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STUDYING THE ACCELERATED PHOTOSTABILITY OF CIPROFLOXACIN AND LOMEFLOXACIN IN TABLETS AND EYE DROPS

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Keywords:

Fluoroquinolones, Ciprofloxacin, Lomefloxacin, Photostability, Stability-indicating methods

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ABSTRACT: The photostability of two fluoroquinolones: ciprofloxacin and lomefloxacin have been detected in tablets, and eye drops formulations using light-stability cabinet. Ciprofloxacin and lomefloxacin were subjected to stress conditions. The degradation products were well separated from the peak of the active substance. The stability of these compounds has been studied both in containers and under direct light in the light-stability cabinet. Samples were assayed immediately and at 1, 3, 6 months by stability-indicating high-performance liquid chromatography methods with a photodiode array detector. The determination was performed on C18 (250 × 4.6 mm, 5 µm). The first mobile phase consisted of 0.025M phosphoric acid, and acetonitrile (87:13) pumped at a flow rate 2ml/min for ciprofloxacin, while the second consisted of water, acetonitrile, triethylamine (80:20:0.3) pumped at a flow rate 1ml/min for lomefloxacin. The UV detector was operated at 278 nm for ciprofloxacin and 288 nm for lomefloxacin. The methods were suitably validated for linearity, accuracy, precision, robustness, and selectivity. All validation parameters were within the acceptance range. Data analysis revealed that plastic and amber containers could not protect either ciprofloxacin or lomefloxacin in eye drops formulation from photodegradation after one month in the cabinet. White blister protects the two agents in tablets formulation after six months in the cabinet.

INTRODUCTION: The fluoroquinolones represent a relatively new class of antibiotics with outstanding therapeutic potential, attributable to their broad spectrum of antimicrobial activity and useful pharmacokinetic properties: they are orally active, their lipophilicity and low degree of plasma protein binding allow for excellent tissue penetration and concentrations [1]. Since quinolones discovery in the early 1960s, the quinolone group of antibacterials has generated considerable clinical and scientific interest [2].

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It has been estimated that over 10000 analogs of naldixic acid have been synthesized [3]. The quinolone pharmacore is represented in **Fig. 1** [3].

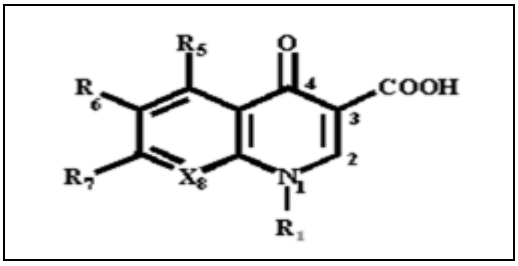

FIG. 1: THE QUINOLONE PHRMACORE

Quinolone phototoxicity is related to the generation of reactive oxygen species, including hydrogen peroxide, since these species cause severe tissue damage [4]. These reactive oxygen species attack cellular lipid membranes, which results in lipid peroxidation and subsequent inflammation [5].

Only a few studies on the photostability of fluoroquinolones have been published [6, 7], and there were no studies on the effect of the packaging on the photostability of pharmaceutical preparations. Therefore, in this study an attempt has been made to determine the effect of light on the stability of ciprofloxacin, the most widely used agent in this class due to its broad spectrum and excellent oral bioavailability [8] and lomefloxacin, the most phototoxicity [5], after both direct exposure and in containers in the light-stability cabinet, using stability-indicating HPLC methods.

MATERIALS AND METHODS: Ciprofloxacin and Lomefloxacin working standards were obtained from the quality control and researches laboratories in the ministry of health- Syria. Tablets and eye drops were obtained from different local manufactures.

Acetonitrile and water of HPLC, phosphoric acid, hydrochloric acid, sodium hydroxide, and hydrogen peroxide were purchased from MERCK, triethylamine from PANREAC.

A Hitachi HPLC system equipped with an L-2130 isocratic and gradient pump, L-2200 auto-injector, L-2450 photodiode array detecto, and Knauer C18 column (250 × 4.6 mm, 5µm) were used.

