Spectrophotometric Determination of Vancomycin Hydrochloride in Pharmaceutical Preparations through Diazotization and Coupling Reactions

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Abstract

Two simple, sensitive, accurate and economic methods A and B have been developed for the quantitative estimation of vancomycin hydrochlorid (VHC) and its formulations using another two drug compounds as a coupling reagents.The proposed methods are based on a coupling reaction between VHC and diazotized procain (method A) or diazotized sulphacetamide sodium (method B) in alkaline medium to form intense yellow, water-soluble dyes that are very stable and have a maximum absorption at 447 and 439 nm for methods A and B respectively. Regression analysis of Beer's law plots showed good correlation in the concentration ranges 1-28 and 1-45 μg ml^{-1} for methods A and B, respectively with a molar absorbtivity of 4.605×10^4 L $mol^{-1}cm^{-1}$ and 4.516×10^4 L $mol^{-1}cm^{-1}$, Sandell's sensitivity of 0.032 μg.cm^{-2} and 0.033 μg.cm^{-2} for methods A and B respectively. Relative standard deviation (RSD%) were less than 2.9 for both methods.The methods were successfully applied to the determination of VHC in bulk drug and its formulations.

Keywords: Vancomycin hydrochlorid, diazotization, spectrophotometry.

التقدير الطيفي للفانكومايسين هيدروكلورايد في المستحضرات الصيدلانية من خلال تفاعلات الازوتة والازدواج

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قسم الكيمياء، كلية العلوم، جامعة بغداد، بغداد، العراق

الخلاصة:

تم تطوير اثنان من الطرق الطيفية البسيطة والسريعة والحساسة للتقدير الكمي للفانكومايسين هيدروكلورايد في مستحضراته الصيدلانية باستخدام دوائين اخرين ككواشف ازدواج. تعتمد الطرق المقترحة على تفاعل الازوته والازدواج بين الفانكومايسبن هيدروكلورايد والبروكائين المؤزوت(الطريقة A) او السلفاسيتامايد الصوديوم المؤزوت(الطريقة B)في الوسط القاعدي لتكوين صبغة صفراء مستقرة اعطت اقصى امتصاص عند447 و 439 نانومترا للطريقتين A وB على التوالي.مدى الخطية لقانون بير كان من 1−28 ومن 1−45 مايكروغرام من الفانكومايسين هيدروكلورايد لكل مللتر من المحلول وبممتصية مولارية 4.605×10^4 و 4.516×10^4 لترمول $سم^{-1}$ $^{-1}$ وحساسية ساندل 0.033 و0.032 مايكروغرام /$سم^2$ للطريقتين A وB على التوالي .الانحراف القياسي النسبي كان اقل من 2.9 للطريقتين. تم تطبيق الطريقتان المقترحتان وبنجاح في التقدير الطيفي للفانكومايسين هيدروكلورايد في مستحضراته الصيدلانية.

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1. Introduction:

Vancomycin is a glycopeptidic antibiotic very efficient against a number of gram positive microorganisms [1]. 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 [2].

