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Stability indicating methods for the determination of acyclovir in the presence of its degradation product

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ABSTRACT

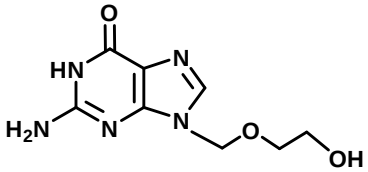
Two sensitive, selective and precise stability-indicating methods are presented for the determination of Acyclovir (ACV) in the presence of its degradate (Guanine). Method A depends on using first derivative of the ratio spectrophotometry (DD^1) by measurement of the amplitude at 265 nm. Method B is based on a high performance liquid chromatographic (HPLC) separation of ACV from its degradation products using an ODS column with a mobile phase consisting of acetonitrile–distilled water (4:96, v/v,) with UV detection at 254 nm. Regression analysis showed good correlation in the concentration ranges 10-60 μgmL^{-1} and 20 – 120 μgmL^{-1} with percentage recoveries of 100.06±0.89and 99.62±0.66 for methods A and B, respectively. These methods are suitable as stability indicating methods for the determination of ACV in presence of its degradation product either in bulk powder or in pharmaceutical formulation. Statistical analysis of the results has been carried out revealing high accuracy and good precision.

KEYWORDS

Acyclovir;
Derivative ratio spectrophotometry;
HPLC;
Stability indicating method.

INTRODUCTION

Chemically, Acyclovir (ACV), known as 2-amino-1,9-dihydro-9-(2-hydroxyethoxymethyl)-6H-purine-6-one[1] (Figure 1).Guanine is a synthetic precursor, degradation product and also metabolite of ACV[2]. ACV is still the first choice for the treatment of the diseases caused by herpes simplex virus (HSV) and herpes zoster virus (HZV). The selectivity and low side effects are due to the mechanism of action of ACV. ACV acts in the replication phase of the virus. ACV acts by inhibiting the viral DNA synthesis[3].



(M.Wt = 225.2)

Figure 1 : Structural formula of Acyclovir

The literature survey reveals several analytical methods for quantitative estimation of ACV in body ?uids and in pharmaceutical formulations. These methods include spectrophotometric methods[4-6], colorimetric methods[7-9], spectrofluorimetric methods[10-12], high performance liquid chromatographic (HPLC) methods[3,13-30], chemiluminescent methods[31], electrochemi-

cal methods[32] and polarographic methods[33].

In modern analytical laboratory, there is always a need for significant stability-indicating methods of analysis. The present work aimed to develop simple instrumental methods for the quantification of ACV in bulk form or in the presence of its main degradation product; which is Guanine. These methods include first-derivative of the ratio spectra (DD^1) and HPLC.

The scientific novelty of the present work is that the methods used are simple, rapid, selective, less-expensive and less time-consuming compared to published methods.

EXPERIMENTAL

Instruments

- A double beam UV / VIS spectrophotometer (Shimadzu, Japan) model UV- 1650 PC with a quartz cell of 1cm path length. The spectral band width is 2nm and the wavelength-scanning speed is 2800nm/min.
- A liquid chromatograph consists of an isocratic pump (Agilent Model G1310A), an ultraviolet wavelength detector (Model G1314A, Agilent 1100 Series), a Rheodyne injector (Model 7725 I, USA) equipped with 20 µl injector loop, An Agilent (USA) column Xterra 5µm C_{18} (250 mm × 4.6 mm.), a Teflon membrane filter of pore size 0.45µm and 47mm diameter for solvent and a Teflon disposable membrane filter of pore size 0.45µm for samples.

Materials

Pure standard

Acyclovir was kindly supplied by NODCAR (Egypt). B.No. ACP1041203. Its purity was found to be 99.99%, according to the official HPLC method[2].

Guanine was kindly supplied by (Sigma-USA). Its purity was found to be 99.00%.

Pharmaceutical dosage form

Acyclovir 400 Stada® tablets, batch no. 716302 and 716303 (manufactured by Global Nabi Pharmaceuticals, Egypt). Each tablet claimed to contain 400mg acyclovir.

