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# ANALYTICAL METHOD DEVELOPMENT AND VALIDATION BY NEW RP-UPLC METHOD FOR THE DETERMINATION OF DOLUTEGRAVIR SODIUM IN TABLET DOSAGE FORM

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**ABSTRACT**

A simple accurate, precise rapid isocratic UPLC method development for the simultaneous estimation of Dolutegravir Sodium in tablet dosage form. The chromatographic system was carried on Acquity BEH C18 (50*3.0mm. 1.7µm) using mobile phase consisting a mixture of 70 volmes of Dipotassium hydrogen orthophosphate of 30 volumes of Methanol, with detection of 260 nm. The retention time of Dolutegravir Sodium was found to be 2.857 min calibration curve was linear over the concentration range of Dolutegravir Sodium, the correlation coefficient for both peak was found to be 0.998 respectively. All the analytical validation parameters were determined and found in the limit as per ICH guidelines.

**Keywords**: Dolutegravir Sodium, UPLC.

## INTRODUCTION

### Ultra Performance Liquid Chromatography

Chromatography is a non-destructive procedure for resolving a multi-component mixture of traces, minor or constituents in to individual fractions [1]. It is a method of separating a mixture of components in to individual components through a porous medium under the influence of solvent [2-5]. For many years, researchers have looked at "fast LC" as a way to speed up analyses. The need for speed, the availability of affordable and easy to use mass spectrometers. Smaller columns and faster flow rates (amongst other parameters) have been used [6]. Elevated temperature, having the dual advantages of lowering viscosity, and increasing mass transfer by increasing the diffusivity of the analytes, has also been investigated [7]. However, using conventional particle sizes and pressures, limitations are soon reached and compromises must be made, sacrificing resolution. HPLC technology simply doesn't have the capability to take full advantages of sub-2µm particles. UPLC can be regarded as new invention for liquid chromatography [8].

## REVIEW OF LITERATURE

Srinivasa Rao Avanapu, A simple and rapid high performance liquid chromatographic method was developed and validated for simultaneous estimation of abacavir, lamivudine and dolutegravir in their tablet dosage form [9]

P. Saidulu The objective of this research was to develop simple, rapid, precise, accurate and economical stability-indicating reversed phase (RP) HPLC assay method and validated for simultaneous estimation of Lamivudine, Abacavir and Dolutegravir in bulk laboratory synthetic mixture and their combined dosage form [10].

Jomol Joseph A new method was established for simultaneous estimation of Dolutegravir and Rilpivirine by RP-HPLC method. The - Validation of Analytical Procedures: Text and Methodology [11].

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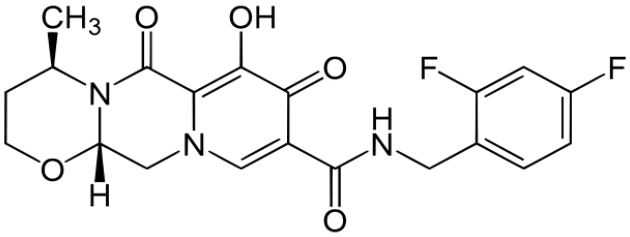
chromatographic conditions were successfully developed for the separation of Dolutegravir and Rilpivirine.

**Drug Profile**

Dolutegravir is a HIV-1 intergrase inhibitor that blocks the strand transfer step of the integration of the viral genome into the host cell




## MATERIALS & METHODS

**Table 1. Instrumentation**

| | |
|---|---|
| UV-Visible | Nicolet evolution 100 |
| UV-Visible | Vision Pro |
| UPLC software | Open lab EZ chrome |
| UPLC | Agilent Technologies |
| Ultra sonicator | Citizen, Digital Ultrasonic |
| pH meter | Global digital |
| Electronic balance | Mettler Toledo |
| Syringe | Hamilton |
| UPLC Column | Inertsil ODS |

**Table 2. Reagents and Chemicals**

| | |
|---|---|
| Water | HPLC Grade |
| Methanol | HPLC Grade |
| Potassium Dihydrogen | AR Grade |
| Acetonitrile | HPLC Grade |
| Dipotassium hydrogen | AR Grade |
| Orthophosphoric acid | HPLC Grade |

**Working/Reference Standards**

Dolutegravir sodium (API) Gift samples obtained from Chandra Labs, Hyderabad.

## MATERIALS & METHODS:

**Preparation of Standard Solution of Dolutegravir Sodium:**

Weigh accurately 10 mg of Dolutegravir sodium in 25 ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 20 µg/ml of Dolutegravir sodium is prepared by diluting 0.5ml to 10ml with mobile phase. This solution is used for recording chromatogram.

