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Original Article

DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING RP-HPLC METHOD FOR THE DETERMINATION OF POTENTIAL DEGRADATION PRODUCTS OF DIFLUPREDNATE IN OPHTHALMIC EMULSION

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ABSTRACT

Objective: The objective of the current study was to develop and validate a simple, robust, precise and accurate RP-HPLC (reverse phase-high performance liquid chromatography) method for the quantitative determination of potential degradation products of Difluprednate (DIFL) in the ophthalmic emulsion.

Methods: Chromatographic separation was achieved on the YMC pack ODS-AQ (150× 4.6) mm, 3µm column with a mobile phase containing a gradient mixture of mobile phase A (0.02M Ammonium formate buffer pH 4.5 adjusted with formic acid) and Acetonitrile as mobile phase B, at flow rate of 1.5 ml/min and with UV detection at 240 nm.

Results: The peak retention time of DIFL was found at about 17.2 min, the RRT of degradation product-1 (DP-1), degradation product-2 (DP-2), and degradation product-3 (DP-3), were found to be about 0.49, 0.65 and 0.79 respectively (calculated with respect to Difluprednate). Stress testing was performed in accordance with an ICH (international council for harmonisation) guideline Q1A (R2) [1]. The method was validated as per ICH guideline Q2 (R1)[2]. The calibration curve was found to be linear in the concentration range of 0.1 to 0.75 µg/ml for Difluprednate, DP-1, DP-2 and DP-3. The LOD (Limit of detection) was found to be 0.1µg/ml and LOQ (Limit of quantification) of 0.15µg/ml for Difluprednate, DP-1, DP-2 and DP-3 respectively. The recovery from LOQ to 150% was within 90-110%. The forced degradation data confirms the stability indicating the nature of the method.

Conclusion: A simple, robust, precise and accurate RP-HPLC method for the quantitative determination of potential degradation products of Difluprednate in the ophthalmic emulsion was developed and validated.

Keywords: Difluprednate, Stability indicating, RP-HPLC, Degradation products, Forced degradation

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INTRODUCTION

Difluprednate (DIFL) [(6S,8S,9R,10S,11S,13S,14S,17R)-17-(2-Acetyl-oxyacetyl)-6,9-difluoro-11-hydroxy-10,13-imethyl-3-oxo-6,7,8,11,12, 14,15,16-octahydrocyclopenta[a] phenanthren-17-yl] butanoate (fig. 1) is a glucocorticoid receptor agonist and a diflurinated derivative of prednisolone having anti-inflammatory activity. DIFL ophthalmic emulsion is a topical formulation of DIFL that is an ophthalmic corticosteroid emulsion for topical instillation [4-10].

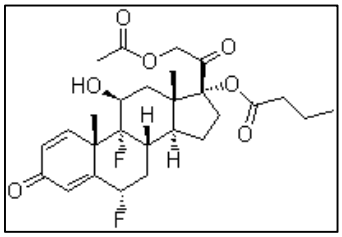

Fig. 1: Chemical structure of DIFL

Literature survey revealed that RP-HPLC method has been reported for the estimation of DIFL from biological matrices, for assay in bulk drug as well as in eye drops [5]. The UV-Visible spectrometric method was also reported for estimation of DIFL [10]. DIFL and its formulations are not official in any pharmacopeia.

To the best of our knowledge, no stability indicating reverse-phase high-performance liquid chromatography [RP-HPLC] method has been reported for the determination of DIFL and its related impurities in the emulsion based eye drops. Very less work has been done on the quantification of related substances of DIFL in the ophthalmic emulsion; hence a simple robust and sensitive stability indicating RP-HPLC method was developed and validated for determination of DIFL and its related impurities in the ophthalmic emulsion as per the ICH guidelines [1-3].

Three potential degradation products were observed in the marketed products and in the degradation studies, the identified degradation products were enriched, isolated and purified. These purified degradation products were degradation product-1(DP-1), degradation product-2 (DP-2) and degradation product-3 (DP-3) which were then used to validate the method. The specification limit for the three degradation products and unknown impurities is considered as 1.0% based on the maximum daily dose as per ICH guideline [11]. The main aim of this research work was to develop a stability indicating the RP-HPLC method for determination of degradation products of DIFL in the ophthalmic emulsion.

Stability indicating RP-HPLC method is defined as a validated method having the ability to specifically quantitate the analyte in the presence of excipients, degradation products, and process impurities. Due to the impact of stress (hydrolysis, oxidation, temperature and photolytic) during shelf life and instability studies, various degradation products shall be formed, specific quantification of these degradation products provides additional assurance of the stability indicating nature of the method [12-14].