Novirus 200 mg® capsules batch no.683500 and 683501 (manufactured by GlaxoWellcome, Egypt). Each capsule claimed to contain 200mg acyclovir.

Acyclovir 5%® cream batch no. 903126 and 903127 (manufactured by Misr Co. for Pharm. Ind. S.A.E, Egypt). Each 100gm claimed to contain 5gm acyclovir

Acyclovir 3%® ointement batch no. 303066 and 303067 (manufactured by Misr Co. for Pharm. Ind. S.A.E, Egypt). Each 100 gm claimed to contain 3gm acyclovir.

Zovirax Suspension® batch no. 071040 and 071041 (manufactured by GlaxoWellcome, Egypt). Each 5ml claimed to contain 400 mg acyclovir.

Degraded sample

Hundred (100) milligrams of Acyclovir were heated with 25-ml of 2M HCl solution for two hours. Complete degradation was confirmed by TLC through the disappearance of drug spot using isopropanol: ammonia: water (7:1:2 by volumes) as a developing system. The solution was neutralized with 2M NaOH. The degradate was separated on preparative TLC plates using isopropanol: ammonia: water (7:1:2 by volumes) as a developing system. The plate was developed in a chromatographic tank previously saturated for one hour with the mobile phase and then air dried. The spots were visualized under UV light at 254nm[34]. The isolated degradate was subjected to IR and mass spectral analysis for subsequent identification.

Chemicals and solvents

All chemicals used throughout this work were of analytical grade and the solvents were of spectroscopic grade.

1. Acetonitrile HPLC grade (Aldrich- USA)
2. Methanol HPLC grade (Aldrich – USA)
3. Conc. HCl and NaOH pellets (Sigma- USA)

Reagents

1. HCl (0.1M) solution was prepared in distilled water.
2. 2M HCl, 2M NaOH were prepared in distilled water

Standard solutions

- Stock standard solutions of ACV and degradate (0.5 $mgmL^{-1}$) in 0.1M HCl, and working standard solutions of ACV and its degradate (0.1 $mgmL^{-1}$) in 0.1 M HCl (for DD^1 method).
- Stock standard solutions of ACV and its degradation product (1 $mgmL^{-1}$) in mobile phase, and working stan-

dard solutions of ACV and its degradate (0.2 mgmL^{-1}) in mobile phase (for HPLC method).

Laboratory prepared mixtures

Solutions containing different ratios of ACV and its degradate were prepared according to molecular weight of ACV and Guanine to contain up to 80% of degradation product (for DD^1 method) and up to 70% (for HPLC method)

PROCEDURES

Construction of calibration graph for DD^1 spectrophotometric method

Transfer accurately aliquot portions equivalent to (50–300 µg) of ACV from its working standard solution (0.1 mgmL^{-1}) into a series of 5 mL calibrated volumetric flasks. Complete to the volume with 0.1M HCl to reach the concentration range of 10–60 µgmL^{-1}. Accurately transfer 100µg of the degradate (Guanine) from its working standard solution (0.1mgmL^{-1}) into a 5- mL calibrated volumetric flask and complete the volume with 0.1M HCl to get a final concentration of 20 µgmL^{-1} to be used as a divisor. Scan the spectra of the prepared solutions (220–280 nm) and store into the PC. Divide the stored spectra of ACV (amplitude at each wavelength) by the spectrum of 20 µgmL^{-1} of the degradation product. Measure the amplitudes of the first-derivative peaks of ACV at 265 nm with $\Delta\lambda$= 2 nm and a scaling factor = 6. Construct calibration graphs relating the peak amplitudes of (DD^1) to the corresponding concentrations. Compute the regression equation for the studied drug at the specified wavelength and determine unknown samples containing ACV.

