INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

STABILITY INDICATING HPTLC METHOD DEVELOPMENT FOR SIMULTANEOUS ESTIMATION OF ACYCLOVIR AND HYDROCORTISONE IN API AND PHARMACEUTICAL DOSAGE FORM

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Article Received on: 26/07/19 Approved for publication: 07/09/19

DOI: 10.7897/2230-8407.1010301

ABSTRACT

An accurate, precise, sensitive, selective stability indicating High Performance Thin Layer Chromatographic method has been developed and validated for simultaneous estimation of Acyclovir and Hydrocortisone in API and Pharmaceutical dosage form. Chromatographic separation of both the drugs were achieved by using TLC aluminum plates precoated with silica gel 60 F 254 as stationary phase and Toluene: Methanol: Glacial acetic acid in the ratio of 6:4:0.05 v/v/v as mobile phase. Densitometric analysis was carried out in absorbance mode at 250 nm. The Rf value was found to be 0.30 ± 0.02 and 0.73 ± 0.02 for Hydrocortisone and Acyclovir respectively. The method was validated in compliance with ICH guidelines for linearity, Precision, Robustness, Repeatability, Limit of detection, Limit of quantification, Accuracy and Assay. Linear regression analysis data for the calibration curves shows good linear correlation relationship over the concentration ranges from 1000-6000 ng/spot with R^2 = 0.9997 and 20-1200 ng/spot with R^2 = 0.9997 for Acyclovir and Hydrocortisone respectively. The Limit of detection and Limit of quantification were 240 and 720 ng/spot for Acyclovir while 400 and 1220 ng/spot for Hydrocortisone. The recoveries of Hydrocortisone and Acyclovir were found to be 98.6 and 99.8%. The drugs were subjected to acidic, basic, neutral, oxidative, thermal and photolytic conditions with below 20% degradation.

Keywords: Acyclovir, Hydrocortisone, HPTLC, Stability indicating study, Validation

INTRODUCTION

Acyclovir is an antiviral drug, viral (HSV-1, HSV-2, and VZV). Thymidine kinase converts acyclovir to acyclovir monophosphate which is then converted to diphosphate by cellular guanylate kinase and finally to triphosphate by phosphoglycerate kinase, phosphoenolpyruvate kinase and pyruvate kinase. Acyclovir triphosphate competitively inhibits viral DNA polymerase and competes with natural deoxyguanosine triphosphate for the incorporation into viral DNA. Once incorporated Acyclovir triphosphate inhibits DNA synthesis by acting as chain terminator[1-2].

The anti-inflammatory actions of corticosteroids are thought to involve lipocortins, phospholipase A2 inhibitory proteins which, through inhibition of arachidonic acid, control the biosynthesis of prostaglandins and leukotrienes. Specifically, glucocorticoids induce lipocortin-1 (annexin-1), synthesis which then binds to cell membrane preventing phospholipase (A2) from coming in contact with arachidonic acid. This leads to diminished eicosanoid production[3-4].

Glucocorticoids also stimulate the lipocortin-1 escaping to the extracellular space, where it binds to the leukocyte membrane receptors and inhibits various inflammatory events: epithelial adhesion, emigration, chemo taxis, phagocytosis, respiratory burst and the release of various inflammatory mediators (lysosomal enzymes, cytokines, tissue plasminogen activator, chemokines etc.) from neutrophils, macrophages and mastocytes.[5]

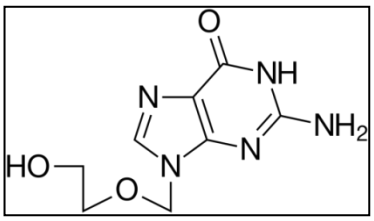

Figure 1: Structure of Acyclovir

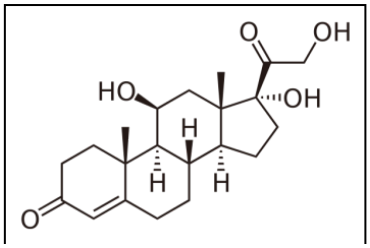

Figure 2: Structure of Hydrocortisone

Literature survey revealed that various analytical methods like HPLC; UV has been reported for simultaneous estimation of Acyclovir and Hydrocortisone in dosage form like tablets, cream etc but so far no HPTLC method has been reported for

simultaneous estimation of Acyclovir and Hydrocortisone and no stability studies are reported.[6-7]

So there was a need to develop HPTLC method because of its enormous flexibility, parallel separation of many samples with minimum time requirement and simultaneous visual evaluation of many samples and its components.[8-9]

The main objective of this method is to develop simple, precise, cost effective stability indicating HPTLC method with a wide linear range and good reproducibility for simultaneous estimation of Acyclovir and Hydrocortisone in API and pharmaceutical dosage forms.

