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Low level detection of *p*-Toluene sulfonic acid in Valganciclovir hydrochloride by RP-HPLC method

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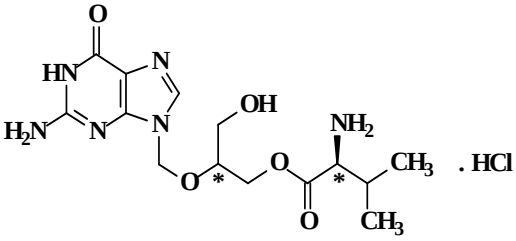
ABSTRACT

The present work describes the development of a reverse phase high performance liquid chromatographic (RP-HPLC) method for the quantification of p-Toluenesulfonic acid in Valganciclovir hydrochloride. The drug substance was subjected to stability studies using optimized method condition to enhance low level of detection with minimum acquisition time. Successful separation of p-Toluenesulfonic acid was achieved from the related impurities of the drug on an Inertsil ODS-3V (150 x 4.6mm) 5µm column. The gradient LC method employs solution A and solution B as mobile phase. The solution A contains 0.1% OPA and solution B contains Acetonitrile. The HPLC method was developed and validated to prove its performance characteristics by demonstrating selectivity, sensitivity (limit of detection and quantification), linearity, accuracy, precision and ruggedness.

Keywords: RP-HPLC, Valganciclovir Hydrochloride, *p*-Toluenesulfonic acid, Validation.

INTRODUCTION

Valganciclovir hydrochloride is L-valine 2-[(2-amino-1,6-dihydro-9-oxo-9H-purin-9-yl)-methoxy]-3-hydroxypropylester, Monohydrochloride is a prodrug for ganciclovir [1]. The molecular formula $C_{14}H_{22}N_6O_5$.HCl, with molecular weight of 390.82 and the chemical structure is shown in the Figure 1.It is an antiviral drug used to treat cytomegalovirus (CMV) retinitis in adult patients with acquired immunodeficiency syndrome (AIDS) and for the prevention of CMV disease in adult kidney, heart, and kidney-pancreas transplant patients [2-4]. Valganciclovir is hydrolyzed to ganciclovir through enzymes in the gut mucosa and hepatic cells. Its properties are, therefore, the same as ganciclovir. The break-down of ganciclovir produces ganciclovir triphosphate, which inhibits viral DNA synthesis producing anti-cytomegalovirus activity [5]. Valganciclovir is available as 450mg tablets and in 50mg/mL powder for oral solution for oral administration. It is marketed under the trade name Valcyte. Valganciclovir is classified as Type II prodrug which occurs extracellularly, in digestive fluids, systemic circulation or other extracellular body fluids [6].



**Asymmetric centre*

Figure 1. Structure of the Valganciclovir Hydrochloride drug substance

In pharmaceutical industry, *p*-Toluenesulfonic acid (pTSA) is used as counter-ions for basic drugs during the synthesis of the drug substance because of hydrophilic nature and strong acidic properties [7-8]. It is also known as tosylic acid (TsOH). It has broad application towards esterification of carboxylic acid [9], transesterification of an ester [10], oxidative degradation [11] and reductive amination of aldehydes and ketones[12].Various analytical techniques has been reported in the literature for the determination of PTSA. The reported works are UV-spectrometric method by H.Cefontain et.al [13], trace determination by J.Dokladalova et.al [14], coupled CE/MS method by Agilent [15], mass spectral determination by R.H.Wiley et.al [16], NMR analysis by G.Shao et.al [17] and SPME coupled GC/SIM-MS by I.Colon et.al [18]. The impurities can arise during the manufacturing process and storage of the drug substance or products, the criteria for their acceptance up to certain limits are based on pharmaceutical studies or known safety data [19]. In view of that, the drug product available in the market is directly consumed by human being based on the prescription, so it should be of good quality and highest purity. So monitoring of *p*-Toluenesulfonic acid (PTSA) in the drug substance is essential for preserving the desired quality of active moiety of the compound. The aim for this paper work is to develop a rapid and economical method for PTSA determination in Valganciclovir Hydrochloride. By employing simple isocratic HPLC method [20], the elution pattern was established without co-elution of other impurities.