The first mobile phase consisted of 0.025M phosphoric acid and acetonitrile (87:13, v/v) [9] pumped at a flow rate 2ml/min for ciprofloxacin, while the second consisted of water, acetonitrile, triethylamine (80:20:0.3, v/v/v) pumped at a flow rate 1ml/min for lomefloxacin[10]. The UV detector was operated at 278 nm for ciprofloxacin and 288 nm for lomefloxacin.

Preparation of stock and standard solutions: Stock solution of ciprofloxacin 1 mg/ml was prepared in the mobile phase and diluted further with mobile phase to obtain the standard solution of 300 µg/ml.

The stock solution of lomefloxacin 0.1 mg/ml was prepared in the mobile phase and diluted further with mobile phase to obtain the standard solution of 60 µg/ml.

Assay methods: Assay methods of ciprofloxacin and lomefloxacin are shown in **Table 1.**

TABLE 1: ASSAY METHODS OF CIPROFLOXACIN AND LOMEFLOXACIN

Light	Heat	H_2O_2	NaOH	HCl	
50%	0%	5%	39%	23%	Degradation percentage
7.61	-	5.2	8.8	14.62	Resolution factor

Method validation: Method validation procedure was based on the recommendations of the international conference on harmonization Q2 (R1) for analytical procedures validation [11].

Stability- Indicating Study: [12] The stability study consists of the following challenges: exposure to hydrogen peroxide, acid and alkali hydrolysis, and exposure to heat and light.

A standard solution at 10 times the normal working concentration was prepared. 10 ml aliquots of the sample were taken into five separate 100 ml volumetric flasks and treated as follows:

20 ml of 5N HCl was added and immersed in a boiling water bath for one hour.

20 ml of 5N NaOH was added and immersed in a boiling water bath for one hour.

10 ml of H_2O_2 20% was added and let stand 30 minutes.

Stored at 60ºC for one week. Stored under white light for one week.

Each sample was diluted to the mark with RO/DI water. The acid sample was neutralized with 20 ml 5N NaOH before dilution and the alkali sample with 20 ml 5N HCl before dilution.

Stability Study: First, samples of pharmaceutical preparations (tablets and eye drops) for each of ciprofloxacin and lomefloxacin have been assayed (time zero).

After that, other samples have been incubated in a light-stability cabinet designed for light-stability tests in compatible with the second option for light sources identified in the international conference on harmonization topic Q1B for the photostability testing [13], so that is maintained at a temperature 25 °C and a relative humidity of 40% during the study period, amounting to six months.

Part of the samples was incubated under direct exposure to light (direct light), another part was incubated within its direct package (white blister, amber, and plastic containers), while the rest of the samples were incubated after suitable protection from light (dark). The changes in the incubated samples have been recorded and studied according to the assay of the active ingredient using validated methods. The results were compared to determine the effect of the light on the stability of the active substances in their dosage forms during storage and consumption, and the effect of the packaging containers on the protection from light.

Statistical Studies:

Fisher Test: Fisher test was used to compare variances between assay results of the active ingredients in each group of samples at 95% confidence limits.

Student Test: Student test was performed for comparison between the average weights of the active ingredients in each of the conditions used (light, dark, white blister, plastic, and amber containers) at 95% confidence limits.

RESULTS AND DISCUSSION:

Verifying for the Assay Methods Stability-Indicating: The assay methods of ciprofloxacin and lomefloxacin were verified for their stability-indicating as the hardest conditions were applied to accelerate the degradation of the active ingredients. The methods proved to be suitable for the use in the stability study of ciprofloxacin and lomefloxacin. They were able to differentiate selectively between the active substance and its degradation products, as the resolution factor was more than 1.5 in all mediums **(Tables 2** & **3, Fig. 2 - 9).**

