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Article

Application of redox reactions for the determination of valganciclovir hydrochloride in pharmaceuticals

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Two simple, selective and sensitive spectrophotometric methods were developed and validated for the determination of valganciclovir hydrochloride (VLGH) in pure drug and tablets. The first method was based on the reduction of iron(III) to iron(II) by VLGH and subsequent formation of iron(III)-ferricyanide complex (Prussian blue) in acid medium which was measured at 730 nm (method A). In the second method (method B), permanganate was reduced by VLGH to bluish green manganate in alkaline medium and the absorbance was measured at 610 nm. The absorbance measured in each case was related to VLGH concentration. The experimental conditions were carefully studied and optimized. Beer's law was obeyed over the concentration ranges of 2.5-20.0 and 2.0-40.0 μg mL^{-1} for method A and method B, respectively, with corresponding molar absorptivity values of 1.28×10^4 and 6.88×10^3 L mol^{-1} cm^{-1}. The limits of detection (LOD) and quantification (LOQ) were 0.11 and 0.33 μg mL^{-1} (method A) and 0.21 and 0.64 μg mL^{-1} (method B). Within-day and between-day relative standard deviations (%RSD) at three different concentrations levels were < 2.4%, and the respective relative errors (%RE) were ≤ 3%. The proposed methods were successfully applied to the determination of VLGH in tablets, and the results confirmed that the proposed methods were equally precise and accurate as the official method.

Keywords: Valganciclovir hydrochloride/determination. Spectrophotometry/method validation. Pharmaceutical formulations.

INTRODUCTION

Valganciclovir hydrochloride (VLGH) is the L-valine, 2-[(2-amino-1,6-dihydro-6-oxo-9H-purin-9yl) methoxy]-3-hydroxypropylester, monohydrochloride (Figure 1), the drug is soluble in water and methanol, insoluble in ether and it's available as white crystals. VLGH is the L-valyl ester of, and product for ganciclovir (Sugawara *et al.*, 2000). Cytomegalovirus infection in transplant recipients usually occurs when latent viruses from a seropositive donor organ are reactivated owing to immunosuppression (Koda-Kimble *et al.*, 2009), due to this matter; numeral drugs were developed and used for cytomegalovirus (CMV) prophylaxis after organ transplants. The most widely used drugs for this case include VLGH, ganciclovir, and acyclovir. Acyclovir drug and its prodrug valacyclovir are not approved by the Food and Drug Administration (FDA)- drugs for prophylaxis of CMV. Ganciclovir is used successfully for prophylaxis, pre-emptive treatment of CMV viremia, and also for therapy of tissue-invasive CMV disease. Unfortunately, ganciclovir has to be administered intravenously, because it is poorly absorbed after oral administration (Chang, 2008; Dagon-Topal, Ozkan, Uslu, 2007a).

VLGH is a FDA approved medication for the prevention of CMV disease in certain transplant recipients at high risk for developing CMV diseases (AIDSinfo, 2016). Activation of ganciclovir requires at first conversion to ganciclovir monophosphate form by viral enzymes: protein kinase pUL97 in CMV (Charles, Robert, 2012).

Due to its medicinal use, VLGH has been determined in pharmaceuticals and body fluids using several techniques. VLGH in blood serum has been assayed using high performance liquid chromatography technique (HPLC) (Dagon-Topal, Ozkan, Uslu, 2007b) and liquid chromarography-tandem mass spectrometry (Singh *et al.*,

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2011; Heinig *et al.* 2011; Xu *et al.*, 2006; Xu *et al.*, 2007). Very few methods for the determination of the drug in bulk and dosage forms are found in the open literature, and include UV-spectrophotometry (Konidala *et al.*, 2014), HPLC (Sanll, Sanll, Lunte, 2017; Sawant, Barge, 2014; Lakshmi, Kumara, 2013; Mathrusri, Lakshmi, Sirichandra, 2013), HPTLC (Barge *et al.*, 2011), capillary electrophoresis (Sanll, Sanll, Lunte, 2017) and voltammetry (Prashanth *et al.* 2014; Dogan-Topal *et al.* 2013; Gholivand, Torkashvand, 2016).

The previously reported methods, particularly chromatographic and voltammetric methods, require sophisticated and expensive instrumentation which are not always available in all laboratories. Despite its simplicity, cost-effectiveness and easy availability, visible spectrophotometric technique was never applied to the determination of VLGH in pharmaceuticals. The aim of the present study was, therefore, to use this technique for the determination of VLGH in bulk drug and tablets, exploiting the reducing characteristic property of the drug molecule.

