Research Article

RP-HPLC Method for Simultaneous Estimation of Nadifloxacin and Mometasone Furoate in Semisolid Dosage From

Naik Shreya*, Dr.Desai Shuchi, Bijal Patel
Department of Pharmaceutical Quality Assurance, ROFEL Shri G. M. Bilakhia College of Pharmacy, Vapi – 396191, Gujarat, India.
***Corresponding author's E-mail:** Shreyanaik321@gmail.com

Received: 16-07-2022; **Revised:** 19-09-2022; **Accepted:** 26-09-2022; **Published on:** 15-10-2022.

ABSTRACT

The objective was to develop and validate an easy, economical, fast, reliable, reproducible, precise and accurate reversed-phase high-performance liquid chromatography (RP-HPLC) method for the estimation of Nadifloxacin and Mometasone furoate in semisolid dosage form. The chromatographic separation was achieved by using the HPLC system equipped with a C-18(2) column (250 × 4.6 mm, Particle size 5µm) at wavelength 254 nm. The mobile phase consisting of Methanol : Acetonitrile : water (pH-4 adjusted with OPA) in ratio of (55:30:15 v/v) was used in isocratic mode. The flow rate was fixed at 1.0 mL/min with a continuous run up to 10 min, while the retention time was located near about 3.870 and 5.377 min for Nadifloxacin and Mometasone Furoate respectively. The dosage form is cream named as NADIREST-M available in market which has Chlorocresol as preservative. So, to see the interference of Chlorocresol it was also estimated and there was no interference in estimation of both drugs by checking system suitability parameters (Chlorocresol peak has resolution more than 2 from the peak of drug). In the concentration range of 50-130 µgmL-1 and 5-13 µgmL^{-1} Nadifloxacin and Mometasone Furoate respectively, the detector response was found linear with linear regressed equation Y=15116X-365319 and Y=52717X-184314. In the assay of NADI, 99.12% and MOM 99.77% of the drug was recovered. This method proved a satisfactory validation for all the parameters such as accuracy, linearity, specificity, precision, range, ruggedness, robustness, reproducibility as per ICH guidelines. The results of the study evidenced that it is useful for the routine determination of Nadifloxacin and Mometasone Furoate in Semisolid pharmaceutical dosage forms like a cream.

Keywords: Nadifloxacin, Mometasone Furoate, Chlorocresol, Nadirest-M, ICH Guidelines, HPLC.

QUICK RESPONSE CODE →

DOI:
10.47583/ijpsrr.2022.v76i02.023

DOI link: http://dx.doi.org/10.47583/ijpsrr.2022.v76i02.023

INTRODUCTION

NADIFLOXACIN is a topical fluoroquinolone antibiotic used to treat bacterial skin infections . It is also used in treatment of acne vulgaris. MOMETASONE FUROATE is medium-strength corticosteroids used to treat skin conditions. It is official in Indian Pharmacopeia 2018. It decreases swelling (inflammation), redness and itching. Nadifloxacin inhibits the enzyme DNA gyrase that is involved in bacterial DNA synthesis and replication, thus inhibiting the bacterial multiplication. Nadifloxacin has chemical formula $C_{19}H_{21}FN_2O_4$ with IUPAC name 9-Fluoro-8-(4-hydroxy-1-piperidinyl)-5-methyl-1-oxo-6,7- dihydro-1H,5H-pyrido [3,2,1- ij]quinoline-2-carboxylic acid and Molecular weight 360.4 g/mol. Characteristic-White amorphous powder. Freely soluble in DMSO, slightly soluble in water, methanol and ethanol. Melting range 244-249 ˚C. Log P value is 2.9. Storage store at room temperature. CAS No 124858-35-1. Mainly used in bacterial skin infections i.e. Acne vulgaris.