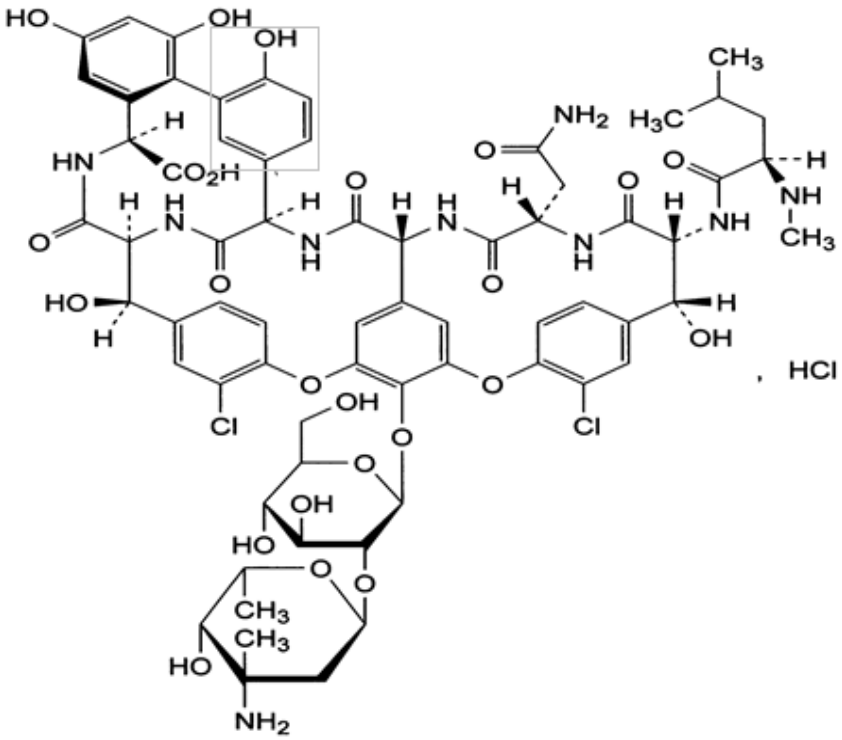


Figure 1- Vancomycin hydrochloride (VHC)

There are, in the literature, several methods for the determination of vancomycin in biological fluids and in pharmaceutical products, include HPLC [3-5], HPLC-tandem mass spectrometry [6], flow injection analysis [7],capillary electrophoresis [8,9], radioimmunoassay [10], however only few spectrophotometric methods are reported for the analysis of vancomycin [11-13]. This paper describes spectrophotometric methods for determination of VHC by the diazotization-coupling reactions with diazotized procain or sulphacetamide sodium in alkaline medium. Procain or sulphacetamide sodium were found to be a useful new coupling reagents for diazotization reaction, because they produced a stable and rapid coupling organic products, furthermore, these reagents are drugs compounds therefore they are easily obtainable, highly purified and are freely soluble in water and therefore the proposed methods are considered as a green methods. In addition these methods have been satisfactorily applied for the determination of vancomycin hydrochloride in pure and pharmaceutical preparations.

2. Experimental

2.1. Apparatus

All spectral and absorbance measurements were carried out on a Shimadzu UV–vis 260 digital double beam recording spectrophotometer.

2.2. Preparation of solutions

- Vancomycin Hydrochlorid (VHC) stock standard solution (1000 μg mL^{-1}) was prepared by dissolving 0.1000 g of pure VHC (SDI) in distilled water and made up to 100 mL volumetric flask

with the same solvent. Working standard solutions were prepared by suitable dilution of the stock standard solution with distilled water.

- Sodium nitrite solution (1×10^{-3} M) was prepared by dissolving 0.0173 g of sodium nitrite (Merck) in distilled water and diluting to the mark in 250 mL volumetric flask.
- Hydrochloric acid solution (1M***)*** was prepared by diluting 43 mL of 11.64 M of concentrated hydrochloric acid (BDH) with distilled water in 500 mL volumetric flask.
- Sulphacetamide sodium (1×10^{-3} M) solution was prepared by dissolving 0.0214 g of Sulphacetamide sodium (SDI) in distilled water and diluting to the mark in 100 mL volumetric flask.
- Procaine HCl:(pure standard drug (SDI) (1×10^{-3} M***)*** was prepared by dissolving 0.0273 g of procaine HCl (SDI) in distilled water and diluting to the mark in 100 mL volumetric flask.
- Amonium hydroxide (Fluka) solution (1M***)*** was prepared by diluting 8.5mL of 11.77M of concentrated ammonium hydroxide with distilled water in 100 mL volumetric flask.
- Sodium hydroxide NaOH (Merck) solution: stock solution of 2 M was prepared by dissolving 20 g of NaOH in 250 mL distilled water, and working solutions were prepared by appropriate dilution of the stock solution.
- Pharmaceutical preparations of vancomycin hydrochloride

Pharmaceutical preparations were obtained from commercial sources.

