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Second Derivative Synchronous Spectrofluorimetric Determination of Lomefloxacin Hydrochloride in Presence of Its Decarboxylated Degradation Product

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ABSTRACT

Lomefloxacin hydrochloride is a third generation fluoroquinolone antibiotic. In the present study, a rapid, specific and highly sensitive second derivative synchronous spectrofluorimetric method has been developed and validated for the determination of lomefloxacin hydrochloride in the presence of its decarboxylated degradation product. The drug was found to be labile to acidic degradation using reflux with 2M HCl for 10 hours. Complete degradation of the drug and the degradation pathway was confirmed using TLC, IR, ^{1}H NMR and mass spectrometry. The linear regression analysis data for the calibration curve shows a good relationship in the range of 0.5 –3.5 µg/ml. The developed method was validated according to the International Conference on Harmonization (ICH) guidelines demonstrating good accuracy and precision. The results were statistically compared with those obtained by the reported method, and no significant difference was found.

INTRODUCTION:

Lomefloxacin hydrochloride is *(RS)*-1-ethyl-6,8-difluoro-1,4-dihydro-7-(3-methylpiperazin-1-yl)-4-oxoquinoline-3-carboxylic acid hydrochloride (Fig. 1). Lomefloxacin hydrochloride is a fluorinated 4-quinolone or fluoroquinolone antibacterial with a wider spectrum of activity than nalidixic acid and more favorable pharmacokinetics allowing its use in systemic infections. It has been used in the treatment of infections including bone and joint infections, gastroenteritis (including travelers' diarrhea and campylobacter enteritis, cholera, salmonella enteritis, and shigellosis), gonorrhea, infections in immune compromised patients (neutropenia), Q fever, lower respiratory-tract infections, typhoid and paratyphoid fever.[1] Lomefloxacin hydrochloride is white powder soluble in water and methanol with 387.81 molecular weight.[2] It's nonofficial drug, but literature survey reveals that many HPLC methods were reported for determination of lomefloxacin hydrochloride in pharmaceutical preparations and biological fluids.[3-11] Also, atomic absorption[11], spectrophotometric[12-19], spectrofluorimetric[20-24] and electrochemical[25-30] methods were reported for determination of lomefloxacin hydrochloride alone or in presence of other fluoroquinolone antibiotics. Reviewing the literature on the determination of lomefloxacin hydrochloride revealed the lack of any spectrofluorimetric method for the determination of the intact drug in presence of its acid-induced degradation product. The aim of this work was to develop and validate simple, sensitive and selective second derivative synchronous spectrofluorimetric method for the determination of lomefloxacin hydrochloride in presence of its decarboxylated degradation product.

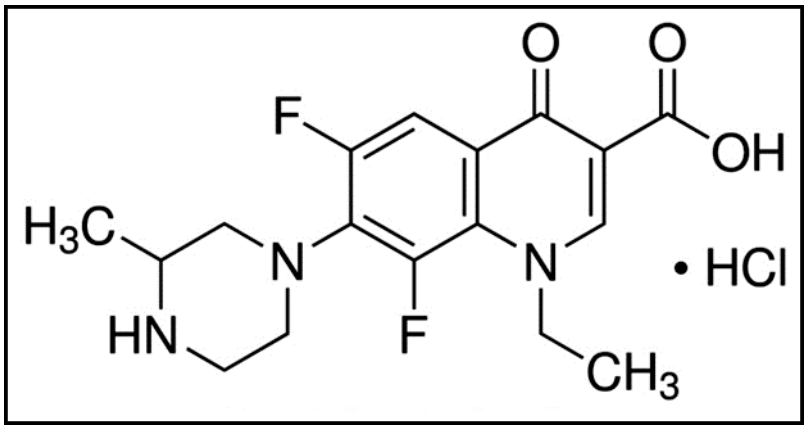

Fig. 1: Structural formula of Lomefloxacin Hydrochloride

MATERIALS AND METHODS

Instruments:

- Jasco FP-6200 Spectrofluorometer (Japan), equipped with 150 Watt Xenon lamp. Slit widths for both monochromators were set at 10 nm. A 1 cm quartz cell was used. All measurements were done with medium sensitivity.
- Hotplate (Torrey Pines Scientific, USA)
- Jenway, 3510 pH meter (Jenway, USA)
- Rotatory evaporator (Scilogex-RE 100-pro, USA)
- NMR, Gemini-400 BB (Agilent, USA)
- FT-IR, Nicolet IR 200 (Thermo electron corporation, USA)
- GCMS-QP-1000 EX mass spectrometer at 70 ev (Shimadzu, Tokyo, Japan)

Materials and reagent:

All reagents used were of analytical grade, solvents were of HPLC grade and water used throughout the procedure was freshly distilled.

- Pure lomefloxacin hydrochloride (99.65 %) was kindly provided by Sigma pharmaceutical industries - Quesna City – Egypt, S.A.E.
- Lomex® tablet: labeled to contain 442 mg lomefloxacin hydrochloride per tablet equivalent to 400 mg lomefloxacin, manufactured by Sigma pharmaceutical industries - Quesna City – Egypt, S.A.E.; (batch number 40357), purchased from local market.
- Acetonitrile, ammonia (30%), chloroform, ethanol, methanol, 1-propanol and tetrahydrofuran, all of HPLC grades (Sigma-Aldrich, Germany).
- Hydrochloric acid and potassium hydroxide (El-Nasr Company, Egypt), prepared as 2M aqueous solutions.
- Ammonium acetate (El-Nasr Company, Egypt), prepared as 0.3M aqueous solution.

- Potassium chloride, potassium biphthalate, sodium acetate, monobasic potassium phosphate, boric acid, glacial acetic acid and sodium hydroxide(El-Nasr Company, Egypt).

- Buffers of different pH values prepared as prescribed in US pharmacopeia:[31]

1. Potassium chloride and hydrochloric acid buffer pH 2.
2. Acid phthalate buffer pH 3.
3. Acetate buffer pH range from 4 to 5.
4. Phosphate buffer pH range from 6 to 8.
5. Alkaline borate buffer pH range from 9 to 10.

Standard solutions:

A stock standard solution of lomefloxacin hydrochloride (1 mg/ml) was prepared by dissolving 100 mg of the drug powder in 50 ml of water and the volume was completed to 100 ml with water. Working standard solution (10 µg/ml) was prepared by dilution of the stock solution with water.

Standard solution of degraded lomefloxacin hydrochloride:

100 mg of pure lomefloxacin hydrochloride powder was treated with 25 ml of 2M HCl in a 100-ml round bottomed flask, the solution was heated under reflux for 10 hours. After cooling, the solution was neutralized to pH = 7-8 using 2M KOH and evaporated to dryness under vacuum. The obtained residue was extracted three times with 25 ml of methanol, filtered into 100-ml volumetric flask and diluted to volume with methanol to obtain a stock solution labeled to contain degradation product derived from 1 mg/ml of lomefloxacin hydrochloride.[17] Working solution of degradation product (10 µg/ml) was obtained by dilution of the stock solution with water.

Procedures:

General procedure:

Aliquots of standard lomefloxacin hydrochloride solution (10 µg/ml) containing (5–35 µg) were transferred into a series of 10 ml volumetric flasks and 1 ml of acetate buffer (pH= 4)

was added. The solutions were diluted with water to 10 ml and mixed well. The synchronous fluorescence spectra were measured by scanning both monochromators at a constant wavelength difference (Δλ) = 160 nm. The second derivative corresponding to each synchronous spectrum was recorded using data points = 15. The peak amplitudes of the second derivative were measured at 446 nm. A blank experiment was performed simultaneously. A calibration graph was constructed by plotting the peak amplitudes of the second derivative at 446 nm versus drug concentrations in µg/ml and the regression equation was computed.

Optimization of experimental conditions:

(i) Selection of optimum Δλ: The general procedure was repeated using a fixed amount of lomefloxacin hydrochloride (25 µg) at different Δλ (100-300 nm), then comparing the synchronous fluorescence intensity values.

(ii) Effect of diluting solvents: The general procedure was repeated using a fixed amount of lomefloxacin hydrochloride (25 µg) and different diluting solvents, then comparing the synchronous fluorescence intensity values at 446 nm.

