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Method Development and Determination of Valacyclovir HCl in Pharmaceutical Dosage Forms by Visible Spectrophotometry

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Abstract: Two simple rapid and sensitive spectrophotometric methods have been developed for determination of the amount of Valacyclovir HCl in pure and pharmaceutical dosage forms. Method-A is based on the reaction of keto group of Valacyclovir HCl with Phenyl hydrazine hydrochloride(PHH) in the presence of an hexacyano ferrate (III) in acidic medium forming red colour product, exhibiting λ_{max} at 520 nm.. In Method-B, determination is based on the reduction of ferric ion into ferrous ion by the mentioned drug in the presence of 1,10-phenanthroline to form a highly stable orange red colored ferrion complex. Beer's law is obeyed in the concentration ranges 2-10 µg/mL; 5-25 µg/mL; for method A&B respectively. The molar absorptivity and %RSD are $2.66x10^4$, $5.06x10^3$ $L.mol^{-1}cm^{-1}$ and 0.8704 and 0.6515 for methods A& B respectively.
Key words: Valacyclovir HCl, PHH, $NaIO_4$, $K_3Fe(CN)_6$, Ferrion complex.

INTRODUCTION

Valacyclovir, L-valine-2-[(2-amino-1, 6-dihydro-6-oxo-9-hipurin-9-yl) methoxy] ethyl ester is the L-valyl ester prodrug of the antiviral drug acyclovir that exhibits activity against herpes siplex virus types,1(HSV–1) and 2(HSV–2) and vericellazoster virus[1]. The mechanism of action of acyclovir involves the highly selective inhibition of virus DNA replication, via enhanced uptake in herpes virus-infected cells and phosphorylation by viral thymidine kinase. The substrate specificity of acyclovir triphosphate for viral, rather than cellular, DNA polymerase contributes to the specificity of the drug[2,3]. Valacyclovir is rapidly converted to acyclovir and further phospharilated to acyclovir triphosphate. The incorporation of acyclovir tri phosphate into the growing chain of viral DNA results in chain termination[4-10]. Very few methods are reported in literature for the assay of Valacyclovir in pharmaceutical dosage forms which includes spectrophotometry[11-14], HPLC[15] and RP-HPLC methods[16].Visible spectrophotometry is the technique of choice in research laboratories and pharmaceutical industries due to its low cost and inherent simplicity. The objective of the work is to develop new spectrophotometric methods for the assay of selected drugs in bulk and tablet dosage forms with good accuracy, simplicity, precision and economy. The present work describes two spectrophotometric methods (A&B) for the assay of Valacyclovir HCl using Phenyl hydrazine

hydrochloride with hexacyano ferrate (III) and 1, 10-phenanthroline with Fe(III).

MATERIALS AND METHODS

Instrumentation and conditions: All spectral and absorbance measurements were made on ELICO SL-159, UV-visible spectrophotometer with 1cm quartz cells was used.

Reagents and Solvents: All chemicals used were of analytical grade

$NaIO_4$ solution (Loba; 0.855%, $4.00x10^{-2}$M): Prepared by dissolving 855mg of $NaIO_4$ in in 100mL of 0.3M HCl.

PHH (Phenyl Hydrazine Hydrochloride) (Loba; 1.0%, $6.90x10^{-2}$M): Prepared by dissolving 1.0g of PHH in 100mL of distilled water and filtered.

$K_3Fe(CN)_6$ solution (Sd-fine; .2.0%, $6.00x10^{-2}$M): Prepared by dissolving 2.0g of $K_3Fe(CN)_6$ in 100mL distilled water.

NaOH solution (BDH, 0.4%, 0.1M): Prepared by dissolving 400mgs of NaOH to 100mL distilled water and standardized.

1,10-phenanthroline solution (Sd.fine; 0.2%): Prepared by dissolving 200mg of 1, 10-phenanthroline in 100 ml of distilled water.

Orthophosphoric acid (OPA) (BDH, 0.1%): Prepared by dissolving 1.27ml orthophosphoric acid with 100ml of distilled water.10ml of the stock solution is diluted to 100ml with distilled water.

Ferric chloride solution (Wison labs; 0.054%): Prepared by dissolving 54 mg of ferric chloride in 100 ml distilled water.

