International Journal of Advanced Chemistry, 8 (2) (2020) 203-208

International Journal of Advanced Chemistry

Website: www.sciencepubco.com/index.php/IJAC

Research paper

Development and validation of valganciclovir hydrochloride in bulk and pharmaceutical dosage form by HPTLC method

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Abstract

A simple, rapid and accurate High performance thin layer chromatography is described for the Development and validation of HPTLC method for Valganciclovir Hydrochloride in bulk and Pharmaceutical dosage form. The separation is carried out on Merck TLC aluminum sheets of silica gel 60 F254 using Chloroform: Methanol: Ammonia (6.5:3.4:0.1v/v) mobile phase. Quantification was done by Densitometric scanning at 254nm. The linearity was found to be the range of 100-500ng/spot for Valganciclovir hydrochloride with the correlation coefficient of 0.9993. The regression equation was found to be Y=10.168x-94.8. The Rf value of Valganciclovir hydrochloride was found to be 0.74. The LOD and LOQ were found to 9.19 and 27.87 respectively. Average recovery was found to be 99.66% which show that the method was free from interference from excipients present in the formulation. Simultaneously the Percentage relative standard deviation was well within the range of 2%. The above method was validated according to the ICH guidelines. The established method enabled accurate, precise and applied to the analysis of Valganciclovir hydrochloride in bulk and Pharmaceutical dosage form.

Keywords: *Valganciclovir Hydrochloride; HPTLC; Validation; ICH; Tablet Dosage Form.*

1. Introduction

Valganciclovir hydrochloride is a hydrochloride salt form of Valganciclovir, a prodrug form of ganciclovir, a nucleoside analogue of 2'-deoxyguanosine, with antiviral activity. The completion of phosphorylation, Valganciclovir is incorporated into DNA, resulting in the inhibition of viral DNA polymerase, and viral replication. Valganciclovir hydrochloride is an antiviral agent that is used to treat cytomegalovirus retinitis in patients with AIDS, and for the prevention of cytomegalovirus infections in organ transplant recipients who have received an organ from a CMV-positive donor. The Valganciclovir acts by slowing the growth of the CMV virus. It helps prevent the spread of infection to other areas of the body. [2]

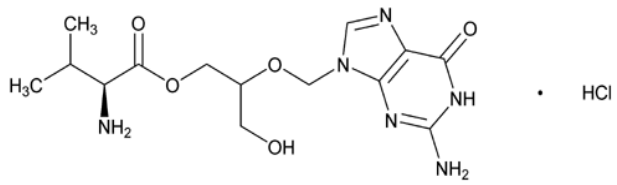

Fig. 1: Chemical Structure of Valganciclovir Hydrochloride.

Valganciclovir hydrochloride contains not less than 97% and not more than 102%. Hydrochloride is calculated based on the anhydrous and solvent free basis. Valganciclovir hydrochloride is available in the form of White to off crystalline powder. The crystals from water+isopropranol undergo phase changes at 142˚C. It is freely soluble in Water, Dimethyl sulfoxide, Methanol, Acetonitrile and Acetic acid. Valganciclovir hydrochloride is available under the brand name of Valcyte, Cymeral, Rovalcyte and Darilin. [3-6].
Literature Survey revealed that the drug has been estimated by UV-Spectrophotomtric [11-13], RP-HPLC [14-17], HPTLC [18] and Liquid chromatographic method [19-21] has been reported so far.
The present study describes a simple, precise and accurate analytical method for the estimation of Valganciclovir hydrochloride in bulk and pharmaceutical dosage forms. The above method was developed and validated according to the ICH guidelines.

2. Experimental

2.1. Chemicals

The Valganciclovir hydrochloride was obtained as a gift sample from the pharmaceutical industry. All the chemicals used were of under analytical grade. The tablet formulation was procured from local pharmacy store. All dilutions were performed in standard volumetric flasks. Chloroform, Methanol, Ammonia were obtained Bharthi College of pharmacy, Bharathinagara, KM Doddi, Maddur Taluk, Mandya District, India.

2.2. Instrumentation

The samples were spotted in the form of bands of 6 mm width with a Camag microliter syringe (100/µl) on pre-coated silica gel aluminum plates 60 F-254 (10 cm × 10 cm with 250 mm thickness, E. Merck, Wilmington, USA) using a Camag Linomat-5 applicator. The plates were pre-washed with methanol and activated at 100°C for 5 min prior to chromatography. The slit dimension was kept at 6.00 mm × 0.45 mm (micro) and 20 mm/s scanning speed was employed. The mobile phase consisted of Chloroform: Methan0l: Ammonia (6.5:3.4:0.1v/v) and 10ml of mobile phase was used. Linear ascending development was carried out in a 10 cm × 10 cm twin trough glass chamber (Camag, Switzerland) saturated with the mobile phase. The chamber saturation time for the mobile phase was 25 min at room temperature (25°C ± 2). The length of the chromatogram run was around 8 cm. Then the thin layer chromatography plates were dried in a current of air with the help of an air dryer. Densitometric scanning was done on a Camag TLC scanner 3 and which was operated by winCATS software.

