

SIMULTANEOUS DETERMINATION OF INTACT LOMEFLOXACIN AND CIPROFLOXACIN IN THE PRESENCE OF THEIR ACID DEGRADATION PRODUCTS

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نم في هذا البحث تحليل هيدروكلوريد اللوميفلوكساسين في وجود ناتج تكسيره باستخدام كروماتوجرافيا السوائل بالضغط العالي بالإضافة إلي طريقتين أخرتين لتحليل كل من هيدروكلوريد اللوميفلوكساسين وهيدروكلوريد السيبروفلوكساسين في وجود ناتج تكسيرهم بالحامض. في طريقة كروماتوجرافيا السوائل بالضغط العالي تم الفصل على عمود كربون والسائل المتحرك المكون من ماء: اسيتونيتريل : تراى إيثيل أمين (: :) والذي تم ضبط الأس الهيدروجيني له إلي باستخدام حامض أورثوفوسفوريك وبمعدل سريان قدره / ثانية وتم القياس عند طول موجة . ن.م وقد تم تطبيق الطريقة على تركيزات تتراوح بين إلي ميكروجرام/ ميكروليتر. الطريقة الثانية: هي طريقة كروماتوجرافيا الطبقة الرقيقة وتم استخدام خليط مكون من ميثانول: محلول أمونيا منظم بنسبة (:) المركبات وقياس المركبات عند أطول موجة . ن.م و . ن.م لكل من هيدروكلوريد اللوميفلوكساسين وهيدروكلوريد السيبروفلوكساسين على التوالي. وقد تم تطبيق الطريقة على تركيزات تتراوح بين إلي ميكروجرام/ ميكروليتر و . إلي , ميكروجرام/ ميكروليتر لكل من هيدروكلوريد اللوميفلوكساسين وهيدروكلوريد السيبروفلوكساسين على التوالي. الطريقة الثالثة: هي طريقة المشتقة الطيفية الأولى وذلك بقياس الأطوال عند أطول موجة . ن.م و . ن.م لكل من هيدروكلوريد اللوميفلوكساسين وهيدروكلوريد السيبروفلوكساسين على التوالي وقد تم تطبيق الطريقة على تركيزات تتراوح بين إلي ميكروجرام/ مل ومن إلي ميكروجرام/مل لكل من هيدروكلوريد اللوميفلوكساسين وهيدروكلوريد السيبروفلوكساسين على التوالي. وقد تم تطبيق الطرق المقترحة بنجاح على أقراص الكافلوكس وسرفيلوكس وكذلك تمت مقارنة الطرق المقترحة بالطرق المرجعية حيث تبين عدم وجود فروق إحصائية من حيث الدقة أو الضبط.

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A reversed phase HPLC method was developed for the determination of lomefloxacin and its degradation product. In addition, two other methods have been developed for the determination of lomefloxacin hydrochloride (LF.HCl) and ciprofloxacin hydrochloride (CF.HCl) in presence of their acid induced degradation products.

For the reversed phase HPLC method (determination of LF.HCl), the mobile phase used was a mixture of water: acetonitrile: triethylamine (80:20:0.6, v/v/v) adjusted to pH 3.0 with o-phosphoric acid. The flow rate was 1.5 ml/min. and the detection was carried out at 328 nm. The linearity range was found to be 0.5-6 µg / 20 µl for LF.HCl. The limits of detection and quantification (LOD & LOQ) were 0.22 µg / 20 µl & 0.74 µg / 20 µl respectively.

The second method was densitometric method for the determination of both LF.HCl and CF.HCl, the developing system used was a mixture of methanol and ammonia buffer (80:20, v/v). Detection was carried out at 288 nm & 279 nm. for intact LF.HCl and CF.HCl respectively. The linearity ranges were found to be 1-6 µg / 10 µl & 0.25-2.5 µg / 10 µl for intact LF.HCl and CF.HCl respectively. LOD & LOQ were 0.1, 0.34 µg / 10 µl & 0.05, 0.18 µg / 10 µl for both drugs, respectively.

The third method was derivative spectrophotometric method for the determination of (LF.HCl) & (CF.HCl). The linearity ranges were found to be 2-8 µg/ml & 5-12 µg/ml for LF.HCl and CF.HCl respectively. LOD & LOQ were 0.39 µg, 1.29 µg/ml & 1.03, 3.45 µg/ml for LF.HCl and CF.HCl, respectively.

Separation and identification of the acid degradation products of lomefloxacin hydrochloride and ciprofloxacin hydrochloride were carried out.

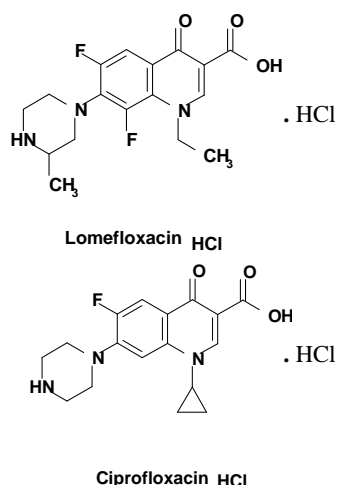
The three described methods proved to be sensitive, precise and applicable to both dosage forms and laboratory prepared mixtures of the intact drugs and their acid degradation products.