MATERIALS AND METHODS

Chemicals and reagents

DIFL (active pharmaceutical ingredient) and DIFL ophthalmic emulsion were procured as a gratis sample from Sun Pharma, Baroda. Market samples of Flupred© were also received from Sun

pharma; other market samples were procured from a local pharmacy. Ammonium formate, formic acid and sodium hydroxide (AR-grade) were purchased from Merck. HPLC grade acetonitrile was purchased from Merck, AR-grade hydrochloric acid and 30% hydrogen peroxide was purchased from Rankem. DP-1, DP-2, and DP-3 were enriched, isolated and purified in-house.

Instruments

Chromatography was performed on Waters HPLC 2695 equipped with quaternary pumps with PDA (Photodiode array) detector. The chromatographic separation was performed using YMC Pack ODS-AQ® C18 reverse phase column (150 × 3) mm, 3 µm, 110Å, YMC Japan. Data acquisition and integrations were performed using empower 3 software.

Methods

Chromatographic conditions

Chromatographic separation was performed in gradient mode with a flow rate of 1.5 ml/min consisting of mobile phase-A (0.02 M ammonium formate, pH 4.5) and an organic phase (Acetonitrile) as mobile phase-B with the column temperature set to 40 °C. The injection volume was 20 µl, and UV detection of DIFL and its degradation products was accomplished at 240 nm. The column was YMC pack ODS-AQ (150 × 4.6) mm, 3µm. The gradients used was 0 min (A-75% and B-25%), 10 min (A-55% and B-45%), 15 min (A-55% and B-45%), 20 min (A-20% and B-80%), 28 min (A-20% and B-80%), 30 min (A-75% and B-25%) and 36 min (A-75% and B-25%).

Preparation of solutions

Preparation of buffer solution

Dissolved 1.29 gm of Ammonium formate in 1000 ml of milli-Q water, sonicated for 5 min to dissolve. Adjusted the pH of the solution to 4.5 with diluted Formic Acid (20%v/v) and filtered through 0.45 µmNylon membrane filter paper.

Preparation of standard solutions

5 mg of DIFL was dissolved in 20 ml of acetonitrile to prepare a standard stock solution of 250 ppm; 2 ml of the standard stock solution was further diluted to 100 ml with acetonitrile (5 ppm system suitability solution).

Preparation of sample solutions

1 ml of DIFL ophthalmic emulsion 0.05% w/v was diluted up to 10 ml with acetonitrile; sonicated the solution for 5 min and centrifuged at 5000 rpm for 10 min and the clear supernatant solution was injected in the chromatograph.

Preparation of placebo solutions

1 ml of placebo was diluted to 10 ml with acetonitrile; sonicated for 5 min and centrifuged at 5000 rpm for 10 min and the clear supernatant solution was injected into the chromatographic system, and the chromatogram was recorded.

Preparation of DP-1 stock solutions

2.5 mg of purified DP-1 was dissolved in 10 ml of acetonitrile and further diluted to 50 ml with acetonitrile.

Preparation of DP-2 stock solutions

2.5 mg of purified DP-2 was dissolved in 10 ml of acetonitrile and further diluted to 50 ml with acetonitrile.

Preparation of DP-3 stock solutions

2.5 mg of purified DP-3 was dissolved in 10 ml of acetonitrile and further diluted to 50 ml with acetonitrile.

Validation

The optimized analytical method was validated for system suitability, linearity and range, precision, limit of detection, limit of quantitation, accuracy from LOQ to 150 % of the specification limit (1% for all impurities) and robustness in accordance with ICH guidelines for analytical procedures Q2 (R1).

System suitability

System suitability parameters were studied to verify the system performance and suitability of the method before every experiment. Six replicate injection of DIFL standard solution [5µg/ml] were analyzed using the developed method. Factors such as theoretical plate count, tailing factor, percent relative standard deviation [% RSD] of peak area and retention time were taken into consideration for testing system suitability.

Linearity and range

The linearity was evaluated at eight concentration levels in the range between 0.05 to 0.75 µg/ml for DIFL and three degradation products. A calibration curve was plotted by plotting concentration against corresponding peak area, and linearity was determined using least square regression analysis. The analytical range was established by the highest and lowest concentrations of analyte where acceptable linearity obtained.