2.3. Stock and working standard solution

Valganciclovir hydrochloride (100 mg) was accurately weighed into a 100 mL volumetric flask and dissolved in a minimum volume of methanol and diluted to the required volume with methanol to furnish a solution of concentration 1000ng/µL. This was used as stock solution. Calibration standards were prepared over the concentration range 100-500ng/band for Valganciclovir hydrochloride by appropriate dilutions of the above-mentioned standard stock solution in a 10mL volumetric flask with methanol.

2.4. Calibration curve

Separate stock standard solutions of Valganciclovir hydrochloride were used for the preparation of calibration standard solutions. All calibration standards were prepared freshly every day and were found to be stable during the analysis time. The plate was developed, dried and scanned as described above. After densitometric scanning, the peak area was recorded for each concentration and a calibration plot was obtained by plotting average peak area against concentration of Valganciclovir hydrochloride (ng/spot). The slope and correlation coefficient were also determined.

2.5. Preparation of sample solution

Brand name: VALCYTE 450mg.
Manufacturer: Roche pharmaceuticals India.
Batch number: VL 30722.
Composition: Each tablet contains 496.3mg Valganciclovir HCL and other inactive ingredients microcrystalline cellulose, povidone K-30, crospovidone and stearic acid.
The film coat applied to the tablets contains Opadry pink.
The marketed tablet formulation of VALCYTE 450mg was purchased in the local Pharmacy store. Twenty tablets of VALCYTE 450mg were accurately weighed and ground to fine powder equivalent to Valganciclovir hydrochloride (100 mg) was accurately weighed into a 100 mL volumetric flask and dissolved in a minimum volume of methanol and diluted to the required volume with methanol to furnish a solution of concentration 1000ng/µL. This was used as stock solution.

3. Method validation

Validation of the above HPTLC method was carried out with respect to the following parameters:

3.1. Precision

Repeatability of sample application and measurement of peak area were carried out using six replicates of the same band (400ng/spot of Valganciclovir Hydrochloride). The intra-and inter-day variation for the determination of Valganciclovir Hydrochloride was carried out at three different concentration levels of 300, 400 and 500ng/spot.

3.2. Limit of detection and limit of quantification

In order to determine the limit of detection (LOD) and limit of quantification (LOQ), Valganciclovir hydrochloride concentrations in the lower part of the linear range of the calibration curve were used. Valganciclovir hydrochloride solutions of 100-500 ng/ band were prepared and applied on the plate. The LOD and LOQ were calculated using the equation LOD = 3.3 × N/B and LOQ = 10 × N/B, where, N is the standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and B is the slope of the corresponding calibration curve.

3.3. Specificity

The specificity of the method was determined by acomparing the standard drug and sample. The spot for Valganciclovir hydrochloride in the sample was confirmed by comparing the R values and spectra of the spot with that of the standard. The peak purity of Valganciclovir

Hydrochloride was determined by comparing the spectra at three different levels, i.e., peak start (S), peak apex (M) and peak end (E) positions of the spot.

3.4. Ruggedness

Ruggedness of the method was performed by spotting 100-500ng/band of Valganciclovir Hydrochloride by three different analysts keeping the same experimental and environmental conditions.

3.5. Accuracy

The samples were spotted with extra 50, 100 and 150% of standard Valganciclovir Hydrochloride, and the mixture was analyzed by the proposed method. This was done to determine the recovery of the drug in the formulations.

3.6. Robustness

By making small & deliberate changes in the environment and the mobile phase composition, the effects on the results were examined. The Mobile phases having different compositions of Chloroform: Methanol: Ammonia (6.5:3.4:0.1v/v) was tried and chromatograms were run. The volumes of mobile phase, temperature and relative humidity were varied in the range of ± 5%. The plates were pre-washed by methanol and activated at 80 ± 10°C for 2, 5 and 7 min prior to chromatography. The spotting Time to chromatography and from chromatography to scanning was varied from 10, 15 and 20 min.

3.7. Application of the proposed method to the tablet formulation

To determine the concentration of Valganciclovir Hydrochloride in tablets. Twenty tablets of VALCYTE 450mg were accurately weighed and ground to fine powder equivalent to 100 mg of Valganciclovir was transferred into a 100ml volumetric flask. The standard drug from the powder was extracted using methanol. The resulting solution was filtered using a 0.45 µm filter (Mill filter, Milford, MA, USA). The above solution (300ng/band) was applied on a TLC plate, followed by development and scanning, as described above.

