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Research Article

NEW ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ACYCLOVIR BY RP-HPLC METHOD

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ABSTRACT

A rapid new analytical method development and validation indicating RP-HPLC method was developed and validated for determination of acyclovir in bulk dosage form. Sample was analyzed on a kromasil ODS C_{18} column (250 × 4.6 mm, 5µ). The mobile phase consists of glacial acetic acid: acetonitrile buffer (pH 3.8) in the ratio of 95:05 at flow rate 1 ml min^{-1} with UV detection wavelength at 253nm. The retention time of acyclovir was 4.070 minutes. The calibration curve was linear over the concentration range of 1-32 µg/ml (r^2=0.999). Acyclovir was found to degrade in alkaline and oxidative stress conditions. However it was stable in acid and dry heat conditions. The validation studies were carried out according to ICH guidelines. The development method was found to be linear, precise, accurate and robust.

Keywords: Acyclovir(ACV), ICH, Validation, RP-HPLC, UV-Detection, precise, 253 *n*m.

INTRODUCTION

Aciclovir (ACV), also known as acyclovir, is an antiviral medication[3]. It is primarily used for the treatment of virus infections, chickenpox, and shingles. Other uses include prevention of cytomegalovirus infections following transplant and infections due to Epstein-Barr virus. It is available by mouth and intravenously[4]. It is generally considered safe for use in pregnancy with no harm having been observed[4,5]. It appears to be safe during breastfeeding . Aciclovir is a nucleic acid analogue made from guanosine. It works by decreasing the production of the virus's DNA[4].

Acyclovir, also called acycloguanosine, antiviral drug used to control the symptoms of infections involving herpes simplex virus (HSV), which causes herpes simplex, or varicella-zoster virus (VZV; a type of herpes virus), which causes shingles and chickenpox. Acyclovir was first discovered in the mid-1970s and is effective against active, replicating HSV or VZV. Acyclovir belongs to a group of synthetic drugs called nucleoside analogs, which are characterized by their similarity to naturally occurring nucleosides—the structural subunits of DNA and RNA—that are found in cells and viruses.

The discovery of aciclovir was announced in 1977[8]. It is on the World Health Organization's List of Essential Medicines, the most important medications needed in a basic health system[9]. It is available as a generic medication and is marketed under many brand names worldwide[1].

Aciclovir is used for the treatment of herpes simplex virus and varicella zoster virus infections, including:[2,10,11].

- Genital herpes simplex (treatment and prevention)
- Neonatal herpes simplex
- Herpes simplex labialis (cold sores)
- Shingles
- Acute chickenpox in immunocompromised patients
- Herpes simplex encephalitis
- Acute mucocutaneous HSV infections in immunocompromised patients
- Herpes of the eye and herpes simplex blepharitis (a chronic (long-term) form of herpes eye infection)
- Prevention of herpes viruses in immunocompromised people (such as people undergoing cancer chemotherapy)[12].

Acyclovir, 9-[(2-hydroxyethoxy)-methyl]-guanosine, is an acyclic guanosine derivative which exhibits a selective inhibition of herpes viruses replication with potent clinical antiviral activity against the herpes simplex and varicella-zoster viruses[13,14]. HPLC methods have been reported for determination of acyclovir in human serum using UV [15-25] or fluorescence detection[26-30]. Thus, it is highly effective in disrupting the formation of herpes virus DNA and has very little activity in uninfected cells, even at high concentrations.

However, mutation of HSV-TK or VZV-TK can cause resistance to acyclovir. Acyclovir may be taken orally, applied topically, or injected intravenously.

MATERIALS AND METHODS

Optimized chromatographic conditions for the determination of ESL by HPLC:

Column: kromasil ODS C_{18} column
Flow rate: 1.0 ml/min
Column temperature: 24^{o} C
Materials required: Acetonitrile, Glacial acetic acid, Water, and Acyclovir.
Injection volume: 20.0µL
Diluent: Glacial acetic acid
Preparation of Mobile phase: A mixture of 0.01M Glacial acetic acid of pH-3.8 and acetonitrile in the ratio of 95:05 (v/v).

Preparation of Stock Solutions:

An ACV stock solution (1 mg/ml) was prepared by dissolving a 25 mg of Acyclovir in a 25ml volumetric flask separately and dissolved in acetonitrile and the volume was made up to the mark with the acetonitrile. From the above 1mg/ml solution, six dilutions in between 1-32 µg/ml of Acyclovir were made with Acetonitrile by pipetting out 0.1-3.2 ml from the 1mg/ml solution.

