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Fluorimetric Determinations of Ciprofloxacin in Presence of Its Metabolite and Degradate in Pharmaceutical Preparations and Human Serum

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Summary: A sensitive fluorimetric method based on measuring the fluorescence intensity after the reaction of ciprofloxacin (CF) with fluorescamine at 485 nm (excited at 390 nm) is described. Optimization of the reaction conditions has been studied and the validity of the suggested procedure was assessed applying the standard addition technique. The effect of fluorescamine concentration, pH of medium, reaction time and stability of the fluorescent product were studied. The investigated method is successfully applied to the determination of ciprofloxacin in bulk powder, pharmaceutical products and serum. Results with mean recovery 100.11±0.86% was obtained. The results of the present method were compared, with the data obtained by using a spectrophotometric (UV-Vis) method. Another variant of the method in micellar medium allows the direct measurement of ciprofloxacin in human serum by standard addition method. The enhanced fluorescence of ciprofloxacin in 10^{-3} mol L^{-1} sodium dodecyl sulfate solution shows a better linear range than the aqueous method with a recovery of 100.34% and a relative standard deviation of 0.65%. Also, it permits the background fluorescence of serum blank to be minimized. Hence, sufficient sensitivity is reached to determine therapeutic concentrations of the drug with an average recovery of 100.65%.

Introduction

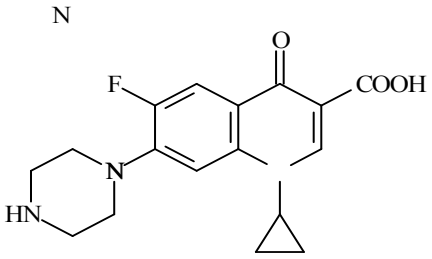

Fig. 1: Chemical structure of ciprofloxacin.

Ciprofloxacin {1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7 (1-piperazinyl)-3-quinolinecarboxylic acid} (Fig. 1), is synthetic broad spectrum antimicrobial agent.

Ciprofloxacin is the most potent quinolone against Gram-negative and Gram-positive bacteria.[1,2] The target of ciprofloxacin in bacteria is DNA gyrase, a type II topoisomerase. DNA gyrase is an essential enzyme which is responsible for negatively supercoiling covalently closed circular DNA and also in the catenation and decantation reactions.[3] The mechanism of action of the ciprofloxacin antibacterial agent involves the inhibition of DNA gyrase resulting in a rapid bactericidal effect.[4, 5] Numerous techniques have been developed for the analysis of ciprofloxacin in biological fluids and pharmaceutical preparations. The analysis of ciprofloxacin has traditionally been performed using microbiological methods. However this technique is slow and suffers from poor precision and specificity. Although numerous chemical and physical techniques have been reported for the assay of ciprofloxacin, they suffer from a variety of disadvantages. Several different methods have been used for the determination of CF, including high-performance liquid chromatography,[6-9] spectrophoto-metry,[10-12] conductimetry,[13] voltammetry,[14(fluorimetry,[15-17] titrimetry,[18] potentiometry,[19] capillary electrophoresis [20] and flow-injection chemiluminescence methods.[21] Native fluorescence of ciprofloxacin has been measured at 420 nm after excitation at 360 nm. The fluorescence of its primary degradate was found to interfere after excitation at 360 nm. Also related compounds like other fluoroquinolones interfere. Fluorescamine, 4-phenylspiro[furan-2(3H),1-phthalan]-3,3-dione, is a fluoregenic reagent that reacts directly with primary or secondary amines to form a fluorophore of high fluorescence intensity.[22] It has been used for the analysis of various pharmaceutical compounds that contain amino groups[23] and can detect nanogram quantities of either amines or amino acids.[24] In analytical chemistry, surfactants have been recognized as being very useful for different types of analytical methodology e.g. electrophoresis,[25] reversed-phase HPLC[26] and luminiescence spectroscopy.[27] In the present work, the spectrofluorimetric method was used for the determination of ciprofloxacin in presence of its metabolite and degradate via measuring the flouresence intensity resulting from its reaction with fluoresccamine at pH 3. The paper describes spectrofluorimetric and micelle-enhanced spectrofluorimetric determina- tions of

ciprofloxacin in spiked serum and also its determination in five commercial pharmaceutical formulations.

