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Simple spectrophotometric method for measuring antibiotics by using $Fe(SCN)^{2+}$ Complex pharmaceutical products

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Abstract

A simple, sensitive and accurate spectrophotometric method of analysis of Clarithromycin in pharmaceutical dosage forms has been developed and validated. Clarithromycin can function as an electron donor in acidic environments and convert Fe^{3+} to Fe^{2+}. So an increase in the concentration of Clarithromycin in solution reduces the value of $Fe^{3+}$and increases the value of $Fe^{2+}$and if this reduction reaction occurs at the presence of SCN- color faintness in $FeSCN^{2+}$will be evident with respect to the degree of Clarithromycin concentration. The optimum wavelength for measuring absorption through spectrophotometer (UV-visible) is 445nm. In this work temperature, time, HCl concentration, KSCN concentration ,pH made optimum have all been. The linear Dynamic range is 2-22 ppm .Detection limit was 0.6 ppm. The relative standard deviation for the determination of 10 µgmL-1of antibiotic was about 0.5 -1.6% .The proposed method was successfully applied to the determination of selected antibiotic from pharmaceutical preparation. The calibration curve has been used in identifying the amount of Clarithromycin in pharmaceutical products.

Keywords: Clarithromycin, UV-visible spectrophotometric method, pharmaceutical products, complex formation

1.0 Introduction

Antibiotics are among the most frequently prescribed medications in modern medicine. Antibiotics cure disease by killing or injuring bacteria. The first antibiotic was penicillin, discovered accidentally from a mold culture. Today, over 100 different antibiotics are available to doctors to cure minor discomforts as well as life-threatening infections. Although antibiotics are useful in a wide variety of infections, it is important to realize that antibiotics only treat bacterial infections. Antibiotics are useless against viral infections (for example, the common cold) and fungal infections (such as ringworm) As irrational use of antibiotics may lead to bacterial resistance, doctors can best determine if an antibiotic is right for particular condition [1-5]. Clarithromycin is a macrolide antibiotic used to treat pharyngitis, tonsillitis, acute maxillary sinusitis, acute bacterial exacerbation of chronic bronchitis, pneumonia (especially atypical pneumonias associated with Chlamydia pneumoniae or TWAR), skin and skin structure infections. In addition, it is sometimes used to treat Legionellosis, Helicobacter pylori, and lyme disease.

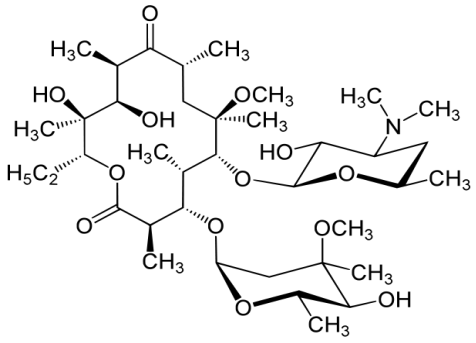

Figure-1: Structure of Clarithromycin

Clarithromycin is almost never used in HIV patients due to significant interaction with HIV drugs. As Clarithromycin is used widely, it is important that it should be detected by using suitable methods. Various methods are available for determination of Clarithromycin. Owing to the vital importance of antibiotic drugs in biological fluids and pharmaceutical preparations, various spectroscopic, chromatographic and electrochemical methods for assay of antibiotics have been reported. Clarithromycin is reported to be determined by different spectrophotometry, HPLC , GC , Electrophoresis, and Circular dichrosim [6-10] methods. In this work similar attempt was made wherein color reduction Of $FeSCN^{2+}$complex with antibiotic for determining amount of antibiotic in pharmaceutical products. Clarithromycin is donor electron and it can convert Fe^{3+} to Fe^{2+} and Fe^{2+} could not make permanent complex. So this property was used for determination of Clarithromycin using $FeSCN^{+2}$ complex.

2.0 Material and methods

The structural formula for Clarithromycin is showing by (Figure-1). Absorbance were measured with a UV-visble model 1240 (Shimadzu) spectrophotometer with 1 cm cells. pH adjustments were made using WTW multilab 540 Ionalyzer (Germany) pH mV-meter. A water thermostat (COOL NISC model CTE21) was used at 62±2ºC.All chemicals were of analytical reagent grade and freshly double distilled water was used throughout. Clarithromycin obtained from Zakaria pharmaceutical company (Tabriz-Iran) was of chemically pure laboratory working standard.

