AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

A Development and Validation of RP-HPLC Method For Simultaneous Estimation of Nadifloxacin and Clobetasol Propionate In Its Pharmaceutical Dosage Form

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ABSTRACT

A novel, simple, precise, rapid, reproducible and cost effective RP-HPLC method was developed and validated for the simultaneous estimation of Nadifloxacin and Clobetasol propionate in its pharmaceutical dosage form. The chromatographic separation was carried out using C_{18} Shim pack XR ODS II (250 mm × 4.6 mm, 5µm) column with mobile phase comprising of Acetonitrile : Water (50:50)(%v/v). Flow rate was maintained 1.0 mL/min and quantitation was carried out using UV detection at 242nm. Retention time of Nadifloxacin and Clobetasol propionate were found to be 2.64 min and 6.19 min respectively. The method was validated by assessing different parameters such as specificity, linearity, precision, accuracy, robustness, LOD and LOQ for the developed method. The linearity range 20-240 µg/mL and 1-12 µg/mL were selected for Nadifloxacin and Clobetasol propionate respectively. The correlation coefficient (r^2) for Nadifloxacin and Clobetasol propionate were found to be 0.9995 and 0.9999 respectively. The limit of detection for Nadifloxacin and Clobetasol propionate were found to be 0.029 µg/mL and 0.21 µg/mL. The limit of quantitation for Nadifloxacin and Clobetasol propionate were found to be 0.089 µg/mL and 0.64 µg/ml respectively. Percentage recovery was found to be 99% to 100.56% for Nadifloxacin and 99.37% to 99.79% for Clobetasol propionate. All the validation parameters were with-in the acceptance limit. The relative standard deviation (%RSD) was found to beless than 2% in all the assessed parameters. The developed HPLC method can successfully used for the quantitative estimation of both the drugs in its formulation.

Keywords: Nadifloxacin, Clobetasol propionate, RP-HPLC, Simultaneous determination, Validation

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Received 17 August 2016, Accepted 31 August 2016

Please cite this article as: Patel N *et al*., A Development and Validation of RP-HPLC Method For Simultaneous Estimation of Nadifloxacin and Clobetasol Propionate In Its Pharmaceutical Dosage Form. American Journal of PharmTech Research 2016.

INTRODUCTION

Nadifloxacin is a potent, newer topical fluoroquinolones broad spectrum antibiotic[1]. It is a second generation fluoroquinolones class of drug. Nadifloxacin is used for treatment acne vulgaris and various bacterial skin reaction. It is a topical agent for short-term treatment of various skin reaction[2]. Chemically Nadifloxacin is (RS)-9-Fluoro-8-(4-hydroxy-piperidin-1-yl)-5-methyl-1-oxo-6,7-dihydro-1H,5H-pyrido[3,2,1-ij]quinoline-2-carboxylic acid. Nadifloxacin have strong and rapid bactericidal action against the Gram-negative aerobic bacteria and limited action against the Gram-positive organisms. It antagonize the bacterial DNA synthesis by inhibiting bacterial DNA gyrase and topoisomerase IV. DNA gyrase inhibition prevents the relaxation of positively super coiled DNA which is necessary for usual transcription and replication. Inhibition of topoisomerase IV interferes in division of replicated chromosomal DNA into the respective daughter cells when cell division proceed[3].It is off white solid powder with $C_{19}H_{21}FN_2O_4$ molecular formula. Structure of Nadifloxacin is depicted in figure 1. From literature survey, it was observed that various spectrophotometric, HPLC and HPTLC methods have been developed for estimation of Nadifloxacin alone and combination with other drugs in pharmaceutical dosage form.[4-11]

