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A New Stability Indicating RP-HPLC Method for Simultaneous Estimation of Voxilaprevir, Sofosbuvir and Velpatasvir in Bulk and Pharmaceutical Dosage Forms.

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ABSTRACT

A new, simple, rapid and stability-indicating RP-HPLC method was developed and validated for the estimation of Voxilaprevir (VOX), Sofosbuvir (SOF) and Velpatasvir (VEL) in pharmaceutical dosage forms. The HPLC method was developed on SHISEIDO C18 column (250 x 4.6 mm i.d, 5µ) using Acetonitrile : 20 mM acetate buffer (pH 3.0±0.1) in the ratio of 85:15 v/v at 245 nm. The retention times for VOX, SOF and VEL were found to be 2.75, 3.37 and 6.49 min respectively. Linearity was established in the range of 2.5-15 µg/ml, 10-60 µg/ml and 2.5-15 µg/ml for VOX, SOF and VEL respectively. The method was precise with %RSD < 2 for both intraday and interday precision. The accuracy of the method was performed over three levels of concentration and the recovery was in the range of 98-102%. The drugs were individually subjected to forced degradation (thermal, photolytic, hydrolytic, and oxidative stress conditions) studies at different strengths and temperatures. VOX showed highest degradation in acidic and basic condition. SOF and VEL showed highest degradation in peroxide and acidic conditions when compared with basic, photolytic and thermal conditions. The method was successfully applied for quantifying the drugs in marketed dosage forms.

Keywords: Voxilaprevir, Forced degradation, Acetate buffer, RP-HPLC, Sofosbuvir.

I. INTRODUCTION:

Voxilaprevir (Fig. 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). HCV is a single-stranded RNA virus that is categorized into nine distinct genotypes, with genotype 1 being the most common in the United States, and affecting 72% of all chronic HCV patients. Sofosbuvir (Fig. 2) is a nucleotide analog inhibitor of HCV NS5B polymerase. It is a direct acting antiviral 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 (Fig. 3) 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). HCV is a single-stranded RNA virus that is categorized into nine distinct genotypes, with genotype 1 being the most common in the United States, and affecting 72% of all chronic HCV patients. Velpatasvir acts as a defective substrate for NS5A (Non-Structural Protein 5A), a non-enzymatic viral protein that plays a key [1-4].

The present study is to develop a stability indicating RP-HPLC method for VOX, SOF and VEL. The objective of the study is to subject the drugs for acid, base, peroxide, light, thermal degradation and estimate its extent of degradation. A Literature survey reveals that several analytical methods for the estimation of VOX, SOF and VEL based on UV [5-8], HPTLC [9], HPLC [10-23], Stability indicating HPLC [24-30], Related substances [19],Bio-analytical [31], UPLC [32], LC-MS [33,34] were reported.

Although different analytical methods are available, a more economical stability indicating analytical method was developed for estimation of VOX, SOF and VEL. We have forcefully degraded the drugs (standards) under different stress conditions and developed an HPLC method that can differentiate the pure drug from its degradants. The International Conference on Harmonization (ICH) guideline entitled “Stability Testing of New Drug Substances and Products” requires that stress

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testing be carried out to elucidate the inherent stability characteristics of the active substance [35].

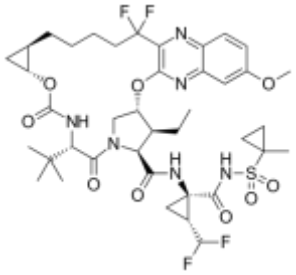

Fig.1 Chemical structure of Voxilaprevir

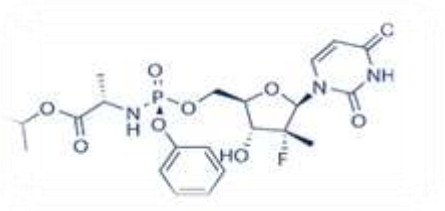

Fig.2 Chemical structure of Sofosbuvir

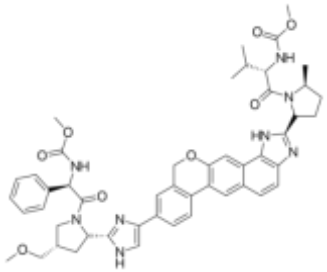

Fig.3 Chemical structure of Velpatasvir

II. MATERIALS AND METHODS:

A. Chemicals and reagents:

Voxilaprevir (VOX), Sofosbuvir (SOF) and Velpatasvir (VEL) working standards were procured from Yarrow Chemicals Pvt. Ltd, Mumbai. Commercially available as Vosevi tablets were procured as gift samples from Gilead sciences Pvt. Ltd. HPLC grade water was purchased from Thermo Fisher Scientifics Ltd., Mumbai. HPLC grade Acetonitrile, Orthophosphoric acid, Hydrochloric acid, Sodium hydroxide pellets purified and Hydrogen peroxide 30% of AR grade were procured from Merck specialties Pvt. Ltd., Mumbai.

B. Instrumentation and analytical conditions:

RP-HPLC method was performed on the HPLC system (Shimadzu) consisting of binary gradient pump with UV detector (LC-20AD). Rheodyne injector with 20 µl fixed loop was used for injecting sample on SHISEIDO C18 column (250 x 4.6 mm i.d, 5µ) in the present study.

C. Preparation of solutions:

i. Preparation of standard stock solutions:

Standard stock solutions were prepared by transferring accurately weighed 10 mg of VOX, SOF and VEL into separate 100 ml volumetric flask. The compounds are then dissolved in diluent (mobile phase) to obtain a standard solution of VOX (100 µg/ml), SOF (100 µg/ml) and VEL (100 µg/ml)

ii. Preparation of the mobile phase:

The mobile phase is a mixture of acetate buffer and acetonitrile. Acetate buffer is prepared by dissolving 1.64 gm of sodium acetate in 1000 ml (20 mM concentration) HPLC grade water. pH of the buffer is adjusted to 3.0 using ortho phosphoric acid. The prepared buffer was filtered through 0.45 µm membrane filter (Millipore) and sonicated before use. Mobile phase is pumped in the ratio of 85 : 15 %v/v (acetonitrile : buffer).

METHOD VALIDATION:

The developed method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures. The developed method was validated with respect to parameters such as linearity, LOD, LOQ, precision, accuracy and specificity. Forced degradation studies were done according to ICH Harmonized Tripartite Guideline, Stability Testing of New Drug Substances and Products: Q1A (R2).

A. System suitability:

The system suitability of the HPLC method was determined by making six replicate injections from freshly prepared standard solutions and analyzing each solute for their retention time, theoretical plates number (N) and tailing factor (T).

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B. Specificity:

It is the ability to assess unequivocally the analyte in the presence of impurities, degradants and matrix. To determine this, 20 µl of blank, standard and sample solutions were injected separately in triplicate and respective chromatograms were recorded under the optimized conditions.

C. Limit of detection and limit of quantification:

Limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the values of Signal to Noise (S/N) ratio for two drugs. For LOD, S/N ratio should be 3:1 and for LOQ, S/N ratio should be 10:1

D. Linearity:

The calibration curves were obtained with concentrations of the standard solutions of 2.5-15 µg/ml, 10-60 µg/ml and 2.5-15 µg/ml for VOX, SOF and VEL respectively. Linearity is evaluated by regression analysis, which was calculated by the least square regression method.

E. Accuracy:

To check the degree of accuracy, recovery studies were performed in triplicate by the standard addition method at 50%, 100% and 150% levels.

F. Precision:

The precision of the analytical method was evaluated by the determination of the repeatability of the method (intra day precision) and intermediate precision (inter day precision) of the sample solutions. Repeatability was calculated by assaying six samples prepared on the same day. Intermediate precision was calculated by assaying 3 days. The relative standard deviation of the area of peaks was calculated.