Experimental

Equipment Kontron spectrofluorophotometer (Switzerland), Model SF 25 connected to IBM compatible computer fitted with WIND 25 spectroscopy software for Windows is used.

Procedure

Reagents All reagents and solvents were of analytical grade and checked before use for the presence of fluorescent contaminants. Ciprofloxacin (CF) hydrochloride $C_{17}H_{18}FN_3O_3.HCl.H_2O$ was provided from Amrya for pharmaceutical Industries (Alexandria, Egypt). Pharmaceutical preparations were purchased from local pharmacies. Fresh serum (HS) was obtained from blood (VACSERA) by centrifugation at 2000 rpm for 2 minutes. These samples were used within 24 h. Fluorescamine was obtained from Sigma Chem. Co. (St. Louis, MO., USA). Sodium dodecyl sulfate (SDS) was purchased from Fluka, and prepared in 10^{-3} mol L^{-1} as aqueous solution. The other chemicals were of the highest quality available. Fluorescamine solution, 1 mg/ml in anhydrous acetone was aged for 24 h (by standing at room temperature) prior to use. McIlvaine's citric acid-phosphate buffer pH 3 was prepared by mixing 79.45 ml of 0.1 mol L^{-1} citric acid monohydrate solution and 20.55 ml of 0.2 mol L^{-1} disodium hydrogen phosphate solution. Stock standard solution for the fluorimetric and enhanced fluorimetric methods; 100 and 10 µg/ml CF in bi-distilled water (aqueous) and in 10^{-3} M SDS solution (micellar) was prepared, respectively.

a- Tablets Weigh and powder 20 tablets. Weigh accurately a quantity of the powdered tablets equivalent to about 500 mg of ciprofloxacin (Table 3), then with three 10 ml portions of methanol. Filter (using Whatmann No. 1) the extracts and transfer into a 50-ml calibrated flask. Complete to the mark with methanol. Dilute 1 ml portion of the resultant solution to 100 ml with bi-distilled de-ionized water. The above fluorimetric procedure using the diluted solution was applied. Calculate the concentration of ciprofloxacin from the corresponding calibration graph.

b- Injection From the mixed contents of five vials (Cipro Injection, 200 mmol/100 ml^{-1}), an accurately measured volume equivalent to 10 mg ciprofloxacin was transferred into 100-ml volumetric flask and the volume was completed to the mark with bi-distilled de-ionized water. The above fluorimetric procedure is then applied.

c- Serum Place 5 ml serum in a 25-ml stopper shaking tube. A 0.5 ml of standard ciprofloxacin solution was added, followed by 10 ml of diethylether. The tube was shaken for 5 min and centrifuged then the organic layer was rejected, followed by addition of 20 ml ethyl acetate, centrifugation and removal of the upper aqueous layer was taken place. Organic phase was vaccum evaporated, and transferred to another shaking tube containing 10 ml 0.1 mol L^{-1} hydrochloric acid, shaken for 10 min and centrifuged. The aqueous layer was finally transferred to 100-ml calibrated flask and completed to the mark with bi-distilled de-ionized water. The above fluorimetric procedure was applied using 0.1, 0.2 and 0.3 ml portions of this solution. Each concentration was calculated from calibration graph prepared similarly using bi-distilled de-ionized water in place of serum.

Enhanced Spectrofluorimetric Determination of CF (CF/F, miscllar)

1. **Tablets and Injection** Apply the procedures of fluorimetric determination of CF and fluorimetric measurement of CF/FS complex in tablets and injection as in aqueous using ciprofloxacin standard micellar solution (10 µg /ml).
2. **Determinarion of CF in Serum using the Suggested Micellar Method.** Plasma was spiked with appropriate amounts on CF standard micellar solution (10 µg/ml). The above procedure of tablets and injection was directly applied in the range of 0.1-0.6 µg /ml CF. No pretreatment was required for the determination of serum samples.

