Vol.1.Issue.1.;2013

Bulletin of Pharmaceutical and Medical Sciences (BOPAMS)
A Peer Reviewed International Journal http://www.bopams.com

RESEARCH ARTICLE

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC METHOD FOR DETERMINATION OF VALGACYCLOVIR IN TABLET DOSAGE FORM

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Article Info
Received: 19/04/2013
Revised from:19/04/2013
Accepted on:22/05/2013

ABSTRACT

The use of organophosphorus pesticides is widespread in developing countries for increasing the yield of agriculture. It has resulted in increased incidence of ingestion of organophosphorus for self harm purpose. This study was aimed to assess the pattern and outcome of acute poisoning cases in a tertiary care hospital .Total of 118 patients consisted of 62 males and 56 females in the age group of 14-60 years were studied. Maximum number of 60 patients were in the age group of 25-49 years while 49 patients in age group of 14-24 years and 9 patients in age group of 50 years and above. Higher number of 62 patients were from the rural area while 56 patients had urban background .History of ingestion of pesticide was present in all cases. In 90 patients poison consumption was suicidal in nature while in 28 patients the poisoning was accidental. Phosphomidones was the major culprit in majority of the patients followed by malathion and dichlorophos. 103 recovered and mortality was observed in 15 patients.

Key words – Valganciclovir, HPTLC, validation, tablet dosage form

INTRODUCTION

Valganciclovir is chemically, L-Valine,2[(2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl)methoxy]-3-hydroxypropyl ester, monohydrochloride 1. Valganciclovir Hcl is a synthetic acyclic Nucleoside analogue of 2-deoxyguanosine Valganciclovir is an anti-viral drug. After oral administration, it is fastly cleaved by esterases in the intestine wall and liver to form active ganciclovir. It is used to prevent disease caused by a virus called cytomegalovirus (CMV) Ganciclovir inhibits replication of cytomegalovirus (CMV) Valganciclovir is a phosphodiesterase type 5-inhibitor, used in the management of erectile dysfunction2. Valganciclovir is not official in any of the pharmacopoeias. The literature survey reveals HPLC [3, 4,5,6,7], LC/MS [8,9,] methods for the determination of Valganciclovir pharmaceutical dosage forms as well as in biological fluids. The literature survey does not reveal any simple HPTLC method for the determination of Valganciclovir in tablet dosage form. The present manuscript describes simple, sensitive, accurate, precise and specific HPTLC method for the estimation of Valganciclovir in tablet.

MATERIALS AND METHODS

Apparatus: A Camag HPTLC system (Switzerland) comprising of Camag Linomat V semiautomatic sample applicator, Camag TLC Scanner 3, Camag (Muttenz, Switzerland) flat bottom and twin-trough developing chamber (10 × 10 cm), UV cabinet with dual wavelength UV lamp, Camag winCATS software, Hamilton syringe (100 µl), Sartorius CP224S analytical balance (Germany), Ultrasonic bath (Frontline FS-4, Mumbai, India) were used in the study.

Reagents and Materials : Pharmaceutical grade of Valganciclovir was kindly supplied as a gift sample from Dr.Reddys Laboratories,Hyderabad, India. Silica Gel 60 F254 TLC plates (10 × 10 cm, layer thickness 0.2 mm, E. Merck, Germany) were used as stationary phase. The pharmaceutical tablet formulation containing 20 mg of Valganciclovir was procured from the local pharmacy. Chloroform, methanol and methanol (HPLC grade, Rankem, India) were used for mobile phase preparation and as solvents.

Preparation of standard: 100 mg of the valganciclovir standard powder is taken and then added to methanol in a 100ml volumetric flask. The contents were thoroughly shaken and then filterde usin whatmann filter paper and finally the volume was made upto the mark with methanol. The stock solution contains 100mg/100ml of valganciclovir.

Preparation of sample solution: Twenty tablets were weighed, their average weight was determined, and crushed in mortar. Powder equivalent to 10 mg of Valganciclovir was weighed and transferred to 100 ml volumetric flask. The drug from powder were dissolved and extracted with methanol. To ensure complete extraction of drugs it was sonicated for 30 min. The extract was filtered through Whatman filter paper No. 41 and residue was washed with methanol. The extract and washing were pooled and transferred to another 100 ml volumetric flask and volume was made with methanol to achieve final concentration of 100 µg/ml of Valganciclovir.