MATERIALS AND METHODS

Chemicals, reagents and samples

Reference samples of Valganciclovir Hydrochloride and its related impurities of USP namely Valganciclovir impurity-A, Valganciclovir impurity-B, Valganciclovir impurity-C, Valganciclovir impurity-D, Valganciclovir impurity-E, Valganciclovir impurity-F, Valganciclovir impurity-G, Valganciclovir impurity-H, Valganciclovir impurity-I, Valganciclovir impurity-J, Valganciclovir impurity-K, Valganciclovir impurity-L, Valganciclovir impurity-M and Valganciclovir impurity-N were synthesized and characterized by LC-MS, NMR and IR in the laboratory was procured from Aurobindo Pharma Limited, Hyderabad, India. The *p*-Toluene sulfonic acid was obtained from Sigma Aldrich Limited. All chemicals and reagents were of analytical purity grade unless stated otherwise. Water obtained from Milli-Q purification system. Orthophosphoric acid and LC grade Acetonitrile purchased from Merck Limited (Darmstad, Germany). The LC system consists of quaternary gradient pumps with auto sampler and auto injector (Alliance 2695, Waters, Milford, USA) controlled with Empower software for data acquisition (Waters).

Preparation of standard solution

Accurately weighed 9mg of *p*-Toluene sulfonic acid (PTSA) reference sample standard was transferred to a 20mL volumetric flask, added about 10mL of diluent and sonicated to dissolve, diluted up to the mark with diluent and mixed. Further 1mL of this solution was diluted to 20mL with diluent and further 1mL of the resulting solution was diluted to 10mL with diluent. This solution contains 2.25µg mL^{-1} of *p*-Toluene sulfonic acid.

Preparation of sample solution

Accurately weighed 20mg of Valganciclovir Hydrochloride was transferred to 10mL volumetric flask and added about 5mL of diluent and sonicated to dissolve, diluted up to the mark with the diluent and mixed.

Preparation of impurities stock solution:

Accurately weighed 1.0 mg of each impurities of Valganciclovir Hydrochloride was transferred to 10mL volumetric flask individually and added about 5mL of diluent and sonicated to dissolve, diluted up to the mark with the diluent and mixed.

Chromatographic condition

The isocratic reverse phase LC method employs solution A and B as mobile phase in the ratio of 30:70 v/v. The solution A contains aqueous 1%v/v Ortho-phosphoric acid and LC grade Acetonitrile as solution-B. The flow rate of the mobile phase was 0.8 mL/min. The chromatographic separation was achieved on a GL sciences column, 150 x 4.6mm, Inertsil ODS-3V, 5µm particle. The acquisition time was 15 minutes. The column temperature was maintained at 40°C and the detection was monitored at a wavelength of 220nm. The injection volume was 10µl. Milli-Q grade water was used as diluent.

RESULTS AND DISCUSSION

Chromatographic method optimization

The scope of the chromatography method was to separate PTSA from Valganciclovir and its IX related impurities. The impurities were co-eluted with PTSA using different makes of stationary phases like C8, C18 and phenyl as well as with different composition of mobile phases and organic modifiers. Hence, 0.1%v/v Ortho-phosphoric acid

was chosen as buffer solution to exclude the precipitation of aqueous salt buffers with combination of higher organic modifier ratios. During the evaluation of various column chemistries, ODS has shown better resolution. In the optimized chromatographic condition the PTSA was well separated and the retention time was found to be 7min. The developed method was found to be specific for PTSA in Valganciclovir drug substance Figure 2.