MATERIALS AND METHODS

ACY and HYDRO were provided as gift samples from Apotex Pharmaceuticals, Bangalore. Marketed formulation consists of 5 mg of Acyclovir and 1 mg of Hydrocortisone was procured from local market of Chhattisgarh. Analytical grade solvents and TLC aluminum plates precoated with silica gel F 254 used for this study were purchased from Merck Pvt. Ltd, Mumbai

Equipments

Chromatographic separation of drug was performed by using precoated silica gel TLC plates F 254, 20 x 20 cm size. Samples were applied on the TLC plates in the form of bands, width 6 mm using Hamilton micro liter syringe (100 µl). Linear ascending development was carried out in 10 × 10 cm twin trough glass chamber (CAMAG) using mobile phase which consists of Toluene: Methanol: Glacial acetic acid in the ratio of 6:4:0.05 v/v/v. The slit dimension was kept as 5 × 0.45 mm. The optimized saturation time for mobile phase was 30 min. Chromatogram was run till the distance of 8 cm. After development the plate was dried and densitometric analysis was performed on a Camag TLC. Scanner equipped with win CATS software version 1.4.4.6337 at 250 nm. The source of radiation utilized was deuterium lamp.

Method development

Preparation of standard stock solution (S1)

10 mg of Acyclovir and 10 mg of Hydrocortisone were weighed accurately and transferred into separate 10 ml volumetric flask and the volume was made up to mark and with methanol to get standard stock solution of Acyclovir A1 (1000 µg/ml) and Hydrocortisone H1 (1000 µg/ml) respectively.[10-19]

Preparation of working standard solution (S2)

5 ml of standard stock solution of Acyclovir A1 (1000 µg/ml) was diluted with methanol up to 10 ml to get working stock solution of ACY (A2, 500µg/ml). 1 ml of standard stock solution of Hydrocortisone H1 (1000 µg/ml) was diluted with methanol up to 10 ml to get working stock solution of HYDRO (H2, 100 µg/ml) solutions.

Preparation of mixed standard solution (S3)

5 ml working stock solution of ACY (A2, 500 µg/ml) and 5 ml of working stock solution of HYDRO (H2, 100 µg/ml) were mixed together to get mixed standard stock solutions.

Optimization of Chromatographic method

The main objective in developing stability indicating HPTLC method is to achieve a good resolution of Acyclovir and Hydrocortisone and its degradation products.

Validation of Analytical Method

The following parameters were used for validation of the developed method. The method was validated as per the ICH guideline for linearity, Precision, Robustness, and Repeatability, limit of detection, limit of quantification, accuracy and assay.[20-21]

Linearity

The mixed standard solution of Hydrocortisone (100 µg/mL) 0.2, 0.4, 0.6, 0.8, 1.2 µl and Acyclovir (500 µg/mL) 1, 2, 3, 4, 5 and 6 µl was applied on TLC plate. The linear regression data for calibration curves (n = 3) showed good linear relationship over a concentration range of 200-1200 ng/spot for Hydrocortisone and 1000-6000 ng/spot for Acyclovir. The calibration curves were developed by plotting peaks of areas vs. concentrations.[22-25]

Precision

The precision of the method was verified by Intraday and Interday precision studies.2, 4 and 6 µl spots of Acyclovir and 0.4, 0.8 and 1.2 µl spot of Hydrocortisone were applied on TLC plate. % RSD was calculated by analyzing standard stock solution of Acyclovir and Hydrocortisone within the calibration range, three times in a day for Intraday precision and three consecutive different days for Interday precision.

LOD and LOQ

The sensitivity measurement of Acyclovir and Hydrocortisone was determined in terms of the LOD and LOQ. The LOD and LOQ were calculated using the equation.

$$LOD = 3.3\ (SD)\ X\ S$$
$$LOQ = 10\ (SD)\ X\ S$$

Where SD is standard deviation and S = slope of the linear regression equation

Robustness

Robustness studies were done by making small, deliberate changes in optimized condition like mobile phase composition; saturation time and wavelength (n = 3). The acceptance criterion for % RSD was NMT 2% which is within the accepted range.

