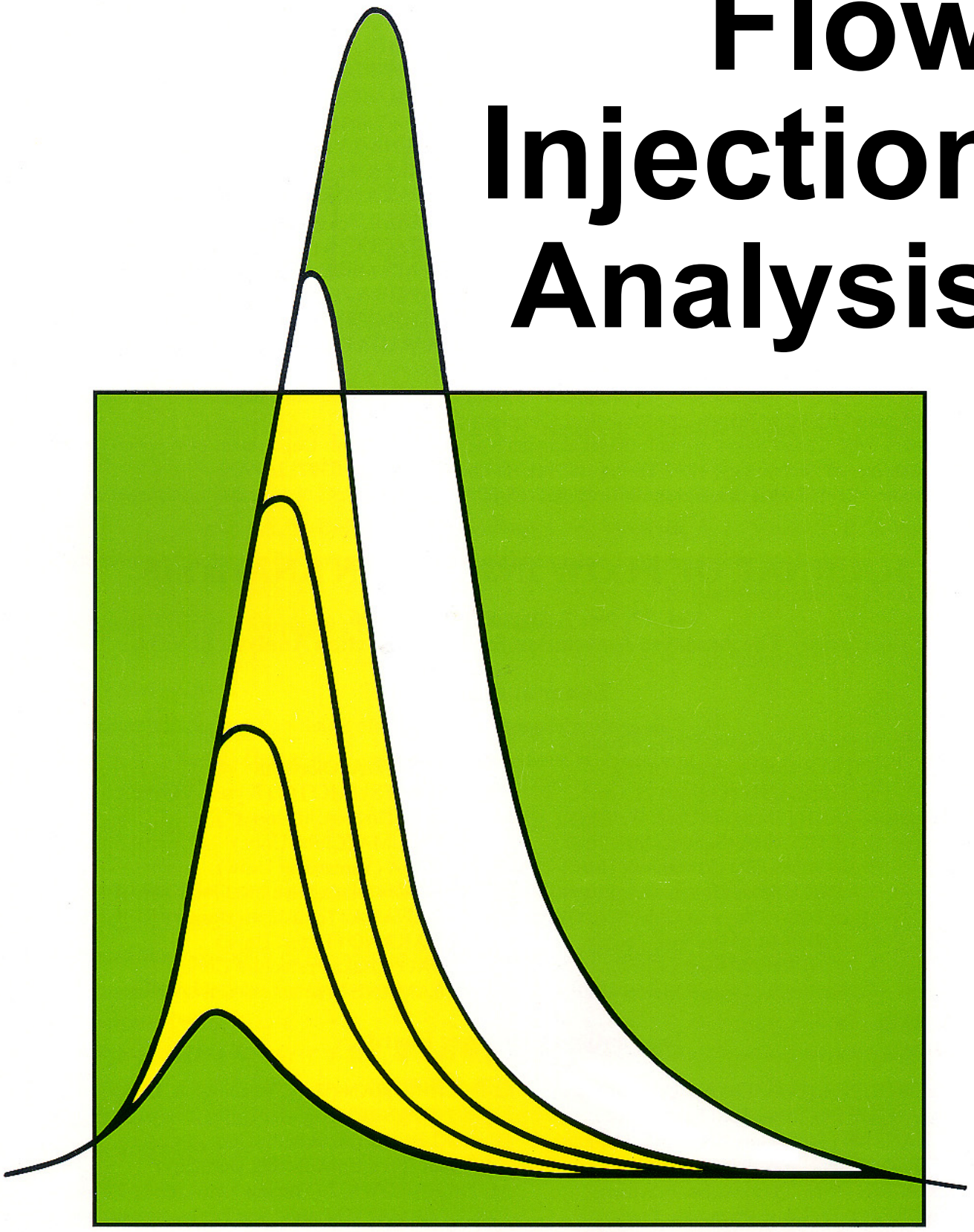
ISSN 0911-775X CODEN: JFIAEA

JOURNAL OF Flow Injection Analysis



FIA 研究懇談会会誌

J. Flow Injection Anal., Vol. 25, No. 2 (2008) 151–155

Flow Injection Spectrophotometric and Chromatographic Determination of Ciprofloxacin and Norfloxacin in Pharmaceutical Formulations

