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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING METHOD FOR THE SIMULTANEOUS ESTIMATION OF ELBASVIR AND GRAZOPREVIRIN PHARMACEUTICAL DOSAGE FORMS BY RP-HPLC

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Keywords:

Elbasvir, Grazoprevir, RP-HPLC, Method development, Validation, Stability indicating method

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ABSTRACT: The present study aimed to develop and validate stability indicating method for the simultaneous determination of Elbasvir and Grazoprevir in its pharmaceutical dosage form using RP-HPLC. Chromatographic separation was done with Discovery C18 (250 mm × 4.6 mm, 5 µ) column using 0.1% ortho-phosphoric acid (OPA) and acetonitrile in the ratio 50:50% v/v as mobile phase on isocratic mode. The column oven temperature was maintained at 30 ºC with a flow rate 1.0 ml/min and components were detected at a wavelength of 315 nm. The retention times for Elbasvir and Grazoprevir was found to be 2.24 min and 3.21 min respectively. The developed method was validated according to ICH guidelines. A good linearity response was observed in the concentration range of 12.5 µg/ml - 75 µg/ml for Elbasvir and 25 µg/ml - 150 µg/ml for Grazoprevir with correlation coefficient of 0.999 for both the drugs. The method was found to be accurate, precise, specific, rugged and robust. The drugs were subjected to stress conditions for testing their stability and found to be stable, with net degradation was within the limits.

INTRODUCTION: Elbasvir **Fig. 1A**, chemically designated as Dimethyl *N*,*N'*-([(6S)-6*H*-indolo[1,2-c][1,3]benzoxazine-3, 10-diyl] bis{1*H*- imidazole-5,2-diyl-(2*S*)-pyrrolidine-2, 1-diyl[(2*S*)- 1- oxo- 3-methylbutane-1,2-diyl]})biscarbamate, is a white to off-white crystalline solid belonging to antiviral category. It is practically insoluble in water and very slightly soluble in ethanol, but is very soluble in ethyl acetate and acetone and has pKa values of 5.39 and 12.42.

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It is used in the treatment of chronic hepatitis C infection [1 - 3]. Grazoprevir **Fig. 1B**, chemically designated as (1*R*,18*R*,20*R*,24*S*,27*S*)-*N*-{(1*R*,2*S*)-1-[(cyclopropylsulfonyl) carbamoyl]- 2- vinylcyclopropyl}- 7- methoxy- 24- (2-methyl-2-propanyl)-22,25-dioxo-2,21-dioxa-4, 11, 23, 26-tetraazapentacyclo[24.2.1.03,12.05,10.0,18,20]nonacosa-3,5,7,9, 11-pentaene-27-carboxamide, is white crystalline solid belonging to antiviral category. It is practically insoluble in water but freely soluble in ethanol and has a pKa value of 5.31. It is used in the treatment of chronic hepatitis C infection [4 - 6]. According to literature survey, very few methods such as two RP-HPLC methods [7, 8] and one LC-MS/MS [9] were developed for the simultaneous estimation of Elbasvir and Grazoprevir in pharmaceutical dosage form.

The present study aimed to develop and validate a stability indicating RP-HPLC method for the simultaneous determination of Elbasvir and Grazoprevir in their tablet dosage form.

