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Estimation of Ciprofloxacin Hydrochloride in Bulk and Formulation by Derivative UV-Spectrophotometric Methods

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Abstract

Simple, fast and reliable spectrophotometric methods were developed for determination of Ciprofloxacin Hydrochloride in bulk and pharmaceutical dosage forms. The solutions of standard and the sample were prepared in Distilled Water. The quantitative determination of the drug was carried out using the zero/0th, first, and second order method values measured at264, 273and 273 nm respectively. Calibration graphs constructed at their wavelengths of determination were linear in the concentration range of Ciprofloxacin Hydrochloride using 2-10µg/ml (r^2=0.9991, r^2=0.9993, r^2=0.9955) for zero, first and second order spectrophotometric method. All the proposed methods have been extensively validated as per ICH guidelines. There was no significant difference between the performance of the proposed methods regarding the mean values and standard deviations. The developed methods were successfully applied to estimate the amount of Ciprofloxacin Hydrochloride in pharmaceutical formulations.

Keywords: Ciprofloxacin Hydrochloride, UV visible spectrophotometry, Zero, first and second order derivative spectrum.

1. Introduction

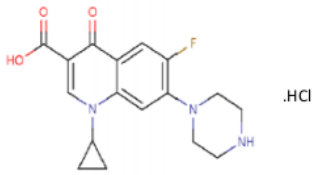
Ciprofloxacin hydrochloride (figure no.1), a fluoroquinolone, is the monohydrochloride monohydrate salt of 1-cyclopropyl -6- fluoro -1, 4-dihydro-4-oxo -7-(1 -piperazinyl) - 3-quinolinecarboxylic acid, Figure no.1 is a fluoroquinolone-type antibiotic agent. It exhibits broad spectrum antimicrobial activity against Gram - positive and Gram-negative bacteria such as Pseudomonas aeruginosa, *Streptococcus faecalis*, *Staphylococcal aureus*, and *Enterobacter aerogenes*[1,2]. It is used in the treatment of a wide range of infectious diseases.[3] Ciprofloxacin is also one of the antibiotics approved by the FDA for patients who have been exposed to the inhaled form of anthrax. Its mode of action depends upon blocking bacterial DNA replication by binding itself to an enzyme called DNA gyrase, thereby preventing the enzyme's ability to untwist the DNA double helix, which is required for DNA replication.[4]

Several analytical methods have been developed for the determination of ciprofloxacin. Inlecturer review, ciprofloxacin was determined by high performance liquid chromatography (HPLC), voltammetry, Spectrofluorimetric method, Biosensors, HPLC–MS/MS, Solid phase spectrophotometry, micro emulsion electro kinetic chromatography (MEEKC) method[5-12],Microbiological turbidimetric method Spectrophotometry, Micellar liquid chromatographic (MLC) electrophoresis, flow injection UV spectrophotometric[12-21], flow injection chemiluminescence (CL), thin-layer chromatography is established, with micelle solutions as mobile phases(Micelle TLC Fluorimetry). The Rayleigh light scattering technique,Derivative spectrophotometertric, and Fourier transform infrared spectrometric (FTIR).[22-25]

To our notice, no UV- spectrophotometric

method using zero, first and second order spectrophotometric method has been reported for the determination of Ciprofloxacin Hydrochloride in bulk and tablets.Hence an attempt has been made to develop new zero, first and second order spectrophotometric method for estimation of Ciprofloxacin Hydrochloride in bulk and pharmaceutical formulations with good accuracy simplicity, precision and economy.

Fig. 1 Structure of Ciprofloxacin Hydrochloride



2. Materials and Methods

2.1 Derivative Spectrophotometric Methods.

Derivative spectrophotometry is a useful means of resolving two overlapping spectra and eliminating matrix interferences or interferences due to an indistinct shoulder on side of an absorption band. Derivative spectrophotometry involves the conversion of a normal spectrum to its first, second or higher derivative spectrum. In the context of derivative spectrophotometry, the normal absorption spectrum is referred to as the fundamental, zeroth order or D^0 spectrum. The absorbance of a sample is differentiated with respect to wavelength λ to generate first, second or higher order derivative.

