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Selective and Validated Spectrophotometric Method for Determination of Acyclovir and Valacyclovir using N-Bromosuccinimide

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ABSTRACT

Simple, sensitive spectrophotometric methods are presented for the assay of acyclovir and valacyclovir in bulk drug and in tablets. The methods employ N-bromosuccinimide (NBS) as the oxidimetric reagent and dye, methyl orange used as a spectrophotometric reagent. The method involves adding a measured excess of NBS to acyclovir and valacyclovir in acid medium followed by determination of residual NBS by reacting with a fixed amount of methyl orange and measuring the absorbance at 508 nm. The methods developed were applied to the assay of acyclovir and valacyclovir in commercial tablet formulations and the results were compared statistically with those of a reference method. Acyclovir and valacyclovir showed maximum absorbance at 508 nm with linearity was observed in the concentration range of 1-5 µg mL^{-1} and 5-10 µg mL^{-1} respectively. The relative standard deviations of 0.024 % for acyclovir and 0.018 % valacyclovir were obtained. The accuracy and reliability of the methods were further ascertained by performing recovery tests via standard-addition method. The recoveries of acyclovir and valacyclovir tablets are in the range 99.26 ±0.52, 99.47±0.96 respectively. The proposed method is simple, rapid, precise and convenient for the assay of acyclovir and valacyclovir in commercial tablet preparations.

Key words: Acyclovir, Valacyclovir, Oxidation, N-Bromosuccinimide (NBS), Spectrophotometry, Pharmaceutical formulation.

INTRODUCTION

Acyclovir (ACV), (9, 2-hydroxyethoxy) methyl guanine, and it is an antiviral drug used extensively in the treatment of skin infections caused by herpes simplex virus[1] . It is official in European Pharmacopoeia[2], British Pharmacopoeia[3] and United States Pharmacopoeia[4]. Acyclovir is highly active *in-vitro* against herpes simplex-b (HSV) type-I and II and varicella viruses, but its toxicity to mammalian cells is low. Acyclovir is phosphorylated to the active compound acyclovir triphosphate after entry into herpes infected cell. Literature survey reveals that few methods like high performance liquid chromatography and few spectrophotometric methods[5-8].

Valacyclovir (VCV), *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 simplex virus types, 1 (HSV-1) and 2 (HSV-2) and vericellazoster virus [9]. The mechanism of action of acyclovir involves the highly selective inhibition of herpes 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 [10-11]. After oral administration, VCV is converted rapidly and extensively to acyclovir as a result of first-pass intestinal and hepatic metabolism through enzymatic hydrolysis[12]. The oral bioavailability of acyclovir is higher after administration of Valacyclovir relative to acyclovir itself. Literature survey reveals that few methods like high performance liquid chromatography[13] and few spectrophotometric methods[14-16]. So there is a need for development of simple and suitable analytical spectrophotometric method for the determination of acyclovir and valacyclovir in bulk and pharmaceutical formulations. UV-Visible spectrophotometry is the technique of choice in research laboratories, hospitals and pharmaceutical industries due to its low cost and inherent simplicity. Hence the present work deals with the spectrophotometric estimation of acyclovir and valacyclovir using N-Bromo succinamide reagent in presence of acidic medium.

However, the reactions of N-Bromo succinamide reagent[17] with acyclovir and valacyclovir have not been investigated so far. The present study describes the evaluation of N-Bromo succinamide as a reagent in the development of simple and rapid spectrophotometric method for the determination ACV and VCV in its pharmaceutical dosage forms.

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METHOD & MATERIALS:

Instruments:

A double-beam Shimadzu 1700 UV spectrophotometer, connected to computer and loaded with UV solution software was used. For an intermediate precision study, a different Shimadzu 1800 UV spectrophotometer UV spectrophotometer connected to computer with UV-PC software was used. Both instruments have an automatic wavelength accuracy of 0.1 nm and matched quartz cells of 10 mm (1.0 cm) cell path length. The absorbance of ACV and VCV in the selected medium at respective wavelength was determined and the apparent molar absorptivity was calculated.

Reagents:

All employed chemicals were of analytical grade and high-purified water was used throughout the study. Acyclovir and valacyclovir pure samples were obtained as a gift samples from Strides Arcolab Limited, Bangalore, India.

N-Bromosuccinimide (NBS) 0.02 %(w/v):

0.02 g of NBS was accurately weighed transferred into a 100 ml calibrated flask and make up the volume up to the mark with distilled water to obtain a solution of 0.02% (w/v). The solution was freshly prepared and protected from light during the use.