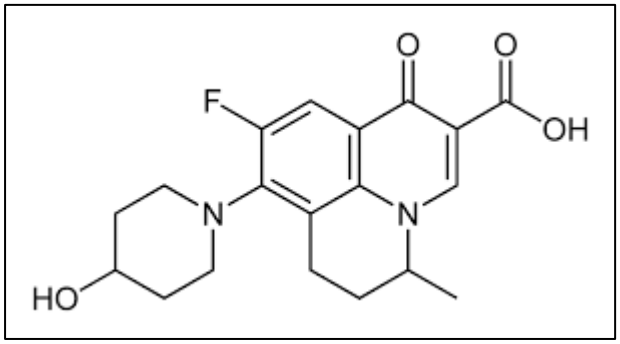

Figure 1: Chemical structure of Nadifloxacin

MOMETASONE FUROATE [3,4] shows anti-inflammatory, antipruritic, and vasoconstrictive properties. Corticosteroids are thought to act by the induction to phospholipase A2 inhibitory proteins, collectively called lipocortin. It is postulated that these proteins control the biosynthesis of potent mediators of inflammation. Mometasone Furoate is official in Indian Pharmacopeia 2014 and United States Pharmacopeia 2004. Mometasone Furoate has chemical formula $C_{27}H_{30}Cl_2O_6$ and IUPAC name 9α,21-Dichloro-11β-hydroxyl-16αmethyl-3,20-dioxopregna-1,4-dien17-yl furan-2-carboxylate. It has Molecular weight 521.4 g/mol. Freely soluble in acetone and dichloro methane. Slightly soluble in alcohol. Insoluble in water. Melting range 244-249 ˚C . Log P value is 3.9. Storage store at room temperature. CAS No 105102-22-5. Mainly used in itching, redness, inflammation.

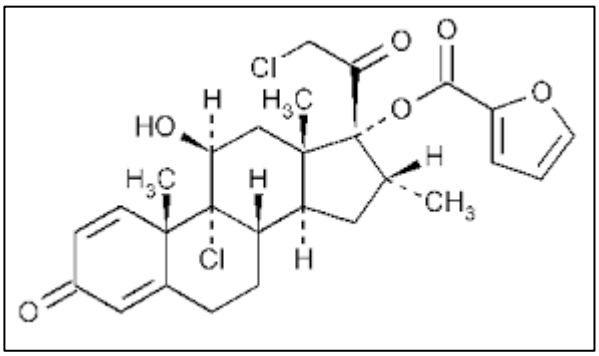

Figure 2: Chemical structure of Mometasone Furoate

Nadifloxacin (10mg/gm of cream) and Mometasone (1mg/gm of cream) combination is approved by CDSCO on 17/08/2006. The combination cream is available in market named as NADIREST-M cream [5].

Detailed Literature review was done for methods already submitted on Nadifloxacin, Mometasone Furoate and with other combination but it was found no RP-HPLC was done for simultaneous estimation of Nadifloxacin and Mometasone Furoate in combination [6-19].

Figure 3: Dosage form Nadirest-M Cream.

MATERIALS AND METHODS

Instruments and Apparatus: Younglin HPLC system was used for method development and validation. Data acquisition was performed on Acme 9000 HPLC with UV730 D detector (Autochrome-3000 software). The separation was achieved on Cosmosil C18 column (250 × 4.6 mm, 5 µm) column. Digital balance (Wensar high precision balance, PGB 100, sensitivity: 0.01 mg), pH meter (Unitech, ME-302), Wensar Ultra Sonicator (WUC-4L) and pipettes and volumetric flasks (Borosilicate) used during the study.

Experimental Section

NADIFLOXACIN was obtained as gift sample from Daiwik Pharmaspheres, Vapi and MOMETASONE FUROATE was obtained as gift sample from Sumit Laboratories, Vapi. Nadirest-M cream was procured from local market. HPLC grade methanol, HPLC grade water, AR grade orthophosphoric acid was procured from Merck Ltd., India.

Chromatographic Conditions

Stationary phase (Column): Cosmosil C18 column (250 × 4.6 mm, 5 µm)

Mobile phase: Methanol : Acetonitrile : water (pH-4 adjusted with 0.1% OPA) (55:30:15 v/v)

Detection wavelength: 254 nm

Flow rate: 1 ml/min

Injection volume: 10 µl

Temperature: Room temperature

Run time: 10 Min

Preparation of 0.1% OPA Solution: Take 0.1 ml of OPA and transfer it into a 100 ml volumetric flask add HPLC grade water in it and dissolve them completely. Then make up the volume by remaining water to get 0.1 % v/v. The resulting solution was filtered through a 0.45µ membrane filter and sonicated for three cycles each of 10 min.

Preparation of Mobile Phase: Methanol : Acetonitrile : water (pH-4 adjusted with 0.1% OPA) (55:30:15). Methanol, acetonitrile and water with 0.1% OPA pH 4 mixed thoroughly and degassed by sonication.

