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New Approach for Determination of Ciprofloxacin by Quenched Fluorescence of Analytically Interested Species Using Continuous Flow Injection Laser Diode Fluorimeter Analyser

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Abstract

The objective of this study was to establish an accurate, precise, sensitive, simple, fast and reliable method for the determination of ciprofloxacin hydrochloride in pure or in pharmaceutical dosage forms using Homemade instrument fluorimeter continuous flow injection analyser with solid state laser (405 nm) as a source. The method is based upon the fluorescence fluorescein sodium salt and its quenching by ciprofloxacin hydrochloride in aqueous medium. The solutions of standard and the sample were prepared in distilled water. The calibration graph was linear in the concentration range of using (10 - 100) $mMol.L^{-1}$ ciprofloxacin hydrochloride (r= 0.9891) with relative standard deviation (RSD%) for 3 $mMol.L^{-1}$ ciprofloxacin HCl solution is lower than 2%(n=6). A comparison was made between the newly developed method of analysis with the quoted value using the standard addition method and no significant difference between the new developed method and the quoted value by the manufacturers companies. This indicates clearly that the new method can be successfully used for the assay of the cited day.

Keywords: Ciprofloxacin hydrochloride, homemade instrument fluorimeter continuous flow injection analyser, flow injection, fluorescein sodium salt

طريقة جديدة لتقدير السايبروفلوكساسين بوساطة تثبيط الفلورة لفصائل تحليلية مهمة وباستخدام محلل الحقن ألجرياني المستمر للفلورة بثنائيات وصلات ليزرية

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الخلاصة:

تم استحداث طريقة دقيقة ، بسيطة ، سريعة و حساسة لتقدير السايبروفلوكساسين بشكله النقي وفي مستحضراته الصيدلانية باستخدام جهاز فلورومیتر مصنع محليا بالاقتران مع تقنية الحقن ألجرياني المستمرذو مصدر ليزري بطول موجي (405 nm) تستند الطريقة على تثبيط الفلورة لمادة الفلورسين باستخدام السايبروفلوكساسين كمادة مثبطة للفلورة في الوسط المائي . تم الحصول على خطية تتراوح بين (10 – 100) مللي مول. لتر$^{-1}$ مع معامل الارتباط r=0.9891 والانحراف القياسي المئوي اقل من 2% لتركيز 3 مللي مول. لتر$^{-1}$ سايبروفلوكساسين ل(n=6). تم تطبيق الطريقة لثلاثة عقاقير دوائية لتقدير السايبروفلوكساسين. كما قدمت مقارنة بين الطريقة المستحدثة والقيمة الفعالة باستخدام اختبار t– المزدوج لوحظ ان القيمة المحسوبة اقل من القيمة الجدولية ، وعلى هذا الأساس بالإمكان استخدام الطريقة المقترحة للتحليل بالحقن ألجرياني كطريقة بديلة لتقدير السايبروفلوكساسين .

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Introduction

Fluoroquinolones are important antibacterial group of drugs developed in recent years which have been widely used for treatment of many bacterial infections. Broad spectrum of activity and good oral absorption have led to extensive clinical use of the newer fluoroquinolones. The ciprofloxacin hydrochloride (CIP) Figure-1 chemically described as 1-cyclopropyl -6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxyclic acid, monohydrochloride. CIP is used to treat or prevent certain infections caused by bacteria. The ciprofloxacin is also used to treat or prevent anthrax in people who may have been exposed to anthrax germs in the air [1].

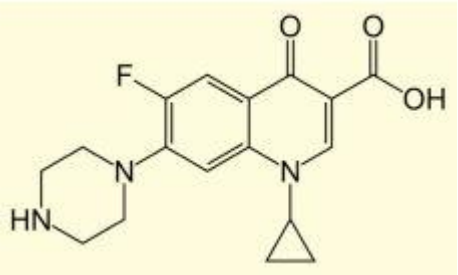

Figure 1- Chemical structure of ciprofloxacin HCl

Different methods were described for quantitative analysis of ciprofloxacin hydrochloride like uv-vis [2-6] and HPLC [7-11]. Differential electrolytic potentiometric titration method for the determination of ciprofloxacin [12] , Titrimetric and spectrophotometric methods are described for the determination of ciprofloxacin in bulk drug and in formulations using cerium (IV) sulphate as the oxidimetric agent and methyl orange and indigo carmine as chromogenic agents [13]. Highly sensitive method for the determination of ciprofloxacin in pure and pharmaceutical preparation were developed by coupling the continuous flow injection analysis via turbidimetric (T180o) and scattered light effect at two opposite position (2N90o) [14]. Sequential injection analysis (SIA) system for the spectrophotometric was used for determination of norfloxacin (NOR) and ciprofloxacin (CIP) in pharmaceutical formulations. [15]. This work describes the development of a new, simple,rapid and selective method to determine the ciprofloxacin concentration in pharmaceuticals by the quenching of fluorescence.

