Flow Injection- Spectrophotometric Determination of Vancomycin Hydrochloride in Pharmaceutical Preparations Using Diazotized Metoclopramide

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Abstract

A batch and flow injection analysis (FIA) spectrophotometric methods have been developed for the determination of vancomycin hydrochloride (VHC) in aqueous solution and in pharmaceutical preparations. The methods are based on the reaction of VHC with diazotized metoclopramide (DMCP) in alkaline medium. The water soluble yellowish-orange color product was measured at λmax 451 nm. Linearity was observed from 0.5 to 100 and 1 to 550 µg mL^{-1} of VHC with detection limits of 0.230 and 0.419 µg mL^{-1} by batch and FIA procedure respectively. The sampling rate was 128 injections per hour for flow injection methods. The effects of chemical and physical parameters have been carefully considered and the proposed procedures were successfully applied to the determination of VHC in pharmaceutical formulations.

Keywords: Spectrophotometric; Flow injection analysis; Vancomycin hydrochloride; Metoclopramide.

1. Introduction

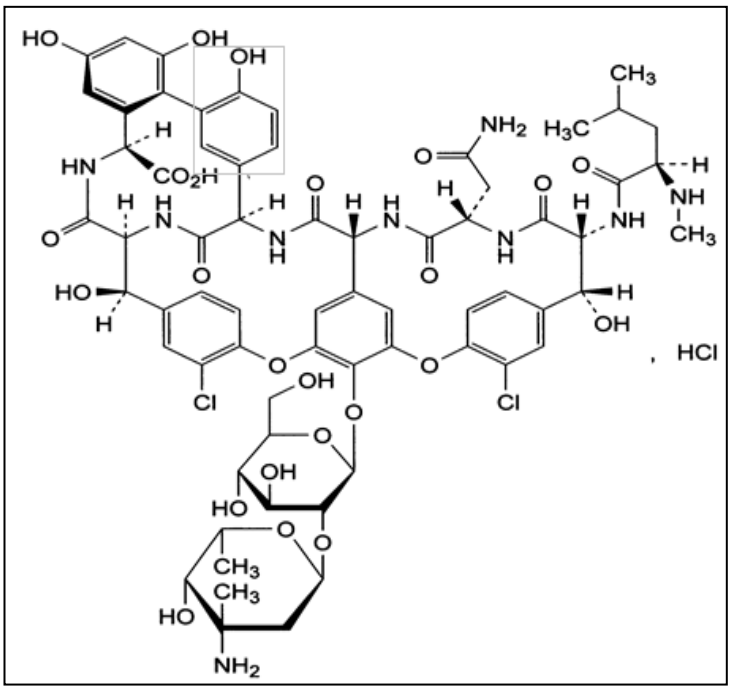
Vancomycin is a glycopeptide antimicrobial substance or mixture of glycopeptides produced by the growth of certain strains of Amycolatopsis orientalis (Nocardia orientalis, Strptomyces orientalis), or by any other means [1]. Vancomycin hydrochloride (VHC) consists principally of the monohydrochloride of (3S,6R,7R, 22R, 23S,26S,30aSa,36R,38aR)-3-(2-amino-2-oxoethyl) -44-[[2-O-(3-amino-, 3,6-trideoxy-3-O-methyl-α-L-*lyxo*- hexopyranosyl) - β - D - glucopyranosyl] oxy]-10,19-dichloro- 7,22, 28, 30,32 - pentahydroxy -6- [[(2R) -4- methyl -2- (methylamino) pentanoyl] amino]-2,5,24,38,39- pentaoxo-2,3,4,5,6,7,23,24,25, 26,36,37,38,38a-tetradecahydro- 22*H*-8, 11:18, 21-dietheno-23, 36(iminomethano)- 13, 16:31, 35– dimetheno-1*H*,13*H* -[1,6,9] oxadiaza-cyclohexadecino [4,5-*m*] [10,2,16] ben-zoxadiazacyclotetracosine-26-carboxylic acid [2]. Vancomycin was introduced in 1958 as an antibiotic active against Gram-positive cocci, particularly streptococci, staphylococci and pneumococci. It is not active against Gram-negativebacteria, Vancomycin hydrochloride is recommended for use when infections fail to respond to treatment with the more common antibiotics or when the infection is known to be caused by a resistant organism, it is particularly effective for the treatment of endocarditis caused by Gram-positive bacteria [3]. VHC is officially recognized in B.P [2] and U.S.P [4].