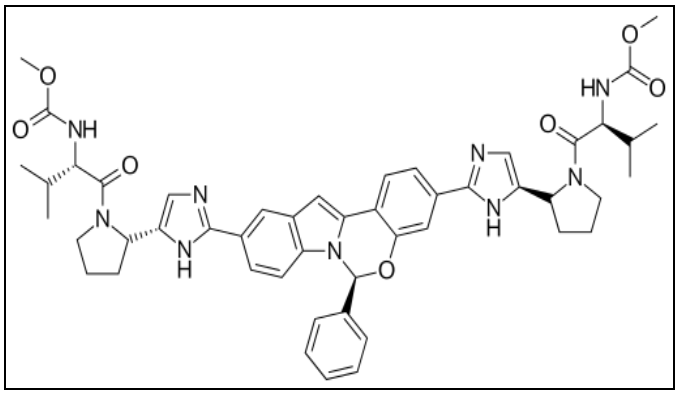

FIG. 1A: CHEMICAL STRUCTURE OF ELBASVIR

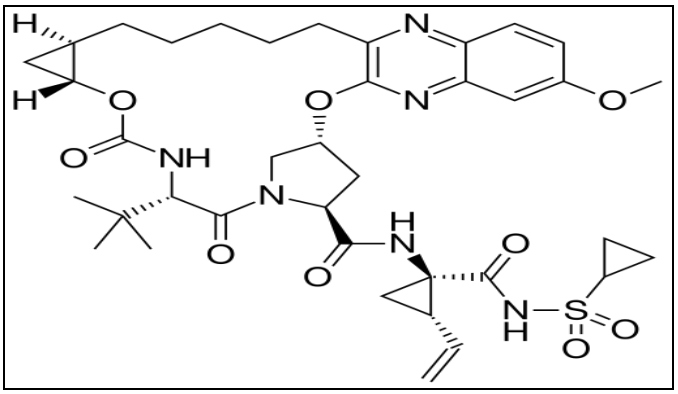

FIG. 1B: CHEMICAL STRUCTURE OF GRAZOPREVIR

MATERIAL AND METHODS:

Reagents and Chemicals: Elbasvir and Grazoprevir working standards were supplied as gift samples by spectrum labs, Hyderabad (India). Elbasvir and Grazoprevir (Zepatier) tablets were purchased from local pharmacy. All the solvents used for the method were of HPLC grade and chemicals were of AR grade.

Instrument and Analytical Conditions: Waters HPLC 2998 model equipped with an auto sampler, Discovery C18 (250 mm × 4.6 mm, 5 µ) column, PDA detection and running on empower 2 software was used for chromatographic separation. An isocratic mode with 0.1% ortho-phosphoric acid and acetonitrile in the ratio 50:50% v/v was used as mobile phase. The detection was done at 315 nm at a flow rate of 1.0 ml/min. The other instruments used were pH meter (EI), Digital Balance (Infra Instruments), Ultrasonic Bath (Wadegati), Hot air oven (Cisco).

Preparation of Mobile Phase: 0.1% OPA buffer was prepared by diluting 1 ml of OPA in 1000 ml of distilled water. Mixture of buffer and acetonitrile in the ratio 50:50% v/v respectively makes mobile phase.

Preparation of Diluent: For the preparation of diluent, water and acetonitrile in the ratio 50:50% v/v were mixed respectively.

Preparation of Standard and Sample Solution: Dissolve 5 mg of Elbasvir standard and 10 mg of Grazoprevir standard in 10 ml of diluent. Dilute 1 ml of the above stock solution to 10 ml with diluent. (50 µg/mL Elbasvir and 100 µg/mL Grazoprevir).

Accurately weigh an amount equivalent to 5 mg of Elbasvir from the powdered tablet dosage form (Zepatier) and dissolve in 10 ml of diluent. Filter the above solution and pipette out 1 ml and make up the volume to 10 ml with diluent.

Method Validation: The developed method was validated as per ICH guidelines [10]. The following parameters were validated; accuracy, precision, linearity, specificity, ruggedness, robustness and stability. Forced degradation studies were also conducted by exposing the drugs solution to various conditions such as acidic, basic, peroxide, thermal, neutral and photolytic conditions.

RESULTS AND DISCUSSION: For the development of the method, initially many mobile phase ratios at different flow rates were tried to elute the drugs. Mixture of 0.1% OPA and Acetonitrile in the ratio 50:50% v/v at 1.0 ml/min in isocratic mode was selected as mobile phase based on peak parameters. Discovery C18 (250 mm × 4.6 mm, 5 µ) column was used for separating the drugs. The column oven temperature was maintained at 30 ºC. From the Overlay, suitable wavelength considered for monitoring the drugs was 315 nm as shown in the **Fig. 2**.

The prepared standard and sample solutions were injected in the chromatographic system and system suitability and % assay were calculated. The standard, sample and blank chromatograms were shown in **Fig. 3, 4** and **5** respectively.