Chromatographic conditions: The chromatographic estimations were performed using following condition; stationary phase, precoated Silica Gel 60 F254 aluminum sheets (10 × 10 cm) (pre-washed with methanol and dried in air); mobile phase, n-Butanol: GAA: Water (45:40:5) ; chamber saturation time, 30 min; temperature, 25 ± 2o, migration distance, 80 mm; wavelength of detection, 254 nm; slit dimensions, 5 × 0.45 mm; scanning speed, 10 mm/s. Following spotting parameter were used - band width, 6 mm; distance from the plate edge, 10 mm; space between two bands, 10 mm and spraying rate, 1 µl/s.

Chromatographic separation: Six microlitres of standard solution of Valganciclovir (100 µg/ml) was applied on TLC plate under nitrogen stream using semiautomatic spotter. The plate was dried in air and developed up to 80 mm at constant temperature using mixture of n-Butanol: GAA: Water (45:40:5) as mobile phase in Camag twin-trough chamber previously saturated with mobile phase for 30 min. The plate was removed from the chamber and dried in air. Photometric measurements were performed at 254 nm in absorbance/reflectance mode with Camag TLC Scanner 3 using winCATS software incorporating the track optimization option.

Validation of the proposed method : The proposed method is validated according to the International Conference on Harmonization (ICH) guidelines12.

Linearity (Calibration curve) : Adequate dilutions were made from the stock (0, 1, 2, 3, 4, 5, 6 µ/spot) and then applied as spots on the HPTLC plate after development of plate on the selected mobile phase, it was dried in the hot air oven. Then areas under curve of valganciclovir in those 5 concentrations were measured at 254nm. The measured peak areas were plotted against concentrations as shown in graphs. The calibration curve was prepared by plotting peak area versus concentration (ng/spot) corresponding to each spot.

Accuracy (% Recovery) : The accuracy of the method was determined by calculating recoveries of Valganciclovir by the standard addition method. Known amounts of standard solutions of Valganciclovir was added at 80,100 and 120 % level to prequantified sample solutions of Valganciclovir (300 ng/spot). The amount of Valganciclovir was estimated by applying obtained values to the regression line equation.

Method Precision (% Repeatability) : The precision of the instrument was checked by repeatedly injecting (n = 6) solutions of Valganciclovir (3 ng/spot) without changing the parameters of the proposed method.

Intermediate Precision (Reproducibility): The intraday and interday precisions of the proposed methods were determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for 3 different concentration of standard solution of Valganciclovir (3, 4 and 5 ng/spot) for the proposed method. The results were reported in terms of relative standard deviation (% RSD).

Limit of detection (LOD) and limit of quantification (LOQ): LOD and the LOQ of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines12. LOD = 3.3 × σ/S LOQ = 10 × σ /S Where σ = Standard deviation of the response S = Slope of calibration curve

Specificity : The specificity of the method was ascertained by analyzing standard drugs and the sample. The spots for deflazacort in the samples were confirmed by comparing the Rf and spectra of the spots with that of the standards.

Analysis of Valganciclovir in tablet : Five microlitres of sample solution was applied to the TLC plate to get 5 ng/spot and followed by development and scanning as described earlier. Analysis was carried out in triplicate, peak areas were measured at 254 nm and sample concentrations calculated. The amount of Valganciclovir present in the sample solution was determined by fitting area values of peak corresponding to Valganciclovir into the equation of line representing calibration curve of Valganciclovir. The potential interference from excipients was also examined.

RESULTS AND DISSCUSION

Valganciclovir is soluble in methanol; therefore methanol was selected as solvent. Several mobile phases were tried to accomplish good separation of Valganciclovir. Using the mobile phasen-Butanol: GAA: Water (15:10:5) and 10 × 10 cm silica gel 60F254 aluminum-backed plates, good separation was attained with retardation factor (Rf) values of 0.78 + 0.008 for Valganciclovir (Figure 1 and 2). A wavelength of 254 nm was used for quantification of the drug. Even saturation of TLC chamber with mobile phase for 30 min assured better reproducibility and better resolution.