Preparation of Sample Solution:

25 mg powder of Acyclovir was weighed. The powder was accurately transferred to 25ml volumetric flask containing 20 ml of the acetonitrile and sonicated for 5-10 min. The above solution was carefully filtered through Whatmann filter paper (No. 41) only if solution remains unclear and then the volume was made up to the mark with Acetonitrile.

METHOD DEVELOPMENT

Selection of wavelength: ACV standard solutions were prepared in diluent at a concentration range of 10µg/ml and scanned in UV detector; all the solutions of ACV were having UV maxima at around 253 nm. Hence detection at 253 nm was selected for method development purpose.

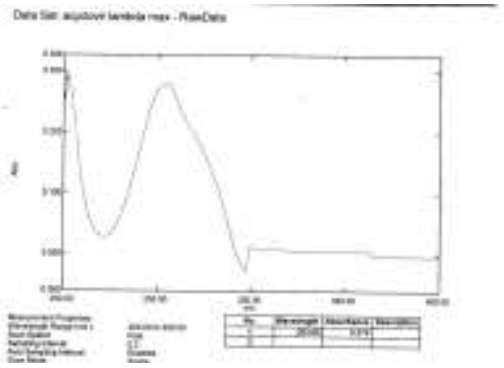


Figure: UV-Spectrum of Acyclovir

Method development approach for the selection of suitable column and mobile phase:

The main aim of the chromatographic method was to separate critical closely eluting compounds of ACV and to elute ACV as a symmetrical peak. ACV spiked solutions were subjected to separation by reversed-phase kromasil ODS C_{18} column, 150 x 4.6mm x 3.5µm and carried out the analysis with the above conditions. The successful separation was observed with resolution greater than 2.0.

To study the importance of the method with respect to mobile phase pH, buffers of various pH were prepared from pH=2.0 to 7.0.

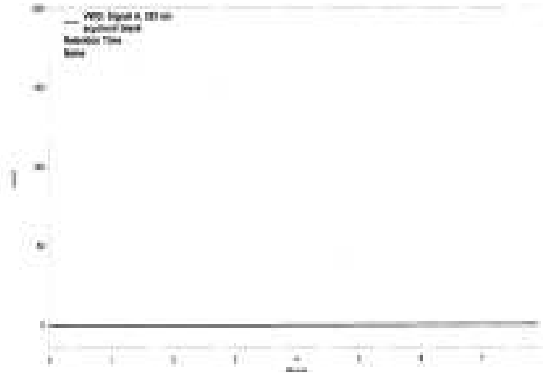


Figure: Typical blank chromatogram of method development

VALIDATION PARAMETERS

Accuracy:

The accuracy of an analytical method is the degree of closeness between the true value of analytes in the sample and the value determined by the method and is sometimes called trueness. Accuracy can be measured by analyzing samples with known concentrations and comparing the measured values with the true values. According to FDA, the accuracy for bio-analysis should be determined by a minimum of five determinations for at least three concentrations (low, medium and high) in the range of expected concentrations.

% Active/impurity content	Acceptable mean recovery
≥ 10	98 –102%
≥ 1	90 –110%
0.1 – 1	80 – 120%
< 0.1	75 – 125%