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Abstract

Methods based on flow injection analysis (FIA) and high-performance liquid chromatography (HPLC) have been developed for the determination of ciprofloxacin (CIP) and norfloxacin (NOR) in pharmaceutical dosage forms. The FIA method is based on the measurement of absorbances of the red complexes formed between iron (III) and the studied drugs at 440 nm. Optimization of chemical and FI variables has been made. Under the optimized conditions, the sampling rate was over 80 h^{-1}. For the chromatographic method, a reverse phase Suplico LC_8 column (7.5 cm * 4.6 mm i.d, 3µm dp) was used at ambient temperature. The mobile phase finally selected was prepared by using ACN: 0.005 M H_3PO_4 (30:70) adjusted to pH = 4.98 by TEA. The developed methods have been successfully applied for the determination of ciprofloxacin and norfloxacin in pharmaceutical formulations. The common excipients and additives did not interfere in their determinations. The results obtained by the proposed methods have been statistically compared by means of Student *t*-test and by the variance ratio *F*-test.

Keywords: Ciprofloxacin, Norfloxacin, FIA, HPLC, pharmaceutical formulations.

1. Introduction

Quinolones are antibacterial agents widely used in the treatment of infections in both humans and animals. They are important drugs for the treatment of respiratory tract, urinary tract, skin and skin-structure infections. Fluoroquinolones are well absorbed from the gastrointestinal tract, have excellent tissue penetration, low protein binding, and long elimination half-lives. These antibiotics are effective in treating various infections and are well tolerated in adults [1,2].

Several methods have been reported in literature for the determination of ciprofloxacin and/or norfloxacin either in pure form, dosage, or in biological fluids. These include high performance liquid chromatography [3-6], spectrophotometry [7-11], spectrofluorometry [12,13] polarography [14] and adsorptive stripping voltammetry [15].

The present paper describes two methods, spectrophotometric and chromatographic for the determination of ciprofloxacin and norfloxacin. The chemical structures of the studied drugs and the suggested general structure of their Fe(III) chelates [16] are given in Figure 1. The FIA method is based upon the reaction of the drug under investigation with Fe^{3+} in acidic medium. Both methods are satisfactorily applied for the determination of the titled antibiotic in the pure form as well as in pharmaceutical preparations.

2. Experimental

2.1. Flow Injection Analysis (FIA)

2.1.1. FIA Apparatus

The proposed FI setup is depicted in Figure 2. It consists of two channels. All measurements were performed with a Varain DMS-100 UV-Visible spectrophotometer connected to a linear 1200 recorder. Teflon tubing of 0.51 mm i.d. was used in the flow system. The sample solution was injected via a Rheodyne 6-way injection valve and merged with the carrier (0.01 M HCl) in the mixing coil (RC1). A home-made confluence point was used to ensure rapid mixing of sample mixture with the reagent (5 mM Fe^{3+}) in the second reaction coil (RC2). A sample injection volume of 60 µl was used. The absorbance of the produced color was monitored at 440 nm.

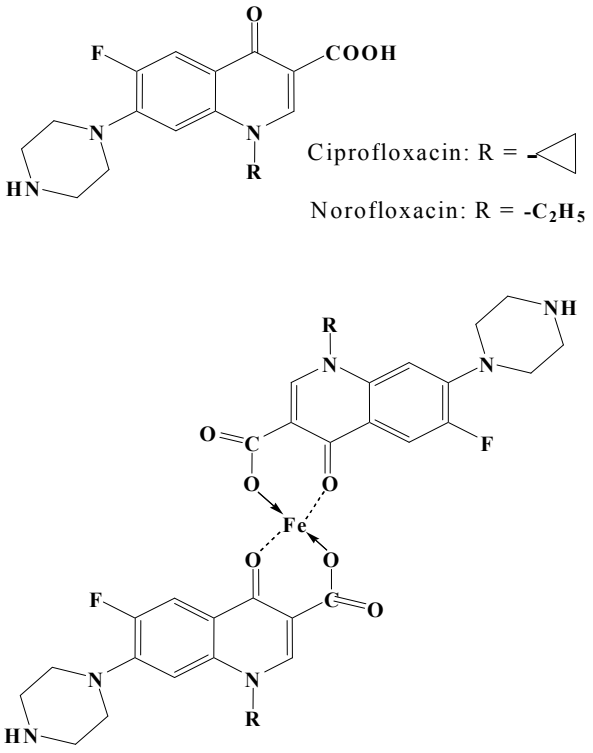

Figure 1: Structures of ciprofloxacin, norfloxacin and the Suggested general structure for their Fe(III) chelates.