G. Robustness:

Robustness was determined by analysis of samples under slight variations in chromatographic conditions. The flow rate of the mobile phase was changed from 0.9 ml/min to 1.1 ml/min. The ratio of the organic phase (acetonitrile) was changed by +2% and -2%. The effect of retention time and peak parameters were studied.

STRESS TESTING STUDIES:

To demonstrate the stability-indicating of the method, forced degradation studies were performed under different stress conditions like acidic, basic, thermal, peroxide, UV and neutral environment. The extent of degradation and the interference of formed degradant peaks with analyte were investigated.

Acidic degradation:

Under the forced degradation studies with acidic environment 1 ml of VOX, 4 ml (SOF) & 1ml (VEL) of stock analyte samples were transferred into three calibrated 10 ml volumetric flasks individually. One ml of 0.1M HCl, 0.5M HCl, 1M HCl respectively were added to them and the flasks were kept under room temperature of 28 ºC for a period of 24 hrs. The solution was then neutralized by adding 1 ml of 0.1M NaOH, 0.5M NaOH, 1M NaOH respectively and then these are made up to the mark using the diluents. The resultant solution was filtered through HPLC grade 0.45 micron syringe filter.

Alkaline degradation:

Under the forced degradation studies with basic environment 1 ml of VOX, 4 ml (SOF) & 1ml (VEL) of stock analyte samples were transferred into three calibrated 10 ml volumetric flasks individually. One ml of 0.1M NaOH, 0.5M NaOH, 1M NaOH are added respectively into three 10 ml volumetric flasks and the flasks were kept under room temperature of 28º C for a period of 24 hours. The solution was then neutralized by adding 1 ml of 0.1M HCl, 0.5M HCl, 1M HCl respectively and then these are made up to the mark using the diluents. The resultant solution was filtered through HPLC grade 0.45 micron syringe filter.

Oxidative degradation:

For doing oxidation 1 ml of VOX, 4 ml (SOF) & 1ml (VEL) of stock analyte samples were transferred into three calibrated 10 ml volumetric flasks individually, added 1 ml of 1%, 3%, 4.5% H_2O_2 for peroxide studies and made up to the mark with diluent.

Thermal degradation:

For thermal studies, 1 ml of VOX, 4 ml (SOF) & 1ml (VEL) of stock analyte samples were transferred into three calibrated 10 ml volumetric flasks, made up to the mark with diluent. The samples were kept under reflux at a temperature of 60˚C for 1hr, 105˚C for a period of 45 min and 150˚C for 30 min. The resultant solution was filtered through HPLC grade 0.45 micron syringe filter.

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Photolytic degradation:

UV studies were done by mixing 1 ml of VOX, 4 ml (SOF) & 1ml (VEL) of stock into three calibrated 10 ml volumetric flasks individually, Samples were kept in an UV chamber for a period of 6 hrs,12 hrs and 24 hrs respectively, then made up to the mark with diluent and then injected into the system after filtration. The final concentrations were adjusted in such a way to attain a concentration of 40 µg/ml of SOF and 10 µg/ml of VEL.

Neutral method

Neutral studies were done by mixing 1 ml of VOX, 4 ml (SOF) & 1ml (VEL) of stock into three calibrated 10 ml volumetric flasks individually, then made up to the mark with diluent and then injected into the system after filtration. The final concentrations were adjusted in such a way to attain a concentration of 40 µg/ml of SOF and 10 µg/ml of VEL.

Data processing:

Control samples were also injected besides degradation samples. The peak areas of the drugs were calculated and compared with controls. The percentage degradation was calculated according to the data obtained. The data was processed using Microsoft® Excel™ sheet to get rate of reaction, half life, time required to degrade 90%.

III. RESULTS AND DISCUSSION:

A. Method development and optimization:

The choice of the detection wavelength was based on the scanned absorption spectrum of Voxilaprevir, Sofosbuvir and Velpatasvir. 10 mg of Voxilaprevir, Sofosbuvir and Velpatasvir were dissolved in 10 ml of Acetonitrile and water (50 : 50) seperately. The UV-spectrum of Voxilaprevir, Sofosbuvir and Velpatasvir was separately scanned in the wavelength range 200-400 nm against blank. After correlation of the spectrums 245 nm wavelength was selected for the analysis (Fig. 4). Trails were performed using different columns (Hypersil BDS C18, Symmetry C18, Phenomenex C18 and Shiseido C18), buffers (Acetate, Phosphate, Ortho phosphoric acid), pH (3-6), organic phases (Acetonitrile, Methanol). Shiseido C18 column (250mm X 4.6 mm, 5 µ) produced good separation with efficient resolution and more theoretical plates. The drugs were eluted at a flow rate of 1.0 ml/min using a mobile phase consisting of acetoniitrile : 20 mM acetate buffer (pH 3.0) in the ratio of 85 : 15 v/v respectively. The retention times for VOX, SOF and VEL were found to be 2.75, 3.37 and 6.49 min respectively.

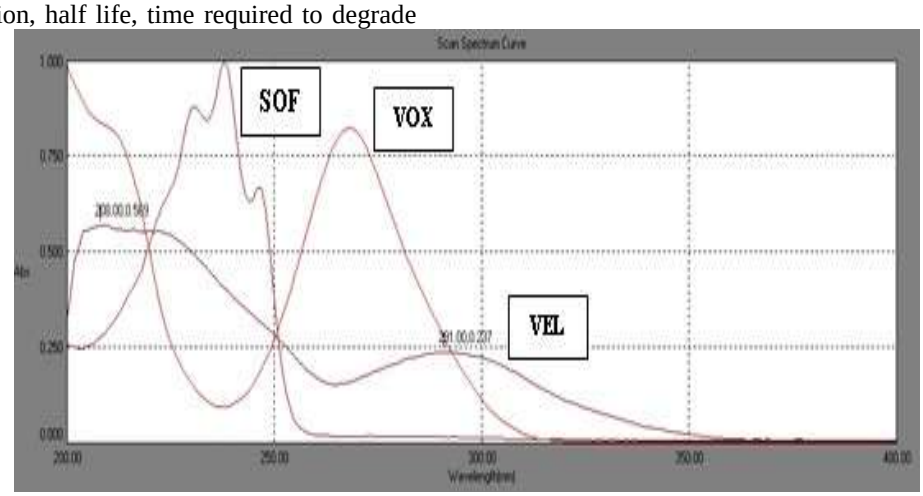

Fig.4: UV Overlay spectrum of VOX, SOF and VEL.

B. System suitability:

Under optimized chromatographic conditions 20 µl of solution containing 10 µg/ml of VOX, 40 µg/ml of SOF and 10 µg/ml of VEL was injected into the system in six replicates. Chromatograms were recorded and studied for different system suitability parameters like retention time, peak area, number of theoretical plates, tailing factor and resolution. The results were shown in table 1.

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Table 1. System suitability results for VOX, SOF and VEL

INJECTION	VOX peak area	SOF peak area	VEL peak area
Injection1	457095	964534	910721
Injection2	456012	963243	910695
Injection3	454854	964634	920657
Injection4	457654	966525	910720
Injection5	456258	964121	920735
Injection6	457592	964734	920726
Average	456577.5	964631.833	915709
Standard deviation	1081.49	1076.46	5474.01
%RSD	0.2368	0.1115	0.5977
Theoretical Plates	5201	5312	11837
Tailing factor	1.01	1.0552	1.021

C. Specificity:

The HPLC chromatograms were recorded for blank (Fig. 5a) and standard (Fig. 5b) under optimized analytical conditions and compared for additional peaks, however no additional peaks were found. The three peaks were completely separated in HPLC chromatogram and the resolution was found to be more than 2.