Construction of calibration graph for liquid chromatographic method

Transfer accurately aliquot portions equivalent to 100–600 µg from ACV working standard solution (0.2mgmL^{-1}) into a series of test tubes, add 3mL 0.1M HCl andsonicate for 5 minutes. Filter the samples through a 0.45 µm membrane filter into 5-mL calibrated volumetric flask complete to volume with the mobile phase. Chromatograph triplicates 20-µL injections of each solution using a Xterra RP18 5µm column (250 mm × 4.6 mm, 5µm) and acetonitrile: water (4:96 v/v) as a mobile phase at a flow rate of 0.6 mL/ min and UV detection of effluent at 254nm. Maintain the column at ambient temperature (~25 °C). Filter the mobile phase through 0.45µm Teflon membrane filter and degas for ~15 min in ultrasonic bath prior to use. To reach good equilibrium, perform the analysis after passing ~50–60 mL of the mobile phase, just for conditioning and prewashing of the stationary phase. Record the peak areas and calculate the average peak areas. Plot the calibration graph representing the relative peak areas against the corresponding concentrations of ACV in µgmL^{-1} and compute the regression equation.

Analysis of laboratory prepared mixtures containing different ratios of ACV and its degradate using the suggested methods

Proceed as mentioned under the calibration procedure of each method. The concentration of Acyclovir was calculated from the corresponding regression equation.

Lab mixtures were prepared according to the molecular weight of ACV and Guanine.

Application to pharmaceutical formulation

Acyclovir 400 stada tablets

Weigh and finely powder twenty tablets. Accurately weigh and transfer a portion of the powder claimed to contain 400mg of ACV into 100-mL beaker, add 15mL 0.1M HCl. Sonicate the solutions for 30 minutes, filter through filter paper into 100-mL calibrated volumetric flask and complete to the volume with 0.1M HCl (for DD^1 method) and with the mobile phase (for HPLC method). Dilute the solution to the same concentration of the working solution and proceed according to the calibration procedure of each method mentioned above. Calculate the concentration of ACV from its corresponding regression equation.

Novirus 200mg capsules

Weigh the content of twenty tablets. Accurately weigh and transfer a portion of the powder claimed to contain 200mg of ACV into 100-mL beaker, add 15mL 0.1M HCl. Sonicate the solutions for 30 minutes, filter through filter paper into 100-mL calibrated volumetric flask and complete to the volume with 0.1M HCl (for DD^1 method) and with the mobile phase (for HPLC method). Dilute the solution to the same concentration

of the working solution and proceed according to the calibration procedure of each method mentioned above. Calculate the concentration of ACV from its corresponding regression equation.

Acyclovir 5% cream

Weigh accurately a quantity of the well-mixed cream claimed to contain about 200 mg of Acyclovir into 100-mL beaker, add 15mL 0.1M HCl. Sonicate the solutions for 30 minutes, filter through filter paper into 100-mL calibrated volumetric flask and complete to the volume with 0.1M HCl (for DD^1 method) and with the mobile phase (for HPLC method). Dilute the solution to the same concentration of the working solution and proceed according to the calibration procedure of each method mentioned above. Calculate the concentration of ACV from its corresponding regression equation.

Acyclovir 3% ointment

Weigh accurately a quantity of the ointment claimed to contain about 200 mg of Acyclovir into 100-mL beaker, add 15mL 0.1M HCl. Sonicate the solutions for 30 minutes, filter through filter paper into 100-mL calibrated volumetric flask and complete to the volume with 0.1M HCl (for DD^1 method) and with the mobile phase (for HPLC method). Dilute the solution to the same concentration of the working solution and proceed according to the calibration procedure of each method mentioned above. Calculate the concentration of ACV from its corresponding regression equation.

Zovirax suspension

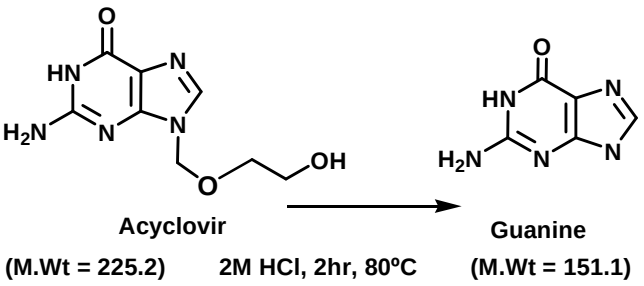
Transfer an accurately measured amount of well shaken oral suspension claimed to contain 200mg of ACV into 100-mL beaker, add 15mL 0.1M HCl. Sonicate the solutions for 30 minutes, filter through filter paper into 100-mL calibrated volumetric flask and complete to the volume with 0.1M HCl (for DD^1 method) and with the mobile phase (for HPLC method). Dilute the solution to the same concentration of the working solution and proceed according to the calibration procedure of each method mentioned above. Calculate the concentration of ACV from its corresponding regression equation.