Preparation of Standard stock solution:
Valacyclovir HCl (100mg) was accurately weighed and dissolved in 20ml of distilled water, transferred to a standard 100ml volumetric flask. The final volume was made up to the mark with distilled water. The final concentration was brought to 100µg/mL with distilled water for both proposed methods (A&B).

Procedure for the assay of Valacyclovir HCl in pharmaceutical dosage forms:

Twenty tablets were weighed accurately and reduced to fine powder, drug equivalent to 100 mg of Valacyclovir HCl taken in a 100 ml volumetric flask, sonicated for about 30 min, and the volume was made up to the mark with distilled water, filtered by using Whattmann-42 filter paper. The filtrate was quantitatively diluted with methanol to yield concentrations in the linear range of the assay of Valacyclovir HCl.

Recommended Procedures for the Determination of Valacyclovir HCl
Method A:

Aliquots of standard Valacyclovir Hcl solution (0.5–2.5ml, 100µg/ml) were transferred into a series of 25mL-calibrated tubes. Then 0.5mL of $NaIO_4$ solution was added to each tube and the volume made upto 5mL with distilled water. After keeping the tubes for 30min. at room temperature, 1.5mL of NaOH, 2.0mL of PHH solution and 1.0mL of $K_3Fe(CN)_6$ solutions were added successively and shaken well. The tubes were kept in ice water for 5min.Later 5.0mL of Conc.HCl was added. Finally the solution in each tube was made up to 25mL with ethanol. The absorbance was measured after 15min. at 520nm against reagent blank.

Method B:

Different volumes of (0.5-2.5ml,100µg/ml) standard Valacyclovir Hcl were transferred into a series of 10ml calibrated test tubes and then a solution of 0.5ml of Fe(III),2ml of 1,10- phenonthraoline is added and total volume is made up to 5.0ml with distilled water.The tubes were kept in a boiling water bath for 30 min. The tubes were removed and cooled .2ml of ortho phosphoric acid was added and the volume in each tube was made up to the 10ml with distilled water. The absorbance of the solution in each tube is measured immediately between 450-550 nm and λmax is found to be 510 nm against a reagent blank prepared similarly.