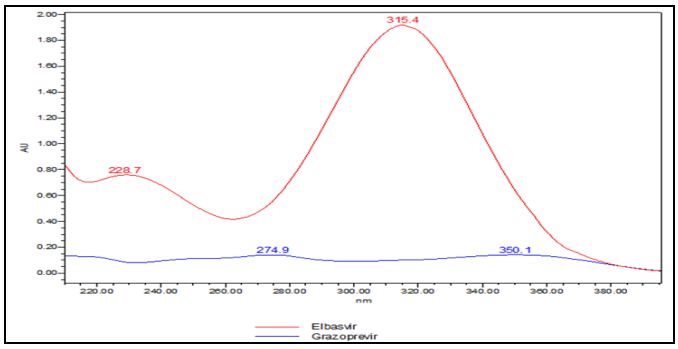

FIG. 2: OVERLAY UV SPECTRUM OF ELBASVIR AND GRAZOPREVIR

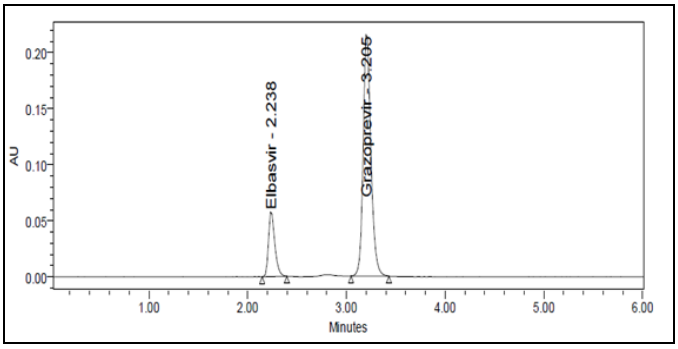

FIG. 3: STANDARD CHROMATOGRAM

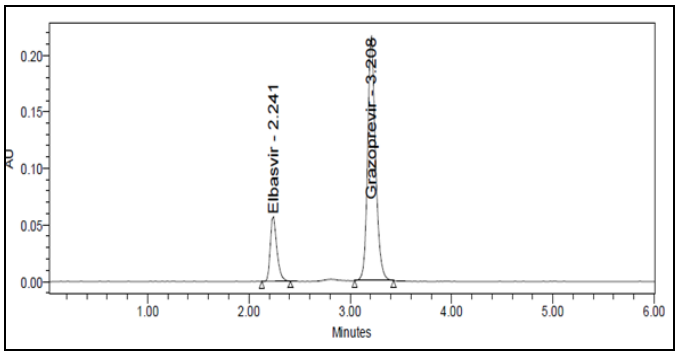

FIG. 4: SAMPLE CHROMATOGRAM

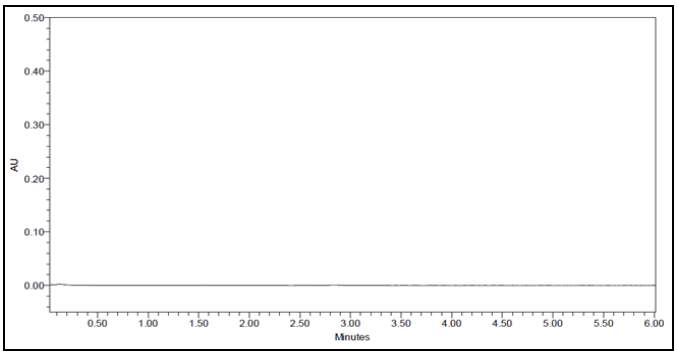

FIG. 5: BLANK CHROMATOGRAM

The developed method was validated with the validation parameters. Linearity of the method was determined by preparing the serial dilutions of the standard solution in the concentration range of 12.5 µg/ml - 75 µg/ml and 25 µg/ml - 150 µg/ml for Elbasvir and Grazoprevir respectively.

