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R E S E A R C H A R T I C L E

Development and Validation of High Performance Liquid Chromatography Method for Simultaneous Estimation of Acyclovir and Hydrocortisone in Their Combined Tablet Dosage Form

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A B S T R A C T

The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Acyclovir and Hydrocortisone in tablet dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statistically according to ICH guidelines. The Sample recoveries in all formulations were in good agreement with their respective label claims. From literature review and solubility analysis initial chromatographic conditions Mobile phase Phosphate buffer: Methanol PH 2.5 (60:30 v/v) were set Potassium dihydrogen orthophosphate PH 2.5 adjusted with Orthophosphoric acid, Xbridge C18 Column (250mm x 4.6mm)5µg, Flow rate 1ml min-1and temperature was ambient, eluent was scanned with PDA detector in system and it showed maximum absorbance at 258 nm. As the methanol content was increased Acyclovir and Hydrocortisone got eluted with good peak symmetric properties. The retention times for Acyclovir and Hydrocortisone was found to be 2.122 min and 3.562 min respectively. System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria. Linearity study was carried out between50% to150 % levels, R2 value was found to be as 0.999.

Keywords: XbridgeC18, Acyclovir and Hydrocortisone, RP-HPLC

A R T I C L E I N F O

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CONTENTS

1. Introduction. 198
2. Materials and Method. 198
3. Results and Discussion. 199

4. Conclusion. 201
5. References. 201

1. 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. It can be taken by mouth, applied as a cream, or injected.

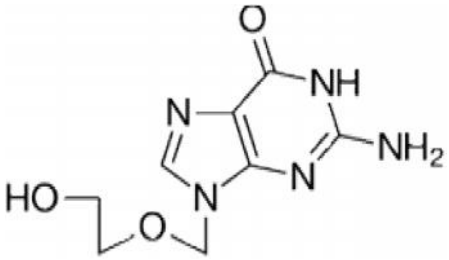

Fig 1: Structure of Acyclovir

Hydrocortisone is the name for the hormone cortisol when supplied as a medication. Uses include conditions such as adrenocortical insufficiency, adrenogenital syndrome, high blood calcium, thyroiditis, rheumatoid arthritis, dermatitis, asthma, and COPD.

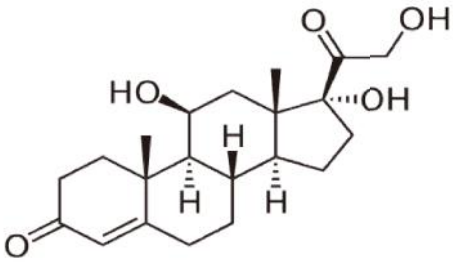

Fig 2: Structure of Hydrocortisone

2. Materials and Method

Instrumentation

System Alliance Waters 2690 separation module, Pump Analytical HPLC isocratic pump, Detector Photo diode array detector, Software Empower 2 software, Column Agilent (250×4.6mm, 5µ) C-18 RP-column, Sonicator Analytical Technologies Limited- Ultrasonic cleaner. U.V double beam spectrophotometer LABINDIA, UV 3000^{+}pH meter, Weighing machine.

Chemicals: Acyclovir and Hydrocortisone, KH_2PO_4, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid, Tri ethyl amine.

Optimized chromatographic conditions:

Parameters	**:Description**
Flow rate	**:**1ml min^{-1}
Column	**:**XbridgeC$_{18}$ Column (250mm x 4.6mm)5µg.
Mobile Phase	**:**Phosphate buffer: Methanol P^{H} 2.5 (60:30 v/v)
Buffer	**:**Potassium dihydrogen orthophosphate PH 2.5 adjusted with Orthophosphoric acid
Detector	**:**PDA
Column temperature	**:**Ambient
Type of elution	**:**Isocratic
Wavelength	**:**258 nm
Injection volume	**:**20µl
Run time	**:**10min

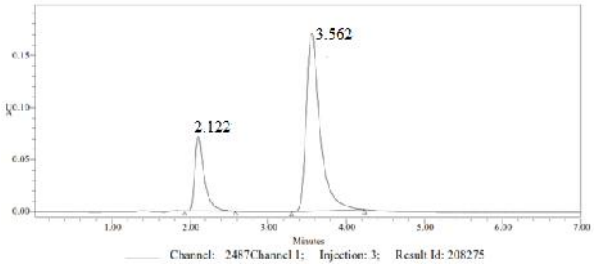

Fig 3: Optimized chromatogram

Observation: The separation of two analytical peaks was good. The plate count also above 2000, tailing factor below 2, and the resolution is above 2. The condition is taken as optimized method.

