A new analytical method for determination of ledipasvir and sofosbuvir in pharmaceutical formulations by HPLC method

K. Swathi*, P. Venkateswara Rao, N. Srinivasa Rao

Department of Pharmaceuticals Analysis, vikas college of Pharmacy, Krishna District, Andhra Pradesh, India

ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Sofosbuvir and Ledipasvir in Tablet dosage form. Chromatogram was run through Std Discovery C8 150 x 4.6 mm, 5µ. Mobile phase containing Buffer 0.1% OPA: Acetonitrile taken in the ratio 60:40 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method was 0.1% OPA buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 260 nm. Retention time of Sofosbuvir and Ledipasvir were found to be 2.367 min and 3.436 min. %RSD of the Sofosbuvir and Ledipasvir were and found to be 0.6 and 0.5 respectively. %Recovery was obtained as 99.61% and 99.80% for Sofosbuvir and Ledipasvir respectively. LOD, LOQ values obtained from regression equation of Sofosbuvir and Ledipasvir were 0.67, 2.02 and 0.70, 2.12 respectively. Regression equation of Sofosbuvir is 
\[ y = 4266.x + 7700, \] and 
\[ y = 4861.x + 2656. \] of Ledipasvir.

Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Keywords: Sofosbuvir; Ledipasvir; RP-HPLC.

INTRODUCTION

Development of the simple and reproducible analytical methods for estimation of multi component drugs is very important part of quality control and for social awareness which was established in present work.

Sofosbuvir\(^{[1-3]}\) is a prodrug nucleotide analog used as part of combination therapy to treat hepatitis C virus (HCV) infection or to treat co-infection of HIV and HCV. After metabolism to the active antiviral agent 2'-deoxy-2'α-fluoro-β-C-methyluridine-5' -triphosphate (also known as GS-461203), the triphosphate serves as a defective substrate for the NS5B protein, an RNA-dependent RNA polymerase required for replication of viral RNA. More recently, sofosbuvir has become available as a fixed dose drug combination product with ledipasvir\(^{[1-3]}\) (trade name Harvoni

Figure 1: chemical structure of sofosbuvir
Ledipasvir, previously known as GS-5885, is an inhibitor of the Hepatitis C Virus (HCV) NSSA protein required for viral RNA replication and assembly of HCV virions. Although its exact mechanism of action is unknown, it is postulated to prevent hyper phosphorylation of NSSA which is required for viral production. It is effective against genotypes 1a, 1b, 4a, and 5a and with a lesser activity against genotypes 2a and 3a of HCV. Ledipasvir is available as a fixed dose drug combination product with sofosbuvir (tradename Harvoni) used for the treatment of chronic Hepatitis C, an infectious liver disease caused by infection with Hepatitis C Virus (HCV). Approved in October 2014 by the FDA, Ledipasvir and sofosbuvir are direct-acting antiviral agents indicated for the treatment of HCV genotype 1 with or without cirrhosis.

**MATERIALS AND METHODS**

**Materials:** Sofosbuvir and Ledipasvir pure drugs (API), Combination Sofosbuvir and Ledipasvir tablets (Harvoni), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphoric acid. All the above chemicals and solvents are from Rankem.\(^3\)\(^4\)

**HPLC method**

**Instrument:** UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 nm and 10 nm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Sofosbuvir and Ledipasvir solutions having universal loop injector of injection capacity 20 µL.\(^4\)\(^6\) The column used was Discovery C18 (4.6 x 250 mm, 5 µm) at ambient temperature. Different mobile phases were tested in order to find the best conditions, for separating both the drugs simultaneously. Optimised Chromatographic conditions The mobile phase having 60% OPA (0.1%): 40% Acetonitrile was selected because it was found that it ideally resolve the peaks with retention time (RT) 2.380 min and 3.449 min for Sofosbuvir and Ledipasvir respectively.\(^7\)\(^8\) Wavelength was selected by scanning all standard drugs over a wide range of wavelength 200 nm to 350 nm. Both the components showed reasonably good response at 260 nm.

