



ISSN: 2306-6091

International Journal of Pharmaceuticals and Health Care Research (IJPHR)

IJPHR | Vol.14 | Issue 4 | Oct - Dec 2026

www.ijphr.com

DOI : <https://doi.org/10.61096/ijphr.v14.iss4.2026.830-850>

Method Development and Validation by RP-HPLC for Estimation of Acyclovir and Hydrocortisone in Bulk and Pharmaceutical Dosage Form

Surkanti Chaithanya Reddy^{*1}, Ch. Sunitha²

¹Department of Pharmaceutical Analysis, Princeton College of Pharmacy, Pocharam, Hyderabad, Telangana

*Corresponding Author: **Surkanti Chaithanya Reddy**

Email: princeton.pharmacy@gmail.com



Published on:
14.10.2026
Published by:
Futuristic
Publications
2026| All rights
reserved.



Creative
Commons
Attribution 4.0
International
License.

Abstract: A rapid and reliable Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Acyclovir and Hydrocortisone in bulk and pharmaceutical dosage forms. Chromatographic separation was achieved using a Waters HPLC system equipped with an auto sampler and PDA detector (Model 996), employing a Gemini C18 column (4.6 × 150 mm, 5 µm) maintained at 30°C. The mobile phase consisted of Acetonitrile and Water in a 50:50 v/v ratio, with a flow rate of 1.0 mL/min. Detection was carried out at 238 nm, with an injection volume of 10 µL and a total run time of 8 minutes. The method was validated as per ICH guidelines, demonstrating excellent linearity, precision, accuracy, specificity, and robustness. The retention times of both drugs were distinct and interference-free. This method is efficient, economical, and well-suited for routine quality control and analysis of Acyclovir and Hydrocortisone in pharmaceutical preparations.

Keywords: RP-HPLC, Acyclovir, Hydrocortisone, Gemini C18 column, simultaneous estimation, validation.

INTRODUCTION

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components.¹

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP,

and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also quality is important in every product or service, but it is vital in medicines as it involves life.

There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these conditions standard and analytical procedures for these drugs may not be available in Pharmacopoeias. In instrumental analysis, a physical property of the substance is measured to determine its chemical composition. Pharmaceutical analysis comprises those procedures necessary to determine the identity, strength, quality and purity of substances of therapeutic importance.²

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs. In brief the reasons for the development of newer methods of drugs analysis are:

1. The drug or drug combination may not be official in any pharmacopoeias.
2. A proper analytical procedure for the drug may not be available in the literature due to Patent regulations.
3. Analytical methods for a drug in combination with other drugs may not be available.
4. Analytical methods for the quantitation of the drug in biological fluids may not be available.
5. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable.^{1,2}

1.1 DIFFERENT METHODS OF ANALYSIS

The following techniques are available for separation and analysis of components of interest.

Spectral methods

The spectral techniques are used to measure electromagnetic radiation which is either absorbed or emitted by the sample.

E.g. UV-Visible spectroscopy, IR spectroscopy, NMR, ESR spectroscopy, Flame photometry, Fluorimetry.²

Electro analytical methods

Electro analytical methods involved in the measurement of current voltage or resistance as a property of concentration of the component in solution mixture.

E.g. Potentiometry, Conductometry, Amperometry.²

Chromatographic methods

Chromatography is a technique in which chemicals in solutions travel down columns or over surface by means of liquids or gases and are separated from each other due to their molecular characteristics.

E.g. Paper chromatography, thin layer chromatography (TLC), High performance thin layer chromatography (HPTLC), High performance liquid chromatography (HPLC), Gas chromatography (GC).²

Miscellaneous Techniques

Mass Spectrometry, Thermal Analysis.

Hyphenated Techniques

GC-MS (Gas Chromatography – Mass Spectrometry), LC-MS (Liquid Chromatography – Mass Spectrometry), ICP-MS (Inductivity Coupled Plasma- Mass Spectrometry), GC-IR (Gas Chromatography – Infrared Spectroscopy), MS-MS (Mass Spectrometry – Mass Spectrometry).

Analytical techniques that are generally used for drug analysis also include biological and microbiological methods, radioactive methods and physical methods etc. are mentioned in Table 1.1.²

Table 1.1: Summary of Hyphenated separation techniques.²

Separation technique	Hyphenated mode
Liquid chromatography	Liquid chromatography-mass spectrometry(LC/MS)
	Liquid chromatography-Fourier-transform infrared Spectrometry(LC-FTIR)
	Liquid chromatography-nuclear magnetic resonance spectroscopy(LC/NMR)
Gas chromatography	Liquid chromatography-inductively coupled plasma mass spectrometry(LC-ICPMS)
	Gas chromatography-mass spectrometry(GC/MS)
	Gas chromatography-Fourier-transform infrared(GC-FTIR)
Capillary electrophoresis	Gas chromatography-FTIR-MS(GC-FTIR-MS)
	Capillary electrophoresis-mass spectrometry(CE/MS)
	Capillary electrophoresis- nuclear magnetic resonance spectroscopy(CE/NMR)
Thin layer chromatography(TLC)	Capillary electrophoresis-surface enhanced Raman spectrometry (TLC-SERS)
	Thin layer chromatography- mass spectrometry(TLC/MS)
	Thin layer chromatography- surface enhanced Raman spectrometry(TLC-SERS)
Superficial fluid chromatography/ extraction(SFC/SFE)	Superficial fluid extraction-capillary gas chromatography-mass spectrometry(SFE-CGC-MS)
	Superficial fluid-Fourier-transform infrared(SFC-FTIR)

1.2 INTRODUCTION TO HPLC

HPLC is also called as high pressure liquid chromatography since high pressure is used to increase the flow rate and efficient separation by forcing the mobile phase through at much higher rate. The pressure is applied using a pumping system. The development of HPLC from classical column chromatography can be attributed to the development of smaller particle sizes. Smaller particle size is important since they offer more surface area over the conventional large particle sizes. The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages.

1. Improved resolution of separated substances
2. column packing with very small (3,5 and 10 µm) particles
3. Faster separation times (minutes)
4. Sensitivity
5. Reproducibility
6. continuous flow detectors capable of handling small flow rates
7. Easy sample recovery, handling and maintenance.⁶

1.2.1 Types of HPLC Techniques

1.2.1.1 Based on Modes of Chromatography

These distinctions are based on relative polarities of stationary and mobile phases

Reverse phase chromatography: In this the stationary phase is non-polar and mobile phase is polar. In this technique the polar compounds are eluted first and non polar compounds are retained in the column and eluted slowly. Therefore it is widely used technique.

Normal phase chromatography: In this the stationary phase is polar and mobile phase is non-polar. In this technique least polar compounds travel faster and are eluted first where as the polar compounds are retained in the column for longer time and eluted.⁴

1.2.1.2 Based on Principle of Separation

Liquid/solid chromatography (Adsorption): LSC, also called adsorption chromatography, the principle involved in this technique is adsorption of the components onto stationary phase when the sample solution is dissolved in mobile phase and passed through a column of stationary phase. The basis for separation is the selective adsorption of polar compounds; analytes that are more polar will be attracted more strongly to the active silica gel sites. The solvent strength of the mobile phase determines the rate at which adsorbed analytes are desorbed and elute. It is widely used for separation of isomers and classes of compounds differing in polarity and number of functional groups. It works best with compounds that have relatively low or intermediate polarity.³

Liquid/Liquid chromatography (Partition Chromatography): LLC, also called partition chromatography, involves a solid support, usually silica gel or kieselguhr, mechanically coated with a film of an organic liquid. A typical system for NP LLC column is coated with β , β' -oxy dipropionitrile and a non-polar solvent like hexane as the mobile phase. Analytes are separated by partitioning between the two phases as in solvent extraction. Components more soluble in the stationary liquid move more slowly and elute later.^{1,2}

Ion exchange: In this the components are separated by exchange of ions between an ion exchange resin stationary phase and a mobile electrolyte phase. A cation exchange resin is used for the separation of cations and anion exchange resin is used to separate a mixture of anions.^{3,16,17}

Size exclusion: In this type, the components of sample are separated according to their molecular sizes by using different gels (polyvinyl acetate gel, agarose gel). ex: separation of proteins, polysaccharides, enzymes and synthetic polymers.^{3,15}

Chiral chromatography: In this type of chromatography optical isomers are separated by using chiral stationary phase.

Affinity chromatography: In this type, the components are separated by an equilibrium between a macromolecular and a small molecule for which it has a high biological specificity and hence affinity.³

1.2.1.3 Based on elution technique

Isocratic separation: In this technique, the same mobile phase combination is used throughout the process of separation. The same polarity or elution strength is maintained throughout the process.

