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EFFICIENCY OF A COST-EFFECTIVE UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF CIPROFLOXACIN HCL IN MARKETED TABLET FORMULATION

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ABSTRACT: A simple, reproducible and economical UV spectrophotometric method was developed for determining the potency of ciprofloxacin HCl in tablet dosage forms marketed in Bangladesh. Standard curves of ciprofloxacin HCl in the media of 0.1N HCl and distilled water were obtained by plotting absorbance versus concentration where calibration curve was found to be linear (r^2>0.99). The average potency value for seven tablet samples (C1, C2, C3, C4, C5, C6 and C7) measured by HPLC method was 99.08±0.60% for 0.1 N HCl taking the absorbance at 277nm and 98.76±0.69% in case of distilled water at 276 nm, whether the value determined by this UV spectrophotometric method was quite satisfactory being 98.15±0.76% and 97.64±0.71%, respectively. In a separate experiment, efficiency of this method in practice was checked by determining the thermostability of ciprofloxacin HCl to find out how severely that degrades in high temperature conditions. Development of such economical UV spectrophotometric method will encourage low-investing pharmaceutical companies to employ this alternative method in quality control laboratories for routine analysis of ciprofloxacin HCl.

INTRODUCTION: Since their discovery in the early 1960s, the quinolone group of antibacterial drugs has generated considerable clinical and scientific interest. Later it became possible to enhance the efficacy by manipulating the basic molecule and it led to the development of ciprofloxacin, a fluoroquinolone known as 1-cyclopropyl-6- fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3- quinolinecarboxylic acid[1-4]. Ciprofloxacin is a broad-spectrum antibiotic, effective against both gram positive and gram negative organisms and widely used both in human and veterinary medicine to treat various infectious diseases[5-11].

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Fluoroquinolones like ciprofloxacins are also capable of exerting immunomodulatory effects on various cell types [12-13]. Ciprofloxacin can enter cells via porin and destroys microorganisms by inhibiting bacterial DNA gyrase, a type II bacterial topiosomerase necessary in replication and transcription stages of DNA synthesis [7, 9, 10, 14] and topoisomerase IV enzymes essential for the separation of bacterial DNA, thereby inhibiting cell division [15]. They are generally well tolerated, safe and adverse effects vary significantly depending on their physicochemical properties [7, 14].

Ciprofloxacin is one of the few broad spectrum antibiotics available in both intravenous and oral formulations. In this respect, it offers the potential for cost savings with sequential intravenous and oral therapy and is considered desirable to develop additional assay methods suitable for the rapid and reliable quality control of its pharmaceutical

formulations. HPLC is regarded as the official method for analysis of ciprofloxacin [3, 4, 16], but for routine analysis, these techniques are often time consuming and expensive. The UV spectrophotometric analysis is often preferred in quality control testing and ordinary laboratories as it is simple, easy, less time consuming and an economical method [17, 18]. Though methods using UV spectrophotometry have been already reported for the determination of ciprofloxacin [19, 20], these methods require expensive reagents, involve tedious extraction steps or need time for the reaction between the analyte and the colorimetric reagent.

For developing nations like Bangladesh, development of even cheaper and simpler UV spectrophotometric method for the determination of active pharmaceutical ingredients (API) in formulated drug products is a necessity as many small companies find it difficult to maintain good manufacturing practices or drug laws as their investment is not so much.

Antibiotics like ciprofloxacin are irrationally used in Bangladesh like many other developing countries [21, 22]. As a third world country, most of the people live under the poverty line and are encouraged to buy drugs from unofficial distributors because drugs are often not available in government hospitals. However, the patients have access to drug, both prescription and non-prescription, including nutritional supplements and over the counter (OTC) drugs from local drug outlets [23].

