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Development and Validation of RP-HPLC Method for Estimation of a Multidrug Marketed Formulation

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Abstract: ***Phenylephrine HCL, Cetirizine HCL and Nimesulide, one of the Nasal decongestant, Antihistaminic, Non-Steroidal Anti-inflammatory, Respectively. In this regard the research& development of reliable analytical methods for self -determination. It is soluble in acetonitrile & insoluble in acetone. The main aim of present research work is to develop & validate a method for estimation of cetirizine HCL, Phenylephrine HCL, & Nimesulide by using UV- visible spectrometry and High-Performance liquid chromatography. RP-HPLC method for estimation of pharmaceutical dosage form was developed successfully. Chromatographic separation was performed on Phenomenex GeminiC18 150X4.60 mm 5µmParticle size. The mobile phase consisting of a Acetonitrile: water PH 3.28. 50:50 was delivered at arate1ml /min. The detection was made 215 nm. Mobile phase was degassed before use & separation was done.***

Keywords: PhenomenexGemini 150x4.60 mm, RP-HPLC, Acetonitrile, PH 3.28 215 wavelength

1. Introduction

HPLC is a modern form of liquid chromatography that uses small-particle column through which the mobile phase is pumped at high pressure. This is chromatographic process, where a mixture of analytes is separated into two distinct bands as they migrate down the column filled with stationary phase. HPLC is a dynamic partitioning process of analytes between the flowing liquid and spherical packing particles. HPLC is used either in the liquid-solid adsorption chromatography mode or the liquid-liquid partition chromatography mode, either normal or reversed-phase. Both partition and adsorption chromatography operates on differences in solute polarity since polarity is important in determining both adsorption and solubility.[1,2]

As a general rule, highly polar materials are best separated using partition chromatography, while very non polar are separated using adsorption chromatography

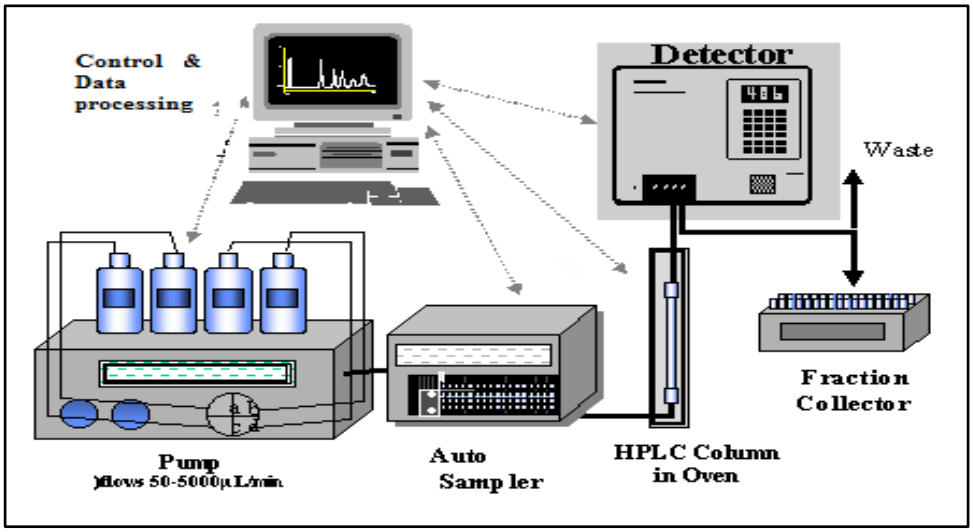


Figure 1: Simplified schematic representation of an HPLC system including PDA detector

Normal phase chromatography
The separation by this method is based on adsorption of the analyte on to polar stationary phase. The typical stationary phases employed in normal phase or adsorption chromatography are common porous adsorbents such as silica and alumina that have polar hydroxyl group on their surface. It can be used for separation of non -polar compound and isomers as well as for the fractionation of complex sample by functional groups or sample clean-up.[5]

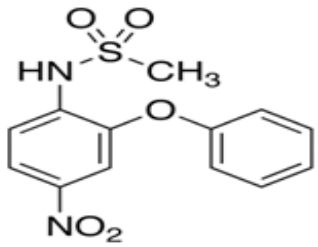
Reverse phase chromatography
The separation is based on analyte partition coefficient between a polar mobile phase and (hydrophobic) nonpolar stationary phase. Stationary phase commonly used is permanently bonding hydrophobic group such as octadecyl (C18) bonded group on silica support. It is most popular HPLC mode and it is used in 70 % of all HPLC analysis. It is suitable for analysis of polar (water-soluble), medium polarity and some non-polar analytes[3-4].