Gradient separation: In this technique, a mobile phase combination of lower polarity or elution strength is followed by gradually increasing polarity or elution strength.³

1.2.1.4 Based on the scale of operation

Analytical HPLC: Where only analysis of samples are done. Recovery of samples for reusing is normally not done, since the sample used is very low. Ex: μ g quantities.

Preparative HPLC: Where the individual fractions of pure compounds can be collected using fraction collector. The collected samples are reused. Ex: separation of few grams of mixtures by HPLC.⁴

1.2.1.5 Based on type of analysis

Qualitative analysis: Which is used to identify the compound, detect the presence of impurities to find out the number of components. This is done by using retention time values.

Quantitative analysis: This is done to determine the quantity of individual or several components of mixture. This is done by comparing the peak area of the standard and sample.³

1.3 INSTRUMENTATION OF HPLC

The basic liquid chromatograph consists of six basic units. The mobile phase supply system, the pump and programmer, the sample valve, the column, the detector and finally a means of presenting and processing the results.

1.3.1 Mobile phase (solvent) reservoirs and solvent degassing

The mobile phase supply system consists of number of reservoirs (200 mL to 1,000 mL in capacity). They are usually constructed of glass or stainless steel materials which are chemically resistant to mobile phase.

Mobile phase

Mobile phases in HPLC are usually mixtures of two or more individual solvents. The usual approach is to choose what appears to be the most appropriate column, and then to design a mobile phase that will optimize the retention and selectivity of the system. The two most critical parameters for nonionic mobile phases are strength and selectivity.^{8,24}

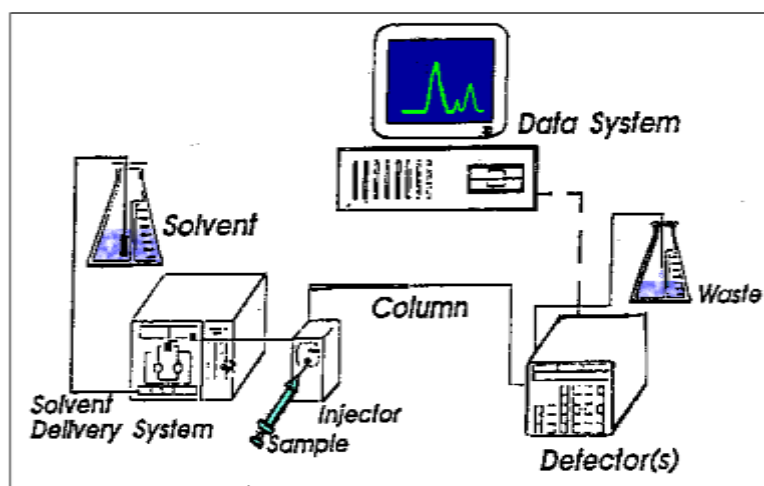


Fig.1.1: Components of HPLC instrument block diagram.²²

Mobile phase preparation

Mobile phases must be prepared from high purity solvents, including water that must be highly purified. Mobile phases must be filtered through $\leq 1 \mu\text{m}$ pore size filters and be degassed before use.

Degassing of solvents

Many solvents and solvent mixtures (particularly aqueous mixtures) contain significant amounts of dissolved nitrogen and oxygen from the air. These gasses can form bubbles in the chromatographic system that cause both serious detector noise and loss of column efficiency. These dissolved gases in solvent can be removed by the process of degassing. Every solvent must be degassed before introduction into pump as it alter the resolution of column and interfere with monitoring of the column effluent.

Degassing is done in many ways:

- By warming the solvents
- By stirring vigorously with a magnetic stirrer
- By subjecting to vacuum filtration
- By ultra sonication (using ultrasonicator)
- By bubbling He gas through the solvent reservoir.⁸

Table 1.2: Physical properties of common HPLC solvents.⁸

Solvent	MW	BP	RI (25°C)	UV Cutoff (nm)	Density g/ML. (25°C)	Viscosity (25°C)	Dielectric Constant
Acetonitrile	41.0	82	1.342	190	0.787	0.358	38.8
Dioxane	88.1	101	1.420	215	1.034	1.26	2.21
Ethanol	46.1	78	1.359	205	0.789	1.19	24.5
Ethyl acetate	88.1	77	1.372	256	0.901	0.450	6.02
Methanol	32.0	65	1.326	205	0.792	0.584	32.7
CH ₂ Cl ₂	84.9	40	1.424	233	1.326	0.44	8.93
Isopropanol	60.1	82	1.375	205	0.785	2.39	19.9
n-propanol	60.1	97	1.383	205	0.804	2.20	20.3
THF	72.1	66	1.404	210	0.889	0.51	7.58
Water	18.0	100	1.333	170	0.998	1.00	78.5

1.3.2 Pumping systems

The pumping system is one of the most important features of an HPLC system. There is a high resistance to solvent flow due to the narrow columns packed with small particles and high pressures are therefore required to achieve satisfactory flow rate.

The main requirements of pumping systems are:

1. Generation of pressures up to 6000 psi.
2. Pulse free output
3. Flow rates ranging from 0.01 to 10 mL/min
4. Flow control and flow reproducibility of $\pm 0.5\%$
5. Corrosion resistant components (seals of Teflon and stainless steel)
6. Should be easy to dismantle and repair.

There are three basic types of pumps in common use.

1. Reciprocating pumps.
2. Displacement pumps or syringe pumps.
3. Pneumatic pumps or constant pressure pumps.⁸

1.3.3 Sample introduction system

Injection ports are of two basic types,

1. The sample is injected directly into the column.
2. The sample is deposited before the column inlet and then swept by a valving action into the column by the mobile phase.

Injectors should provide the possibility of injecting the liquid sample within the range of 0.1 to 100 mL of volume with high reproducibility and under high pressure (up to the 4000psi). They should also produce minimum band broadening and minimize possible flow disturbances. The most useful and widely used sampling device for modern LC is the micro sampling injector valve. With these sampling valves, samples can be introduced reproducibly into pressurized columns without significant interruption of flow, even at elevated temperatures. High-performance valves provide extra column band-broadening characteristics comparable or superior to that of syringe injection.⁸

1.3.4 Columns

Typical analytical columns are 10, 15 and 25 cm in length and are fitted with extremely small diameter (3, 5 or 10 μm) particles. The internal diameter of the columns is usually 4 or 4.6 mm; this is considered the

best compromise among sample capacity, mobile phase consumption, speed and resolution. Preparative columns are of larger diameter. Packing of the column tubing with the small diameter particles requires high skill and specialized equipment. For this reason, it is generally recommended that the most experienced chromatographers purchase prepacked columns, since it is difficult to match the high performance of professionally packed LC columns without a large investment in time and equipment. The column can be classified based on the material bonded to the silica packed surface such as C₄, C₈, C₁₈, phenyl, chiral, cyanomicrobore columns (1mm to 100cm), U shaped and coiled columns are available. Guard columns are used before the analytical columns to increase the life of analytical columns by retaining non eluted components and particulate matter. The flow chart in the Table 1.3 can assist one in determining which columns to examine.

Table 1.3: Column Selection Flow Chart.⁸

Sample	LC mode	Column choice
Basic or acidic	Reverse Phase-ion pair (allows neutral and charged compounds to be simultaneously analyzed)	C ₁₈ , C ₈ , C ₆ , C ₄ , C ₂ , TMS, CN, amino (not for carbonyl compounds), phenyl, Hamilton PRP-1 (pH 1-13).
Ionizable	Ion Exchange Anionic, cationic	Strong cations exchange, Strong anion exchange.
Neutral	a) Normal phase b) Reverse phase	a)Increasing the polarity of bonded phases diol, CN, NH ₂ , silica alumina b) C ₁₈ , C ₈ , phenyl, C ₂ .