[A]= f(λ): zero order

[dA/dλ= f(λ): first order

[$d^2A/d\lambda^2$]= f(λ): second order

The first derivative spectrum of an absorption band is characterized by a maximum, a minimum, and a cross-over point at the λ max of the absorption band. The second derivative spectrum is characterized by two satellite maxima and an inverted band of which the minimum corresponds to the λ max of the fundamental band[26].

2.2 Apparatus and instrumentation

A shimadzu 1800 UV/VIS double beam spectrophotometer with 1cm matched quartz cells was used for all spectral measurements. Single Pan Electronic balance (CONTECH, CA 223, India) was used for weighing purpose. Sonication of the solutions was carried out using an Ultrasonic Cleaning Bath (Spectra lab UCB 40, India). Calibrated volumetric glassware (Borosil®) was used for the validation study.

2.3 Materials

Reference standard of Ciprofloxacin Hydrochloride API was supplied as gift sample by Marksan Pharmaceutical Ltd., Verna, and Goa. Tablet sample with label claim 500 mg per tablet were purchased from local market Pune.

2.4 Method development

2.4.1 Preparation of Standard and Sample Solutions

Stock solution of 10µg/ml of Ciprofloxacin Hydrochloride was prepared in Distilled Water, for zero, first and second order spectrophotometric analysis. The standard solutions were prepared by dilution of the stock solution with Distilled water in a concentration range of 2, 4, 6, 8, and 10µg/ml with Distilled water for zero order and area under the curve spectrophotometric methods. Distilled water was used as a blank solution.

Fig. 2: Zero/0th order derivative spectrum of Ciprofloxacin Hydrochloride in Distilled water (10µg/ml).

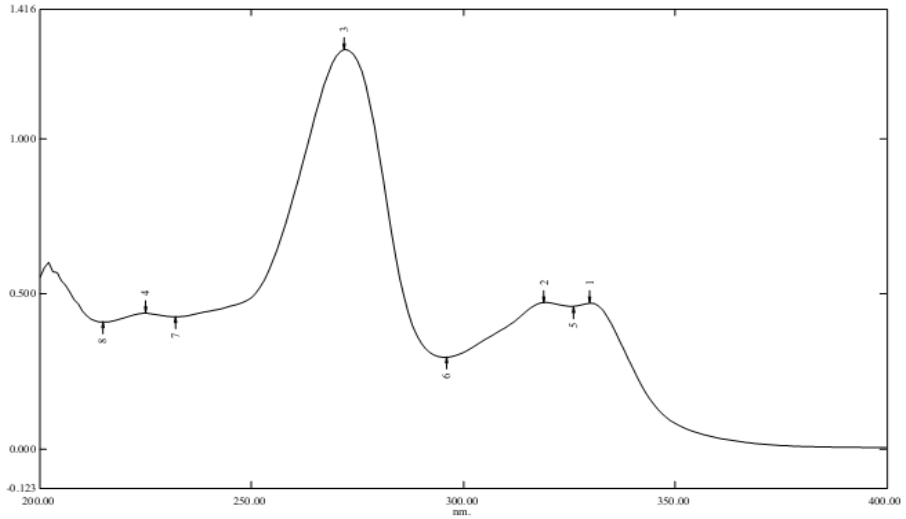


Fig. 3: First order derivative spectrum of Ciprofloxacin Hydrochloride in Distilled water (10µg/ml).