RESULTS AND DISCUSSION

ACV was subjected to acid and alkaline hydrolysis, oxidation degradation. The drug was found to be degraded under acidic condition, the guanine was obtained, and being inactive and impurity[35], the determination of ACV in the presence of its degradation product was essential.

It was found that complete acid hydrolysis of ACV was achieved by heating at 80°C with 2M HCl for 2hr yielding the degradation product[35].

Scheme of degradation



The International Conference on Harmonization (ICH) guideline entitled "stability testing of new drug substances and products" requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance[36]. An ideal stability-indicating method is the one that quantifies the standard drug alone and also resolves its degradation product.

Method development

First derivative of ratio spectra (DD^1) method

The zero order spectra of ACV shows overlap with its degradate (Figure 2). In order to improve the selectivity of the analysis of ACV in the presence of its degradation product, DD^1 spectrophotometric method was established. The main advantage of the method is that the whole spectrum of the interfering substance is cancelled. Accordingly, the choice of the wavelength se-

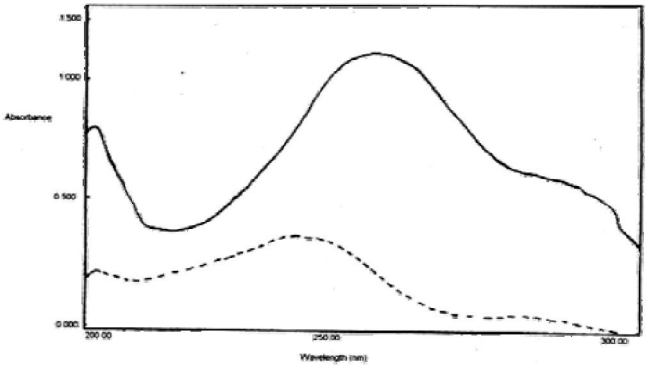


Figure 2 : Zero order spectrum of ACV 60 µgmL^{-1} (—) and zero order spectrum of Guanine 20 µgmL^{-1} (…).

lected for calibration is not critical as in the D^1 method.

In order to optimize DD^1 method, several divisor concentrations (10, 20, 30 and 40 μgmL^{-1}) of the degradate were tried, the best result was obtained when using 20 μgmL^{-1} of the degradation product as a divisor. Different smoothing and scaling factors were tested, where a smoothing factor ?λ = 2 and a scaling factor = 6 were suitable to enlarge the signal of ACV to facilitate its measurement and to diminish error in reading the signal (Figures 3, 4).

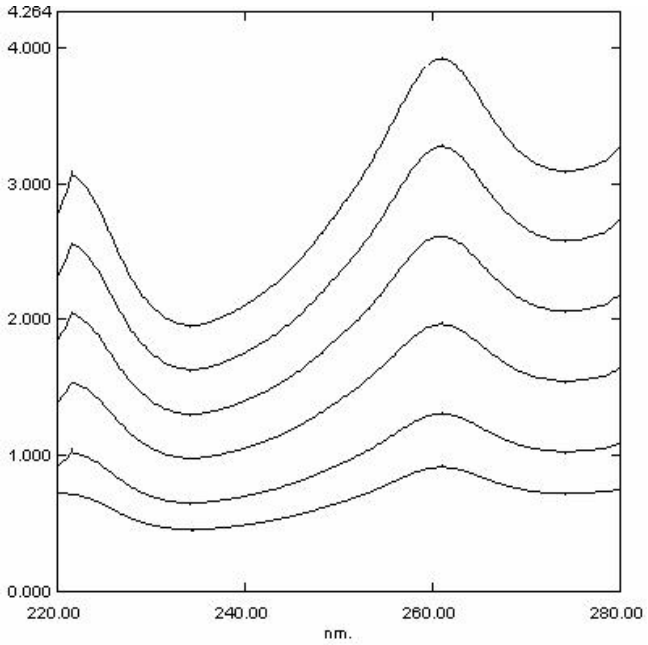


Figure 3 : Zero order of ratio spectra of ACV (10–60 μgmL^{-1}) using 20 μgmL^{-1} of degradate as a divisor.

