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Research

Method development and validation for ciprofloxacin and ornidazole in its bulk and combined dosage forms by RP–HPLC

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Abstract

A new method was established for simultaneous estimation of Ciprofloxacin and Ornidazole by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Ciprofloxacin and Ornidazole by using Inertsil C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH 4.6: ACN (30:70%v/v) (pH was adjusted with orthophosphoric acid), detection wave length was 255nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2. The retention times were found to be 2.399mins and 3.907mins. The % purity of Ciprofloxacin and Ornidazole was found to be 100.7% and 101.4% respectively. The system suitability parameters for Ciprofloxacin and Ornidazole such as theoretical plates and tailing factor were found to be 1.3, 5117.5 and 1.4, 3877.3 the resolution was found to be 8.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Ciprofloxacin and Ornidazole was found in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient (r2) was found to be 0.9988 and 0.999, % mean recovery was found to be 100% and 100.5%, %RSD for repeatability was 0.2 and 0.4, % RSD for intermediate precision was 0.5 and 0.1 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.95 and 3.04, and LOQ value was 9.87 and 10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Ciprofloxacin and Ornidazole in API and Pharmaceutical dosage form.

Keywords: Ciprofloxacin and Ornidazole, InertsilC18, 5µm, RP-HPLC

INTRODUCTION

Ciprofloxacin

Ciprofloxacin is chemically known as 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4dihydroquinoline-3-carboxylic acid. Ciprofloxacin is a potent broad-spectrum antibiotic effective against a wide range of bacterial infections, particularly those caused by Gram-negative organisms. It is frequently used in the treatment of UTIs, respiratory, gastrointestinal, and skin infections. While generally well tolerated, ciprofloxacin has significant side effects and should be used cautiously, particularly in older adults and those with

a history of tendon disorders, CNS issues, or cardiac arrhythmias. Ciprofloxacin inhibits bacterial DNA gyrase and topoisomerase IV, enzymes crucial for bacterial DNA supercoiling and replication. This action disrupts DNA replication and cell division, leading to bacterial death.

Ornidazole

Ornidazole is chemically known as 1-chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl) propan-2-ol. Ornidazole is an antibiotic and antiprotozoal medication used to treat infections caused by bacteria and protozoa. It belongs to the nitroimidazole class of drugs. It has a broad spectrum of activity. Ornidazole disrupts the DNA structure of anaerobic organisms. Once the drug enters the cells of bacteria or protozoa, it is reduced to reactive metabolites that bind to the DNA, leading to the inhibition of nucleic acid synthesis and subsequent cell death. It is commonly prescribed for conditions such as amoebiasis, giardiasis, trichomoniasis, and certain anaerobic bacterial infections. Ornidazole is available in oral, injectable, or topical forms, and its dosage depends on the type and severity of the infection. Common side effects may include nausea, dizziness, headache, and gastrointestinal discomfort.

MATERIALS AND METHODS

Chemicals and reagents

All reagents used were analytical grade, and solvents used were HPLC grade; Methanol and Water was procured from MERCK Pharmaceuticals. Potassium dihydrogen orthophosphate was purchased from MERCK Pharmaceuticals. Acetonitrile for HPLC was purchased from MERCK Pharmaceuticals. Ciprofloxacin was (100% pure) procured from MYLAN. Ornidazole (100% pure) was procured from CIPLA.

Instrumentation and software

Waters HPLC system Alliance 2695 separation module with an auto-injector, temperature controller for sample storage, and Empower Software were used to monitor the signal output. Feature UV double beram spectrophotometer -Model UV 3000 and Software UV Win 5. Digital Weighing balance -Model BSA224SCW, Ultra sonicator (model: SE60US), pH Meter (model: AD102U), Suction pump (model:VE115N) Thermal oven (make: NEWTRONIC) were employed in this work.

Chromatographic conditions

The chromatographic conditions were successfully developed for the separation of Ciprofloxacin and Ornidazole by using Inertsil C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH 4.6: ACN (30:70%v/v) (pH was adjusted with orthophosphoric acid). Detection and quantitation of the main active pharmaceutical ingredients were achieved using a PDA detector at 255 nm.