- Vancolon / Injection (Julphar UAE-500 mg).
- Vondem/ Solution for Infusion (DEMO S.A. Greece-500mg).
- Samples of vancomycin hydrochloride VHC solution (obtained from commercial sources): the contents of five vials (two commercial sources) were mixed. An aliquot corresponding to 0.1000 g of VHC (0.1019 and 0.1098g from vancolon and vondem respectively) was dissolved and diluted to 100 ml with distilled water in a volumetric flask to obtain 1000 $\mu g.mL^{-1}$ of VHC. More dilute solutions of pharmaceutical preparations were made up by simple dilution with distilled water.

3. Methodology

3.1. Method A

In method (A), a 0.5mL of (1×10^{-3} M) procaine hydrochloride was transferred into a series of 25mL calibrated flask. To this solution equimolar of sodium nitrite solution (1×10^{-3} M) was added and the acidity was adjusted with 0.5 mL of 1 M hydrochloric acid solution. The solution was shaken thoroughly. Then, An aliquot of a standard solution (500 $\mu g\ mL^{-1}$) (3.36×10^{-4}M) containing 25-700 μg of VHC was transferred into this series of 25 mL calibrated flasks and 1.5 mL of 1 M ammonium hydroxide 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 azo dye was measured at 447nm against the corresponding reagent blank.

3.2. Method B

In method (B), a 1mL of (1×10^{-3} M) sulphacetamide sodium was transferred into a series of 25mL calibrated flask. To this solution equimolar of sodium nitrite solution (1×10^{-3} M) was added and the acidity was adjusted with 0.5 mL of 1 M hydrochloric acid solution. The solution was shaken thoroughly. Then, An aliquot of a standard solution (500 $\mu g\ mL^{-1}$) (3.36×10^{-4}M) containing 25-1125 μg of VHC was transferred into this series of 25 mL calibrated flasks and 1.5 mL of 1 M sodium hydroxide solution was added and the contents were diluted to the mark with distilled water and mixed well. After 10min, the absorbance of the colored azo dye was measured at 439 nm against the corresponding reagent blank.

4. Results and discussion

4.1. Absorption spectra

When a diluted aqueous solution of VHC was mixed with diazotized procaine (method A) or diazotized sulphacetamide sodium (method B) in alkaline medium, an intense yellow azo dyes formed immediately, which became stable after 10min and remain stable for 2 hour at least. The yellow products have a maximum absorption at 447 nm and 439 nm for method A and B respectively. Figure-2 shows the spectra of the products formed and the reagent blank under optimum conditions (section 4.4), the maximum absorption at 447 nm (method A) and 439 nm (method B) respectively.

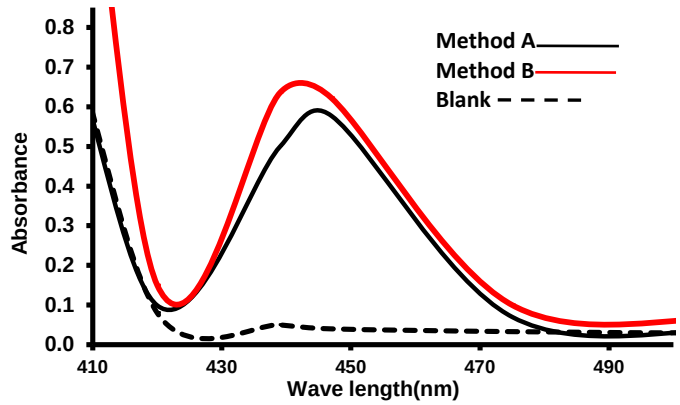


Figure 2- Absorbance spectra of VHC (20µg mL^{-1}) treated as described under procedure above (method A & B) and measured against blank, the reagent blank measured against distilled water

4.2.Optimization of the experimental conditions

The effects of various parameters on the absorption intensity of the formed products were optimized.For optimization of conditions and in all subsequent experiments, a solution of 500 µg of VHC was used and the final volume was 25 ml (i.e.20 µg mL^{-1}).

Effect of order of addition

Different orders of addition of reagents were examined and it was found that the order of addition of reagents by mixing procaine hydrochloride (method A) or sulphacetamide sodium (method B) with sodium nitrite then HCl, VHC and ammonium hydroxide (method A) or sodium hydroxide (method B) gave the highest absorbance and were used in all subsequent experiments.

