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# ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF VALACYCLOVIR IN DOSAGE FORMS BY UPLC TECHIQUE

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**ARTICLE INFO**

**Key Words**

Ultra Performance Liquid Chromatography (UPLC), Acetonitrile, Potassium dihydrogen ortho phosphate, valacyclovir

**ABSTRACT**

The aim of the technique was to develop a simple, accurate, fast, accurate and validated Ultra Performance Liquid Chromatographic (UPLC) method for coinciding valacyclovir analysis in the form of pharmaceutical dosing. The technique was enhanced and the chromatograms also showed smart resolution, retention period, peak response and lowest noise control line ratio by working 70 percent OPA (0.1 percent): 30% Acetonitrile at a wavelength of 254 nm with UV detector usage. The valacyclovir retention time was 1.069 min with a flow rate of 0.3 ml / min. The present technique was valid for Percent Assay, precision, accuracy, linearity, LOD, and LOQ. The percentage of the valacyclovir assay was 99.80%. The one-dimensionality shown by the medication at a level varies valacyclovir (12.5-75µg / ml) shows severe regression coefficient of 0.999. UPLC technique was developed and valid as per the ICH guidelines for coinciding valacyclovir estimation in Pharmaceutical.

## INTRODUCTION

Velpatasvir are white and light tan crystalline powder, both the medicine are freely soluble in methanol and practically insoluble in water[1,2] . It inhibits genotype one to six hepatitis c virus (HCV) RNA replicons in-vitro and has high sustained virologic response (SVR) rates[3,4]. The literature survey on UPLC and spectrophotometric methodology for Velpatasvir and with alternative medicine has been extensively studied[5,6,11]. From the literature survey, it has been noted that High performance liquid chromatographic activity were reportable within the estimation of Velpatasvir[7,9,10]. Thus this analysis paper describes the assessment of Velpatasvir tablet dosage form adopting Ultra performance liquid chromatographic (UPLC) methodology.

## MATERIALS AND METHOD:

**Instrumentation:** Electronics Balance, Denver meter -BVK enterprises, IndiaUltrasonicator-BVK enterprises, WATERS UPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software, UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Valacyclovir solution[8].

**Materials and Reagents**: Valacyclovir pure drugs (API), Single drug Valacyclovir tablets (VALTREX), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium

dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Ranke

**Stock and Sample solution preparations:** Valacyclovir accurately weighed 25 mg was transferred to 50ml clean dry volumetric flask, 3/4th of the diluents were added to the volumetric flask and sonicated for 10 minutes. Flask was made of diluents and branded as Standard Stock Solution. (Valacyclovir: 500µg / ml).

Preparation of sample solution: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred to a 100 ml volumetric flask, 50 ml of diluents were added and sonicated for 25 minutes, and the volume was diluted and filtered with UPLC filters (500 µg / ml Valacyclovir)

## RESULTS AND DISCUSSION:

10µL of the blank, stock and sample were administered into the chromatographic system and areas for the peak were used for computation.

**Assay:** Six linear concentrations of **Valacyclovir** (12.5-75µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Valacyclovir was y = 19604x + 5864.5 Correlation coefficient obtained was 0.9993 for the drug.

**PROCESS VALIDATION:** The projected Ultra Performance liquid chromatographic (UPLC) process valid in sight of ICH pointers concerning following aspects: System suitableness, linearity, accuracy, precision, limit of quantification (LOQ), limit of detection (LOD), specificity and robustness.

**System suitability:** All the system suitability parameters were within the range and satisfactory as per ICH guidelines[10].

**Specificity:** Validation technique was checked for interference. This optimized technique was specific since at the time of retention of each medicine there were no interference peaks noted in blank and placebo.

**Linearity:** Six linear Valacyclovir concentrations (12.5-75 µg / ml) were injected in duplicate form. The above mentioned average areas were given and the linearity equations obtained for Valacyclovir were y = 19604x + 5864.5 for the drug was 0.9993 for the correlation coefficient.