Preparation of Diluent: Mobile phase was used as diluent.

Preparation of Standard Stock Solution:Preparation of Nadifloxacin Standard Stock Solution (1000 µg/ml): Standard solution was prepared by dissolving 10 mg of Nadifloxacin in 10 ml methanol in clean and dry volumetric flask, add and make volume up to the mark with the mobile phase.

Preparation of Mometasone Furoate Stock Solution (100 µg/ml): Standard solution was prepared by dissolving 1 mg of Mometasone Furoate in 10 ml methanol in a clean and dry volumetric flask, add and make the volume up to the mark with the mobile phase.

Working Standard Solution of Nadifloxacin and Mometasone Furoate (50:5 µg/ml): A 0.5 ml (standard stock solution of Nadifloxacin 1000 µg/ml) and 0.5 ml of (standard stock solution of Mometasone Furoate 100 µg/ml) were pipetted out and transferred into 10 ml volumetric flask, volume was made up to the mark with the mobile phase.

Preparation of Sample Solution: Preparation of Sample Stock of Nadifloxacin and Mometasone Furoate (50:5 µg/ml): Cream equivalent to 10mg of NADI was taken into 100 ml of volumetric flask and added 10 ml of Tetrahydrofuran, the solution was warmed for 5-10 mins, ultrasonicated for 20 mins to completely disperse the cream, followed by addition of 50ml methanol and ultrasonicated for 15min and was makeup upto the mark with methanol. The Solution was filtered through Whatmann filter paper no. 41. Thus, resulting solution gave 100 µg/ml of NADI and 10 µg/ml of MOM respectively. From the above solution, 5 ml was pipette out and transferred to 10 ml volumetric flask and volume was made upto mark with methanol in order to give a solution containing NADI (50 µg/ml) + MOM (5µg/ml).

Method Validation[40]

Specificity

Specificity of the method can be termed as the absence of any interference at a retention time of samples. Specificity was performed by injecting blank and standard preparations. Chromatograms were recorded and retention times from sample and standard preparations were compared for identification of analytes.

Linearity and Range

A series of standard solutions 50-130 µg/ml of Nadifloxacin and 5-13 µg/ml of Mometasone Furoate were prepared. An aliquot of 10µl of each solution was injected 3 times for each standard solutions, and peak area was observed. The plot of average peak area versus the concentration is plotted, and from this, the correlation coefficient and regression equation were generated. The calibration data of Nadifloxacin and Mometasone Furoate is given in Table 3, while Fig. 4 represents overlain Fig. 5 and 6 represent linearity graphs of both drugs respectively.

Limit of Detection

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. The limit of detection (LOD) of the drugs was derived by calculating the signal to noise ratio (S/N, i.e., 3.3. Limit of detection can be calculated using the following equation as per ICH guidelines.

$$LOD = 3.3 \times \sigma/S$$

Where, σ = the standard deviation of response and S = Slope of calibration curve. Shown in Table 4.

Limit of Quantification

It is the lowest concentration of an analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions. The limit of quantification (LOQ) of the drugs was derived by calculating the signal to noise ratio (S/N, i.e., 10 for LOQ) using the following equation as per International Conference on Harmonization (ICH) guidelines.

$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of response and S = Slope of the calibration curve. Shown in Table 4.

Method Precision

The method was validated regarding intra-day and inter-day precision. The intra-day and inter-day study were performed by injecting 70, 90 and 110 µg/ml of Nadifloxacin and 7, 9 and 11 µg/ml of Mometasone Furoate three times for each aliquot. The % RSD for the precision study was found less than 2% as shown in Table 5.

Accuracy (% Recovery)

The recovery of an analytical method is determined by applying the method to analyze samples to which known amounts of analyte have been added. The recovery is calculated from the test results as the percentage of analyte recovered by the assay. The known amounts of standard solutions of Nadifloxacin (40, 50 and 60 µg/ml) and Mometasone Furoate (4., 5 and 6 µg/ml) were added to a pre quantified test solutions of Nadifloxacin and Mometasone Furoate (50 µg/ml) and (5 µg/ml). Each solution was injected in triplicate, and the recovery was calculated by measuring peak areas. Results are shown in Table 6.

