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Difference Spectroscopic Method for the Estimation of Ciprofloxacin Hydrochloride in Bulk and in its Formulation

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ABSTRACT

A simple, precise and accurate difference spectroscopic method has been developed for the estimation of Ciprofloxacin Hydrochloride in bulk drug form by difference spectrophotometric method. Ciprofloxacin Hydrochloride has exhibited maximum absorbance at about 272nm and 278nm in acidic and basic solution respectively. Beer's law was obeyed in the concentration range of 2-10 µg/ml in both the cases. The regression of coefficient was found to be r^2=0.9982. The LOD and LOQ value were found to be 0.5140ppm and 0.5577ppm respectively. The proposed method was successfully applied for the determination of Ciprofloxacin Hydrochloride in bulk drug. As per ICH guidelines the result of the analysis were validated statistically and were found to be satisfactory.

Keywords: Ciprofloxacin Hydrochloride, Validation, Spectrophotometer

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-quinoline carboxylic 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 Enterobacteraerogenes[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. In lecturer 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, Microbiological turbidimetric method Spectrophotometry, Micellar liquid chromatographic (MLC) electrophoresis, flow injection UV spectrophotometric, 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). [5-14]

To our notice, no UV- spectrophotometric method using Difference Spectroscopic Method has been reported for the determination of Ciprofloxacin Hydrochloride in bulk and tablets. Hence an attempt

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has been made to develop new Difference Spectroscopic Method for estimation of Ciprofloxacin Hydrochloride in bulk and pharmaceutical formulations with good accuracy simplicity, precision and economy.

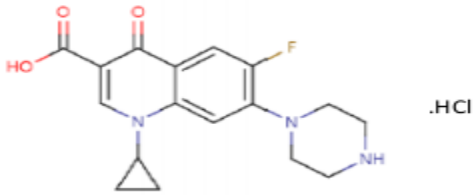

Fig. 1 Structure of Ciprofloxacin Hydrochloride.

Objective:

Ciprofloxacin hydrochloride shows improved absorbing interference by the technique of different spectrophotometry. Thus the objective of the present study was to develop new analytical difference spectrophotometry method and its validation parameters for the proposed method according to ICH guidelines for the estimation of Ciprofloxacin hydrochloride bulk drug.

MATERIALS AND METHODS

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.

Materials:

Reference standard of Ciprofloxacin Hydrochloride API was supplied as gift sample by Lupin Laboratory Park, Aurangabad. Tablet sample with label claim 500 mg per tablet were purchased from local market Pune.

Selection of common solvents:

1N HCL and 1N NaOH were selected as a common solvent for developing spectral characteristics of drug.

Preparation of solution:

Standard stock solution containing Ciprofloxacin hydrochloride was prepared by dissolving 10mg in 100ml of methanol and then diluted with 1N NaOH and 1N HCL separately to get series of dilution ranging from 2-10 µg/ml and then absorbance recorded at 272 nm and 278 nm respectively against reagent blank. Calibration curve was prepared by plotting concentration versus difference in absorbance and found to be linear in the concentration range of 2-10 µg/ml.

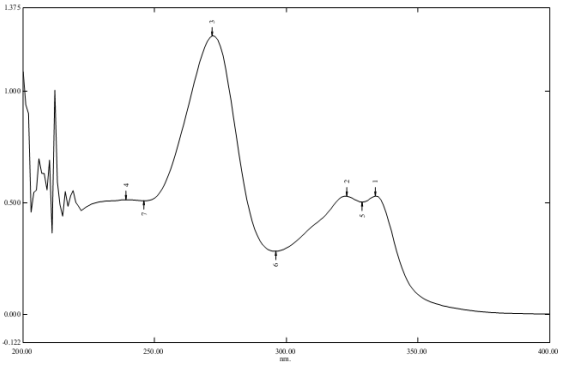

Fig. 2: 1N NaOH with λMax 272 nm

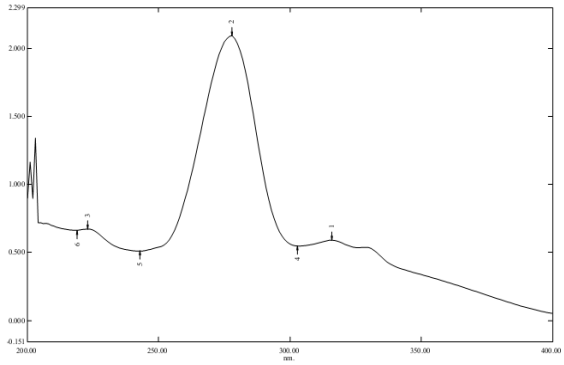

Fig. 3: 1N HCL with λMax 278 nm

VALIDATION:

The proposed method was validated according to ICH (Q2) R1 guidelines for validation of analytical procedures. As per the ICH guidelines the method validation parameters checked were Selectivity, linearity, precision and accuracy.

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Selectivity:
The selectivity of the method was assessed by analyzing standard drug, and pharmaceutical product, comparing the maxima and minima of the standard with that of the sample to determine whether the pharmaceutical product and excipient lead to interfere in the estimation.