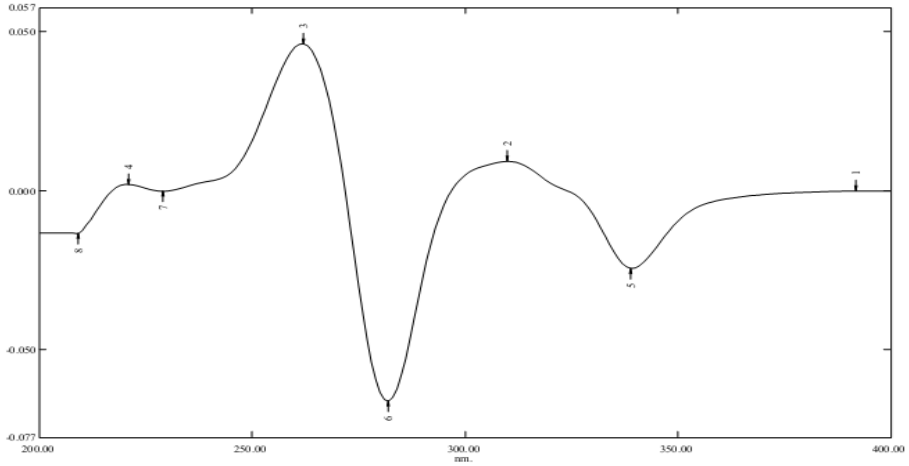
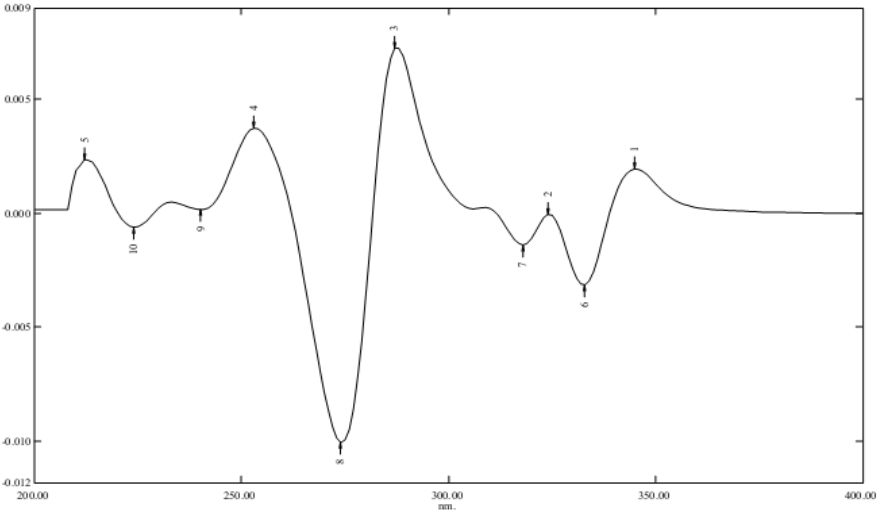


Fig. 4: Second order derivative spectrum of Ciprofloxacin Hydrochloride in Distilled water (10µg/ml).



2.4.2 Assay Procedure

Twenty tablets each containing 500 mg of Ciprofloxacin Hydrochloride were weighed crushed to powder and average weight was calculated. Powder equivalent to 10 mg of Ciprofloxacin Hydrochloride was transferred in 100ml of volumetric flask. A 50 ml of distilled water was added and sonicated for 15 minutes. Then solution was further diluted up to the mark with distilled water. The solution was filtered using Whatmann filter paper no. 41; first 5 ml of filtrate was discarded. This solution was further diluted to obtain 10µg/mL solution with water subjected for UV analysis using distilled water as blank. Appropriate dilutions were made with Distilled water from stock solution for zero, first and second order spectrophotometric methods.

Table 1: Assay of tablet dosage form

Sr. No.	Methods	Sample Sol. Conc. (µg/ml)	Amnt found (%)*			Mean % Found	%RSD*
1	Zero order	10	97.14	97.22	97.13		
2	First order	10	98.04	98.07	98.08	98.06	0.021
3	second order	10	98.17	98.22	98.21	98.02	0.027

*n=3, % RSD = % Relative Standard Deviation.