INTRODUCTION

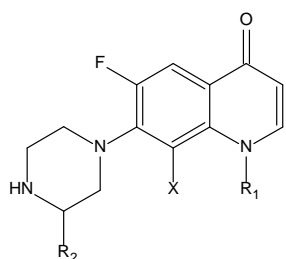
Fluoroquinolone antibacterial agents, which are relatively late arrivals on the antibacterial scene, proved to be very useful therapeutic agents. They are particularly efficient in the treatment of complicated

urinary tract infections and also in the treatment of infections resistant to the traditional antibacterial agents¹. The drugs proved to be effective in the treatment of respiratory infections, external otitis, chronic gram negative bacillary osteomyelitis, gonorrhoea, cervicitis, and bacterial prostatitis.

They also helped greatly in eradication of *Salmonella typhi* in carriers and anthrax². This investigation is concerned with the analysis of LF.HCl and CF.HCl, either alone or in the presence of their acid degradation products, Fig. (1).



The chemical structure of lomefloxacin HCl and ciprofloxacin HCl.



Compound	X	R ₁	R ₂
Ciprofloxacin	H	cyclopropyl	H
Lomefloxacin	F	ethyl	CH ₃

The identified chemical structures of the acid degradation products of lomefloxacin HCl and ciprofloxacin HCl.

Fig. 1

Numerous analytical methods had been reported for the determination of CF.HCl, while few methods were described for LF.HCl hydrochloride. For example, volumetric^{3&4}, spectrophotometric⁵⁻¹⁵, fluorimetric¹⁶⁻²³ and chromatographic methods²⁴⁻⁴¹ were reported.

These drugs are characterized by their susceptibility to photo-degradation into inactive products. This fact encouraged the authors to propose stability-indicating procedures for the determination of the intact drug in the presence of its acid degradation product. New methods were proposed in this work for the estimation of the components of the above mentioned mixtures. The proposed methods were, HPLC, densitometric TLC and derivative spectrophotometric methods.

EXPERIMENTAL

Materials and reagents

All chemicals and reagents used were of analytical or pharmaceutical grade. Distilled water was used throughout. Solvents were of HPLC grade or spectroscopic grade.

Lomefloxacin hydrochloride (LF.HCl): It was supplied by Alkan Pharma S.A.E., 6 October city, Egypt. It was analyzed spectrophotometrically⁴²⁾ and found to contain 99.17±0.60 Lomefloxacin hydrochloride.

Ciprofloxacin hydrochloride: It was supplied by Biochemie, Vienna,

Austria. It was analyzed by HPLC⁴³ and found to contain 99.43 ± 0.55 CF.HCl.

“Alkaflox” tablets: (batch no. 001) nominally containing 400 mg of LF.HCl hydrochloride, produced by Alkan Pharma S.A.E., 6 October City, Egypt.

“Serviflox” tablets: (batch no. 030) nominally containing 291.5 mg of CF.HCl hydrochloride (equivalent to 250 mg of CF.HCl), produced by Biochemie, Vienna, Austria.

Methanol HPLC grade, 2M Hydrochloric acid, ammonia buffer: 10% v/v ammonium hydroxide in 1.605% m/v aqueous ammonium chloride solution, diethyl ether (BDH), 2 M sodium hydroxide, acetonitrile HPLC grade, triethylamine, o-phosphoric acid, double distilled water.

Mobile phase for HPLC

A mixture of water: acetonitrile: triethylamine (80:20:0.6, v/v/v) adjusted to pH 3.0 with o-phosphoric acid.

Developing system for densitometry

A mixture of methanol and ammonia buffer (80:20, v/v) was freshly prepared prior to analysis.

Preparation of stock solutions

Preparation of lomefloxacin hydrochloride and ciprofloxacin hydrochloride stock solutions

A solution of LF.HCl (0.5 mg/ml) or CF.HCl (1 mg/ml) was prepared in the specified solvent.

Preparation of degraded lomefloxacin hydrochloride and ciprofloxacin hydrochloride stock solutions

An accurate weight of LF.HCl or CF.HCl (250 mg) was refluxed in 2 M hydrochloric acid (40 ml) for 48 hrs^{44&45}. After cooling, the solution was neutralized with 2M sodium hydroxide, and then concentrated to a small volume. The concentrated solution was extracted with diethyl ether (10 ml x 3). The ether extract was evaporated to dryness on a warm water bath. The obtained residue was used for preparing the degraded LF.HCl solution (0.5 mg/ml) or CF.HCl solution (1 mg/ml) in the specified solvent.

Preparation of internal standard stock solution

A solution of enrofloxacin (0.5 mg/ml) was prepared in the mobile phase.

Apparatus

For HPLC

HPLC apparatus consisted of Waters autosampler injector, Waters isocratic 610 pump, Waters 486 uv detector adjusted at 328 nm. and μ Bondapak C₁₈ reversed-phase column (25 cm x 4.6 mm i.d.).

For densitometry

CS-9301 PC Dual wavelength. Flying Spot Scanning Shimadzu densitometer, Japan.

For spectrophotometry

Shimadzu uv-visible recording spectrophotometer UV-265/ Japan.

For identification of degraded structure

- a- For infra red spectra: Shimadzu Infra red spectrometer (IR- 435), Japan.
- b- For ¹H- NMR spectra: Joel, FX 90Q.NMR spectrometer.
- c- For mass spectra: Finnigan SSQ 7000 Gas chromatograph- mass.