Accuracy and method precision

The method precision and accuracy were carried out by preparing and analyzing the sample as per the method and spiking the sample with three impurities at 4 different level (LOQ, 50%, 100% and 150% of specification level) in three replicates preparation at each level. The concentration for each impurity at LOQ, 50%, 100% and 150% was 0.15µg/ml, 0.25µg/ml, 0.5µg/ml and 0.75µg/ml respectively. The percentage recovery and percentage relative standard deviation were taken into consideration for testing accuracy and precision at each level.

Intermediate precision (Ruggedness)

The ruggedness of the method was carried out by spiking the sample preparation with the three impurities at 100% level in six replicates. The samples were analyzed by the different analyst using the different column on a different system. The % RSD of the 9 determination; three from method precision at 100% level and six from ruggedness has been reported.

LOD and LOQ

LOD and LOQ of the developed method were calculated from the standard deviation of the y-intercepts and slope of the calibration curve of DIFL and three impurities using the following formula:

$$LOD=\frac{3.3\alpha}{S} \quad \ldots\ldots 1$$

$$LOQ=\frac{10\alpha}{S} \quad \ldots\ldots 2$$

Where α is the standard deviation of the y-intercepts and *S* is the slope of the calibration curve.

Relative response factor (RRF)

Relative response factor of the three impurities was calculated using slope of the regression line for each impurity using the following formula:

$$RRF=\frac{Si}{Ss} \quad \ldots\ldots 3$$

Where *Si* is slope of impurity standard and *Ss* is slope of drug standard.

Forced degradation study

Acidic degradation

Accurately pipetted and transferred 1 ml sample solution into a 10 ml volumetric flask, added 1 ml acetonitrile mixed and added 0.5 ml of 0.2M Hydrochloric acid solution and refluxed at 60 °C for 10hr, cooled the resulting solution at room temperature and neutralized the sample using 0.5 ml of 0.2M sodium hydroxide solution and diluted up to 10 ml with acetonitrile (50 µg/ml of DIFL). Centrifuged at 5000 rpm for 10 minute and the supernatant solution was

injected into the chromatographic system and the chromatogram was recorded.

Alkaline degradation

Accurately pipetted and transferred 1 ml sample solution into a 10 ml volumetric flask, added 1 ml acetonitrile mixed and added 0.5 ml of 0.025M sodium hydroxide solution and refluxed it at 40 °C for 6hr, cooled the resulting solution at room temperature and neutralized the sample using 0.5 ml of 0.025M hydrochloric acid solution and diluted up to 10 ml with acetonitrile (50 µg/ml of DIFL). Centrifuged at 5000 rpm for 10 min and the supernatant solution was injected into the chromatographic system, and the chromatogram was recorded.

Thermal degradation

Accurately pipetted and transferred 1 ml sample solution into a 10 ml volumetric flask, added 1 ml acetonitrile; mixed and kept in a preheated oven at 80 °C for 72 hr, cooled the resulting solution at room temperature and diluted it up to 10 ml with acetonitrile (50 µg/ml of DIFL). Centrifuged at 5000 rpm for 10 minute and the supernatant solution was injected into the chromatographic system, and the chromatogram was recorded.

Oxidative degradation

Accurately pipetted and transferred 1 ml sample solution into a 10 ml volumetric flask, added 1 ml acetonitrile mixed and added 1 ml of 6% hydrogen peroxide solution and refluxed it at 60 °C for 3hr, cooled the resulting solution at room temperature and diluted up to 10 ml with acetonitrile (50 µg/ml of DIFL). Centrifuged at 5000 rpm for 10 minute and the supernatant solution was injected into the chromatographic system and the chromatogram was recorded.

Photolytic degradation

5 ml of the sample was exposed to UV light at 254 and 360 nm for 7 d. accurately pipetted and transferred 1 ml of the exposed sample into a 10 ml volumetric flask, diluted up to 10 ml with acetonitrile (50 µg/ml of DIFL). Centrifuged at 5000 rpm for 10 min and the supernatant was injected into the chromatographic system and the chromatograms were recorded.

RESULTS

Method development and optimization

The purpose of this method was to separate the potential known and unknown degradation products of DIFL in shorter run time. C18 column from various manufacturers was tried, and good peak separation was observed in YMC pack ODS-AQ which is polar imbedded reverse phase column. Various buffer including phosphate and formate were tried in the pH range of 3.0 to 7.0. The gradient has been optimized after selection of suitable buffer, pH and column. Based on the optimization trial final method was developed and validated as per ICH guideline [2].

The optimized chromatographic condition was shown in table 1 and the typical HPLC chromatogram of standard and sample spiked with degradation product were shown in (fig. 2 and 3) and its combined PDA spectrum was shown in fig. 5.