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the method was studied by changing flow rate (±0.2ml/min-1), mobile phase composition. After each changes sample solution was injected and system suitability parameters were observed. The results were shown in Table 7.

Analysis of Combined Dosage Forms of Nadifloxacin and Mometasone Furoate

Pharmaceutical formulation of Nadifloxacin and Mometasone Furoate in combined dosage form was purchased from local market. The response of combined dosage form was measured at 254 nm for quantification of Nadifloxacin and Mometasone Furoate by using RP-HPLC. The amount of Nadifloxacin and Mometasone Furoate present in sample solution were determined by the responses into the regression equation for Nadifloxacin and Mometasone Furoate in the method. Results are given in Table 8. The experiments mentioned above were carried out in a controlled environment during the academic year 2018-2020 at the Quality Assurance Laboratory of ROFEL Shri G. M. Bilakhia college of Pharmacy, VAPI, India.

RESULTS AND DISCUSSION

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry were found in a mixture of 55:30:15 methanol, acetonitrile and water with pH 4 adjusted with 0.1% OPA and flow rate 1 ml/min proved to be better than the other compositions regarding resolution and peak shape. The effluent was monitored at 254 nm using a UV detector. The retention times of Nadifloxacin and Mometasone Furoate were found to be 3.870 and 5.377 min respectively. A chromatogram of the mixture in optimized conditions is shown in Fig. 3 and the system suitability parameters are shown in Table 2 . To check interference of Chlorocresol peak was compard with standard solution of chlorocresol for confirmation which was observed at 2.790 min and as the resolution

was more than 2 from other two drug peak, we can say there was no interference of chlorocresol in selected mobile phase.

Table 1: System suitability parameters specifications

SST	Limits
Resolution (Rs)	> 2.0
% RSD	<2.0%
Plate count (N)	>2000
Tailing factor (Tf)	< 2.0

System Suitability Study: System suitability study shown in Table 2.

Table 2: System suitability parameter study

Parameter	Nadifloxacin (n=5)	Mometasone Furoate (n=5)
Retention time (Rt) (min)	3.780	5.175
Resolution (R) s	2.550	
Theoretical plates (N)	6786.15	7395.49
Tailing factor (N) f	1.644	2.550

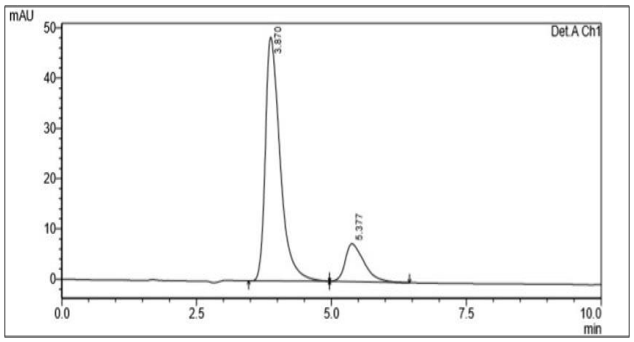

Figure 3: Optimized chromatogram of Nadifloxacin and Mometasone Furoate

Specificity

The method was found to be specific as there was no interference observed in any of the parameters under observation.

Linearity and Range

The linearity of Nadifloxacin and Mometasone Furoate were found between 50-130 µg/ml and 5-13 µg/ml respectively. The results are shown in Figure 4.

