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Original article

Quantification of Clioquinol in Bulk and Pharmaceutical Dosage Forms by Stability Indicating LC Method

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A rapid, specific and sensitive stability indicating reverse phase high performance liquid chromatographic method has been developed and validated for analysis of clioquinol in both bulk and pharmaceutical dosage form. A Sunfire C_{18}, 4.5µm column with mobile phase containing acetonitrile-water pH 3 adjusted with 1% o- phosphoric acid (90:10, v/v) was used. The flow rate was 1.0 mL/min and effluents were monitored at 254nm. The retention time of clioquinol was 6.1 min. Clioquinol pure drug were subjected to acid and alkali hydrolysis, chemical oxidation, dry heat degradation, and sun light degradation. The degraded product peaks were well resolved from the pure drug peak with significant difference in their retention time values. Stressed samples were assayed using developed LC method. The proposed method was validated with respect to linearity, accuracy, precision and robustness. The method was successfully applied to the estimation of clioquinol in pharmaceutical dosage forms. The method is suitable for the routine analysis of clioquinol in tablets and ointment.

Key words: Clioquinol, Forced degradation, Reversed phase liquid chromatography, Validation

Kliokinol'ün Ham Halde ve Farmasötik Dozaj Formlarında Analizi İçin Stabilite Belirtmeli LC Yöntemi

Kliokinol'ün ham halde ve farmasötik dozaj formlarında analizi için hızlı, spesifik ve duyarlı bir stabilite belirtmeli ters faz yüksek performanslı sıvı kromatografi yöntemi geliştirilmiş ve valide edilmiştir. Bir Sunfire C_{18} kolonu (4.5 µM), asetonitril – su (pH'ı % 1'lik fosforik asit ile 3'e ayarlanmış) (90:10, h/h) içeren hareketli faz ile birlikte kullanılmıştır. Akış hızı 1 mL/dak'dır ve elüatlar 254 nm 'de gözlenmiştir. Kliokinol'ün alıkonma zamanı 6.1 dakikadır. Kliokinol'ün saf hali, asit ve alkali hidrolizine, kimyasal oksidasyona, kuru ısı parçalanmasına ve gün ışığı parçalanmasına maruz bırakılmıştır. Parçalanan ürünlerin pikleri, alıkonma zamanı değerlerindeki belirgin farklılık ile saf madde pikinden iyi bir biçimde ayrılmıştır. Strese maruz bırakılmış örnekler, geliştirilen sıvı kromatografi yöntemi kullanılarak analiz edilmiştir. Geliştirilen yöntem doğrusallık, doğruluk, kesinlik ve sağlamlılık açısından valide edilmiştir. Yöntem, kliokinol'ün farmasötik dozaj formlarından tayini için başarıyla uygulanmıştır. Yöntem, tablet ve merhem içindeki kliokinol'ün tabletlerden ve merhemlerden rutin analizi için uygundur.

Key words: Kliokinol, Zorlamayla parçalanma, Ters faz sıvı kromatografi, Validasyon

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INTRODUCTION

Chemically Clioquinol (CLQ) is 5-chloro-7-iodo-8-quinolinol shown in Figure 1 and acts as a zinc and copper chelator. Metal chelation is a potential therapeutic strategy for Alzheimer's disease because the interaction of zinc and copper is involved in the deposition and stabilization of amyloid plaques, and chelating agents can dissolve the amyloid deposits by preventing metal-A-beta interactions (1-3). As Alzheimer's disease and prion disease are CNS degenerative disorders characterized by amyloid deposits, it is conceivable that some drugs may be active in preventing both. Transmissible spongiform encephalopathies (TSE) form a group of progressive, fatal neurodegenerative diseases affecting the central nervous system of humans (kuru, Creutzfeldt-Jacob disease) and animals (scrapie, bovine spongiform encephalopathy) (4-6). It is believed (7) that the causative agents are proteinaceous infectious particles ("prions") completely devoid of any nucleic acids that represent the altered counterpart of a cell protein, and are resistant to proteolytic digestion, high temperatures, denaturating agents and the disinfectants usually used for sterilisation. The pathological protein (PrP^{sc}) is the protease-resistant isoform of a GPI-anchored cell transmembrane molecule (PrP^{c}) that is mainly expressed in CNS neurons, but also in many other cell types. As it is the main component of amyloid deposits, and the cause of neurodegenerative CNS lesions, PrP^{sc} is the primary target for therapeutic strategies (8, 9). The hamster model is particularly suitable for TSE studies because the period required for the development of experimental scrapie is shorter than in mouse; when hamsters are intracerebrally infected by the 263K prion strain, the incubation period lasts 2 months and death occurs after about 1 month (10, 11). Preliminary results indicate that clioquinol may improve cognitive symptoms and prolong the survival of infected animals (12).

