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HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHIC METHOD FOR QUANTITATIVE ESTIMATION OF VALACYCLOVIR HYDROCHLORIDE IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, sensitive, precise and specific high-performance thin-layer chromatographic (HPTLC) method for the determination of valacyclovir both in bulk drug and pharmaceutical dosage form was developed and validated. The method employed aluminium plates precoated with silica gel G60 F254 as the stationary phase. The solvent system consisted of n-butanol: methanol:Water in the proportion of 3:1:1, v/v/v. This solvent system was found to give compact spots for valacyclovir with *Rf* value 0.22 ± 0.01. Densitometric analysis of valacyclovir was carried out in the absorbance mode at 254 nm. Linear regression analysis showed good linearity (r^2 =0.998) with respect to peak area in the concentration range of 100–700 ng/spot. The method was validated for precision, limit of detection (LOD), limit of quantitation (LOQ), accuracy and specificity. Statistical analysis proved that the method is repeatable and specific for the estimation of the said drug.

KEYWORDS: Valacyclovir, HPTLC, Method Development, ICH guidelines.

1. INTRODUCTION

Valaciclovir hydrochloride [(S)-2-[(2-amino-6-oxo-6,9-dihydro-3H-purin-9-yl)methoxy] ethyl-2-amino-3-methylbutanoate] (Figure 1) is a hydrochloride salt of L-Valyl ester of acyclovir.[1-3] It is an oral antiviral drug used to treat infections with herpes zoster (shingles), herpes simplex genitalis (genital herpes), and herpes labialis (cold sores). It inhibits the

replication of viral DNA. It is a prodrug intended to increase the bioavailability of acyclovir by increasing lipophilicity. Valacyclovir is converted by esterase to active drug acyclovir via hepatic first pass metabolism3.

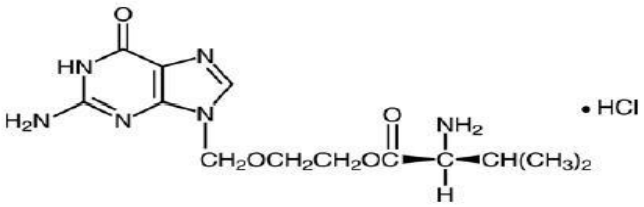

Figure 1: Structure of valacyclovir hydrochloride

Literature survey revealed that few spectrophotometrc methods[4-11], HPLC methods[12-18], and LC-MS methods for biological fluids[19-23] are reported in the literature for the determination of valacyclovir in Bulk, pharmaceutical formulations and serum samples. To our knowledge, no article related to the high-performance thin-layer chromatographic (HPTLC) determination of valacyclovir in pharmaceutical dosage forms has been reported in the literature. The aim of the present work was to develop an economic, precise, accurate, specific, HPTLC method using densitometric detection for the determination of valacyclovir in bulk and pharmaceutical dosage form.

2. EXPERIMENTAL

2.1. Materials

The standard drug Valaciclovir hydrochloride was supplied as a gift sample by GlaxoSmithKline Ltd, Nashik, India, Valcivir® tablets 500 mg (Cipla Pharmaceutical Ltd., Goa, India) were used for the assay. All chemicals and reagents used were of analytical grade (Merck Chemicals, Mumbai, India).

2.2. HPTLC Instrumentation and Chromatographic Conditions

The HPTLC plates were prewashed with methanol and activated at 110℃ for 5 min prior to chromatography. The samples were spotted in the form of bands 8 mm width with a Camag 100 microlitre sample syringe (Hamilton, Bonaduz, Switzerland) on silica gel precoated HPTLC aluminum plate G60 F254, [(20 ×10cm) with 250μm thickness; E. Merck, Darmstadt, Germany, supplied by Anchrom Technologists, Mumbai] using a Camag Linomat V applicator (Switzer-land). A constant application rate of 0.2 μL/s was used and the space between two bands was 16 mm. Linear ascending development was carried out in 20 cm×10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile

phase. The mobile phase was consisted of n-butanol: methanol:Water (3:1:1, v/v/v) and 20 mL were used per chromatography run. The optimized chamber saturation time for mobile phase was 10 min using saturation pads at room temperature. The length of chromatogram run was 8 cm. Densitometric scanning was performed using a CAMAG TLC operated by CATS software (V 3.15, Camag). The slit dimension was kept at 6 mm×0.45 mm and the scanning speed was 100 nm/s. The source of radiation used was a deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm. All determinations were performed at detection wavelength of 254 nm.

