Spectrophotometric determination of Puromycin(A) & Pristinamycin(B) in pharmaceutical products with turbidimetry method by using Cu^{2+} and tangesto phosphoric acid

BAKHTIAR KHODAVIRDILO[1*], NASER SAMADI[2] ,SAMANEH KHODAVIRDILO [3]

[1] Department of chemistry,Azarbaye jane Gharby&Centeral Education ,Urmia, Iran

[2] Department of chemistry,Urmia university, Urmia, Iran

[3] Cenral research education(Moallem) Azarbayjane Gharbi, Urmia,Iran,Iran

Coresponding author. Address : Shahrake Behdary Koye anaam 12 metry avval Plake 7 Urmia Iran

E-Mail: b.khodavirdilo@yahoo.com

ABSTRACT

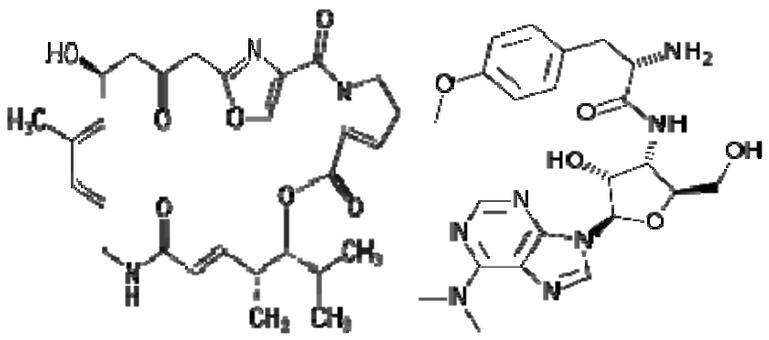
A simple, sensitive,fast and accurate spectrophotometric Turbidimetery method of analysis of antibiotics in pharmaceutical dosage forms has been developed and validated. Unfortunately, usage of these methods in measuring an organic composition, especially in medicines has not been considered. In this method, the necessary and optimum conditions for measuring a antibiotics by using a mixture of reagents deposition of Cu^{2+} and tangesto phosphoric acid have been reported and to following that, the suggestion method of measuring a antibiotics in pharmaceutical production has been developed. The linear Dynamic range for Puromycin(A)is 2-25 ppm and for Pristinamycin(B) is 2.5 -24ppm .Detection limit for Puromycin(A) is 1.5 ppm and Detection limit for Pristinamycin(B) is 1.6 ppm. This method is like that antibiotics which is a electron donor cause changing Cu^{2+} to Cu^{+} and Cu^{+} is made a sediment with Cl^{-} and create a colloid and every 1 mol of antibiotic is made a mol of Cu^{+}. In this study,the turbidance of homogenous CuCl producted from the above mentioned oxidation-reduction is proportional to concentration of antibiotic,and therefore,we developed an indirect simple,fast,and inexpensive turbidimetric method for the determination of antibiotics in pharmaceutical preparations. By measuring an CuCl , the content of existent antibiotics in pharmaceutical production has been calculated.

Keywords: spectrophotometric; pharmaceutical dosage; tangesto phosphoric acid; electron donor; Turbidimetery

INTRODUCTION

Antibiotics are compounds of natural or synthetic origin that selectively kill or inhibit the growth of microorganisms, and many of them are sufficiently specific to be useful for the treatment of bacterial infections. A large fraction of these compounds, including many that are used in the clinic, are inhibitors of bacterial protein synthesis,and almost all such antibiotics interact with the ribosome. The peptidyl transferase center is the target of many of these antibiotics, which is surprising given the high-degree of conservation in that region of the ribosome, and given the need for antibiotics to target specific organisms. Since publication of atomic resolution structures of both the large and small ribosomal subunits[1–3] several papers have appeared describing the structures of complexes between ribosomal subunits andantibiotics[4–8].Puromycin is an aminonucleoside antibiotic, derived from the Streptomyces alboniger bacterium,[9] that causes premature chain termination during translation taking place in the ribosome. Part of the molecule resembles the 3' end of the aminoacylated tRNA. It enters the A site and transfers to the growing chain, causing the formation of a puromycylated nascent chain and premature chain release[10]. The exact mechanism of action is unknown at this time but the 3' position contains an amide linkage instead of the normal ester linkage of tRNA. That makes the molecule much more resistant to hydrolysis and stop the ribosomeIt is not selective for either prokaryotes or eukaryotes.Also of note, puromycin is critical in mRNA display. In this reaction, a puromycin molecule is chemically attached to the end of an mRNA template, which is then translated into protein. The puromycin can then form a covalent link to the growing peptide chain allowing the mRNA to be physically linked to its translational product. Antibodies that recognize puromycylated nascent chains can also be used to purify newly synthesized polypeptides[11]. and to visualize the distribution of actively translating ribosomes by immuno fluorescence [12]. Puromycin is a reversible inhibitor of dipeptidyl-peptidase II (serine peptidase) and cytosol alanyl amino peptidase (metallo peptidase). The mechanism of inhibition is not well understood, however puromycin can be used