**Preparation of Sample Solution of Dolutegravir Sodium**

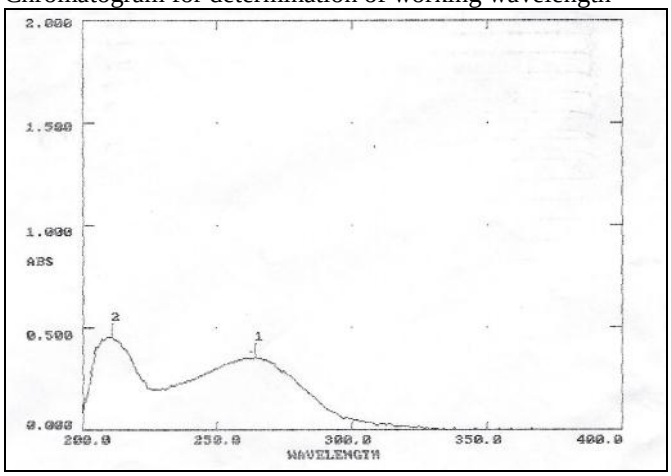
Weigh accurately 10 Tablets Instgra – 50 mg weigh accurately 10 mg of Dolutegravir sodium in 25 ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 20 µg/ml of Dolutegravir sodium is prepared by diluting 0.5ml to 10ml with mobile phase. This solution is used for recording chromatogram.

**Table 3. Chromatographic Conditions**

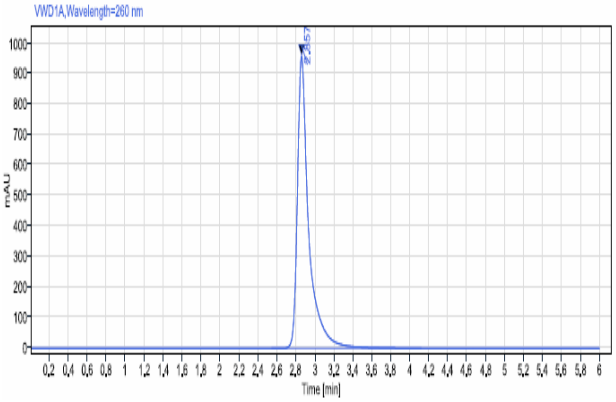
| | |
|---|---|
| Mobile phase | $K_2$ $HPO_4$: Methanol (70 : 30) |
| pH | 3.0 |
| Column | Acquity BEH C18 (50*3.0mm. 1.7µm) |
| Flow rate | 1.0 ml/min |
| Column temperature | Room temperature(20-25ºC) |
| Sample temperature | Room temperature(20-25ºC) |
| Wavelength | 260 |
| Injection volume | 20 µl |
| Run time | 6 min |

## RESULT AND DISCUSSION

Chromatogram for determination of working wavelength

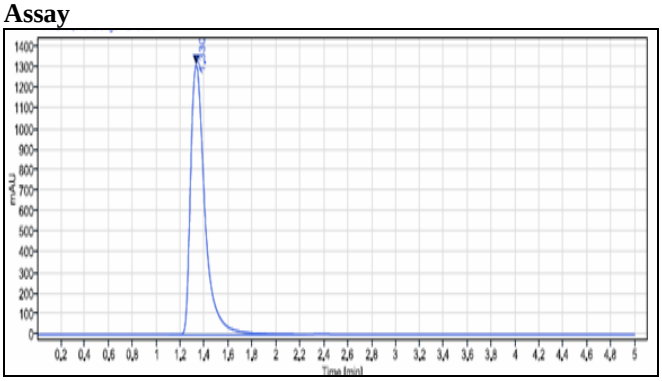



**Chromatogram For Optimized Concentration**



| S.No. | Name | Rt (min) | Peak Area | Theoritical Plates | Tailing Factor | Resolution |
|---|---|---|---|---|---|---|
| 1 | **DOLUTEGRAVIR SODIUM** | 2.857 | 7974.22 | 3875 | 1.65 | - |

**Assay**



**Chromatogram of Assay sample preparation**

**Table 4. Assay Results**

| **Dolutegravir sodium** | | |
|---|---|---|
| | **Standard Area** | **Sample Area** |
| **Injection-1** | 7972.48 | 7955.89 |
| **Injection-2** | 7967.99 | 7957.5 |
| **Injection-3** | 7968.81 | 7961.37 |
| **Injection-4** | 7970.96 | 7952.04 |
| **Injection-5** | 7978.3 | 7960.09 |
| **Average Area** | 7971.71 | 7957.38 |
| **Standard deviation** | 4.09 | |
| **%RSD** | 0.05 | |
| **Assay(%purity)** | 99.82 | |