Chemicals

- Stock solution of ciprofloxacin hydrochloride($C_{17}H_{18}FN_3O_3.HCl.H_2O$ M.wt=385.80 $g.mol^{-1}$) (SDI) (0.1M) was prepared by dissolving 3.358g of CIP in 100mL distilled water, and fluorescein sodium salt (uranine) ($C_{20}H_{10}Na_2O_5$ M.wt=376.27 $g.mol^{-1}$) **(**Hopking & William**)** (0.01M) was prepared by dissolving 3.7627 g in 1L of distilled water**.**
- Twenties tablets were weighted crushed and grinded tablets containing 500 mg of Ciprofoxacin hydrochloride were weighted, 2.9784 g of ciprova- India, 2.9745 g of cipropharm- Jordan, and 2.9900 g of ciproxene- Syria, equivalent to 1.9290 g of active ingredient to obtain 50 $mMol.L^{-1}$. The powder was dissolved in little distilled water followed by filtration to get rid of undissolved materials, and then complete the volume to 100 mL with distilled water.

Apparatus and manifold

A Homemade fluorimeter continuous flow injection analyser using a solid phase laser (405 nm) as a source. One channel of peristaltic pump was used (Ismatec type ISM 796), with a rotary 6-port injection valve (Rheodyne, U.S.A) , a sample loop (0.5 mm id, Teflon, variable lengths) for sample injection, reaction coil (2 mm id, 50cm length) to complete the quenching process were used . The output signals was recorded by x-t potentiometric recorder (kompenso graph C-1032 Siemens - Germany). Peak height was measured for each signal. Figure-2 shows the flow diagram that was used for the determination of ciprofloxacin.

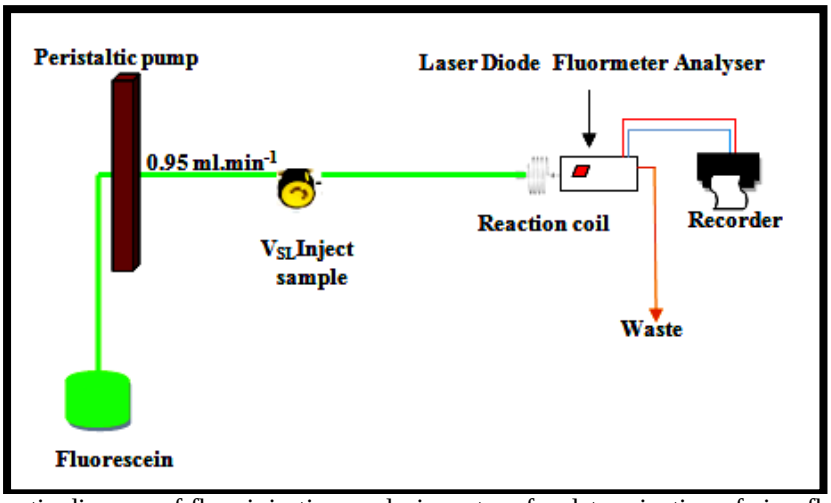

Figure 2- A schematic diagram of flow injection analysis system for determination of ciprofloxacin using laser diodes CFIA analyser

Methodology

The flow diagram shown in Figure-2 is used for the determination of ciprofloxacin hydrochloride by the quenching fluorescence of fluorescein sodium salt which is formed in the first line. The carrier stream (fluorescein sodium salt (uranine)) (1mMol.L^{-1}) at the flow rate 0.95 mL.min^{-1} to react with the injected sample volum (35µl) ciprofloxacin.HCl to complete the reaction , with an outlet for reactants product to pass through homemade instrument fluorimeter continuous flow injection analyser. The responses were recorded on x-t potentiometric recorder which measure the quenched fluorescence signals.

Variable optimization

Chemical variables

Manifold reaction design coupled with laser diode fluorimeter CFIA analyser:

The study was carried out using the following experimental conditions, 10 mMol.L^{-1} of ciprofloxacin.HCl solution with 35 µL as a sample volume and (0.01,0.05,0.1,0.5,1,5) mMol.L^{-1} fluorescein sodium salt with flow rate of 0.95and 1.3 mL.min^{-1} for the two carrier streams (H_2O) and fluorescein solution respectively. This study was carried out to optimize the best manifold that can be used.