Column Thermostats

Control of column temperature is important in liquid chromatography. The effect of temperature on retention times and reproducibility is quite significant, especially when using the reverse phase models.⁸

1.3.5 Detectors

Optical detectors are most frequently used. These detectors pass a beam of light through the flowing column effluent as it passes through a low volume (~ 10 mL) flow cell. The most commonly used detector in LC is the ultraviolet absorption detector. A variable wavelength detector of this type, capable of monitoring from 190 to 460-600 nm, will be found suitable for the detection of the majority samples. Other types of Detectors:

1. UV detector
2. Refractive index detector
3. Fluorimetric detector
4. Conductivity detector
5. Amperometric detector
6. Photodiode array detector (PDA detector).⁸

1.3.6 Data handling

Data handling in chromatography now ranges from a simple pen recorder to complicated computer integration and computerized data handling systems. Several manufacturers today offered microprocessor controlled chromatographs. Thus the solvent delivery system, injector, oven, detector, fraction collector and data reduction can be carried under the control of a central microprocessor with the capability to program sequential parameters.⁸

1.4. CHROMATOGRAPHIC PARAMETERS

1.4.1 Capacity factor (k')

The capacity factor is a measure of the degree of retention of an analyte relative to an unretained peak. It is affected by solvent composition, separation, aging and temperature of separation.

$$k' = \frac{(t_R - t_0)}{t_0}$$

Where, t_R = retention time for the sample peak.

t_0 = retention time for the unretained peak.

The peak should be well-resolved from other peaks and the void volume. Generally the value of k' is > 2 .³

1.4.2 Resolution (R_s)

Resolution is ability of a column to separate Chromatographic peaks. Resolution can be improved by increasing column length, decreasing particle size, increasing temperature, changing the eluent or stationary phase.

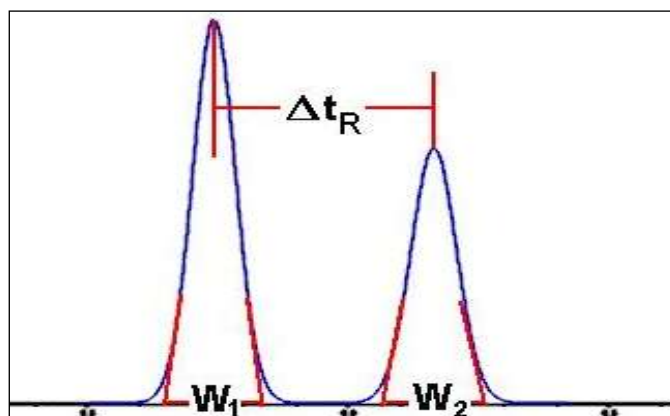


Fig 1.2: Resolution of peaks.³

$$R_s = \frac{(t_{R1} - t_{R2})}{0.5(t_{W1} + t_{W2})}$$

Where,

t_{R1} , t_{R2} are retention times of the first and second adjacent bands.

t_{W1} and t_{W2} are baseline at bandwidth of the peaks for reliable quantitation well-separated peaks is essential for quantitation.

R_s of > 2 between the peak of interest and the closest potential interfering peak (impurity, excipient, degradation product, internal standard, etc.) are desirable.⁴

1.4.3 Theoretical plate number / Efficiency (N)

A measure of peak band spreading determined by various methods, some of which are sensitive to peak asymmetry. Smaller the band spread higher the theoretical plate indicates good column and system performance. A theoretical plate is an imaginary or hypothetical unit of a column where equilibrium has been established between stationary phase and mobile phase. Theoretical plate number is a measure of column efficiency that is, how many peaks can be located per unit run-time of the Chromatogram.

$$N = 16[t/w_b]^2$$

Where, t = retention time for the sample peak,

w_b = peak width.

$$H = \frac{L}{N}$$

N is fairly constant for each peak on a Chromatogram with a fixed set of operating conditions. H (height) or HETP (height equivalent of a theoretical plate) measures the column efficiency per unit length (L) of the column. Parameters which can affect N or H include peak position, particle size in column, flow rate of mobile phase, column temperature, viscosity of mobile phase, and molecular weight of the analyte. The theoretical plate number depends on elution time but in general should be > 2000 .⁴

1.4.4 Tailing factor (T) or Asymmetry factor (AF)

It is a measure of the symmetry of a peak.

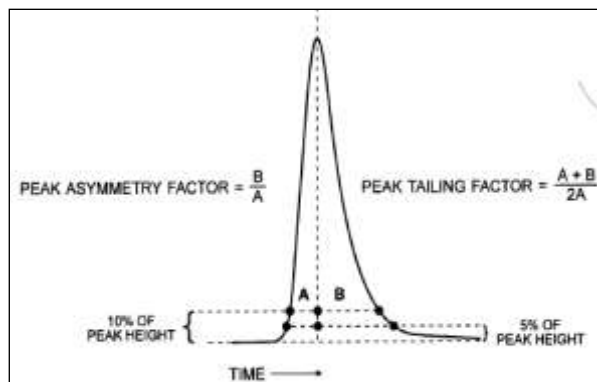


Fig 1.3: Peak Asymmetry

$$T = \frac{W}{2f}$$

Where, W= peak width at 5 % height,

f = distance from peak front to apex point at 5% height.

The accuracy of quantitation decreases with increase in peak tailing because of the difficulties encountered by the integrator in determining where/when the peak ends and hence the calculation of the area under the peak.⁴

MATERIALS AND METHODS

1. Collection of literature for the selected drug.
2. Extensive literature survey for selection of appropriate solvents to dissolve respective selected drug and preparation of stock solution.
3. Study of drug profile.
4. Procurement of samples, standards and other chemicals.
5. Selection of chromatographic conditions.
6. Selection of mobile phase.
7. Method trials on HPLC by using different solvents and columns.
8. Development of RP-HPLC method which is different from the finished articles.
9. Optimization of the developed method by varying mobile phase conditions, temperature.
10. Validation of the developed method for the following parameters:
 - Accuracy
 - Precision
 - Specificity
 - Limit of Detection
 - Limit of Quantitation
 - Linearity
 - Robustness
 - System Suitability

RESULTS AND DISCUSSION

Trails for Method Development:

Trail 1:

Column : Symmetry C18 (4.6×250mm) 5μ

Column temperature : 30°C

Wavelength : 235nm

Mobile phase ratio : Methanol: water (10:90% v/v)

Flow rate : 0.6ml/min

Injection volume : 40.00μl

Run time : 10 Minutes

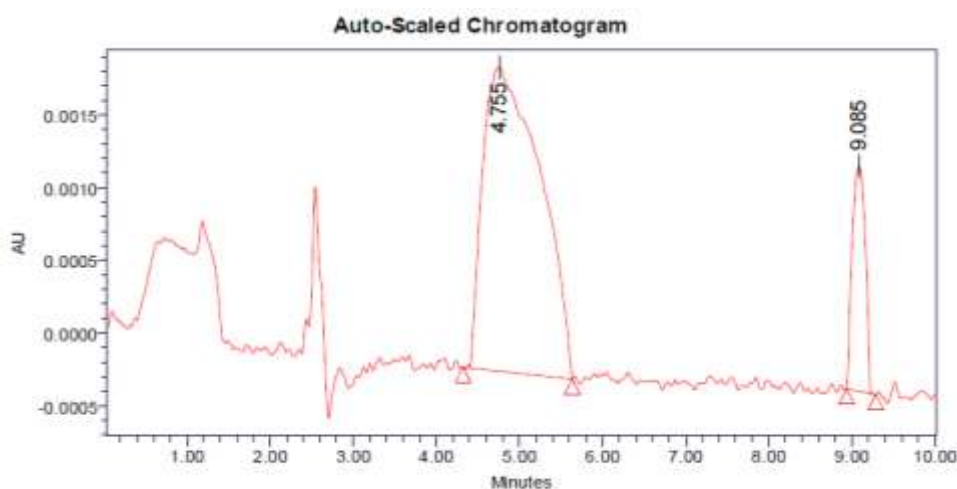


Fig.14: chromatogram for trail 1

Table 14: peak results for trail 1

S.No	Peak Name	R _t	Area	Height	USP Tailing	USP Plate count
1	Acyclovir	4.755	100588	2092	1.81	180
2	Hydrocortisone	9.085	17050	1554	0.99	14429

Observation:

In this trial it shows less plate count and improper separation of two peaks in the chromatogram. So it's required more trials to obtain good peaks.

