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Development of Green Bromination Titration for Determination of Ciprofloxacin Hydrochloride in Different Pharmaceutical Dosage Forms

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Abstract

Ciprofloxacin Hydrochloride (CIP-HCl) is a second-generation synthetic fluoroquinolone used for a wide range of infectious diseases. This study set out to provide a handy, cost-effective, facile, and eco-friendly analytical technique titrimetric method (method A). The developed titrimetric method is based on the bromination of CIP-HCl by bromine produced in situ by the reaction of acid on the bromate-bromide mixture. Alongside, simple UV-Spectroscopic (method A) from previous literature was conducted. Titrimetric allows the determination over the range of 5.0-70.0 mg CIP-HCl while in UV-spectroscopic, Beer's law is obeyed in the concentration ranges of 3.5-11.5 μ g mL^{-1}. The result of the proposed method was compared statistically with the reference UV-spectroscopic method from the literature. Student's t-test and F-ratio at 95% confidence level were used for comparison. The result showed no significant differences between the developed method (method A) and the reference method (method B) in regards to accuracy and precision. The developed method and UV-spectroscopic were applied effectively for the determination of the CIP-HCl in three different dosage forms (tablet, infusion, and eyedrops), with three brands for each dosage form.

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Introduction

Ciprofloxacin hydrochloride (CIP-HCl), the salt of 1-cyclopropyl-6-fluoro1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid. It is classified as a second-generation synthetic fluoroquinolone with a broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria. The Structure-Activity Profile of CIP-HCl is related to the presence of the piperazine moiety which is responsible for the antipseudomonal activity. The fluorine ion is responsible for expanding the antimicrobial activity to include both gram-positive and negative bacteria. It has been used for several types of infection including; urinary tract, gastrointestinal, skin, and respiratory (Tripathi, 2013).

The literature review showed the application of several analytical methods for the determination of the CIP-HCl in pharmaceutical dosage forms including UV-spectroscopy, Derivative UV-VIS Spectrophotometry, and the HPLC method. However, there is a flood of publications that cannot be limited here. Within this huge number of publications, there is a scarce number of articles focused on the simple titration method for the determination of CIP-HCl. A titrimetric method based on oxidation-reduction back-titration with the Cerium (IV) Sulphate was applied for the analysis of tablets and injection of CIP-HCl. Alongside this, another two spectrophotometric were also developed in the same study and the result of the three methods was comparable to each other (Basavaiah, *et al.,* 2006a). Another non-aqueous titration was based on the reaction of CIP-HCl with Mercuric (II) acetate and acetic anhydride and the solutions were titrated against acetous perchloric acid. The method was successfully used to determine the amount of CIP-HCl in the tablet's dosage form (Adegbolagun, *et al.,* 2007). A redox reaction of CIP-HCl was carried out by back titration of the excess amount of the $KMnO_4$ with the $FeSO_4$. In the same study, a UV-spectroscopic method was developed and there was no significant difference in the results of both methods. Both methods were applied for the analysis of the CIP-HCl tablets (Ebeshi, *et al.,* 2011). A study was carried out in Nigeria to determine CIP-HCl in five brands of tablets. This method is based on Acid-base back-titration. The result of this study showed that only two of the five brands were within the British Pharmacopeia (BP) description which was owed to the reliance on the individual decision of the analyst carrying out the titration (Ejele *et al.,* 2015).

The concept of the titrimetric method based on reaction with the liberated Br_2 was applied previously in the determination of some pharmaceuticals such as Astemizole (Nagegowda, Basavaiah, 2005), cyproheptadine (Basavaiah *et al.,* 2006b) Atenolol (Basavaiah, *et al.,* 2006a), Dothiepin (Abdulrahman & Basavaiah 2011), carbamazepine (Basavaiah & Abdulrahman 2014) Fexofenadine (Raghu, *et al.,* 2018). Other studies based on the concept of bromination and followed by spectroscopic analysis for some pharmaceuticals (Anastas & Warner, 1998; Prashanth &Basavaiah 2012; Devi, *et al.,* 2011).