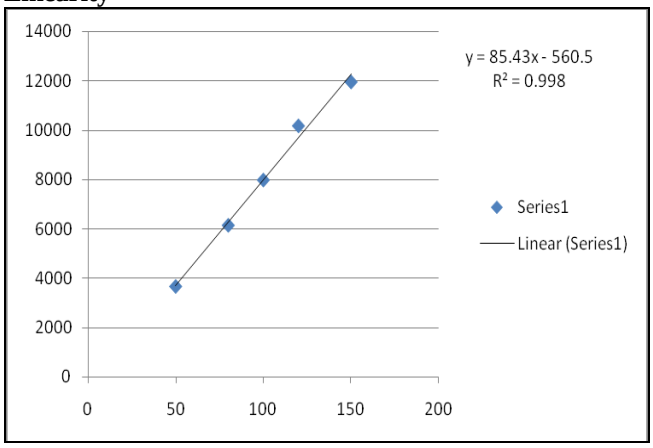
**Table 5. Accuracy**

| % Recovery Level | Area | Concentration Added | Concentration Recovered | %Recovery | Average |
|---|---|---|---|---|---|
| 50% _01 | 7004575 | 250 | 252.18 | 100.9 | |
| 50% _02 | 7020900 | 250 | 252.77 | 101.1 | |
| 50% _03 | 7002470 | 250 | 252.11 | 100.8 | |
| 100% _01 | 13910853 | 500 | 500.83 | 100.2 | |
| 100% _02 | 13902676 | 500 | 500.53 | 100.1 | 100.5 |
| 100% _03 | 13701006 | 500 | 493.27 | 98.7 | |
| 150% _01 | 21010188 | 750 | 756.42 | 100.9 | |
| 150% _02 | 21026894 | 750 | 757.02 | 100.9 | |
| 150% _03 | 21021825 | 750 | 756.84 | 100.9 | |

**Table 6. Method precision**

| Dolutegravir sodium | | |
|---|---|---|
| S.No. | RT | AREA |
| 1 | 2.857 | 7984.67 |
| 2 | 2.857 | 7975.66 |
| 3 | 2.856 | 7972.43 |
| 4 | 2.857 | 7970.17 |
| 5 | 2.856 | 7967.67 |
| 6 | 2.857 | 7975.88 |
| AVG | 2.856 | 7974.41 |
| SD | 0.0004 | 5.93 |
| %RSD | 0.014 | 0.074 |

**Linearity**




**ROBUSTNESS**

**Table 7. Result of Robustness Study**

| **Chromatographic changes** | | **Rt(min)** | **Tailing Factor** | **Theoretical Plates** | **%RSD for Standard areas** |
|---|---|---|---|---|---|
| Flow rate (mL/min) | 0.8 | 4.290 | 1.58 | 4686 | 0.16 |
| | 1.2 | 2.143 | 1.66 | 3339 | 0.24 |
| Temperature | 35 | 2.882 | 1.67 | 3943 | 0.08 |
| | 45 | 2.839 | 1.68 | 3807 | 0.02 |

**Table 8. Ruggedness**

| **DOLUTEGRAVIR SODIUM** | **%Assay** |
|---|---|
| **Analyst 01** | 99.77 |
| **Analyst 02** | 99.80 |
| **%RSD** | 0.13 |

## DISCUSSION

### Assay

The amount of Dolutegravir sodium present in the taken dosage form was found to be 99.82 % respectively.

### Accuracy

The percentage mean recovery of Dolutegravir sodium is 100.50%.

### System Suitability

The % RSD for the retention times and peak area of Dolutegravir sodium were found to be less than 2%.

**Linearity and Range**

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of Dolutegravir sodium is 0.998.

**Precision**

Test results for Dolutegravir sodium are showing that the %RSD of Assay results are within limits.

**Robustness**

The system suitability parameters were within limit at all variable conditions.

**Ruggedness**

The % RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged.

## CONCLUSION

The validated method is found to be Specific, Linear, Precise, Accurate, Robust and Rugged for the estimation of Dolutegravir sodium in tablet dosage form.

Hence it is concluded that the assay method is found to be valid in terms of reliability, precision, accuracy and specificity for routine analysis as well as for stability analysis.

## ACKNOWLEDGEMENT

Nil

## CONFLICT OF INTEREST

No interest

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