**Methods:**

**Diluent:** Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50

**Preparation of Standard stock solutions:**

Accurately weighed 40 mg of Sofosbuvir, 9 mg of Ledipasvir and transferred to 25 ml & 25 ml volumetric flasks and 3/4th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labelled as Standard stock solution. (1600 µg/ml of Sofosbuvir and 360 µg/ml of Ledipasvir).

**Preparation of Sample stock solutions:** 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 50 ml volumetric flask; 50 ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (800 µg/ml of Sofosbuvir and 1800 µg/ml of Ledipasvir).

**Preparation of Standard working solutions (100% solution):** 0.2 ml of each stock solution was pipetted out and taken into a 10 ml volumetric flask and made up with diluent (160 µg/ml of Sofosbuvir and 36 µg/ml of Ledipasvir).

**Preparation of Sample working solutions (100% solution):** 0.2 ml of filtered sample stock solution was transferred to 10 ml volumetric flask and made up with diluent (160 µg/ml of Sofosbuvir and 36 µg/ml of Ledipasvir).

**Preparation of buffer:**

0.1% OPA Buffer: 1 ml of ortho phosphoric acid was diluted to 1000 ml with HPLC grade water.

**Typical Chromatogram**

Retention times of Sofosbuvir and Ledipasvir were 2.369 min and 3.436 min respectively. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

---

Figure 2: Chemical structure of ledipasvir

Figure 3: Typical Chromatogram of Sofosbuvir and Ledipasvir
Linearity: Six linear concentrations of Sofosbuvir (40-240 µg/ml) and Ledipasvir (9-54 µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Sofosbuvir was \( y = 4266x + 7700 \) and of Ledipasvir was \( y = 4861x + 2656 \). Correlation coefficient obtained was 0.999 for the two drugs.

### Table 1: System suitability parameters for Sofosbuvir and Ledipasvir

| S.no | RT (min) | USP Plate Count | Tailing | RT (min) | USP Plate Count | Tailing | Resolution |
|------|----------|-----------------|---------|----------|-----------------|---------|-------------|
| 1    | 2.366    | 5341            | 1.08    | 3.434    | 9522            | 1.09    | 7.5         |
| 2    | 2.367    | 5497            | 1.09    | 3.436    | 9659            | 1.09    | 7.6         |
| 3    | 2.367    | 5685            | 1.08    | 3.436    | 9776            | 1.08    | 7.5         |
| 4    | 2.369    | 5082            | 1.04    | 3.436    | 9731            | 1.10    | 7.5         |
| 5    | 2.369    | 5104            | 1.03    | 3.438    | 10083           | 1.09    | 7.6         |
| 6    | 2.372    | 5095            | 1.03    | 3.447    | 9852            | 1.05    | 7.7         |

### Table 2: Accuracy table of Sofosbuvir and Ledipasvir

| % Level | Amount Spiked (µg/mL) | Amount recovered (µg/mL) | % Recovery | Amount Spiked (µg/mL) | Amount recovered (µg/mL) | % Recovery |
|---------|-----------------------|--------------------------|------------|-----------------------|--------------------------|------------|
| 50%     | 80                    | 79.779                   | 99.72      | 18                    | 17.986                   | 99.92      |
| 100%    | 160                   | 159.392                  | 99.62      | 36                    | 35.902                   | 99.73      |
| 150%    | 240                   | 239.079                  | 99.62      | 54                    | 53.976                   | 99.95      |

Mean % Recovery: 99.61% for Sofosbuvir and 99.80% for Ledipasvir.

**System suitability parameters:**

The system suitability parameters were determined by preparing standard solutions of Sofosbuvir (160 ppm) and Ledipasvir (36 ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should not be more than 2%.

**Specificity:** Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

**Precision:**

**Preparation of Standard working solutions (100% solution):** 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask.
and made up with diluent. (160µg/ml of Sofosbuvir and 36µg/ml of Ledipasvir).