Standard preparation:

Weigh accurately 10mg Hydrocortisone Working Reference Standard and 400mg of Acyclovir Working Reference Standard is taken in to 100ml volumetric flask and then it was dissolved and diluted to volume with mobile phase up to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase. (Stock solution)Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

Sample preparation:

40.02mg Acyclovir 10.02 mg Hydrocortisone and 10 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 10 tablets was transferred into a 100ml standard flask. A volume of 70ml of mobile phase was added and sonicate for 30min.Then the solution was cooled and diluted to volume with mobile phase and filtered through 0.45µm membrane filter. (Stock solution)Further pipette 0.25ml of Acyclovir and Hydrocortisone of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

Method Validation

- System Suitability
- Linearity
- Specificity
- Precision
- Intermediate Precision
- Accuracy
- Limit of Detection and Limit of Quantification
- Robustness

3. Results and Discussion

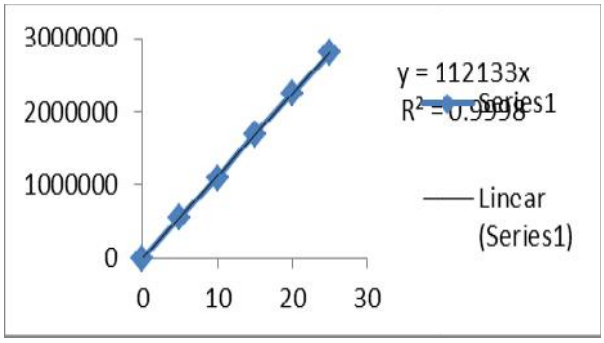

Fig 4: Linearity Graph of Acyclovir

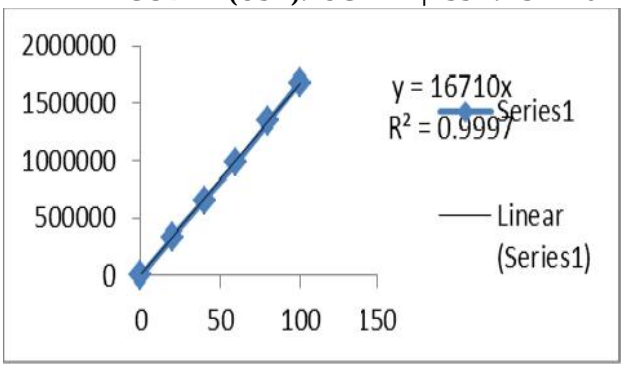

Fig 5: Linearity Graph of Hydrocortisone

Table 2: Peak results of Standard & Test Chromatograms for Assay

Parameter	Standard		Test	
	Hydrocortisone	Acyclovir	Hydrocortisone	Acyclovir
Retention time	2.102	3.537	2.120	3.536
Peak Area	2008408	1185786	2005829	1189695
USP Plate Count	5167	5389	5752	5187
Tailing Factor	1.3	1.3	1.4	1.2
USP Resolution	-	6.6	-	8.3

Table 3: Results of Assay

Parameters	Hydrocortisone	Acyclovir
Standard peak area	2008408	1185786
Test peak area (mean)	2005829	1189695
Average Weight	694.2mg	694.2mg
Label claim	0.5 mg	50 mg
% Purity of Standard	99.50	99.58
Amt obtained	99.88 mg	150.10 mg
% Assay	99.77%	100.12%

Table 4: Preparation of working standard solutions for Linearity

Sample ID	Acyclovir		Hydrocortisone	
	Concentration (mcg/ml)	Area	Concentration (mcg/ml)	Area
20% of operating concentration	5	554140	20	340046
40% of operating concentration	10	1095681	40	660204
60% of operating concentration	**15**	**1692966**	**60**	**983023**
80% of operating concentration	20	2256546	80	1339886
100% of operating concentration	25	2797214	100	1682302
Correlation Coefficient			0.999	