In the current study, the developed titrimetric method is based on the bromination of CIP-HCl by bromine produced in situ by the reaction of acid on the bromate-bromide mixture. Bromine produced in situ by the effect of the acid on bromate– bromide mixture was attained on the principles of green chemistry (El-Didamony, 2010). CIP-HCl is reacted with an excess quantity of bromate-bromide mixture in an acid medium, followed by an iodometric back reaction of unreacted bromine (method A). The parameters affecting the reaction were evaluated and under the optimum reaction conditions, the results were precise and accurate. The result obtained from method A was compared with the simple UV-spectroscopic method (method B) (Naveed & Waheed, 2014). The application of the titrimetric method for analyzing the different dosage forms of CIP-HCl (tablets, infusion, and eye drops) revealed that there is no interference from the excipients.

The application of the titration method for pharmaceutical analysis has been reduced recently due to the presence of more accurate instrumental analytical methods. However, using the electronic device to measure the consumed quantities of reagents can be significantly increased the accuracy and costs, especially in quality control laboratories in low-income countries. It is considered cost-effective, simpler, and more rapid than the most sophisticated analytical techniques which are not easily available in most developing countries. The main objective of the current study was to develop a simple, rapid, and environmental-friendly analytical method for the determination of CIP-HCl in bulk and different forms of dosage forms, as well as to compare the result of the development with the UV-spectroscopic method. The developed method could be recommended as a suitable and promising perspective for the analysis of CIP-HCl in poor-resource economies.

Method

Instrument

UV-Visible Spectroscopy (Lasany® advanced microprocessor UV-VIS-L1-295), was used in the examinations made with UV-spectrophotometry.

Reagents and materials

The standard of CIP-HCl was supplied from the Modern pharma -in Yemen as a gift. All reagents and chemicals used were of analytical grade (Merck, Labtech, Flukachemika, and LOBACHEMIE), and distilled water was used to prepare the solutions. Three pharmaceutical dosage forms were analyzed (tablets, infusion, and eye drops), with three brands for each dosage form. Tablet of 500 mg, infusion 2 mg mL^{-1}, and eye drop of 0.3% CIP.

Bromate-bromide mixture

Stock standard solution of $KBrO_3$-KBr equivalent to 0.05 M Br_2 was prepared by dissolving 2.7835 g of $KBrO_3$ and 13 g KBr in a 1000 ml calibrated flask. Sodium Thiosulphate (0.06M): Prepared by dissolving 14.8902 g in a 1000 ml calibrated flask. Potassium Iodide 2% solution was prepared. Starch Solution Take 1 g of starch and rub with a small amount of H_2O then add the paste to 100 ml boiling water with constant stirring and boil for 1 min then cool and add 3 g of KI. Hydrochloric Acid Concentrated acid was diluted appropriately with water to get 5 M HCl and used.

Developed Method

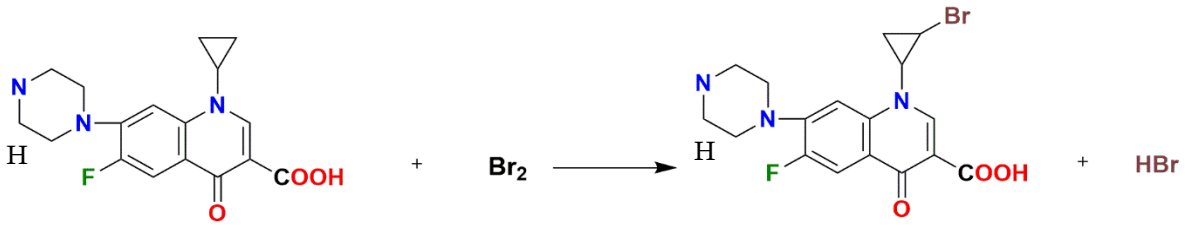
Method A (titrimetric)

