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RESEARCH ARTICLE

UPLC–Q-TOF-MS/MS Method Development and Validation for the Identification of Forced Degradation Products of Velpatasvir

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Abstract: The aim of the present investigation was to show how to utilize liquid chromatography (LC) and liquid chromatography-mass spectrometry (LC-MS) to separate, identify, and characterize extremely minute amounts of velpatasvir degradation products (DPs). Chromatographic separation was carried out using an Acquity H-Class UPLC system (Waters, Milford, MA, USA) equipped with a conditioned auto sampler and an Acquity BEH C18 column (100 mm X 2.1mm i.d.,1.7 m particle size) (Waters, Milford, MA, USA). The temperature of the column was kept constant at 40 °C. The mobile phase was pumped at a flow rate of 0.40 mL/min and included water with 0.1% formic acid in water (solvent A) and acetonitrile with 0.1% formic acid (solvent B). Forced degradation of velpatasvir was studied under circumstances of hydrolysis (neutral, acidic, and alkaline), oxidation, photolysis, and thermal stress, as recommended by the ICH Q1A (R2). Under hydrolysis (acidic, alkaline) and oxidation (peroxide stress) conditions, the drug degraded significantly. Under thermal and photolytic stress conditions, the drug remained stable. Some unique degradation products were discovered in this investigation under a variety of circumstances that had not before been reported.

Keywords: Velpatasvir, Stress testing, Degradation pathway, UPLC-QTof-MS/MS

INTRODUCTION

Velpatasvir (Figure.1) is a Direct-Acting Antiviral (DAA) medication used as part of combination therapy to treat chronic Hepatitis C, an infectious liver disease caused by infection with Hepatitis C Virus (HCV). Velpatasvir acts as a defective substrate for NS5A (Non-Structural Protein 5A), a non-enzymatic viral protein that plays a key role in Hepatitis C Virus replication, assembly, and modulation of host immune responses. Since June 2016, Velpatasvir has been available as a fixed dose combination product with Sofosbuvir, as the commercially available product Epclusa. Epclusa is the first combination HCV product indicated for the treatment of all genotypes of Hepatitis C with or without cirrhosis.

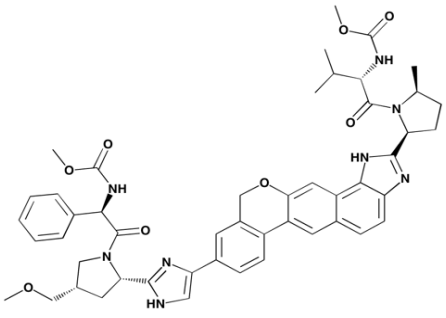


Fig .1: Chemical structure of Velpatasvir

Review of literature

An extensive literature survey was carried out and found that various analytical methods like Several High-Performance Liquid Chromatography (HPLC) methods with UV detection for estimation of velpatasvir in pharmaceutical dosage forms[3-6] as well as in plasma[2] were reported with combination. El-Wekil MM, et al, reported enhanced dispersive solid phase extraction assisted by cloud point strategy prior to fluorometric determination of anti-hepatitis C drug velpatasvir in pharmaceutical tablets and body fluids[7]

Recently, liquid chromatography–mass spectrometry LC–MS/MS, evolved as versatile tool for the characterization of drug impurities, degradation products. Recent advances in liquid chromatographic mass spectrometric detectors like ion trap, time of flight, flow injection analysis tandem mass, quadrupole time of flight resulted in successful characterization of drug substances and drug products. Recently design of experiment tools for method development on LCMS/MS for trace level identification of impurities also evolved as an efficient tool. However, so far, no study has been reported on the systematic characterization and mechanistic pathway of degradation products of velpatasvir under stress conditions prescribed by ICH Q1A R2. The main aim of the present study was to investigate the complete degradation behavior of the drug and to characterize the degradation products. It was accomplished by exposing the drug to ICH-recommended stress conditions of light, thermal, hydrolysis, oxidation, acidic and basic conditions, analysing the resultant solutions to optimized LC-MS,

MS/MS, and accurate mass measurements to establish the fragmentation pattern of the drug and its degradation products.

OBJECTIVES OF RESEARCH

Ultra-pressure liquid chromatography (UPLC) method has been developed for the identification of various stability indicating degradation products formed under various conditions i.e., acidic, alkaline, neutral and oxidative, thermal and photolytic condition. In continuation the degradation products formed under the above stated conditions has been tentatively identified (qualitative aspect) with high resolution UPLC-PDA-QTOF-MS/MS spectrometry. The current study was performed to investigate Velptasvir under the hydrolytic & oxidative, thermal and photolytic conditions, further stability indicating degradation products were analyzed by considering their fragment ions observed in MS/MS studies. Tentative identification of degradation products was observed.

MATERIAL AND METHODS

Chemicals and reagents

Velptasvir, (99% purity) was a gift sample from Shilpa Medicare Limited, manufacturing unit in Hyderabad, India. LCMS-grade acetonitrile was obtained from Biosolve ChimieSARL (Dieuze, France), formic acid (Optima LC/MS grade) from Fisher Scientific (Geel, Belgium) and methanol (LiChrosolv) was purchased from Merck (Darmstadt, Germany). Ultra-pure water was used throughout study which was produced by Milli-Q water (18.2 mΩ resistance) purification system (Millipore, Milford, MA, USA). Sodium hydroxide (LR Grade) from SD Fine chemicals (Mumbai, India) Hydrochloric acid (AR Grade) obtained from Sigma-Aldrich (U.S.A), Hydrogen peroxide (AR Grade) (30% v/v) obtained from Avra chemicals (Hyderabad, India). LC-MS Grade formic acid solution obtained from Fisher Scientific (Geel, Belgium).

Instrumentation

High-resolution masses of degradation products were measured after UPLC separation. Chromatographic separation was performed on Acquity H-Class UPLC system (Waters, Milford, MA, USA) with a conditioned auto sampler, using an Acquity BEH Phenyl Hexyl column (150 mm X 2.1mm i.d.,1.7 µm particle size) (Waters, Milford, MA, USA).