Methyl orange 0.01%:

0.01 g of methyl orange is accurately weighed and transferred into a 100.0ml volumetric flask and dissolve it in 10 ml of water, then made up to the mark with distilled water.

Hydrochloric acid 1M:

8.5 ml of con.hydrochloric acid is accurately measured and transferred into a 100.0 ml volumetric flask and made up to the mark with distilled water.

Selection of Analytical Wavelengths for ACV and VCV:

A 1 ml quantity of 0.02% NBS solution, 1.0 ml 0f 1M HCl were added into two test tubes and 3.5 ml of acyclovir and valacyclovir stock solutions were added and kept it aside for 20 minutes then add 1 ml of 0.01% methyl orange dye which results in te formation of pink color complex. The sol tions were made up to 10ml with water. The absorption spectrums of the complex were determined against blank

solution and the wavelengths of maximum absorption at 508nm of the products of the reactions were noted. The results obtained were showed in fig 2.

Effect of reagent concentration:

The effect of varying the concentration of NBS was carried out using reagent concentrations of 0.01, 0.02, 0.03, 0.04, 0.05% in water and 1 ml of 1M HCl. After mixing 1.0 ml of each reagent concentration with the drug solutions of ACV and VCV kept it aside for 20 minutes and then add 1 ml of methyl orange and made up to 10 ml with water, the absorbance readings of the complex formed were made at 508 nm on the UV-visible spectrophotometer.

Optimization Studies:

Effect of NBS Concentration:

The studying of NBS concentrations revealed that the reaction was dependent on NBS reagent. The absorbance of the reaction solution increased as the NBS concentration increased, and the highest absorption intensity was attained at NBS concentration of 0.02 % (w/v). Higher NBS concentrations up to 0.05 % had no effect on the absorption values. Further experiments were carried out using 0.02 %. Results obtained were showed in fig 3.

Preparation of calibration curve:

Standard solutions of ACV and VCV in water, having final concentrations in the range of 1-5 µg/ml and 5-10 µg/ml, were transferred into a series of 10 ml volumetric flasks, to these solutions 1.0 ml of 0.02% NBS, 1.0 ml of 1M HCl were added and kept it aside for 20 minutes and then add 1 ml of methyl orange indicator. The mixture was then gently shaken until the appearance of color chromogen. The contents were diluted up to 10 ml with distilled water. The absorbance of each solution was measured at 508 nm against the reagent blank prepared in the same manner, without the analyte and the calibration curve of ACV and VCV is shown in figure 1.

Analysis of commercial pharmaceutical preparations:

Twenty tablets of ACV and VCV were weighed accurately and ground into a fine powder. An amount of the powder equivalent to 100 mg of acyclovir 100 mg of VCV were weighed and transferred into two separate 100 ml volumetric flasks, 60 ml of water added and shaken thoroughly for about 20 min. Then, the volume was made up to the mark with water, mixed well and filtered using a quantitative filter paper. First 10 ml portion of the filtrateof both the drugs were rejected and 2.5 ml of the tablet extract was subjected to analysis using the procedure described above.

General procedure:

Several standard solutions of acyclovir and valacyclovir were taken in individual standard flasks. To each standard flask, 1.0 ml of 0.02% NBS, 1.0 ml of 1M HCl and kept it aside for 20 minutes then add 1 ml of methyl orange indicator and mixtures were then shaken until the appearance of colour chromogen and dilute the solution upto the mark with distilled water. The absorbance was measured at ? max at 508 nm for acyclovir and valacyclovir respectively against a blank similarly prepared by omitting the drug solution with water. The concentration of acyclovir and valacyclovir in each standard flask was obtained by interpolating the corresponding absorbance value from Beer's plot of standard acyclovir and valacyclovir solutions.

Quantification:

The limits of the Beer's law, the molar absorptivity and the Sandell's sensitivity values were evaluated. Regression analyses of the Beer's law plots at their respective *l* max values revealed a good correlation. Graphs of absorbance versus concentration showed zero intercept, and are described by the regression equation, $Y = bX + c$ (where Y is the absorbance of a 1 cm layer, b is the slope, c is the intercept and X is the concentration of the drug in µg/ml) obtained by the least-squares method. The results are summarized in Table 1.

Validation of the method:

The validity of the method for the assay of ACV and VCV were examined by determining the precision and accuracy. This was determined by analyzing six replicates of the drug within the Beer's law limits. The low values of the relative standard deviation (R.S.D.) indicate good precision of the methods. To study the accuracy of the methods, recovery studies were carried out by the standard calibration curve method. For this, known quantities of pure acyclovir and valacyclovir were mixed with definite amounts of pre-analyzed formulations and the mixtures were analyzed as before. The total amount of the drug was then determined and the amount of the added drug was calculated by difference. The results are given in Table No.3 and 4. The average present recoveries obtained were quantitative results are given in Table 3.