The present method defines the determination of CIP-HCl using bromine generated in situ as a green brominating agent and is based on the bromination reaction of CIP-HCl with a known excess of the bromate-bromide mixture in an acid medium via electrophilic substitution reaction. The main benefit of this reagent is using a bromate-bromide mixture instead of using extremely noxious and harmful liquid bromine, no formation of toxic by-products, and using green and readily available reagents. The current methods involve the addition of 5 M HCl to the CIP-HCl (5-70 mg) to acidify the solution, followed by the addition of an excess amount of Bromate-bromide mixture (20 mL) which produces Br_2 equivalent to 0.05 M. The mixture was mixed well and the flask was reserved aside for 15 min with periodic shaking. Then, 20 mL of 2 % (w/v) potassium iodide was added by pipette to the flask and the released iodine was titrated with 0.06M sodium thiosulphate to a starch endpoint. A blank titration was performed under the same conditions. The reaction was found to follow a 1:1 (CIP: Br_2) stoichiometry as represented in Figure 1. Parameters such as; contact time, the molarity of reagents, and the concentration of acid media were estimated. The best result was obtained as mentioned previously in the section on the titration method.

Reaction:

1) $BrO_3^- + 5\,Br^- + 6H^+ \longrightarrow 3Br_2 + 3H_2O$

2)



3) $Br_2 + 2KI \longrightarrow I_2 + 2KBr$

4) $I_2 + 2Na_2S_2O_3 \longrightarrow 2NaI + Na_2S_4O_6$ (Sodium tetrathionate)

Figure 1. Possible Reaction Pathway for Bromination of CIP in HCl Medium.

The quantity of CIP-HCl was calculated from the following equation:

$$\text{Amount (mg)} = \frac{(B - S)}{n} \times \text{Mol. wt}$$

where B is the mmole of the titrant in the absence of the drug (Blank reading), and S is the mmole of the titrant in the presence of the drug, Mol. wt. is the relative molecular mass of the drug, and n is the reaction stoichiometry of the reaction of thiosulfate with the liberated iodine (2:1).

Method B (UV-Spectroscopic)

A stock standard solution containing 50 μ g mL^{-1} CIP-HCl was prepared by dissolving 10 mg of standard in water and diluting to the mark in a 200 ml calibrated flask. A series of CIP-HCl (3.5-11.5 μ g mL^{-1}) were prepared and examined at max absorbance of 278 nm (Naveed & Waheed, 2014). This method was selected from the literature because it is suitable for our laboratory resources.

Assay procedure for tablets

Ten tablets of CIP-HCl were exactly weighed and ground into a fine powder. An amount of the powder equivalent to 50 mg of CIP was accurately weighed into a 100 ml calibrated flask and the steps mentioned above in the case of the titrimetric method were followed, while in the spectroscopic method quality of the powdered tablet was diluted to get the final concentration equal to 7.5 μ g mL^{-1}. The content was mixed well for 5–10 min, filtered, and examined by spectroscopy.

Assay procedure for infusion

A volume of infusion equivalent to 60 mg of CIP was taken in the case of titrimetric analysis while in method B, a volume of infusion was diluted to get the final concentration equal to 6.0 μ g mL^{-1}. The content was mixed well and examined by spectroscopy.

Assay procedure for eye drops

Exactly 5 mL of eye drop is equivalent to 15 mg of CIP was taken in the case of titrimetric analysis while in method B, a volume of eye drop was diluted to get the final concentration equal to 6.0 μ g mL^{-1}. The content was mixed well and examined by spectroscopy.

Results and Discussion

Various organic pharmaceutical compounds undergo bromination by Bromate-bromide mixture in an acid medium as mentioned above. Also, CIP-HCl shows a tendency to undergo a reaction with the bromine. The current study focused on this principle to develop a simple titrimetric method for the determination of CIP-HCl and the method was successfully applied for the determination of the cited drug in three different dosage forms without interfering with the excipients. Meanwhile, a simple UV-spectroscopic method was used as a standard method to compare the result obtained from method A.