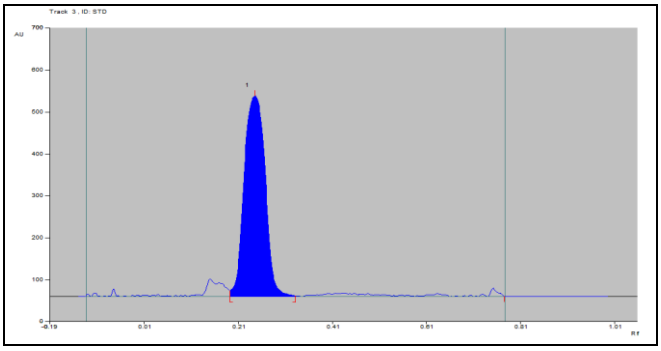

Figure 1: HPTLC chromatogram of Valganciclovir with corresponding R_f value at 254 nm

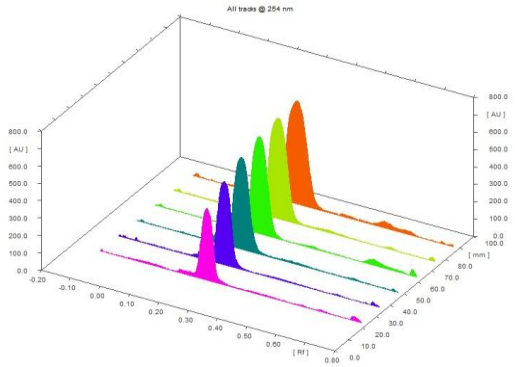

Figure 2: 3D-Chromatogram show peaks of Valganciclovir in different concentrations at 254 nm

Linearity range for Valganciclovir was found in the concentration range of 100 to 800 ng/spot, with a correlation coefficient of 0.9970. The average linear regression equation was represented as Y = 2.351X + 68.996, where X = concentration of Valganciclovir in ng/spot and Y = peak area. The limit of detection and limit of quantification for Valganciclovir were found to be 28.11 ng/spot and 93.45 ng/spot, respectively indicate sensitivity of the method.

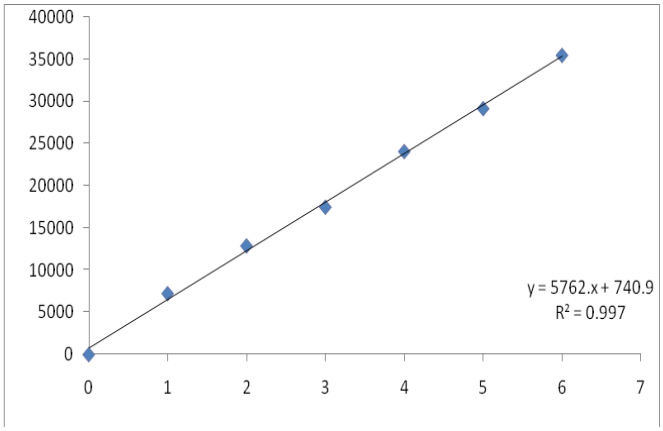

Fig. 3.Calibration graph of Valganciclovir

The intra-day precision (% RSD) was calculated for standard Valganciclovir solutions (300, 400 and 500 ng/spot) for 3 times on the same day. The inter-day precision (% RSD) was calculated for standard Valganciclovir solutions (300, 400 and 500 ng/spot) for 3 times over a period of one week. The intra-day and inter-day variation (% RSD) were found to be in the range of 0.38-0.81 and 0.45-1.90, respectively. These values indicate that the method is precise. Precision of the instrument was checked by repeated scanning of the same spot (500 ng/spot) of Valganciclovir six times without changing position of the plate and % RSD for measurement of peak area was found to be 0.24. The % RSD for measurement of peak area ensures proper functioning of HPTLC system indicates repeatability of the proposed method. Different validation parameters for the proposed HPTLC method for determining Valganciclovir content are summarized in Table 1.

Accuracy of the method was evaluated by calculating recovery of Valganciclovir by standard addition method at 4 different levels of the calibration curve (n = 6). The % mean recovery was found to be 100.98± 0.76 ensuring that the method is accurate (Table 2).