Results and Discussion

In the present work, the fluorimetric method depends on the use of highly sensitive reagent, fluorescamine which react directly with ciprofloxacin to form a fluorophore of high fluorescence intensity. The formation of a complex between CF and FS resulted in physico-chemical modification of intrinsic fluorescence of ciprofloxacin, the emission becoming higher and the emission intensity lower. These

results accord with literature[27] of interamolecular transition leading to a shift in photoluminescence of ciprofloxacin. Thus, CF/FS complex can be measured at 485 nm after excitation at 390 nm without any interference of its degradate and metabolite. The investigated procedure depends on the use of fluorescamine as amine fluorogenic derivative.

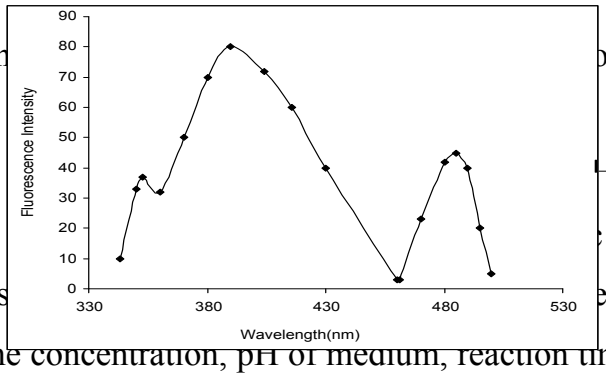

Fig. 2: Excitation an ol ml^{-1}) fluoroscamine

Fluorescamine amino groups of ciprofloxacin in the s escent derivative. The effect of fluorescamine concentration, pH of medium, reaction time and stability of the fluorescent product were studied. The influence of reagent concentration on the fluorescence developed at the selected wavelength was obtained by changing the concentration of fluorescamine over the range 2.5-25 µg/ml and showed that the optimum concentration to be used is 10 µg/ml. Changing the pH of the final solution over the range 1-6 showed that, the optimum pH was found to be 3. The maximum fluorescence intensity resulting from the reaction of ciprofloxacin with fluorescamine was developed within 5 min and stable for 1 h at room temperature. With the aim of studying the influence of ionic strength of aqueous solution of ciprofloxacin containing buffer at various concentrations of KCl were prepared. The results showed no fluorescence intensity variations for concentrations <1 mol L^{-1} KCl. The influence of the temperature on the fluorescence intensity shows a nearly linear (negative) relationship between temperature and fluorescence intensity for CF/FS complex. When temperature is decreased, the fluorescence is enhanced with temperature coefficient of 1.06%. This value showed that internal conversion was probable.[28] Hence samples were thermostated at 25±0.1^{o}C. The fluorescence of CF/FS complex in micellar media was studied by preparing 10 µmol ml^{-1} CF standard solutions with increasing concentrations of SDS. The fluorescence intensity increased with increasing of SDS concentrations until it reached a stable level with 10^{-3} mol L^{-1} SDS concentrations

more than 10^{-3} mol L^{-1} SDS provoked, no increment of fluorescence intensity. A micellar medium was demonstrated to be more suitable for serum samples determinations as fluorescence blanks were minimal under these working conditions. Nevertheless, the method of choice for pharmaceutical samples is that in micellar media, which ought to be more sensitive. Calibration graph was constructed for the proposed spectrofluorimetric method from five points over the concentration range 1-6 µmol ml^{-1} as cited in Table 1. Regression analysis indicated a linear relationship. A summary of the results of the regression data is also presented in Table 1.

Table 1. Analytical parameters of the fluorimetric and enhanced fluorimetric determination of ciprofloxacin.

Parameter	Value	
	Aqueous	Micellar
Maximum wavelength (nm)	390 nm (Ex.), & 485 nm (Em.)	390 nm (Ex.), & 485 nm (Em.)
Concentration range	1-6 µmol ml^{-1}	0.1-0.6 µmol ml^{-1}
Intercept	0.84	0.13
Slope	15.52	166.2
Correlation coeff.	0.999	0.999
Recovery (%)[a]	100.11	100.34
RSD (%)[a]	0.85	0.65

