ANALYTICAL METHOD DEVELOPMENT FOR SIMULTANEOUS ESTIMATION OF CIPROFLOXACIN HCL AND OLOPATADINE HCL IN BULK DRUG AND FORMULATION BY RP-HPLC METHOD

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ABSTRACT

A reverse phase chromatography technique to simultaneously estimate ciprofloxacin HCl and olopatadine HCl as bulk drugs and in formulation has been developed. The chromatographic separation of drugs and formulation was accomplished on C18 Agilent Zorbax column (250 x 4.6 mm, 5 µm) using acetonitrile: TFA water (40:60, V/V). A 10 µL volume was injected with flow rate 1 mL min^{-1}, and peaks were detected. The retention periods for ciprofloxacin HCl and olopatadine HCl were 2.92 and 5.10 minutes respectively. The method was linear in the concentration ranges 24-36 µg mL^{-1} and 56-84 µg mL^{-1} for ciprofloxacin HCl and olopatadine HCl, with regression coefficient correlation values of 0.9999 and 0.9996, respectively. Relative standard deviation of precision, accuracy, and ruggedness was below 2 %. Forced degradation studies were performed under different conditions which were in an admissible range. The established method was simple, accurate, and precise. Therefore, it can be applied for the routine quantification and degradation of these drugs and formulation.

Keywords: Reverse phase- High-performance liquid chromatography, Ciprofloxacin HCl, Olopatadine HCl, Simultaneous estimation, Method validation, Force degradation study

INTRODUCTION

Ciprofloxacin hydrochloride (CFH) is an antibiotic utilized to treat eye infections (conjunctivitis)[1]. Chemically it is denoted as 1-cyclopropyl-6-fluro-1,4-dihydro-4-oxo-7-(piperazine-1-yl)-3-quinoline carboxylic acid (Fig. 1 A). This drug is incorporated in oral, ophthalmic, and otic administration to rehabilitate conjunctivitis. Conjunctival mucosal inflammatory diseases are characterized by inflammation of the conjunctiva and a yellow-white mucopurulent discharge, both of which may lead to significant visual impairments and abnormalities. Several investigations have revealed that 50-60 % of conjunctivitis cases were bacterial infections[2]. CFH is an active molecule with a potent mechanism against gram-positive and gram-negative bacteria, that inhibits topoisomerase II, IV, and DNA-gyrase to suppress cell division in bacteria[3]. Olopatadine hydrochloride (OLH) is an antihistaminic used to treat allergic conjunctivitis by inhibiting the discharge of histamine from mast cells[4]. It is chemically 11-[(*Z*)-3-(dimethylamino) propylidene]-6,11-dihydrodibenzo [b,e] oxepin-2-acetic acid hydrochloride (Fig. 1 B). OLH plays a significant role in prohibiting type 1 immediate hypersensitivity reactions[5]. Acute and subacute conjunctivitis triggered by susceptible strains of aerobic gram-positive and gram-negative bacteria such *S. aureus*, *S. pneumonia*, *S. epidermidis*, and *Haemophilus influenza* is rehabilitated with CFH and OLH[6,7].

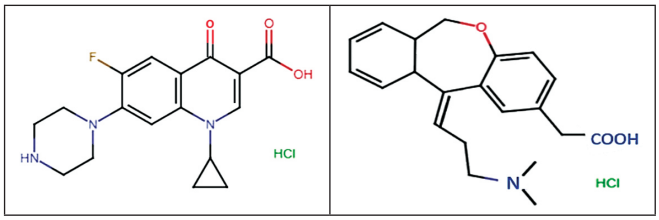

Fig. 1 A Fig. 1 B

Fig. 1: Chemical structures of A. CFH and B. OLH

The literature survey revealed numerous methods for liquid chromatography assessment of CFH and OLH in aqueous samples and biological fluid[8–11]. Several methods were also demonstrated to estimate CFH and OLH with other drugs such as dexamethasone, ambroxol hydrochloride, chloramphenicol, ornidazole and tinidazole[12–15]. However, the Reverse Phase-High Performance Liquid Chromatography (RP-HPLC)

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technique for simultaneous determination of CFH & OLH has not yet been disclosed. Hence, the current research focused on developing and validating a simple, sensitive, and cost-effective RP-HPLC technique to determine CFH and OLH in bulk drugs and the formulation. The established method has been validated for system suitability, specificity, linearity, limit of detection and qualification, precision and accuracy. The forced degradation study was performed for CFH and OLH formulation in different conditions, including oxidative, acidic, base, dry heat, and photolytic degradation.

MATERIALS AND METHODS

Materials

CFH was obtained as a sample from Aadhaar Life Sciences Pvt. Ltd., India and OLH from Indoco Pvt. Ltd., Mumbai, India. Acetonitrile and trifluoroacetic acid (analytical grade) were procured from Merck Specialties Pvt. Ltd., Mumbai, India. The RP-HPLC grade ultra-pure water was acquired through the in-house Milli-Q water system.

Instruments

The RP-HPLC system utilized for the method development comprised of Agilent 1260 Infinity II with a G7111A pump with degasser, G7115A detector, and G7129A autoinjector. Analysis and separation have been done on C18: (Agilent Zorbax SB-Aq (250 x 4.6 mm, 5µm) column. The mobile phase comprised of acetonitrile (ACN):0.1 % trifluoroacetic acid water (TFA water) (40:60, V/V), the injection volume was set to 10 µL and in an isocratic mode the flow rate was set at 1mL min^{-1} for all samples.