Effect of type of acid used in diazotization process

The diazotization reaction should be accomplished in acidic medium. Therefore the effects of different acids solutions (1 M) were studied such as hydrochloric acid, sulfuric acid, phosphoric acid and acetic acid. It was found that hydrochloric acid was the most suitable acidic medium for a maximum absorbance and was used in all subsequent experiments.The effect of different volumes of hydrochloric acid (1M) were studied on the maximum absorbance by varying the volume of HCl between (0.5-2.5mL) and fixing the other parameters:(procaine hydrochloride (1×10^{-3} M) (method A), sulphacetamide sodium(1×10^{-3} M)(method B) ,$NaNO_2$(1×10^{-3} M) and highest absorbance was obtained with 0.5 mL of acid for both methods and was chosen for further use figure-3.

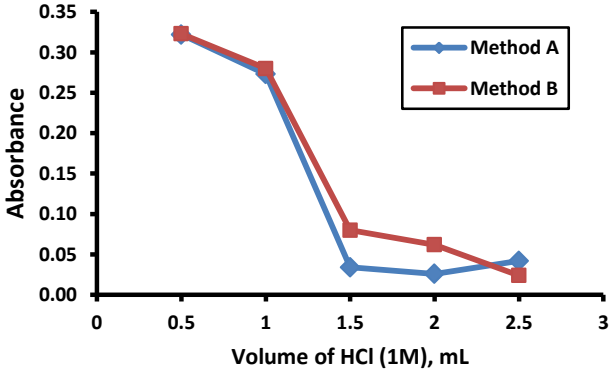


Figure 3- Effect of the volume of HCl (1M)

Effect of alkaline solution

Preliminary results indicated that the presence of an alkaline in the reaction mixture is essential for developing a more intense yellow color. The colored products were only formed in alkaline medium since VHC is converted into its salt (phenoxide ion). The later is more stable than phenol leading to a more coupling reactivity. In this respect, an alkline solutions (1M) of sodium hydroxide, potassium hydroxide, sodium acetate, ammonium hydroxide and

sodium carbonate were examined .It was found that ammonium hydroxide and sodium hydroxide were the most suitable alkaline medium for a maximum absorbance for methods A and B respectively and was used in all subsequent experiments.The effect of different volumes of ammonium hydroxide (1M) or sodium hydroxide (1M) was studied on the maximum absorbance by varying the volume of the base solution between (0.5-3mL) with fixing the other parameters. A volume of 1.5mL of ammonium hydroxide (1M) or sodium hydroxide (1M) for methods A and B respectively were enough to obtain the maximum absorbance figure-4.

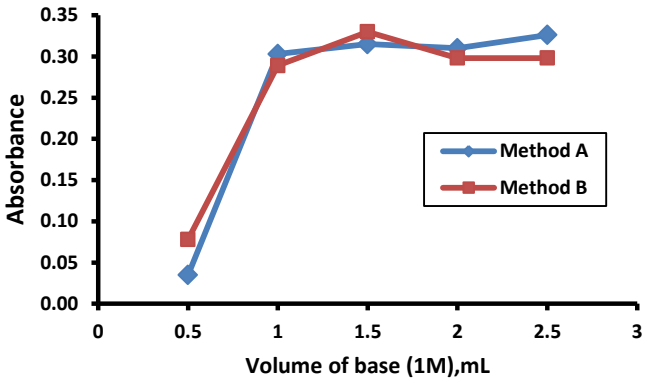


Figure 4- Effect the volume of NaOH and NH_4OH (1M)

Effect of diazotized reagent concentration

Effect of reagents (procaine hydrochloride (1×10^{-3}M) (method A), sulphacetamide sodium (1×10^{-3}M) (method B) were studied in the range of (0.25-2.5mL) with fixing the volumse of HCl , NH_4OH and NaOH.The greatest absorbance intensity were obtained with 0.5mL of procaine hydrochloride and 1 mL of sulphacetamide sodium for both methods respectively, figure-5.

