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A REVIEW FOR ESTIMATION OF ACYCLOVIR DRUG USING DIFFERENT ANALYTICAL METHODS

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ABSTRACT

Acyclovir, 9-(2-hydroxyethoxy) methyl guanine is a nucleoside analogue with antiviral activity against herpes viruses. This drug is an effective agent in the treatment of herpes virus infections and may also used in the prophylaxis of cytomegalovirus infections in immune compromised patients. The food and drug administration (FDA) approved treatment of genital herpes and HSV encephalitis. ACV is the first line treatment for HSV encephalitis. Currently there are no other medications indicated for the treatment of the condition. ACV incorporate itself into viral deoxyribonucleic acid (DNA), preventing further synthesis as it inhibits DNA synthesis and viral replication after it is converted to Acyclovir Triphosphate by viral and cellular enzymes and is available in both oral and Intravenous dose. This review article represents the collection and discussion of various analytical methods available in the literature for the determination of Acyclovir in the pharmaceutical and biological samples consisting of HPLC method, HPTLC method, RP-HPLC method, UV-spectroscopy method, UHPLC-HESI-MS/MS method. These methods were validated according to ICH guidelines for the range linearity, accuracy, precision, robustness, ruggedness, LOD, LOQ, and sensitivity This review provides details about the comparative utilization of various analytical techniques for the determination of Acyclovir. The present review article can be effectively explored to conduct future analytical investigation for Acyclovir.

INTRODUCTION

Acyclovir (ACV), also known as acyclovir, is an antiviral medication. It is primarily used for the treatment of herpes simplex virus infections, chickenpox and shingles. Other uses include prevention of cytomegalovirus infections following transplant and severe complications of Epstein Barr virus infection. It can be taken by mouth, applied as a cream or injection.

Chemical Characteristics: Acyclovir is a crystal powder, white to almost white with solubility soluble in dilute hydrochloric acid, difficult to dissolve in water, insoluble in ethanol. The melting point of acyclovir drug is 256.5^{o}C (493.7^{o}F) and boiling point of acyclovir is 595°C. The partition coefficient value in n-octanol at 22°C is – 1.57 at 25°C with a pH of 6.8 is-1.8 and the acyclovir pKa value at room temperature is 2.16 and 9.04 at 37°C.

Chemical taxonomy: Acyclovir is chemically known as 2-Amino-1,9-dihydro-9-((2-hydroethoxy) methyl)-3H-p urin-6-one is a member of class of antiviral medications called synthetic nucleoside analogues.

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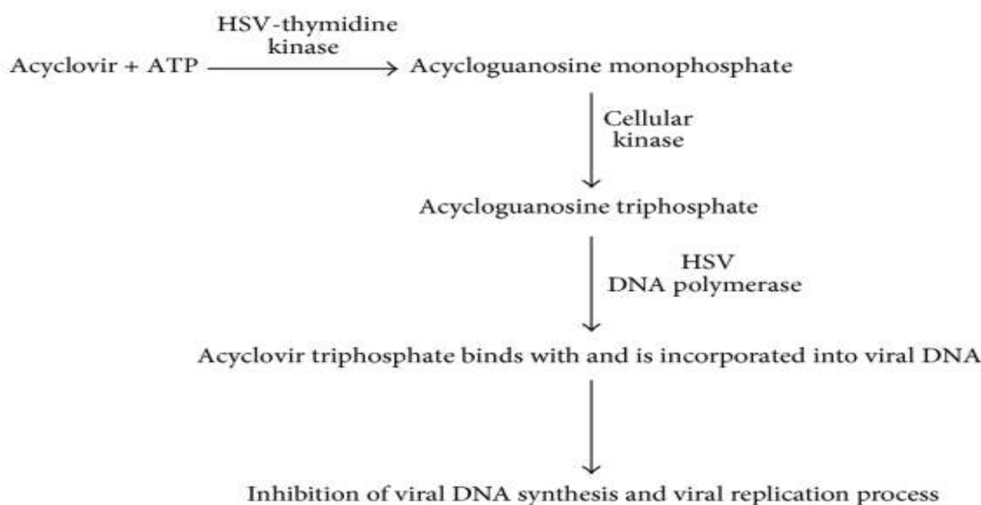