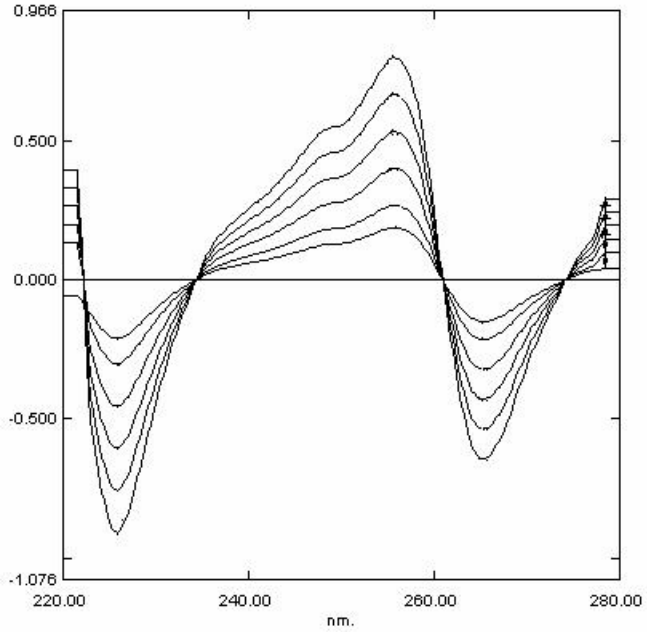


Figure 4 : First derivative of ratio spectra of ACV (10-60 μgmL^{-1}) using 20 μgmL^{-1} of Guanine as a divisor.

Dividing the absorption spectra of ACV in the range of 10-60 μgmL^{-1} by the absorption spectrum of the degradate 20 μgmL^{-1} (as a divisor); the obtained ratio spectra were differentiated with respect to wavelength.

DD^1 values showed good linearity and reproducibility at 265nm, the linear regression equation was computed and found to be

DD^1 = 0.0104C + 0.0148 r = 0.9995 at 265 nm,

Where DD^1 is the peak amplitude of the first derivative curve for (ACV / its degradate), C is the concentration of ACV (μgmL^{-1}) and r is the correlation coefficient.

The method was checked by analysis of laboratory prepared mixtures of ACV and its degradate in different ratios as presented in TABLE 2, where at 265nm, ACV could be determined in presence of up to 80%of degradate with mean percentage recovery 100.53±0.86.

High-performance liquid chromatography

A simple isocratic high-performance liquid chromatographic method was developed for the determination of ACV in pure form and in pharmaceutical preparation.

To improve separation of peaks it was necessary to investigate the effect of different variables. Studying the optimum parameters for maximum separation was carried out as follows:

Stationary phase

Different types of stationary phase C8 and C18 columns with different dimensions and particles sizes were tried (Agilent C8 Zorbax, Agilent C18Zorbax TM, Agilent C8 Eclipse, Agilent C18 Eclipse and Xterra RP18 columns), to get the best stationary-mobile phase match.

It was clearly found that Xterra RP18 [(ODS), 5 µm (250 mm x 4.6mm, i.d.)], Agilent (USA) gave the most suitable resolution for quantification of ACV and its degradate.

Mobile phase

Different mobile phases with varying organic modifiers including acetonitrile and methanol have been tested for optimizing the HPLC-separation. The mobile phase selection was based on peak parameters (symmetry,

tailing), run time, ease of preparation and cost. The mobile phase containing methanol:water in the proportion (10:90, v/v), (20:80, v/v), (30:70, v/v) and (70:30, v/v), and acetonitrile:water in the proportions (15:85, v/v), (10:90, v/v), (8:92, v/v), (6:94,v/v), (4:96,v/v) and (2:98, v/v) were tried. The mobile phase containing acetonitrile:water (4:96, v/v), flowing at 0.6mL/min was found to be quite satisfactory for the good resolution and determination of the studied drug substance in the presence of its degradate.