Many of these outlets cannot maintain proper storage conditions by keeping drugs in freezer. Regulations for the use of prescription antibiotics like ciprofloxacin usually specify that they should be stored between the temperatures of approximately 18-29°C. But in warm countries like Bangladesh, the room temperature often reaches 40-42°C in summer and exposure to this temperature could lead to degradation of the antibiotic and reduce its effectiveness. Besides, prescription antibiotics are often inadvertently set aside in locations such as personal bags, cars, window sills, stovetops, or outdoors. Therefore, checking the thermostability of ciprofloxacin to determine how severely that degrades in high temperature conditions seems to be important. The aim of the present investigation is to develop an economical UV spectrophotometric method for determination of ciprofloxacin HCl in marketed tablet formulations in Bangladesh. After crosschecking the efficiency of this method by HPLC, thermostability of standard ciprofloxacin HCl was analyzed by this method.

MATERIALS AND METHODS:

Reagents and Instruments:

Reference standard of ciprofloxacin HCl was collected from Pharmadesh Laboratories Ltd, Dhaka, Bangladesh. Ciprofloxacin tablets from seven pharmaceutical companies were bought from pharmacy outlets. All other reagents used were of analytical grade. Millex-GP 0.22µ filter disk was purchased from Millipore (Sigma-Aldrich, Japan). All the absorption spectral measurements were carried out using a SPD10A VP UV-visible spectrophotometer with 1 cm matched quartz cells with a path-length of 10 mm (Shimadzu Corp., Kyoto, Japan) and an HPLC instrument (LC-MS 2020, Shimadzu Corp., Kyoto, Japan).

Determination of wavelength of maximum absorption in 0.1N HCl and distilled water:

2 mg/ml of stock solution was prepared by dissolving 100 mg of ciprofloxacin HCl in 50 ml of 0.1N HCl (pH 1.0 to 1.2) and in distilled water (pH 4.5 to 6.0). Various concentrations (5, 10, 15, 20, 25 µg/ml) of standard solutions were prepared from the stock solution. The wavelength of maximum emission (λmax) of ciprofloxacin HCl in each media was found by scanning them over the UV range of 190nm to 400nm.

Potency of ciprofloxacin HCl frommarketed tablet preparations analyzed by UV-spectrophotometer using 0.1N HCl and distilled water as solvents:

Standard preparation: 100 mg of ciprofloxacin HCl was taken into a 100 ml volumetric flask, water was added and sonicated for 5 minutes. 5 ml was taken into another volumetric flask.

Sample preparation: 20 pieces of ciprofloxacin HCl tablets from each company were grinded in a mortar by using pestle. From that powder,

equivalent weight of the standard ciprofloxacin HCl was taken in a 100 ml volumetric flask, diluted and sonicated for 20 minutes. Then 5 ml was taken into another 100 ml volumetric flask. The final concentration of this solution was 50µg/ml. The potency was determined by an UV spectrophotometer using 0.1N HCl acid and distilled water as diluting solvents at 277 nm and 276 nm respectively.

Calculating percentage drug recovery by HPLC to verify the validated UV spectrophotometric method:

The applicability of the validated method was tested by analyzing ciprofloxacin HCl in pharmaceutical dosage form. Moreover, the same product batches were analyzed by using HPLC method. The result obtained from samples measured by spectrophotometric measured (n = 6) was compared with the same obtained by HPLC method (n = 6) at the same concentration level.

HPLC Conditions: The column was a Nucleosil C18, 250×4.6 mm, 5µ or equivalent, 125/4.0 mm. The solvents in the mobile phase were (A) 0.5% aqueous phosphoric acid (v/v), and (B) Acetonitrile 87:13 (v/v). Retention time was 20 min whereas the detection wavelength was 278 nm. Flow rate was 1.5 ml/min, column temperature was 30°C, sample temperature was ambient, and the injection volume was 10 µl.

Standard Preparation: 27.5 mg of reference standard of ciprofloxacin HCl was weighed into a 100 ml volumetric flask, diluted and sonicated for 5 minutes. The resultant solution (Solution B) was filtered through a 0.22µ-disk filter.