After oral administration in rodents (mice and rats, but not hamsters), clioquinol is extensively metabolised to glucuronate and sulfate metabolites (13-18), but these animal and human studies made use of relatively insensitive and nonspecific HPLC methods with UV detection, and thus required complex extraction procedures in order to determine tissue clioquinol levels. An even more complex GC method with electron-capture detection after acetylation has been developed by Jack and Riess (19), which also used solvent extraction with a sensitivity of 50 ng/ml. Finally, a highly sensitive GC–MS method has been developed that uses benzene extraction and the conversion of clioquinol nto pentafluorobenzyl ether (20). As studying the pharmacokinetics of clioquinol and its tissue distribution may be relevant to understanding its targets and its mechanism of inhibiting prion infection, we have developed a simple, sensitive and specific method of determining clioquinol in pharmaceutical dosage forms by means of HPLC. Clioquinol is official in Indian Pharmacopoeia and European Pharmacopoeia. A literature survey regarding quantitative analysis of these drugs revealed that attempts have been made to develop analytical method for the estimation of clioquinol by liquid chromatographic method (LC) (21-29). Specially, stability indicating RP-HPLC method is routinely used for analysis of clioquinol in pharmaceutical dosage form as per ICH guidelines (30).

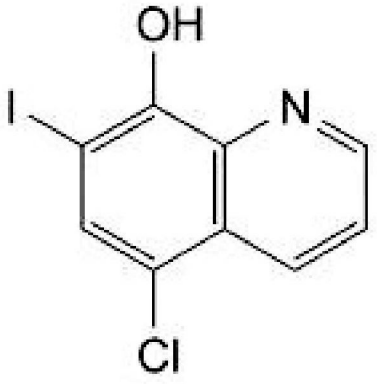

Figure 1. Structure of Clioquinol

MATERIALS AND METHODS

Apparatus

HPLC

The liquid chromatographic system of waters (Calcutta, India) containing 515 HPLC isocratic pump, variable wavelength programmable 2998 photodiode array detector and rheodyne

injector with 20 µL fixed loop was used. A Sunfire C_{18} column (waters, Ireland) with 250×4.6 mm i.d. and 5 µm particle size was used as stationary phase.

Electronic balance.

All the drugs and chemicals were weighed on Shimadzu electronic balance (AX 200, Shimadzu Corp., Japan).

Reagents and Materials

Pure samples

Analytically pure CLQ was obtained as gift sample from Vishal Laboratories, Rajkot, India. The purity of CLQ was declared to be 98.72% according to the manufacturer's analysis certificates.

Market samples

Tablet formulation (ENTEROQUINOL, East India Pharmaceutical works Ltd., Hyderabad, India) (Formulation 'A') containing labeled amount of 320 mg of clioquionol and Ointment formulation (DERMOQUINOL 8%, East India Pharmaceutical works Ltd., Hyderabad, India) (Formulation 'B') was used for the study.

Chemicals and Reagents

Acetonitrile, water (E. Merck, Mumbai, India) used as a solvent was of HPLC grade, while *o*- phosphoric acid (S.D. fine chemicals, Mumbai, India) were of analytical grade and used for the preparation of mobile phase.

Preparation of mobile phase and stock solution

Mobile phase was prepared by mixing 900 mL of acetonitrile with 100 mL of deionised water. The pH of mobile phase was adjusted to 3 with 1% solution of *o*-phosphoric acid. The mobile phase was filtered through Whatman filter paper No. 42 (0.45 µm). The mobile phase was sonicated for 10 min prior to use for degassing.

CLQ (25.0 mg) was accurately weighed and transferred to 25 mL volumetric flask containing a few mL of methanol. The solid was dissolved by swirling and volume was adjusted to the mark with the same solvent which gave 1000 µg/mL of the drug. Aliquot from the above solution was appropriately diluted with methanol to obtain standard stock solution of 100 µg/mL of drug.

Chromatographic conditions

A reversed phase C_{18} column (Sunfire) equilibrated with mobile phase comprising of acetonitrile:deionized water (90:10, v/v) and pH of mobile phase was adjusted with *o* – phosphoric acid. Mobile phase flow rate was maintained at 1 mL/min and eluents were monitored at 254 nm. A 20 µL of sample was injected using a fixed loop, and the total run time was 10 min. All the chromatographic separations were carried out at controlled room temperature (25 ± 2 °C).

Analysis of Marketed Formulations

Twenty tablets were weighed accurately and finely powdered. Tablet powder equivalent to 25 mg CLQ was taken in 25 mL volumetric flask containing few mL of methanol and the flask was sonicated for 5 minutes. The solution was filtered in another 25 mL volumetric flask using Whatman filter paper (No. 42) and volume was adjusted to the mark with the same solvent. Appropriate aliquot was transferred to a 10 mL volumetric flask and the volume was adjusted to the mark with the mobile phase to obtain a solution containing 10 µg/mL of CLQ. The solution was sonicated for 10 min. It was analysed under proposed chromatographic conditions and chromatogram recorded. The amount of CLQ was computed using regression equation.

