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Novel Analytical Design of Ganciclovir

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Abstract

A rapid, simple, precise and accurate high performance thin layer chromatographic method has been developed and validated for the estimation of Ganciclovir in bulk and capsule dosage form. The Ganciclovir was chromatographed on silica gel 60 F_{254} HPTLC plate as a stationary phase. The mobile phase was ethyl acetate: methanol: gla.acetic acid in the volume ratio of 6.0:2:0:2.0 respectively. It gave a dense and compact spot of Ganciclovir with an R_f value of 0.36. The quantitation was carried out at 250 nm. The method was validated in terms of linearity, accuracy, precision and specificity. The statistical analysis proved that the developed method is accurate and reproducible with linearity in the range of 100 to 500 ng/spot. The limit of detection and limit of quantitation for Ganciclovir were 30 and 90 ng/spot respectively. The developed method can be employed for the routine analysis of Ganciclovir in the capsule dosage form.

Keywords: Ganciclovir, HPTLC, Validation, Capsule dosage form.

Introduction

Ganciclovir, chemically known as 2-amino-9- { [(1 , 3 - d i h y d r o x y p r o p a n - 2 - l o x y] m e t h y l } -6,9-dihydro-3H-purin-6-one, is a nucleoside analogue widely used in the treatment of cytomegalovirus infections[1]. Various techniques have been developed for the quantitation of Ganciclovir in pure and pharmaceutical formulations. It is official in the United States Pharmacopoeia[2], which describes an HPLC method for its determination in injections and in oral suspension. The literature is enriched with several methods for the determination of Ganciclovir in pharmaceutical dosage forms including body fluids. The most extensively used technique for the quantitation of Ganciclovir is HPLC; other reported methods include visible spectrophotometry, flow injection luminescence spectrometry and radioimmunology[3-12]. The aim of this study was to develop and validate analytical HPTLC method for sensitive, specific, and rapid determination of Ganciclovir in bulk and capsule dosage form.

Materials and Methods

Ganciclovir was procured as a gift sample from Natco Pharmaceuticals Ltd., Chennai. Silica gel 60F_{254} TLC plates (10×10 cm, layer thickness 0.2 mm, E. Merck, Darmstadt, Germany) were purchased from Merck Ltd India, Mumbai and used as stationary phase. Analytical grade methanol, ethyl acetate and glacial acetic acid were all obtained from Qualigens Fine Chemicals, Mumbai, India. Thin layer chromatography was performed on 10×10 cm aluminium backed TLC plates coated with 250 µm layer of silica gel 60F254 (E. Merck, Darmstadt, Germany). The plates were prewashed by methanol and activated at 105-110° for 15 min prior to use for chromatography. The solutions were spotted at a distance of 1 cm from the edge of the plate by means of a Camag Linomat applicator. The plates were developed for 20 minutes in a camag chamber, previously equilibrated 15 minutes with the mobile phase of ethyl acetate: methanol: gla.acetic acid in the volume ratio of 6.0:2:0:2.0 respectively. After development,

plates were air dried and densitometric scanning was performed at a wavelength of 250 nm with camag scanner controlled by CATS software.

A fresh stock solution of Ganciclovir was prepared in methanol (1000 µg/ml). The standard solutions were prepared by dilution of the stock solution with the methanol to give solution containing 100 µg/ml (100 ng/µl). 1.0, 2.0, 3.0, 4.0 and 5.0 µl/spot were done on the plate to obtain a final concentration range of 100-500 ng/spot.

Results and Discussion

Table 1: Optimized Chromatographic Conditions

S.no	Variables	Values/medium
1	Stationary Phase	Silica gel 60 F254 HPTLC plate
2	Mobile Phase	Ethyl acetate:Methanol:Gl.acetic acid (6:2:2)
3	Densitometry Detection Wavelength	250 nm
4	Correlation Coefficient	0.987*
5	R_f	0.36*
6	Solvent	Methanol

*Average of six determinations

Table II: Method Validation Parameter

S.no	Parameters	Values
1	Linearity	100-500ng/spot
2	LOD	30ng/spot
3	LOQ	60 ng/spot
4	Precision	
	Inter day (%RSD)	0.23
	Intraday (% RSD)	0.33
5	Ruggedness	
	Analyst I	0.42
	Analyst II	0.38
6	Robustness	

	Mobile Phase	034
	Detection Wavelength	028

Table III: % Content Analysis of Formulation

Drug (Brand)	Label Claim(mg)	Amount found	%RSD*
Natclovir	250mg	249.92	0.72

*Average of six determinations

Table IV: % Recovery of Formulation

Brand used	Std.drug added	Theoretical content(ng/spot)	%Recovery*	% RSD
Natclovir (capsule)	0% level	100	99.54	0.24
	50 %level	150	99.44	0.32
	100%level	200	99.62	042
	150%level	250	99.18	0.46

*Average of six determinations

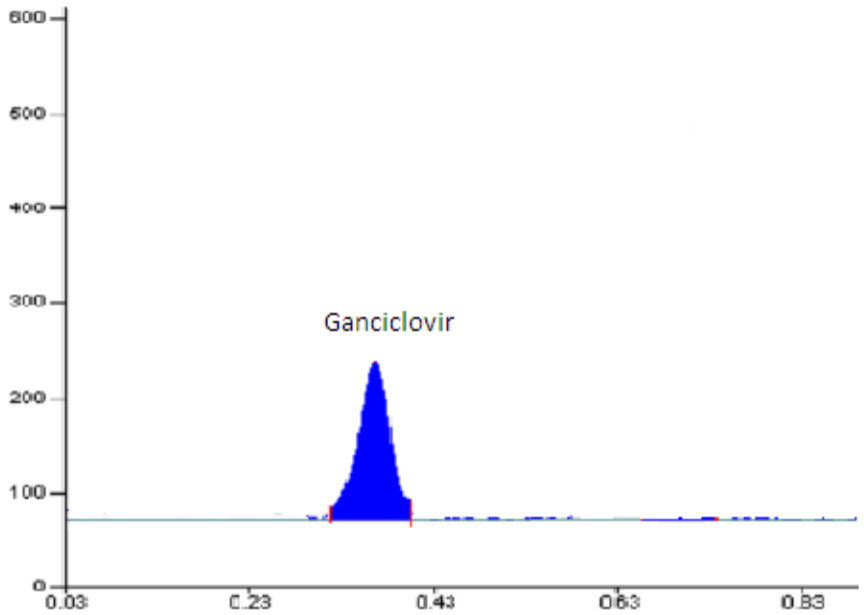

Fig I: Typical Chromatogram of Ganciclovir.

The selectivity confirms that no component other than the analytes contributes to the result. In order to confirm the sensitivity limit of detection (LOD) and limit of quantification (LOQ) were calculated. The specificity was confirmed by observing that the excipients present in the solution do not interfere in the response of analyte.

To confirm the suitability and repeatability, the % RSD of the response such as peak area/ Rf value were calculated and it was less than ±2. The Ruggedness was studied by changing the analyst and observes the %RSD, it was less than ±2. The Robustness was performed with the assistance of changing the chromatographic condition, the corresponding %RSD values are within the limit. The %content and % Recovery were performed on formulation such as Natclovir 250 mg capsule.

Conclusion

The developed HPTLC method combined with densitometric analysis was found suitable for determination of ganciclovir. Statistical data analysis proves that the method is precise and reproducible for the analysis of ganciclovir. The system being economical and can be employed for the routine estimation of the drug in capsule as well as in bulk drug analysis.

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