The first manifold design:

One line system was used. Figure-3 shows the flow diagram. Fluorescein sodium salt of various concentrations was used at flow rate 0.95 mL.min^{-1} to carry the injected ciprofloxacin. HCl which quenches the fluorescent signal as shown the in the response profile in Figure-4.

The second manifold design:

The manifold system is composed of two lines Figure-5. The first line supplies distilled water as a carrier stream at flow rate 0.95 mL.min^{-1} which leads to the injection valve for carrying ciprofloxacin. HCl sample. The second line supplies fluorescein solution at flow rate 1.3 mL.min^{-1}. Both of lines merges via Y- junction and the merged passes through laser diode fluorimeter continuous flow injection analyser. This process leads to quenching the fluorescent signal as shown in the response profile in Figure-6.

As a final conclusion was that the one line system gave regular and smooth response profiles at fluorescein concentration (1 mMol.L^{-1}).

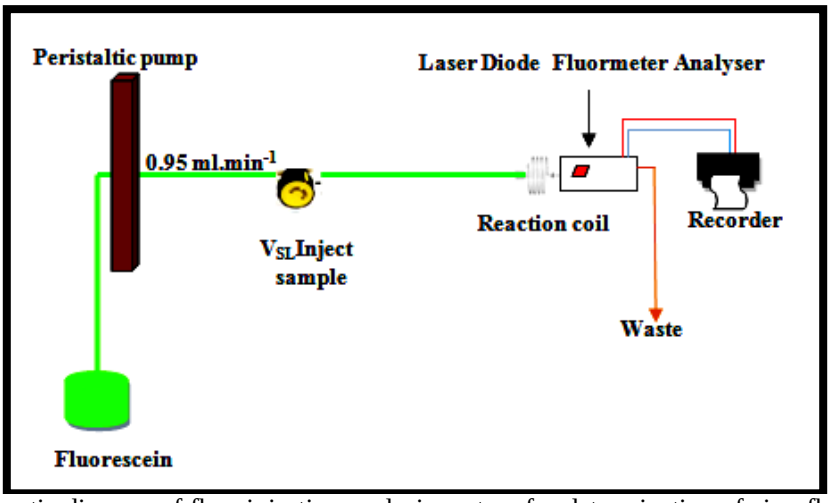

Figure 3- A schematic diagram of flow injection analysis system for determination of ciprofloxacin using laser diodes CFIA analyser (Single line mode).

