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Validated stability Spectrophotometric process for estimation vancomycin antibiotic degradation behavior in pharmaceutical kinds

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Abstract

Objective: To estimate the dissolution behavior of the antibiotic vancomycin hydrochloride in pharmaceutical formulations, a novel, quick as well as innovative stabilization implying spectrophotometric process has been created and certified. Using the spectrophotometric process to calculate the constant acid dissociation (Ka).

Material and methods: The process focused on the observed drug oxidation by a documented excess of Ceric ammonium nitrate in hydrochloride acid solution as well as corresponding identification of unreacted oxidant by combining it with methylene blue respectively. The oxidant reacted correlates to the substance of the compound. As well as wave length absorbance calculation 588 nm. This approach follows Beer-Lambert plot regression showed remarkable concentration range 1-50 ppm associations. It was a coefficient of correlation of 0.9982. Calculated the limits of molar absorptivity 6.9117× 104 (L . mol-1. cm-1) , sandal sensitivity 0.02 µg. cm-2, identification 0.410 ppm, as well as quantification 0.731 ppm. The method's precision and accuracy was established and checked.

Results: The stoichiometric proportions were analyzed for the aforementioned product. This measured the growing conditions of reaction as well as other analytical variables. The technique has decent repeatability with little than two percent relative standard deviation (RSD percentage).

Conclusion: The impact of the material typically often used in such medications as excipients was examined. The suggested technique have been implemented in pharmaceutical products to assess such drugs. The findings showed that the technique is as reliable and repeatable as the approved technique.

Keywords: validated stability, spectrophotometric, vancomycin, antibiotic, pharmaceutical forms

ДӘРІЛІК ФОРМАЛАРДАҒЫ ВАНКОМИЦИН АНТИБИОТИКТЕРІНІҢ НАШАРЛАУЫН БАҒАЛАУҒА АРНАЛҒАН ТҰРАҚТЫЛЫҚТЫҢ СПЕКТРОФОТОМЕТРИЯЛЫҚ ПРОЦЕСІ

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ТҰЖЫРЫМДАМА

Мақсаты: Антибиотик ванкомицин гидрохлоридінің ерітіндінің қасиеттерін фармацевтикалық құрамда бағалау үшін спектрофотометриялық процесстің қатысуымен жаңа, жылдам және инновациялық тұрақтандыру процесі құрылды және сертификатталды. Қышқылдың диссоциациялану тұрақтысын (Ка) есептеу үшін спектрофотометриялық процесті қолдану.

Материалдар мен тәсілдер: Бұл процесс препараттың тұз қышқылының ерітіндісіндегі церий-аммоний нитратының құжатталған мөлшерден асып кетуімен, сонымен қатар оны метилен көкпен сәйкестендіру арқылы реакцияланбаған оксиданттың сәйкестендірілуіне бағытталған.

Әсет ететін тотықтырғыш қосылыстың затымен байланысады. Жұту толқыны ұзындығын есептеу 588 нм құрайды. Бұл тәсіл Бер-Ламберт кестесінің регрессиясына сәйкес келеді, ол миллионға 1-50 бөліктерге шоғырланудың керемет ауқымын көрсетті. Бұл корреляция коэффициенті 0,9982 болды. Молярлық жұту қабілетінің шегі есептелді: 6.9117×104 (литр моль-1 см-1), Санделге сәйкес сезімталдығы 0,02 мкг. см-2, идентификациясы 0,410 б/млн, ал сандық көрсеткіші 0,741 б/млн. Әдістің дәлдігі мен сенімділігі анықталды және тексерілді.

Нәтижелер: Жоғарыда аталған өнім үшін стехиометриялық пропорциялар талданды. Реакцияның өсу шарттары, сондай-ақ басқа аналитикалық айнымалылар өлшенді. Бұл әдіс салыстырмалы стандартты ауытқумен екі пайыздан (салыстырмалы стандартты ауытқудың пайызы) сәйкес лайықты жаңғыртылуға ие.