to distinguish between aminopeptidase M (active) and cytosol alanyl aminopeptidase (inhibited by puromycin) Puromycin is used in cell biology as selective agent in cell culture systems. It is toxic to prokaryotic and eukaryotic cells. Resistance to puromycin is conferred by the Pac gene encoding a puromycin N-acetyl-transferase (PAC) that was found in a Streptomyces producer strain. Puromycin is soluble in water (50 mg/ml) as colorless solution at 10 mg/ml. Puromycin is stable for one year as solution when stored at -20 °C. The recommended dose as a selection agent in cell cultures is within a range of 1-10 µg/ml, although it can be toxic to eukaryotic cells at concentrations as low as 1 µg/ml. It acts quickly and can kill up to 99% of nonresistant cells within 2 days. Puromycin is poorly active on *E.* coli. Puromycin-resistant transformants are selected in LB agar medium supplemented with 125 µg/ml of puromycin. But use of puromycin for *E.* coli selection requires precise pH adjustment and also depends on which strain is selected. For hassle–free selection and optimum results the use of special modified puromycin is possible. Plates containing puromycin are stable for 1 month when stored at 4 °C.Pristinamycin (INN), also spelled pristinamycine, is an antibiotic used primarily in the treatment of staphylococcal infections, and to a lesser extent streptococcal infections. It is a streptogramin group antibiotic, similar to virginiamycin, derived from the bacterium Streptomyces pristinaespiralis. It is marketed in Europe by Sanofi-Aventis under the trade name Synercid and Pyostacine.Pristinamycin is a mixture of two components that have a synergistic antibacterial action. Pristinamycin IA is a macrolide, and results in pristinamycin having a similar spectrum of action to erythromycin. Pristinamycin IIA (streptogramin A) is a depsipeptide[13]. PI and PII are coproduced by S. pristinaespiralis in a ratio of 30:70. Each compound binds to the bacterial 50 S ribosomal subunit and inhibits the elongation process of the protein synthesis, thereby exhibiting only a moderate bacteriostatic activity. However, the combination of both substances acts synergistically and leads to a potent bactericidal activity that can reach up to 100 times that of the separate components.The pristinamycin biosynthetic gene cluster is the largest antibiotic supercluster known so far with a size of ~210 kb, where the PI and PII biosynthetic genes are not clustered individually but are scattered across the complete sequence region[14]. Furthermore, this biosynthetic gene region is interrupted by a cryptic type II PKS gene cluster.Despite the macrolide component, it is effective against erythromycin-resistant staphylococci and strepcococci[15]. Importantly, it is active against methicillin-resistant Staphylococcus aureus (MRSA). Its usefulness for severe infections, however, may be limited by the lack of an intravenous formulation owing to its poor solubility[16]. Nevertheless it is sometimes used as an alternative to rifampicin+fusidic acid or linezolid for the treatment of MRSA.The lack of an intravenous formulation led to the development of the pristinamycin-derivative quinupristin/dalfopristin (i.e., Synercid), which may be administered intravenously for more severe MRSA infections[17-18].In figure(1) shown structure formula of Puromycin(A) & Pristinamycin(B).