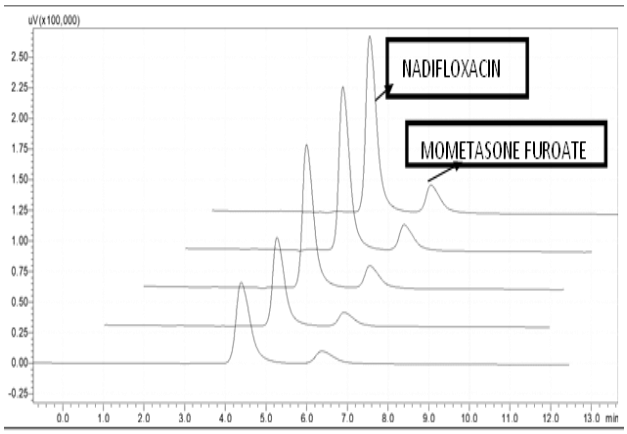

Figure 4: Overlain chromatogram of linearity study

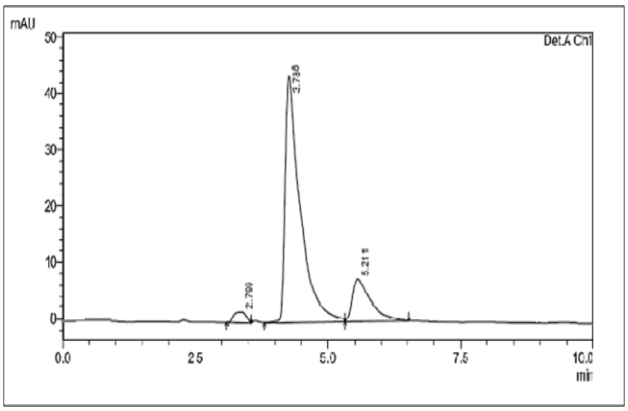

Figure 5: Chromatogram of Marketed formulation (cream)

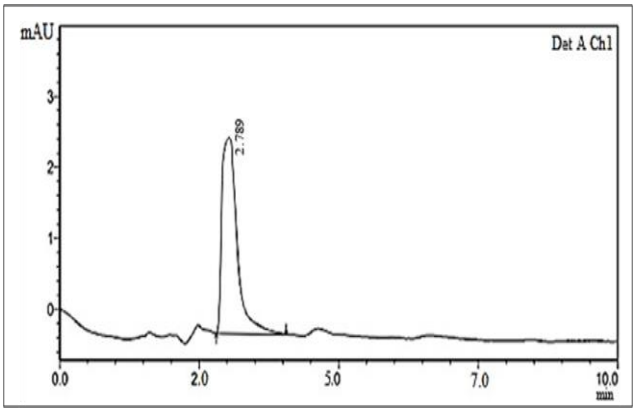

Figure 6: Conformation of Chlorocresol at 2.789 min

LOD and LOQ

The LOD was found to be 5.1686 µg/ml for Nadifloxacin and 0.1053 µg/ml for Mometasone Furoate, while the LOQ was found to be 15.6626 µg/ml for Nadifloxacin and 0.3191 µg/ml for Mometasone Furoate shown in Table 3.

Precision

The % RSD for repeatability study for Nadifloxacin and Mometasone Furoate was found to be 0.4963 and 1.3279 respectively. The Inter-day and Intra-day study also show % RSD value for Nadifloxacin and Mometasone Furoate within the acceptable limit. Results for precision study are shown in Table 4.

Accuracy

Accuracy of the method was confirmed by recovery study at three levels (80%, 100%, and 120%) of standard addition. Percentage recovery for Nadifloxacin was found to be 99.04% to 101.08%, while for Mometasone Furoate it was found to be 99.20% to 101.66% as shown in Table 5.

Robustness

The typical variations studied under this parameter were flow rate and mobile phase composition. Overall % RSD was found to be less than 2% for all variations which indicates that the proposed method is robust. Robustness data are shown in Table 6.

Table 3: Results of LOD and LOQ

Sr.No	Drug	LOD	LOQ
1.	Nadifloxacin	5.1686	15.6626
2.	Mometasone Furoate	0.1053	0.3191

Table 4: Precision study for Nadifloxacin and Mometasone Furoate

Parameters	Nadifloxacin			Mometasone Furoate		
	Conc (µg/ml)	Area Mean ± SD	% RSD	Conc (µg/ml)	Area Mean ± SD	% RSD
Repeatability	90	986169 ± 4894.63	0.4963	9	296424 ± 3963.30	1.3279
Intraday	70	735348 ± 2377.23	0.3232	7	184323 ± 1528.50	0.8293
	90	985468 ± 6784.12	0.6884	9	298192 ± 1651.40	0.5538
	110	1315436 ± 1825.69	0.1387	11	387698 ± 2730.10	0.7092
Interday	70	715985 ± 3576.63	0.4995	7	77078 ± 867.53	1.1255
	90	984512.3 ± 7875.14	0.7999	9	295933 ± 5472.16	1.8491
	110	1313460 ± 7171.47	0.3459	11	391930 ± 3688.27	0.9410