TABLE 2: LOMEFLOXACIN UNDER STRESS CONDITIONS

Light	Heat	H_2O_2	NaOH	HCl	
56%	0%	21.5%	47.5%	46%	Degradation percentage
9.31	-	9.42	9.54	2.91	Resolution factor

TABLE 3: CIPROFLOXACIN UNDER STRESS CONDITIONS

Assay methods of ciprofloxacin	
30ºC	Column temperature
0.025M phosphoric acid and acetonitrile (87:13). The PH was adjusted to 3 using triethylamine [9]	Mobile phase
2 ml/min	Flow rate
278 nm	Wavelength
300 µg/ml	Standard concentration
Assay methods of lomefloxacin	
24ºC	Column temperature
Water, acetonitrile, triethylamine (80:20:0.3). The PH was adjusted to 3.3 using phosphoric acid [10].	Mobile phase
1 ml/min	Flow rate
288 nm	Wavelength
60 µg/ml	Standard concentration

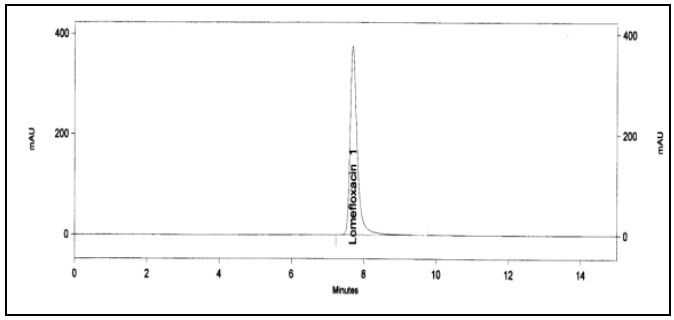

FIG. 2: HPLC CHROMATOGRAM OF LOMEFLOXACIN (STANDARD SOLUTION)

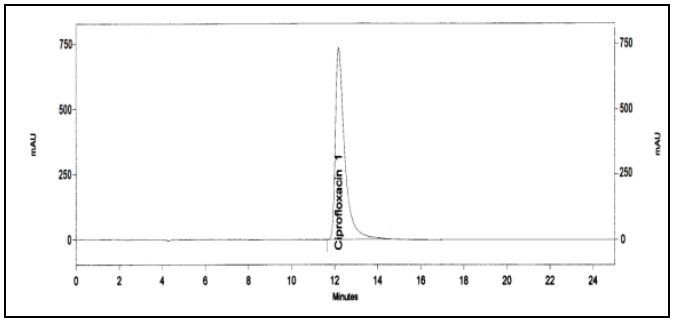

FIG. 3: HPLC CHROMATOGRAM OF CIPROFLOXACIN (STANDARD SOLUTION)

