Improving Drug Quality Monitoring in Ghana by Ion-pair Complexes: The Case of Ciprofloxacin Hydrochloride and Bromophenol Blue

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ABSTRACT: *Purpose:* The mechanics of ion-pair complex formation between 1-cyclopropyl- 6-fluoro- 4-oxo- 7-piperazin- 1-yl- quinoline- 3-carboxylic acid monohydrochloride (CPF) and 4,4'-(1,1-dioxido-3*H*-2,1-benzoxathiole-3,3-diyl)bis(2,6-dibromophenol) (BPB) and its application in Ghana for quality control of some medicinal agents have been examined. *Method:* CPF was allowed to react with BPB in aqueous acetate buffer of pH 2.8 at room temperature (29±1 °C), extracting the ion-pair complex into $CHCl_3$ and determining the absorbance at 420nm. The optical characteristics, conditional stability constant and free energy of formation of the complex were also evaluated, finally applying the method to the assay of six brands of CPF tablets. *Results:* Effective linear dynamic range according to the Ringbom's plot was 5.0–27.5 μgmL^{-1}. Molar absorptivity, Sandell's sensitivity index and degree of dissociation of the complex were $1.06x10^4$ $Lmol^{-1}cm^{-1}$, 0.036 μgcm^{-2} and 0.163 respectively. The logarithmic conditional stability constants and Gibb's function determined by the continuous variations and mole ratio methods were respectively 5.95±0.110;-34,401.00$Jmol^{-1}$ and 5.76±0.214;-33,326.83$Jmol^{-1}$. *Conclusion:* The CPF tablets were successfully assayed and the method showed a great deal of precision and accuracy making its application suitable for regular quality monitoring of CPF and its congeners in Ghana.

Keywords: Ion-pair, ciprofloxacin, bromophenol blue, drug quality, counterfeit, fluoroquinolone

INTRODUCTION

The burden of counterfeit, substandard, or adulterated medicines has been an issue of grave concern world-wide. In recent years, many countries, irrespective of their development, have been challenged by episodes of economically-motivated adulteration. Melamine, diethylene glycol, and over-sulfated chondroitin sulfate have created morbidity and mortality within countries and across regions of the globe [1]. It has been reported that about 15% of all drugs in circulation are likely to be substandard or counterfeit, with the clinical and financial burdens falling most heavily on developing countries [2] because of weak regulatory systems, poorly staffed and equipped national drug control laboratories and poor enforcement due to corruption or lack of political will [1]. Medicines can also be substandard because of heat and humidity common to many developing countries. Drug quality can reduce during manufacture, storage, and distribution as a result of high temperatures and humidity. A study reported in 1997 indicated one-third of chloroquine-containing and antibacterial medicines collected in Nigeria and Thailand as below compendia standards as a result of degradation and poor manufacturing [3].

Notwithstanding the above, rising cost of drugs generally creates a corresponding increase in incentive to produce counterfeit drugs because of profit margin. Production cost of counterfeits can be very low because cheap substitutes or none at all, are used. Neither huge infrastructures nor facilities are needed; and there are no expenses incurred for quality assurance or meeting Good Manufacturing Practice requirements [4]. Economically constrained patients seeking relief from diverse ailments and diseases may prefer cheaper options to probably more expensive and

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quality medicines prescribed by health care providers. However, use of poor quality drugs bears serious health implications such as treatment failure, adverse reactions [5, 6], drug resistance [7], increased morbidity, and mortality. [8, 9]. Poor quality medicines can also erode public confidence in a country's health programme and waste scarce resources [10, 11]. It therefore becomes necessary in the interest of consumers/ patients, prescribers, pharmaceutical companies and governments to safeguard the quality of medicines by having available and affordable techniques for quality monitoring. The benefits are that patients will receive cure and value for money; prescribers will earn the trust of patients; pharmaceutical companies will have a reputation because consumers have confidence in their products leading to more profits and governments will have the health of the public protected, preventing increased public expenditure [12] in controlling the menaces of unwholesome medicines.

Ciprofloxacin hydrochloride (CPF) is the monohydrochloride monohydrate salt of 1-cyclopropyl- 6-fluoro- 4-oxo- 7-piperazin- 1-yl-quinoline- 3-carboxylic acid. It is a broad-spectrum antimicrobial agent belonging to the fluoroquinolone group, showing good activity against both gram-negative and gram-positive bacteria by inhibition of their DNA gyrase [13]. Its clinical applications in Ghana include treatment of typhoid fever, urinary tract, pelvic, bone and joint infections. A recent survey of 50 pharmacies in Kumasi (the Ashanti regional capital and 2nd largest city in Ghana) by the authors indicated 16 different brands of CPF tablets. Usually, influx of diverse and multi-source pharmaceutical products generates quality issues. Poor quality antimicrobial agents can be a major threat to the economy of Ghana because of development of drug resistance with attendant health implications. Therefore, monitoring anti-infectives to preserve their therapeutic values, especially, in situations where cross resistance is possible as is the case of the fluoroquinolones [14] is a relevant strategy. Newman *et al.*, 2006, reported in their study 'resistance to antimicrobial drugs in Ghana' that prevalence of resistance to ciprofloxacin is about 10% [15]. However, Nankanishi *and co-workers*, had earlier reported increasing bacterial resistance to the fluoroquinolones [14] and a later study in Bangladesh in 2001 by other researchers involving a sample of 15 brands of ciprofloxacin collected for assay by HPLC and bioassay corroborated the findings of Nankanishi *et al*. In the latter report, seven out of the 15 brands of medicines assayed had active ingredient less than the USP specification [16] and this is one of the main factors of drug resistance development.