Қорытынды: Қосымша заттар сияқты препараттарда жиі қолданылатын материалдың әсері зерттелді. Ұсынылған әдістеме фармацевтикалық препараттарға осындай дәрі-дәрмектерді бағалау үшін енгізілген. Нәтижелер көрсеткендей, әдіс бекітілген әдіс секілді сенімді және жаңғыртылуға жатады.

Негізгі сөздер: дәлелденген тұрақтылық, спектрофотометрикалық, ванкомицин, антибиотик, дәрілік форма

СПЕКТРОФОТОМЕТРИЧЕСКИЙ ПРОЦЕСС ПОДТВЕРЖДЕННОЙ СТАБИЛЬНОСТИ ДЛЯ ОЦЕНКИ УХУДШЕНИЯ ХАРАКТЕРИСТИК АНТИБИОТИКОВ ВАНКОМИЦИНА В ЛЕКАРСТВЕННЫХ ФОРМАХ

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РЕЗЮМЕ

Цель: Для оценки свойств растворения антибиотика гидрохлорида ванкомицина в фармацевтических составах был создан и сертифицирован новый, быстрый и инновационный процесс стабилизации, подразумевающий спектрофотометрический процесс. Использование спектрофотометрического процесса для расчета постоянной кислотной диссоциации (Ка).

Материалы и методы: Данный процесс фокусировался на наблюдаемом окислении лекарственного средства документированным избытком нитрата церия-аммония в растворе хлористоводородной кислоты, а также на соответствующей идентификации непрореагировавшего окислителя путем объединения его с метиленовым синим, соответственно. Реагирующий окислитель коррелирует с веществом соединения. Расчет длины волны поглощения составляет 588 нм. Данный подход соответствует регрессии графика Бера-Ламберта, показавшей замечательный диапазон концентрации 1-50 частей на миллион. Это был коэффициент корреляции 0,9982. Рассчитаны пределы молярной абсорбционной способности 6,9117×104 (л. моль-1. см-1), чувствительность по Санделу 0,02 мкг. см-2, идентификация 0,410 ч/млн, а также количественная оценка 0,731 ч/млн. Точность и достоверность метода были установлены и проверены.

Результаты: Стехиометрические пропорции были проанализированы для вышеупомянутого продукта. Измерены условия роста реакции, а также другие аналитические переменные. Методика имеет достойную воспроизводимость с относительным стандартным отклонением менее двух процентов (процент относительного стандартного отклонения).

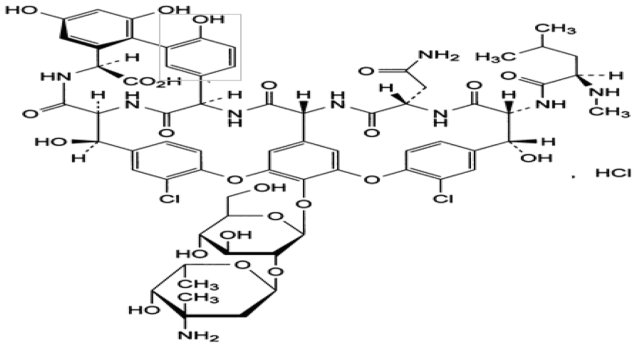
Заключение: Изучено влияние материала, обычно используемого в таких препаратах, как вспомогательные вещества. Предложенная методика была внедрена в фармацевтические препараты для оценки таких лекарств. Результаты показали, что методика такая же надежная и воспроизводимая, как и утвержденная методика.

Ключевые слова: подтвержденная стабильность, спектрофотометрический, ванкомицин, антибиотик, лекарственная форма

Introduction

Vancomycin (VAN) is an antibiotic glycopeptide obtained from Streptomyces orientalis as well as Nocardia lucida (Figure1).