A B

Fig .1. Structure of Puromycin(A) & Pristinamycin(B)

We used pharmaceutical products are a class of antibiotics that have found wide use in therapy. The mentioned antibiotics are very reactive against gram positive, gram negative and some anaerobic organisms. Determinations of mentioned antibiotics have been carried out in pharmaceuticals and biological samples using several methods. Assay of ciprofloxacin alone and along with some other quinolones has been done using techniques such as HPLC [5-10], capillary electrophoresis[19], LC-MS[20],UV-spectrophotometric methods[21] and ion-association complexes with aluminium/erythrosine[25], ternary complex formation with eosin and palladium[23], luminescence[24-29]. Metal chelation has been used for several fluoroquinolones. This work we determined antibiotics in pharmaceutical products by tubidimetry method. antibiotics which are electron donor cause changing Cu^{2+} to Cu^{+} and Cu^{+} is made a sediment with Cl^{-} and create a colloid and every 1mol of antibiotic is made a mol of Cu^{+}. In this study,the turbidance of homogenous CuCl producted from the above mentioned oxidation-

reduction is proportional to concentration of antibiotic,and therefore,we developed an indirect simple,fast,and inexpensive turbidimetric method for the determination of antibiotics in pharmaceutical preparations. By measuring an CuCl , the content of existent antibiotics in pharmaceutical production has been calculated.

EXPERMENTAL

Reagents and Instrumentals

First. Dissolve the 0.288gr of tangesto phosphoric acid in a quantity of distilled water. In crease the volume of solution to 50ml. – then dissolved the 1.35g of $CuCl_2$ in a quantity of distilled water. Increase the volume of solution to 50ml.

Dissolve the 50mg of standard Puromycin & Pristinamycin in some distilled water. With some drop of Hydrochoric acid (HCl) and in crease the volume of solution to 50ml.

syringe of Hamilton (50 & 100 µ L)

Magnet mixer machine, model ZMS – 74 manufactures by Zak shimi – Tehran – Iran.

Absorbances 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 20-50°C.All chemicals were of analytical reagent grade and freshly double distilled water was used throughout. Antibiotics obtained from Zakaria pharmaceutical company (Tabriz-Iran) was of chemically pure laboratory working standard.

DESCUSSION

Optimization of conditions

In order to optimized the proposed turbidimetric method the effect of some experimental variable such as temperature,pH,cocentration of reagents,and stiring were studied . Altering each variable in turn while keeping the other constant was studied .The effect of temperature on the quantity of the proposed precipitates was studied between 20-50°C. The obtained results showed that the reaction temperature has considerable effect on the amount of CuCl in method is widely related to temperature and depending on the nature and composition of studied drugs solution. The quantity and quality of the producted CuCl as a colloidal inorganic precipitate was disorderly altered with increasing temperature .Therefore,due to relative large absorbance values, high repeatability,and simplifying of procedures, room temperature was selected for further investigations in proposed method.The changes of turbidance values with- pH,in method turbidimetry are very sharp,as seen, the turbidance values decrease with increasing pH,may be due to large hydrolysis Cu(II) and it was formed $Cu(OH)_2$.At pH>6,the turbidance values increase probably due to considerable hydrolysis of Cu(II)and low solubility of Puromycin(A) & Pristinamycin(B) drugs.Therefore, the pH 1 was selected for this method.

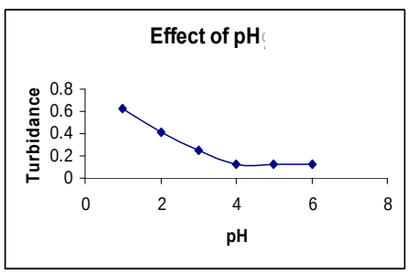


Fig. 2. Optimization of pH

Determination of turbidance in different wave lengths

Generally, in turbidimetric determinations,a quantity called turbidance coresponding to absorbance ,can be defined that follows a relation analogousto Beer's law $S=\log\frac{p_o}{p}$ =kbN where S represents turbidance ,k is a proportionality factor called turbidity coefficient, b is the path length,and N is the number of scattering particales per mili Liter.The theoretical treatment shows that:

$$k=0.434\{0.67d^6\pi^{-5}\lambda^{-4}\frac{(m-2)^2}{(m+2)^2}\}(1)$$

Where d is the partical diameter, λ is the wave length,and m is ratio of the refractive index of the particales to that of the solvent.This relation holds for dialute suspensions in which the partical size is on uniform and small compared to the wave length.According to Eq.(1),when spectrophotometer is used for turbidimetric ,a wave length in the blue near-UV(short wave length)should be selected for maximum sensitivity .It can be expected that the turbidance values decrease by increasing wave lengths. Therefore ,in the present work,all of the turbidance values was measured at 400nm.