2.3. Preparation of Standard Solution

Accurately weighed valacyclovir hydrochloride (equivalent to 100 mg of valacyclovir) was transferred to a 100 mL volumetric flask and dissolved in and diluted up to the mark with methanol to obtain a standard solution of valacyclovir (1000 μg/mL). The aliquot of 10 mL from this solution was diluted to 100 mL with methanol to obtain working standard solution of 100 µg/mL.

2.4. Method Validation

The HPTLC method was validated as per the ICH guidelines[24-26].

2.4.1. Linearity

The standard solution was spotted on the HPTLC plate (1µL to 7 µL) to obtain the spots in the concentration range of 100–700 ng/spot. Each concentration was spotted six times on the HPTLC plate. The plate was developed using the previously described mobile phase and scanned. The peak areas were plotted against the corresponding concentrations to obtain the calibration graph. Linear calibration curve was generated using least-squares linear-regression analysis.

2.4.2. Precision

Precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analyses of (500 ng/spot) of the drug in hexaplicate on the same day. The %RSD of six determinations was calculated. Intermediate precision of the method was checked by repeating studies on two different days. The %RSD of twelve determinations was calculated.

2.4.3. Limit of Detection and Limit of Quantitation

The sensitivity of the method was determined in terms of limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ were calculated by using the formula, LOD = 3.3 × σ/S and LOQ = 10 × σ/S, where σ is residual standard deviation of regression line and S is slope of corresponding regression line.

2.4.4. Accuracy

Accuracy of the method was determined by standard addition method in which the known amount of standard valacyclovir solutions were added to pre-analyzed sample solution. These amounts corresponded to 80, 100, and 120 % of the sample concentration. The amount of valacyclovir was estimated by comparing the peak area of sample with that of standard. Accuracy study was performed in triplicate, and % recovery of valacyclovir was calculated.

2.4.5. Specificity

Specificity of the method was determined by comparing the chromatogram of sample with the chromatograms of standard.

2.4.6. Solution Stability

The stability of standard solutions was tested after 1, 6 and 24 h of storage. The stability of the solutions was determined by comparing peak area with that of freshly prepared standard and peak purity at 500 ng/spot.

2.5. Analysis of Marketed Pharmaceutical Dosage Form.

To determine the content of valacyclovir in marketed pharmaceutical dosage form, twenty tablets were accurately weighed, their average weight was determined and they were finely powdered. The powder equivalent to 100 mg of valacyclovir was weighed and transferred into a 100 mL volumetric flask containing 50 mL methanol, sonicated for 15 minute, and diluted to 100 mL with methanol. The above solution was filtered through the whatmann no. 41 filter paper. From this solution 1 mL solution was transferred into 10 mL volumetric flask and diluted to volume with methanol. 3 µL volume was spotted for six times on the TLC plates followed by the development and measured at 254nm. The amount of valacyclovir was estimated by comparing the peak area of sample solution with that of standard.

3. RESULTS AND DISCUSSION

3.1. Selection of Analytical Wavelength

The VU absorption spectrum of Valacyclovir showed maximum absorbance at 254 nm so it was selected as detection wavelength.