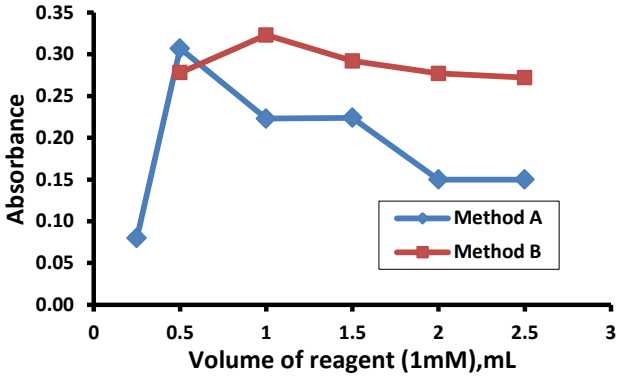


Figure 5- Effect of the volume of reagent (mL)

Effect of reaction time

The resultant colored products of the proposed methods were found to be formed rapidely and immediately,but the color intensity reached a maximum after VHC solution had been reacted with diazotized procaine hydrochloride (method A), diazotized sulphacetamide sodium (method B) in alkaline medium for 10min, therefore a 10 min development time was selected as optimum in the general procedures. The color obtained was stable for 2hr.

4.3. Compositions of the products

The composition of the formed complexes between VHC and diazotized procaine hydrochloride (method A), diazotized sulphacetamide sodium (method B) had been established under the recommended optimum conditions by using continuous variation and mole ratio methods[14].

4.3.1.Determination of the composition of the azo dyes in solution:

Mole-ratio method

In method A or B, a 0.5mL of (1× 10^{-3} M) VHC solution was transferred into a series of 25mL volumetric flask. To this solution 0.05,0.1,0.25,…….1.5mL of (1× 10^{-3} M) diazotized procaine (or diazotized sulphacetamide sodium for method B) and 1.5mL of 1M ammonium hydroxide solutions (or 1.5 mL of 1 M sodium hydroxide solution for method B) were added, the contents were diluted to the mark with distilled water and mixed well. After 10min for both methods, the absorbance of the colored azo dye was measured at 447 and 439 nm for both methods respectively against the corresponding reagent blanks.

Continuous variations method

In method A or B, different volumes (0-5 mL) of VHC solution (1× 10^{-3} M) were transferred to separate 25mL volumetric flask. To each flask, respectively was added 5,4,3,2,1,0 mL of the (1×10^{-3} M) diazotized procaine (or diazotized sulphacetamide sodium for method B) and 1.5mL of 1M ammonium hydroxide solutions(or 1 M sodium hydroxide solution for method B) were added, the contents were diluted to the mark with distilled water and mixed well. After 10min for both methods, the absorbance of the colored azo dye was measured at 447 and 439 nm for both methods respectively against the corresponding reagent blanks.

The results obtained in figure-6 and figure-7, show that a 1:1 azo dye was formed between VHC and diazotized reagents for both methods at 447nm and 439 nm respectively.