The column temperature was maintained at 40 °C. The mobile phase consisting of water with 0.1% formic acid in water (solvent A) and acetonitrile with 0.1% Formic acid (solvent B) was pumped at a flow rate of 0.40 mL/min. The gradient elution program was as follows: 0-1.5 min, 5%B; 3.20 min, 15% B; 6.30min, 35%B; 7.50min, 50%B; 9.60 min, 65% B; 11.00 min, 95% B; 12.00 min 95% B, 15.00 min 5% B Equilibration time was 2.50 min and the injection volume was 1.0 µL. The optimized chromatographic conditions and gradient elution program was shown in table 1.

Table 1: Optimized Chromatographic conditions of Velpatasvir

Column	Acquity BEH C_{18} column (100mm X 2.1mm, 1.7 µm)
Mobile phase	water with 0.1% formic acid (solvent A) and acetonitrile with 0.1% Formic acid (solvent B)
Flow rate	0.40mL/min
Column temperature	40^0C
Injection volume	0.5 µL
Detection Wavelength	295 nm
Run time	15 min
Retention time	7.49 min

LC-MS/MS mode was applied to analyze the samples in both TIC as well as MS/MS mode where collision energy was at 30 eV. Eluted compounds were detected from m/z 30 to m/z 700 using Xevo G2-XS QTof mass spectrometer (Waters, Manchester, UK), which was connected to Electrospray ionization (ESI) interface with negative ion mode using the following instrument settings, capillary voltage, 2.0KV; sample cone, 40V; source temperature, 120 °C; desolvation temperature 350 °C; cone gas flow rate 50 L/Hr; desolvation gas (N_2) flow rate 850 L/Hr, Argon as CID gas for MS/MS experiments.

All analyses were performed using the lock spray, which ensured accuracy and reproducibility. Leucine - Enkephalin (5ng/mL) was used as lock mass generating a reference ion in negative mode at m/z 554.2615 and introduced by a lock spray at 10 µL/min for accurate mass acquisition.

Data acquisition was achieved using Mass Lynx v 4.1. Acquiring data in this manner provided the collection of information of intact precursor ions as well as fragment ions.

Preparation of Solutions

Mobile phase

Mobile phase consisting of water with 0.1% formic acid in water (solvent A) and acetonitrile with 0.1% Formic acid (solvent B).

Degradation experiments

For the purpose of stability indicating studies degradation of Velptasvir was carried out under various stress conditions. The process of degradation is shown below

Acid degradation studies

5mg of VPTS was added to 2.5mL ACN and refluxed with 2.5mL 0.1M Hydrochloric acid at 60^{0}C for 24 Hrs. The samples were collected at a time intervals of 0, 0.5, 1.0, 3.0, 6.0, 12.0 and 24 Hrs. Solutions were neutralized with NaOH.

Alkali induced degradation study

5mg of VPTS was added to 2.5mL ACN and refluxed with 2.5mL 0.1 M Sodium hydroxide at 60^{0}C for 24 Hrs. The samples were collected at a time intervals of 0, 0.5, 1.0, 3.0, 6.0, 12.0 and 24 Hrs. Further collected samples at different time intervals were neutralized with HCl.

Oxidative induced degradation study

Accurately weighed 5 mg of VPTS dissolved in 2.5mL ACN and 2.5mL H_2O_2 0.3 % (v/v) and then refluxed under 60^{0}C for 24 Hrs. The samples were collected at a time intervals of 0, 0.5, 1.0, 3.0, 6.0, 12.0 and 24 Hrs. Further solutions were diluted with Acetonitrile.

Thermal degradation study

Drug sample of 10mg each was taken in two10mL volumetric flasks and sealed. One flask was exposed to dry heat in hot air oven for specified temperature and time interval and the other was kept as control.

Photolytic degradation study

The drug layer of 1mm thickness was prepared in a petridish and exposed to ICH recommended photo stability conditions with the overall illumination of not less than 1.2 million lx h along with the integrated near ultraviolet energy of not less than 200 Wh/m2. Another petridish containing the drug (1mm layer thickness) was wrapped with aluminum foil and kept as control.

Sample solutions

The collected samples under various stress conditions at different time intervals were appropriately diluted to 200 ppm concentrations with Hyper grade methanol as diluent and subjected to UPLC-Q-TOF-MS/MS analysis.

Samples were prepared by filtering the solution through 0.22µ filter paper prior to UPLC and UPLC-MS analysis. Samples were withdrawn at different time intervals and diluted with mobile phase before injection.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

To achieve acceptable separation between the drug and its degradation products, water with 0.1% formic acid (solvent A) and acetonitrile with 0.1% Formic acid (solvent B) was used as mobile phase in gradient elution mode and Acquity BEH C_{18} column (100mm X 2.1mm, 1.7 µm) column was used for successful separation of velpatasvir and its degradation products.

Well resolved peaks with acceptable symmetry were achieved by employing a mobile phase composed of (A) water with 0.1% formic acid and (B) acetonitrile with 0.1% Formic acid in a gradient program as follows (T min / %B): 0/5, 0.8/5, 1.5/20, 4.8/25, 7.2/30, 8.1/40, 9/60, 10.5 to 11.5/95,12/5. The flow rate was 0.4 mL/min and detection wavelength was 295 nm.

The runtime was 15 min and the temperature of the column oven was set at 40 °C. These optimized chromatographic conditions were used for separation of velpatasvir and its degradation products. The method was validated with respect to the parameters outlined in ICH guidelines Q1A R2. For LC-MS studies, same method was used as for UPLC.

The Q-TOF ESI source conditions were also optimized to obtain a good signal and high sensitivity (Figure 2). The conditions such as drying gas flow, nebulizing gas flow, drying gas temperature, capillary voltage, spray voltage, and skimmer voltage were optimized to maximize the ionization in the source and sensitivity even at a very low concentration to identify and characterize the degradation products.

