

Three Simple Spectrophotometric Methods Using for Estimation of Vancomycin in Pure and Vial Injection

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Articles Information

Received:
27, 11, 2019
Accepted:
04, 02, 2020
Published:
01, March, 2020

Keywords:

Vancomycin
Zero-order
Area under curve
First derivative method
Spectrophotometric

Abstract

Three rapid, accurate and ecological friendly spectrophotometric methods are used for determination of Vancomycin at wavelength 200 nm for the zero-order method, 195-205 nm for the area under the curve (AUC) method and 200 nm for the first derivative method(D1). A calibration graph shows that Beer's law is obeyed over the concentration range between 4-16 mg.L⁻¹ for all three methods with a correlation coefficient of 0.994, 0.998 and 0.997 for zero-order, AUC and D1 at valley 200 nm, respectively. Average of Rec.% were found to be of 100.870, 101.425 and 99.515 for zero-order, AUC and D1 at valley 200 nm, respectively. Statistical analysis of data reveals that all three methods are precise, accurate and suitable for the application Vancomycin in pure and pharmaceutical preparations.

DOI: 10.22401/ANJS.23.1.05

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1. Introduction

Vancomycin Figure1 is a glycopeptides antimicrobial material or blend of glycopeptides produced by the growth of certain strains of amycolatopsis orientalis "Strptomyces Orientalies and Nocardia Orientalis"[1]. It is used to treating bacterial infections in patients allergic to β - lactam antibiotics[2]. There are many procedure for the estimation of Vancomycin in biological fluids and in pharmaceutical forms, include flow injection analysis [3,4], voltametric method[5], HPLC[6,7,8], capillary electrophoresis[9] and spectrophotometric method[10]. The aim of this research is to improved rapid, simple, accurate, economic and also requires less time for analysis and minimum solvent [11]. In this work, The zero-order, area under curve (AUC) and the first derivative (D1) spectrophotometric method were applied for assessment of Vancomycin in pure and vial injection forms.

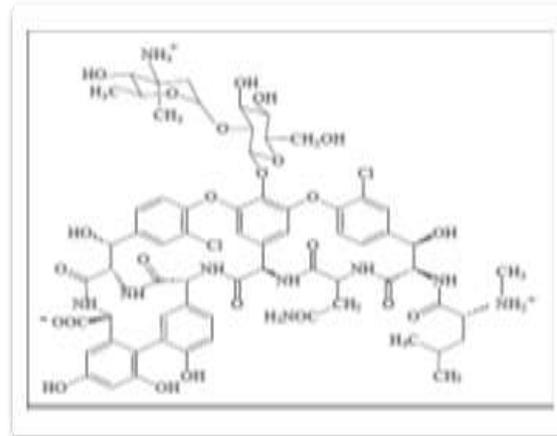


Figure1. Chemical structure of Vancomycin[12].

2. Materials and methods

UV-Visible spectrophotometer SHIMADZU (Japan) with computer program for recording and analysis of data, using 1 cm quartz cells.

Reagent and standard solution

Vancomycin powder pure (99.99%) was provided as a gift from the state company for the drug manufacture Samara-Iraq (SDI), Vancomycin pure stock standard solution (100 mg. L⁻¹) (C₆₆H₇₅Cl₂N₉O₂₄ f.w=1449.3 gm. mol⁻¹), was prepared by dissolving 0.01 gm of Vancomycin drug in small amount of distilled water and then

diluted to 100 ml with distilled water, more diluted solutions were prepared by using distilled water from Vancomycin pure stock solution.

Assay of pharmaceutical preparations

Three Vancomycin vial injection were take (label claim 0.5 gm/vial), gulf pharmaceutical manufacture, Ras al Khaimah, U.A.E, equivalent to 0.01 gm of Vancomycin drug was diluted with small amount of distilled water then transferred to 100 ml volumetric flask and completed to the market to get 100 mg. L⁻¹ of Vancomycin drug.

Procedure

I. The Zero-order method[13]

The standard Vancomycin solutions 4-16 mg.L⁻¹ were scanned between "190-360 nm" in UV-Visible spectrophotometer, and the peak was given maximum absorbance at "200 nm".