Fig 1: Calibration curve for Acyclovir and Valacyclovir

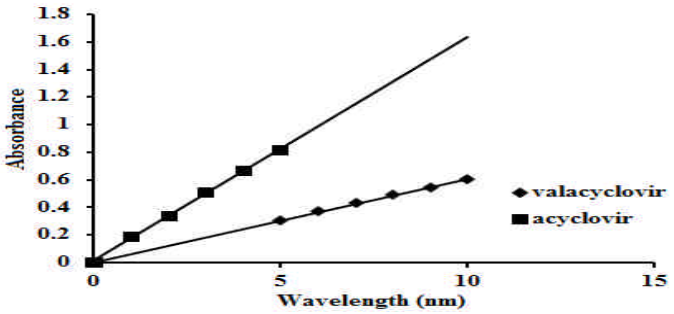


Fig 2: Absorption spectrum of Acyclovir Valacyclovir

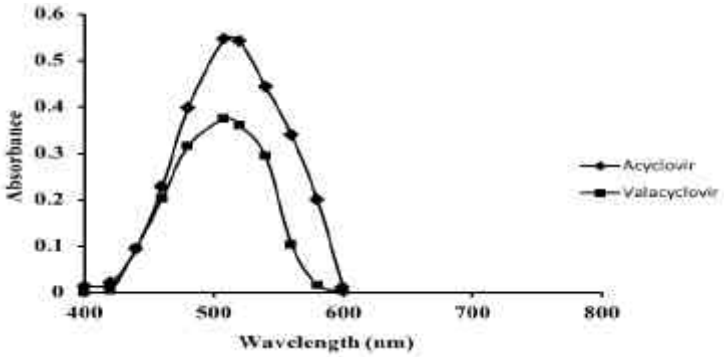


Fig 3: Effect of volume of HCl and N-Bromosuccinimide percentage on the formation of colored product

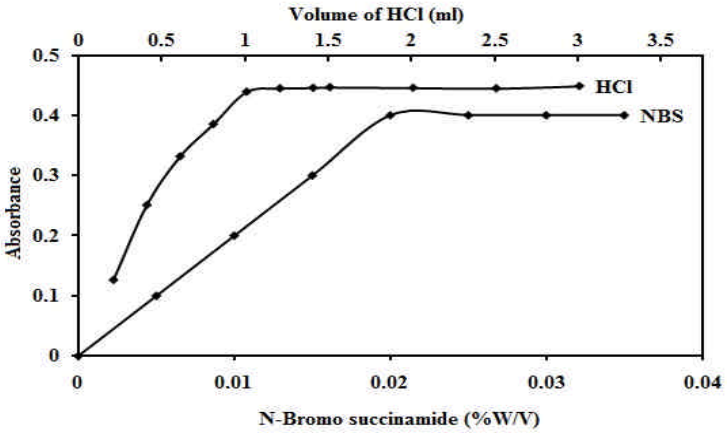


Fig 4: Effect of time on the formation of colored product

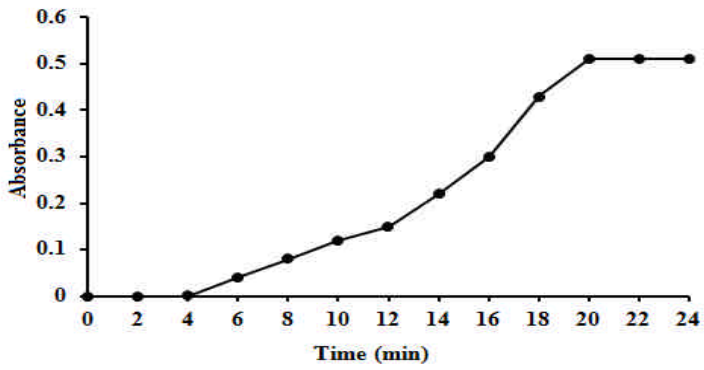


Table 1. Optical characteristics of spectrophotometric method

S.No	Parameter	Values	
	Acyclovir	Valacyclovir	
1.	λ_{max} nm	508nm	508nm
2.	Beers law limits (µg/ml)	1-5	5-10
3.	Molar absorptivity (1 /mol/cm)	0.46 x 10^3	0.77 x 10^3
4.	Correlation coefficient (R)	0.9992	0.9996
5.	Sandell's sensitivity(ng cm^{-2})	0.000549	0.016778
6.	Regression equation (y)	0.1621x+0.0109	y = 0.0605x + 0.0010
7.	Slope, *b*	0.1621	0.0605
8.	Intercept, *c*	0.0109	0.0010
9.	Relative standard deviation%	0.00903	0.65
10.	Limit of detection (µg/ml)	0.087	0.325
11.	Limit of quantification (µg/ml)	.446	0.9872

