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Development and validation of an extractive ion-pair spectrophotometric method for the determination of ciprofloxacin hydrochloride

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ABSTRACT

A new, rapid and sensitive assay is proposed for the spectrophotometric determination of Ciprofloxacin in pure form and in pharmaceutical formulation. The proposed method involves formation of purple coloured, Butanone extractable ion-pair complex of Ciprofloxacin with Eriochrome Black T (EBT) in acidic medium provided by phthalate buffer (pH 2). The extracted ion pair complex showed maximum absorbance at 514 nm and Beer's law was obeyed in the concentration range of 5 – 25 µg/mL. The molar absorptivity and Sandells sensitivity were determined to be 65,491.02 L mol^{-1} cm^{-1} and 1.90 µg cm^{-2} respectively. The detection limit for the method was found to be 1.09 µg/mL while limit of quantitation was 2.27 µg/mL. Other validation parameters for the method were similarly established. The method was successfully applied for the determination of commercial brands of the drug. Statistical comparison of the results with the official titrimetric method showed good agreement and indicated no significant difference in accuracy and precision.

Key words: Ciprofloxacin; spectrophotometry; ion pair; pharmaceutical preparation; validation

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INTRODUCTION

Ciprofloxacin (1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1- yl)-quinoline-3-carboxylic acid) is a 4-quinolone derivative, derived from nalidixic acid [1]. It is a broad spectrum antibiotic active against both gram positive and gram negative bacteria and acts via inhibition of two essential bacterial enzymes - DNA gyrase and DNA topoisomerase IV, resulting in the degradation of chromosomal DNA and interference with bacterial cell division and gene expression [2, 3]. The drug is particularly effective against a variety of infections such as those of the urinary tract, respiratory tract, gastrointestinal tract, skin and soft tissues [4].

The USP [5] and BP [6] both recommend liquid chromatography as the official method for the determination of ciprofloxacin while the International Pharmacopoeia [7] describes a non-aqueous titrimetric method. Various non-official analytical methods have also been reported in scientific literature for the analysis of the drug in pharmaceutical formulation and biological fluids including UV-Visible spectrophotometry [8, 9, 10, 11], FTIR [12, 13], Mass spectrometry [14], Fluorimetry [15], Capillary electrophoresis [16, 17], HPLC [18, 19, 20, 21, 22], RP-HPLC [23, 24], HPTLC [25, 26, 27], Potentiometry [28, 29], AAS [30], Rayleigh light scattering [31], Voltammetry [32, 33, 34]. Some of the above mentioned techniques are highly sophisticated with good sensitivity but may be relatively expensive, requiring equipment not commonly available in all laboratories. Others have limitations such as the need for an internal standard, multiple extraction steps and technical complexity of the analytical procedure. In contrast, ultraviolet-visible (UV-Vis) spectrophotometry has the advantages of simple instrumentation which is

cheap and easily operated. UV spectrophotometers are also readily available in most laboratories providing acceptable analytical results and are thus very useful in determining organic compounds in different matrices.

Ciprofloxacin possesses a wide spectrum of activity and favourable pharmacological properties which make it one of the most widely used quinolone antibiotics despite the development of newer derivatives (third and fourth generation quinolones). This therefore engenders the need to develop alternative methods of analysis that do not require highly sophisticated equipment and which are simple enough to be carried out in moderately equipped laboratories without compromising the accuracy and sensitivity required for analytical methods that are used in routine quality control of pharmaceuticals. The present study is therefore aimed at developing and validating a simple and cost-effective colorimetric method for determination of ciprofloxacin in bulk and marketed formulation that utilizes a readily available dye.

EXPERIMENTAL SECTION

Equipment

A Shimadzu UV-Visible spectrophotometer with matched 1 cm quartz cells was used for all spectral measurements with Shimadzu UV Probe system software (version 2.1). pH measurements were carried out using a calibrated digital pH meter (GallenKamp, England).

Chemicals and reagents

All chemicals used were of analytical reagent grade and procured as follows: Acetic Acid, Butanone, Methanol, Ethyl acetate, Diethyl ether, Dichloromethane, Hydrochloric acid, Potassium hydrogen phthalate (Sigma Aldrich, Germany), Sodium hydroxide pellets (Avondale Laboratory, England). Double distilled water was used to prepare all solutions. Freshly prepared solutions were used for method development and validation. Eriochrome Black T (Fischer Scientific, UK). Pure Ciprofloxacin reference material was obtained from CHAN Medi-Pharm, Jos, Nigeria. Various brands of Ciprofloxacin tablets were obtained from a Pharmacy retail outlet in Jos, Nigeria.