Figure 1 - Chemical structure of vancomycin HCl



It was first used in the 1950s to combat staphylococcal illnesses and was rapidly confined to the position of replacement medication given the high occurrence of erotogenic effects and ototoxicity [1]. However, the increasing number of Staphylococcus infections as well as Streptococcus, immune to different antibiotics, have led to regeneration of vancomycin. In fact, the majority of particles were clear of fresh preparation as well as showed limited side effect [2]. Nonetheless, if serum concentrations were maintained beneath 40 mg/l and therapeutic drug management (TDM) was required, it was generally assumed that drug toxicity could be prevented, vancomycin is a first edical glycopeptide antibiotic clinic in 1958. Elucidated at the middle of the 70's, is tricyclic as well as the molecular weight was around 1500Da. Vancomycin works toward gram-positive coccus as well as bacillus inhibiting the biosynthesis of the mucopeptide membrane, contributing to a stagnation of productivity [3]. In particular, RNA composition is prevented. Vancomycin is immune to proteolytic enzyme activity related to molecular complexity [2,4]. Several techniques for determining vancomycin have been developed, including capillary electrophoresis [5,6], HPLC [7-11]. Given its reduced cost as well as broad variety of applications, the right technique.

Evaluation on conventional HPLC, furthermore, liquid chromatography processes with fluorescence detection [12], ultraviolet detection [13-15], mass spectrometry detection [16,17] as well as electrochemical detection [18], other chemical methods also utilized for the assay of VAN drug like radioimmunoassay [19], flow injection analysis [20], fluorescence polarization immunoassay [21], polarography [22] additionally few spectrophotometric processes have been for VAN drug determination as pure drug and in different pharmaceutical bands of VAN drug [23-26]. This article outlines spectrophotometric approach for assessing VAN by oxidizing the product analyzed by a documented excessive of Ceric ammonium nitrate in the solution of hydrochloride acid as well as corresponding measurement of unreacted oxidant by interacting with methylene blue simultaneously. Such reagents, which are helpful for estimating the deterioration activity of vancomycin hydrochloride antibiotics in pharmaceutical products because they also developed reliable and fast organic binding products, are easily accessible, easily filtered and are dissolved in ethanol and are therefore deemed to be a green process.

Material and methods

Chemicals, materials as well as specimens, typical bulk specimens of antibiotic vancomycin hydrochloride (purity 100%) were obtained as a donation test from SDI (State Drug as well as Clinical Appliances Organisation) Samara-Iraq. This solution was compelled by disbanding 0.025g of vancomycin in 100ml of deionized water in the volumetric flask solution Ceric ammonium nitrate 0.001M. Evaporating prepared this solution 0. 0177g of ceric ammonium nitrate (Fluke) in a volumetric flask of 100ml distilled water. Chemicals Ltd solution of methyl blue dye (BDH), 1.6×10-3M: this formula was formulated by disintegrating 0.005g in 100ml of deionized water in the volumetric flask, diluting 10ml for the above solution to 100ml in a volumetric flask with distilled water. Hydrochloric acid eluent (BDH) Chemicals Ltd 2M. The remedy was formed by diminishing 16.4 ml of condensed HCl in the volumetric flask into 100ml of distilled water.

Equipment and apparatuses

Spectrophotometric calculations were performed on UV-visible double beam spectrophotometer (UV-1800, Shimadzu Corp., Japan) connected to computer enforced UV probe 2.0 software with an adjusted spectral bandwidth of 2nm and 10mm.

Table 1 Tested pharmaceutical preparation for vancomycin hydrochloride

Company preparation	Composition	Pharmaceutical
Zermacin	Per vial 0.5 g Vancomycin hydrochloride	(Arwan pharmaceutical Industries Lebanon S.a.l, jadra, Lebanon)
Vancomicina	Per vial 1g Vancomycin hydrochloride	Barcelona, spain
Vancolon	Per vial 0.5 g Vancomycin hydrochloride	julphar, Gulf pharmaceutical Industries, Ras Al Khaimah, (U.A.E.)