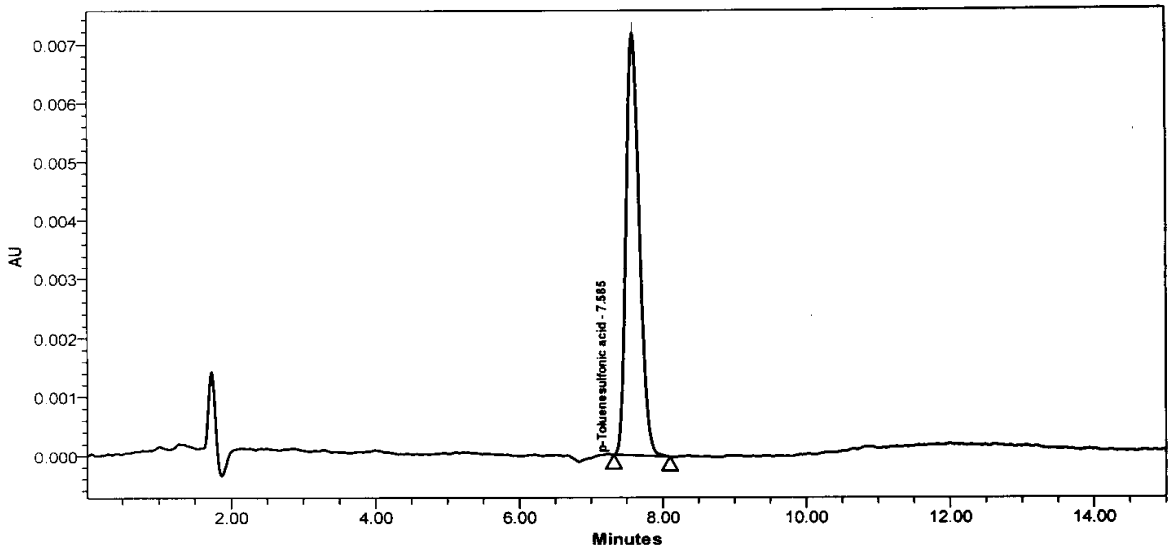


Figure 2. Zoomed chromatogram of PTSA standard

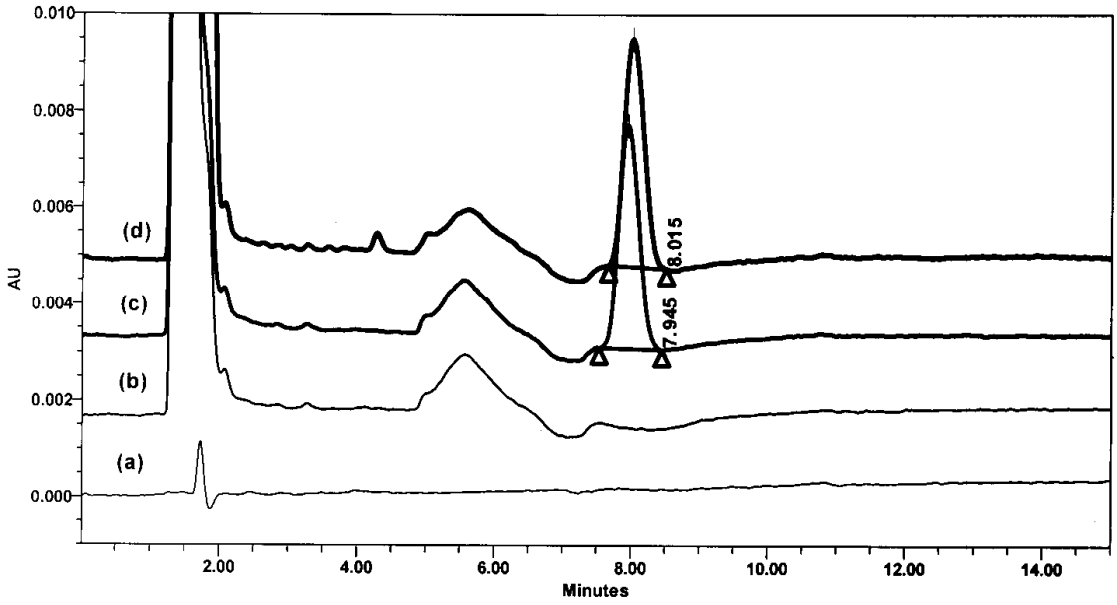


Figure 3. Representative chromatogram obtained from (a).Diluent, (b).As such sample, (c).Control sample and (d).Spiked sample.

Method Validation

The proposed HPLC method was validated [21] for selectivity, sensitivity, linearity, accuracy, limit of detection and limit of quantification, intermediate precision, sample solution stability, forced degradation and stability studies.

Selectivity

The sample solutions of impurities, sample and standard were prepared at 0.1%w/w concentration based on Valganciclovir and injected into the chromatographic system to identify the retention time. The retention time of PTSA was found to be about 8.0 min. The sample was found to contain PTSA at very low level, and therefore, the sample (Valganciclovir) was spiked with PTSA at 0.1% w/w level (Control sample) and sample spiked with PTSA

along with other known impurities of Valganciclovir including PTSA (spiked sample). It is confirm that no co-eluting peak was observed due to other known related impurities of Valganciclovir drug substance with the analyte peak under investigation, thereby indicating that the method is selective for determining the content of PTSA. In view, Figure 3 describes the representative chromatogram obtaind from Diluent, As such sample, sample spiked with PTSA (Control sample) and sample spiked with PTSA along with known related impurities of Valganciclovir (Spiked sample).

