ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: https://ijrps.com

A novel stability indicating method development and validation for the simultaneous estimation of Velpatasvir & Sofosbuvir in bulk and its pharmaceutical formulations

Harshalatha P*[1], Chandrasekhar KB[2] and Chandrasekhar MV[3]

[1]Research Scholar, Jawaharlal Nehru Technological University Anantapur, Anantapuramu, A.P., India
[2]Department of Chemistry, Jawaharlal Nehru Technological University Anantapur, Anantapuramu, A.P., India
[3]Department of Chemistry, Government College (UG & PG), Anantapuramu, A.P., India

Article History:

Received on: 12.01.2018
Revised on: 22.04.2018
Accepted on: 27.04.2018

Keywords:

Velpatasvir,
Sofosbuvir,
Sodium dihydrogen orthophosphate,
Validation

ABSTRACT

A simple and rapid RP–HPLC method, for the simultaneous determination of Velpatasvir and Sofosbuvir, was developed in bulk and its tablet dosage form. Separation was performed on a Water's C18 4.6 mm × 15 cm, five µm particle column at ambient temperature. The mobile phase consisted of Sodium dihydrogen orthophosphate buffer (pH 4.2, adjusted with OPA) and ACN in the ratio of 85:15 v/v at a flow rate of 1ml/min. Both the analytes were determined using a photodiode array at 292 nm. The retention time of Velpatasvir and Sofosbuvir was found to be 2.89 and 3.84 min, respectively. The validation of the proposed method was carried out for specificity, linearity, accuracy, precision, limit of detection, limit of quantitation and robustness. The linear dynamic ranges were from 32.5 – 97.5 µg/ml and 125 – 375 µg/ml for Velpatasvir and Sofosbuvir, respectively. The percentage recovery obtained for Velpatasvir and Sofosbuvir was 99.53 and 100.26%, respectively. Limit of detection and quantification for Velpatasvir were 0.0068 and 0.029 µg/ml, for Sofosbuvir 0.104 and 0.347 µg/ml, respectively. The developed method can be used for routine quality control analysis of titled drugs in combination in tablet formulation.

* Corresponding Author

Name: Harshalatha P
Phone: +91-9949632906

Email: harsha.pankaj@yahoo.in

ISSN: 0975-7538
DOI: https://doi.org/10.26452/ijrps.v9i2.1563

Production and Hosted by

IJRPS | https://ijrps.com

INTRODUCTION

In recent times, there is an increased tendency towards the development of stability-indicating assays (Mohammadi *et al.,* 2006; Ivana *et al.,* 2006), using the approach of stress testing as enshrined in the international conference on Harmonization (ICH) guidelines Q1AR2 (ICH guidelines, 2003). Even the approach is being extended to drug combinations (Grosa *et al.,* 2006) to allow accurate and precise quantitation of multiple drugs, their degradation products, and interaction products if any.

The combination of Sofosbuvir (SOF) (Figure 1) chemically is Isopropyl (2S)-2-[[[(2R,3R,4R,5R)-5-(2,4-dioxopyrimidin-1-l)- 4-fluoro-3 hydroxy-4-methyl- tetrahydro furan-2-yl] methoxy-phenoxy phosphoryl] amino] propanoate and Velpatasvir (VEL) (Figure 2) chemically is Methyl {(2S)-1-[(2S,5S)-2-(9-{2-[(2S,4S) 1-{(2R)-2- [(methoxy carbonyl) amino]-2- phenyl acetyl}- 4(methoxy methyl)- 2-pyrrolidinyl] -1H- imidazol-4 -yl}- 1, 11-dihydro isochromeno [4',3':6,7] naphtha [1,2-d] imidazol-2-yl) -5-methyl-1 pyrrolidinyl] -3-methyl-1-oxo-2-butanyl}carbamate is a novel for the prescribed for Hepatitis C virus (HCV) infection. SOF is a nucleotide hepatitis C virus non structural protein 5 B polymerase inhibitor and VEL is HCV

NS5A replication complex inhibitor (Martindale, 2018).