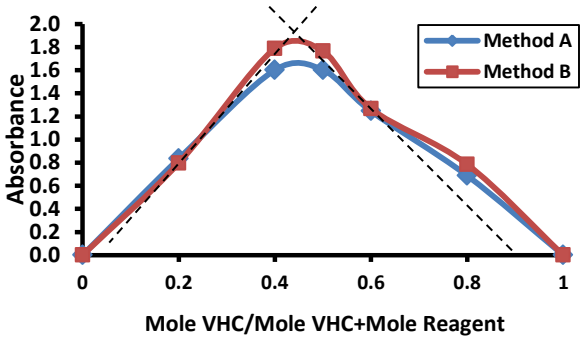


Figure 6- Continuous variation plot

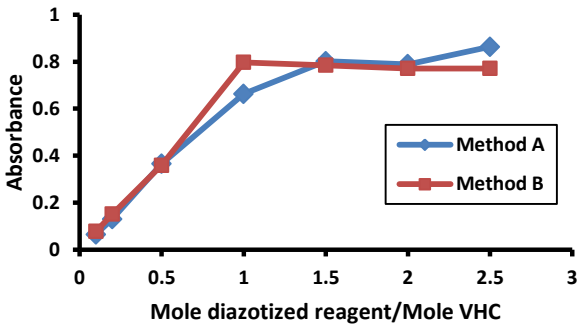
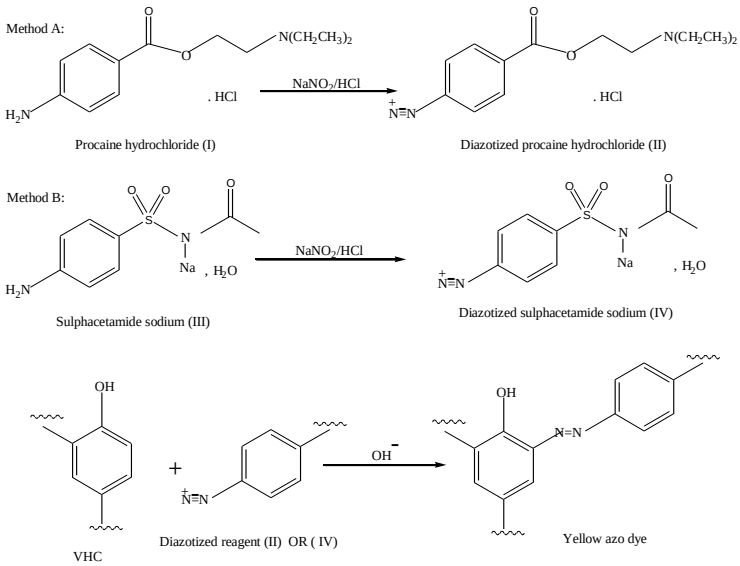


Figure7-Mole ratio plot

Diazotization coupling reactions are usually occurs in two steps one of them is reaction of amino reagent (procaine or sulphacetamide sodium) with sodium nitrite in an acidic medium producing the diazo compound and the other one is a coupling between diazotized reagent and the phenolic drug. Due to the phenolic nature of VHC, it can readily be coupled with a chromogenic reagent (diazotized reagents) according to scheme1. The yellow dye product was only formed in alkaline medium (ammonium hydroxide or sodium hydroxide for methods A and B respectively) since VHC is converted into its salt (phenoxide ion). The later is more stable than phenol (resonance) leading to a more stable intermediate with diazotized reagents .The reaction schemes are given below [15]:



Scheme 1: proposed mechanism of the reaction between VHC and diazotized procaine and diazotized sulphacetamide sodium

4.4. Selected optimum conditions

The unvaried optimization method which was used above to study the effect of variables on the absorbance intensity that gave the optimum conditions in both methods were shown in table-1.

Table 1- The optimum conditions for the determination of VHC using methods A and B

Variable	Studied range	Optimum value	
		Method A	**Method A**
Order of addition	Different orders	Procaine HCl+$NaNO_2$ +HCl+VHC+NH_4OH	Sulphacetamide.Na+$NaNO_2$ +HCl+VHC+NaOH
Volume of HCl(1M),mL	0.5-2.5	0.5	0.5
Type of base	Different bases	NH_4OH	NaOH
Volume of Base(1M),mL	0.5-3	1.5	1.5
Diazotized reagent(1×10^{-3}M).mL	0.25-2.5	0.5	1

4.5. Analytical characteristics of spectrophotometric method

For the proposed methods and under the optimum conditions table-1, the calibration graphs, figure-8 & 9, for VHC were obtained by the procedures described previously in which a series of standard solutions were analyzed in triplicates to test the linearity. The slope (a), the intercept (b), the correlation coefficient (r) and the correlation of determination (r^2) were evaluated by a least-squares regression analysis and are included in table-2. The obtained r value is highly significant. Statistical evaluation [16] of the regression line gave the values of standard deviations for residuals (Sy/x), slope (Sa), intercept (Sb) at 95% confidence are shown in the same table. These small figures point out to the high precision of the proposed method.In addition both methods have a good sensitivity (limit of detection (LOD) were 0.51 and 0.67 µg mL^{-1} for methods A and B respectively)and also a good precision (RSD less than 2.9 for both methods).