Table 2. Results of determination of acyclovir and valacyclovir in formulations and statistical comparison with the reference method

Pharmaceutical dosage form	Labelled Amount	Amount found by proposed methods (mg)	Recovery of Reference Method (%)	Recovery of proposed methods (%) A B
T1 (Zovirax) (ACV)	200mg	198.92	99.12	99.46 *t*=0.352 *f*=1.02
T2 (Valcivir) (VCV)	500 mg	249.12	99.28	99.64 t=0.316 *f*=1.08

Table 3. Accuracy and method precision data for the developed method

Drug	S.N	Label Claim (mg)	Amount found*	% Purity*	Average (%)	S.D	R.S.D[a]	RSD[b]	S.E.M
	1	200	198.24	99.12	99.15	0.016	0.0846	0.0824	0.0094
	2		198.16	99.08					
T1 Zovirax	3		200.12	100.06					
(ACV)	4		197.64	98.82					
	5		198.08	99.04					
	6		197.64	98.82					
	1	500	498.12	99.62	99.65	0.018	0.0802	0.07094	0.0082
	2		496.98	99.39					
T2 (Valcivir)	3		497.12	99.42					
(VCV)	4		498.88	99.77					
	5		499.98	99.99					
	6		498.46	99.69					

SD. Standard deviation; SEM. Standard error of mean; RSD.relative standard Deviation; a. intraday precision, b. inter day precision.

Table 4. Standard addition of acyclovir and valacyclovir for accuracy

formulation Studied	Amount taken(mcg/ml)	Amount added	Total found(mcg)	Recovery (%)
Zovirax (ACV)	2	2	4.96	99.26
Valcivir (VCV)	6	2	7.98	99.68

indicating good accuracy of the methods.

Specificity and selectivity:

ACV and VCV solutions were prepared in the selected media with and without common exicipients separately. All solutions were scanned from 800 to 200 nm at a speed of 200 nm min^{-1} and checked for change in the absorbance at respective wavelengths. In a separate study, drug concentration of 20µg/ml was prepared independently from pure drug stock solution in selected media and analysed paired *t* test at 95 % level of significance was performed to compare the means of absorbance.

Linearity:

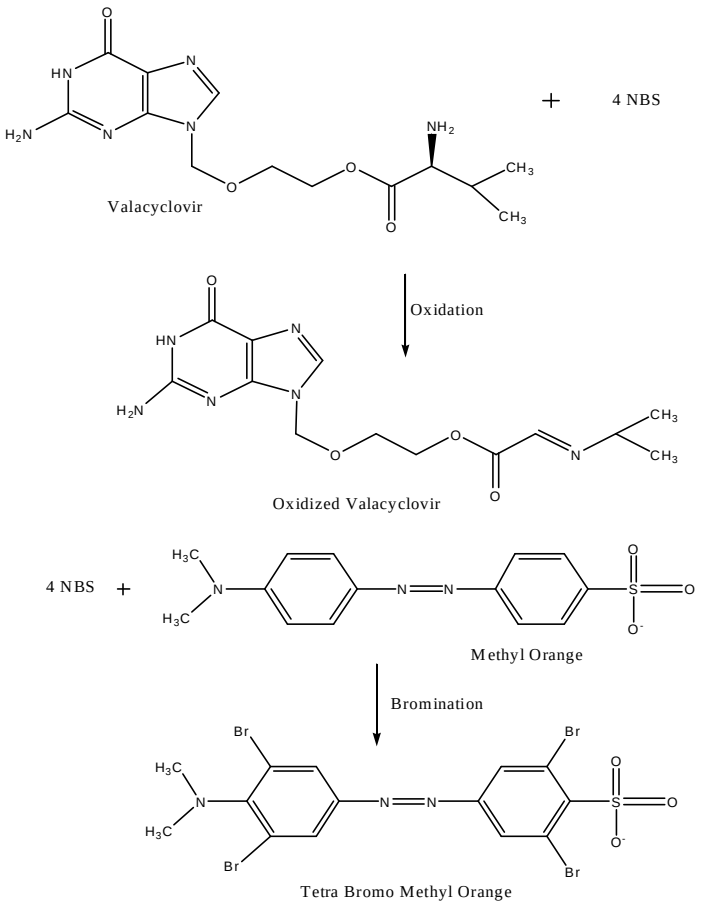
To establish linearity of the proposed methods, a separate series of solutions of acyclovir and valacyclovir were prepared from the stock solutions and analysed. Least square regression analysis was performed on the obtained data.