**Precision:** From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.3% for Valacyclovir and .As the limit of Precision was less than "2" the system precision was passed in this method

**Accuracy:** Three levels of Accuracy samples were prepared using normal method of addition. For each level of accuracy, triplicate injections were given and mean percent recovery for Valacyclovir was obtained as 100.24 per cent.

**Robustness:** Robustness conditions like Flow minus (0.2ml/min), Flow plus (0.4ml/min), mobile phase minus (75B:25A), mobile phase plus (65B:35A), temperature minus (27°C) and temperature plus(33°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

## CONCLUSION:

The predicted UPLC methodology was found to be straightforward, precise, reliable, accurate, fast, and economical for concurrent vlacyclovir dose type estimation. The Sample recovers all of the said formulations in smart compliance with their individual label statements, and the predicted methodology was accurate as all of the correct parameters were specified according to ICH tips. This methodology may be suggested in forms of pharmaceutical dose valacyclovir for internal control testing method.

**Table no.1: Assay Data of valacyclovir**

| S.no | Standard Area | Sample area | % Assay |
|---|---|---|---|
| 1 | 967834 | 961299 | 99.61 |
| 2 | 966062 | 965044 | 100.00 |
| 3 | 964949 | 966652 | 100.17 |
| 4 | 960516 | 964144 | 99.91 |
| 5 | 964920 | 961469 | 99.63 |
| 6 | 960187 | 960083 | 99.49 |
| Avg | 964078 | 963115 | 99.80 |
| Stdev | 3077.0 | 2549.1 | 0.3 |
| %RSD | 0.3 | 0.3 | 0.3 |

**Fig.No.1 Optimized Chromatogram**

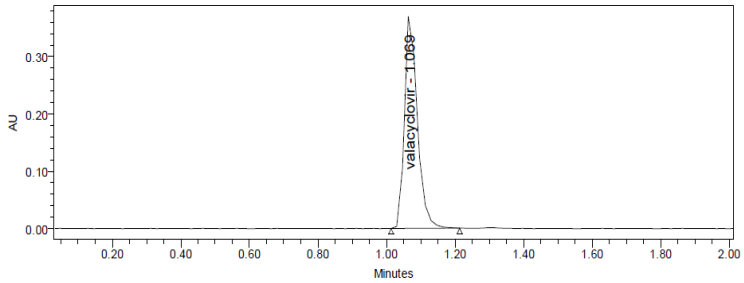



**Table no.2: System suitability**

| S no | Valacyclovir | | |
|---|---|---|---|
| Inj | RT(min) | USP Plate Count | Tailing |
| 1 | 1.069 | 3887 | 1.28 |
| 2 | 1.069 | 3954 | 1.29 |
| 3 | 1.070 | 4004 | 1.28 |
| 4 | 1.071 | 3715 | 1.29 |
| 5 | 1.071 | 3558 | 1.28 |
| 6 | 1.071 | 4055 | 1.27 |