Extraction and analysis of CLQ from ointment

Take 1 gm of ointment containing 80 mg of CLQ in 100mL of beaker was warmed on water bath until the ointment had melted. 25 mL methanol was added, heated on water bath for 5 min. The sample was extracted with sonication, the solution cooled and filtered Whatman filter paper (No.42) into 100 mL volumetric flask, washed the residue retained on filter paper with 20 mL of methanol twice. The extracts were combined, cool, and volume was adjusted to the mark with methanol.

Appropriate volume of the aliquot was transferred to a 10 mL volumetric flask and the volume was adjusted to the mark with the mobile phase to obtain a solution containing 12 µg/mL of CLQ. The solution was sonicated for 10 min. It was analysed under proposed chromatographic conditions and chromatogram

was recorded. The amount of CLQ was computed using regression equation.

Validation

The method was validated for accuracy, precision, specificity, detection limit, quantitation limit and robustness.

Linearity of calibration curve

Appropriate aliquots of CLQ standard stock solution were taken in a series of 10 mL volumetric flasks. The volumes were made up to the mark with mobile phase to obtain final concentrations of 0.1, 1, 5, 10, 20, and 30 µg/ mL of CLQ. Linearity of the method was evaluated by constructing calibration curves at six concentration levels over a range of 0.1- 30 µg/mL of CLQ. The solutions were injected using a 20 µL fixed loop system and chromatograms were recorded. Calibration curves were constructed by plotting average peak area versus concentrations (n = 5) and regression equations were computed for CLQ.

Precision

The instrumental precision was evaluated by injecting the solution containing three different concentrations of CLQ (0.5, 5, 30 µg/ mL) six times repeatedly and peak areas were measured. The results are reported in terms of percentage relative standard deviation (% RSD).

The intra-day and inter-day precision study of CLQ was carried out by estimating the corresponding responses three times on the same day and on three different days for three different solutions containing CLQ (0.5, 5, 30 µg/ mL) and the results are reported in terms of percentage relative standard deviation (% RSD).

Accuracy

The accuracy of the method was determined by calculating recoveries of CLQ in tablet dosage form and in ointment dosage form by method of standard additions. In tablet dosage form known amount of CLQ (0, 5, 10, 15 µg/ mL) and in ointment dosage form known amount of CLQ (0, 6, 12, 18 µg/ mL) was added to a pre quantified sample solutions and the amount of CLQ was estimated by proposed method, measuring the peak area and by fitting these values to the straight-line equation of calibration curve.

Specificity

The specificity study has been carried out by commonly used excipients present in selected tablet formulation. They were mixed with a pre weighed quantity of drug. A solution of the mixture was prepared and appropriately diluted to obtain a solution of 10 µg/mL CLQ. The solution was analysed by proposed method and chromatogram recorded. The amount of CLQ was computed using regression equation. The excipients used were talc, micro crystalline cellulose, starch, and carboxy methyl cellulose.

Detection limit and Quantification limit

The detection limit is defined as the lowest concentration of an analyte that can reliably be differentiated from background levels. Limit of quantification is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated using following equation as per ICH guidelines.

$$LOD = \frac{3.3 \times \sigma}{S} \qquad LOQ = \frac{10 \times \sigma}{S}$$

Where σ is the standard deviation of y-intercepts of regression lines and S is the average slope of the calibration curves.

Robustness

Robustness of the method was studied by observing the stability of the sample solution at 25 ± 2°C for 24 h, change in flow rate at 1±0.1 mL, change in pH of mobile phase, temperature of working area ± 5 °C, and change in mobile phase ratio.

Forced degradation study

Forced degradation study using acid and alkali hydrolysis, chemical oxidation, dry heat degradation and photo degradation studies were carried out and interference of the degradation products were investigated.

Alkali hydrolysis

To study forced degradation in basic medium 10 mg of CLQ was transferred to 25 mL volumetric flask and 3 mL of 1 N Sodium hydroxide (NaOH) was added to flask. The content of the flask was heated in a water bath at 80 °C for 72 h and allowed to cool to room temperature. Solution was neutralized with 1 N HCl us-

ing pH meter and volume was adjusted to the mark with methanol. Appropriate aliquot was taken from the above solution into another 10 mL volumetric flask and diluted with mobile phase to obtain final concentration of 10 µg/ mL of CLQ. The solution was analysed under proposed chromatographic condition and chromatogram recorded. The amount of CLQ was computed using regression equation.

Acid hydrolysis

To study forced degradation in acidic medium 10 mg of CLQ was transferred to 25 mL volumetric flask and 3 mL of 1 N Hydrochloric acid (HCl) was added to flask. The content of the flask was heated in a water bath at 80 °C for 72 h and allowed to cool to room temperature. Solution was neutralized with 1 N NaOH using pH meter and volume was adjusted to the mark with methanol. Appropriate aliquot was taken from the above solution into another 10 mL volumetric flask and diluted with mobile phase to obtain final concentration of 10 µg/ mL of CLQ. The solution was analysed under proposed chromatographic condition and chromatogram recorded. The amount of CLQ was computed using regression equation.