Current pharmacopoeia assay method (British Pharmacopoeia (BP) and United States Pharmacopoeia (USP)) for CPF is High Performance Liquid Chromatography (HPLC). Despite its usefulness in drug analyses, the technique is resource intensive in terms of equipment, operation, accessories and consumables including energy. Inexpensive but comparable assay technique(s) will therefore enhance the capacity of industry, regulators (national and quality control laboratories) and researchers in emerging economies such as Ghana, to effectively control the quality of drugs, especially, agents for infectious and non-infectious diseases.

Our study therefore seeks to invigorate the application of extractive spectrophotometric methods in Ghana for monitoring the content of active ingredients in pre- and post-marketing pharmaceutical products that are suitable for acid-dye ion-pair complex formation reactions. Ion-pair complexes have been widely studied and utilized in the assay of diverse pharmaceuticals in the bulk and formulations [17, 18, 19]. However, it is difficult to find reports on specific application of CPF:BPB ion-pair complex in the assay of CPF and its formulations. Unsuccessful attempt was made at getting the report of Sedai and Nihai in this respect [20]. Consequently, the study took into account the optimization of reaction conditions and development of an assay procedure for the application of the proposed method to CPF containing pharmaceutical agents.

THEORY

Fluoroquinolones ionize in acidic solution with the secondary piperazinyl nitrogen bearing a positive charge. 4,4'-(1,1-dioxido-3*H*-2,1-benzoxathiole-3,3-diyl)bis(2,6-dibromophenol) commonly called bromophenol blue is a sulphonphthalein dye usually used as an acid- base indicator whose useful range lies between pH 3.0 and 4.6. As an anionic dye, BPB in an aqueous buffered acidic

medium forms an ion-pair complex with CPF which can be extracted into a suitable organic solvent such as trichloromethane (chloroform) or dichloromethane (methylene chloride) in order to separate the ion-pair complex from the excess acid dye. The extracted chromogene can then be determined spectrophotometrically at the wavelength of maximum absorption. If the concentration of dye is constant and in sufficient excess to make dissociation negligible, the equilibrium concentration of the complex will essentially be proportional to the concentration of CPF in the reaction. The pH of the aqueous solution, when necessary, can be modified to keep the analyte and acid dye in ionised forms. Neutral electrolytes such as KCl, K_2SO_4 and NH_4NO_3 are commonly used when needed to control the ionic strength of the reaction to keep the dissociation of the ion-pair complex to the minimum. The extensive conjugation in the structure of the complex as proposed in Figure 1 makes absorption in the visible range of the electromagnetic spectrum possible, giving the procedure the advantage of avoiding irrelevant absorption from other excipients and unwanted contents.

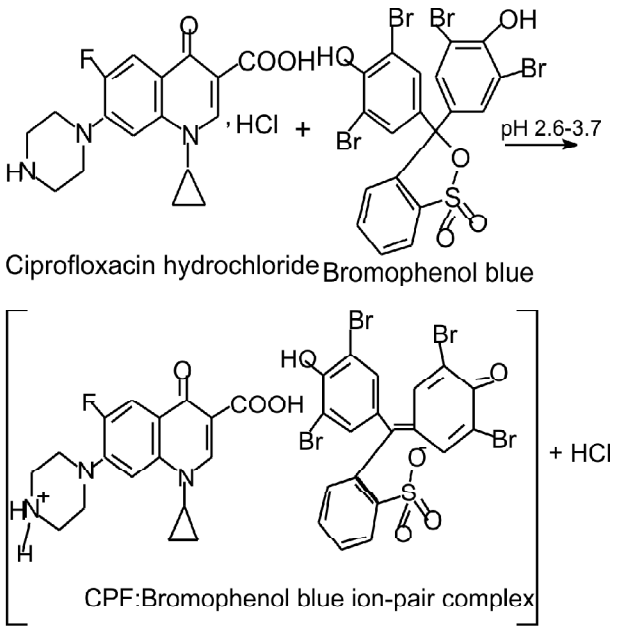

Figure 1: Proposed Reaction Scheme for CPF:BPB ion Pair Complex

EXPERIMENTALS

Materials, Reagents and Apparatus

Following are details of materials, reagents and apparatus utilised in the study. Ethanol (96%), chloroform and glacial acetic acid were manufactured by BDH, UK, while anhydrous sodium acetate, bromophenol blue powder and perchloric acid were from Sigma-Aldrich, USA. They were all of analytical grade obtained from the Department of Pharmaceutical Chemistry, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. The distilled water used was doubly distilled. Ciprofloxacin hydrochloride pure powder (Pharmaceutical grade) was obtained from a local pharmaceutical manufacturing company and was manufactured by Zhejiang Create Bio Chemical Co. Ltd., China. This pure powder was calibrated against Ciprofloxacin Hydrochloride USP which is a reference standard and was obtained from the Food and Drugs Board, Ghana. Commercially available ciprofloxacin hydrochloride tablets bought from pharmacies in Kumasi, Ghana for application of the method were; Ciprolet (Dr Reddy's Laboratory, India), Cipflox (Ernest Chemist Ltd., Ghana), Quintor (Torrent Pharmaceuticals Ltd., India), Ciprinol (KRKA, Slovenia), Ciprokam (XL Laboratory Ltd, India) and Ciprofloxacin (Phyto-Riker Pharmaceuticals Ltd., Ghana). Common laboratory glassware including 50mL separatory funnels was used. Where necessary, filtration was done using the Whatman's No. 1 filter paper. All measurement of weights was done with Libror (AEG-220) Analytical Electronic Balance. Other equipment include Stuart Scientific (SF1) Flask Shaker, Buchi (B-480) Water Bath and Kent (EIL 7020) pH meter. Absorbance of solutions was measured with Cecil (CE 7200) Double Beam UV-Visible Spectrophotometer with 1 cm matched glass cuvettes.