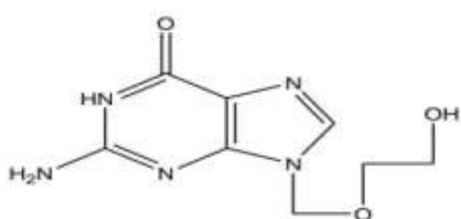

Chemical structure of Acyclovir

Molecular Formula: $C_8H_{11}N_5O_8$

Molecular Weight: 225.21

Mechanism of action: Acyclovir (9-[2-hydroxymethyl] guanine) is a nucleoside analogue that selectively inhibits the replication of herpes simplex virus types 1 and 2 (HSV-1, HSV-2) and varicella-zoster virus (VZV). After intracellular uptake, it is converted to acyclovir monophosphate by virally-encoded thymidine kinase. This step does not occur to any significant degree in uninfected cells and thereby lends specificity to the drug's activity. The monophosphate derivative is subsequently converted to acyclovir triphosphate by cellular enzymes. Acyclovir triphosphate competitively inhibits viral DNA polymerase by acting as an analogue to deoxyguanosine triphosphate (dGTP). Incorporation of acyclovir triphosphate into DNA results in chain termination since the absence of a 3' hydroxyl group prevents the attachment of additional nucleosides. Acyclovir triphosphate has a much higher affinity for viral DNA polymerase than for the cellular homolog, yielding a high therapeutic ratio.

PHARMACOKINETIC STUDIES:

The oral bioavailability of acyclovir ranges from 10 to 30 % and the percentage decreases with increasing dose. Peak plasma concentration average 0.4 to 0.8 µg/ml after 200mg and 1.6µg/ml after 800mg dose. The oral route is preferred to parenteral administration due to the risk of local toxicity at the injection site. Acyclovir is widely distributed attaining CSF concentration that is 50 percent of plasma concentration. It penetrates cornea well. Acyclovir is 9 to 33% protein bound in plasma. Acyclovir is metabolised in liver and 90-92 % of acyclovir is excreted in the urine as unchanged drug through glomerular filtration and tubular secretion. Plasma t1/2 is 2-3 hours. Renal impairment necessitates dose reduction.

Drug interaction associated the acyclovir are with the combination of Abatacept the metabolism of acyclovir can be increased when combined with Abatacept. The serum concentration of acyclovir can be increased when it is combined with Abiraterone.

Analytical Methods for Estimation of Acyclovir Drug in Pharmaceutical Dosage Form and Biological Samples.

RP-HPLC Method for Quantitative Estimation of Acyclovir In Bulk Drug And Tablets: The present work is aimed to develop a simple, rapid, reproducible, reliable, and efficient reversed phase high performance liquid chromatographic (RP-HPLC) method

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for estimation of an antiviral drug Acyclovir in raw material and its tablet dosage form. Separation was done by using mobile phase consisting of HPLC grade water and methanol in the ratio of 50:50. The separations were carried out on Welcome C_{18} column (250 into 4.6mm; 5µm) Shimadzu LC-20AT Prominence Liquid Chromatography. The flow rate was set at 1mL/min. The injection volume was 20µL and the UV detector was operated at 250nm using Shimadzu SPD-20A Prominence UV-Visible detector. The retention time of Acyclovir was found to be 3.077 minutes. The standard calibration plot was found linear over the range of 2 to 10 µg/mL and the coefficient of correlation was found to be (r^2 =0.9999). The %RSD values of intraday and interday precision were found below two which indicates that the method was highly precise. The LOD and LOQ were found to be 0.247µg/mL and 0.74µg/ml. The run time of Acyclovir is 5 minutes and the temperatures are ambient temperature (25ºC). The developed method was eventually applied for quantification of marketed formulation. The developed method was validated according to international conference on harmonization (ICH) guidelines for specificity, linearity, range, accuracy, precision, detection limit, quantitation limit, robustness, and system suitability parameters and results obtained were satisfactory. The method can be successfully utilized for the reliable quantification of Acyclovir in bulk and tablet dosage form.

RP-HPLC Method to Quantitative Acyclovir in Cross-Linked Chitosan Microspheres Produced By Spray Drying: An accurate, simple, reproducible, and sensitive liquid chromatographic method is developed and validated to qualitative acyclovir (ACV) in cross-linked chitosan microspheres produced by spray drying. The analysis is carried out using a reversed- phase C18 column with UV-vis detection at 254nm. The mobile phase is diluted with pure water and acetonitrile (95:5v/v) at a flow rate of 0.8mL/min. The parameters used in the validation process are: linearity, range, quantitation limit, detection limit, accuracy, specificity precision, ruggedness. The retention time of acyclovir is approximately 3.5min with symmetrical peaks. The linearity in the range of 1-10 µg/mL presents a correlation coefficient of 0.9999. The chitosan and the tripolyphosphate in the formulation do not interfere with the analysis, and the recovery proves to be suitable to quantitate ACV in cross-linked chitosan microspheres.