Repeatability

Repeatability of sample application was assessed by spotting 2 µl spot of Acyclovir and 0.4 µl spot of Hydrocortisone on TLC plate. This procedure was repeated 6 times. The repeatability of method was evaluated by calculating % RSD.

Recovery

The % Recoveries obtained were 98.4 to 99.89% for Hydrocortisone and 98.6 to 99.9% for Acyclovir and Hydrocortisone respectively.

Assay

The percentage purity is not less than 98.5% and not more than 101.0% for Acyclovir while for hydrocortisone it is not less than 96% and not more than 104.0% as per Indian Pharmacopeia

Forced degradation studies

Forced degradation studies for various parameters were performed as per ICH guidelines. According to ICH guidelines the degradation should be between 5 to 20%.

Following procedures were applied for all the studies after carrying out acidic, basic, oxidative, thermal and photolytic degradation as per ICH guidelines.

Acid Degradation

5 ml of mixed standard solution of Acyclovir and Hydrocortisone (S3) and 5 ml of 0.1N HCl was kept at room temperature for 8 hrs. Standard solution of Acyclovir (500 µg/ml), standard solution of Hydrocortisone (100 µg/ml) and the degraded respective samples of Acyclovir and Hydrocortisone were spotted on TLC plates of size 7 x 10 cm and the plate was run with mobile phase consisting of Toluene: Methanol: Glacial Acetic acid in the ratio of 6:4:0.05 v/v/v. The plate was dried and scanned at 250 nm. Densitogram was recorded and % degradations were calculated.

Base degradation

5 ml of mixed standard solution of Acyclovir and Hydrocortisone (S3) and 5 ml of 0.1N NaOH was kept at room temperature for 8 hrs. Standard solution of Acyclovir (500 µg/ml), standard solution of Hydrocortisone (100 µg/ml) and the degraded respective samples of Acyclovir and Hydrocortisone were spotted on TLC plates of size 7 x 10 cm and the plate was run with mobile phase consisting of Toluene: Methanol: Glacial Acetic acid in the ratio of 6:4:0.05 v/v/v. The plate was dried and scanned at 250 nm. Densitogram was recorded and % degradations were calculated.

Oxidative degradation

5 ml of mixed standard solution of Acyclovir and Hydrocortisone (S3) and 5 ml of 3% H_2O_2 was kept at room temperature for 8 hrs. Standard solution of Acyclovir (500 µg/ml), standard solution of Hydrocortisone (100 µg/ml) and the degraded respective samples of Acyclovir and Hydrocortisone were applied on TLC plate and the plate was run with mobile phase consisting of Toluene: Methanol: Glacial Acetic acid in the ratio of 6:4:0.05 v/v/v. The plate was dried and scanned at 250 nm. Densitogram was recorded and % degradations were calculated.

Thermal degradation

10 mg of Acyclovir and 10 mg of Hydrocortisone powder was kept in a petridish. Petridish was kept in hot air oven at 60˚C. Powdered sample of 1 mg were withdrawn after 30 min, 1 h, 2 h, 3 h and 4 h till 8 h intervals and diluted with methanol. Spot of mixed standard solution and resultant degraded solution was applied on TLC plate and the plate was run with mobile phase consisting of Toluene: Methanol: Glacial Acetic acid in the ratio of 6:4:0.05 v/v/v. The plate was dried and scanned at 250 nm. Densitogram was recorded and % degradations were calculated.

Photolytic degradation

The photochemical stability of the drug was studied by exposing the working standard solution of Acyclovir and Hydrocortisone to direct sunlight for 8 h. Powdered sample of 1 mg were withdrawn after 30 min, 1 h, 2 h, 3 h and 4 h till 8 h intervals and diluted with methanol. The resultant solution was applied on TLC plate and the plate was run with mobile phase consisting of Toluene: Methanol: Glacial Acetic Acid in the ratio of 6:4:0.05v/v/v. The plate was dried and scanned at 250 nm. Densitogram was recorded and % degradations were calculated.