2.1.2. Reagents and Solutions for FIA

Generally, all chemicals used were in pure grade available and as received without further purification. Water was always distilled and de-ionized.

Ferric (III) solution: A 0.1 M Fe(III) solution was prepared by dissolving 4.040 g of the solid $Fe(NO_3)_3.9H_2O$ in 100 ml of 0.01 M HCl. Working solutions of Fe(III) in the range 0.001-0.01 M were prepared by successive dilution.

Norfloxacin standard solution: A stock solution of 1000 ppm of norfloxacin was prepared by dissolving 0.1 g in 100 ml of 0.01M HCl. The solution was stirred for 10 min before dilution to volume to ensure complete dissolution.

Ciprofloxacin standard solution: A stock solution of 1000 ppm of ciprofloxacin solution was prepared same as norfloxacin solution.

Sample solutions: Ten tablets were weighed and ground into a fine powder. A portion of the powder equivalent to 500 mg of active ingredient was transferred to 100 ml volumetric flask, stirred for 20 min with 0.01 M HCl to dissolve the analyte and diluted to 100 ml by 0.01 M HCl. About 10 ml of this solution was filtered through a 0.2µm cellulose acetate syringe filters. From this solution, different concentrations were prepared by proper dilution with 0.01 M HCl.

2.1.3. FIA procedure

A volume of 60 µl of the prepared sample was injected into the sample loop by means of a syringe. Samples were injected into the carrier stream pumped at a flow rate of 1.3 ml/min. The Fe (III) was added at a rate of 1.3 ml/min in a confluence manner to ensure rapid and adequate mixing of the sample with the reagent. After injection, the valve was returned to the load position when the maximum change in absorbance has been reached. The absorbance was monitored at 440 nm. When the base line was reached, another sample was injected.

2.2. High Performance Liquid Chromatography (HPLC)

2.2.1. Apparatus

A Knauer model-501 liquid chromatograph was used throughout this work. The system is equipped with a 20 µl manual injector and a programmable variable wavelength UV detector. The whole system is connected to a Pentium-4 computer and full control was made using the Eurochrom software. A reversed-phase C8 column was used for separation.

2.1.2. Reagents and Solutions

All chemicals used were of analytical reagent grade, and the solvents were HPLC grade. Pure ciprofloxacin and norfloxacin were kindly provided by Jordanian Pharmaceutical Manufacturing (Amman, Jordan). Pharmaceutical preparations containing the studied compounds were obtained from commercial sources.

***Mobile phase*:** The mobile phase was prepared by mixing 300 ml of acetonitrile with 700 ml of 0.005 M H_3PO_4 which was already adjusted to pH = 5.0 with triethyl amine. Before mixing, the aqueous solvent was filtered using 0.45 µm cellulose nitrate membrane filters, and the acetonitrile was pre-filtered with a 0.45 µm PTFE filters. The two solvents are mixed together, stirred and degassed by vacuum prior to use.

***Internal standard solution*:** The internal standard solution was prepared by dissolving 200 mg of naphazolin nitrate in 1 L of the mobile phase. This solution was then used to prepare all other solutions to be analyzed by HPLC.

***Standard solutions of norfloxacin and ciprofloxacin*:** A stock solution of 1000 ppm was prepared by dissolving 100.0 mg of ciprofloxacin or norfloxacin in the internal standard solution. Working standard solutions in the range 2.5 – 500 ppm were prepared by dilution.

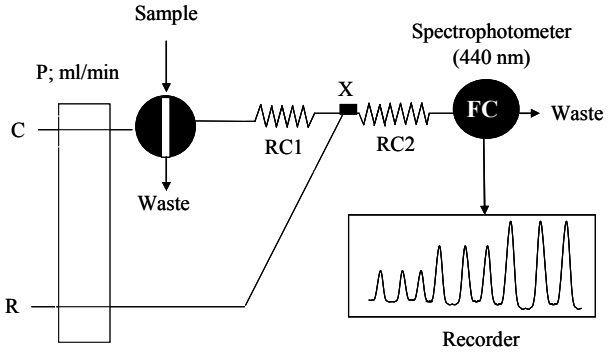

Figure 2: Schematic diagram of the proposed FIA system. P, peristaltic pump; C: carrier solution, 0.01M HCl; R, reagent, 0.005M Fe^{3+} in 0.01 M HCl; RC1 and RC2 are the reaction coils; X, confluence point; FC, Flow cell.