Procedure

HPLC method for the determination of Lomefloxacin

General procedure and linearity

A solution containing intact LF.HCl (0.5 mg/ml) and degraded LF.HCl (0.4 mg/ml) was prepared in the mobile phase. Different aliquots (0.5-6 ml) of this solution were introduced into a series of 10 ml volumetric flasks, followed by internal standard solution (1 ml) to each flask. The volume of each flask was completed with the mobile phase. Twenty-micro liters of each solution were injected onto the column. The chromatograms were recorded using flow rate of 1.5 ml/min. and wavelength of detection at 328 nm.

The resulting chromatograms (Fig. 2), retention times (t_R) of the peaks and the areas under the peaks (AUPs) were recorded. The ratios (R) of the recorded AUPs of intact LF.HCl or of its degradation product to that of the internal standard were plotted versus the concentration ($\mu\text{g}/20 \mu\text{l}$) and the calibration graphs for intact and degraded LF.HCl were obtained.

The following regression equations (1,2) were computed:

$$\begin{aligned} \text{Concentration of intact LF.HCl} \\ (\mu\text{g}/20 \mu\text{l}) = \\ 0.5496 R + 0.0859 \dots\dots\dots \text{Eq. (1)} \\ r^2 = 0.9989. \text{ or} \\ R = 1.8195 X C (\mu\text{g}/20\mu\text{l}) - 0.1563 \end{aligned}$$

$$\begin{aligned} \text{Concentration of degraded LF.HCl} \\ (\mu\text{g}/20 \mu\text{l}) = \\ 0.4499 R + 0.1102 \dots\dots\dots \text{Eq.(2)} \\ r^2 = 0.9983. \text{ or} \\ R = 2.2227 X C (\mu\text{g}/20 \mu\text{l}) - 0.2449 \end{aligned}$$

Where C is the concentration in $\mu\text{g}/20 \mu\text{l}$, R is the ratio of AUP of drug/AUP of IS and r^2 is the correlation coefficient.

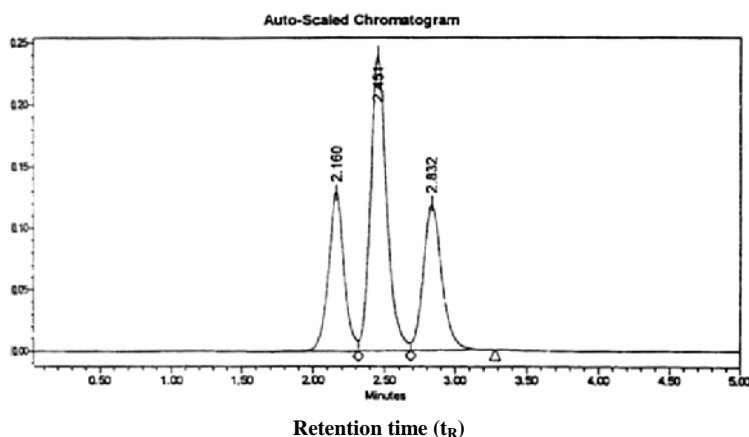


Fig. 2: HPLC chromatogram of degraded LF.HCl ($t_R = 2.16$ min.), intact LF.HCl ($t_R = 2.451$ min.) and enrofloxacin ($t_R = 2.832$ min.).

Simultaneous determination of intact and degraded lomefloxacin hydrochloride in laboratory prepared mixtures by RP-HPLC

Two solutions of intact LF.HCl and degraded LF.HCl (0.6 mg/ml) each in the mobile phase were prepared. Different aliquots (1.5-4.5 ml) and (0.5-3.5 ml) of both intact and degraded LF.HCl solutions, respectively, were introduced into a series of 10 ml volumetric flasks. One- ml aliquot of internal standard solution was added to each flask and the volumes were completed with the mobile phase. Twenty-micro liters of each mixture solution was injected onto the column and the chromatograms were recorded using the same parameters mentioned under general procedure and linearity. The concentrations of intact and degraded LF.HCl were calculated using the regression equations (1&2).

Determination of intact lomefloxacin hydrochloride in "Alkaflox" tablets by RP-HPLC

An accurate weight of the powdered twenty tablets equivalent to (35 mg) of intact LF.HCl was introduced into a 100 ml volumetric flask. The mobile phase (80 ml) was added and the solution was shaken (using a mechanical shaker) for 10 minutes and the volume was completed with the mobile phase. The solution was filtered through a dry filter paper. The first 10 ml of the filtrate was rejected and the filtrate was refiltered through 0.45 µm millipore teflon filter. Different aliquots (2-4 ml) of this solution were

transferred into a series of 10 ml volumetric flasks, one ml aliquot of the internal standard solution was added to each flask and the volume was completed with the mobile phase. Twenty-micro liters of each solution were injected onto the column and the chromatograms were recorded using the same parameters used under general procedure and linearity on page. The procedure was repeated using the standard addition technique. The ratio (R) of AUP of the drug to the AUP of the internal standard was used in calculating the recovered concentrations of labeled and added LF.HCl.

Densitometric simultaneous determination of intact lomefloxacin hydrochloride and ciprofloxacin hydrochloride and their acid degradation products

General procedure and linearity

Different aliquots of intact LF.HCl stock solution (1-6 ml) or CF.HCl stock solution (0.5-5 ml) were transferred into two series of 10 ml volumetric flasks. Also, several aliquots of degraded LF. HCl stock solution (0.5-6 ml) or degraded CF.HCl stock solution (0.5-5 ml), were transferred into two series of 10 ml volumetric flasks. Each flask was completed to volume with methanol.

