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**Spectrofluorimetric Study of the Charge-transfer Complexation of Certain Fluoroquinolones with 2,3,5,6-tetrafluoro-p-bezoquinone**

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**Abstract:** A highly sensitive spectrofluorimetric method was developed for the first time, for the analysis of ten fluoroquinolones (FQs) antibacterials, namely amifloxacin (AMI), ciprofloxacin (CIP), difloxacin (DIF), enoxacin (ENO), enrofloxacin (ENR), lomefloxacin (LOM), levofloxacin (LEV), norfloxacin (NOR), ofloxacin (OFL) and pefloxacin (PEF) in their pharmaceutical dosage forms or in biological fluids through charge transfer (CT) complex formation with fluoranil (TFQ). The TFQ was found to react with these drugs to produce stable complexes and the fluorescence intensity of the complexes was greatly enhanced. The formation of such complexes was also confirmed by both infrared and ultraviolet-visible measurements. The different experimental parameters that affect the fluorescence intensity were carefully studied. At the optimum reaction conditions, the drug-TFQ complexes showed excitation maxima ranging from 270 to 285 nm and emission maxima ranging from 450 to 460 nm. Rectilinear calibration graphs were obtained in the concentration range 0.02 to 3.1 µg mL⁻¹ for the studied drugs. The method has been successfully applied to determine their pharmaceutical dosage forms with good precision and accuracy compared to official and reported methods as revealed by t- and F-tests. They also applied for the determination of studied drugs in human urine samples.

**Key words:** Fluoroquinolones, p-fluoranil, charge-transfer complexes, spectrofluorimetry

**INTRODUCTION**

Fluoroquinolones (FQs) are a class of important synthetic antibiotics, which are active against both Gram (+) and Gram (-) bacteria through inhibition of their DNA gyrase[1], also they have some activity against myobacteria, mycoplasmas and rickettsias.

Several chromatographic methods have been reported for determination of these compounds, amifloxacin (AMI)[2], ciprofloxacin (CIP)[3], difloxacin (DIF)[4], enoxacin (ENO)[5], enrofloxacin (ENR)[6], lomefloxacin (LOM)[7], levofloxacin (LEV)[8], norfloxacin (NOR)[9], ofloxacin (OFL)[10] and pefloxacin (PEF)[11] were determined by high-performance liquid chromatography (HPLC). Various spectrophotometric methods were described for determination of CIP, ENR and PEF[12], NOR[13] and LEV[14] by charge-transfer complex formation with chloranilic acid, tetracyanoquinodimethane and tetracyanoethylene. In addition, several methods have been reported for their determination such as spectrophotometry[15,16], fluorimetry[17-19], polarography[20], voltametric[21] and capillary electrophoresis[22]. HPLC methods generally require complex and expensive equipment, provision for use and disposal of solvents, labor-intensive sample preparation procedure and personal skilled in chromatographic techniques. Charge-transfer spectrofluorimetry (CTF) has been found to be useful for the determination of quinolone in real samples (23-27), showing several advantages such as low interference level, low detection limit, high sensitivity, good analytical selectivity, easy and less time consuming comparing with the above methods. A new spectrofluorimetric method for determination of AMI, CIP, DIF, ENO, ENR, LOM, LEV, NOR, OFL and PEF was reported through CT complexation with TFQ in this paper, having been satisfactory applied to the determination of studied drugs in commercial formulations and urine samples.

**MATERIALS AND METHODS**

**Reagents:** All solvents used were of analytical reagent grade. Methanol, ethanol, isopropanol, acetone, acetonitrile and chloroform (Merck KGaA, Germany). TFQ (Sigma Chemical Co.,USA) was prepared 4 x 10⁻⁴ mol L⁻¹ in acetone, solution was found be stable for at least 1 week at 4 °C. Samples of FQs were generously supplied by their respective manufactures: AMI (Sterling Winthrop Inc., USA); DIF (Abbott Laboratories, North Chicago, USA); NOR (Eipico, Cairo, Egypt); OFL (Hoechst AG, Frankfurt, Germany); CIP (Miles Inc. Pharmaceutical Division,
Working standard solutions were prepared by dilution of stock standard solution with acetone. Stock standard solutions were stable for several weeks at room temperature.