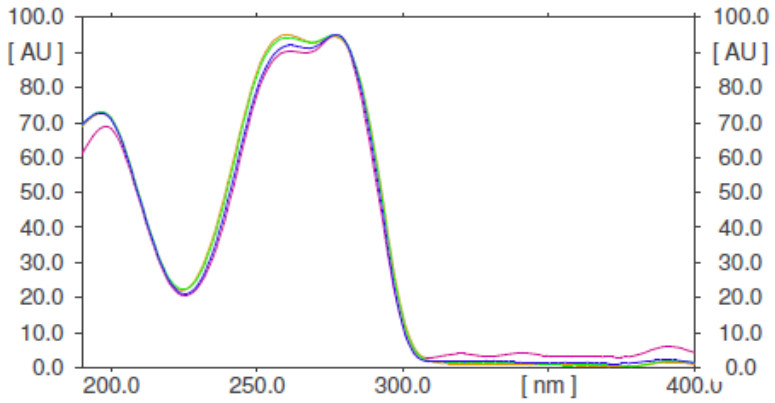

Figure 2: UV spectrum of standard valacyclovir hydrochloride

3.2. Optimization of the Chromatographic Conditions

The HPTLC procedure was optimized with a view to develop simple HPTLC method. The pure drug was spotted on HPTLC plates and run in different solvent systems. Initially, n-butanol, methanol and water were tried in different ratio. The optimum mobile phase was found to be consisted of n-butanol: methanol: Water (3:1:1 v/v/v). The sharp peak was obtained with *Rf* value of 0.22 ±0.01 (Figure 3). In order to reduce the neckless effect, the TLC chamber was saturated for 10 minute using saturation pads. The mobile phase was run upto distance of 8cm, which takes approximately 20 minute for development of HPTLC plate.

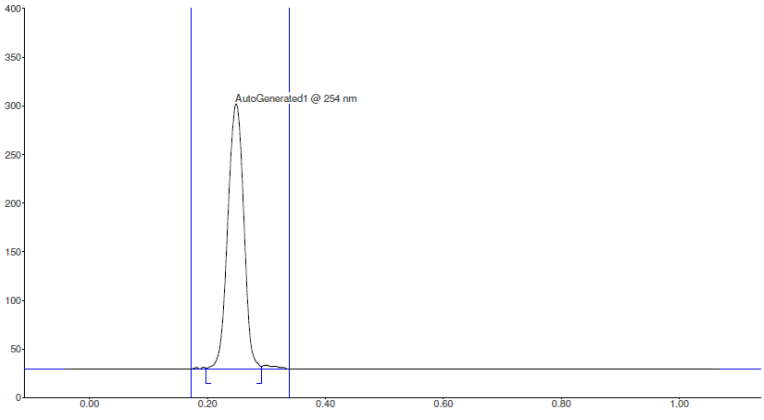

Figure 3: Densitogram of standard valacyclovir hydrochloride; (*Rf*: 0.22 ±0.01), mobile phase n-butanol: methanol: Water (3:1:1 v/v/v).

3.3. Validation of the Method

3.3.1. Linearity

Linear relationship was observed by plotting drug concentration against peak areas. valacyclovir showed linear response in the concentration range of 100–700 ng/spot (figure 4). The corresponding linear regression equation was Y = 367.1 + 6.72 * X with square of correlation coefficient (r^2) of 0.998 for valacyclovir. Linear regression data is shown in table 1.

Table 1: Linear regression data for valacyclovir

Parameter	Result
Linearity range	100-700 ng/spot
Regression equation	Y = 6.72 X +367.1
Correlation coefficient (r^2)	0.998
Slope	6.72
Y-Intercept	367.1

