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Development of new RP-HPLC method for simultaneous estimation of Luliconazole and Naproxen sodium in the formulated gel

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ABSTRACT

The objective of the study was to develop a new simple, accurate, sensitive, reproducible RP-HPLC method for the simultaneous estimation of Luliconazole and Naproxen Sodium in formulated gel.The resolution of two drugs was achieved on enable C18 (4.6× 150mm, 5µm) column using mobile phase phosphate buffer pH 5.8 : methanol in the ratio 60:40v/v with isocratic elution, flow rate 1mL/min.Retention time of Luliconazole and Naproxen Sodium were4.2 min and 6.8 min respectively, using analytical wavelength 220 nm. The percentage recovery for Luliconazole and Naproxen Sodium was found to be 97.8% and 99.3% respectively. The method was validated according to the ICH guidelines and it can be used for routine analysis in simultaneous estimation of luliconazole and naproxen sodium in pharmaceutical gel.

INTRODUCTION

Gel is preferred for topical application due to more stability and better application property.Film forming gels are a novel approach in this area that might present an alternative to the conventional dosage forms used on the skin, such as ointments, creams, gels or patches. The polymeric solution is applied to the skin as a liquid and forms an almost invisible film in situ by solvent evaporation.A diabetic foot is any pathology that results directly from peripheral arterial disease (PAD) and sensory neuropathy affecting the feet in diabetes mellitus; it is a long-term (or "chronic") complication of diabetes mellitus[1, 2].The present study is to formulate a gel which can treat diabetic foot in diabetic patients. In such cases, one can use pharmaceutical gel containing Luliconazoleand Naproxen Sodium for the purpose of curing diabetic foot inflammation and fungal infections. With this aim and objectives, an attempt was made to formulate and evaluate a pharmaceutical gel.

MATERIALS AND METHODS

Chemicals and reagents:

Luliconazole-gift sample from Hetero Hyderabad. Naproxen sodium- yarrow chem. Mumbai.Methanol, sodium chloride, dibasic and mono basic potassium phosphate were used for analysis along with distilled water, acetonitrile of HPLC grade.

Formulation of a pharmaceutical gel

Pharmaceutical gel containing Luliconazoleand Naproxen Sodium was formulated by heating Propylene glycol to 65°C then methyl parabenandpropyl paraben were added along with water and carbopol, and kept for 24 hours for adequate swelling of polymer. Triethanolaminewas then added to neutralize the carbopol and pH was adjusted to 6.7 6.9. Propylene glycol at 65°C was heated in anothervessel, Luliconazole (2%) and Naproxen sodium (2%) were added. The above mixture was allowed to cool at room temperature and added to above mixture [3, 4].

Evaluation of pharmaceutical gel

pH measurement: The pH of various gel formulations are determined by using digital pH meter. 1 g of gel is dissolved in 100 ml. freshly prepared distilled water and stored for two hours. The measurement of pH of each formulation is done in triplicate and average values are calculated.

Viscosity measurement: Brookfield digital viscometer can be used to measure the viscosity of prepared gel formulations.

The gels are rotated at 0.3, 0.6 and 1.5 rotations per minute. At each speed, the corresponding dial reading is noted. The viscosity of gel is obtained by multiplication of dial reading with factor given in the Brookfield viscometer catalogues.

Spreadability: Spreadability refers to the extent of area to which gel readily spreads on application. It is determined by wooden block and glass slide apparatus. The time in sec. taken by two slides to slip off from gel which is placed in between the slides under the direction of certain load is expressed as spreadability. Lesser the time taken for the separation of two slides, better the spreadability. Spreadability is calculated by using the formula:

S = M.L / T

Where,S = Spreadability.

M = Weight tide to the upper slide L = Length of a glass slide.

T = Time taken to separate the slide completely from eachother.

Homogeneity: All developed gels are tested for homogeneity by visual inspection after the gels have been set in the container. They are tested for their appearance and presence of any aggregates.

Drug content: 1 g gel is dissolved in 100 ml. of suitable solvent. The aliquots of different concentrations of gel are prepared by suitable dilutions, filtered and absorbance is measured spectrophotometrically. Drug content is determined from the linear regression analysis of calibration curve of drug.

Grittiness: All the gel formulations are checked microscopically for the presence of any particulate matter.

Extrudability: The gel formulations are filled in collapsible tubes, after being set in the containers. The extrudability of gel formulations are determined in terms of weight required in grams to extrude 0.5 cm. ribbon of gel in 10 sec.