Quartz cells have been utilized for analytical system production across the 200-700nm scope. Medicines as well as substances were weighted on electronic analytical weighted equilibrium Susceptible balance (BL 210S from Sartorius, Thermal-cooling of the water bath (Haake, Fe3).

Assay procedure for pharmaceutical preparations

A handful of vancontaining preparations were evaluated as an active ingredient. This is summarized in Table 1, 2 [27].

General procedure and calibration

Suitable aliquots of antibiotic operating standard solutions for vancomycin hydrochloride were inserted into a system for 25ml volumetric flasks with 250ppm. Instead 1ml of HCl 2M as well as 1ml of ceric ammonium nitrate 0.001M were applied to the solutions for 25 minutes. At room temperature 25ml, eventually add 2ml of methyl blue, instead diminish the flasks to the distilled water mark after 5min.

Around 800-200nm, spectra for the solutions were scanned. The absorption of solutions toward distilled water was calculated at 588nm as water blank. The processed a certain way, but it did not contain any substance [28].

Standard solution preparation and calibration graph

To achieve a concentration of 250ppm, stock solution was produced by disintegrating 250mg of VAN in 100ml of deionized water. Significant volumes were collected from the inventory solution to create the calibration as well as weaken in 25ml volumetric flask simultaneously. The absorbance of each specimen was evaluated on a UV visible spectrophotometer at λmax, towards a blank solution. Perform the latter process for three times as well as estimate a median of three absorbance levels. Taking the notes, the calibration curve was charted using the X-axis VAN concentration as well as the subsequent Y-axis absorption (Figure 2).

Figure 2 - Calibration curve of VAN assay

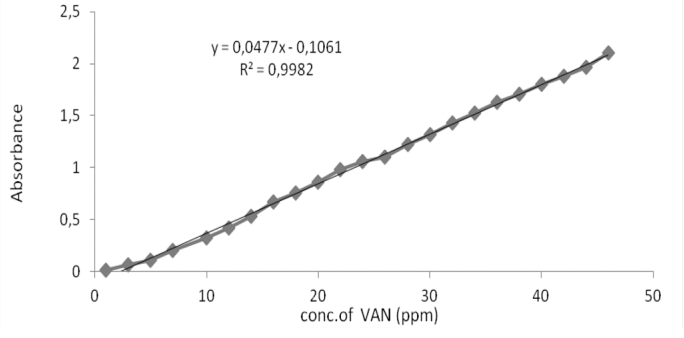


Table 2 Solvent influence on the absorbance

Solvent	Absorbance	ε ,L.mol-1.cm-1
chloroform	0.332	6.8001× 102
ethanol	0.711	3.5290× 104
methanol	0.651	1.1997× 104
dichloromethane	0.221	2.9247× 103
dimethylsulphoxide	0.231	6.9117× 104
acetone	0.432	1.2610× 103
acetonitrile	0.520	1.8017× 102
Dimethyl formamide	0.421	2.8210× 103
Teri butyl alcohol	0.542	1.3401× 104
Dimethyl sulphoxide	0.188	1.6022× 103
Formic acid	0.259	1.2721× 102
2- propanol	0.471	0.9605× 104
Di ethyl ether	0.302	1.2551× 103
Benzene	0.171	0.1027× 101
Water	0.738	6.9117× 104

Determination in pharmaceutical preparations

The pharmaceutical preparations were received in different kinds (vials) from official sources. Reliably measured amounts of compounded vials, vials or equipment were moved to 100ml volumetric flasks for a loan of 250 mg/l of drugs. Otherwise the spectrophotometric technique implemented was carried out on aliquot sections of the consequent specimen solutions [29].

