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RP-HPLC METHOD DEVELOPMENT & VALIDATION OF ACYCLOVIR (ACY) IN PHARMACEUTICAL FORMULATION

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ABSTRACT

Acyclovir is a deoxynucleoside analog antiviral used to treat herpes simplex, Varicella zoster, herpes zoster, herpes labialis, and acute herpetic keratitis herpesvirus. It has a wide therapeutic window and is slightly soluble in water and organic solvents like DMSO and dimethyl formamide. Acyclovir is converted into acyclovir monophosphate by viral thymidine kinase, and then into acyclovir triphosphate by various enzymes. Acyclovir triphosphate has a higher affinity for viral DNA polymerase than cellular DNA polymerase, incorporating it into the DNA where the missing 2' and 3' carbons cause DNA chain termination. The present study focuses on developing and validating a RP-HPLC method for estimating Acyclovir in pharmaceutical formulations, aiming to apply suitable analytical techniques, optimize them, validate the method according to ICH guidelines, and select the drug. Research shows that the RP-HPLC method is a viable option for accurately measuring Acyclovir (ACY) in medicinal dose tablet forms. Reproducibility is a strong suit of the RP-HPLC technique, which also happens to be sensitive, accurate, precise, and repeatable. Acyclovir (ACY) tablet dose formulation analysis may also be executed effectively. These techniques do not experience any influence from additives, matrices, etc. These results could be better understood with more research on other pharmacological formulations.

KEYWORDS: Acyclovir; RP-HPLC; Validation; ICH guidelines; antiviral drug.

1. INTRODUCTION

Acyclovir is a deoxynucleoside analog antiviral used to treat herpes simplex, Varicella zoster, herpes zoster, herpes labialis, and acute herpetic keratitis herpesvirus. It has a wide therapeutic window and is slightly soluble in water and organic solvents like DMSO and dimethyl formamide.[1-2] Acyclovir is converted into acyclovir monophosphate by viral thymidine kinase, and then into acyclovir triphosphate by various enzymes. Acyclovir triphosphate (Figure 1) has a higher affinity for viral DNA polymerase than cellular DNA polymerase, incorporating it into the DNA where the missing 2' and 3' carbons cause DNA chain termination.[3,4]

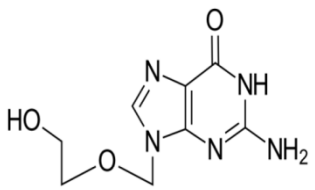

Figure 1: Chemical Structure of Acyclovir Triphosphate.

It is 9-33% protein bound in plasma. Acyclovir is oxidized to 9-carboxymethoxymethylguanine by alcohol dehydrogenase and aldehyde dehydrogenase, and 1% 8-hydroxylated to 8-hydroxy-acyclovir by aldehyde oxidase.[5,6] The majority of acyclovir is excreted in urine as unchanged drug, with 90-92% excreted unchanged through glomerular filtration and tubular secretion. Approximately 2% of the drug is recovered in feces, and less than 0.1% is expired as CO2.[7-10]

The present study focuses on developing and validating a RP-HPLC method for estimating Acyclovir in pharmaceutical formulations, aiming to apply suitable analytical techniques, optimize them, validate the method according to ICH guidelines, and select the drug.

2. MATERIALS AND METHODS

2.1 Procurement of the Drug

Acyclovir, a 10g tablet from Arch Pharma labs Ltd. Thane, is available in 99.8 percent purity. Zovirax 200 procured from the Glaxo SmithKline Pharmaceuticals Ltd containing 200mg of Acyclovir.

2.2 Method and Procedure

2.2.1 Identification and characterization of drug

Acyclovir was selected as model drug candidate for method development and validation. The drugs were kindly gifted from Pharmaceutical industry India. The

procured drug was analyzed for different physical properties viz. color, odor, melting point, etc. The IR absorbance spectrum of Acyclovir was recorded using FTIR 8400S spectrometer (Shimadzu) over range of 4000 to 400 cm^{-1}.[11-15]

2.2.2 Selection of Mobile Phase

The mobile phases tested include methanol with water in various ratios, such as 90:10, 80:20, 70:30, 60:40, and 50:50, with varying pH levels.