Preparation of Standard Solutions

The CPF, BPB and anhydrous sodium ethanoate (sodium acetate) powders were dried in an oven at 100 °C for 4hrs and kept in a dessicator before preparing solutions. A stock CPF solution of concentration 1mgmL^{-1} was prepared in distilled water by weighing an amount of powder equivalent to 100mg of ciprofloxacin into a 100mL volumetric flask. About 30mL of distilled water was initially added, content shaken till all powder dissolved and solution made up to 100mL with distilled water. A 100µgmL^{-1} CPF solution was further prepared by dilution from the stock with distilled water. Six acetate buffer solutions of different pH ranging

between 2.0 and 5.0 were prepared following the method of the British Pharmacopoeia, 2007[21]. A 500µgmL^{-1} solution of BPB was also prepared by dissolving a quantity of crystalline BPB powder equivalent to 50.0 mg of BPB in 20mL of ethanol (96%v/v). The alcoholic solution was quantitatively transferred into a 100mL volumetric flask and made up to the mark with distilled water. Further 1 in 100 dilution of the stock was made to obtain the 500µgmL^{-1} solution. All solutions were properly labeled and kept in a cool dry place away from light.

Recommended General Method

Into a separatory funnel was measured 2mL of 100µgmL^{-1} CPF, acetate buffer of pH 2.8 and 3 mL 500µgmL^{-1} BPB solutions. The total volume was adjusted to 10mL with doubly distilled water, mixing well and allowing the reaction to equilibrate for 10min at room temperature of 29±1°C. Two 5mL portions of $CHCl_3$ were successively added to the content of the separatory funnel, shaking for about 2min each time and allowing the flask to stand for clear separation of the organic and aqueous layers. The yellowish chloroformic layer containing the ion-pair complex was separated, pooled together and dried over anhydrous sodium tetraoxosulphate(vi) crystals. The absorbance of the chloroformic solution was determined at 420nm against a reagent blank after checking the absorbance and wavelength scales of the spectrophotometer.

Application of Method to Commercially Available Tablet Samples

Twenty tablets of each brand (6) were weighed together and the average weight of a tablet for each brand calculated. The set of twenty for each brand was then powdered in a porcelain mortar and a quantity of powdered tablets equivalent to 100mg CPF transferred into a 100mL volumetric flask. About 30mL of distilled water was transferred into the flask and the content was shaken for 15min in a flask shaker at moderate speed. The flask was made up to volume with distilled water with intermittent manual shaking. The solution was filtered with Whatman.s no.1 filter paper, discarding the first few milliliters of filtrate. Each brand was assayed with solutions respectively containing 75, 100 and 125µg (7.5-12.5µgmL^{-1}) of CPF. The rest of the procedure was the same as already described under general procedure, making triplicate determinations for each analytical solution.

STATISTICAL ANALYSES

Graph Pad Prism Version 5.0 was used for the descriptive statistics and the analysis of variance while Microsoft Excel 2007 was used for graphs and regression analyses.

RESULTS AND DISCUSSION

Preliminary Assessment of Experimental Samples and Investigation of Ion-pair Reaction Conditions

Preliminary Assessment of Samples

The identity, strength, and stability of pharmaceutical formulations are usually the elements assessed in controlling the quality of drugs using specific monograph requirements of pharmacopoeias. Chemical samples therefore used in designing assay methods for active pharmaceutical ingredients are evaluated for compliance with specific pharmacopoeia standards in order to make valid the analytical procedure developed. In view of this, the pure CPF powder, commercial tablets and BPB were characterized according to the specifications of BP 2007. The respective identities were thus confirmed as positive. To further rule out the possibility of formulation factors affecting the performance of the proposed procedure when it is applied to the assay of the tablets, uniformity of weight test according to BP 2007 was carried out on each brand of tablets. The variations in a random sample of 20 tablets for each brand were within pharmacopoeia limits. The average weight of a tablet for each brand determined from the uniformity of weight test was used with the labeled content to define equivalent quantities of powdered CPF tablets to be taken for assay by the proposed method.

Investigation of Reaction Conditions

The UV-visible characteristics of CPF, BPB, $CHCl_3$ and $CHCl_3$ that has been used to extract BPB were obtained to define baselines for the method development process. Advantage was taken of previous studies involving other fluoroquinolones such as ofloxacin, lomefloxacin hydrochloride and enoxacin [19, 22] to determine the basic conditions for the formation of the CPF:BPB ion-pair complex.

An ion pair reaction was established by taking 1mL of the 100µgmL^{-1} CPF solution into a 50mL separatory funnel, adding 1mL of acetate buffer of pH 3.5 and 4mL of 0.50mg/mL BPB solution. The content of the flask was shaken for about five minutes and 4mL of distilled water added to bring the total volume to 10mL. The aqueous solution was then extracted with 10mL of $CHCl_3$, drying the organic layer over anhydrous sodium tetraoxosulphate (vi) crystals and determining the UV-vis characteristics several times against reagent blanks. Wavelength of maximum absorption (λ_{max}) of the extractive was 420±1.08nm (n = 6). The procedure was repeated two more times with 2 and 4 mL respectively of the 100µg/mL CPF solution, keeping the volumes of BPB and acetate buffer constant and making the volume up to 10mL with distilled water. This was done to examine the effect of increasing concentration of analyte on the consistency of the λ_{max} of the extractive. All the values obtained were within 95% confidence interval (418.53-420.80nm), indicating how constant the λ_{max} can be in replicate determinations.