II. AUC method[14]

Standard solutions of Vancomycin 4-16 mg.L⁻¹ were scanned between "190-352 nm" in UV-Visible spectrophotometer, and AUC was measured at wavelength ranges of "195-205 nm".

III. D1 method[15]

4-16 mg.L⁻¹ standard solution of Vancomycin were scanned in the first derivative spectra at scan rate speed 600 nm/min, scan range "190-360 nm" and slit width 2.00 nm, using " $\Delta\lambda=2\text{nm}$ " and scaling factor=20. The absorbance was measured at "200 nm valley" and was plotted against concentration to get calibration curve^[16].

3. Results and discussion

i. The Zero-order method

The calibration curve for Vancomycin standard solution in the concentration range 4-16 mg.L⁻¹ was measured, and Figure2 has shown the absorbance of Vancomycin was measured at a wavelength of "200 nm". The absorbance was plotted versus concentration Figure 3 and statistical parameters are shown in Table (1).

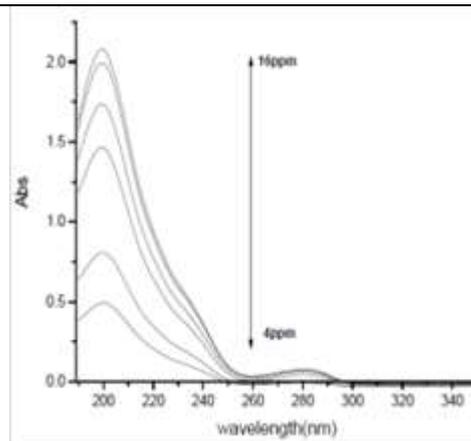


Figure2. Zero-order of Vancomycin(4-16 mg.l⁻¹)

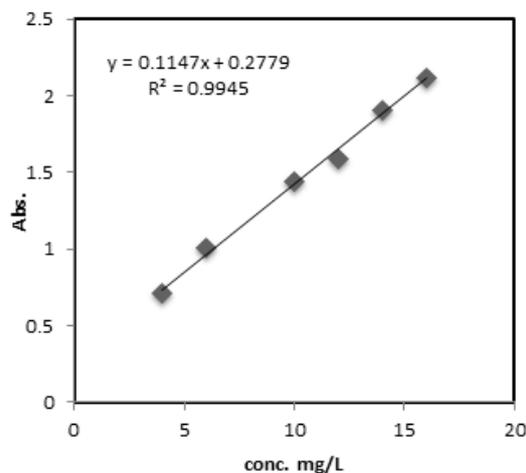


Figure3. Calibration curve of zero-order spectra for Vancomycin 4-16 mg.L⁻¹ at peak"200 nm".

ii. AUC method:

This method depends on the calculation integrated value of absorbance and two selected wavelength λ_1 and λ_2 ^[17]. AUC method based on the calculation of the area covered the curve and horizontal axis, the horizontal axis refer to baseline^[18]

$$AUC = \int_{\lambda_2}^{\lambda_1} Ad\lambda = \int_{205}^{195} Ad\lambda$$

$\lambda_1(195)$ and $\lambda_2(205)$ = the wavelength refers to the start and end points of curve reign.

A is absorbance of standard solutions of Vancomycin. Figure 4 shows the area under the curve spectra for Vancomycin standard solutions at (4, 6, 10, 12, 14 and 16 mg. L⁻¹), a calibration curve for this method plotted between AUC against concentration as shown in Figure 5, and statistical parameters recorded in Table(1).