**Pharmaceutical formulations:** The following available commercial preparations were analyzed: Spectrama® tablets (Amoun Pharmaceutical Industries Co., Cairo, Egypt) labeled to contain 400 NOR per tablet; Neofloxacin® tablets (Alexandria Co. for Pharmaceuticals, Alexandria, Egypt) labeled to contain 400 mg NOR per tablet; Norbactin® (Chem. Ind. Co., Giza, Egypt) labeled to contain 400 mg NOR per tablet; Tarivid® tablets (Hoechst Orient, Cairo, Egypt, under license of Hoechst AG, Frankfurt, Germany) labeled to contain 200 mg OFL per tablet; Kirof® tablets (Amoun Pharmaceutical Industries Co., Cairo, Egypt) labeled to contain 200 mg OFL per tablet; Mefoxin® tablets (Misr Co. for Pharmaceutical Industries, Cairo, Egypt) labeled to contain 250 mg CIP per tablet; Serviflox® tablets (Under Licence from Biochemie Kundi Austria), labeled to contain 250 CIP per tablet; Ciprohexal® tablets (Hexal Co., Germany) labeled to contain 500 mg CIP per tablet; Cipro® otic drops (Chem. Indus. Develop. Co., Giza, Egypt) labeled to contain 3.5 mg CIP per each mL; Globacin® tablets (Global Napi Pharm. Egypt) labeled to contain 400 mg PEF per tablet; Pefflacin® ampoules (Rhone-Poulenc Rorer, Neuilly/Seine, France) labeled to contain 400 mg PEF and 15.3 mg sodium ascorbate per 5 mL ampoule; Tavanic® tablets (Under Licence of Aventis Pharma Germany) labeled to contain 500 mg LEV per tablet.

**Apparatus:** Fluorescence signals were measured on an FP-750 spectrophluorometer (Jasco, Germany) connected to an IBM computer loaded with FLwinlab™ application software 4.00.02 version, was used. All the measurements took place in a standard 10 mm path-length quartz cell, thermo stated at 25.0±0.5 °C, with 2.5 nm bandwidth for the emission and excitation monochromators.

A UV-Lambda 40 ultraviolet-visible spectrophotometer (Perkin Elmer instruments, USA) was used for the absorbance measurements.

An infrared spectrometer FT-400/600Plus series (Jasco, Germany) was used for recording IR spectrum.

**General procedure:** A suitable amount of drug solution was pipetted into a 10 mL volumetric flask. 1.0 mL of TFQ solution was added and the solution was diluted to volume with acetone and mixed thoroughly. The solution were thermo stated at 25.0±0.5 °C and the fluorescence intensities of CT complexes of AMI, CIP, DIF, ENO, ENR, LOM, LEV, NOR, OFL and PEF were measured at 456, 460, 458, 450, 452, 453, 456, 455, 455 and 451 nm using an excitation wavelength of 280, 270, 279, 282, 283, 285, 284, 276, 271 and 274 nm against a blank solution, respectively. The calibration graph was constructed in the same way with studied drugs solutions of known concentrations. The amount of drugs was computed from their calibration graphs.

**Analysis of tablets:** An accurately weighed amount, equivalent to 10 mg of each drug from composite of 20 powdered tablets, was transferred into a 100 mL calibrated flask and diluted to the mark with the appropriate solvent, sonicated for 20 min and filtered off to obtain solutions of 100 µg mL⁻¹. Further dilutions were made to obtain sample solution and the general procedures were proceeding described earlier.

**Analysis of ampoules:** A volume equivalent to 10 mg of each drug was transferred into 100 mL calibrated flask and diluted to the mark with the appropriate solvent to obtain solution of 100 µg mL⁻¹. Further dilutions were made to obtain sample solution and the general procedures were proceeding described earlier.

**Analysis of drops:** One milliliter of the drops was transferred into a 100 mL calibrated flask and diluted to the mark with the appropriate solvent to obtain a solution of 30 µg mL⁻¹. Further dilutions were made to obtain sample solution and the general procedures were proceeding described earlier.

**Preparation of the complexes for infrared:** To 5 mL of 0.05 mol L⁻¹ TFQ in acetone and 5 mL of 0.05 mol L⁻¹ each investigated drug in acetone was added in around bottom flask containing 50 mL of acetone and stirred for 30 min. The solvent was evaporated under reduced pressure and the resulting oily residues were dried over calcium chloride.