***Sample solutions of norfloxacin and ciprofloxacin*:** The same solutions used in FIA method were used. From these solutions, different concentrations (100, 50, and 25 ppm) were prepared by proper dilution with 0.01 M HCl and mixed with proper volumes of the internal standard.

2.2.3. Chromatographic conditions

A reverse phase Suplico LC_8 column (7.5 cm * 4.6 mm i.d, 3µm dp) was used at ambient temperature. The mobile phase finally selected was prepared by using ACN: 0.005 M H_3PO_4 (30:70) adjusted to pH= 4.98 by TEA. The mobile phase was filtered, stirred and degassed to remove air bubbles. The flow rate was 2 ml/min, the wavelength was 273 nm and the injection volume was 20 µl.

Results and Discussion

3.1. Development of the FIA Method

The idea of this work is based on the reaction of the drugs under investigation with Fe^{3+} in acidic medium. The intensity of the formed red complex was found to be linearly related to the concentration of the analyte. Therefore, this method was adapted for automation using flow injection analysis (FIA) technique. Consequently, in order to achieve reasonable sampling rate, reproducible measurements and low detection limits, several factors have been optimized.

The reaction between Fe^{3+} and ciprofloxacin was carried out in HCl solution. Therefore, different concentrations of HCl were prepared ranging from 0.005 M to 0.050 M. Maximum analytical signal was obtained at 0.01 M HCl. Similarly, the effect of Fe^{3+} concentration was also studied and the results are presented in (Fig 3a). Different concentrations of Fe^{3+} ranging from 0.001 M to 0.01 M were tested. A gradual increase in the absorbance was observed with increasing Fe^{3+} concentrations. Maximum absorption was obtained at 0.005 M. At higher Fe^{3+} concentrations, a slight decrease in the absorbance values was observed. Therefore, a concentration of 0.005 M of Fe^{3+} in 0.01 M HCl was chosen.

The flow rate of the carrier and the reagent streams were optimized with 0.01 M HCl as carrier and 0.005 M Fe^{3+} solution in 0.01 M HCl as the colorimetric reagent. The sample volume was 100 µl. The length of the first reaction coil (RC_1) was 40 cm and the second reaction coil (RC_2) was 30 cm. The effect of the total flow rate was changed over the range 0.8 to 3.6 ml/min while all other variables were kept constants. Throughout this study, the two lines were always pumped at equal flow rates. The effect of changing the flow rate on the peak height of the signal is shown in (Fig 3b). The highest signal was obtained at a flow rate of a 1.3 ml/min for each reagent. At higher flow rates, irreproducible results and unstable baseline were observed. At lower flow rates, on the other hand, the residence time (time needed to reach maximum absorbance and then return to the base line) was increased, and the peak height was decreased due to the increase in the dispersion of the sample zone. So a flow rate of a 1.3 ml/min for each reagent was chosen. At this flow rate about 45 seconds are needed for one determination, which means that the average sample rate using 100 µl sample injections is about 80 samples per hour.

The effect of the reaction coil length on the analytical signal was investigated. Various coils with increasing lengths were investigated. As expected, no significant change on the analytical signal was observed upon changing RC_1 over the range 30 – 100 cm. Therefore, the shortest possible length (30 cm) was used to minimize the dispersion of the sample and consequently enhance the sensitivity of the determination. However, a gradual increase in the analytical signal was observed when the length of RC2 was increased from 15 cm to 75 cm. Coils larger than 75 cm caused peak broadening due to sample dispersion and decrease in the peak height. The maximum change in peak height was obtained when the coil length was 75 cm. Therefore, a 75 cm reaction coil was chosen as the optimum to ensure high sensitivity and a high measurement rate Figure (3c).

The effect of the injected volume on the peak height was investigated by injecting different volumes using different lengths of the sample loop. As expected, an increase in the volume of injected sample solution leads to an increase in peak height. Consequently, the sensitivity of measurement can be improved by increasing sample volume. However, increasing the sample volume lead to an increase in the peak width and time for the signal to retain to the baseline. So a 100 µl volume was chosen which produces a reasonable sensitivity and sampling rate.