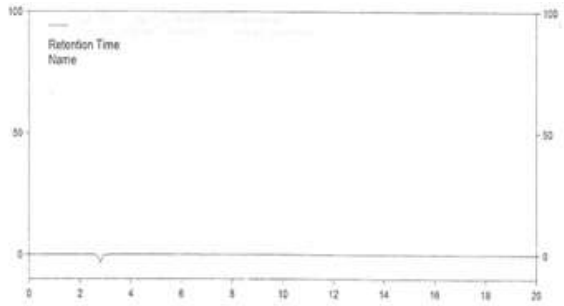

Fig 5a.Blank chromatogram

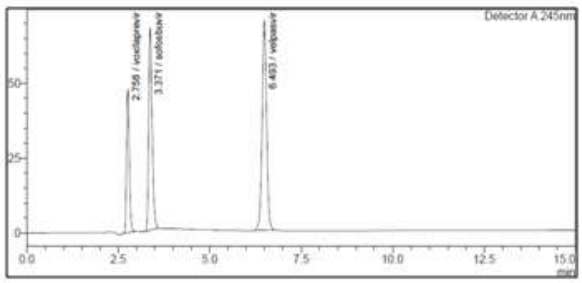

Fig. 5b: Chromatograms for specificity of VOX, SOF and VEL, which were injected in combination.

D. Linearity:

Linearity was established over the range of 2.5 µg/ml to 15 µg/ml for Voxilaprevir, 10 µg/ml to 60 µg/ml for Sofosbuvir and 2.5 µg/ml to 15 µg/ml for Velpatasvir using the weighted least square regression analysis and the results were shown in table 2 and 3, linearity graphs were down as fig 6A, 6B, 6C.

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Table 2. Linearity of VOX, SOF and VEL

Conc. VOX (µg/ml)	Mean Area* ± S.D	%RSD	Conc. SOF (µg/ml)	Mean Area* ± S.D.	%RSD	Conc. VEL (µg/ml)	Mean Area* ± S.D.	%RSD
2.5	131601.7±583.12	0.4	10	201595±556.50	0.2	2.5	342371±1623.1	0.4
5	244455±1160.4	0.4	20	401558.3±545	0.1	5	601134±637.2	0.1
7.5	358526±5776.3	1.7	30	655448.7±1589.3	0.2	7.5	906773.7±1709.	0.1
10	457071.3±1737.82	0.3	40	857362.3±1050.25	0.1	10	1233693±20615	1.6
12.5	578387.7±995	0.7	50	1094410±6035	0.5	12.5	1505074±6997	0.4
15	687360.3±1148.92	0.1	60	1260711±5492	0.4	15	1775992±2303	0.1

***Mean of six determinations**

Table 3. Linearity parameters of VOX, SOF and VEL

Drug	R^2	Slope	Conc. range (µg/ml)
VOX	0.999	44333	2.5-15
SOF	0.997	21646	10-60
VEL	0.998	11665	2.5-15

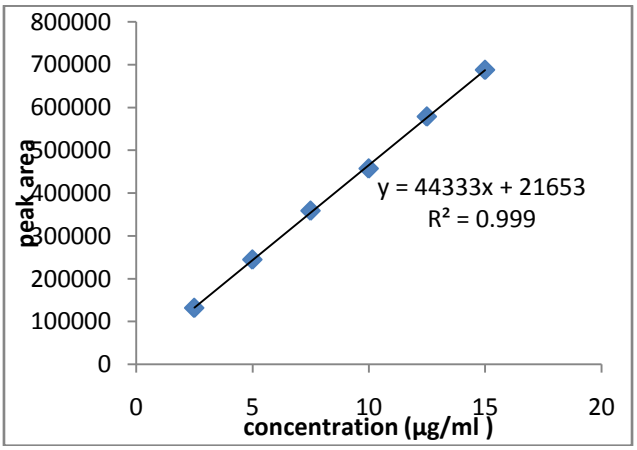

Figure 6A: Linearity plot of VOX

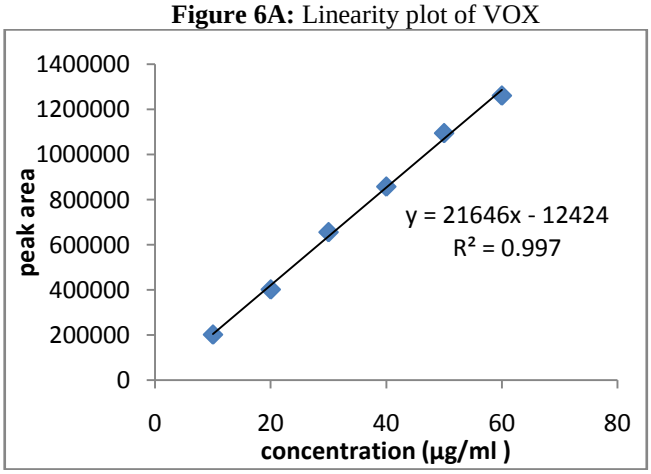

Figure 6B: Linearity plot of SOF

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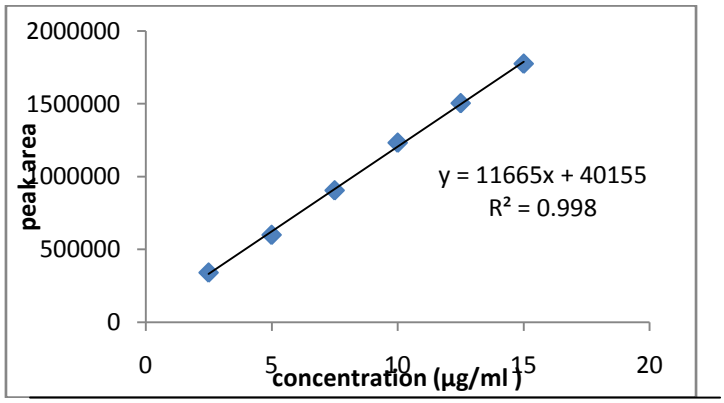

Figure 6C: Linearity plot of VEL

E. Accuracy:

The accuracy for proposed method was determined, recovery studies were performed in mentioned levels and recorded (Table 4), Obtained results were found to be within the limits of 98-102%, indicating an agreement between the true value and found value.

Table 4. Recovery studies of VOX, SOF and VEL

Drug	Conc. Standard (µg/ml)	Conc. Added (µg/ml)	Amount Recovered (µg/ml)	% Recovery* ±S.D.	%RSD
VOX	5	2.5	7.61	101.4±560.4	0.1
	5	5	9.99	99.90±1148.0	0.2
	5	7.5	12.62	100.98±1130.0	0.2
SOF	20	10	30.47	101.57±2274	0.2
	20	20	39.88	99.71±1148	0.1
	20	30	49.78	99.55±5549	0.4
VEL	5	2.5	7.39	98.55±5790	0.5
	5	5	9.88	98.77±2311	1.4
	5	7.5	12.42	99.37±2274	1.5

*Mean of six determinations

F. Precision:

Precision was calculated as intra-day and inter-day variations for the drugs. Percent relative standard deviations for estimation of VOX, SOF and VEL under intra-day and inter-day variations were found to be less than 2. Results were showed in Table 5 & 6

Table 5. Precision values of VOX, SOF and VEL

Drug	Conc. (µg/ml)	Intra-Day		Inter-Day	
		Mean Area*±S.D.	%RSD	Mean Area*±S.D.	%RSD
VOX	5	244221.6±663.35	0.2	244243.3±650.42	0.26
	10	451728±2892.52	0.6	657458±1168.3	0.50
	15	657693±1726.2	0.2	657458±1168.37	0.10
SOF	20	431925±571.57	0.1	431963±553.05	0.12
	40	918496±949.22	0.1	919259±1264.7	0.13
	60	1270365±23088.2	1.8	1273555±20328.1	1.50
	5	601281±609.17	0.1	601594.7±693.4	0.14
	10	1247027±5712.8	0.4	12833933±11573	0.90

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VEL	15	1881332±11552.7	0.6	1884875±9991.7	0.50

*average of 6 determinations

Table 6. Precision results of VOX, SOF and VEL

Parameter	VOX	SOF	VEL
Intra-Day Precision (%RSD)	0.2-0.6	0.1-1.8	0.1-0.6
Inter-Day Precision (%RSD)	0.26-0.1	0.12-1.5	0.14-0.5
Analyst Precision (%RSD)	0.6-0.2	0.1-1.8	0.4-0.6
Injection Repeatability For R_t (%RSD)	0.1-0.5	0.13-1.5	0.5-0.9
Injection Repeatability For Area (%RSD)	0.2-0.1	1.5-1.8	0.5-0.6

G. Sensitivity

It is expressed as Limit of detection and Limit of quantitation. LOD is the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) with a stated confidence level (generally 99%). LOQ is the lowest concentration at which the analyte can not only be reliably detected but at which some predefined goals for bias and imprecision are met.