A survey of literature revealed that few methods based on visible spectrophotometry for VHC [5, 6, 7] have been reports. Other methods include HPLC [8,9,10,11], Polarography [12], Capillary electrophoresis [13], Radioimmunoa- ssay [14], Fluorescence polarization immunoassay [15] and Flow injection analysis [16, 17]. This paper describes the batch and flow injection methods for spectrophotometric determination of vancomycin hydrochloride (VHC) by the diazotization-coupling reaction between VHC and diazotized metoclopramide (DMCP) in the presence of sodium hydroxide. It has been satisfactorily applied for the determination of vancomycin hydrochloride in pure and injections preparations, the reaction can be carried out in batch and FIA and the two approaches were compared.

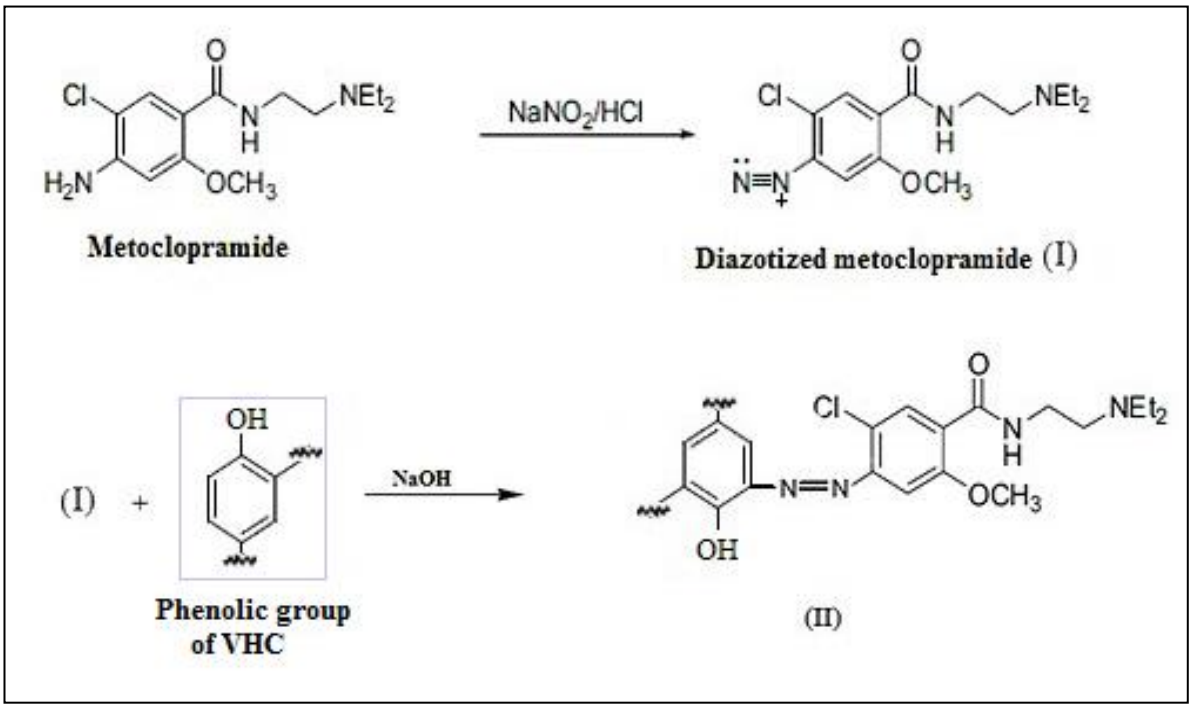
1.1. *Reaction mechanism of the method*

Vancomycin hydrochloride forms a yellowish-orange colored product (λ max at 451 nm with a molar absorption coefficient of 4.620×10^4 $L.mol^{-1}.cm^{-1}$) with DMCP in alkaline medium. The aromatic amino group

present in metoclopramide is diazotized with nitrous acid ($NaNO_2$/HCl) and the resultant diazonium salt (I) is coupled with VHC at room temperature to form yellowish-orange colored product (II) in alkaline medium (sodium hydroxide) according to scheme.1. The yellowish-orange dye product was only formed in alkaline medium since VHC is converted into its salt (phenoxide ion). The latter is more stable than phenol (resonance) leading to a more stable intermediate with DMCP [18].



Vancomycin hydrochloride (VHC).



Scheme (1) Proposed mechanism of the reaction between VHC and DMCP in alkaline medium.

2.1. *Apparatus*

All spectral and absorbance measurements were carried out using a digital double beam spectrophotometer (shimadzu, UV-vis 260). A silica cells were used for the absorbance measurements of the batch procedure. A flow cell 50 µL internal volume and 1 cm bath length was used for the absorbance measurements of FIA. A peristaltic pump (Ismatec, Laborechnik Analytik, CH8152, Zurich, Switzerland) was used to transport the solution.

