International Journal of Research and Reviews in Pharmacy and Applied science

www.ijrrpas.com

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SPECTROPHOTOMETRIC DETERMINATION OF ANTIBIOTICS IN PHARMACEUTICAL PRODUCTS WITH TURBIDIMETRY METHOD BY USINGPB^{4+} AND TANGESTO PHOSPHORIC ACID

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 Pb^{4+} 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 Tobramycin is 2-23 ppm and for azithromycin is 2.2 -24ppm .Detection limit for Tobramycin is 1.6 ppm and Detection limit for azithromycin is 1.7 ppm. This method is like that antibiotics which is a electron donor cause changing Pb^{4+} to Pb^{2+} and Pb^{2+} is made a sediment with Cl^- and create a colloid and every 1 mol of antibiotic is made a mol of Pb^{2+}. In this study,the turbidance of homogenous $PbCl_2$ 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 $PbCl_2$, the content of existent antibiotics in pharmaceutical production has been calculated.

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

INTRODUCTION

We used pharmaceutical products are a class of antibiotics that have found wide use in therapy. The mentioned antibiotics (Tobramycin(A) and azithromycin (B)) are very reactive against gram positive, gram negative and some anaerobic organisms. They are the latest class of antibacterial agents developed, the origin of which is accidental. mentioned antibiotics are a family of synthetic broad spectrum antibiotics. In analytical chemistry, method for determining the amount of cloudiness, or turbidity, in a solution based upon measurement of the effect of this turbidity upon the transmission and scattering of light. Turbidity in a liquid is caused by the presence of finely divided suspended particles. If a beam of light is passed through a turbid sample, its intensity is reduced by scattering, and the quantity of light scattered is dependent upon the concentration and size distribution of the particles[17]. In this case, activity of that has been increased substantially. The profitable antibacterial traits of this composition cause a substantial overused of that as an edible drug or covering in treatment of skinny putrefactions[16-17]. 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[11,12], LC-MS[13,14],UV-spectrophotometric methods[15-19] and ion-association complexes with aluminium/erythrosine[20], ternary complex formation with eosin and palladium[21], luminescence[22]. Metal chelation has been used for several fluoroquinolones[23,24]. Derivatization for spectrofluorimetric analysis has also been carried out using 4-chloro-7-nitrobenzofurazan in borate buffer pH 9.0[25]. Sparfloxacin, on the other hand, has been assayed by such techniques as UV using bromothymol blue[26], non-aqueous titration[27]and microbiological method[28]. ^{1}H NMR technique has also been reported for the analysis of Tobramycin(A) and azithromycin (B) [29]. Antibiotics: in this case Tobramycin as very important Antibacterial drugs has so extensive usage in the entire world. There fore, measuring this Drug and generally qualitative Expand of that and preparation of primeval supplies of that have a precious concern[9-11]. Standard methods (such as Created by HPLC) of survey of measuring a Tobramycin in a medicine production need a lot of time and expensive. There fore, researchers are attempt to present a quick, simple and cheap methods to decrease the problem of these kinds of measuring. So we are attempting to present simple methods to measuring Tobramycin(A) and azithromycin (B) too. Its needs to say that the number of reported Spectrophotometric methods to measuring Tobramycin(A) and azithromycin (B) are so limited. And Those are themself a binary motive to do this research projection[12]. To avoid this problem, do not mix clindamycin phosphate and gentamicin sulfate prior to dilution. Rather, dilute one drug or the other, agitate the solution and then add the second antibiotic .Tobramycin sulfate is 0 - 3 - amino - 3 - deoxy - a - D - glucopyranosyl - (1→4) - 0 - [2,6 - diamino - 2,3,6 - trideoxy - a - D - ribo - hexopyranosyl - (1→6)] - 2 - deoxy - L - streptamine, sulfate (2:5)(salt) and has the chemical formula$(C_{18}H_{37}N_5O_9)_2 \bullet 5H_2SO_4$. The molecular weight is 1425.45. The structural formula for Tobramycin(A) and azithromycin (B) shown in figure 1.The pharmacy bulk package of Tobramycin(A) and azithromycin (B) for injection USP and BP is a container of a sterile preparation for parenteral use that contains multiple single doses. It is intended for use in a pharmacy admixture program. Package use is restricted to the preparation of admixtures for intravenous infusion or to the filling of empty sterile syringes for intravenous injections for patients with individualized dosing requirements. Tobramycin sulfate, a water-soluble antibiotic of the aminoglycoside group, is derived from the actinomycete Streptomyces tenebrarius. This work we determined antibiotics in pharmaceutical products by tubidimetry method. antibiotics which are electron donor cause changing Pb^{4+} to Pb^{2+} and Pb^{2+} is made a sediment with Cl^- and create a colloid and every 1mol of antibiotic is made a mol of Pb^{2+}. In this study,the turbidance of homogenous $PbCl_2$ 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 $PbCl_2$, the content of existent antibiotics in pharmaceutical production has been calculated.

