ISSN : 0974-7419 Volume 16 Issue 7

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 16(7) 2016 [295-304]

Batch and flow-injection spectrophotometric determination of vancomycin hydrochloride in pharmaceutical preparations using diazotized procaine penicillin

Sadeem subhi abed, Omar T.Hussein
Department of Chemistry, College of science, University of Baghdad, Baghdad, (IRAQ)
E-mail: sadeem.subhi@yahoo.com

ABSTRACT

New, simple and sensitive batch and Flow-injection spectrophotometric methods for the determination of Vancomycin Hydrochloride in pure form and in pharmaceutical preparations were proposed. These methods were based on diazotization and coupling reaction between Vancomycin Hydrochloride and diazotized Procaine penicillin in alkaline medium to form an intense yellow water-soluble dye that is stable and has a maximum absorption at 446nm. A graphs of absorbance versus concentration show that Beer's law is obeyed over the concentration range of 0.8-40 and 5-700 $\mu g.mL^{-1}$ of Vancomycin Hydrochloride with detection limits of 0.066 and 0.707 $\mu g.mL^{-1}$ of Vancomycin Hydrochloride for batch and FIA methods, respectively. The FIA procedure sample throughput was $124h^{-1}$. All different chemical and physical experimental parameters affecting on the development and stability of the colored product were carefully studied and the proposed methods were successfully applied to the determination of Vancomycin Hydrochloride in pharmaceutical preparations.

KEYWORDS

Vancomycin hydrochloride;
Spectrophotometric determination;
Procaine penicillin;
Diazotization and coupling;
Flow injection.

INTRODUCTION

Vancomycin is a glycopeptidic antibiotic very efficient against a number of gram positive microorganisms[1]. Vancomycin was introduced in 1958 as an antibiotic active against Gram-positive cocci, particularly streptococci, staphylococci and pneumococci. It is not active against Gram-negative bacteria, Vancomycin hydrochloride is recommended for use when infections fail to respond to treatment with the more common antibiotics[3]. Its molecule shows a complex tricycle structure containing amino acids and sugars, Figure-1. Its mode of action is inhibition of cell wall synthesis of susceptible bacteria. The main target of this antibiotic is the (L-Lys)-D-alanyl-Dalanine terminal peptide of the cell wall precursor. In addition vancomycin alters the bacterial cell membrane permeability and RNA synthesis. vancomycin is used clinically as a result of high activity against gram positive pathogens such as many coagulase negative Staphylococcus(CNS), Corynebacterium, Clostridium difficile, multi-resistant Staphylococcus aureus and gentamicin resistant Enterococcus which are refractory to established drugs[4].

Full Paper

Vancomycin was introduced in 1958 as antibiotic active against Gram-positive cocci, particularly streptococci, staphylococci and pneumococci. It is not active against Gram-negative bacteria, Vancomycin hydrochloride is recommended for use when infections fail to respond to treatment with the more common antibiotics[5]. VHC is officially recognized in B.P[2] A survey of literature revealed that few methods based on visible spectrophotometry for VHC[6-8] have been reported. Other methods include HPLC[9-11], HPLC-tandem mass spectrometry[12], flow injection analysis[13], Polarography[14], Radioimmunoassay[15], Fluorescence polarization immunoassay[16]. however only few spectrophotometric methods are reported for the analysis of vancomycin[17-19]. This paper describes spectrophotometric methods for determination of VHC by the diazotization-coupling reactions with diazotized O-nitro aniline in alkaline medium. O-nitro aniline was found to be a useful new coupling reagents for diazotization reaction, because they produced a stable and rapid coupling organic products furthermore, these reagent is easily obtainable, highly purified and are soluble in ethanol therefore the proposed methods are con-

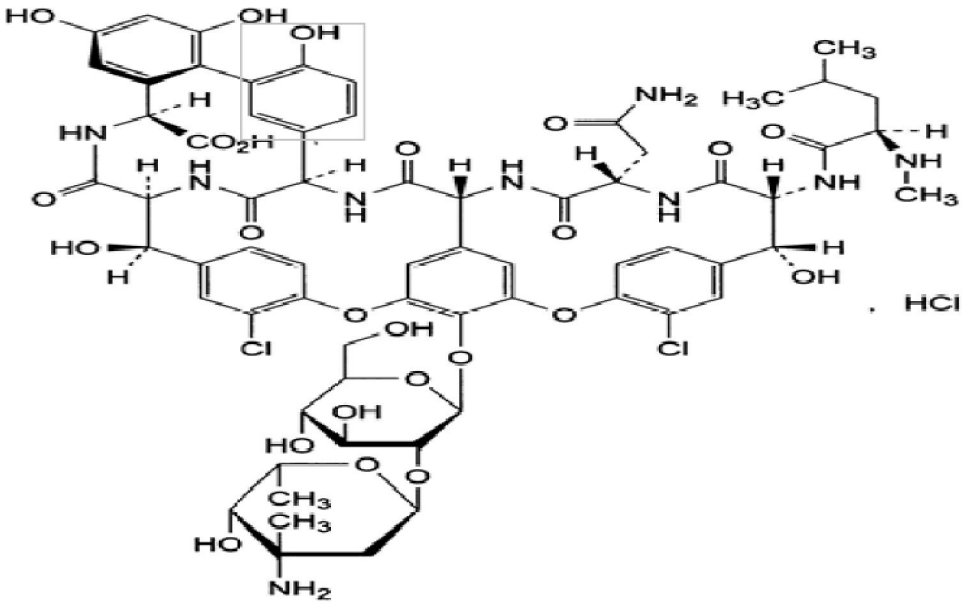


Figure 1 : Vancomycin hydrochloride (VHC)