Preparation of Reagents

Preparation of Ciprofloxacin standard solution

The stock solution of 1 mg/mL was prepared by weighing and dissolving 100 mg of Ciprofloxacin reference powder in Acetic acid (0.05 M), and making up the volume to mark in a 100 mL volumetric flask. Working standards were prepared by appropriate dilution of the standard stock solution.

Preparation of Potassium Hydrogen Phthalate buffer solution (pH 2.0)

A 2.553 g potassium hydrogen phthalate was weighed and dissolved in 0.25 ml of 0.1 M HCl. The volume was then made up to 250 mL with distilled water in a volumetric flask. The pH was then adjusted to 2.0.

Preparation of Eriochrome Black T (0.01 % w/v)

A 0.01 g of Eriochrome Black T powder was accurately weighed and dissolved in 10 mL methanol and this was made up to 100 mL with distilled water in a volumetric flask. Working solutions were always freshly prepared.

Titrimetry

Non-aqueous acid-base titration against per chloric acid is the official assay method [7] and this was employed to determine the drug content in the five brands.

Extractive ion-pair spectrophotometric method

Determination of wavelength of maximum absorbance (λ_{max}) and Construction of calibration curve

Aliquots of Ciprofloxacin hydrochloride standard solution (100 μg/mL) were transferred into a series of 100 mL separating funnels. To each separating funnel 1.0 mL of Eriochrome black T solution (0.01% w/v) and 4.0 mL potassium hydrogen phthalate–HCl buffer of pH 2.0, were added and the contents vortexed mixed and allowed to react for two (2) minutes. The mixture was extracted twice with 5.0 mL Butanone and the organic phases were combined. Absorption spectrum of the purple coloured Ciprofloxacin-Eriochrome Black T ion- pair complex was obtained by scanning the extracted complex between 350 - 800 nm. Subsequent absorbance measurement were carried out at the determined max of 514 nm against the reagent blank similarly prepared. All measurements were made at room temperature (25 ± 2°C). The procedures were repeated for other analyte aliquots and calibration plot was plotted using Absorbance versus concentration.

Analysis of Pharmaceutical Formulation

An accurately weighed quantity of the pulverized tablets equivalent to 100 mg of the Ciprofloxacin was extracted with 0.05 M Acetic acid and sonicated for about 10 min. The extract was filtered into a 100 mL volumetric flask and the volume made up to mark. Suitable aliquots of the solution were then taken and the analysis was completed using the previously described procedure. The nominal content of the tablets was then determined with the aid of the previously plotted calibration graph. Five different brands of Ciprofloxacin tablets were assayed using the proposed method.

Method Validation

The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures [35].

Precision and Accuracy

Calibration standards of Ciprofloxacin at three different concentration levels were analysed in five replicates on the same day to determine the intra-day precision and accuracy, and on each of five separate days to determine inter-day precision and accuracy. Precision was expressed as the relative standard deviation (RSD %). Accuracy was expressed as the mean relative error (RME %)

Linearity

The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. The calibration curves were constructed by plotting concentration versus absorbance, using linear regression analysis.

LOD and LOQ

The limit of detection (LOD) and limit of quantitation (LOQ) of the method was established using the formula: LOD = 3 *s*/*k* and LOQ = 10 *s*/*k*, where *s* is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte, and *k* is the slope of the calibration curve.

Optimization of Reaction Conditions

The optimal reaction conditions for complex formation and stability were also investigated. Experiments were therefore conducted to study the effects of pH of the buffer, reaction time, reagent concentration, extraction solvent and reagent volume. The stability of the ion pair complex was also evaluated over 24 hours.

