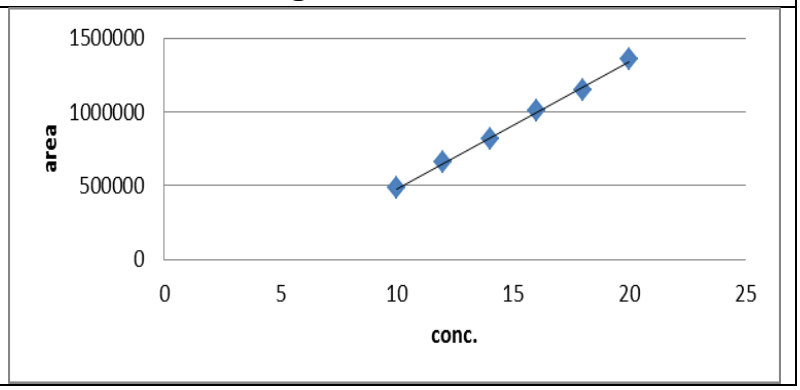


Table No. 19 Calibration curve data & Figure 10 of Cetirizine HCl

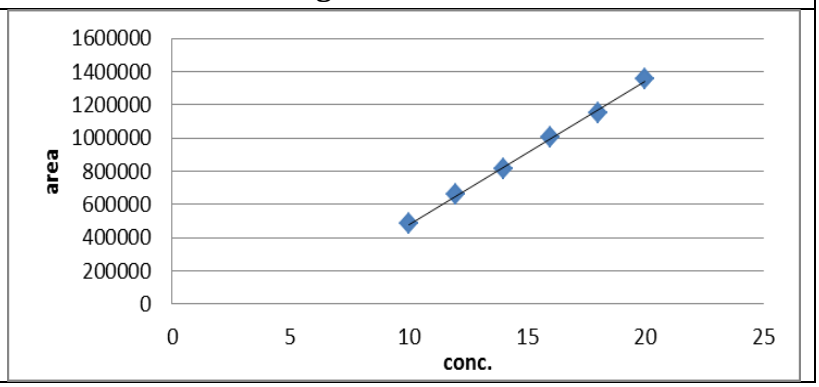
Conc. (µg/ml)	Peak Area
10	468794.4
12	654789.7
14	824578.1
16	1014705
18	1145745
20	1368778



Calibration curve of NIM

Table No. 20 Calibration curve data & Figure 11 of Nimesulide

Conc. (µg/ml)	Peak Area
10	497845.2
12	691478.5
14	1025771
16	1014705
18	1314784
20	1447898



Application of proposed method to laboratory mixture

In order to see the feasibility of the method in the marketed formulation, it was first tried in physical laboratory mixture.

- **Standard solution (100 µg/ml of CET, PHE and 2000 µg/ml of NIM):** Accurately weighed quantity of 10 mg CET, 10 mg PHE and 200 mg of NIM was transferred to 100 ml volumetric flask. The volume was adjusted up to mark with mobile phase.
- **Working standard solution mixture (14 µg/ml of CET, PHE and 280 µg/ml of NIM):** From this stock solution 1.4 ml of aliquot transferred into 10 ml volumetric flask. The volume was adjusted up to the mark with mobile phase to obtain 10 µg/ml of CET, PHE and 200 µg/ml of NIM.
- **Procedure**
  Each sample was analyzed as per the optimized chromatographic condition as mentioned in Table No. 16 The peak area was noted with respect to concentration.
- **Calculation**
  The % drug content was calculated using formula 2 and 3 as mention below.

$$Y = mx + c \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$x = \frac{Y-c}{m} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where, Y= Peak area of sample solution x= Found concentration

$$\% \, drugcontent = \frac{foundconc.inppm}{actualconc.inppm} \times 100 \ldots\ldots\ldots (3)$$

The results of laboratory physical mixture analysis are shown in Table No. 21

Table No. 21 Analysis of physical mixture

Drug	Amt. taken	Amt. found	%recovery
PHE	14	14.27	101.94
CET	14	13.48	96.33
NIM	280	294.33	105.11

Analysis of tablet formulation

Standard solution (100 µg/ml CET, 100 µg/ml PHE and 2000 µg/ml NIM): To determine the content of CET, PHE and NIM from tablet formulation, a quantity of powder equivalent to 10 mg of CET, 10 mg PHE and 200 mg of NIM was weighed and transferred into 100 ml volumetric flask. The solution was dissolved in mobile phase, sonicated for 10 min and volume was made up to the mark.