Optimisation of Reaction Conditions

Optimized reaction conditions were obtained by varying one parameter at a time while keeping others constant and monitoring the effect on the absorbance of the extractive. The parameters optimized were reaction time, critical pH of acetate buffer, volume of acetate buffer, order of addition of reactants and reagents, shaking time, reaction stoichiometry and stability of extractive in chloroform. Appropriate volumes of distilled water were added in each case to keep the reaction mixture at a total volume of 10mL. Critical values of test parameters were graphically determined.

Reaction Time

Figure 2 illustrates the effect of equilibration time on the formation of the CPF: BPB ion-pair complex. Different sets of reaction monitored over 150min revealed that ion-pairs were formed before 5min of equilibration, peaking off by 10min. Time required for maximum reaction was monitored by constant maximum absorbance of the extractive in $CHCl_3$. The graph indicates maximum reaction almost to have taken place by 5min. Extraction of the complex into the organic solvent could therefore be done after 5min but the reaction time allowed for the study was 10min. The relatively short reaction time was good for the procedure because in most quality control applications, short sample work-up times are preferable for efficiency. Even though absorbance of complex appeared fairly constant between 10 and 150min of reaction, a slight decrease in absorbance was observed between 120 and 150min of reaction. This was probably due to the dissociation of the complex within the reaction mixture. This postulate was later supported by the ion-pair complex stability studies where the complex was found stable in $CHCl_3$ at room temperature even at 48hrs after extraction. This observation therefore makes the extractive nature of the procedure useful, especially in situations where large numbers of analytical samples have to be analysed and sample storage cannot be avoided.

Effect of pH and Volume of Acetate Buffer on Formation of Ion-pair Complex

Optimum pH of buffer was studied by adding to reaction mixture same volume of acetate buffer of different pH (2.0-5.0) at room temperature. The other components were 2mL and 4mL of CPF and BPB solutions respectively, making up to volume with doubly distilled water. Allowing a reaction time of 10min, the absorbance of each chloroformic extract was obtained in replicates against reagent blanks. Figure 3a indicates that a maximum and relatively constant absorbance of ion-pair complex was obtained between pH 2.8 and 4.0 suggesting an evidence of moderate acidity as a requirement for the maximum formation of CPF:BPB complex. Related studies have reported similar observations using sulphonphthalein dyes and buffers such as acetate, potassium hydrogen phthalate and McIlvaine [17, 19, 22]. Reaction environments containing buffers of pH less than 2.8 and greater than 4.0 as shown in Figure 3a probably did not support formation of maximum ion-pairs because the reaction environment failed to keep both the anionic dye and counter-ion fully in ionized forms. This assumption was supported by the fact that modern spectroscopic methods have proven ion-pair formation as involving electrostatic, hydrophobic and charge transfer interactions [23]. Therefore, a reaction environment that is unable to maintain maximum ionized species fails to provide maximum complex formation. In that respect, an optimum pH of 2.8 was used for this work but any other pH between 2.8 and 4.0 could provide similar results as the current study.

Variations of volume of buffer between 0.5 and 5.0mL did not produce significant difference (p = 0.05) between the absorbances recorded. As depicted by Figure 3b, 1mL of buffer was enough to produce fairly maximum and constant amount of ion-pair complex but we used 2mL in the study.

It was also observed during variation of pH of the buffer that, the nature of the absorption spectra and λ_{max} of the extracted complex did not vary. Ibrahim *et al*. had demonstrated the formation of different ion-pair complex at different pH and applied the concept to the determination of khellin in compound pharmaceutical preparations containing either atropine or papaverine [17]. Turner and Anderson had also reported a similar phenomenon in their study on complex formation of sulfosalicylic acid with copper (II) [24]. Since absorption spectra and λmax did not vary with pH in the current study, we inferred that only one kind of ion-pair complex was formed.

Effects of sequence of addition of reactants, shaking time and number of extractions

Though in some cases the sequence of addition of reactants in complex forming reactions becomes important because the sequence can affect the intensity of light absorption and stability of the complex [25], the results was different in the study being reported. Reactants added in the order

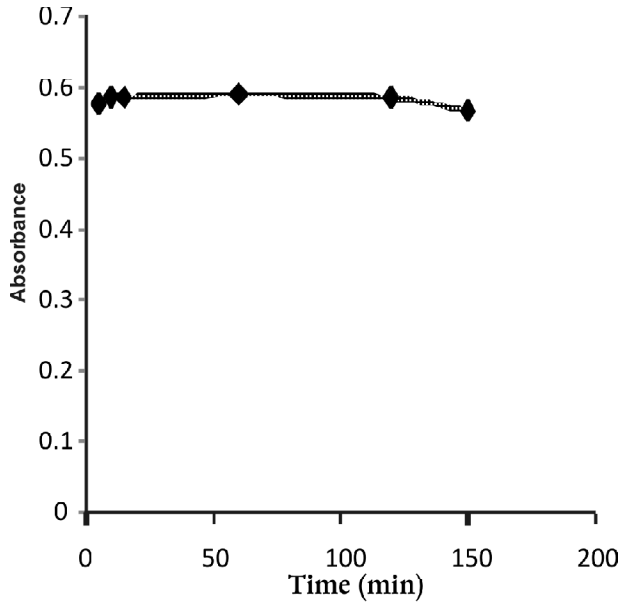

Figure 2: Establishing Optimum Reaction time for CPF:BPB Complex with 2mL of 100µgmL^{-1} CPF, Acetate Buffer of pH 2.8 and 500 µgmL^{-1} BPB