Trail 2:

Column : Xterra C18 (4.6×250mm) 5μ

Column temperature : 30°C

Wavelength : 235nm

Mobile phase ratio : Methanol: Water (35:65% v/v)

Flow rate : 0.8ml/min

Injection volume : 10.00μl

Run time : 10.0 Minutes

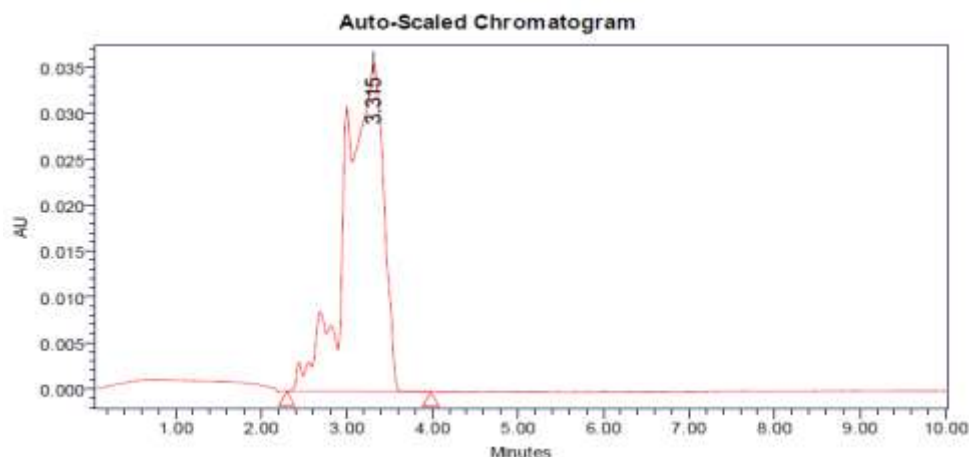


Fig 15: chromatogram for trail 2

Table 15: peak results for trail 2

S.No	Peak name	R _t	Area	Height	USP Tailing	USP plate count
1	Hydrocortisone	3.315	1107312	35849	0.64	454

Observation: In this above trail it shows one sample peak and less plate count, improper baseline in the chromatogram. So its required more trails to get optimized chromatogram.

Trail 3:

Column : Xterra C18 (4.6×250mm) 5μ
 Column temperature : 30°C
 Wavelength : 235nm
 Mobile phase ratio : ACN: Water (10:90% V/V)
 Flow rate : 0.7ml/min
 Injection volume : 20μl
 Run time : 9minutes

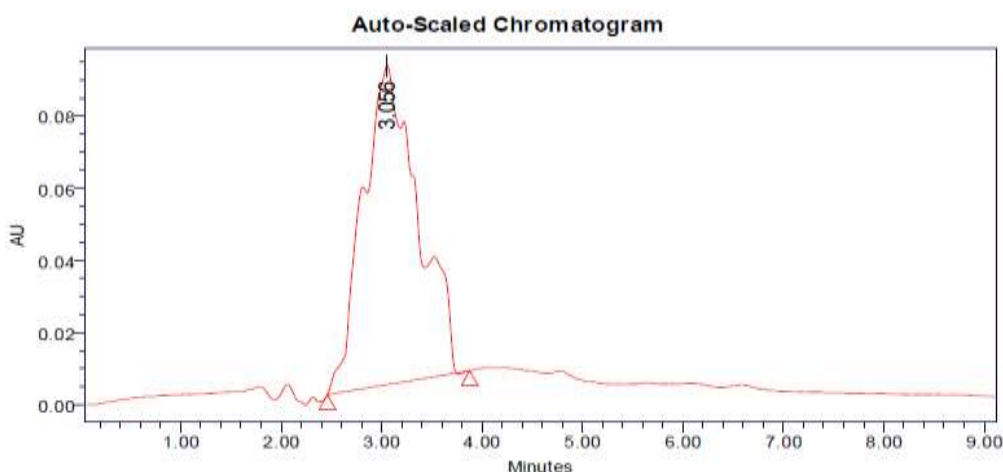


Fig 16: chromatogram for trail 3

Table 16: peak results for trail 3

S.No	Peak name	R _t	Area	Height	USP Tailing	USP plate count
1	Hydrocortisone	3.056	3346687	88477	1.10	51

Observation:

In this trail only one sample peak is eluted. And it shows less plate count and improper baseline in the chromatogram. More trails required to get perfect peak.

Trial 4:

Column : Hypersil C18 (4.6×250mm) 5μ
 Column temperature : 35°C
 Wavelength : 235nm
 Mobile phase ratio : ACN: Water (40:60) V/V
 Flow rate : 1.1ml/min
 Injection volume : 10μl
 Run time : 10minutes

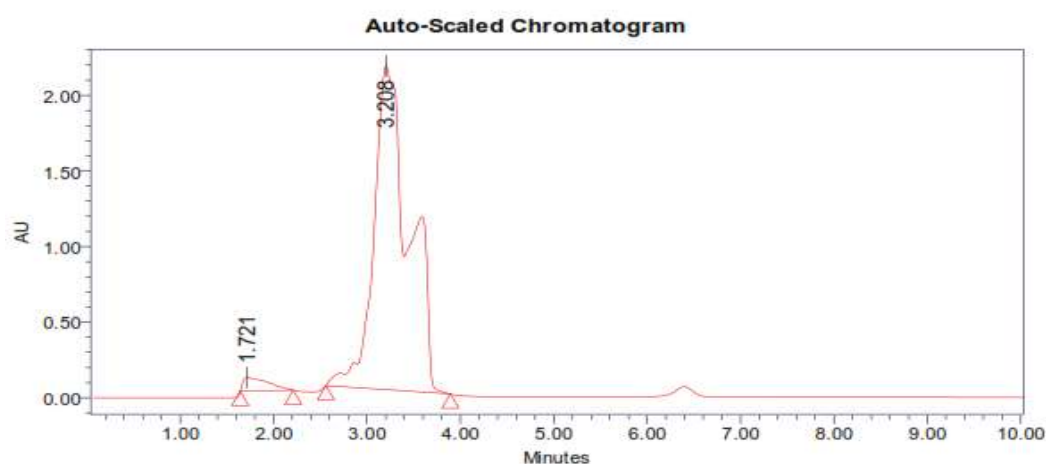


Fig 17: chromatogram for trail 4

Table 17: peak results for trail 4

S. No.	Peak name	R _t	Area	Height	USP Tailing	USP plate count
1	Acyclovir	1.721	1660846	92369	3.56	168
2	Hydrocortisone	3.208	56288276	2140306	1.12	886

Observation:

In this above trail more tailing shows and improper peak shape in the chromatogram. More trails required to get optimized peaks.

Trial 5: Optimized Chromatogram (Standard)

Mobile phase ratio : Acetonitrile: Water (50:50v/v)
 Temperature : 30°C
 Column : Gemini C18 (4.6×150mm, 5μ)
 Flow rate : 1ml/min
 Wavelength : 238 nm
 Injection volume : 10 μl
 Run time : 8 min

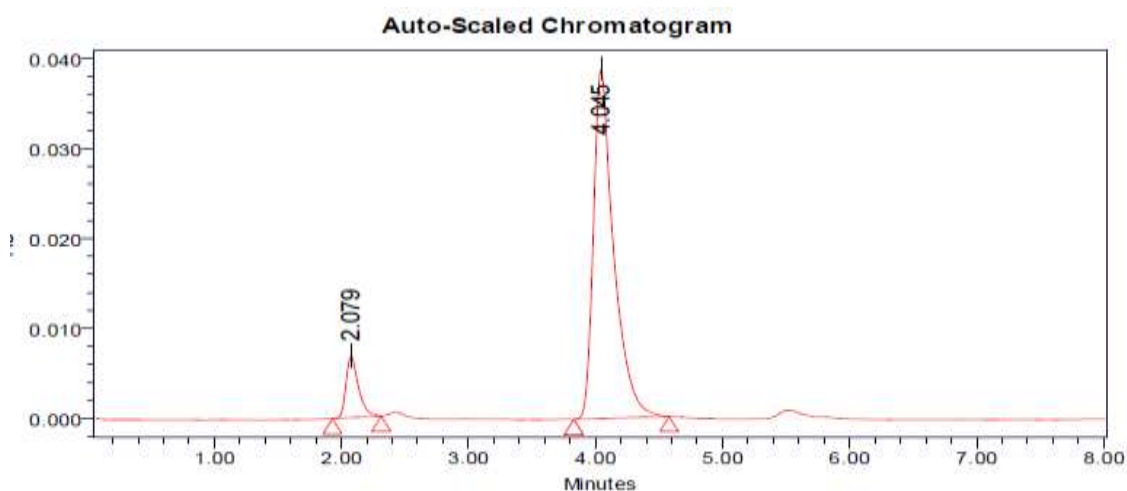


Fig.18: Optimized Chromatogram (Standard)

Table 18: Optimized Chromatogram (Standard)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Acyclovir	2.079	46168	6841	1.33	4251
2	Hydrocortisone	4.045	429069	38885	1.59	5224