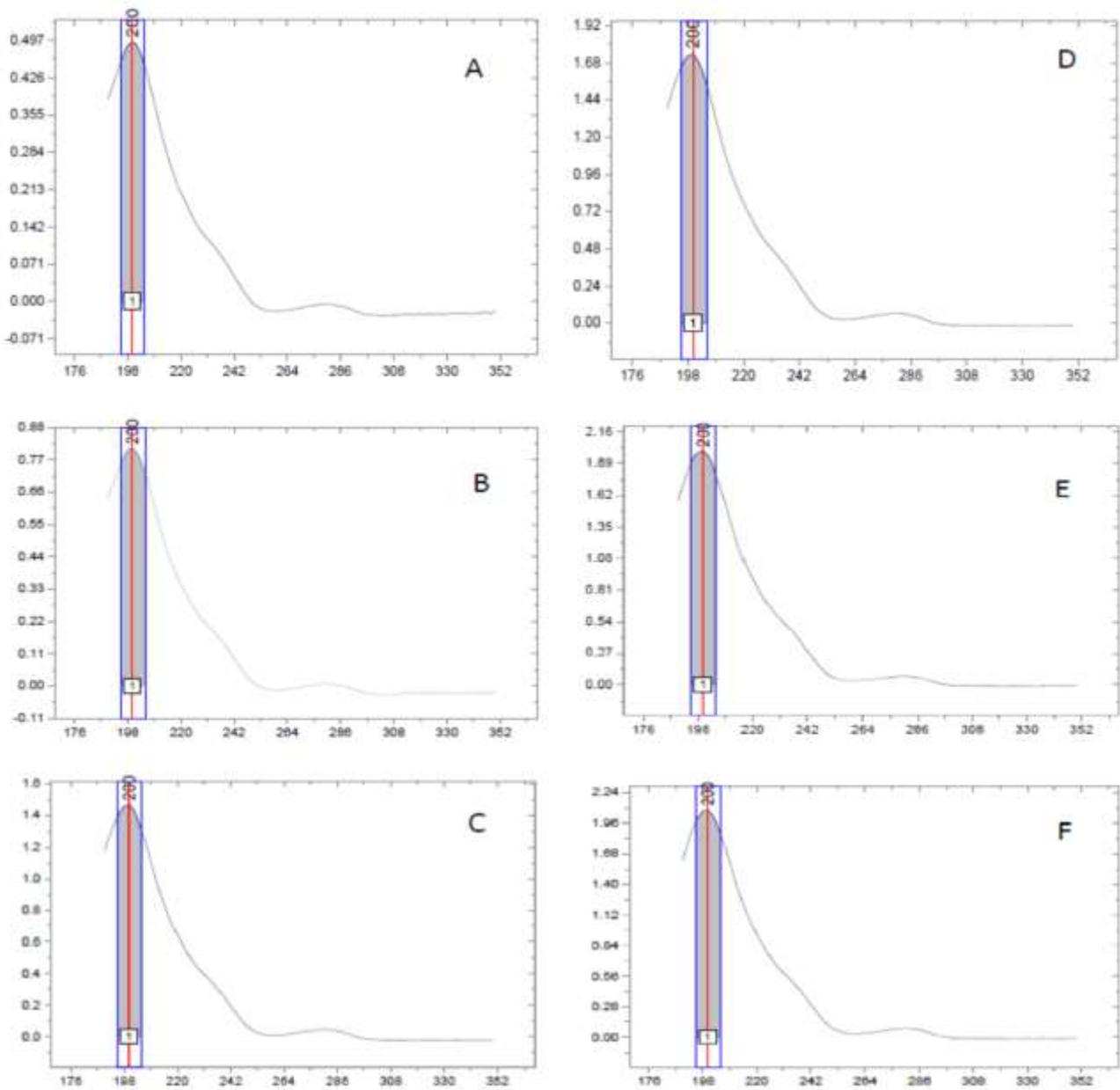


Figure 4. AUC for Vancomycin drug, (A=4mg.L⁻¹), (B=6 mg.L⁻¹), (C=10 mg.L⁻¹), (D=12 mg.L⁻¹), (E=14 mg.L⁻¹) and (F=16 mg.L⁻¹).