**Repeatability:** Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.5% and 0.6% respectively for Sofosbuvir and Ledipasvir. As the limit of Precision was less than “2”, the system precision was passed in this method.

**Accuracy:**

**Preparation of 50% Spiked Solution:** 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

**Preparation of 100% Spiked Solution:** 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

**Preparation of 150% Spiked Solution:** 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

**Accuracy:** Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.61% and 99.80% for Sofosbuvir and Ledipasvir respectively[9-10].

**Table 4: Sensitivity table of Sofosbuvir and Ledipasvir**

| Molecule | LOD | LOQ |
|----------|-----|-----|
| Sofosbuvir | 0.67 | 2.02 |
| Ledipasvir | 0.70 | 2.12 |

**Precision:** Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.5% and 0.6% respectively for Sofosbuvir and Ledipasvir. As the limit of Precision[11] was less than “2”, the system precision was passed in this method.

**Table 5: System precision table of Sofosbuvir and Ledipasvir**

| S. No | Area of Sofosbuvir | Area of Ledipasvir |
|-------|-------------------|-------------------|
| 1.    | 698943            | 177127            |
| 2.    | 695463            | 176673            |
| 3.    | 693621            | 177445            |

**Table 6: Intermediate precision table of Sofosbuvir and Ledipasvir**

| S. No | Area of Sofosbuvir | Area of Ledipasvir |
|-------|-------------------|-------------------|
| 1.    | 695241            | 176978            |
| 2.    | 695200            | 176075            |
| 3.    | 694259            | 176873            |
| 4.    | 694723            | 176902            |
| 5.    | 697754            | 176596            |
| 6.    | 696181            | 177083            |
| Mean  | 695560            | 176751            |
| S.D   | 1250.9            | 368.9             |
| %RSD  | 0.2               | 0.2               |

**Robustness:** Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

**Table 7: Robustness data for Sofosbuvir and Ledipasvir**

| S. No | Condition | %RSD of Sofosbuvir | %RSD of Ledipasvir |
|-------|-----------|--------------------|--------------------|
| 1     | Flow rate (-) 0.9ml/min | 0.5          | 0.4          |
| 2     | Flow rate (+) 1.1ml/min | 0.5          | 0.7          |
| 3     | Mobile phase (-) 65B:35A | 0.5          | 0.4          |
| 4     | Mobile phase (+) 55B:45A | 0.5          | 0.5          |
| 5     | Temperature (-) 25°C | 0.3          | 0.3          |
| 6     | Temperature (+) 35°C | 0.1          | 0.1          |

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus(35°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.

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (65B:35A), mobile phase plus (55B:45A), temperature minus (25°C) and temperature plus(35°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.

**LOD sample Preparation:** 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks.

---

© Rubatosis Publications | International Journal of Research in Pharmaceutical Chemistry and Analysis
and made up with diluents. From the above solutions 0.1ml each of Sofosbuvir, Ledipasvir, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents.

**LOQ sample Preparation:** 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Sofosbuvir, Ledipasvir, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

**Assay:** Radha Krishna Pharmaceuticals, (Hepcvir L) bearing the label claim Sofosbuvir 400mg, Ledipasvir 90mg. Assay was performed with the above formulation. Average % Assay for Sofosbuvir and Ledipasvir obtained was 99.32 and 98.47% respectively.

### Table 8: Assay Data of Sofosbuvir

| S.no | Standard Area | Sample area | % Assay |
|------|---------------|-------------|---------|
| 1    | 698943        | 695241      | 99.28   |
| 2    | 695463        | 695200      | 99.27   |
| 3    | 693621        | 694259      | 99.14   |
| 4    | 704923        | 694723      | 99.20   |
| 5    | 698452        | 697754      | 99.63   |
| 6    | 693668        | 696181      | 99.41   |
| Avg  | 697512        | 695560      | 99.32   |
| Stdev| 4288.8        | 1250.9      | 0.18    |
| %RSD | 0.6           | 0.2         | 0.18    |