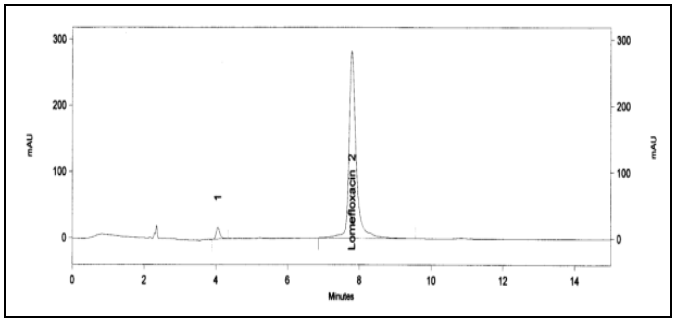

FIG. 4: HPLC CHROMATOGRAM OF LOMEFLOXACIN TREATED WITH 5N HCl

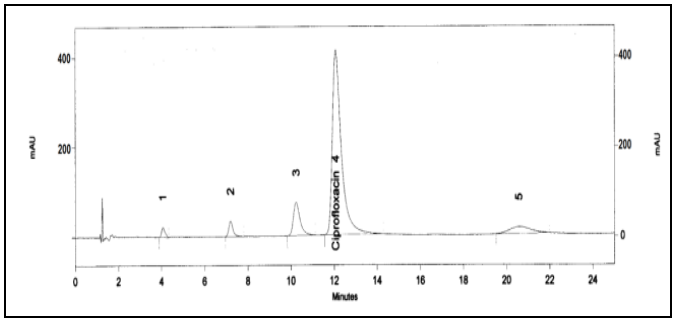

FIG. 5: HPLC CHROMATOGRAM OF CIPROFLOXACIN TREATED WITH 5N HCl

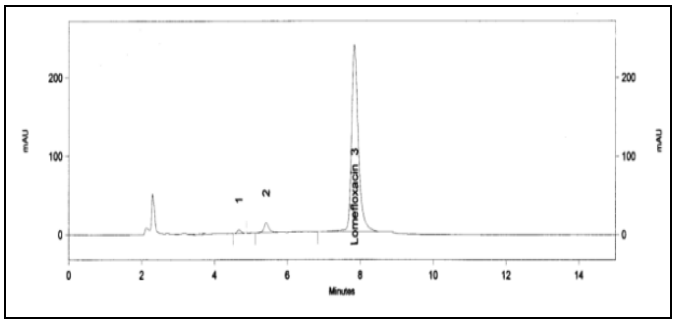

FIG. 6: HPLC CHROMATOGRAM OF LOMEFLOXACIN TREATED WITH 5N NaOH

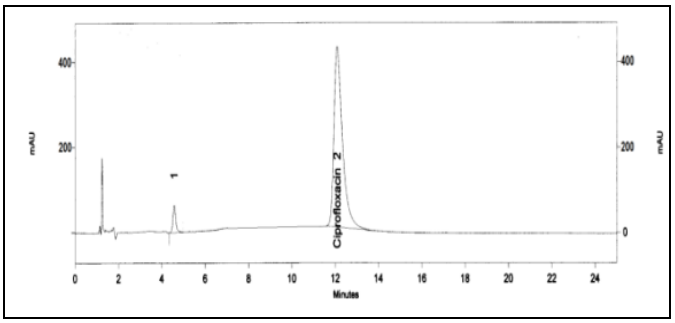

FIG. 7: HPLC CHROMATOGRAM OF CIPROFLOXACIN TREATED WITH 5N NaOH

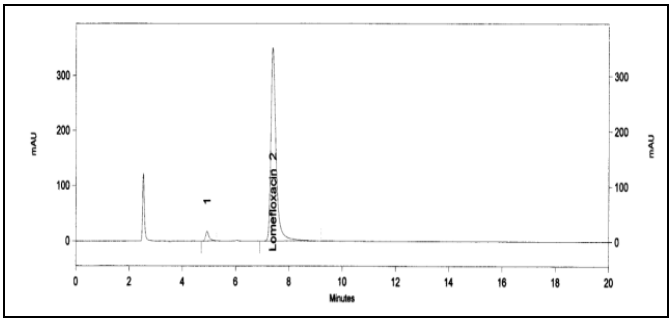

FIG. 8: HPLC CHROMATOGRAM OF LOMEFLOXACIN TREATED WITH H_2O_2 20%

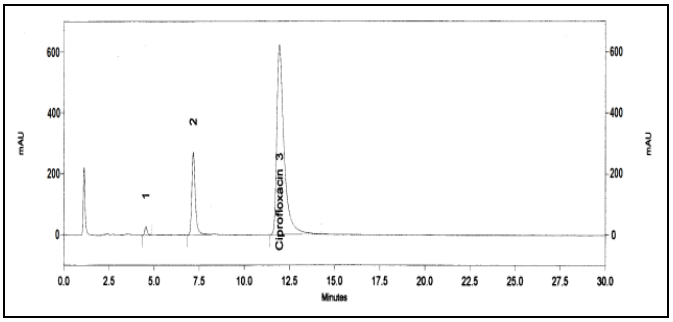

FIG. 9: HPLC CHROMATOGRAM OF CIPROFLOXACIN TREATED WITH H_2O_2 20%

The methods also showed the stability of both compounds at high temperatures where there has been no damage after incubation at a temperature of 60 ºC for a week as it appears in the **Fig. 10** and **11** as well as their sensitivity against the light, which turned out through the significant contraction in their concentrations after exposure to white light for one week, as shown in **Fig. 12** & **13**.