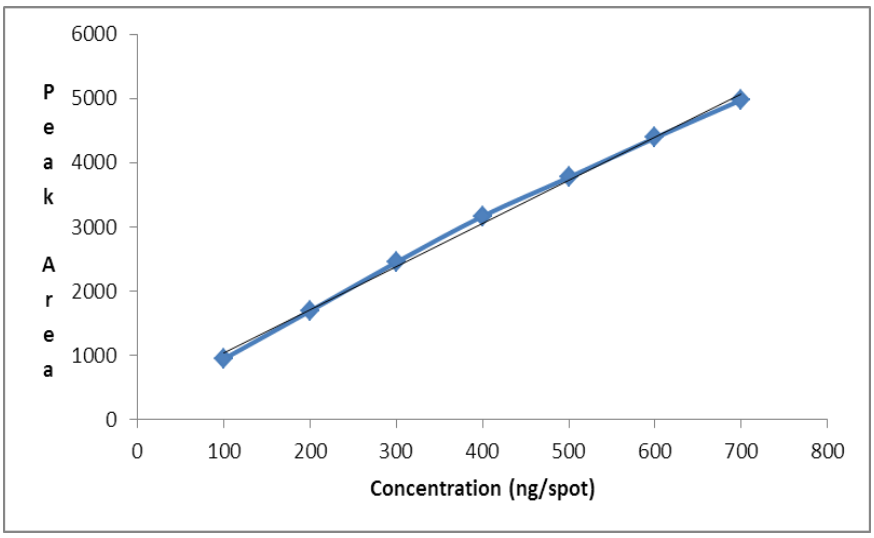

Figure 4: Plot of Concentration versus Peak area of valacyclovir

3.3.2. Precision

The results of the repeatability and inter-mediate precision experiments are shown in Table 2. The developed method was found to be precise as the % RSD values for repeatability and intermediate precision studies were < 2% respectively.

Table 2: Results of precision studies of valacyclovir

Concentration applied (ng/spot)	Repeatability (Intraday)		Intermediate precision (Interday)	
	Concentration found* ± SD (ng/spot)	% RSD (n=6)	Concentration found ± SD (ng/spot)	% RSD (n=12)
500	508.00 ± 1.75	0.34	507.24±6.40	1.26

n= number of determinations

3.3.3. Limit of Detection and Limit of Quantitation

The LOD and LOQ were found to be 42.34 ng/spot and 128.30 ng/spot respectively.

3.4.4. Accuracy

The developed method showed high and consistent recoveries at all studied levels. The results obtained from recovery studies are presented in Table 3. The mean % recovery ranged from 99.95 % to 101.92 %. Additionally, the obtained recoveries were found to be normally distributed with low % RSD at all concentration levels.

Table 3: Results of recovery studies of valacyclovir

Level	Standard Drug Added (ng/spot)	Drug Recovered* ± SD (ng/spot)	%Recovery	%RSD
80%	240	239.87 ± 2.98	99.95	1.24
100%	300	305.76 ± 4.33	101.92	1.42
120%	360	363.26 ± 4.81	100.91	1.33

*Mean of three determinations.

3.3.5. Specificity

A single peak of valacyclovir in tablet solution was observed at Rf 0.22 (figure 5). No interference of excipients with the valacyclovir peak was observed.

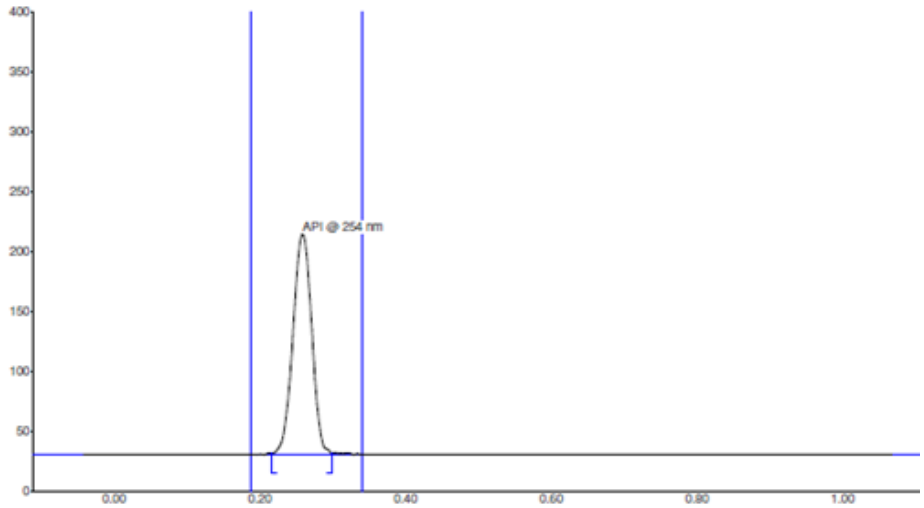

Figure 5: Chromatogram of tablet solution

3.4. Solution Stability

There was no indication of degradation in sample solutions of valacyclovir as revealed by peak purity data of solution stored at different times. The solution was found to be stable at

ambient temperature for 24h, and no unknown peaks were observed. The stability data is given in table 4.