3.2. Evaluation of the FIA method

The calibration curves for the determination of the investigated fluoroquinolones were obtained under the optimum conditions. Calibration graphs were obtained by injecting standard solutions of NOR and CIP in the range of 5 to 750 µg/ml. The linearity is good in each instance and Beer's low is obeyed for the tested drugs. The calibration curves were linear up to 500 µg/ml for both drugs with correlation coefficients of 0.9997 or better.

The limit of detection (LOD) was calculated statistically as the concentration of analyte leading to a signal that is three times the blank standard deviation. The LOD values were 1.25 and 1.10 ppm for CIP and NOR, respectively. The overall system precision was also calculated and found to be <5%.

The intra-day (within-day) precision was evaluated by replicate analysis of two different concentrations of norfloxacin within the linearity range at different time intervals. The inter-day (different days) precision was similarly evaluated on several days up to 3 days. Every day, a new calibration graph was constructed. The results in both cases indicated high precision, as the percent RSD did not exceed 5%. The precision of the measurements ranged from a RSD of 1.4 – 4.4% (n =6).

3.3. Optimization of the HPLC method

Chromatographic separation was carried out using Lichrosorb C8 column. Different ratios of ACN and phosphate buffer (pH = 2.5 to 6) were tested as mobile phases. However, the separation time was long (>10 min) with a considerable peak tailing. Therefore, in order to minimize the analysis time and to improve the separation efficiency, triethylamine (TEA) was added to the mobile phase in different ratios. Optimum separation was achieved using 30% of acetonitrile and 70 % of 5 mM H_3PO_4 adjusted to pH 5.0 with TEA. Naphazoline nitrate was used as the internal standard throughout this work (Figure 4).

3.4. Evaluation of the HPLC method

The linearity of the detector response was determined by preparing calibration standard solutions of pure analyte in the range 2.5 to 500 ppm. Plots of peak area ratios versus amounts injected were linear up to 130 and 200 ppm, with correlation coefficients of 0.9987 and 0.9985 for norfloxacin and ciprofloxacin, respectively. The LOD values were 0.80 and 0.60 ppm for CIP and NOR, respectively. The overall system precision was also calculated and found to be <3%.

3.5. Interferences

In order to examine the applicability of the proposed FIA and HPLC methods to routine pharmaceutical analysis, the effect of common excipients normally used in pharmaceutical formulations was studied. Synthetic mixtures containing different concentrations of ciprofloxacin and norfloxacin in the presence of more than 100 folds of common additives were prepared. The most common additives that have been used in this study are; sodium saccharide, sodium benzoate, magnesium stearate, xanthate and stearic acid. The undissolved material was filtered off before injection. The results obtained were compared with expected values. No significant changes were observed on

the results and obtained recoveries were in the range of 96.5 to103.2% in all cases. These results prove that the proposed FIA and HPLC methods are applicable for pharmaceutical analysis and that no interferences due to excipients are present.

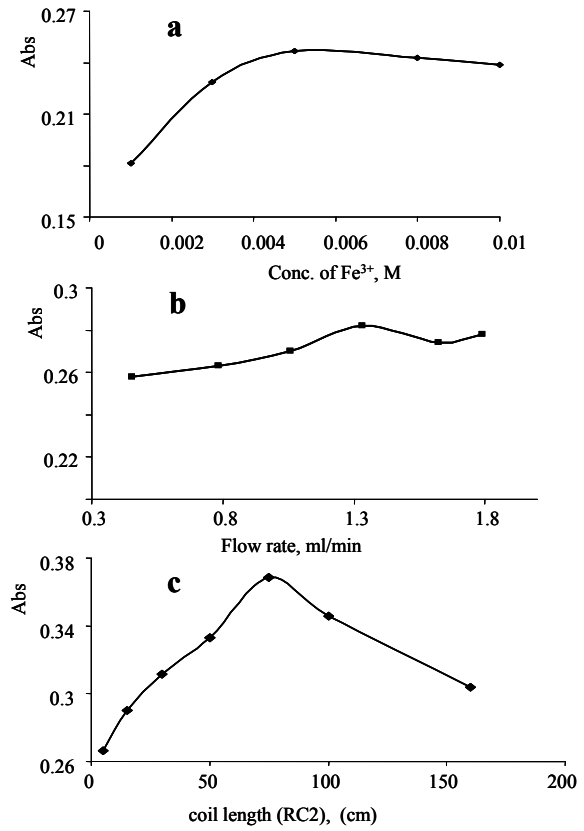

Figure 3: Effect of chemical and FIA variables on the analytical signals.

