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Development and Validation of Chromatographic Method for the Determination of Valganciclovir Hydrochloride Form

Dr. Rajendra B. Patil [1], Preeti Shinde [2], Suvarna S. Vanjari [3], Mnjiri Shastri [4], Swati Kshirsagar [5], Anjali Kale [6], Chetan Pulate[7]

[1-6]JSPM's Rajarshi Shahu College of Pharmacy and Reasearch, Tathawade, Pune-33, Maharashtra, India.
[7]Sharadchandra Pawar College of Pharmacy, Otur, Tal. Junnar, Pune-410504, Maharashtra, India

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ABSTRACT
This study was primarily aimed at designing and verifying a High-Performance Thin-Layer Chromatography (HPTLC) technique specifically designed for accurate quantification of Valganciclovir Hydrochloride in both API as well as formulated medical preparations. Chromatographic method utilized silica gel 60 F_{254} plates as the fixed and eluent phase comprising chloroform, methanol, and ammonia in a volumetric ratio of 6.5:34:0.1. A well-defined spot consistently appeared at an Rf value near 0.74, indicating strong specificity of the method. In accordance with ICH guidelines, the developed HPTLC procedure was fully validated for parameters including specificity, linearity, accuracy, precision, sensitivity (LOD and LOQ), and robustness. Quantification was carried out using densitometric detection at 246 nm. Procedure exhibited outstanding proportionality over a range of 200 to 1000 ng per band, with a r of 0.998. Derived regression equation was y = 10.837x + 4270.4. Sensitivity evaluations revealed an LOD of 38.361 ng per band and an LOQ of 116.246 ng per band, confirming the method's high analytical sensitivity. Its validation underscores the technique's efficiency in routine quality control, characterized by its operational simplicity, high precision, accuracy, speed, and robustness. As such, it serves as a dependable tool for the analysis of Valganciclovir Hydrochloride in both its pure and formulated states.

INTRODUCTION:

Valganciclovir Hydrochloride is the hydrochloride salt form of Valganciclovir, a prodrug that converts into ganciclovir in vivo. Ganciclovir, a nucleoside analogue structurally similar to 2′-deoxyguanosine, exhibits broad- spectrum virus-blocking properties, particularly against cytomegalovirus (CMV). Valganciclovir has received FDA approval for treating cytomegalovirus (CMV) retinitis in immunocompromised individuals with AIDS and for preventing CMV infection in organ transplant recipients who receive grafts from CMV-positive donors[1]. Its therapeutic action involves blocking the replication of CMV, effectively limiting the virus's ability to proliferate within the body[2]. Appearing white to off-white crystalline powder as well as is highly water-dissolvable, with a solubility of approximately 70 mg/mL at 25 °C and a pH of 7.0. Valganciclovir Hydrochloride is officially listed in the USP[3]. Study of the available literature indicates showing that a variety of analytical approaches have been employed to estimate Valganciclovir, including High-Performance Liquid Chromatography (HPLC)[4-6], Ultraviolet (UV)[7-8] spectroscopy, and Liquid Chromatography–Mass Spectrometry (LC-MS)[9-10]—have been effectively used for its quantification in pharmaceutical formulations. However, only one HPTLC-based method using chloroform in the mobile phase has been reported. Given the environmental and health concerns associated with chloroform, significant crucial to design an alternative HPTLC method that minimizes or eliminates the use of such hazardous

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solvents[11]. The present research addresses this gap by proposing a modified, validated HPTLC approach for the analysis of Valganciclovir Hydrochloride.

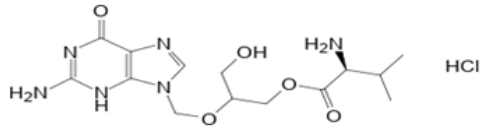

Fig no.1: Valganciclovir Hydrochloride

MATERIALS AND METHODS:

Chemicals & Reagent:

Valganciclovir Hydrochloride were acquired from Sujalam Chemicals, Hadapsar, Pune. and Reagents like MeOH (HPLC grade) were secured from Mumbai, India's Merck Life Science Pvt. Ltd. Silica gel 60 F_{254} precoated TLC plates (Merck) were used for chromatographic separation. All glassware used in the experiment was properly calibrated prior to use. Dichloromethane, methanol, and ammonia were procured from the laboratory Belonging to JSPM's Rajarshi Shahu College of Pharmacy and Research, Tathawade.