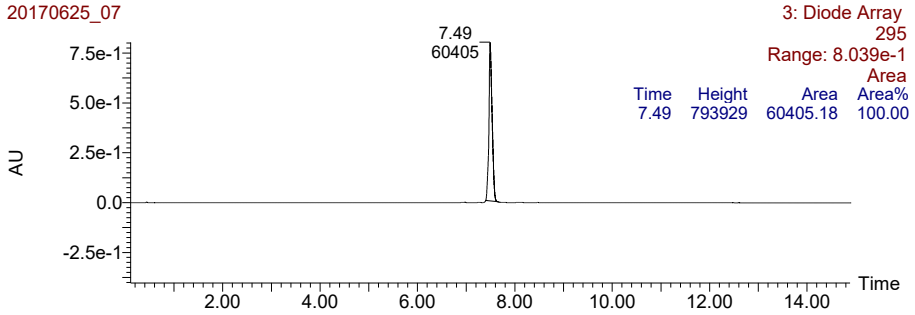


Figure 2: Optimized chromatogram of Velpatasvir

Validation of proposed method

Specificity

Specificity is the ability of the analytical method to measure the analyte concentration accurately in the presence of all the potential impurities**.** The specificity was determined by subjecting Velpatasvir to stress under various conditions. All the degradation products were well separated from the Velpatasvir and the method was found to be specific.

Linearity

The method was found to be linear over a concentration range of 5-25 µg mL of Velpatasvir (60–140 % of the nominal concentration). Each concentration was injected in triplicate (n3). Standard calibration curve was plotted by taking concentration of velpatasvir (µg/mL) on x-axis and mean area response (AU) on y-axis. The proposed method was found to be linear with correlation coefficient of 0.999 and linear regression equation of y = 3062.x - 5.714. The obtained calibration curve of velpatasvir was shown in figure 3.

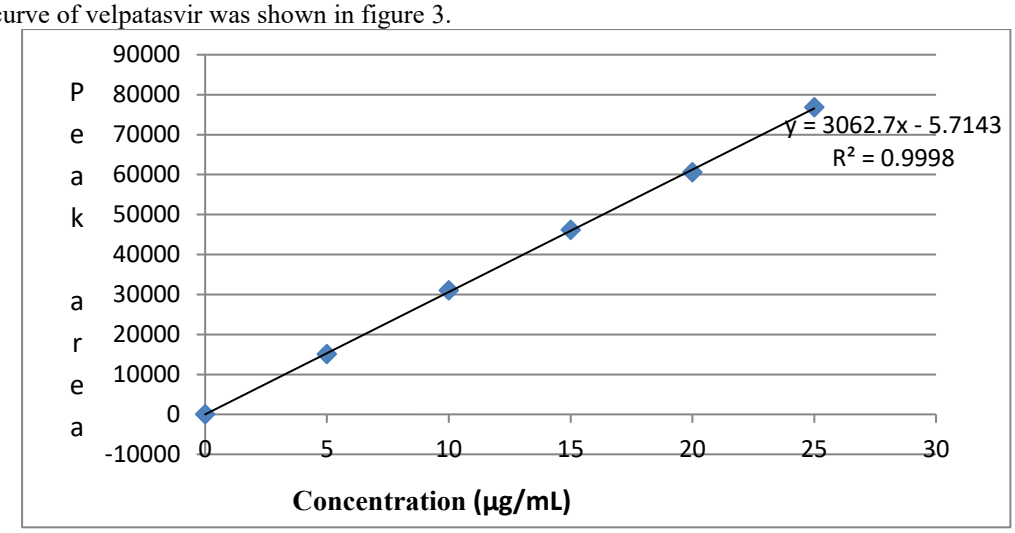


Figure 3: Calibration curve of velpatasvir

Accuracy

The accuracy was established by the addition of known quantities of standard to the synthetic mixture of excipients which are taken in the formulation of the drug product. Each solution was injected in triplicate and the percentage recovery was calculated. The percentage recovery was found to be 99–101 %. Results are given in Table 2.

Table 2: Accuracy data of Velpatasvir

Spiked concentration (µg/ml)	Amount found in µg/ml (mean ± SD; %RSD)	Mean recovery (%)
5	5.00 ±0.17; 0.05	100
15	15.04 ±0.37; 0.07	100.2
25	24.93 ±0.13; 0.11	99.7

Precision

The repeatability of the method was established by analyzing six injections of the standard drug at 100 % level and the %RSD was found to be 0.2. The intermediate precision of the method was investigated by analyzing the drug at three different concentrations (60, 100 and 140 % of the nominal concentration) on different days (interday precision), different column (with different lot number), different analyst and different instrument within the same laboratory. %RSD was determined for the concentration of velpatasvir found at each level as shown in Table 3. The developed method has good precision as low %RSD values were obtained.

Table 3: Precision data of Velpatasvir

Concentration of Rilpivirine (µg/ml)	Concentration of Rilpivirine found (µg/ml) ± SD; %RSD			
	Day 1	Day 2	Different analyst	Different instruments
5	5.01±0.11; 0.14	5.05±0.35;0.40	5±0.10;0.21	4.9±0.17;0.26
15	15.03±0.04; 0.05	14.96±0.13; 0.09	14.9±0.09;0.12	14.8±0.07;0.09
25	25.01±0.06; 0.05	24.98±0.31; 0.21	25±0.28;0.23	24.9±0.21;0.23

Robustness

Robustness is a measure of reliability of the method to small and deliberate changes made to the parameters of the developed analytical method. This study involved small changes in column temperature (40 ± 5 °C) and flow rate (0.4 ± 0.05 mL/ min). The peak area of the injections (n = 3) was taken as a measure for calculation for determining the robustness of the method. The %RSD for the pH change and flow rate was less than 1 %. In addition, there was no significant variation in the assay of the components indicating the method to be robust as shown in Table 4.

Table 4: Robustness data of velpatasvir

Parameter	Condition	Tailing	Theoritical plates	Resolution	% assay of drug
Column temperature	45°C	1.16	60893	1.63	100.04
	35°C	1.22	60418	1.54	100.02
Flow rate	0.35mL/min	1.21	61032	1.71	99.97
	0.45mL/min	1.15	62163	1.79	99.89

The Limit of Detection (LOD) and Limit of Quantification (LOQ)

The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed UPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to-noise ratio of 3). The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal-to-noise ratio of 10). The results were shown in table 5.