Table 1: Optimised chromatographic condition for the estimation of DIFL and potential degradation products

Parameter	Condition
Mobile phase	0.02 M ammonium formate buffer pH 4.5: acetonitrile [80:20 v/v].
Gradient Program	The gradients used are 0 min (A-75% and B-25%), 10 min (A-55% and B-45%), 15 min (A-55% and B-45%), 20 min (A-20% and B-80%), 28 min (A-20% and B-80%), 30 min (A-75% and B-25%) and 37 min (A-75% and B-25%).
Diluent	Acetonitrile
Column	YMC pack ODS AQ (150 mm × 4.6 mm) 3 µm
Column temperature	40 °C
Detection wavelength	240 nm
Injection volume	20 µl
Flow rate	1.5 ml/min
Runtime	36 min

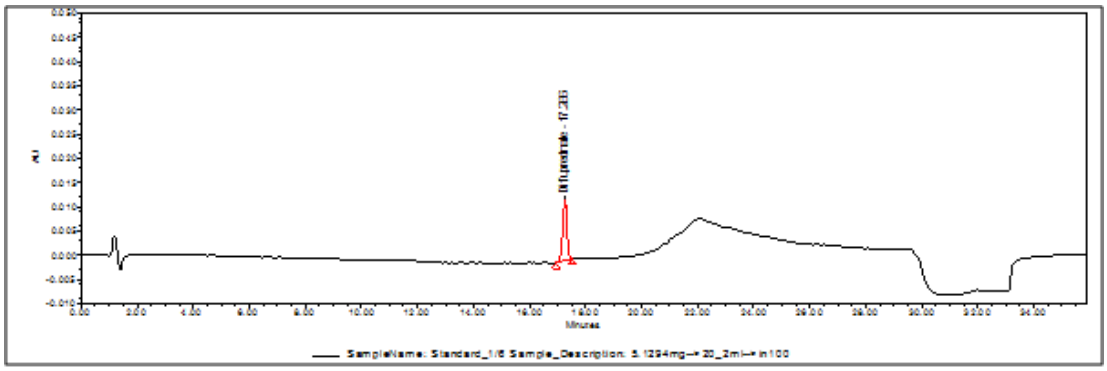

Fig. 2: HPLC chromatogram of standard

Method validation

The developed method was validated as per ICH guideline [1-3].

System suitability

The developed method has produced a theoretical plate above 5000 for DIFL and three impurities with tailing factor less than 2. Similarly, the %RSD of peak area and retention time for DIFL peak was less than 5, which ensure the suitability of the developed method. The results of the system suitability study were summarised in table 2.

Acceptance criteria

The relative standard deviation of six replicate injections for peak area should not be more than 5.0%. The tailing factor should not be more than 2. The theoretical plates should not be less than 5000.

Linearity and range

Eight point calibration curve was obtained in concentration ranges from 0.10-0.75 µg/ml for DIFL and its three impurities. The response of the drug was found to be linear in the selected concentration range and the correlation coefficient for DIFL, DP-1, DP-2 and DP-3 were

found to be 0.99686, 0.99837, 99818 and 0.99802 respectively. The results of linearity were summarised in table 3.

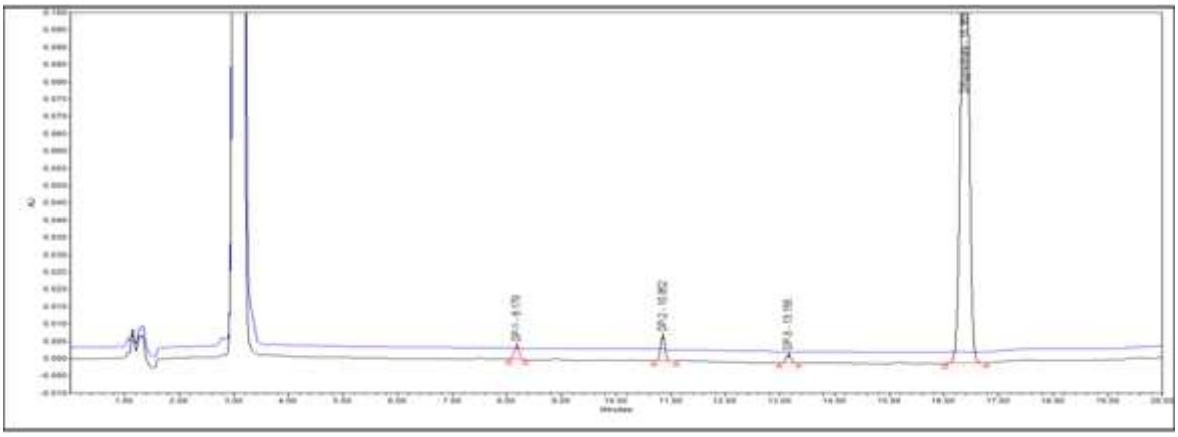

Fig. 3: HPLC chromatogram of sample spiked with impurities (DP-1, DP-2 and DP-3)