Table 7. LOD and LOQ of VOX, SOF and VEL

Parameter	VOX	SOF	VEL
LOD (µg/mL)	0.12	0.16	0.28
LOQ (µg/mL)	0.38	0.48	0.81

H. Stock solution stability

i. Short term stock dilution stability at room temperature

The stability of stock dilutions of analyte was evaluated at room temperature.

Acceptance Criteria: % stability should be within 90 to 110% or the % change should be ± 10%.

%Stability = (Mean response of stability samples / Mean response of comparison samples) × 100%.

%Change = 100 – (Mean response of stability samples / Mean response of Comparison samples × 100)

Table 8: Short term stock solution stability study

Stock dilutions at room temperature	LQC	% Change	MQC	% Change	HQC	% Change
	5*		10*		15*	
VOX						
After 1hr	4.998	0.04	9.996	0.04	14.996	0.026
After 2hr	4.991	0.18	9.992	0.08	14.990	0.066
After 3hr	4.981	0.38	9.988	0.12	14.985	0.100
SOF						
	20*		40*		60*	
After 1hr	19.997	0.015	39.997	0.007	59.987	0.021
After 2hr	19.964	0.180	39.855	0.365	59.868	0.220
After 3hr	19.934	0.330	39.819	0.452	59.749	0.418
VEL						
	5*		10*		15*	
After 1hr	4.999	0.020	9.998	0.020	14.997	0.020
After 2hr	4.987	0.260	9.994	0.060	14.991	0.209
After 3hr	4.979	0.420	9.989	0.110	14.984	0.255
N	3		3		3	

*nominal concentrations in µg/ml

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ii. Short term stock dilution stability in refrigerator

Acceptance Criteria: % change should be ± 10%.

%Stability = (Mean response of stability samples/Mean response of comparison samples) × 100%.

%Change = 100 – (Mean response of stability samples / Mean response of Comparison samples × 100)

Table 9. Short term stock solution stability study in refrigerator

Stock dilutions at room 2-8°C	LQC	% Change	MQC	% Change	HQC	% Change
	5*		10*		15*	
VOX						
After 1hr	4.999	0.020	9.999	0.010	14.998	0.013
After 2hr	4.997	0.060	9.995	0.050	14.994	0.040
After 3hr	4.994	0.120	9.991	0.090	14.991	0.060
SOF						
	20*		**40***		**60***	
After 1hr	19.999	0.005	39.999	0.002	59.998	0.003
After 2hr	19.984	0.080	39.975	0.062	59.958	0.070
After 3hr	19.975	0.125	39.959	0.102	59.919	0.135
VEL						
	5*		**10***		**15***	
After 1hr	4.999	0.020	9.999	0.010	14.998	0.013
After 2hr	4.997	0.060	9.995	0.050	14.994	0.040
After 3hr	4.994	0.120	9.991	0.090	14.991	0.060
N	3		3		3	

*nominal concentrations in µg/ml

iii. Long term stock solution stability

The stability of the stock solution when stored in the refrigerator for a given period of time was determined. Stock solutions of the analyte was prepared and stored in the refrigerator between 2 – 8 °C for 3 weeks (stability stock).

Correlation factor (CF) = Concentration of comparison stock / Concentration of stability stock

%Stability = (Mean response of stability samples / Mean response of comparison samples) × CF x 100%

Table 10. Long term stock solution stability study

Stock dilutions at room 2-8°C	LQC	% Change	MQC	% Change	HQC	% Change
	5*		10*		15*	
VOX						
After 1st week	4.988	0.240	9.985	0.150	14.954	0.306
After 2nd week	4.965	0.700	9.954	0.460	14.901	0.660
After 3rd week	4.942	1.160	9.892	1.080	14.868	0.880
SOF						
	20*		**40***		**60***	
After 1st week	19.959	0.205	39.899	0.252	59.898	0.170
After 2nd week	19.884	0.580	39.775	0.562	59.658	0.570
After 3rd week	19.775	1.125	39.659	0.852	59.419	0.963
VEL						

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	5*		10*		15*	
After 1st week	4.989	0.220	9.989	0.110	14.958	0.280
After 2nd week	4.967	0.660	9.955	0.450	14.904	0.640
After 3rd week	4.944	1.120	9.901	0.990	14.871	0.860
N	3		3		3	

I. Robustness:

Robustness of the method was studied by injecting the standard solutions with slight variations in the optimized conditions namely, ± 1% in the ratio of acetonitrile in the mobile phase, varying wavelength and ± 0.1 ml of the flow rate.

Table 11. Robustness Parameters of VOX, SOF and VEL

Sample	VOX			SOF			VEL		
	R_t	Area	Tailing Factor	R_t	Area	Tailing Factor	R_t	Area	Taling Factor
Standard	2.753	456068	1.20	3.435	918399	1.14	6.563	1256330	1.030
0.8 ml/min	3.358	555524	1.23	4.119	1133955	1.03	7.773	1527650	1.032
1.2 (ml/min)	2.234	398747	1.20	2.737	814156	1.16	5.184	1109782	1.04
Org. Phase (+5%)	2.710	1.9999	1.24	3.082	399588	1.20	6.077	562453	1.008
Org.Phase (-5%)	2.811	209915	1.25	3.117	424168	1.30	7.863	576100	0.960
243 nm	2.736	383157	1.10	3.370	975475	1.17	6530	1399743	1.031
247 nm	2.760	582956	1.10	3.391	837695	1.16	6556	1080897	1.027

*average of 3 determinations

J. Ruggedness

Ruggedness of the method was studied by changing the experimental conditions such as operators, instruments, source of reagents, solvents and column of similar type.

Table 12. Results for ruggedness of VOX, SOF and VEL

Drug	Analyst	Retention time (min)	Peak Area	RSD (%)	System suitability results	
					Plate count	Tailing factor
VOX	Analyst 1	2.71	456879	0.05	5212	1.02
	Analyst 2	2.72	456991	0.04	5124	1.03
SOF	Analyst 1	3.21	854495	0.03	5314	1.13
	Analyst 2	3.28	854587	0.05	5318	1.24
VEL	Analyst 1	6.37	1233421	0.02	11854	1.12
	Analyst 2	6.29	1233712	0.03	11845	1.05

K. Assay:

The marketed formulation used was vosovi (Gilead) 100 mf of Voxilaprevir, 400 mg sofosbuvir and 100 mg Velpatasvir**.**

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Table 13. Assay of VOX, SOF and VEL in pharmaceutical formulation

Drug	Label claim	Amount found	Mean* %Recovery ± S.D.	%RSD*
VOX	100 mg	99.25	99.25±0.254	0.457
SOF	400 mg	398.79	99.46±0.692	0.693
VEL	100 mg	99.54	99.54±0.963	0.963

*values are expressed as mean ±SD (n= 3)

L. Stress testing studies:

Stress studies were performed to the analyte by exposing the drug sample to acidic, alkali, peroxide, UV and thermal environment. The degradation peaks were confirmed by witnessing different peaks at different R_t and also there was a decrement in the peak area of the analyte. The forced degradation data of VOX, SOF and VEL was given in Table 14, 15 and 16.