Fig. 5: Zero order derivative spectrum of Ciprofloxacin Hydrochloride dosage form (10µg/ml).

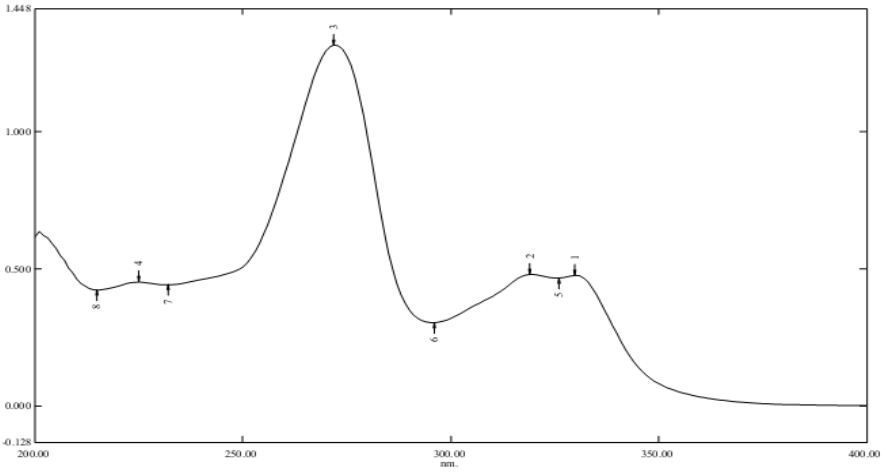


Fig. 6: First order derivative spectrum of Ciprofloxacin Hydrochloride dosage form (10µg/ml).

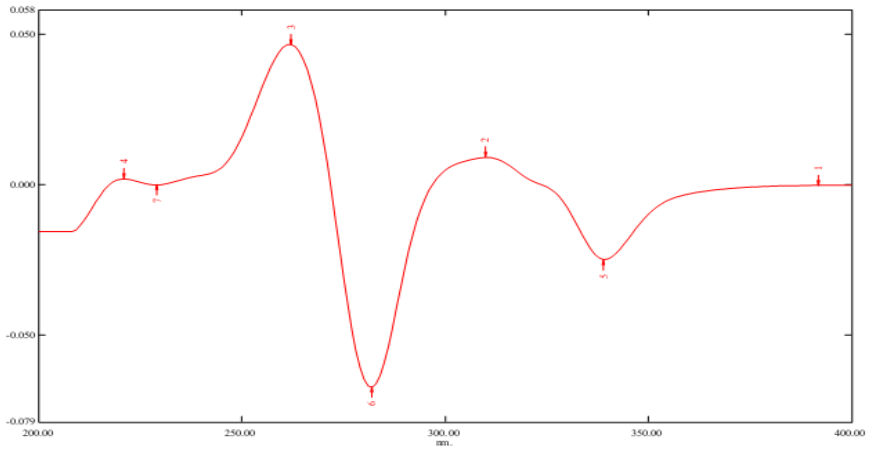
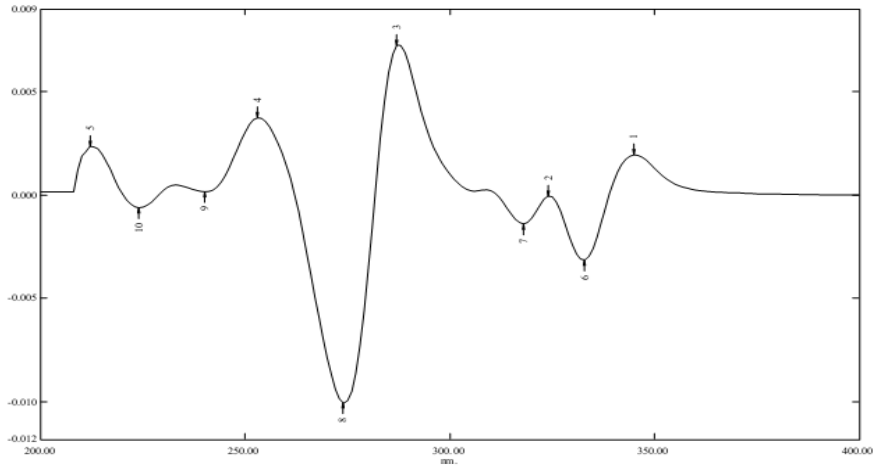


Fig. 7: Second order derivative spectrum of Ciprofloxacin Hydrochloride dosage form (10µg/ml).