Two methods presented here based on either reduction of iron(III) by VLGH in acid medium and subsequent formation of iron(II)-ferricyanide complex, which was measured at 730 nm (method A) or the reduction of permanganate in alkaline medium to intense bluish-green colored manganite with an absorption maximum at 610 nm (method B). Both the methods were demonstrated to be more convenient and facile compared to the previously reported methods.

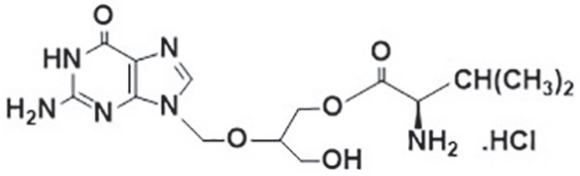

FIGURE 1 - Chemical structure of valganciclovir hydrochloride.

MATERIAL AND METHODS

Instrumentation

A Systronics model 166 digital spectrophotometer (Systronics, Ahmedabad, Gujarat, India) with 1-cm matched quartz cells was used for absorbance measurements.

Material

Pure valganciclovir HCl (VLGH) sample, certified to be 99.85% pure was donated by Apotex Pharmachem India Pvt. Ltd., Bangalore, India, as gift and was used as received. VLGH-containing tablets: Valgan (Cipla Ltd., Patalganga, Maharashtra, India), Valcyte (Roche, Chennai, India), each containing 450 mg of active ingredient were purchased from local commercial sources.

Regents and chemicals

Iron(III) solution: A 0.5% (w/v) solution of iron(III) alum (Indian Drugs and Pharmaceuticals Ltd., Hyderabad, India) was prepared in 0.1 M HCl. Potassium Ferricyanide solution: A 0.5% (w/v) solution was prepared by dissolving of 0.5 g of the chemical (Glaxo Laboratory, Mumbai, India) in water. Hydrochloric acid: Concentrated hydrochloric acid (Merck Pvt. Ltd., Mumbai, India, sp. gr. 1.18), was diluted appropriately with water to get 2 M HCl. Potassium permanganate (0.1% w/v): One gram of the pure compound (Merck, Mumbai, India) was dissolved in 50 mL of water; the solution was boiled for 15-30 minutes to remove any residual manganese (IV) ions, allowed to cool at room temperature, and then filtered. The filtrate was diluted to 100 mL (Mendham *et al.*, 2006), and standardized using the Vogel procedure (Vogel, 1961). The stock solution (1.0 % w/v) was then diluted to get 0.1% w/v concentration. Sodium hydroxide: A 5 M NaOH solution was prepared by dissolving the required quantity of the chemical (Merck, Mumbai, India) in water.

Standard drug solution

Exactly 25 mg of pure VLGH was dissolved in water and diluted to the mark with the same solvent in a 250 mL calibrated flask to get 100 µg mL^{-1} VLGH solution.

General procedures

Preparation of calibration curves

Prussian blue method (method A)

Different aliquots (0.25, 0.5, 0.75, 1.0, 1.25, 1.5 and 2.0 mL) of 100 µg mL^{-1} standard VLGH solution were accurately transferred into a series of 10 mL calibrated flasks and the volume was brought to 2.0 mL with water. Subsequently, 0.5 mL each of iron(III) and potassium ferricyanide reagent solutions were added to each flask and the volume was brought to mark with 2 M HCl and mixed well. After 15 min, the absorbance of each solution was measured at 730 nm *versus* the reagent blank.

Potassium permanganate method (method B)

Different aliquots (0.2,0.5,1.0,1.5,2.0,2.5,3.0,3.5 and 4.0 mL) of 100 µg mL^{-1} VLGH solution were placed

in a series of 10 mL calibrated flasks and the volume brought to 4.0 mL with water. One milliliter of 0.1% w/v permanganate solution was added to each flask followed by the addition of 1.0 mL of 5 M NaOH. Each flask was made up to the mark and mixed well. The flasks were kept aside for 15 min, and the absorbance of each flask was then measured at 610 nm *versus* the reagent blank.

In each case, the standard graph was established by plotting the measured absorbance values *versus* the concentration values of VLGH. The concentration of the unknown was computed using the regression equation derived from the Beer's law data.