2.2.3 Chromatographic Conditions

The chromatographic conditions were established through trial and error, maintaining constant consistency throughout the method. The column was Inertsil 4.6 x 250 mm, with a particle size of 5 µm, stationary phases of C18 Inertsil, mobile phase of Acetonitrile: Phosphate Buffer (75:25), pH 4.5, and a sample size of 20 µL.

2.2.4 Validation of the Method

Adjusting several UFLC settings (FDA, 1995, 1997, 2000, 1994, 1987; USP, 2000) confirmed the reliability of the UFLC approach.[16] Calibration plot least-squares linear regression analysis verified the UFLC method's linearity[17], the limits of detection and quantification for the medicines mentioned were determined to be three and five epochs, respectively, above and below the baseline noise, The process adhered to the guidelines established by the United States Pharmacopoeia (USP, 2000), specificity[17], precision[18] accuracy[19], robustness[20] and ruggedness[21] were determined.

3. RESULTS AND DISCUSSION

3.1 Characterization of the Drug

Acyclovir, an antiviral drug, is characterized by its poor water solubility and low oral bioavailability. It is a white, crystalline powder with a molecular weight of 225.20. Its solubility in water is around 0.2 g/100 mL at 25°C, and it varies slightly with pH. Acyclovir is also soluble in dilute aqueous solutions of alkali hydroxides and mineral acids, and freely soluble in dimethyl sulfoxide. Melting point was found to be 256.48 ± 0.568 ºC, which was found to be relevant according to Indian Pharmacopeia.

3.2 Identification of the Drug

3.2.1 UV Spectra Analysis

The ultraviolet absorption spectrum of Acyclovir was obtained using Shimadzu1800- UV visible spectrophotometer and 1cm quartz cells, over a wavelength range of 400 to 200 nm, which was found to be 255 nm (Figure 2).

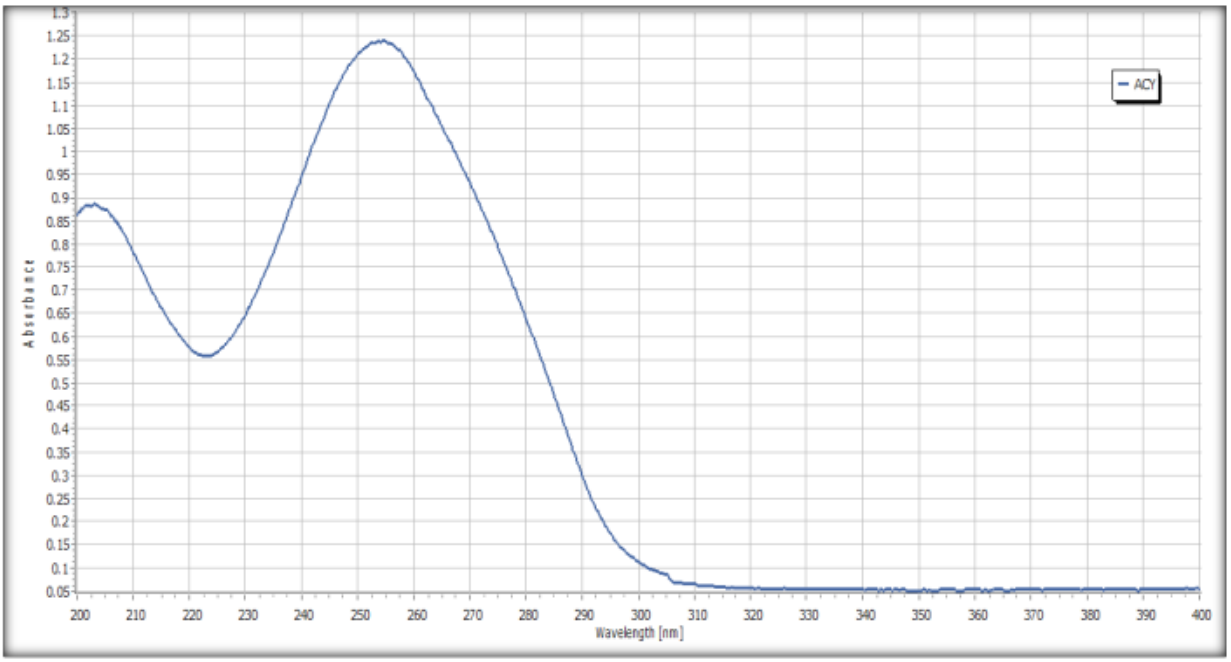

Figure 2: UV Spectra Analysis.