A system suitability rule has been established from the above experiment for the following parameters, retention time at about 8.0 minutes, peak tailing should not be more than 1.5 and plate counts should not be less than 3000. Therefore, the Table 1 summarized the system suitability and peak purity results obtained from the above experiment.

Table 1. Experimental data of system suitability and selectivity

System suitability [a]		
Components	PTSA	
Retention Time (R_T)	8.0 minutes	
Peak Tailing	1.1	
Plate counts	7126	
Selectivity [b]		
Peak Purity		
	Peak Angle	Purity Threshold
Standard	0.532	0.641
Control sample	0.676	0.721
Spiked sample	0.578	0.608

[a] *Average experimental observation,*
[b] *Criteria for peak purity: Purity angle should be less than purity threshold*

Linearity:
By measuring area responses at different levels of PTSA over the range of 5% to 150% of analyte concentration the linearity data were validated. Required concentrations of solutions were prepared from stock solution for different level of 0.050µg mL^{-1}- 1.665 µg mL^{-1}, correlation co-efficient was found to be 1.000. The statistical parameters slope, intercept, residual standard on deviation response and correlation co-efficient values were calculated in Table 2.

Table 2. Experimental data obtained from Linearity analysis

Component	PTSA
Calibration range (µg mL^{-1})	0.050 – 1.665
Calibration Points	7
Slope	86153
Intercept	98
STEY X	491
Correlation co-efficient (CC)	0.99996
Residual sum of square (r^2)	0.99992

The area and concentration were treated by least square linear regression analysis plot [Area count in terms of Area count (AU) at Y-axis Vs Concentration (µg mL^{-1}) at X-axis] as shown in Figure 4.

Sensitivity:
To predict the limit of detection (LOD) and limit of quantification (LOQ) the solutions were prepared from known stock concentration. The predicted values obtained from a linear regression line performed at lower concentration levels using slope (S) and residual standard deviation (S.D).The limit of detection and quantification predicted was found to be 0.019 µg mL^{-1} and 0.057 µg mL^{-1} respectively, by using the calculation 3.3*STEY.X/SLOPE* 100/Sample concentration (for LOD) and 10*STEY.X/SLOPE*100/Sample concentration (for LOQ), and each predicted level was verified for precision by analyzing six replicate measurements. The percentage relative standard deviation for six replicate measurements at predicted LOD and LOQ concentration levels was found to be 11.2 and 3.8 respectively, verifying the predicted values.

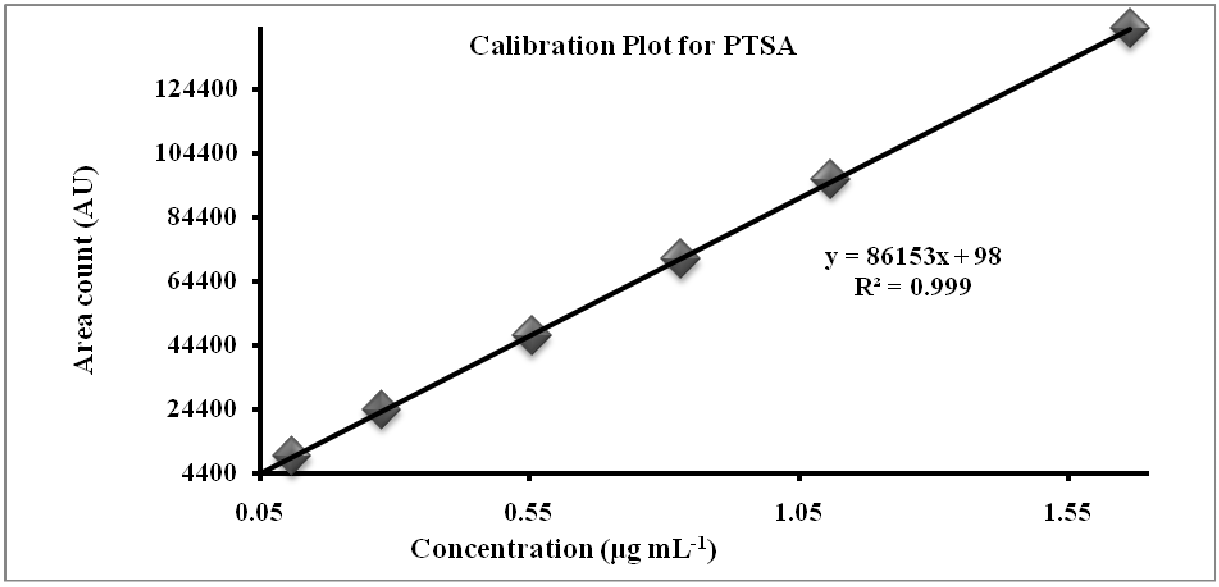


Figure 4. Correlation plot obtained from the linearity experiment.

