New Method for the Estimation and Validation of Fostemsavir and Ganciclovir by using RP-HPLC

Srinivasa Rao Surabhi*, Neelu Jain
Department of Chemistry, Sri Satya Sai University of Technology and Medical Sciences, Sehore-466001, Madhya Pradesh, India

ABSTRACT

We have evolved a completely unique, reliable HPLC technique for simultaneous quantification of Fostemsavir and Ganciclovir. The chromatographic detachment was attained on an X-Bridge phenyl column (150x4.6mm, 3.5 μ) using isocratic elution with a buffer containing 0.1% OPA and acetonitrile proportion of 50:50 as movable part with a stream of 1 mL/min at room temperature. The maximum absorbance of the drugs was observed at 236 nm. Dissolve 1mL of orthophosphoric acid in 1 lt of HPLC marked water and sieved by using 0.45 μ filter paper, this solution was used as a buffer. 10 min run time was used to separate Fostemsavir and Ganciclovir. Analysis was achieved within 15 min over honest linearity within the concentration range from 6-90 μg/mL of Fostemsavir and 2.5-37.5 μg/mL of Ganciclovir. To check the system suitability parameters, the normal solution was injected six times, from the outcomes, it was concluded that all the outcomes were well under the acceptable range. Precision and recovery study results were well under a suitable range. From this technique, an assay of Fostemsavir and Ganciclovir was performed and the results were well under the acceptable range. Degradation studies were carried out on Fostemsavir and Ganciclovir, with a purity threshold greater than the purity angle in all conditions and within the acceptable range. The above-mentioned technique was validated according to ICH guidelines.

*Corresponding Author
Name: Srinivasa Rao Surabhi
Phone: 7659827321
Email: srini.analytical@gmail.com

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INTRODUCTION

Ganciclovir, marketed under the brand name Cytovene, among others, is an antiviral (Hayden and de Jong, 2011; Pillay and Zambon, 1998) drug used to treat cytomegalovirus (Marti-Carreras and Maes, 2019; Mattes et al., 2000) (CMV) infections. Ganciclovir is generally associated with a variety of serious drugs. Common drug reactions (≥ 1% of patients) include granulocytopenia (Breedveld et al., 2017), neutropenia (Donadieu et al., 2017), anaemia (Stein et al., 2016), thrombocytopenia (Ahmed et al., 2007), fever, nausea, vomiting, dyspepsia (Duncanson et al., 2018), diarrhoea, abdominal pain, flatulence (Bailey et al., 2009), anorexia (Espie and Eisler, 2015), increased liver enzymes, headache, confusion, hallucination (Chaudhury et al., 2009), seizures (Greenhalgh et al., 2020), pain and phlebitis (Nisio et al., 2015) at the injection site (due to high pH), sweating, rash, itching, increased levels of serum creatinine (McDonald et al., 2012) and blood urea.

Fostemsavir, marketed with a trade name of Ruko-
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It is an antiretroviral (Cohen et al., 2016) medication for adults living with HIV/AIDS who have tried multiple HIV medications (Savarino and Shytaj, 2015) and whose HIV infection cannot be effectively treated with other therapies due to resistance, aversion or safety considerations. Nausea is the most common adverse reaction. Significant adverse reactions included rises in liver enzymes among participants already infected with the hepatitis B or C virus and changes in the immune system (immune reconstitution syndrome) (Krueger and Majde, 2003). Fostemsavir is an inhibitor of HIV and a prodrug of temsavir (BMS-626529). Fostemsavir is a type 1 human immunodeficiency virus (HIV-1) gp120-directed attachment inhibitor. Figure 1 shows the chemical structures of Fostemsavir and Ganciclovir.

**EXPERIMENT**

**Chemicals and Reagents**

Acetonitrile, Orthophosphoric acid and water (HPLC mark) took from Merck (Mumbai, India). The APIs (Fostemsavir and Ganciclovir) took from Dr Reddy’s Laboratories, Hyderabad.

**Equipment**

An HPLC system (Waters alliance e2695 model) consisting of a quaternary pump, PDA detector-2998 was used. Data processing was performed with Empower 2.0 software.

**Chromatographic Conditions**

The chromatographic detachment was achieved in isocratic mode at room temperature using an X-bridge phenyl column (150x4.6 mm, 3.5 μ). A mixture of acetonitrile and 0.1 percent orthophosphoric acid (OPA) in 50:50 v/v at a stream of 1 mL/min was used as a movable phase. The total time of 10 min was used to separate the selected drugs Fostemsavir and Ganciclovir.

**Preparation of Buffer**

1 mL of OPA is dissolved in 1 liter water (HPLC mark) and filtered by using 0.45 μ filter paper.