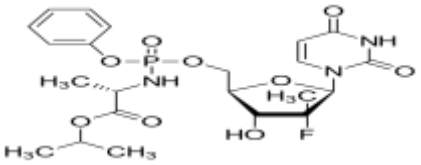

Figure 1: Chemical structure of Sofosbuvir

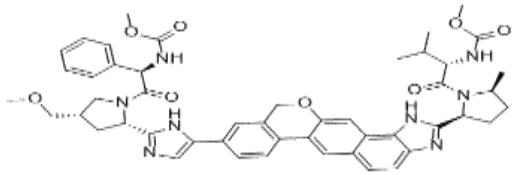

Figure 2: Chemical structure of Velpatasvir

A thorough literature survey has been done and found that two RP-HPLC (Sarath Nalla *et al.,* 2017 & Jahnavi Bandla *et al.,* 2017) methods were reported for the simultaneous estimation of SOF and VEL in pharmaceutical dosage forms.

EXPERIMENTAL

Instrumentation

The HPLC system consisted of an LC Waters (Waters, Milford, MA, USA) using a Water's C18 250 x 4.6 mm, 5µ column, a quaternary gradient system (600 Controller), in line degasser (Waters, model AF). The system was equipped with a photodiode array detector (Water, 2998 model) and autosampler (Waters, model 717 plus). Data were processed using Empower Pro software (Waters, Milford, MA, USA). Sodium dihydrogen orthophosphate buffer (pH 4.2, adjusted with OPA) and ACN in the ratio of 85:15 v/v was used as mobile which was pumped at a flow rate of 1.0 mL min−1. The detection wavelength for SOF and VEL was 292 nm.

Chemicals and Reagents

Reference standards of SOF and VEL were kindly supplied by TSK laboratories, Hyderabad, India with a purity of 99.85% and 99.99% respectively. Tablet formulation containing 400 mg of SOF and 100 mg of VEL was procured from Anukar pharmacy, Hyderabad. Acetonitrile (HPLC grade) was purchased from Spectrochem (Mumbai, India). All other reagents and chemicals used in this study were of analytical grade. Sodium dihydrogen orthophosphate and orthophosphoric acid were purchased from SD Fine Chemicals (Mumbai, India). Water was purified using a Millipore system (Millipore Corp., Bangalore, India).

Preparation of Stock and Standard Solutions

The standard stock solutions containing 1 mg mL-1 each of SOF and VEL was prepared separately by dissolving reference standards in mobile phase (Sodium dihydrogen orthophosphate buffer (pH 4.2 , adjusted with OPA) and ACN in the ratio of 85:15 v/v) and diluting with the same diluent. 2.5 mL aliquots of VEL and 10 ml of SOF were transferred to 25 mL calibrated volumetric flask and the volume was made up to the mark with the same solvent mixture to prepare standard preparation having a concentration of 100µg/ml of VEL and 400 µg/ml of SOF respectively. Calibration standards containing 32.5 to 97.5 µg/ml of VEL and 125 to 375 µg/ ml of SOF were prepared by diluting the standard stock solution to the appropriate volume with the same diluent.

Preparation of Test Solution

Twenty Velasof tablets were weighed and finely powdered in a mortar. Tablet powder equivalent to 10 mg (91.052mg) was accurately weighed and transferred to a 10 mL calibrated volumetric flask dissolved in mobile phase mixture, and the solution sonicated for 10 min. Volume was made up to the mark with the same solvent. The solution was filtered through a 0.45 µm membrane filter, if necessary. This solution contains 100 µg/ml of VEL and 400 µg/ml of SOF.

Stability studies

All forced degradation studies (Stability studies) were performed at an initial drug concentration. Acid hydrolysis was performed in 0.1 N HCl at 85°C for 5 hrs. Alkali hydrolysis was carried out in 0.1 N NaOH at 85°C for 6 hrs. Oxidative studies were performed at 55° C in 3% hydrogen peroxide for 8 hrs. UV degradation studies were carried out at 256nm for 30 hrs. For thermal degradation drug powder was heated to 85°C for 30hrs. Samples were withdrawn at appropriate times and subjected to HPLC analysis after suitable dilution.

Validation of the Method

The developed method was validated as per the International Conference on Harmonization (ICH) guidelines with respect to Precision, Accuracy, Linearity, robustness, LOD & LOQ.