3.2.2 FT-IR of Acyclovir

The IR absorbance spectrum of Acyclovir was recorded using FTIR 8400S spectrometer (Shimadzu) over range of 4000 to 400 cm-1 (Figure 3). The IR spectroscopy theory utilizes the concept that molecules tend to absorb specific frequencies of light that are characteristic of the corresponding structure of the molecules. The energies are reliant on the shape of the molecular surfaces, the associated vibronic coupling, and the mass corresponding to the atoms. The FTIR spectra of a sample of pure ACY is shown in figure above. The FTIR spectrum of ACY showed a distinct peak at 3437.71 cm−1 (N–H stretching vibrations), 3177.72 cm−1 (O–H stretching vibrations), 2979.51 cm−1 (C-H stretching vibrations), 1628.31 cm−1 (C=O stretch). This FTIR spectra confirmed the drug.

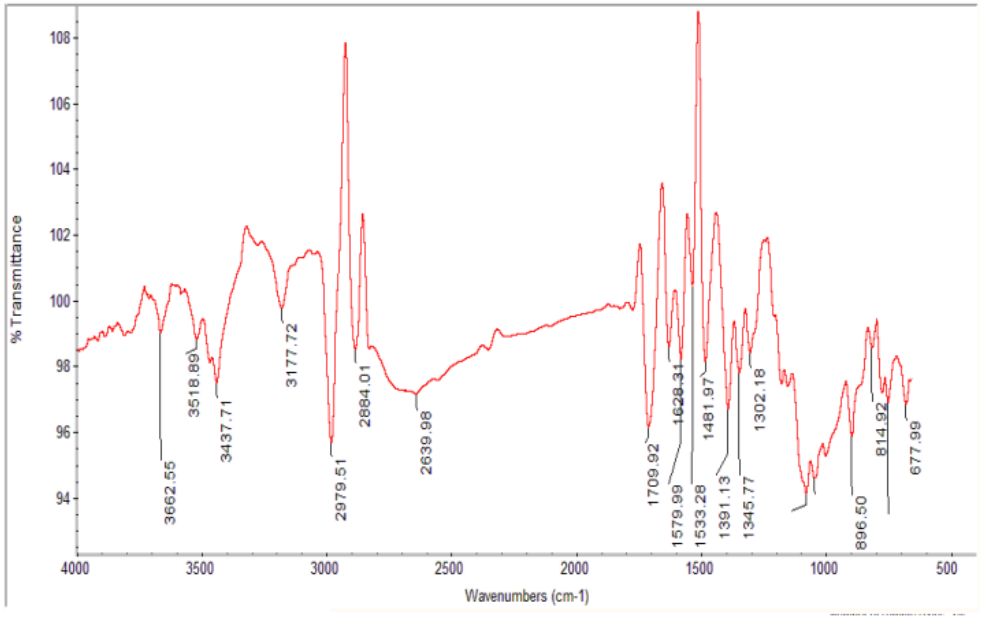

Figure 3: FT-IR study of Acyclovir.

3.3 Selection of the Mobile Phase

From various mobile phases tried, mobile phase containing Methanol: Phosphate Buffer (50:50) pH 5 was selected, since it gives sharp reproducible retention time for ACY (Figure 4-7).

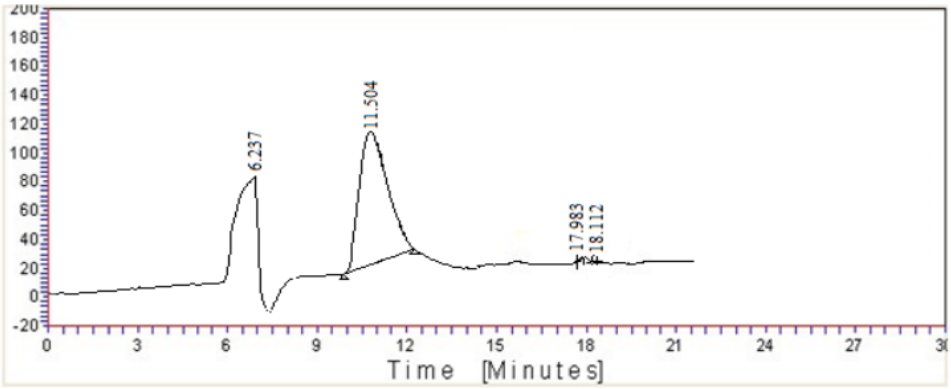

Figure 4: Trial Chromatogram obtained by using Methanol: water (90:10) as mobile phase.

