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A Simple, Sensitive, Specific Reversed-Phase Ultra Performance Liquid Chromatographic UV Method For Estimation Of Paratoluenesulfonic Acid In Valacyclovir Hydrochloride Sample

Authors & Affiliation:

U. Satyanarayana[1], P. Rakesh Reddy[2], K. Kanthi Reddy[2], K. Rama Krishna[3*].

[1] Waters India Pvt Ltd, H.No-2-11-30, Plot No 11, Rukmini Towers, Paigah Colony, S.P. Road, Secunderabad, 500003, India.

[2] Heterodrugs ltd (R&D), Plot No 80 & 81, APIE, Balanagar, Hyderabad, India.

[3] Department of Chemistry, GITAM University, Rishikonda, Visakhapatnam, India.

Correspondence To:
U. Satyanarayana

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ABSTRACT

A simple, sensitive, accurate and precise Reversed Phase-UPLC- method was developed for quantitave determination of paratoluenesulfonic acid (PTSA) content in Valacyclovir Hydrochloride pharmaceutical drug substance. The RP-UPLC method was developed by using Acquity UPLC BEH C18 100mm x 2.1mm, 1.7µ column and mobile phase consisted of Tetra butyl ammonium hydrogen phosphate buffer and Acetonitrile in the ratio of 79:21v/v, the column temperature was maintained at 25º C. The mobile phase flow rate maintained at 0.20ml/min. The retention time of PTSA is about 8.6 min. The linearity in the concentration range of 0.1ppm to 4.0ppm with R^2 =0.999 (n=5), Limit of detection (LOD) and limit of Quantification (LOQ) values were 0.03ppm and 0.10ppm for PTSA respectively with respect to test concentration of 2.0mg/ml. The validation study was carried out as per international conference on harmonization (ICH) guidelines. The proposed method was found to be specific, precise, linear and accurate and can be applied to determine paratoluenesulfonic acid in commercial batch samples of Valacyclovir Hydrochloride.

INTRODUCTION

Paratoluensulfonic acid (PTSA) is a strong organic acid and is a common reagent used in pharmaceutical industry as acid catalyst for chemical synthesis of drug substances. PTSA is comparable in strength to mineral acids such as sulphuric acid, but are especially suitable for organic reactions where an inorganic, mineral acid could cause charring, oxidation, or an unwanted chemical reaction. The chemical structure of paratoluenesulfonic acid is shown in Figure No -1. The presence of trace level of alcohols like methanol, ethanol and propanol etc., from synthetic reactions will result in the formation of corresponding alkyl tosylates [1-2]. Due to the increasing concern from the regulatory agencies with respect to potential impurities[3-5], a number of sophisticated analytical techniques such as HPLC [6], GC-MS and LC-MS methods are available for estimation of impurities in Valacyclovir HCl drug substances and drug products.

UPLC methods are more sensitive than HPLC because of the holistic design of the system[7], at low volume injections samples in corresponds to HPLC. In the open literature, few RP-UPLC UV detection methods were reported. Hence the aim of the present work is development and validation of simple, accurate and precise reversed phase UPLC UV detection method for determination of PTSA in Valacyclovir Hydrochloride API based on ICH guidelines [8].

MATERIALS AND REAGENTS

Samples of paratoluenesulfonic acid and Valacyclovir Hydrochloride (API) were received from Hetero Drugs Limited, (Balanagar, Hyderabad, India). Tetra butyl ammonium Hydrogen Sulfate AR grade, anhydrous Orhtophosphoric acid (solid) AR grade, Isopropyl alcohol (IPA) HPLC grade, Acetonitrile (ACN) HPLC grade purchased from Rankem (Mumbai), India. High purity water collected using Millipore Milli-Q plus water purification system (Millipore, MA, USA).

CHROMATOGRAPHIC CONDITIONS

The Chromatographic separations were performed by using Acquity Ultra Performance Liquid Chromatography system (Acquity UPLC ™) equipped with Binary Gradient solvent manager and Acquity UPLC PDA detector. The system control, Data acquisition and the collected data were processed through Waters Empower 2 software version Build number 2154. Acquity UPLC BEH C18, 2.1 x 100mm, 1.7µm column (Purchased form Waters Corporation USA). The mobile phase consisted of Tetra butyl ammonium hydrogen phosphate buffer and Acetonitrile in the ratio of 79:21v/v [*]. Mobile phase was degassed and filtered through 0.2µm nylon 66 membrane filter. The mobile phase flow rate was kept at 0.20mL min^{-1}. The column temperature was maintained at 25ºC. Detection was carried out at a wavelength of 222 nm and injection volume was 1.0 µL under PLUNO (Partial Loop with Needle Overfill) mode and the run time is 20.0 minutes.