Procedure

Aliquot portion of drug standard solution 250 mgL^{-1} were transferred into 10 mL volumetric flasks. 2 mL of HCl (0.3M) was added and the resulting solutions were placed in a thermostat adjusted at 62 ºC for 60 minutes. After this period of time,0.5mL of 2.5×10^{-2}M $FeCl_3$ and 2 mL of 5×10^{-3}M KSCN were added and diluted to the volume with double distilled water .Absorbance values were measured at λ_{max} = 445nm(Figure-2) against a distilled water blank after 10 minutes. The calibration curve was drawn or regression equation calculated.

Parameter	SCN^-(0.005M)	Fe^{3+}(0.025M)	HCl (0.33M)	T(ċ)	T (minute)
Optimized amount	2mL	0.5mL	2mL	62	60

Table-1: Optimized of parameters

Determination in pharmaceutical preparation

The pharmaceutical preparation were obtained from local sources in various forms (Tablet, capsule, oral suspension). An accurately weighed amount equivalent to 100.0 mg of each drug from composite of 20 powdered tablets was transferred into a 100 mL volumetric flask. The residue was washed thoroughly with about 1 mL HCl (0.3M) and the combined filtrate as well as washing solutions was subjected to evaporation under vacuum till dryness.

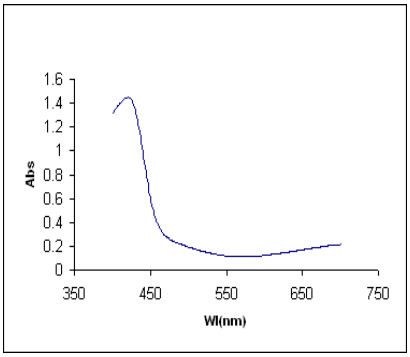

Figure-2: λ_{max} =445nm constant for this study

The residue lifted was dissolved in about 20 mL distilled water and filtered into 100 mL volumetric flask. The filter paper was washed thoroughly with double distilled water, and then the combined filtrate as well as washing solutions was mixed well and completed to volume with the same solvent to obtain solution of 1.0 mg mL−1.

Drug	Regression Equation	L.R(ppm)	R	n	D.L(ppm)	Q.L(ppm)
Clarithromycin	Y=0.056X+0.503	2-22	0.9924	6	0.6	1.75

Y=Absorbance, X= mL(antibiotic 10ppm),L.R= Linear Range, D.L=Detection Limit, Q.L=Quantification Limit

Table-2: assay parameters and regression analysis

Pharmaceutical	Labeled (mg)	Added (mg)	Found[a] (Recovery) Proposed method	RSD	Official method[b]	RSD
Clarithromycin tablet [b]	250	0	98.4	1.6	99.6	0.8
		100	96.8	1.1	98.2	1
Clarithromycin tablet [b]	500	0	98.2	1.3	99.3	0.6
		100	97.2	1.4	98.6	0.9
Suspension (5mL) [c]	250mgL^{-1}	100	101.2	1.2	102.8	0.8
Clarithromycin capsule[c]	500	0	98.2	1.6	99.1	0.7
		100	97.8	1.1	99.4	0.6

[a] Average of six replicate measurements, [b]Proposed method has compared with HPLC(USP)
[c]Proposed method has compared with GC(BP)

Table-3: Determination of Antibiotic in Some Pharmaceutical products

The final solution was diluted quantitatively with the same solvent to obtain working standard solution of 250mgL−1, then the general procedure was followed. The contents of 20 capsules were evacuated and well mixed. Then an accurately weighed amount equivalent to 100.0 mg evacuated capsules or dry powder suspension of each drug was transferred into a 100 mL beaker, and then the procedure was continued as described under tablets.