Solvent effect

Table 2 used both polar as well as nonpolar solvents to choose tasteful solvents for drug evaluation. Water is discovered to be an appropriate solvent for Van, producing optimum absorbance with a specified concentration of drugs, while another solvents generated reduced absorption due to improper complex dissociation [30].

Reaction time influence

Ceric ammonium nitrate engagement with drug stemmed in complex creation that stabilized within 25 minutes of stirring. Starting to add blue methylene after 5 minutes (Table 3). Then the flasks watered down to the limit with distilled water then Spectra was digitized for solution at optimum absorption. The established color stayed stable at room temperature for almost one hour. After 3 days both those solutions are given independence [31].

Validation of the proposed method

The approaches generated were evaluated in terms of international guidelines viz., selectivity, specificity, linearity, LOD, LOQ, precision, accuracy, Shandell's sensitivity as well as reliability (Table 4). The accuracy is tested by rehashing every other project at least 6 times whilst the accuracy was evaluated by

Table 3 The effect of time on oxidation and bleaching of the dye

Time of oxidant addition min	Standing time before dilution, min.							
	5	10	15	20	30	40	50	60
5	0.490	0.566	0.433	0.661	0.455	0.526	0.582	0.525
10	0.716	0.516	0.510	0.705	0.571	0.629	0.592	0.565
15	0.722	0.522	0.620	0.719	0.611	0.620	0.624	0.620
20	0.726	0.675	0.685	0.662	0.645	0.665	0.661	0.652
25	0.733	0.711	0.689	0.677	0.661	0.623	0.678	0.681
30	0.721	0.701	0.692	0.688	0.645	0.660	0.681	0.684

Table 4 Experimental limitation qualities from the consistent method of the calibration curve

Parameter	Values of method
Regression equation	Y= 0.0477X - 0.1061
Molar absorptivity	6.9117× 104 (L . mol-1. cm-1)
Correlation coefficient	0.9982
limits of Beer's law (linearity)	(1 – 50) (µg/ml or ppm)
Sandell's sensitivity	0.02 µg . cm-2
Slope,	0.0477
Limit of detection	0.410 (ppm)
Limit of quantitation	0.731 (ppm)
Intercept,	0.1061
Intraday Precision RSD*	% 0.917
Inter day Precision RSD*	% 1.208
Accuracy(% mean recovery)*	% 99.450

Where * average of six determinations

bringing established test weight as well as conducting recovery studies. The technique created was also used for pharmaceutical research. The recovery tests carried out produce high accuracy and precision as well as the findings are contrasted to the verified strategies published on these drugs available. The percentage beliefs of RSD, t-test additionally F-test examinations are within the allowed scope of experiments.

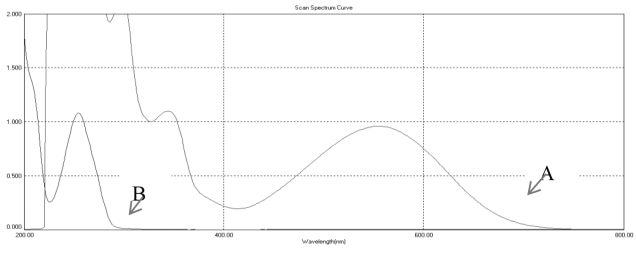
Suitable statistical evaluation was carried out to test the degree of repeatability of the methods. For the repeatability analysis, six specimens of tablet formulations were analyzed. Calculation of standard deviation and RSD percentage [32,33].

Results and discussion

Absorption spectra

When a purified aqueous solution of VAN 1ml has been combined with 1ml HCl and 1ml ceric ammonium nitrate as well as continues for 25 minutes after the combination has been introduced methyl blue with a volume of 2ml and stays for 5 minutes, an extreme blue produced spontaneously. The maximum absorption was shown at 588 nm (Figure 3) [29].

Figure 3 - (A) the maximum absorption for dye (methyl blue), (B) the maximum absorption for Drug



Optimizing of the controlled conditions

Segmentation of the effects of different factors on the absorption rate of the formulated substances. For conditions as well as all future experiments to be configured.