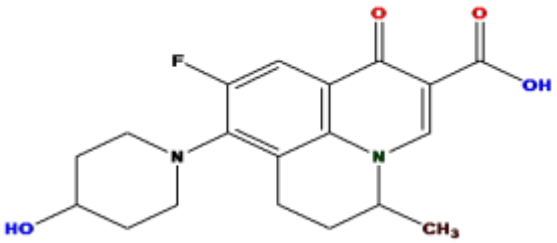

Figure: 1 Chemical structure of Nadifloxacin

Clobetasol propionate is the very potent glucocorticosteroids for topical use. It is used for the short term relief of inflammation and itching caused by a number of skin conditions such as allergic reactions, eczema and psoriasis[12]. Chemically Clobetasol propionate is 17-(2'-chloroacetyl)- 9-fluoro-11-hydroxy-10,13,16-trimethyl- 3-oxo- 6,7,8,11,12,14,15,16 octahydrocyclopenta [a phenanthren-17-yl] propanoate. Clobetasol propionate induces peptide lipocortins which reduce the enzyme Phospholipase A2 which is required for the conversion of membrane phospholipids to Arachidonic acid. From this Arachidonic acid various inflammatory mediators such as prostaglandins, kinins, histamine and liposomal enzymes are produced. By inhibiting Phospholipase A2, inflammatory mediators are not released[13].It is White to cream–colored crystalline powder with $C_{25}H_{32}ClFO_5$ molecular formula. Structure of Clobetasol propionate is depicted in Figure 2. Literature survey revealed various spectrophotometric, HPLC and HPTLC

methods for estimation of Clobetasol propionate alone and combination with other drugs in pharmaceutical dosage form.[14-28]

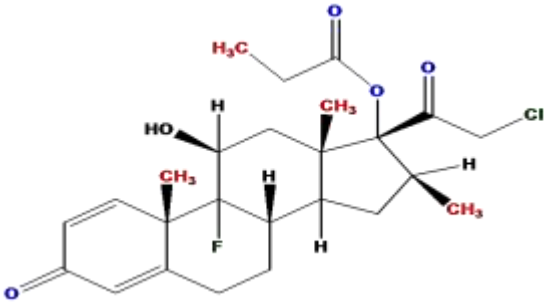

Figure 2: Chemical structure of Clobetasol propionate

Not a single method is available for simultaneous determination of Nadifloxacin and Clobetasol propionate in its pharmaceutical dosage form. So aim of present work is to develop and validate a rapid, precise and reproducible reverse phase high performance liquid chromatographic method for simultaneous determination of Nadifloxacin and Clobetasol propionate in its pharmaceutical dosage form according to ICH guideline.

MATERIALS AND METHOD

Chemicals and reagents

Nadifloxacin was obtained as a gift sample from Wockhardt Ltd., Aurangabad, India. Clobetasol propionate was also obtained as a gift sample from Envee drugs Pvt. Ltd., Nadiad, India. Acetonitrile (HPLC grade), Water (HPLC grade) and Methanol (HPLC grade) were obtained from Merck Chemicals, Mumbai. Nylon filters (0.45 µm) were obtained from Pall india Pvt. Ltd, Bengaluru.

Instrumentation

The chromatographic separation was obtained by using HPLC system (Shimadzu- LC 20AD binary pump system equipped with a UV and PDA detector). The output signal was monitored using LC Solution software. Bath Sonicator (Toshcon instrument) and digital balance (Shimadzu Model ATX 224, Japan) were also used in research.

Chromatographic Conditions

The HPLC method was developed on column C_{18} (Shim pack XR ODS II, 250 mm × 4.6 mm, 5µm particle size) used as the stationary phase. The separation was achieved by using a Acetonitrile: Water (50:50)(%v/v) as the mobile phase at a flow rate 1.0 mL/min. The detection was carried out by UV detection wavelength at 242 nm. The volume of injection loop was 20 µL. The run time was set at 10 min. Before the injection of the drug solution, the column was

equilibrated for at least 30 min with mobile phase. Optimized chromatographic conditions were shown in Table 1.

Preparation of diluent

The diluent consists of mixture of Acetonitrile and Water in the ratio of 50:50 (%v/v).

Preparation of Standard stock solutions

Accurately weighed 50 mg of NADI and 10 mg of CLO were taken into 100 mL volumetric flask. The volume was made upto the mark using diluent to obtain a standard stock solution containing 500 µg/mL NADI and 100 µg/mL CLO.

Preparation of working standard solutions

Appropriate aliquots of 0.4, 0.8, 1.6, 3.2 and 4.8 mL were withdrawn from the standard stock solution of NADI (500 µg/mL) in different 10 mL volumetric flasks. Appropriate aliquots of 0.1, 0.2, 0.4, 0.8 and 1.2 mL were withdrawn from the standard stock solution of CLO (100 µg/mL) and added to 10 mL volumetric flasks. The volume were made up to the mark with diluent to obtain the final concentrations of 20, 40, 80, 160 and 240 µg/mL of NADI and 1, 2, 4, 8 and 12 µg/mL of CLO.