Formulation Analysis: 20 pieces of ciprofloxacin HCl tablets from each company were grinded to powder by using mortar and pestle. An equivalent weight of the standard ciprofloxacin HCl was taken from the powder in a100 ml volumetric flask, diluted and shaked at 250 rpm for 10 minutes by a rotary shaker and sonicated for 15 minutes.

The resultant solution (solution A) was filtered through a 0.22µ-disk filter. The vials containing solution B and solution A were loaded on the auto sampler and then analyzed by HPLC.

Comparative analysis of standard, light-exposed and heat-degraded ciprofloxacin HCl by UV spectrophotometer using 0.1 N HCl and distilled water as diluting solvents:

100 mg of standard ciprofloxacin HCl was dissolved in 100 ml volumetric flask using 0.1N HCl and distilled water separately. 10 ml of solution was taken into 15 test tubes. 5 of those tubes were exposed in sunlight for 14 hours and the other 5 were kept in 100ºC in a water bath for 6 hours. A control group of 5 test tubes were preserved in a refrigerator. Potency of all the samples was analyzed by a UV-spectrophotometer.

Statistical analysis:

All results were expressed as mean ±S.D of three experiments. All data were analyzed with two-way analysis of variance (ANOVA) followed by Dunnett's test using statistical software SPSS 15.0. The level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION:

The maximum absorption of different concentrations of ciprofloxacin HCl was found at wavelength 277nm and 276nm by using 0.1N HCl and distilled water respectively (Table 1). In both cases, the calibration curve was found to be linear (**Fig. 1**).

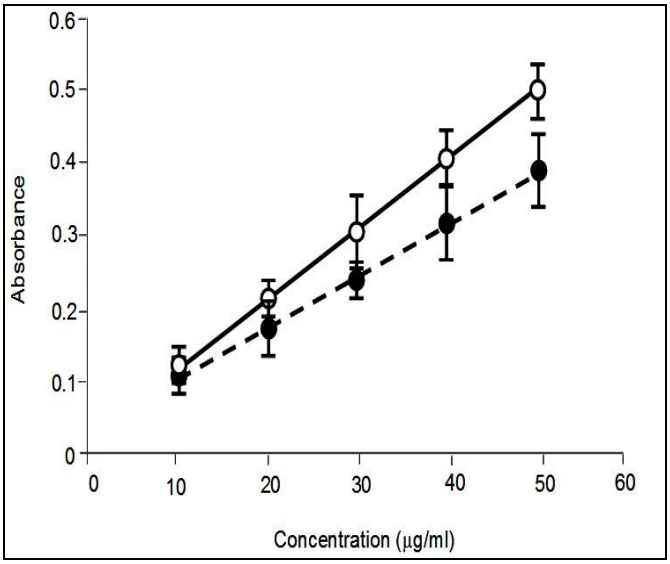


FIG.1: CALIBRATION CURVE OF CIPROFLOXACIN HCLIN THE MEDIA OF 0.1N HCL (-o-) AND DISTILLED WATER (-•-). *P<0.05, VS THE RESPECTIVE CONTROL.

The correlation coefficient of these drugs was found to be close to 1.00 (0.999), indicating good linearity **(Table 2).** The plot of the residuals was normally distributed around the regression line, reflecting

accuracy of the method as Beer's law was obeyed in the working concentration range (10-50 µg/ml). The assay results obtained by the proposed method as shown in **Table 2** are in fair agreement. Similar value with the correlation coefficient (r^2=0.999) was found to determine Levofloxacin in tablet dosage form using a UV spectrophotometric method [24].

TABLE 1: ABSORBANCE OF STANDARD CIPROFLOXACIN HCL INDIFFERENT MEDIA AND AT DIFFERENT CONCENTRATIONS

Concentration (µg/ml)	Absorbance (in 0.1N HCl at λ_{277})	Absorbance (in distilled water at λ_{276})
10	0.101±0.04	0.080±0.03
20	0.213±0.02	0.162±0.07
30	0.326±0.05	0.241±0.06
40	0.435±0.01	0.326±0.05
50	0.535±0.06	0.412±0.07