Standard preparation

10mg of Ciprofloxacin and Ornidazole working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2 ml of DMF is added. Then it is sonicated to dissolve it completely and make volume up to the mark with the diluent. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted up to the mark with diluent.

Sample preparation

Accurately 10 tablets are weighed and crushed in mortar and pestle and weight equivalent to 10 mg of Ciprofloxacin and Ornidazole (marketed formulation) sample into a 10mL clean dry volumetric flask and about 7mL of Diluents is added and sonicated to dissolve it completely and made volume upto the mark with the same solvent. (Stock solution) Further 3 ml of above stock solution was pipetted into a10ml volumetric flask and diluted upto the mark with diluent.

RESULTS AND DISCUSSION

Method development strategy and optimization

To provide a suitable method for the routine quality control analysis of this multi component drug mixture. The developed process was carefully planned and optimized to separate the cited compounds. The most critical aspect in RP-HPLC method development is the achievement of sufficient resolution of the analytes with good peak symmetry in a reasonable analysis time. Many experiments were conducted to optimize both the stationary and mobile phases for better results. In these trials, estimation was based on efficient resolution between the two analytes peaks. For optimization of the mobile phase, different types of mobile phase were tested entirely, such as Methanol: water (60:40 v/v), water: Methanol (40:60 v/v), Phosphate buffer (0.05m) pH 5.0: Methanol (50:50%v/v), Phosphate buffer (0.05M) pH 4.6: MeOH. The most desirable clear separation between the two

primary compounds within a relatively short run time was obtained. Method Development Trails are done using the Inertsil C18 (4.6 x 250mm) column; consequently, it became the Mobile phase of choice for this mixture. Other Mobile phase exhibited poor separation between the peaks of the target compounds. The excessive tailing for the peaks was another disadvantage of using the Methanol: water (60:40 v/v), water: Methanol (40:60 v/v), Phosphate buffer (0.05m) pH 5.0: Methanol (50:50%v/v), Phosphate buffer (0.05M) pH 4.6: MeOH. The multi-wavelength ranges were evaluated to measure each analyte at its maximum wavelength to verify the sensitivity. Ciprofloxacin and Ornidazole show stronger UV absorption with prominent peaks at 255 nm. Further optimization was carried eluting peaks with optimal separation by the flow rate 1 mL/min and column temperature (range from 25°C to 40°C).Estimation of Ciprofloxacin and Ornidazole in different mobile phases, solvent-buffer ratios were tried to propose final chromatographic conditions. The shape of the peaks, the symmetry, and resolution of Ciprofloxacin and Ornidazole were good with mobile phase containing phosphate buffer (0.05M) pH 4.6 and ACN in a ratio of (30:70v/v). Isocratic elution at a 1mL/min flow rate, sample, and column temperature was Ambient. The developed method was successfully helpful to estimate the amount of Ciprofloxacin and Ornidazole in bulk and tablet dosage form. The Optimised Chromatographic Conditions are shown in table 2, Chromatogram was illustrated in (Figure 3)

Method validation

Analytical method validation is necessary to ensure that the analytical method employed for a specific test is appropriate for its intended purpose. After method development, analytical techniques were validated before the duration of routine use. The parameters evaluated during contemporary method development include specificity, linearity, range, accuracy, robustness, & precision. The proposed method was to conform based on the International Conference on Harmonization (ICH) Q2 (R1) guidelines.

Specificity

An essential obligatory ICH guideline for method validation is specificity or selectivity. In other words, specificity is the ability to evaluate the purity of the analyte in the presence of the co-eluting or co-migrating impurity. The method specificity was demonstrated by demonstrating that no excipients mediate with the retention time of both drugs in the assay sample chromatogram.

Method precision

In method precision, a homogenous test of a single batch was analyzed six times. The results assure whether a method produces consistent results for a single batch. Calculate the percent relative standard deviation (%RSD). The suggested method was found to be precise since the RSD values method precision was below 1.0. The summary results were shown in table3.

Intermediate precision

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of the same dimensions. The % RSD for the area of five standard injections results should not be more than 2%.The summary results were shown in table 3.

LOD (Limits of Detection) and LOQ (Limits of Quantification)

The LOQ and LOD are calculated using signal-to-noise ratios at analytical responses of 3×10 times the background noise. The method validation results were shown in table 3.