In addition, an injection valve (Rheodyne, Altex 210, Supelco, USA) was employed to provide appropriate injection volumes of standard solutions and samples while a flexible vinyl tubing (0.5 mm internal diameter) was used for the peristaltic pump. The reaction coil (RC) was of Teflon material with an internal diameter of 0.5 mm. The solutions were propelled by peristaltic pump with initial total flow rate of 1.5mL.min^{-1} in the flow injection method, and the absorbance was measured at 451nm.

2.2. *Reagent and materials*

All the chemicals used were of analytical grade and all the solutions were prepared with distilled water, freshly prepared solutions were always used.

Standard vancomycin hydrochloride VHC solution: stock solution (1000 µg.mL^{-1}) was prepared daily by dissolving 0.1 g of the pure compound (Molecular weight of VHC is 1485.71 g.mol^{-1}) in 100 mL of distilled water and serial dilutions with distilled water were made.

Sample vancomycin hydrochloride VHC solution: the contents of five vials (three commercial sources) were mixed. An aliquot corresponding to 0.1 g of VHC was diluted to 100 ml with distilled water in a volumetric flask to obtain 1000 µg.mL^{-1} of VHC. More dilute solutions of pharmaceutical preparations for batch and FIA procedures were made up by simple dilution with distilled water.

Diazotized metoclopramide (DMCP) (Industries and Medical Appliance, SDI, Samara, Iraq): a solution of 2 mM was prepared daily by dissolving 0.0708 g of metoclopramide hydrochloride (Molecular weight of metoclopramide hydrochloride is 354.3 g.mol^{-1}) [2] in distilled water.Then, a volume of 1 mL of 1M hydrochloric acid (HCl (BDH)) was displaced in a 100 mL volumetric flask. The mixture was then cooled to a bout 0 – 5 ºC using an ice bath for 5 min. This was followed by adding 0.0138 g of sodium nitrite (Molecular weight of sodium nitrite is 69 g.mol^{-1}, Merck) to the mixture with continuous stirring. After 5 min, the volume was made up to the 100 mL mark with distilled water and several dilutions were prepared with distilled water.

Sodium hydroxide NaOH (Merck) solution: stock solution of 1 M was prepared by dissolving 10 g of NaOH in 250 mL distilled water, and working solutions were prepared by appropriate dilution of the stock solution.

2.3. *Procedure*

2.3.1. *General batch procedure*

Aliquots of standard VHC solution containing 12.5-2500 µg of VHC was transferred into a series of 25 mL standard flasks. A volume of 2 mL of 2 mM of DMCP and 3 mL of 50 mM NaOH solution were added. The contents of the flasks were diluted to the mark with distilled water, mixed well and left for 15 min. The absorbance was measured at 451 nm (at room temperature 25°C) against reagent blank containing all materials except VHC. A calibration graph was drawn and the regression equation was calculated. For the optimization of conditions and in all subsequent experiments, a solution of 500 µg was used in a final volume of 25 mL (20 µg.mL^{-1}).

2.3.2. *General FIA procedure*

Working solutions of VHC in a range of 1–550 µg.mL^{-1} (6.730×10^{-4}–0.370 mM) were prepared from the stock solutions (1000 µg.mL^{-1}). A 200 µL portion of VHC was injected into the stream of 1 mM DMCP and was then combined with a stream of 10 mM NaOH solution with a total flow rate of 2.5 mL.min^{-1} and reaction coil length of 100 cm Fig.(1). The resulting absorbance of the produced was measured at 451 nm. Moreover, optimization of conditions was carried out using 50 µg.mL^{-1} of VHC.

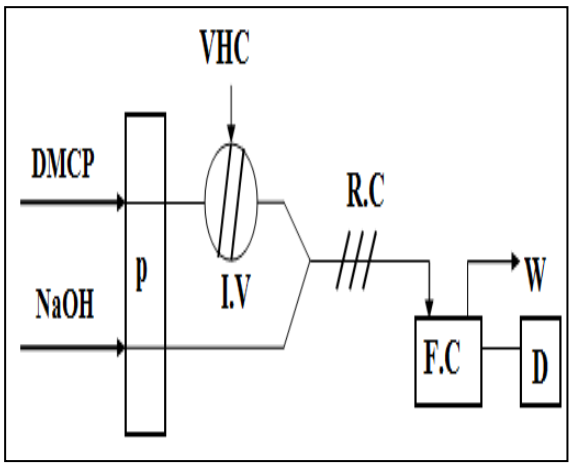


Fig.(1) Schematic diagram of flow injection-Spectrophotometric analysis P, Peristaltic pump; I.V, Injection valve; R.C, Reaction coil; F.C, Flow cell; D, Detector (vis-spectrophotometric); W, Waste.

3. Results and Discussion

3.1. *Absorption spectra*

VHC forms a yellowish-orang colored product (λ_{max} of 451 nm with a molar absorption coefficient of 4.620 × 10^4 L.mol^{-1}. cm^{-1}) with DMCP in alkaline medium. The absorption spectra of the colored product are given in Fig.(2).