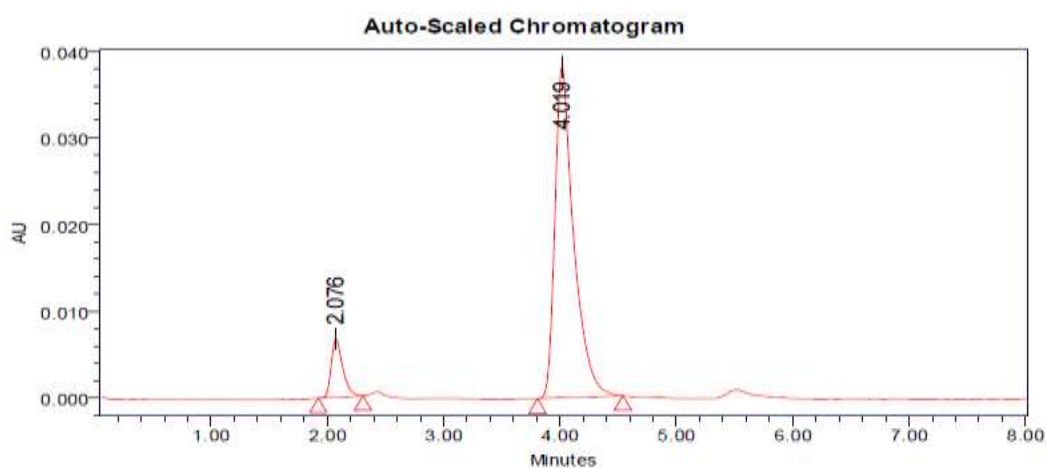
Optimized Chromatogram (Sample)

Fig 19: Optimized Chromatogram (Sample)

Table 19: Optimized Chromatogram (Sample)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Acyclovir	2.076	46150	6766	1.36	5152
2	Hydrocortisone	4.019	427826	38246	1.58	6071

Acceptance Criteria:

- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

VALIDATION

Blank:

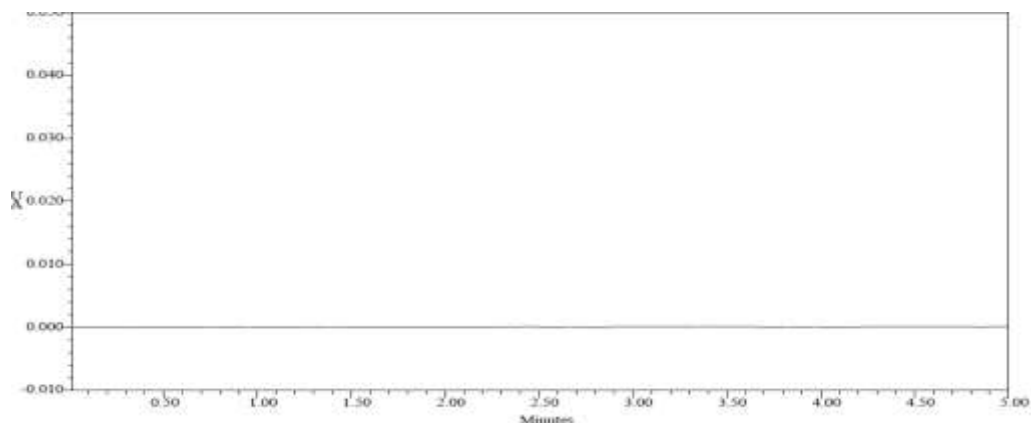


Fig 20: Chromatogram showing blank (mobile phase preparation)

SPECIFICITY

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.

Analytical method was tested for specificity to measure accurately quantities Acyclovir and Hydrocortisone in drug product.

Assay (Standard):