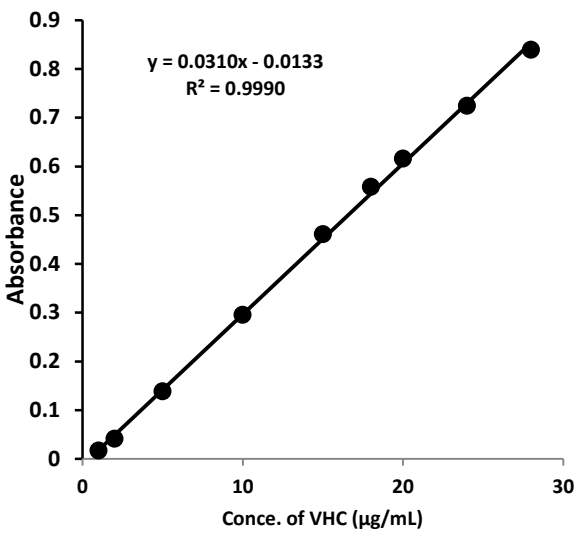


Figure 8- Calibration graph of VHC for method A

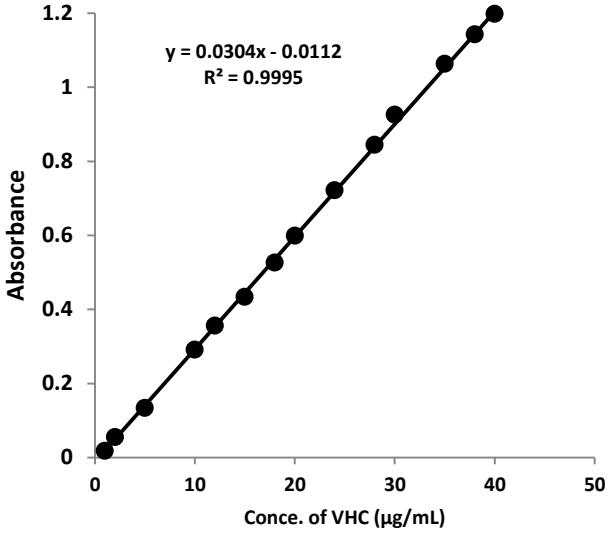


Figure 9- Calibration graph of VHC for methodB

Table 2- Analytical parameters of spectrophotometric methods

Parameters	**Method A**	**Method B**
λ_{max} (nm)	447	439
Linearity range, µg mL^{-1}	1-28	1-45
Molar absorbtivity,ε (L mol^{-1} cm^{-1})	4.605×10^{4}	4.516×10^{4}
Sandell's sensitivity(µg mL^{-1})	0.032	0.033
Regression equation	y = 0.0310x - 0.0133	y = 0.0304x - 0.0112
Correlation coefficient, r	0.9995	0.9998
Linearity percentage, r^2%	99.900	99.960
Slope, b	3.096×10^{-2}	3.039×10^{-2}
Intercept, a	-1.327×10^{-2}	-1.1203×10^{-2}
Standard deviation of the residuals, $S_{y/x}$	1.010×10^{-2}	9.35322×10^{-3}
Standard deviation of the slope, S_b	3.6696×10^{-4}	1.7608×10^{-4}
Standard deviation of the intercept, S_a	6.04096×10^{-3}	4.1433×10^{-3}
Relative standard deviation (RSD%)	<2.9	<1.6
Average of recovery%	99.81	100.22
Stability (hr)	2	2
Molar ratio (D:R)	1:1	1:1
Limit of detection(µg mL^{-1})	0.51	0.67

The validity of the method was evaluated by Statistical evaluation of the regression lines. The small values of the standard deviation of the residuals ($S_{y/x}$), standard deviation of the intercept (S_a) and standard deviation of the slope (S_b) pointed out to the high precision of the proposed method and low scattering of the points of the calibration curve and high accuracy.

5. Pharmaceutical applications

The proposed methods were applied for the determination of VHC in its pharmaceutical forms by the analysis of two concentrations for each sample using the recommended procedures. Solutions of pharmaceutical forms were prepared as given under the section 2.2, and the results obtained are summarized in table-3. The applicability of the proposed methods for the determination of VHC in commercial dosage forms were statistically compared with those obtained by the British Pharmacopoeia procedure(HPLC method)[16] and also compared with each other using the Student t-test and variance ratio F-test at 95% confidence level [17]. The results obtained are summarized in table-4 and table-5. In all cases, the calculated F and t values for comparison between proposed methods and classical method and also that obtained from comparison of two methods with each other, did not exceed the theoretical values, indicating that there is no significant difference between the performance of the two methods as regard accuracy(t-test) and precision (F-test).