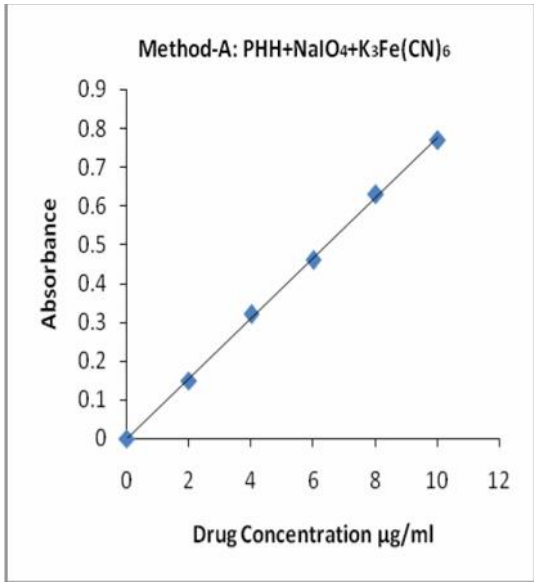


Fig.1 Beer's plot of valacyclovir HCl with PHH, $NaIO_4$, $K_3Fe(CN)_6$

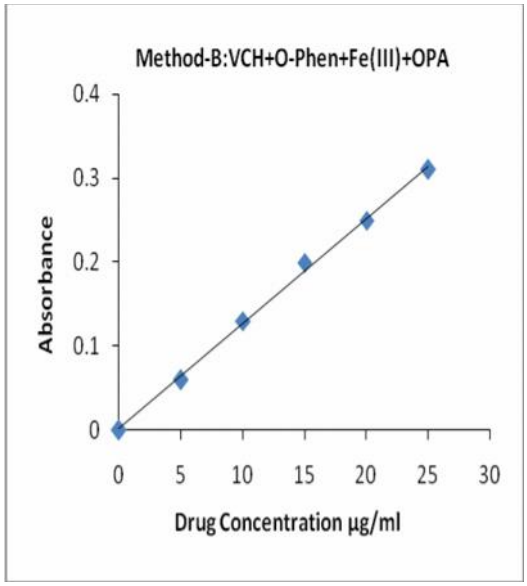
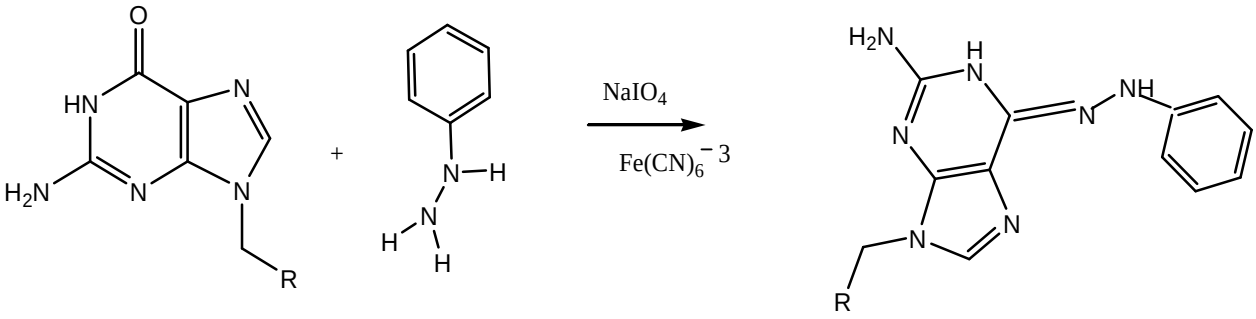
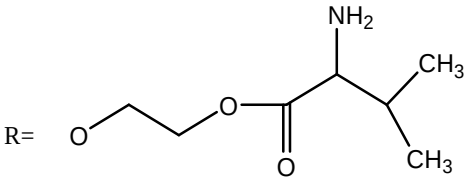


Fig.2 Beer's plot of valacyclovir HCl with O-Phen, Fe (III), OPA

Scheme-A



Valacyclovir Hcl PHH Colour Product



Scheme-B

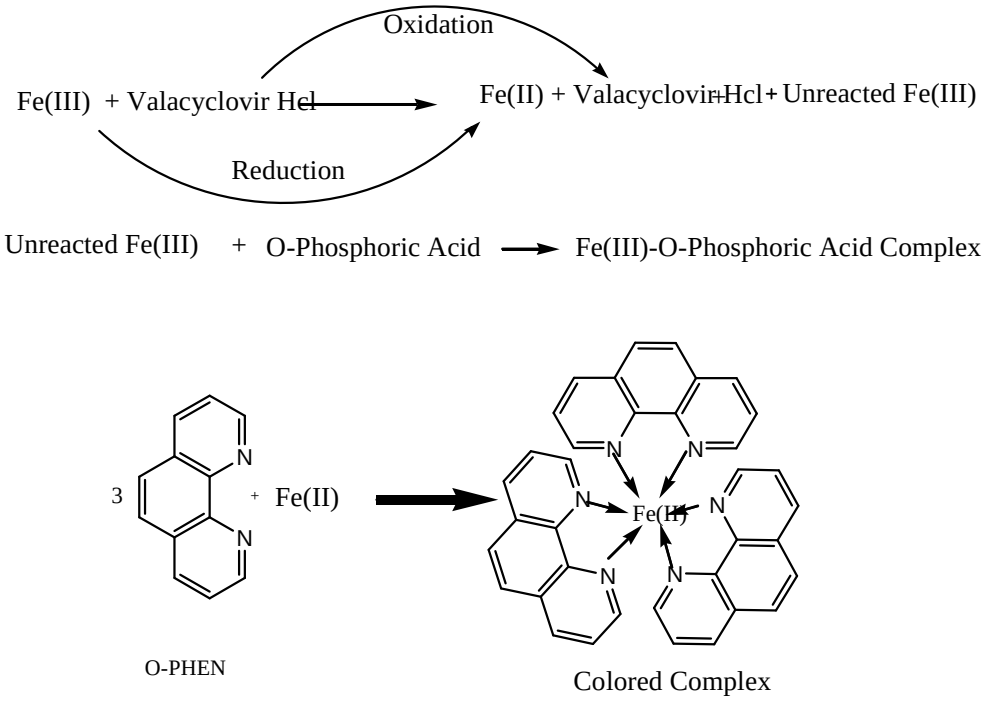


Table-1: Optical Characteristics, Precision, Accuracy of the Methods proposed for the determination of Valacyclovir