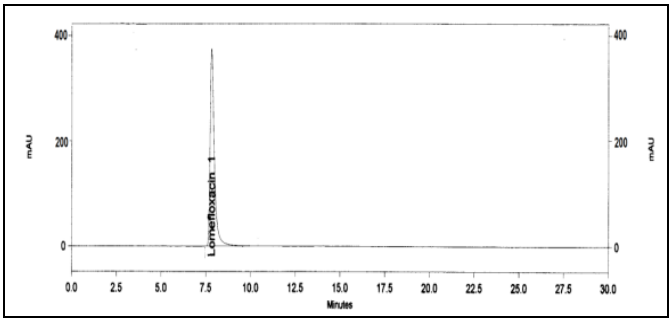

FIG. 10: HPLC CHROMATOGRAM OF LOMEFLOXACIN AT 60ºC FOR ONE WEEK

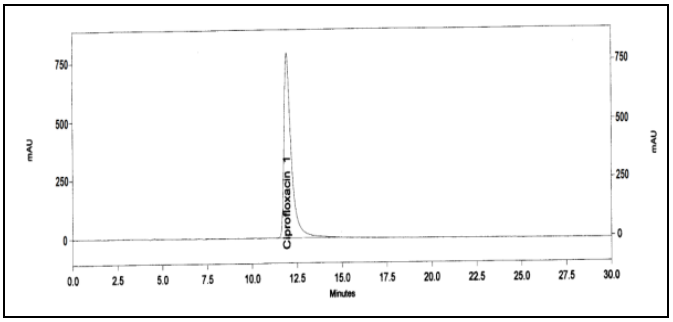

FIG. 11: HPLC CHROMATOGRAM OF CIPROFLOXACIN AT 60ºC FOR ONE WEEK

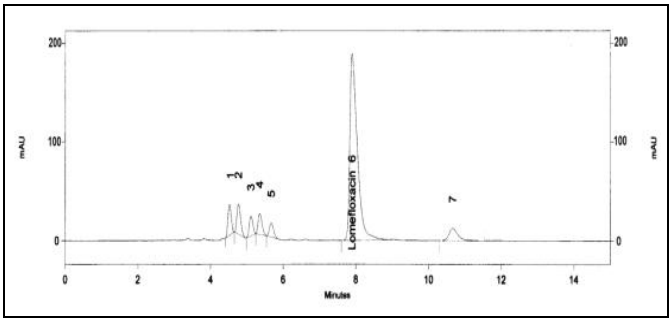

FIG. 12: HPLC CHROMATOGRAM OF LOMEFLOXACIN UNDER WHITE LIGHT FOR ONE WEEK

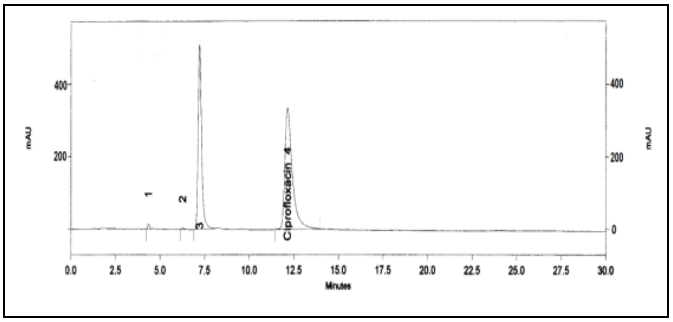

FIG. 13: HPLC CHROMATOGRAM OF CIPROFLOXACIN UNDER WHITE LIGHT FOR ONE WEEK

For acid and alkaline mediums, no damages were noticed when using 0.5 N concentrations of hydrochloric acid and sodium hydroxide, which has necessitated a gradually increased until 5N concentration where we got a suitable degradation.