A ten-microliter aliquot of each solution was applied on thin-layer chromatographic plates (20x20 cm) using a micropipette. Spots were spaced 2.5 cm apart and 2 cm from the bottom edge of the plate. The plates were developed for at least one

hour in a chromatographic tank, previously saturated with the specified developing system, by ascending chromatography to a distance of 15 cm. The plates were left to dry at room temperature. The spots were detected under a uv lamp (254 nm) and the two drugs were scanned under the following conditions:

Photo mode: Reflection, scan mode: Zigzag, swing width: 12 mm.

Wavelength:

- i) 288 and 328 nm for intact and degraded LF.HCl, respectively.
- ii) 279 and 323 nm for intact and degraded CF.HCl, respectively.

Chromatograms, result outputs and the areas under the peaks (AUPs) were recorded. The recorded AUPs were plotted versus the concentration used ($\mu\text{g}/10 \mu\text{l}$) and the calibration curves for both intact and degraded LF.HCl or CF.HCl were obtained. The following regression equations (3,4,5 and 6) were computed for calculating the concentrations of intact LF.HCl, degraded LF.HCl, intact CF.HCl, and degraded CF.HCl, respectively.

Concentration of intact LF.HCl ($\mu\text{g}/10 \mu\text{l}$) =
 $0.0287 \text{ AUP} - 0.0022 \dots \dots \text{Eq. (3)}$
 $r^2 = 0.9998$. Or
 $\text{AUP} = 34.843 \text{ X C } (\mu\text{g}/10\mu\text{l}) + 0.0767$

Concentration of degraded LF.HCl ($\mu\text{g}/10 \mu\text{l}$) =
 $0.0388 \text{ AUP} - 0.1587 \dots \dots \text{Eq. (4)}$
 $r^2 = 0.9982$. Or
 $\text{AUP} = 25.773 \text{ X C } (\mu\text{g}/10\mu\text{l}) + 4.0902$

Concentration of intact CF.HCl: ($\mu\text{g}/10 \mu\text{l}$) =
 $0.0194 \text{ AUP} - 0.0398 \dots \dots \text{Eq. (5)}$
 $r^2 = 0.9991$. Or
 $\text{AUP} = 51.546 \text{ X C } (\mu\text{g}/10\mu\text{l}) + 2.0515$

Concentration of degraded CF.HCl ($\mu\text{g}/10 \mu\text{l}$) =
 $0.0246 \text{ AUP} - 0.0758 \dots \dots \text{Eq. (6)}$
 $r^2 = 0.9980$. Or
 $\text{AUP} = 40.650 \text{ X C } (\mu\text{g}/10\mu\text{l}) + 3.0813$

Where C is the concentration in $\mu\text{g}/10\mu\text{l}$, AUP is the area under the peak and r^2 is the correlation coefficient.

Densitometric determination of intact and degraded lomefloxacin hydrochloride and ciprofloxacin hydrochloride in laboratory prepared mixtures

Four solutions of intact and degraded LF.HCl (1.2 mg/ml), of intact and degraded CF.HCl (0.4 mg/ml) were prepared in methanol.

Different aliquots of both intact LF.HCl (1-4.5 ml) or of intact CF.HCl (1.5-4 ml), were transferred into two series of 10 ml volumetric flasks. Different aliquots of degraded LF.HCl (0.5-4 ml) or degraded CF.HCl (1-3.5 ml) were added to intact LF.HCl series or to intact CF.HCl series, respectively [series (1) and (2)] for LF.HCl and CF.HCl, respectively. Each volumetric flask was completed to volume with methanol. Ten-microlitre aliquots of each mixture solution were applied on thin-layer chromatographic plates using a micropipette and the procedure was completed using the

same parameters mentioned under general procedure and linearity.

The recorded AUPs were used for calculating the concentrations of the two components (intact and degraded drug) in each mixture solution, using the regression equations (3,4,5 and 6) for intact and degraded LF.HCl and CF.HCl, respectively.

Densitometric determination of lomefloxacin hydrochloride and ciprofloxacin hydrochloride in “Alkaflox” and “Serviflox” tablets, respectively

An accurate weight of the powdered twenty tablets equivalent to LF.HCl (35 mg) or CF.HCl (30 mg), was extracted with methanol (10 ml x 3). Each extract was filtered into a 50ml volumetric flask. The residue was washed with methanol (5 ml x 3), the volume was completed with methanol. Aliquots (2-4 ml) of LF.HCl solution or (1-2 ml) of CF.HCl solution, were transferred into two series of 10 ml volumetric flasks and completed to volume with methanol. Ten-microlitre aliquot of each solution was applied on thin-chromatographic plates and the procedure was completed using the same parameters mentioned under general procedure for linearity. This experiment was repeated applying the standard addition technique. The recorded AUPs for the spots were used for calculating the concentration of labelled and added intact LF.HCl or CF.HCl, from regression equations (3 and 5).

Simultaneous derivative spectrophotometric determination of intact lomefloxacin hydrochloride or ciprofloxacin hydrochloride and their acid degradation products

General procedure and linearity

An aqueous solution of intact LF.HCl (0.5 mg/ml) or CF.HCl (1 mg/ml) was prepared. A ten-ml aliquot of this solution, containing LF.HCl or CF.HCl was introduced into a 100 ml volumetric flask and the volume was completed with 2 M hydrochloric acid.