VOX showed highest degradation in acidic and basic condition. SOF and VEL showed highest degradation in peroxide and acidic conditions when compared with basic, photolytic and thermal conditions. SOF showed least degradation in acidic condition compared with VOX and VEL. SOF and VEL showed approximately equal degradation in peroxide condition. All the drugs showed good stability under photolytic, thermal and neutral conditions with very less degradation.

The pattern of degradation of the drugs individually in all the conditions and in different strengths were well portrayed in the figure 7 A to 7C. From the graphs it was evident that VOX showed highest degradation in acidic, SOF showed high degradation in peroxide and acidic conditions while VEL showed high degradation in acidic condition and moderate degradation in peroxide condition.

Table 14. Degradation data of VOX, SOF and VEL in different conditions

Condition		% Degraded ± SD of VOX	% Degraded ± SD of SOF	% Degraded ± SD of VEL
Acid	0.1M	36.931	0.413	12.371
	0.5M	52.352	8.416	32.577
	1M	76.589	17.06	48.113
Basic	0.1M	12.442	0.580	0.561
	0.5M	28.731	1.257	1.057
	1M	55.829	2.102	1.395
Peroxide	1%	0.173	1.222	1.390
	3%	2.367	10.475	9.450
	4.5%	4.745	18.30	17.466
UV	6hrs	0.162	0.124	1.114
	12hrs	0.423	0.528	4.005
	24hrs	1.032	1.71	8.783
Thermal	60°C	1.124	0.470	0.971
	105°C	2.774	1.433	4.179
	150 °C	3.660	2.715	8.291

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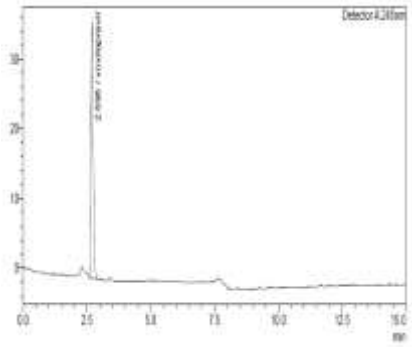
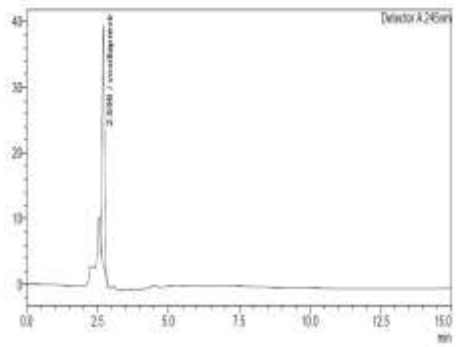
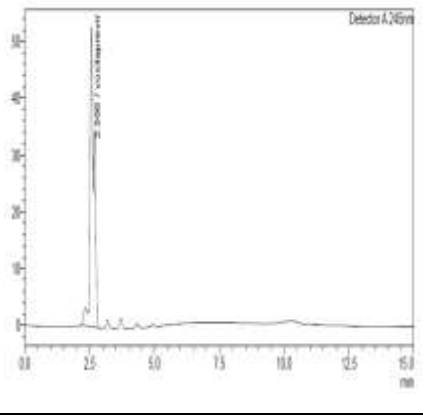
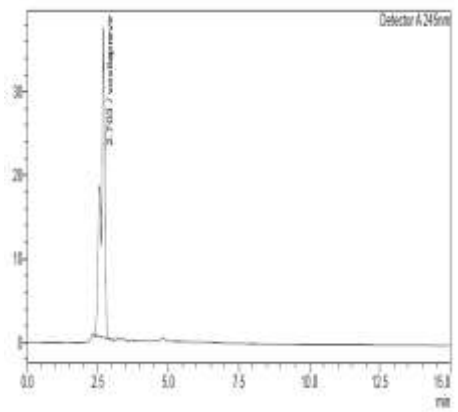
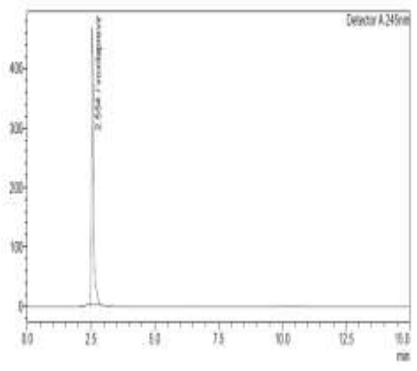
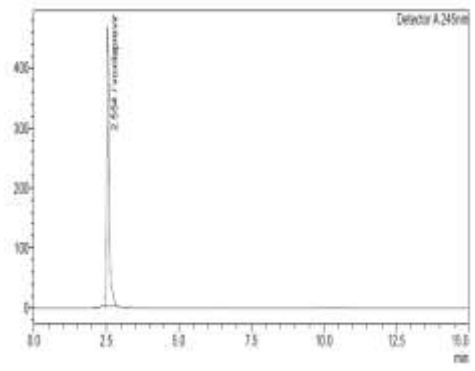
Neutral	15min	0.174	0.382	0.198
	30min	0.286	0.588	0.243
	45min	0.315	0.785	0.402

Table 15. Degradation chromatograms of VOX in different conditions

Condition	0.1M	1M
HCl		
	0.1M	1 M
NaOH		
	1%	4.5%
Peroxide		
	6 hrs	24 hrs

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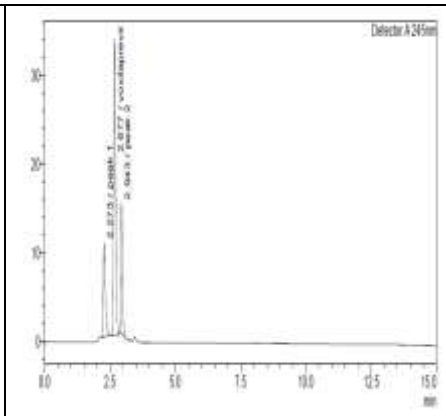
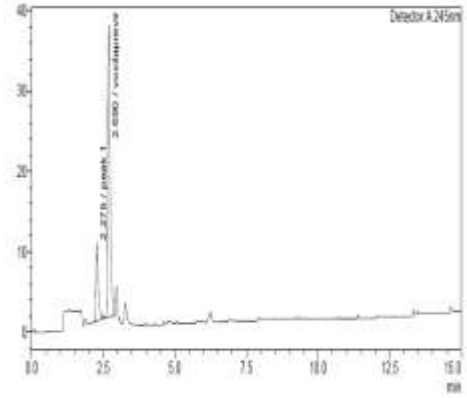
UV		
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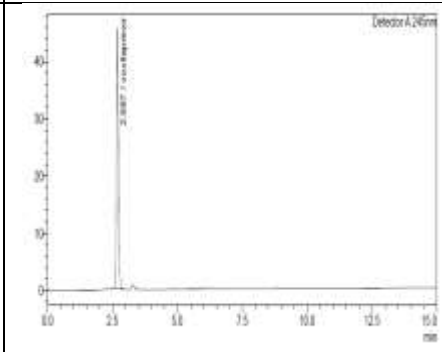
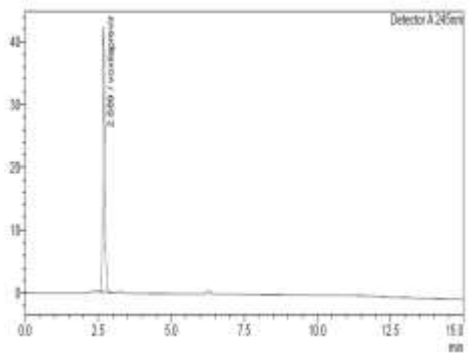
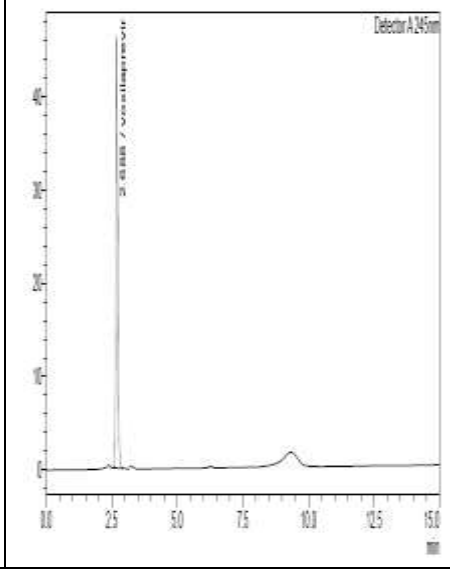
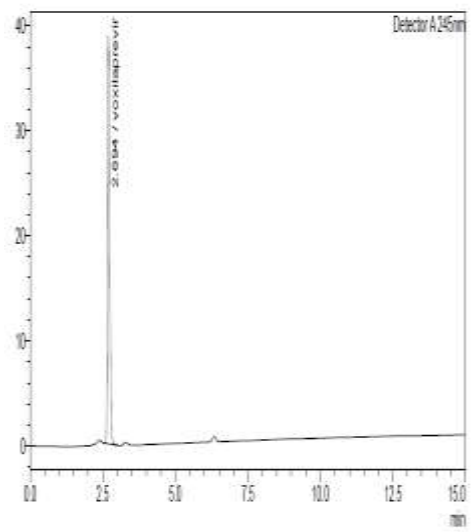
Thermal		
	15 min	45 min
Neutal		