Methods

Selection and optimization of chromatographic condition for RP-HPLC[16]

Standard stock solutions (A and B) development

A stock solution of CFH was developed in a 10 mL volumetric flask by dissolving accurately weighed 5 mg of CFH. A diluent mixture of ACN: water (50:50, V/V) was utilized to dissolve it and sonicated for 10 minutes, later with a diluent mixture the final volume was added up to 10 mL in flask (A) (500 µg mL^{-1}). Using the methodology above, a stock solution of 5 mL OLH in 10 mL of diluent (500 µg mL^{-1}) in a separate volumetric flask (B) was prepared.

Preparation of working solution

Working solutions were prepared from corresponding stock solutions. In 10 mL volumetric flask, 0.6 mL solution from flask A and 1.4 mL from flask B were pipetted out and diluted with a diluent mixture up to 10 mL to obtain the concentration of working solution of CFH (30 µg mL^{-1}) and OLH (70 µg mL^{-1}).

Placebo solution

It was developed by measuring 2.0 mL placebo in a volumetric flask, and volume completed with the diluent mixture. From the above, 0.5 mL of solution was pipetted out, placed into a volumetric flask and, further volume adjusted with the diluent mixture up to 10 mL.

Sample solution

Precisely measured 2.0 mL of CFH and OLH formulation solution was transfered into a volumetric flask, and volume was adjusted with the diluent mixture. From this, 0.5 mL was pipetted out, transferred into a volumetric flask, and volume was made up with the diluent mixture up to 10 mL.

Development of mobile phase

It was developed by integrating ACN and 0.1 % TFA water (40:60 V/V) which was filtered via nylon filter paper of 0.45 µm and degassed in a sonicator for 15 minutes before use.

Development of optimized chromatographic condition

For attaining simultaneous estimation of bulk drug and formulation, various RP-HPLC parameters like analytical column type, mobile phase ratio, rate of flow, spectrum range for detection, injection volume and run time were optimized and the parameters are presented in Table I.

Table I: Optimized chromatographic conditions for RP-HPLC analysis

Analytical column	C18 Agilent Zorbax SB-Aq (250 x 4.6 mm, 5µm)
Mobile phase	Acetonitrile: 0.1 % TFA water (40:60, V/V)
Flow rate	1 mL min^{-1}
Column temperature	30°C
Injection volume	10 µL
Runtime	17 minutes
Detection wavelength	260 nm

Assay of CFH and OLH in the formulation

Accurately measured 2.0 mL of CFH and OLH

formulation solutions were poured into a flask. The volume was adjusted with the diluent mixture and sonicated for 1 minute to mix the contents. From the solution described above, 0.5 mL was taken out and deposited in a volumetric flask and volume was made with the diluent mixture up to 10 mL. Further it was filtered out by 0.45 µm nylon syringe filter before injecting it into a RP-HPLC column.

RP-HPLC method validation[17]

The optimized method has been established by the ICH Q2 (R1) guidelines for assessing system suitability, specificity, accuracy, linearity, robustness, precision, limit of detection (LOD), limit of quantitation (LOQ) and forced degradation study. The detailed method validation parameters are discussed in a subsequent section.

System suitability study

System suitability parameters concerning tailing factor, theoretical plate quantity, resolution and repeatability among CFH & OLH peaks were evaluated by injecting a blank mobile phase preceded by five CFH and OLH mixture replicates.

Specificity

Specificity of proposed RP-HPLC method was performed by injecting diluent, placebo solution, CFH, and OLH solutions and their formulation into the RP-HPLC system. Then, each sample's retention time was recorded.

Accuracy

This parameter of the developed method was established by the placebo spiking method, completed by spiking CFH and OLH separately at three significant levels 80, 100, and 120 %. Duplicate determination of these three levels has been calculated to achieve the mean of % recovery and % RSD.

Precision

Method precision

Five composite samples of CFH (30 µg mL^{-1}) and OLH (70 µg mL^{-1}) were analyzed for method precision on the same day. It was ascertained by calculating % RSD.

Intermediate precision

Intermediate precision was conducted on five composite samples CFH (30 µg mL^{-1}) and OLH (70 µg mL^{-1}) were analyzed on different days. Peak areas (PA) were calculated, and the repeatability was evaluated by % RSD.

Repeatability

The study was conducted on five working standard solution samples of CFH (30 µg mL^{-1}) and OLH (70 µg mL^{-1}) were analyzed on the same day. PA were recorded, and the repeatability was assessed by % RSD.

Linearity

Linearity was calculated by diluting the standard stock solution of CFH and OLH in the spans of (24-36 µg mL^{-1} and 56-80 µg mL^{-1}), respectively, in the volumetric flask. The calibration plots were generated by depicting the PA of both drugs against the concentrations with least-square linear regression analysis.

Limit of detection and limit of quantification

The LOD and LOQ for CFH and OLH were determined using a standard deviation (SD) of the intercept and the slope from the linear regression equation.

LOD = 3.3 x σ/ S and LOQ = 10 x σ/ S, where σ: the SD of the response, S: is the slope of the calibration curve.