Precision

Six injections, of optimized concentrations of both the analytes (Vel-100µg/ml and SOF-400 µg/ml), were given on the same day and the values of relative standard deviation (%RSD) were calculated to determine intra-day precision. These studies were also repeated on different days to determine inter-day precision.

Accuracy

Accuracy was evaluated by fortifying a mixture of the dosage form with three known concentrations of the drug. The percent recovery of the drug from the dosage form was determined.

Table 1: Optimized chromatographic conditions of VEL and SOF

S. No	Parameter	Description/Value
1.	Stationary Phase	Water's C18 (250X4.6X5)
2.	Mobile Phase	Sodium dihydrogen orthophosphate buffer (pH 4.2, adjusted with OPA) and ACN in the ratio of 85:15 v/v
3.	Flow rate	1ml/min
4.	Detection Wavelength (Isosbestic Point)	292 nm
5.	Detector	Photodiode array
6.	Injection	Autosampler -Waters, model 717 plus
7.	RT's	Velpatasvir: 2.889 min Sofosbuvir: 3.837 min
8.	Injection volume	20 µl
9.	Column Temperature	Ambient
10.	Run time	6 mins
11.	Diluent	Mobile Phase

Table 2: System suitability data

S. No	Parameter*	VEL	SOF
1.	Theoretical Plate Count	5792	6565
2.	Average Peak Area	484229	471750
3.	Peak Height	77213	59501
4.	RT	2.889	3.837
5.	Tailing	1.0	0.9
6.	Resolution	-	8.66
7.	S/N	3603	1954

* Average of 6 replicates

Table 3: Accuracy data of Velpatasvir and Sofosbuvir

S. No	Accuracy Level	Peak Area	Amount Added	Amount Found	% Recovery	Mean % Recovery
Velpatasvir						
1.	50%	236743*	48.97	48.89	99.84	99.53
2.	100%	472019#	97.93	97.48	99.53	
3.	150%	705803*	146.90	145.76	99.22	
Sofosbuvir						
4.	50%	231570	195.76	196.35	100.30	
5.	100%	461352.3#	391.52	391.18	99.91	100.26
6.	150%	696438.7*	587.28	590.92	100.55	

Table 4: Shows the results of precision

S. No	Intraday Precision				Inter-day Precision			
	VEL		SOF		VEL		SOF	
	Peak Area	% Assay	Peak Area	% Assay	Peak Area	% Assay	Peak Area	% Assay
1.	475549	100.23	463235	100.22	471766	99.44	461543	99.85
2.	473511	99.80	462215	100.00	474755	100.07	463311	100.24
3.	474845	100.08	460173	99.56	475123	100.14	462746	100.11
4.	475072	100.13	461841	99.92	475440	100.21	460936	99.72
5.	472170	99.52	461288	99.80	472449	99.58	462752	100.12
6.	474229	99.95	461750	99.90	472785	99.65	461602	99.87
Avg	474229.33	99.95	461750.33	99.90	473719.67	99.85	462148.33	99.99
SD	1231.87	0.26	1012.93	0.22	1568.81	0.33	917.40	0.20
%RSD	0.26	0.26	0.22	0.22	0.33	0.33	0.20	0.20

Linearity

A stock solution of the drug was prepared at the strength of 1 mg ml^{-1}. It was further diluted to prepare solutions containing 32.5 – 97.5 µg/ml and 125 – 375 µg/ml for Velpatasvir and Sofosbuvir, respectively. The solutions were injected in triplicate into the HPLC column, keeping the injection volume constant (20µl).

Table 5: Linearity Data of Velpatasvir and Sofosbuvir

S. No.	Linearity Level (%)	Velpatasvir		Sofosbuvir	
		Concentration (µg/ml)	Peak Area	Concentration (µg/ml)	Peak Area
1.	50	32.5	2813066	125	3077228
2.	75	48.75	4253268	187.5	4629475
3.	100	65	5613521	250	6143585
4.	125	81.25	7052657	312.5	7688257
5.	150	97.5	8406053	375	9285177
Regression equation		y = 86064x + 33568		y = 24759x - 25128	
Slope		86064		24759	
Y-intercept		33568		25128	
R^2		0.9999		0.9999	