Method development

Titrimetric

Several reaction conditions were optimized. The reaction between CIP-HCl and bromine was performed in H_2SO_4 and HCl. There was no significant difference between the two acids, so HCl was used in further analysis. Also, the effect of the acid concentration was verified over the range of (1, 2.5, and 5 M of HCl) The optimum result was obtained at 5 M of HCl. The effect of time on the reaction was studied by keeping all other reaction parameters constant. The titration was carried out after (5,10,15 and 20 min) and the results revealed that the reaction was complete in 15 min. There was no significant effect on the result by extending the contact time up to 20 min. About 20 volume of $KBrO_3$–KBr equivalent to 0.05 M Br_2 was found suitable for a stoichiometric bromination of CIP in the investigated range.

UV-Spectroscopic

UV spectrophotometric method is suitable for the analysis of CIP-HCl since it contains a suitable chromophore, the quinoline ring system shows, due to chromophore C=C-C=C-C=C-C=O, intense absorption in UV. The wavelength of maximum absorbance was determined by scanning the standard solution over 200-400 nm. The maximum absorbance was at the wavelength (λ max) of 278 nm. This method was selected because of several factors for instance; using water as a solvent and using the simple method without the need for derivatization of the formation of a complex.

Method validation

Quantitative data

Validation of the applied analytical methods was carried out according to the ICHQ2 (R) 1Guidelines. The titrimetric method is applicable over the concentration range of 5.0 –70.0 mg of CIP and the reaction stoichiometry was calculated to be (1:1) for the reaction between CIP and Br_2. In the spectrophotometric method, Beer's law was obeyed over the concentration ranges 3.5-11.5 μ g mL-1. The limits of detection (LOD) and limits of quantification (LOQ) for method B were 0.093, and 0.311 μ g mL^{-1} respectively. The straight-line equation for the spectroscopic method is given below:

$$Y = 0.0746\,x + 0.0422 \qquad (r^2=0.9999) \ldots\ldots\ldots (1)$$

Precision and Accuracy

The precision and accuracy of the titrimetric method were evaluated at three-level concentrations (within the working range) three times. The RSD was below 2% which indicated the precision of the developed method. In the case of the spectroscopic method, they were evaluated at three different concentrations,6 times for three days. The RSD The RSD was below 2% which indicated the precision of the developed method. The reproducibility of the developed method was evaluated by examining the drug solution at three different levels for 3 days. Inter-day precision and accuracy result was acceptable. The data related to the precision and accuracy of both methods is summarized in Table 1.

Table 1: Intra-day and inter-day precision and accuracy studies.

Method	CIP-HCl	Intra-day			Inter-day		
		Found	Precision (RSD)	Accuracy[a]	Found	Precision (RSD)	Accuracy[a]
Titrimetric	5 mg	5.10	1.59	2.0	5.13	1.38	2.6
	30 mg	29.97	1.43	0.10	30.18	1.43	0.6
	60 mg	59.34	0.66	1.10	59.92	0.61	0.13
Spectroscopic	3.5 µg mL^{-1}	3.58	1.04	2.18	3.50	1.05	0.76
	7.5 µg mL^{-1}	7.66	1.08	2.13	7.44	0.96	0.8
	11.5µgmL^{-1}	11.73	1.169	2.0	11.84	0.95	2.39

a Bias (%) = [(found - taken)/taken] · 100.

Table 2: Results of determination of CIP-HCl in the three dosage forms by the developed titrimetric method and the reference spectroscopic method with statistical evaluation.