(iii) Effect of pH and buffer: The general procedure was repeated using a fixed amount of lomefloxacin hydrochloride (25 µg) and different buffers with different pH, then comparing the synchronous fluorescence intensity values at 446 nm.

(iv) Effect of buffer volume: The general procedure was repeated using a fixed amount of lomefloxacin hydrochloride (25µg) and different volumes of acetate buffer (pH 4), then comparing the synchronous fluorescence intensity values at 446 nm.

(v) Effect of time: The general procedure was repeated using a fixed amount of lomefloxacin hydrochloride (25 µg)at different time interval (0-60 min.), then comparing the synchronous fluorescence intensity values at 446 nm.

Application to laboratory prepared mixtures:

The general procedure was repeated using aliquots of lomefloxacin hydrochloride solution (10 µg/ml) containing (30–5 µg) with aliquots of lomefloxacin hydrochloride degradation product solution (10 µg/ml) containing (5–30 µg). Lomefloxacin hydrochloride concentrations were calculated from the corresponding regression equation.

Application to pharmaceutical formulation:

Ten **Lomex**® tablets (442 mg/tablet) were weighed and finely powdered. Appropriate weight of powder equivalent to 10 mg of lomefloxacin hydrochloride was accurately weighed, transferred to 100-ml volumetric flask and the volume was made up to 75 ml with water. The solution was shaken vigorously for 15 min then sonicated for 30 min and then filtered. The volume was completed to 100 ml with water to obtain a concentration of 100 µg/ml then diluted with the same solvent to obtain a concentration of 10 µg/ml. Repeat the general procedure using aliquots covering the working concentration range.

RESULTS AND DISCUSSION:

In the present study, a simple and sensitive second derivative synchronous spectrofluorimetric procedure was suggested for selective quantitative determination of lomefloxacin hydrochloride in presence of its decarboxylated degradation product without previous separation. Synchronous fluorescence spectroscopy (SFS) was first described by Lloyd in 1971.[32] It involves the simultaneous scan of the excitation and emission monochromators. Depending on the scan rate, three basic types of SFS technique are possible: constant-wavelength, constant-energy and variable-angle. Constant-wavelength SFS is the basic type and the most widely used of all synchronous modes, where a constant wavelength interval (Δλ) was kept between the excitation and emission monochromators.[32]

Synchronous fluorescence spectroscopy has several advantages over conventional fluorescence, including; narrowing of spectral band, simplification of emission spectra and contraction of spectral range. The sharpness and narrowness of the peak of a SFS spectrum, compared to those of conventional spectrum, make it more selective and useful to analyze multi-component mixtures without pre-separation procedures.[33]

Degradation of lomefloxacin hydrochloride:

It was reported that complete degradation of lomefloxacin hydrochloride was achieved upon heating under reflux with 2M hydrochloric acid for 10 hours to give its decarboxylated degradation product as shown in the following scheme. [17]

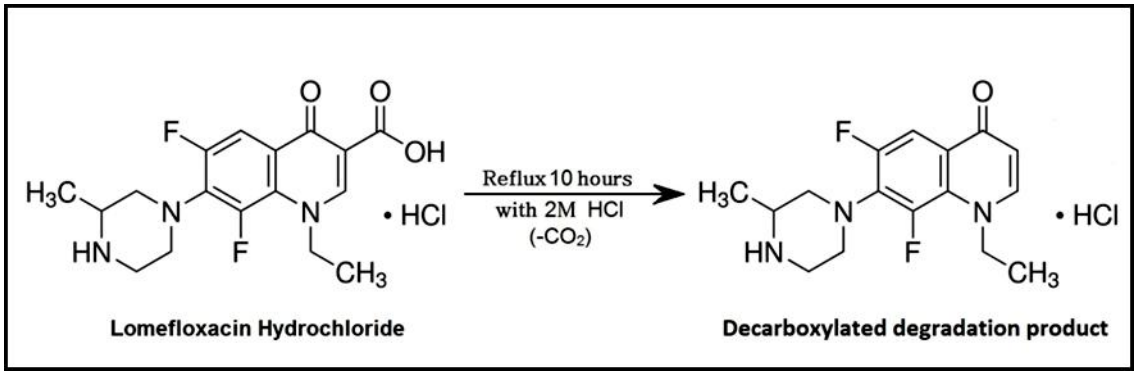

Fig. 2: Proposed degradation pathway of intacat lomefloxacin hydrochloride

Confirmation of complete degradation using TLC technique:

Time required for complete degradation was exactly determined by spotting on TLC plates every 30 minutes using mobile phase system consists of 0.3 M ammonium chloride solution: conc. ammonia solution: 1-propanol (1:1:8, by volume), complete degradation of lomefloxacin hydrochloride was confirmed by absence of spot in the region of the degradation product corresponds to the spot of the intact drug.

Confirmation of degradation product using IR techniques:

IR spectrum of the intact lomefloxacin hydrochloride in Fig. 3, showed peak of (C=O) of carboxyl group (-COOH) at 1726.31 cm^{-1}, while IR spectrum of degradation product in Fig. 4, showed disappearance of (C=O) stretch of carboxyl group which indicates the cleavage of carboxylic acid linkage.

Confirmation of degradation product using ^{1}H NMR techniques:

The ^{1}H NMR of the intact lomefloxacin hydrochloride in dimethyl sulfoxide (DMSO) in Fig. 5, showed triplet signal of three protons of aliphatic ($-CH_3$) in ethyl group at 1.323 - 1.358 ppm, doublet signal of three protons of methyl group ($-CH_3$) attached to piperazine ring at 1.745 - 1.762 ppm, multiplet signals of seven protons in piperazine ring at 3.546 – 4.206 ppm, multiplet signal of two protons ($-CH_2-$) in ethyl group at 4.632 - 4.685 ppm, singlet signal of one proton in benzene ring at 7.796 ppm, singlet signal of one proton in pyridine ring at 8.902 ppm, doublet signal of two protons (NH_2) in piperazine ring at 10.198 - 10.315 ppm and singlet signal of one proton (-COOH) in carboxyl group at 11.783 ppm.

The ^{1}H NMR of the degradation product in dimethyl sulfoxide (DMSO) in Fig. 6, showed disappearance of (-COOH) carboxyl group singlet signal at 11.783 ppm indicating the cleavage of carboxylic acid linkage.

Confirmation of degradation product using mass spectrometry:

Mass spectrometry was performed for the intact drug and its degradation product and molecular ion peak was obtained at m/z = 387.12 and m/z = 343.13, respectively indicating that the molecular weight of the degradation product is 343.13 as shown in Fig. 7 and 8.