TABLE 2: LINEARITY PARAMETERS OF CIPROFLOXACIN HCL INDIFFERENT MEDIA

Parameter	0.1 N HCl	Distilled water
λ_{max} (nm)	277 nm	276nm
RSD% for Extinction coefficient	0.6335	0.6296
Regression Equation	Y=0.0109x-0.0005	Y=0.0083x-0.0082
Correlation co-efficient (r^2)	0.999	0.999
Slope	0.0109	0.0083
Intercept	-0.005	-0.0082

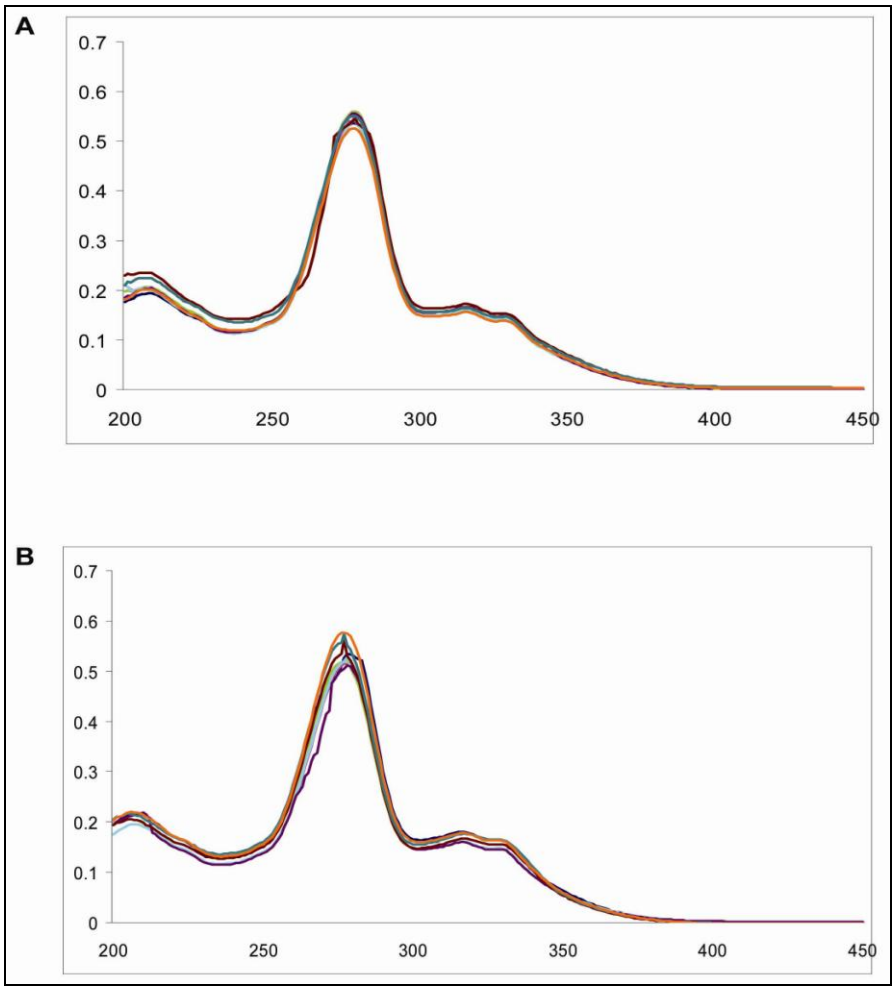


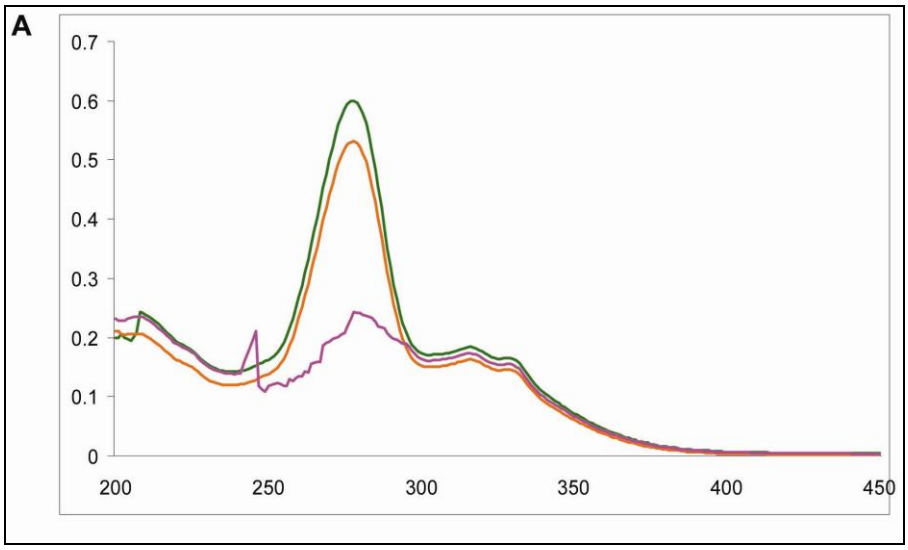
FIG.2: UV-ABSORPTION SPECTRA OF CIPROFLOXACIN HCL TABLET SAMPLES FROM SEVEN COMPANIESUSING 0.1N HCL AT 277NM (A) AND DISTILLED WATER AT 276NM (B). STANDARD CIPROFLOXACIN HCL (—), C1 (—), C2 (—), C3 (—), C4 (—), C5 (—), C6 (—) AND C7 (—)

The values of absorbance and calculated potency of ciprofloxacin HCl tablet samples marketed by pharmaceutical companies were observed to remain parallel to that of the standard ciprofloxacin HCl when measured by the UV-spectrometric method (**Fig. 2**). Simultaneously this method was compared to HPLC method to highlight its validity. When 0.1N HCl was used as solvent, the average measured percentage of potency was 98.15±0.84% for ciprofloxacin HCl tablet samples and 99.77±0.72% for standard ciprofloxacin HCl, which is quite satisfactory comparing the values obtained (99.22±2.38%for tablet samples and 102.65±3.64% for standard) in case of Levofloxacin [24]. In the same condition, an average value of 99.08±0.54% ciprofloxacin HCl tablet samples was found using HPLC. When distilled water was used as the solvent, the average percentages of potency measured by UV-Spectrophotometer and HPLC were 97.64±0.78% and 98.76±0.55%, respectively (**Table 3**). Though methods using UV spectrophotometry have been already reported for the determination of ciprofloxacin [19, 20], the method on study showed satisfactory results using distilled water, the cheapest reagent.

TABLE 3: COMPARISON OF THE PERCENTAGES OF ESTIMATED POTENCY OF CIPROFLOXACIN HCl TABLET SAMPLES MEASURED BY UV-SPECTROPHOTOMETER AND HPLC METHOD

Company	% of Potency measured by UV- spectrophotometer		% of Potency measured by HPLC	
	0.1N HCl	Distilled water	0.1N HCl	Distilled water
C1	99.91±0.60	98.77±0.71	100.12±0.76	99.66±0.69
C2	98.53±0.96	98.60±0.61	98.76±0.69	98.79±0.54
C3	98.00±1.18	96.26±0.99	98.57±0.54	97.46±0.73
C4	96.87±0.51	97.27±0.74	97.46±0.73	99.11±0.62
C5	98.71±1.00	98.10±0.73	99.11±0.62	98.54±0.07
C6	96.76±1.17	95.93±1.16	98.24±0.07	97.31±0.38
C7	99.06±0.46	97.60±0.57	99.61±0.38	98.61±0.82

Previously, it was reported that the reaction rate of thermal decomposition of ciprofloxacin HCl increased at 30, 40, 50, 60 and 70ºC when the temperature rises up [25]. Previously ciprofloxacin in aqueous solution were found to degrade to an ethylenediamine derivative of ciprofloxacin when exposed to natural sunlight [26]. In this study, it became evident that, λ_{max} for sunlight-and heat-induced solution became reduced from its actual value. It was found that the peak from heat-degraded sample in 0.1N HCl appeared much smaller than the standard but no interference was observed with the peaks of the parent compound. But in distilled water, the same peak looked shattered and almost non-existent (**Fig.3**).