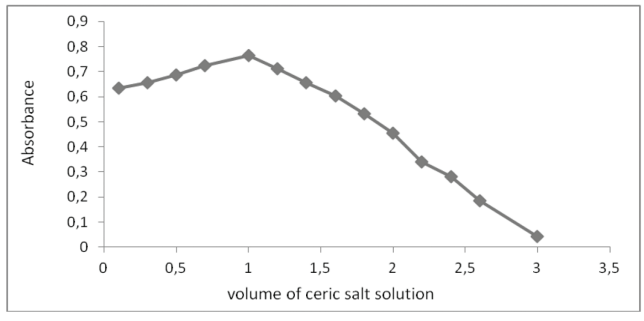
The selected dye and concentration

Provisional tests were conducted to improve the effective and optimal dye concentration (methyl blue, that can be spectrophotometric ally calculated). The findings indicate that it was found that methyl blue was a helpful reaction agent, 2ml volume from the dye was the best that given the maximum absorption with 1ml drug, 1ml ceric salt and 1ml of acidic solution [31].

Oxidant amount influence

The impact of various volumes ml of 0.001M of ceric ammonium nitrate on methylene blue dye color without Vancomycin has been reported. Figure 5 demonstrates that 1 ml of ceric ammonium nitrate solution was adequate to acquire full staining or methyl blue dye color, that given highly selectivity for the product, which it was suggested in subsequently tests [29].

Figure 5 - Demonstrates that 1ml of ceric salt solution was the prefect volume on the absorbance level



Order of addition influence

Separate orders of attachment of reagents were investigated and it was observed that the sequence of introduction of materials by combining drug and acid hydrochloride with ceric ammonium nitrate after 25min methyl blue solution that provided the strongest absorption after 5min and was used in certain ensuing tests [32].

Type of acid utilized influence in determination process

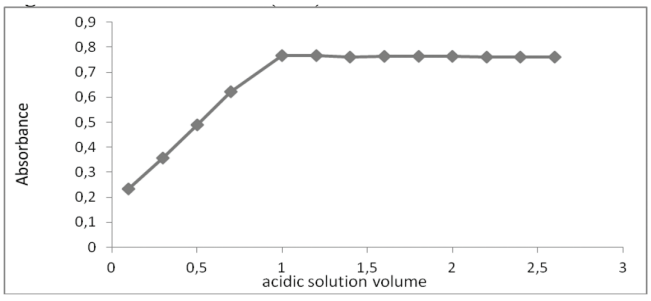
The reaction studied should be carried out in an acidic medium. The impacts of various acid solutions 2M sulfuric

acid, hydrochloric acid, phosphoric acid, nitric acid as well as acetic acid have therefore been researched. It was noticed that hydrochloric acid was used in all ensuing studies as the most appropriate acidic media for optimum absorption. In another parameters, the influence of different volumes of hydrochloric acid 2M on maximal absorbance was examined by adjusting the volume of HCl between 0.5-2.5ml as well as 1ml was the best (Table 5, Figure 6) [31].

Table 5 Acid type effect on the absorbance level

Absorbance	Acid Type
0.745	HCl
0.642	H3PO4
0.698	HNO3
0.713	H2SO4
0.511	CH3COOH

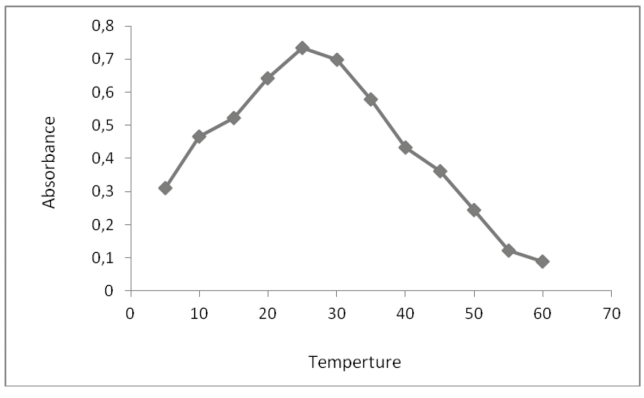
Figure 6 - Volume of HCl (ml) effect on the absorbance of color product