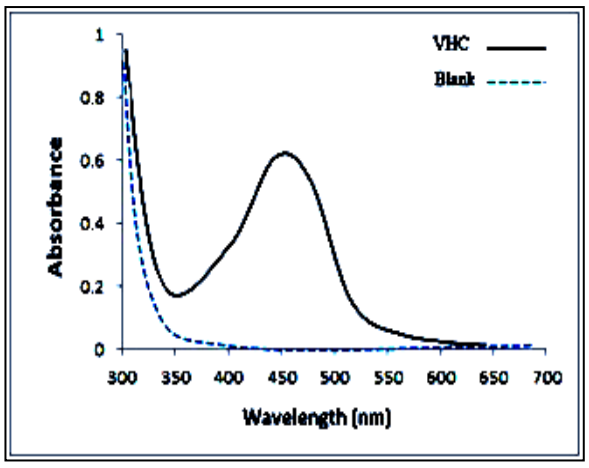


Fig.(2) Absorption spectra of (20 µg.mL^{-1}) VHC treated as described under procedure (1 mL of HCl (1 M), 2 mL of DMCP (2 mM) and 3 mL of NaOH (50 mM) in final volume 25 mL) and measured against reagent blank and the reagent blank measured against distilled water.

The stoichiometry of the reaction between each VHC and DMCP was investigated under the recommended optimum conditions by Job's method [19] according to the following procedure: into a series of 25 mL volumetric flasks, a decreasing volumes (10 to 0.0 mL) of 3.365×10^{-4} M of DMCP were added, followed by adding increasing volumes (0.0 to 10 mL) of VHC of same concentration, and 3 mL of 50 mM NaOH solution. The solutions were diluted to the mark with distilled water, allowed to stand for 15 min, and the absorbance was measured versus reagent blank at 451 nm. The plot Fig.(3) reached a maximum value at a mole fraction of 0.5 indicating that the reaction proceeds with mole ratio 1:1 for DNPH: VHC.

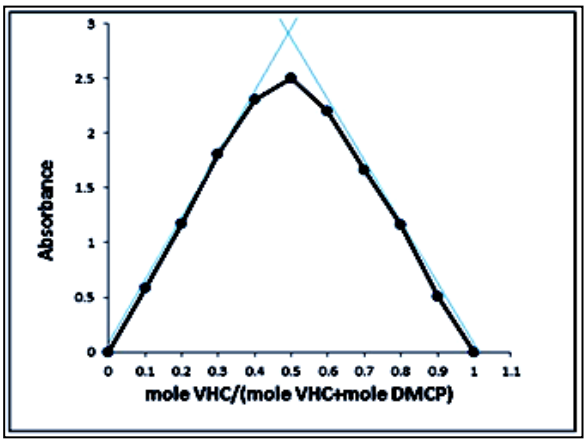


Fig.(3) The mole ratio of the reaction between VHC and DMCP.

3.2. *Batch spectro photometric determination*

The parameters affecting mainly the sensitivity and stability of the colored product were studied and optimized. Optimum conditions were established by changing one-factor-at-a-time (OFAT) and keeping the others fixed by observing the effect produced on the absorbance of the colored species. The yellowish-orange colored product which was formed between VHC and DMCP had developed only in alkaline medium; therefore, the effects of different alkaline solutions were studied such as sodium acetate, sodium carbonate, ammonium hydroxide and sodium hydroxide. The maximum sensitivity and stability were obtained only when the reaction was carried out in the presence of sodium hydroxide solution. The best experimental conditions for the determination of VHC were established for HCl 1 M (from 0.3 to 6 mL) which was used in diazotization of reagent, DMCP 2 mM (from 0.3 to 6 mL) and sodium hydroxide 50 mM (from 0.3 to 6 mL) by varying OFAT to a fixed concentration of

VHC (20 µg.mL^{-1}) while the others were kept constant in a final volume of 25 mL and measuring the absorbance at 451 nm as shown in following Fig.(4).

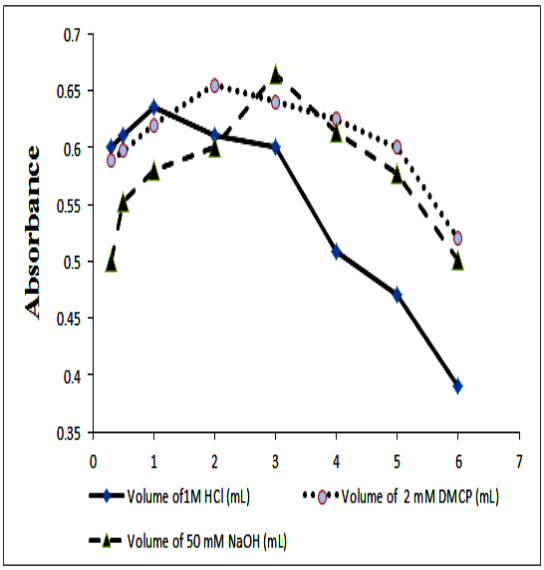


Fig.(4) Effect of volume of HCl, DMCP and NaOH on the absorbance of the colored product.