The method was found to be specific for Valganciclovir. The specificity of the method was ascertained by analyzing standard drug and the samples. The spot for Valganciclovir in the sample was confirmed by comparing the Rf value and spectra of the spot with that of standard.

Table 1: Regression Analysis Data and Summary of Validation Parameters for Proposed HPTLC Method

S.NO	PARAMETER	RESULTS
1.	Linearity range (ng/spot)	1 - 6
2.	Slope	5695.
3.	Intercept	800.0
4.	Correlation co-efficient (r2)	0.9970
5.	Precision (% RSD) Intra-day (n = 3) Inter-day (n = 3)	0.38 - 0.81 0.45 - 1.90
6.	Repeatability of peak area (% RSD) (n = 6)	0.24
7.	Accuracy (% Recovery) (n = 5)	100.98± 0.76
8.	Limit of detection (LOD) (ng/spot)	0.1449µg/Spot
9.	Limit of quantification (LOQ) (ng/spot)	0.4392µg/Spot
10.	Specificity	Specific

n is number of determination and RSD is relative standard deviation.

Table 2: Recovery data for the Proposed Method

Concentration (µg/Spot)of sample present	**Concentration (µg/Spot)of standard present**	**Peak area**	**% Recovery**
1	0	6256.4	104.9%
1	1	12150.6	103.7%
1	2	17865.8	99.6%
1	3	24073.3	103.7%
1	4	29445.3	93%
Mean % recovery			100.98%

The peak purity of Valganciclovir was assessed by comparing the spectra of standard at peak start, peak apex and peak end positions of spot. Good correlation was also found between standards and sample spectra (Figure 4). None of the formulation excipients were interferes in the quantification of Valganciclovir at this Rf value.

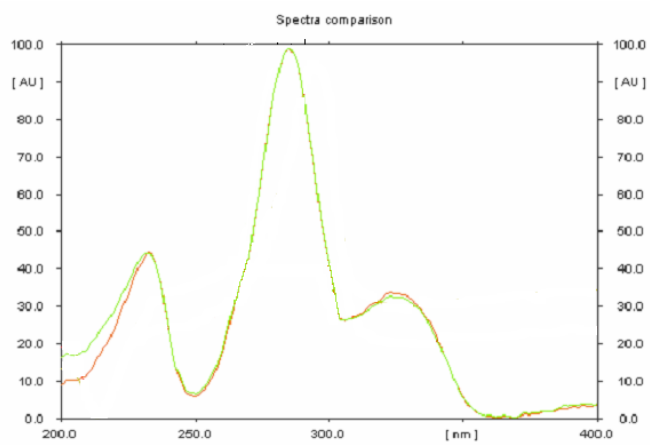

Figure 4: Overlain UV absorption spectrum of standard and sample Valganciclovir

This method was applied to determine the content of Valganciclovir in market sample of single component Valganciclovir tablet. The average percentage of Valganciclovir in market sample was found to be 99.98 ± 0.75 (n = 6). The results are in agreement with the labeled value of Valganciclovir in tablet dosage form (Table 3). The results indicate that the proposed HPTLC method was found to be simple, sensitive, specific, precise and accurate for the estimation of Valganciclovir in tablet formulations.

Table 3: Analysis of Tablet Formulation of Valganciclovir by Proposed HPTLC Method (n = 6)

Tablet	Label claim (mg)	Parameters MEAN S.D	% amount found (n = 6) HPTLC METHOD
BRAND (VALCEPT)	450	0.85	99.85
BRAND B (Valstead)	450	0.76	98.67

CONCLUSION

The results of the analysis of pharmaceutical formulation by the proposed method are highly reproducible and reliable and are in good agreement with the label claim of the drug. The additives usually present in the pharmaceutical formulations of the assayed samples did not interfere with determination of Valganciclovir. The observations and results obtained from this study, including specificity, linearity and range, accuracy, precision (method precision as repeatability and intermediate precision as intra and inter day precision) are lie well within acceptable results. From the experimental studies it can be concluded that proposed method can be adopted for the routine analysis of Valganciclovir in tablets without interference of excipients.