Robustness

The robustness of the RP-HPLC technique was assessed by examining samples under experimentally changed chromatographic circumstances, like column temperature, flow rate, pH and mobile phase composition. The standard and test samples of CFH and OLH were assessed under various conditions. The requirements for robustness are mentioned in Table II.

Table II: Robustness conditions

Condition changes	Column temperature (°C)	Mobile phase composition (change in % M.P-A and M.P-B)
Decreased	28	58 % M.P-A: 42 % M.P-B
Normal	30	60 % M.P-A: 40 % M.P-B
Increased	32	62 % M.P-A: 38 % M.P-B

Forced degradation studies[18,19]

Force degradation is the deterioration of a drug or its product by exposing it to more severe conditions than accelerated degradation. These studies demonstrate the molecule's chemical behaviour, which leads to the development of the formulation and packaging of the product. Therefore, oxidative degradation (H_2O_2), acid degradation (0.2 N HCl), base degradation (0.1 N NaOH), dry heat degradation (70 °C for 5 h) and photolytic degradation (254 nm for 5 h) for CFH and OLH in the formulation have been investigated.

Oxidative degradation

To assess the oxidative degradation of the drugs in prepared ocular formulation, 2 mL of the formulation solution and placebo solution were combined with 1 mL of 30 % hydrogen peroxide (H_2O_2) in a flask. The volume was made up with the diluent mixture. Above 0.5 mL solution was mixed with the diluent mixture in a flask to attain a concentration of CFH 30 µg mL^{-1} and OLH 70 µg mL^{-1}, of which 10 µL was inoculated into the RP-HPLC system to examine the extent of degradation.

Acid degradation

Accurately measured 2 mL of formulation solution and placebo solution were combined with 1 mL of 0.2 N hydrochloric acids in a round bottom flask and refluxed for 30 minutes at 60˚C. Volume was later adjusted with the diluent mixture. Above 0.5 mL solution was mixed with the diluent mixture in a volumetric flask to acquire a concentration of CFH 30 µg mL^{-1} and OLH 70 µg mL^{-1}, of which 10 µL was inoculated into the RP-HPLC system to determine the extent of degradation.

Base degradation

To determine base degradation, 2 mL of formulation solution and placebo solution were combined with 1 mL of 0.1 N NaOH separately and refluxed at 60˚C for 30 minutes. The volume was made with the diluent mixture. Above 0.5 mL was mixed with the diluent mixture in a flask to acquire a concentration of CFH 30 µg mL^{-1} and OLH 70 µg mL^{-1}, of which 10 µL was injected into the RP-HPLC system to estimate the extent of degradation.

Dry heat degradation

To examine dry heat degradation, 2 mL of the formulation and placebo solutions were poured into a flask and left in an oven for 5 h at 80 °C, and the volume was made with the diluent mixture. Above 0.5 mL was mixed with the diluent mixture in a flask to acquire a concentration of CFH 30 µg mL^{-1} and OLH 70 µg mL^{-1}, of which 10 µL was infused into the RP-HPLC system to determine the amount of degradation.

Photolytic degradation

The photochemical stability of drugs was examined under UV light. 2 mL of formulation and placebo solutions were taken in separate beakers and kept under the UV light of 254 nm for 5 h in the photostability chamber. Volume was made with the diluent mixture up to 10 mL. From the above 0.5 mL solution was mixed with the diluent mixture in a flask attain an concentration of CFH 30 µg mL^{-1} and OLH 70 µg mL^{-1}, of which 10 µL was uploaded into the RP-HPLC system to determine the % of degradation.

RESULTS

Development of RP-HPLC method for CFH and OLH

For the RP-HPLC method development of the CFH and OLH, mobile phase was selected based on its polarity, solvents was run with different ratios of solvents at 1 mL min^{-1} of flow rate at 260 nm. The retention times of CFH and OLH were observed at 2.92 and 5.10 minutes, respectively, with a resolution of 12.36. The obtained peaks of CFH and OLH showed that the solvent utilized in method development depicts better separation and resolution. Hence ACN: 0.1 % TFA water (40:60, V/V) was used for further analysis. The optimized chromatographic conditions and chromatogram are illustrated in Table III and Fig. 2.

Table III: Result of optimization of chromatographic conditions

Sr. No.	MP & Ratio	Diluent	CFH	OLH	
			RT (minute)	RT (minute)	Resolution
1	40 ACN-60 0.1 % TFA	50 ACN:50 water	2.92	5.10	12.36

Assay of CFH & OLH

The % assay of CFH and OLH was determined as 99.03 and 100.12 %, respectively, within 98.0 to 102.0 % of drugs. Results and chromatogram of assay are given in the Table IV and Fig. 3.