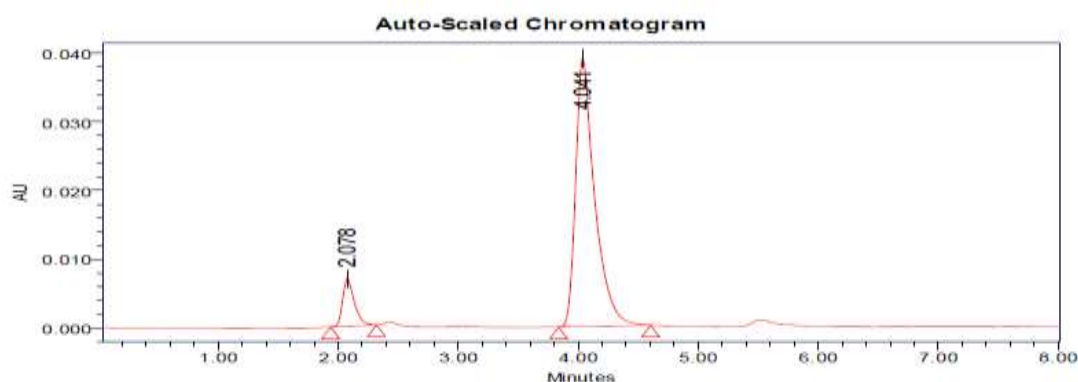


Fig 21: Chromatogram showing assay of standard injection -1

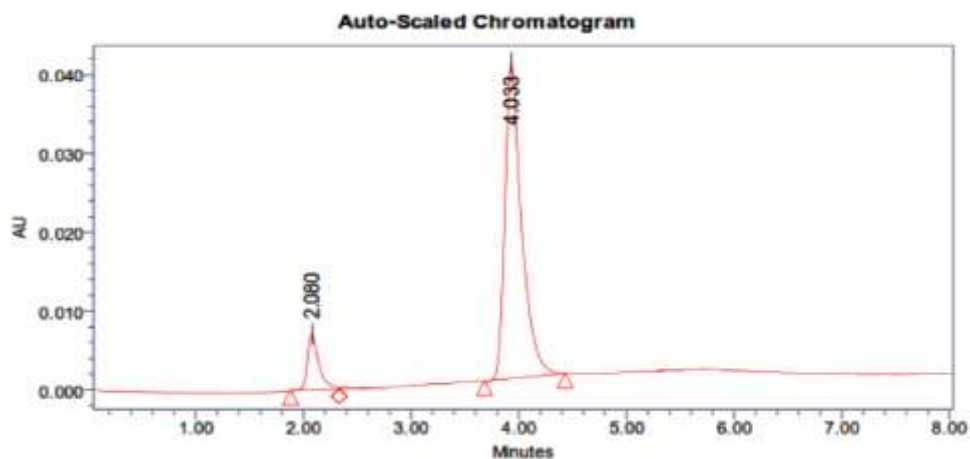


Fig 22: Chromatogram showing assay of standard injection -2

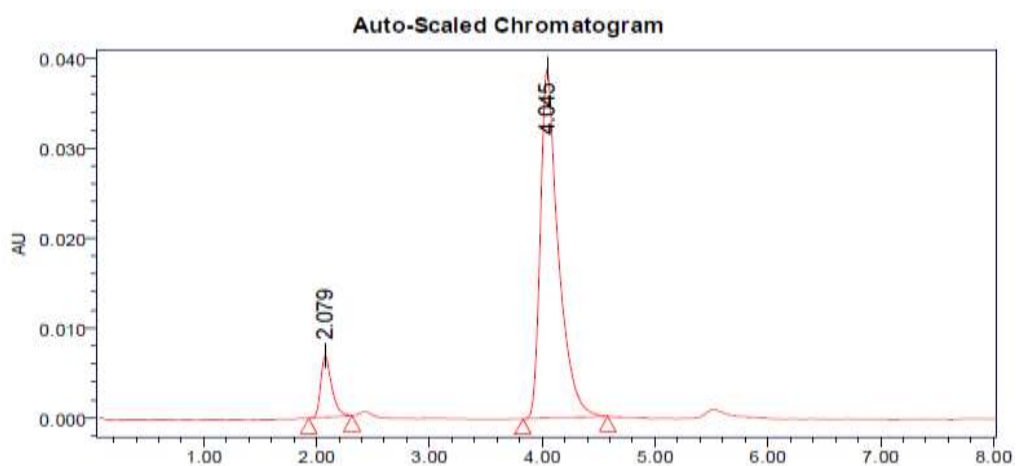


Fig 23: Chromatogram showing assay of standard injection -3

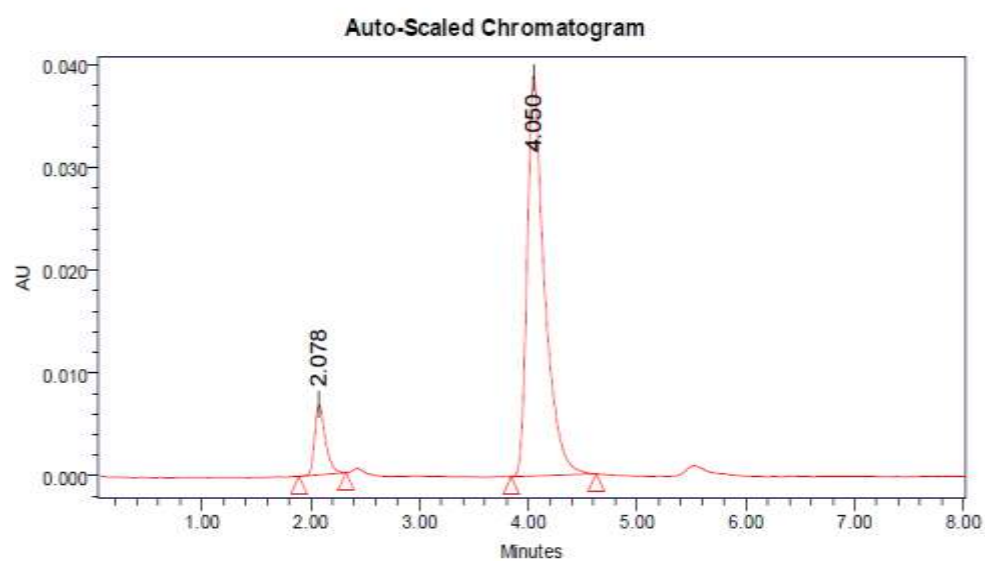


Fig.24: Chromatogram showing assay of standard injection -4

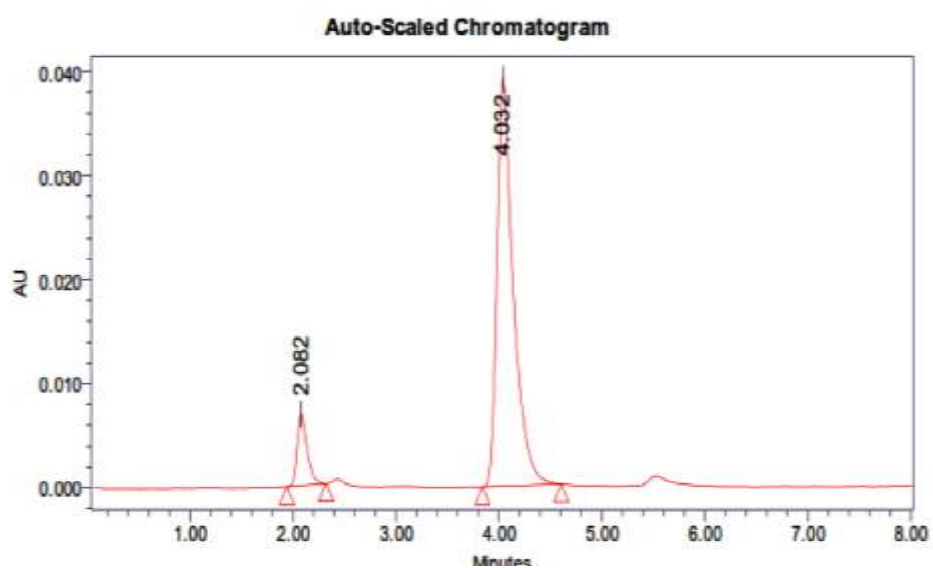


Fig 25: Chromatogram showing assay of standard injection -5

Table 20: Peak results for assay standard of Acyclov

S.No.	Peak Name	RT	Area ($\mu\text{V}\cdot\text{sec}$)	Height (μV)	USP Plate Count	USP Tailing
1	Acyclovir	2.078	49569	6811	6945	1.51
2	Acyclovir	2.080	49649	6999	6149	1.57
3	Acyclovir	2.078	49731	6972	6473	1.49
4	Acyclovir	2.079	49479	6971	6190	1.49
5	Acyclovir	2.082	49684	6841	6294	1.49
Mean			49607			
Std. Dev.			107.963			
% RSD			0.217637			

Acceptance criteria:

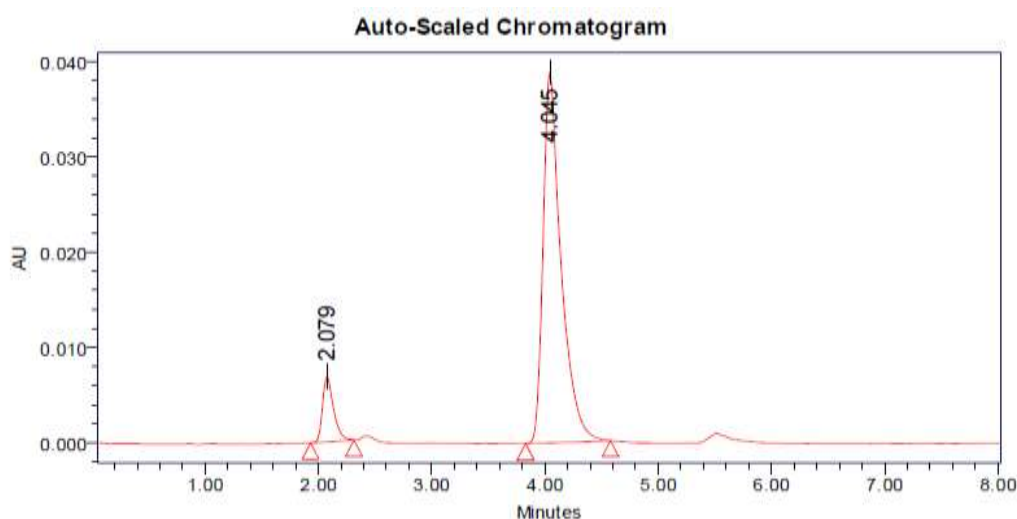
- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

Table 21: Peak results for assay standard of Hydrocortisone

S.No.	Peak Name	RT	Area ($\mu\text{V}\cdot\text{sec}$)	Height (μV)	USP Plate Count	USP Tailing
1	Hydrocortisone	4.041	423328	44147	7672	1.35
2	Hydrocortisone	4.033	423805	44538	7786	1.13
3	Hydrocortisone	4.050	423229	44964	5772	1.34
4	Hydrocortisone	4.045	423876	44959	5191	1.35
5	Hydrocortisone	4.032	423575	38885	5137	1.35
Mean			423559.5			
Std. Dev.			328.2606			
% RSD			0.0775			