ACKNOWLEDGEMENT

The author is thankful to Dr.Reddys Laboratories Hyderabad, India for providing the gift sample of Valganciclovir and Trinity College of pharmaceutical Sciences, peddapalli-Karimnagar for providing all the facilities to carry out the research work.

REFERENCES:

[1]. Maryadele JO Neil. The Merck Index: An Encyclopedia of chemicals, drugs and biologicals. 14th ed., Whitehouse station, New Jersey: Published by Merck Research Laboratories, Division of Merck and Co., Inc; 2006: 1550.

[2]. Sweetman SC. Martindale: The Complete Drug Reference, 35th ed., London, UK: Pharmaceutical Press; 2007: 1990.

[3]. Hassan Y, Aboul E, Ali I. Determination of Valganciclovir in pharmaceutical preparation by HPLC using monolithic silica column. Talanta 2005; 65: 276-280.

[4]. Rabbaa-Khabbaz L, Abi Daoud R. A sensitive and simple high performance liquid chromatographic method for quantification of Valganciclovir in human serum. J Appl Res 2006; 6: 170-175.

[5]. Ching-Ling C, Chen-Hsi C. Determination of Valganciclovir in small volumes of plasma by high-performance liquid chromatography with UV detection. J Chromatogr B 2005; 822: 278-284.

[6]. Shakya AK, Abu-Awwad AN, Arafat TA, Munther M. Validated liquid chromatographic-ultraviolet method for the quantitation of Valganciclovir in human plasma using liquid-liquid extraction. J Chromatogr B 2007; 852: 403-408.

[7]. Subba Rao DV, Radhakrishnan P, Himabindu V. Development of a validated LC assay for Valganciclovir bulk drug and pharmaceutical dosage form. Chromatographia 2008; 67: 183-188.

[8]. Ramakrishna NVS, Vishwottam KN, Puran S, Koteshwara M, Manoj S, Santosh M, Chidambara J, Wishu S, Sumatha B. Quantitation of Valganciclovir in human plasma by liquid chromatography–tandem mass spectrometry with electrospray ionization. J Chromatogr B 2004; 809: 243-249.

[9]. Xiaolan Z, Xiao X, Song S, Chen B, Zhang F, Shouzhuo Y, Zutian Z, Dajin D, Hongwei H. Simultaneous determination of sildenafil, vardenafil and Valganciclovir as forbidden components in natural dietary supplements for male sexual potency by high-performance liquid chromatography-electrospray ionization mass spectrometry. J Chromatogr A 2005; 1066: 89-95.

[10]. Ali I, Aboul-Enein HY. Validated method for Valganciclovir analysis in pharmaceutical preparations by capillary electrophoresis. Chromatographia 2004; 60: 187-191.

[11]. Abdel-Hamid ME. Determination of sildenafil, Valganciclovir and vardenafil in tablets and adulterated herbal products by ESI-MS-MS. J Liq Chromatogr Rel Technol 2006; 29: 591-603.

[12]. The International Conference on Harmonization, Q2 (R1), Validation of Analytical Procedure: Text and Methodology: 2005.

[13]. Sugawara M, Huang W, Fei YJ, et al. Transport of valganciclovir, a ganciclovir prodrug, via peptide transporters PEPT1 and PEPT2. J Pharm Sci. 2000;89(6):781-9.

[14]. Kogelnik AM et al. Use of valganciclovir in patients with elevated antibody titers against Human Herpesvirus-6 (HHV-6) and Epstein - Barr virus (EBV) who were experiencing central nervous system dysfunction including long-standing fatigue. J Clin Virol. 2006;37(S1):S33-S38.

[15]. Paltiel AD et al. Preevaluation of clinical trial data: the case of preemptive cytomegalovirus therapy in patients with human immunodeficiency virus. Clin Infect Dis. 2001;32(5):783-93.

[16]. Pescovitz MD et al. Valganciclovir results in improved oral absorption of ganciclovir in liver transplant recipients. Antimicrob Agents Chemother. 2000; 44(10):2811-5.

[17]. Reusser P. Antiviral therapy: current options and challenges. Schweiz Med Wochenschr. 2000;130(4):101-12.