Oxidative stress degradation

To perform oxidative stress degradation study, 10 mg of CLQ was transferred to 25 mL volumetric flask and 3 mL of 6 % hydrogen peroxide was added. The content of the flask was heated in a water bath at 80 °C for 72 h. Solution was allowed to cool to room temperature and volume was adjusted to the mark with methanol. Appropriate aliquot was taken from the above solution into another 10 mL volumetric flask and diluted with mobile phase to obtain final concentration of 10 µg/mL of CLQ. The solution was analysed under proposed chromatographic condition and chromatogram recorded. The amount of CLQ was computed using regression equation.

Dry heat degradation

To study dry heat degradation, 10 mg of CLQ was transferred to 25 mL volumetric flask and was exposed in oven at 80 °C for 72 h. The solid was allowed to cool and dissolved in few mL of methanol by swirling and volume was adjusted to the mark with the methanol. Appropriate aliquot of the solution was transferred to 10 mL volumetric flask and diluted with mobile phase to obtain final concentration of 10 µg/ mL of CLQ. The solution was analysed under proposed chromatographic condition and chromatogram recorded. The amount of CLQ was computed using regression equation.

Photolytic degradation

To study photostability of the CLQ, the solid drug was exposed to sunlight for 24 h. 10 mg of this drug was transferred to 10 mL volumetric flask containing few mL of methanol. The solid was dissolved by swirling and volume was adjusted to the mark with the same solvent. Appropriate aliquot of the solution was transferred to 10 mL volumetric flask and diluted to the mark with mobile phase to obtain the final concentration of 10 µg/ mL of CLQ. The solution was analysed under proposed chromatographic condition and chromatogram recorded. The amount of CLQ was computed using regression equation.

RESULTS AND DISCUSSION

Optimization of mobile phase

The objective of the method development was to resolve chromatographic peaks for active drug ingredients and degradation products produced under stressed conditions with less asymmetry factor.

Various mixtures containing water, methanol, and acetonitrile were tried as mobile phases in the initial stage of method development. Mixture of methanol: water (90:10, v/v), methanol-water (60:40, v/v), acetonitrile-water (50:50, v/v), were tried as mobile phase but satisfactory resolution of drug and degradation peaks were not achieved.

The mobile phase acetonitrile: water (90:10) was found to be satisfactory and gave symmetric peak for CLQ. The retention time for proposed method was found to be 6.1 min as shown in "Figure 2 (A)" and chromatogram of placebo was shown in "Figure 2 (B)". The system suitability parameters like theoretical plates per meter and asymmetry factor for CLQ were found to be 5805 and 0.86, respectively. The mobile phase flow rate was maintained at

1 mL/min. The UV spectra of the drug showed that CLQ absorbed appreciably at 254 nm, so detection was carried out at 254 nm.

Validation of the Proposed Metods

Linearity: The calibration curve for CLQ was found to be linear in the range of 0.5 - 30 µg/mL with a correlation coefficient of 0.9986. The standard deviation value of slope and intercept of CLQ was found to be 1875.86 and 10311.47, respectively which indicated strong correlation between peak area and concentration. The regression equation of calibration curves was obtained as y=81037x-68225 as shown in "Figure 3".

Precision: Instrument precision was determined by performing injection repeatability test and the % RSD value for CLQ was found to be 0.64 as shown in Table 1. The intra-day and inter-day precision studies were carried out and the % RSD value was found to be 0.76-1.06 and 1.12-1.36, respectively. The low RSD values indicate that the method is precise as shown in Table 2.

Accuracy: The accuracy of the method was de-

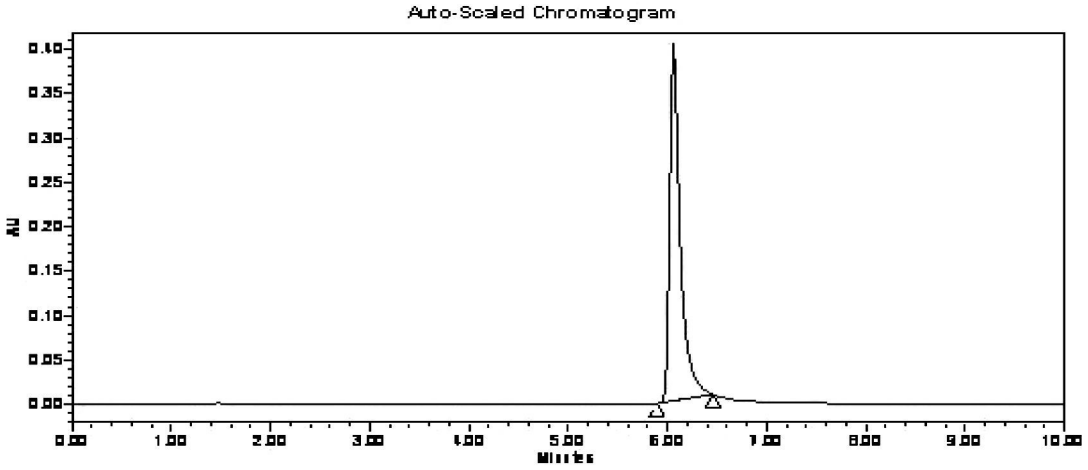

Figure 2 (A). Liquid chromatogram of CLQ (30µg/mL; 6.19 min).