Table 5: LOD and LOQ data of Velpatasvir

Parameter	LOD (µg/ml)	LOQ (µg/ml)
Velpatasvir	0.16	0.43

Forced degradation behavior of Velpatasvir

Alkali induced degradation study

The stability indicating degradation products (DP-1 to DP-4) observed under the alkaline hydrolytic conditions in which one of the degradation product (DP-3) was showing the same HRMS and MS/MS fragmentation information equivalent to the velpatasvir drug substance. So the DP-3 was claimed as the diasteriomer of the velpatasvir drug substance. Remaining three degradation products MS/MS data were depicted in the following table 6 contains the information regarding the fragment ions observed from the degradation products and the RDBE values and the observed molecular ions data as follows. Here the UPLC achieves the better chromatographic resolution which leads to separation of the diasteriomeric degradation products along with three other degradation products observed in the alkaline hydrolytic conditions. In continuation to sthe chromatographic separation the mass spectrometric detection of molecular ions which further subjected to fragmentation of observed precursor ions leads to the formation of daughter ions which are unique to specific molecular ion. The TOF-MS/MS instrument was operated at 50,000 resolution and CID-MS/MS mode was applied. The obtained chromatograms and degradation product mass spectrums were shown in figure 4 and 5.

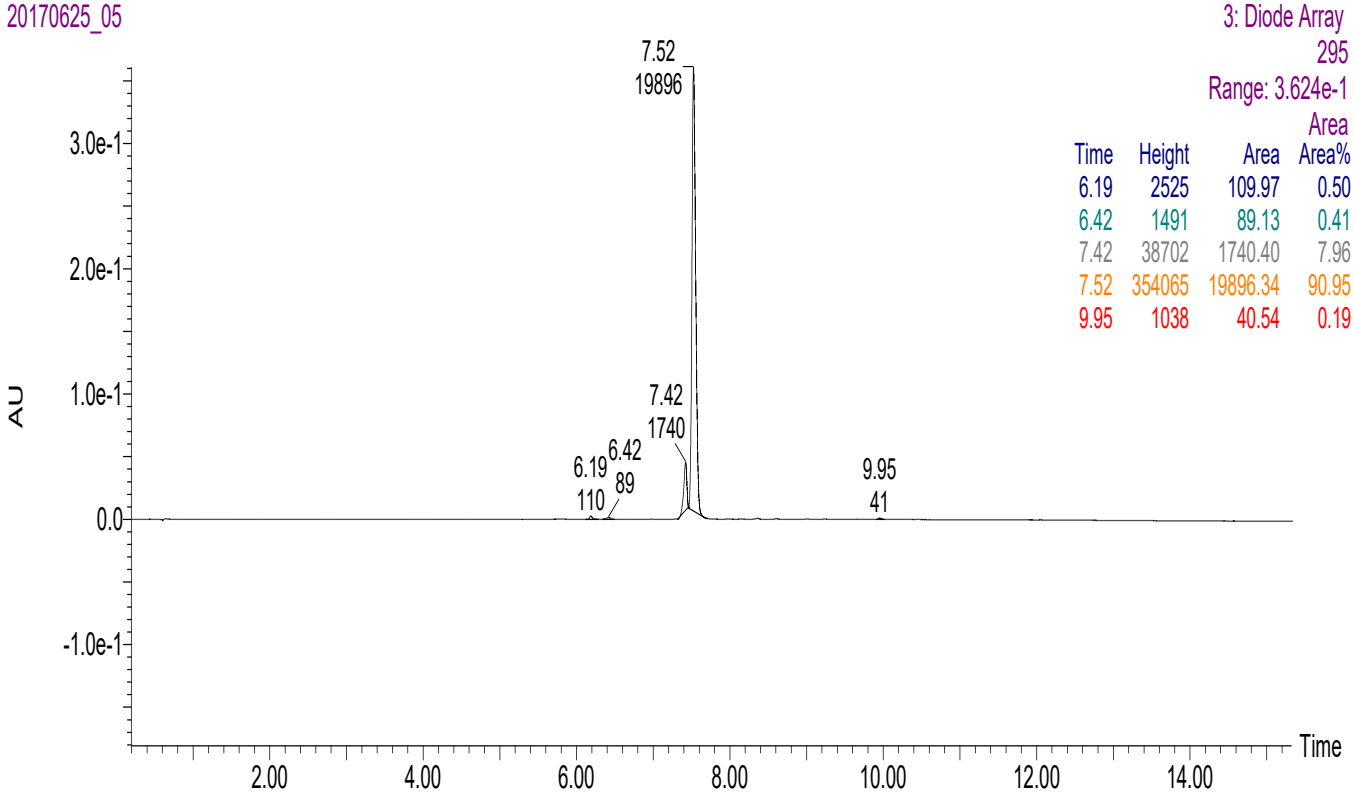


Figure.4: UPLC chromatogram of Velpatasvir under alkaline hydrolysis conditions (0.1 N NaOH, 60 ^{0}C)