Procedure for tablets

Twenty tablets were ground after accurately weighed into fine powder. A portion of the powder equivalent to 10 mg of VLGH was accurately weighed and transferred into a 100 mL calibrated flask. A 50 mL of water was added and the content was shaken for 20 min. The content was diluted with water to the mark, and mixed well; the insoluble residue was removed by filtration with Whatman 42 filter paper. Known aliquots (1 mL in method A and 2 mL in method B) of the tablet extract were subjected to analysis following the procedures for method A and method B.

Procedures for method validation

Procedures for method validation were carefully carried out according to the international conference on harmonization guidelines ICH (ICH, 2005), which include linearity, limits of detection (LOD) and quantification (LOQ), precision and accuracy, robustness and ruggedness and selectivity.

Linearity

The linearity of both the methods (A and B) was determined by plotting the absorbance *vs* concentration. Calibration measurements were subjected to least square regression analysis to obtain information related to slope, the Y-intercept and the correlation coefficient (*r*). The detection limits LOD and quantification LOQ values were calculated according to the formulae:

$$\text{LOD} = \frac{3.3\ \text{S}}{b} \text{ and LOQ} = \frac{10\ \text{S}}{b}$$

where S is the standard deviation of replicate (n=7) blank absorbance, and *b* is the slope of the calibration curve.

Accuracy and precision

Intra-day and inter-day precision values were determined at three different concentrations levels of VLGH seven times at the same day for intra-day variation and on separate five successive days for inter-days variation to obtain the relative standard deviation (%RSD). Accuracy was evaluated as percentage relative error between the measured concentrations and taken concentrations for VLGH (Bias %).

Robustness and ruggedness

Robustness was tested by evaluating the effect of small variation in method experimental variables, including the volume of reagents and the contact time, on the performance of the proposed methods. In these tests, one parameter of variables was changed while the other variables were constant and the %RSD was calculated each time. Ruggedness was also tested by employed the proposed methods to determine VLGH using the same conditions of experimental by three different analysts using the same cuvettes (inter-chemists) and also by a single analyst using three different cuvettes (inter-instrument).

Procedure for selectivity

The selectivity of the proposed methods was examined using placebo blank and synthetic mixture analyses. The composition of placebo blank was: 20 mg starch, 20 mg sucrose, 10 mg talc, 10 mg lactose, 20 mg sodium alginate, 20 mg methyl cellulose and 20 mg magnesium stearate. It was prepared by homogeneous mixing in a mortar. Ten milligrams of the placebo was placed in a 100 mL calibration flask, and its extract was prepared as described under procedure for tablets. Two milliliters of the extract were subjected for assay according to general procedures. Ten milligrams of pure VLGH was added to 10 mg of the previously prepared placebo blank. The mixture was quantitatively transferred into a 100 mL calibrated flask, and followed the steps described under procedure for tablets.

RESULTS AND DISCUSSION

Absorption spectra

Many compounds of pharmaceutical importance were previously determined using iron(III) and ferricyanide as chromogenic agents (Ayman, Wafaa, 2008; Al-Okab, Syed, 2012; Manoranjani, Karuna, 2011; Nagendra, 2011; Basavaiah, Chandrashekar, 2005; Hosny, 2014). The method A in the present study is based on the oxidation-reduction process followed by complexation reaction. This process involved the reduction of iron(III) by VLGH and the resulting iron(II) reacted with ferricyanide forming intense, Prussian blue similar to previous reports which

was measured at 730 nm (Figure 2).

Potassium permanganate finds some application in strongly alkaline solutions. $KMnO_4$ is a good oxidizing agent and the Mn-containing products from the redox reactions depend on the pH values. Acidic solution of $KMnO_4$ is reduced to the faintly pink $[Mn(H_2O)_6]^{2+}$, and in an alkaline solution it is reduced to bluish-green colored (manganate ion) MnO_4^{2-}, in which manganese is in +6 oxidation state (Basavaiah, Devi, 2010).

The method B was based on the reduction of $KMnO_4$ by VLGH in alkaline medium. The resulting manganate ion, which is a bluish-green colored chromogen with a strong absorption maximum, was measured at 610 nm (Figure 2) similar to the previous reports (Basavaiah, Devi, 2010; Rajendraprasad, Basavaiah, 2009; Devi, Basavaiah, Vinay, 2012). The possible reaction pathways for two methods (A and B) are presented in Figure 3.

