Abstract

From the Literature study it is unable to find the combined HPLC methods for Acyclovir and Valacyclovir using HPLC-UV detector. However, an analytical method for individual analysis for Acyclovir and Valacyclovir was published. So It is felt necessary that to develop HPLC method for quantitative determination of Acyclovir and Valacyclovir. It was also found that there are some analytical methods reported for the Acyclovir and valacyclovir separately and most of the works reported were done on the biological fluids.

Based upon the data of previous literature review and results of published methods, the trials of method development taken and finalized the method for validation of assay and related substances with degradants study. The finalized method is achieved on HPLC column Inertsil cyno column, (250×4.6mm dimensions) having particle size 5µm, with flow rate as 0.8 ml/ min and column oven temperature as 50º C. The buffer was prepared by dissolving 1.00 g of Ammonium acetate in 1000 ml water, filtered through 0.45µm membrane filter and degassed in ultrasonic bath prior to use as mobile phase A. Acetonitrile is the organic solvent used as mobile phase B in isocratic mode. The injection volume amounted to 5µl. The analysis was carried out under isocratic condition as mobile phase A: Mobile phase B is (95:5). Detection was monitored at the wavelength of 254 nm. Diluent used was a mixture of acetonitrile and water in the ratio of (50:50) in the preparation of analytical solutions.

According to the International Conference on Harmonization (ICH) guidelines taking into consideration the specified limits for Acyclovir and Valacyclovir Tablets and based upon the results obtained from the method validation are summarized below,

The method of assay was validated for Specificity and system suitability was demonstrated by comparing chromatograms of diluent, actives, the related impurities and the placebo (containing all the ingredients of the formulation except the analytes) of the tablets as per the procedure applied to sample solution. System suitability was performed by injecting resolution solution and determining resolution between closely eluting peak of Acyclovir, Acyclovir impurity A, Valacyclovir and Valacyclovir related compound C. The parameters such as tailing factor, theoretical plates were checked in resolution solution. The standard solution for Acyclovir and Valacyclovir was prepared twice and injected. The parameters such as similarity factor, and % RSD for peak area responses and retention time of Acyclovir and Valacyclovir were determined. Results for resolution, system suitability are checked and found within limit.
In the chapter of linearity the concentration of standard solution is serially diluted to 50, 60, 80, 100, 120, 140 & 150% of assay concentration (working level 200 ppm for Acyclovir and 500 ppm for Valacyclovir) was prepared and injected. The method is linear in the range of 100.0 µg/ml to 300.0 µg/ml for Acyclovir and 250.0 µg/ml to 750.0 µg/ml for Valacyclovir of the specified limit of the respective analyte. The linearity curve is plotted between y-axis in mean response of injection replicate (peak area of Acyclovir and Valacyclovir) and x-axis in respective serial concentration (in ppm). The Linear co-efficient (R2) and the % y-intercept are to be determined and found within limit.

In accuracy and recovery chapter working level concentration of standard solution that is 50, 100 & 150 % of assay concentration prepared and injected. Both the Analytes showed the recovery between 98% to 102 % concluding the accuracy of the method. In precision chapter method was studied for repeatability and intermediate precision. Samples of zovirax and valtrax tablets showed assay value within the limit, hence the precision of the method was done and % RSD of by analyzing sample of zovirax and valtrax tablets six times separately. The RSD for recovered Acyclovir and Valacyclovir were well within the limit of 2.00% confirming the precision of the method. It was determined on six separate sample solutions prepared from same batch by a different analyst using different mobile phase, diluents preparation and same instrument on a different day with different lot of same brand column. The overall RSD was evaluated and were within the acceptance criterion of NMT 2.0%.

In solution stability, Stability of solutions in Room temperature from 0th hr ,2nd hr,4th hr,6th hr,8th hr, 12th hr, 16th hr, 20th hr and then up to 24th hrs. System suitability solution, Standard solution, sample solution and Placebo solution preparation is as per specificity chapter and the results of stability of sample solutions were determined at time periods representative for storage. The stability of sample solution was confirmed on the sample by comparing the values of 0th sample at different time interval to its initial value. The % relative difference should not be more than 2.00. The standard solution was found to be stable till 24 hrs since the % RSD of the peak area response of Acyclovir and valacyclovir is not more than 2.0 %. The absolute difference of % assay in sample solution was not more than 2.0 %. Indicating that the sample solution is also stable for 24 hrs.

