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A Validated Stability Indicating HPTLC Method for Estimation of Acyclovir in Tablets in Presence of its Alkaline Hydrolysis Degradation Product

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ABSTRACT

A sensitive, selective, precise and stability indicating high performance thin layer chromatographic method of analysis of in bulk drug and in formulations was developed and valiidated in bulk and pharmaceutical dosage form. This method employed TLC aluminum plates precoated with silica gel 60F-254 as stationary phase. The solvent system consisted of Toluene: n-Butanol: Methanol: Water (5.0:3.0:1.0:1.0 v/v/v/v) . This system was found to give compact spots for Acyclovir(retention factor value of 0.28). Densitometric analysis of Acyclovir was carried out in the absorbance mode a 259 nm. The linear regression data for the calibration plots showed good linear relationship with r2=0.997 in the concentration range of 100-500 ng/band. The method was validated for precision, accuracy, robustness and specificity. The limits of detection and limits of quantitation were 08.08 ng/spot and12.16 ng/spot respectively. The drug undergoes Acyclovir in acid, alkaline, neutral oxidation, dry heat, and photo degradation treatment. Statistical analysis proved the method is an economic, reproducible accurate and selective for estimation of ACY. Because the method could effectively separate the drug from its degradation products, it can be employed as a stability indicating method.

Keywords: Acyclovir ,HPTLC, force degradation studies

INTRODUCTION

Acyclovir (Fig no. 1) (9-[(2-hydroxyethoxy) methyl] guanine, zovirax} is a guanosine analogue with anacyclic side chain at the 9 position. it is used orally for the treatment and prophylaxis of initials and recurrent episodes of genital and labial herpes and for the acute treatment of herpes zoster for the treatment varicella (chickenpox) in immunocompetent individuals.[1-3] The chemical name for acyclovir is 2-amino-1,9-dihydro-9- [(2-hydroxyethoxy) methyl]-6H-purine-6-one, or 9- [(2-hydroxyethoxy) methyl]- guanine. Its molecular formula is C8H11N5O3, and molecular weight 225.21 g/mol .[4]Acyclovir is commonly used as the free acid form in solid oral dosage forms, whereas the sodium salt is used in parenteral dosage forms.[5, 6]

Literature survey also revealed HPLC methods have been reported for estimation of Aciclovir in pharmaceutical formulations [7] These methods are based on developed to separate the drug from the degradation products, (HPLC) assay method was developed and validated for quantitative determination of Acyclovir in bulk drugs.[8] These methods are based on developed An isocratic reversed-phase HPLC method with PDA detector has been developed for the assay and purity evaluation of Acyclovir and Valacyclovir in bulk drug. [9]

The objective of the proposed method is to develop simple and accurate method for the estimation of Acyclovir in pharmaceutical dosage forms by HTPLC.

MATERIAL

A Reagents and Chemicals: Toluene: n-Butanol: Methanol: Water (5.0:3.0:1.0:1.0 v/v/v/v) from S. D. Fine chemicals, Mumbai. Reference standard Acyclovir was procured from Cipla Ltd., Kumrek, Rangpo, Sikkim, India) for the gift sample of Acyclovir

B Sample Application: The standard and formulation samples of ACY were spotted on Precoated TLC plates in the form of narrow bands of lengths 6 mm, with 10 mm from the bottom and left margin and with 9 mm distance between two bands

C Selection of Mobile Phase: Aliquot portion of standard stock solutions (5 µL each) were applied on TLC plates in the form of band (band size: 6mm). Different solvents with varying polarity as well as combination of solvent were tried to get well separated bands of the drugs. After trying several permutations and combinations, the solvent system containing Toluene: n-Butanol: Methanol: Water (5.0:3.0:1.0:1.0 v/v/v/v) were found to be most satisfactory as it gave good resolution of both drugs

METHODS

Instrumentation and chromatographic conditions are as below Camag HPTLC system,Sample application Camag linomate IV automatic sample, Scanner Camag TLC scanner, Software Camag wincats software , Camag Twin trough chamber (10x10) and (20x20) Merck HPTLC plate coated with silica Tab 60 F 254(0.2mm thickness) on aluminium sheet Hamilton syringe (100µl)

Selection of wavelength for densitometric evaluation of separated bands: Standard stock solution was applied on TLC plate with the help of CAMAG LINOMAT-V automatic sample applicator, the plate was chromatographed in twin-through glass chamber saturated with mobile phase for 30 minute. After chromatographic development, the plate was removed and air dried. The separated bands on the TLC plate were scanned over the wavelength range of 200-700 nm. The wavelength 254nm was selected for densitometric evaluation of separated bands.