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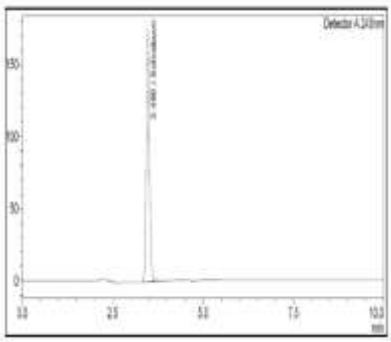
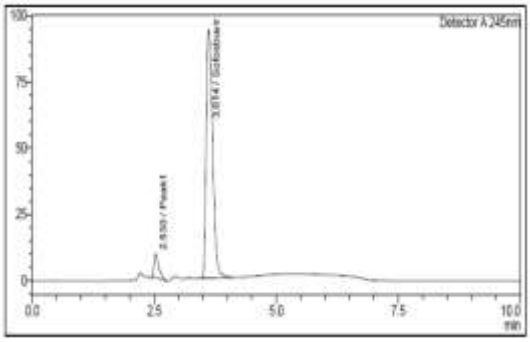
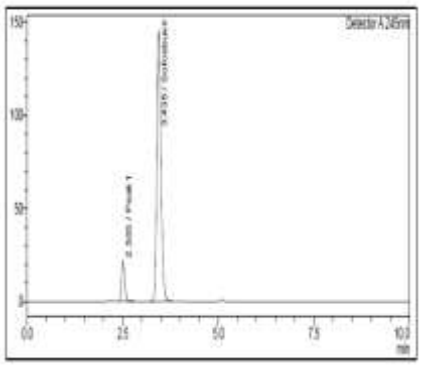
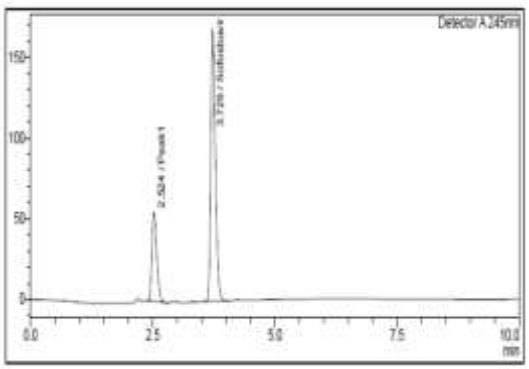
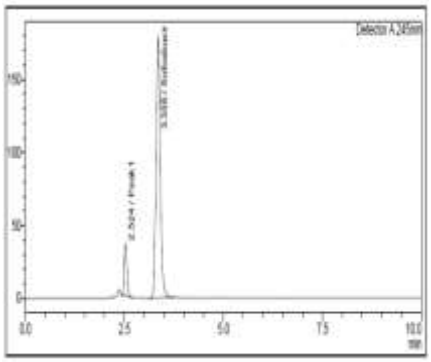
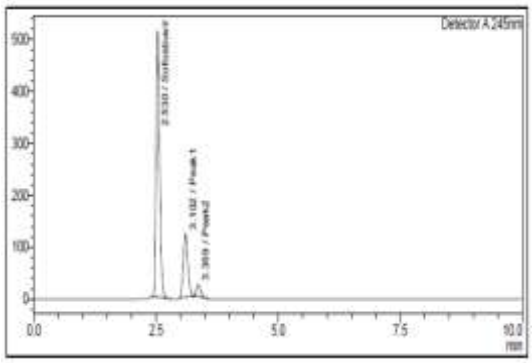
Table 16. Degradation chromatograms of SOF in different conditions

Condition	0.1M	1M
HCl		
	0.1M	1 M
NaOH		
	1%	4.5%
Peroxide		
	6 hrs	24 hrs

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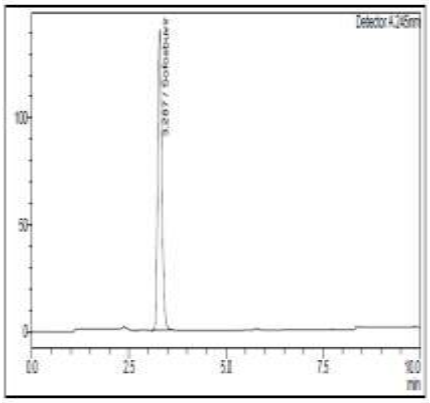
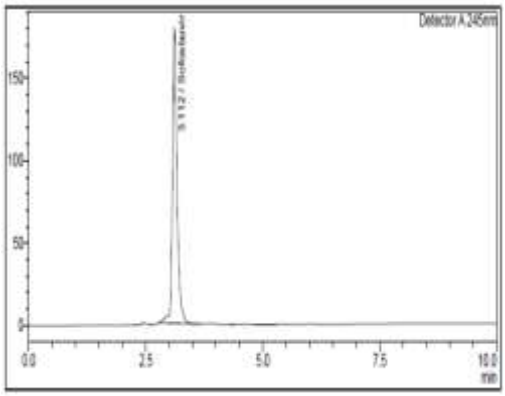
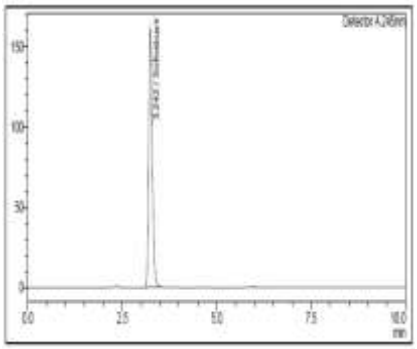
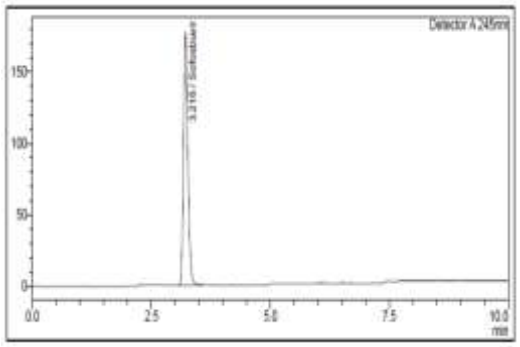
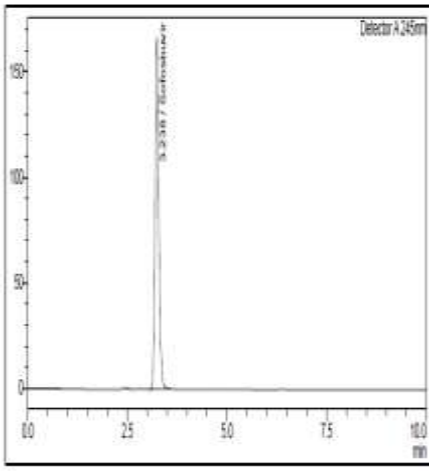
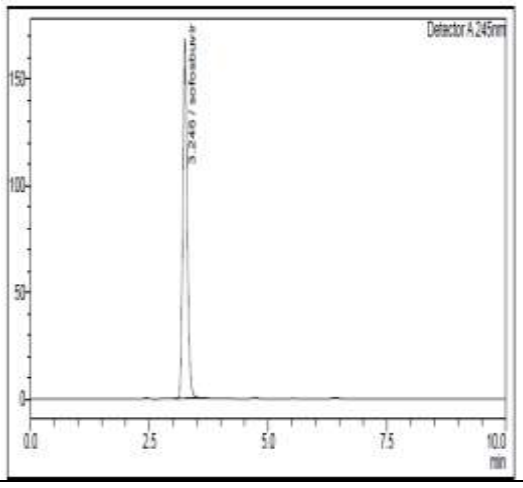
UV		
	60°C	150°C
Thermal		
	15 min	45 min
Neutal		