Table 6: Robustness data

S. No	Parameter	Condition	Velpatasvir			Sofosbuvir		
			RT	Peak Area	% Assay	RT	Peak Area	% Assay
1.	Flow	0.8 ml/min	3.590	473726	99.85	4.758	473406	100.26
2.		1 ml/min	2.889	474229	99.95	3.837	462302	100.02
3.		1.2 ml/min	2.512	471186	99.31	3.414	460579	99.65
4.	Temp	25°C	2.980	471452	99.37	3.840	461658	99.88
5.		30°C	2.889	474229	99.95	3.837	461750	99.90
6.		35°C	2.895	471938	99.47	3.869	461880	99.93

Table 7: Assay results of VEL and SOF

S. No	Sample Wt. (mg)	Velpatasvir		Sofosbuvir	
		Peak area	% Assay	Peak area	% Assay
1	99.969	471766	99.44	461543	99.85
2	99.969	474755	100.07	463311	100.24
3	99.969	475123	100.14	462746	100.11
4	99.969	475440	100.21	460936	99.72
5	99.969	472449	99.58	462752	100.12
6	99.969	472785	99.65	461602	99.87
Average		473719.67	99.85	462148.33	99.99
STDEV		1568.81	0.33	917.40	0.20
% RSD		0.33	0.33	0.20	0.20

Table 8: Conditions and results of forced degradation studies

S. No	Condition	Velpatasvir			Sofosbuvir		
		Peak Area	% Assay	% Degradation	Peak Area	% Assay	% Degradation
1.	Acid	415549	87.59	12.41	403235	87.24	12.76
2.	Base	423511	89.26	10.74	412215	89.18	10.82
3.	H2O2	444845	93.76	6.24	410173	88.74	11.26
4.	UV	435072	91.70	8.30	421841	91.26	8.74
5.	Heat	435487	91.79	8.21	428457	92.70	7.30

Specificity and selectivity

The specificity of the method was established through the study of resolution factors of the drug peak from the nearest resulting peak, and also among all other excipients.

Robustness

Robustness of the study was evaluated by changing the parameters of the method like flow rate (±0.2 ml) and column temperature (± 5°C).

Detection and quantitation limits

Combined standard solutions were prepared by sequential dilutions and injected on the chromatograph, at decreasing concentrations. The limit of detection was defined as the concentration for which a signal to noise ratio of 3 was obtained and for quantitation limit, a signal to noise ratio of 10 was considered.

RESULTS AND DISCUSSION

The chromatographic conditions were optimised for the simultaneous determination of SOF and VEL within a short analysis time (< 6 mins) and an acceptable peak resolution (Rs> 2). To accomplish these objectives, the chromatographic column was first chosen based on peak shapes and resolution. In preliminary experiments, the sample retention time increased with an increase in column length. In order to avoid long run times, a C_8 column was

initially used. This, however, resulted in peak overlap between SOF and VEL. Therefore, a waters C_{18} column (4.6 mm × 15 cm, 5 µm), which permits the use of high flow rate with consequent low increase in back pressure, was subsequently used to resolve better both the analytes, reducing elution time and obtain sharp peaks for individual drugs. Table 1 shows the optimised chromatographic conditions of VEL and SOF.

System suitability

The system suitability was evaluated by six replicate analyses of analytes at 100 µg/ml of VEL and 400 µg/ml of SOF. Average theoretical plate count was found to be 5729 and 6565 for VEL and SOF respectively. Retention times of VEL and SOF were found to be 2.889 and 3.837 mins respectively. Results were shown in table 2.

Specificity

In order to confirm the specificity of the method for VEL and SOF in the presence of excipients, triplicate solutions were injected into HPLC. Specificity is the ability of the method to detect the analytes under research in the presence of other ingredients, such as excipients. Figure 3 & 4 shows the chromatograms of Blank, standard and samples.