Validation of the Assay Methods: The assay method of lomefloxacin was validated, and all the parameters met the recommendations of ICH for analytical method validation **Table 4**.

The linear equation and correlation coefficient were: Y=3163697X+359440, 0.9994, respectively **Fig. 14**. This demonstrates the linearity of this method and its suitability for the analysis of lomefloxacin.

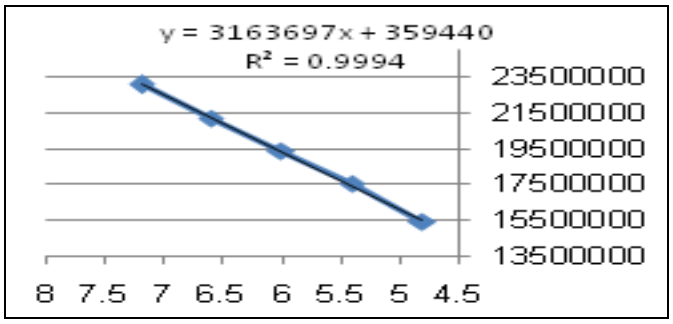

FIG. 14: RELATION BETWEEN CONCENTRATION AND PEAK AREA OF LOMEFLOXACIN

The results of accuracy showed that the method is accurate, with an average percentage recovery 102.18.

The relative standard deviation for repeatability was 0.71, and for intermediate precision was 1.17.

The purity of analyte peak and the RSD value of < 2% (0.22) indicate that the method is selective for analysis of lomefloxacin in its dosage forms.

The results of the robustness test indicate that the method is robust. The percentage recovery for the assay values (n=5) were 98.72, 100.24, 99.32. The relative retention time of samples was 0.998, 0.997, and 0.997.

The assay method of ciprofloxacin was validated, and all the parameters met the recommendations of ICH for analytical method validation **Table 4**.

The linear equation and correlation coefficient were: Y=1652531X-4109032, 0.9983, respectively **Fig. 15**. This demonstrates the linearity of this method and its suitability for the analysis of ciprofloxacin.

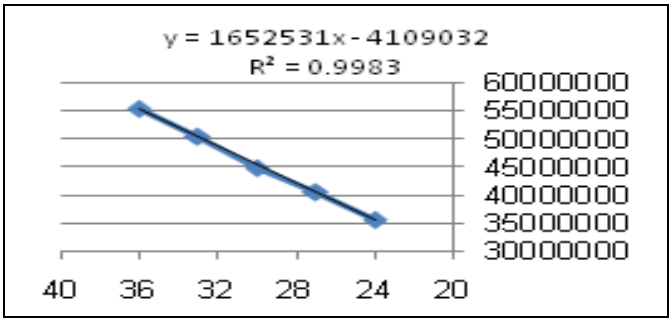

FIG. 15: RELATION BETWEEN CONCENTRATION AND PEAK AREA OF CIPROFLOXACIN

The results of accuracy showed that the method is accurate, with an average percentage recovery 101.68. The relative standard deviation for repeatability was 1.19, and for intermediate precision was 0.78.

The purity of analyte peak and the RSD value of < 2% (0.18) indicate that the method is selective for analysis of lomefloxacin in its dosage forms.

The results of the robustness test indicate that the method is robust. The percentage recovery for the assay values (n=5) were 97.71, 100.39, 97.72. The relative retention time of samples was 0.998, 1, and 0.996.