3. Results and Discussion

The zero, first and second order method values spectra for Ciprofloxacin Hydrochloride were recorded at the wavelength of 272nm, 274nm, 274nm respectively.

3.1 Linearity and Range

Under the experimental conditions described, the graph obtained for zero, first and second order method spectra showed linear relationship. Regression analysis was made for the slope, intercept and correlation coefficient values. The regression equations of calibration curves were y=0.0411x+0.015 (r^2=0.9991) at 272 nm for zero order derivative spectrophotometry, y=0.002x-0.0022 (r^2=0.9993) at 274 nm for first order derivative spectrophotometry and y=0.0009x+0.0014 (r^2=0.9955) at 274 nm for second order derivative spectrophotometry. The range was found to be 5-25µg/ml for all zero, first and second order spectrophotometric methods.

Fig.8: Linearity of Ciprofloxacin Hydrochloride by zero/0th order spectrophotometric methods

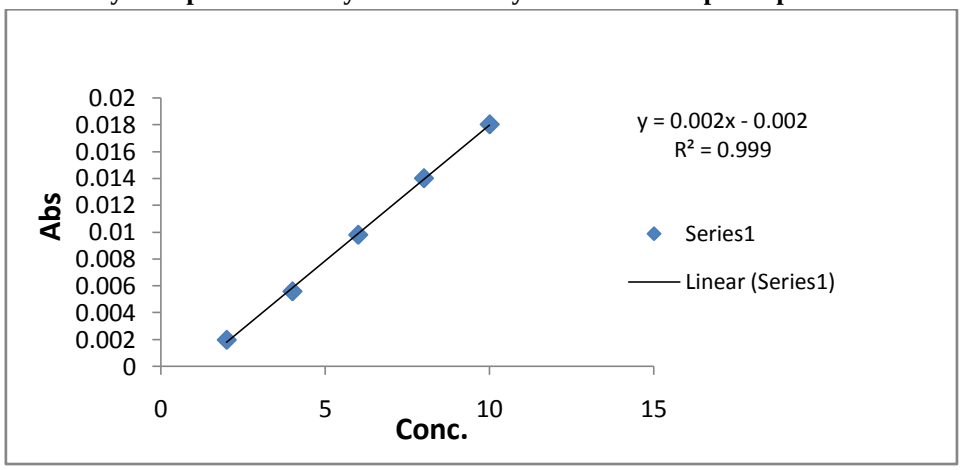


Fig.9: Linearity of Ciprofloxacin Hydrochloride by first order spectrophotometric methods.