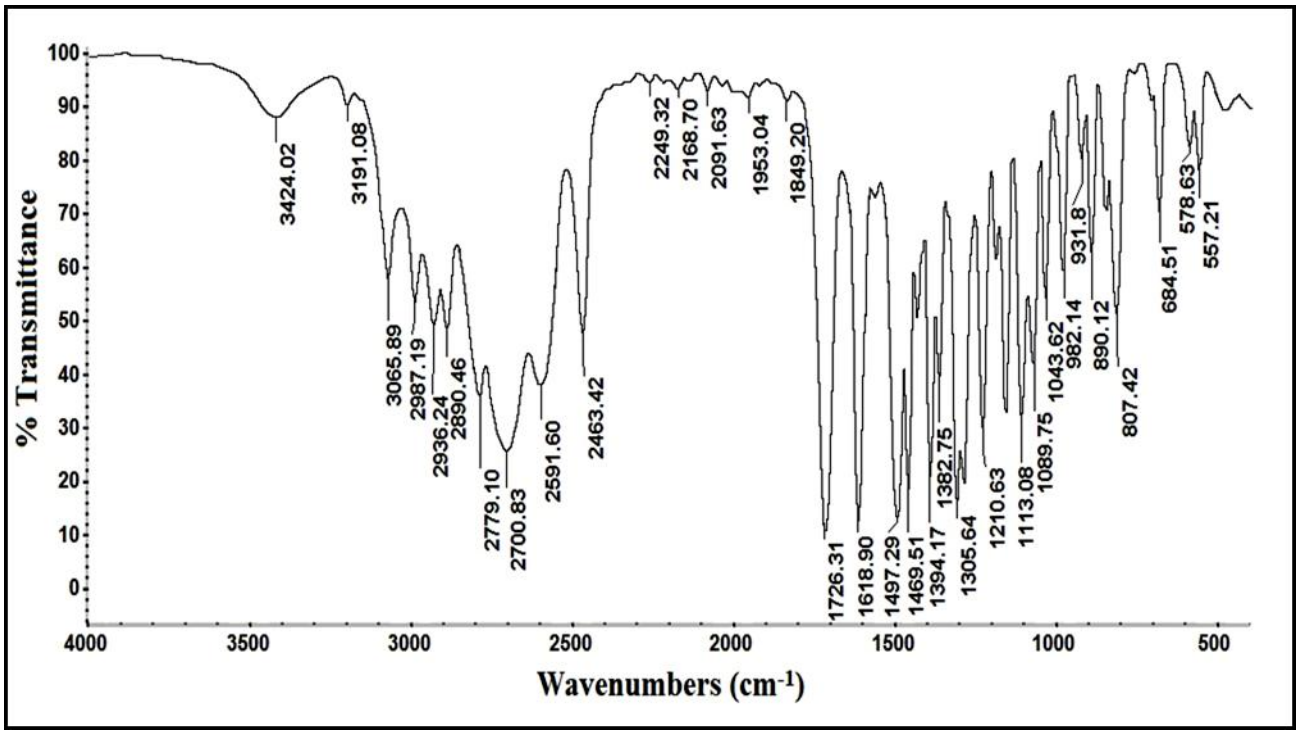

Fig. 3: IR spectrum of intact lomefloxacin hydrochloride

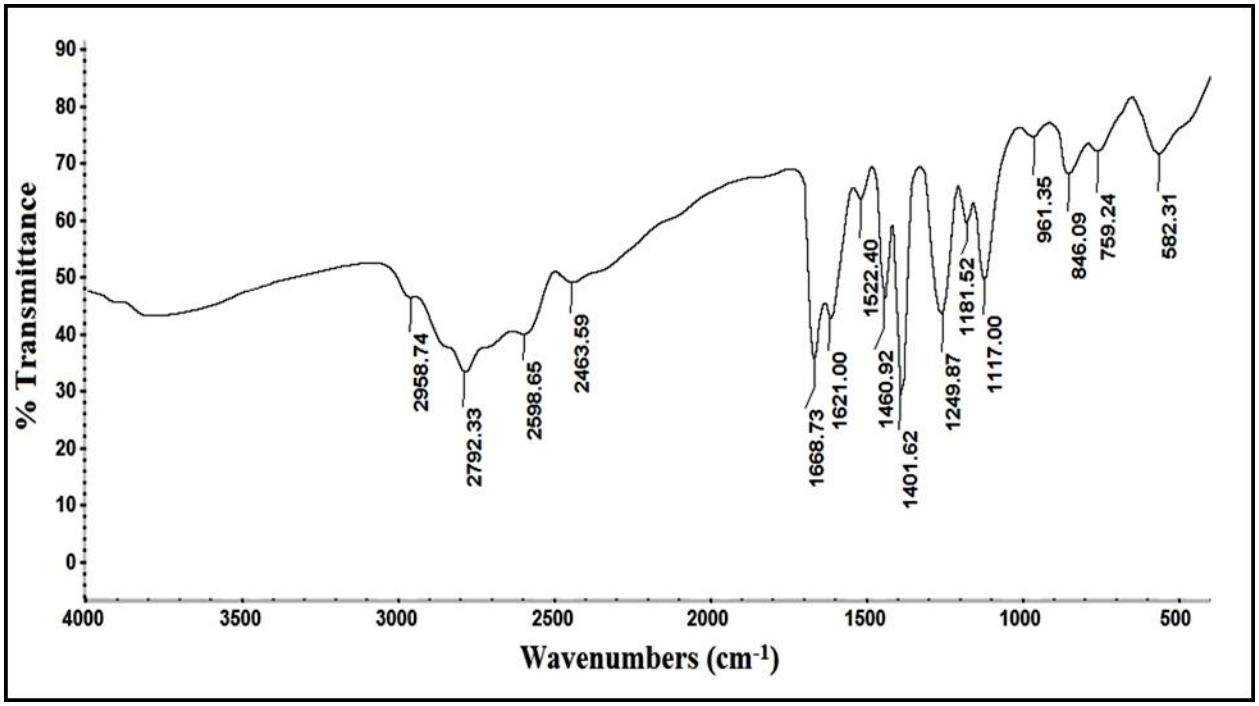

Fig. 4: IR spectrum of lomefloxacin hydrochloride degradation product

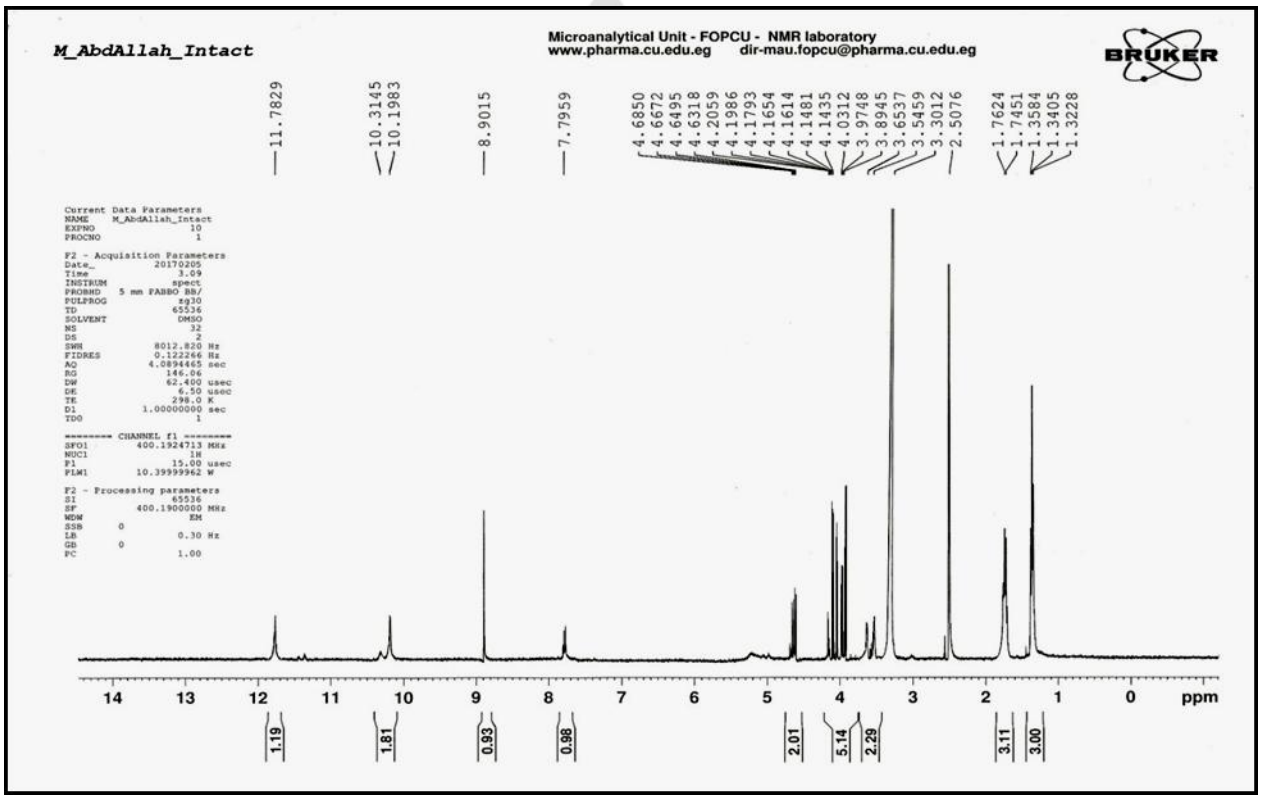

Fig. 5: ^{1}H NMR spectrum of intact lomefloxacin hydrochloride in (DMSO).