3.4 Application of proposed method for estimation of Acyclovir in formulation

Equal volume (20µL) of standard and sample solution were injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured. The content Acyclovir was calculated by comparing a sample peak with that of standard (Figure 8).

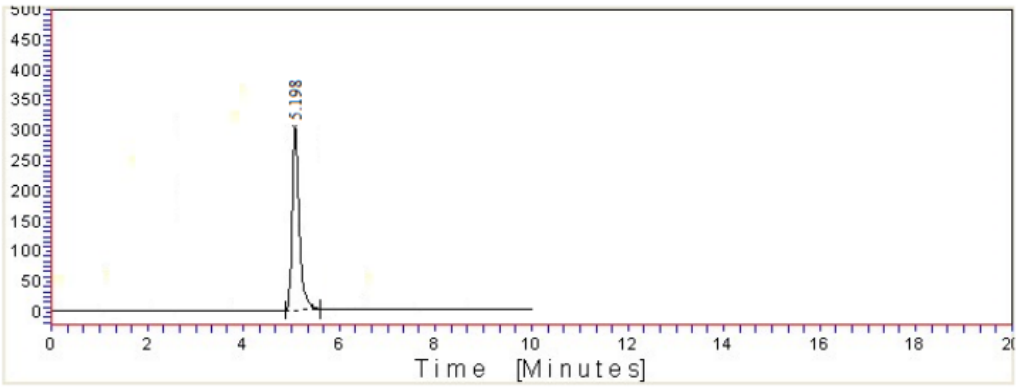

Figure 8: Chromatogram obtained by formulation of Acyclovir.

3.5 Validation of the Method

Table 1 shows the results of the accuracy tests that were conducted using the usual addition technique for recovery. The standard deviation or root-mean-square of a set of data is a measure of an analytical method's precision. Replicate drug estimate using the suggested approach confirmed it (Table 2). In order to determine the method's specificity, we looked at how effectively it could isolate the ACY peak from the matrix component peaks. Average time spent in retention for -ACY - 3.981 There was no influence from the matrix component as the values were so similar to those in the typical laboratory combination. Range and linearity: A concentration ranging from 80% to 120% of the test concentration was achieved by dissolving and diluting tablet powder according to USP standards, which is comparable to 80, 90, 100, 110, or 120% of the label claim. The solutions that were produced had their chromatograms documented. According to Table 3, the marketed ACY formulation was determined to be linear within ± 20% of the drug's test concentration. A slight change in the organic content of the mobile phase, wavelength, or flow rate had no effect on the chosen parameters, according to the robustness analysis. Within this range, you should see system suitability results. Table 4 shows that the technique was resilient. The minimum concentration of analyte in a sample that can be detected, but not necessarily quantified to an exact number, is called the limit of detection. The limit of quantitation is the smallest concentration of analyte in a sample that can be accurately and precisely measured using quantitative methods (Table 5). The experimental and results sections detail the steps used to generate the standard laboratory mixture and conduct the analysis once the chromatographic conditions had been established. Table 6 shows that it was extended for drug estimation in commercialised tablet formulation and delivered accurate and reliable results.

Table 1: Results and statistical data for Recovery study of ACY.

Sr. No	wt. of formulation	Amount of Drug Added in (µg/ml)	Peak Area of stand.	Peak Area of sample	% Recovery
	ACY	ACY	ACY	ACY	ACY
1	20.5	1	225587.1	224910.3	99.7
2		1		224684.8	99.6
3		1		227391.8	100.8
4		2		226263.9	100.3
5		2		226489.4	100.4
6		2		224233.6	99.4
7		3		228068.6	101.1
8		3		228970.9	101.5
9		3		226038.3	100.2

Table 2: Results and statistical data of Precision Study.