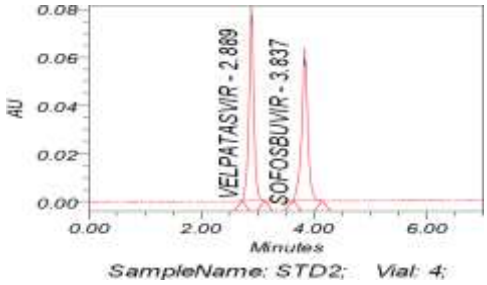

Figure 3: Standard Chromatogram

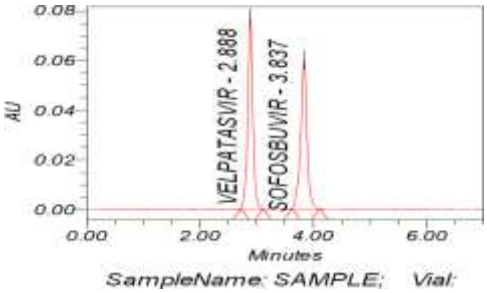

Figure 4: Sample Chromatogram

Accuracy

The accuracy of VEL and SOF tablets method was evaluated by the determination of % recovery of VEL and SOF from their combined dosage form. The Mean % recovery for each compound was calculated at three concentration levels, i.e., 50, 100 & 150%. Mean % recover for 99.53 % for VEL and 100.26 for SOF in all the three levels of concentrations. Table 3 shows the results of accuracy.

Precision

Inter and Intraday precision are expressed as the percentage relative standard deviation (%RSD) for VEL and SOF. The % RSD of VEL and SOF for intraday precision was found to be 0.26 and 0.22% respectively and for inter-day precision %, RSD of VEL & SOF was found to be 0.33 and 0.20% respectively. Table 4 shows the results of precision.

Linearity

The linearity of the proposed method was evaluated by the analysis of working standard solutions of VEL and SOF at five different serially concentrated solutions. The linear ranges were 32.5 – 97.5 µg/ml and 125 – 375 µg/ml for Velpatasvir and Sofosbuvir, respectively. Linearity data was given in Table 5. Figure 7 shows the linearity graphs.

Limits of detection (LOD) and quantification (LOQ)

LOD/LOQ parameters are not a requirement for the drug assay, however, it is always useful to demonstrate the analysis is being conducted in a region which is above the LOQ value and they are the measure of the sensitivity of the method. The LOD and LOQ are established from the standard deviation of the response and the slope of a calibration curve prepared with reference sample solutions. LOD is calculated by the formula LOD = 3.3 (SD/Slope) and LOQ by 10 (SD/Slope). Limit of detection and quantification for Velpatasvir were 0.0068 and 0.029 µg/ml, for Sofosbuvir 0.104 and 0.347 µg/ml, respectively.

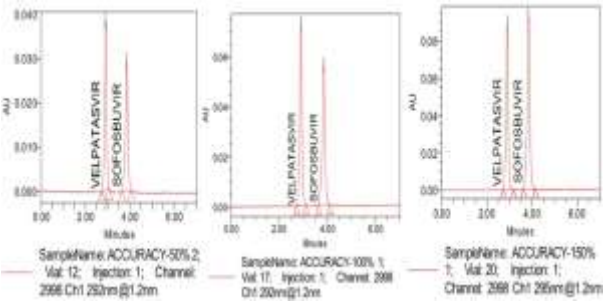

Figure 5: Accuracy chromatograms of VEL and SOF

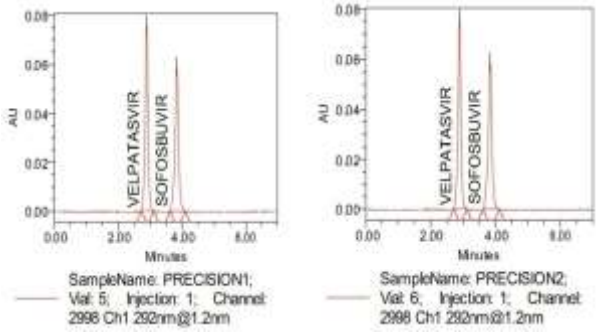

Figure 6: Intra and Inter day precision chromatograms of VEL and SOF

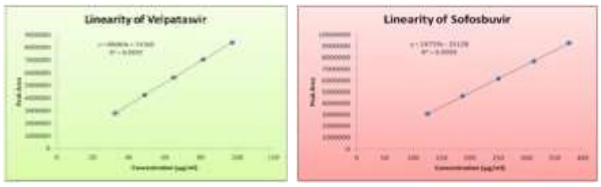

Figure 7: Linearity curve of VEL & SOF

Robustness

The proposed method was found to be robust as the change in the flow rate of the mobile phase and column temperature did not show any effect on the method performance. Results were tabulated in table 6.