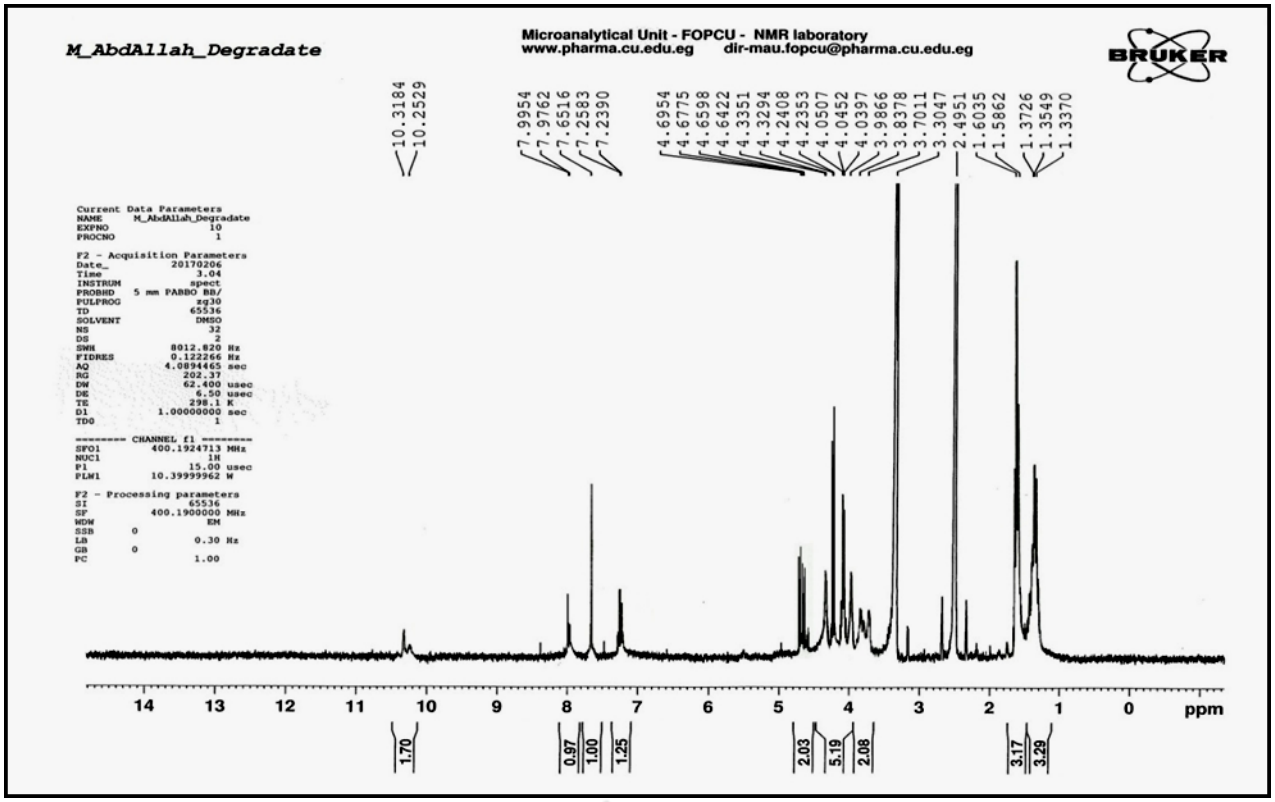

Fig. 6: ^{1}H NMR spectrum of lomefloxacin hydrochloride degradation product in (DMSO)

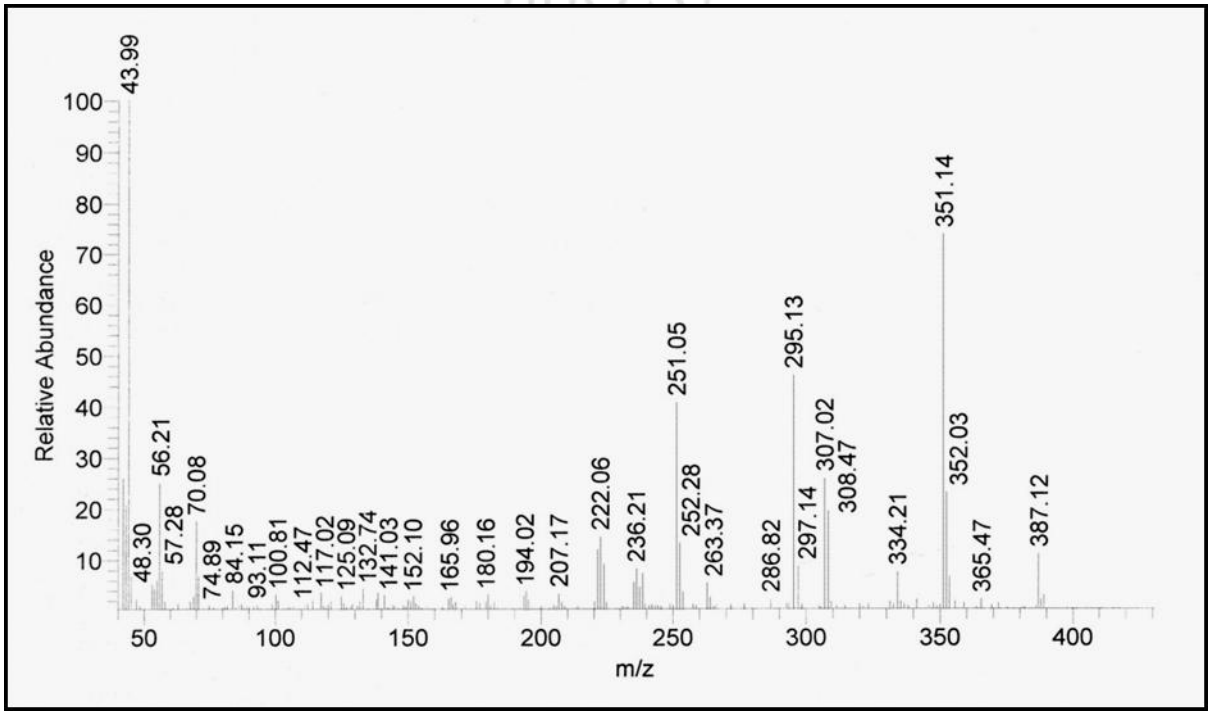

Fig. 7: Mass spectrum of intact lomefloxacin hydrochloride

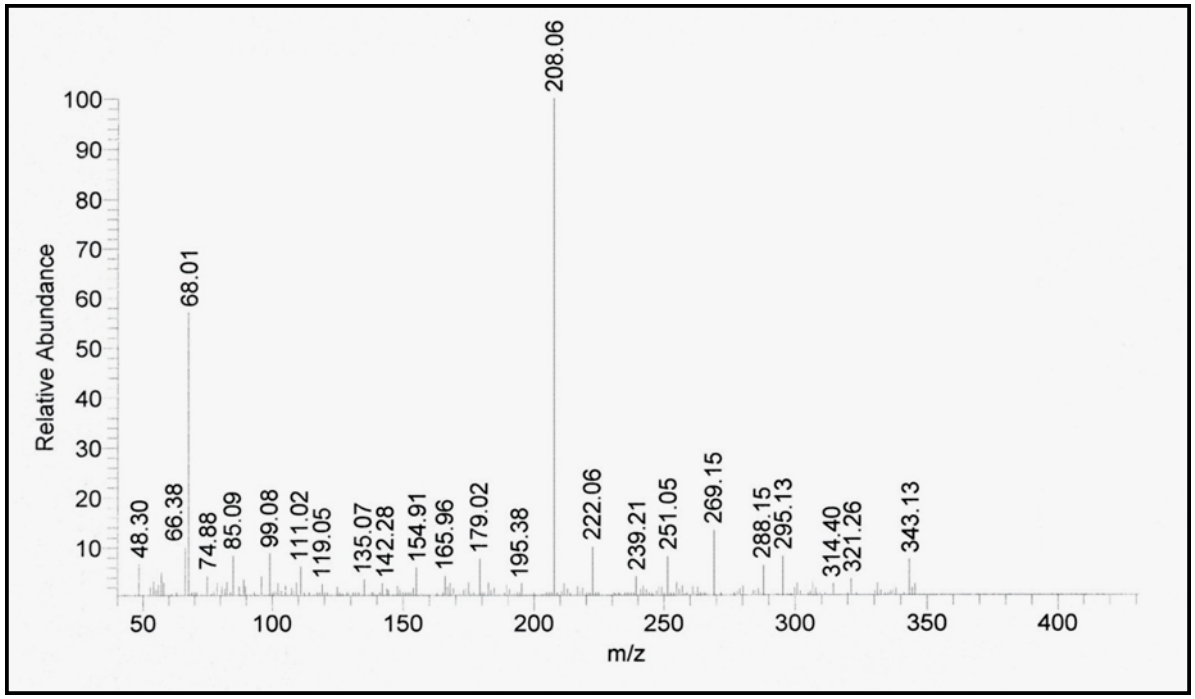

Fig. 8: Mass spectrum of lomefloxacin hydrochloride degradation product

Spectral characteristics:

Lomefloxacin hydrochloride and its degradation product exhibit native fluorescence. Lomefloxacin hydrochloride emission can be measured at 452 nm ($\lambda_{ex.}$ = 284 nm) and the excitation and emission spectra of lomefloxacin hydrochloride in water are shown in Fig. 9. The emission spectra of lomefloxacin hydrochloride and its degradation product are overlapped, as shown in Fig. 10 which hindered the use of normal fluorescence for determination of lomefloxacin hydrochloride in presence of its degradation product. Moreover, the derivatization of normal fluorescence spectra does not solve the problem. Such overlapping can be resolved by measuring the synchronous fluorescence at $\Delta\lambda$=160 nm, then deriving the second derivative (the first derivative is not suitable). The resulted in second derivative synchronous fluorescence spectra of lomefloxacin hydrochloride and its degradation product allow the determination of lomefloxacin hydrochloride in presence of its degradation product at 446 nm, as shown in **Fig. 11-13**.

Optimization of experimental conditions:

Different parameters which affect the synchronous fluorescence intensity of lomefloxacin hydrochloride were carefully studied and optimized. Such factors were changed individually

while others were kept constant. These factors included; Δλ selection, pH, buffer volume, type of the diluting solvent and stability time.

The optimum Δλ value is important for performing the synchronous fluorescence scanning technique with regard to its resolution, sensitivity and features. It can directly influence spectral shape, bandwidth and signal value. Δλ of 160 was chosen as optimal for the separation since it resulted in two distinct peaks with good regular shapes and reduced the spectral interference caused by each compound.

Water was found to be the best diluting solvent and maximum synchronous fluorescence intensity was achieved by using 1 ml of acetate buffer (pH 4) to adjust pH, as shown in **Fig. 14-16**. The synchronous fluorescence was immediately developed and remained stable at least for one hour, as shown in **Fig. 17**.

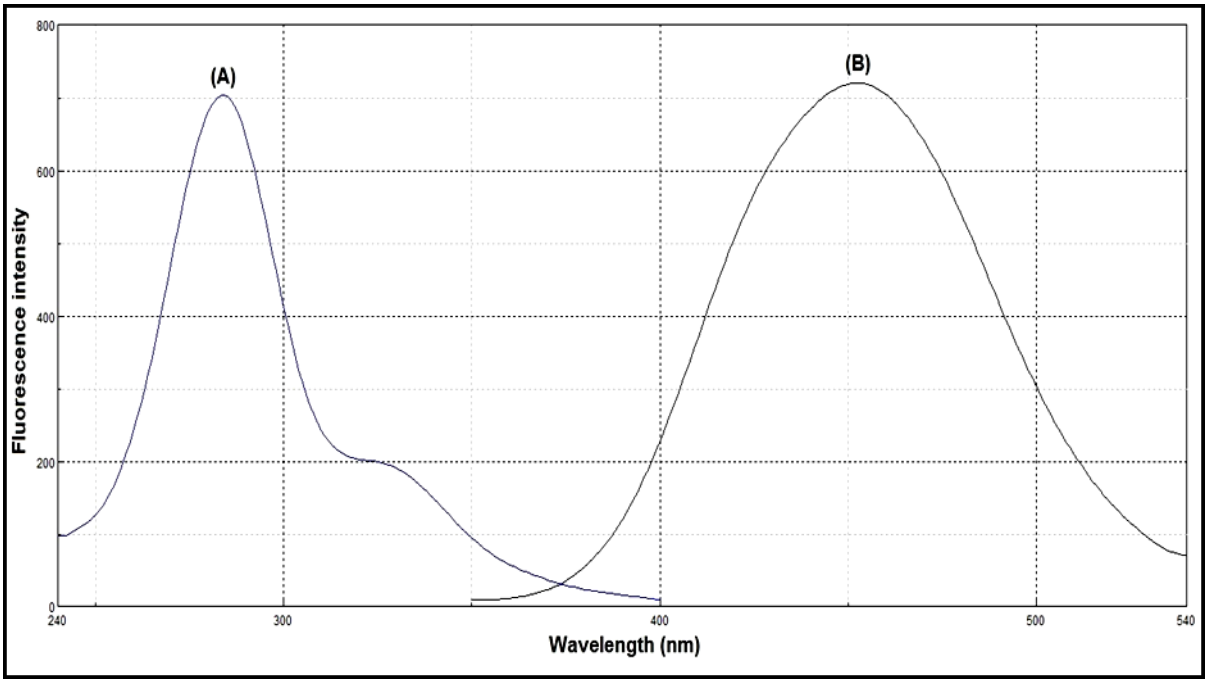

Fig. 9: (A) Excitation and (B) emission spectra of lomefloxacin hydrochloride (2.5 µg/ml) in water

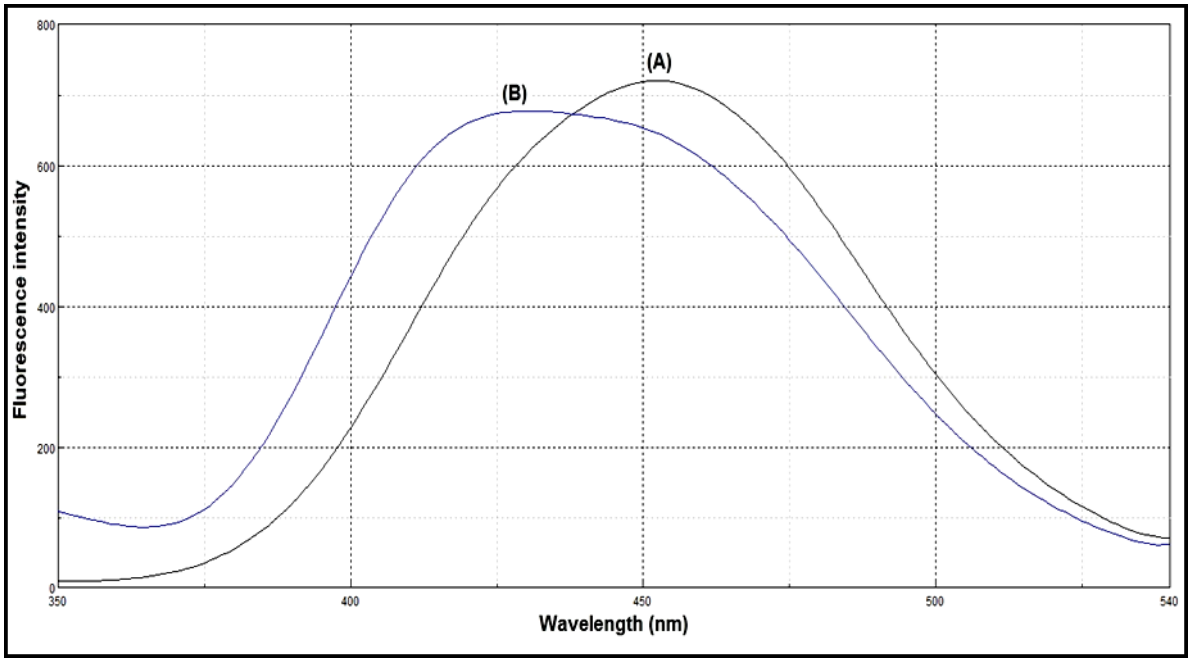

Fig. 10: Emission spectra of (A) lomefloxacin hydrochloride (2.5 µg/ml) and (B) lomefloxacin hydrochloride degradation product (2.5 µg/ml), in water