Instruments:

HPTLC System : CAMAG HPTLC applicator system and CAMAG densitometer
Data processing tool: winCATS 1.4.2
UV-Vi's spectrophotometer: Jasco Model V-730
Balance: Mettler Toledo AB54S
Separation Parameters:
Adsorbent layer: Merck TLC plate (silica gel60 f254)
Syringe: The Hamilton microliter syringe
Application: Bands of 6 mm **Development Distance**:80mm
Preparation of Reagents:

Base and Final analytical solution*:*

A precisely measured 10 mg quantity of Valganciclovir Hydrochloride was placed in 10 mL volumetric container. Initially diluted using a small amount of methanol, and the solution was subsequently diluted the final volume with methanol, yielding primary standard mixture of 1000 µg/mL. Subsequently, 1 mL of this mixture was transported into a separate 10 mL volumetric flask and further mixed with methanol creating a working solution of 100 µg/mL. Calibration samples were then formulated by serially diluting the working solution with methanol to produce final concentrations ranging from 200 to 1200 ng per band.

Formulation of Sample Solution:

Twenty Valganciclovir Hydrochloride tablets (Valgan, Cipla Ltd; 450 mg each) were weighed, powdered, and a portion equivalent to 10 mg of the drug was transported to a 10 mL volumetric flask. Diluted in methanol, sonicated for 10 minutes, and diluted to volume to get a 1000 µg/mL stock mixture. After filtration via Whatman No. 41, 1 mL of the filtrate was mixed to 10 mL with methanol to prepare a 100 µg/mL working solution. From this, 4 µL was used for the TLC plate to achieve 400 ng/band.

Wavelength Selection for Analysis:

Additional concentrations derived from the primary solution were formulated using methanol and analyzed within the wavelength span of 200 to 400 nanometers to obtain the spectral profile.

Selection of mobile phase and Chromatographic Parameters:

Samples were applied as 6 mm-wide strips, spaced 8 mm apart, using a 100 µL microsyringe onto aluminum- backed silica gel plates (60 F254, dimensions 10 × 10 cm, Thickness: 250 µm). The detection utilized a slit size of 5 mm × 0.45 mm and a scanning rate of 20 mm/sec. Chromatographic separation followed an upward linear technique within a 10 × 10 cm dual-trough chamber, using a designated solvent system. A 15-minute saturation interval was allotted for chamber equilibration with the mobile phase. The chromatographic migration spanned 8 cm, completed over a 15-minute timeframe. Plates were air-dried post-development. Densitometric evaluation was executed using a CAMAG TLC scanner set at 246 nm, controlled via winCATS software for all analyses.

Method Validation:

Linearity: To evaluate the linear response between analyte concentration and signal intensity, six varied levels of Valganciclovir Hydrochloride were examined. A working standard of 100 µg/mL was used to dispense precise volumes onto TLC plates. Each concentration level was spotted in quintuplicate. The detected peak responses were charted against their respective concentrations to generate a calibration graph and determine the linear behavior of the analytical method.

Range: The method's working range defines the interval from the minimum to the maximum concentration at which the substance can be consistently quantified with satisfactory precision, accuracy, and a linear response.

Specificity: Specificity denotes the capability of the procedure to distinctly detect and quantify the target compound amidst other interfering agents like excipients, matrix elements, possible contaminants are included. This characteristic was

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examined via a peak purity analysis. Comparative spectral evaluations of both the reference and test samples of Valganciclovir Hydrochloride revealed no overlapping signals, confirming the method's strong specificity.

Precision: Evaluation was analyzed using examining both Day-to-day and hourly fluctuations. The intra-day study, three distinct concentration levels were tested three times within a single date, as well as the %RSD was computed. Inter-day precision was determined at consistent concentration levels were analyzed in triplicate over 3 successive dates, and resulting %RSD values were used to evaluate the consistency and reliability of the method over time.

Accuracy: For assessing the analytical accuracy, yield experiments involved the addition of known quantities of the pure drug to previously analyzed tablet powder at levels corresponding to 50%, 100%, and 150% of the nominal concentration. Average % of drug recovered was determined using the linear regression equation, with 400 ng per band serving as the reference concentration.

Robustness: Robustness was evaluated by intentionally applying small changes to experimental parameters, including slight adjustments in scanning wavelength and the timing between sample application, chromatographic development, and detection. The impact of these modifications on the peak area was analyzed to verify the method's consistency and dependability under mildly fluctuating conditions.