The colored product is formed immediately and becomes stable after 15 min and remains for more than 180 min. The order of addition of the reagents is an essential part of the experiment, it was found that the order of addition of the reagent cited under general procedure (2.3.1) gave maximum color intensity and a minimum absorbance of the blank and was used in all subsequent experiments. The effect of temperature on the color intensity of the dye was studied. A high absorbance was obtained when the color is developed at room temperature (25 ºC) than the calibrated flasks were placed in an ice bath at (0 ºC) or in a water bath at (45 ºC).

The stability constant of the dye products was calculated [20] by comparing the absorbance of a solution containing stoichiometric amount of VHC and DMCP (3.365×10^{-4} M) with that of solution containing five-fold excess of DMCP reagent. The stability constant of the dye products in water under the described experimental conditions was 1.303×10^{5} L.mol^{-1}.

The optimum conditions for batch method are incorporated in Table.1, and the effect of excipients on the recovery of VHC is incorporated in Table (2). The regression equation obtained from a series of VHC standards, and the analytical figures of merits of this procedure are summarized in Table (3).

3.3. *Influence of excipients*

Despite the fact that vancomycin is more used in the pure form, however, in order to assess the possible analytical applications of the proposed method, the influence of four common excipients: starch, talc, lactose and poly vinyl pirrolidone (pvp) was studied by analyzing synthetic sample solutions containing 20 µg.mL^{-1} of VHC and excess amounts (10-fold excess) of each excipient, none of these substances interfered seriously in the determination of VHC by the proposed methods Table (2).

Table (1)
Optimum conditions established in batch method.

Parameter	*Optimum range*	*Conditions in procedure*
λ_{max} (nm)	300 - 700	451
Effect of volume of (1M) HCl solution required	0.3 - 6 mL	1 mL
Effect of volume of (2 mM) DMCP solution required	0.3 - 6 mL	2 mL
Effect of volume of (50 mM) NaOH solution required	0.3 - 6 mL	3 mL
Type of reaction medium	Alkaline, acidic, and neutral	Alkaline
Type of alkaline medium	NaOH, NH_4OH, Na_2CO_3, CH_3COONa	NaOH
Effect of Addition Order	VHC, DMCP and NaOH	VHC + DMCP + NaOH
Effect of temperature	0 - 45 ºC	25 ºC
Stability period after final dilution	1 - 200 min	The colored product is formed immediately and becomes stable after 15 min and remains for more than 180min.

Table (2)
Effect of excipients (200 µg.mL^{-1}) on the recovery of VHC (20 µg.mL^{-1}).

Excipient	*Conc. µg.ml^{-1}*	*Error %*	*Recovery %*
Starch	19.710	- 1.438	98.561
Talc	19.726	- 1.366	98.633
Lactose	19.801	- 0.991	99.008
PVP	19.610	- 1.950	98.050

3.4. *FIA Spectrophotometric determination*

The batch method for the determination of VHC was adopted as a basis to develop FIA procedure. The manifold used for the determination of VHC was designed to provide different reaction conditions for magnifying the absorbance signal generated by the reaction of VHC with DMCP and in sodium hydroxide medium. Maximum absorbance intensity was obtained when the VHC solution was injected into a stream of DMCP and was then combined with the stream of NaOH Fig.(1). The influences of different physical or chemical parameters on the intensity of the colored product were optimized as follows:

3.4.1. *Optimization of reagents concentration*

The effects of various concentrations of DMCP in the range (0. 01-4 mM) were investigated. A concentration of 1 mM of DMCP gave the highest absorbance and was chosen for further use Fig.(5 a). HCl acid was found necessary for diazotization of DMCP. The effect of HCL was studied in the volume range of 1 M HCl (from 0.3 to 6 mL) and greatest absorbance intensity was obtained with 1 mL Fig.(5 b). Therefore, the effect of various concentrations of NaOH was similarly studied in the concentration range of (1-80 mM) and the greatest absorbance intensity was obtained with 10 mM Fig.(5 c).