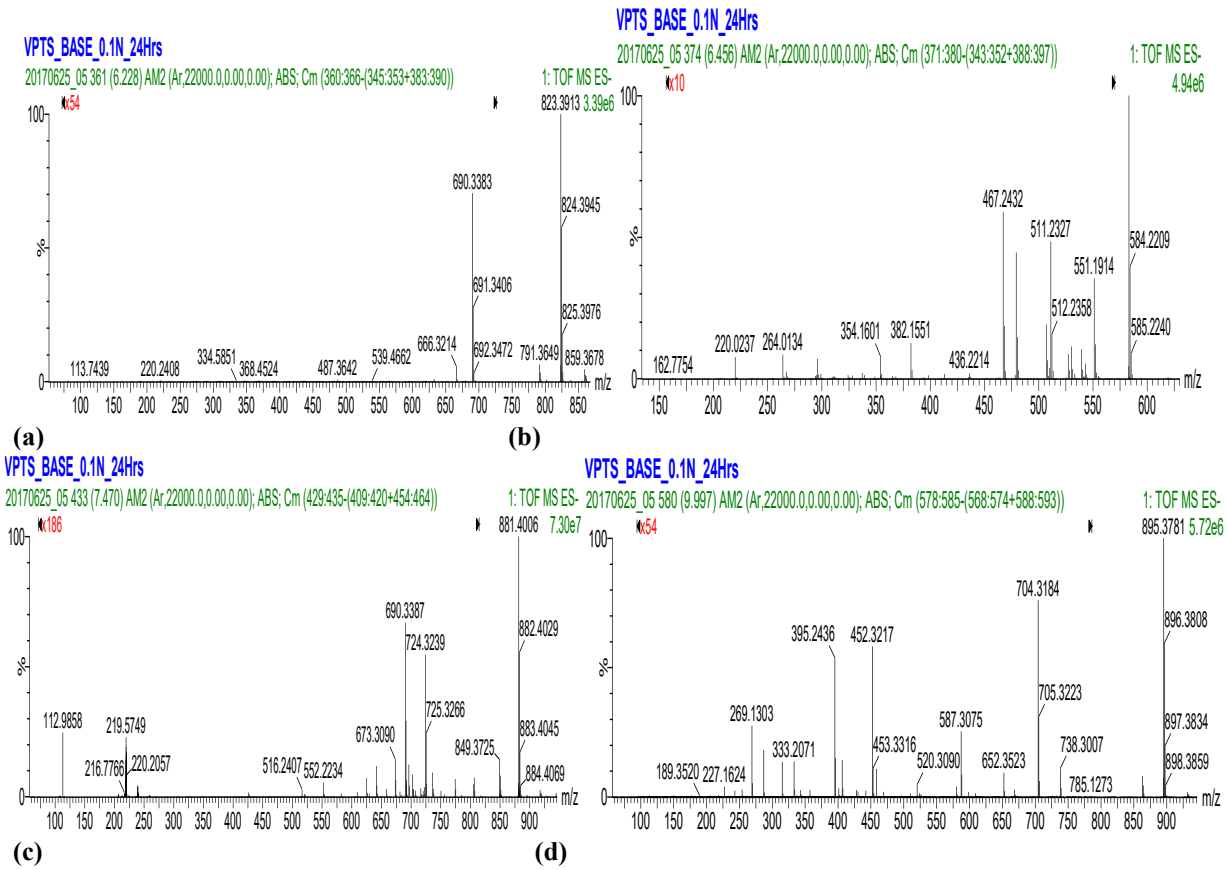


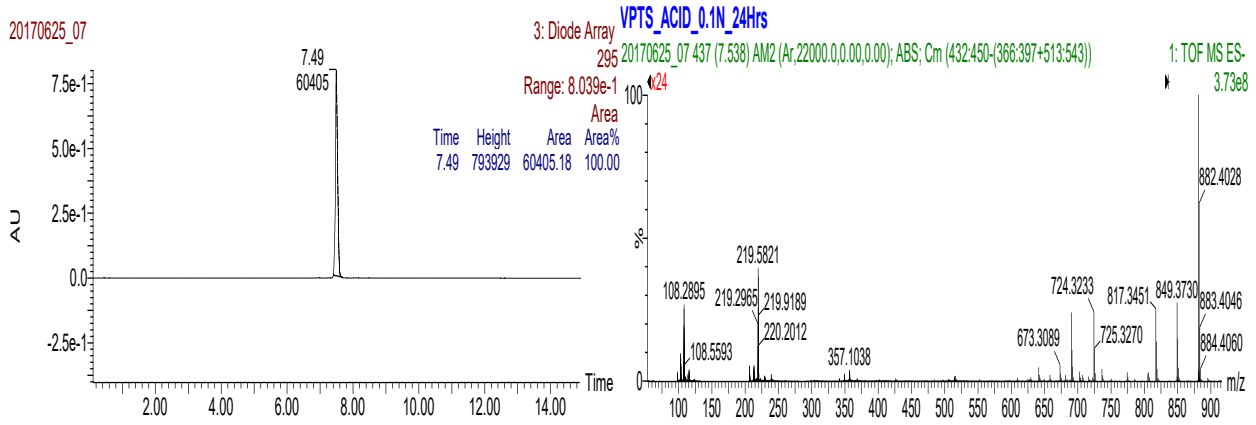
Figure 5: MS/MS spectra for (a) DP-01 (b) DP-02 (c) DP-03 (d) DP-04 formed under alkaline conditions (0.1 N NaOH, 60 ^{0}C)

Table.6: The fragment ions observed from the alkali degradation products and the RDBE values and the observed molecular ions data as follows.

S No	Observed HRMS	RDBE	Mol. Formula	Error
1	823.3913	26.5	$C_{47}H_{51}N_8O_6$	-2.3
	791.3649	27.5	$C_{46}H_{47}N_8O_5$	-2.5
	690.3383	21.5	$C_{39}H_{44}N_7O_5$	-3.0
2	583.2180	19.5	$C_{32}H_{31}N_4O_7$	-2.2
	551.1914	20.5	$C_{31}H_{27}N_4O_6$	-3.1
	511.2327	17.5	$C_{30}H_{31}N_4O_4$	-3.5
	479.2066	18.5	$C_{29}H_{27}N_4O_3$	-3.5
	467.2432	16.5	$C_{29}H_{31}N_4O_2$	-3.2
	382.1551	16.5	$C_{24}H_{20}N_3O_2$	-1.3
	351.1601	15.5	$C_{23}H_{20}N_3$	-1.4
3	881.4006	27.5	$C_{49}H_{53}N_8O_8$	2.3
	849.3725	28.5	$C_{48}H_{49}N_8O_7$	0.1
	817.3456	29.5	$C_{47}H_{45}N_8O_6$	-0.7
	690.3387	21.5	$C_{39}H_{44}N_7O_5$	-2.5
	641.2866	19.5	$C_{33}H_{37}N_8O_6$	4.7
4	895.3781	28.5	$C_{49}H_{51}N_8O_9$	0.2
	863.3514	29.5	$C_{48}H_{47}N8O_8$	-0.3
	820.3444	28.5	$C_{47}H_{46}N_7O_7$	-1.8
	704.3184	22.5	$C_{39}H_{42}N_7O_6$	-1.8
	452.3217	5.5	$C_{23}H_{42}N_5O_4$	-4.4
	395.2436	10.5	$C_{23}H_{31}N_4O_2$	-2.8

Acid degradation studies

The Velpatasvir was highly stable under the acid hydrolytic conditions where the degradation was observed in various concentrations from 0.1M HCl to 1M HCl for about 24 Hrs under heating/reflux conditions. With the above studies we can promptly claim that velpatasvir was highly stable under the acidic hydrolysis conditions. To observe any forced degradation products under the acid hydrolysis conditions better to go through the forced degradation study in stability chambers where the temperature and humidity conditions can be controlled without any deviations. All the reactions were kept without exposing to the light conditions which could be prone to formation of uneven/unstable degradation products. The UPLC chromatogram and mass spectrum were shown figure 6, which contains there is no observation of degradation products.