**Fig no.2: System suitability chromatogram**

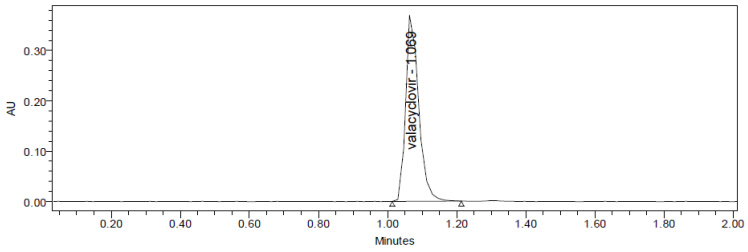


**Fig no.3: Calibration curve of valacyclovir**

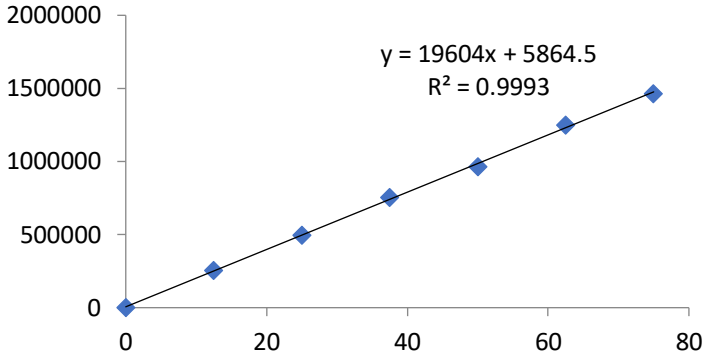



**Table no.3: Precision**

| S. No | Area Of Valacyclovir |
|---|---|
| 1. | 967834 |
| 2. | 966062 |
| 3. | 964949 |
| 4. | 960516 |
| 5. | 964920 |
| 6. | 960187 |
| Mean | 964078 |
| S.D | 3077.0 |
| %RSD | 0.3 |

**Table no.4: Accuracy**

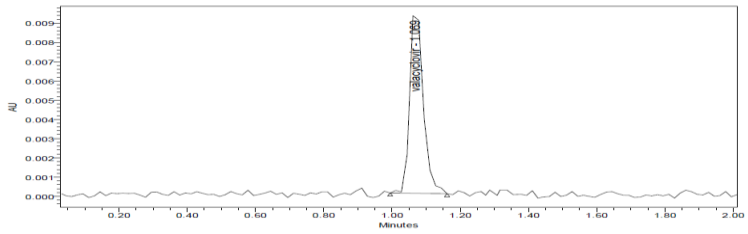
| % Level | Amount Spiked (µg/mL) | Amount recovered (µg/mL) | % Recovery | Mean %Recovery |
|---|---|---|---|---|
| 50% | 25 | 25.00 | 100.01 | 100.24% |
| | 25 | 25.18 | 100.72 | |
| | 25 | 24.75 | 99.00 | |
| 100% | 50 | 50.44 | 100.89 | |
| | 50 | 50.54 | 101.08 | |
| | 50 | 50.37 | 100.75 | |
| 150% | 75 | 74.62 | 99.50 | |
| | 75 | 74.88 | 99.84 | |
| | 75 | 75.28 | 100.37 | |

**Table no.5: Robustness**

| S.no | Condition | %RSD of Valacyclovir |
|---|---|---|
| 1 | Flow rate (-) 0.2ml/min | 0.3 |
| 2 | Flow rate (+) 0.4ml/min | 0.4 |
| 3 | Mobile phase (-) 75B:25A | 0.1 |
| 4 | Mobile phase (+) 65B:55A | 0.4 |
| 5 | Temperature (-) 27°C | 1.0 |
| 6 | Temperature (+) 33°C | 1.0 |

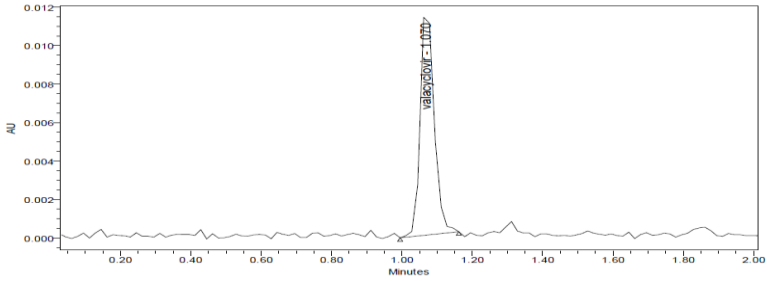
**LOD sample Preparation:** LOD was reported as 0.39µg / ml for valacyclovir

**Fig no.4: LOD Chromatogram**




**LOQ sample Preparation:** The valacyclovir LOQ was noted as 1.17 µg / ml

**Fig no.5: LOQ Chromatogram**

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**Conflict of interest:** The authors declare no conflict of interest.

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