Precision:

The precision of the proposed methods was ascertained by actual determination of six replicates of fixed concentration of the drug within the Beer's range and finding out the absorbance by the proposed method in all the three drugs. The

Reaction:

Scheme: 1 Mechanism of reaction of Acyclovir with NBS



Accuracy:

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts of bulk samples of ACV and VCV within the linearity range were taken and added to the pre-analyzed formulation.

Ruggedness:

To ascertain the ruggedness of the methods, six replicate determinations at different concentration levels of the drugs were carried out. The intra-day RSD values were less than 1%. The values of between-day RSD for different concentrations of drugs obtained from the determinations and indicate that the proposed method has reasonable ruggedness. The within-day RSD values were less than 1%. The values of inter-day RSD for different concentrations of drugs obtained from the determinations and indicate that the proposed method has reasonable ruggedness.

Limit of detection (LOD) and limit of quantitation (LOQ):

The LOD and LOQ for ACV and VCV by the proposed method were determined using calibration standards. LOD and LOQ were calculated as 3.3 *s*/*S* and 10 *s*/*S*, respectively, Where S is the slope of the calibration curve and *s* is the standard deviation of *y*-intercept of regression equation.

RESULTS AND DISCUSSION:

The methods described here is based on the oxidation reaction. It innvoles the reaction of ACV and VCV with NBS in acidic medium. The method is based on the the determination of residual concentration of NBS after completion of the oxidation process under specified experimental conditions.The amount of NBS reacted corresponds to the drug content in the method. The ability of NBS to oxidize acyclovir and valacyclovir and bleach the colors of methyl orange dye has been used for the indirect spectrophotometric assay of the drug. In this method, the drug is reacted with a known excess of NBS in acid medium, and the unreacted oxidant is determined by reacting with a fixed amount of methyl orange and measuring the absorbance at 508 nm. In this method, the absorbance increased linearly with increasing concentration of drug. ACV and VCV, when added in

increasing amounts to a fixed amount of NBS, consume the latter and there will be a concomitant fall in its concentration. When a fixed amount of dye is added to decreasing amounts of NBS, a concomitant increase in the concentration of dye results. This is observed as a proportional increase in the absorbance at the respective wavelengths of maximum absorption with increasing concentration of acyclovir and valacyclovir as indicated by the correlation coefficients of 0.9992 and 0.9996 respectively. Preliminary experiments were performed to determine the maximum concentrations of the dye spectrophotometrically, and these were found to be 0.01% for acyclovir valacyclovir respectively. Hydrochloric acid was the ideal medium for the oxidation of ACV and VCV by NBS. The reaction between drug and NBS was unaffected when 1.0 – 2.5 ml of 1 M hydrochloric acid in a total volume of about 7 ml was used. Hence, 1.0 ml of 1 M hydrochloric acid is used for both steps in the assay procedures. For a quantitative reaction between ACV and VCV and NBS, a contact time of 20 min was found necessary in both procedures and constant absorbance readings were obtained when contact times were extended upto 20 minutes. A standing time of 5 min was necessary for the bleaching of the dye color by the residual NBS. The measured color was found to be stable for several hours in the presence of the reaction product.

Selection of reaction medium:

To find a suitable medium for the reaction, different aqueous acids were used, such as sulphuric acid, nitric acid, hydrochloric acid and acetic acid. The best results were obtained when hydrochloric acid was used. In order to determine the optimum concentration of hydrochloric acid, different volumes of 1N hydrochloric acid solutions (0.5, 1.0, 2.0, 2.5, 3.0 ml) were used to a constant concentration of ACV and VCV and the results of the observation are plotted in Fig. 2. From the figure, it is evident that 1 ml of 1 N HCl was found optimum. Larger volumes up to 3 ml had no effect on the absorbance of the colored species.

CONCLUSION:

The reagents utilized in the proposed methods are cheap, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. Moreover, the methods are free from interference by common additives and excipients. The wide applicability of the new procedures for routine quality control was well established by the assay of acyclovir and valacyclovir in pure form and in pharmaceutical preparations.

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