Acceptance Criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

Assay (Sample):**Fig.26:** Chromatogram showing assay of sample injection-1

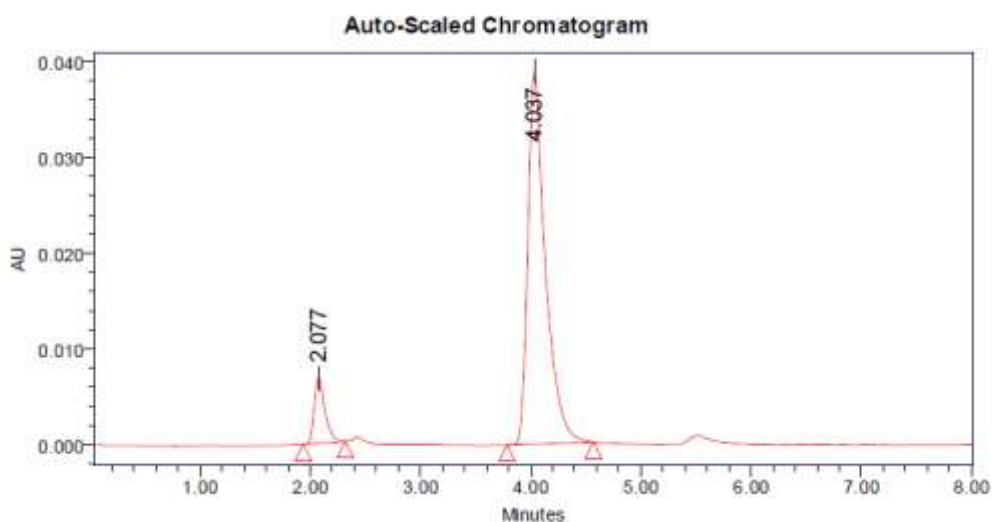


Fig.27: Chromatogram showing assay of sample injection-2

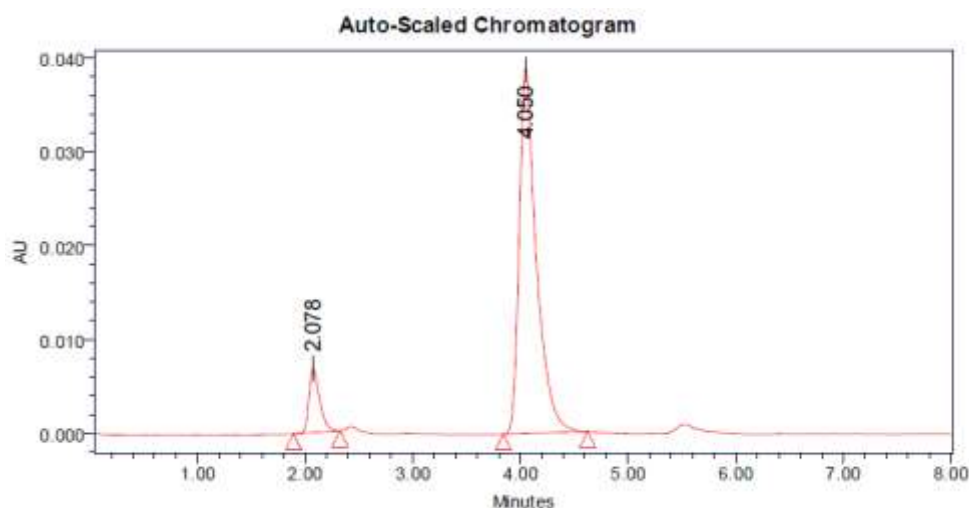


Fig.28: Chromatogram showing assay of sample injection-3

Table 22: Peak results for Assay sample of Acyclovir

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Acyclovir	2.078	46684	6918	1.34	5217	1
2	Acyclovir	2.079	46168	6841	1.33	5251	2
3	Acyclovir	2.077	46088	6851	1.37	7127	3

Table 23: Peak results for Assay sample of Hydrocortisone

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Hydrocortisone	4.050	430575	39127	1.60	6197
2	Hydrocortisone	4.045	429069	38885	1.59	6224
3	Hydrocortisone	4.037	429543	38892	1.58	8203

%ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Acyclovir and Hydrocortisone in pharmaceutical dosage form was found to be 98.2%