RP-HPLC method validation

System suitability study

The system suitability study demonstrates the proficiency of the column. It was analyzed by estimating peak area, asymmetry factor, and % RSD, which must be under 2 %. The % RSD of CFH and OLH system

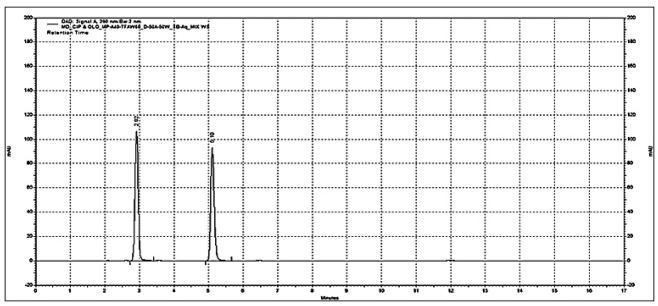


Fig. 2: RP-HPLC chromatogram of CFH and OLH

Table IV: Assay of CFH and OLH in the formulation

Drugs	Concentration of sample (µg mL^{-1})	PA of standard drug	PA of formulation	% Assay	Specified limit
CFH	30	1475888	1461572	99.03	98.0 to 102.0 %
OLH	70	1378434	1380088	100.12	98.0 to 102.0 %

Table V: Summary of system suitability study

Standard (100% Level)	CFH			OLH		
	PA	Asymmetry	T. Plates	PA	Asymmetry	T. Plates
Standard-1	1456662	0.94	3214	1361574	**0.88**	**11736**
Standard-2	1457718	0.97	3174	1365497	**0.88**	**11697**
Standard-3	1457913	0.96	3219	1363867	**0.86**	**11538**
Standard-4	1457745	0.9	3229	1364493	**0.87**	**11785**
Standard-5	1457214	0.98	3176	1363478	**0.86**	**11595**
Average	1457450.4			833293.3		
SD	512.31			421.5		
% RSD	0.03			0.05		

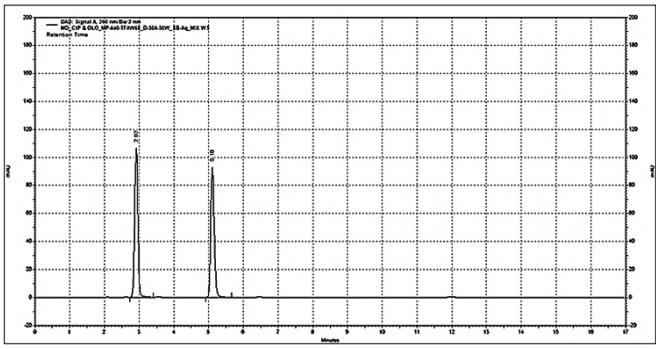


Fig. 3: RP-HPLC chromatogram of CFH and OLH in the formulation

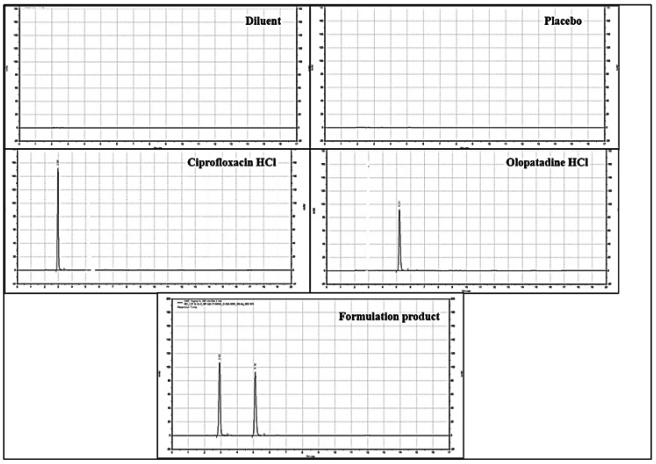


Fig. 4: RP-HPLC chromatogram of CFH and OLH samples for specificity

suitability studies were found to be 0.03 % for CFH and 0.05 % for OLH. The outcomes of the system suitability study are summarized in Table V.

Specificity

The specificity study of CFH, OLH, and their formulation show no significant interference in chromatograms due to diluent, mobile phase and excipients. The retention time for CFH, OLH and their formulation was 2.92 and 5.10 minutes. The representative chromatograms are given in Fig. 4, and the specificity results are given in the Table VI.

Table VI: Summary of specificity study

Samples	Retention time (minutes)	
	CFH	OLH
Diluent	-	-
Placebo	-	-
CFH	2.92	-
OLH	-	5.10
Formulation product	2.92	5.10

Accuracy

The proposed technique's accuracy established at 3 concentration levels (80, 100, and 120 %) is represented in Table VII. The % RSD data indicates that the % recovery for CFH was found to be 0.04, 0.05, 0.01 % and

0.07,0.20, 0.01 % for OLH, exhibiting that the method has acceptable accuracy within 2 %. High-accuracy results from the proposed method demonstrate that it can be used for analysis. The chromatograms of the accuracy result are shown in Fig. 5.

Precision

Method precision

Method precision for 5 sample preparations was found to be 0.08 % for CFH and 0.06 % for OLH, demonstrating the exceptional precision of the established method. The analysis and chromatogram of the precision study are given in the Table VIII and Fig. 6.