Accurately measured aliquots (2-8 ml) or (2.5-6 ml) of intact solution of LF.HCl or CF.HCl, respectively, were transferred, into two series of 50 ml volumetric flasks, followed by the addition of accurately measured volumes (2-8 ml) or (4-8 ml) of degraded solution of LF.HCl or CF.HCl, respectively. Each flask was completed to volume with the same solvent.

The first and second derivative spectra for each solution were recorded against a solvent blank, using the following parameters:

Real time date :2, speed: fast, slit: 1, cyc.T(min): 0, ordinate range limit: - 0.3 to + 0.3 or - 0.4 to + 0.4 for LF.HCl or CF.HCl, respectively.

Wavelength range: 200-400 nm or 200-350 nm for LF.HCl or CF.HCl, respectively.

Also, the second derivative spectrum for each flask solution was recorded against a solvent blank, using the same above parameters except for the following:

- 1- Ordinate range limit: - 0.09 to + 0.06 or -0.12 to + 0.08 for degraded LF.HCl or CF.HCl, respectively.
- 2- Wavelength range: 250-350 nm or 225-325 nm for degraded LF.HCl or CF. HCl, respectively.

The heights (mm) of the first derivative spectra at 292 nm for LF.HCl or at 260 nm for CF.HCl were measured and the calibration graph for each drug was plotted. The heights (mm) of the second derivative spectra at 272 or at 262 nm for degraded LF.HCl or CF.HCl, respectively, were measured and the calibration graphs for the degraded drugs were constructed.

The following regression equations 7,8,9 and 10 were computed for calculating the concentration of intact and degraded lomefloxacin and ciprofloxacin, respectively.

Concentration of intact LF.HCl ($\mu\text{g/ml}$) =
 $0.1631 H_{292} + 0.0394 \dots \dots \dots \text{Eq.}(7)$
 $r^2 = 0.9998$. Or
 $H_{292} = 6.1312 \times C (\mu\text{g/ml}) - 0.2416$.

Concentration of intact CF.HCl ($\mu\text{g/ml}$) =
 $0.1916 H_{260} + 0.2480 \dots \dots \dots \text{Eq.}(8)$
 $r^2 = 0.9984$. Or
 $H_{260} = 5.2192 \times C (\mu\text{g/ml}) - 1.2944$.

Concentration of degraded LF.HCl ($\mu\text{g/ml}$) =
 $0.1534 H_{272} + 0.0359 \dots \dots \dots \text{Eq.}(9)$
 $r^2 = 0.9998$. Or
 $H_{272} = 6.5189 \times C (\mu\text{g/ml}) - 0.2340$.

Concentration of degraded CF.HCl ($\mu\text{g/ml}$) =
 $0.2123 H_{262} + 0.5092 \dots \dots \dots \text{Eq.}(10)$
 $r^2 = 0.9963$. Or
 $H_{262} = 4.7103 \times C (\mu\text{g/ml}) - 2.3985$.

Where C is the concentration in $\mu\text{g/ml}$, H is height of the peak and r^2 is the correlation coefficient.

Derivative spectrophotometric determination of lomefloxacin hydrochloride and ciprofloxacin hydrochloride in “Alkaflox” and “Serviflox” tablets, respectively

An accurate weight of the powdered tablets equivalent to LF.HCl or CF.HCl (20 mg) was extracted with methanol (15 ml x 3) then filtered into a 100 ml volumetric flask. After complete washing of the residue with methanol (10 ml x 3), the solution was diluted to volume with methanol. A twenty-ml aliquot of this solution, equivalent to LF. HCl or CF.HCl (4 mg) was evaporated on a warm water bath. The residue was dissolved in 2 M hydrochloric acid and quantitatively transferred into a 100 ml volumetric flask, and the volume was completed with 2 M hydrochloric acid. Accurately measured aliquots of the above solution (2-6 ml) or (6-9 ml) were introduced into two series of 50 ml volumetric flasks. Each flask was completed to volume with 2M hydrochloric acid.

The first derivative spectrum of each drug was recorded against a blank using the same parameters mentioned under general procedure and linearity. This procedure was

repeated using the standard addition technique.

The heights (mm) at 292 and 260 nm were measured and used for calculating the corresponding labeled and added concentrations of intact LF.HCl and CF.HCl, respectively.

RESULTS AND DISCUSSION

HPLC method

In this study different mobile phases were attempted for the separation of LF.HCl in presence of its degradation product(s). However, Thoppil & Amin³⁸ reported a stability-indicating reversed-phase liquid chromatographic procedure for the determination of CF.HCl as bulk drug, in pharmaceutical preparations and in the presence of its acid and alkaline degraded solutions. The mobile phase consisted of water-acetonitrile-triethylamine (80:20:0.6, v/v/v) adjusted with o-phosphoric acid to pH 3.

The above mobile system previously applied for CF.HCl was successfully used for the separation and determination of intact and degraded LF.HCl, since it showed good resolution between the eluted peaks. Detection was carried out at 328 nm. The polarity of the system was calculated⁴⁶ and found to be (10.9). The flow rate was set at 1.5 ml/min. Enrofloxacin was used as an internal standard with the elution order: degraded LF.HCl ($t_R = 2.160$), intact LF.HCl ($t_R = 2.451$), and enrofloxacin ($t_R = 2.832$) Fig. (2). The chromatogram of the mixture. shows

complete separation and good resolution.