LOD (mg/L) =3 × Noise/signal × Lowest concentration of the linearity samples

LOD (mg/L) =10 × Noise/signal × Lowest concentration of the linearity samples

Linearity

An assay can obtain test results directly proportional to the concentration of an analyte in the sample. The determination of this parameter will describe the range of the analytical assay. The linearity of the method was intended by drawing the calibration curves. Standard solutions of Deferasirox and Deferiprone of different concentration levels (10%-150%) prepared by serial dilution of standard stock solution) were used for this purpose. The summary results were presented in table 3, and the linearity curve was shown in (Figure 4,5).

Accuracy

The accuracy of an analytical method is the closeness of the test results obtained by the process to the actual value. Accuracy may often be expressed as a percent of recovery by testing known added amounts of analyte. Accuracy was the measurement of the exactness of the analytical method. In this HPLC method, the recovery of the samples was verified with three concentration levels (50%, 100% & 150%). The recovery was performed by API + placebo and injected into the HPLC (triplicate).The summary results were presented in table 3.

Robustness

To demonstrate the robustness of the method, changes were made to the chromatographic conditions and system suitability parameters, such as tailing factor (<2.0), theoretical plate counts (>3000), and resolutions were between the nearest peaks (>2.0). Based on the results, the maximized method was proved robust, even under changed conditions. The outline results were presented in table 4.

Filter validation and solution stability

Two different types of 0.45µm filters (Nylon and PVDF) were used to determine the filter's effect on the sample. Concentrations of both types of filtered samples were calculated and compared against the centrifuged sample and showed no difference in results. The sample solution was stable for up to 24 h on the bench.

Ciprofloxacin:

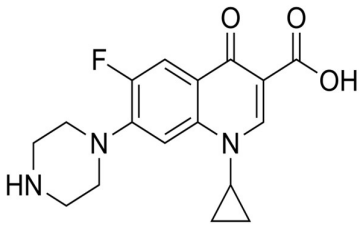
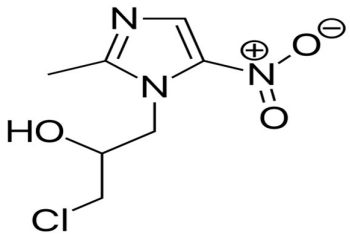


Fig 1: Chemical Structures of Ciprofloxacin and Ornidazole.

Ornidazole:



Chromatogram for Ciprofloxcin and Ornidazole Standard Preparation

Retention time of Ciprofloxacin – 2.237 min

Retention time of Orfloxacin - 4.342 min.