PREPARATION OF SOLUTIONS

Preparation of Weak needle wash solution
Prepare a mixture of Water and Acetonitrile in the ratio of 90:10 v/v degassed and filtered the mixture through 0.22µ filter.

Preparation of Strong needle wash solution
Prepare a mixture of Acetonitrile and Water in the ratio of 90:10 v/v degassed and filtered the mixture through 0.22µ filter.

Preparation of Buffer
Weighed and transferred about 1.695g of Tetrabutylammonium hydrogen sulfate and 0.49 g of Ortho-Phosphoric acid (Solid) in 1000mL of water mixed and dissolved the contents.

Preparation of Mobile phase
Mobile phase was prepared by mixing Buffer and Acetonitrile in the ratio of 79:21 v/v and the contents were degassed and filtered through 0.22µ filter.

Preparation of Diluent solution
Prepared a 0.2% v/v of Isopropyl alcohol (IPA) in Water and sonicated for 10 minutes in ultrasonic bath.

Preparation of Test solution
Accurately weighed and transferred about 250mg of test sample into a 10mL volumetric flask, and dissolved and made up to the mark with diluent solution .

Preparation of paratoluenesulfonic acid stock solution (2.0 ppm)

Accurately weighed and transferred about 2.0 mg of paratoluenesulfonic acid standard into a 100 mL volumetric flask and dissolved and made up to the mark with diluent solution. Accurately transferred 1.0mL of above solution into a 100mL volumetric flask and diluted up to the mark with diluent solution.

Preparation of paratoluenesulfonic acid standard solution (0.2ppm):

Accurately transferred 0.5mL of above paratoluenesulfonic acid stock solution into a 20mL volumetric flask and diluted up to the mark with diluent solution and the resulting solution is 0.2ppm of paratoluenesulfonic acid.

RESULTS AND DISCUSSION

Experimental design and Validation

The following experimental study parameters were set, such as Limit of detection (LOD), Limit of Quantitation (LOQ), Linearity, Accuracy of paratoluenesulfonic acid content in Valaciclovir Hydrochloride drug substance (API). The specifications set for paratoluenesulfonic acid content in the API should be not more than 0.2ppm. The method was validated in compliance with ICH guidelines (Q2B).

System Suitability parameters

The system suitability test of the proposed chromatographic method was performed before validation runs. Typical UPLC chromatogram is shown in Figure no - 2. The paratoluenesulfonic acid standard solution was injected and confirmed the retention time, theoretical plates and tailing factor and are summarized in Table no-1

LIMIT OF DETECTION (LOD)

Preparation of Limit of detection Solution

Prepared the LOD Limit solution by diluting stock solution of known concentration of paratoluenesulfonic acid near by the expected LOD Limit as signal to noise (s/n) ratio is about 3.0.

Based on the Signal to Noise ratio results obtained from different preparations, the required S/N (about 3.0) corresponding solution concentration is 0.03ppm paratoluenesulfonic acid. Injected 1.0µL of LOD solution for six times into the system and record the Chromatograms and average s/n is found to be 3.57 shown in table no-2.

LIMIT OF QUANTITATION (LOQ)

Based on the Signal to Noise ratio results obtained from LOD solution, LOQ concentration of paratoluenesulfonic acid was prepared by diluting PTSA stock solution appropriately to obtain 0.10ppm concentration, which gave a Signal to Noise ratio of about 10. Injected 1.0µL of LOQ solution for six times into the system and record the Chromatograms and calculated the average s/n is found to be 9.27 shown in table no -3.

LINEARITY

Linearity was established by using 5 point calibration.

The linearity solutions were prepared by appropriate dilution of paratoluenesulfonic acid standard 2.0 ppm solution.

Level -1 the final concentration of 0.10ppm (0.25ml of paratoluenesulfonic acid standard solution in 20ml standard volumetric flask).

Level -2 the final concentration of 0.16ppm (0.40ml of paratoluenesulfonic acid standard solution in 20ml standard volumetric flask).