RESULTS

Fig.No 1: Typical Densitogram of ACY,1M NaOH,1 M HCL,H2O2 3%, Dry Heat

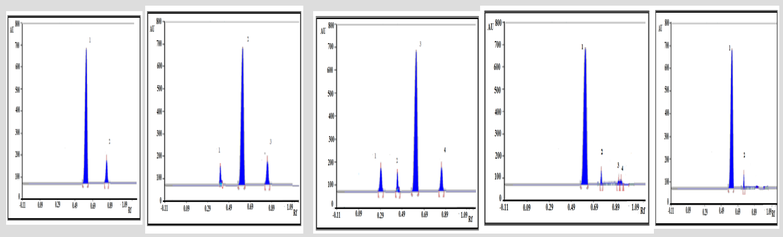

Table no 1:Result of Analysis tablet

Marketed tablet			Average Weight: 100mg
Sr.No.	Weight of Tab taken (mg)	Amount of drug estimated (mg/Tab taken)	% Label Claim
		ACY	ACY
1.	100	100.09	100.90
2.	100	99.98	99.91
3.	100	98.78	98.79
4.	100	99.04	99.06
5.	100	100.00	100.00
6.	100	101.10	101.13

Table No.2: Statistical Valiidation for Analysis of Tablet

Sr.no	Drug	Amount of drug estimated (mg/Tab)*	% Label Claim *	S.D	C.V	S.E
1.	ACY	99.83	99.79	±1.5128	±1.6281	0.5181

Table No. 3: Results of Recovery Studies

Level of recovery	Weight of Tab taken (mg)	Amount of drug added (mg)	Amount of drug recovered (mg)	% Recovery
		ACY	ACY	ACY
80 %	100	80	79.77	99.69
	100	80	80.03	100.03
	100	80	80.11	100.24
100 %	100	100	99.15	99.77
	100	100	99.68	99.78
	100	100	100.11	100.18
120 %	100	120	120.18	100.24
	100	120	120.01	100.04
	100	120	119.78	99.94

Table No.4: Statistical Validation

Level of recovery	%Mean Recovery*	Standard Deviation	% R.S.D.	S.E
	ACY	ACY	ACY	ACY
80 %	99.97	± 0.2523	1.2030	0.4821
100 %	99.95	± 0.1692	1.2012	0.3123
120 %	100.02	± 0.0721	1.2112	0.5155

Table No.5: Results of Degradation Study

Sr. No.	Stress Condition	Percent assay of active substance (ACY)	Rf value of degraded product
1.	Alkali(1M NaOH)	98.65	0.41,0.62 ,0.87
2.	Acid (1 M HCl)	99.83	0.61,0.85
3.	Oxide(3% H_2O_2)	97.76	0.29,0.45 ,0.62,0.87
4.	Heat (60^0C)	99.02	0.61,0.72 ,0.89
5.	UV (240nm)	99.19	0.62,0.73

DISCUSSION

A new HPTLC method has been developed for the identification and quantification of Acyclovir. Low cost, faster speed, and satisfactory precision and accuracy are the main features of this method. Method was successfully validated as per ICH guidelines and statistical analysis proves that method is sensitive, specific, and repeatable. It can be conveniently employed for routine quality control analysis of Acyclovir bulk drug and in marketed formulations without any interference from excipients The low values of %RSD obtained after introducing small deliberate changes in the developed HPTLC method confirmed the robustness of the method and structural elucidation of the degradation product were confirmed by the IR and mass spectral data. The IR spectrum (KBr) of DG was characterized by the absorption frequency of NH2-band as a doublet at 3476.44 cm_1 and –OH band at 3182.71cm^{-1},C-H at 3306 cm-^{1}The mass spectrum of DG was characterized by the appearance of the molecular ion peaks at 227.11 m/z and 267.24 m/z (M & M+2) which confirm the molecular weight of the suggested degradation product. Identified ACY and degradant.

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