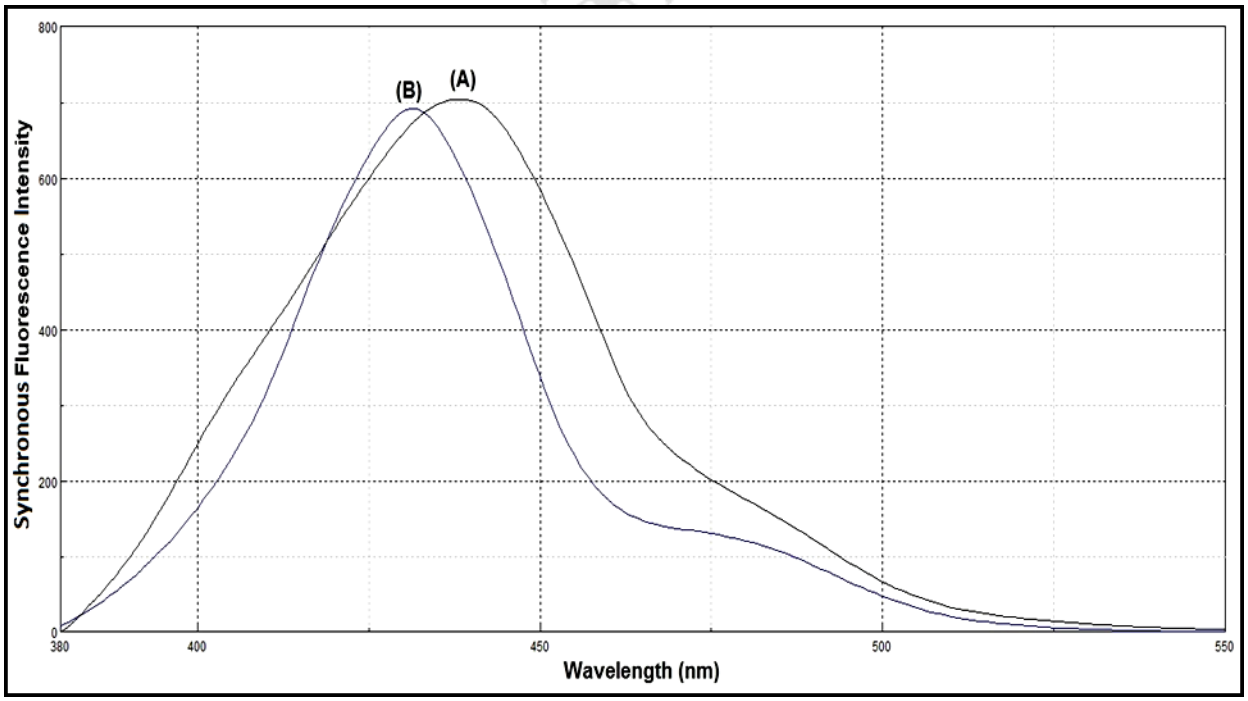

Fig. 11: Synchronous fluorescence spectra of (A) lomefloxacin hydrochloride (2.5 µg/ml) and (B) lomefloxacin hydrochloride degradation product (2.5 µg/ml), in water using Δλ=160 nm

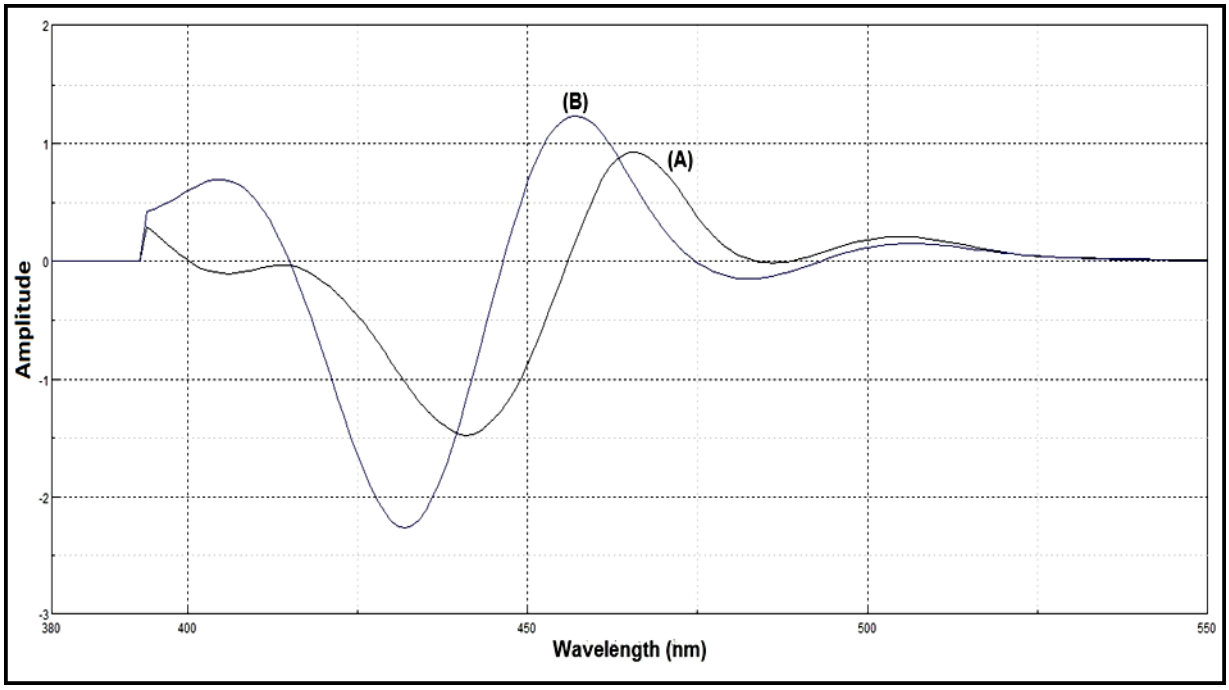

Fig. 12: Second derivative synchronous fluorescence spectra of (A) lomefloxacin hydrochloride (2.5 µg/ml) and (B) lomefloxacin hydrochloride degradation product (2.5 µg/ml) in water using Δλ=160 nm

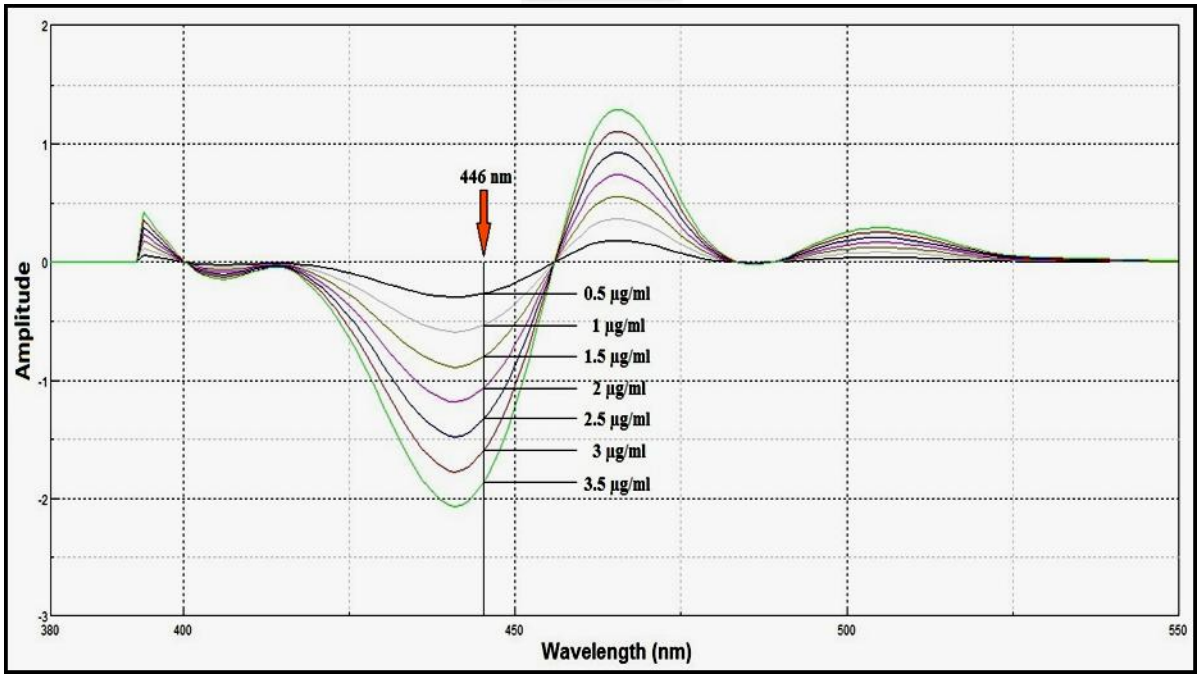

Fig. 13: Second derivative synchronous fluorescence spectra of lomefloxacin hydrochloride at different concentrations (0.5 – 3.5 µg/ml) in water using Δλ=160 nm