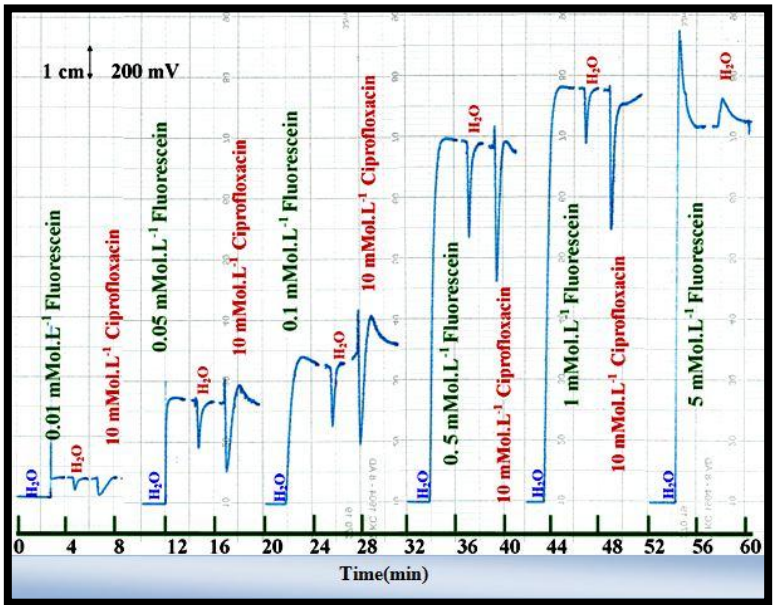

Figure 4- Effect of the variation of fluorescein concentration on response

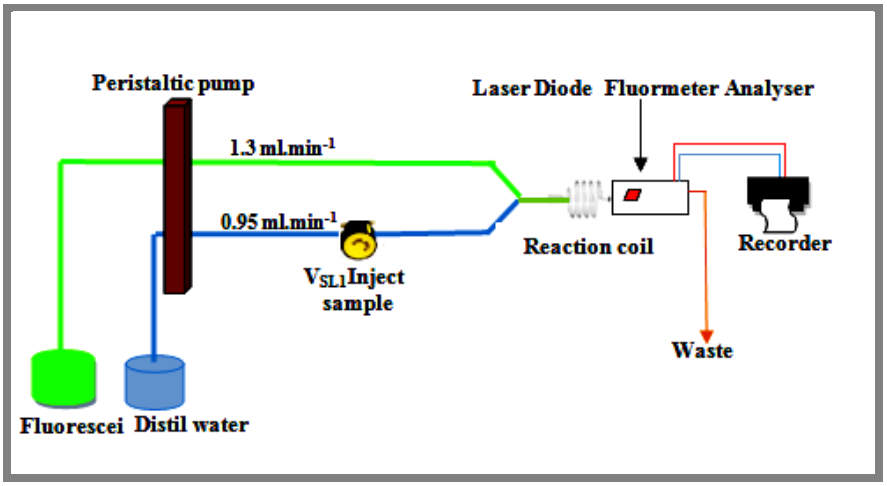

Figure 5- A schematic diagram of flow injection analysis system for determination of ciprofloxacin using laser diodes CFIA analyser (Two line system).

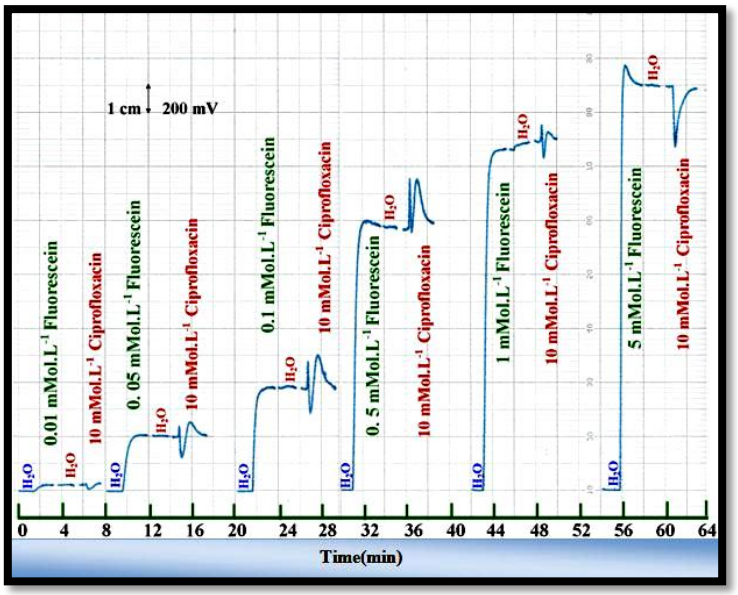

Figure 6- Effect of the variation of fluorescein concentration on response.

Physical variables

Physical parameters (flow rate, sample volume, purge times and volume of coil) were studied using one line manifold system Figure-2.

Flow rate

Conducting a flow rate study on the inrestigated system using 10 $mMol.L^{-1}$ of ciprofloxacin with a carrier stream of fluorescein reveals different kinds of energy transducer response output *vs.* time profile as shown in Figure-7. The obtained results were tabulated in Table-1. Flow rates of 0.2 to 0.9$mL.min^{-1}$ gave distorted profiles with a time consuming measurements and there were difficulties in restoring the background. While a flow rate of 0.95 $mL.min^{-1}$ could be considered as an acceptable flow as it can be seen in Figure-7, taking into account measurement time, response profile and returning to the background.