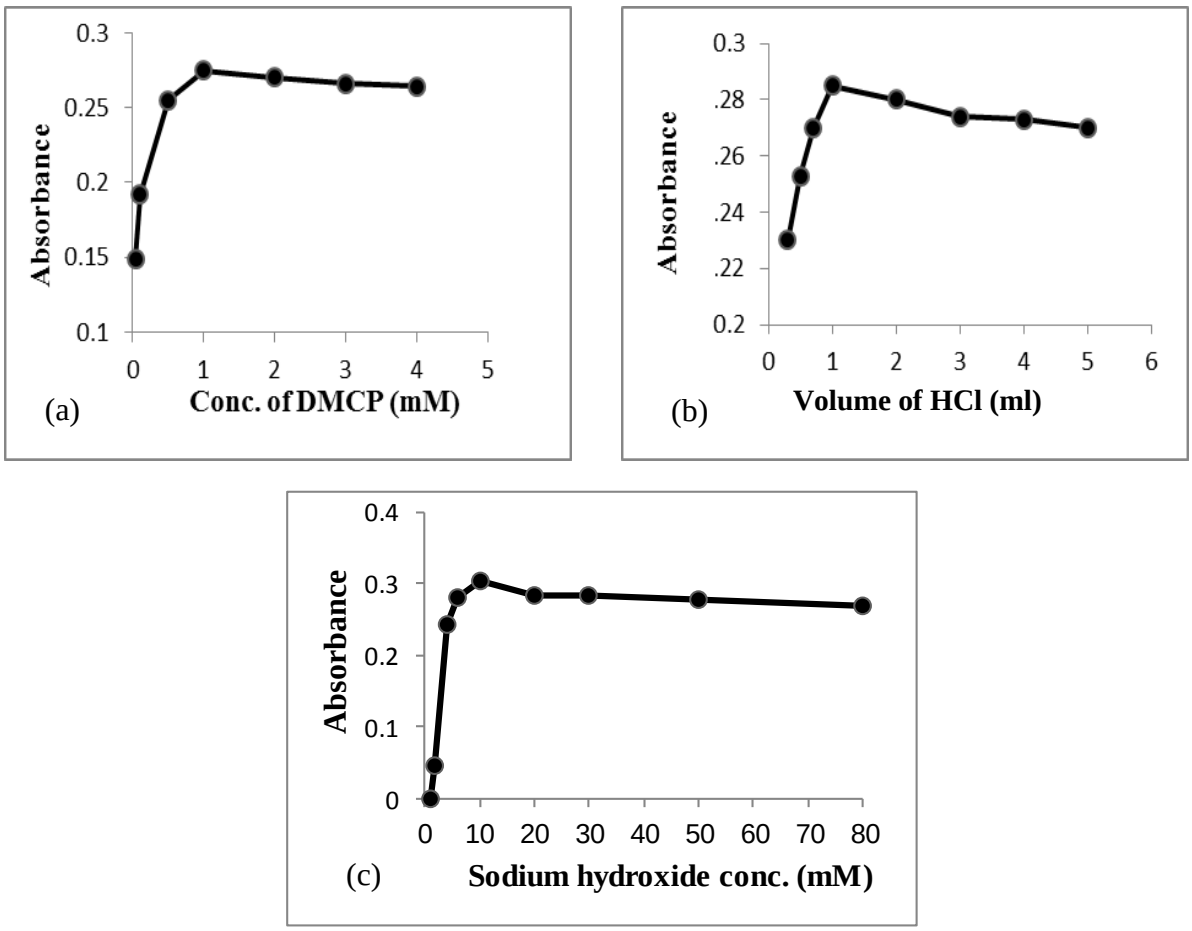


Fig.(5) Effect of reagents concentration on FIA (a) Effect of concentration of DMCP (1 mL of HCl (1M) and NaOH (50mM)) (b) Effect of volume of 1 M HCl (DMCP (1mM) and NaOH (50mM)) (c) Effect of concentration of NaOH (1 mL of HCl (1M) and DMCP (1mM)).

3.4.2. *Optimization of manifold parameters*

The variables studied under the optimized reagents concentrations were the flow rate, the injected sample volume and the reaction coil length. The results showed that a flow rate of 2.5mL.min^{-1} gave the highest absorbance Fig.(6 a) and it was used in all subsequent experiments. The volume of the sample was varied between 50 and 250 µL using different lengths of sample loop and showed that a sample of 200 µL gave the best absorbance Fig.(6 b). Moreover, a coil length of 100 cm gave the highest absorbance Fig.(6 c) and was used in all subsequent experiments. A standard calibration graph, obtained from a series of VHC standards and the main analytical figures of merits of the developed procedures are indicated and compared in Table (3).

3.5. *Analytical application*

The precision of the methods was evaluated by analyzing pure samples of VHC and a good recovery was obtained Table (3). The proposed methods were applied successfully to the analysis of some pharmaceutical preparations containing VHC (Injection and oral use), and they gave a good accuracy and precision as shown in Table (4). The results obtained by the proposed and reference methods [2] for dosage forms were compared statistically by means of the F-test and t-test [21] and were found not to differ significantly in precision and accuracy between the proposed methods and the official methods Table (5).