Table 5: Accuracy study for Nadifloxacin and Mometasone Furoate

Drug	Conc level (%)	Sample amount (µg/ml)	Standard amount added (µg/ml)	Total amount found (µg/ml)	Total amount recovered (µg/ml)	%Recovery
Nadifloxacin	0%	50	0	0	49.66	99.32
	80%	50	40	90	89.14	99.04
	100%	50	50	100	100.45	100.45
	120%	50	60	110	111.19	101.08
Mometasone Furoate	0%	5	0	0	4.96	99.20
	80%	5	4	9	9.15	101.66
	100%	5	5	10	10.08	100.80
	120%	5	6	11	10.93	99.36

Table 6: Robustness study for Nadifloxacin and Mometasone Furoate

Parameter	Change level	Area(n=2)		Area(n=2)	
		Nadifloxacin		Mometasone Furoate	
		Area (Mean ± SD)	%RSD	Area (Mean ± SD)	%RSD
Flow rate (± 0.2 ml/min-1)	0.8 ml/min	416243 ± 2564.30	0.3625	2.780 ± 0.0090	0.3257
	1.2 ml/min	349658 ± 2588.90	0.4022	3.997 ± 0.0156	0.4049
Mobile phase composition (± 2 ml/min-1)	52:37:17 v/v	396834 ± 533.88	0.1812	4.247 ± 0.0100	0.2359
	48:32:13 v/v	359656 ± 496.94	0.1954	3.316 ± 0.0060	0.1820

Table 7: Analysis of marketed formulation of Nadifloxacin and Mometasone Furoate by proposed method

NADIREST-M Cream	Amount taken (µg/ml)		Amount Obtained (µg/ml)		% NADI ± S.D. (n=3)	% MOM ± S.D. (n=3)
	90	9	89.19	8.98	99.12 ± 1423.30	99.77 ± 1956.45

Analysis of Sample Cream by Proposed Method

Applicability of the proposed method was tested by analyzing the commercially available marketed formulation. The percentage of Nadifloxacin and Mometasone Furoate were found to be 99.12% and 99.77% respectively. Results as % assay are shown in Table 7.

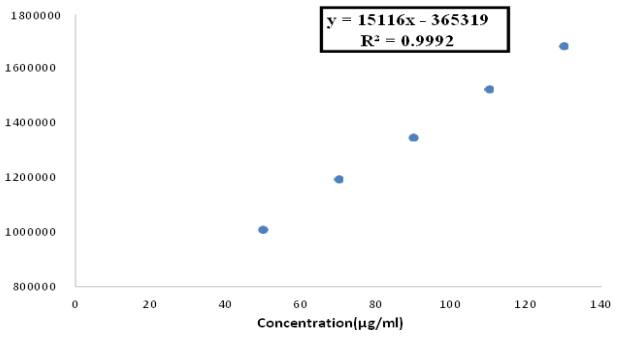

Figure 7: Calibration curve of Nadifloxacin

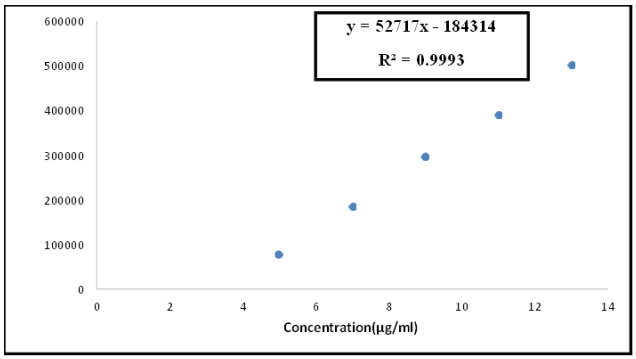

Figure 8: Calibration curve of Mometasone Furoate

CONCLUSION

The method provides selective quantification of Nadifloxacin and Mometasone Furoate. This developed RP-HPLC method for estimation of Nadifloxacin and Mometasone Furoate is accurate, precise, robust and specific. The method has been found to be better, because of its isocratic mode and use of an economical and readily available mobile phase, readily available column, UV detection and better resolution of peaks. All results were found satisfactory. So, this newly developed method can be suitable for routine analysis of combined dosage forms due to easily available reagents and which are cost effective.

Acknowledgement: Special acknowledge to ROFEL, SHRI G. M. Bilakhia College of Pharmacy, Vapi, INDIA which provided us with the required equipments, chemicals needed to achieve this work.