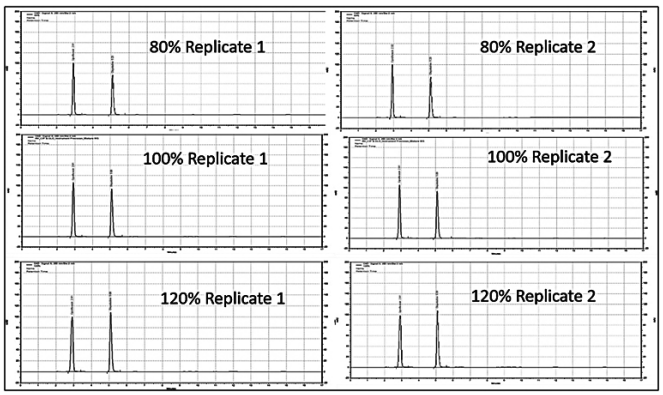


Fig. 5: RP-HPLC chromatogram of CFH and OLH samples for accuracy

Table VIII: Summary of precision study

Samples	CFH		OLH	
	PA	% Assay	PA	% Assay
Working standard	1453186	-	1367732	-
MP-1	1456834	99.25	1369507	99.13
MP-2	1457630	99.30	1369601	99.14
MP-3	1455448	99.15	1369626	99.14
MP-4	1455083	99.13	1367879	99.01
MP-5	1455234	99.14	1368287	99.04
Average		99.19		99.09
STDEV		0.076731		0.060268
% RSD		0.08		0.06

Intermediate precision

Intermediate precision for five sample preparation was found to be 0.02 % for CFH and 0.12 % for OLH,

Table VII: Summary of accuracy study

Accuracy of CFH								
% Level	**Replicate**	**Concentration spiked (µg mL^{-1})**	**PA**	**Amount recovered (µg mL^{-1})**	**% recovery**	**AVG**	**STDEV**	**% RSD**
80%	1	23.93	1164122	23.89	99.84	99.82	0.035417	0.04
	2	23.93	1163538	23.88	99.79			
100%	1	29.91	1456662	29.89	99.95	99.98	0.051234	0.05
	2	29.91	1457718	29.92	100.02			
120%	1	35.89	1741852	35.75	99.59	99.60	0.012736	0.01
	2	35.89	1742167	35.75	99.61			

Accuracy of OLH								
% Level	**Replicate**	**Concentration spiked (µg mL^{-1})**	**PA**	**Amount recovered (µg mL^{-1})**	**% recovery**	**AVG**	**STDEV**	**%RSD**
80%	1	56.00	1090475	55.97	99.95	99.90	0.06883	0.07
	2	56.00	1089413	55.92	99.85			
100%	1	70.00	1361574	69.89	99.84	99.98	0.203403	0.20
	2	70.00	1365497	70.09	100.13			
120%	1	84.00	1632517	83.79	99.75	99.75	0.013005	0.01
	2	84.00	1632216	83.78	99.74			

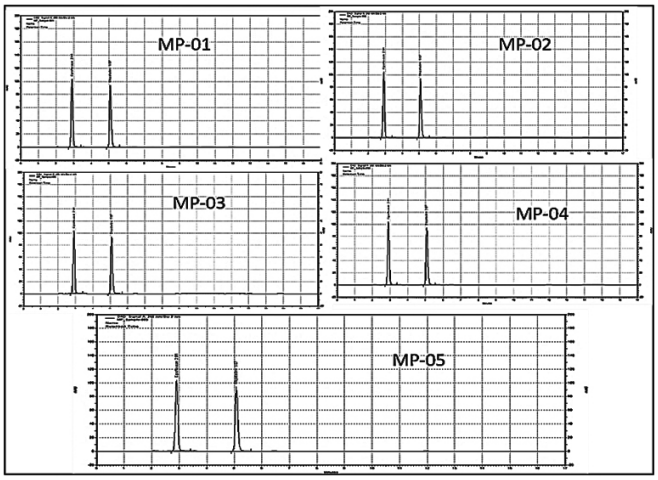


Fig. 6: RP-HPLC chromatogram for method precision study of CFH and OLH

respectively, confirming the better precision of the developed method. The results and chromatogram of the intermediate precision study are indicated in Table IX and Fig. 7.

Table IX: Summary of the intermediate precision study

Sample	CFH		OLH	
	PA	% Assay	PA	% Assay
Working standard	1453186	-	1367732	-
IP-1	1457016	99.26	1371556	99.28
IP-2	1457328	99.28	1373057	99.39
IP-3	1456766	99.24	1370525	99.20
IP-4	1456496	99.23	1371139	99.25
IP-5	1456781	99.24	1374615	99.50
Average		99.25		99.32
STDEV		0.021257		0.119543
% RSD		0.02		0.12

Repeatability

Repeatability for five sample preparation was found to be 0.04 % for CFH and 0.11 % for OLH, respectively, confirming the excellent precision of the developed method. The obtained outcomes and chromatogram of the repeatability study are mentioned in Table X and Fig. 8.

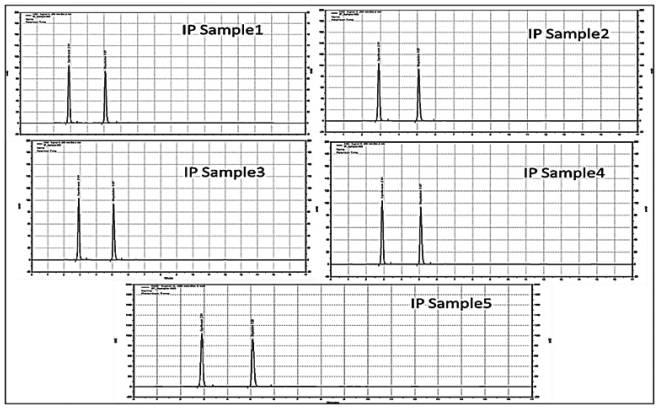


Fig. 7: RP-HPLC chromatogram of CFH and OLH samples for intermediate precision

Table X: Summary of repeatability study

CFH		OLH	
RP	**PA**	**RP**	**PA**
RP-1	1456662	RP-1	1361574
RP-2	1457718	RP-2	1365497
RP-3	1457913	RP-3	1363867
RP-4	1457745	RP-4	1364493
RP-5	1457214	RP-5	1363478
Average	1457450	**Average**	1363782
STDEV	512.3127	**STDEV**	1451.004
% RSD	0.04	**% RSD**	0.11