EXPERMENTAL AND METHOD

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 4.25g of $PbCl_4$ in a quantity of distilled water. Increase the volume of solution to 50ml.
- Dissolve the 50mg of standard Tobramycin 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 20-60°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.

RESULTS AND 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 20-60°C. The obtained results showed that the reaction temperature has considerable effect on the amount of $PbCl_2$ 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 $PbCl_2$ 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 Pb(II) and it was formed $Pb(OH)_2$.At pH>6,the turbidance values increase probably due to considerable hydrolysis ofPb(II)and low solubility of Tobramycin and Azithromycin drugs.Therefore, the pH 1 was selected for this method.

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.

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 tangestophosphoric acid is 15ppm.

Optimum making of concentration of $PbCl_4$ except the concentration of $pbCl_4$.

All other parameters have been poised and by changing the concentrations of $PbCl_4$, suctions have been changed too.The maximum suction is for content of 271ppm from $PbCl_4$. So the optimum quantity of $PbCl_4$ is 271ppm.Table 2 shape 6 show the result of usage Mix the 0.5mL of tangesto phosphoric acid with 0.5mL $PbCl_4$ then add different density of Tobramycin 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 5 volumetric flasks. Then add 0.5, 0.75, 1, 1.5mLof Tobramycin standard solution to every 5 volumetric flasks from 1 till 5 and read absorption in λ= 400nm .The Blank in this work was distilled water. This work repeated with Azithromycin and drown calibration curve for anyone of antibiotics of Beer،s law in an offered turbidimetery method[29-30].

After drawing the curve of calibration, the content of existent Tobramycin in real samples has been calculated. measuring an existent Tobramycin 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 $PbCl_4$ 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 Tobramycin in capsule has been calculated[13-18]. Measuring an existent Tobramycin 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 $PbCl_4$. 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 Tobramycin in Gel has been calculated.Measuring the existent Tobramycin in Solution %3 (w/w%)Take 0.2ml of solution and add it to 1mL mixture of tangesto phosphoric acid and $PbCl_4$. 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 Tobramycin in solution has been calculated.Summary of acquired result from Turbidimetry method of table 2 has been seen and we act for determining Azithromycin such as Tobramycin.

CONCLUSIONS

A simple , cheap, precise and sensitive spectrophotometric method is proposed for the determination of Tobramycin and azithromycin in compare with HPLC and GC. In this study,the turbidance of homogenous $PbCl_2$ 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 $PbCl_2$, 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.

ACKNOWLEGMENTS

This work was helped Professor Dr. Naser Samadi (Urmia, Iran).We thank Mr. Elia Abrahimi and Ali Khodavirdilo for their helpful computer work.

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Tables:

character	pH	W.L*(nm)	V_{PbCl4}(282ppm)	$V_{Tangestophos.}$(15ppm)	T(^{0}C)
Optimum value	1	400	0.5	0.5	25

*W.L=wave length

Table 1.Optimization conditions

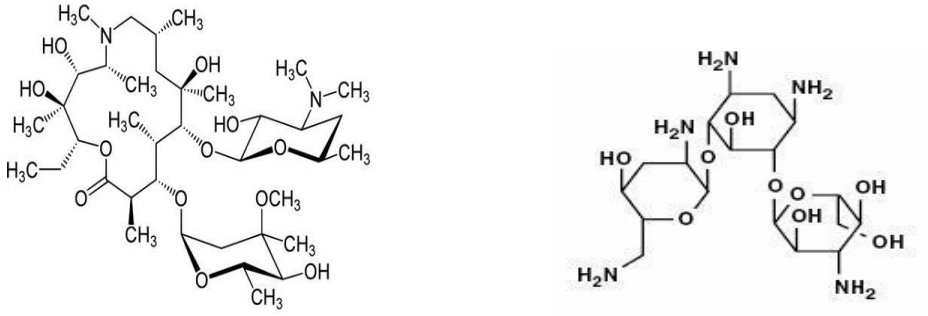
Drug	Regression Equation	R	n	Dynamic range linear
Tobramycin	Y = 0.1019x-0.0147	0.9997	5	2-23 (ppm)
azithromycin	Y = 0.12x-0.022	0.9936	6	2.2-24 (ppm)