**Analysis of human urine:** Dilute urine samples of a healthy subject who has taken, orally, FQs tablets at specific times, in 5 mL sample solution were transferred into a separating funnel and shaken well for 3 min. Then 5 mL of 0.2 mol L⁻¹ phosphate buffer solution (pH 7.0) was added and the mixture shaken and extracted with 3 x 10 mL of dichloromethane chloroform (1:1 v/v) mixture. The organic layer was filtered over anhydrous sodium sulfate. The extract was dried under nitrogen gas at room temperature and residue dissolved in least amount of acetone, transferred into a 10 mL volumetric flask and procedures were proceeding described earlier.
RESULTS AND DISCUSSION

Excitation spectra and emission spectra: Solution of the studied drugs have native fluorescence, however in presence of TFQ, the fluorescence intensity increases substantially, the sensitivity is enhanced by 22-54 fold (Fig. 1). Indicated CT complexes formation between the investigated drugs and TFQ, these drugs were probably through the lone pair of electron donated by the N atom in piperazinyl of FQs (n-donor) to TFQ (π-acceptor).

Effect of reaction temperature: The effect of temperature on the formed CT complexes was studied in the range of 10-60 °C. All the formed complexes were stable up to 40 °C, at temperatures higher than 40 °C, the relative fluorescence intensity decreases due to dissociation of the complexes at higher temperatures. Similarly, the fluorescence intensity was found to depend on temperature of CT complexes with studied drugs is small in the range of 10-40 °C, thus the determination of studied drugs were carried out at 25±0.5 °C. It was further found that takes 30 min to form the complexes completely which were stable for at least 24 h.

Effect of TFQ concentration: The influence of CT reagent concentration was studied in the range 4 x 10^{-5} mol L^{-1} - 4 x 10^{-3} mol L^{-1}. The relative fluorescence intensity increased with increasing TFQ concentration up to 4 x 10^{-4} mol L^{-1} but leveled off at higher concentrations. Experiment indicated that 1.0 mL TFQ solution is enough for each drug, thus the final TFQ concentration of 4 x 10^{-4} mol L^{-1} was used for all of the studied drugs.

Effect of solvent: Fluorescence spectral characteristics of AMI, CIP, DIF, ENO, ENR, LOM, LEV, NOR, OFL and PEF in different solvents are compared. The studied solvents involved water, methanol, ethanol, isopropanol, acetone, acetonitrile and chloroform. Experimental results indicated that acetone gave the maximum and stable fluorescence emission for studied drugs.

Effect of CT reagent: The influence of the CT reagent on the relative fluorescence intensity of all the formed CT complexes with FQs was studied at their respective maxima using TFQ, TCNQ, TCNE, CL and DDQ as model electron acceptors. The results show that TFQ is most sensitive CT reagent for the studied drugs. In general, the order of decreasing sensitivity is TFQ > TCNQ > TCNE > DDQ > Cl (Fig. 2).

Investigations on the structure of the charge-transfer complexes: The fluorescence intensity increases substantially indicated the possible CT complexes formation of the type n-π complexes.

Fig. 1: Fluorescence spectra of CIP (c and d, 0.8 µg mL^{-1}), TFQ (e and f, 4x10^{-4} mol L^{-1}) and CIP-TFQ (a and b). Excitation spectra (a, c and e) and emission spectra (b, d and f).

Fig. 2: Fluorescence emission spectra of: (1) CIP (0.8 µg mL^{-1}) with TFQ (4x 10^{-4} mol L^{-1}). (2) CIP (0.8 µg mL^{-1}) with TCNQ (4x 10^{-3} mol L^{-1}). (3) CIP (0.8 µg mL^{-1}) with TCNE (4x 10^{-3} mol L^{-1}). (4) CIP (0.8 µg mL^{-1}) with DDQ (4x 10^{-4} mol L^{-1}). (5) CIP (0.8 µg mL^{-1}) with CL (4x 10^{-4} mol L^{-1}).

Fig. 3: Absorption spectra: (1) CIP (0.8 µg mL^{-1}); (2) TFQ (4x 10^{-4} mol L^{-1}) and (3) CIP-TFQ against acetone blank.