Stability test: Stability study is carried out by freeze-thaw cycling. The product is subjected to a temperature of 4 ^{0}C for one month, then at 25 ^{0}C for one month followed by 40 ^{0}C for one month. Syneresis is observed. Finally, the gel is exposed to ambient room temperature and the separating liquid exudates are noted.

***In-vitro* drug diffusion study:** In-vitro drug release studies are performed by using a Franz diffusion cell. 0.5 g of gel is taken in cellophane membrane. Diffusion studies are conducted at 37 ^{0}C ± 1 ^{0}C employing 250 ml. phosphate buffer, pH 7.4 as the dissolution medium.

Determination of absorption maximum (λ_{max})

Luliconazole (fig 1) and Naproxen sodium (fig 2) are soluble in methanol hence the absorption characteristics of Luliconazaole and Naproxen sodium were determined in the methanol. Absorption maxima of Luliconazole and Naproxen sodium at 298 nm and 238nm[5, 6, 7]

Selection of analytical concentration ranges (10-50µg/mL) and Construction of Calibration Curve to check Linearity:

The linearity of response for Luliconazole and Naproxen sodium was determined in the range of 10-50µg/mL. The calibration curve of analytical method was assessed by plotting concentration versus peak areaand represented graphically.

Analytical method validation

Linearity and range:

The linearity of analytical methodis ability to elicit test results that are directly proportional to the concentration of analyte in the sample within the range. The range of analytical method is the interval between upper and lower levels of analyte that have been demonstrated within a suitable level of precision, linearity and accuracy.Aliquots of Mixed standard solution were prepared in the concentration range 1-50 µg/ml.

Precision

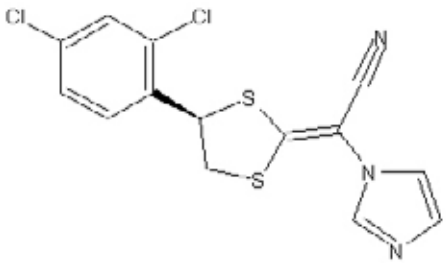
The precision of an analytical method is the degree of agreement among individual test results, when the method is applied repeatedly to multiple samplings of homogeneous sample. It provides an indication of random errors in results and expressed as relative standard deviation (%RSD). Precision of the method is reported as repeatability, intraday and inter day precision.

Repeatability

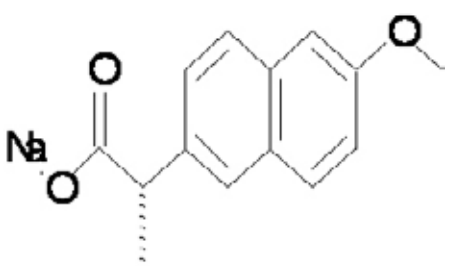
Repeatability assessment of an analytical method is performed by analyzing six replicates of single concentration that is 30 µg/mL of the mixed standard solution .Absorbance of samples were recorded at 220 nm.The % relative standard deviation (RSD) was calculated.

Intraday and Inter day precision

Variations of results within the same day (intraday) and variation of results between days (inter day) were analyzed. The



Structure of Luliconazole



Structure of Naproxen Sodium

Fig. 1 : Structure of Luliconazole and Naproxen Sodium

Intra-assay precision of the proposed method was determined on samples of drug solutions at varying concentration levels (20µg/ml, 30µg/ml and 40µg/ml for mixed standard solutions) by analyzing three replicates of each sample as a batch in a day.The Inter-day precision was determined by analyzing the same samples (20µg/ml, 30µg/ml and 40µg/ml for mixed standard solutions) in three consecutive days.

Accuracy

Accuracy is the closeness of test results obtained by the method to the true value.Accuracy was determined by the recovery studies. The method was performed at 3 levels that are 80%, 100% and 120% of the assay (30µg/mL) concentration, in triplicate at each level. These solutions are prepared equivalent of 0.3 ml of mixed standard solution containing (0.3 mg of Luliconazole and naproxen sodium) 30µg/mL and directly transfer into 10ml volumetric flasks, about 5ml of methanol is added and sonicated for about 5 minutes. After sonication make up the volume with mobile phase upto the mark.Such that final concentrations become 80%, 100% and 120%. Calculate the mean percentage for the recovery. The two standard solutions of concentration, each 3 times were injected into HPLC system and% Recovery was calculated.