"Best column, best mobile phase, best detection wavelength, efforts in separation can make a world of difference while developing HPLC method for routine analysis. Determining the ideal combination of these factors assures faster delivery of desired results- a validated method of separation." [6,7,8]

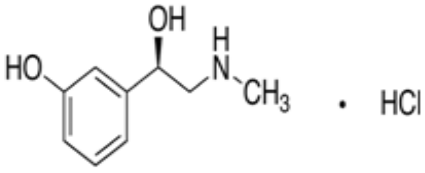
Phenylephrine (PHE) chemically is (1R)-1-(3hydroxy-phenyl)2-(methylamino) ethanol hydrochloride and is used as sympathomimetic (decongestants). Cetirizine Dihydrochloride (CET) provides prompt relief of itchy watery eyes, runny nose, sneezing, itching of the nose or throat due to respiratory allergies 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 formulas of PHE, CET and NIM are given in drug profile.[16,13]

The mixture of 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 antibacterials in sinusitis, tonsillitis, and otitis media.[15]

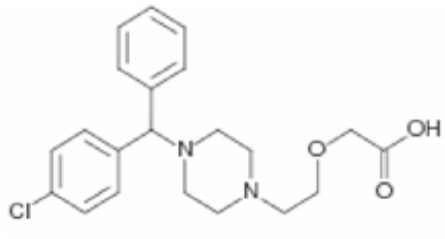
Drug Profile



Nimesulide



Phenylepherine HCL



Cetrizine HCL

2. Method Development

Instruments

Table 1: List of instruments

S. No.	Name of Instruments	Make	Model
1	Analytical balance	Sansui	Sansui-vibra DJ-150S-S
2	UV-Spectrophotometer	Shimadzu	Shimadzu-1700
3	HPLC	Shimadzu	Systronic LC 6600
4	Digital pH Meter	Milwaukee	Milwaukee pH 600

Selection of wavelength:
Solution of Nimesulidephenylephrine HCl cetirizine HCl standard was scanned in range of 300-200 nm.The wavelength 215 nm was selected for further experiment because the drug shows good response.[17]

Selection of common solvent (diluents)
Acetonitrile of HPLC grade was selected as common solvent for preparation of stock solution & developing spectral characteristics of drugs, further dilutions from stock solution were made in Acetonitrile. The selection was made after assessing the solubility of both the drugs in different solvents.[17]

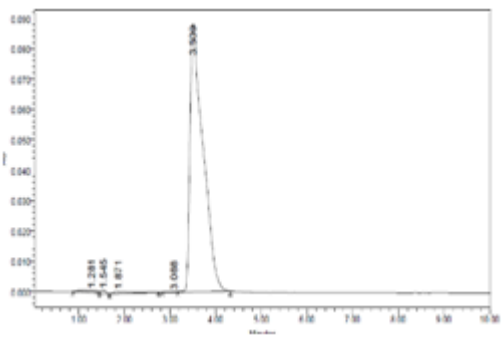


Figure 2: Nimesulide Standard **R.T.**: 3.509

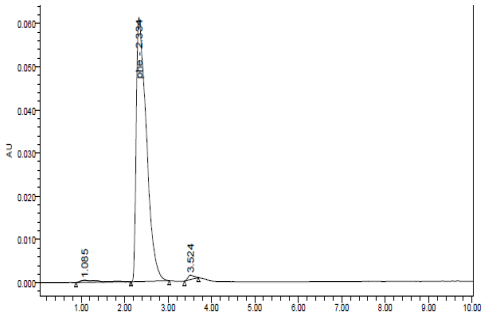


Figure 3: Phenylephrine Standard **R.T.**: 2.334SampleName:Nim+A60+W40+C18+R1+PH3.28 Sample Name:phy+A60+W40+C18+R1+PH3.28