Table: Acceptance Criteria for Accuracy

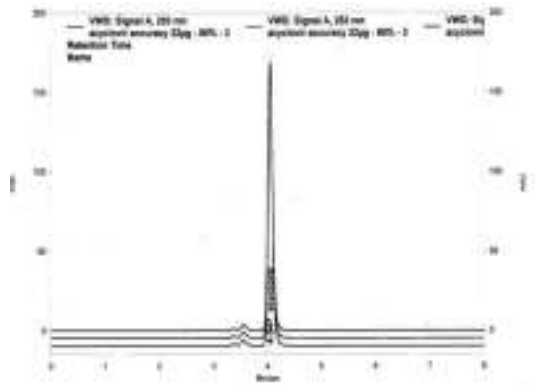


Figure: accuracy of Acyclovir at 80%

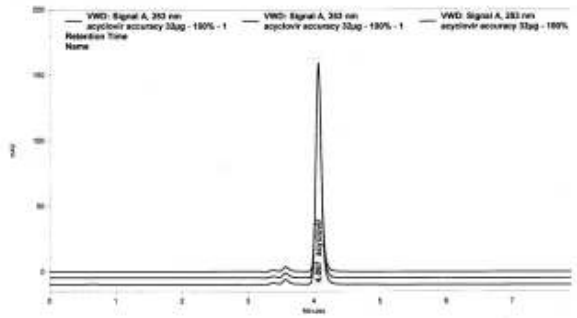


Figure: accuracy of Acyclovir at 100%

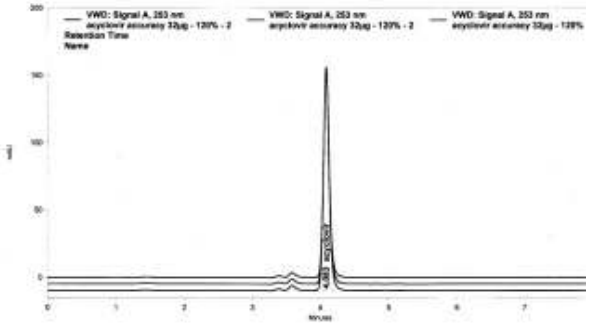


Figure: accuracy of Acyclovir at 120%

Precision:

The precision of an analytical method is the closeness of a series of individual measurements of an analyte when the analytical procedure is applied repeatedly to multiple aliquots of a single homogeneous volume of biological matrix. The precision is calculated as coefficient of variation (C.V.) i.e., relative standard deviation (RSD). The measured RSD can be subdivided into three categories: repeatability (intra-day precision), intermediate precision (inter-day precision) and reproducibility (between laboratories precision). Repeatability should be tested by the analysis of a minimum of five determinations at three different concentrations (low, medium and high) in the range of expected concentrations, according to FDA.

Component measured in sample	Precision
≥ 10.0%	2%
1.0 up to 10.0%	≤ 5%
0.1 up to 1.0%	≤ 10%
<0.1%	≤20%

Table: Acceptance Criteria for Precision

Precision may be measured at three levels:

- Repeatability,
- Intermediate precision and
- Reproducibility.

It is normally expressed as RSD %.

Repeatability is the results of a method operated over a short interval of time under the same conditions.

Inter-day precision

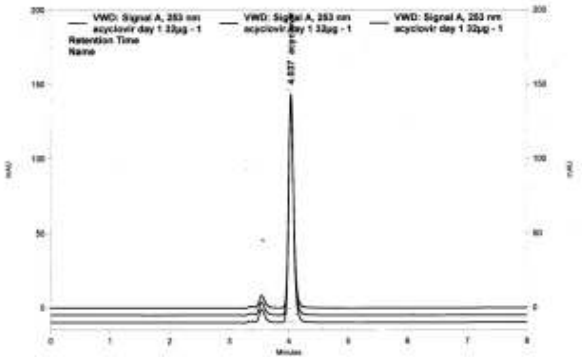


Figure: Chromatogram for precision ACV Day1

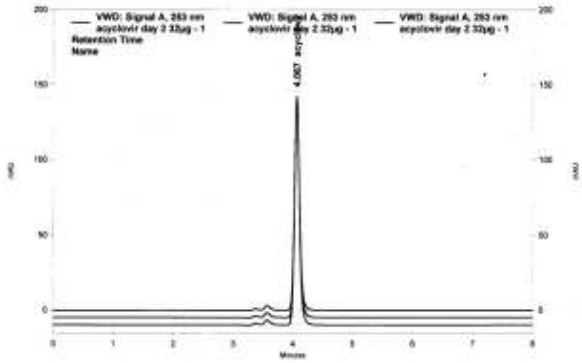


Figure: Chromatogram for precision ACV Day2

Intraday precision

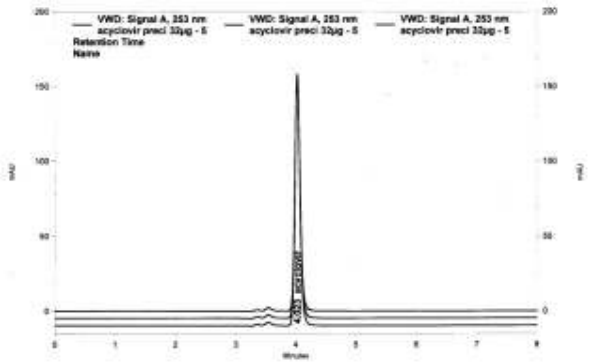


Figure: Chromatogram for precision ACV Morning

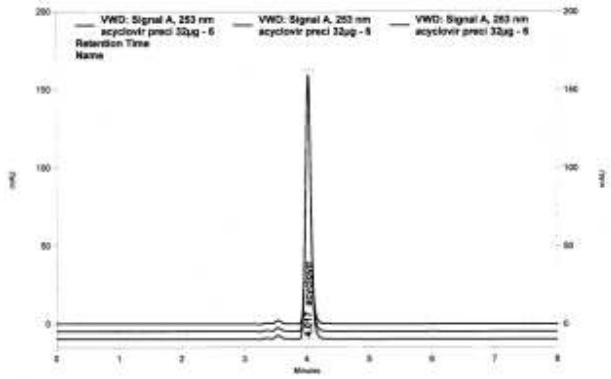


Figure: Chromatogram for precision ACV After noon

Reproducibility is determined by testing the homogeneous samples in different laboratories. It is a measure of precision between laboratories.