A Validated Stability Indicating Hptlc Method for Estimation of Acyclovir In Tablets In Presence Of Its Alkaline Hydrolysis Degradation Product: A sensitive stability indicating high-performance thin-layer chromatographic method was developed and validated for quantitative determination of Acyclovir in tablets. Chromatographic separation was performed on a precoated silica gel $60F_{254}$ HPTLC plates using Toluene: n-Butanol: Methanol: Water (50:30:10:10 v/v/v/v) as a mobile phase. A TLC scanner set at 259 nm was used for direct evaluation of the chromatograms in reflectance/absorbance mode. Acyclovir and degradant were satisfactorily resolved with Rf values of 0.62± 0.05, 0.78 ± 0.05, respectively. Calibration curve was a polynomial in the concentration range of 200-1000 ng/band. The high correlation coefficient ($r^2 > 0.9991$) values indicated clear correlations between the investigated compound concentrations and their peak areas within the test ranges. The method was validated according to ICH guidelines. The repeatability and intermediate precision, expressed by the RSD, were less than 2.0%. The accuracy and validity of the method were further ascertained by performing recovery studies via a standard addition method. The accuracy of the method expressed as percent recovery was satisfactory (99.85%). The drug was subjected to the International Conference on Harmonization (ICH)-prescribed hydrolytic, oxidative, photolytic and thermal stress conditions. The method was validated according to ICH guidelines. The drug showed instability in alkaline and oxide

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while it remained stable in heat and UV radiation conditions. The proposed. HPTLC method was utilized to investigate the alkaline degradation of Acyclovir (ACV). The performance of the method was validated according to the present ICH guidelines for specificity, limit of detection, limit of quantification, linearity, accuracy, precision, ruggedness and robustness.

High pressure liquid chromatographic method for estimation of acyclovir in bulk and marketed formulation: A simple, selective, rapid and economical High-Performance Liquid Chromatography (HPLC) method was developed for estimation of Acyclovir in bulk and marketed formulation. Chromatographic separation and estimation were achieved in a run time of 6.482 minutes on Hemsil C_{18}-5U (250 × 4.6 mm, 5μm) using Acetonitrile and pH adjusted water (6.74) in the ratio of 10:90 v/v at flow rate of 0.5ml/min. UV detector set at 254nm was used for detection. The linearity range for Acyclovir was found to be 4-40 µg/ml with coefficient of linear regression greater than 0.9948. The method was validated as per International Conference on Harmonization (ICHQ2 (R1) 2005) guidelines for accuracy, precision, specificity, LOD & LOQ, linearity and robustness. The method was successfully applied for estimation of Acyclovir from bulk and marketed formulation for routine analysis.

Quantification of Acyclovir in Human Plasma by Ultra-High-Performance Liquid Chromatography - Heated Electrospray Ionization - Tandem Mass Spectrometry for Bioequivalence Evaluation: A rapid, sensitive, and robust method for the determination of acyclovir in human plasma and its validation towards evaluating the bioequivalence of drug formulations. After a simple liquid-liquid extraction from plasma, acyclovir is quantified using ultra-high-performance liquid chromatography - heated electrospray ionization - tandem mass spectrometry (UHPLC-HESI-MS/MS). The assay has a total analysis time is 5 min, a linear y of 1.0 - 2000 ng/mL, a lower limit of detection of 0.5 ng/mL, and a lower limit of quantification of 1.0 ng/ml. Intra- and inter-day precision is no more than 10.3% and intra-and inter-day accuracy was within 13% at various concentrations in human plasma. Validation according to FDA guidelines for bioanalysis indicates that the described UHPLC-HESI-MS/MS method provides rigorous quantification of acyclovir in human plasma and representative data demonstrates successful application towards the determination of pharmacokinetic profiles as part of an evaluation of drug formulation bioequivalence.