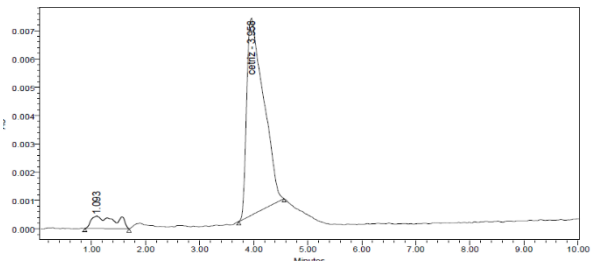


Figure 4: Cetrizine standard **R. T. 1.093** Sample Name: cetri+A60+W40+C18+R2+PH3.28

Figure 2: Nimesulide Standard having retention time 3.509 minute in which the mixture of solvent acetonitrile and water and a Modulator Ph solution is added as a mobile phase and Phenomenex Gemini C18 150X4.60 mm 5µm Particle size is used as a stationary phase Sample Name: Nim+A60+W40+C18+R1+PH3.28 **Figure 3: Phenylpherine HCl** Standard having retention time 2.334 minute in which the mixture of solvent acetonitrile and water and a Modulator Ph solution is added as a mobile phase and Phenomenex Gemini C18 150X4.60 mm 5µm Particle size is used as a stationary phase Sample Name:

Phy+A60+W40+C18+R1+PH3.28**Figure 4:cetirizine HCl** Standard having retention time **1.093** minute in which the mixture of solvent acetonitrile and water and a Modulator Ph solution is added as a mobile phase and Phenomenex Gemini C18 150X4.60 mm 5µm Particle size is used as a stationary phase Sample Name: cetri+ A60+ W40+ C18+ R1+ PH3.28

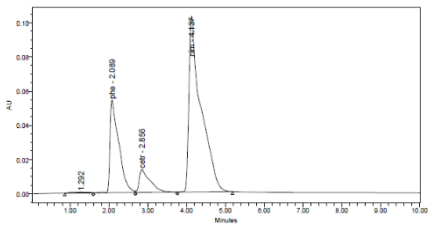
Mobile phase buffer:

Solution A: 0.1M phosphoric acid: 6.9 ml of ortho phosphoric acid into 500 ml volumetric flask &make up the volume up to the mark with HPLC water.

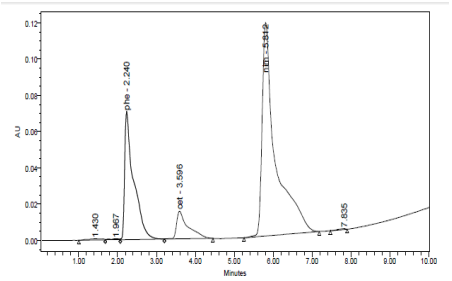
Solution B: 0.1M monobasic sodium mono phosphate 13.8gm of monobasic sodium mono phosphate in 1000ml HPLC water.
Mix the reported quantity of solution A & B in 100ml volumetric flask so as to get buffer of 3.5[18]

Procedure: The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. The standard solution containing mixture of NIM, PHP, CTZ was injected in different individual solvents as well as combination of solvents have been tried to get a good separation and stable peak. Each solution was filtered through Whatman filter paper no. 45. Well resolved peaks with symmetry within limits & significant based on sample solubility, various mobile phase compositions were evaluated to achieve acceptable separation using selected chromatographic condition

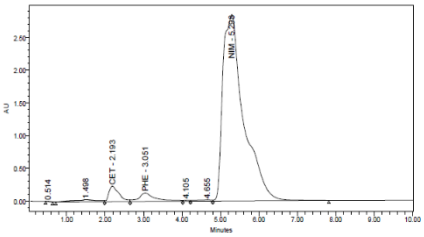
Mix working standard solution: 1ml NIM, 1ml PHP & 1ml CTZ were Pipetted out from above standard stock solution respectively into a 10ml volumetric flask, and diluted up to mark by ACN & to obtained resultant concentration of 1000ppm, 50ppm, 50ppm, of Nimesulide PhenylephrineHcl cetirizine Hcl respectively.
Principle: RP-HPLC elution and PDA detection.