Both IR and UV measurements also confirmed the formation of such complexes. The majority of infrared measurements on such CT complexes have been concerned with the shifts in the vibrational frequencies of donors or acceptors. Decreases in the vibration frequency of a particular band have been used as evidence for a particular site of a CT interaction. The infrared spectra of the complexes show some difference compared with the sum of the spectra of the two components. This was used to distinguish between weak CT complexes and the products of electron-transfer.
Mechanism of reaction: TFQ is an $\pi$-acceptor, AMI, CIP, DIF, ENO, ENR, LOM, LEV, NOR, OFL and PEF are nitrogenous compounds. So CT complexes can be formed with these drugs. Molar ration of the reactants in the CT complex was determined by Job's method of continuous variation\[29\] and Yoe and Jones method of mole ratio\[30\] and it was found to be 1:1 for studied drugs with TFQ. This ratio may be due to the presence of the fluorine atom acting as an electron drawing group in the molecule of FQs. The benzene ring has lower electron density, but nitrogen atom in 4 of piperazinyl has more electron density and less sterically hindered. So n-$\pi$ CT complexes were formed (Table 3).

Analytical parameters: Under the experimental conditions described, standard calibration curves of CT complexes for AMI, CIP, DIF, ENO, ENR, LOM, LEV, NOR, OFL and PEF with TFQ were constructed by plotting fluorescence intensity versus concentration, the linear regression equation for each method are listed in Table 4. The correlation coefficients ranged from 0.9993 to 0.9999, indicating good linearity. The small value of variance confirmed the small degree of scattering of the experimental data points around the regression line.

Precision of the proposed methods was determined in each concentration range, by 10 measurements carried out on different days within 1 week of different solution of AMI, CIP, DIF, ENO, ENR, LOM, LEV, NOR, OFL and PEF. Target concentrations corresponded to middle values in each range. Table 4 gives a R.S.D. (within-day and between-day) of solutions of 0.01, 0.10 and 1.00 µg mL$^{-1}$ were determined by using the proposed procedure.

**Analysis of pharmaceutical formulations:** The proposed methods were applied to the determination of the studied drugs in their pharmaceutical formulations. Five replicate determinations were made. Satisfactory results were obtained for studied drugs (Table 6). Moreover, to check the validity of the proposed methods, the standard addition method was applied by adding AMI, CIP, DIF, ENO, ENR, LOM, LEV, NOR, OFL and PEF to the previously analyzed pharmaceutical formulations. The recovery of each drug was calculated by comparing the concentration obtained from the (spiked) mixtures with those of the pure drugs. Table 5 shows the results of analysis of the commercial pharmaceutical formulations and the recovery study (standard addition method) of studied drugs. In comparison of the results obtained by the proposed method with those obtained by literature method\[31\] indicated that the accuracy is satisfactory. The obtained high-intensity fluorescence bands and the very low reagent background make these procedures suitable for the routine quality control analysis of the investigated compounds with minimum interference. The proposed and reference methods were applied to the determination of the studied drugs in pharmaceutical formulations containing different FQs (Table 6). In the $t$ and $F$ tests, no significant differences were found between the calculated and the theoretical values (95% confidence) of both the proposed and the reference methods. This indicates similar precision and accuracy.

**Analysis of human urine:** The proposed method was applied to determine CIP as a representative example of the studied drugs in human urine samples from healthy volunteers who received a single oral dose of 500 mg CIP. The urine samples of individuals were collected at 6, 12, 24 and 36 hr after oral administration of CIP tablets. In this case, the high performance liquid chromatography (HPLC) method proposed by Wong et al. was used as a reference method\[31\]. The results obtained summarized in Table 7, show that both methods (spectrofluorimetric and chromatographic)
Table 3: Structures of the investigated drugs CT complexes with TFQ

| Compound     | R₁ | R₂ | R₃ | R₄ |
|--------------|----|----|----|----|
| 1- Amifloxacin | NHCH₂ | H | CH₃ | H |
| 2- Ciprofloxacin | H | H | H | H |
| 3- Difloxacin | CH₂-F | H | CH₃ | H |
| 4- Enoxacin | CH₂ | H | H | H |
| 5- Enrofloxacin | H | CH₃ | H |
| 6- Lomefloxacin | C₂H₅ | F | H | CH₃ |
| 7- Levofloxacin | CH₂ | CH₃ |
| 8- Norfloxacin | C₂H₅ | H | H | H |
| 9- Ofloxacin | C₂H₅ | H |
| 10- Pefloxacin | C₂H₅ | H | CH₃ | H |