Precision

The method was assessed by six replicate injections of PTSA standard solution (0.1%w/w) into chromatographic system, and the percentage relative standard deviation of response for six replicate measurements was found to be 0.2 for the repeatability of the system. Reproducibility of the method (Method precision) was demonstrated by preparing six replicate sample preparations by spiking known concentration (0.1% w/w) of PTSA in random selection of one batch of Valganciclovir drug substance. The samples were analyzed as per method, and the content of PTSA was determined. The values obtained from the above experiment were found to be 0.099%w/w with %RSD value of 1.1 has shown good repeatability for analytical experiment. The degree of reproducibility is known as ruggedness, obtained by the analysis of the same sample concentration (which is used in the method precision) under a variety of conditions using different series of column, with different user on different day by using new standard also found to be 0.097%w/w with %RSD value of 1.8, also proves that the method is rugged for the determination of PTSA under the experimental conditions.

Stability of sample solution

The Valganciclovir drug substance was spiked with known concentration of PTSA with respect to sample concentration (0.1% w/w) was stored at 25 ± 2°C temperature conditions, were injected into chromatographic system at different time intervals. The content of PTSA was determined at each interval, the sample solution was found to be stable over a period of 15 hours. The % difference between the peak area obtained at initial and different time interval was found to be less than 0.1. However, it is observed from the experimental condition the stability of the sample was found to be stable for at least 15 hour at room temperature (~25°C).

Accuracy

The recovery studies during the method was evaluated by preparing sample solution spiked with known amount of PTSA at different concentration levels in the range between 50%, 100% and 150% with respect to Valganciclovir concentration. Each concentration of sample solution was prepared in triplicate and analyzed as per the method. The percent recovery of PTSA was found to be in the range of 92.2 to 96.7, mean percent recovery was 95.0, when calculated against the known added amount, indicating that the method is accurate. Table 3, describes the experimental results obtained from accuracy analysis.

Robustness

To assess the robustness of the method, experimental conditions were altered for the following parameters between the specified range for temperature (±5°C), flow of mobile phase (±10%) and wavelength (±5nm). The result obtained from the robustness indicated that, the experimental method parameters were tolerance limit with minor changes to optimize the method.

Table 3, Experimental data obtained from Accuracy analysis

Component	Specification level [c]		
Amount (% w/w)	50%	100%	150%
Added [d]	0.051	0.102	0.151
Found [d]	0.047	0.099	0.146
% Recovery [e]	92.2	97.1	96.7
%R.S.D [f]	2.1	1.8	3.1
Overall statistical data			
Mean		95.3	
SD		1.2	
% R.S.D		2.3	
95% Confidence level (±)		± 3	

[c] specification level 0.1%, [d] n=3, average of three determinations
[e]Average experimental determination, [f] overall %R.S.D.

Stability Studies

To present stability studies on Valganciclovir drug substance for the determination of PTSA content, the analysis were conducted on samples from variable sources of temperature and humidity storage of accelerated (40°C/75%RH), long term (25°C/60%RH) and refrigerated (5°C±3°C) storage condition [22]. The results obtained from the above storage conditions are found to below the limit of detection. Hence formation of PTSA in Valganciclovir drug substances resulting as process related impurity, in view of that the sample shows no degradation profile with respect to storage at different conditions of temperature and humidity. The experimental condition shows precise results with good repeatability on inter and intra day with other analyst and different chromatograph shows the method is rugged for the determination of PTSA content.

CONCLUSION

The proposed simple methodology for quantitative determination of *p*-Toluene sulfonic acid in Valganciclovir drug substance is rapid, accurate, precise and selective .The method provided satisfactory validation data for the tested parameters as per the ICH guidelines. Hence the proposed method may be conveniently used in bulk manufacturing laboratories.

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