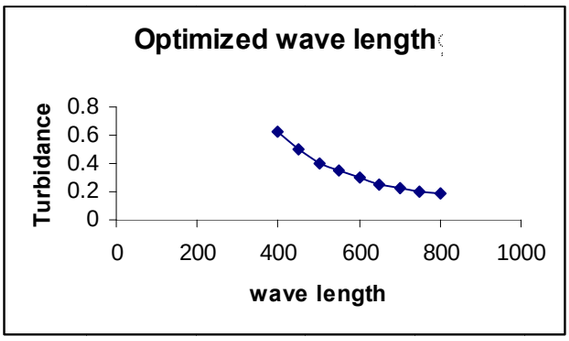


Fig.3. Optimization of wave length

Optimum making of concentration of tangesto phosphoric acid

Except the concentration of tangesto phosphoric acid, all sensational parameters of suction in Turbidimetery have been poised and by changing the concentration of tangesto phosphoric acid, suctions have been changed too. The maximum absorption was in volume 0.5. Of 15ppm solution of tangesto phosphoric acid. So the optimum quantity of tangesto phosphoric acid is 15ppm.

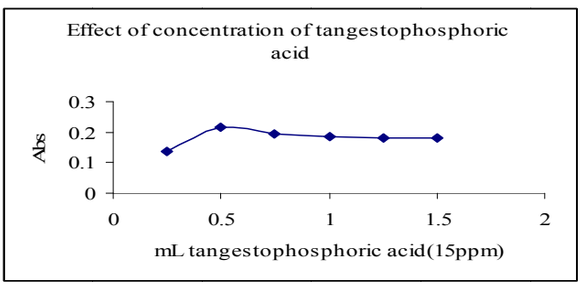


Fig.4. Optimization of tangesto phosphoric acid

Optimum making of concentration of $CuCl_2$ except the concentration of $CuCl_2$.

All other parameters have been poised and by changing the concentrations of $CuCl_2$, suctions have been changed too.The maximum suction is for content of 275ppm from $CuCl_2$. So the optimum quantity of $CuCl_2$ is 275ppm.Table 2 shape 5 show the result of usage Mix the 0.5mL of tangesto phosphoric acid with 0.5mL $CuCl_2$ then add different density of Puromycin to prepared mixture and read suctions in length wave was 400nm. And draw the curve of calibration by direction below. First prepare 5 volumetric flasks and add 1mL of tangesto phosphoric acid mixture to every 6 volumetric flasks. Then add 0.5, 0.75, 1, 1.5,2, 2.5 mLof Puromycin standard solution to every 5 volumetric flasks from 1 till 6and read absorption in λ= 400nm .The Blank in this work was distilled water. This work repeated with Pristinamycin and drown calibration curve for anyone of antibiotics of Beer's law in an offered turbidimetery method[30-32]

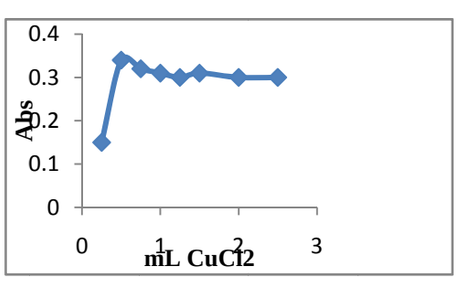


Fig.5. Optimization of $CuCl_2$

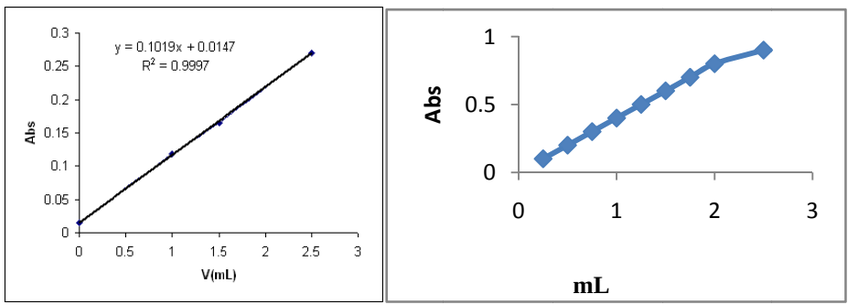
character	pH	W.L*(nm)	V_{CuCl_2}(271ppm)	$V_{Tangestophos.}$(15ppm)	T(°C)
Optimum value	1	400	0.5	0.5	25