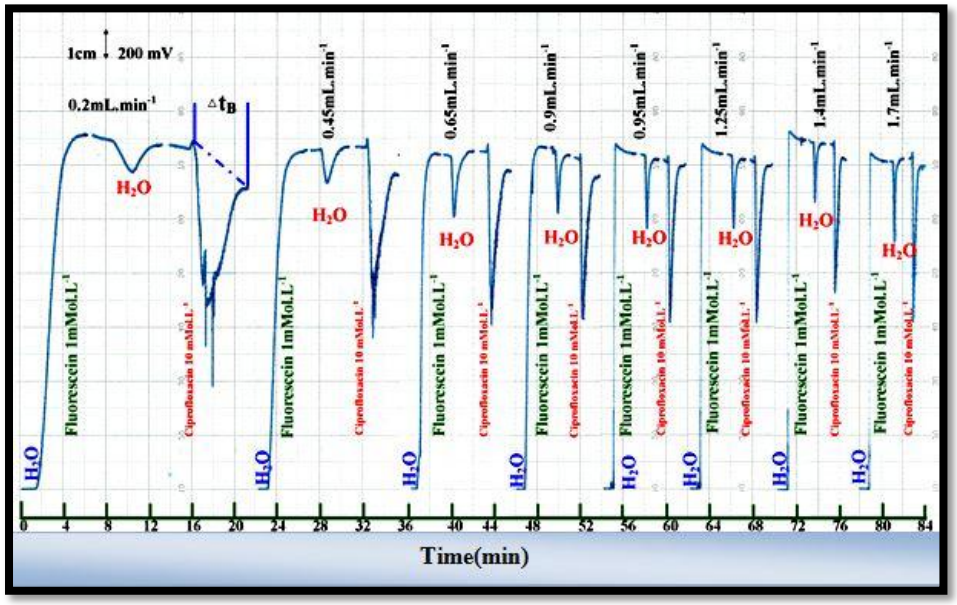

Figure 7- Effect of the variation of the flow rate on quenched fluorescence response profile

Table 1- Effect of flow rate on the quenched fluorescence response

Flow rate mL.min^{-1}	Quenched fluorescence response of blank (mV)	Quenched fluorescence response expressed as an average peak heights (n=3) in $\bar{y}_i$ (mV)	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2,n-1}\, \sigma_{n-1}/\sqrt{n}$	Base width Δt_B (sec)
0.2	320	1160	0.43	1160±12.42	312
0.45	320	1280	0.78	1280±24.85	192
0.65	580	1040	0.96	1040±24.85	132
0.9	620	880	0.57	880±12.42	108
0.95	700	820	0.61	820±12.42	96
1.25	620	820	2.44	820±49.69	78
1.4	580	820	1.83	820±37.26	72
1.7	760	780	1.28	780±24.85	66

Sample volume

The injected volume of sample was varied from 18 to 39 µL by changing the length of the sample loop in the injection valve, while the other chemical and physical parameters were remained fixed. An increase in the injection volume leads to a significant increase in sensitivity and to more fluorescence that can be shown in Figure-8 and Table -2 (35 µL) can be described as sample injection volume for the manifold system used.

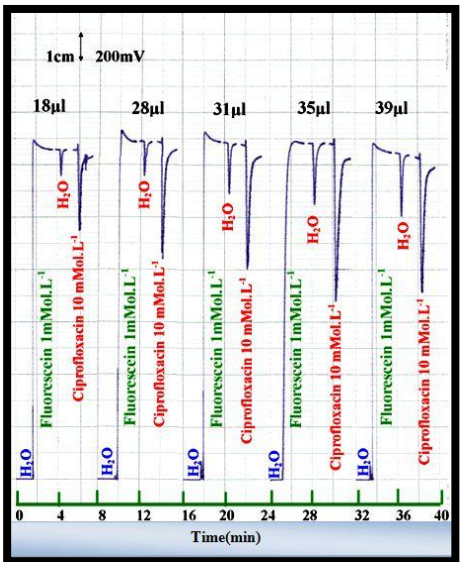

Figure 8- Effect of the variation of the sample volume on quenched fluorescence response profile

Table 2- Effect of the variation of sample volume on the quenched fluorescence response

Sample volume µl	Quenched fluorescence response of blank (mV)	Quenched fluorescence response expressed as an average peak heights (n=3) in $\bar{y}_i$ (mV)	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2,n-1}\, \sigma_{n-1}/\sqrt{n}$	Base width Δt_B (sec)
18	260	520	1.92	520±24.85	90
28	340	760	0.66	760±12.42	90
31	500	700	2.86	700±49.69	84
35	600	900	1.11	900±24.85	84
39	610	690	0.73	690±12.42	84

Purge time

Variable purge times for the sample segment of 5 to 30 seconds were used. The injection valve was in the open mode to allow enough time for the carrier solution to pass through the set in injection mode followed by turning the injection valve to the load position. Sample volume of 35 µL was used with concentration of ciprofloxacin HCl of 10 mMol.L^{-1}. Figure-9 shows the continuation of the increase in quenched response with increasing of injection time. The obtained results were tabulated in Table-3 that can be described by the manifold system used.

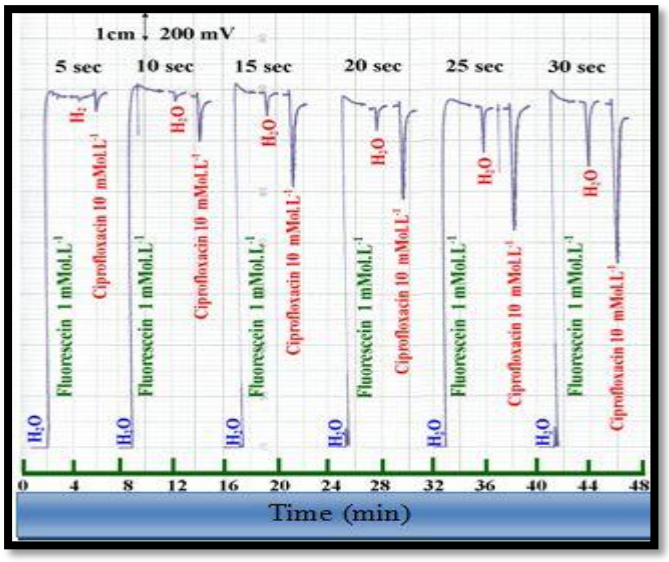

Figure 9- Effect of the variation of the purge time on fluorescence response profile.

