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Original Article

A New Stability Indicating Ultra Performance Liquid Chromatography-PDA Method for the Estimation of Valganciclovir in Bulk and Tablet Dosage Form

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ABSTRACT

Background: The present article described about the systemic development of ultra-performance liquid chromatography (UPLC) method for the quantitative determination of valganciclovir in bulk and dosage form. The subsequent validation and degradation study was also performed. **Methods:** The chromatographic Separation was achieved with an HSS (100x2.1 mm, 1.8m).column with an isocratic mobile phase containing a mixture of 0.01N potassium dihydrogen orthophosphate and acetonitrile (55:45 v/v). The flow rate of the mobile phase was 0.3 ml/min with a column temperature of 30°C and detection wavelength at 254 nm. The developed method was validated according to the ICH guidelines with respect to linearity, accuracy, precision, specificity, detection limits and robustness. **Results:** The precision of the results, stated as the %RSD was below 1.0%. The accuracy of the method demonstrated at three levels in the range of 50%, 100% and 150% of the specification limit. The calibration curve was linear over a concentration range from 25 to 150 µg/ml with a correlation coefficient of 0.9997. The recovery of valganciclovir was found to be in the range of 98 to 102%, whereas the detection limits were found to be 0.933 and 2.827 µg/ml. Forced degradation study was carried out under acidic, alkaline, oxidative, photolytic and thermal conditions to prove the stability-indicating ability of the developed UPLC method. **Conclusion:** The method is validated according to the ICH guidelines and it is applied successfully for the determination of valganciclovir in tablets.

Key words: Valganciclovir, UPLC, Validation, Degradation studies.

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INTRODUCTION

Today's pharmaceutical industries are looking for new ways to cut cost and shorten time for development of drugs while at the same time improving the quality of their products and analytical laboratories are not exception in this trend. Though high performance liquid chromatography (HPLC) is a well-established reliable technique used in controlling the quality and consistency of active pharmaceutical ingredients (API's) and dosage forms, it is often a slow technique because of the complexity of some of the samples, it could still be improved. Ultra-performance liquid chromatography (UPLC), has proven one of the most promising developments in the area of fast chromatographic separations with its unique characteristics of high chromatographic resolution, speed, and sensitivity analysis.[1] UPLC instrumentation involves a Binary solvent manager, sample manager, detector. There are various types of UPLC techniques were found, which include Normal phase chromatography (NP-UPLC), Size exclusion chromatography, Ion exchange chromatography, Reverse phase chromatography (RP-UPLC) and Bio-affinity chromatography. Chromatographic methods are generally used for the quantitative and qualitative estimation of raw materials, drug products, drug substances and compounds in biological fluids.[2]

Valganciclovir HCl (VGC), chemically L-Valine, 2[(2-amino-1, 6-dihydro-6-oxo-9H-purin-9-yl) methoxy]-3-hydroxypropyl ester, monohydrochloride is a white to off-white crystalline powder with a molecular formula of $C_{14}H_{22}N_6O_5 \cdot HCl$ and a molecular weight of 390.83 It is an antiviral medication used to treat cytomegalovirus infections.[3] The mechanism of action of valganciclovir is that exists as a mixture of two diastereomers. After oral administration, both diastereomers are rapidly converted to ganciclovir by intestinal and hepatic esterases, which have demonstrated anti-viral activity against cytomegalovirus infections. Valganciclovir is available as tablet dosage form in the market. The drug was official in USP NF. Literature study on the reactivity of the valganciclovir in various solutions proved that the drug is susceptible to degradation in solutions with different pH. Extensive iterature survey reveals that various analytical methods *viz* UV Spectrophotometry and isocratic RP-HPLC were reported for the estimations of valganciclovir in bulk and pharmaceutical dosage form.[4] There are very limited works that have been done on this drug by HPLC. The every reported methods have its own limitations related to retention time, sensitivity, involvement of costly mobile phase and no method has been stated by UPLC technique. Thus the objective of work was to minimize the retention time using ultra performance technique with short length column and to so that develop a sensitive reliable, validate and stability indicating RP-UPLC method for the determination of valganciclovir in bulk drug and dosage form.[5]

MATERIALS AND METHODS

Instrumentation

The analysis of the drug was carried out on a Waters Acquity UPLC system (Milford, MA, USA)[6] with Auto Injector and TUV Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Valganciclovir solutions. The pH of the solutions was measured by a pH meter (thermo scientific), sonicator (ultrasonic sonicator), microbalance (sartorius) and vacuum filter pump were used.