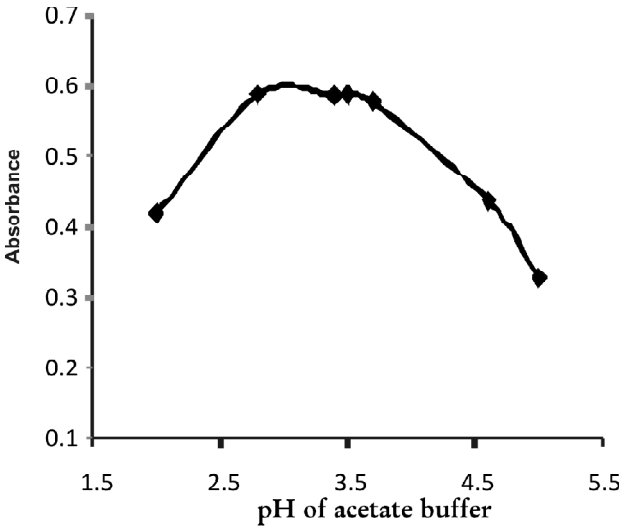

Figure 3a: Effect of pH on the Absorbance of the CPF: BPB Complex at 420nm

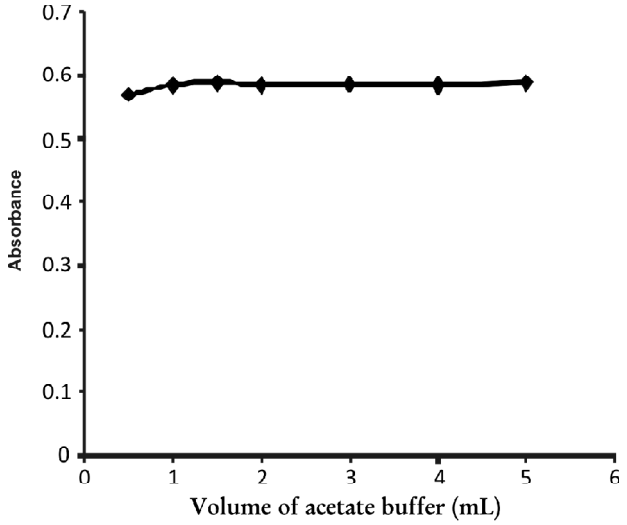

Figure 3b: Effect of the Volume of Acetate Buffer of pH 2.8 on the Absorbance of CPF: BPB Complex

CPF:BPB:Buffer and CPF:Buffer:BPB using optimum conditions showed no significant difference in absorbance of complex extracted. Similarly, shaking the reaction with $CHCl_3$ over a period of 5min before separating the organic layer gave a constant maximum absorbance between 1 and 5min (Figure 4). The organic/aqueous layers were therefore shaken for 2min in our work before separation. In investigating the number of extractions, a reaction was extracted three times consecutively with 5ml portions of the chloroform, drying over anhydrous Na_2SO_4 and determining the absorbance of each extractive. It was realized that the absorbance of the third extract was negligible while that of the first was highest,

pooling over 90% of the three extractions. Since the second extract gave absorbance values that could not be neglected, two 5mL aliquot extractions was adopted and applied.

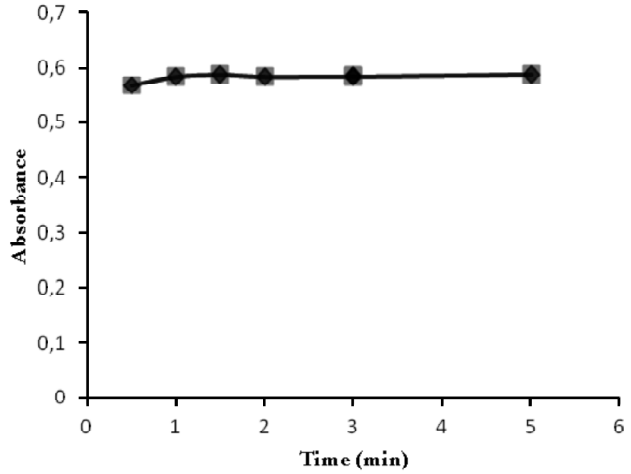

Figure 4: Effect of Shaking Time on the Efficiency of Extraction of CPF: BPB Complex into $CHCl_3$

Stoichiometry of Ion-pair Reaction, Stability Constant of Complex and Determination of Optimum Volume of BPB (500µgmL^{-1})