RESULTS

Formulations of gel:

Formulation of gel with Luliconazole and Naproxen sodium were shown in the Table 1 as picture wise. These formulations were further used for evaluation tests and validation process.

Evaluation parameters

Various parameters were evaluated for gel formulation and the results were given in the Table 2

Determination of absorption maximum (λmax):

From standard solution of Luliconazole (10µg/ml) and Naproxen sodium (10µg/ml) were prepared. The scanning for solution of Luliconazole (10µg/ml) and Naproxen sodium (10µg/ml) was carried out in the range of 200-400 nm against using methanol as a blank. The maximum absorption (λmax) of Luliconazoleand Naproxen sodium wasfound at298 nm and 238 nm. The spectrum is shown in fig 2. Isobestic point 220nm was selected as detection wavelength Overlay of both the spectrums shown in fig 3

Optimized chromatographic conditions

Chromatographic separation was carried using ODS Enable C18 4.6×150mm, 5 µm column. Methanol: pH 5.8 buffer (60:40%v/v) at flow rate 1ml/min was used as mobile phase.the injection volume was 20µl and eluents were monitored at 220nm[8.] Optimized chromatographic conditions were shown in the table 3. Chromatogram was shown in the fig 4.

METHOD VALIDATION

System suitability parameters

The HPLC system was stabilized for 60 min to get a stable base line all the parameters were found within the limits and were shown in the Table 4

Table 1 : Binding affinity and number of hydrogen bond interaction of different coformers with Etodolac

FORMULATION	TRIAL 1	TRIAL 2	TRIAL 3	TRIAL 4
IMAGE				
TRIAL WITH	Carbapol, methyl paraben, propyl paraben, triethanol amine, glycerol.	Addition of carbapol by dissolving in water for 24 hrs.	Luliconazole, Naproxen sodium.	Luliconazole, Naproxen sodium
RESULT	Coloration of gel due to oxidation. Carbapol lumps were formed.	Carbapol is completely soluble and no lumps were seen.	Consistency of the gel was not good.	No draw back

Table 2 : Evaluated parameters of formulated pharmaceutical gel.

S.NO	EVALUATION PARAMETERS	Formulation 1	Formulation 2	Formulation 3	Formulation 4
1	Colour	Brownish red	Wheatish	White	White
2	Skin irritation	No	No	No	No
3	Homogeneity	-	+	+++	+++
4	Spreadability	Poor	Good	Good	Good
5	Stability test	-	+	+++	+++
6	Solubility test	Water	Water	Water	Water
7	Grittiness	+	-	-	-