TABLE 1 : Optimum conditions established in batch method

Parameter	Range selected	Optimum Conditions in procedure
λmax (nm)	350 – 700	446
Effect of volume of (3mM) Procaine penicillin solution required	0.1 - 5 mL	0.2 mL
Effect of volume of (1M) HCl solution required	0.5 - 5 mL	2 mL
Effect of volume of (0.1M) Na_2CO_3 solution required	0.25 - 5 mL	4 mL
Type of reaction medium	Alkaline, acidic, and neutral	Alkaline
Type of alkaline medium	NaOH, NH_4OH, Na_2CO_3, CH_3COONa	Na_2CO_3
Effect of Addition Order	PP, VHC, Na_2CO_3	VHC +PP + Na_2CO_3
Effect of temperature	0 - 45°C	25°C
Stability period after final dilution	1 - 150 min	The colored product is formed immediately and becomes stable after 10min and remains for more than 150 min.

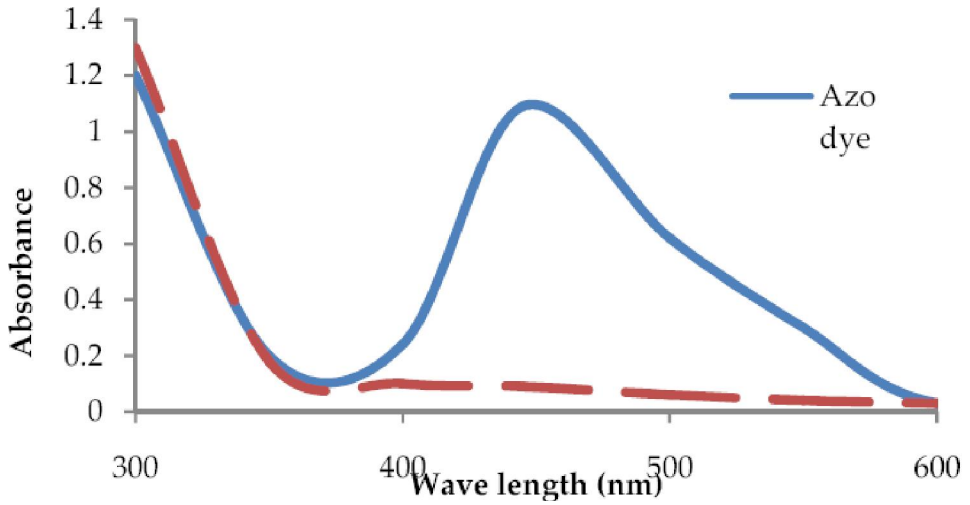


Figure 2 : Absorption spectra of (40 µg.mL^{-1}) VHC treated as described under procedure and measured against reagent blank(Diazotized Procaine penicillin and sodium carbonate anhydrous) and the reagent blank measured against distilled water

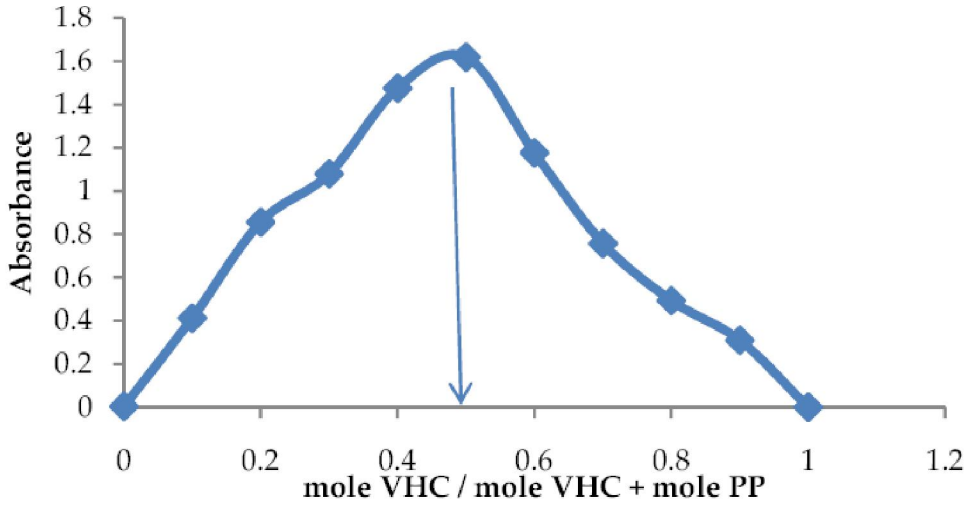


Figure 3 : Continuous variation plot of the reaction between VHC and diazotized Procaine penicillin (3x10^{-3}M)

sidered as a green methods. In addition these methods have been satisfactorily applied for the determination of vancomycin hydrochloride in pure and pharmaceutical preparations.