The stoichiometry of the reaction was determined by Job's method of continuous variations [26] and Yoe and Jones' mole ratio method [27]. Using Job's method, equimolar solutions ($2.718x10^{-4}$ M) of CPF and BPB were prepared and mixed in the volume ratios 1:9 to 9:1. Total volume of CPF/BPB was 5mL but in each case, acetate buffer and distilled water were added to the separatory funnel to bring the total volume to 10mL, following optimum reaction conditions. From Figure 5a, it is realized that maximum absorbance of the extracted complex occurred at a mole fraction of 0.5 indicating the stoichiometric ratio of the complex as 1:1. With the mole ratio method, a series of reactions containing a constant concentration of CPF ($5.44x10^{-4}$ M) and varying concentrations of BPB ($7.46x10^{-6}$-$3.73x10^{-4}$M) were prepared and analysed under optimal conditions. Figure 5b describes the profile of ion-pair associate formation with the varying BPB concentrations. It is clear from the graph that the mole ratio of reactants that gave a constant maximum absorbance of extracted ion-pair associates was 1:1. Earlier studies involving other fluoroquinolones had reported same ion-pair composition [19, 22]. In both methods, the fact that change in concentration of one of the reactants resulted in change in absorbance of the chloroformic extractive suggested the formation of the ion-pair complex. Looking at Figure 5b, beyond the equivalence point, further increase in concentration of BPB did not yield significant increase in absorbance of the extractives. Obradovic *et al*. had explained that a continual increase of absorbance beyond the equivalence point in Yoe and Jones' method indicates the formation of a complex with low stability [28]. Since Figure 5b virtually leveled off after the equivalence point, it was inferred that the CPF:BPB complex was not weak in stability. This position was later supported by the calculation of the logarithmic stability constants from the Harvey and Manning method [29] using the data of continuous variations and the molar ratio methods. For the continuous variations method, the mathematical relationship used was; $K_f = (A/A_m)/[(1-A/A_m)]^{n+1}C_m(n)^n$ where A and A_m are respectively the observed maximum absorbance and the absorbance value when all the drug present in the reaction is associated. C_m is the molar concentration of CPF at the maximum absorbance and n is the number of moles of BPB required to form complex with 1 mol of CPF. With the mole ratio method; $K_f = (1-\pm)/(\pm^2C)$ where C is the concentration of the complex usually defined by that of the limiting reagent and ± is the degree of dissociation given by; $\pm = (A_m-A)/A_{m.}$ A_m is the absorbance of the complex when excess BPB is present and A is the absorbance of the complex at the equivalence point. Logarithmic values obtained for both methods were respectively 5.95±0.110 and 5.76±0.214 (n=3) at 29±1 °C. The free energies of formation calculated by $\Delta H = RTInK$ using dissociation constants from both the continuous variations and mole ratio approaches were -34,401.00 Jmol^{-1}(-8217.11 Calmol^{-1}) and -33,326.83Jmol^{-1}(-7960.54Calmol^{-1}) respectively. These values suggested that the CPF:BPB complex had reasonable stability. The degree of dissociation (±) of the complex was found as 0.163.

In determining the optimum volume of BPB required for maximum complex formation, 2mL of 100µgmL^{-1}CPF was used. Volumes of BPB ranging between 0.1-5.0mL (500µgmL^{-1}) were individually used and their respective effect on absorbance of the complex monitored. Figure 5c showed that an approximate volume of 1mL gave an absorbance beyond which further increase in volume of BPB did not yield proportional increase

in absorbance of complex formed. Since the curve appeared saturated beyond this volume, 3mL of BPB solution was used to provide excess reagent. The optimized conditions were used to prepare a reaction mixture and had the ion-pair complex extracted into chloroform and the stability of the complex examined over 48hrs at average room temperature of 29±1 °C. Figure 6 shows that the complex was stable in chloroform over the period and conditions of the stability test. This supports the earlier findings about the stability of the complex. However, in keeping the extractive for such a long time, proper storage should be provided to avoid evaporating off some of the chloroform as that can change the concentration of the extractive and thereby, affect assay values.

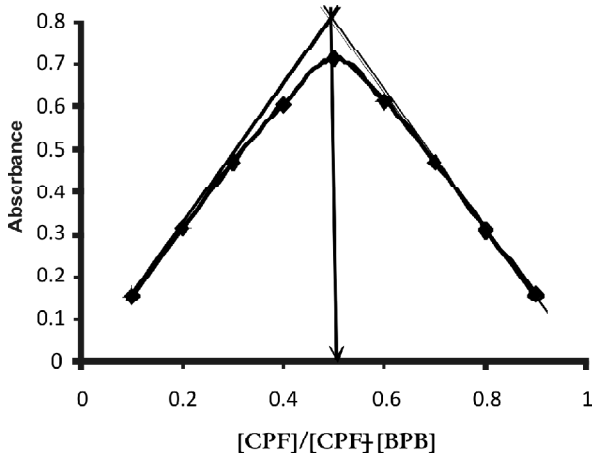

Figure 5a: Determination of the Stoichiometry of the CPF: BPB ion-pair Associate Extracted into $CHCl_3$ using Job's method of Continous Variations at 29 ± 1°C [CPF] + [BPB] = 1.359×10^{-4}M

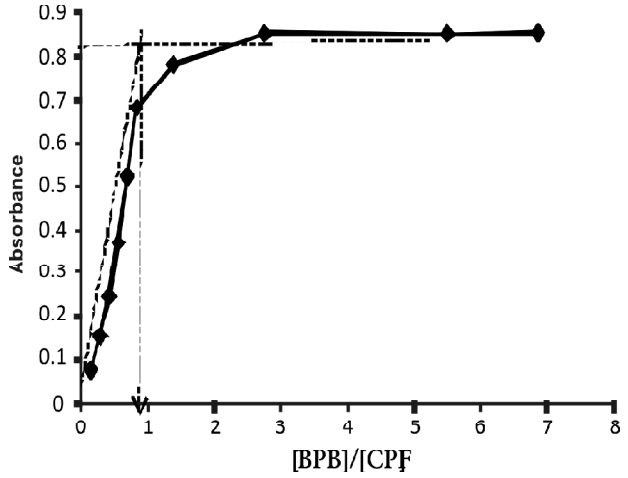

Figure 5b: Determination of the Stoichiometry of the CPF-BPB Complex by the Mole Ratio Method [CPF] = 5.43×10^{-5} M and [BPB] Ranged between 7.46×10^{-6} M-3.73×10^{-4}M