Working solution mixture (14 µg/ml of CET, PHE and 280 µg/ml of SULB): From this stock solution transferred 1.4 ml of aliquot into 10 ml volumetric flask. The volume was adjusted up to the mark with mobile phase.

Procedure

The sample was analyzed for five times as per the optimized chromatographic condition as mentioned in Table No. 16. The peak area was noted with respect to concentration. The % drug content was calculated. The results of tablet analysis are shown in Table No. 22.

Table No. 22: Analysis of tablet formulation

Sr.no	Amount of drug present (µg/ml)			Amount taken			Peak Area			Amount found			%recovery		
	PHE	CET	NIM	PHE	CET	NIM	PHE	CET	NIM	PHE	CET	NIM	PHE	CET	NIM
1	10	10	10	14	14	280	200561	468794.4	497845.2	14.27235	13.487	294.33	101.945	96.3356	105.119
2	12	12	12	14	14	280	214552	654789.7	691478.5	14.11582	13.8392	293.5901	100.827	98.8514	104.853
3	14	14	14	14	14	280	248974	824578.1	1025771	13.98246	13.60936	291.9065	99.8747	97.2097	104.252
4	16	16	16	14	14	280	287416	1014705	1014705	13.85237	13.71704	287.3115	98.9454	97.9788	102.611
5	18	18	18	14	14	280	298745	1145745	1314784	13.88271	13.66521	289.9306	99.1622	97.6086	103.546
												Mean	100.15	97.59	104.07
												S.D.	1.24	0.93	1.01
												%RSD	1.24	0.95	0.97

The proposed method was validated as per ICH Q2 (R1) guidelines for different parameters like accuracy, precision and robustness.

2. Precision

In order to validate and prove the applicability of the method, a laboratory mixture of CET, PHE and NIM was prepared from the stock solutions in the ratio corresponding to amounts in the dosage form. For quantitative estimation of the mixture, three series (10, 12, 14 µg/ml for cetirizine and Phenylephrine, 240, 280, 320 µg/ml for nimesulide) were prepared, with three solutions for each concentration. The result was shown in table.

Table no. 23 precision: Interday precision (n=6)

Drug	Conc.	Mean area	% RSD
PHE	10	199097.7	1.64
	12	219894.8	1.90
	14	247671.2	1.50
CET	10	479389	1.44
	12	657534.3	1.76
	14	824241.5	0.93
NIM	200	4110936	0.45
	240	4248279	0.94
	280	4490540	1.04

Intraday precision (n=6)

Drug	Conc.	Mean area	%RSD
PHE	10	211703.7	1.67
CET	10	491693.8	1.88
NIM	200	4213392	0.82

All factors which are consider in Precision and results are found within limits.

3. Accuracy

For CET, PHE and NIM recovery study was carried out at 80, 100 and 120 % level of label claim.

Standard stock solution (100 µg/ml of CET and PHE, 2000 µg/ml of NIM): Accurately weighed quantity of a tablet powder equivalent to 7 mg of CET and PHE, 140 mg of NIM was transferred to three 100 ml volumetric flask. To the first flask add 1mg CET, 1 mg PHE and 20 mg NIM. To the second flask add 3mg CET, 3mg PHE and 60 mg NIM. To the third flask add 5mg CET, 5 mg PHE and 100 mg NIM bulk drug. Adjust the volume up to mark with mobile phase.

Working sample solution (12 µg/ml of CET and PHE, 240 µg/ml of NIM): From the above stock solution take 6 ml of aliquot and transferred into 10 ml volumetric flask. Adjust the volume up to mark with mobile phase. The solution was sonicated for 10 min.

Procedure

The sample was analysed as per the optimized chromatographic condition as mentioned in Table No. 16 The peak area was noted with respect to concentration.

Calculation

The % drug content was calculated using formula 2 and 3 as mentioned in the application of method to laboratory mixture. % recovery was calculated using formula.

$$\%recovery = \frac{S}{A+B} \ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Where, S = Recovered concentration in µg/ml A = Actual concentration of sample solution in µg/ml

B = Added concentration of standard stock solution in µg/ml

Table no. 24 Recovery study

Level of recovery	**Drug**	**Amount of drug present (µg/ml)**	**Amount of drug found(µg/ml)**	**%recovery**	**%RSD**
80%	CET	9.6	9.50	98.99	1.23
	PHE	9.6	9.31	97.04	1.22
	NIM	192	189.31	98.60	1.25

100%	CET	12	11.66	97.23	1.76
	PHE	12	11.61	96.79	1.01
	NIM	240	234.53	97.72	1.01
120%	CET	14.4	14.0	97.25	1.47
	PHE	14.4	14.17	98.41	1.42
	NIM	288	283.20	98.33	1.59

All factors which are consider in Accuracy and results are found within limits.