Preparation of sample solution

The sample solution was prepared by accurately weighing and transferring about 1gm of cream (NADOXIN-C) that (containing 10mg of Nadifloxacin and 0.5mg of Clobetasol propionate) into a 50 mL clean and dry volumetric flask. About 20 mL diluent was added into volumetric flask and mixed for 2-5 minutes on vortex mixture. The obtained solution was sonicated for 5-7 minutes to dissolve the cream. Then volume was made up to the mark with mobile phase to get 200 µg/mL NADI and 10 µg/mL CLO. The obtained solution was filtered through 0.45 µm membrane filter. From the filtrate, 4 mL was pipetted out into 10 mL clean and dry volumetric flask and diluted up to mark with mobile phase to obtain a concentration of NADI (80 µg/mL) and CLO (4 µg/mL). The both solutions were filtered through the 0.45 µm nylon filter paper. Prepared solution were analyzed for 3 replicates and results were obtained in terms of % label claim as shown in Table 12.

METHOD VALIDATION

The developed RP-HPLC method was validated as per International Conference on Harmonization (ICH) guidelines.[29-30.]Various validation parameters such as linearity, precision, accuracy, Limit of detection (LOD), Limit of quantitation (LOQ), Specificity and robustness were performed.

System suitability testing

System suitability testing is an integral part of many analytical procedures. The different parameters were checked by injecting the standard solutions. The chromatogram was recorded

under finally optimized conditions. The parameters such as theoretical plates (N), tailing factor(As), retention time (Rt), resolution and capacity factor (k') were checked for developed RP-HPLC method. Results are shown in Table 2.

Specificity

The specificity of developed method was analyzed by injecting a diluents: ACN: Water (50:50 %v/) and placebo solutions. Thus interference of matrix components on this study was estimated. Also presences of extra peaks were identified by taking chromatograms of suspected std. preservatives. Results are shown in Figure 5, 6, 7, 8, 9.

Linearity

Linearity was determined by a series of three injections of different standards concentration using a fixed 20 µL loop. The solution of increasing concentration in the range of 20-240 µg/mL of NADI and 1-12 µg/mL of CLO were prepared from the standard stock solution of NADI (500 µg/mL) and CLO (100 µg/mL). Peak areas were recorded for each concentration. Solutions of all concentrations were injected to obtain a linear regression equation. A graph of concentration vs. Peak area was plotted as shown in Figure 3, 4 and regression line equation was obtained. Linearity results are shown in terms of %RSD in Table 3,4.

Precision

The precision was determined in terms of Repeatability, Intra and Inter day variation in the peak area.

Intraday precision

Intraday precision of the proposed method was resolute by analyzing the corresponding responses of three different concentrations (3 times) on the same day. Standard solution of NADI containing 40, 80 and 160 µg/mL and CLO containing 2, 4 and 8 µg/mL were analyzed three times on the same day at different time intervals. The result was reported in terms of % RSD of peak areas in Table 5 and for repeatability it has been shown in Table 7.

Interday precision

Interday precision of the proposed method was resolute by analyzing the corresponding responses of three different concentrations on three different days. Standard solution of NADI containing 40, 80 and 160 µg/mL and CLO containing 2, 4 and 8 µg/mL were analyzed on three different days. The result was reported in terms of % RSD of peak areas in Table 6.

Recovery studies (Accuracy)

Accuracy of the method was performed by calculating the recovery of NADI and CLO by the standard addition method. The recovery study was performed by adding a working standard

solution of NADI and CLO into a preanalyzed sample solution at three levels i.e., 80%, 100% and 120%. From the sample solution, 4 mL sample was taken in 10 mL volumetric flask and increasing aliquots of combined working standard (1.26 mL, 1.58 mL and 1.90 mL from 500 µg/mL of NADI and 0.32 mL, 0.4 mL and 0.48 mL from 100 µg/mL of CLO) were added and diluted upto 10 mL with mobile phase. The concentration of sample solution was constant at all three level. Three replicates are injected for each sample. The result is reported in term of % recovery in Table 8. Recovery study was calculated by using the following formula:

% Recovery = (A - B) / C × 100

Where, A = Total amount of drug estimated (mg)

B = Amount of drug found on reanalyzed basis (mg)

C = Amount of standard drug added (mg)

Limit of detection and limit of quantitation

Limit of detection (LOD) and limit of quantitation (LOQ) was calculated from the linearity studies. LOD detects the lowest amount of analyte in the sample but not necessarily quantified. LOQ measure the lowest amount of analyte in the sample which can be quantitatively determined with precision and accuracy. For each of the three replicates, slope was determined and standard deviation of intercepts was calculated. The values of LOQ and LOD were calculating using following mathematical equation as the data were given in Table 9.