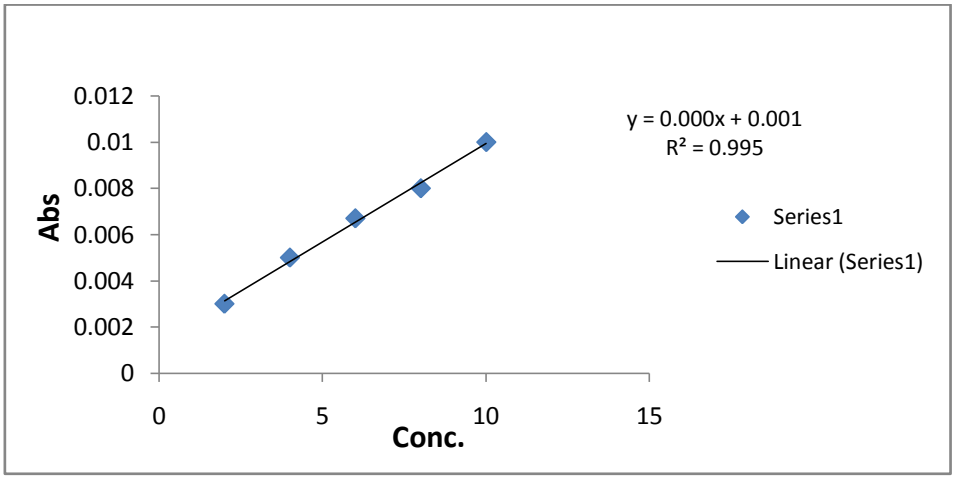


Table 2: Statistical data for the calibration graphs for determination of Ciprofloxacin Hydrochloride by Proposed methods.

Parameters	Zero order	First order	Second order
Linearity range (µg/ml)*	2-10	2-10	2-10
r^2± S.D*		0.9993	0.9955

3.2 Accuracy

To study the accuracy of the proposed methods, and to check the interference from excipients used in the dosage forms, recovery experiments were carried out by the standard addition method. The accuracy for the analytical method was evaluated at 80%, 100% and 120% levels of 20µg/ml standard solution. For Zero, first and second order derivative were measured in wavelength range at 272, 274 and 274nm respectively and results were obtained in terms of percent recovery. Three determinations at each level were performed and % RSD was calculated for each level.

Table 3: Accuracy results for Ciprofloxacin Hydrochloride.

Accuracy level	Method	Samp Conc.	Std. conc	Total amnt.	%Recovery			%Recovery (mean)	% RSD
80	Zero	15	12	27					
100	First	15	15	30	99.29	99.42	99.35	99.35	0.065
120	second	15	18	33	99.22	99.13	99.02	99.12	0.101

*n=3, % RSD = % Relative Standard Deviation.

3.3 Precision

To determine the precision of the method, Ciprofloxacin Hydrochloride solutions at a concentration of 10µg/ml were analysed each three times for all zero, first and second order spectrophotometric methods. Solutions for the standard curves were prepared fresh every day.

Table 4: Results of Intra and Inter Day Precision

Parameters	Intra Day Precision		Inter Day Precision	
	S.D*	% RSD*	S.D*	% RSD*
Zero order	0.2903	0.2901	0.2569	0.2571
First order	0.0001	0.6438	5.7700	0.3201
second order	5.7712	0.5701	0.0003	1.8601

3.4 Sensitivity

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula

$$LOD = 3.3\sigma/S$$

The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives response that can be accurately quantified. LOQ was calculated using the following formula

$$LOQ = 10\sigma/S$$

Where, σ is standard deviation of the response and

S is the slope of the calibration curve.

The LOD and LOQ were found to be 0.40µg/ml and 1.21µg/ml for zero order derivative, 0.315µg/ml & 0.956µg/ml for first order derivative and 0.814µg/ml &2.462µg/ml for second order derivative respectively.

3.5 Analysis of the Marketed Formulation:

There was no interference from the excipients commonly present in the tablets. The drug content was found to be 99.58%, 99.10% and 99.20% for zero, first and second order derivative spectrophotometric methods respectively. It may therefore be inferred that degradation of Ciprofloxacin Hydrochloride had not occurred in the marketed formulations that were analysed by this method. The low % R.S.D. value indicated the suitability of this method for routine analysis of Ciprofloxacin Hydrochloride in pharmaceutical dosage form.