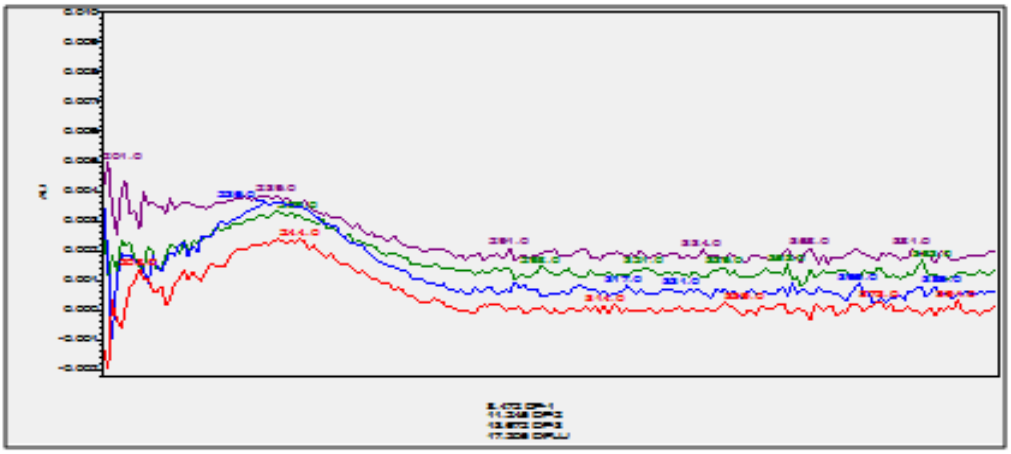

Fig. 4: PDA spectrum of DIFL, DP-1, DP-2 and DP-3

Precision

The developed method had shown % RSD less than 5 for both method precision (precision and recovery study) and intermediate precision study, which ensures precision of the developed method. The results of the precision study were summarised in table 4 and 7.

Limit of detection and limit of quantification

LOD and LOQ were estimated from the standard deviation of the Y-intercepts and slope of the calibration curve of DIFL and its impurities. This showed that the developed method can detect and quantify very low concentrations of DIFL in the presence of its degrading products; therefore obtained data proves the sensitivity of the developed method for quantification of impurities.

Accuracy and method precision

The percentage recovery of the sample spiked with the known impurities was within 100±10%, which ensures the accuracy of the developed method. The results of recovery studies were summarised in table 5-8.

Robustness

As per ICH guideline, the prepared solution was analyzed as per the method with a small but deliberate change in chromatographic conditions as listed below table 9. Change in flow rate, temperature and pH were studied.

Table 2: System suitability of the developed RP-HPLC method

Parameters	Name of drug	Acceptance criteria			
	DIFL	DP-1	DP-2	DP-3	
Retention time	17.24	8.48	11.27	13.70	---
Theoretical plates [N]	109364	16524	43369	44606	>5000
Tailing factor	0.93	0.84	0.96	0.94	<2
Resolution	NA	11.59	10.13	12.91	>2
% RSD of Peak area*	0.41	NA	NA	NA	<5
% RSD of Retention time*	0.26	NA	NA	NA	<2

*N (Number of measurements) =6, NA: Not applicable

Table 3: Linearity, range and RRF of the developed RP-HPLC method

% of specification level	Conc. in µg/ml	Calculated area*			
		DIFL	DP-1	DP-2	DP-3
10	0.05	ND	NA	NA	NA
20	0.1	4.4	2	3.61	1.86
30	0.15	4.5	3.8	4.43	3.99
50	0.25	7.86	6.31	7.45	6.24
80	0.4	12.81	12.04	13.03	10.97
100	0.5	15.66	14.52	16.62	13.72
120	0.6	17.33	18.74	20.32	17.24
150	0.75	23.35	23.99	25.77	21.63
Correlation Coefficient (r)		0.99686	0.99837	0.99818	0.99802
Y intercept		1.10345	-1.14013	-0.1899	-0.60816
%Y-Intercept		27.4132	31.6629	28.5376	27.3023
Slope		0.56207	0.46273	0.5065	0.4774
RRF		1	1.13	1.18	1.10

*Calculated Area= Area count/1000, Conc: concentration, RRF: relative response factor, NA: Not applicable, ND: Not detected.