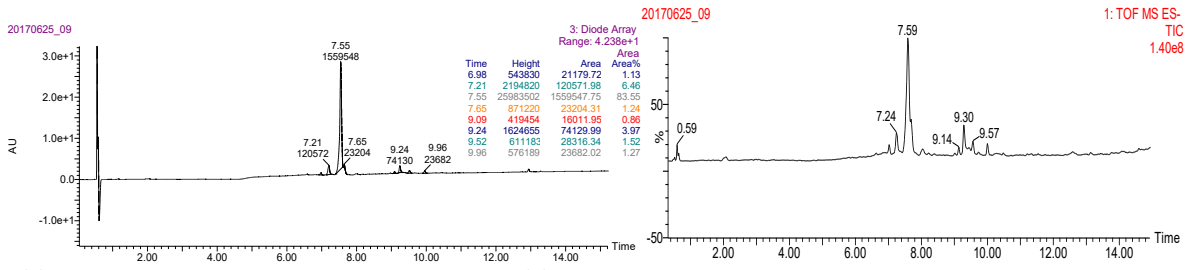


(a) **(b)**

Figure 6: (a) UPLC chromatogram (b) MS/MS spectra of Velptasvir under acidic hydrolysis conditions (0.1 N HCl, 60 ^{0}C)

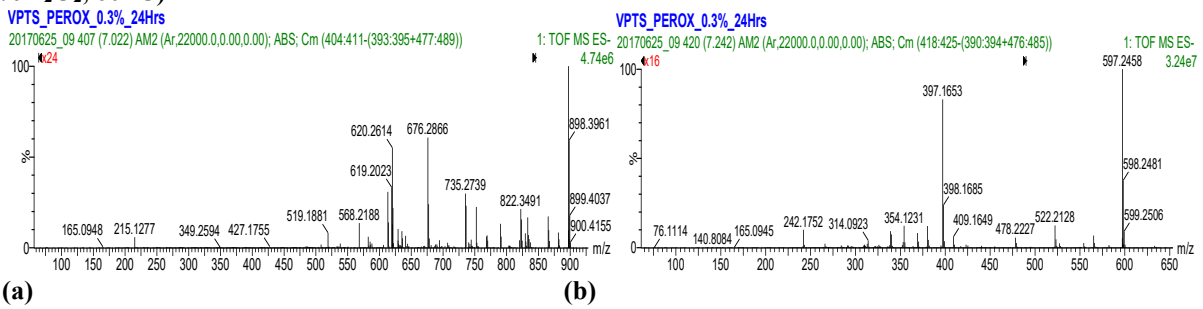
Oxidative induced degradation study

For the observation of degradation products under oxidative conditions generally either Hydrogen peroxide or Azaiso bis-nitrile (AIBN) will used in the experments. In this condition hydrogen peroxide has been used for the oxidative stability indicating conditions. Under these conditions seven degradation products has been observed. The degradation products of velpatasvir MS/MS data were depicted in the following table.2 contains the information regarding the fragment ions observed from the degradation products and the RDBE values and the observed molecular ions data as follows. Further all the degradation products which were separated on reverse phase chromatography were tentatively identified using the Time-of-flight mass spectrometry. Because of the high-resolution capacity of the instrument with the observed data molecular ions/formulas has been generated and depicted in the following table 7 and the obtained chromatograms and degradation products mass spectrums were shown in figure 7 and 8.



(a) **(b)**

Figure 7: (a) UPLC chromatogram (b) TIC-MS chromatogram of Velpatasvir under peroxide conditions (0.3%H_2O_2, 60 ^{0}C)