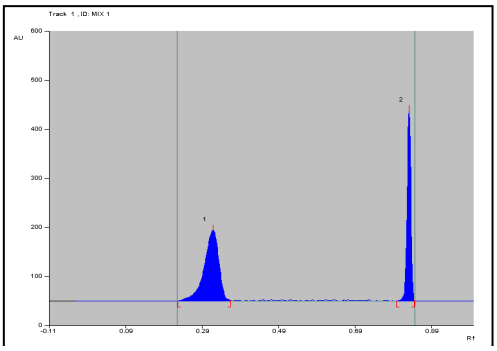

Figure 3: Representative Densitogram of Acyclovir and Hydrocortisone by HPTLC method

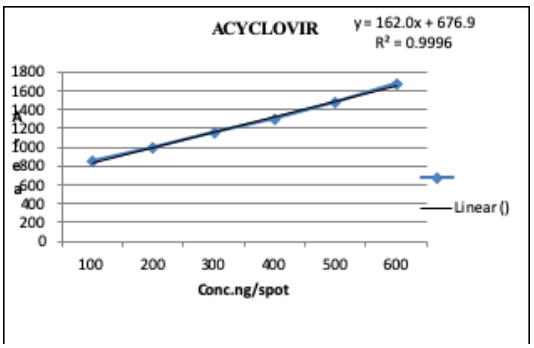

Figure 4: Linearity Graph of Acyclovir

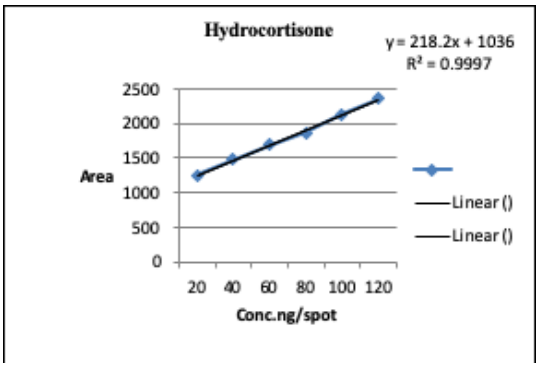

Figure 5: Linearity graph of Hydrocortisone

Table 1: Linearity data of Acyclovir and Hydrocortisone

Parameter	Acyclovir	Hydrocortisone
Linearity ng/spot	1000-6000 (ng/spot)	200-1200 (ng/spot)
R^2	0.9996	0.9997
Slope	162.0	218.2
Intercept	679.9	1036

Table 2: Intra-day precision details of Acyclovir and Hydrocortisone

Drug	CONC. (ng/spot)	Avg. Area (n = 3)	% RSD
HYDRO	400	936.57	1.24
	800	982.66	1.61
	1200	1017.13	1.4
ACY	2000	1254.13	1.4
	4000	1366.3	0.65
	6000	1568.06	1.71

Table 3: Inter-day precision details of Acyclovir and Hydrocortisone

Drug	CONC. (ng/spot)	Avg. Area (n = 3)	% RSD
HYDRO	400	952.9.	1.22
	800	982.73	1.5
	1200	1036.13	1.2
ACY	2000	1285.1	1.1
	4000	1477.03	1.3
	6000	1635.13	1.2

Table 4: LOD and LOQ details of Acyclovir and Hydrocortisone

Drug	LOD (ng/spot)	LOQ (ng/spot)
ACY	240	720
HYDRO	400	1220

Table 5: Repeatability details of Acyclovir and Hydrocortisone

S. No.	Drug	Conc. (ng/spot)	Avg. Area (n = 6)	%RSD
1.	HYDRO	400	936.57	1.2
2.	ACY	2000	1254.1	1.4

Table 6: Robustness details of ACY and HYDRO

Conditions	Rf		Avg. Area (n = 3)		%RSD	
	HYDRO	ACY	HYDRO	ACY	HYDRO	ACY
Mobile Phase Composition (± 0.5 ml Toluene)						
Toluene :Methanol: GAA(5.5:4:0.5v/v/v)	0.29	0.71	951.43	1377.53	1.4	1.67
Toluene :Methanol: GAA(5.5:4:0.5v/v/v)	0.30	0.79	978.2	1478.2		
Toluene :Methanol: GAA(6.5:4:0.5v/v/v)	0.31	0.70	982.6	1568.06		
Wavelength (± 1 nm)						
249 nm	0.28	0.68	957.83	1344.73	1.4	1.3
250 nm	0.30	0.73	978.2	1478.2		
251 nm	0.31	0.74	983.03	1537.16		
Duration of Saturation (± 5 min)						
25	0.28	0.71	976.3	1444.6	1.11	1.4
30	0.30	0.73	978.2	1478.2		
35	0.31	0.75	986.7	1549.5		