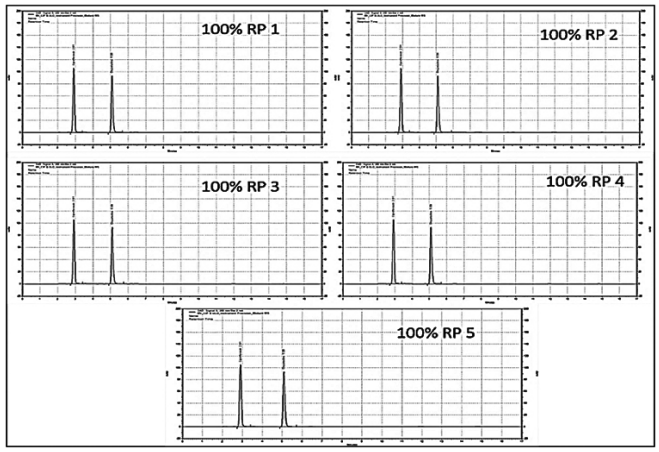


Fig. 8: RP-HPLC chromatogram of CFH and OLH samples for repeatability

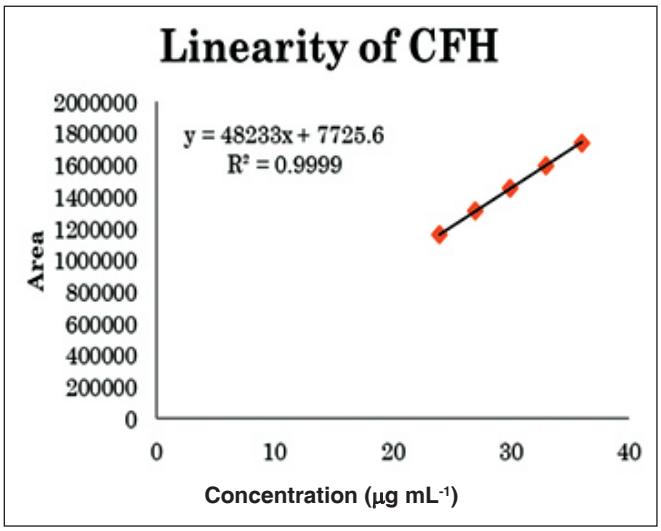
Linearity

The linearity was demonstrated within the ranges of 24-36 µg mL^{-1} and 56-84 µg mL^{-1} for CFH and OLH, calculated by linear regression analysis. The correlation coefficient for the calibration curve was

determined to be 0.9999 and 0.9996, respectively. The PA of each concentration is given in the Table XI and the chromatogram and overlay of linearity are shown in Fig. 9 and Fig. 10.

Table XI: Summary of linearity study

CFH			OLH	
% Level	Concentration (µg mL^{-1})	PA	Concentration (µg mL^{-1})	PA
80	24	1164122	56	1090475
90	27	1309718	63	1230883
100	30	1456662	70	1361574
110	33	1601254	77	1506502
120	36	1741852	84	1632517