Specificity

It is defined as the instrument's ability to measure or identify the analyte without any interference from sample matrix, impurities, precursors or degradation products.

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedures.

Linearity:

The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analytes in samples within a given range or proportional by means of well-defined mathematical transformations. Linearity may be demonstrated directly on the test substance (by dilution of a standard stock solution) and/or by using separate weighing of synthetic mixtures of the test product components, using the proposed procedure.

Linearity is determined by a series of 3 to 6 injections of 5 or more standards whose concentrations span 80–120 percent of the expected concentration range. The response should be directly proportional to the concentrations of the analyte or proportional by means of a well-defined mathematical calculation. A linear regression equation applied to the results should have an intercept not significantly different from 0. If a significant nonzero intercept is obtained, it should be demonstrated that this has no effect on the accuracy of the method.

Linearity of Acyclovir

The linearity of an analytical method is the ability to attain test results which are directly proportional to the concentration of analyte with in the given range.

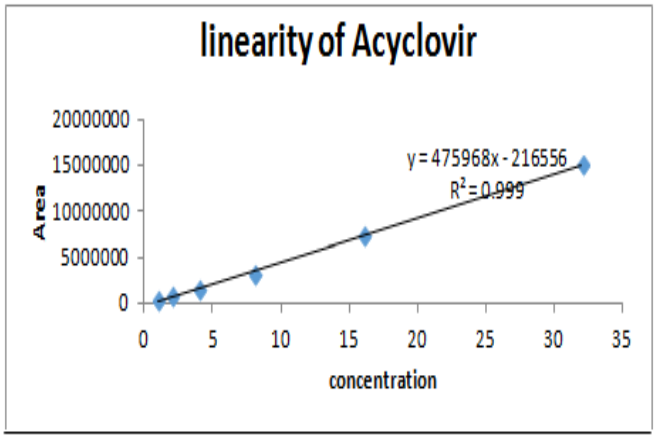


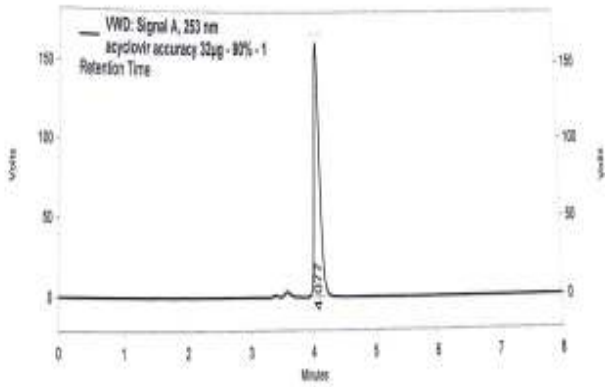
Fig No: 2.3 linearity of Acyclovir

Linearity coefficient (r^2) = 0.999

System suitability test:

System suitability testing is an integral part of chromatographic method. The tests are based to ensure that the equipment, analytical operations, electronics and samples to be analyzed make an integral system and it can be calculated as such.

Tailing factor and theoretical plates for ACV was calculated. System suitability results were tabulated.



Theoretical plates per column were calculated from the data obtained from the peak using the following expression

$n = (5.54Vr2)/LWh2$

Theoretical plates per meter were calculated from the data obtained from the peak.

$$n = (5.54Vr2)/Wh2$$

Where, 'n' is number of theoretical plates per meter, 'Vr' is the distance along the base line between the point of injection and a perpendicular dropped from the maximum of the peak of interest and 'Wh' is the width of the peak of interest at half peak height.

Tailing factor is also known as symmetry factor symmetry factor of peak was calculated from the following expression.

Symmetry factor = a/b

Where,

a = ½ width of the peak at one twentieth of the peak height,

b = ½ width of the peak at one twentieth of the peak height.

Limit of Quantification (LOQ) and Limit of Detection (LOD):

LOQ and LOD established for all impurities based on the impurities dilution linearity method.