Chemicals and reagents

Valganciclovir was obtained as a gift sample from Mylan Laboratories Ltd., Hyderabad. Potassium di-hydrogen orthophosphate and acetonitrile were purchased from RFCL, Rankeem Limited. HPLC grade water, glacial acetic acid, and acetonitrile were obtained from Rankem, Avantor

Performance Material India Limited. High purity water was obtained by using Millipore Milli Q Plus water purification system.

Chromatographic conditions

The method was developed by using an HSS 100 x 2.1 mm, 1.8µ. column with an isocratic mobile phase containing a mixture of 0.01N potassioum dihydrogen orthophosphate and acetonitrile (55:45v/v). The mobile phase was filtered through the 0.22µ filter under vacuum filtration. Flow rate of the mobile phase was 0.3 ml/min. The column temperature was maintained at 30°C and the eluted compounds were monitored at the wavelength of 254 nm. The sample injection volume was 3µl.

Preparation of diluents

The diluent used for the analysis was prepared by using homogenous mixture of 500 ml of water and 500 ml acetonitrile.

Preparation of mobile phase

A mixture of 0.01N potassium dihydrogen orthophosphate and acetonitrile in the ratio of 55:45 (v/v) and the mixture was filtered through 0.22µ membrane filter under vacuum filtration.

Preparation of standard solution (Valganciclovir 450µg/mL)

45mg valganciclovir standard was weighted and transferred into a 10 ml clean and dry volumetric flask, add 7ml of diluent, sonicated for 30 min and make up to the final volume with diluents. 1 ml was pipette out from the above stock solution and transferred in to a 10ml volumetric flask and volume was made up to the mark with diluent.

Preparation of sample solution

5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 5ml of diluent was added and sonicated for 30 min, volume was made up using diluent and filtered. From the above filtered solution 1ml was pipette out into a 10 ml volumetric flask and volume was made upto mark using diluent.

Method validation

As per ICH guidelines for the determination of valganciclovir the described method has been validated for the related substances by UPLC determination.[7]

Linearity

Linearity test solutions for related substance were prepared by diluting the impurity stock solution to the required concentrations. The solutions were prepared at six concentration levels ranging from 25µg/ml to 150µg/ml. The data were subjected to statistical analysis using a linear-regression model.[8]

Precision

Precision was determined as repeatability and intermediate precision by analyzing the samples in accordance with ICH guidelines. The Precision of the method was determined by injecting standard solution of Valganciclovir for six times and measure the area for all six injections in UPLC chromatographic system.[9]

Accuracy

The accuracy of the method was determined for valganciclovir by recovery experiments. Known amount of valganciclovir bulk sample (test preparation) in triplicate at three concentration levels 50%, 100%, and 150% of the specified limit were taken for analysis and the percentage of recoveries were calculated.[10]

LOD and LOQ

The LOD and LOQ of valganciclovir were determined by using signal to noise approach as defined in ICH guidelines. The LOD and LOQ were assessed at signals to noise ratio of 3:1 and 10:1 respectively by injecting dilute solution of drug was injected into the chromatograph and signal to noise (S/N) ratio was calculated.[11]

Robustness

This parameter is used to measure the capacity of the developed to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness was performed by injecting the valganciclovir standard solution in to the UPLC by altering the flow rate, column temperature and also by changing the composition of the organic solvent from the normal chromatographic conditions.[12]

Degradation studies

All forced degradation studies were performed at an initial drug concentration of 450µg/ml^{-1} in mobile phase and the degradation studies of valganciclovir was carried out under conditions of acid degradation studies were performed in (1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60c), alkali degradation studies were performed in (1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60c), oxidative studies were performed in (1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60c), dry heat degradation studies were performed in (The standard drug solution was placed in oven at 105^{0}C for 1 h to study dry heat degradation) and photo stability studies were performed in (The photochemical stability of the drug was also studied by exposing the 4500µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1hrs or 200 Watt hours/m^2 in photo stability chamber.) Neutral degradation studies were performed in (Stress testing under neutral conditions was conducted by refluxing the drug in water for 1 hr at a temperature) Samples were withdrawn at proper time, cooled, and neutralized by adding base or acid and subjected to UPLC analysis after suitable dilution.[13]

Assay of Marketed dosage form

Twenty tablets from each brand (VALGAN) were procured, weighed and crushed to a fine powder. The powder equivalent to 25 mg Valganciclovir was accurately weighed into a 25 ml volumetric flask and made up to volume with mobile phase. The contents of the volumetric flask were sonicated for 30 min to enable complete dissolution of Valganciclovir. The solution was filtered and the filtrate was diluted with mobile phase. 10µL of these solutions were injected into the system, chromatogram was obtained and result was calculated.

