Determination of Teicoplanin via Spectrophotometric and High Performance Liquid Chromatographic Methods

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Abstract

Two methods have been suggested for the quantitative estimation of Teicoplanin (TCP) as pure and in its dosage forms. The first: spectrophotometric method included reaction of TCP with Fe (III) in acidic medium and the liberated Fe (II) coupled with potassium ferricyanide, the reaction needs heating for 30 minutes at 40o C to form Prussian blue complex. The maximum absorption of resulting product was measured at 767 nm with a molar absorptivity of 6.39 x 105 l.mol-1.cm-1.Linearity according to Beer's law is in the concentration range from 0.4 to 7.5µg . Ml-1. The relative standard deviation% (RSD %) of the method was better than 2.9 % .The LOD and LOQ values have been calculated and equal to 0.1541 and 0.5136 µg.ml-1 respectively. The second method was reverse phase high performance liquid chromatographic (RP-HPLC) using C18 (150 mm length x 4.6 mm ID, 5 µm) column with 20 µl injection volume. The mobile phase was consisting of methanol: Acetonitrile in ratio (90:10 V /V), and the flow rate was maintained at 2.5 ml.min-1.TCP was monitored using Agilent 1200 series equipped with photo diode array detector. Different analytical parameters were determined according to International Conferences on Harmonization (ICH).Linearity was observed in concentration range from 4 to 200. The suggested methods are simple and sensitive and the RP-HPLC can be used as a quality-control tool for routine quantitative analysis of TCP in pharmaceutical dosage form.

Keywords: Teicoplanin • Spectrophotometry • Prussian blue • RP-HPLC • Pharmaceutical dosage

Introduction

TCP is considering as an important antibiotic used in the prophylaxis and also treatment of serious infection which is the gram-positive bacteria is the responsible. A topical research showed that in 2005 an approximation of 18,650 in hospital deaths according to methicillin resistant Staphylococcus aureus toxicities in the United States [1]. Teicoplanin is a glycopeptide antibiotic and it has a good activity towered MRSA [2].Teicoplanin is not official yet neither in the United States Pharmacopeia (USP 35) nor B.P. 2009.

Various methods have been used in assay of TCP these methods included: HPLC [3-7] , high-performance liquid chromatography with electrochemical detection[8], LC/MS[9], SPE and micellar electrokinetic chromatography [10], LC, MEKC, and CEC[11] and fluorescence polarization immunoassay[12,13]. Through the literary survey and the proven methods above, we did not find many different methods for estimating TCP, specifically spectrophotometric methods, as there is only one spectrophotometric method using the diazo-coupling reaction of TCP with diazotized p-nitroaniline[14]. The main objective of the current work is to suggest two methods, one in spectroscopy and the other in HPLC, to estimate the TCP in its pharmaceutical product.

Experimental

Apparatus

Agilent UV- DAD 8453 spectrophotometer with1 cm bath quartz cells were used for all measurements of absorbance and for constructing absorption spectra and, also1200 Agilent HPLC apparatus was used.

Reagents and solutions

All chemicals used in the present study are of analytical grade reagents and all solvents used of HPLC grade.

Ferric solution (0.03M) was prepared by dissolving 0.8109 g of ferric chloride hexahydrate in 0.5 ml of concentrated hydrochloric acid then the volume of 100 ml was completed with D.W. in a volumetric flask.

Teicoplanin(50 µg.ml-1) potassium ferricynide (0.01M) and 2% ethylendiaminetetracetic acid(EDTA) were prepared by dissolving 0.0050 g , 0.3292 g and 2 g ethylenediamine-tetraacetic acid disodium salt dehydrate - disodium in 100 ml distilled water in a volumetric flasks respectively .Also acetic acid solution(1M) was prepared by diluting 5.7 ml of concentrated acetic acid to 100 ml with distilled water.

Pharmaceutical preparation solution ,50 µg.ml-1

The contents of two injection vials (Targocid) were mixed; An accurately weighted amount of powder equivalent to 0.0050 g of TCP was dissolved in 100 ml distilled water in a volumetric flask. A suitable aliquot of solution was taken and the recommended procedures for spectrophotometric and HPLC were followed to analyse the drug.