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

Pharmaceutical producted	Labeled(cantained Antibiotics)	Calculated by proposed method (Recovery) ± SD	RSD	Calculated by official method (Recovery) ± SD	RSD
Capsule(Tobramycin)	150mg	151.4 ± 1.5 mg	1.4	150.5 ± 1.7 mg	1.3
Solution(Tobramycin)	3%(w/w%)	%3.04 ± 0.31	1.6	%3.02 ± 0.22	1.4
Gel (Tobramycin)	3%(w/w%)	%3.12 ± 0.15	1.5	%3.04 ± 0.153	1.3
Capsule(Azithromycin)	250mg	251.3± 2.5	1.4	249.6± 2.5	1.6
Tablet(Azithromycin)	100mg	99.8±2	1.2	101±2	1.1
Suspension(Azithromycin)5mL	250mgL^{-1}	3±0.3	1.4	%3±0.3	1.4

Table 3. Determination of Antibiotic in Some Pharmaceutical products

Figures



A B

Figure.1.Structure formula of Tobramycin(A) and azithromycin (B)

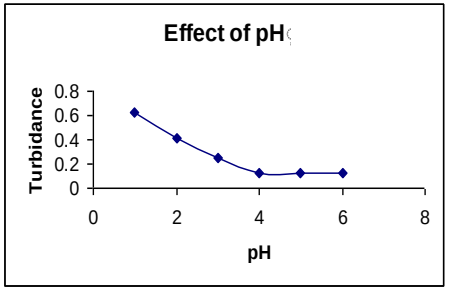


Figure. 2. Optimization of pH

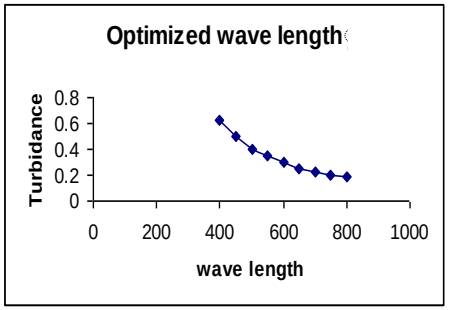


Figure.3. Optimization of wave length

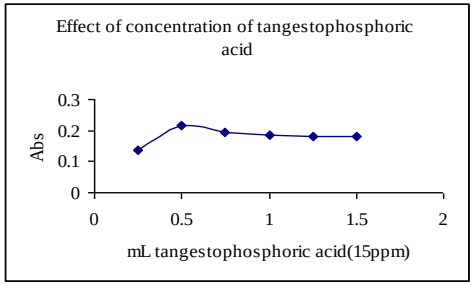


Figure.4. Optimization of tangesto phosphoric acid

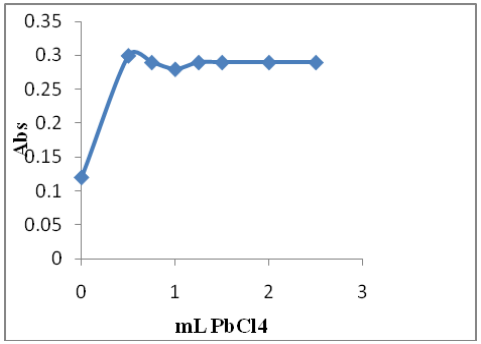


Figure.5. Optimization of $PbCl_4$

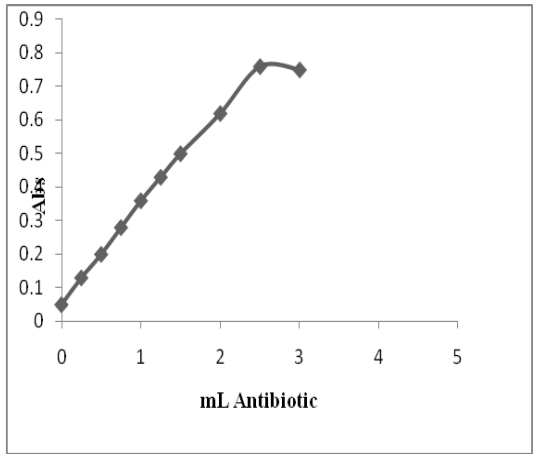


Figure.6. Calibration curve for Tobramycin