Assay: The content analysis of Valgan® tablets (450 mg) was carried out following the established sample preparation protocol. To ensure reproducibility, the procedure was performed six times. Once the test solution was transferred to the TLC plate, the corresponding peak area was recorded. A dose of 400 ng per band was applied for the assay evaluation.

RESULT AND DISCUSION:

Selection of Wavelength: A prominent absorbance peak was observed at 246 nm, confirming the drug's optimal detection wavelength.

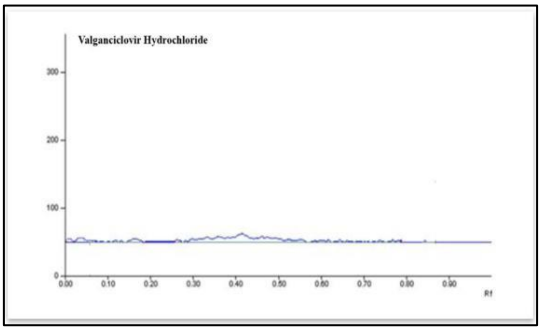

Fig no.2: UV Spectrum of Valganciclovir hydrochloride (10µg/ml)

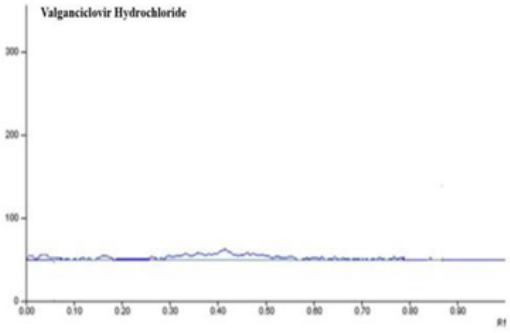

Fig no.3: Densitogram of blank (Methan

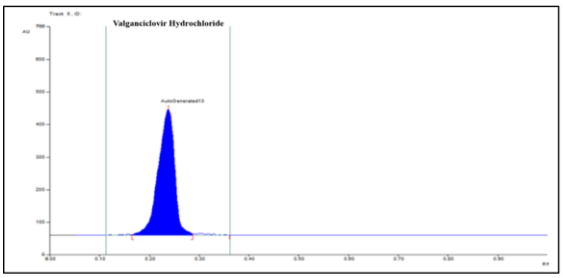

Fig.4: Densitogram of standard solution of Valganciclovir hydrochloride

Specificity: Evaluates the method's capability to precisely quantify the target compound despite the presence of excipients, matrix substances, and possible interfering agents. Peak purity values greater than 0.996 confirmed the absence of overlapping signals at the analyte's retention point.

Linearity: The method's linearity was established from 200 up to 1000 ng per band. A consistent linear relationship was observed across this range.

Table no.1: Linearity Study of Valganciclovir Hydrochlorride

Replicate	Concentrations of Valganciclovir hydrochloride(ng/band)				
	200	**400**	**600**	**800**	**1000**
1	5749.2	8871.6	11008.5	13079.3	14222.9
2	5525	9204.9	11028.5	13039.9	14226
3	5574.7	9008.8	11418.1	12718.6	13950.1
4	5776.1	8936.3	11216.6	13053.6	14130.7
5	5722.2	8799.1	11258	13017.4	14548.3
Average	5669.44	8964.14	11185.94	12981.76	14215.6
SD	112.205	155.352	170.516	148.811	217.111
% RSD	1.979	1.733	1.524	1.146	1.527

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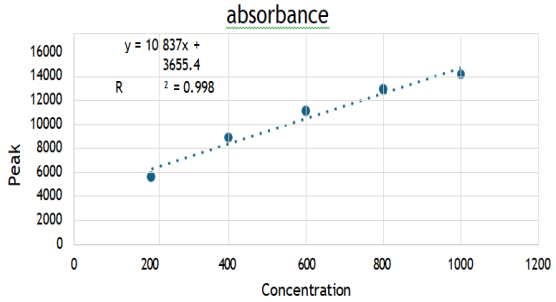

Fig no.4: Linearity of Valganciclovir Hydrochloride

Precision: The intraday and intraday precision study was performed for valganciclovir hydrochloride results are shown below table. % RSD value is less than 2, hence method is precise.