(a) **(b)**

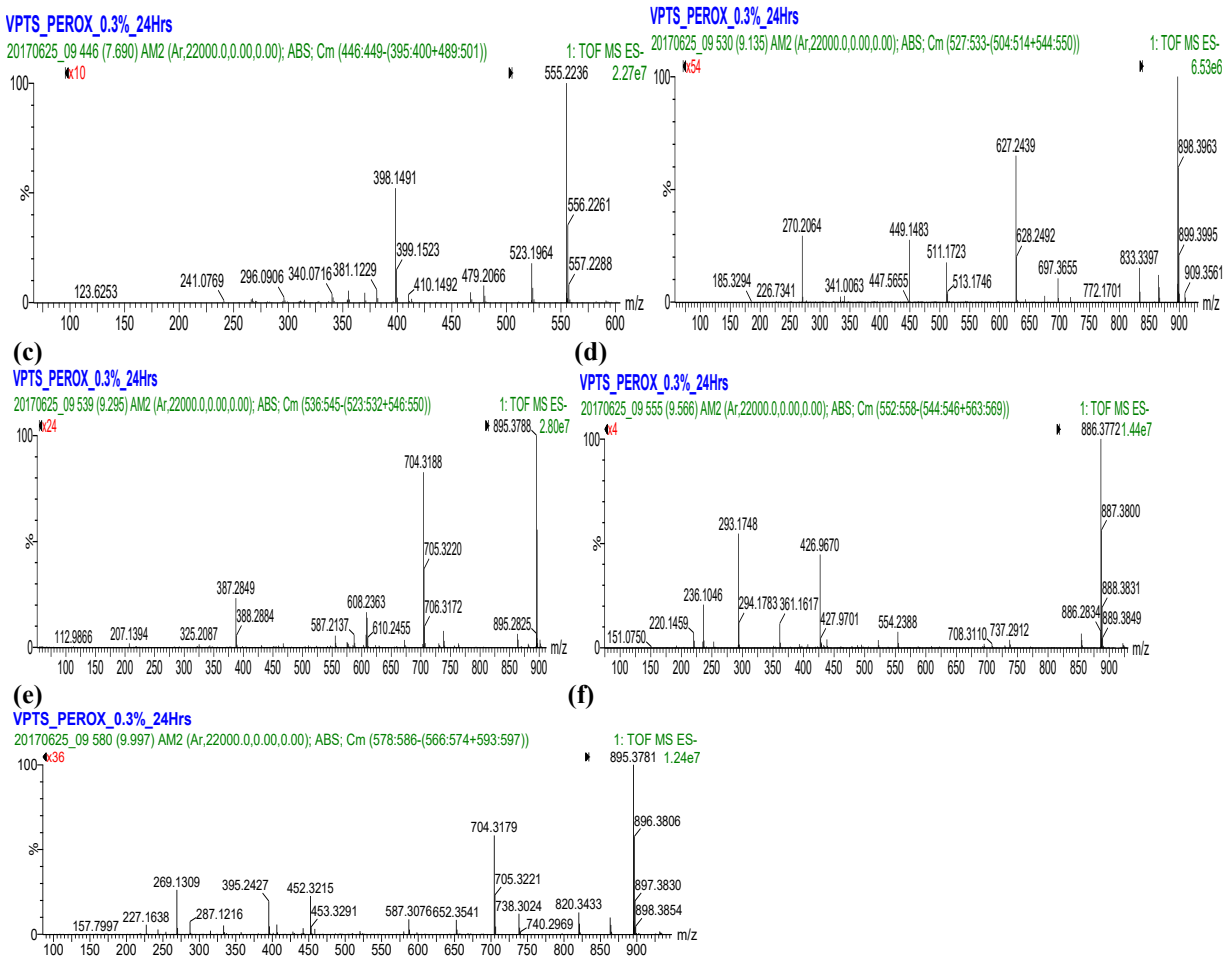


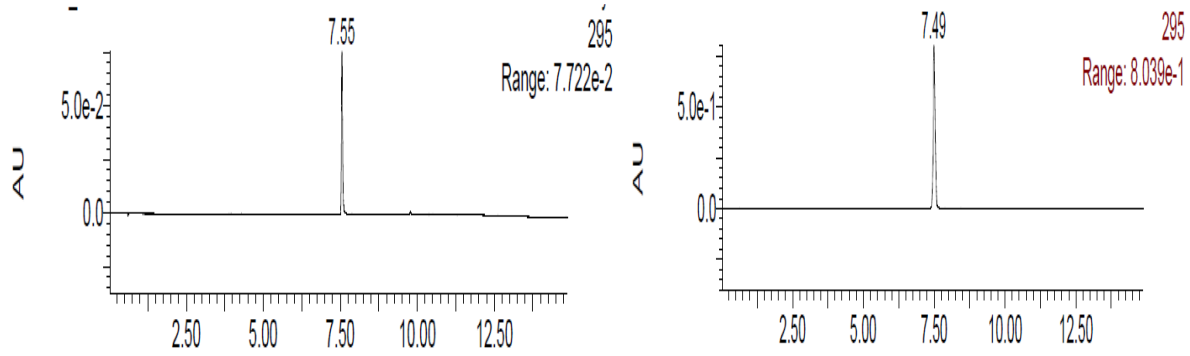
Figure 8: MS/MS spectra for (a) DP-05 (b) DP-06 (c) DP-07 (d) DP-08 (e) DP-09 (f) DP-10 (g) DP-11 formed under peroxide conditions (0.3% H_2O_2, 60 ^{0}C)

Table 7: The fragment ions observed from the peroxide degradation products and the RDBE values and the observed molecular ions data as follows.

S No	Observed HRMS	RDBE	Mol. Formula	Error
1	897.3934	27.5	$C_{49}H_{53}N_8O_9$	-0.2
	865.3665	28.5	$C_{48}H_{49}N_8O_8$	-0.9
	833.3431	29.5	$C_{47}H_{45}N_8O_7$	2.4
	676.2866	22.5	$C_{37}H_{38}N_7O_6$	-2.7
	620.2614	21.5	$C_{34}H_{34}N_7O_5$	-1.1
	519.1881	16.5	$C_{27}H_{27}N_4O_7$	0.2
2	597.2458	19.5	$C_{32}H_{33}N_6O_6$	-0.7
	565.2184	20.5	$C_{31}H_{29}N_6O_5$	-2.7
	522.2128	19.5	$C_{30}H_{28}N_5O_4$	-2.5
	397.1653	16.5	$C_{24}H_{21}N_4O_2$	-3.0
3	555.2236	18.5	$C_{31}H_{31}N_4O_6$	-1.4
	523.1964	19.5	$C_{30}H_{27}N_4O_5$	-3.2
	398.1491	16.5	$C_{24}H_{20}N_3O_3$	-3.5
	381.1229	17.5	$C_{24}H_{17}N_2O_3$	-2.6
4	897.3937	27.5	$C_{49}H_{53}N_8O_9$	0.1
	865.3665	28.5	$C_{48}H_{49}N_8O_8$	-0.6
	627.2439	19.5	$C_{34}H_{35}N_4O_8$	-2.6
	511.1723	19.5	$C_{27}H_{23}N_6O_5$	-1.4
	443.1483	15.5	$C_{23}H_{21}N_4O_6$	4.9
	270.2064	2.5	$C_{15}H_{28}NO_3$	-1.9
5	895.3788	28.5	$C_{49}H_{51}N_8O_9$	1.0

	863.3514 704.3188 387.2849	29.5 22.5 2.5	$C_{48}H_{47}N_8O_8$ $C_{39}H_{42}N_7O_6$ $C_{20}H_{39}N_2O_5$	-0.3 -1.3 -2.6
6	886.3772 854.3502 554.2388 361.1617 293.1748	26.5 27.5 18.5 9.5 5.5	$C_{48}H_{52}N_7O_{10}$ $C_{47}H_{48}N_7O_9$ $C_{31}H_{32}N_5O_5$ $C_{16}H_{21}N_6O_4$ $C_{17}H_{25}O_4$	-0.5 -1.4 -2.7 -1.9 -1.7
7	**Same as 5**	-	-	-