MATERIALS AND METHODS

Apparatus

All spectral and absorbance measurements were carried out by using a shimadzu UV – visible – 260 digital double beam recording spectrophotometer (Tokyo – Japan), and using 1 cm quarts cells. A quartz flow cell with 50 µL internal volume and 1 cm bath length used for the absorbance measurements. A two channel manifold (Figure 3) was employed for the FIA spectrophotometer determinations of VHC. A peristaltic pump (IsmatecLobortechnik–Analytic, CH – 8512, Glatbragg–Zurich, Switzerland, Sixchannels) was used to transport the reagents solutions. Injection valve (Rheodyne, Altex 210, supeko use) was employed to provide appropriate injection volumes of standard solutions and samples, flexible vinyl tubing of 0.5 mm internal diameter was used for the peristaltic pump. Reaction coil (RC) was of Teflon with internal diameter of 0.5 mm. The diazotized Procaine pencilline (A) stream was combined (Figure 3) with injected sample (vancomycin hydrochloride) and they merged with sodium carbonate (B) stream at T – link then mixed in reaction coil (RC) with length (75 cm), injection loop (200

Full Paper

μL), total flow rate 4 mLmin^{-1}, the absorbance was measured at 446 nm at temperature 25 C°.

Standard vancomycin hydrochloride VHC solution

Stock solution (500 μg.mL^{-1}) was prepared daily by dissolving 0.05 g of the pure VHC in 100 mL of distilled water and serial dilutions with distilled water were made.

Sodium nitrite solution (3×10^{-3} M) was prepared by dissolving 0.0207 g of sodium nitrite (Merck) in distilled water and diluting to the mark in 100 mL volumetric flask.

Hydrochloric acid solution (1M) was prepared by diluting 86 mL of 11.64 M of concentrated hydrochloric acid (BDH) with distilled water in 1000 mL volumetric flask.

Diazotized Procaine penicillin solution (3×10^{-3}M)

Prepared daily by dissolving 0.1766gm of Procaine penicillin in 5 ml ethanol, 20 ml distilled water and 2 ml of 1M hydrochloric acid in a 100ml volumetric flask. Cool the mixture to 0-5°C for 5 min using an ice-bath, add 0.0207gm amount of sodium nitrite and stir the mixture. After 5min the volume is made up to the mark with addition of cooled distilled water. More dilute solutions were prepared by suitable dilutionwith distilled water.

Sodium Carbonate anhydrous (BDH) solution

Stock solution of 0.1 M was prepared by dissolving 2.65 g of Na_2CO_3 in 250 mL distilled water, and working solutions were prepared by appropriate dilution of the stock solution.

Sample vancomycin hydrochloride VHC solution

The contents of five vials were mixed (two commercial sources vancolon VHC injection Julphar company UAE 500mg and vancorin VHC injection CheilJedang corporation company Republic of Korea 1g). An aliquot corresponding to 0.05 g of VHC was diluted to 100 mL with distilled water in a volumetric flask to obtain 500 μg.mL^{-1} of VHC. More dilute solutions of pharmaceutical preparations for batch and FIA procedures were made by simple dilution with distilled water.

General procedure for calibration

a. General batch procedure

In method a 0.2mL of (3×10^{-3} M) Diazotized Procaine penicillin solution was transferred into a series of 25mL calibrated flask. Then, An aliquot of a standard solution (500 μg mL^{-1}) (3.36×10^{-4}M) containing 0.8-40 μg mL^{-1} of VHC was transferred into this series of 25 mL calibrated flasks and 4mL of 0.1 M sodium carbonate anhydrous solutions was added and the contents were diluted to the mark with distilled water and mixed well. After 10 min, the absorbance of the colored was measured at 446nm against the corresponding reagent blank TABLE-1 summarized the studied optimum conditions.