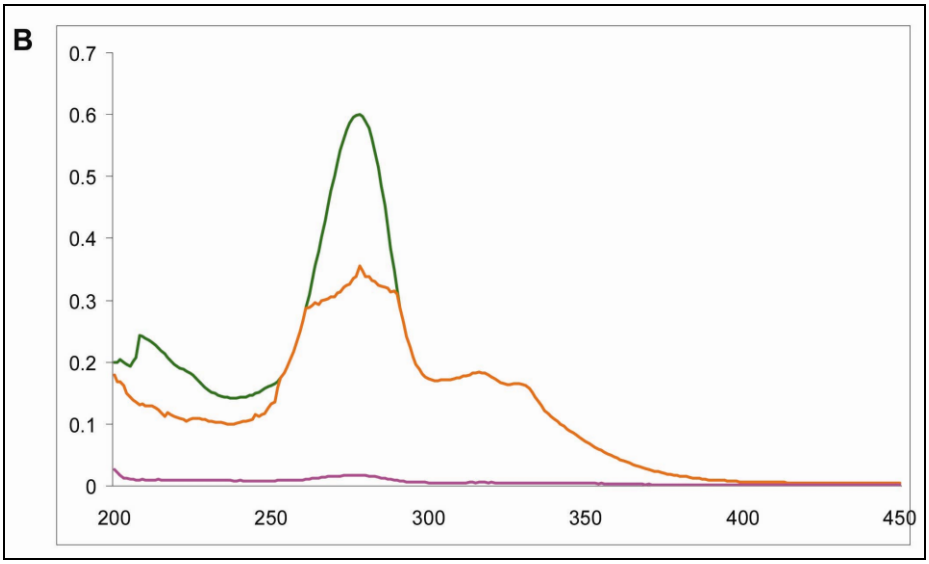


FIG. 3: UV-ABSORBANCE SPECTRA OF CIPROFLOXACIN HCL USING 0.1 N HCL (A) AND DISTILLED WATER (B) AS SOLVENTS. TOP, MIDDLE AND BOTTOM PEAKS ARE SHOWING THE SPECTRA FOR STANDARD (—), SUNLIGHT-INDUCED (—) AND HEAT-DEGRADED (—) CIPROFLOXACIN HCL, RESPECTIVELY.

Perhaps the presence of HCl in the molecule of ciprofloxacin HCl plays a minor role to protect the degradation of ciprofloxacin HCl to some extent. This might be the reason of finding lesser degradation of ciprofloxacin HCl in 0.1N HCl than in distilled water. For the standard ciprofloxacin HCl solution, average absorbance value and percentage of potency became 0.597 and 99.77±0.70%. In case of sunlight-induced samples in 0.1N HCl, those values became reduced to 0.530 and 88.28±0.87% whereas in distilled water, much reduced values like 0.335 and 56.17±0.56% were found. For heat-degraded samples in 0.1N HCl, average values for absorbance and percentage of potency dramatically lowered down to 0.231 and 38.44±0.85%, and became lowest in case of distilled water, 0.017 and 2.85±1.17%, showing the importance of maintaining proper storage conditions by keeping drugs in freezer.

CONCLUSION: It may be concluded that the proposed UV spectrophotometric method can be successfully applied for the analysis of ciprofloxacin HCl in tablet dosage forms. It is cost-effective, accurate, sensitive and reliable method that does not require an elaborate treatment offering distinct advantages of simplicity. The interference from excipients was eliminated by selecting the most adequate wavelength. Comparative analysis of standard ciprofloxacin HCl with light-exposed and heat-degraded ciprofloxacin HCl using UV spectrophotometric method also showed the efficiency in practice. It can encourage the low-investing pharmaceutical companies to employ this alternative method in quality control laboratories for routine analysis of ciprofloxacin HCl.

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