Temperature influence

The impact of temperature on methyl blue color strength has been reported. In reality, maximum absorption was achieved when t in the room (25oC), coloring density and stability were detected at low or high temperatures, so room temperature is indicated for subsequent tests (Figure 7) [34].

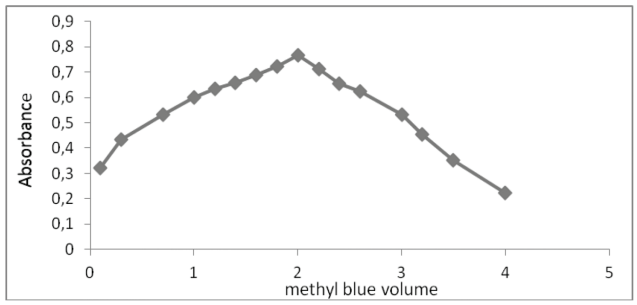
Figure 7 - Temperature influence on the absorbance of color product



Linearity

The calibration curve was developed through calculating the absorption of vancomycin hydrochloride serial dilutions in distilled water as shown in Figure 4. Vancomycin dilutions were primed in the 1-50ppm concentration range, Beer-Lambert law was compiled in the concentration scope being observed. The calibration curve regression equation was Y=0.477X-0.1061 with a correlation coefficient value of 0.9982, as well as the

Figure 4 - Demonstrates that 2ml of methyl blue color was the finest volume



Y-Intercept standard deviation was discovered to be 0.1061 as shown in Table 4. A correlation coefficient (R2) level for the calibration curve with 0.9982 is called linear. The relative standard deviation levels (percentage RSD) of range 2 are deemed acceptable [22], these levels are shown in Table 4.

Repeatability (precision of intra-assay) to examine the level of repeatability of the processes, prefect statistical assessment was carried out. Six specimens of the vials formulations were analyzed for the repeatability test. The standard deviation additionally % RSD was calculated [35].

The reaction stoichiometry

The stoichiometry of the reaction between VAN as well as the oxidant ceric ammonium nitrate was evaluated utilizing Job's method as well as method of the mole ratio; the conclusions drawn that 1:1 drug to oxidant complex created, the unreacted ceric ammonium nitrate reacting with the methyl blue dye and measuring the absorbance at 588 nm. The product established in distilled water was soluble in the acidic medium according to the process studied [30].

Method for pKa estimation

The dependent or evident stabilization constant of the 1:1 (D:R) substance was tested as obeys: two collections of solutions were formulated, the first collection of solutions was designed to include stoichiometric quantities of the phenolic drug (VAN) to the oxidant. The second set was produced with an oxidant reagent fivefold.

Phenolic product (D) association with the oxidant reagent (R)

D + R → DR

proceeds according to the equation:

and K= [DR]/[D][R]; where K= stability constant

If α is the level of dissociation as well as C is the product's molar concentration (M) equal to the phenolic drug concentration, the latter equation may be as obeys:

K= [1-α]C/[αC][αC] ……(1)

= (1- α)/ α2C ……..(2)

Provided α=Am-As/Am; where Am and As involve an excess and stoichiometric quantity of oxidant reagent, accordingly. The impact of temperature on the stabilization constant was analyzed as well as the findings showed that the stabilization constant levels at room temperature 25ºC were greater than that of 45 ºC and 5 ºC (Table 6), suggesting that

Table 6 Stability constant at different temperature

Temp., Co	Am*	As*	α	C (M)	l1.mol-
5	0.519	0.401	0.227	2.5×10-4 M	6.03×104
25	0.679	0.548	0.192		8.78 ×104
45	0.577	0.372	0.355		2.04×104

a highly stable compound was produced 25ºC. VAN as well as an appropriate amount reagent 1ml of 2.5×10-4M of and other methyl blue solution with five times the concentration of the principal concentration. Under the rendered experimental circumstances, the stable average of the coloring component in water was 5.61 ×104 11.mol-1 [36,37].