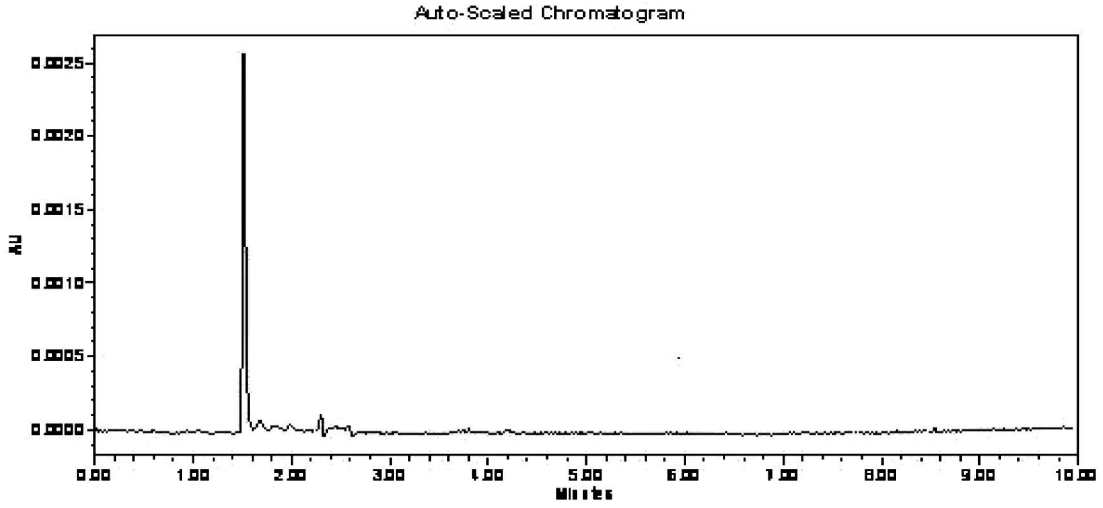

Figure 2 (B). Liquid chromatogram of placebo.

termined by calculating recoveries of CLQ by method of standard addition. For formulation 'A' recoveries was found to be 97.70 – 99.13 % for CLQ as shown in Table 3. For formulation 'B' recoveries was found to be 95.31 – 98.32 % for CLQ as shown in Table 4. The high values indicate that the method is accurate.

Limit of detection and limit of quantification: By calculation method, the detection limit and quantitation limit for CLQ was found to be 0.42 µg/mL and 1.27 µg/mL, respectively. The above data shows that a microgram quantity of the drug can be accurately and precisely determined.

Specificity: The specificity study was carried out to check the interference from the excipients used in the formulation by preparing synthetic mixture containing the drug and excipients. The chromatogram showed peaks for the drug without any interfering peak.

Robustness: The method was found to be robust, as small but deliberate changes in the

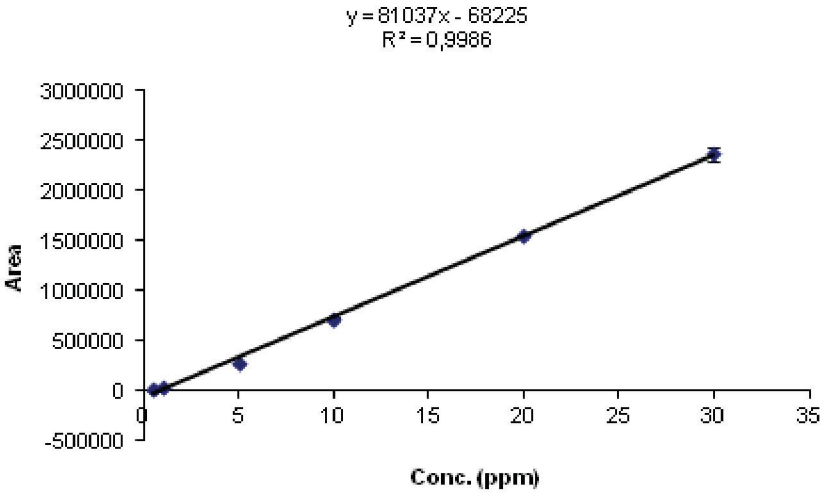

Figure 3. Calibration curve of CLQ (0.5-30 µg/mL).

Table 1. Instrumental precision data of proposed method.

Concentration (µg/mL)	Clioquinol (CLQ)		
	0.5 (µg/mL)	10 (µg/mL)	30 (µg/mL)
Peak Area	10586.3	730622	2440742
	10411.2	726370	2417900
	10648.6	735732	2374716
	10528.1	718786.2	2408969
	10592.3	724216.7	2414555
	10371.2	727377.2	2425241
Mean	10522.95	727184	2413687
Std. Dev.	109.6843	5743.1	22022.76
% RSD	1.04	0.79	0.91

Table 2. Intra-day and inter-day precision data for CLQ.