* Ex.: Excitation, Em.: Emission
[a] Average of five measurements

Five replicate determinations at different concentration levels were carried out to test the accuracy of the method. The relative standard deviations were found to be less than 2%, indicating excellent reproducibility of the selected method. The proposed method was successfully used for the determination of the studied drug in pharmaceutical dosage form (Table 2) and in human serum (Table 3) with good accuracy and precision. The interference of foreign compounds (soluble expiates used in Rancif, Ciproby, Ciprofar and Bactflux and some typical co-administerod drugs) and ciprofloxacin metabolite and degradate was studied by increasing concentrations of these compounds to 1 µmol ml^{-1} CF solution until a greater than 5% variation in

fluorescence intensity was achieved. Table 4, shows the maximum tolerable weight ratio for these compounds when using both the aqueous and micellar suggested methods. As can be seen, the selectivity achieved by the micellar method is better than, or similar to that of the aqueous fluorescence method, but both have sufficient selectivity to determine ciprofloxacin in the presence of the tested compounds.

Table 2. Determination of ciprofloxacin in pharmaceutical formulations by the suggested fluorimetric methods.

Preparation	Aqueous			Micellar			UV-Vis (12)
	Found[a] %	Added µg/ml	R(%)	Found[a]%	Added µg/ml	R(%)	Found %
Rancif Tablets (250 mg)	10.13±0.77	5 10 15	99.5 103 98.5 100.33±0.65	100.3±0.41	0.5 1.0 1.5	100.6 100.4 100.8 100.6 ± 0.65	97.3±1.34
Ciprobay Tables (250 mg)	102.1±0.78	5 10 15	100.8 101.0 100.5 100.77±0.25	100.8±0.38	0.5 1.0 1.5	100.3 101.2 100.7 100.73±0.25	98.4±0.98
Ciprofar Tablets (250mg)	100.5±0.73	5 10 15	99.6 99.8 97.7 99.03±1.17	99.7± 0.68	0.5 1.0 1.5	99.9 100.1 99.7 99.90±1.17	96.8±0.68
Bactiflox Tablets (250 mg)	101.3±0.96	5 10 15	100.9 100.3 99.6 100.27±0.64	100.5± 134	0.5 1.0 1.5	98..3 96.5 99.7 99.5±0.64	95.4±0.74
Cipro Injection (200 mmol/100 ml^{-1})	99.95±0.88	5 10 15	100.3 102.4 101.9 101.53±1.08	100.2± 1.26	0.5 1.0 1.5	98.7 100.5 100.3 99.83±1.08	97.4±0.89

[a] Average of five measurements

The validity of the proposed procedure was assessed by applying the standard addition technique (Table 2), The results obtained indicate that different additives with ciprofloxcin do not interfere. Statistical comparison shows that there is no significant difference between the results obtained and the reference method.(13)

Table 3. Precision and accuracy of ciprofloxacin in spiked human serum by the suggested procedures.

Aqueous Fluorimetry		Micellar Fluorimetry	
Added µmol ml^{-1}	R%±SD*	Added µmol ml^{-1}	R%±SD*
1	102.8±2.51	0.1	100.1±1.78
2	103.0±1.90	0.2	100.0±1.26
3	101.5±0.89	0.3	100.1±1.25
4	105.1±2.00	0.4	99.9±0.86
5	101.7±1.80	0.5	100.2±0.65
6	103.6±0.81	0.6	101.0±0.97

* Mean of five separate determinations

The proposed method was found to be accurate and precise since the calculated t and F values are less than the tabulated ones. The developed procedures showed working characteristics adequate for the determination of ciprofloxacin in presence of its metabolite and degradate in pharmaceutical preparations and human serum. Table 3 shows that the results obtained for determination of ciprofloxacin in tablets and injection. Table 4 shows good recoveries of ciprofloxacin in spiked human serum by the proposed procedures. The recovery values and relative standard deviations, which were calculated after standard additions, are also presented in Table 4. The average recoveries for both procedures are, 102.95±1.65 and 100.21±1.13% for aqueous and micellar methods respectively.

Conclusion

The results of this study show that, the proposed flourimetric procedures are advantageous for the determination of ciprofloxacin in presence of its metabolite and

degradate because they are satisfactory, reliable, sensitive and selective. The previous spectrofluorimetric methods have some disadvantages, e.g., sophisticated procedure,[17] interference of other fluoroquinolone compounds[16] and low detection limit[15] The described spectrofluorimetric methods have simple work-up procedure, require no sophisticated instrumentation and no poisonous organic solvents .No pretreatments even for the serum samples by using micellar method.

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