**Diluent**

Movable part was used as diluent.

**Standard Preparation**

Carefully weigh and transfer weight similar to 60 mg of Fostemsavir tablet powder and 25 mg of Ganciclovir capsule powder in a flask of 100 mL and add 70 mL of diluent. Sonicated to melt and dilute up to the mark with diluent. 5 mL of the above prepared stock solution was taken and diluted to 50 mL with diluents.

**RESULTS AND DISCUSSION**

Figure 1: Structural representations of (A) Fostemsavir and (B) Ganciclovir

Figure 2: Chromatogram of system suitability

Figure 3: Chromatogram of blank

Figure 4: Calibration plots of (A) Fostemsavir and (B) Ganciclovir

The current study was planned to develop an easy, accurate and fast systematic HPLC method for the simultaneous estimation of Fostemsavir and Ganciclovir. The chromatographic conditions have been
### Table 1: Optimized Chromatographic Conditions

| Parameter                          | Proposed Method                                      |
|------------------------------------|------------------------------------------------------|
| Column                             | X-Bridge phenyl (150x4.6 mm, 3.5 μ)                  |
| Mobile Phase                       | 0.1% Ortho phosphoric acid: Acetonitrile (50:50)     |
| Injection capacity                 | 10 μl                                                |
| Stream                             | 1.0 mL/min                                           |
| Temperature of column              | 25°C                                                 |
| Absorbance                         | 236 nm                                               |
| Run Time                           | 10.0 min                                             |
| Elution time of Fostemsavir        | 6.932 min                                            |
| Elution time of Ganciclovir        | 3.152 min                                            |

### Table 2: Results of System Suitability

| Parameter     | Fostemsavir | Ganciclovir |
|---------------|-------------|-------------|
| Theoretical plates | 6261        | 3395        |
| USP Tailing   | 1.02        | 1.01        |
| USP Resolution | 15.14       | -           |
| Elution time  | 6.932       | 3.152       |

### Table 3: Outcomes of Linearity

| S. No | Fostemsavir | Ganciclovir |
|-------|-------------|-------------|
|       | Concentration (μg/mL) | Area | Concentration (μg/mL) | Area |
| 1     | 6.00        | 358697      | 2.50        | 164921 |
| 2     | 15.00       | 915146      | 6.25        | 435210 |
| 3     | 30.00       | 1585421     | 12.50       | 865142 |
| 4     | 60.00       | 3452174     | 25.00       | 1754821 |
| 5     | 75.00       | 4265201     | 31.25       | 2150364 |
| 6     | 90.00       | 5104273     | 37.50       | 2581637 |

### Table 4: Outcomes of Method Precision

| S. No | Region of Fostemsavir | Region of Ganciclovir |
|-------|------------------------|------------------------|
| 1     | 3452187                | 1745289                |
| 2     | 3452610                | 1725104                |
| 3     | 3415827                | 1798658                |
| 4     | 3432506                | 1748562                |
| 5     | 3455852                | 1752379                |
| 6     | 3462516                | 1732605                |
| Average | 3445250              | 1750433                |
| Std. dev | 17555.234          | 25763.520              |
| % RSD  | 0.51                  | 1.47                   |
Table 5: Results of Intermediate Precision

| S.No. | Area of Fostemsavir | RSD | Region of Ganciclovir | RSD |
|-------|---------------------|-----|-----------------------|-----|
| 1     | 3451271             |     | 1725936               |     |
| 2     | 3456238             |     | 1765234               |     |
| 3     | 3422562             | 0.63| 1732569               | 1.25|
| 4     | 3425174             |     | 1785642               |     |
| 5     | 3465923             |     | 1745216               |     |
| 6     | 3475824             |     | 1748575               |     |

Table 6: Results of Accuracy

| Accuracy | Amount of Fostemsavir | % Recovery | Amount of Ganciclovir | % Recovery |
|----------|-----------------------|------------|-----------------------|------------|
| 50*      | 30                    | 99.8       | 12.5                  | 98.9       |
| 100*     | 60                    | 99.6       | 25                    | 99.4       |
| 150*     | 90                    | 99.9       | 37.5                  | 99.7       |

* Results are mean recovery of three sample preparations

Table 7: Outcomes of robustness

| Variable             | % RSD of Fostemsavir | % RSD of Ganciclovir |
|----------------------|----------------------|----------------------|
| Stream (0.8 mL/min)  | 0.48                 | 0.54                 |
| Stream(1.2 mL/min)   | 0.35                 | 0.72                 |
| Org part (45:55)     | 0.79                 | 0.28                 |
| Org part (55:45)     | 0.26                 | 0.61                 |