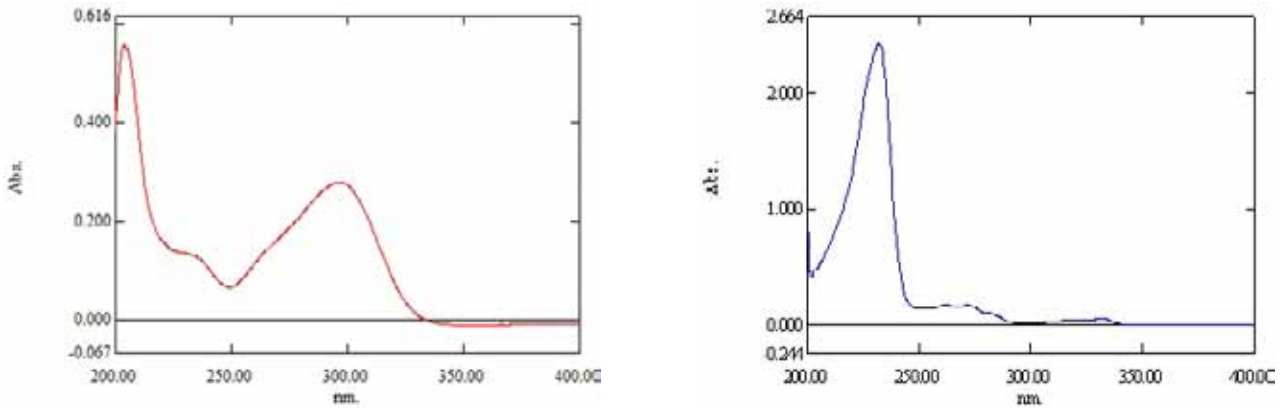


Fig. 2 : Determination of Absorption Maximum of Luliconazole and Naproxen sodium

Absorption maxima of Luliconazole and Naproxen sodium at 298 nm and 238nm

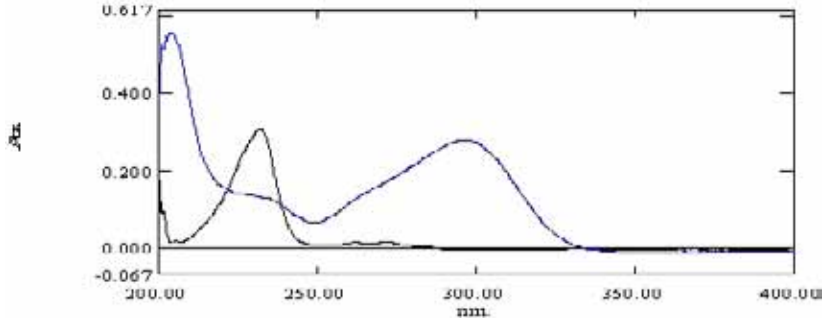


Fig. 3 : Overlay UV-spectrum of Luliconazole and Naproxen sodium

Table 3 : Optimized RP-HPLC conditions

Parameters	Conditions
Column	Enable C18 4.6×150mm, 5 µm
Mobile Phase	Methanol : pH 5.8 buffer(60:40%v/v)
Flow Rate	1 ml/min
Run Time	10 min
Volume of injection	20 µL
Detection Wavelength	220 nm

Linearity

Linear relationship was found in the concentration range of 10-50 µg/mL for Luliconazole and Naproxen sodium and results are shown in Table 5and the calibration curve is shown in graph no 1.

PRECISION: Repeatability

Repeatability was performed by analyzing six replicates of single concentration that is 30µg/mL of Luliconazole and 30µg/mL Naproxen sodium. Results are recorded in the Table 6

Intra-day and Inter-day precision

The results of intra-day and inter-day precision were expressed as % RSD and it was found to be NMT 2. The results of intra and inter day precision are shown in Table 7

Accuracy: Accuracy for drug substance was determined on samples of drug solutions for Luliconazole and Naproxen sodium. The %RSD was calculated and reported in Table 8.It was found that the %RSD values are within the acceptance limits. Recovery studies for drug product were carried out by adding known amount of standard drug (30µg/mL) to the sample solution

Table 4 : System suitability parameters for optimized chromatographic condition.

System suitability parameter	Luliconazole	Naproxen sodium	Acceptance criteria
Tailing factor	1.91	0.93	NMT 2.0
Theoritical plates	34996	10450	NLT 2000
%RSD for 6 injections	0.51	0.73	NMT 2.0

Table 5 : Linearity of Luliconazole and Naproxen sodium

Linearity of Luliconazole

Concentration (µg/mL)	Area
10	214359
20	265860
30	325148
40	379453
50	428612

Linearity of Naproxen Sodium

Concentration (µg/mL)	Area
10	91354
20	125523
30	161450
40	196770
50	235644

Table 6 : Repeatability data for Luliconazole and naproxen sodium

Conc (µg/mL)	Luliconazole Peak area ratio	Naproxen Peak area ratio	Mean[a]± Standard Deviation	Mean[a]± Standard Deviation	%RSD	%RSD
30	325148	161450	324271.3 ± 1669.808	162150 ± 1191.638	0.51	0.73
	325198	161450				
	322146	162450				
	322140	161650				
	325148	164450				
	325848	161450				

Table 7 : Intra-day precision data for Luliconazole and naproxen sodium

Conc.	Luliconazole Peak area ratio	Naproxen Peak area ratio	Mean[a]± Standard Deviation	Mean[a]± Standard Deviation	%RSD	%RSD
20	265860	125523	265590 ± 547.4486	125170.3 ± 527.428	0.20	0.42
	264960	125424				
	265950	124564				
30	325148	161460	324548 ± 519.6152	161766.7 ± 592.8181	0.16	0.36
	324248	162450				
	324248	161390				
40	379453	196970	379086.7 ± 550.9994	196503.3 ± 650.7176	0.14	0.33
	378453	196780				
	379354	195760				

Acceptance criteria
The % RSD for area of sample injections should not be more than 2%.