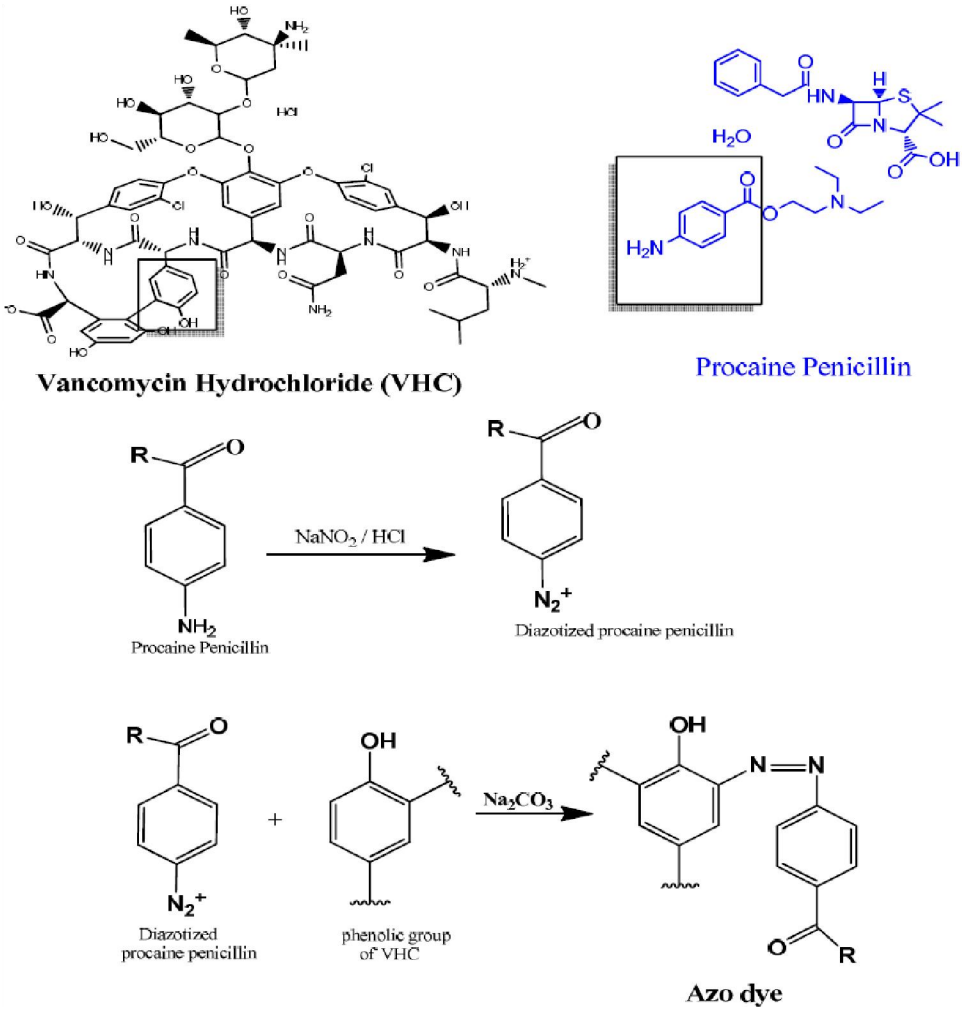
b. General FIA procedure

A Vancomycin Hydrochloride solution in the range of 5-700μg.mL^{-1} was prepared from the standard working solution of 500 μg.ml^{-1}. A 200 μl portion of Vancomycin Hydrochloride VHC was injected into the stream of diazotized Procaine penicillin (3 x 10^{-3}M) then the mixture combined with (0.1 M) Na_2CO_3 at T-link with a total flow rate of 4ml min^{-1} for the two channels, the resulting absorbance of the Yellow product was measured at 446 nm and a calibration graph was constructed. Optimization of conditions was carried out on 50 μg.ml^{-1} of VHC.

RESULTS AND DISCUSSION

Batch spectrophotometric determination

The factors affecting on the sensitivity and stability of the colored diazotization coupling reaction between diazotized Procaine penicillin and VHC in an alkaline medium were carefully studied. A typical spectrum for the azo dye formed was measured versus reagent blank which has negligible absorbance at λmax 446 nm (Figure 2). The experimental conditions for the determination of VHC were established. The diazotization coupling reaction occurred in an acidic medium and a hydrochloric acid of concentration 1M was selected, the effect of different volumes of 1 M of HCl were studied and 2 ml volume seems to be optimum for an intense azo dye color. Effect of the volumes of reagent (Procaine penicillin 3×10^{-3}M) were studied in the range of 0.1-5 ml and 0.2 ml was found to be optimum. The ab-



Scheme 1 : The proposed reaction between VHC and PP

sorbance of the dye formed increased and became more stable in alkaline medium, therefore, the effect of different alkaline solutions(0.1M) on the colored product was studied such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, sodium acetate and sodium carbonate. Maximum sensitivity and stability were obtained only when the reaction was carried out in the presence of sodium carbonate solution. The effect of different volumes (0.25-5 ml) of Na_2CO_3 (0.1 M) was studied. A volume of 4mL was found enough to obtain a maximum absorbance. Experimental results revealed that the color intensity reach maximum after diazotized Procaine penicillin solution had been reacted with VHC in alkaline medium for 10 min, therefore, a 10 min development time was suggested as the optimum reaction time and remain stable for 200 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 gave maximum color intensity and the minimum absorbance of the blank and was used in all subsequent experiments.

The stoicheiometry of the reaction between VHC and diazotized Procaine penicillin was investigated using continuous variation method. The results obtained (Figure 2) shows that a (1:1) azo dye was formed between VHC and diazotized Procaine penicillin according to scheme-1.