RESULTS AND DISCUSSION

Method Development

Based on drug solubility and Pka value following conditions has been used to develop the method estimation of valganciclovir**.** Different chromatographic conditions were experimented to achieve better efficiency of the chromatographic system. Parameters such as mobile phase composition, column, detection wavelength, column temperature, pH of mobile phase and diluents were optimized. Various proportions of buffer, and solvents (water, acetonitrile and methanol) were evaluated in order to achieve suitable composition of the mobile phase. Choice of retention time, tailing, theoretical plates, and run time were the major tasks while

developing the method. Finally valganciclovir eluted with good peak shape and retention time and tailing was passed using 0.01N KH_2PO_4: Acetonitrile (55:45), with a flow rate of 0.3mL/min. The retention time obtained for valganciclovir is 0.855. Quantification was achieved at UV detection at 254 nm based on peak area. The method was validated as per ICH guidelines. The optimised chromatogram was shown in Figure 1.

Method validation

The developed method was applied for the estimation of drug in marked dosage form, and the average percentage assay was found 99.29, with relative standard deviation 0.19 which is found within the limit and shows the suitability of developed method for the estimation of dosage form. The assay chromatogram was depicted in Figure 2 and result was shown in Table 1. The System suitability parameters such as tailing factor (1.5), retention factor (0.855), plate number (9195.3), RSD (1.0), standard deviation (771.8) were evaluated for six replicate injections of the drug. Which indicates the suitability of the system for this optimised condition. The results were given in Table 2. The linearity was determined for six concentrations and the correlation coefficient was found to be 0.999 for valganciclovir which is within the specified limits. It showed that the developed method followed Beer-Lambert's law within the range of 25–150µg/ml. And it follows linear regression equation y=291.4x+789.3.The least squares method was used to establish the regression line and the curves were linear. The linearity data was

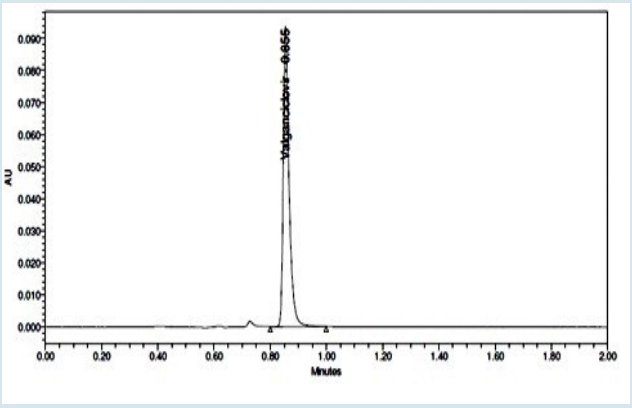

Figure 1: Optimized chromatogram of valganciclovir.

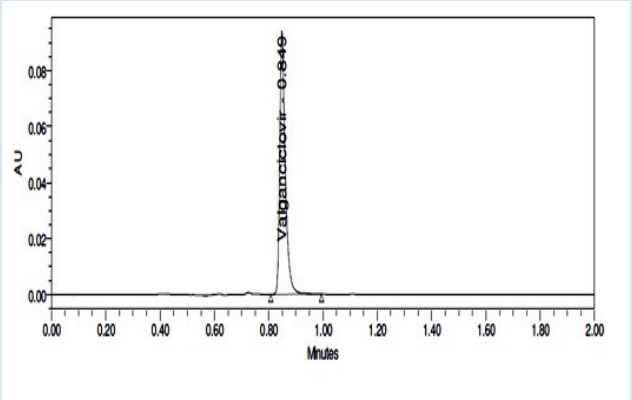

Figure 2: Assay chromatogram of marketed dosage form of valganciclovir.

Table 1: Assay results of marketed dosage form.

Formulation	Content	Amount found	% Recovery
Valgan (cipla)	450mg	445.63	98.44

Table 2: System suitability study of valganciclovir.

Peak name	Valganciclovir
RT	0.855
Area(mean)	134251
USP plate count	9195.3
USP tailing	1.5
Standard deviation	771.8
%RSD	0.6

Table 3: Linearity data of valganciclovir.