LINEARITY

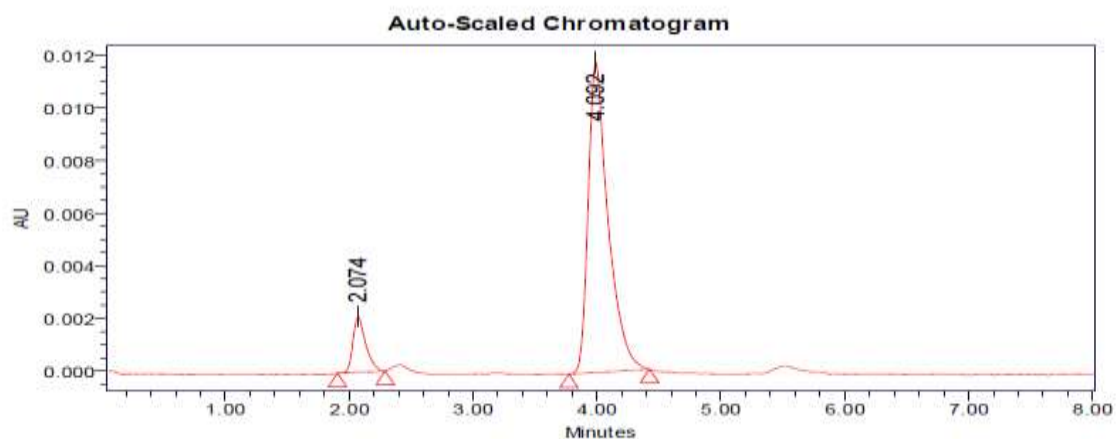


Fig.34: Chromatogram showing linearity level-1

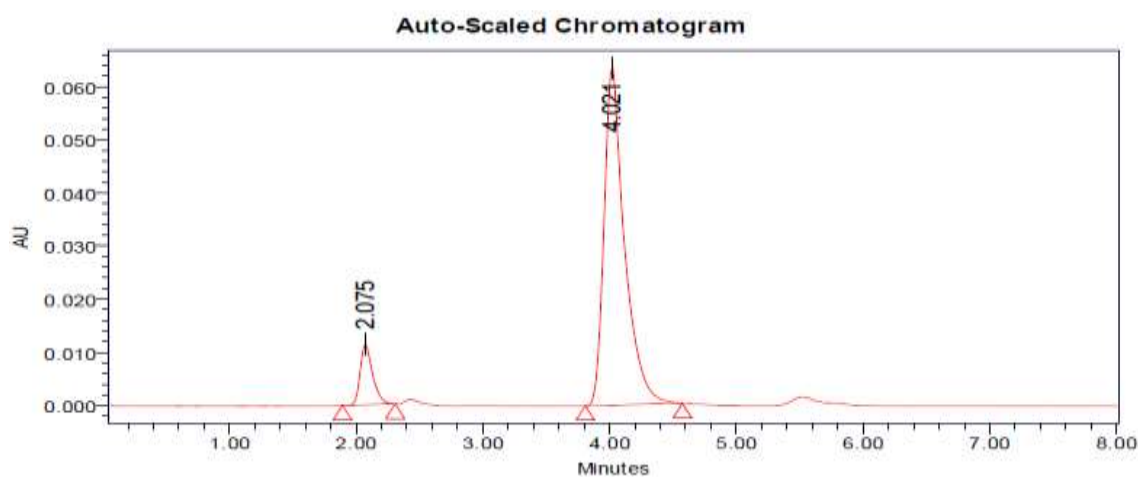


Fig.35: Chromatogram showing linearity level-2

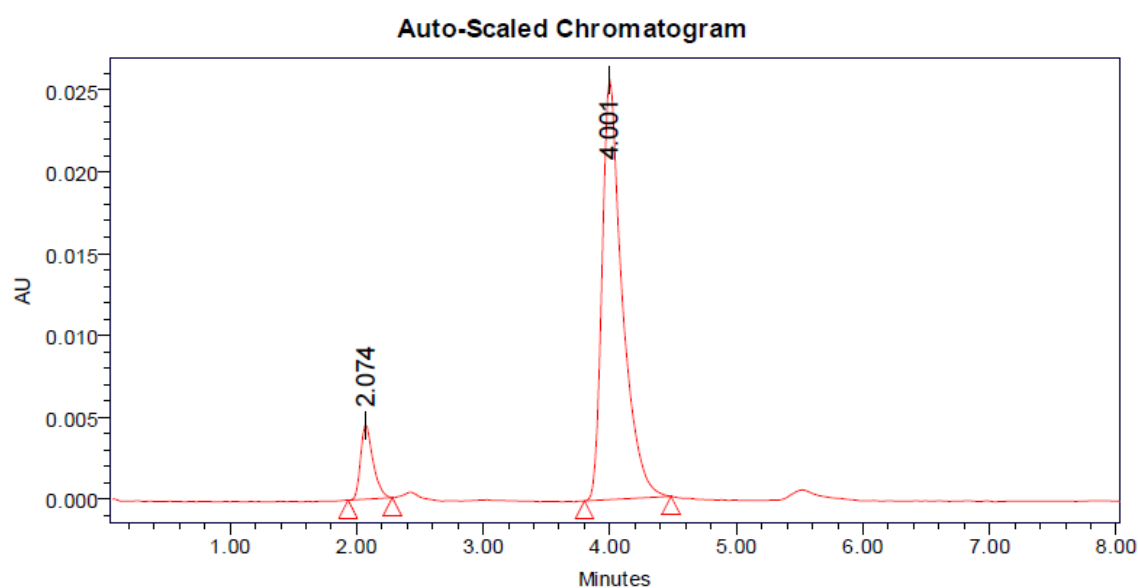


Fig 36: Chromatogram showing linearity level-3

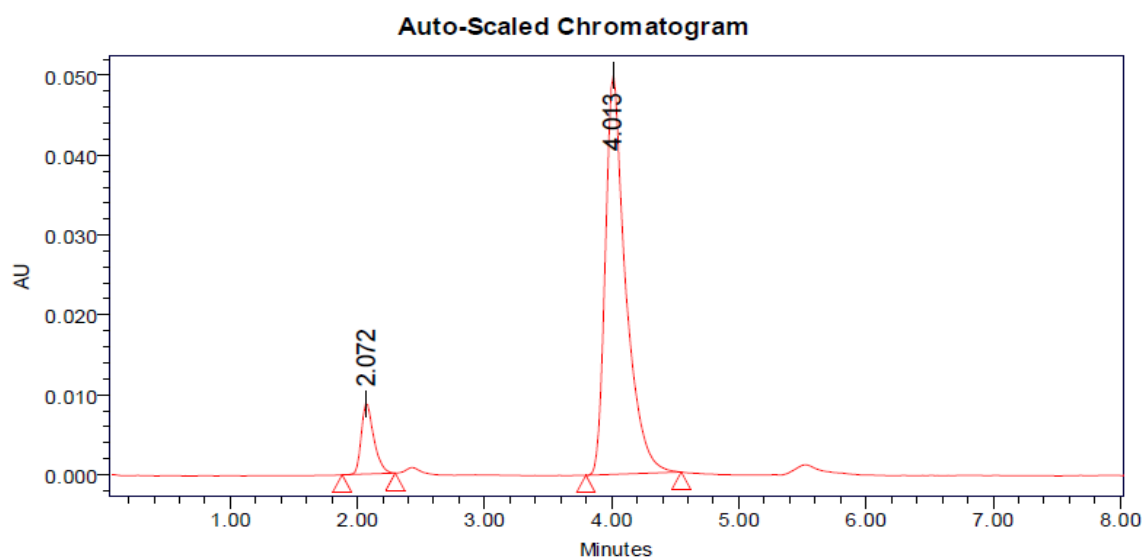


Fig 37: Chromatogram showing linearity level-4

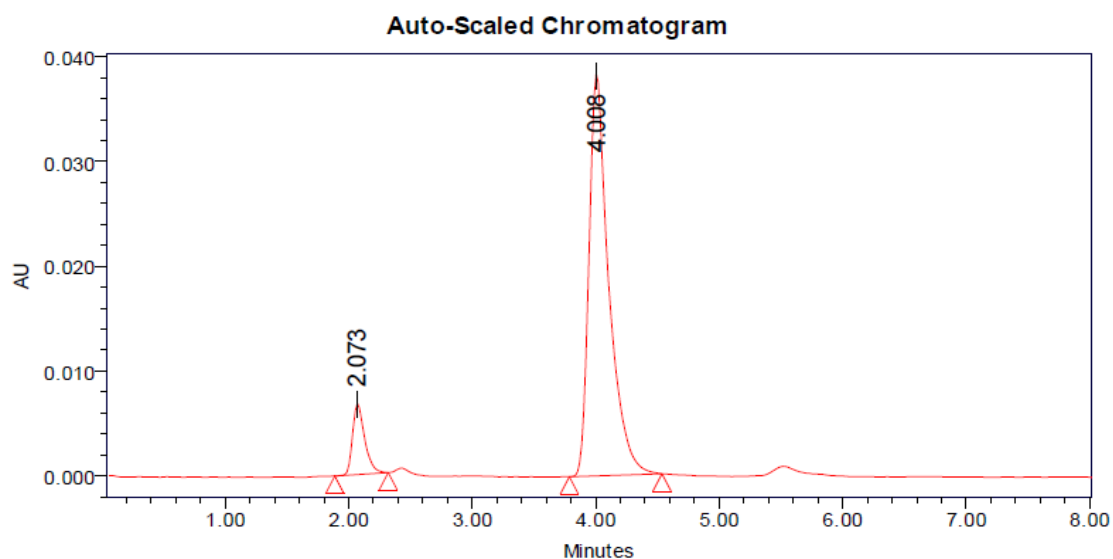


Fig 38: Chromatogram showing linearity level-5

Table 25: CHROMATOGRAPHIC DATA FOR LINEARITY STUDY FOR ACYCLOVIR:

Concentration µg/ml	Average Peak Area
20	15065
40	31009
60	46166
80	60569
100	76862

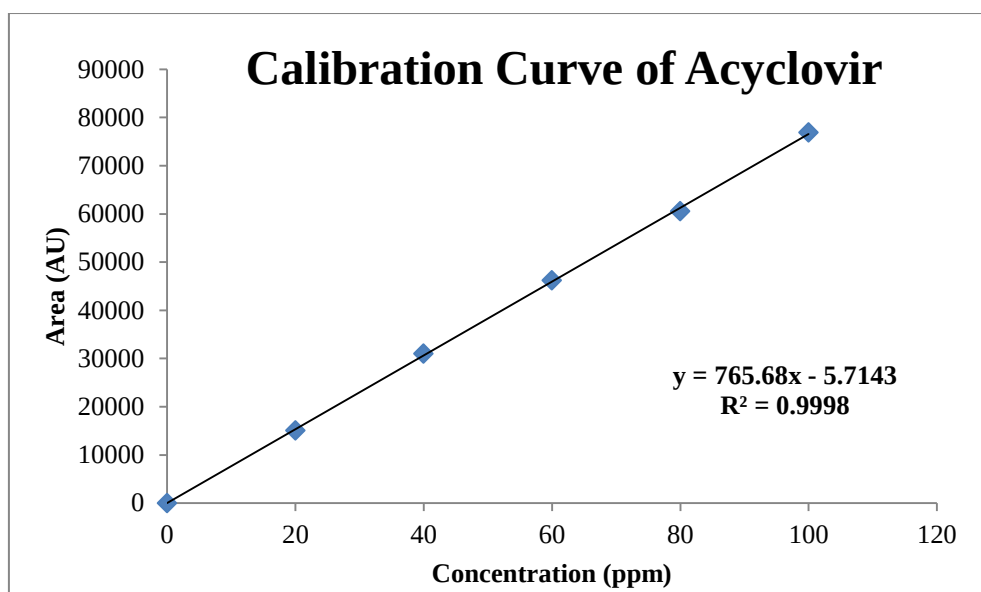


Fig 39: Chromatogram showing linearity level

LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Acyclovir is a straight line.

$$Y = mx + c$$

Slope (m) = 765.68

Intercept (c) = 5.7143

Correlation Coefficient (r) = 0.9998

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 5.7143. These values meet the validation criteria.

Table 26: CHROMATOGRAPHIC DATA FOR LINEARITY STUDY FOR HYDROCORTISONE:

Concentration µg/ml	Average Peak Area
40	131289
80	284775
120	427559

CONCLUSION

In the A simple, precise, and reliable Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Acyclovir and Hydrocortisone in bulk and pharmaceutical dosage forms. The method employed a Waters HPLC system with an auto sampler and PDA detector (model 996), using a Gemini C18 column (4.6 × 150 mm, 5 µm) maintained at 30°C. The mobile phase consisted of Acetonitrile and Water in a 50:50 v/v ratio, delivered at a flow rate of 1.0 mL/min. Detection was carried out at 238 nm with an injection volume of 10 µL, and the total run time was 8 minutes. The method was validated according to ICH guidelines and demonstrated excellent linearity, accuracy, precision, specificity, and robustness. The retention times for both Acyclovir and Hydrocortisone were well-resolved, with no interference from excipients or degradation products. In conclusion, the developed RP-HPLC method is accurate, reproducible, and efficient, making it highly suitable for routine quality control and analysis in pharmaceutical formulations

BIBLIOGRAPHY

1. H.H. Willard, L.L. Merritt, J.A. Dean, F.A. Settle. Instrumental methods of analysis, 7th edition, CBS publishers and distributors, New Delhi. 1986, P.518-521, 580-610.
2. John Adamovics, Chromatographic analysis of pharmaceutical, Marcel Dekker Inc. New York, 2nded, P.74, 5-15.
3. Gurdeep Chatwal, Sahm K. Anand. Instrumental methods of chemical analysis, 5th edition, Himalaya publishing house, New Delhi, 2002, P.1.1-1.8, 2.566-2.570
4. D. A. Skoog. J. Holler, T.A. Nieman. Principle of instrumental analysis, 5th edition, Saunders college publishing, 1998, P.778-787.
5. Sharma BK. Instrumental methods of chemical analysis, Introduction to analytical chemistry, 23th ed .Goel publishing house meerut, 2004, P12-23.
6. Skoog, Holler, Nieman. Principals of instrumental analysis 5thed, Harcourt publishers international company, 2001, P.543-554.
7. William Kemp. Organic spectroscopy, Palgrave, New York, 2005, P.7-10, 328-330
8. P.D. Sethi. HPLC: Quantitative analysis pharmaceutical formulations, CBS publishers and distributors, New Delhi (India), 2001, P.3-137.
9. Michael E, Schartz IS, Krull. Analytical method development and validation. 2004, P. 25-46.
10. R. Snyder, J. Kirkland, L. Glajch. Practical HPLC method development, 2nded, A Wiley international publication, 1997, P.235,266-268,351-353.653-600.686-695.
11. Basic education in analytical chemistry. Analytical science, 2001:17(1).
12. Method validation guidelines international onference on harmonization; GENEVA; 1996
13. Berry RI, Nash AR. Pharmaceutical process validation, Analytical method validation, Marcel Dekker Inc. New work, 1993; 57:411-28