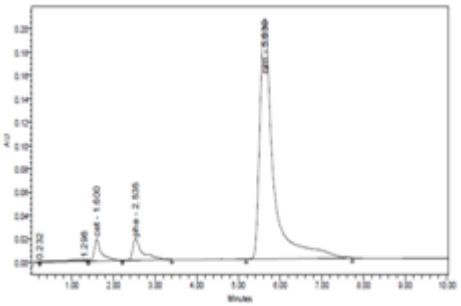
Sample Name: N+P+C+A55+W45+C18+R1+PH3
Figure 5: Nimesulide +phenylephrine Hcl +cetirizine HCl mixture of sample is separated in which the mixture of solvent acetonitrile 55% and water 45% a Modulator as sodium phosphate buffer of Ph 3. as a mobile phase and Phenomenex Gemini C18 150X4.60 mm 5µm Particle size is used as a stationary phaseas trial 1.



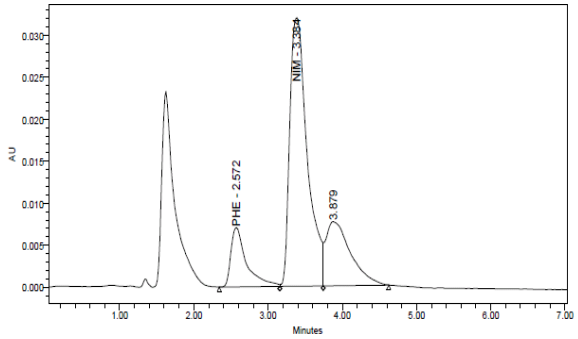
Sample Name: N+P+C+A50+W50+C18+R1+PH3.75+1% Triethanol amine
Figure 6: Nimesulide + phenylephrine Hcl +cetirizine HCl mixture of sample is separated in which the mixture of solvent acetonitrile 50% and water 50% a Modulator as sodium phosphate buffer of Ph 3.75& 1% Triethanol amine as a mobile phase and Phenomenex Gemini C18 150X4.60 mm 5µm Particle size is used as a stationary phase as trial 2.



Sample Name: N+P+C+A50+W50+C18+R1+PH3.20+2% Triethanol amine
Figure 7: Nimesulide + phenylephrine Hcl +cetirizine HCl mixture of sample is separated in which the mixture of solvent acetonitrile 50% and water 50% a Modulator as sodium phosphate buffer of Ph 3.20 & 2% Triethanol amine as a mobile phase and Phenomenex Gemini C18 150X4.60 mm 5µm Particle size is used as a stationary phase as trial 3



Sample Name -N+P+C+A50+W50+C18+R2+PH3.5+0.2% Triethanol amine
Figure 8: Nimesulide +phenylephrine HCl +cetirizine HCl mixture of sample is separated in which the mixture of solvent acetonitrile 50% and water 50% and a Modulator as sodium phosphate buffer of Ph 3.5 & for adjusting acidic nature of Ph a basic 0.2%Triehanol amine is added as a mobile phase and Phenomenex Gemini C18 150X4.60 mm 5µm Particle size is used as a stationary phase.and a sharp peak obtained .



Sample Name -N+P+C+A50+W50+C18+R2+PH3.5+0.2% Triethanol amine.

Figure 9: Nimesulide + phenylephrine HCl + Cetirizine HCl Separation of the three drugs in marketed preparation having brand name- Amma cold manufactured by –Onenass pharmaceutical Content NIM-100mg, PHP-5mg,CTZ - 5mgAverg weight of tablet 638.2mgin selected mobile phase showing

Table 2: Chromatographic Condition

Mobile Phase	Water:Acetonitrile (50:50) PH 3.5+0.2%Triethanol amine
Column	Phenomenex C15 150x 4.6mm, 5µm
Flow Rate	1 ml/min.
Wavelength	215 nm.
Column Temp.	40^{O}C
Injection Volume	20 µL
Sample Temp.	Ambient
Retention Time NIM PHP CTZ	 5.6min 2.5min 1.6min
Run Time	10.0 min.