Table 4: Stability data

Time (h)	Concentration applied (ng/spot)	Concentration found* ± SD (ng/spot)	%RSD
1	300	297.23 ± 2.19	0.74
6	300	294.07 ± 5.20	1.77
24	300	294.91 ± 2.78	0.94

* Mean of three determinations

The data of summary of validation parameters are listed in Table 5.

Table 5: Summary of validation parameters

Parameters	Results
Linearity	100-700 ng/spot
LOD	42.34 ng/spot
LOQ	128.30 ng/spot
Precision	% RSD = 0.34 (Repeatability) % RSD = 1.26 (Intermediate)
Recovery	99.95 % to 101.92 %

3.5. Analysis of Marketed Pharmaceutical Dosage Form

A single spot at *Rf* value of 0.22 was observed in the chromatogram of the drug samples extracted from capsules. There was no interference from the excipients that are commonly present in the formulations. The drug content was found to be 99.95%. the results are summarized in table 6. The good performance of the method indicated the suitability of this method for routine analysis of valacyclovir in pharmaceutical dosage form.

Table 6: Analysis of Tablet formulation

Labeled claim (mg)	Amount found* ± SD (mg)	% labeled claim	%RSD
500	499.75 ± 5.78	99.95	1.16

*Mean of six determinations

4. CONCLUSION

The developed HPTLC technique was found to be precise, specific, and accurate. Statistical analysis proved that the method was repeatable and selective for the analysis of valacyclovir hydrochloride in bulk drug and pharmaceutical dosage form. The proposed HPTLC method is suitable for routine determination of valacyclovir hydrochloride in pharmaceutical formulation in quality-control laboratories.

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REFERENCES

1. United States Pharmacopeia, National Formulary-29, Rockville, USA: United States Pharmacopeial convention, 2011.
2. Maryadele J. The Merck Index. In: O'Neil, editor. 13th ed. Whitehouse Station, NJ, USA: The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals; 2001, 9966.
3. Martindale. The Extra Pharmacopoeia. Published by direction of the Council of Royal Pharmaceutical Society of Great Britain. 34 th ed, Vol. 11. London Royal Pharmaceutical Society., 1996. 2005; 653-656.
4. Reddy JS, Maqsood Ahmed Md. S, Chakravarth IE, Prabhavathi K, Spectrophotometric estimation of Valacyclovir in pharmaceutical preparations, Journal of Chemical and Pharmaceutical Research, 2011; 3(4): 773-776.
5. Bhavar GB, Gosavi SA, Shewale PB, Shinde MA, Development and validation of spectroscopic method for estimation of valacyclovir in tablet dosage form, Analytical Chemistry:An Indian Journal, 2008; 8(7): 1-3.
6. Aswani Kumar CH, Anil Kumar T, Gurupadayya BM, Sloka SN, Rahul Reddy MB, Novel spectrophotometric determination of valacyclovir and cefotaxime using 1, 2-napthaquinone-4-sulfonic acid sodium in bulk and pharmaceutical dosage form, Archives of Applied Science Research, 2010; 2(4): 278-287.
7. Srihari G, Reddy Uma Maheswar, Rami Reddy N, Chakravarthi IE, A Simple Spectrophotometric Determination Of Valacyclovir In Pharmaceutical Preparations, Journal of Current Chemical & Pharmaceutical Sciences, 2011; 1(1): 15-18.
8. Reddy S, Maqsood Ahmed Md. S, Chakravarth, IE, Prabhavathi K, Spectrophotometric Estimation of Valacyclovir In Pharmaceutical Preparations, Journal of Chemical and Pharmaceutical Research, 2011; 3: 773-776.
9. Sriharia G, Rami Reddya N, Nagarajasetty K, Chakravarthic IE, Spectrophotometric Determination of Valacyclovir In Pharmaceutical Formulations, Chemical Science Transactions, 2013; 2: 61-64.