4. Robustness

Robustness of the method was assessed by making variation in flow rate and proportion of mobile phase. An appropriate concentration of 12 µg/ml of CET, PHE and 240 µg/ml of NIM

Table no.25 Robustness study of PHE

Chromatographic condition	Retention time	Peak area	Theoretical plate	Asymmetry	Capacity factor
A: Flow rate					
0.9 ml	2.69	225792	2754.316	0.00	0.00
1 ml	2.39	195678	2609.189	0.00	0.00
1.1 ml	2.08	219635	2366.342	0.00	0.00
Wavelength					
225 nm	2.35	218774	2598.656	0.00	0.00
229 nm	2.39	195678	2609.189	0.00	0.00
233 nm	2.36	219608	2155.127	0.00	0.00

Table no.26 Robustness study of CET

Chromatographic condition	Retention time	Peak area	Theoretical plate	Asymmetry	Capacity factor
A: Flow rate					
0.9 ml	3.86	971372	2376.229	0.00	0.43
1 ml	3.39	974034	4317.811	0.00	0.41
1.1 ml	3.0	975942	2476.083	1.32	1.29
Wavelength					
225 nm	3.39	980241	2488.139	1.75	0.44
229 nm	3.39	974034	4317.811	0.00	0.41
233 nm	3.39	983204	2864.837	1.54	0.43

Table no. 27 Robustness study of NIM

Chromatographic condition	Retention time	Peak area	Theoretical plate	Asymmetry	Capacity factor
A: Flow rate					
0.9 ml	9.96	1361372	17456.183	1.11	1.89
1 ml	8.48	4101042	16721.175	1.12	1.54
1.1 ml	7.75	1372014	18511.630	1.12	1.71
Wavelength					
225 nm	8.70	1383673	17440.051	1.06	1.7
229 nm	8.48	4101042	16721.175	1.12	1.54
233 nm	8.72	1355782	18953.739	1.17	1.69

Acceptance criteria

Theoretical plate = not less than 2000

Asymmetry = not more than 2

Capacity factor = not more than 2

All factors which are consider in Robustness and results are found within limits.

5. LOD and LOQ

The quantitation limit is a parameter of quantitative assay for low levels of compounds in sample matrices. The limit of detection (LOD) and limit of quantitation (LOQ) were determined using following formulae.

$$LOD = \frac{3.3 \times SD}{S}$$

$$LOQ = \frac{10 \times SD}{S}$$

Where, SD = Standard deviation S = Slope

For PHE, CET and NIM limit of detection was found to be 0.32, 0.20, and 0.73 and limit of quantitation was found to be 0.97, 0.60, 2.22 respectively.

All factors which are consider in LOD and LOQa nd results of LOD and LOQ was found to be0.001139 g/ml and0.00356 g/ml within limits.

Conclusion

Present investigation is an attempt to develop analytical method to determine the related substances in PHE, CET and NIM tablet by the use of UV visible spectrophotometry and High Performance Liquid Chromatography.

Double beam UV –visible spectrophotometer with 10 mm matched quartz cell (Shimadzu -1700) one is sample and other is reference were used for quantitative estimation of Phenylephrine HCL, Cetirizine HCL and Nimesulide. The various trials were carried out by varying parameters.

RP-HPLC method for estimation of pharmaceutical dosage form was developed successfully.

Chromatographic separation was performed on Phenomenex Gemini C18 stainless steel column with dimension 250 × 4.6 mm, 5 µm particle size. The mobile phase consisting of a acetonitrile : water (pH 3) 60:40 v/v) was delivered at rate of 1 ml/min. The detection was made at 229 nm. Mobile phase was filtered through a 0.45 µ membrane filter paper and degassed before analysis. Separation was performed at ambient temperature. They are used to reproducibility of the chromatographic system.

On the basis of the above it was concluded that the developed method of assay for candidate drug was validated as per the ICH guideline.

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