RESULTS AND DISCUSSION

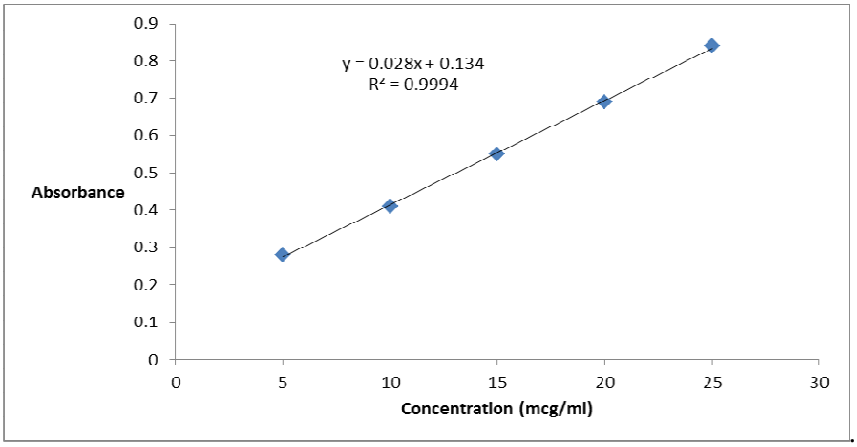

Figure 1: Calibration plot for Ciprofloxacin-Eriochrome Black T ion pair complex

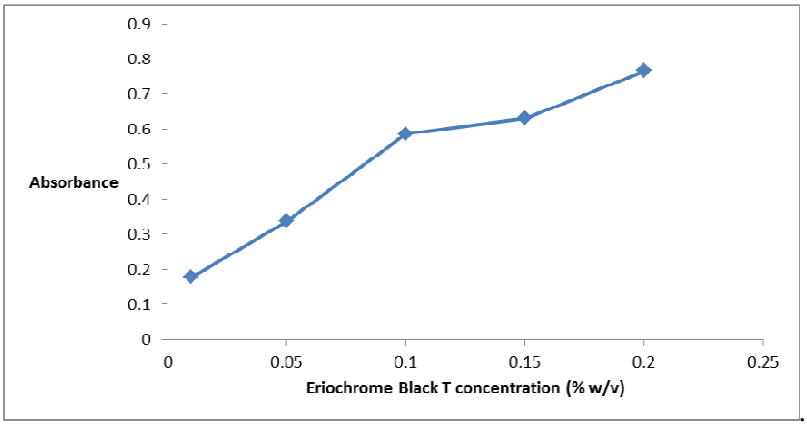

Figure 2: Effect of reagent concentration on Ciprofloxacin-Eriochrome Black T ion pair complex

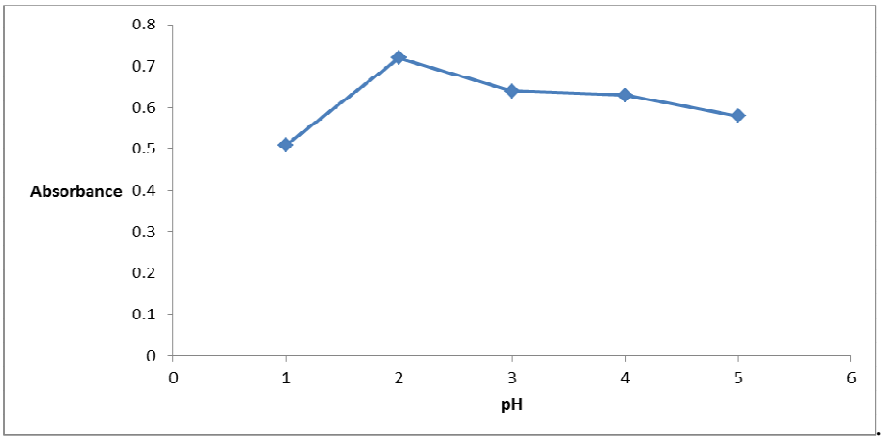

Figure 3: Effect of pH on Ciprofloxacin-Eriochrome Black T ion pair complex

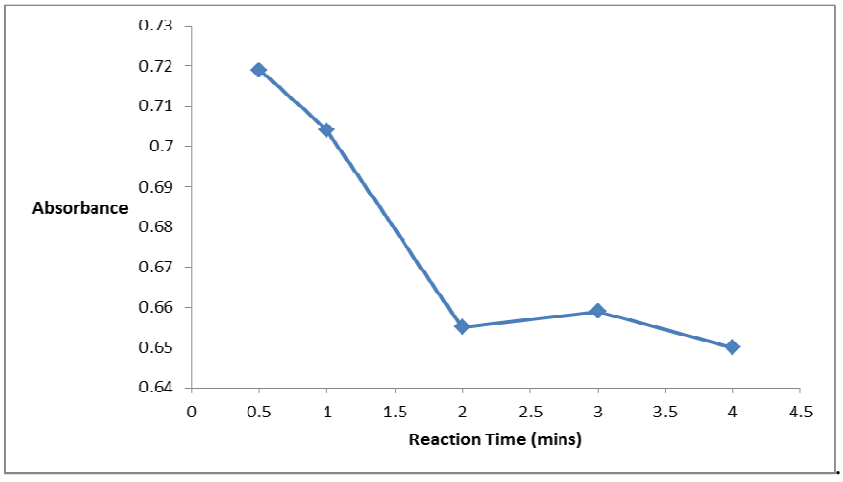

Figure 4: Effect of reaction time on Ciprofloxacin-Eriochrome Black T ion pair complex