Table 5: Summary of validation parameters

Parameter	0^{th}/First derivative	1^{st} derivative	2^{nd} derivative
λ range	200-400 nm	200-400 nm	200-400 nm
Regression Equation (y=mx+c)	Y=	Y=0.002x0.0022	Y=0.0009x+0.0014
Measured wavelength	272 nm	274nm	274nm
Linearity range	2-10µg/ml	2-10µg/ml	2-10µg/ml
Slope	0.0411	0.002	0.0009
Intercept	0.015	0.0022	0.0014
Correlation coefficient (R^2)	0.999	0.9993	0.9955
Limit of Detection (LOD) µg/ml	0.6294	0.31	0.81
Limit of Quantitation (LOQ) µg/ml	1.9075	0.95	2.46
Accuracy (Mean % Recovery)	98.72	99.35	99.12
Precission (%RSD)	0.2901	0.6438	0.5701

4. Conclusion

No UV/ zero, first and second order spectrophotometric methods have been described for the determination of Ciprofloxacin Hydrochloride. Therefore simple, fast and reliable derivative spectrophotometric methods were developed for the routine determination of Ciprofloxacin Hydrochloride. The developed methods can be concluded as accurate, sensitive and precise and can be easily applied to the pharmaceutical formulation.

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References

[1] Chin N. X. and Neu H. C. Ciprofloxacin, a quinolone carboxylic acid compound active against aerobic and anaerobic bacteria. Antimicrob. *Agents Chemother*. 1984; 25: 319–326.

[2] Eliopoulos G.M., Gardella A., and Moellering R.C. *In vitro* activity of ciprofloxacin, a new carboxyquinoline antimicrobial agent". *Antimicrob. Agents Chemother*. 1984; 25: 331–335.

[3] Nix D.E. and DevitoJ.M. Ciprofloxacin and norfloxacin, two fluoroquinolone antimicrobials. *Clin. Pharm.* 1987; 6:105–117.

[4] Syeda, K.; Rahul, R.; M. K. Durga and, M.Padmalatha. A simple and validated re-HPLC method for the simultaneous estimation of tinidzole and ciprofloxacin in bulk and pharmaceutical dosage forms. *Int. J. Res. Dev. Pharm. L. Sci*., 2012; 2(1): 238-243.

[5] Matthieu, T. and Carlos, A.R. Flow injection visible diffuse reflectance quantitative analysisof total sulfur in biodiesel, in plant leaves and in natural waters 2009; 34(2): 29-36.

[6] Mike, S. "Turbidity instrumentation an overview of today's available techenology" Turbidity and Other Sediment Surrogates Workshop, 2002, April 30 – May 2.

[7] KrugF. J, FilhoH. B, Zagatto E. A. G. and JørgensenS. S. Rapid determination of su lphate in natural water and plant digests by continues flow injection turbidimetry. *Analyst*, 1977; 102(8): 503-508.

[8] Cicero, O. C.; Airton V.P.; Clezio A. and Orlando F. Flow injection turbidimetric determination of thiamine in pharmaceutical formulations using silicotungstic acid as precipitant reagent, *Talanta*, 1999;48 (3): 659-667 .

[9] Pellegrino, R.M.; Segoloni, F. and Cagini, C. Simultaneous determination of ciprofl oxacin and the active metabolite of prulifloxacin in aqueous human humor by high -performance liquid chromatography. *J. Pharm. Biomed. Anal*, 2008; 3: 567.

[10] Aksoy, B.; Kucukgüzel, I. and Rollas, S. Development and validation of stability - indicating HPLC method for determination of ciprofloxacin hydrochloride and its related compounds in film coated tablets. *Chromatographia*, 2007; 66: 57.

[11] Dincel, A.; Yıldırım, A.; Çalayan, F. and Bozkurt, A.Determination of ciprofloxacin in human gingival creicular fluid by high performance liquid chromatography. *Acta Chromatographica*, 2005; 15: 308.

[12] ConkleJ. L., LattaoC. V. White J. R. and Cook R. L. "Individual and Simultaneous Determination of Ciprofloxacin, Ofloxacin and Norfloxacin Using an HPLC with Fluorescence and UV Detection with a Wetland Soil Matrix, *Analytical Letters*, 2009; 42(18): 2937-2950.