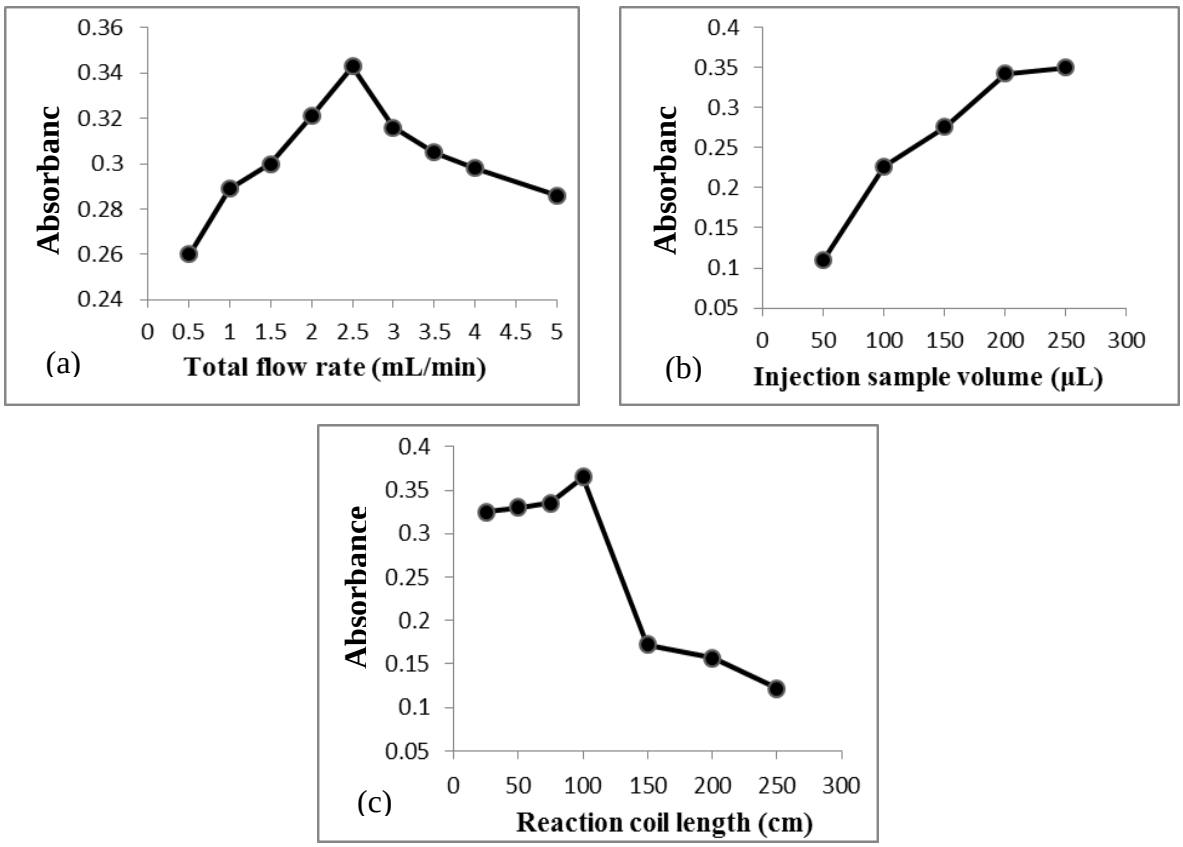


Fig.(6) Effect of manifold parameters on FIA in the presence of 1 mL of HCl (1M), DMCP (1mM) and NaOH (10mM) (a) Effect of total flow rate (b) Effect of injection sample volume (c) Effect of reaction coil.

Table (3)
Analytical characteristics of the procedures developed for the determination of VHC.

Parameter	*Batch procedure*	*FIA procedure*
Regression equation	y = 0.0311 x – 0.0095	y = 0.0058 x + 0.0402
Molar absorption coefficient ($L.mol^{-1}cm^{-1}$)	4.6205×10^4	8.6171×10^3
Linearity range ($\mu g.mL^{-1}$)	0.5 – 100	1 - 550
Correlation coefficient	0.9999	0.9995
Sy/x	1.12276×10^{-2}	3.8146×10^{-2}
Sa	5.0405×10^{-3}	1.7450×10^{-2}
Sb	9.25922×10^{-5}	5.9675×10^{-5}
Sandell's sensitivity ($\mu g.cm^{-2}$)	0.03215	0.1724
Reproducibility (%)* (RSD %)	0.363	1.164
Recovery%*	100.789	99.591
Error %*	+ 0.789	- 0.407
Limit of detection ($\mu g.mL^{-1}$)**	0.2306	0.4192
Through-put (1/h)	4	128