Sr. No.	Weight of Standard (mg)	Weight of Sample (mg)	Peak Area of Stand.	Peak Area of Sample	% Label claim
	ACY	ACY	ACY	ACY	ACY
1	10	20.5	225587.1	224684.8	99.6
2		20.5		227391.8	100.8
3		20.5		227617.4	100.9

Table 3: Observations of Linearity and range study for ACY.l

Sr. No.	% Label claim	Peak area ACY
1	80	180469.68
2	90	203028.39
3	100	225587.1
4	110	248145.81
5	120	270704.52

Table 4: Result of Robustness study of ACY.

Sr. No.	Condition	Parameter	Peak Area	RT
01	Change of wavelength	253 nm	225587.1	5.187
02		255 nm	225509.4	5.198
03		257 nm	225511.9	5.174
04	Change in Temperature	30 °C	225601.2	5.182
05		25 °C	225587.1	5.198
06		20 °C	225511.3	5.188
07	Change in Flow rate	0.8 ml/min	225555.4	5.201
08		1ml/min	225587.1	5.197
09		1.2 ml/min	225687.1	5.103

Table 5: Limit of detection (LOD) and Limit of quantitation (LOQ).

Sr. No.	Drug Name	LOD µg/ml	LOQ µg/ml
1	ACY	0.128	1.986

Table 6: Summary of laboratory mixture and marketed formulation analysis by RP-HPLC Method.

Sr. no.	Sample	Statistical data	ACY	
			% Estimation	%Recovery
1.	Standard Laboratory mixture	Mean	100.93	-
		S.D.	0.153	-
		C.V.	0.002	-
2.	Zovirax 200	Mean	100.00	100.33
		S.D.	0.361	0.707
		C.V.	0.004	0.007

4. CONCLUSIONS

Research shows that the RP-HPLC method is a viable option for accurately measuring Acyclovir (ACY) in medicinal dose tablet forms. Reproducibility is a strong suit of the RP-HPLC technique, which also happens to be sensitive, accurate, precise, and repeatable. Acyclovir (ACY) tablet dose formulation analysis may also be executed effectively. These techniques do not experience any influence from additives, matrices, etc. These results could be better understood with more research on other pharmacological formulations.

5. Conflict of Interest

None.

6. REFERENCES

1. Kłysik K, Pietraszek A, Karewicz A, Nowakowska M. Acyclovir in the treatment of herpes viruses–a review. Current medicinal chemistry, Jul. 1, 2020; 27(24): 4118-37.
2. Schalkwijk HH, Snoeck R, Andrei G. Acyclovir resistance in herpes simplex viruses: Prevalence and therapeutic alternatives. Biochemical Pharmacology, Dec 1, 2022; 206: 115322.
3. Wei YP, Yao LY, Wu YY, Liu X, Peng LH, Tian YL, Ding JH, Li KH, He QG. Critical review of synthesis, toxicology and detection of acyclovir. Molecules, Oct. 29, 2021; 26(21): 6566.
4. Brandariz-Nuñez D, Correas-Sanahuja M, Maya-Gallego S, Martín Herranz I. Neurotoxicity associated with acyclovir and valacyclovir: A systematic review of cases. Journal of Clinical Pharmacy and Therapeutics, Aug. 2021; 46(4): 918-26.
5. Wang M, Hou J, Yu DG, Li S, Zhu J, Chen Z. Electrospun tri-layer nanodepots for sustained release of acyclovir. Journal of Alloys and Compounds, Dec. 15, 2020; 846: 156471.
6. Lv H, Guo S, Zhang G, He W, Wu Y, Yu DG. Electrospun structural hybrids of acyclovir-polyacrylonitrile at acyclovir for modifying drug release. Polymers, Dec. 7, 2021; 13(24): 4286.
7. Schlüter-Vorberg L, Prasse C, Ternes TA, Mückter H, Coors A. Toxification by transformation in conventional and advanced wastewater treatment: the antiviral drug acyclovir. Environmental Science & Technology Letters, Dec. 8, 2015; 2(12): 342-6.
8. Jacob S, Nair AB, Al-Dhubiab BE. Preparation and evaluation of niosome gel containing acyclovir for enhanced dermal deposition. Journal of liposome research, Oct. 2, 2017; 27(4): 283-92.
9. Rao S, Abzug MJ, Carosone-Link P, Peterson T, Child J, Siparksy G, Soranno D, Cadnapaphornchai MA, Simões EA. Intravenous acyclovir and renal dysfunction in children: a matched case control study. The Journal of pediatrics. Jun 1, 2015; 166(6): 1462-8.
10. Russo D, Siciliano A, Guida M, Galdiero E, Amoresano A, Andreozzi R, Reis NM, Puma GL, Marotta R. Photodegradation and ecotoxicology of acyclovir in water under UV254 and UV254/H2O2 processes. Water research, Oct. 1, 2017; 122: 591-602.
11. Gupta S, Verma P, Mishra AP, Omar N, Mathur R. A review on novel analytical method development and validation by RP-HPLC method. Indian Journal of Forensic Medicine & Toxicology, Sep. 5, 2021; 15(4): 3479-86.
12. Chaudhari VS, Borkar RM, Murty US, Banerjee S. Analytical method development and validation of reverse-phase high-performance liquid chromatography (RP-HPLC) method for simultaneous quantifications of quercetin and piperine in dual-drug loaded nanostructured lipid carriers. Journal of Pharmaceutical and Biomedical Analysis, Jul. 15, 2020; 186: 113325.
13. Attimarad M, Venugopala KN, Islam MM, Shafi S, Altaysan AI. Rapid simultaneous quantitative analysis of Hypoglycemic agents by RP HPLC: Development, validation and application to