FIA-spectrophotometric determination

The batch method for the determination of VHC was adopted as a basis to develop a 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 the diazotized Procaine penicillin with

Full Paper

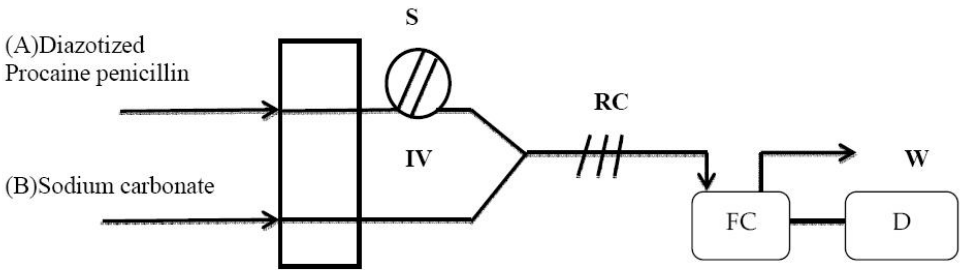


Figure 3 : A schematic diagram of FIA manifold Where: (A) and (B), solutions of Diazotized Procaine penicillin and sodium carbonate respectively; PP =peristaltic pump; S= injection sample VHC; IV= injection valve; T= T-link; RC= reaction coil; FC= flow cell; D= detector; W= waste

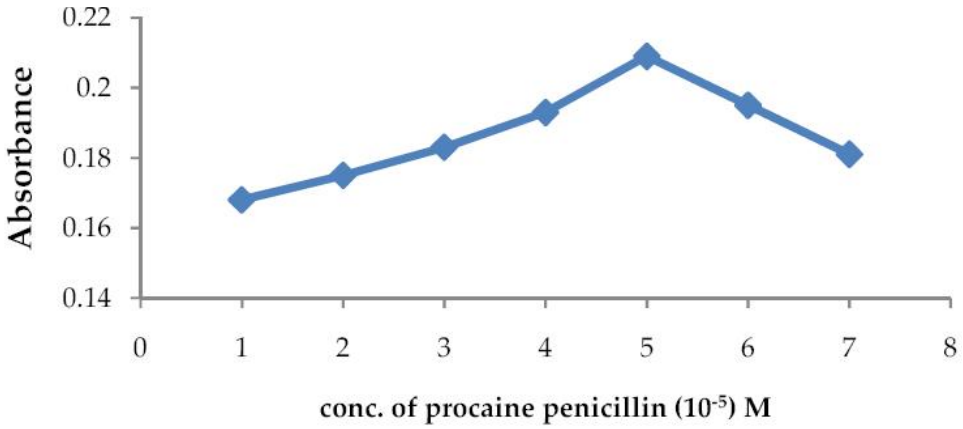


Figure 4 : Effect of the concentration of procaine penicillin reagent

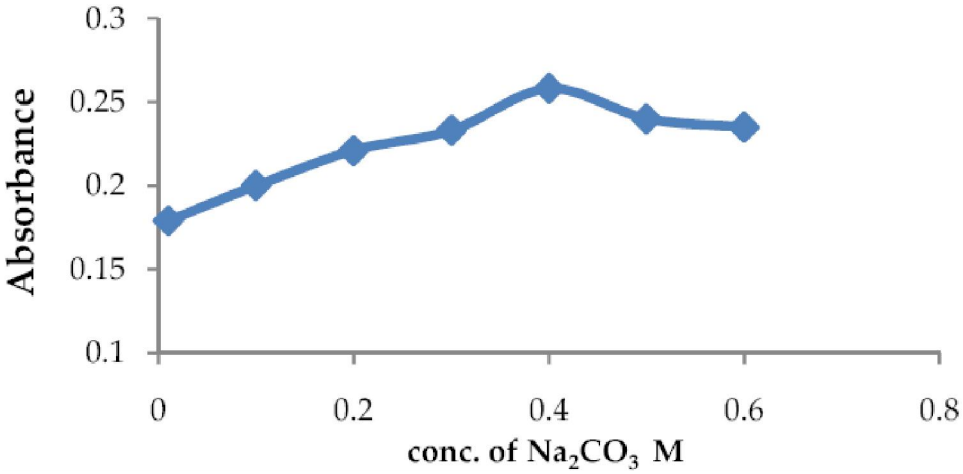


Figure 5: Effect of the concentration of Na_2CO_3 in (M)

VHC in sodium carbonate medium. Maximum absorbance intensity was obtained when the sample (VHC 50 $\mu g.ml^{-1}$) was injected into a stream of diazotized Procaine penicillin and then mixed with sodium carbonate as given in (Figure 3). The influence of different chemical and physical FIA parameters on the absorbance of the colored product was optimized as follows:

Optimization of chemical parameters

The effect of various concentrations of Procaine penicillin was investigated. A concentration of (5x 10^{-5} M) Procaine penicillin, gave the highest absorbance and was chosen for further experiments as shown in (Figure 5).