Detector wavelength

For determination of the optimum HPLC-UV detector wavelength, the method was repeated using the same chromatographic conditions at different wavelengths (200-300 nm), where, the optimum wavelength with ideal sensitivity and low noise was at 254 nm and is quite far from the cut-off of water and acetonitrile. Upon applying the previously mentioned chromatographic conditions, well resolved sharp peaks of ACV

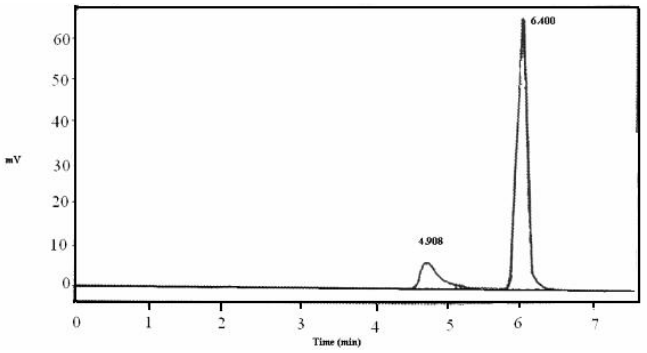


Figure 5 : Liquid chromatographic separation of ACV (6.4 min) from Guanine (4.9min), experimental conditions.

and its degradation product appeared at retention times of 6.4 min and 4.9 min in order. The total run time for a complete quantification of the drug and its degradate was ~7 min. A typical chromatogram was obtained from the analysis of a laboratory prepared mixture of ACV and its degradation product by using the proposed method (Figure 5).

System suitability

System suitability parameters[37,38] were calculated under the optimized experimental conditions. The ACV and the degradation product could be eluted in the form of symmetrical peaks quite away from each other and the retention time values of the separated peaks together with other chromatographic parameters are shown in TABLE 2.

The table describes the calculated resolution value (Rs) as well as selectivity factor (α), which ensures complete or 100% separation of the components under investigation. The Tailing factor of the studied drug peak also revealed a linear isotherm peak elution without tailing. (TABLE 1a)

TABLE 1a : Parameters required for system suitability test of HPLC method

Parameter	Obtained value	Reference value
Resolution (R)	1.43	R > 0.8
T (tailing factor)	0.83	T = 1 for a typical symmetric peak
α (relative retention time)	1.35	> 1
K' (column capacity)	9.66	1- 10 acceptable
N (column efficiency)	2718	Increases with efficiency of the separation
HETP	0.0092	The smaller the value, the higher the column efficiency

Robustness

The robustness of the HPLC method was investigated by the analysis of samples under a variety of experimental conditions such as small changes in proportions of different components, by up to ±0.5 % mainly of the organic part of the mobile phase. The effect on retention time and peak parameters were studied. It was found that the method was robust when the column and the mobile phase ratio were varied. During these investigations, the retention times were modified, however the areas and peaks symmetry were conserved.(TABLE 1b)

This HPLC method has an advantage over the of-

TABLE 1b : System suitability parameters after small change in some working parameters

Parameters	Changed parameter	
	Flow rate[¤]	Mobile phase composition[Ψ]
t_R (min)*	6.55±0.21	6.36±0.85
Number of theoretical plates (N)	2059	1989
HETP	0.013	0.026
Tailing factor (T)	0.76	0.91

***Triplicate run per a sample.**

[¤] Flow rate was 0.6±0.1 mL/min.

[Ψ] Mobile phase composition was acetonitrile: water (4:96 v/v) ± 0.5% change of the organic part in the mobile phase.

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ficial HPLC method[39]; as it allows better separation of ACV and its degradate at retention times of 6.4 and 4.9 minutes respectively, while in the official HPLC method the retention times are 1 minute and 0.6 minute for ACV and its degradate respectively.