Table 4: Statistic and analytical parameters

| Drugs | λₑₓ / λₑₘ (nm); L.R. (linear range µg ml⁻¹); L.D. (Limit of detection µg ml⁻¹); L.Q. (Limit of quantitation µg ml⁻¹); b (Slope); S.D. (Standard deviation of slope); a (Intercept of the ordinate); Sa (Standard deviation of the intercept of the ordinate); V (Variance); r (Correlation coefficients). |
|-------|--------------------------------------------------------------------------------------------------|
| AMI   | 280/456 | 0.08-2.5 | 0.008 | 0.025 | 423.77 | 1.14 | 1.32 | 0.12 | 0.018 | 0.9998 |
| CIP   | 270/460 | 0.04-1.4 | 0.012 | 0.039 | 387.96 | 1.17 | 1.00 | 0.87 | 0.123 | 0.9999 |
| DIF   | 279/458 | 0.07-3.1 | 0.022 | 0.070 | 287.35 | 0.94 | 0.92 | 0.02 | 0.024 | 0.9993 |
| ENO   | 282/450 | 0.03-2.0 | 0.004 | 0.015 | 619.48 | 1.16 | 1.11 | 0.01 | 0.053 | 0.9999 |
| ENR   | 283/452 | 0.04-2.8 | 0.006 | 0.021 | 496.00 | 1.11 | 1.27 | 0.06 | 0.025 | 0.9994 |
| LOM   | 285/453 | 0.02-2.7 | 0.008 | 0.031 | 145.97 | 1.02 | 1.62 | 0.04 | 0.062 | 0.9995 |
| LEV   | 284/456 | 0.04-2.8 | 0.004 | 0.015 | 299.03 | 1.21 | 1.04 | 0.06 | 0.058 | 0.9996 |
| NOR   | 276/455 | 0.02-2.9 | 0.012 | 0.041 | 321.98 | 1.12 | 1.21 | 0.06 | 0.082 | 0.9994 |
| OFL   | 271/455 | 0.03-2.2 | 0.009 | 0.080 | 762.93 | 1.11 | 1.71 | 0.01 | 0.027 | 0.9996 |
| PEF   | 274/451 | 0.05-2.6 | 0.005 | 0.015 | 265.87 | 0.99 | 1.81 | 0.07 | 0.012 | 0.9999 |

Table 5: Precision results of FQs (n=11)

| Concentration (µg ml⁻¹) | Within-day R.S.D. (%) | Between-day R.S.D. (%) |
|-------------------------|-----------------------|------------------------|
|                         | 0.01 | 0.10 | 1.00 | 0.01 | 0.10 | 1.00 |
| AMI                     | 1.2  | 1.1  | 1.9  | 1.0  | 1.8  | 1.5  |
| CIP                     | 0.8  | 1.8  | 1.6  | 1.3  | 1.4  | 1.7  |
| DIF                     | 1.3  | 1.8  | 1.5  | 0.8  | 1.5  | 1.3  |
| ENO                     | 1.7  | 1.4  | 1.4  | 1.2  | 1.0  | 1.5  |
| ENR                     | 1.5  | 1.9  | 0.9  | 1.5  | 1.0  | 1.1  |
| LOM                     | 1.9  | 1.5  | 1.4  | 1.7  | 1.6  | 1.3  |
| LEV                     | 1.6  | 1.2  | 1.5  | 1.5  | 1.4  | 1.9  |
| NOR                     | 1.6  | 1.4  | 1.8  | 1.2  | 1.9  | 1.2  |
| OFL                     | 1.1  | 1.6  | 1.5  | 1.5  | 1.3  | 1.2  |
| PEF                     | 1.7  | 1.8  | 0.9  | 1.1  | 1.8  | 1.5  |

yield values within the same range when tested by using statistical procedures.

The accuracy was assessed by investigating the recovery of each of the studied drugs at four concentration levels covering the specified range (five replicates of each concentration). The results showed average percentage recoveries were 95.9±1.1 with standard deviations less than 2.0 for human urine, indicating both good accuracy and precision (Table 8).