A graph was plotted between peak areas and concentration for both the drugs, where correlation coefficient was found to be 0.9998 for Elbasvir and 0.9997 for Grazoprevir, indicating that the method obeys Beer's law. The linearity plots were shown in the **Fig. 6A** and **6B**.

TABLE 1: SYSTEM SUITABILITY AND VALIDATION PARAMETER RESULTS

Parameters	Elbasvir	Grazoprevir
Specificity	Specific	Specific
Precision (% RSD)	0.8	0.8
Accuracy (% Recovery)	99.90% - 100.53%	99.48% - 100.00%
Linearity range (µg/ml)	12.5 - 75	25 – 150
Correlation coefficient (r)	0.9998	0.9997
Limit of Detection (µg/ml)	0.086	0.45
Limit of Quantitation (µg/ml)	0.261	1.38
Ruggedness (% RSD)	0.2	0.9
Robustness	Robust	Robust
Stability	Stable	Stable
USP tailing factor	1.31	1.18
USP Plate count	5686	8401
USP Resolution	7.3	

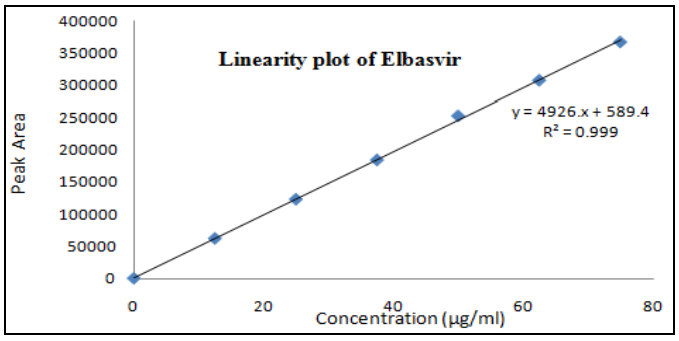

FIG. 6A: LINEARITY PLOT OF ELBASVIR

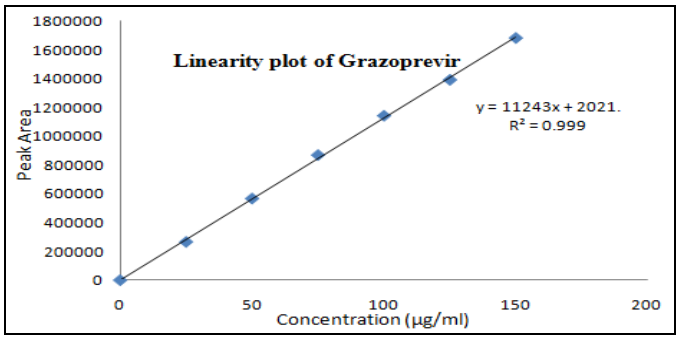

FIG. 6B: LINEARITY PLOT OF GRAZOPREVIR

The % relative standard deviation (% RSD) for Elbasvir was found to be 0.8 and for Grazoprevir was found to be 0.8, indicates that the method is precise. The % recovery for Elbasvir was found to be 99.90% - 100.53% and for Grazoprevir was found to be 99.48% - 100.00%, indicates that the method is accurate. Method was found to be specific as there is no interference of excipients with the retention time of both the drugs. The placebo chromatogram was shown in the **Fig. 7**.

The method was found to be rugged and robust. The standard drugs were subjected to forced degradation conditions in order to check the stability of the drugs. The drugs were found to be stable as the degradation of drugs at various stress conditions was within the net degradation limits. The forced degradation study results and chromatograms were shown in **Table 2** and **Fig. 8** respectively.