A linear relationship was observed when the ratios of the AUPs of each component to the AUP of the internal standard were plotted versus the concentration of each component. The concentration range for each component in the mixture, regression equations, mean percentage recoveries, limit of detection (LOD) and limit of quantification (LOQ) when using the proposed methods are displayed in Table (1).

Densitometric method

Few developing systems were used for TLC separation and solvent polarity optimization of LF.HCl⁴⁷ and CF.HCl^{27&48} in the presence of either related impurities or photodegradation products in irradiated solutions. Seija Tammilehto *et al.*⁴⁸ studied the photodegradation of CF.HCl irradiated in aqueous solutions through using a mixture of acetonitrile and ammonia buffer (pH 10.6-11.1) as a developing system (6.5:3.5, v/v) for the separation of the compound and its photodegradation products.

Using methanol, instead of acetonitrile, in a different proportion in the present work, offered better separation of the drug and its acid degradation product. The polarity of the used system was calculated and found to be (5.15)⁴⁶.

The TLC chromatogram of both intact and degraded LF.HCl showed complete separation with ($R_f = 0.66$) for the intact drug and ($R_f = 0.83$) for

Table 1: Results obtained in the chromatographic methods for the determination of intact and degraded lomefloxacin hydrochloride and ciprofloxacin hydrochloride.

Item	TLC method				HPLC method	
	LF.HCl		CF.HCl		LF.HCl	
	Intact	Deg.	Intact	Deg.	Intact	Deg.
Concentration range	1-6 μg/10 μl	0.5-6 μg/10 μl	0.25-2.5 μg/10 μl	0.25-2.5 μg/10 μl	0.5-6 μg/20 μl	0.4-4.8 μg/20 μl
Mean percentage recoveries 1-Laboratory prepared mixtures:	99.60 ± 0.55	99.51 ± 0.67	99.55 ± 0.72	99.22 ± 0.69	99.68 ± 0.77	100.30 ± 0.89
2-Added standard to 'Alkaflox' or 'Serviflox' tablets	99.25 ± 0.80		99.63 ± 0.64		99.38 ± 0.70	
LOD	0.10 (μg /10μl)		0.05 (μg /10μl)		0.22 (μg /20μl)	
LOQ	0.34 (μg /10μl)		0.18 (μg /10μl)		0.74 (μg /20μl)	

the degraded product. The separated spots of both components were scanned on the same plate at 288 and 328 nm for the intact and degraded LF.HCl, respectively.

Upon applying this modified developing system to the analysis of both intact and degraded CF.HCl, the TLC chromatogram showed complete resolution with ($R_f = 0.53$) for the intact drug and ($R_f = 0.75$) for the degraded drug. The separated spots of both components were scanned on the same plate at 279 and 323 nm for the intact and degraded CF.HCl, respectively.

The concentration range for each component in the two mixtures, regression equations, mean percentage recoveries, limit of detection and limit of quantification when using the proposed methods are displayed in Table (1).

The results obtained in the determination of intact LF.HCl and CF.HCl in the presence of their acid degradation products using HPLC and densitometric methods were statistically compared to the reported methods (spectrophotometric⁴² and HPLC⁴³, respectively) showing no significant difference concerning accuracy and precision, Table (2).

Table 2: Statistical analysis for the chromatographic methods for the determination of lomefloxacin hydrochloride and ciprofloxacin hydrochloride.

Statistical Term	Lomefloxacin hydrochloride			Ciprofloxacin hydrochloride	
	HPLC method	TLC method	Reference 42	TLC method	Reference 43
Mean	99.68	99.60	99.17	99.55	99.43
SD	±0.77	±0.55	±0.60	±0.72	±0.55
%RSD within day	0.77	0.55		0.72	
%RSD between days	1.17	1.16		1.19	
S.E.	±0.31	±0.22	±0.24	±0.29	±0.23
n.	6	6	6	6	6
V	0.59	0.30	0.36	0.52	0.30
L.O.D.	0.22 (µg /20µl)	0.10 (µg /10µl)		0.05 (µg /10µl)	
L.O.Q.	0.74 (µg /20µl)	0.34 (µg /10µl)		0.18 (µg /10µl)	
t*	1.28	1.30		0.32	
F*	1.64	1.20		1.73	

*The corresponding theoretical values for t and F at p = 0.05 are 2.23 and 5.05, respectively..

Derivative spectrophotometric method

The zero-order spectra of intact and degraded LF.HCl and CF.HCl, Figs. (3&4), show complete overlapping which prevents the determination of the intact and degraded drugs in presence of each other using this technique.