It was observed that the reaction between diazotized Procaine penicillin and VHC depends on alkaline medium, therefore the effect of different concentrations of sodium carbonate was studied and 0.4M was found to be the optimum as shown in (Fig-

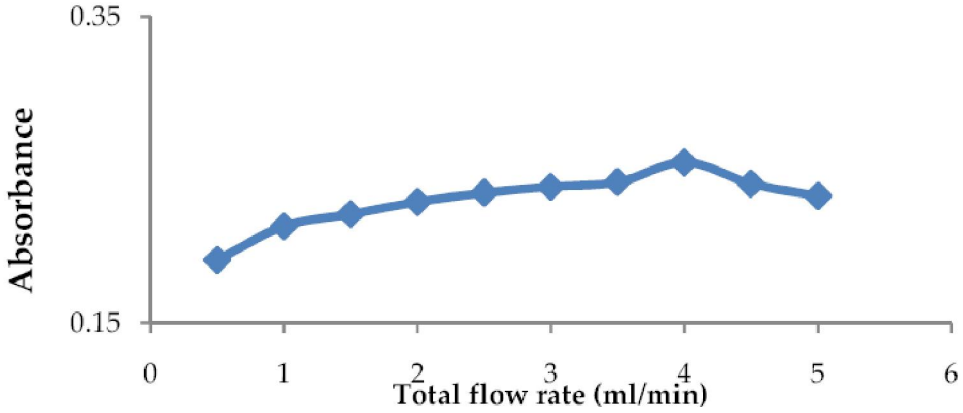


Figure 6 : Effect of total flow rate

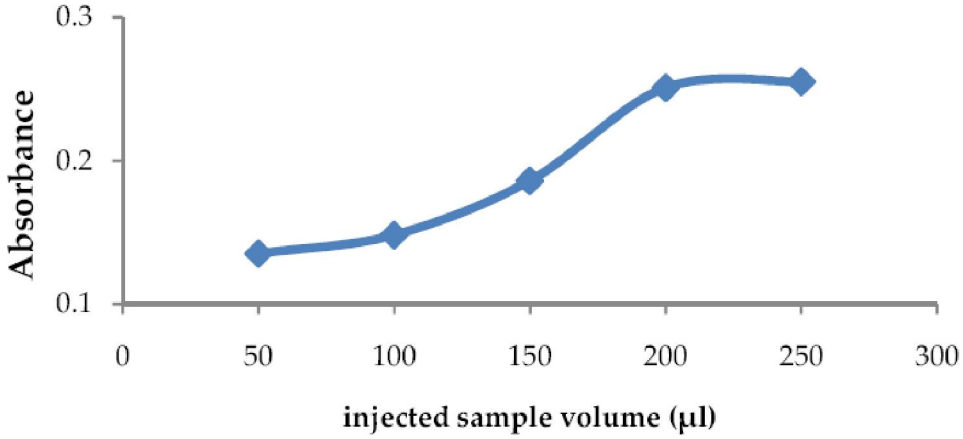


Figure 7 : Effect of injection sample volume (µl)

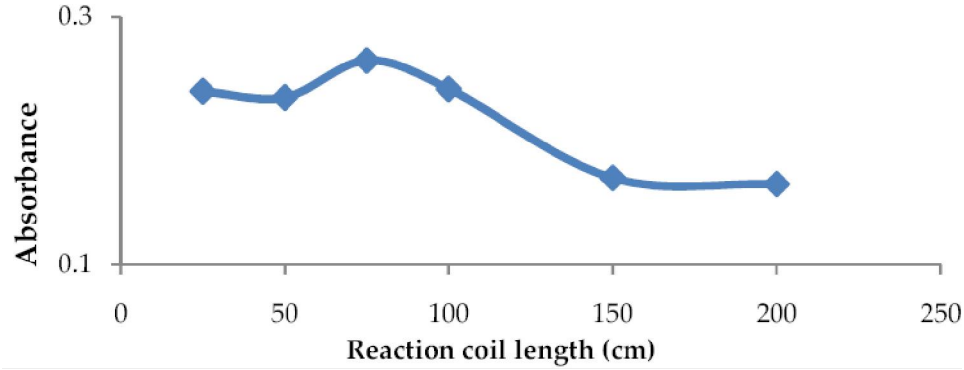


Figure 8 : Effect of reaction coil (cm)

ure 6).

Optimization of manifold parameters

The effect of total flow rate on the sensitivity of the colored reaction product was investigated in the range of 0.5-5ml min^{-1}. The results obtained showed that a total flow rate of 4 ml min^{-1}, gave the highest absorbance as shown in (Figure 7), and was used in all subsequent experiments.

The volume of the injection sample was varied between 100 and 250 µl using different lengths of sample loop. The results (Figure 8) obtained showed that injected sample of 200 µl gave the best absorbance.

The coil length is an essential parameter that affects on the sensitivity of the colored reaction product and was investigated in the range of 25-250 cm. the results obtained showed that a coil length of 75 cm gave the highest absorbance as shown in (Figure 9) and was used in all subsequent experiments.