[13] Liangqia, G.; Zenghong, X; Xucong, L., Xiaoping, W.; Bin, Q; Yubin, Z. Hainan, Y. and Guonan, C. Pharmacokinetics of ciprofloxacin in eels by high-performance liquid chromatography with fluorescence detection, *Analytical Biochemistry*, 2005; 341 (2): 275–279.

[14] Anastasia, Z. and Niki, M. Sensitive LC determination of ciprofloxacin in pharmaceutical preparations and biological fluids with fluorescence detection" *Journal of Pharmaceutical and Biomedical Analysis*, 2002; 28(3-4): 559 –568.

[15] Najla, M. K.; Anil, K. S.; Erika, R. M. and Maria, I. R, Quantitative determination of ciprofloxacin and norfloxacin in pharmaceutical preparations by high performance liquid chromatography, *Brazilian Journal of Pharmaceutical Sciences*, 2005; 41(4):509 -513.

[16] BannefeldK. H, Stass H. and Blaschke G. Capillary electrophoresis with laser -induced fluorescence detection, an adequate alternative to high -performance liquid chromatography, for the determination of ciprofloxacin and its metabolite desethyleneciprofloxacin in human plasma. *Journal of Chromatography B: Biomedical Sciences and Applications*, 1997; 692(9): 453–459.

[17] Hongyuan, Y. and Kyung H. R. Molecularly Imprinted Solid- Phase Extraction for Determination of Enrofloxacin and Ciprofloxacin in Chicken Muscle. Molecularly Imprinted Solid-Phase Extraction *Bull. Korean Chem. Soc*., 2008; 29(6): 1173.

[18] Haeseker M. B, Verbon A. Welzen J, Neef C. Bruggeman C.A. and Stolk L. M.L.A Simple and Rapid RP-HPLC Method to Determine Ciprofloxacin Levels In Human Serum. *Asian J Pharm Biol Res* 2011; 1(3):350 -354.

[19] Prafulla, M. S., Jignesh C. and Roshni P. Development and validation of RP –HPLC Method for Bismuth Ciprofloxacin2, 2-Bipyridyl Metal Complex. *Pharmagene*, 2013; 1(2): 42-47.

[20] Shoulian, W.; Jiesheng, L.; Haifang L. And Jin-Ming L. Separation of seven fluoroquinolones by microemulsion electrokinetic chromatography and application to ciprofloxacin, lomefloxacin determination in urine" *Journal of Chromatography*. 2007; 1163(1–2): 333 –336.

[21] QiangC. Rui W. and F. Yi, Microbiological turbidimetric method in determination of serum concentrations of ciprofloxaxin. Chinese Journal of Clinical Pharmacology and Therapeutics, 1998, 3.

[22] Amina, M. E. Mohamed, E. M. and Fawzi A. E. Spectrophotometric determination of some fluoroquinolone antibacterials by binary complex formation with xanthene dyes. 11 Farmaco, 2004, 59(10): 809–817.

[23] Fakhr E.; O. Suliman and Salah M. S, Sequential injection technique employed for stoichiometric

studies, optimization and quantitative determination of some fluoroquinolone antibiotics complexed with iron (III) in sulfuric acid media. *Talanta*, 1996; 43(4): 559 -68.

[24] Nikita V. P .and Arun M. P. First derivative spectrophotometric method for the simultaneous estimation of ciprofloxacin and metronioxacin in their combind dosage form" *International Journal of Universal Pharmacy and Life Sciences*, 2012; 2(2): 281- 288.

[25] Pandey S, Pandey P, Tiwari G, Tiwari R. and Rai A. K.FTIR spectroscopy: A tool for quantitative analysis of ciprofloxacin in tablets. *Indian J Pharm Sci*, 2012; 74 (1): 86 -90.

[26] Shiga T, Shiga K, Kuroda M. An online differential converter, for obtaining the first derivative of absorption spectra. *Anal Biochem* 1971; 44:291-7.