Table 5: Precision data of Acyclovir

S. No	NAME	RT	AREA
1	Acyclovir	2.108	602223
2	Acyclovir	2.105	607748
3	Acyclovir	2.113	607302
4	Acyclovir	2.109	608674
5	Acyclovir	2.109	607376
Mean			606665
Std.Dev.			2542.3
%RSD			0.42

Table 6: Precision data of Hydrocortisone

S.No	Name	RT	Area
1	Hydrocortisone	3.552	2220333
2	Hydrocortisone	3.550	2221573
3	Hydrocortisone	3.564	2215483
4	Hydrocortisone	3.564	2217379
5	Hydrocortisone	3.565	2211255
Mean			2217205
Std.Dev.			4100.8
%RSD			0.18

Table 7: Intermediate Precision data for Acyclovir

S.NO	NAME	RT	AREA
1	Acyclovir	2.108	596608
2	Acyclovir	2.105	598959
3	Acyclovir	2.113	595728
4	Acyclovir	2.109	594485
5	Acyclovir	2.109	595267
Mean			596209
Std.Dev.			1718.7
%RSD			0.29

Table 8: Intermediate Precision data for Hydrocortisone

S.NO	NAME	RT	AREA
1	Hydrocortisone	3.552	2207732
2	Hydrocortisone	3.550	2202266
3	Hydrocortisone	3.564	2209375
4	Hydrocortisone	3.564	2204037
5	Hydrocortisone	3.565	2204466
Mean			2205575
Std.Dev.			2899.8
%RSD			0.13

Table 8: Accuracy Study of Hydrocortisone

Sample Id	Conc found (µg/ml)	Concn Obtained (µg/ml)	%Recovery	Mean recovery	Statistical Analysis
50%	5	5.01	100.2		
50%	5	4.96	99.2	99.73	
50%	5	4.99	99.8		**%RSD= 0.505**
100%	10	9.95	99.5		
100%	10	9.87	98.7	98.8	
100%	10	9.82	98.2		**%RSD=0.66**
150%	15	14.64	97.6		
150%	15	14.76	98.4	98.8	
150%	15	15.06	100.4		**%RSD=1.45**

Table 9: Accuracy Study of Acyclovir

Sample Id	Concn Obtained(µg/ml)	%Recovery of drug	Mean accuracy	%RSD
50%	4.92	98.0		
50%	4.96	99.2		
50%	5.02	100.4	**99.2**	**1.2**
100%	9.95	99.5		
100%	9.94	99.4		
100%	9.98	99.8	**99.5**	**0.2**
150%	14.78	98.6		**0.530**

150%	14.94	99.6	**99.0**	
150%	14.83	98.8		

Table 10: LOD and LOQ Data of Acyclovir and Hydrocortisone

Hydrocortisone			**Acyclovir**		
Conc.(x) (µg/ml)	**Peak Areas (y)**	**Statistical Analysis**	**Conc.(x) (µg/ml)**	**Peak Areas (y)**	**Statistical Analysis**
5	1196	S = 39092 c = 618048 LOD: 0.001µg/ml LOQ: 0.004µg/ml	20	1661	S = 39092 c =369381 LOD:0.005 µg/ml LOQ: 0.015µg/ml

Table 11: Robustness data for Hydrocortisone

Std. Replicate	**Variation in flow rate**		**Variation in Mobile phase composition**	
	Flow Rate 0.8ml/min	**Flow Rate 1.2ml/min**	**Buffer: Methanol (40:60)**	**Buffer: Methanol (30:70)**
1	674735	2057613	606093	603559
Retention time	2.330	1.950	2.290	1.998
Tailing factor	1.7	1.3	1.4	1.5
Theoretical plates	2673	2452	2642	2599

Table 12: Robustness data for Acyclovir

Parameter	**Variation in flow rate**		**Variation in Mobile phase composition**	
Standard	Flow Rate 0.8ml/min	Flow Rate 1.2ml/min	Buffer: Methanol (40:60)	Buffer: Methanol (30:70)
1	2505636	2057613	2239255	2300346
Retention time	3.885	3.263	4.435	3.009
Tailing factor	1.7	1.3	1.2	1.6
Theoretical plates	2522	2452	2310	2299

4. Conclusion

The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Acyclovir and Hydrocortisone in tablet dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statistically according to ICH guidelines. The Sample recoveries in all formulations were in good agreement with their respective label claims.

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