Level -3 the final concentration of 0.20ppm (0.50ml of paratoluenesulfonic acid standard solution in 20ml standard volumetric flask).

Level -4 the final concentration of 0.30ppm (0.75ml of paratoluenesulfonic acid standard solution in 20ml standard volumetric flask).

Level -5 the final concentration of 0.40ppm (1.00ml of paratoluenesulfonic acid standard solution in 20ml standard volumetric flask).

Procedure

Injected 1.0µL of each level solutions in triplicate and recorded the chromatograms. Overlaid chromatograms shown in Figure No -3. The linearity curve was obtained by plotting the concentration on paratoluenesulfonic acid on X-axis against mean peak area response on Y-axis. The linearity plot shown in Figure No -4. Determined the correlation co-efficient and calculated the %RSD of peak responses from triplicate injections at each level. The linearity correlation co-efficient was found to be 0.999 and the %RSD of peak area responses were less than 5.0 and the results are shown in table no - 4.

ACCURACY

Preparation of Accuracy test solutions

Accurately weighed and transferred about 250 mg of Valacyclovir Hydrochloride in 10 ml standard volumetrric flasks and separately spiked LOQ level-2 (0.16ppm) solution and made up to the mark with diluent in triplicate preparations. Each preparation was injected in to the system and recorded the chromatograms. Accuracy test chromatogram shown in Figure no 5. Calculated the % recoveries of paratoluenesulfonic acid from each preparation. The % recoveries are shown in table no-5.

CONCLUSIONS

The developed reversed-phase UPLC method for estimation of PTSA in Valacyclovir Hyderochlride Active Pharmaceutical Ingredient was validated according to ICH guidelines. The proposed method is 20 minutes run time and is free from interference with respect to the test sample. The validation data indicative of good precision and accuracy and proves the reliability of the method. The developed method can be used to monitor the PTSA content in production batch samples.

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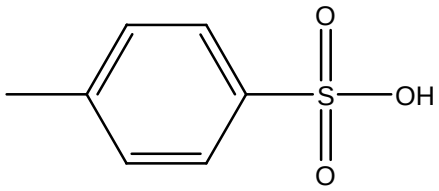
FIGURES



para toluenesulfonic acid

Figure No -1 Structure of Para Toluenesulfonic acid.

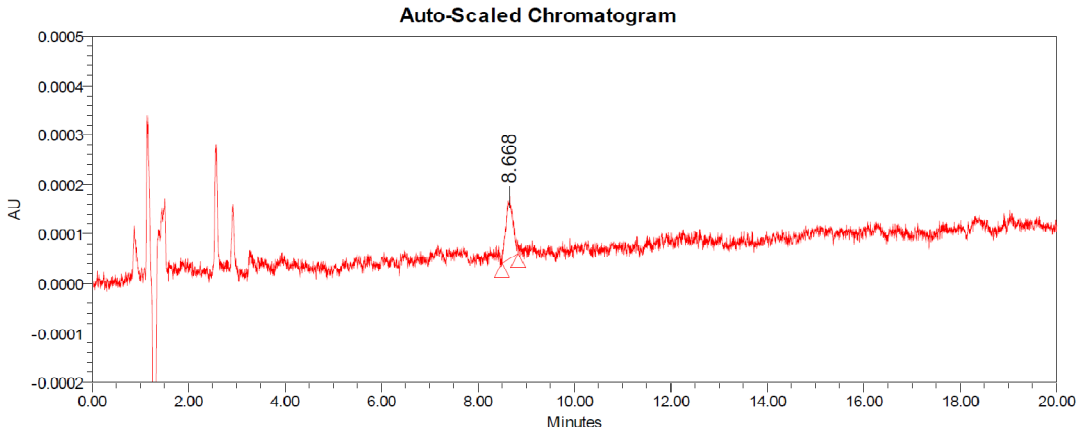


Figure No -2 (Typical UPLC chromatogram)