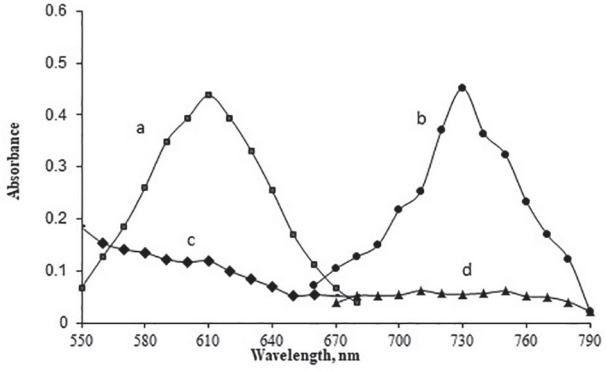

FIGURE 2 - Absorption spectra of: (a) Manganate ion (method B) [20 µg mL^{-1} VLGH]; (b) Prussian blue (method A) [12.5 µg mL^{-1} VLGH]; c and d are the respective blanks.

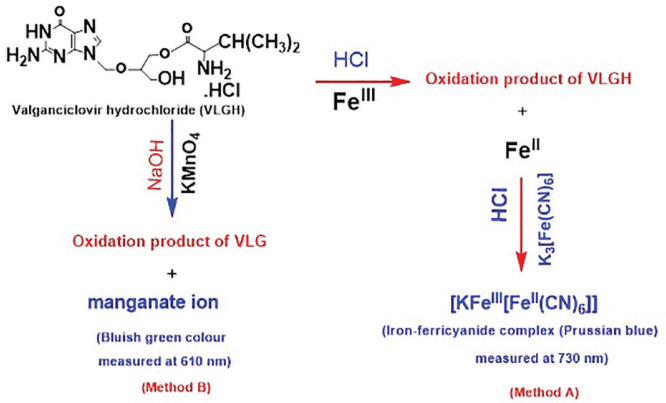

FIGURE 3 - The possible reaction pathways.

Method development

In method A, several experimental variables, such as reagents' concentration and contact time were carefully studied and optimized. The volumes of potassium ferricyanide and iron(III) solutions were found optimum at equal volumes of 0.5 mL (0.5%) in the total volume of 10 mL as shown in Figure 4. Maximum absorbance was reached in 15 min after mixing the reagents and remained stable for 30 min.

In method B, optimum concentration of permanganate was determined by varying the volume of 0.1% w/v $KMnO_4$ from 0.5-3.0 mL in the total volume of 10 mL. It was found that as the concentration of $KMnO_4$ increased, the method sensitivity also found increased, and the respective blank absorbance also increased concomitantly at the same time. The difference in absorbance between sample and blank was maximum when 1.0 mL of $KMnO_4$ was used. Therefore, one milliliter of 0.1% w/v $KMnO_4$ in a total volume of 10 mL was fixed as optimum taking into consideration the sensitivity of the reaction and minimum blank absorbance. One milliliter of 5 M NaOH in the total volume of 10 mL was optimum as shown in Figure 5. The reaction was completed in 15 min and the color remained stable for the next 45 min (Figure 6).

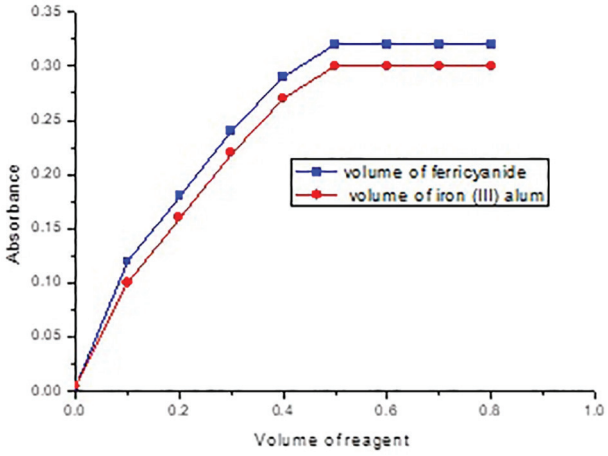

FIGURE 4 - Effect of iron(III) and ferricyanide (method A) (VLGH: 10 µg mL^{-1}).

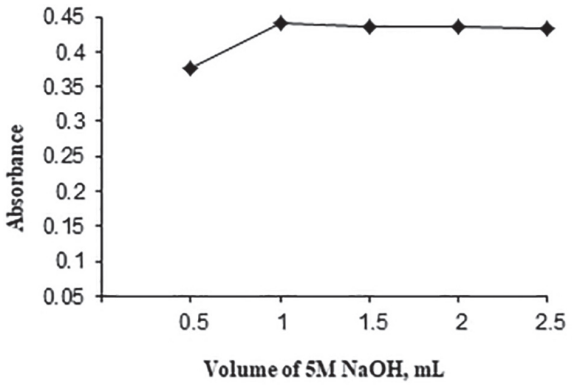

FIGURE 5 - Effect of NaOH in method (B) (VLGH: 20 µg mL^{-1}).