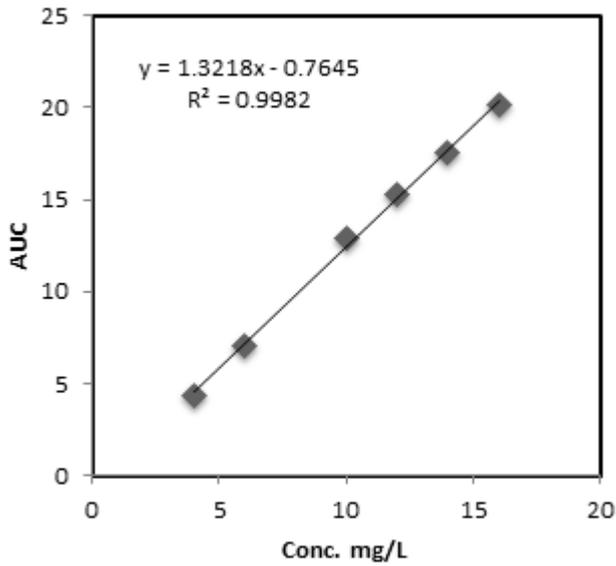


Figure 5. Calibration curve of AUC method for Vancomycin 4-16 mg.L⁻¹ at wavelength range "195-205 nm".

iii. D1 method

Figure 6 shows the first derivative spectra for Vancomycin standard solutions(4-16 mg.L⁻¹), this study selects one valley at "200 nm" for determination Vancomycin, calibration curve and statistical parameters given in Figure 7 and Table 1, respectively.

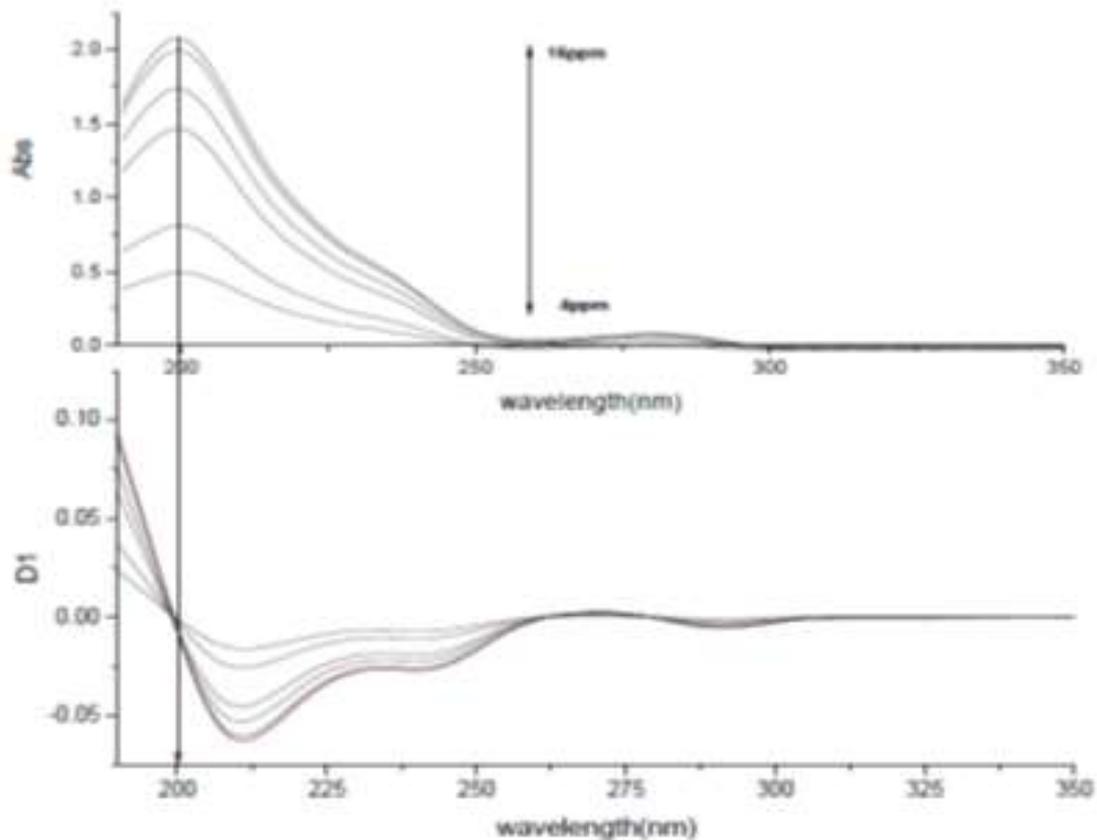


Figure 6. First derivative spectra of Vancomycin(4-16 mg.l⁻¹)