*W.L=wave length

Table 1.Optimization conditions

RESULTS

After drawing the curve of calibration, the content of existent Puromycin in real samples has been calculated. measuring an existent Puromycin in a capsule which manufactured by yasmin Pharmacia company. Weigh 0.1g capsule and dissolve by some drop of concentration HCl in some distilled water. Then increased the volume of solution to 100ml. take 2ml of that and add it to 1ml mixture of tangesto phosphoric acid 0.001m and $CuCl_2$ 0.1m and some drop of concentration HCl . Increase the volume of acquired solution to 5 ml. and put it to UV – visible machine and read the absorption in λ=400 nm. By using an acquired (regression equation) from the calibration curve, the amounts of existent Puromycin in capsule has been calculated[33-47]. Measuring an existent Puromycin in Gel 3%(w/w)%dissolve 1g Gel by some drop of density hydrocholoric acid (HCl) in some distilled water. Increase the volume of solution to 10mL. Take 2ml of that and add it to 1mL mixture of tangesto phosphoric acid and $CuCl_2$. Then increase the volume of solution to 5ml. and after that put it in UV-visible machine and read the suction. By using the acquired (regration equation) from the calibration curve, the amounts of existent Puromycin in Gel has been calculated.Measuring the existent Puromycin in Solution %3 (w/w%)Take 0.2ml of solution and add it to 1mL mixture of tangesto phosphoric acid and $CuCl_2$. Increase the volume of solution to 5ml by adding some distilled water then put the acquired solution in the UV-visible machine and read the suction. And by using an acquired (regration equation) from the calibration curve, the amounts of existent Puromycin in solution has been calculated.Summary of acquired result from Turbidimetry method of table 3 has been seen and we act for determining Pristinamycin such as Puromycin .



A B

Fig.6. Calibration curve for Puromycin(A)& Pristinamycin(B)

Drug	Regression Equation	R	n	Dynamic range linear
Puromycin	Y = 0.1019x-0.0147	0.9997	5	2-25 (ppm)
Pristinamycin	Y = 0.12x-0.022	0.9936	6	2.5-24(ppm)

Table 2.. Measuring parameters, linear equation, correlation coefficient, sample quantity .

CONCLUSION

A simple , cheap, precise and sensitive spectrophotometric method is proposed for the determination of Puromycin and Pristinamycin in compare with HPLC and GC. In this study,the turbidance of homogenous CuCl producted from the above mentioned oxidation-reduction is proportional to concentration of antibiotics,and therefore,we developed an indirect simple,fast,and inexpensive turbidimetric method for the determination of antibiotics in pharmaceutical preparations. By measuring an CuCl , the content of existent antibiotics in pharmaceutical production has been calculated . The other advantages of the present method in 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[48-51].

A CKNOWLEGMENTS

This work was helped Professor Dr. Wan Aini Wan Ibrahim (UTM, Malaysia).we thank MS. Kazem Najafi and Ali Khodavirdilo and Mr. Elia Ebrahimi for their helpful computer work.

Pharmaceutical producted	Labeled(cantained Antibiotics)	Calculated by proposed method (Recovery) ± SD	RSD	Calculated by official method (Recovery) ± SD	RSD
Capsule(Puromycin)	150mg	152.6± 2.5 mg	1.4	152.5 ± 2.7 mg	1.4
Solution(Puromycin)	3%(w/w%)	%3.01 ± 0.22	1.6	%3.02 ± 0.21	1.6
Gel (Puromycin)	3%(w/w%)	%3.04 ± 0.143	1.7	%3.04 ± 0.153	1.5
Capsule(Pristinamycin)	250mg	249.4± 2	1.5	249.6± 2.5	1.5
Tablet(Pristinamycin)	100mg	102±2	1.4	101±2	1.3
Suspension(Pristinamycin) 5mL	250mgL^{-1}	3±0.3	1.3	%3±0.3	1.4

Table 3. Determination of Antibiotic in Some Pharmaceutical products

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