Table 4: Intermediate precision (Ruggedness) of the developed RP-HPLC method

Sample ID	Component		
	DP-1	DP-2	DP-3
MP Set-1	98.8	104.0	94.1
MP Set-2	96.4	106.1	93.8
MP Set-3	98.8	100.2	97.3
IP Set-1	101.3	98.2	96.6
IP Set-2	98.7	97.4	97.0
IP Set-3	102.9	98.1	97.2
IP Set-4	100.3	97.5	96.9
IP Set-5	102.0	97.0	97.1
IP Set-6	102.3	97.7	96.4
***Mean recovery**	100.17±2.14	99.58±3.29	96.27±1.40
% RSD	2.14	3.30	1.45

MP=Method precision, IP= Intermediate precision,* mean±SD (Standard deviation), N=9.

Table 5: Accuracy at LOQ of the developed RP-HPLC method

Component	Recovery at LOQ level				
	% Recovery			% Avg. recovery*	% RSD
	Set-1	Set-2	Set-3		
DP-1	95.0	95.4	90.4	93.6±2.81	3.0
DP-2	88.5	93.7	92.1	91.4±2.69	2.9
DP-3	95.8	94.7	96.7	95.7±0.97	1.0
DIFL	104.4	101.4	99.9	101.9±2.31	2.3

*N=3, mean±SD, Avg= Average

Table 6: Accuracy at 50%

Component	Recovery at 50% of specification level				
	% Recovery			% Avg. recovery*	% RSD
	Set-1	Set-2	Set-3		
DP-1	103.1	104.3	98.6	102.0±3.00	2.9
DP-2	101.3	104.3	105.3	103.6±2.08	2.0
DP-3	102.1	104.3	96.9	101.1±3.80	3.8
DIFL	98.1	99.9	98.1	98.7±1.04	1.1

*N=3, mean±SD, Avg= Average

Table 7: Accuracyat 100%

Component	Recovery at 100% of specification level				
	% Recovery			% Avg. recovery*	% RSD
	Set-1	Set-2	Set-3		
DP-1	98.8	96.4	98.8	98.0±1.39	1.4
DP-2	104.0	106.1	100.2	103.4±2.99	2.9
DP-3	94.1	93.8	97.3	95.1±1.94	2.0
DIFL	101.5	101.7	101.7	101.6±0.12	0.1

*N=3, mean±SD, Avg=Average

Table 8: Accuracy at 150%

Component	Recovery at 150% of specification level				
	% Recovery			% Avg. recovery*	% RSD
	Set-1	Set-2	Set-3		
DP-1	101.2	97.5	107.2	102.0±4.90	4.8
DP-2	98.8	102.4	99.0	100.1±2.02	2.0
DP-3	98.2	97.9	96.0	97.4±1.19	1.2
DIFL	101.1	101.1	101.3	101.2±0.12	0.1

*N=3, mean±SD, Avg= Average.

Table 9: Robustness of the developed RP-HPLC method

Parameter	Mean area*	% RSD (area)	Retention* time	%RSD for RT	USP plate count	USP tailing
Column temperature 45◦C(+)	132.93±0.90	0.68	16.9±0.03	0.20	40207	0.87
Column temperature 35◦C(-)	139.21±0.77	0.55	17.77±0.05	0.26	105038	0.94
Flow rate 1.7 ml/min(+)	121.39±1.22	1.00	16.06±0.01	0.06	38335	0.93
Flow rate 1.3 ml/min(-)	156.66±0.81	0.52	18.25±0.01	0.06	119926	0.92
pH 4.7 (+)	150.09±2.07	1.38	16.27±0.02	0.14	65417	0.98
pH 4.3 (-)	151.8±5.47	3.6	16.58±0.04	0.25	65305	0.95

N=6, RT=Retention time, *mean±SD

Application of the validated method for analysis of a market sample

The developed method was used to determine the % impurities of the marketed samples. The results of the assay were summarised in table 10 and the summary of the validation parameter was summarised in table 11, it was confirmed that the market sample has the impurities. Amongst it, the major impurities were DP-1, DP-2 and DP-3.