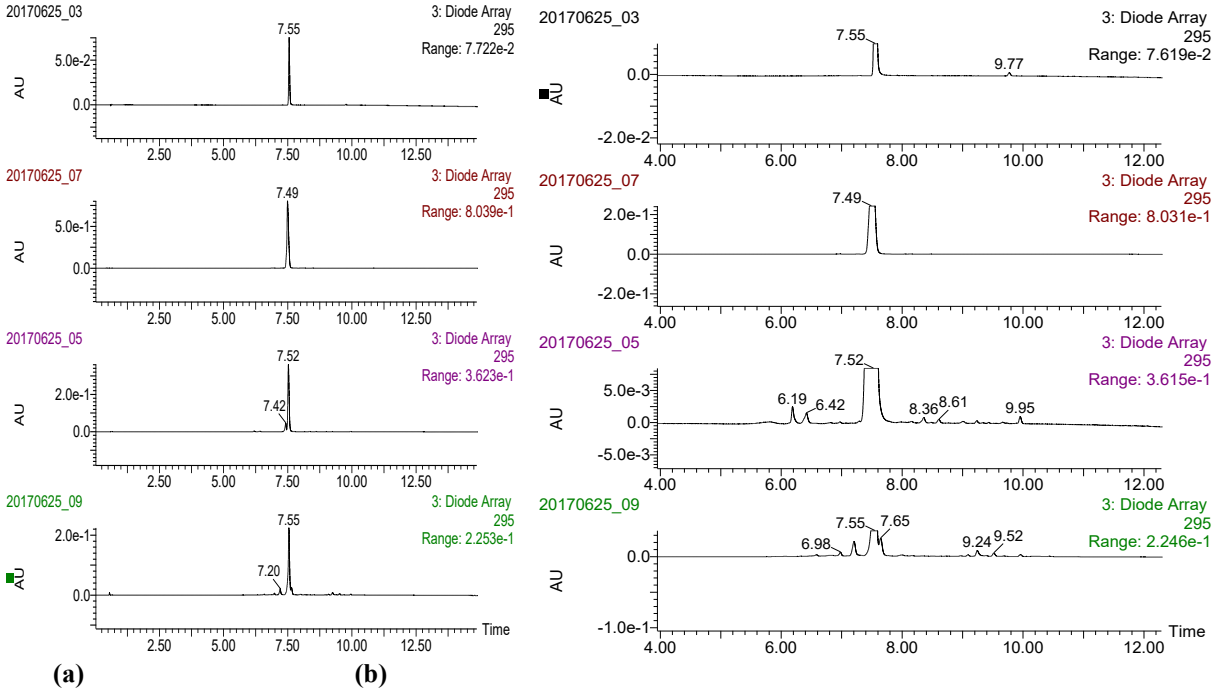
Degradation products were not observed in photolytic and thermal conditions. UPLC chromatograms of Velptasvir under photolytic and thermal conditions were shown in figure 9. So the drug remained stable under thermal and photolytic stress conditions.



(a) **(b)**

Figure 9: UPLC chromatogram of Velpatasvir under (a) photolytic conditions (b) thermal conditions

That study indicates the formation of stability indicating degradation products which were formed under the alkaline & oxidative conditions. In comparison to the alkaline and acidic hydrolysis conditions for the drug substance the acidic conditions were showing that the drug substance was highly stable in the above specified conditions but not the alkaline and Peroxide conditions. The Comparitive UPLC chromatogram of Velpatasvir (1) Acid Hydrolysis (2), Alkaline Hydrolysis (3) and Peroxide conditions (4) were shown in figure 10.



(a) **(b)**

Figure 10: (a) Comparitive UPLC chromatogram of 1. Velpatasvir 2. Acid Hydrolysis 3. Alkaline Hydrolysis and 4. Peroxide conditions (b) Zoom in View

CONCLUSION

The study indicates the formation of stability indicating degradation products which were formed under the hydrolytic & oxidative conditions. In comparison to the alkaline and acidic hydrolysis conditions for the drug substance the acidic conditions were showing that the drug substance was highly stable in the above specified conditions but not the alkaline

conditions. In alkaline conditions four degradation products in which one was diasteriomeric form for the drug substance. Apart from the hydrolytic conditions under oxidative peroxide conditions it was observed that formation of seven degradation products in which four were oxidative `degradation products which were formed by the addition of oxygen atom by the elimination of two hydrogen atoms. The drug remained stable under thermal and photolytic stress conditions. For further characterization of the above formed degradation products to proceed for the purification via Prep HPLC & toxicity for the identification of genotoxic impurities.

REFERENCES

1. https://www.drugbank.ca/drugs/DB11613
2. Phani RS, Prasad KS, Mallu UR. A Bioanalytical Method Development and Validation for Simultaneous Determination of Velpatasvir and Sofosbuvir in Spiked Human Plasma. Asian J. Chem. 2017; 29:2565-2569.
3. Sridevi, Marakada; Rao, T. Siva; Naidu, Challa Gangu. Development and validation of liquid chromatographic method for simultaneous determination of sofosbuvir, velpatasvir and voxilaprevir in fixed tablet dosage form. European Journal of Biomedical and Pharmaceutical Sciences. 2018; 5(5):1-10.
4. Bandla J, Ganapaty S. Stability indicating RP-HPLC method development and validation for the simultaneous determination of Sofosbuvir and Velpatasvir in tablet dosage forms. Indian Journal of Pharmaceutical and Biological Research. 2017; 5(4):10-16.
5. Nalla S, Seshagiri Rao JV. A Stability indicating RP-HPLC method for simultaneous estimation of Velpatasvir and Sofosbuvir in combined tablet dosage forms. World Journal of Pharmacy and Pharmaceutical Sciences. 2017; 6(9):1596-1611.
6. Jyothi U, Umadevi P. Analytical Method Development and Validation for the Simultaneous Estimation of Sofosbuvir and Velpatasvir Drug Product by RP-HPLC Method. Indo American Journal of Pharmaceutical Research. 2017; 7(8).
7. El-Wekil MM, Ali HR, Marzouk AA, Ali R. Enhanced dispersive solid phase extraction assisted by cloud point strategy prior to fluorometric determination of anti-hepatitis C drug velpatasvir in pharmaceutical tablets and body fluids. RSC Advances. 2018; 8(24):13292-13300