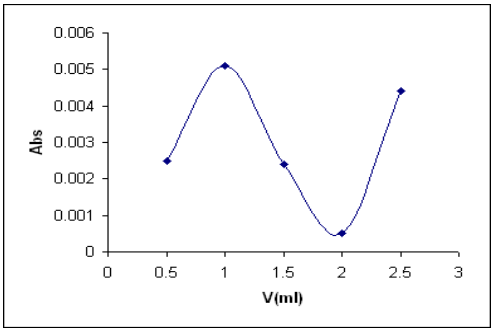

Figure-3: Effect of HCl concentration(2mL of HCl 0.33M) used for the hydrolysis of 10 ppm Clarithromycin within period of 60minute at 62 ºC on the absorbance of $FeSCN^{2+}$ complex.

3.0 Results and discussion

Preliminary studies were designed to examine the reaction between the selected antibiotic and chromogenic reagents at room temperature. The obtained results confirmed that no considerable interactions occurred under these conditions, but by increasing the temperature, the color of $FeSCN^{2+}$ complex gradually decrease in solution, because this reaction is exothermic. Among HNO_3 , H_2SO_4 and HCl , hydrochloric acid was selected for further experiments because it provided low intensity color and also nearly fast reaction. The effect of various concentration of HCl used in the acidic hydrolysis step of antibiotic is shown in Figure-3. As it can be seen, by increasing the concentration of HCl, the absorbance values decrease and level off at about 0.3M.

Therefore, the 0.33M of HCl was selected as optimum acid concentration. Figure-4 shows that the hydrolysis of antibiotic is completed at 62 ºC after 60minutes (Figure-5) and increasing of heating time and temperature did not considerably alter the color. In this work, in all experiments Fe^{3+} concentration has been considered 0.5 mL 2.5×10 -2M, and blank was distilled water.

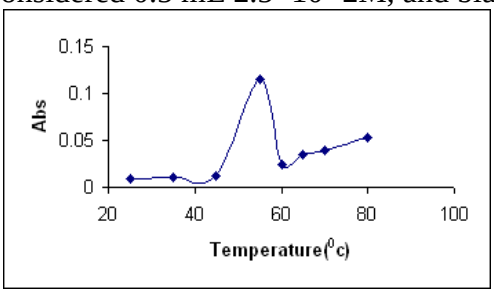

Figure-4: Effect time of acidic hydrolysis at 62 ºC on the absorbance of $FeSCN^{2+}$ complex, related to the reaction of hydrolyzed Clarithromycin with chromogenic mixture.

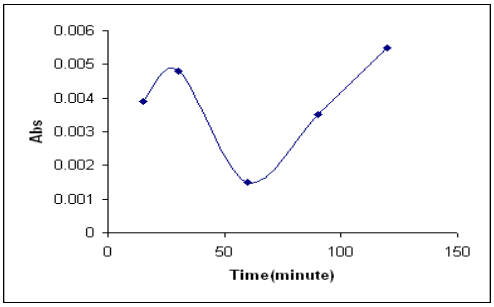

Figure-5. Effect of acidic hydrolysis temperature within a period of 60minute on the absorbance of $FeSCN^{2+}$ complex,related to the reaction of hydrolyzed Clarithromycin with chromogenic mixture.

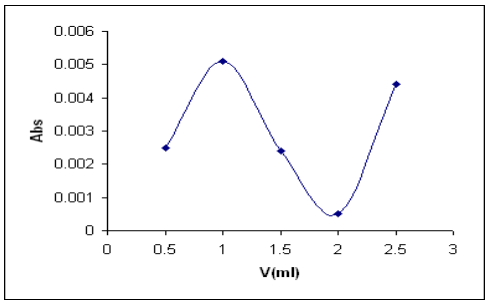

Figure-6: Effect of different concentration of KSCN on the absorbance of $FeSCN^{2+}$ complex produced corresponding to Clarithromycin.