Table 10: Related substances of DIFL in ophthalmic emulsion

Sample details	DP1 (%)	DP2 (%)	DP3 (%)	% Unknown	%Total impurities
DIFL Ophthalmic emulsion 0.05%w/v	0.42	0.59	0.15	0.30	1.46

Table 11: Summary of validation parameters of developed RP-HPLC method

S. No.	Validation parameter	DIFL	DP-1	DP-2	DP-3
1	Linearity range (µg/ml)	0.10-0.75	0.10-0.75	0.10-0.75	0.10-0.75
2	Precision				
	Method precision(%RSD)*	NA	1.41	2.89	2.04
	Intermediate precision(%RSD)*	NA	1.38	0.42	0.29
3	Accuracy (% recovery)*	101.6±0.12	98.0±1.39	103.4±2.99	95.1±1.94
4	LOD (µg/ml)	0.1	0.1	0.1	0.1
5	LOQ (µg/ml)	0.15	0.15	0.15	0.15
6	Specificity	Specific	Specific	Specific	Specific
7	Robustness	Robust	Robust	Robust	Robust

*mean±SD, Sr. No=Serial number

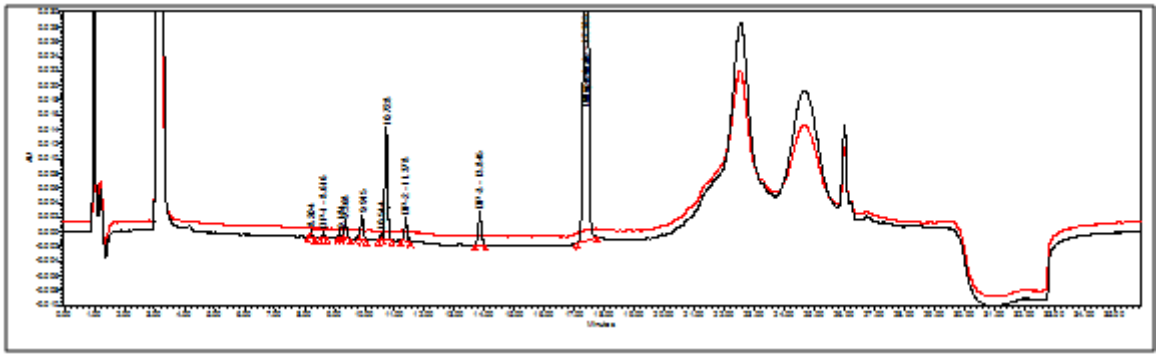

Fig. 5: Chromatogram of alkali hydrolysis sample and placebo

Forced degradation studies

Forced degradation studies were carried under conditions of acid/base hydrolysis, oxidation, heat and photolysis. For each study, samples and placebo were subjected to different stress conditions. The concentration of the reagents and the time of exposure was optimized to obtain degradation within the range of 5-20%. During optimization of degradation conditions, excessive degradation was

observed in the acid, base and peroxide degradation; the conditions were then optimized to get degradation between 5-20% which shall avoid generation of secondary degradation products.

During the forced degradation studies the sample was found to be very susceptible to base-catalyzed hydrolysis. The sample was also prone to acid catalysed hydrolysis and oxidation, which is shown in fig. 5-7. Throughout the degradation study, three major peaks were observed, which were also observed in the market sample too, these degradation products are then named as DP-1, DP-2 and DP-3. Further work in future needs to be done for the identification of these impurities. The degradation products observed under these conditions were well resolved from the drug peak. Thus the developed method was considered stability indicating. The optimized degradation conditions and results were summarised in table 12.