****The reproducibility, recovery and error of each method was tested by analyzing five replicate samples containing 20, 40, 80 µg.mL^{-1} of pure VHC for batch method and 50, 300,500 µg.mL^{-1} of pure VHC for FIA method.***
RSD Relative standard deviation.
Sy/x Standard deviation of the residuals.
Sa Standard deviation of the intercept.
Sb Standard deviation of the slope.
*****Limit of detection=3SD_B/b, SD_B is the standard deviation of the absorbance (n=10) of the blank determinations ($SD_B=2.39\times10^{-3}$ and 8.104×10^{-4} for batch and FIA methods respectively), b is the slope of the corresponding calibration curve).***

Table (4)
Application of the proposed methods to the determination of VHC in dosage forms.

Pharmaceutical preparation	*Proposed methods*	*Conc. µg.mL^{-1}*		*E%*	*Rec. %*	*RSD%*
		Present	*Found**			
Vancomycin(1) Hydrochloride For Solution For Infusion Wockhardt UK 500mg and 1g	Batch	20	19.622	- 1.688	98.311	0.262
		50	48.762	- 2.475	97.524	0.193
		80	77.700	- 2.873	97.126	0.713
	FIA	50	49.965	- 0.068	99.931	0.507
		100	99.620	- 0.379	99.620	0.408
		300	299.275	- 0.241	99.758	0.960
Vancolon(2) Vancomycin Hydrochlorid Injection Julphar UAE 500 mg and 1 g	Batch	20	20.112	+ 0.562	100.562	0.516
		50	49.790	- 0.418	99.581	0.183
		80	79.083	- 1.145	98.854	0.548
	FIA	50	51.344	+ 2.689	102.689	0.223
		100	102.206	+ 2.206	102.206	0.435
		300	303.586	+ 1.195	101.195	1.085
Vondem(3) Vancomycin Hydrochloride For Solution For Infusion DEMO S.A. Greece 500mg	Batch	20	20.209	+ 1.045	101.045	0.255
		50	48.665	- 2.668	97.331	0.239
		80	78.376	- 2.029	97.970	0.296
	FIA	50	49.793	- 0.413	99.582	0.604
		100	99.793	- 0.206	99.793	0.679
		300	302.034	+ 0.678	100.678	0.402

**** Mean of five measurements of each method, (1), (2), (3) three commercial sources of VHC.***

Table (5)
The comparison of the proposed method with standard method.

Pharmaceutical preparation	*Proposed methods*						*Standard method[2]*
	Batch			*FIA*			
	Rec.%	*t**	*F**	*Rec.%*	*t*	*F*	*Rec. %*
VHC pure	100.789	1.273	2.141	99.591	0.330	2.953	100.000
Vancomycin(1) Hydrochloride	97.653			99.769			98.343
Vancolon(2)	99.665			102.03			102.697
Vondem(3)	98.782			100.017			101.853

**** Theoretical values at 95% confidence limit, $n_1 = n_2 = 4$, $t = 2.45$ where t has $v = n1 + n2 - 2$ degrees of freedom = 6 , $F = 9.277$ where F has $v_1 = n_1 - 1$, $v_2 = n_2 - 1$ degrees of freedom = 3***

4. Conclusions

The proposed methods are simple and cost-effective for determination of VHC. They are adequate in aqueous solution and in pharmaceutical samples at a concentration level of traces ($\mu g.mL^{-1}$) without the need for previous separation steps, temperature or pH control. The procedures have also good linearity, rapid, through-put 128 sample of FIA at hour, sensitivity and economical value compared to othermethods.

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الخلاصة

يتضمن البحث تطوير طريقتين طيفيتين باستخدام نظام الدفعة والتحليل بالحقن الجرياني لتقدير الفانكومايسين هيدروكلورايد في المحاليل المائية والمستحضرات الصيدلانية. تعتمد الطريقتان على تفاعل الفانكومايسين هيدروكلورايد مع كاشف الميتوكلوبرامايد المؤزوت في الوسط القاعدي. تم قياس الناتج البرتقالي المصفر الذائب في الماء عند طول موجي 451 نانومتر. بلغت مديات الخطية من 0.5 الى 100 ومن 1 الى 550 مايكروغرام.مل$^{-1}$ وبحدود كشف 0.230 و0.419 مايكروغرام.مل$^{-1}$ لطريقتي الدفعة والحقن الجرياني على التوالي. بلغ معدل النمذجة 128 نموذج بالساعة لطريقة الحقن الجرياني. تمت دراسة تاثير جميع المتغيرات الفيزيائية والكيميائية على الطرق المقترحة وتم تطبيق الطريقة بنجاح في تقدير الفانكومايسين هيدروكلورايد في مستحضراته الصيدلانية.