LOD = 3.3 × σ / S

LOQ = 10 × σ / S

Where, σ = Standard deviation of response (Intercepts)

S = Slop of calibration curve

Robustness

Robustness of an analytical method is a measure of its capacity to remain unaffected by small variations in method parameters. Robustness study was carried out by deliberately changing the chromatographic parameters such as flow rate, mobile phase composition and wavelength by ±2 units. The results were evaluated in terms of %RSD of Assay values as depicted in Table 10 and Table 11.

RESULTS AND DISCUSSION

Table 1: Optimized chromatographic parameters

Parameters	Optimized condition
Elution mode	Binary mode
Stationary phase	C_{18} (Shim pack XR ODS II, 250 mm x 4.6 mm, 5µm)

Mobile phase		ACN: Water (50:50 % v/v)
Flow rate (ml/min)		1 mL/min
Detection		UV detector at 242 nm
Injection volume		20µL
Run time		10 min
Retention time	Nadifloxacin	2.64 min
	Clobetasol propinate	6.19 min

Table 2: System suitability parameters of proposed method

Parameters	Observed results		Acceptance criteria
	NADI	CLO	
Retention time	2.646	6.198	% RSD < 2
Theoretical plates (N)	13426.537	36141.983	> 2000
Tailing factor (T)	0.8	1.1	T ≤ 1.5
Capacity factor (k')	8.95	9.61	K' > 2
Resolution	9.58		> 2

Table 3 Linearity of NADI

Sr No	Conc. (µg/ml)	Mean area ± SD (n=3)	% RSD
1	20	2184303 ± 6688.49	0.30
2	40	3458760 ± 11808.45	0.34
3	80	6627365 ± 61180.12	0.92
4	160	12898836 ± 222964.7	1.72
5	240	19534103 ±15663.41	0.08

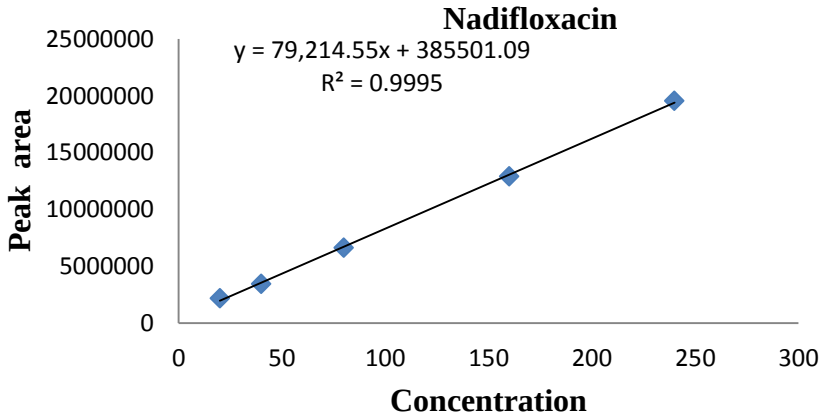

Figure 3: Calibration curve of Nadifloxacin

Table 4:Linearity of CLO

Sr. No	Conc. (µg/ml)	Mean area ± SD (n=3)	% RSD
1	1	83683 ± 663.15	0.79
2	2	172552 ± 2356.01	1.36
3	4	327094 ±2558.45	0.78
4	8	648546 ±8753.61	1.34
5	12	959910 ±18150.76	1.89