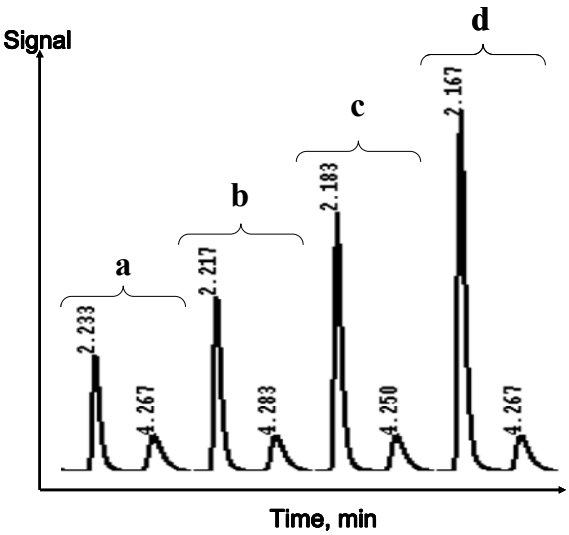

Figure 4: Representative chromatograms obtained with calibration standards containing 20 (a), 30 (b), 50 (c) and 70 (d) ppm of ciprofloxacin (t_R = 2.2-2.4 min). The IS eluted at about 4.1 min.

3.6. Applicability of the proposed FIA and HPLC methods

In order to evaluate the applicability of the proposed FIA and HPLC methods to routine pharmaceutical analysis, different commercial pharmaceutical product were purchased from the local market and analyzed by the proposed methods. The results obtained were compared with the expected values and presented in Table 1. In all cases the RSD was less than 5.0% for methods, indicating good accuracy and precision. The results obtained by the FIA procedure were also compared with the results obtained by the HPLC procedure for the same set of samples by means of t- and F-tests at 95% confidence level. No significant differences were found between the results of the two methods was found. The closeness of the results to the label claim supports the accuracy of the method. No interferences were observed from any additives in any of the products analyzed.

4. Conclusion

The proposed FIA and HPLC methods provide simple, accurate and reproducible quantitative analyses for the assay of ciprofloxacin and norfloxacin in pharmaceutical formulations. The proposed methods can be used for the quantification of other fluoroquinolones. The average sampling rate of the FIA method is about 80 samples per one hour. The HPLC method needs about 5 minutes for complete separation of the target compound and the internal standard. The proposed methods showed good linearity, precision and reproducibility and were successfully applied for the analysis of the above-mentioned drugs in pharmaceutical dosage forms without any interferences.

Table 1: FIA and HPLC results for the analysis of CIP and NOR in pharmaceutical preparations.

Drug	Trade Name	Taken	%Recovery ± RSD, (n = 6)	
		(μg/ml)	FIA	HPLC
Norfloxacin	Noracin-400 [1]	25	100.1 ± 1.7	99.1 ± 0.5
		50	99.0 ± 1.7	101.8 ± 0.6
		100	97.4 ± 0.8	100.6 ± 1.1
Norfloxacin	Noroxin-400 [2]	25	101.3 ± 1.8	99.5 ± 1.0
		50	101.0 ± 1.1	101.2 ± 0.3
		100	97.7 ± 1.0	101.4 ± 0.4
Ciprofloxacin	Ciprodar-250[3]	25	100.0 ± 0.3	102.0 ± 4.8
		50	99.4 ± 1.8	102.4 ±3.9
		100	101.9 ± 0.5	97.9 ± 0.0
Ciprofloxacin	Floroxine -500[4]	25	102.6 ± 2.5	104.6 ± 3.1
		50	97.9 ± 1.9	102.7 ± 1.4
		100	99.0 ± 0.9	97.7 ± 0.9

1. Tablets (400 mg/T), Merck Sharp & Dohme, Nertherlands.
2. Tablets (400 mg/T), The Jordanian Pharm. Man. Co. Ltd.
3. Tablets (250 mg/T), Dar Al Dawa, Naur, Jordan.
4. Tablets (500 mg/T), The United Pharm. Mtg., Jordan.

Acknowledgments

The financial support from Yarmouk University is gratefully acknowledged. The author would like to thank Jordanian Pharmaceutical Manufacturing for providing the standard and the excipients.

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(Received August 4, 2008)
(Accepted September 4, 2008)