10. Sugumaran M, Jothieswari D, Development and validation of spectroscopic method for estimation of valacyclovir in tablet dosage form, Oriental Journal of Chemistry, 2010; 26: 163-165.
11. Siva Ramakrishna V, Ravi Kumar D, Malleswara Rao NVN, Ram Babu C, Method development and determination of valacyclovir HCL in pharmaceutical dosage forms by visible spectrophotometry, International Journal of PharmTech Research, 2012; 4(3): 1009-1014.
12. Palacios ML, Demasi G, Pizzorno MT, Segall AI, Validation of an HPLC Method for the Determination of Valacyclovir in Pharmaceutical Dosage, Journal of Liquid Chromatography & Related Technologies, 2005; 28 (5): 751 –762.
13. Rao PN, Rjayathirtharao K, Vseshagirirao JV, RP-HPLC Estimation Of Valacyclovir In Tablets, Asian Journal of Chemistry, 2006; 18: 2552-2556.
14. Jadhav AS, Pathare DB, Shingare MS, Development and validation of enantioselective high performance liquid chromatographic method for Valacyclovir, an antiviral drug in drug substance, Journal of Pharmaceutical and Biomedical Analysis, 2007; 43(4): 1568-1572.
15. Srinivasa Rao K, Sunil M, Stability-indicating liquid chromatographic method for Valacyclovir, International Journal of ChemTech Research, 2009; 1(3): 702-708.
16. Rasool SK, Naik DV, Prasad Babu D, Buchi N, RP-HPLC method for the estimation of valacyclovir in bulk and pharmaceutical formulations, International Journal of Pharmacy and Pharmaceutical Sciences., 2012; 4(1): 214-218.
17. Sugumaran M, Bharathi V, Hemachander R, Lakshmi M, RP-HPLC method for the determination of Valacyclovir in bulk and Pharmaceutical formulation, Der Pharma Chemica, 2011; 3(4): 190-194.
18. Sultana Y, Agarwal NK, Safiakhanam P, Development and validation of stability indicating rp-hplc method for estimation of valacyclovir in pharmaceutical dosage forms, International Journal of Pharmaceutical and Clinical Research, 2013; 5(1): 7-12.
19. Savaser A, Ozkan CK, Ozkan Y, Uslu B, Ozkan SA, Development and Validation of an RP-HPLC Method for the Determination of Valacyclovir in Tablets and Human Serum and Its Application to Drug Dissolution Studies, Journal of Liquid Chromatography & Related Technologies, 2003; 26(11): 1755-1767.
20. Yadav M, Upadhyay V, Singhal P, Goswami S, Shrivastav PS, Stability evaluation and sensitive determination of antiviral drug, valacyclovir and its metabolite acyclovir in

human plasma by a rapid liquid chromatography–tandem mass spectrometry method, Journal of Chromatography B, 2009; 877(8–9): 680-688.

21. Kasiari M, Gikas E, Kazanis M, Panderi I, Selective and rapid liquid chromatography/negative-ion electrospray ionization mass spectrometry method for the quantification of valacyclovir and its metabolite in human plasma, Journal of Chromatography B, 2008; 864(1–2): 78-86.
22. Heon-Woo L, Ji-Hyung Seo, Kyung-Tae Lee, Development and validation of a high-performance liquid chromatography–tandem mass spectrometry for the determination of penciclovir in human plasma: Application to a bioequivalence study, Journal of Chromatography B, 2007; 852(1–2): 382-388.
23. Sasanya JJ, Abd-Alla AM, Parker AG, Cannavan A, Analysis of the antiviral drugs acyclovir and valacyclovir-hydrochloride in tsetse flies (Glossina pallidipes) using LC-MS, Journal of Chromatography B- Analytical Technologies in the Biomedical and Life Sciences, 2010; 878(26): 2384-2390.
24. International Conference on Harmonization ICHQ1A, Stability testing of New Drug Substances and Products, ICH, Geneva, Switzerland, 1993.
25. P. D. Sethi, High Performance Thin Layer Chromatography: Quantitative Analysis of Pharmaceutical Formulations, CBS Publishers and Distributors, New Delhi, India, 2nd edition, 1996.
26. International Conference on Harmonization ICH Q2 (R1), Validation of Analytical Procedures: Text and Methodology, the International Federation of Pharmaceutical Manufacturers & Associations, Geneva, Switzerland, 2005.