Clobetasol propionate

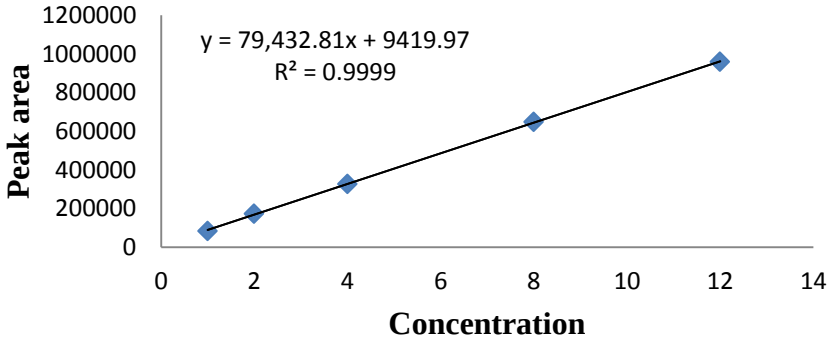

Figure 4: Calibration curve of Clobetasol propionate

Table 5: Intraday precision

Sr.No	Conc. (µg/ml)		Peak area (n=3)		%RSD	
	NADI	CLO	NADI	CLO	NADI	CLO
1	40	2	3453346	171385	0.07	1.91
2	80	4	6671082	325856	0.05	0.54
3	160	8	12948038	653078	0.49	0.71

Table 6: Interday precision

Sr.No	Conc. (µg/ml)		Peak area (n=3)		% RSD	
	NADI	CLO	NADI	CLO	NADI	CLO
1	40	2	34651387	168754	0.23	1.62
2	80	4	6535408	327729	0.49	1.83
3	160	8	12623350	641896	0.19	1.03

Table 7: Repeatability

Sr.No	Concentration (µg/mL)		Peak area		SD		% RSD	
	NADI	CLO	NADI	CLO	NADI	CLO	NADI	CLO
1	80	4	6557410	320250	20519.87	3523.08	0.31	1.1
2			6552102	321654				
3			6545546	315178				
4			6540687	319625				
5			6533497	324254				
6			6499980	315550				

Table 8: Recovery studies

Drug	Level	Amount contributed by cream(µg/ml)	Amount Spiked(µg/ml)	Amount recovered(µg/ml)	% Recovery(n=3)	% RSD
NADI	80%	79.28	63.42	63.67	100.39	0.41
	100%		79.28	78.49	99.00	0.91
	120%		95.13	95.67	100.56	0.04
CLO	80%	4	3.2	3.18	99.37	0.18
	100%		4	3.98	99.50	0.14
	120%		4.8	4.79	99.79	0.12

Table 9: Limit of detection and limit of quantitation

Drugs	LOD (µg/ml)	LOQ (µg/ml)
Nadifloxacin	0.029	0.089
Clobetasol propionate	0.21	0.64

Table 10: Robustness study of Nadifloxacin

Change in Parameters		NADI		
		Retention time (min)	Peak area	Assay (%)
Flow rate(ml/min)	0.8	2.69	19236194	99.15 %
	1.0	2.63	19167759	98.79 %
	1.2	2.59	18818693	97.48 %
% RSD		1.9	0.87	0.89
Wavelength (nm)	240	2.59	19265650	99.30 %
	242	2.63	19167759	98.79 %
	244	2.64	19063520	98.24 %
% RSD		1.00	0.52	0.54
Mobile phase composition (% v/v)	48:52	2.67	19331287	99.65 %
	50:50	2.63	19167759	98.79 %
	52:48	2.60	18759120	97.16 %
% RSD		1.33	1.25	1.28

Table 11: Robustness study of Clobetasol propionate

Change in Parameters		CLO		
		Retention time (min)	Peak area	Assay (%)
Flow rate(ml/min)	0.8	6.25	962250	99.91
	1.0	6.10	959910	99.66
	1.2	5.98	940210	97.58
% RSD		1.8	1.26	1.29
Wavelength (nm)	240	5.98	961120	99.83
	242	6.10	959910	99.66
	244	6.00	963152	100.00
% RSD		1.06	0.17	0.17
Mobile phase composition (% v/v)	48:52	6.2	955899	99.25
	50:50	6.10	959910	99.66
	52:48	5.99	961120	99.83
% RSD		1.72	0.28	0.29

Table 12: Analysis of Marketed formulation

Formulation	Labeled amount (mg)		Amount found (mg)		% Label claim ± SD (n = 3)		% RSD	
	NADI	CLO	NADI	CLO	NADI	CLO	NADI	CLO
NADOXIN-C Cream	10	0.5	9.86 9.87 9.99	0.497 0.495 0.496	99.1 ± 0.072	99.2 ± 0.001	0.7	0.2

Table 13:Summary of validation parameters developed by RP-HPLC

Parameters	NADI	CLO
Concentration range	20-240 µg/ml	1-12 µg/ml
Slope	79214	79432