4. Results and discussion

4.1. Development of optimum mobile phase

The TLC procedure was optimized with a view to developing a stability-indicating assay method. Initially, Chloroform: methanol (5:5v/v) gave good resolution but high Rf value of 0.95 for Valganciclovir hydrochloride, to reduce the Rf value then the mobile phase was optimized to Chloroform: Methanol: Ammonia (6.5:3.4:0.1v/v) Finally, the mobile phase consisting of gave a sharp and well-defined peak at Rf value of 0.74. The spots were obtained when the chamber was saturated with the mobile phase for 15 min at room temperature.

4.2. Calibration curve

The linear regression data for the calibration curves showed a good linear relationship over the concentration range 100-500ng/band. The results were depicted in (table 1).3D view (Fig.2) Linear regression equation was found to be Y= 10.168x + 94.8 and R^2 = 0.9993. The calibration curve was shown in the (Fig.3).

Table 1: Linearity

SI.NO	CONCENTRATION	PEAK AREA
1	100ng/spot	1076
2	200ng/spot	2143
3	300ng/spot	3169
4	400ng/spot	4213
5	500ng/spot	5125

*Mean of six estimations.

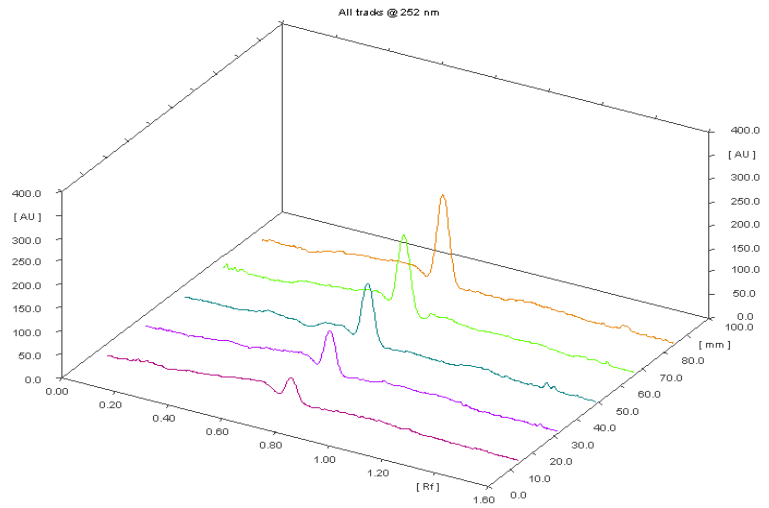

Fig. 2: 3D-Chromatogram Show Peaks of Valganciclovir in Different Concentrations at 254 Nm.

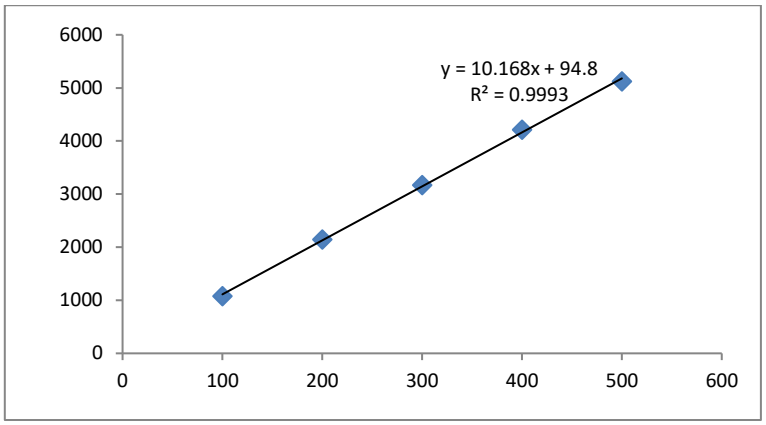

Fig. 3: Calibration Graph of Valganciclovir Hydrochloride.

4.3. Validation of the method

The summary of validation parameters is shown in the (Table 2).

Table 2: Summary of Validation Parameters

S.NO	PARAMETER	RESULTS
1	Linearity	100-500ng/spot
2	Regression equation	Y= 10.168x + 94.8
3	Slope	10.168
4	Intercept	94.8
5	Correlation coefficient(R^2)	R^2 = 0.9993
6	Limit of detection	9.19
7	Limit of quantification	27.87
8	Specificity	Specific
9	Recovery (n=6)	99.66

4.4. Precision

The precision of the above method was expressed in terms of %relative standard deviation (%RSD). The results depicted in (Table 3).