Table 3- Effect of the variation of purge time on the quenched fluorescence response.

Purge time (Sec)	Quenched fluorescence response of blank (H_2O)(mV)	Quenched fluorescence response expressed as an average peak heights (n=3) in $\bar{y}_i$ (mV)	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2,n-1}\, \sigma_{n-1}/\sqrt{n}$
5	60	120	0.0	120±0.0
10	100	350	1.43	350±12.42
15	240	670	1.49	670±24.85
20	240	700	0.71	700±24.85
25	460	740	1.35	740±12.42
30	620	940	1.06	940±12.42

Effect of coil length:

Variable coil lengths 0 – 100 cm were studied, this range of length comprises a volume of 0.00 – 0.785 mL which is connected after injection valve directly in flow system .Optimum concentrations of fluorescein sodium salt (1mMol.L^{-1}) and ciprofloxacin.HCl(10 mMol.L^{-1}) with sample volume of 35 µL were used. A study shows that when the injector was directly corrected to the flow cell (when no coil is used) gave better response profiles. Figure-10 describes the detailed profile which gave most acceptable result and a better response profile. Therefore no coil was used.

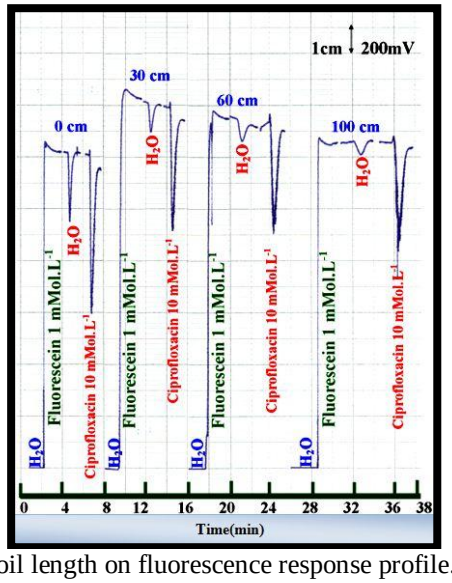

Figure 10- Effect of the variation coil length on fluorescence response profile.

Calibration graph

A series of variable concentrations ranging from 0.1 to 100 mMol.L^{-1} for ciprofloxacin HCl were prepared and injected at the established optimum conditions. Table-4 tabulates the results obtained and variation of ciprofloxacin HCl concentration at 35 µL sample volume to the injected *vs*. quenched response of laser diode fluorimeter analyser as shown in Figure-11. A scatter plot diagram was constructed between the variations of the quenched responses *vs*. concentration of ciprofloxacin HCl showing a linear dynamic range from 10 to 100 mMol.L^{-1}. Figure-12, Table-5 tabulates the correlation coefficient, linear percentage, straight line equation and the calculated t-value at 95% confidence; which is larger than tabulated t-value indicating clearly that the linearity against non-linearity is accepted to quenched response of laser diode fluorimeter analyser, when using (1mMol.L^{-1}) fluorescein sodium salt.

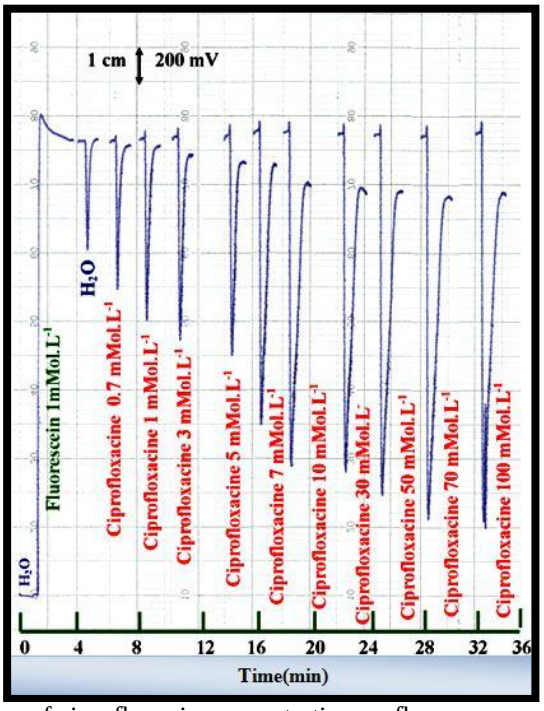

Figure 11- Effect of the variation of ciprofloxacin concentration on fluorescence response.