A linear relationship was obtained by plotting the relative peak area ratio to the concentration of ACV (μgmL^{-1}). The linearity range was found to be 20–120 μgmL^{-1} using the following regression equation

A = 0.01C + 0.192 r = 0.9995 at 254 nm

Where A is the peak area ratio, C is the concentration of ACV (μgmL^{-1}) and r is the correlation coefficient.

The proposed method is valid for the determination of ACV in presence of up to 70% of its degradation product in different laboratory prepared mixtures as presented in TABLE 2, with mean percentage recovery 99.72±0.66.

Application to pharmaceutical preparation

The suggested methods are valid and applicable for the analysis of ACV in different pharmaceutical dosage forms. The validity of the proposed methods was assessed by applying the standard addition technique, which showed accurate results with no interference from excipients as shown in TABLE 3.

Method validation

Method validation was performed according to USP guidelines[39] for all the proposed methods as follows:

Range and linearity

The linearity of the method was evaluated by

TABLE 2 : Determination of Acyclovir in the presence of its degradate in laboratory prepared mixtures by the proposed methods

Method	Percentage%		Recovery%
	ACV	*Degradate	ACV
DD[1] method	90	10	99.58%
	80	20	100.00%
	60	40	100.20%
	40	60	101.40%
	20	80	101.50%
Mean±S.D			100.53±0.86
HPLC method	90	10	99.18%
	80	20	99.28%
	70	30	99.57%
	50	50	99.75%
	30	70	100.83%
Mean±S.D			99.72±0.66

***Lab mixtures are prepared according to the molecular weight of ACV and Guanine**
*** Gunaine**

TABLE 3 : Comparison of the results obtained by the proposed methods and the official method[2] for the analysis of ACV in its pharmaceutical formulations and application of the standard addition technique

Pharmaceutical dosage form	DD[1] method	HPLCmethod	** Official HPLC method[2]
	Found% ± S.D	Found% ± S.D	Found% ± S.D
(a) Acyclovir ® tablets			
B.No. 716302	99.19±0.48	99.74±0.92	99.23±0.54, 99.98±0.35
B.No. 716303	99.44±0.61	100.41±0.81	99.42±0.65, 100.23±0.55
(b) Novirus ® capsule			
B.No. 683500	100.67±0.77	100.53±1.03	100.16±1.02, 100.20±1.12
B.No. 683501	100.58±0.88	98.72±0.97	99.99±0.78, 99.99±0.63
(c) Acyclovir 5%® cream			
B. No. 603126	98.94±0.57	100.09±0.62	100.81±0.59, 99.05±0.68
B.No. 603127	99.99±0.81	100.26±0.84	98.57±0.77, 100.57±0.81
(d) Acyclovir 3% ® ointment			
B.No. 303066	100.91±0.95	99.14±0.54	100.81±0.59, 100.19±1.10
B.No. 303067	100.39±0.97	99.36±0.71	98.57±0.77, 100.28±0.93
(e) Zovirax ® susp			
B.No. 071040	100.06±0.95	100.71±1.07	99.38±0.93, 99.06±0.59
B.No. 071041	100.18±0.8905	100.36±0.79	101.33±1.21, 100.36±0.91

**** Official HPLC: using glacial acetic acid in water (1 in 1000) and a 4.2mm x 25 cm column at a flow rate 1.5 mL/min. Retention time for ACV and Guanine was 1 min and 0.6 min respectively.**

processing 6-point calibration curves on 3 different days. The calibration curves were constructed within concentration ranges that were selected on the basis of the anticipated drug concentration during the assay of the dosage form. A linear least-squares regression analysis was conducted to determine slope, intercept, and coefficient of determination to demonstrate linearity of the method. The goodness of fit in all cases was found to be >0.9966, indicating a functional linear relationship. The relevant slope values were statistically different from zero ($P < 0.05$), and though the intercepts of the calibration curves were significantly different from zero, they did not affect the accuracy of the method. The linear regression analysis data is summarized in TABLE 4.