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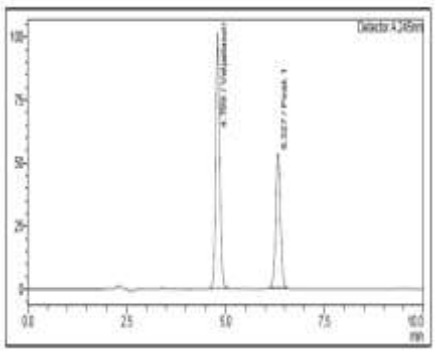
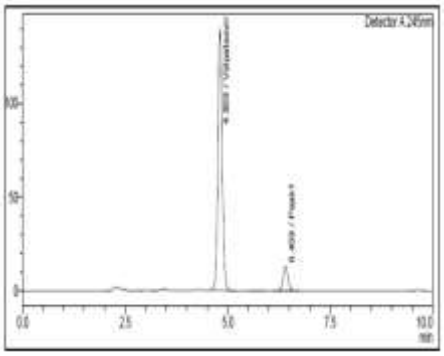
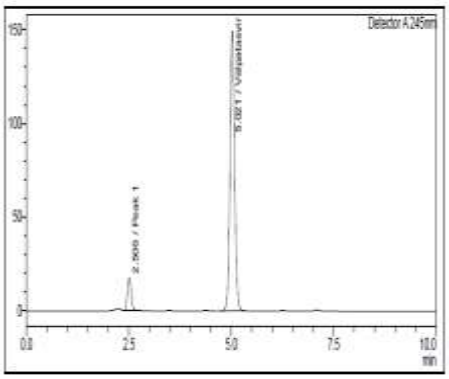
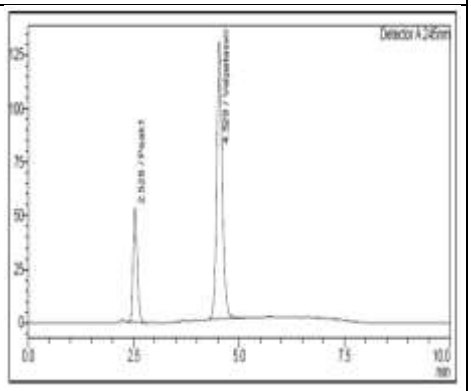
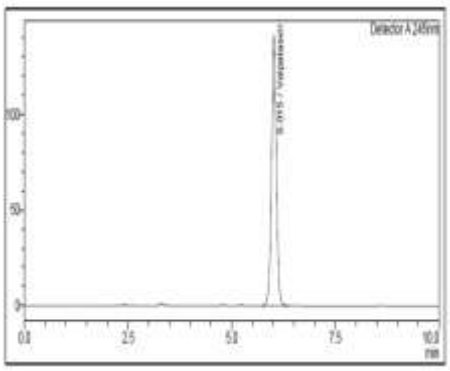
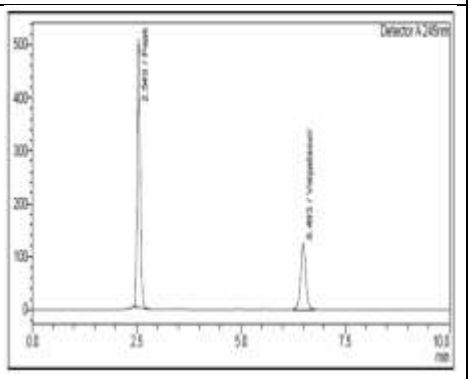
Table 17. Degradation chromatograms of VEL in different conditions

Condition	0.1M	1M
HCl		
	0.1M	1 M
NaOH		
	1%	4.5%
Peroxide		
	6 hrs	24 hrs

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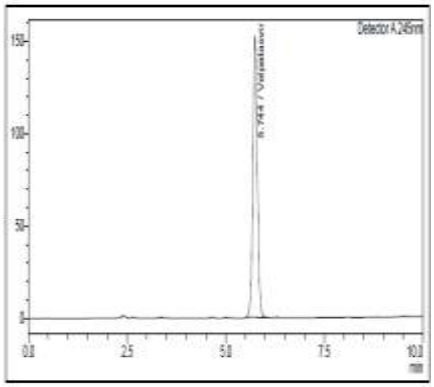
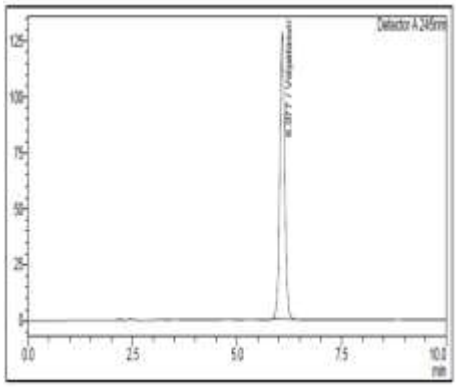
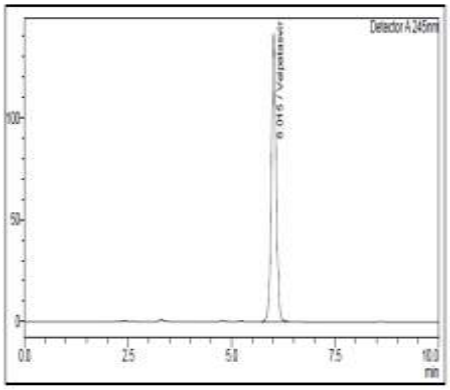
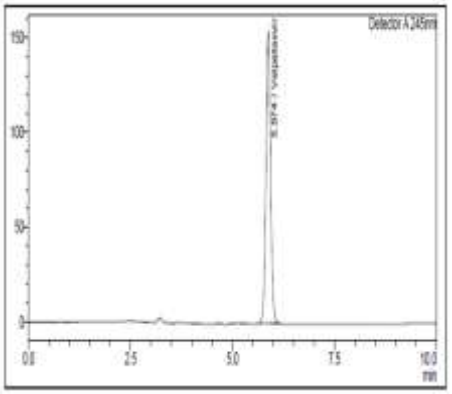
UV		
	60°C	150°C
Thermal		
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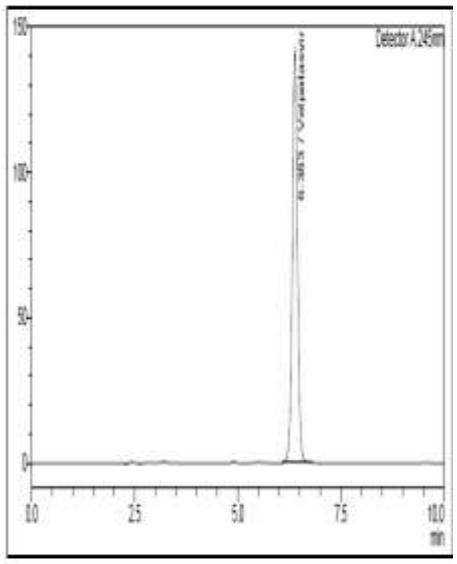
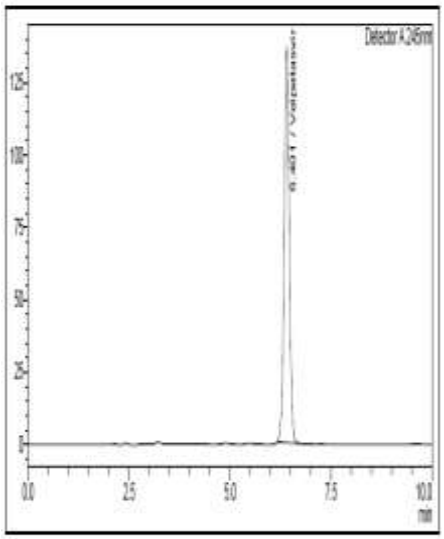
Neutal		