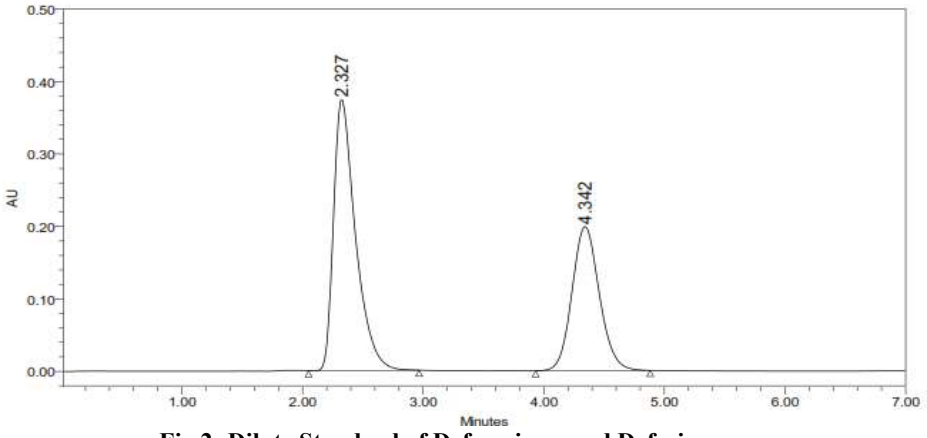


Fig 2: Dilute Standard of Deferasirox and Deferiprone

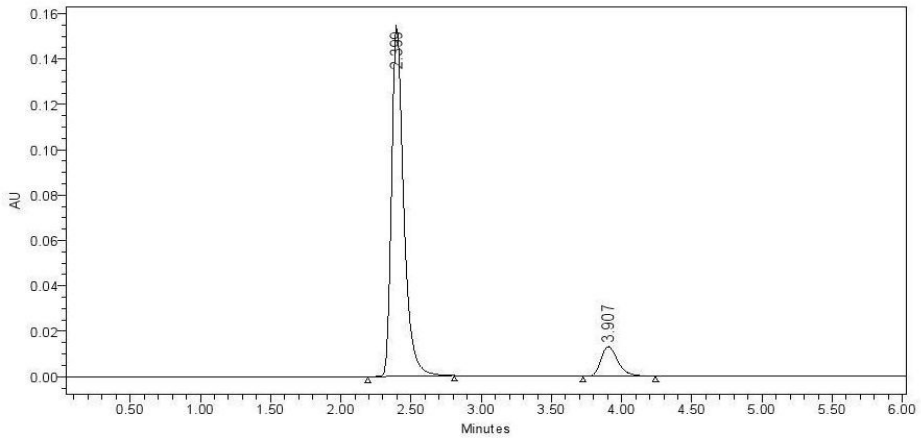


Fig 3: Optimized Chromatogram

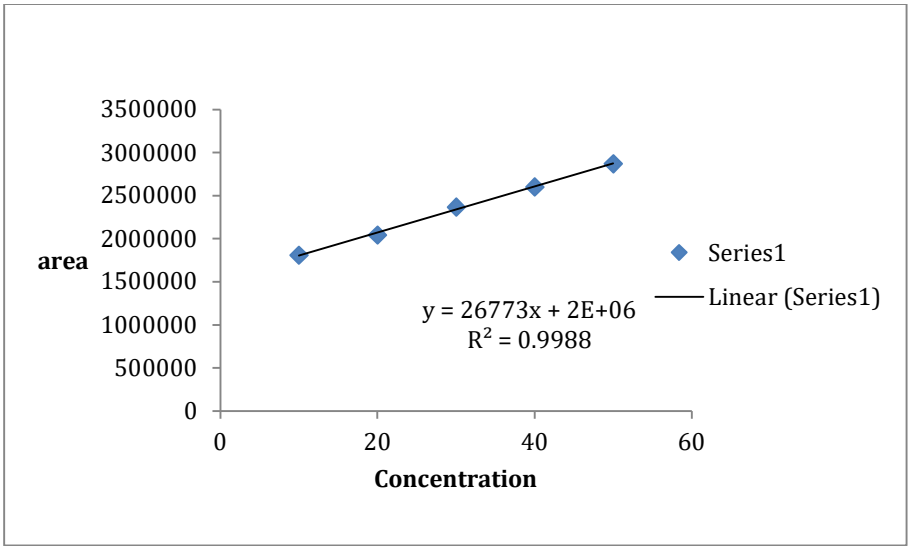


Fig 4: Calibration curve of Ciprofloxacin at 260 nm

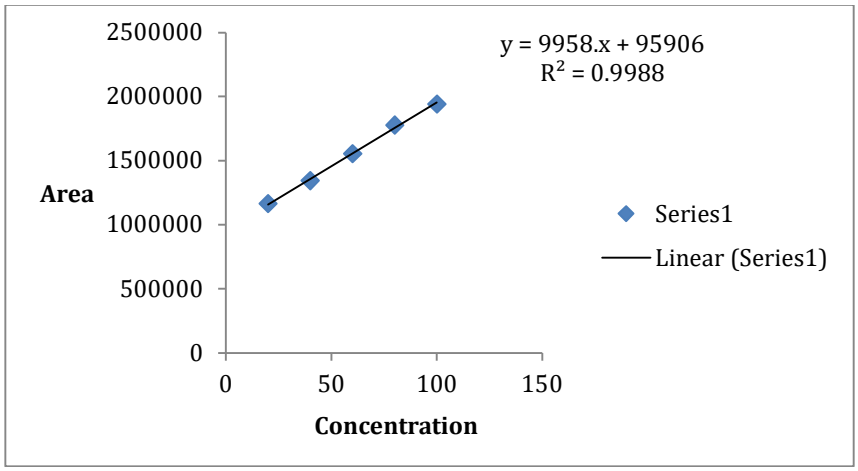


Fig 5: Calibration curve of Ornidazole at 260 nm

Table 1: System suitability evaluation.