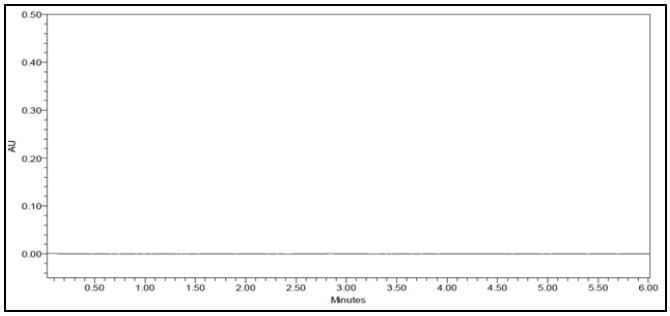

FIG. 7: PLACEBO CHROMATOGRAM

TABLE 2: RESULT OF FORCED DEGRADATION STUDIES

S. no.	Stress condition	Elbasvir			Grazoprevir			% area of degradation peak
		% Assay	Peak purity Angle	Peak purity threshold	% Assay	Peak purity angle	Peak purity threshold	
1	2N HCl for 30 mins at 60 °C	95.37	0.062	0.075	95.98	0.093	0.279	-
2	2N NaOH for 30 mins at 60 °C	97.24	0.022	0.052	97.01	0.091	0.278	-
3	20% H_2O_2 for 30 mins at 60 ºC	98.45	0.272	0.306	98.73	0.090	0.280	-
4	Water for 6 hrs at 60 ºC	99.64	0.291	0.332	99.22	0.153	0.279	-
5	UV light 200 wts/hr or 7 days	99.89	0.244	0.322	99.34	0.132	0.280	-
6	105 ºC for 6hrs	99.83	0.184	0.294	99.80	0.130	0.282	-