Table 8 : Accuracy data

Conc level (%)	Amount added((µg/mL)	Response	Amount Recovered (µg/mL)	% Recovery	Mean %Recovery
Luliconazole (40µg/mL)					
80	32	323420	31	97	91
100	40	361250	37.47	94	
120	48	372250	39.3	82	
Naproxen sodium (40µg/mL)					
80	32	189876	35.3	110	99
100	40	209970	39.6	99	
120	48	221879	42.5	88	

The mean percentage recovery for 80%, 100%, 120% level was found to be 97%, 94%, 82% for Luliconazole, 110%, 99%, 88% for Naproxen sodium. There are within acceptance limits. Therefore, the HPLC method for the determination of assay of two drugs in formulation was found to be accurate.
Acceptance criteria: % Recovery for analyte concentration in three replicate standard samples should be in the range of 80-120%.

Table 9 : Assay data for two drugs

	Area (30µg/mL)	Amount found (30µg/mL)	% Purity
Luliconazole	325148	4.89	97.8 %
Naproxen sodium	161450	4.93	99.3%

(80µg/mL, 100µg/mL, and 120µg/mL). The % recovery were calculated for Luliconazole and Naproxen sodium and recorded in the Table 9 respectively.

Assay

A quantity (5 mg) of the gel containing (0.5mg of Luliconazole and 0.5mg Naproxen sodium) was dissolved in 60 ml of methanol and allow thedrugs to dissolve completely. The volume was made up to mark with phosphate buffer of pH 5.8. The solution was filtered. From this the working standard solution 30 µg/mL was prepared and injected into HPLC system under optimized chromatographic conditions. Area of each peak was measured at selected wavelength 220nm. The amount of each drug present in the sample was determined using the prepared standard calibration curves of Luliconazole and Naproxen Sodium. The results were shown in Table 9.

DISCUSSION

Gel is preferred for topical application due to more stability and better application property.Film forming gels are a novel approach in this area that might present an alternative to the conventional dosage forms used on the skin, such as ointments, creams, gels or patches. The polymeric solution is applied to the skin as a liquid and forms an almost invisible film in situ by solvent evaporation.

Diabetes is a chronic metabolic disorder characterized by high levels of glucose in the blood (hyperglycemia) resulting from defects in insulin secretion, insulin action or both. A diabetic foot is any pathology that results directly from peripheral arterial disease (PAD) and/or sensory neuropathy affecting the feet in diabetes mellitus; it is a long-term (or "chronic") complication of diabetes mellitus. Presence of several characteristic diabetic foot

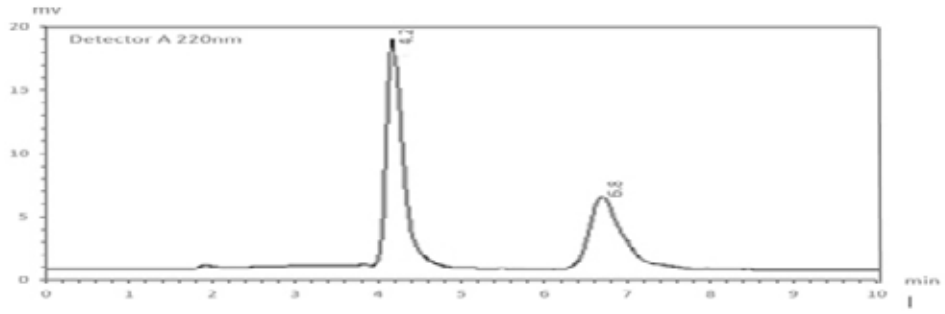
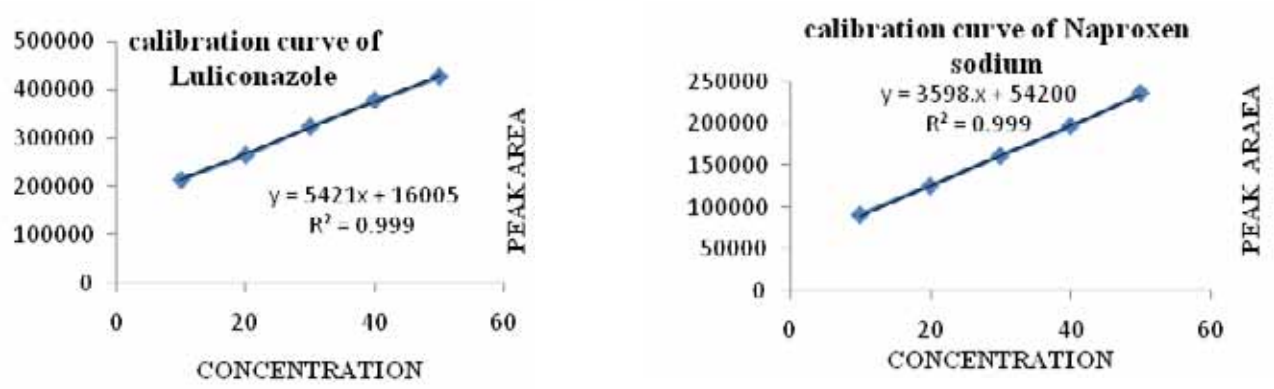


Fig. 4 : Optimized RP-HPLC conditions

Graph 1 : Calibration curve of Luliconazole and Naproxen sodium



The standard graph was plotted between peak area and concentration and is shown as graph.

pathologies such as infection, diabetic foot ulcer and neuropathic osteoarthropathy is called diabetic foot syndrome.