Linearity Level (%)	Concentration (µg/mL)	Area
0	0	0
25	12.5	34860
50	25	67159
75	37.5	96134
100	50	132753
125	62.5	165064
150	75	197103

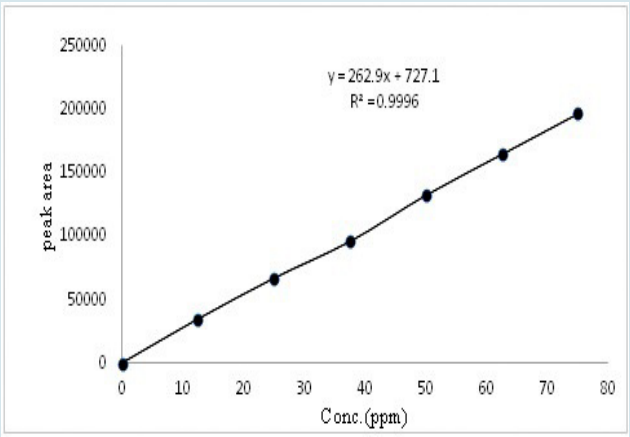

Figure 3: Linearity of the developed method for valganciclovir.

shown in Table 3 and linearity graph was shown in Figure 3. Both precision and accuracy were determined with standard quality control samples. And known samples of valganciclovir prepared in triplicates at three different concentration levels 50%, 100%, 150%, covering the linearity range. And subjecting the samples to the proposed UPLC method. The % recovery was found to be 99.13 to 99.90 which is within the limits as shown in Table 4 and the % RSD was not more than 2.0%, indicates the proposed method is highly accurate. For the study of repeatability under precision six working sample solutions of 450 µg/mL are injected and the % Amount found was calculated and %RSD was found to be 0.2 and the results of precision were shown in Table 5. For the intermediate precision the % amount found was calculated and %RSD was found to be 0.6 and the results of precision were shown in Table 6. And the minimum variation in % RSD indicates the present

Table 4: Accuracy results of valganciclovir.

% Level	Amount spiked (µg/ml)	Amount Recovered (µg/ml)	Mean % Recovery
	50	99.45	
50%	50	99.77	
	50	99.13	
	100	99.74	
100%	100	99.90	99.44%
	100	98.99	
	150	99.19	
150%	150	99.52	
	150	99.26	

Table 5: Repeatability data of the precision

S.NO	Peak area
1	128339
2	127290
3	128758
4	128742
5	127148
6	128627
Average	128151
Standard deviation	738.5
%RSD	0.6

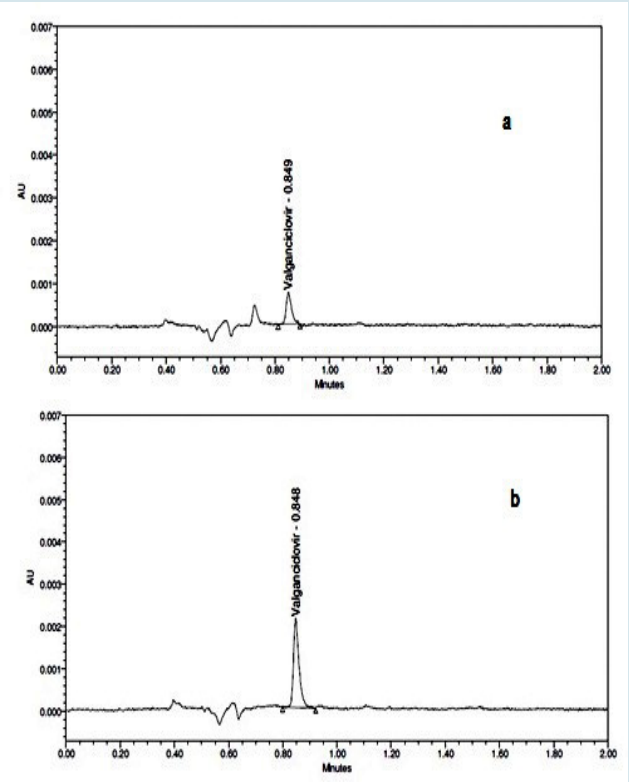

Figure 4: LOD (a) and LOQ (b) chromatogram of valganciclovir.

Table 6: Intermediate precision of valganciclovir.