medicine. Indian Journal of Pharmaceutical Education and Research, Apr. 1, 2022; 56(2): 564-72.
14. Raju VB, Gandhi BM, Sumanth KS, Srinivas K, Neeraja TN. RP-HPLC method development and validation for simultaneous estimation of telmisartan and ramipril in pure and pharmaceutical dosage forms. Asian Journal of Research in Chemistry, 2017; 10(2): 179-85.
15. Annadi AM, El Zahar NM, Abdel-Sattar NE, Mohamed EH, Mahmoud SA, Attia MS. Development and validation of molnupiravir assessment in bulk powder and pharmaceutical formulation by the RP-HPLC-UV method. RSC advances, 2022; 12(53): 34512-9.
16. Mangrio GR, Maneengam A, Khalid Z, Jafar TH, Chanihoon GQ, Nassani R, Unar A. RP-HPLC method development, validation, and drug repurposing of sofosbuvir pharmaceutical dosage form: a multidimensional study. Environmental research, Sep. 1, 2022; 212: 113282.
17. Haq N, Shakeel F, Alanazi F, Alshora DH, Ibrahim MA. Development and validation of a green RP-HPLC method for the analysis of rosuvastatin: a step towards making liquid chromatography environmentally benign. Green Processing and Synthesis, Apr. 25, 2018; 7(2): 160-9.
18. Dongala T, Katari NK, Palakurthi AK, Jonnalagadda SB. Development and validation of a generic RP-HPLC PDA method for the simultaneous separation and quantification of active ingredients in cold and cough medicines. Biomedical Chromatography, Nov. 2019; 33(11): e4641.
19. Sravanthi G, Gandla KS, Repudi L. New analytical method development and validation for estimation of molnupiravir in bulk and tablet dosage form by RP-HPLC method. Cellular, Molecular and Biomedical Reports, Sep. 1, 2023; 3(3): 130-6.
20. Araujo P. Key aspects of analytical method validation and linearity evaluation. J Chromatogr B., 2009; 877: 2224-34. 16. Goyal D, Maurya S, Verma C. Cleaning validation in the pharmaceutical industry-an overview. Pharma Tutor., 2016; 4: 14-20.
21. Mahmoud MR, Mahgoub SM, Abdelazeem R, Abdelsatar MM, Allam AA, Alfassam HE, Radalla AM, Mahmoud R. RP-HPLC method development and validation for the quantification of prednisolone and salbutamol with their simultaneous removal from water using modified clay–activated carbon adsorbents. RSC advances, 2025; 15(11): 8675-95.