Table 8: Outcomes of forced degradation

| Stress Parameter                               | % of Degradation |       |
|------------------------------------------------|------------------|-------|
| Acid deg (1N HCl)                             | 11.6             | 13.9  |
| Alkali deg (1N NaOH)                          | 11.9             | 13.1  |
| Peroxide degradation (30% Peroxide)           | 13.4             | 12.5  |
| Reduction degradation (30% sodium bi sulphate)| 12.2             | 12.1  |
| Thermal (sample, 70°C, 6 Hrs)                  | 10.8             | 11.2  |

optimised to provide good assay efficiency. Various combinations of Fostemsavir and Ganciclovir have been tried to optimise the mobile process. The final mobile step of the activity is orthophosphoric acid (0.1 per cent) and acetonitrile (50:50 v/v). The mobile step of each drug was selected on the basis of its polarity. A wavelength of 236 nm was chosen because the two drugs showed strong absorption at this 236 nm wavelength. The rate of flow was 1.0 mL/min. Fostemsavir and Ganciclovir peaks were eluted at 3.152 min, 6.932 min, respectively. The proposed approach is checked by all analyses within limits set out in the ICH guidelines. 10 min run time was used to separate Fostemsavir and Ganciclovir peaks. The optimised chromatography conditions are shown in Table 1.

System Suitability

The normal solution has been introduced into the HPLC system and the system suitability parameters are within the range. Table 2 shows the effects of the device suitability and Figure 2 shows the typical chromatogram.

Specificity

From the specificity, it was concluded that no blank interference was observed at the retention times of Fostemsavir, Ganciclovir. Figure 3 is a null chromatogram.

Linearity

The linearity concentration of Fostemsavir was prepared in the concentration range of 6-90 μg/ml. The regression equation was estimated to be Y=
56757.94x + 2245.96 and the correlation coefficient ($R^2$) was 0.9995.

The linearity concentration of Ganciclovir was prepared in the concentration range of 1-15 µg/ml.

The regression equation was estimated to be $Y= 69075.53x + 1201.30$ and the correlation coefficient ($R^2$) was 0.9999. Linearity results were shown in Table 3, calibration plots of Fostemsavir and Ganciclovir were shown in Figure 4.

**Precision**

The accuracy of this method has been tested for intraday and intermediate precision variations. Six separate injections of the test solution of Fostemsavir and Ganciclovir was analyzed on the same day under the same experimental conditions were used to test intraday studies. Six separate injections of the test solution of Fostemsavir and Ganciclovir was analyzed on different days, different analyst and different instruments were used to test intermediate precision. Since percentage RSD values were found to be <2%, the method is highly accurate. A good recovery of the drug was gained at individual concentrations, suggesting that the procedure was successful. Outcomes of the precision were given in Table 4.

**Intermediate Precision (Ruggedness)**

In Table 5, intermediate precision results were given.

**Accuracy**

The accuracy of the system was achieved by measuring the recovery experiments at three stages (50 percent, 100 percent and 150 percent). APIs were prepared at concentrations of 30, 60, 90 µg/mL of Fostemsavir and 12.5, 25, 37.5 µg/mL of Ganciclovir. For each spike stage, the test solution was injected three times and the test was performed according to the test process. The recovery percentage, mean and RSD values were determined. The percentage recovery values were found to be in the range of 98-102 percent. Accuracy findings have been shown in Table 6.

**Robustness**

In robustness, there is a minor variation in the flow rate (±0.2 ml) and the organic solvent (±10 percent) in their chromatographic state and there is no substantial improvement in the percent RSD. The robustness findings have been shown in Table 7.

**Degradation Behavior**

Fostemsavir and Ganciclovir samples were exposed to different conditions of forced degradation in order to include partial degradation of the compound. Forced degradation studies have been conducted to demonstrate the method is acceptable for deteriorated goods. In addition, the studies provide details on the conditions under which the drug is unstable such that steps can be taken during formulation to prevent possible instability. The outcomes of forced degradation have been shown in Table 8.

**CONCLUSIONS**

Degradation activity of the drug was studied under hydrolysis (acid, baseline, and neutral) oxidation, photolysis and thermal stress conditions. The drug was best capable under simple, neutral conditions and unstable under oxidative conditions. An isocratic RP-HPLC method for the determination of fostemsavir and Ganciclovir has been and is accurate and is effective. The regression line equation is capable of predicting the drug concentration in the range of 6-90µg/ml of fostemsavir and 1-15µg/ml ganciclovir, respectively, from the peak area obtained. The method has been successfully validated and has allowed accurate, sensitive, robust and precise detection of fostemsavir and Ganciclovir in a popular marketed preparation.

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**Conflict of Interest**

The authors declare that they have no conflict of interest for this study.

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