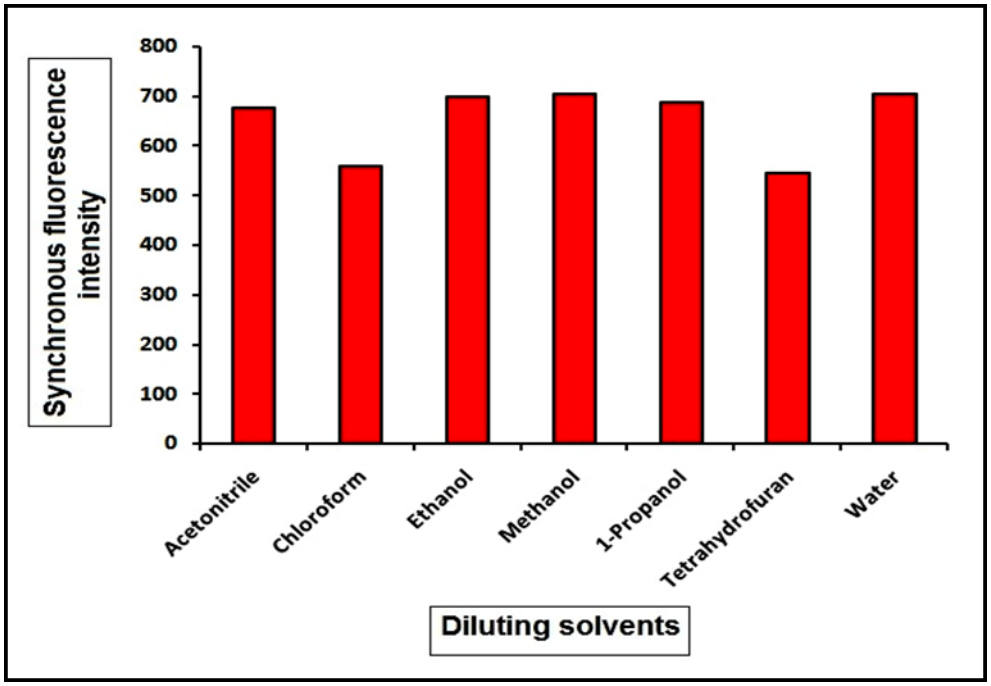

Fig. 14: Effect of different diluting solvents on synchronous fluorescence intensity of lomefloxacin hydrochloride (2.5 µg/ml)

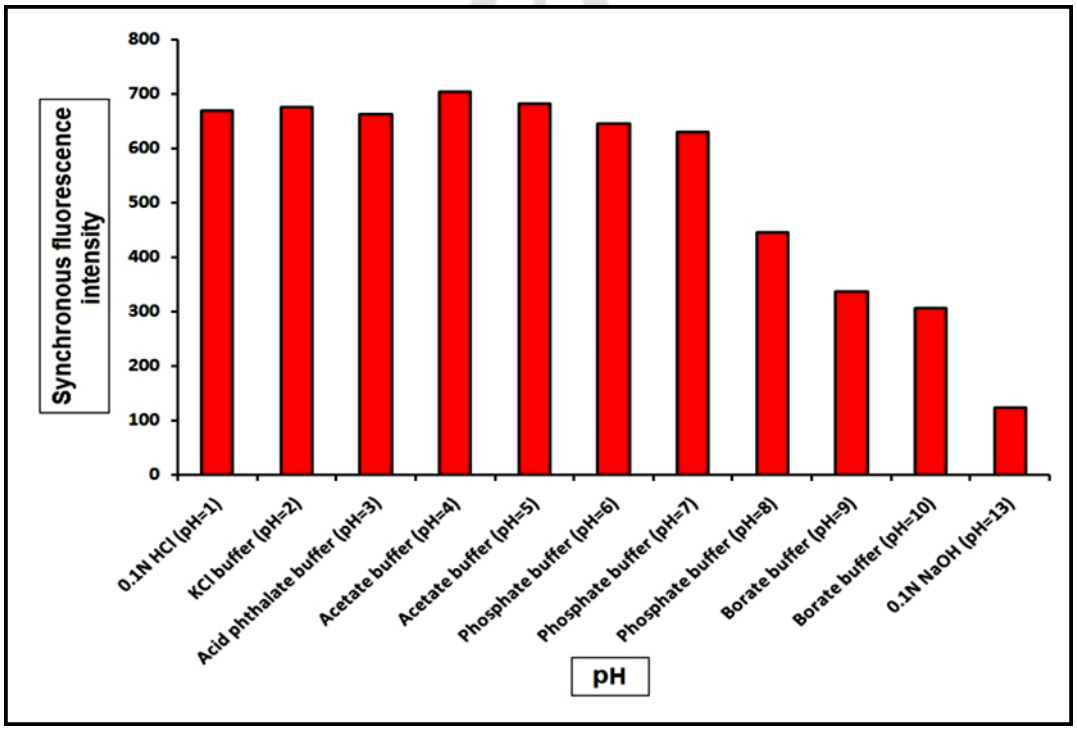

Fig. 15: Effect of pH on synchronous fluorescence intensity of lomefloxacin hydrochloride (2.5 µg/ml)

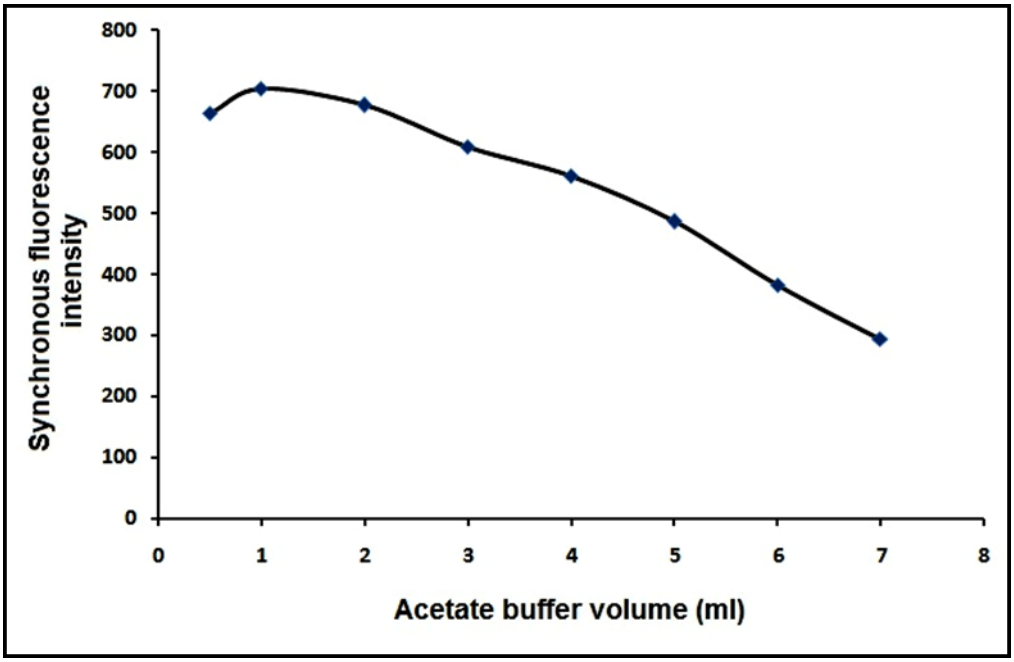

Fig. 16: Effect of acetate buffer volume on synchronous fluorescence intensity of lomefloxacin hydrochloride (2.5 µg/ml)