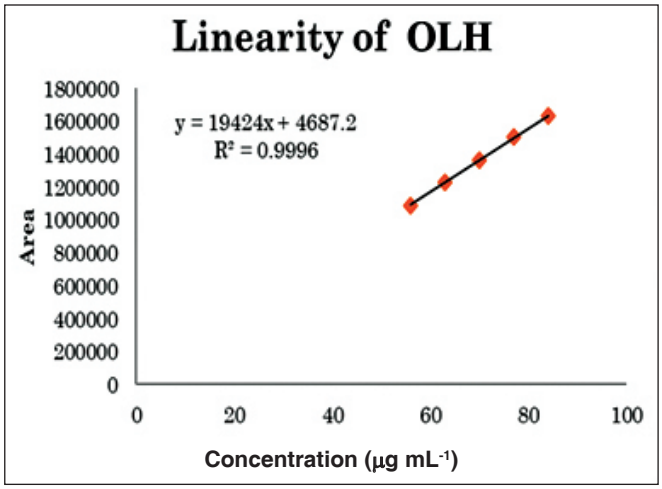


Fig. 9: Calibration graph of CFH and OLH for linearity

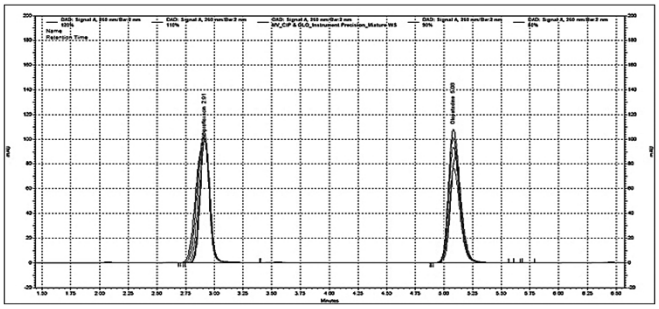


Fig. 10: The linearity overlay of CFH and OLH

Table XII: Summary of robustness study

Column temperature	Samples	CFH			
		RT (minutes)	Resolution	PA	% Assay
28 °C	Blank	-	-	-	-
	Working standard	2.91	0.00	1455439	-
	Formulation product	2.91	0.00	1439326	97.90
30 °C	Blank	-	-	-	-
	Working standard	2.91	0.00	1453186	-
	Formulation product	2.91	0.00	1454320	99.08
32 °C	Blank	-	-	-	-
	Working standard	2.91	0.00	1454187	-
	Formulation product	2.90	0.00	1453850	98.98
OLH					
28 °C	Blank	-	-	-	-
	Working standard	5.13	12.46	1367586	-
	Formulation product	5.14	12.41	1354479	99.04
30 °C	Blank	-	-	-	-
	Working standard	5.07	12.18	1367732	-
	Formulation product	5.07	12.18	1367815	100.01
32 °C	Blank	-	-	-	-
	Working standard	5.03	12.03	1369709	-
	Formulation product	5.03	12.07	1370275	100.04

Limit of detection and limit of quantification

The LOD and LOQ were found to be 0.46, 1.42 µg mL^{-1} for CFH and 4.15, 7.95 µg mL^{-1} for OLH, representing the method's sensitivity.

Robustness

All robustness conditions met the system suitability criteria. The % assay of CFH and OLH has met the CFH and OLH sample specification under all robustness conditions. The resolution of known components in all robustness conditions is more significant than 1.0 in the spiked samples. The data demonstrate the method's robustness shown in Table XII.

Forced degradation studies

These studies were accomplished on CFH and OLH prepared formulation solutions. The detailed results of the study are explored below section.

Oxidative degradation

The mixture of formulation solution and placebo with hydrogen peroxide was incorporated separately into the RP-HPLC system to evaluate the degradation. In oxidation degradation, the placebo and formulation solution show the initial peak of H_2O_2. The % degradation of OLH and CFH in the formulation was 6.94 % and 4.43 %, respectively. The chromatograms of oxidative degradation are given in Fig. 11.

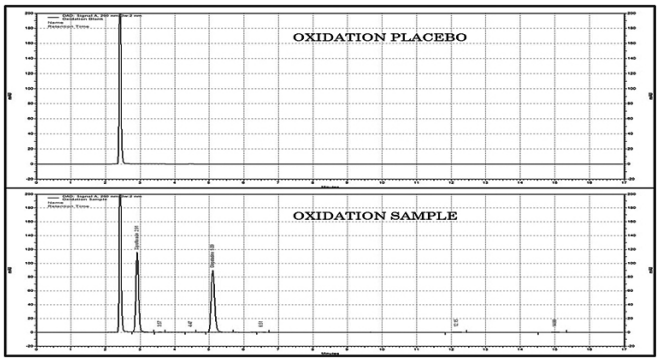


Fig. 11: RP-HPLC chromatogram representing oxidative degradation of CFH and OLH

Acid degradation

The formulation solution and placebo mixture with 0.1N HCl were infused into the RP-HPLC system separately to evaluate the degradation. The chromatogram shows 12.94 % degradation for the CFH and 2.75 % for OLH, and no peak was observed in the placebo solution. The chromatograms of acid degradation are represented in Fig. 12.

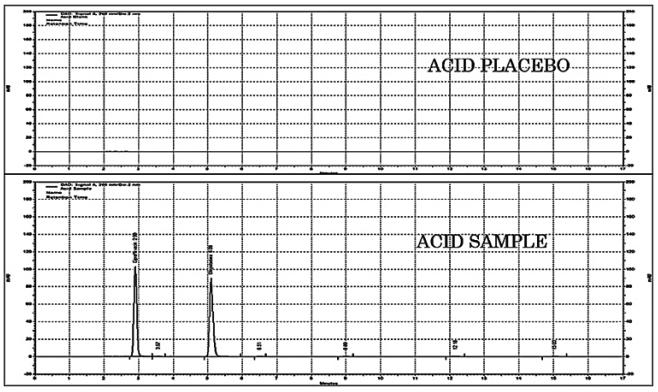


Fig. 12: RP-HPLC chromatogram representing acid degradation of CFH and OLH

Base degradation

The formulation solution and placebo mixture with 0.1N NaOH were infused into the RP-HPLC system separately to evaluate the degradation. The chromatograms indicated to that the 10.06 % CFH and 10.18 % OLH degraded in sodium hydroxide, whereas the placebo solution did not show any peak. Base degradation chromatograms are indicated in Fig. 13.