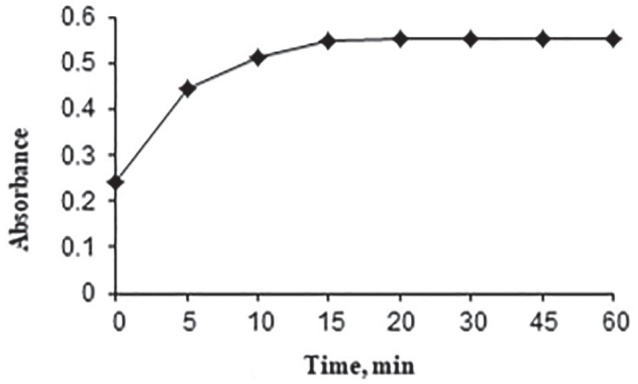

FIGURE 6 - Effect of contact time (method B) (VLGH: 25 μg mL^{-1}).

METHOD VALIDATION

After optimizing the experimental variables for maximum product formation, the proposed methods were validated according to the current ICH guidelines (ICH, 2005).

Linearity

From the respective plot of absorbance and concentration, the linearity was found over the concentration ranges of 2.5-20 and 2.0-40 μg mL^{-1} for method A and method B, respectively. The parameters of the regression equation were calculated using the calibration graphs along with the standard deviations of intercept and slope. Parameters such as LOD, LOQ, molar absorptivity (ε) and also Sandell sensitivity values are presented in Table I.

Accuracy and precision

The proposed methods were validated for both intra-day and inter-day accuracy and precision. The precision ranged from 0.98 to 2.39%, which should be satisfactory to determine the drug in the sample matrix used. The %RE for three levels of drug varied from 0.91 to 2.97% which showed good accuracy. These results are summarized up in Table II and reveal fair accuracy and precision of the proposed methods.

TABLE I - Sensitivity and regression parameters

Parameter	Method A	Method B
λ_{max}, nm	730	610
Linear range, μg mL^{-1}	2.5-20	2.0-40
Molar absorptivity (ε), L mol^{-1} cm^{-1}	1.28×10^{4}	6.88×10^{3}
Sandell sensitivity*, μg cm^{-2}	0.0306	0.0568
Limit of detection (LOD), μg mL^{-1}	0.11	0.21
Limit of quantification (LOQ), μg mL^{-1}	0.33	0.64
Regression equation, y**		
Intercept (a)	-0.0054	0.0207
Slope (b)	0.0358	0.019
Standard deviation of a (S_a)	9.98×10^{-2}	0.0998
Standard deviation of b (S_b)	0.00515	0.00236
Regression coefficient (r)	0.9977	0.9997

*Limit of determination as the weight in μg mL^{-1} of solution, which corresponds to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area 1 cm^2 and l = 1 cm. **y=a+bx, where y is the absorbance, x concentration in μg mL^{-1}, a intercept and b slope.

Robustness and ruggedness

These robustness and ruggedness tests were performed at three concentrations levels of VLGH. The results expressed as the percentage of relative standard deviation %RSD and are computed in Table III and reveal

TABLE II - Evaluation of intra-day and inter-day accuracy and precision

Method	VLGH taken (μg mL^{-1})	Intra-day accuracy and precision (n=7)			Inter-day accuracy and precision (n=5)		
		VLGH found[a] (μg mL^{-1})	RSD[b] %	RE[c] %	VLGH found (μg mL^{-1})	RSD[b] %	RE[c] %
A	6	5.87	0.98	2.17	5.89	1.94	1.83
	12	11.77	1.57	1.92	11.81	2.12	1.58
	18	18.48	1.22	2.97	18.44	1.83	1.87
B	10	9.91	1.27	0.91	10.23	1.67	2.30
	20	20.32	1.16	1.60	20.27	1.57	1.35
	30	30.44	1.75	1.47	30.56	2.39	1.87

[a] Mean value of seven determinations; [b] Relative standard deviation (%); [c] Relative error (%).