In this work, a derivative spectrophotometric procedure is presented to solve this problem

through measurements in both the first and second derivative spectra. The first derivative spectra, Figs. (5&6), show that intact LF.HCl displays a peak at 272 nm and a valley at 292 nm, while the degraded product has zero crossing at both 292 and 271 nm. Thus, the ordinate value at 292 nm was chosen for the determination of the intact drug in the presence of its degradation product. In addition, the first derivative

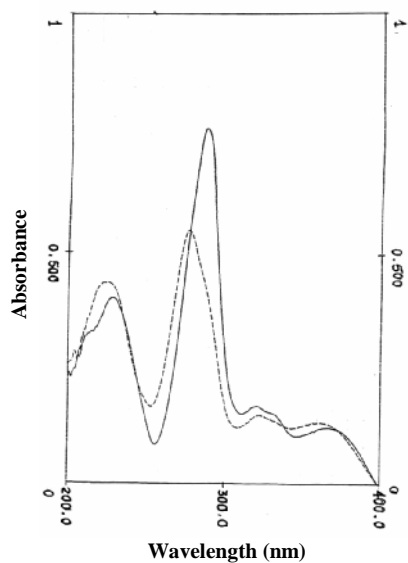


Fig. 3: Zero-order spectra of intact lomefloxacin hydrochloride (0.24 mg/50ml) (—) and of degraded drug (0.24 mg/50 ml) (---) in 2N HCl.

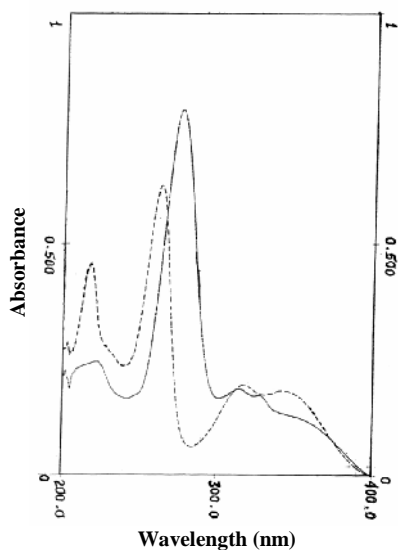


Fig. 4: Zero-order spectra of intact ciprofloxacin hydrochloride (0.56 mg/50ml) (—) and of degraded drug (0.56 mg/50 ml) (-----) in 2N HCl.

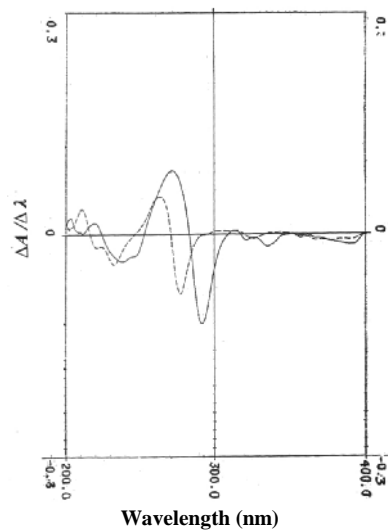


Fig. 5: First-derivative spectra of intact lomefloxacin hydrochloride (0.24 mg/50ml) (—) and of degraded drug (0.24 mg/50 ml) (---) in 2N HCl.

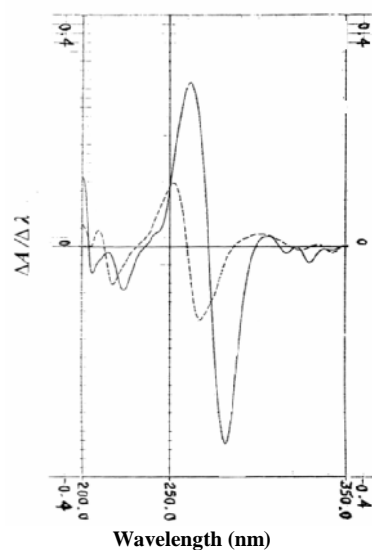


Fig. 6: First-derivative spectra of intact ciprofloxacin hydrochloride (0.56 mg/50 ml) (—) and of degraded drug (0.56 mg/50 ml) (-----) in 2N HCl.

spectrum of intact CF.HCl, Fig. (6), exhibits a peak at 262 nm and a valley at 282 nm, while its degradation product has zero crossing at both 260 and 286 nm. Consequently, the ordinate value at 260 nm was preferred for calculating the concentration of the intact drug in the presence of its degradation product.

Figures (5&6) show that the degradation product of LF.HCl or CF.HCl could not be determined through measurements in the first derivative spectra. On the other hand, in the second derivative spectra, Fig. (7), the degraded LF.HCl shows a valley at 272 nm, at which the intact drug has a zero crossing. Thus, the ordinate value at 272 nm was used for computing the concentration of the degradation product in the presence of the intact drug.

Similarly, the second derivative spectra, Fig. (8), show that the degraded CF.HCl exhibits a valley at 262 nm at which the intact drug shows no interference. Therefore, the ordinate value at 262 nm was used for calculating the concentration of the degradation product in the presence of the intact drug.

Linearity was shown over the concentration range (1.2-9.6 $\mu\text{g/ml}$) or (2.8-12.6 $\mu\text{g/ml}$) of intact LF.HCl or CF.HCl, respectively. (HPLC and Densitometric), linearity was also observed over the concentration range (1.2-9.6 $\mu\text{g/ml}$) or (6.4-16 $\mu\text{g/ml}$) of degraded LF.HCl or CF.HCl, respectively.