In robustness chapter the method is deliberate varied chromatographic conditions carried out (mobile phase flow rate is varied by +0.1 ml and -0.1 ml and column temperature is varied by +2
and -2 degree), The resolution had no significant changes, when the parameters were modified. Method robustness shows that the minor change of the operational parameters does not affect the chromatographic separation. It was determined on three separate sample solutions prepared from same batch. The % RSD was evaluated and was within the acceptance criterion of NMT 2.0%. The cumulative % RSD was evaluated with the method precision samples and were within the acceptance criterion of NMT 2.0%.

Acyclovir drug substance, Valacyclovir drug substance, Zovirax and Valtrax tablets, (200/500) mg, and Placebo for Acyclovir and Valacyclovir tablets were individually subjected to acidic, basic, oxidative, thermal and photolytic degradation to obtain degradants impurities in at least one of the forced degradation conditions. The Purity Angle value is required to be less than Purity threshold value in order to demonstrate the absence of co-elution and specificity of the method. The chromatograms acquired were processed for Peak Purity of the Acyclovir and Valacyclovir peak in the range of 210-400 nm to demonstrate the specificity. Based upon the observations, Zovirax and Valtrax tablets, 200/500 mg, are comparatively stable in Oxidation, Hydrolysis and Photolytic degradation conditions. The peak purity is confirmed from the peak angle and peak threshold value for Acyclovir and Valacyclovir peak in degradation samples indicates absence of co-elution (Peak angle should be less than peak threshold). Thus, illustrating the stability indicating nature of the method for the determination of Assay in Zovirax and Valtrax tablets, also specific and selective.

In related substances and degradation of impurities of acyclovir and valacyclovir the method is validated for specificity and system suitability, there were no peaks due to the Mobile phase, Diluent and Placebo at the retention time of Acyclovir, Valacyclovir and their respective impurities. Thus, illustrating the HPLC method is specific. In LOD and LOQ, a series of Standard Solutions of Acyclovir, Acyclovir imp A, Valacyclovir and Valacyclovir related comp. C were prepared. The results for limit of detection are given in method validation report, which are well within the limit. i.e. signal to noise ratio is equal to or above 3. (concentration is 0.15 ppm for Acyclovir, 0.1 ppm for Acyclovir imp A, 0.15 ppm Valacyclovir and 0.1 ppm Valacyclovir related comp. C). The results for limit of quantitation are given in method validation report, which are well within the limit. i.e. signal to noise ratio is equal to or above 10. (concentration is 0.5 ppm for Acyclovir, 0.3 ppm for Acyclovir imp A, 0.5 ppm Valacyclovir and 0.3 ppm Valacyclovir related comp. C). In linearity, the concentration of serial standard solution
is LOQ, 50, 60, 80, 100, 120, 140 & 150% of assay concentration (working level 5 ppm for Acyclovir, 3 ppm of Acyclovir impurity A, 5 ppm of valacyclovir and 3 ppm for Valacyclovir related compound C) was prepared and injected. The linearity curve is plotted between y-axis in mean response of injection replicate (peak area of Acyclovir, Acyclovir impurity A, Valacyclovir and Valacyclovir related comp C) and x-axis in respective serial concentration (in ppm). The Linear co-efficient (R2) and the % y-intercept are to be determined. The regression coefficient ($r^2$) for Acyclovir, Acyclovir impurity A, Valacyclovir and Valacyclovir related compound C was determined as 1.00, 1.00, 1.00 and 1.00 respectively, the % Y-intercept for Acyclovir, Acyclovir impurity A, Valacyclovir and Valacyclovir related compound C was 1.85%, -0.94%, 2.38% and 0.95% respectively, the Response factor for Acyclovir, Acyclovir impurity A, Valacyclovir and Valacyclovir related compound C was 5.55%, 5.61%, 6.64% and 6.42% respectively. In accuracy and recovery impurity is spiked at concentration of standard solution is LOQ, 50,100 & 150 % of concentration (working level) prepared in sample solution and injected. The accuracy was expressed as percent recovered at each level of concentration and as overall accuracy when the data are normalized to percent theoretical. The accuracy study of the Acyclovir impurity A and Valacyclovir related compound C was determined for the following levels, LOQ, 50%, 100% and 150% of the specified limit. Both the impurities showed the recovery between 85% to 115 % for LOQ and 90% to 110% for 50%, 100% and 150% level concluding the accuracy of the method. The results for both the analytes and impurities are within the acceptance criteria.