Luliconazole is topical Azole Antifungal available as amorphous powder, soluble in Aqueous Methanol, DMSO and Acetonitrile. One must avoid use in patients with a history of azole antifungals hypersensitivity and its ocular exposure. Luliconazole exact mechanism of action against dermatophytes is unknown, it appears to inhibit ergosterol synthesis by inhibiting the enzyme lanosterol demethylase. Inhibition of this enzymes activity by azoles results in decreased amounts of ergosterol, a constituent of fungal cell membranes, and a corresponding accumulation of lanosterol. It works by slowing the growth of fungi that cause infection. It was approved by FDA (USA) in November 2013 and is marketed under brand name Luzu [9,10,11,12.]

Naproxen sodium is white to creamy in color available ascrystalline powder. It is soluble in methanol and water. It is an NSAID. Naproxen is contraindicated in the patients with history of *asthma*, *urticaria*, or other allergic-type reactions after taking. Naproxen works by blocking the effects of cyclo oxygenase enzymes, which help produce prostaglandins. Prostaglandins are produced at sites of injury or damage, causing pain and inflammation, blocking cyclooxygenase enzymes results in fewer prostaglandins, thus reducing pain and inflammation. It was approved by FDA (USA) in 1994. [13,14,15,16.]

From the literature survey, it was observed that luliconazole is an anti-fungal and used to treat fungal infections. Naproxen drug is a Non-Steroidal Anti Inflammatory drug (NSAID) and used to treat pain and inflammation[17]. Combination of Luliconazole and Naproxen drugs can be used to treat fungal infections with inflammation and pain. ButTill date there is no literature for the current two drugs Luliconazole and Naproxen sodium, hence we have developed an RP-HPLC method for simultaneous estimation of Luliconazole and Naproxen Sodium in the formulated gel.

The aim of the study is to treat diabetic foot in diabetic patients. In such cases, one can use pharmaceutical gel containing Luliconazole and Naproxen Sodium for the purpose of curing diabetic foot inflammation and fungal infections. With this aim and objectives, an attempt was made to formulate and evaluate a pharmaceutical gel. Naproxen sodium was chosen in combination with Luliconazole because it's low cardiac toxicity in prolonged usage.

A pharmaceutical gel consisting of Luliconazole and Naproxen sodium was formulated and evaluated for organoleptic parameters like pH measurement, Viscosity measurement, spreadability, Homogeneity, Drug content, Grittiness, Extrudability, Stability test *in-vitro* drug diffusion study. The results were found to be within the limits as shown in the Table 2. The linearity was found in the range of 10-50 µg/mL, shown in Table 5. The r^2 value was found to be 0.9991 for Luliconazole and 0.9995 for Naproxen sodium shown in the graph 1. The recovery studies revealed that the proposed method was accurate to determine small change in concentration of the solution.

Precision was determined by studying the repeatability and intermediate precision. %RSD was calculated for Luliconazole and Naproxen sodium, results are shown in the Table 6

CONCLUSION

Thepresent work for evaluation and simultaneous estimation of Luliconazole and Naproxen sodium by RP-HPLC method in formulated gel was aimed, To formulate a pharmaceutical gel with Luliconazole and Naproxen sodium with a hope to show and anti fungal activity and reduce inflammation and pain in diabetic foot patients. The prepared formulations (F1, F2, and F3) were evaluated for physical properties.

A new, Simple, Reproducible, Sensitive RP-HPLC method was developed and validated for simultaneous estimation of Luliconazole and Naproxen Sodium. The analytical method was validated as per ICH guidelines with respect to various parameters- Linearity, Accuracy, Precision[18]From the results, it can be concluded that the developed method is effective for simultaneous estimation of Luliconazole and naproxen sodium from the formulated gel, which can be used to cure anti-fungal infection, pain and inflammation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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