Limit of Detection and Limit of Quantification:
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 σ /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 σ/S
Where, σ is standard deviation of the response and S is the slope of the calibration curve.
LOD& LOQ of Ciprofloxacin hydrochloride was found to be 0.5140µg/ml & 1.5577µg/ml respectively.

Linearity:
Different volumes of stock solutions were suitably diluted with corresponding medium (2, 4, 6, 8, and 10 µg/ml) to get the desired concentrations. Each solution was analyzed in triplicate. The amplitude values were plotted against the corresponding concentrations to obtain the linear calibration curve.

Table 1: Difference of Ciprofloxacin hydrochloride.

Sr. No	Concentration Of Ciprofloxacin hydrochloride (µg/ml)	Absorbance at 233 nm (1N NaOH)	Absorbance at 234 nm (1N HCl)	Difference in absorbance
1	2	0.315	0.184	0.131
2	4	0.529	0.267	0.262
3	6	0.804	0.43	0.374
4	8	1.084	0.484	0.524
5	10	1.25	0.592	0.658

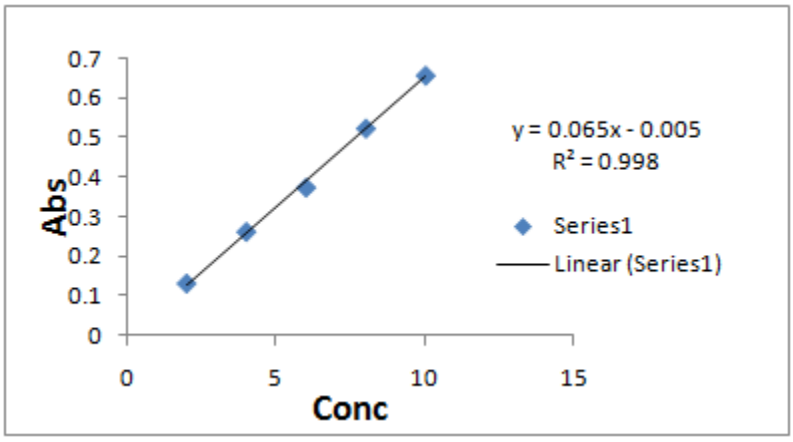

Fig. 4Showing linearity of Ciprofloxacin

Range: 2-10 µg/ml.

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Precision:

Precision of analytical methods were expressed in relative standard deviation (RSD) of a series of measurements. The intra-day and inter-day precisions of the proposed methods were determined by estimating the corresponding responses (i.e. three concentrations / three replicates each) of the sample solution on the same day and on three different days respectively. Precision was calculated as inter-day and intra-day coefficient of variation.

Table 2: Precision study of ciprofloxacin with 1N HCL

Drug	Conc. [µg/mL]	Trial	Trial	Trial	SD	%RSD
Ciprofloxacin	4	0.464	0.459	0.455	0.0045	0.9816
Ciprofloxacin	6	0.482	0.485	0.479	0.0031	0.6224
Ciprofloxacin	8	0.520	0.537	0.526	0.0086	1.6339

Table 3: Precision study of ciprofloxacin with 1N NaoH

Drug	Conc. [µg/mL]	Trial	Trial	Trial	SD	%RSD
Ciprofloxacin	4	0.383	0.388	0.384	0.0026	0.6872
Ciprofloxacin	6	0.809	0.821	0.818	0.0062	0.7653
Ciprofloxacin	8	1.013	1.024	1.027	0.0073	0.7217

Accuracy:

The accuracy of the method was determined by recovery experiments. A known amount of standard Ciprofloxacin hydrochloride corresponding to 2, 4, 6 and 8, 10% of the label claim (standard addition method) was added to pre-analyzed sample of tablet. The recovery studies were carried out in triplicate at each level.

Table 4: Recovery study of ciprofloxacin

Standard concentration [µg/mL]	Difference in Abs $\times 10^2$	found concentration [µg/mL]	Recovery %
2	0.131	1.914894	95.74468
4	0.262	3.905775	97.64438
6	0.374	5.607903	93.46505
8	0.524	7.887538	98.59422
10	0.658	9.924012	99.24012

RESULT AND DISCUSSION

Estimation of Ciprofloxacin Hydrochloride was found to be simple, accurate and reproducible; beer lambert law was obeyed in the concentration range of 2-10mg/ml. The optical characteristics such as percent relative standard deviation and percent range of error were found to be within the limit and satisfactory. All of the analytical validation parameter for the proposed method was determined according to ICH guidelines. And all validation parameters results were getting within the range of ICH standards.

The recovery studies showed that the result were within the limit indicating no interference. The proposed method is simple, sensitive, accurate and precise and can be successfully employed for the routine analysis of the Ciprofloxacin hydrochloride in bulk drug.

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CONCLUSION

The statistical analyses showed that the data from the proposed methods are in good agreement for the estimation of Ciprofloxacin hydrochloride in bulk drug. The method is economical, rapid and do not require any sophisticated instruments contrast to chromatographic method. Thus it can be effectively applied for the routine analysis of Ciprofloxacin hydrochloride in bulk drug.

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