Full Paper

TABLE 2 : Analytical values of the calibration graphs for the determination of VHC

Parameter	Batch procedure	nFIA procedure
Regression equation	y = 0.03x - 0.035	y = 0.002x + 0.204
Molar absorption coefficient ($L.mol^{-1}.cm^{-1}$)	4.4565×10^4	2.971×10^3
Linearity range ($\mu g.mL^{-1}$)	0.8 – 40	5 – 700
Correlation coefficient	0.998	0.999
Linearity percentage r^2%	99. 6	99.8
Sandell's sensitivity ($\mu g\ cm^{-2}$)	0.0333	0.5
Reproducibility (%)* (RSD %)	1.23	1.21
Recovery%*	100.62	100.28
Limit of detection** ($\mu g.mL^{-1}$)	0.333	0.707
Through-put (1/h)	8	124

***The Average of reproducibility, recovery and error of each method was tested by analyzing five replicate samples containing 8, 16, 28 µg.mL^{-1} of pure VHC for batch method and 40,80,150 µg.mL^{-1} of pure VHC for FIA method.**

TABLE 3 : 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			
Vancolon Vancomycin Hydrochloride Injection Julphar UAE 500 mg	Batch	8	7.86	-1.75	98.25	2.48
		16	15.96	-0.25	99.75	0.90
		28	28.20	0.71	100.71	1.10
	nFIA	40	40.40	1	101	2.92
		80	82.4	3	103	0.41
		150	152.9	1.93	101.93	0.22
Vancorin Vancomycin Hydrochloride Injection CheilJedang corporation Republic of Korea 1g	Batch	8	7.80	-2.50	97.50	1.53
		16	16.10	0.62	100.62	0.44
		28	28.76	2.71	102.71	1.93
	nFIA	40	39.70	-0.75	99.25	2.50
		80	78.20	-2.37	97.62	1.69
		150	151.10	0.73	100.73	1.28

The reaction time is also an important parameter that affected on the sample throughput and was investigated by calculating the interval time between the sample injection and the appearance of the end of the signal. The reaction time of each sample was 29 sec, therefore the sample through put was 124 samples per hour.

Calibration graphs

After fixing the optimum conditions of both batch and FI methods for the determination of VHC, calibration graphs were constructed. The analytical values of statistical treatments[19,20] for the calibration graphs are summarized in TABLE-2. The accuracy of the methods was evaluated by analyzing pure samples of VHC and a good recovery was obtained TABLE-2.

RSD Relative standard deviation.

**Limit of detection=3SDB/b, SDB is the standard deviation of the absorbance (n=10) of the blank determinations (SDB=3.33×10^{-3} and 4.714×10^{-4} for batch and FIA methods respectively), b is the slope of the corresponding calibration curve).

Analytical application

The proposed methods were applied successfully to the analysis of some pharmaceutical preparations containing VHC (Injection), and they gave a good Reproducibility and Recovery as shown in

TABLE 4 : The comparison of the proposed method with standard method

Pharmaceutical preparation	Proposed methods	Conc. µg.mL^{-1}		E%	Rec.%	RSD%
		Present	Found			
Vancolon Vancomycin Hydrochloride Injection Julphar UAE 500 mg	Batch	8	7.86	-1.75	98.25	2.48
		16	15.96	-0.25	99.75	0.90
		28	28.20	0.71	100.71	1.10
	nFIA	40	40.40	1	101	2.92
		80	82.4	3	103	0.41
		150	152.9	1.93	101.93	0.22
Vancorin Vancomycin Hydrochloride Injection CheilJedang corporation Republic of Korea 1g	Batch	8	7.80	-2.50	97.50	1.53
		16	16.10	0.62	100.62	0.44
		28	28.76	2.71	102.71	1.93
	nFIA	40	39.70	-0.75	99.25	2.50
		80	78.20	-2.37	97.62	1.69
		150	151.10	0.73	100.73	1.28

***Theoretical values at 95% confidence limit, $n_1 = n_2 = 3$, t = 2.123 where t has $v = n_1 + n_2 - 2$ degrees of freedom = 4, F = 19.0 where F has $v1 = n_1 - 1$, $v2 = n_2 - 1$ degrees of freedom = 2.**

TABLE-3. The results obtained by the proposed and reference methods[2, 18] for dosage forms were compared statistically by means of the F-test and t-test[17] and the proposed methods and the reference methods were found no significant differences in precision and accuracy between the proposed methods and the reference methods TABLE-4.

CONCLUSION

The application of diazotization–coupling reaction of diazotized Procaine penicillin in sodium carbonate medium to the spectrophotometric determinations of the vancomycin hydrochloride in pharmaceutical preparation's was described by batch and nFIA systems, Although the batch system has the advantage's of higher sensitivity and lower limit of detection over the nFIA system, the nFIA system has several advantage's over the batch system simplicity, reproducibility time saving, low reagent consumption need of small sample volume, large dynamic range and high sample throughput (124 sample h^{-1} for VHC) is important feature of the nFIA system.

The proposed methods offer a good linearity and precision and can be applied to the analysis of a wide concentration range of VHC in real samples with satisfactory results.

The proposed methods are simple and inexpensive since it requires simple instrumentation.

REFERENCES

[1] B.Cavalleri, F.Parenti; Encyclopaedia of chemical technology, 4th Edition, **2**, John-Wiley, NY **(1984)**.

[2] The stationary office on behalf of the medicines and healthcare products regulatory agency (MHRA), British Pharmacopeia, London, **(2009)**.

[3] J.H.Block, Jr.J.M.Beale; Wilson and gisvold's textbook of organic medicinal and pharmaceutical chemistry, Eleventh Edition, Lippincott Williams and Wilkins, 355 **(2004)**.