Intercept		385501	9419
Correlation coefficient		0.9995	0.9999
Accuracy ± %RSD	80 %	100.39 ± 0.41	99.37 ± 0.18
(n=3)	100 %	99.00 ± 0.91	99.50 ± 0.14
	120 %	100.56 ± 0.04	99.79 ± 0.12
Precision (% RSD)	Repeatability (% RSD, n=6)	0.31	1.1
	Intraday	0.055-0.49	0.54-1.91
	Interday	0.19-0.49	1.03-1.83
LOD (µg/ml)		0.029	0.21
LOQ (µg/ml)		0.089	0.64
Assay ± SD		99.1 ± 0.072	99.2 ± 0.001

The chromatographic method was developed for the separation of the target analytes in the sample by trying different solvent and their different ratios. The chromatograms obtained had good shape and better resolution qualifying all the system suitability parameters with ACN: Water (50:50 % v/v) as mobile phase. The retention time of were found to be 2.64 min and 6.19 min receptively. The developed method was applied for determination of the target analytes from its cream formulations and the assay values were found to be 99.1 % and 99.2 % Nadifloxacin and Clobetasol propionate respectively. Linearity studies over the range of 20-240 µg/mL for NADI and 1-12 µg/mL for CLO showed good co-relation between concentration of the drug and peak area. The correlation coefficient for Nadifloxacin and Clobetasol propionate was found to be 0.9995 and 0.9999 respectively. The developed method gives precise results under optimized chromatographic parameters with % RSD value less than 2. Recovery study for Nadifloxacin and Clobetasol propionate were found to be 99.0% to 100.56% and 99.37% to 99.79% respectively at three different levels. The results obtained indicate that the excipients used in cream formulation do not interfere with accurate quantitation of the target analyte from its formulation. The chromatogram of placebo proves the method is specific. Extra peaks in the chromatograms were also identified and confirmed by injecting std. solution of suspected preservatives. Limit of detection values for Nadifloxacin and Clobetasol propionate were found to be 0.029 µg/mL and 0.21 µg/mL respectively. Limit of quantitation values for Nadifloxacin and Clobetasol propionate were found to be 0.089 µg/mL and 0.64 µg/mL respectively. For robustness, variation in flow rate, wavelength and mobile phase composition, it was observed that the no major variation is obtained in chromatographic parameters, so concluded that the develop method is robust. The summarized results shown in Table 13 indicate that the method can be routinely applied for determination of the target analytes from its formulation.