Table 3: Separation goal and its remarks in Chromatography[9]

Characteristics	Acceptance Criteria
Accuracy/trueness	Recovery 98-102% (individual) with 80, 100, 120% spiked
Precision	RSD < 2%
Repeatability	RSD < 2%
Intermediate Precision	RSD < 2%
Specificity / Selectivity	No interference
Detection Limit	S/N > 2 or 3
Quantitation Limit	S/N > 10
Linearity	Correlation coefficient r > 0.999
Range	80 –120 %
Sample solution stability	> 24 h or >12 h

Table 4: Characteristics to be validated in HPLC[8,10,12]

Aim	Remarks
Resolution (Rs)	For precise and accurate quantitative method, Rs should be> 1.5
Separation time	For routine procedure<5-10 min.
Quantitation Pressure	RSD <2.0%
Pressure	<150 bars
Peak height	Narrow peaks for large S/N ratio
Solvent consumption	Minimum per sum is desirable

3. Result

Table 5: System Suitability

Sr. No.	Asymmetry			Theoretical plates		
	NIM	PHP	CTZ	NIM	PHP	CTZ
1	1.20	1.58	1.01	658718	1528384	104850
2	1.16	1.62	1.03	653679	1553139	101093
3	1.18	1.60	1.02	660328	1513254	100944
MEAN	1.18	1.6	1.02	657575	1531592	102295
+- S.D.	0.02	0.02	1.01	3468.73	20135.13	2213.37
%RSD	1.69	1.25	0.98	0.52	1.31	2.16

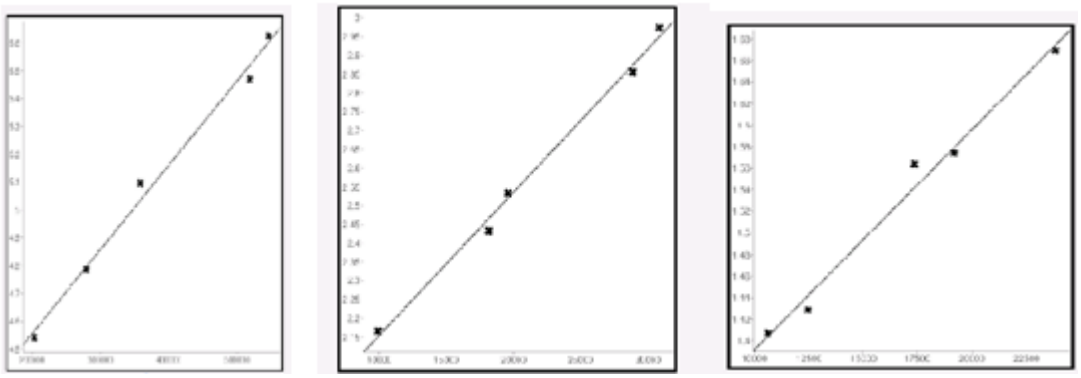
Table 6: Peak Area

Sr. No.	Peak Area			Retention time		
	NIM	PHP	CTZ	NIM	PHP	CTZ
1	4618287	300812	223654	5.630	2.535	1.6
2	4613385	300238	225818	5.630	2.535	1.6
3	4664035	308154	221318	5.630	2.535	1.6
MEAN	4631902.3	303068	223596.6	5.630	2.535	1.6
+- S.D.	27935.43	4413.94	2250.54	5.630	2.535	1.6
%RSD	0.60	1.46	1.01	5.630	2.535	1.6

Table 7: Linearity and range study

Acceptance criteria - the Correlation coefficient shall be NLT 0.98.[15,11]

Sr.no.	Addition level range Labelled claim	NIM			PHP			CTZ		
		Amt. ug/ml	mean peak area response	Amt. recovered	Amt. ug/ml	mean peak area response	Amt. recovered	Amt. ug/ml	mean peak area response	Amt. recovered
1	50%	50	202933	49.2	2.5	9880	2.49	2.5	10625	2.43
2	80%	80	279111	78.1	4.0	18183	3.97	4	17389	3.98
3	100%	100	357876	98.2	5.0	19608	4.98	5	19196	4.97
4	120%	120	518511	118.1	6.0	28861	5.42	6	23849	5.45
5	150%	150	545885	149.5	7.5	30847	7.42	7.5	24731	7.46
Correlation coefficient			0.9955		0.997			0.997		
Slope			3.025789		3.812055			2.0439131		
Intercept			3.951588		1.773330			1.1871356		