Application of the proposed method for the assay of VEL and SOF

The validated HPLC method was used for the simultaneous determination of VEL and SOF in their combined dosage form. Six samples were weighed separately and analysed. The results expressed as percentage purity of API. Table 7 indicates the results of the assay of the drugs by the proposed method.

Forced degradation studies

The proposed method was applied for the determination of stability samples. Table 8 shows the conditions and results of acid, base, peroxide, UV and Heat degradation. Figure 10 shows the chromatograms of forced degradation studies.

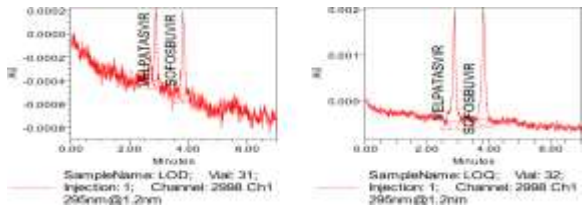

Figure 8: LOD and LOQ chromatograms of VEL and SOF

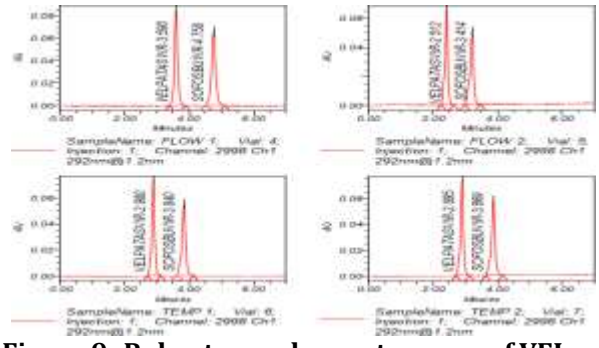

Figure 9: Robustness chromatograms of VEL and SOF

CONCLUSION

Simple and efficient stability indicating RP-HPLC method has been developed, optimised and validated for the isocratic separation, simultaneous determination of Velpatasvir and Sofosbuvir in their combined dosage form. The proposed method, suitable for routine quality control, has been successfully applied to the determination of both analytes in commercial brands of tablets.

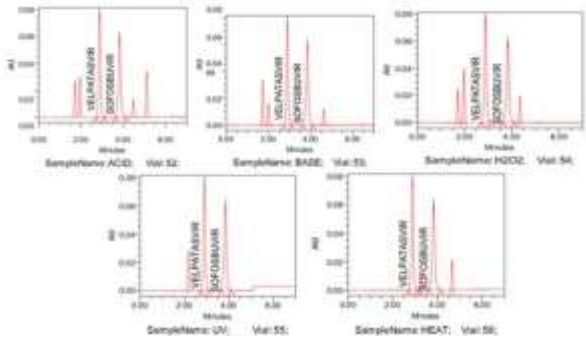

Figure 10: Degradation chromatograms of VEL and SOF

REFERENCES

Grosa G, Del Grosso E, Russo R, Allegrone G. Simultaneous, stability indicating, HPLC-DAD determination of guaifenesin and methyl and propylparabens in cough syrup. J Pharm Biomed Anal. 2006, 41(3),798-803.

ICH, Q1A(R2) Stability testing of new drug substances and products, in International Conference on Harmonization, IFPMA, Geneva, 2003.

Ivana I, Ljiljana Z, Mira Z. Stability indicating assay method for cefuroxime axetil and its application to the analysis of tablets exposed to accelerated stability test conditions. J Chromatogr A. 2006, 1119(1-2), 209-15.

Jahnavi Bandla, S. Ganapathy. 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.

Martindale: The Complete Drug Reference http: //online.lexi.com (browsed on 9th Apr 2018).

Mohammadi A, I. Haririan, N. Rezanour, L. Ghiasi, R.B. Walker. A stability-indicating high-performance liquid chromatographic assay for the determination of orlistat in capsules. Journal of Chromatography A, 2006, 1116 (1) 153–157.

Sarath Nalla and Seshagiri Rao J. V. L. N. 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.

Singh S, Singh B, Bahuguna R, Wadhwa L, Saxena R. Stress degradation studies on Ezetimibe and development of validated stability indicating HPLC assay. J. Pharm. Biomed. Anal, 2006, 41, 1037-1040.