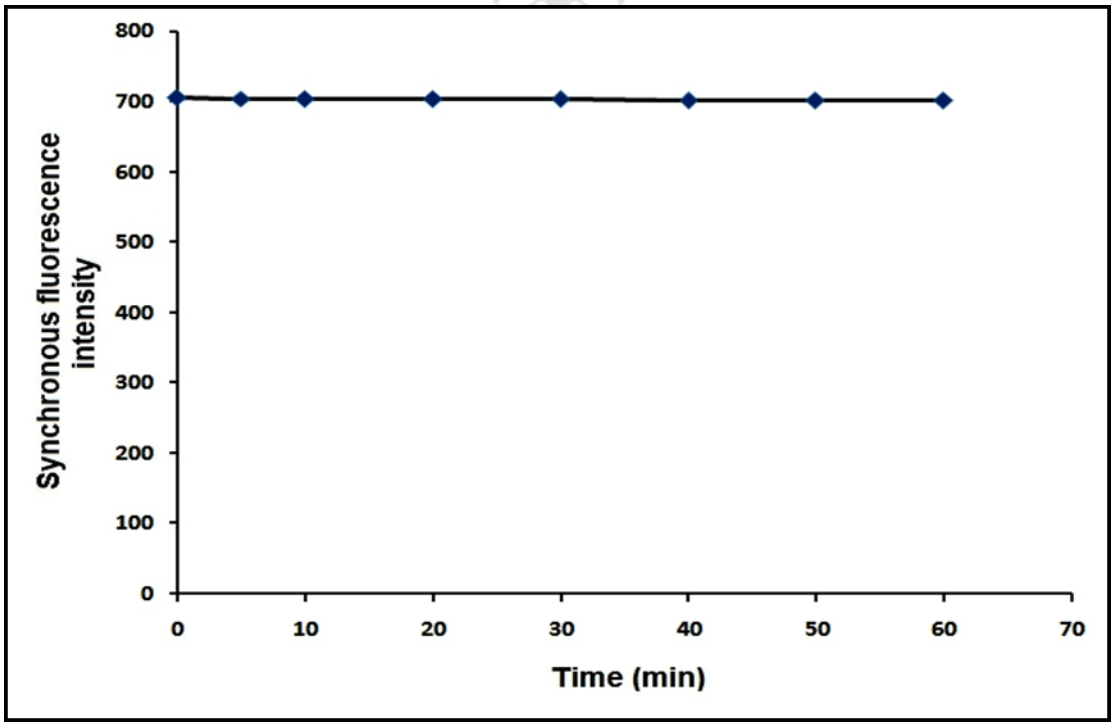

Fig. 17: Effect of time on synchronous fluorescence intensity of lomefloxacin hydrochloride (2.5 µg/ml)

3.4. Methods validation:

Validations of the proposed method were assessed as per the ICH guidelines.[34]

- **Linearity and range:**

Calibration graph was constructed by plotting the amplitudes of the second derivative of the synchronous spectra at 446 nm versus drug concentrations in µg/ml. The regression plot was found to be linear over the range of 0.5-3.5 µg/ml. The linear regression equation for the graph was:

$$y = 0.5047x + 0.0116 \quad (r = 0.9996)$$

Where ***y*** is the peak amplitude of the second derivative of the synchronous fluorescence spectrum at 446 nm, ***x*** is the drug concentration in µg/ml and ***r*** is the correlation coefficient. Linearity range, regression equation, intercept, slope and correlation coefficient for the calibration data were presented in **table (1)**.

- **Limits of detection and quantitation:**

The limits of detection (LOD) and the limits of quantitation (LOQ) were calculated according to ICH guidelines from the following equations:

$$LOD = 3.3\ \sigma / S \qquad LOQ = 10\ \sigma / S$$

Where σ is the residual standard deviation of a regression line and S is the slope of the calibration curve. LOD and LOQ values were mentioned in **table (1)** and indicate good sensitivity of the method.

- **Accuracy and precision:**

Accuracy and precision of the methods were determined by applying the proposed procedure for determination of three different concentrations, each in triplicate, of lomefloxacin hydrochloride in pure form within linearity range in the same day (intraday) and in three successive days (interday). Accuracy of percent recovery (%R) and precision as percent relative standard deviation (%RSD) was calculated and results are listed in **table (1)**. To ascertain the accuracy of the suggested methods, recovery studies were carried out by

standard addition technique at four different levels in **table (2)**. From these data, the method shows high accuracy and precision.

Specificity:

The specificity of the proposed procedure was assured by applying it to laboratory prepared mixtures of lomefloxacin hydrochloride and its degradation product. The proposed procedure was adopted for the selective determination of lomefloxacin hydrochloride in presence of up to 85.71 % of its degradation product. The percentage recovery ± RSD % was 99.78 ± 1.539, as shown in **table (3)**.

- **Robustness:**

The robustness of the method was evaluated by slight changes in the optimum conditions such as Δλ (±1 nm), pH (±0.1) and acetate buffer volume (±0.1 ml). In each case, only one parameter was changed while other parameters were kept constant. These minor changes did not have any significant effect on the synchronous fluorescence intensity and % RSD of the responses were <2 % confirming robustness of the procedure, as shown in **table (1)**.

Table (1): Regression and validation data for the determination of lomefloxacin hydrochloride by the proposed second derivative synchronous spectrofluorimetric method:

Parameters	**Second derivative synchronous spectrofluorimetric method**
Wavelength (nm)	446
Linearity range	0.5 ─ 3.5 (µg/ml)
LOD	0.076 (µg/ml)
LOQ	0.229 (µg/ml)
- Regression Equation - Slope (b) - Intercept (a)	$y^{a} = b\,x^{b} + a$ 0.5047 0.0116
Correlation coefficient (r)	0.9996
Accuracy (% R)	99.87
Precision (% RSD) Repeatability[c] Intermediate precision[d]	 1.406 1.117
Robustness (% RSD) - Δλ (±1 nm) - pH (±0.1) - Acetate buffer volume (±0.1 ml)	 1.356 1.089 0.572

[a] The peak amplitude of the second derivative of synchronous fluorescence spectra.

[b] Concentration in µg/ml.

[c] The intraday (n = 3), average of three concentrations of lomefloxacin hydrochloride (1, 2 and 3µg/ml) repeated three times within the day.

[d] The interday (n = 3), average of three concentrations of lomefloxacin hydrochloride (1, 2 and 3µg/ml) repeated three times in three days.

Table (2): Recovery study of lomefloxacin hydrochloride by adopting standard addition technique using the proposed second derivative synchronous spectrofluorimetric method:

Pharmaceutical taken (µg/ml)	Pure added (µg/ml)	Pure found (µg/ml)	% Recovery
1	0.5	0.51	101.83
	1	0.99	99.20
	1.5	1.51	100.54
	2	1.99	99.47
Mean			100.26
% RSD			1.193

Table (3): Determination of lomefloxacin hydrochloride in mixtures with its degradation product by the proposed second derivative synchronous spectrofluorimetric method:

Intacat (µg/ml)	Degradate (µg/ml)	% Degradate	Intacat found (µg/ml)	% Recovery of Intacat
3	0.5	14.29	2.94	98.11
2.5	1	28.57	2.51	100.33
2	1.5	42.86	1.98	99.05
1.5	2	57.14	1.53	101.64
1	2.5	71.43	1.01	100.92
0.5	3	85.71	0.49	98.63
Mean				99.78
% RSD				1.539

Pharmaceutical applications:

The proposed method was applied for the determination of lomefloxacin hydrochloride in **Lomex®** tablet. Satisfactory results were obtained in good agreement with the label claim, indicating no interference from excipients and additives. The obtained results were statistically compared to those obtained by the reported method [17] indicating good accuracy and precision of the proposed methods for the analysis of the studied drug in its pharmaceutical dosage form, as shown in **table (4)**. No significant differences were found by applying student's *t*-test and *F*-test at 95 % confidence level.

Table (4): Determination of lomefloxacin hydrochloride in Lomex® tablet by the proposed second derivative synchronous spectrofluorimetric method and the reported method:

Parameters	**Proposed method**	**Reported method* [17]**
Number of measurements	5	5
Mean % recovery of lomefloxacin hydrochloride	99.83	100.48
% RSD	1.128	1.314
Student's *t*-test**	0.828 (2.306)	——
F-value**	1.373 (6.388)	——

* Reported method is using first derivative spectrophotometric method with zero crossing point at 303.4 nm.

** The values in parenthesis are tabulated values of "*t* " and "*F* " at (P = 0.05)

CONCLUSION:

In present work, a highly sensitive, accurate and precise second derivative synchronous spectrofluorimetric method was developed and applied for the determination of lomefloxacin hydrochloride in bulk powder and in pharmaceutical preparation in the presence of its acid-induced degradation product. The method was validated according to the ICH guidelines and can be used for the routine analysis and for checking quality of pharmaceutical preparations containing lomefloxacin hydrochloride.

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