Average of three determinations

Additives influence

Additives studies included talc, acacia, lactose, sucrose, magnesium stearate, starch, glucose, aspartate, polyvinylpirrolidone PVP, mannitol, Vitamin C as well as benzoic acid. There is no impact on the observations, the solution VAN was established in this operation and each of the additives was acquired individually at concentrations ten times higher than VAN by the same method in the Calibration Curve 2ml of 250ppm VAN additionally 2ml. For each form of additives, dilution to the volumetric flask mark 25ml was introduced for this test. The level of interference should be appropriate if the error was not more than ± 2% as predicted No interference was shown in the VAN test protocol in the additives being tested (average of three examinations) (Table 7) [38,39].

Table 7 Estimations of VAN 20 ppm in the existence of Additives

Additives	% Error	% Recovery
Sucrose	+1.370	101.370
Glucose	- 0.740	99.260
starch	- 1.010	98.990
Glycerin	- 1.230	98.770
Aspartate	+ 1.460	101.460
mannitol	- 1.600	98.400
lactose	-.1.110	98.890
magnesium stearate	+ 1.210	101.210
Vitamin C	+1.620	101.620
benzoic acid	-1.250	98.750
PVP	+ 1.210	101.210
Talc	- 1.310	98.690
Acacia	- 1.330	98.670

Application of the procedure

The processes were used to study the medicine's assay of pharmaceutical preparations. Concerning the examination of affordable formulations, the following Table 8 appears that.

Table 8 Assessment of VAN was conducted in pharmaceutical products as well as in combination with the official system

Pharmaceutical preparations containing VAN	Average recovery % average RSD%			
	Proposed procedure		Standard procedure	
Pure VAN	99.450	0.917	100.550	1.310
Vancomycin hydrochloride Vial (0.5g) (Lebanon) VAN	99.660	1.030	98.560	1.450
Vancomycin hydrochloride Vial (1g) (Spain)VAN	100.310	0.930	99.170	1.291
Vancomycin hydrochloride Vial(0.5g) (U.A.E)VAN	100.440	1.071	100.290	1.225

Wherever the three normal determinations, also, the standard strategy was gotten from (2009) British Pharmacopeia. The outcomes were Replicable and the determination procedures of formulations were through examined by the Standard procedure the studied approaches was felicitously utilized to estimate VAN as pure form and in its pharmaceuticals types. The outcomes given were checked statistical comparison by an F-test variance ratio for precision additionally for accuracy by utilizing Student's t-test with the common approach [5]. The outcomes seen that the values of the F-test additionally t-test were (F=1.33, t=0.47) for medicine lower than the fundamental value (F=9.28 , t=2.45), there was no clear distinction between the tested approaches and common approaches (Three estimations average). Ultimately, F-test as well as t-test values (statistical analysis) seem that there is no considerable variation in accuracy between the tested procedure additionally the common BP procedures. [2,40,41].

Conclusion

The experimental spectrophotometric procedure for determining pKa (i.e. acid dissociation constant) of VAN drug was considered to be quick, sensitive, precise, accurate as well as economical. The concentration drug form was defined utilizing absorbance principles, absorptivity values and continuous equation. The process uses readily available and inexpensive solvent for evaluation, therefore the procedure was also cost-effective. For linearity, accuracy, reproducibility, precision, LOQ, LOD and robustness the system was further tested. The approach could therefore be used efficiently for daily analysis.

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