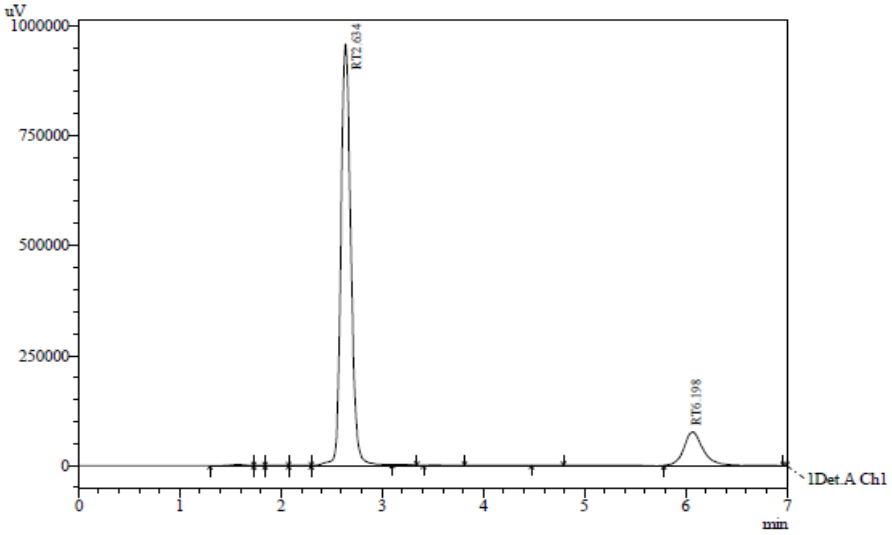

Figure 5: Chromatogram of mixture of standard solution

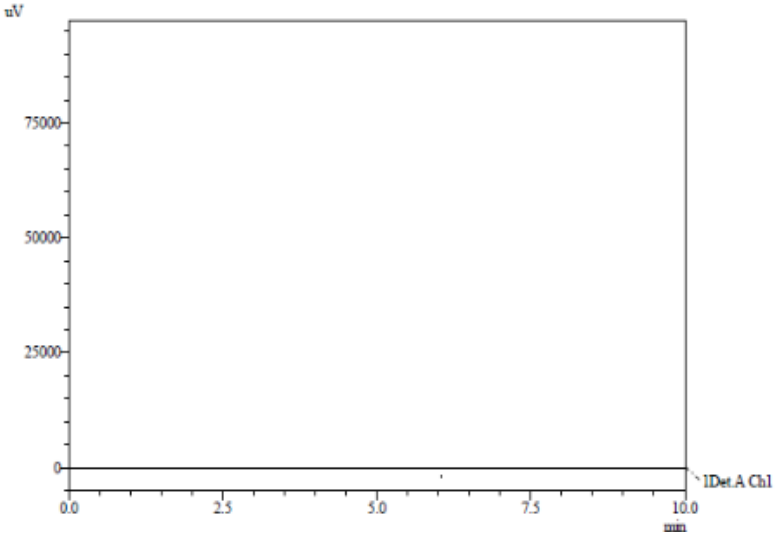

Figure 6: Chromatogram of Diluent

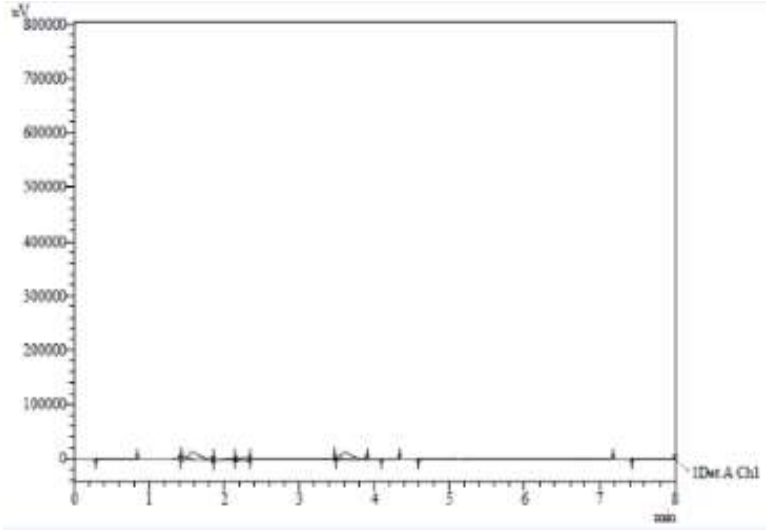

Figure 7: Chromatogram of Placebo

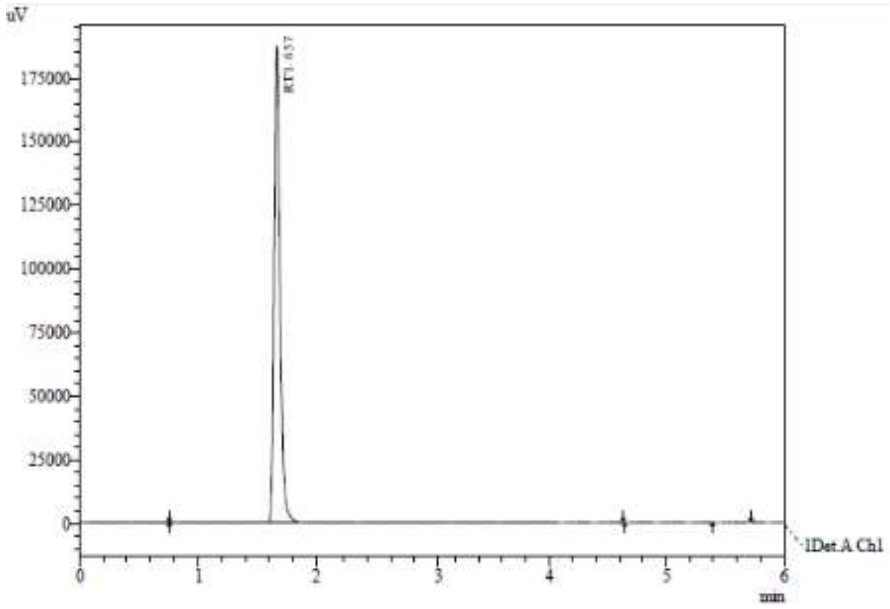

Figure 8: Chromatogram Methyl paraben(10 µg/mL)