Conc. (µg/mL)	Intra-day		Inter-day	
	Mean (Peak area) ± SD (n=3)	% RSD	Mean (Peak area) ± SD (n=3)	% RSD
0.5	10907 ± 115.10	1.06	11125.67 ± 147.19	1.32
5	274995.3 ± 2079.29	0.76	276626 ± 3091.33	1.12
30	2464932 ± 23220.44	0.94	2400736 ± 32632.23	1.36

method parameters have no detrimental effect on the method performance as shown in Table 5. The low value of relative standard deviation was indicating that the method was robust.

The chromatogram of acid hydrolysis performed at 80°C for 72 h reflux showed degradation of CLQ with degradation product peak at retention time (RT) 4.47, 5.306, 7.193 min and 8.256 min Figure 5. The chromatogram of oxidized CLQ with 6% hydrogen peroxide at 80°C for 72 h reflux showed degradation of CLQ with degradation product peak at retention time (RT) 2.677, 5.328 min and 7.183 min Figure 6. The chromatogram of photo-stability of CLQ with exposure to sun light for 24 h showed degradation of CLQ with degradation product

Table 3. Accuracy study of the proposed method for tablet formulation.

Amount of Sample (µg/mL)	Sets	Amount drug of spiked (µg/mL)	Area	Amount recovered (µg/mL)	Average amount recovered (µg/mL)	% Recovery	Average % recovery
10	1	0	715736	9.67	9.77	96.74	97.70
	2	0	720424	9.73		97.32	
	3	0	734361	9.90		99.04	
10	1	5	1131597	14.81	14.91	98.06	99.13
	2	5	1138695	14.89		98.93	
	3	5	1150516	15.04		100.39	
10	1	10	1535147	19.79	19.82	97.86	98.23
	2	10	1545695	19.92		99.16	
	3	10	1533714	19.77		97.67	
10	1	15	1938356	24.76	24.83	97.61	98.31
	2	15	1950556	24.91		99.12	
	3	15	1942970	24.82		98.18	

Table 4. Accuracy study of the proposed method for ointment formulation.

Amount of Sample (µg/mL)	Sets	Amount drug of spiked (µg/mL)	Area	Amount recovered (µg/mL)	Average amount recovered (µg/mL)	% Recovery	Average % recovery
12	1	0	850685	11.34	11.44	94.50	95.31
	2	0	870854	11.59		96.57	
	3	0	854308	11.38		94.87	
12	1	5	1382771	17.91	17.80	99.21	98.32
	2	5	1374547	17.80		98.37	
	3	5	1365029	17.69		97.39	
12	1	10	1826291	23.38	23.65	94.82	97.08
	2	10	1867760	23.89		99.08	
	3	10	1850780	23.68		97.33	
12	1	15	1938356	29.52	29.78	96.04	98.16
	2	15	1950556	29.85		98.73	
	3	15	1942970	29.97		99.73	

peak at retention time (RT) 3.214, 3.642, 4.224, 5.345 min and 7.111 min Figure 7. The drug was found to be stable and the chromatogram of CLQ with dry heat at 80^{0}C for 1 week.

Forced degradation study

Chromatogram of base hydrolysis performed at 80^{0}C for 72 h reflux showed degradation of CLQ with degradation product peak at retention time (RT) 2.445, 2.813, 4.495, 5.296 min and 7.192 min Figure 4.

The degradation study thereby indicated that CLQ was found to be stable to dry heat degradation study while it was susceptible to base hydrolysis, acid hydrolysis, oxidation (6 % hydrogen peroxide), and photo degradation as shown in Table 6. No degradation products from different stress conditions affected determination of CLQ.

The degradation study thereby indicated that CLQ was found to be stable to dry heat degradation study while it was susceptible to base hydrolysis, acid hydrolysis, oxidation (6% hydrogen peroxide), and photo degradation as shown in Table 6. No degradation products from different stress conditions affected determination of CLQ.

Solution stability: The solution stability study showed that CLQ was evaluated at room temperature for 24 hr. The relative standard deviation was found below 2.0%. It showed that solution were stable up to 24 hrs at room temperature.

Table 5. Data from robustness for proposed method.

Parameters	Conc. (µg/mL)	Normal Condition	Change in condition	Area ± SD (n =3)	Amount recovered (µg/mL)	% Recovery	% RSD
Flow rate	10	1.0 mL/min	0.9 mL/min	725289 ± 5290.30	9.73	97.25	0.73
			1.1 mL/min	716760.7 ± 5968.42	9.68	96.83	0.83
Mobile phase ratio	10	Acetonitrile: Water (90:10)	(87:13)	725631± 8965.62	9.89	98.94	1.24
			(93:07)	729122.3 ± 8080.61	9.74	97.35	1.11
pH of mobile Phase	10	pH 3.0	2.5	732169 ± 8152.67	9.88	98.80	1.11
			3.5	728948 ± 9476.83	9.86	98.57	1.30
Temperature of working area	10	25 °C	20 °C	723593.5 ± 8392.27	9.76	97.58	1.16
			30 °C	729893.8 ± 10132.98	9.98	99.76	1.39

Analysis of marketed formulations: The proposed method was successfully applied to the determination of CLQ in their tablet and ointment dosage form (Formulation 'A' and Formulation 'B'). The % recovery for CLQ for formulation 'A' and formulation 'B' was found to be 98.61 ± 0.69 and 97.81 ± 0.92 % mean value ± standard deviation of six determinations which was comparable with the corresponding labeled amounts.