[4] P.S.Avinash, J.Lee, K.Song, T.Panda, C.Kim, S.Rhee, B.Gokul; Analytical techniques for vancomycin -A Review, Biotechnology and Bioprocess Engineering, **5**, 153-158 **(2000)**.

[5] J.H.Block, Jr.J.M.Beale; Wilson and gisvold's textbook of organic medicinal and pharmaceutical chemistry, 11th Edition, Lippincott Williams and Wilkins, 355 **(2004)**.

[6] J.R.Fooks, I.J.McGilveray, R.D.Strickland; Colorimetric assay and improved method for identification of vancomycin hydrochloride, Journal of Pharmaceutical Sciences, **57(2)**, 314-317 **(1968)**.

[7] S.M.El Ashry, F.Belal, M.M.El Kerdawy, D.R.Elwasseef; Spectrophotometric determination of some phenolic antibiotics in dosage forms, Mikrochimica Acta, **135**, 191-196 **(2000)**.

[8] C.S.P.Sastry, T.S.Rao, P.S.N.H.Rao, U.V.Prassa;

Assay of vancomycin and dobutamine using sodium metaperiodate, Mikrochimica Acta, **140**, 109-118 **(2002)**.

[9] S.Putty, H.S.Vemula, W.G.Bobba; A liquid chromatography–tandem mass spectrometry assay for D-Ala-D-Lac:A key intermediate for vancomycin resistance in vancomycin-resistant enterococci, Analytical Biochemistry, **442(2)**, 166-171 **(2013)**.

[10] K.Boronová, J.Lehotay, K.Hroboňová, D.W.Armstrong; Study of physicochemical interaction of aryloxyaminopropanol derivatives with teicoplanin and vancomycin phases in view of quantitative structure–property relationship studies, Journal of Chromatography A,**1301(2)**, 38-47 **(2013)**.

[11] Y.Bijleveld, T.Haan, J.Toersche, S.Jorjani, J.Lee, F.Groenendaal, P.Dijk, A.Heijst, A.W.D.Gavilanes, R.Jonge, K.P.Dijkman, H.Straaten, M.Rijken, I.Zonnenberg, F.Cools, D.Nuytemans, R.Mathôt; A simple quantitative method analysingamikacin, gentamicin, and vancomycin levels in human newborn plasma using ion-pair liquid chromatography/tandem mass spectrometry and its applicability to a clinical study, Journal of Chromatography B, **951–952,** 110-118 **(2014)**.

[12] R.C.Reyes, R.R.González, A.G.Frenich, M.A.R.Maresca, J.L.M.Vidal; Simultaneous analysis of antibiotics in biological samples by ultra high performance liquid chromatography–tandem mass spectrometry, Journal of Pharmaceutical and Biomedical Analysis, **89**, 203-212 **(2014)**.

[13] A.R.Khataee, A.Hasanzadeh, M.Iranifam, M.Fathinia, Hanifehpour, J.S.W.Yand; CuOnanosheets-enhanced flow-injection chemiluminescence system for determination of vancomycin in water, Pharmaceutical and human serum, Spectrochimica Acta Part A: Molecular BiomolecularSpectroscopy, **122**, 737-743 **(2014)**.

[14] F.Belal, S.MEl-Ashry, M.M.El-kerdawy, El D.E.Wasseef; Voltametric determination of vancomycin in dosage forms through treatment with nitrous acid, Arzneimittelforschung, **51(9)**, 763-768 **(2001)**.

[15] B.H.Ackerman, H.G.Berg, R.G.Strate, J.C.Rostschafer; Comparison of radioimmunoassay and fluorescent polarization immunoassay for quantitative determination of vancomycin concentrations in serum, Journal of Clinical Microbiology, **18(4)**, 994-995 **(1983)**.

[16] J.Hermida, S.Zaera, J.C.Tutor; Therapeutic drug monitoring in the COBAS Integra 400 Analyzer, Therapeutic Drug Monitoring, **23(6)**, 725 **(2001)**.

[17] J.R.Fooks, I.J.McGilveray, R.D.Strickland; Colorimetric assay and improved method for identification of vancomycin hydrochloride, Journal of Pharmaceutical Sciences, **57**, 314-317 **(1986)**.

[18] S.M.El-Ashry, F.Belal, M.M.El-Kerdawy, D.R.Elwasseef; Spectrophotometric determination of some phenolic antibiotics in dosage forms, Mikrochim Acta, **135**, 191-196 **(2000)**.

[19] C.S.P.Sastry, T.S.Rao, P.S.N.H.Rao, U.V.Prassa; Assay of vancomycin and dobutamine using sodium metaperiodate, Mikrochim Acta.**140**, 109-118 **(2002)**.

[20] J.N.Miller, J.C.Miller; Statistics and chemometrics for analytical chemistry, 4th Edition, Pearson education limited, London, **(2000)**.

[21] U.S.Pharmacopeial Convention, United states pharmacopeia 30, National formulary 25, Rockville, USA, **(2007)**.