Table 7: Recovery details of HYDRO and ACY

Label Claim	% level	Initial amount added (ng/spot)	Amount added (ng/spot)	%Recovery
HYDRO	80	800	640	98.4
	100	800	800	99.6
	120	800	960	99.89
ACY	80	4000	3200	98.6
	100	4000	4000	99.4
	120	4000	4800	99.9

Table 8: Assay details of HYDRO and ACY

Drug	Rf	Drug Content (%)	Mean %
HYDRO	0.30	99.8	99.76
		99.6	
		99.89	
ACY	0.73	98.6	99.3
		99.4	
		99.9	

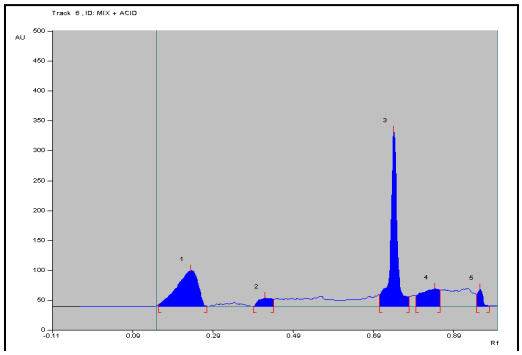

Figure 6: Acid Degradation of Hydro cortisone and Acyclovir with 0.1N HCl

Table 9: Details of acid degradation study

Peak	Rf	%
Std Hydrocortisone (**H1**)	0.30	-
Degradant peak (**H2**)	0.42	13.8
Std Acyclovir (**A1**)	0.72	-
Degradant Peak (**A2**)	0.84	8.4

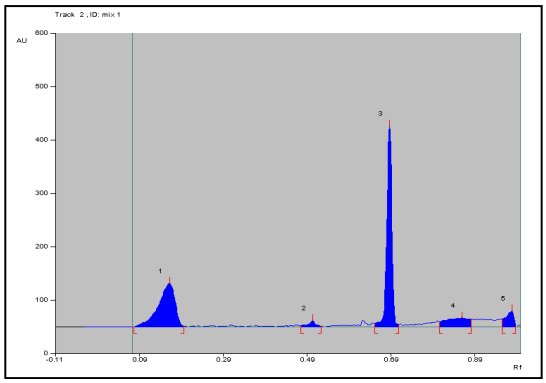

Figure 7: Base Degradation of Hydrocortisone and Acyclovir with 0.1N NaOH

Table 10: Details of base degradation study

Peak	Rf	%
Std Hydrocortisone (**H1**)	0.30	-
Degradant peak (**H2**)	0.42	7.10
Std Acyclovir (**A1**)	0.73	
Degradant Peak (**A2**)	0.85	16.07

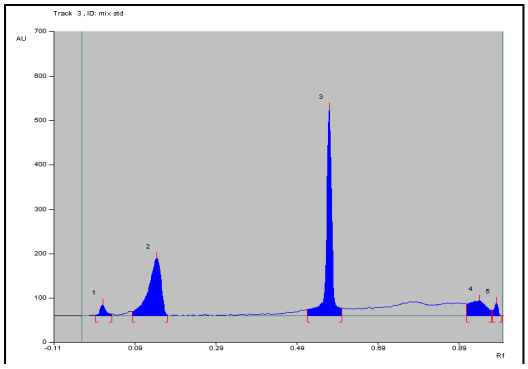

Figure 8: Oxidative degradation of Hydrocortisone and Acyclovir with 3% Hydrogen peroxide

Table 11: Details of oxidative degradation study

S. No.	Peak	Rf	%
1.	Std Hydrocortisone (**H1**)	0.30	-
2.	Degradant peak (**H2**)	0.43	8.8
3.	Std Acyclovir (**A1**)	0.73	-
4.	Degradant Peak (**A2**)	0.84	16.4

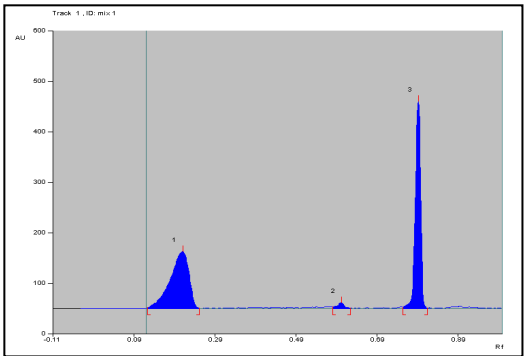

Figure 9: Thermal Degradation of Hydrocortisone and Acyclovir

Table 12: Details of thermal degradation study

S. No.	Peak	Rf	%
1.	Std Hydrocortisone (**H1**)	0.30	-
2.	Degradant peak (**H2**)	0.42	6.06
3.	Std Acyclovir (**A1**)	0.73	-
4.	Degradant Peak(**A2**)	0.85	2.3