### Table 9: Assay Data of Ledipasvir

| S.no | Standard Area | Sample area | % Assay |
|------|---------------|-------------|---------|
| 1    | 177127        | 176978      | 99.59   |
| 2    | 176673        | 176075      | 99.09   |
| 3    | 177445        | 176873      | 99.53   |
| 4    | 179081        | 176902      | 99.55   |
| 5    | 176591        | 176596      | 99.38   |
| 6    | 177154        | 177083      | 99.65   |
| Avg  | 177345        | 176751      | 99.47   |
| Stdev| 908.6         | 368.9       | 0.2     |
| %RSD | 0.5           | 0.2         | 0.2     |

**Degradation studies:** Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

**Oxidation:** To 1 ml of stock solution of Sofosbuvir and Ledipasvir, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions we are kept for 30 min at 60º. For HPLC study, there solution was diluted to obtain 160µg/ml and 36µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Acid Degradation Studies:** To 1ml of stock solution Sofosbuvir and Ledipasvir, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60ºC. The resultant solution was diluted to obtain 160µg/ml & 36µg/ml solution and 10µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Alkali Degradation Studies:** To 1 ml of stock solution Sofosbuvir and Ledipasvir, 1ml of 2N sodium hydroxide was added and refluxed for 30mins at 60ºC. There resultant solution was diluted to obtain 160µg/ml & 36µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Dry Heat Degradation Studies:** The standard drug solution was placed in oven at 105ºC for 1hr to study dry heat degradation. For HPLC study, the resultant solution was diluted to 160µg/ml & 36µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

**Photo Stability studies:** The photochemical stability of the drug was also studied by exposing the 160µg/ml stock solution of Sofosbuvir & 360µg/ml Ledipasvir µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1days or 200 Watt hours/m² in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 160µg/ml & 36µg/ml solutions and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Neutral Degradation Studies:** Stress testing under neutral conditions was studied by refluxing the drug in water for 1hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to 160µg/ chromatograms were recorded ml & 36µg/ml solution and 10µl were injected into the system and the to assess the stability of the sample.

### Table 10: Degradation Data of Sofosbuvir

| S.NO | Degradation Condition | % Drug Degraded | Purity Angle | Purity Threshold |
|------|-----------------------|-----------------|-------------|----------------|
| 1    | Acid                  | 4.21            | 0.051       | 0.296          |
| 2    | Alkali                | 3.96            | 0.124       | 0.252          |
| 3    | Oxidation             | 3.89            | 0.159       | 0.304          |
| 4    | Thermal               | 2.61            | 0.197       | 0.294          |
| 5    | UV                    | 1.48            | 0.133       | 0.280          |
| 6    | Water                 | 1.48            | 0.044       | 0.287          |

### Table 11: Degradation Data of Bromhexine

| S.NO | Degradation Condition | % Drug Degraded | Purity Angle | Purity Threshold |
|------|-----------------------|-----------------|-------------|----------------|
| 1    | Acid                  | 4.62            | 0.187       | 0.320          |
| 2    | Alkali                | 4.22            | 0.162       | 0.587          |
| 3    | Oxidation             | 3.78            | 0.171       | 0.328          |
| 4    | Thermal               | 2.78            | 0.197       | 0.297          |
| 5    | UV                    | 1.22            | 0.130       | 0.296          |
| 6    | Water                 | 0.90            | 0.123       | 0.299          |
Regarding the pH adjustment in mobile phase for the acid and base degradation studies have movement in retention time of drugs. But due to neutralized acid sample with 2N Base solution and base sample with 2N Acid solution there will be no change in retention time.