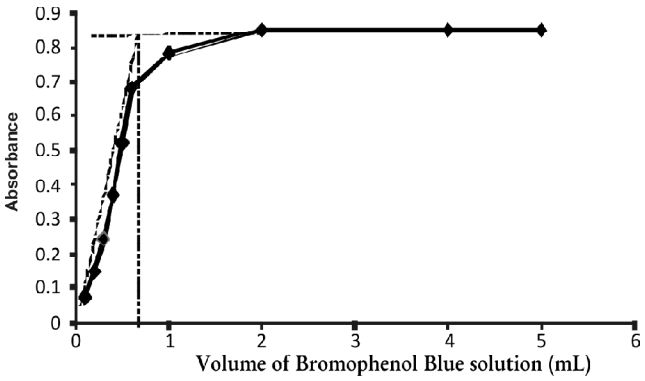

Figure 5c: Determination of Critical Volume of 500 µgmL^{-1} BPB Required to Produce Maximum and Constant Absorbance with 200µg of CPF

Assessment of Analytical Performance of Proposed Method

Verification of Beer's Law and Determination of Detecting Limits and Level of Sensitivity

Beer's law was verified using the method of linear least squares. Applying the general procedure, a series of reactions containing between1.0-37.5µg/mL of CPF were analysed in replicates of six against reagent blanks. As shown in Table 1, the linear dynamic range of the procedure was between 2.5-27.5µgmL^{-1}. Ringbom's plot, the established standard adopted to know the optimum range of concentration for a system that obeys Beer's law was used to define the effective region of linearity of the procedure. The log of concentration of CPF was plotted against (1-*T*) where *T* is the transmittance of the extracted complex. The plot had a sigmoid shape with a linear segment between CPF concentrations of 5.0-27.5µgmL^{-1} (Table 1). Hence, all assays were prepared with sample concentrations within this range. Detailed regression analyses of the calibration graph are presented in Table 1. Following the ICH guidelines on determination of detecting limits [30], the limits of detection and quantitation of the procedure were calculated. Limit of detection (LOD) was calculated by the equation; LOD = 3.3Ã/s while the limit of quantitation (LOQ) was calculated by; LOQ = 10Ã/s. Ã and s are respectively, the standard deviation of absorbance of reagent blanks (n = 7) and slope of the calibration curve. The molar absorptivity and Sandell's sensitivity index were also calculated as a further measure of the sensitivity of the method and results are presented in Table 1. The Sandell's sensitivity represents the

number of micrograms of the analyte per mL of a solution having an absorbance of 0.001 for a pathlength of 1cm (0.001/A(1%, 1cm). Considering all the evaluations of sensitivity, it can be realized from Table 1 that the method was sensitive and sound for its purpose. It may be possible to adopt it for determining some fluoroquinolones in biological fluids such as serum and urine.

Table 1
Optical Characteristics, Detecting Limits and Linear Regression Data for Proposed Method

Parameter	*Value*
Wavelength of maximum absorption (nm)	420
Beer's Law range (μgmL^{-1})	2.5-27.5
Ringbom limits (μgmL^{-1})	5.0-27.5
Molar absorptivity ($Lmol^{-1}cm^{-1}$)	$1.06x10^4$
Sandell's Sensitivity Index(μgcm^{-2})	0.036
LOD (μgmL^{-1})	0.383
LOQ (μgmL^{-1})	1.16
Slope±sd	0.028±0.0002
Intercept±sd	0.021±0.0034
R^2	0.9996

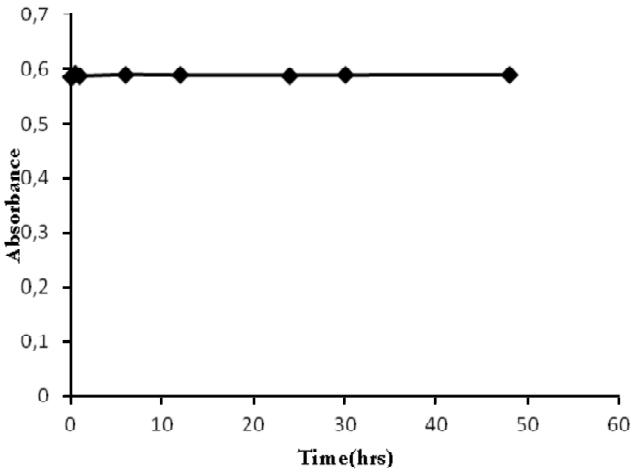

Figure 6: Stability of CPF: BPB Complex in $CHCl_3$ over a Period of 48hrs

Recovery Studies, Intra- and Inter-day Variations

Before the assay of the CPF tablets, the accuracy, repeatability and intermediate precision of the proposed method were evaluated. Recovery studies were done by the standard addition method. To each of a series of solutions already containing 100µg of CPF from a preanalysed tablet matrix, was added between 25.0-275.0µg of pure CPF in solution. The general procedure as already described was followed, determining the absorbance of each different concentration thrice and calculating the respective recoveries. The same procedure was repeated on two other days. From Table 2, the relative standard deviation for the six determinations in each of the three occasions was between 0.357-0.554%, indicating a good measure of intra-day precision or repeatability of the procedure. The standard error of the mean and confidence interval at 95% presented in Table 3 also indicate good intra-day precision. Using one-way analysis of variance, Bartlett's test for equal variances and Bonferroni's Multiple Comparison Test, there was no significant difference among the recovery data obtained over the three different occasions of assay. This implies that analyses done with the procedure on different occasions under similar conditions can have comparable results in terms of accuracy and inter-day precision The average recovery of the proposed method over the three days was 101.4±0.44 (n=18), further demonstrating the accuracy of the method. It also meant that components of the tablet matrix added by standard addition did not have observable interference with formation of the CPF:BPB complex.