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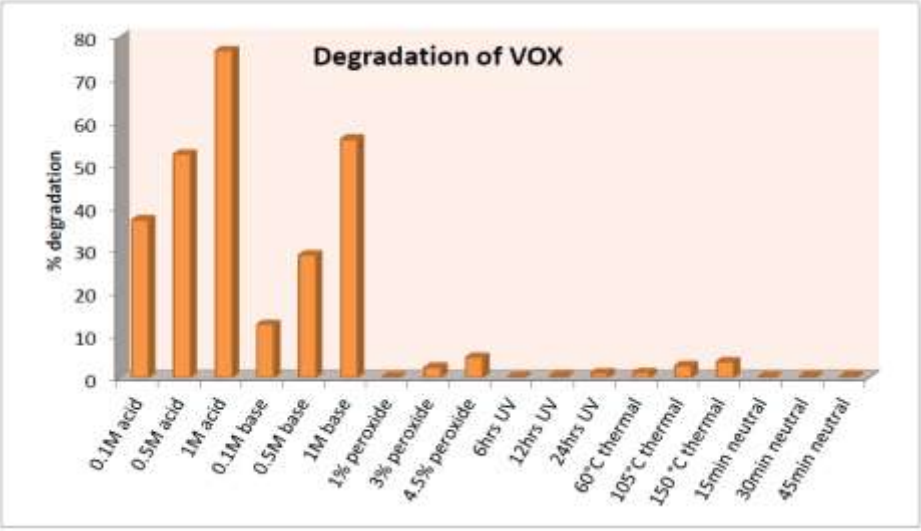

Fig. 7A showing degradation pattern of VOX

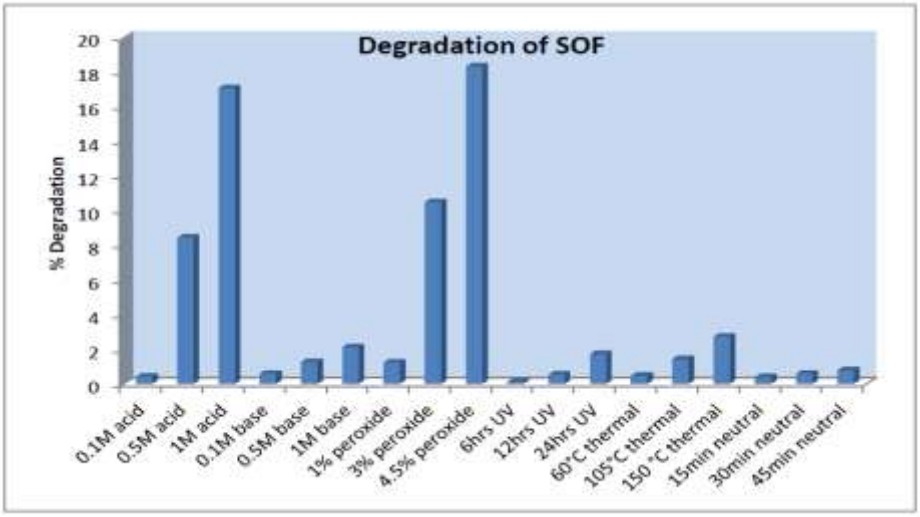

Fig. 8 showing degradation pattern of SOF

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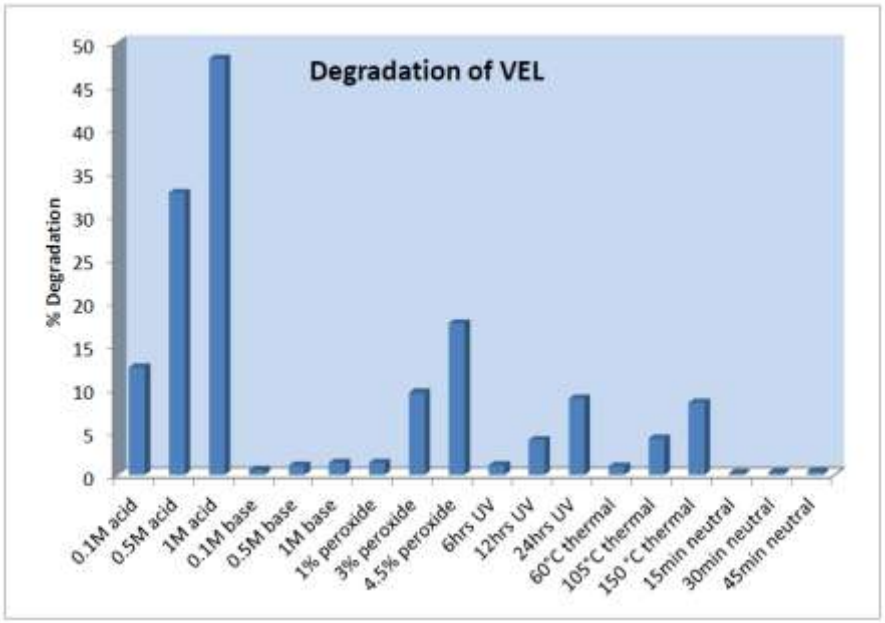

Fig. 9 showing degradation pattern of VEL

IV. CONCLUSION:

A new stability indicating reverse-phase HPLC method was developed and validated for the estimation of Voxilaprevir, Sofosbuvir and Velpatasvir in bulk and pharmaceutical dosage forms. The method was used for the estimation of amount of degradation for the three drugs. The developed method was used to estimate the percentage degradation in all the stress conditions. VOX, SOF and VEL showed highest degradation in peroxide and acidic conditions when compared with basic, photolytic and thermal conditions. VOX showed highest degradation in acidic and basic condition. SOF and VEL showed highest degradation in peroxide and acidic conditions when compared with basic, photolytic and thermal conditions. SOF showed least degradation in acidic condition compared with VOX and VEL. SOF and VEL showed approximately equal degradation in peroxide condition. All the drugs showed good stability under photolytic, thermal and neutral conditions with very less degradation.

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