Nimesulide Phenylpherine HCL Cetrizine HCL

Figure 10: Linearity and range study

Table 8: Accuracy

	NIM			PHP			CTZ		
	Levels			Levels			Levels		
	50%	80%	100%	50%	80%	100%	50%	80%	100%
Amt added in ug/ml	50	80	100	2.5	4	5	2.5	4	5
	50	80	100	2.5	4	5	2.5	4	5
	50	80	100	2.5	4	5	2.5	4	5
Amt taken in ug/ml	50	80	100	2.5	4	5	2.5	4	5
	50	80	100	2.5	4	5	2.5	4	5
	50	80	100	2.5	4	5	2.5	4	5
AMT recovered ug/ml	49.2	78.1	98.2	2.37	3.91	4.98	2.32	3.98	4.90
	48.7	77.8	97.9	2.42	3.98	4.97	2.33	3.95	4.95
	48.5	77.7	97.8	2.30	3.96	4.96	2.31	3.94	4.98
% Recovery	98.4	97.62	98.2	94.8	97.75	99.6	92.8	99.5	98
	97.4	97.25	97.9	94.8	99.5	99.4	93.2	98.75	99
	97.0	97.125	97.8	95.2	99	99.2	92.4	98.5	99.6
Mean	97.6	97.331	97.966	95.63	98.75	99.4	92.8	98.91	98.86
%RSD	0.74	0.26	0.21	1.11	0.91	0.20	0.43	0.53	0.82

Acceptance criteria:

1) The % RSD for the triplicate at each spike level shall be NMT 2.0.
2) The overall %RSD for % recovery for all spike levels shall be NMT 2.0.
3) The % recovery at each spike level shall be NLT 98.0 and NMT 102.0 of the added amount.[14]

Table 9: System precision

Sr.no.	Parameter	Observation			Limits
		NIM	PHP	CTZ	
1	%RSD of peak area response for three replicate injections of std	0.21	0.98	0.16	NMT 2.0
2	Theoretical plates	3468.73	20135.13	2213.37	NLT2000
3	Tailing factor	1.69	1.25	0.98	NMT2.0

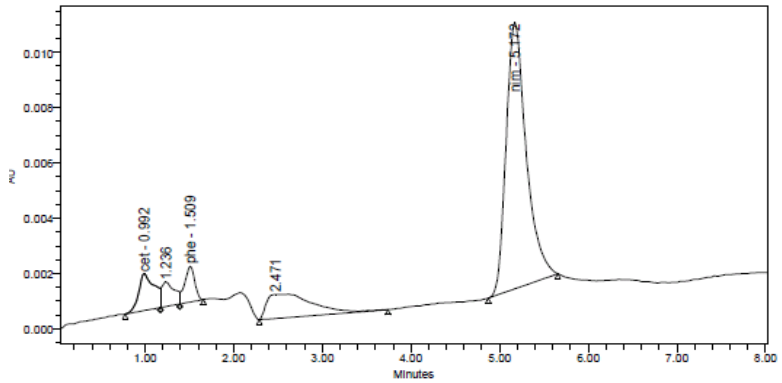
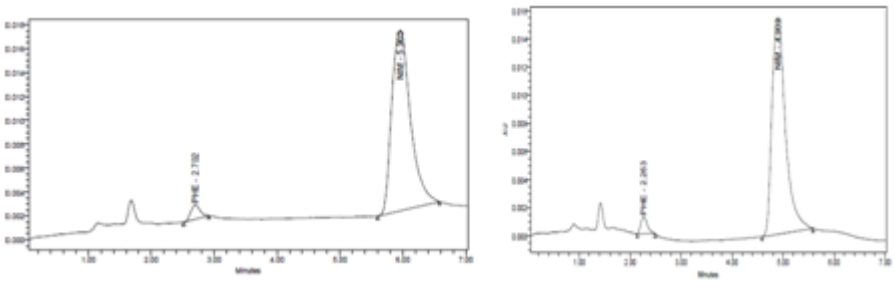