S.No	Peak name	Rt	Area	Height	USP Plate count	USP Tailing	USP Resolution
1	Ciprofloxacin	2.237	7913799	394185	2632	1.8	
2	Ornidazole	4.342	1853381	162758	2614	1.6	5.23

Table 2: Summary of Optimized Chromatographic Conditions

Parameters	Description
Flow rate	1 ml/min
Column	Inertsil C18 5µm (4.6 mm x 250mm)
Mobile Phase	Phosphate buffer (0.05M) pH 4.6: ACN (30:70%v/v)
Buffer	pH was adjusted with orthophosphoric acid (pH 4.6)
Type of elution	Isocratic
Detector	PDA
Column temperature	Ambient
Wavelength	255 nm
Injection volume	20µl
Run time	10min

Table 3: Method validation results

Parameters	Ciprofloxacin	Ornidazle
Linearity		
Range (µg ml^{-1})	10-50µg/ml	20-100µg/ml
Slope	26773	9958
Intercept	2E+06	95906
Correlation Coefficient	0.9988	0.9988
LOD (µg/mL-1) S/N Ratio	2.95	3.04
LOQ (µg/mL-1) S/N Ratio	9.87	10.0
Accuracy(a) (% of Recovery)		
50%	101.8	101.3
100%	99.9	99.4
150%	99.1	99.2
Precision(b)(%RSD)	0.3	0.3
Intermediate precision	0.1	0.1

** All the parameters R^2, S/N Ratio, %RSD should be within limits.*

Table 4: Robustness evaluation.

Parameter	Ciprofloxacin		Ornidazole	
	USP Tailing	USP Plate Count	USP Tailing	USP Plate Count
Normal (1.0 ml/min)	1.1	1234.0	1.2	1548.2
Less Flow (0.8 ml/min)	1.56	883.3	1.22	1748.5
More Flow (1.2 ml/min)	1.2	969.2	1.2	1948.0
*Actual Organic Composition	1.1	1234.0	1.2	1548.2
High Organic (10% More)	1.6	969.2	1.2	1948.0
Low Organic (10% Less)	1.56	883.3	1.22	1748.5

** Results for actual Mobile phase composition (55:45 Buffer: ACN) have been considered from the Accuracy standard.*

CONCLUSION

A sensitive & selective stability indicating RP-HPLC method has been developed & validated for the analysis of Ciprofloxacin and Ornidazole in bulk and pharmaceutical dosage form. Based on peak integrity results,

obtained from the analysis of samples using demonstrated method, it can be certified that the absence of co-eluting peak along with the main peak of Ciprofloxacin and Ornidazole suggested that the developed method is precise for the analysis of Ciprofloxacin and Ornidazole in the bulk and pharmaceutical dosage forms. Further the proposed RP-HPLC method has acceptable sensitivity, precision and reproducibility.

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Abbreviations
HPLC: High-performance liquid chromatography; ICH: International conference on harmonization; LOD: Limit of detection; LOQ: Limit of quantification; Rt: Retention time; RSD: Relative Standard Deviation.