CONCLUSION
A simple, Accurate, precise method was developed for the simultaneous estimation of the Sofosbuvir and Ledipasvir in Tablet dosage form. Retention time of Sofosbuvir and Ledipasvir were found to be 2.367 min and 3.436 min. %RSD of the Sofosbuvir and Ledipasvir were and found to be 0.6 and 0.5 respectively. %Recovery was obtained as 99.61% and 99.80% for Sofosbuvir and Ledipasvir respectively. LOD, LOQ values obtained from regression equations of Sofosbuvir and Ledipasvir were 0.67, 2.02 and 0.70, 2.12 respectively. Regression equation of Sofosbuvir is y = 4266.x + 7700, and y = 4861.x + 2656 of Ledipasvir. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

ACKNOWLEDGEMENT
We are thankful to Krishna teja Pharmacy College, Tirupati, for providing us necessary facilities to carry out our research work.

REFERENCES
1. Sharma, B. K. (1981). Instrumental methods of chemical analysis. Krishna Prakashan Media.
2. Lindholm, J. (2004). Development and validation of HPLC methods for analytical and preparative purposes (Doctoral dissertation, Acta Universitatis Upsaliensis).
3. Rashmin, (2012) An introduction to analytical Method Development for Pharmaceutical formulations. Indoglobal Journal of Pharmaceutical Sciences, Vol 2, Issue 2, 191-196.
4. Malviya, R., Bansal, V., Pal, O. P., & Sharma, P. K. (2010). High performance liquid chromatography: a short review. Journal of global pharma technology, 2(S), 22-26.
5. Skoog, D. A., Holler, F. J., & Crouch, S. R. (2017). Principles of instrumental analysis. Cengage learning.
6. Dr.S. Ravi Shankar, Text book of Pharmaceutical analysis, Second edition, pp. 13.1-13.2
7. David G.Watson. Pharmaceutical Analysis, A text book for Pharmacy students and Pharmaceutical Chemists. 2nd Ed., Harcourt Publishers Limited; pp 221-232.
8. Remington's (2000) The Sciences and Practise of Pharmacy, 20th Edition.
9. Connors, K. A. (1994). A textbook of pharmaceutical analysis. 3rd Ed, Wiley intersciences Inc; Delhi, pp. 373-421.
10. Chatwal, G. R., & Anand, S. K. (2007). Instrumental Methods of Chemical Analysis. Himalaya publishing house, pp 2.566-2.638.
11. David G. Watson Pharmaceutical Analysis, A text book for pharmacy students and Pharmaceutical Chemists. Harcourt Publishers Limited; 2nd Ed., Pp- 267-311.
12. Nasal, A., Siluk, D., & Kalisz, R. (2003). Chromatographic retention parameters in medicinal chemistry and molecular pharmacology. Current medicinal chemistry, 10(5), 381-426.
13. Ashok Kumar, Lalith Kishore, navpreet Kaur, Anroop Nair. (2012) Method Development and Validation for Pharmaceutical Analysis. International Pharmaceutical Science, Vol 2, Issue 3, Jul-Sep.
14. Chandrul Kaushal, K., & Srivastava, B. (2010). A process of method development: A chromatographic approach. J. Chem. Pharm. Res, 2(2), 519-545.
15. Gupta, V., Jain, A. D. K., Gill, N. S., & Gupta, K. (2012). Development and validation of HPLC method-a review. Int. Res J Pharm. App Sci, 2(2), 17-25.
16. Hokanson, G. C. (1994). A life cycle approach to the validation of analytical methods during pharmaceutical product development, part i: The initial validation process.
17. Green, J. M. (1996). Peer reviewed: a practical guide to analytical method validation. Analytical chemistry, 68(9), 305A-309A.
18. ICH (Q2B) (1996). Validation of analytical procedures: text and methodology Q2 (R1). In International conference on harmonization, Geneva, Switzerland.
19. Rutkowska, E., Pająk, K., & Jóźwik, K. (2013). Lipophilicity—methods of determination and its role in medicinal chemistry. Acta poloniae pharmacuetica, 70(1), 3-18.
20. IUPAC. Compendium of Chemical Terminology, 2nd ed. (The Gold Book). PAC69, 1137 (1997)
21. KD Tripathi, Essentials of Medical Pharmacology, 6th Edition, Jaypee brother’s medical publishers (P) LTD, p-254-255.
22. Indian Pharmacopoeia, Indian Pharmacopoeial Commission, Controller of Publication, Government of India, Ministry of health and Family Welfare, Ghaziabad, India, 2 (2010) 1657-1658.
23. British Pharmacopoeia, The British Pharmacopoeial Commission, the stationary office, UK, London, 1408-1409 2 (2011).
24. Grempler, R., Thomas, L., Eckhardt, M., Himmelsbach, F., Sauer, A., Sharp, D. E., & Eickelmann, P. (2012). Empagliflozin, a novel selective sodium glucose cotransporter-2 (SGLT-2) inhibitor: characterisation and comparison with other SGLT-2 inhibitors. Diabetes, Obesity and Metabolism, 14(1), 83-90.