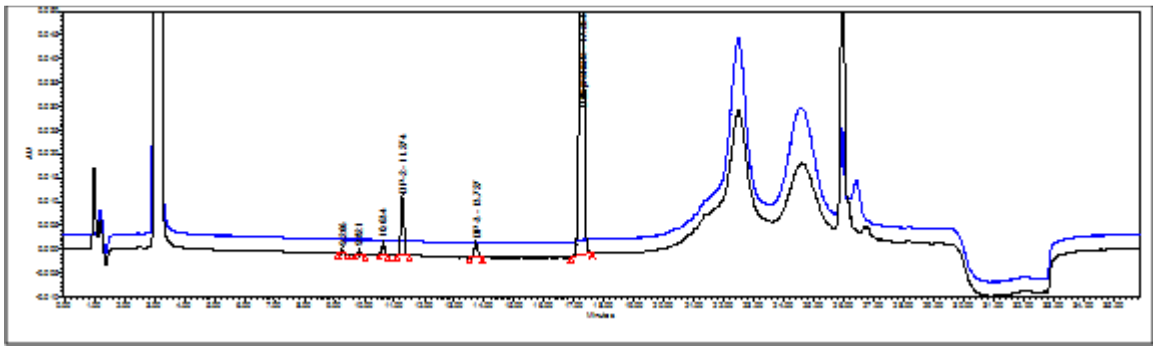

Fig. 6: Chromatogram of acid hydrolysis sample and placebo

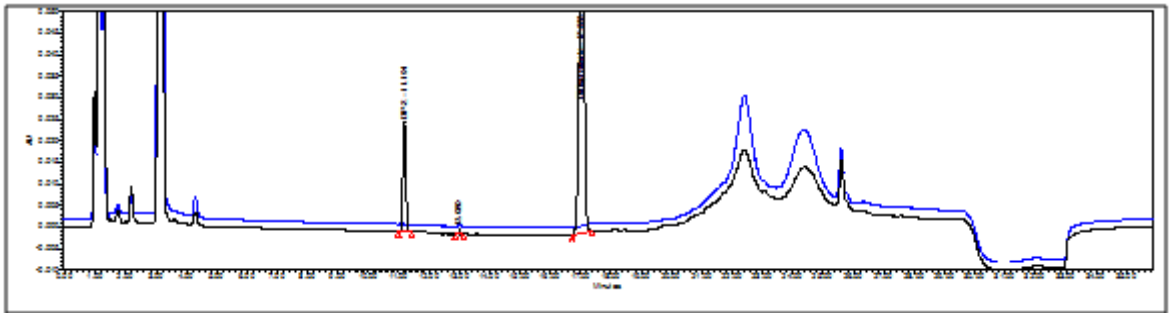

Fig. 7: Chromatogram of peroxide degradation sample and placebo

Table 12: Degradation behaviour of DIFL

Condition for degradation	DP1 (%)	DP2 (%)	DP3 (%)	%Unknown	% Total degradation
0.2M HCl at 60 °C for 10h	0.00	6.67	1.91	1.82	10.40
0.025M NaOH at 40 °C for 6h	0.25	1.37	2.10	9.40	13.12
Thermal degradation for 3 d	2.23	1.18	1.54	3.73	8.68
6% Peroxide at 60 °C for 3 h	0.00	18.87	0.23	0.530	19.64
UV light at 254 for 7d	0.00	0.00	0.00	0.00	0.00

*HCl: Hydrochloric acid, NaOH: Sodium hydroxide.

DISCUSSION

Based on the results of forced degradation in the present study, three major degradation products were identified; these degradation products were also observed in the marketed products. The validation data indicates that the present work complies as per the ICH guideline [2]. The method was found specific, accurate, precise and robust for determination of all degradation products. The LOD and LOQ value were 0.1 and 0.15 respectively for the three degradation products (DP-1, DP-2 and DP-3). Low LOD and LOQ value indicates the sensitivity of the method for determination of degradation product in the ophthalmic emulsion. The high value of the correlation coefficient (more than 0.99) for DIFL and its degradation products indicates good linearity. The recovery of the method for the DIFL and the three impurities was found to be within 90-110%, showing good recovery of the impurities. The precision of the method shows the reproducibility and repeatability of the method. The % RSD value for the impurities was found to be less than 5%, confirming the precision of the method for the quantitative determination of degradation products of DIFL. The specificity of the method to accurately quantify the degradation products of DIFL in the presence of the formulation components was proved by the forced degradation studies. The robustness data of the analytical method proves that the method was robust enough to deal with small changes in the method parameters. The methods available in the literature mostly focused on the assay of DIFL in the ophthalmic emulsion and in rabbit aqueous humor [4-10]. The present work specifically focused on the identification of degradation products and its quantification. This method can be used for evaluation of impurities in the market samples.

CONCLUSION

The developed method was stability indicating where well-resolved peaks were observed for analyte and degradation product. The method was specific, accurate, precise, and robust and can be used for routine quality control as well as accessing the stability of DIFL in bulk and in pharmaceutical dosage forms.

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AUTHORS CONTRIBUTIONS

Conception or design of the work has been done by Murlidhar V. Zope; data Collection has been done by Ms Ashwinikumari Patel and Mr Murlidhar V. Zope; data Analysis and interpretation has been done by Mr Rahul M Patel and Mr Murlidhar V Zope; drafting the article has been done by Mr Rahul M Patel and Mr Murlidhar V Zope; Critical revision of the article has been done by Mr Murlidhar V Zope; final approval of the version to be published was provided by Dr Samir G. Patel.

CONFLICT OF INTERESTS

Declared none

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