Spectrophotometric method

Recommended procedure and calibration graph

An aliquot (4 - 75) µg of TCP was transferred to serious 10-ml volumetric flasks .To each flask 2.5 ml of FeCl3(0.03M),0.25 ml of acetic acid(1M),and finally 3.5 ml of potassium ferricynide (0.01M) were added .The solution was stand for 30 minutes in water bath at 40 oC to allow the oxidation –reduction reaction to go to completed ,before dilution to mark with distilled water 2 ml of 2% ethylenediaminetetraacetic acid was added. After 5 minutes standing the absorbance of the coloured PB at 767 nm was measured .The calibration graph in Fig. 1 was linear from 4 to75 µg.10 ml-1(0.4 -7.5 µg.ml-1) ,the value of molar absorptivity was 6.39 x 105 l. mol. -1.cm-1.

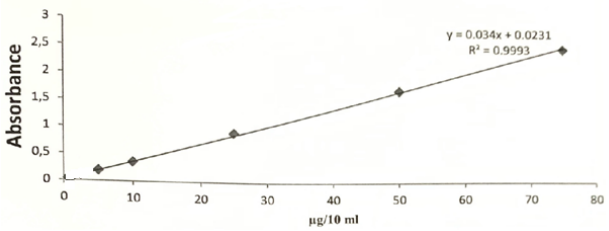

Figure 1: Calibration graph for the suggested spectrophotometric method

The LOD and LOQ have been calculated using equations in literature [15].Table 1 contained the main analytical parameters of present method.

Parameter	Optimum
Max. wavelength, nm	767
Linearity (µg mL-1)	0.4 -7.5
Sensitivity(ε, L mol-1 cm-1)	6.39 x 105
Sensitivity (Sandell's, µg cm-2)	0.00298
Regression equation	Y = 0.034 x [TCP µg.10 ml-1] + 0.0231
The slope	0.034
The intercept	0.0231
Determination coefficient (R2)	0.9993
RSD %	Not more than 2.9 %
LOD (µg mL-1)	0.1541
LOQ (µg mL-1)	0.5136

Table 1: The main analytical parameters.

All parameters affected the intensity of PB complex have been studied and the optimum result of each parameter has been recommended in the next experiment. Table 2 contain the optimum results.

Variable (Parameter	Optimum
Type of acid ,amount	Acetic acid,0.25 ml
Amount of FeCl3,M	2.5 ,0.03 M
Amount of potassium ferricyanide, M.	3.5 ,0.01 M
Temperature ,time of standing	40 OC,30 minutes
Amount of EDTA,%	2 ml, 2%

Table 2: The optimum conditions of suggested method.

The results presented in the Table 3 show that adding 2 ml of 2% EDTA is very important for improving stability.

	Absorbance of µg TCP in 10 ml / minutes with and without EDTA			
Time, mins.	12.5		25	
	Without EDTA	With	Without EDTA	With
Immediately	0.4115	0.4615	0.7295	0.9295
10	0.4152	0.4652	0.7309	0.9309
20	0.4072	0.4672	0.7368	0.9368
30	0.2911	0.4679	0.6701	0.9357
40	0.0672	0.468	0.0804	0.9339
50	……	0.4668	…….	0.9309
60	……	0.4682	……	0.9284

Table 3: The stability without and in presence of EDTA.

The absorption spectrum

Figure 2 shows a maximum absorption at 767 nm of PB complex result from applying the optimum condition of suggested method mentioned above(Table 2), and this wavelength(767 nm) is recommended in the subsequence experiments.

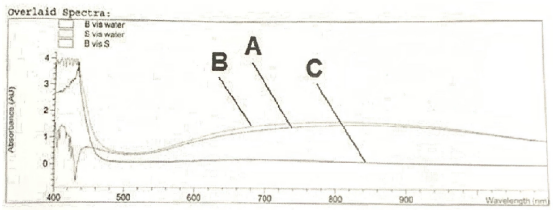

Figure 2: Absorption spectrum of 50 µg TC treated according to the suggested procedure.(A) the PB complex against blank, (B) PB complex against D.W. and (C) blank against D.W.