The increase in sensitivity obtained with the proposed method, compared with other methods is very substantial. Comparison with other proposed method
Table 6: Determination of drugs in pharmaceutical formulation using TFQ

| Drug                  | Proposed method | Reference method [16] |
|-----------------------|-----------------|-----------------------|
| Spectrama® Tablets    | X±SD 97.21±0.60 | 97.00±0.66            |
| V                     | 0.35            | 0.40                  |
| t                     | 0.91            | 0.02                  |
| F                     | 1.10            | 1.20                  |
| Neofloxacin® Tablets  | X±SD 100.11±0.62| 99.11±0.59            |
| V                     | 0.45            | 0.35                  |
| t                     | 0.41±1.11       | 0.31                  |
| F                     | 1.21            | 1.03                  |
| Norbactin® Tablets    | X±SD 99.92±1.00 | 99.00±0.90            |
| V                     | 1.00            | 0.81                  |
| t                     | 0.65            | 0.03                  |
| F                     | 1.01            | 1.13                  |
| Tarivid® Tablets      | X±SD 99.89±0.79 | 100.01±0.70           |
| V                     | 0.55            | 0.49                  |
| t                     | 0.87            | 0.04                  |
| F                     | 1.02            | 1.11                  |
| Kirol® Tablets        | X±SD 97.6±0.53  | 100.0±0.48            |
| V                     | 0.34            | 0.23                  |
| t                     | 0.59            | 0.00                  |
| F                     | 1.21            | 1.09                  |
| Mefoxin® Tablets      | X±SD 100.11±0.56| 99.98±0.54            |
| V                     | 0.41            | 0.29                  |
| t                     | 0.29            | 0.35                  |
| F                     | 1.61            | 1.21                  |
| Serviflox® Tablets    | X±SD 100.7±0.90 | 101.1±0.88            |
| V                     | 0.61            | 0.77                  |
| t                     | 0.81            | 1.41                  |
| F                     | 1.09            | 1.01                  |
| Cipro otic® Drops     | X±SD 98.86±0.69 | 100.0±0.90            |
| V                     | 0.56            | 0.81                  |
| t                     | 0.83            | 0.20                  |
| F                     | 1.57            | 1.02                  |
| Globacin® Tablets     | X±SD 100.51±0.71| 99.11±0.50            |
| V                     | 0.48            | 0.25                  |
| t                     | 0.57            | 0.29                  |
| F                     | 1.15            | 1.32                  |
| Peflacin® Ampoules    | X±SD 99.20±0.51 | 99.99±0.39            |
| V                     | 0.38            | 0.15                  |
| t                     | 0.89            | 0.04                  |
| F                     | 1.99            | 1.07                  |
| Tavanic® Tablets      | X±SD 100.2±0.61 | 99.80±0.50            |
| V                     | 0.45            | 0.25                  |
| t                     | 0.79            | 0.53                  |
| F                     | 1.81            | 1.44                  |
| Ciprohexal® Tablets   | X±SD 100.0±0.55 | 95.9±1.1              |

Three and six determinations were used for the reported and the reference methods, respectively. The tabulated values of t and F at 95% confidence limit are t=2.23 and F=5.79.

Table 7: Determination of CIP in human urine samples

| Sample | CIP found (µg ml⁻¹) | Proposed method | HPLC method | t | P value (%) |
|--------|----------------------|-----------------|--------------|---|-------------|
| 1 (6 hr) | 18.12 ± 0.46 | 17.91 ± 0.58 | 1.56 | 45.1 |
| 2 (12 hr) | 8.22 ± 0.29 | 8.11 ± 0.17 | 1.03 | 32.2 |
| 3 (24 hr) | 2.99 ± 0.09 | 2.24 ± 0.08 | 0.89 | 30.1 |
| 4 (36 hr) | 0.40 ± 0.01 | 0.41 ± 0.02 | 0.55 | 30.0 |

Average ± S.D. of six determinations. The urine samples are collected at 6, 12, 24 and 36 hr after oral administration of CIP tablets in a clinic, done by a healthy subject. P value of the comparison test.