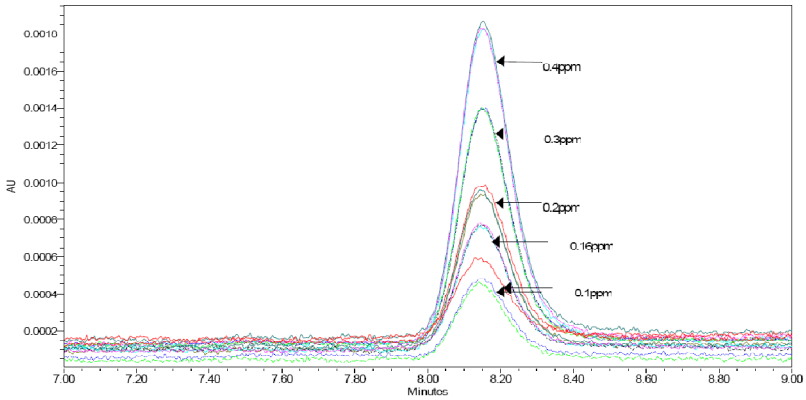


Figure No -3 (linearity overlaid chromatogram).

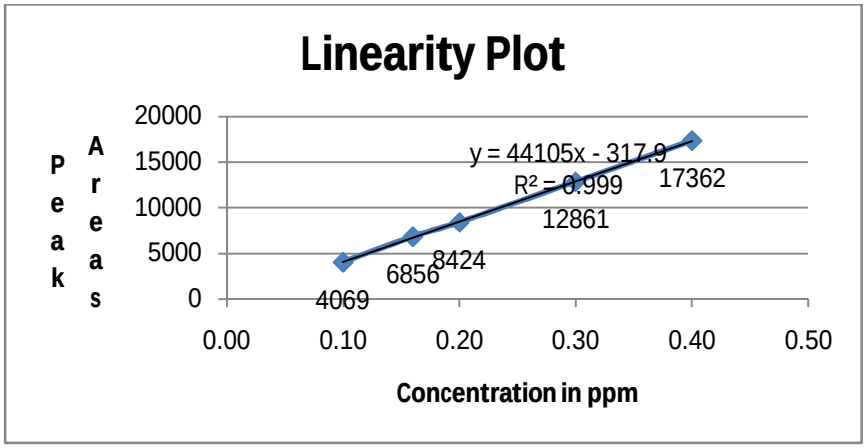


Figure No - 4 Linearity plot

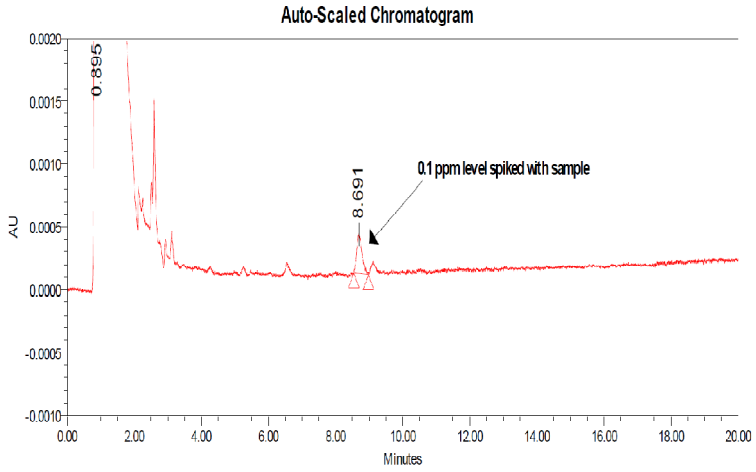


Figure No -5 (Accuracy test chromatogram).

TABLES

Table No-1 System Suitability Test data

Parameter	Value
Retention time	8.6min
Theoretical plates (N)	8430 / column
Tailing factor (T)	1.10

Table No-2 Limit of Detection test data

Component name	Concentration of LOD test solution (in ppm)	Signal to Noise (S/N) ratio
PTSA	0.03	3.57

Table No-3 Limit of Quantitation test data

Component name	Concentration of LOQ test solution (in ppm)	Singal to Noise (S/N)ratio
PTSA	0.10	9.27

Table No - 4 Linearity test data

Level	Concentration in (ppm)	Area of Replicate-1	Area of Replicate -2	Area of Replicate-3	Average	%RSD
Level-1	0.10	4184	4035	3989	4069	2.50
Level-2	0.16	6866	6935	6768	6856	1.22
Level-3	0.20	8257	8452	8564	8424	1.84
Level-4	0.30	12797	12937	12849	12861	0.55
Level-5	0.40	17652	17230	17203	17362	1.45
Correlation Coefficient				0.999		

Table No -5 Accuracy test data

Accuracy at QL Level	**% of Recovery**
Preparation-1	83
preparation-2	17
preparation-3	80

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