Methodology for establishment of LOQ and LOD:

LOD and LOQ are determined by injecting linear solutions from 1µg/mL to 32µg/ml. The calculation method is based on the standard deviation (SD) of the response and the slope (S) of the calibration plot and using the formula;

LOQ = 10 x SD/S and

LOD = 3 x SD/S.

RESULTS AND DISCUSSION

The method that was developed and optimized in HPLC was considered for method validation. The analytical method validation was carried out in accordance with ICH guidelines. The results are discussed in the following section.

Accuracy:

The accuracy of an analytical method is measure of the closeness of test results obtained to the true value.

Table: Accuracy results of ACV

S.no	Area of the % level concentrations, mAU		
	80 %	100 %	120 %
1	15037607	15037607	15037607
2	15033201	15032506	15037323
3	15035667	15033465	15037432
Average	15035492	15034526	15037454
SD	2208.2267	2710.967	143.2725
% RSD	0.0146868	0.018032	0.000953

Precision results:

The precision of the method was also ensured by injecting six individual preparations of ACV. Upon repetitive injections at quantification limit, the peak properties like retention time, area were not influenced. Results have shown negligible variation in measured responses which revealed that the method was repeatable with RSD below 2 %.

Table: Interday precision for acyclovir

S.no	injection	Morning peak area	Noon peak area
		Acyclovir	Acyclovir
1.	Injection-1	15037607	15037607
2.	Injection-2	15036506	15033606
3.	Injection-3	15037307	15034607
4.	Injection-4	15037465	15035465
5.	Injection-5	15037356	15037236
6.	Injection-6	15037497	15034386
Average		15037290	15035485
SD		398.3123	1617.319
%RSD		0.002649	0.010757

Table: Interday precision for acyclovir

S.no	*injection*	*Day-1 peak area*	*Day-2 peak area*
		Acyclovir	*Acyclovir*
1.	*Injection-1*	15037607	15037607
2.	*Injection-2*	15038652	15033400
3.	*Injection-3*	15038600	15033465
4.	*Injection-4*	15037300	15035265
5.	*Injection-5*	15037201	15036426
6.	*Injection-6*	15032605	15035007
Average		15036994	15035195
SD		2240.704	1648.531
%RSD		0.014901	0.010964

Linearity:

Table: Linearity results of Acyclovir

S.No	Concentration in µg/ml	Peak Area*
1.	1	405875
2.	2	812599
3.	4	168209
4.	8	3285012
5.	16	7512854
6.	32	15037607
Linearity coefficient		**0.999**

System suitability test:

System suitability testing is an integral part of chromatographic method. The tests are based to ensure that the equipment, analytical operations, electronics and samples to be analyzed make an integral system and it can be calculated as such.

Table: Result of system suitability

S.No	Parameter	Acyclovir
1.	Theoretical plates per column	10778
3.	Capacity Factor	0.01158
4.	Asymmetry	1.20577
5.	S/N(ASTM)	239.146383

Limit of Quantification (LOQ) and Limit of Detection (LOD):

Table: LOD & LOQ of ACV

Parameter	Acyclovir
LOD(µg/ml)	0.4100
LOQ(µg/ml)	1.3667

Table: recovery results of ACV

S.no	Label	Amt.added	Amt.recoverd	%recovery	%RSD
1	400	320	400.056	100.014	0.002084
2	400	400	399.98	99.97	0.01002
3	400	480	400.04	100.1	0.434153

Acceptance criteria: The individual and the mean recovery value should be within 98 to 102%.

Conclusion: The result obtained in this method was within the limit of 98.0% to 102%.

The % RSD is less than 2.0.

SUMMARY AND CONCLUSION

The quick and effective RP-HPLC method developed for quantitative estimation of Acyclovir is accurate, precise, linear, and specific.

Table: summary of validation results by RP-HPLC

Validation parameters	Acyclovir
Mobile phase	Glacial acetic acid: acetonitrile
Flow rate	1ml/min
Detection wavelength	253 nm
Rt	4.070
Run time	8 min
Theoretical plates	10778
LOD(µg/ml)	0.4100
LOQ(µg/ml)	1.3667
Linearity (r^2)	0.999
Accuracy(%RSD)	0.0112237
Precision(%RSD)	0.009818

The proposed method does not require any laborious clean up procedure before measurement. Acceptable results were obtained from validation of the method. This method revealed an excellent performance in terms of sensitivity and speed.

The method is proven as stability-indicating and can be used for routine analysis of production samples and to quantify the samples of ACV in drug substances and pharmaceutical dosage forms.

Thus the method was validated as a safe, precise, fast, a much specific approach and accurate.

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