Application of Method to Commercially Available CPF Tablets

The method has successfully been applied to the assay of six brands of CPF tablets. From Table 4, the percentage content of each brand fell within the monograph specification of the British Pharmacopoeia (95.0-105.0%w/w). Even though the HPLC method recommended by the BP was not applied to the samples, the accuracy of the method was validated by the recovery studies [30]. The HPLC facility was not readily available at the time of the study and the lack of access was a major reason behind the study design. The method has however proven to be simple, cost effective and user-friendly. This is because the process does not require laborious sample preparations but uses simple laboratory reagents, glass ware and equipment and yet as sensitive as already described by the detection limits, molar absorptivity and the Sandell's sensitivity index. It does not require too much skill to apply but produces accurate results that can be replicated. In addition, either silica or glass cuvettes can be used for absorbance measurements, providing further flexibility in its application.

Table 2
Recovery Studies, Intra-day and Inter-day Variations of Proposed Method

Sample Taken (µg)	*Sample Added (µg)*	**Total Found(µg)*			**Recovery (%)*		
		Day 1	*Day 2*	*Day 3*	*Day 1*	*Day 2*	*Day 3*
100.0	25.0	125.16	125.47	125.43	100.64	101.88	101.72
100.0	50.0	150.82	150.30	150.27	101.64	100.60	100.54
100.0	75.0	176.18	176.71	176.07	101.57	102.23	101.43
100.0	100.0	201.24	201.41	201.34	101.24	101.41	101.34
100.0	125.0	226.82	227.31	226.79	101.46	101.85	101.43
100.0	150.0	252.06	252.24	252.05	101.37	101.49	101.37
				Mean	101.32	101.58	101.31
				RSD	0.357	0.554	0.393
				SEM	0.148	0.230	0.163
				CI (p =0.05)	100.94-101.70	100.99-102.17	100.89-101.72

*Each value is the mean of three replicate determinations

Table 3
Statistical Evaluation of Intra- and Inter-day Variations using One-way Analysis of Variance, Bartlett's Test for Equal Variances and Bonferroni's Multiple Comparison Test

Parameter	*Statistical value & inference*			
One-way analysis of variance				
P value	0.5159			
P value summary	ns			
Are means signif. different? (P < 0.05)	No			
Number of groups	3			
F	0.6920			
R squared	0.08447			
Bartlett's test for equal variances				
Bartlett's statistic (corrected)	1.037			
P value	0.5955			
P value summary	ns			
Do the variances differ signif. (P < 0.05)	No			
Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary
Day 1 vs Day2	-0.2567	0.9887	No	ns
Day 1 vs Day 3	0.01500	0.05778	No	ns
Day2 vs Day 3	0.2717	1.046	No	ns

In spite of likely different excipients used by diverse manufacturers, assay results did not appear to have had interference from the excipients, probably because absorbance in longer wavelengths usually filters off background irrelevant absorption that could have taken place at shorter wavelengths. The pH dependency of the complex also makes the procedure useful in multi-component applications as well as differentiating analyte from any excipients with unrelated physicochemical properties.

The demonstration of mechanics of the CPF:BPB ion-pair complex and success of its application to assay CPF tablets suggest that the technique can be useful in low technically resourced environments for quality monitoring of CPF and its analogues. The technique can also be extended to cover all such medicinal agents with the chemical moiety to pair with sulponphthalein dyes under the necessary conditions.

Table 4
Assay of Ciprofloxacin Hydrochloride Tablets with Proposed Method

Brand	*Quantity Taken (μg)*	*Mean Quantity Found (μg)(n=3)*	*Mean % content (n=3)*	*Overall Mean % content for Brand*
CIPLOX	75.0	77.02	102.69	102.7±1.12
	100.0	101.56	101.56	
	150.0	155.70	103.80	
CIPROLET	75.0	75.11	100.15	101.8±1.44
	100.0	102.67	102.67	
	150.0	153.92	102.61	
QUINTOR	75.0	73.90	98.53	99.6±1.002
	100.0	99.65	99.65	
	150.0	150.79	100.53	
CIPRINOL	75.0	73.90	98.53	98.4±1.09
	100.0	97.31	97.31	
	150.0	149.22	99.48	
CIPROKAM	75.0	73.10	97.46	99.6±4.02
	100.0	97.11	97.11	
	150.0	156.36	104.24	
CIPROFLOXACIN	75.0	74.24	98.99	102.3±2.95
	100.0	103.12	103.12	
	150.0	157.06	104.71	

CONCLUSION

Ion-pair complex formation and extractive spectrophotometry is a useful, cheaper and accurate analytical alternative for monitoring the quality of susceptible drug candidates in the pure and formulations in places where recommended methods with highly sophisticated and capital intensive equipment cannot be afforded. In this regard, CPF and its congeners on the Ghanaian market can be regularly assessed to remove substandard products to avoid treatment failures and development of resistance and cross resistance to chemotherapeutic agents.

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