CONCLUSION

Proposed study describes stability indicating LC method for the estimation of CLQ in bulk and their pharmaceutical dosage forms. The method was validated and found to be selective, sensitive, accurate and precise. Statistical analysis proved that method was repeatable and selective for the analysis of CLQ without any

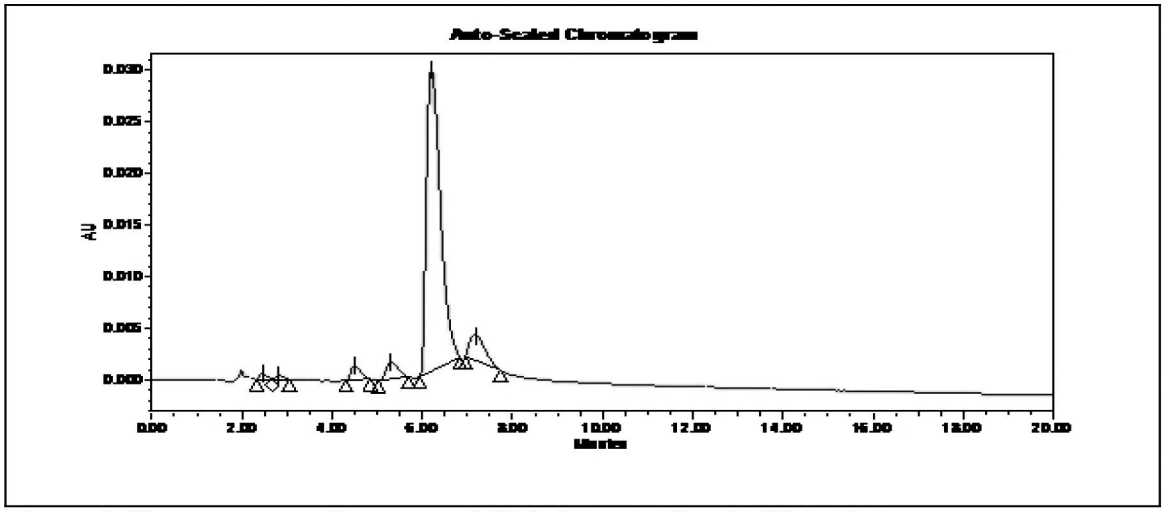

Figure 4. Chromatogram of base treated CLQ (10 µg/mL) at 80 °C for 72 h.

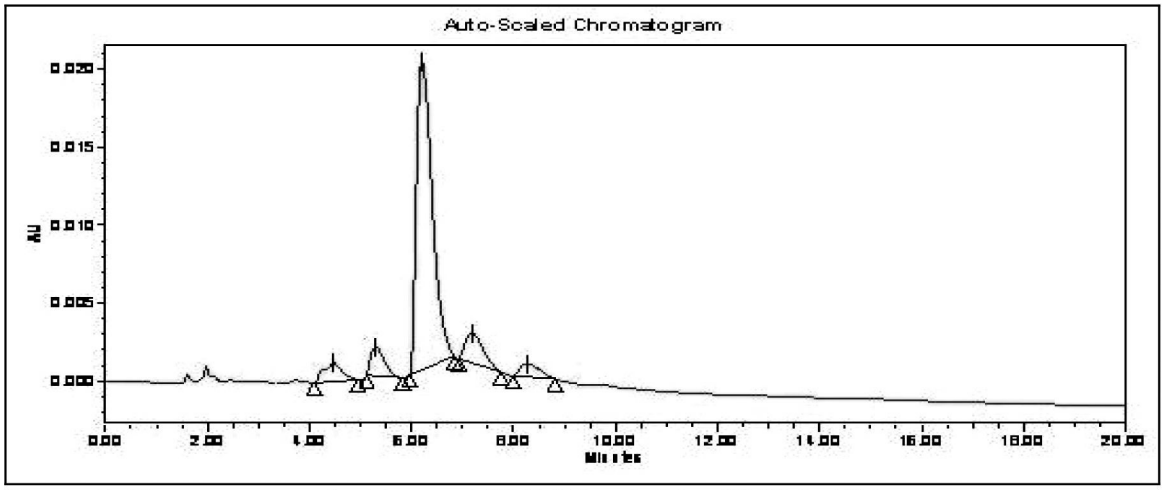

Figure 5. Chromatogram of acid treated CLQ (10 µg/mL) at 80 °C for 72 h.