S.NO	Peak area
1	131927
2	131966
3	132028
4	132192
5	132559
6	131868
Average	132092
Standard deviation	254.7
%RSD	0.2

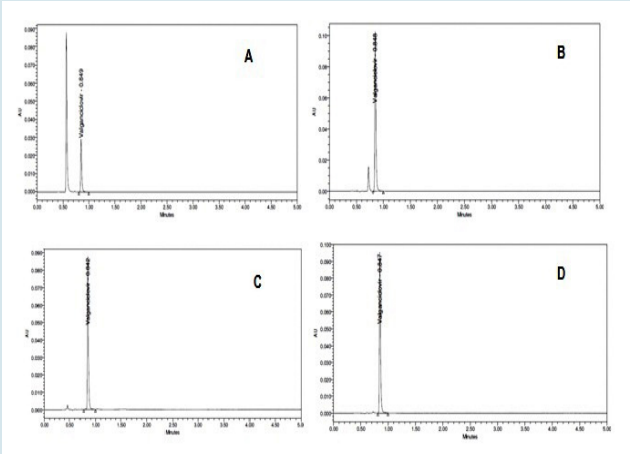

Figure 5: Chromatogram of acid (A), alkali (B), oxidation (C) and thermal (D) degradation samples of valganciclovir.

Table 7: Degradation study results.

S.NO	Degradation condition	% drug degraded
1	Acid	4.63
2	Alkali	3.94
3	Oxidation	3.11
4	Thermal	2.16
5	UV	1.22
6	Water	0.30

method was precise. LOD and LOQ are based on analyte concentration of signal to noise ratios of 3:1 for LOD and 10:1 for LOQ respectively. And the LOD and LOQ were found to be 0.933µ/ml and 2.827µg/ml respectively. The injection volume used in this method was only 3.0µl. It proves the effectiveness and sensitivity of the method. The LOD and LOQ chromatogram were shown in Figure 4a and 4b. The ability of this method to separate and accurately measure the peak of interest indicates the specificity of the method Robustness was carried out by changing three parameters from the chromatographic conditions such as changes in mobile phase composition (±5%), changes in flow rate (±0.1ml/min), and column temperature(±5°C). And the tailing factor was found to be less than 2.0. And the results were proved the method was robust. Degradation studies of valganciclovir were done and drug was degraded under the influence of acid, alkali, oxidation, thermal, photolytic and

water were found to be 4.53, 3.94, 3.11, 2.16, 1.22, 0.30, respectively and in all conditions purity threshold was more than purity angle and within the acceptable range. The chromatograms shown in Figure 5. And the degradation data was shown in Table 7. It indicates the method was selective.

CONCLUSION

In this present study the adopted chromatographic conditions were, stationary phase HSS (100mm x 2.1mm1.8µ), Mobile phase 0.01N KH_2PO_4: acetonitrile in the ratio of 55:45 and flow rate was maintained at 1ml/min, detection wave length was 254 nm, column temperature was set to 30°C and diluent was mobile phase Conditions were finalized as optimized method. System suitability study was performed by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 25% to 150 % levels, R^2 value was found to be as 0.999. Precision was found to be 0.2 for repeatability and 0.6 for intermediate precision.LOD and LOQ are 0.933µg/ml and 2.827µg/ml respectively. By using above method assay of marketed formulation was carried out 99.29% was present. In the degradation studies of valganciclovir, all conditions purity threshold was better than purity angle and found within the acceptable range. The Proposed method was found to be simple, accurate, precise, sensitive, quick and this method can be used for routine analysis of valganciclovir.

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CONFLICT OF INTEREST

The authors declared no conflict of interest in the manuscript

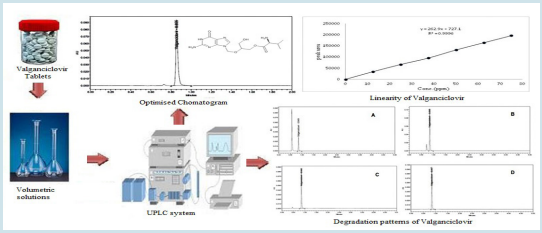
ABBREVIATIONS

UPLC: Ultra Performance Liquid Chromatography; **VGC:** Valganciclovir; **ICH:** International Council for Harmonization; **LOD:** Limit Of Detection; **LOQ:** Limit Of Quantitation; **RSD:** Relative Standard Deviation.