Table 3: Precision

	INTRA-DAY STUDIES		INTER-DAY STUDIES	
SI.NO	Concentration (ng / spot)	AREA	Concentration (ng/spot)	AREA
1	400	4213	400	4213
2	400	4089	400	4139
3	400	4156	400	4259
4	400	4156	400	4332
5	400	4243	400	4246
6	400	4223	400	4254
AVG		4180	AVG	4240.5
STDEV		57.1664	STDEV	63.2226
%RSD		1.37%	%RSD	1.49%

4.5. LOD and LOQ

Detection limit and quantification limit was calculated by the method described above. The LOQ and LOD were found to be 9.19 and 27.87 respectively. This indicated adequate sensitivity of the method.

4.6. Specificity

The peak purity of Valganciclovir Hydrochloride was assessed by comparing the spectra at peak start, peak apex and peak end positions of the spot, i.e., r (S, M) = 0.999 and r (M, E) = 0.999. Good correlation (r^2 = 0.9993) was also obtained between standard and sample spectra of Valganciclovir Hydrochloride.

4.7. Ruggedness

When the method was performed by two different analysts under the same experimental and environmental conditions, it was found to be rugged (Table 4).

Table 4: Ruggedness

Analysts	Mean area* ± Standard deviation	%RSD
Analyst 1	4217±64.264	1.52%
Analyst 2	4223±36.9143	0.87%

*indicates average of six determinations.

4.8. Recovery study

The proposed method when used for extraction and estimation of Valganciclovir Hydrochloride from the pharmaceutical dosage form after overspotting with 50, 100 and 150% of additional drug, affording good recovery of Valganciclovir Hydrochloride. The % recovery is listed in (Table 5).

Table 5: Recovery Studies

Tablet	Spiked levels	Amount of sample (ng/spot)	Amount of standard (ng/spot)	Amount recovered	%Recovery ±SD**	%RSD
VALCYTE 450mg	50	300	150	447.02	99.66±1.035	1.03
	100	300	300	597.07	99.51±0.32	0.335
	150	300	450	752.39	99.74±0.74	0.75

4.9. Robustness of the method

The standard deviation of peak areas was calculated for each parameter, and the %RSD was found to be less than 2% The low values of %RSD (Table 6) indicated that the method was robust.

Table 6: Results of Robustness for Valganciclovir Hydrochloride

Parameters	%RSD
Mobile phase volume(±2ml)	1.52
Development distance (±0.5cm)	1.80
Duration of saturation (±10min)	0.76
Time from spotting to chromatography(±10min)	0.33
Time from chromatography to Scanning(±10min)	0.12

*Mean of three estimations.

4.10. Analysis of the marketed formulation

A single spot at Rf 0.49 was observed in the chromatogram of the drug samples extracted from the tablets. There was no interference from the excipients. The %drug content and %RSD were calculated. The low %RSD value indicated the suitability of this method for the routine analysis of Valganciclovir Hydrochloride in pharmaceutical dosage forms (Fig. 5).

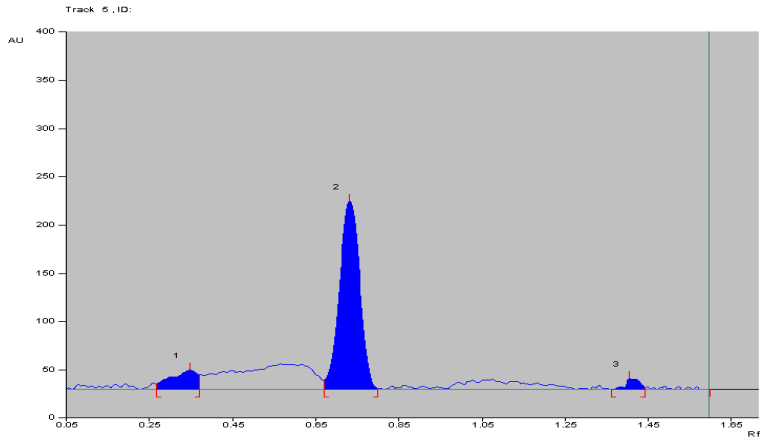

Fig. 5: HPTLC Chromatogram of Valganciclovir (TABLET) with Corresponding Rf Value at 254 Nm.

5. Conclusion

This HPTLC method is simple, precise, specific and accurate. Statistical analysis proved that the method is reproducible and selective for the analysis of Valganciclovir Hydrochloride as the bulk drug and in tablet formulations

Acknowledgment

We like thanks to Management, Principal, Teaching staff, non-teaching staff and my dear Friends of Bharathi College of pharmacy for their continues co-operation and support.

Abbreviations

HPTLC: High performance thin layer chromatography.
ICH: International council for Harmonization.
LOD: Limit of detection.
LOQ: Limit of quantitation.
RSD: Relative standard deviation.

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