Table no.2: Precision

Concentration (µg/ml)	Intra-day Precision			Inter-day Precision		
	Average area	%Recovery	Mean ±%	Average area	% Recovery	Mean ± % RSD
400	8549.1	98.926	99.538 ±1.381	8619.1	100.846	99.449 ±1.229
	8536.3	98.575		8536.3	98.575	
	8628.8	101.112		8549.1	98.926	
600	10408.5	99.941	100.069±0.967	10471.6	101.094	100.808 ±1.122
	10366.4	99.171		10387.8	99.562	
	10471.6	101.094		10508.5	101.769	
800	12279.3	100.604	100.598	12318.6	101.143	100.715 ±1.173
	12339.9	101.435		12353.6	101.623	
	12217.4	99.756		12189.9	99.379	

LOD and LOQ Determination: LOD for Valganciclovir Hydrochloride was calculated as 29.839 ng per band, while the LOQ was found to be 90.421 ng per band.

Assay: Analysis of Valgan tablets containing 450 mg of valganciclovir hydrochloride was conducted in line with the stated procedure in the Preparation of Sample Solution section. Assay was performed in 6 replicates to ensure accuracy and reproducibility. The chromatographic response was recorded after application of the sample solution. A concentration of 400 ng/band, derived from the tablet formulation, was selected for the assay.

Table no.3: Assay

Sr. No.	Peak Area	Amount Recovered (µg/ml)	% Recovery	Mean ± %RSD
1	8619	403.372	100.843	99.814 ±0.995
2	8535.1	394.169	98.542	
3	8549.5	395.749	98.937	
4	8575.7	398.622	99.656	
5	8584	399.533	99.883	
6	8625.5	404.084	101.021	

Table no.4: Accuracy Recovery studies of Valganciclovir hydrochloride

Level	Amount of sample taken (ng/band)	Amount standard spiked (ng/band)	Area	% Recovery	Mean ±% RSD
50 %	400	200	10346.1	98.800	100.172 ±1.196
			10466.8	101.007	
			10450.6	100.710	
100 %	400	400	12307.6	100.992	99.779 ±1.325
			12116.4	98.371	
			12233.4	99.975	
150 %	400	600	14046.4	99.865	99.222 ±0.660
			13989.9	99.245	
			13927	98.556	

Robustness: The method's robustness was assessed by intentionally applying slight changes to analytical parameters, including scanning wavelength, the interval between sample application and development, and the time between development and scanning. The findings showed that these minor adjustments had no significant impact on the results, confirming the method's reliability and stability under varied conditions.

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Table no.5: Robustness

Sr. No.	Parameters	Variation	Concentration (ng/band)	%RSD
1.	Variation in Scanning wavelength	246± 1 nm	400	1.391-1.909
			800	0.427-0.695
			1200	1.624-1.993
2.	Time from application to development	(0, 30, 60 min.)	400	1.235-1.951
			800	0.427-1.183
			1200	1.993-2.009
3.	Time from development to scanning	(0, 30, 60 min.)	400	1.391-1.774
			800	0.427-0.937
			1200	1.369-1.993

Table no.6: Summary of validation study

Sr. No.	Parameter	Valganciclovir hydrochloride
1	Linearity	y = 10.837x + 3655.4; R2= 0.998
2	Range	200 – 1200 ng / band
3	Precision	%RSD
	Intraday	0.835 – 1.381
	Inter day	1.122 – 1.229
4	% Assay	99.814 ±0.995
5	Accuracy	% Recovery
	50%	100.172
	100%	99.779
	150%	99.222
6	LOD	38.361ng / band
7	LOQ	116.246ng /band
8	Specificity	Specific
9	Robustness	Robust

CONCLUSION:

The newly established HPTLC approach to estimate Valganciclovir Hydrochloride has proven to be dependable, accurate, and effective. It met all validation parameters—specificity, linearity, accuracy, precision, robustness, and sensitivity—in full alignment with ICH Q2(R1) guidelines. Owing to its rapid execution and cost-effectiveness, this method is particularly well-suited for routine quality control of pharmaceutical formulations. It's simple yet highly reproducible protocol offers strong potential for broad application in both industrial quality assurance and research environments. Additionally, its consistent recovery rates and low relative standard deviations further support its suitability for use in regularly evaluating the content of Valganciclovir Hydrochloride in tablet and bulk pharmaceutical formulations.

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