Table 3- Application of the proposed methods for determination of VHC in pharmaceutical forms

Proposed method	Drug form	Conc. of VHC,µg mL^{-1}		E%*	Rec.%*	RSD%*
		Present	Found			
Method A	Vancolon / Injection Julphar UAE-500 mg	5.000	4.966	-0.685	99.315	2.076
		10.000	9.979	-0.213	99.787	3.393
	Vondem/ Solution For Infusion DEMO S.A. Greece-500mg	5.000	5.029	0.599	100.599	1.575
		10.000	10.155	1.553	101.553	2.595
Method B	Vancolon / Injection Julphar UAE-500 mg	5.000	5.009	0.176	100.176	3.003
		10.000	9.932	-0.685	99.315	1.951
	Vondem/ Solution For Infusion DEMO S.A. Greece-500mg	5.000	5.112	2.229	102.229	1.483
		10.000	10.031	0.309	100.309	1.062

***Average of four determinations**

Table 4- The comparison of the proposed methods with standard method using t- and F-statistical tests

Drug form	Proposed method				Standard method	
	Method A		Method B			
	Rec.% $(Xi)_1$	$(Xi - \bar{X})_1^2$	Rec.% $(Xi)_i$	$(Xi - \bar{X})_i^2$	Rec. $(Xi)_2$	$(Xi - \bar{X})_2^2$
VHC pure	99.811	0.1122	100.413	0.0039	100.000	2.3013
Vancolon(Injection)/UAE	99.551	0.3540	99.746	0.5329	102.697	1.3924
Vondem(Solution For Infusion)/Greece	101.076	0.8649	101.269	0.6289	101.853	0.1129
S**	1.133(S_1^2=0.666)		1.115(S_2^2=0.583)		(S_2^2=1.903)	
t (2.776)*	1.481		1.143		$(n_1 + n_2 - 2) = 4$	
F (19.000)*	2.859		3.265		n_1= 3 , n_2=3	

***Table value.**

****s = pooled standard deviation**= $\sqrt{\frac{(n_1-1)S_1^2+(n_2-1)S_2^2}{n_1+n_2-2}}$,t = $\frac{|\bar{X}_1-\bar{X}_2|}{S\sqrt{\left(\frac{1}{n_1}+\frac{1}{n_2}\right)}}$, $S_1^2 = variation = \frac{\sum(Xi-\bar{X})_1^2}{n_1-1}$ *and* $S_2^2 = \frac{\sum(Xi-\bar{X})_2^2}{n_2-1}$

Table 5- The comparison between the proposed methods A and B using t- and F-statistical tests

Drug form	Proposed method	
	Method A	Method B
	Rec.% $(Xi)_1$	Rec.% $(Xi)_i$
VHC pure	99.811	100.413
Vancolon(Injection)/UAE	99.551	99.746
Vondem(Solution For Infusion)/Greece	101.076	101.269
S	0.790	
t (2.776)*	0.591	
F (19.000)*	1.142	

***Table value.**

6.Conclusions

Until now the literature contains only a few spectrophotometric methods for determination of VHC. This work offers new spectrophotmetric methods using other drugs (procaine and sulphacetamide sodium) as chromogenic agents. The developed methods are very simple and adequate for the determination of VHC in aqueous solution and in pharmaceutical preparation samples at a concentration level of traces (µg mL^{-1}) and without requiring any previous separation step, a temperature or a pH control .Moreover, the proposed procedures are very economical and cheap especially when compared to other methods such as fluorimetry, electrosensors and capillary electrophoresis and have a good linearity and sensitivity. Finally, the proposed method was applied to the analysis of VHC in injections, and statistical analysis reveals that there is no significant difference in precision and accuracy between the proposed methods and the official method.

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