Figure 11: Chromatogram system precision showing repeatability

Effect of variation in flow rate



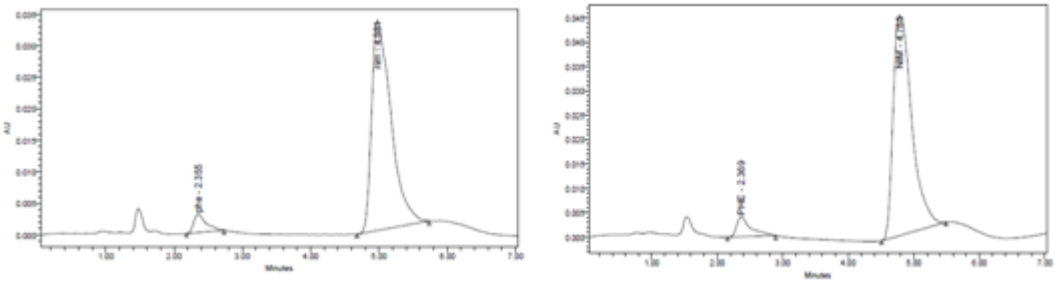
Flow 1.1ml/min Flow 1.3 ml/min

Figure 12: Effect of variation in flow rate

Table 10: Robustness: Flow rate

Sr no	System suitability parameter		Observations for flow rate			Limits
			Unchanged	1.1 ml	1.3ml	
1	% RSD of peak area response for five replicate injections	NIM	0.15	1.77	0.68	NMT 2.0
		PHP	0.87	0.08	1.42	
		CTZ	0.24	0.02	0.07	
2	Therotical plates	NIM	657575	586557	691029	NLT 2000
		PHP	1531592	1173880	1976612	
		CTZ	102295	92604	12435	
3	Tailing factor	NIM	1.92	1.87	1.8	NMT 2.0
		PHP	1.95	1.92	1.95	
		CTZ	1.93	1.8	1.9	
4	Retention time(min)	NIM	5.172	5.960	4.902	
		PHP	1.509	2.702	2.263	
		CTZ	0.992	1.6	1.4	

Change in mobile phase pH



Buffer PH 3.4 Buffer PH 3.6

Figure 13: Change in mobile phase pH

Table 11: Robustness: Change Ph

Sr no	System suitability parameter		Observations for flow rate			Limits
			Unchanged	3.4	3.6	
1	% RSD of peak area response for five replicate injections	NIM	1.82	0.38	0.01	NMT 2.0
		PHP	0.98	0.57	0.12	
		CTZ	0.07	1.26	0.82	
2	Therotical plates	NIM	657575	586557	691029	NLT 2000
		PHP	1531592	1173880	1976612	
		CTZ	102295	92604	12435	
3	Tailing factor	NIM	1.92	1.87	1.8	NMT 2.0
		PHP	1.95	1.92	1.95	
		CTZ	1.93	1.8	1.9	
4	Retention time(min)	NIM	3.821	4.981	4.785	
		PHP	2.2	2.355	2.369	
		CTZ	1.5	1.4	1.5	

Table 12: LOD AND LOQ

S. No.	API	LOD	LOQ
1	NIM	13.82917	41.98658
2	PHP	11.54075	41.4204
3	CTZ	11.38677	34.50538

LOD-3.3*Avg sd/slope
LOQ-10* Avg sd/slope
Application of proposed method for estimation of NIM PHP CTZ on marketed tablet formulation

Preparation of test solution:
For the test solution 10 tablets (Marketed preparation contains NIM 100 mg and PHP 5 mg CTZ 5 mg) were weight and the average weight was determined 10 tablet were triturated and powder . equivalent to 100 mg of NIM 5mg of PHP And CTZ was added into a 100 ml volumetric flask the contain were mixed with diluents and sonicate for 15 min and same contains were filtered through 0.45u membrane filter.1ml of resultant was taken in a 10 ml of volumetric flask and volume was made up to the mark.

Procedure:
Equal volume of 20ul of standard and sample solution was injected separately after equilibrium of stationary phase. The chromatogram was recorded and the response i.e peak area of major peaks were measured. The contain of NIM PHP CTZ were calculated by comparing sample peak with that of standard Amount of drug in tablet was calculated

% estimation = A_t/A_s x D_S/D_t x W_s/W_t x 100

4. Conclusion

Developed method was successfully applied to pharmaceutical formulation. No chromatographic interferences were found in tablet.The present study was undertaken with an objective of developing suitable, sensitive and simple analytical method like RP-HPLC method for simultaneous estimation of Nimesulidephenylephrine and cetirizine in marketed formulation. The result of analysis in all the method was validated as per ICH guidelines in terms of accuracy, precision, ruggedness, linearity and range. The method was found to be sensitive, reliable, reproducible, rapid and economic also Hence this method can be employed for routine quality control analysis of Nimesulidephenylephrine and cetirizine in solid dosage form.

5. Future Scope

- Comparative estimation of different brands can be conducted.
- The method can be developed for estimation of the proposed drugs in biological fluid

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