REFERENCES

1. G.R. Chatwal, S.K. Anand, Text book of Instrumental Methods of Chemical Analysis, Himalaya Publishing House, 5th Ed, 2002, p.2.566-2.570.
2. G.W.Ewing, Text book of Instrumental Methods of Chemical Analysis, Mc Graw-Hill Book Company, 5th Ed, p.375-385.
3. B.K. Sharma, Textbook of Instrumental Methods of Chemical Analysis, GOEL publishing house, Meerut, 23rd Ed, p.288-289.
4. G.Vidya sagar, Textbook of Instrumental Methods of Drug Analysis, Pharmamed Press, 2009,p.106-120.
5. H.H Willard, L. L Merritt, J.A Dean and F.A Settle, Textbook of Instrumental Methods of Analysis, CBS publishers and distributors, New Delhi, 7th Ed, 1986, p.592-596.
6. H.H.Tackett, J.A.Cripe, G.Dyson, Positive displacement reciprocating pump fundamentals-power and directacting types, Proceedings of the twenty-fourth international pump user's symposium, 2008, p.45-58.
7. D.A. Skoog, F.J. Holler, S.R. Crouch, Text book of Instrumental Analysis, Brooks/Cole, Cengage Learning India Private Limited, 2007, p.900-906.
8. R.E. Schirmer, Text book of Modern Methods of Pharmaceuticals, CRC press, 2nd Ed, P.242-244.
9. LR. Snyder, JJ Kirkland, LG. Joseph, Practical HPLC Method Development, Wiley Inter Science, New York, 2nd Ed, 1997, p.1-56.
10. Ranjith singh, HPLC Method Development and Validation- an Overview. J.Pharm.Educ. Res.4(2013)26-33.
11. ICH: Q2B, Analytical Validation–Methodology (1996).
12. Brij Bhushan, Uttam Singh Baghel, Ramandeep Singh, RP-HPLC method development for the estimation of Ciprofloxacin and Phenylephrine hydrochloride in combined dosage form. International Journal of Pharmaceutical and Medicinal Research, 2013;1(2):85-90.
13. T. Raja and A. Lakshmana Rao, Development and Validation of a Reversed Phase HPLC Method for Simultaneous Determination of Ciprofloxacin and Montelukast Sodium In Tablet Dosage Form. International Journal of Research in Pharmacy and Chemistry, 2012, 2(4).
14. Kaminee Parmar, Sunil Baldania, Dimal Shah, Usmangani Chhalotiya, and Naimin Parmar, Development and Validation of First-Order Derivative Spectrophotometry for Simultaneous Determination of Ciprofloxacin Dihydrochloride and Phenylephrine Hydrochloride in Pharmaceutical Dosage Form. International Journal of Spectroscopy. 2013. Article ID 502310, 6 pp.
15. Patel, Nilam K.; Patel, Shirish; Pancholi, S.S., HPLC Method Development and Validation for Simultaneous Estimation of Montelukast Sodium and Ciprofloxacin Dihydrochloride In Pharmaceutical Dosage Forms. International Journal of Pharmacy & Pharmaceutical Sciences; Mar2012,. 4(2), p241
16. Hashem AlAani, Iman Alashkar, Development and Validation of Stability-Indicating RP-HPLC Method for the Analysis of Ciprofloxacin Dihydrochloride and Fexofenadine Hydrochloride in the Presence of Parabens in Liquid Dosage Forms .Int. J. Pharm. Sci. Rev. Res., 23(2), Nov – Dec 2013; nº 12, 64-71
17. Deepali A.Nanaware, Vidhya K. Bhusari , Sunil R. Dhaneshwar, Validated HPLC Method for Simultaneous Quantitation of Levocetrizine Dihydrochloride and Phenylephrine Hydrochloride In Bulk Drug and Formulation. IAJPR. 2013; 3(5): 3484-3495.
18. Arindam Basu, Krishnendu Basak, Mithun Chakraborty, Inder Singh Rawat, Simultaneous RP-HPLC Estimation of Ciprofloxacin Hydrochloride and Montelukast Sodium in Tablet Dosage Form. International Journal of PharmTech Research, Vol.3, No.1, pp 405-410, Jan-Mar 2011.

19. Kumar A, Sharma R, Nair A, Saini G. Development and validation of RP-HPLC method for simultaneous estimation of nimesulide, phenylephrine hydrochloride, chlorpheniramine maleate and caffeine anhydrous in pharmaceutical dosage form.Acta Pol Pharm. 2012 Nov-Dec;69(6):1017-22.
20. Manoj S. Charde, R. A. Kundu, M. H. Ghante, R. D. Chakole,Development and validation of RP-HPLC method for simultaneous estimation of nimesulide, and Drotavirine HCL in pharmaceutical dosage form.international journal of pharmaceutical chemistry.
21. Rajeev Kumar R. Singh, Manapragada V. Rathnam, Sangeeta J. Singh, andRaju V. K. Vegesna, Stability Indicating Method for Simultaneous RP HPLC Determination of Camylofin Dihydrochloride and Nimesulide in Pharmaceutical Preparations. ISRN Analytical Chemistry,Volume 2012 (2012), Article ID 586415, 7 pages
22. Deepali Gharge, Pandurang Dhabale, Simultaneous Estimation of Nimesulide and Paracetamol in Solid Dosage Form by Rp-Hplc Method, International Journal of PharmTech Research, Vol.2, No.2, pp 1330-1333, April-June 2010.