TABLE 4 : Assay parameters and validation sheet for determination of ACV

Parameters	DD[1] method	HPLC method
Range	10– 60 μg.mL^{-1}	20 – 120 μg.mL^{-1}
Regression equation		
Slope	0.0104	0.01
Intercept	0.0148	0.192
Correlation coefficient	0.9995	0.9995
Mean ± S.D	100.06 ± 0.89	99.62 ± 0.66
RSD%[a]	0.900%	0.600%
RSD% [b]	1.110%	0.800%
LOD	1.12	1.39
LOQ	1.97	2.08

[(a)] Intraday relative standard deviation, [(b)] Interday relative standard deviation

Limits of detection and quantification

The limit of detection (LOD) and limit of quantification (LOQ) were calculated respectively according to the current ICH guidelines[40,41] with a ratio of 3.3 and 10 standard deviations of the blank and the slope of the calibration line (TABLE 4).

Accuracy

To study the accuracy of the proposed methods, procedures under study of linearity were repeated three times for the determination of six different concentration of pure ACV. The accuracy expressed as percentage recoveries is shown in TABLE 4. Good accuracy of the developed methods was indicated by the results obtained.

Precision

The precision of the proposed method, expressed as RSD, was determined by the analysis of three different concentrations of pure ACV within the linearity range of ACV. The intraday precision was assessed from the results of three replicate analyses of pure ACV on a single day. The inter-day precision was determined from the same samples analyzed on three consecutive days. The results of intraday and inter-day precisions are illustrated in TABLE 4.

Statistical analysis

TABLE 5 shows statistical comparison of the results obtained by the proposed methods and the official HPLC method[39]. The calculated t and F values are less than the theoretical ones indicating that there is no signi?cant difference between the proposed methods and the official HPLC method[39] with respect to accuracy and precision.

TABLE 5 : Statistical comparison for the results obtained by the proposed methods and the official method[2] for the analysis of pure powder form of Acyclovir

Parameter	DD[1]-Method At 265nm	HPLC method	**Official HPLC method
Mean	100.06	99.62	99.99
S.D.	0.89	0.66	0.57
Variance	0.79	0.43	0.32
n	6	6	6
F-test	2.47(5.05)*	1.34 (5.05)*	
Student's t-test	0.164(2.228)*	1.05 (2.228)*	

*** The values in the parenthesis are the corresponding theoretical t- and F-values at P = 0.05.**

**** HPLC method, using glacial acetic acid in water (1 in 1000) and a 4.2mm x 25 cm column at a flow rate 1.5 mL/min. Retention time for ACV and Guanine was 1 min and 0.6 min respectively.**

One-way ANOVA was applied for the comparison of those methods, where there was no signi?cant difference between the proposed methods and the official HPLC method[39] (TABLE 6).

TABLE 6 : Statistical comparison for the results obtained by the proposed methods and the official method[2] for the determination of pure powder form of Acyclovir using one way ANOVA

Source	DoF	Sum of Squares	Mean Square	F Value	P Value
Model	2	1.36297778	0.681488889	0.77871	0.47669
Error	15	13.1272000	0.875146667		

At the 0.05 level, the population means are not significantly different.

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CONCLUSION

The present work is concerned with the determination of ACV in the presence of its degradation product. In this paper, simple, sensitive and rapid methods are described for the determination of ACV in pure form or in pharmaceutical formulations.

The DD[1] spectrophotometric methods is well-established techniques that is able to enhance the resolution of overlapping bands. This method is simple, more convenient, less time-consuming and economic stability-indicating method compared to other published methods.

The proposed HPLC method is simple and rapid (total run time ~ 7min.) for separation of ACV and its degradation product.

High values of correlation coefficients and small values of intercepts validated the linearity of the calibration graphs and the obedience to Beer's law. The RSD values, the slopes and the intercepts of the calibration graphs indicated the high reproducibility of the proposed methods.

From the results obtained, it is concluded that the suggested methods show high sensitivity, accuracy, reproducibility and specificity and can be used as stability indicating methods. Moreover, these methods are simple and inexpensive, permitting their application in quality control laboratories.

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