TABLE 4: VALIDATION RESULTS OF THE HPLC METHOD OF LOMEFLOXACIN & CIPROFLOXACIN

	Retention time	Linearity	Accuracy Mean Recovery%	Precision RSD%		Selectivity RSD%	Robustness Mean Recovery% / RRT		
Lome-floxacin	7.7 min.	R^2 =0.9994	102.18	Intermediate precision	Repeatability	0.22	1.1	1 ml/min	0.9
				1.17	0.71		99.32	100.24	98.72
							0.997	0.997	0.998
Cipro-floxacin	12.2 min.	R^2 =0.9983	101.68	0.78	1.19	0.18	1.9	2 ml/min	2.1
							97.71	100.39	97.72
							0.996	1	0.998

Stability Studies on Dosage Forms: When exposing the tablets (Ciprofloxacin and Lomefloxacin) directly to light within the light-stability cabinet, there was no decrease in the concentration of the active substances after 3 months, in other words, the difference between concentrations of the active ingredients in the dark and the light was not statistically significant. At the

end of the exposure period after 6 months, the decrease in the concentrations of the active substances was substantially and statistically significant (around 1.5%, 4.5%, respectively), as a result of photodegradation and not return to the forces of luck and coincidence or analytical errors. No decrease was noticed in the concentration of the active ingredients when the tablets exposed to light within the white blister even after 6 months in the cabinet, and the differences were insignificant **Table 5**.

TABLE 5: COMPARING STATISTICAL RESULTS IN TABLETS AFTER LIGHT EXPOSURE

Average of concentrations									
Ciprofloxacin					Lomefloxacin				
	Time zero	1 month	3 months	6 months		Time zero	1 month	3 months	6 months
Dark	99.89	98.85	98.12	98.65	Dark	103.18	103.3	99.73	99.15
Direct light	99.89	98.13	98.06	97.11	Direct light	103.18	102.85	99.64	94.93
Difference	-	insignificant	insignificant	Significant	Difference	-	insignificant	Insignificant	Significant
Dark	100.87	101.43	100.94	101.15	Dark	99.78	98.72	97.94	94.83
Blister	100.87	100.98	101.03	100.64	Blister	99.78	98.84	97.93	95.03
Difference	-	insignificant	insignificant	Insignificant	Difference	-	insignificant	Insignificant	Insignificant

For the eye drops, the degradation was clear since the first month of the direct exposure to light (about 30% .84%, respectively), as well as when exposing the drop to light within both the amber and plastic containers, where the decrease in the concentration of the active substances was substantially and statistically significant after one month (about 2% in both containers for ciprofloxacin, 1.5% in the amber container respectively), as well as when exposing the drop to light within both the amber and plastic containers, where the decrease in the concentration of the active substances was substantially and statistically significant after one month (about 2% in both containers for ciprofloxacin, 1.5% in the amber container and 9% in the plastic container for Lomefloxacin) as a result of photodegradation **Table 6**.

TABLE 6: COMPARING STATISTICAL RESULTS IN EYE DROPS AFTER LIGHT EXPOSURE

Average of concentrations									
Ciprofloxacin					Lomefloxacin				
	Time zero	1 month	3 months	6 months		Time zero	1 month	3 months	6 months
Dark	102.54	101.03	100.86	100.63	Dark	101.57	99.22	98.83	96.34
Direct light	102.54	70.83	51.56	10.33	Direct light	101.57	14.85	4.42	1.45
Difference	-	significant	significant	Significant	Difference	-	significant	significant	Significant
Dark	101.2	100.3	100.01	100.15	Dark	100.94	99.33	99.14	100
Amber	101.2	98.33	93.35	88.11	Amber	100.94	97.93	96.31	90.09
Plastic	101.2	97.96	92.63	91.16	Plastic	100.94	90.26	85.83	82.82
Difference	-	significant	significant	Significant	Difference	-	significant	significant	Significant

CONCLUSION: A reverse phase high-performance liquid chromatography methods have been used for the determination of ciprofloxacin and lomefloxacin in tablets and eye drops dosage forms. The methods were suitably validated and studied for their stability-indicating. The degradation products were well separated from the peak of the active substance. The results of the stability study indicate that white blister can protect tablets from degradation induced by light. They also show that transparent containers should be avoided in eye drops formulations, instead of amber or plastic containers can be used, with an emphasis on the need to keep the eye drops within the outer package and not only within the inner package.

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CONFLICT OF INTEREST: Nil

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