25. Abdul-Ghani, M., & DeFronzo, R. (2008). Inhibition of renal glucose reabsorption: a novel strategy for achieving glucose control in type 2 diabetes mellitus. Endocrine Practice, 14(6), 782-790.

26. Nair, S., & Wilding, J. P. (2010). Sodium glucose co-transporter 2 inhibitors as a new treatment for diabetes mellitus. The Journal of Clinical Endocrinology & Metabolism, 95(1), 34-42.

27. https://www.drugbank.ca/drugs/DB08934.

28. http://www.rxlist.com/jardiance-drug/overdose-contraindications.html

29. Terashima, H., Hama, K. A. Z. U. A. K. I., Yamamoto, R. Y. U. Z. O., Tsuboshima, M. A. S. A. M. I., Kikkawa, R. Y. U. I. C. H. I., Hatanaka, I. K. U. O., & Shigeta, Y. U. K. I. O. (1984). Effects of a new aldose reductase inhibitor on various tissues in vitro. Journal of Pharmacology and Experimental Therapeutics, 229(1), 226-230.

30. Hassouna, M. E. K. M., Abdelrahman, M. M., & Mohamed, M. A. (2017). Assay and dissolution methods development and validation for simultaneous determination of sofosbuvir and ledipasvir by RP-HPLC method in tablet dosage forms. J Forensic Sci & Criminal Inves, 1(3), 001-11.

31. Vikas, P. M., Satyanarayana, T., Kumar, D. V., Mounika, E., Latha, M. S., Anusha, R., & Sathish, Y. (2016). Development and validation of new RP-HPLC method for the determination of sofosbuvir in pure form. World Journal of pharmacy and pharmaceutical Sciences, 5(5), 775-781.

32. Rani, J. S., & Devanna, N. (2017). A new RP-HPLC method development and validation for simultaneous estimation of sofosbuvir and velpatasvir in pharmaceutical dosage form. Int. J. Eng. Technol. Sci. Res., 4, 145-152.

33. Zaman, B., Siddique, F., & Hassan, W. (2016). RP-HPLC method for simultaneous determination of sofosbuvir and ledipasvir in tablet dosage form and its application to in vitro dissolution studies. Chromatographia, 79(23-24), 1605-1613.

34. Nagaraju, T., Vardhan, S. V. M., Kumar, D. R., & Ramachandran, D. (2017). A new RP-HPLC method for the simultaneous assay of sofosbuvir and ledipasvir in combined dosage form. Intern. J. ChemTech Res, 10(7), 761-768.

35. Rao, B. S., Reddy, M. V., & Rao, B. S. (2017). Simultaneous analysis of Ledipasvir and Sofosbuvir in bulk and tablet dosage form by a stability indicating High-Performance Liquid Chromatographic Method. Global Journal for Research Analysis, 6(4), 505-509.

36. Kumar, B. R., & Subrahmanyam, D. K. (2016). A new validated RP-HPLC method for the simultaneous determination of simeprevir and sofosbuvir in pharmaceutical dosage form. Indo American Journal of Pharmaceutical Research, 6(02), 4508-4520.