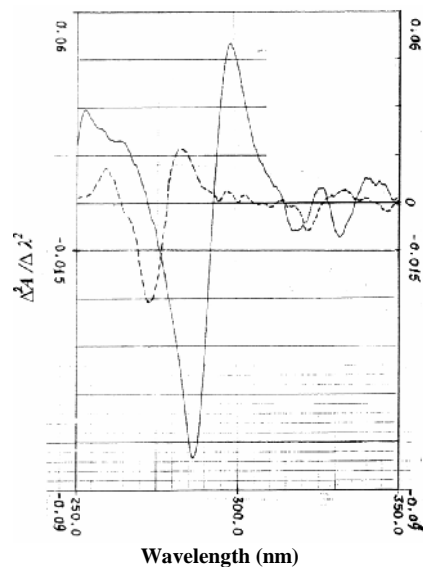


Fig. 7: Second-derivative spectra of intact lomefloxacin hydrochloride (0.24 mg/50 ml) (—) and of degraded drug (0.24 mg/50 ml) (----) in 2N HCl.

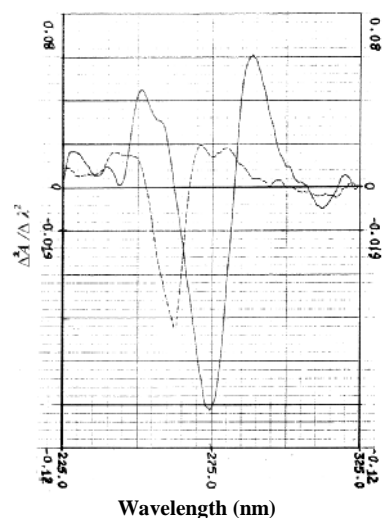


Fig. 8: Second-derivative spectra of intact ciprofloxacin hydrochloride (0.56 mg/50 ml) (—) and of degraded drug (0.56 mg/50 ml) (----) in 2N HCl.

The mean percentage recoveries of intact and degraded LF.HCl were found to be 99.72 ± 0.56 and 98.93 ± 0.85 , respectively (Table 3). Also, the mean percentage recoveries of intact and degraded CF.HCl were 99.62 ± 0.47 and 99.67 ± 0.39 , respectively (Table 3).

The method was successfully applied for the quantitative estimation of LF.HCl or CF.HCl in “Alkaflox” or “Serviflox” tablets respectively, without interference from additives. The standard addition technique was applied. The mean percentage recoveries of labelled and added LF.HCl were found to be 99.76 ± 0.75 and 99.58 ± 0.64 , respectively. Also, the mean percentage recoveries of labelled and added CF.HCl were

99.49 ± 0.66 and 99.78 ± 0.34 , respectively. The second derivative spectra of “Alkaflox” and “Serviflox” tablets showed complete absence of the corresponding acid degradation product.

The proposed methods are valid and applicable for the determination of intact LF.HCl or CF.HCl in laboratory prepared mixtures containing (20-80%) or (40-75%) of the degraded LF.HCl or degraded CF.HCl, respectively.

Statistical comparison between the proposed and the reported methods (spectrophotometric⁴² and HPLC⁴³, for LF.HCl and CF.HCl, respectively) showed no significant difference between them concerning accuracy and precision (Table 3).

Table 3: Statistical analysis for the first derivative spectrophotometric determination of lomefloxacin hydrochloride and ciprofloxacin hydrochloride.

Statistical Term	Lomefloxacin hydrochloride		Ciprofloxacin hydrochloride	
	First Derivative method	Comparison method ¹³	First Derivative method	Comparison method ¹²
Mean	99.72	99.17	99.62	99.43
SD	± 0.56	± 0.60	± 0.47	± 0.55
%RSD	0.56		0.47	
S.E.	± 0.21	± 0.24	± 0.18	± 0.22
n	7	6	7	6
V	0.31	0.36	0.22	0.30
LOD ($\mu\text{g/ml}$)	0.39		1.03	
LOQ ($\mu\text{g/ml}$)	1.29		3.45	

Identification of the degradation products of lomefloxacin hydrochloride and ciprofloxacin hydrochloride

The degradation product of each of LF.HCl and CF.HCl was separated and crystallized from diethylether and has a melting point at 100°C or 192°C, respectively.

The structure for each degradation product was confirmed by spectral analysis (I.R., KBr disc), ¹H NMR, (DMSO) and MS.

Degradation product of LF.HCl

IR: 1620 cm⁻¹ (C=O of pyridone).

¹H NMR (DMSO): 0.9 (d, 3H), 1.3 (t, 3H), (2.7- 3.1) (m, 7H), 4.2 (q, 2H), 6 (d, 1H), 7.6 (d, 1H), 7.9 (d, 1H); M S (M⁺, 307).

Degradation product of CF.HCl

IR: 1620 cm⁻¹ (C=O of pyridone).

¹H NMR (DMSO): (1-1.2) (m, 4H), (2.8-3.1) (m, 8H), 3.4 (t, 1H), 5.8 (d, 1H), 7.3 (d, 1H), 7.6 (d, 1H), 7.8 (d, 1H); MS (M⁺, 287).

The previous data revealed the absence of carboxylic acid group in both degraded LF.HCl and CF.HCl (Fig. 1) which played a vital role in the antibacterial activity of the drugs.

The following structures for the degradation products were suggested (Fig. 1).

Conclusion

A reversed phase HPLC method has been developed for the determination of lomefloxacin and its acid degradation product. In addition, two other methods have been

developed for the determination of lomefloxacin hydrochloride (LF.HCl) and ciprofloxacin hydrochloride (CF.HCl) in presence of their acid induced degradation products. The developed methods are rapid, simple and accurate. The developed methods are recommended for the routine analysis of cited drugs in dosage forms and in drug control laboratories.

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