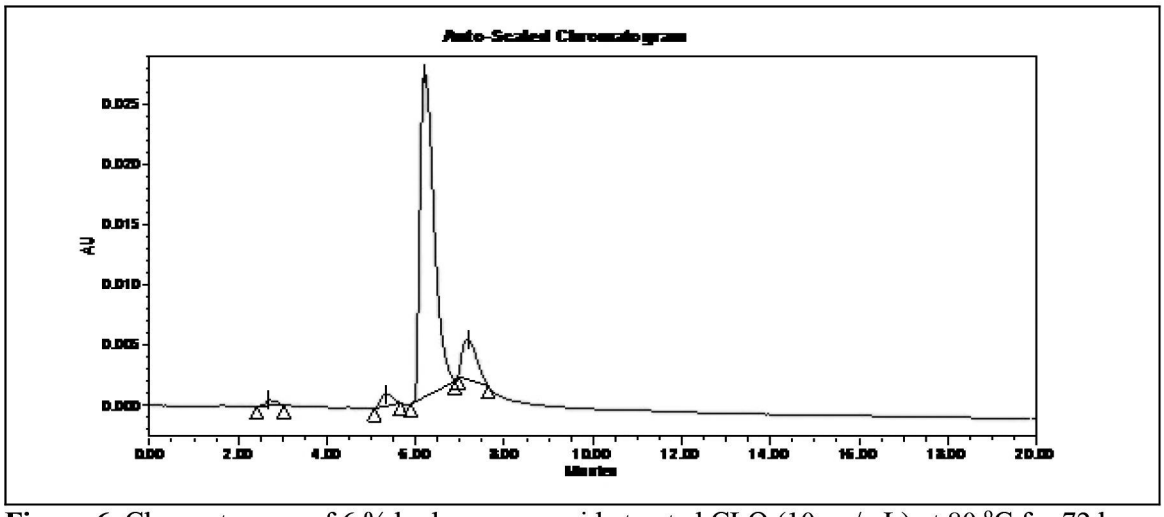

Figure 6. Chromatogram of 6 % hydrogen peroxide treated CLQ (10 µg/mL) at 80 °C for 72 h.

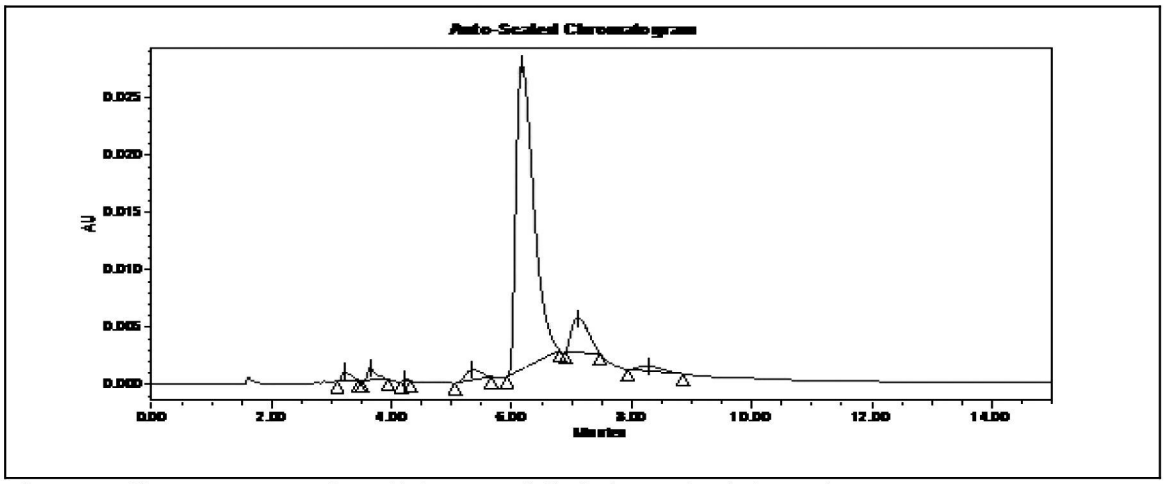

Figure 7. Chromatogram of sun light treated CLQ (10 µg/mL) for 24 h.

Table 6. Forced degradation study of CLQ.

Condition	Time (h)	% Recovery	Retention time of degradation products (min)
Base1 N NaOH[a]	72 h	49.61	2.445, 2.813, 4.495, 5.296, 7.192
Acid 1 N HCl[a]	72 h	47.97	4.47, 5.306, 7.193, 8.256
6% Hydrogen peroxide[a]	72 h	63.15	2.677, 5.328 ,7.183
Dry heat[a]	72 h	97.18	--
Light degradation	24 h	45.35	3.214, 3.642, 4.224, 5.345, 7.111

[a]Samples were heated at 80°C for specified period of time.

interference from the excipients. The method was successfully used for determination of drug in their tablets as well as ointment formulation for the routine analysis. Also the above results indicate the suitability of the method for acid, base, oxidation, wet, dry heat and photolytic degradation study. As the method separates the drugs from its degradation products, it can be used for analysis of stability samples. In addition, the HPLC procedure can be applied to the analysis of samples obtained during accelerated stability experiments to predict expiration dates of pharmaceuticals.

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