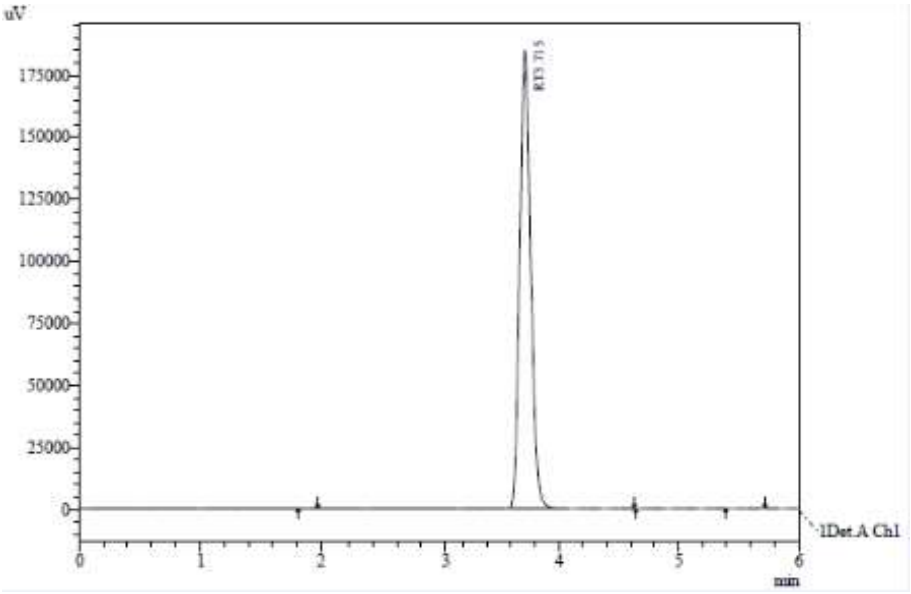

Figure 9: Chromatogram Propyl paraben(10 µg/mL)

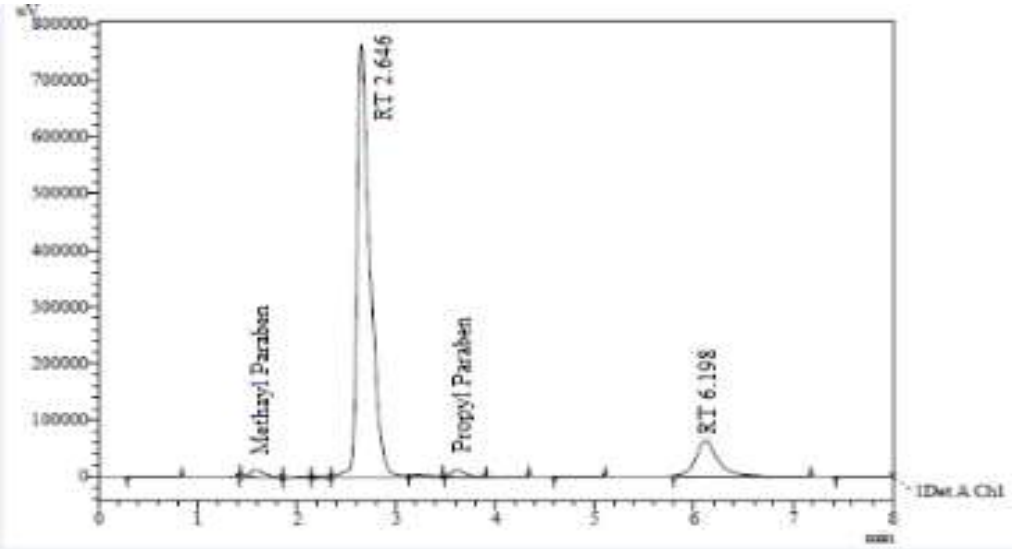

Figure 10: Chromatogram of Sample solution

CONCLUSION

Simple, specific, accurate, precise and reproducible reverse phase high performance liquid chromatography method was developed and validated for the simultaneous estimation of Nadifloxacin and Clobetasol propionate in its pharmaceutical dosage form. Validation of the developed method was carried out as per the ICH guidelines. Hence, developed RP-HPLC method can be useful for quantitative determination of Nadifloxacin and Clobetasol propionate in its pharmaceutical dosage form.

ACKNOWLEDGEMENT

I am thankful to Shri D.D. Patel, President OGECT and Pioneer Pharmacy Degree College for providing necessary facilities to carry out this research work.

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