TABLE III - Method robustness and ruggedness expressed as intermediate precision (% RSD)

Method	VLGH taken (μg mL^{-1})	Robustness (%RSD)			Ruggedness (%RSD)	
		Parameters altered			Inter-analysts (n=3)	Inter-cuvettes (n=3)
		Contact time*	Volume of $[Fe(CN)_6]^{3-}$ / Iron(III)** reagent	Volume of $KMnO_4$/ NaOH***		
A	6	1.36	1.23	0.99	1.24	1.85
	12	1.21	1.62	1.31	1.76	2.13
	18	1.09	1.52	1.09	1.28	1.59
B	10	1.66	1.57	1.25	1.40	1.82
	20	1.54	1.51	1.36	0.89	1.75
	30	2.21	1.44	1.58	1.48	1.67

*Contact time used:13, 15 and 17 min in both methods. **Volumes of $[Fe(CN)_6]^{3-}$ /Iron(III) reagent were: 0.4, 0.5 and 0.6 mL in method A. *** Volume $KMnO_4$ and NaOH were: 0.8, 1.0 and 1.2 mL in method B.

that the performance characteristics of the methods remain largely independent of the deliberate changes effected in the experimental variables as well as analysts and instruments.

Selectivity

The selectivity of the described methods was examined by placebo and synthetic mixture analyses. The placebo blank in method A did not show any measurable absorbance whereas in method B there was no decrease in absorbance of blank in the presence of placebo. Synthetic mixture was subjected by proposed methods to analysis at three concentrations levels, and the percentage recoveries values of pure drug ranged from 99.66±1.13 to 101.45±1.24, indicating non-interference from the inactive ingredients.

Application to tablets

Two brands of VLGH tablets (450 mg strength) were analyzed by the proposed methods and the results are presented in Table IV. The same tablets were also analyzed by the USP reference method (USP, 2016) for comparison, which is recommended HPLC for assay of drug. Statistical analysis of the results obtained applying Student's *t*-test and variance-ratio *F*-test revealed that no significant difference in the performance of the proposed and the USP reference methods with respect to precision and accuracy.

Accuracy by recovery study

The accuracy of the proposed methods was further confirmed by recovery study according to the standard-addition procedure. Pre-analyzed powder of tablet was spiked with the pure VLGH at three concentration levels and the total was found by the proposed methods. The percent recovery values of pure VLGH (Table V) indicate excellent accuracy of the proposed methods and absence of interferences of the inactive ingredients.

TABLE IV - Results of analysis of tablets by the proposed methods and statistical comparison of the results with the official method

Tablet brand name	Nominal amount	Found* (% of nominal amount ± SD)		
		Official method	Proposed methods	
			Method A	Method B
Valgan	450 mg	99.12±0.76	98.78±1.56 t = 0.44 F= 4.21	98.84±1.22 t = 0.43 F= 2.58
Valcyte	450 mg	101.40 ± 0.85	102.35±1.35 t = 1.33 F= 2.52	101.44±1.51 t = 0.05 F= 3.16

*Mean value of five determinations. (Tabulated t-value at the 95% confidence level and for four degrees of freedom is 2.78). (Tabulated F-value at the 95% confidence level and for four degrees of freedom is 6.39).

TABLE V - Results of recovery experiment through standard-addition method

Method	Tablet studied	VLGH in Tablet, µg mL^{-1}	Pure VLGH added, µg mL^{-1}	Total found, µg mL^{-1}	Pure VLGH recovered Percent±SD*
A	Valgan 450	4.44	4.5	9.12	102.0±1.12
		4.44	9.0	13.88	103.3±1.34
		4.44	13.5	17.55	97.85±1.41
B	Valgan 450	8.89	9.0	18.14	101.4±1.88
		8.89	18.0	26.76	99.55±1.67
		8.89	27.0	35.98	100.3±1.82

*Mean value of three determinations.

CONCLUSION

The redox reactions between VLGH drug and iron(III) in acid medium, and $KMnO_4$ in alkaline medium were beneficially used for the development of two methods for the determination of VLGH in pharmaceutical formulations. The present work is free from undesirable steps such as heating or extraction steps, or use of organic solvents. Both systems have wide linear dynamic ranges of applicability, and method A with an ε value of 1.28×10^4 L $mol^{-1}cm^{-1}$ is the most sensitive. The proposed methods use cheap and easily available chemicals and equipment which is available in most routine industrial quality control laboratories of developing and under developed countries which would ill-afford the expensive techniques like HPLC and others.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest regarding this manuscript.

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