Table 4- Effect of the variation of ciprofloxacin concentration on the measurement of quenched fluorescence response. Energy transducer response of fluorescein = 3320 mV.
Measurement of quenched fluorescence response of blank= 820mV

Concentration (mMol.L^{-1})	Quenched fluorescence response expressed as an average peak heights (n=3) in $\bar{y}_i$ (mV)	RSD%	Confidence interval at (95%) $\bar{y} \pm t_{0.05/2,n-1}\ \sigma_{n-1}/\sqrt{n}$
0.7	300	0.0	300 ±0.0
1	480	1.72	480 ±24.85
3	680	1.25	680 ±24.85
5	800	0.0	800 ±0.0
7	1380	0.46	1380 ±12.42
10	1600	0.61	1600±24.85
30	1680	0.52	1680 ±24.85
50	1820	0.94	1820 ±49.69
70	1980	0.0	1980 ±0.0
100	2080	1.72	2080 ±24.85

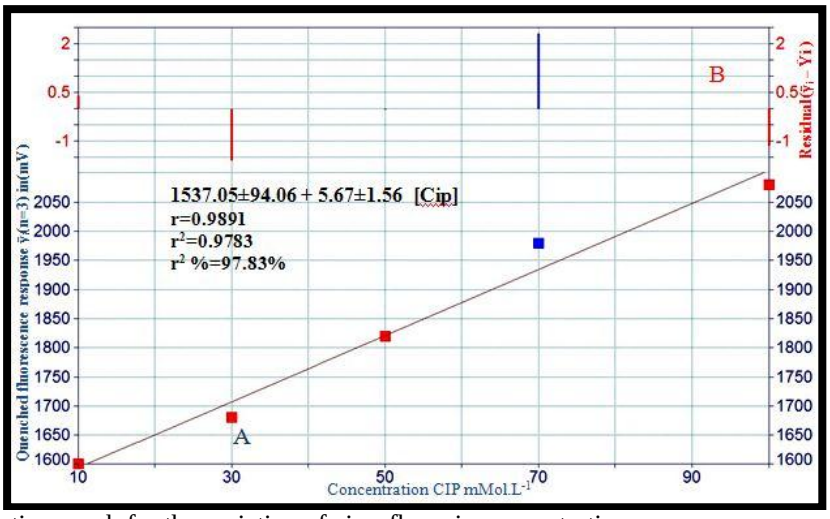

Figure 12- Calibration graph for the variation of ciprofloxacin concentration on:
A: Quenched fluorescence response expressed by linear equation using laser diode fluorimeter analyser,
B: Residual ($\bar{y}_i - \hat{Y}i$), $\bar{y}$: practical value, $\hat{Y}i$: estimated value,

Table 5- Summary of linear regression for the variation of quenched fluorescence response with ciprofloxacin concentration using simple regression line of the from ($\hat{Y}$ = a+bx) at optimum conditions.

Measured [CIP] $mMol.L^{-1}$	Linear dynamic range [CIP] $mMol.L^{-1}$ n= 7	$\hat{y}= a \pm S_a t + b \pm S_b t$ [CIP]$mMol.L^{-1}$ at confidence interval at 95%, n-2	r r^2 r^2 %	$t_{table\ no.}$ at 95%, n-2	Calculated $t_{-value} = \frac{/r/\sqrt{n-2}}{\sqrt{1-r_2}}$
0.7-100	10 - 100	1537.05±94.06 + 5.67±1.56 [CIP]	0.9891 0.9783 97.83%	3.182 << 11.642	

$\hat{Y}$(mV) = Estimated response measurement (n=3) for each single concentration, r=correlation coefficient, r^2%:linearity percentage

Limit of Detection (L.O.D)

A study was carried out to determine the limit of detection of ciprofloxacin HCl via successive gradual dilution of the minimum concentration in the linear range. Table-6 shows the limit of detection conducted by linear range equation and corrected limit of detection (LOD) based on dilution factor (DF).