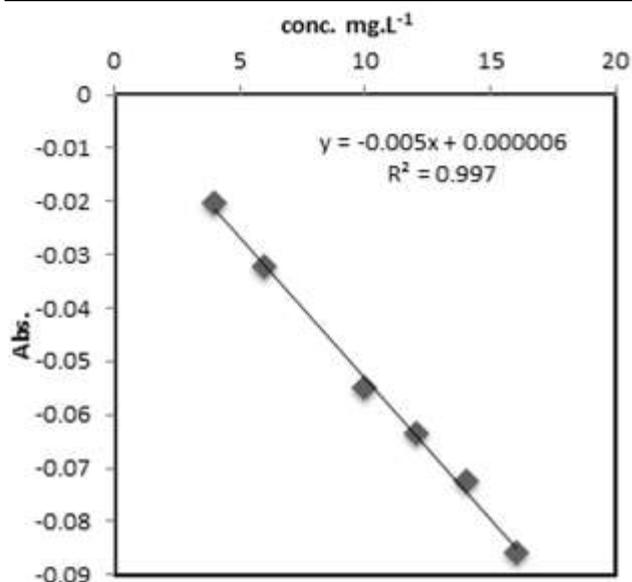


Figure 7. Calibration curve of D1 spectra for Vancomycin (4-16 mg.L⁻¹) at valley "200 nm".

Table 1. Statistical parameters obtain from the calibration curve for Vancomycin

Parameters	Zero-order	AUC	D1 at valley "200 nm"
Wavelength nm	200	195-205	210.6
R ²	0.994	0.998	0.997
Linearity range (mg.L ⁻¹)	4-16	4-16	4-16
Equation for linearity	Y=0.114x+0.277	Y=1.321x-0.764	Y=0.005x+6×10 ⁻⁵
Slope, b	0.114	1.321	0.005
Intercept, a	0.277	-0.764	6×10 ⁻⁵
(S _a) Standard deviation of intercept	0.047	0.312	0.002
(ε) Molar absorptivity (L. mol ⁻¹ . cm ⁻¹)	4.8 ×10 ⁺⁴	-	-
Sandell's sensitivity (mg. cm ⁻¹)	0.0301	-	-
LOD (mg.L ⁻¹)	1.360	0.779	1.392

of the points of the calibration curves and height accuracy.

"LOD = limit of detection = 3.3×SD/S, SD is the standard deviation of the intercept, ε = Molar absorptivity and Sandell's sensitivity = M.wt/ ε". The small values of the slope, intercept, standard deviation the intercept (S_a) refer to the height precision of the three proposed methods (the zero order, AUC and D1 methods) and weak scattering

iv. Accuracy and precision

The accuracy and precision is tested by analyzing five replicate of Vancomycin using the Zero-order, AUC and D1 spectrophotometric method for two different concentrations. Table 2 has recorded the relative error RE% and recovery %.

Table 2. Accuracy and precision for the methods.

Method	Amount of Vancomycin (mg. L ⁻¹)		RE%	Rec.%	Average of Rec.%
	Taken	found			
Zero-order	5	4.962	-0.760	99.24	100.870
	8	8.200	+2.500	102.50	
AUC	5	5.092	+1.840	101.84	101.425
	8	8.081	+1.012	101.01	
D1 at valley "200 nm"	5	4.893	-2.140	97.86	99.515
	8	8.094	+1.175	101.17	

"*Average of five determination, RE*% = $\frac{x-x^o}{x^o} \times 100$, Rec*% = E%+100, x= measured value (found), x^o= true value (taken)"

The low values of relative error RE% show a good recoveries of the zero order, AUC and the first derivative methods are 100.870%, 101.425%, and 99.515% for D1 at valley at "200 nm" which show a good reliability.

v. Application

One type of Vancomycin vial injection has been analyzed using the zero-order, AUC and D1 spectrophotometric method. There is no difference

accuracy and precision and the average of in wavelength of all spectra are observed between the spectra of Vancomycin standards Figures 2, 4 and 6 and Vancomycin vial injection Figure (8, 9 and 10). Two different concentrations of Vancomycin vial injection (8 and 11 mg. L⁻¹) are analyzed five time to obtain relative error and recovery as shown in Table 3.