REFERENCES

1. Raja AP, Venkateshwar RJ. Development and validation of new RP-UPLC method for the quantitative determination of olanzapine in tablet dosage form. Asian J Pharm Clin Res. 2013;6(3):178.
2. Garg R, Singh N, Srinivas SK, Deb B, Ahmed A. UPLC method development and validation for Cefditoren Pivoxil in active pharmaceutical Ingredient. J Appl Pharm Sci. 2011;1(7):149 .
3. Annapurna MM, Lakshmi SK, Sirichandra M. Stability indicating liquid chromatographic method for the quantitative determination of valganciclovir in pharmaceutical dosage forms. J Drug Deliv and Ther. 2013;3(3);64.
4. Krishna VN, Gowramma B, Madhuri L, Gouthami B, Sindhur NN, Meyyanathan SN. Development and Validation of a Stability Indicating RP-HPLC Method for the Determination of Valganciclovir Hydrochloride (RS). Research and Reviews. J Pharm Anal. 2014;3:19.
5. Ponnekanti K, Sundararajan R. Development and validation of new RP-UPLC method for the determination of cefdinir in bulk and dosage form. Int J Pharm Pharm Sci. 2018;10:178.
6. Antil P, Kaushik D, Jain G, Srinivas KS, Thakur I. UPLC Method for Simultaneous Determination of Valsartan and Hydrochlorothiazide in Drug Products. J Chromatogr Sep Tech. 2013;4(5):1. http://dx.doi.org/*10.4172/2157-7064.*
7. Rohith T, Ananda S, Sajan PG, Gowda NM. Development and Validation of UPLC Method for the Determination of Duloxetine Hydrochloride and Its Impurities in Active Pharmaceutical Ingredient. J Anal and Bioanal Tech. 2013;6:1. http://dx.doi.org/10.4172/2155-9872.1000234.
8. Shashikant BL, Jadhav SA, Vishwambar SP, Solanki PV, Bembalkar RS. Vijayavitthal T. Mathad Development and Validation of RP-UPLC Method for the Determination of Iloperidone, Its Related Compounds and Degradation Products in Bulk and Dosage Form. Am J Anal Chem. 2014;5(14)969. http://dx.doi.org/10.4236/ajac.2014.514104.
9. Reddy NN, Rao JV. Uplc method development and validation for the estimation of risedronate in formulation. Asian J Pharm Clin Res. 2013;6:124.
10. Gupta H, Aqil M, Khar RK, Ali A, Sharma A, Chander P. Development and Validation of a Stability-Indicating RP-UPLC Method for the Quantitative Analysis of Sparfloxacin. J Chromatogr Sci. 2010;48(1):1. http://dx.doi.org/1013144.
11. Seshukumar D, Abhishek GG, Kona SS, Gupta RS, Semwal VP. Development and Validation of Stability Indicating RP-UPLC Method for Simultaneous Determination in Fixed Dose Combination of Ezetimibe and Simvastatin. J Chromatogr Sep Tech. 2012;3(131):1. doi:10.4172/2157-7064.1000131.
12. Reddy GN, Rajendra PVVS, Maharana PK. Development and validation of a stability indicating UPLC method for determination of erlotinib in pharmaceutical formulations. Der Pharm. Chem 2012;4(6):2288.
13. Kanakapura BV, Hosakere DR, Xavier CM, Pavagada JR, Raghu MS. A Stability Indicating UPLC Method for the Determination of Tramadol Hydrochloride: Application to Pharmaceutical Analysis. Chromatogr Res Int. 2012;14:1. http://dx.doi.org/10.1155870951.

PICTORIAL ABSTRACT



SUMMARY

- The present research work was designed to develop a UPLC method for the estimation of Valganciclovir. The chromatographic Separation was achieved with an HSS (100x2.1 mm, 1.8m), column with an isocratic mobile phase containing a mixture of 0.01N potassium dihydrogen orthophosphate and acetonitrile (55:45 v/v). The developed method was validated as per ICH guidelines. The result (%RSD) of the each validated parameters were within the limit. Force degradation studies show various degradation patterns of Valganciclovir. The developed method was applied successfully for the determination of Valganciclovir in tablet dosage form.

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Dr. Prasenjit Mondal is an Associate Professor at the Vaageswari College of Pharmacy, Ramakrishna Colony Karimnagar. His doctoral work focused on the Bioanalytical method development and validation in human plasma using LC-MS/MS and pharmacokinetic study. He has published more than 25 research articles in various high impact international and national journals. He guided more than 23 M.Pharm students.

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