Optical characteristics	Method A	Method B
λ_{max} (nm)	520	510
Beer's law limits (µg/ml)	2-10	5-25
Molar absorptivity ($Lmol^{-1}.cm^{-1}$)	$2.66x10^{4}$	$5.06x10^{3}$
Sandell's sensitivity (µg.cm^{-2}/0.001 abs. Unit)	$1.324x10^{-2}$	$8.474x10^{-2}$
Regression equation (Y=a+bc) ;Slope (b)	$7.375x10^{-2}$	$1.194x10^{-2}$
Standard deviation on slope (S_b)	$4.113x10^{-4}$	$1.03x10^{-4}$
Intercept (a)	$1.70x10^{-3}$	$-3.0x10^{-4}$
Standard deviation on intercept (S_a)	$2.728x10^{-3}$	$1.702x10^{-3}$
Standard error on estimation (S_e)	$2.601x10^{-3}$	$1.622x10^{-3}$
Correlation coefficient (r)	0.9999	0.9998
standard deviation	$6.986x10^{-2}$	$6.573x10^{-2}$
Relative standard deviation (%)	$8.704x10^{-1}$	$6.515x10^{-1}$
% Range of error (confidence limits)		
0.05 level	0.1734	0.1632
0.01 level	0.2877	0.2706
LOD	0.1110	0.4276
LOQ	0.3699	1.4254

* Average of six determinations considered

Table-2: Assay of Valacyclovir hydrochloride in dosage forms

Sample	Labelled amount (mg)	Amount found by proposed methods*		Ref. Method[17]	% Recovery by proposed methods**	
		Method A	Method B		Method A	Method B
Tablet	500	487.9 ±0.09 F = 1.78 t = 0.49	488.5 ±0.10 F = 1.44 t = 0.31	499.8 ± 0.12	97.62 ±0.09	97.74±0.04

* Average ± standard deviation of six determinations, the t-and F-test values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit, F = 5.05, t = 2.228

** Recovery of 10 mg added to the preanalysed pharmaceutical formulations (average of three determinations).

RESULTS AND DISCUSSION:

The two methods described for the determination of Valacyclovir Hcl in Tablet were specific. No interferences with excipients were encountered. Under the optimized experimental conditions, linear calibration graphs (Fig-1 and Fig-2) for method A and B respectively were obtained, over the working concentration range of 2-10μg/mL, and 5-25μg/mL with molar absorptivities of $2.66x10^4$ and $5.06x10^3$ for the method-A and method-B respectively. The equations of calibration curves were obtained using the least square regression equation. The slope and the coefficient of correlation (γ) were presented in Table-1. Beer's law limits, molar absorptivity, and Sandell's sensitivity for Valacyclovir HCl with each one among mentioned reagents were calculated. The optical characteristics are presented in Table-1. The correlation coefficient was found to be 0.9999 and 0.9998 for methods A and B, respectively, indicating the good linearity of both the calibration graphs and the intercepts are all close to zero.

The precision of each one among two proposed spectrophotometric methods were ascertained separately from the absorbance value obtained by actual determination of six replicates of a fixed amount of Valacyclovir HCl in total solution. The percent relative standard deviation and percent range of error (at 0.05 % level and 0.01% level confidence limits) were calculated for the proposed methods and are presented in Table -1.Limit of detection(LOD) and limit of quatification (LOQ)of each method were given in Table-1.

Commercial formulations containing Valacyclovir HCl were successfully analysed by each proposed method. The values obtained by the proposed and reference methods for formulations were compared statistically by the t- and F-tests and are found not to differ significantly. The results are incorporated in Table-2.The probable schemes of proposed methods(A and B) were presented in scheme-A and scheme-B respectively.

CONCLUSIONS:

The proposed two methods were found to be simple, selective and sensitive. The statistical parameters and recovery study data clearly indicate the reproducibility and accuracy of the methods. Analysis of the authentic samples containing Valacyclovir HCl showed no interference from the common excipients. The applicability of the proposed procedures for routine quality control is well established by assay of Valacyclovir HCl in bulk form and pharmaceutical preparations.

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