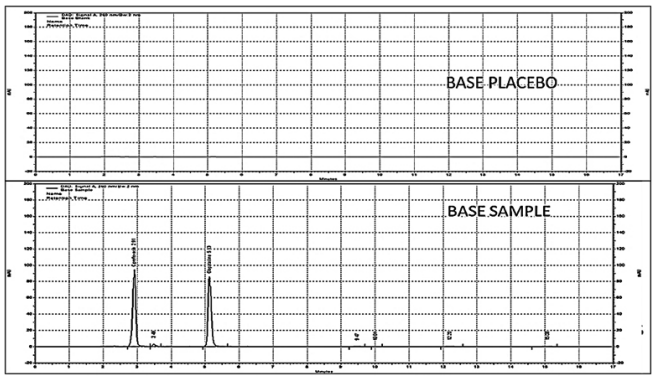


Fig. 13: RP-HPLC chromatogram representing base degradation of CFH and OLH

Dry heat degradation

To estimate dry heat degradation, formulation and placebo solutions were placed separately in the oven at 70 °C and injected into the RP-HPLC system. The chromatogram shows 2.57 % degradation for the CFH and 2.21 % for OLH, while no peak was observed in the placebo solution. A dry heat degradation chromatogram is shown in Fig.14.

Photolytic degradation

The mixture of formulation and placebo were exposed to UV light 254 nm in a UV chamber separately for 5 h and incorporated into the RP-HPLC system. It was found that 66.80 % CFH and 4.59 % OLH were degraded, and there

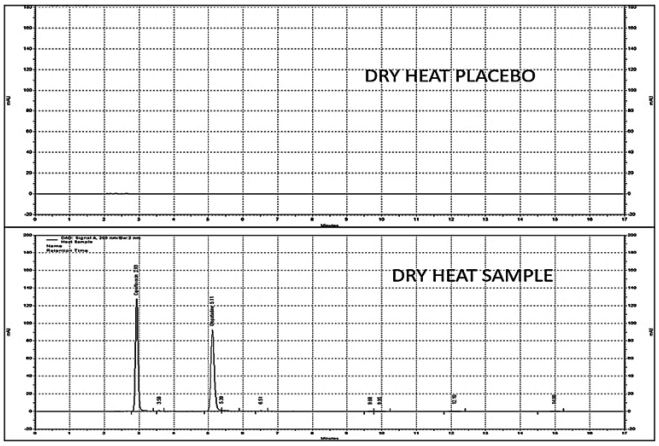


Fig. 14: RP-HPLC chromatogram representing dry heat degradation of CFH and OLH

was no peak in the placebo solution. The chromatograms of the photolytic degradation study are given in Fig. 15. The summary of the degradation study is illustrated in Table XIII.

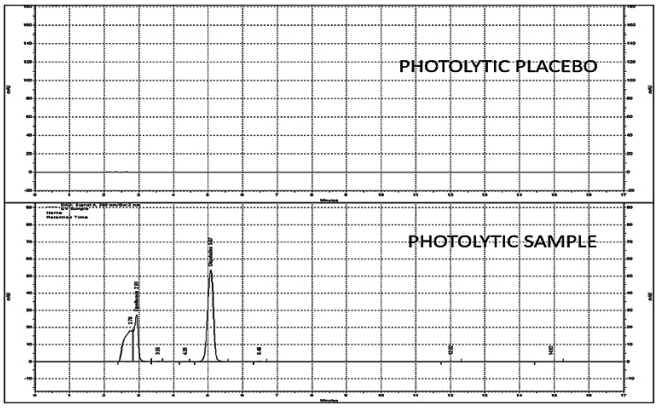


Fig. 15: RP-HPLC chromatogram representing photolytic degradation of CFH and OLH

Table XIII: Summary of forced degradation study

Conditions	CFH			OLH		
	PA	% Assay	% Degradation	PA	% Assay	% Degradation
Acid	1264379	86.14	12.94	1330195	97.26	2.75
Base	1306657	89.02	10.06	1228558	89.82	10.18
Peroxide	1352384	92.13	6.94	1307158	95.57	4.43
UV	473859	32.28	66.80	1304984	95.41	4.59
Heat	1416528	96.50	2.57	1337531	97.79	2.21

According to the general degradation study, CFH and OLH were more degraded in acid, base, and photolytic conditions. The pattern of degradation in all the conditions applied for OLH was within the 5-25 % specification. In the case of CFH acid, base, oxidative and heat degradation system was in the range of 2-25 %, but in the case of photolytic degradation, it was beyond the limit, i.e., 66.80 %; therefore, CFH was stored in an ambered colour container to avoid UV degradation.

DISCUSSION

The established method is simple, precise, quick, robust, accurate, and efficient. The RP-HPLC method separates CFH and OLH using mobile phase of acetonitrile: 0.1 % TFA water (40:60, V/V). The developed method was linear at 0.9999 and 0.996 for CFH and OLH. Accuracy study performed at 80,100, and 120 % for CFH and OLH concentration showed % RSD 0.04, 0.05, 0.01 % and 0.07,0.20, 0.01 %. It was found that all ascertained accuracy data are within the admissible range, affirming the method's accuracy. The robustness studies showed that the applied method was robust to deliberate alteration. The LOD and LOQ were found to be 0.46, 1.42 µg mL^{-1} for CFH and 4.15, 7.95 µg mL^{-1} for OLH, which was within the specified range.

Moreover, the system suitability, specificity, and precision study show that the method was precise and economical. The CFH and OLH peaks observed in the forced degradation study were well separated and found within the specification range of 5-25 % for OLH and 2-25 % for CFH. The forced degradation study performed by RP-HPLC illustrates that the formulation of CFH and OLH was susceptible to oxidative, photolytic, alkaline, acid, and thermal degradation conditions. The proposed technique may be employed in routine analysis for quantitative estimation and degradation evaluation of drugs and formulation by the RP-HPLC.

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