The effect of the concentration of KSCN was studied for solutions containing a fixed concentration of Clarithromycin and varying amounts of KSCN. The effect of KSCN concentration was studied in the range 0.5, 1.0 , 1.5 ,2 , 2.5 mL from KSCN 5×10^{-3} M (Figure-6). The minimum color intensity remained constant at concentration higher than 10ppm for Clarithromycin .Therefore, the concentration selected was10ppm.(Table-1 shows optimization of parameters). In this method we used form of reaction of color reduction $FeSCN^{2+}$. Clarithromycin causes the Fe^{3+} converts to Fe^{2+}, because Clarithromycin is electron donor . Fe^{3+}

with SCN^- makes $FeSCN^{2+}$ complex with red color but Fe^{2+} cannot form with SCN^- to make a permanent complex. Whatever the more Clarithromycin concentration the less complex color, and it causes decrease absorption. When Fe^{3+} is reduced to Fe^{2+} by Clarithromycin which is obtained from acidic hydrolysis of β-lactam antibiotic .

$$Fe^3+ + SCN- \; FeSCN^{2+} \; \Delta H< 0 \ldots\ldots 1$$

$$\text{Clarithromycin} + Fe+ \; Fe^{2+} \longrightarrow \text{Clarithromycin+} \ldots\ldots..2$$

The acidic hydrolysis of antibiotic drugs containing β-lactam ring have been extensively studied. Antibiotic dissolved in water very little, but when we added a few drops of HCl antibiotic is hydrolyzed and dissolved in water fast and that performance is very good .

Analytical applications

In the present work optimization of all parameters which effect on acidic hydrolysis and $FeSCN^{2+}$ reaction for determination of selected antibiotic has been carried out. The relation between absorbance and concentration of drugs was studied. The beer's law limit, regression equation y= - 0.056x +0.5032 and correlation coefficient for the system is given in Table-2. A linear relationship was found between the absorbance at λmax= 445 nm and the concentration of the colored $FeSCN^{2+}$ complex in the concentration range 2-22ppm. Also, the calculated detection (3σ) Equation[3] and Quantification (10 σ)limits Equation[4] (19) of the analysis of samples are presents in Table-2 , and indicate proposed method and official method compared using student –test (27) Equation [1]and F-test (28) Equation [2] and differences between two methods are not meaningful.

$t=\bar{A}\ \sqrt{n}/S\_A \ldots\ldots 3$ $\bar{A}$ is mean and SA is standard deviation of A.

$F=(S\_1\hat{}2)/(S\_2\hat{}2) \ldots . 4$ S is standard deviation.

Sensitivity of the proposed method

A regression analysis of Beer's law plots reveals a good correlation .The resulting calibration curve for Clarithromycin is shown in (Table-2) and Equation (5 and 6) .The effect of common excipients and other substance were tested for possible interferences in the assay . It was observed that talk, glucose, starch, lactose and magnesium stearate did not interfere with the determination at the levels found in dosage forms. In order to establish the validity of analytical method, proprietary drugs containing antibiotic were analyzed . The same samples were also assessed by the official BP (20) or USP (21) methods(HPLC) (Table3) . The recoveries and relative standard deviation raging from 0.5- 1.6% reveal that similar degrees of accuracy and precision are afforded by both method in addition to test accuracy of proposed method, recovery experiments were performed on the samples prepared from dosage forms and pure (Table-3.). The results were found to be satisfactory and confirmed that the proposed method is free from interferences by capsule and tablet fillers or vial addition usually formulated with examined drugs.

$$D.L = A + 3S \ldots\ldots..5$$

$$Q.L = A + 10S \ldots\ldots\ldots\ldots 6$$

A is answer by system and S is standard deviation .Proposed method is very simple , cheap, fast, and precise , because a little time spends for determination of drug in pharmaceutical products and used materials are inexpensive , therefore the other methods are worthwhile and expensive . we added 100mg from pure Clarithromycin in 5mL of oral suspension (250mgL-1), then by proposed method and official method, we found amount of added antibiotic(Table-3) .

4.0 Conclusion

A simple, cheap, precise and sensitive spectrophotometric method is proposed for the determination of Clarithromycin in comparison with HPLC and GC. The decreasing intensity of color of the $FeSCN^{2+}$ complex formed is proportional to the amount of Clarithromycin drug in sample. The other advantages of the present method over the previously described method include low detection limit with high accuracy, precision and noun –interference from the associated substances in the dosage forms. Therefore, the proposed method is suitable for the analysis of the mentioned antibiotic, in pharmaceutical products.

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