UV Spectrophotometric of Cyclovir in Solid Dosage Form: A new, economical, sensitive, simple, rapid UV spectrophotometric method has been developed for the estimation of Acyclovir in pure form and pharmaceutical formulation. This UV method was developed using distilled water as a solvent. In the present method, the wavelength selected for analysis was 254nm.UV-Visible double beam spectrophotometer (Systronic 2201) was used to carry out the special analysis. The ICH guidelines were used to validate the method. The method was validated for linearity, range, accuracy, precision, robustness, LOD and LOQ. Linearity was performed by using a recovery study. The amount of drug recovered was found to be in the range of 100.1 to 100.5%. The % RSD value was found to be less than 2.

CONCLUSION: In the above review numerous analytical techniques for determining Acyclovir in bulk, pharmaceutical formulations and biological samples are summarised from the literature. For the determination of acyclovir analytical methods include HPLC, RP-HPLC, HPTLC, UV spectroscopy, UHPLC-HESI-MS methods were used. Hence these all methods are used to estimate and validate acyclovir in simple, economic, precise, accurate and reproducible ways. This review suggested that liquid chromatographic methods are widely used for the estimation of drug acyclovir.

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S. No	METHOD	DESCRIPTION	REFERENCE
1	RP-HPLC (Bulk drug and tablets)	Stationary phase: Welchrom C_{18} column (250× 4.6 mm; 5μm) Shimadzu LC-20AT Prominence Liquid Chromatography Mobile phase: water: methanol (50:50) Flow rate: 1ml/min Injection volume: 20μl UV detector wavelength: 250nm using Shimadzu SPD-20A Prominence UV visible detector Retention time: 3.077 minutes Linearity over the range: 2 - 10μg/ml Coefficient of correlation: (r^2= 0.9999) LOD: 0.2470 μg/ml LOQ: 0.7486 μg/ml Run time: 5 min Temperature: Ambient temperature (25°C) Theoretical plates [th.pl] (efficiency): 7568 Theoretical plates per meter (t.p/m): 151357 Tailing factor (asymmetry) :1.068 Intraday precision (%RSD) (n=3): 0.075 Interday precision (%RSD) (n=3) :0.089 Percentage recovery: 99.82	[3]
2	RP-HPLC (Cross linked chitosan microspheres produced by spray drying)	Stationary phase: Reversed phase C_{18} column Mobile phase: water: Acetonitrile (95:5v/v) Wavelength of detection: 254nm Flow rate: 0.8ml/ min Retention rate: Approximately 3.5min with. symmetrical peaks Linearity in range: 1-10 μg/ml Correction coefficient: 0.9999 pH of mobile phase: 2.5 +/- 0.5	[6]
3	HPTLC	Stationary phase: precoated silica gel $60F_{254}$ HPTLC Plate Mobile phase: Toluene: n-Butanol: Methanol: Water (50:30:10:10v/v/v/v) Wavelength: 259 nm $R_{f\ value}$ of acyclovir: 0.62 +/- 0.05 Saturation time for chamber: 25 minutes Saturation temperature for chamber: 25+/- 2°C Linearity range: 100-600 ng/band LOD: 8.08 ng/band LOQ: 12.16 ng/band	[1]

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4	HPLC	Stationary phase: Hemsil C_{18} -5U (250 × 4.6 mm, 5µm) Mobile phase: Acetonitrile: pH adjusted water (6.74) (10:90 v/v) Run time: 6.482 mins Flow rate: 0.5 ml/min Wavelength: 254 nm Linearity range: 4-40 µg/ml LOD: 0.016 µg/ml LOQ: 0.0486 µg/ml Precision (%RSD): Intraday Precision- 0.016 Interday precision- 0.04	[4]
5	UHPLC-HESI-MS/MS	Stationary phase: Waters BEH C18 column (50 mm × 2.1 mm, 1.7 µm) Mobile phase: linear gradient of the following mobile phases: A. 2 mM aqueous ammonium acetate :0.1% formic acid B. 100% methanol :0.1% formic acid Retention time: 1.2 mins Chromatographic run time: 5 min Linearity: 1.0 to 2000 ng/mL LOD: 0.5 ng/mL LOQ: 1.0 ng/ mL Intraday and Interday precision: not > 10.3 % Intraday and interday accuracy: <13%	[2]
6	UV spectroscopy	Solvent: Distilled water Wavelength: 254nm Instrument model: UV-Visible double beam spectrophotometer (Systronic 2201) Linearity: 5-30 µg/ml Accuracy % of drug recovered):100.1 to 100.5 % Precision (% RSD): <2 Robustness (%RSD): 0.179445 LOD: 0.862 LOQ: 2.163	[5]

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