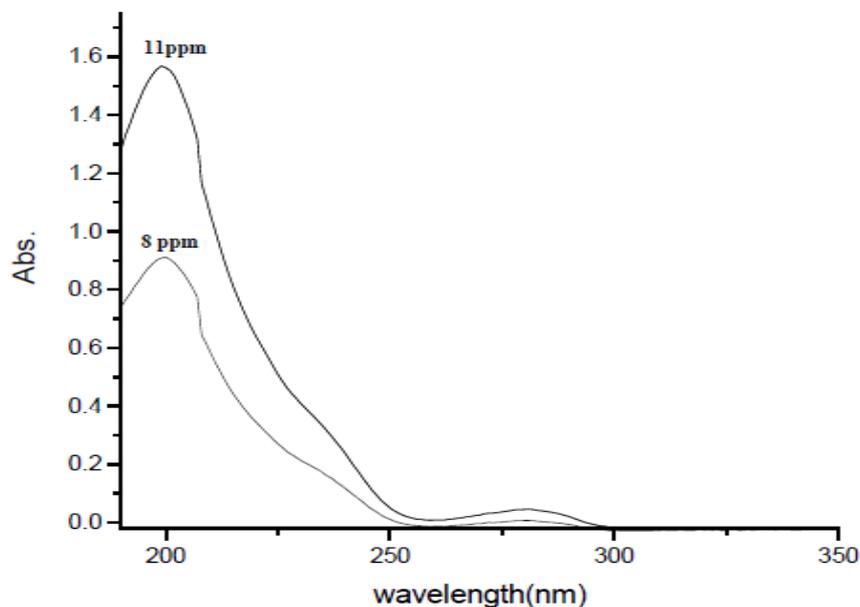


Figure 8. Zero-order Vancomycin vial injection at 8 and 11 mg.L⁻¹

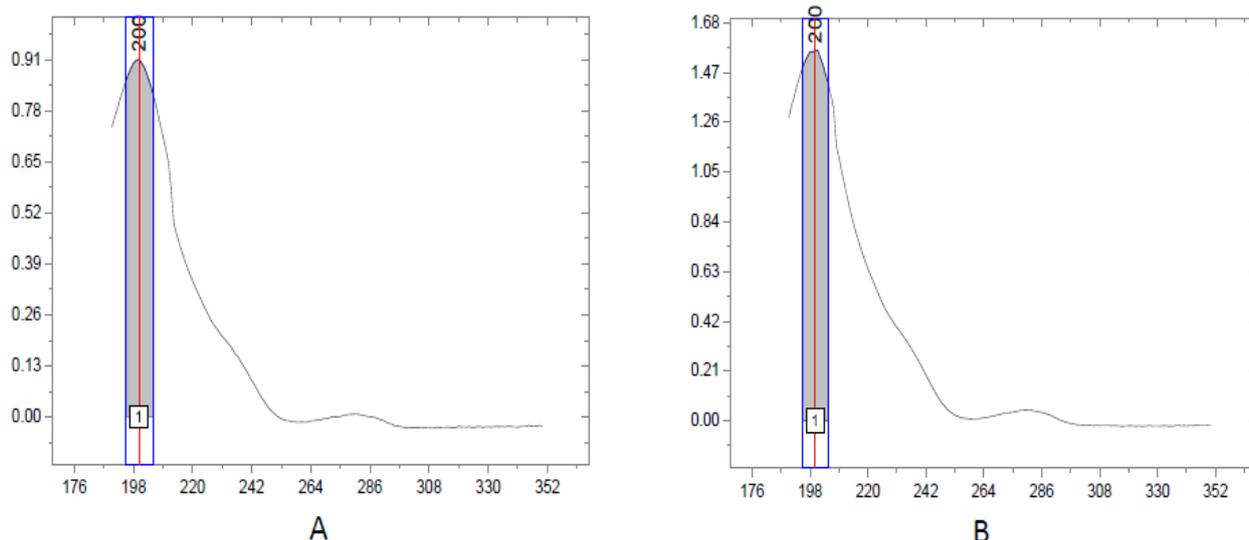


Figure 9. Area under curve of Vancomycin vial injection at (A= 8 mg.L⁻¹) and (B=11 mg.L⁻¹)

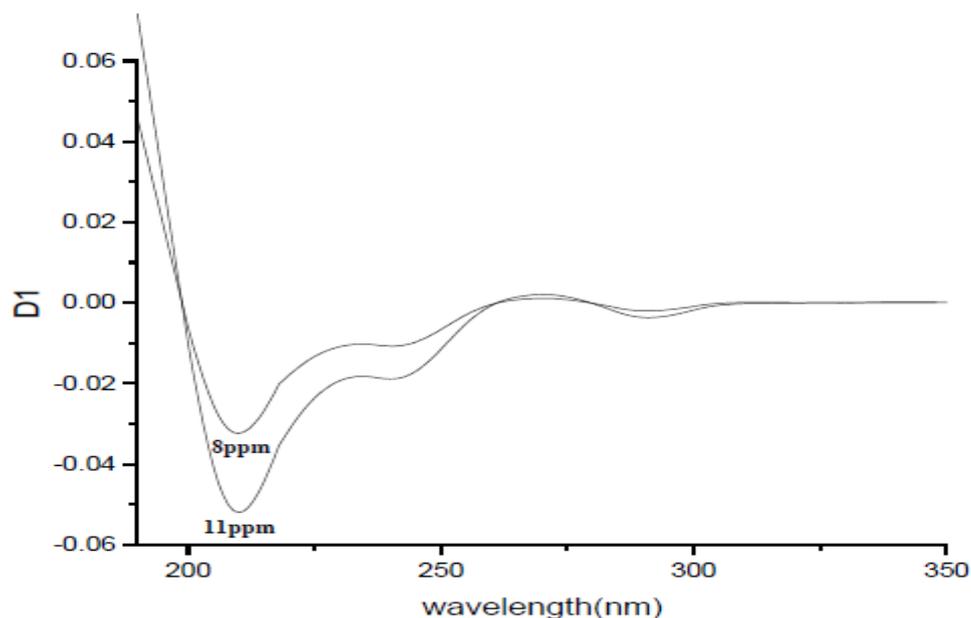


Figure 10. First derivative spectra of Vancomycin vial injection (8 and 11 mg.L⁻¹)

Table 3. The relative error and recovery for assessment of Vancomycin drug in vial injection

Pharmaceutical vial injection	Method	Conc. Of Vancomycin mg. L ⁻¹		RE*%	Rec.*%	Average of Rec.%
		taken	found			
Vancolon (Vancomycin) vial 0.5g.	Zero-order	8	7.841	-1.987	98.013	98.255
		11	10.836	-1.490	98.51	
	AUC	8	7.950	-0.625	99.375	99.585
		11	10.985	-0.136	99.864	
	D1 at valley 200 nm	8	7.910	-1.125	98.875	98.220
		11	10.377	-2.427	97.573	

"*Average of five determination"

Low values of relative error RE% have no significance difference in the average of recoveries and show a good efficiency and selectivity of the present methods (zero order, AUC and D1 methods) without any interferences effects.

4. Conclusions

This work was applied three methods for the simultaneous estimation of vancomycin in pure and vial injection, these methods are simplicity, cost-effectiveness and sensitivity. The percentage recoveries for the all three methods were within the acceptable limits, thus, these methods can be used in quality control laboratories to analyze Vancomycin.

Acknowledgements

The author thanks the University of Mustansiriyah, College of Science, Department of Chemistry for the helpfulness of doing the "UV-Visible" spectra.

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