Table 6- Limit of detection of ciprofloxacin at optimum parameters

Practically based on the gradual dilution for the minimum concentration	Corrected limit of detection (LOD) based on dilution factor (DF).
8.120 µg/sample	0.186 µg/sample

Repeatability

The repeatability of measurement and the efficiency of homemade laser diode fluorimeter analyser were studied at fixed concentration of ciprofloxacin HCl (3mMol.L^{-1}), using the optimum parameters. Repeated measurements for six successive injections were measured, and the obtained results are tabulated in Table-7 which shows that the percentage relative standard deviation indicate clearly the trustability of the adopted methodology using laser diode fluorimeter analyser. Figure-13shows a kind of response-time profile of successive repeatability measurements.

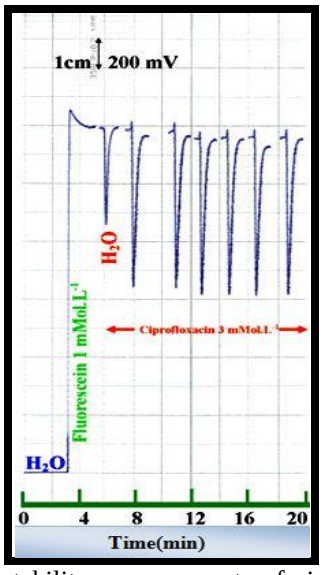

Figure 13- A Profile of successive repeatability measurements of ciprofloxacin using laser diode fluorimeter analyser.

Table 7- Repeatability of successive measurements for ciprofloxacin at (35 µL).
Energy transducer response of fluorescein = 2980 mV
Measurement of quenched fluorescence response of blank= 840 mV

Type of Measured	No. of measurements	Incident light response expressed as peak height (mV)	Average $\bar{y}_i$ mV	RSD %	confidence interval of the mean $\bar{y} \pm t_{0.05/2,\ n-1}\ \sigma n\text{-}1/\sqrt{n}$
Ciprofloxacin	6	530, 520, 520, 540, 520, 540	528.333	1.86	528.333 ±10.32

Application

Three different samples of pharmaceutical preparations (Ciprova- India, Cipropharm – Jordan and Ciproxene - Syria) were used to determine of ciprofloxacin HCl by continuous flow injection analysis using homemade laser diode fluorimeter analyser -continuous flow injection analyser,. A series of sample solutions were prepared by transferring 10 mL to each of the five volumetric flask (50 mL), followed by the addition of (0,5,10,15,20,25) mL from 10 $mMol.L^{-1}$ standard solution of ciprofloxacin HCl in order satisfy the concentration range from (0-5) $mMol.L^{-1}$. Flask no.1 is the sample flask. Table-8 shows the summary of standard additions method results from the three samples with the amount of ciprofloxacin HCl in samples. Using paired t-test between the newly developed method and quoted value; as shown in Table-9, it can be noticed that there was no significant difference between the new developed method and the quoted value by the manufacturers companies. A calculated t-value is less than critical t-value which indicate clearly the new method can be used as well as the method adopted by the manufacture companies.

Table 8- Results for determination of Ciprofloxacin HCl in pharmaceutical preparations

Commercial content country	Confidence interval for average volume at 95%	Quoted content of active ingredient at 95% n=∞ $mMol.L^{-1}$	Found content of active ingredient at 95 n= ∞ $mMol.L^{-1}$	Recovery %	Relative Error % (RE %)
Ciprova(India)	0.772±0.004	500±2.59	508.79±14.00	101.76	1.76
Cipropharm (Jordan)	0.771±0.004	500±2.59	499.51±14.43	99.90	0.10
Ciproxene (Syria)	0.775±0.009	500±5.81	490.50±10.35	98.10	1.90

Table 9- Summarize Paired t-test results for laser diode fluorimeter analyser CFIA method with quoted value using standard addition method for the determination of Ciprofloxacin HCl in pharmaceutical preparations.

Type of Sample	Practical content ($mMol.L^{-1}$). New method		Quoted value μ	$\bar{x}$- μ	$\bar{x}$d	σ_{n-1}	Paired t-test $t=\frac{\bar{x}d\sqrt{n}}{\sigma n-1}$	confidence interval $t_{table.}$ at 95%,
	x	$\bar{x}$						
Ciprova (India)	508.99 494.69 522.69	508.79	500	8.79	0.4	9.20	0.08 << 4.303	
Cipropharm (Jordan)	501.28 484.28 512.98	499.51	500	-0.49				
Ciproxene (Syria)	490.40 500.90 480.20	490.50	500	-9.5				

Conclusions

The proposed method shows interesting features such as rapidity, simplicity, high sensitivity and low cost per analysis. The above data proved that the analytical method developed and validated for assay determination of ciprofloxacin HCl in pharmaceutical formulations is more precise, accurate, robust and rugged throughout its range and can be more useful for commercial applications and as an alternative analytical method.

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