Precision of the method was studied for repeatability and intermediate precision. Samples of zovirax and valtrax tablets does not shows any one of the impurity present in the sample hence in the accuracy and recovery chapter these known impurities were spiked for six replicates and the %RSD of that results are calculated for method precision chapter. The RSD for recovered Acyclovir impurity A and Valacyclovir related compound C were well within the limit of 5.00% confirming the precision of the method. It was determined on six separate sample solutions prepared from same batch by a different analyst using different mobile phase, diluents preparation, same instrument on a different day with different lot of same brand column. The overall RSD of % recovery of known impurity was evaluated and were within the acceptance criterion of NMT 5.00%. Stability of solutions in Room temperature from 0th hr, 2nd hr, 4th hr, 6th hr, 8th hr, 12th hr, 16th hr, 20th hr and then up to 24th hrs. The stability of sample solutions were
determined at time periods representative for storage. The stability of sample solution was confirmed on the sample by comparing the values of 0th sample at different time interval to its initial value. The % difference for recovery of impurity should not be more than 5.00%.

In robustness chapter the method is deliberate varied chromatographic conditions carried out (mobile phase flow rate is varied by +0.1 ml and -0.1 ml and column temperature is varied by +2 and -2 degree), The resolution had no significant changes, when the parameters were modified. Method robustness shows that the minor change of the operational parameters does not affect the chromatographic separation. It was determined on three separate sample solutions prepared from same batch. The % RSD was evaluated and was within the acceptance criterion of NMT 5.0%. The cumulative % RSD was evaluated with the method precision samples and were within the acceptance criterion of NMT 5.0%.

Acyclovir drug substance, Valacyclovir drug substance, Zovirax and Valtrax tablets, (200/500) mg, and Placebo for Acyclovir and Valacyclovir tablets were individually subjected to acidic, basic, oxidative, thermal and photolytic degradation to obtain degradants impurities in at least one of the forced degradation conditions. The Purity Angle value is required to be less than Purity threshold value in order to demonstrate the absence of co elution and specificity of the method. The chromatograms acquired were processed for Peak Purity of the Acyclovir and Valacyclovir peak in the range of 210-400 nm to demonstrate the specificity. Based upon the observations, Zovirax and Valtrax tablets, 200/500 mg, are comparatively stable in Oxidation, Hydrolysis and Photolytic degradation conditions. The peak purity is confirmed from the peak angle and peak threshold value for Acyclovir and Valacyclovir peak in degradation samples indicates absence of co elution (Peak angle should be less than peak threshold).

The proposed HPLC-MS/MS method can be regarded as selective, accurate, precise, and valid for determination of acyclovir with a total running time of 2.0 min. Through this method it was possible to evaluate, acyclovir quantification in human plasma and offers advantages over methods previously reported. The present method is more sensitive, while the analytical run is shorter, permitting a high throughput. A simple, sensitive and selective LCMS/MS method has been developed for the determination of acyclovir in human plasma. Since acyclovir is a polar compound and soluble in aqueous medium and practically insoluble in most of organic solvents its analysis in biological fluids in currently published HPLC methods, involve pre-treatment of acyclovir plasma sample including deproteinization or solid phase extraction, Acyclovir in plasma were concentrated by solid phase extraction and chromatographed on a C18 column using
a mobile phase of 0.1% formic acid: methanol (30: 70% v/v). The method was validated over a linear range of 20-1000 ng/ml for acyclovir. The LOQs were 60.0 and 900 ng/ml. The validated method was applied for the quantitation of acyclovir from plasma samples in a pharmacokinetic study.

The analytical method used for the Assay and Related substances of Acyclovir and Valacyclovir in Zovirax and Valtrax tablets complied with the acceptance criteria set for the analytical parameters: Specificity & System suitability, Limit of detection and Limit of quantitation, Linearity, Precision-Repeatability, Accuracy and Recovery, Precision-Intermediate precision, Stability of sample and standard solution and Robustness studies. The analytical method used for the force degradation study of Assay and Related substances of Acyclovir and Valacyclovir in Zovirax and Valtrax tablets complied with the acceptance criteria. Hence the method could be used for routine analysis.