Table 1: Determination of Ciprofloxacin content in Tablet Formulation

Brand	Official Titrimetric method - IP, 2006 (n = 5)	Proposed Method (n = 5)
Ciprokris®	101.61 ± 0.52	103.43 ± 0.82
Vitapro®	99.84 ± 0.79	102.19 ± 0.11
Ciprovam®	99.40 ± 0.13	100.00 ± 0.42
Cenox®	103.62 ± 0.21	105.31 ± 0.16
Grakkoflo®	102.28 ± 0.55	101.40 ± 0.32

Eriochrome Black T is a classical reagent in pharmaceutical analysis been commonly used as an indicator in water hardness determination via complexometric titration. The formation of ion-pairs between the amino group of many drugs and this reagent has found wide applications in the field of drug analysis [36, 37, 38]. Ciprofloxacin similarly possesses an amino group which is protonated in acidic medium and forms ion pair complex with Eriochrome black T. The purple coloured ion-pair complex was quantitatively extracted with Butanone and a scan of the absorption spectra revealed that the ion-pair complexes absorbed maximally at 514 nm.

Optimization of reaction conditions

Effect of pH

The results shown in figure 3 were obtained by varying the pH for the aqueous phase within the range of 2.0 - 5.0 and keeping other conditions constant. A maximum absorbance was clearly obtained at pH value of 2 at which the drug was found to be maximally protonated thus helping the ion-pair formation. Consequently, Phthalate buffer solution of pH 2.0 was chosen for further investigation

Effect of reagent concentration

The influence of the concentration of Eriochrome Black T was examined by addition of different concentrations of the dye in the range of 0.01 – 0.2 % w/v. The maximum absorbance was obtained when 0.2 % w/v Eriochrome Black T solution was utilized and it is believed that this concentration brings about maximum stabilization of the ion-pair associates.

Effect of reagent volume

The influence of the volume of the reagent was evaluated by addition of different volumes of 0.2 % w/v reagent in the range of 0.5–2.5 mL. The maximum absorbance was obtained when 1.0 mL Eriochrome Black T solution was utilized.

Effect of number of extractions, shaking time and stability

Reproducible absorbance readings were obtained after two extractions with 5 mL of the solvent and a 3 min shaking time. The studied ion-pairs were stable for more than 24 hours at 25° C in the organic solvent.

Effect of extraction solvent

Absorbance spectral characteristics of Ciprofloxacin-Eriochrome Black T ion pair complex after extraction with different solvents were examined. Dichloromethane, Ethyl acetate, Diethyl ether, methanol, Butanone, acetonitrile and chloroform were tested for this investigation. Most of the common organic solvents failed to extract the ion pair complex due to insolubility. Experimental results indicated that Butanone gave the maximum absorbance and most stability among the studied solvents.

Effect of time

The optimum reaction time was determined by monitoring the colour development at room temperature (25±5 ◦C). Complete colour intensity was attained 2 min.

Stoichiometry

Job's method of continuous variation was used for determining the molar ratio of Ciprofloxacin to Eriochrome Black T in the ion pair complexation reaction. The ratio was found to be 1:1. This stoichiometric ratio indicates that the interaction between Ciprofloxacin and the reagent used took place at only one site i.e. the terminal basic aliphatic amino group which enjoys more steric freedom.

Method validation

Linearity, detection and quantification limits

Under the specified optimum reaction conditions, the calibration curves for Ciprofloxacin complexation with the reagent employed in the present work were constructed. The regression equations for the results were derived using the least-squares method. In all cases, Beer's law plots (n = 3) were linear with very small intercepts and good correlation coefficients in the general concentration range of 5–25 µg/mL. The LOD and LOQ were found to be 1.09 and 6.27, respectively µg/mL.

Precision and accuracy

The intra-day and inter-day relative standard deviation (RSD) values obtained by the proposed method were found to be within 0.42–0.55%. Accuracy of the methods expressed as relative mean error (RME) was below 4.6%.

Application to Analysis of Pharmaceutical Formulation

Commercially available Ciprofloxacin in tablet formulation was subjected to analysis both by the proposed method and by a reference method [7]. Statistical comparison of the results by the Student's t-test revealed there was no significant difference between the accuracy and precision of the two methods (Table 1).

CONCLUSION

The proposed method for Ciprofloxacin determination has many advantages over other analytical methods due to its speed, lower cost and versatility of application. All statistical values (percentage recoveries, RSD, confidence limits of the slope and intercept, LOD and LOQ) were within acceptable limits. Also, all the analytical reagents are relatively cheap and available in any analytical laboratory. The method is therefore considered appropriate for routine quality control analyses of the active drug in industry, hospitals and for research purposes.

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