	% of Label Claim ±SD	
	Titrimetry (n=3)	Spectroscopic (n=6)
Tab-I	103.1± 1.178	102.0±0.77
	t=2.284 df=7 and *F*, DFn, Dfd = 2.355, 2,5	
Tab-II	101.9 ±1.446	101.6 ± 0.8897
	t=0.3556 df=7 and *F*, DFn, Dfd = 2.641, 2,5	
Tab-III	102.8 ± 1.287	100.9 ±1.207
	t=2.275 df=7 and *F*, DFn, Dfd= 1.137, 2, 5	
Infusion-I	101.3 ± 0.6285	101.9 ± 0.5747
	t=1.236 df=7 and *F*, DFn, Dfd = 1.196, 2, 5	
Infusion-II	101.7 ±1.287	101.6 ± 0.5604
	t=0.2468 df=7 and *F*, DFn, Dfd = 5.276, 2, 5	
Infusion-III	101.0 ± 1.352	101.9 ± 0.5934
	t=1.450 df=7 and *F*, DFn, Dfd = 5.192, 2, 5	
Eyedrop-I	102.5 ± 1.205	102.4 ± 0.7212
	t=0.2141 df=7 and *F*, DFn, Dfd = 2.790, 2, 5	
Eyedrop-II	102.8 ± 0.6717	103.6± 0.6135
	t=1.732 df=7 and *F*, DFn, Dfd = 1.199, 2, 5	

Eyedrop-III	101.1± 1.577	102.1± 1.033
	t=1.229 df=7 and *F*, DFn, Dfd = 2.331, 2, 5	

Application to the assay of pharmaceutical formulations

The titrimetric method was applied for the determination of CIP-HCl in different dosage forms (table, infusion, and eyedrops). There was no interference from the common excipients present in all examined dosage forms. The obtained result was statistically compared with the spectroscopic method (Naveed & Waheed, 2014) by applying the student's t-test for accuracy and F-test for precision. To compare the result the analysis was carried out on the same batch of dosage forms by both methods. From Table 2, it is clear that the calculated t-value and F-value at a 95% confidence level did not exceed the tabulated values of 2.36 and 5.79, respectively. There were no statistically significant differences in terms of accuracy and precision of the developed method from the existing reference spectroscopic method.

Recovery Study

The accuracy of the established method was further studied by the standard addition method. Three different concentration levels (50%, 100%, and 150% of the standard were added to the tablet powder, infusion, and eyedrop) were analyzed by both methods, and the recoveries percentage was calculated. The % of the recovery in the titrimetric analysis ranged from 96.73to 102.86% and in the spectroscopic method was 97.80-103.14%The result of the recovery study is represented in Table 3.

Table 3: The result of the recovery study for the three dosage forms.

Dosage form	Titrimetric (method A)				Spectroscopic (method B)			
	CIP-HCl in formula mg	Added amount	Total found	% of recovery	CIP-HCl in formula µg	Added amount	Total found	% of recovery
Tablet	10	5	15.2	101.33	3	2	5.11	102.2
	10	20	29.02	96.73	3	4	7.23	103.29
	10	40	50.20	100.40	3	8	11.2	101.82
Infusion	10	5	14.87	99.13	3	2	4.89	97.80
	10	20	30.12	100.40	3	4	7.17	102.43
	10	40	49.56	99.12	3	8	11.3	102.73
Eye drop	2	5	7.20	102.86	3	2	4.9	98.00
	2	20	21.54	97.91	3	4	7.22	103.14
	2	40	42.15	100.36	3	8	10.88	98.91

The current method required no extraction step for the dosage forms and provide an acceptable % of recovery which indicated that there is no interference from the co-formulated compounds. The applicable range of the titrimetric method is wider than the study that is based on titration of cited drug with the cerium (IV) sulfate where the range was 2-12 mg which was applied for tablet and injection forms (Basavaiah *et al.,* 2006b). The study based on non-aqueous titration of CIP-HCl was applied only to tables (Adegbolagun *et al.*, 2007). The content of the CIP-HCl table was also determined by titration with $KMNO_4$ (Ebeshi et al, 2011) and by acid-base back titration (Ejele, *et al.,* 2015). There was no report on the precision or accuracy of the titration methods in these studies.

Conclusion

The literature review showed that the present study is the first study based on titration CIP-HCl with the in situ liberated bromine and applied successfully for three different pharmaceutical dosage forms. The proposed method is handy, facile, and uses available reagents that require no heating or extraction with relatively acceptable accuracy, and precision, and no significant differences from the reference UV-spectroscopic method. Consequently, the developed method could be a suitable and promising alternative for quality control laboratories in low-income countries with the same accuracy and precision as the UV-spectroscopic method.

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