Table 8: Fluorimetric determination of CIP in human urine (n = 5)

| Human urine | Amount added (µg ml⁻¹) | Amount found (µg ml⁻¹) | Recovery (%) ± S.D. | X ± S.D. |
|-------------|------------------------|------------------------|---------------------|---------|
| Sample      |                        |                        |                     |         |
| Urine 1     | 0.002                  | 0.001                  | 97.22 ± 1.1         |         |
| Urine 2     | 0.031                  | 0.029                  | 94.98 ± 0.8         |         |
| Urine 3     | 0.120                  | 0.111                  | 95.32 ± 1.3         |         |
| Urine 4     | 0.559                  | 0.528                  | 96.01 ± 1.4         |         |
| X ± S.D.    |                        |                        | 95.9 ± 1.1          |         |

Average ± S.D. of five determinations.
Table 9: Comparison with other proposed methods for the determination of ten FQs

| Technique               | Studied | L.r^a | L.D^b | Application        | Reference |
|-------------------------|---------|-------|-------|-------------------|-----------|
| Spectrophotometry       | AMI     | 10-60 | 2.2   | Commercial        | [16]      |
| Spectrophotometry       | CIP     | 10-70 | 2.1   | Commercial        | [16]      |
| Spectrophotometry       | OFL     | 5-35  | 3.7   | Commercial        | [15]      |
| Atomic spectrometry     | PEF     | 10-80 | 4.8   | Commercial        | [16]      |
| Spectrofluorimetry      | LEV     | 20-300| 10    | Serum and urine   | [35]      |
| Spectrofluorimetry      | NOR     | 10^-3  | 2740  | Commercial        | [36]      |
| HPLC – FD               | LOM     | 10-1000| 10    | Plasma            | [34]      |
| HPLC – FD               | LEV     | 15-5000| 16    | Plasma            | [32]      |
| HPLC – FD               | NOR     | 70-2109| 70    | Plasma            | [33]      |
| Capillary electro.      | NOR     | 800-4500| 200   | Plasma            | [38]      |
| Capillary electro.      | NOR     | 4-800 | 1.0   | Plasma            | [38]      |
| Spectrofluorimetry      | CIP     | 4-140 | 0.7   | Commercial        | This work |
| Spectrofluorimetry      | NOR     | 2-290 | 1.2   | formulations      |           |
| Spectrofluorimetry      | OHL     | 3-220 | 0.9   | formulations      |           |
| Spectrofluorimetry      | LEV     | 4-280 | 0.4   | formulations      |           |

^a Linear dynamic range (ng ml^-1)  ^b Limit of detection (ng ml^-1)

Table 10: Effect of commonly used excipients on the determination of CIP (0.1 µg mL^-1)

| Drug (0.1 µg mL^-1) | Excipients (50 µg mL^-1) | Recovery (%±S.D.)^a |
|---------------------|-------------------------|--------------------|
| Ciprohexal          | Starch                  | 100.9 ± 1.22       |
| Ciprohexal          | Lactose                 | 99.54 ± 0.94       |
| Ciprohexal          | Glucose                 | 99.32 ± 1.19       |
| Ciprohexal          | Fructose                | 99.09 ± 1.12       |
| Ciprohexal          | Sucrose                 | 100.7 ± 1.31       |
| Ciprohexal          | Magnesium stearate      | 100.1 ± 0.98       |

^a Average value ± S.D. of five determinations

Effect of interfering substances: The assay results were unaffected by the presence of excipients as shown by the excellent recoveries obtained when analyzing the studied drugs in presence of commonly encountered excipients. Samples containing a fixed amount of the FQs (0.1 µg mL^-1) and excipients (50 µg mL^-1) were measured. No interference was observed from commonly used excipients such as starch, lactose, glucose, fructose, sucrose and magnesium stearate (Table 10). This fact indicates good selectivity of the method for determination of the studied drugs in raw material and their dosage forms. Also, the fluorescence of 6 ng mL^-1 for CIP solution was measured in the presence of some cations, typically in urine sample. Ca^{2+}, Mg^{2+}, Na^+ and K^+ do not cause interference at concentration ratio of cation /CIP > 1000 and Zn^{2+}, Cu^{2+}, Al^{3+} and Fe^{3+} interfere negatively at concentration ratio over 500, 300, 200 and 4, respectively, so no interference from the cations presence is expected.

CONCLUSION

The results obtained from the present study indicate that complex formation between the studied FQs and TFQ be employed in the spectrofluorimetric assay of AMI, CIP, DIF, ENO, ENR, LOM, LEV, NOR, OFL and PEF in dosage forms and human urine. The proposed methods are suitable for the routine quality control of the drug alone, in different pharmaceutical formulations and in human urine without fear of interference caused by the excipients expected to be present in pharmaceutical formulations or components of human urine.

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