Application of the method

The present method was succefully applied in determination of TCP in its dosage(vial for injection).The performance of the present method was evaluated by calculation of the t-test compared with literature method(14) for 95% confidence with eight degree of fredom.The results in Table 4 indicated that there is no signified different in appling the two methods.

Pharmaceutical formulation	Recovery % proposed method	RSD%	Recovery % Literature method(14)	RSD%	t-value
Targocid Sanofia - Italian	98.94	2.9	99.12	2.7	0.4191

Table 4: The results of application part.

The comparison of the method

Table 5 contains the comparison of the various analytical parameters of the proposed method with another literature method.

Analytical parameter	Proposed method	Literature method(14)
Type of reaction	Oxidation-reduction	Diazo-coupling
Maximum wavelength, nm	767	490
Temp ,heating time(mins.)	40 oC, 30	RT
The medium(pH)	1.8	11.02
Molar absorpitivity,l.mol.-1 cm.-1	6.39 x105	2.8 x10 4
Linearity, µg. ml-1	0.4- 7.5	Feb-80
RSD %	2.9	2.7
Application	Pharmaceutical formulation (vial for injection)	Pharmaceutical formulation (vial for injection)

Table 5: The results of comparison

The results in Table 5 indicated that the proposed method is more sensitive ,but there is no need heating and waiting time for literature method.

High performance liquid chromatography method

In this method 20 µl of TCP solution injected by micro-syringe.

Linearity of the method

The linearity was analysed through the standard curve ranging from 4 to 200 µg. ml-1.By diluting appropriate amount of TCP stock solution(200 µg. ml-1) with distilled water and prepared in triplicate. The calibration curve was prepared in the same day with the following concentration(4,8.20,50,80,100 and 200 µg. ml-1).the determination coefficient equal to 0.9996 (Fig. 3).

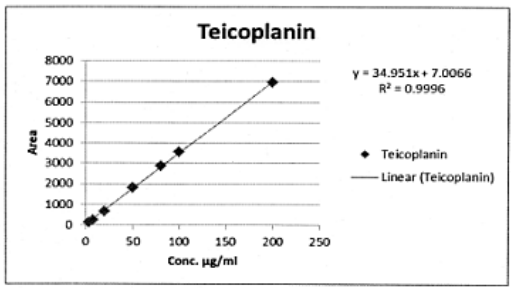

Figure 3: The linearity of RP-HPLC method.

Results and discussion

All parameters affected the shape of peak and sensitivity according to area under the peak has been studied respectively and the optimum condition fixed in the next experiment.

Selection of wave length of determination

Figure 4 and Table 6 indicated that the mixture of acetonitrile: methanol gives the maximum absorbance at 202 nm based in measuring 40 µg. ml-1 of TCP.UV spectra at 202 nm was chosen for their detection, in order to improve selectivity and sensitivity and according to high energy of this region and to avoid any cut-off of mobile phase of this wavelength it will be study chromatogram on both wavelengths 202 and 220 nm.

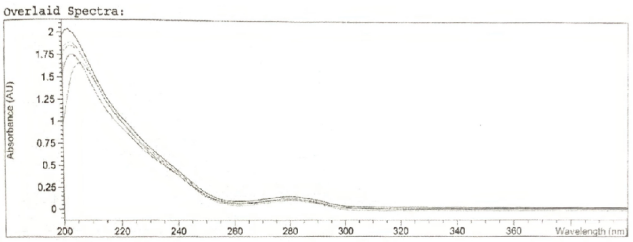

Figure 4: The selection of wavelength of determination.

Composition of mobile phase	Maximum wavelength	Absorbance
ACN:MeOH (1:1)	202	2.039
CAN:EtOH (1:1)	206	1.674
ACN:MeOH:Buffer(2:2:1)	202	1.883
ACN: EtOH: Buffer (2:2:1)	202	1.853

Table 6: The selection of wavelength

Column selection

It was made different runs with different columns. It was found from the results obtained, that C18 give best result comparing to L 57 and C8 (Fig. 5).