REFERENCES

1. Drug profile, "Nadifloxacin", October 2020. https://pubchem.ncbi.nlm.nih.gov/compound/Nadifloxacin.
2. Drug profile, "Nadifloxacin", October 2020. https://www.drugbank.ca/drugs/DB12447.
3. Drug profile, "Momoteasone furoate", October 2020. https://pubchem.ncbi.nlm.nih.gov/compound/Mometasone-furoate.
4. Drug profile, "Momoteasone furoate", October 2020. https://www.drugbank.ca/drugs/DB14512.
5. Drug Information, "CDSCO approval". https://cdsco.gov.in/opencms/resources/UploadCDSCOWeb/2018/UploadApprova lMarketingFDC/Approvedfdcde18.pdf.
6. Kulkarni A, Nanda R, Ranjane M, Ranjane P: Spectrophotometric Estimation of Nadifloxacin in Pharmaceutical Dosage form. Research J. Pharm. and Tec 2010; 3: 429-431.
7. Devhadrao N, Bansode A, Bansode A, Pasthe H: Ultraviolet spectrometric method for estimation of nadifloxacin in dosage forms and bulk drug. Inventi Journals. Pvt Ltd 2004.
8. Kalantre U, Pishwikar S: Development and Validation of Multiwavelength Method for Simultaneous Estimation of Nadifloxacin and Ibuprofen In Formulated Hydrogel. Int. J. Pharm Tech Res 2012; 4(4): 1575-1580.
9. Murthy A, Raghu Babu K, Yekariya N: Analytical method development and validation of nadifloxacin by high performance liquid chromatography. Int. J. Pharm. and Pharm. Sci 2014; 6(9): 344-346.
10. Nanda R, Kulkarni A, Ranjane M, Ranjane P: A Stability-indicating HPTLC method for estimation of Nadifloxacin in topical cream. Res. J. Topical and Cosmetic Sci 2010; 1(1): 25-29.
11. Patel K, Bendale A, Luhar S, Narkhede: HPLC Method Development and Validation for Simultaneous Estimation of Nadifloxacin and Adapalene in Bulk and Dosage Form. J. Pharm. Sci. Bioscientific Res 2016; 6(3): 276-282.
12. Patel N, Patel P, Meshram D: A Development and validation of RP-HPLC method for simultaneous estimation of Nadifloxacin and Clobetasol Propionate in its dosage form. Am. J. Pharm. Tech. Res 2016; 6(5): 289-304.
13. Kumar A, Sinha S, Agarwal S, Ali J, Ahuja A, Baboota S: Validated Stability- Indicating Thin Layer Chromatographic Determination of Nadifloxacin in Microemulsion and Bulk Drug Formulations. Journal of food and drug analysis. 2010; 18(5): 358-365.
14. Patel K, Shah P, Shah P, Gandhi T: Validated high-performance thin-layer chromatographic (HPTLC) method for simultaneous determination of nadifloxacin, mometasone furoate, and miconazole nitrate cream using fractional factorial design. Journal of Food and Drug Analysis 2016; 24(3): 610-619.
15. Bhosale D, Nikalje A: Stability-Indicating UPLC Method for the Estimation of Nadifloxacin, Terbinafine Hydrochloride, Mometasone Furoate, Methyl Paraben, and Propyl Paraben in Topical Pharmaceutical Dosage Form. J. AOAC Int 2017; 100(5): 1407-1413.
16. Ourique A, Contri R, Guterres S, Ruver Beck R, Pohlmann R, Ulrich F, Schaefer: Set-up of a method using lc-uv to assay mometasone furoate in pharmaceutical dosage forms. Quim Nova 2012; 35(4): 818-821.
17. Patel H, Patel M: Development and Validation of UV Spectrophotometric Method for Simultaneous estimation of Terbinafine hydrochloride and Mometasone furoate in Combined Dosage Form. Asian J. Research Chem 2013; 6(1): 29-34.
18. Vichare V, Choudhari VP, Reddy MV: Simultaneous Estimation of Mometasone Furoate and Salicylic Acid in Topical Formulation by UV-Visible Spectrophotometry. Int. J. Chem. Sci 2017; 15(2): 129.
19. Parmar A, Maheshwari D: Simultaneous estimation of mupirocin and mometasone furoate in pharmaceutical dosage form by q-absorption ratio method. Int. Res. J. Pharm. App. Sci 2015; 5(2): 1-7.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.