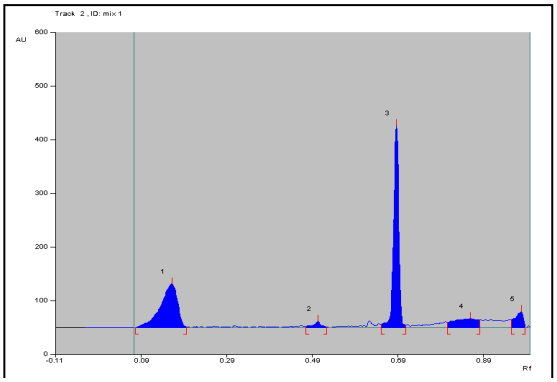

Figure 10: Photolytic Degradation of Hydrocortisone and Acyclovir

Table 13: Details of Photolytic degradation study

S. No.	Peak	Rf	%
1.	Std Hydrocortisone	0.30	-
2.	Degradant peak	0.42	9.6
3.	Std Acyclovir	0.73	
4.	Degradant Peak	0.81	6.8

RESULTS AND DISCUSSION

The separation was achieved by linear ascending development in 10 cm × 10 cm twin trough glass chamber using Toluene: methanol: Glacial acetic acid in the ratio of 6:4:0.05 v/v/v as mobile phase and detection was carried out at 250 nm. The retention factors were found to be 0.30 ± 0.02 and 0.73 ± 0.02 for Hydrocortisone and Acyclovir respectively.

The developed HPTLC method was validated as per ICH guidelines. The developed method found to be linear within the range of 1000-6000 ng/spot with R^2 = 0.9997 and 200-1200 ng/spot with R^2 for Acyclovir and Hydrocortisone respectively. The accuracy of the method was determined at 80%, 100% and 120% level. The percentage recoveries of Acyclovir and Hydrocortisone were found to be 99.7 and 99.3% respectively. The Limit of detection and Limit of quantification of Acyclovir were found to be 240 and 720 ng/spot while Limit of detection and Limit of quantification of Hydrocortisone was 400 and 1220 ng/spot. The developed method was found to be precise as the % RSD values were not more than 2%. The method was found to be robustness as indicated by % RSD values were not more than 2%. The content of Acyclovir and Hydrocortisone in cream has 5 mg of Acyclovir and 1 mg of Hydrocortisone shows no interference from the excipients commonly present in cream.

Forced Degradation study for Acyclovir and Hydrocortisone such as acid degradation shows % degradation at 8.4 and 13.8%, base degradation shows at16.07 and 7.10%, oxidative degradation shows at 16.4 and 8.8%, Thermal degradation shows at 2.3 and 6.06% and photolytic degradation shows at 6.8 and 9.6% degradations.

Densitogram of Acyclovir and Hydrocortisone was shown in Figure 3 whereas densitograms for forced degradation studies were given in Figure 6-10 and results were given in Table 9-13.

CONCLUSION

A simple, specific, precise and accurate HPTLC method has been established as stability indicating method for simultaneous estimation of HYDRO and ACY respectively in API and pharmaceutical dosage form. ICH guidelines were followed throughout the study for method validation and stress testing. Developed HPTLC method effectively separate principle drug peak from degradation product peaks; it can be employed as a stability indicating method.

The intrinsic stability of Acyclovir and Hydrocortisone are established using various ICH recommended stress conditions. The drug Acyclovir was found to be degraded in acid, base, and oxidative conditions, while the drug Hydrocortisone was degraded in acid, alkali, oxidative, neutral and thermal conditions. As the method could effectively separate principle drug peak from degradation product peak, it can be employed as a stability indicating method.

ACKNOWLEDGEMENTS

I am thankful to Bharati Vidyapeeth (Deemed to be university), Poona College of Pharmacy for providing necessary facilities which helped me a lot in successful completion of project.

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Cite this article as:

Pallavi R Chopra *et al*. Stability indicating HPTLC method development for simultaneous estimation of Acyclovir and Hydrocortisone in API and Pharmaceutical dosage form. Int. Res. J. Pharm. 2019;10(10):70-76 http://dx.doi.org/10.7897/2230-8407.1010301

Source of support: Nil, Conflict of interest: None Declared