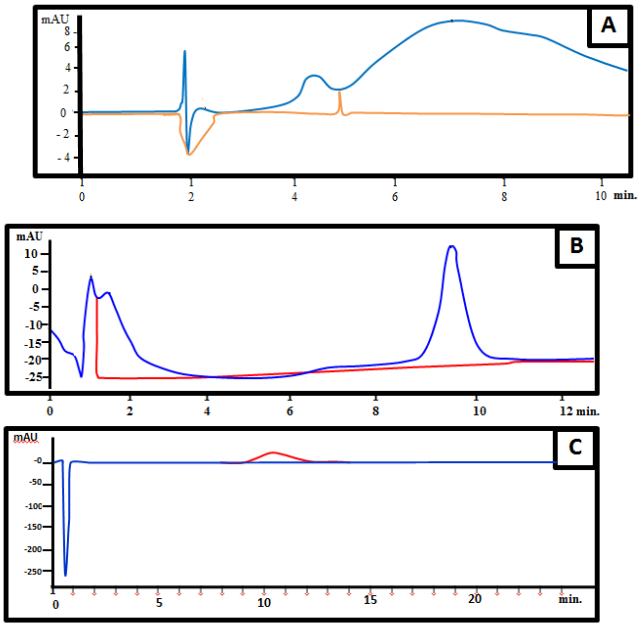

Figure 5: Column selection: (A) using L 57,(B) using C8 and (C) using C18

Selection of mobile phase matrix

After selecting the column and the appropriate wavelength, it was made different runs to figure out the most suitable mobile phase matrix. It was found that the mixture of methanol: acetonitrile (90:10) gives the best results (Fig. 6)

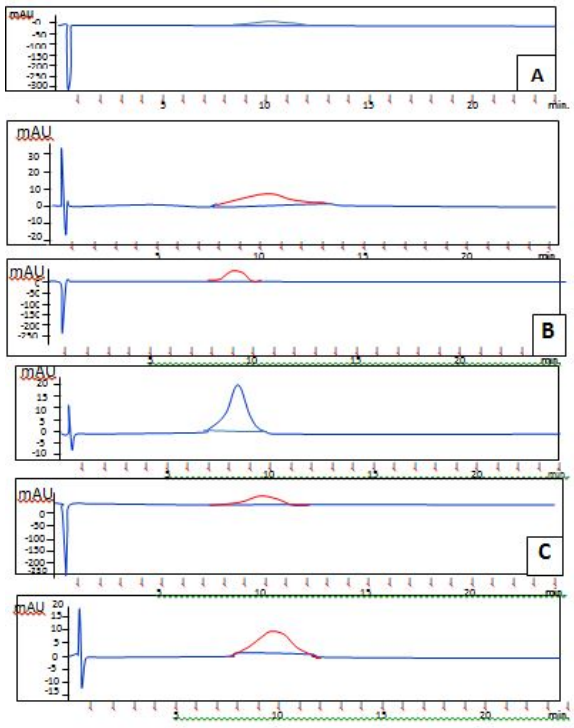

Figure 6: The selection of optimum mobile phase matrix.

(A) 40:60 MeOH:ACN,(B) 90:10 MeOH: ACN and (C) 60:40 MeOH:ACN

Flow rate selection

The effect of flow rate was investigated and its found that 2.5 ml/ minute is the most appropriate flow rate according to the shape of peak(well-shaped symmetrical peak).

Effect of temperature

The injection of TCP into column at different temperature (20 -50 0C) and the effect on area of the peak has been studied .The results indicated that 25 0C was the most suitable temperature to give almost the highest area of peak.

Precision

To ensure assay precision with day (5 injections were performed) and between 5 days(5 injections were randomly), precision was assessed at sample concentration 100 µg.ml-1. RSD% values in Table 7 indicated that the current method is repeatable.

Injection date	Average of area	RSD%
Injection in one day	3932.24	0.17
Injection in five days	3921.47	0.55

Table 7: The precision of the method.

Application of the method

Table 8 shows that the method can be applied in assay TCP in its pharmaceutical formulation TCP injection with satisfactory results.

Pharmaceutical preparation	Amount taken(µg)	Recovery%
TCP injection	50	99.17
(targocid)	100	99.52

Table 8: The results of application of the method.

Conclusions

The suggested spectrophotometric and HPLC methods are simple, precise and accurate in assay TCP as pure and in pharmaceutical formulation (injection).