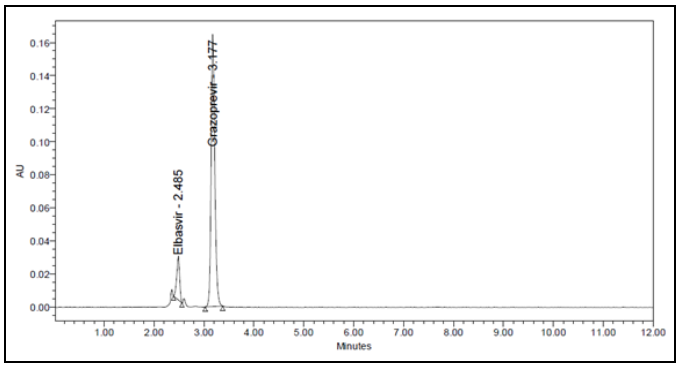

FIG. 8A: ACID DEGRADATION CHROMATOGRAM

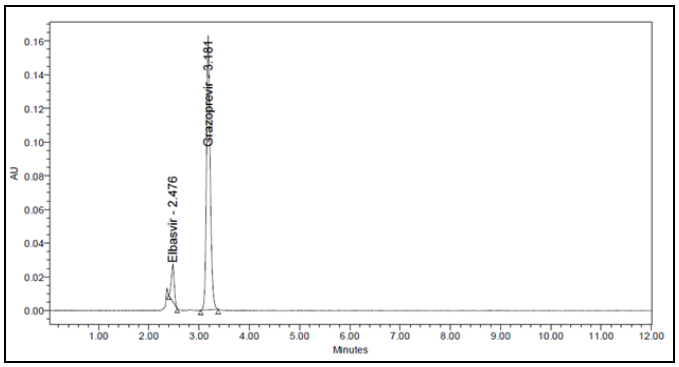

FIG. 8B: BASE DEGRADATION CHROMATOGRAM

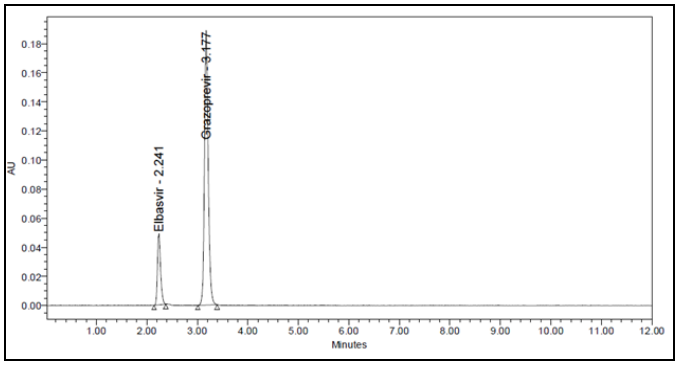

FIG. 8C: PEROXIDE DEGRADATION CHROMATOGRAM

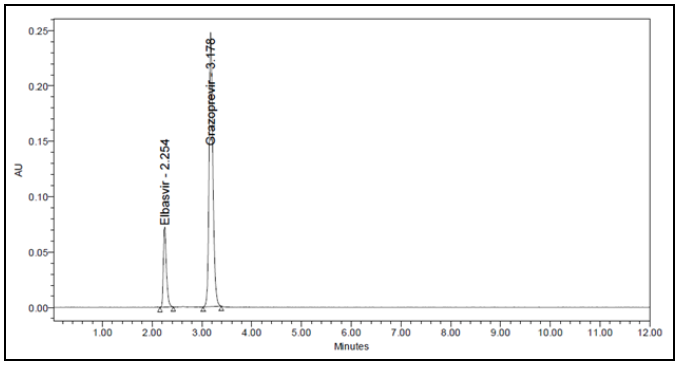

FIG. 8D: WATER STRESS STUDY CHROMATOGRAM

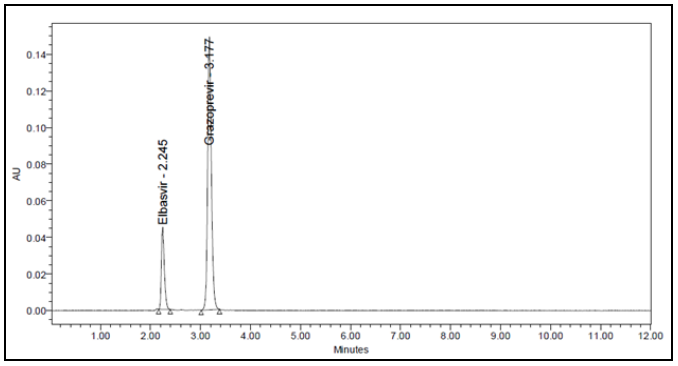

FIG. 8E: PHOTO STABILITY DEGRADATION CHROMATOGRAM

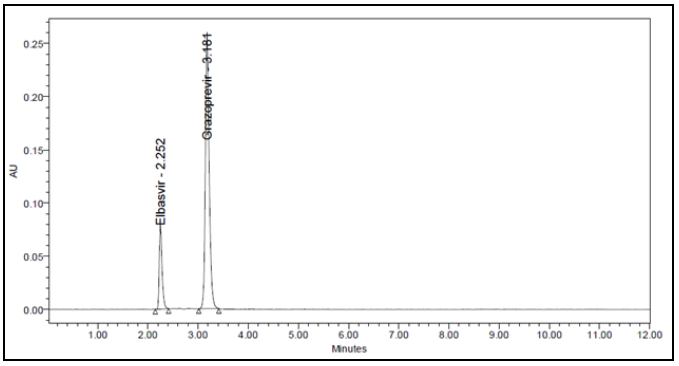

FIG. 8F: DRY HEAT STUDY CHROMATOGRAM

CONCLUSION: Stability indicating method was developed for the simultaneous determination of Elbasvir and Grazoprevir in tablet dosage form using RP-HPLC. The developed method was validated and was accurate, precise, specific, linear, rugged, robust and stable. The forced degradation studies concluded that the drugs were stable at forced degradation conditions. This method is applicable for the simultaneous determination of Elbasvir and Grazoprevir in its dosage form for routine analysis.

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CONFLICT OF INTEREST: Nil

REFERENCES:

1. Carrion AF and Martin P: Safety and efficacy of Elbasvir and Grazoprevir for treatment of hepatitis C. Expert Opin Drug Saf. 2016; 15: 883-90.
2. Coburn CA, Meinke PT and Chang W: Discovery of MK-8742: an HCV NS5A inhibitor with broad genotype activity. Chem Med Chem. 2013; 8: 1930-40.
3. Dore GJ, Altice F and Litwin AH: Elbasvir-Grazoprevir to Treat Hepatitis C Virus Infection in Persons Receiving Opioid Agonist Therapy: A Randomized Trial. Ann Intern Med. 2016; 165: 625-634.
4. Forns X, Gordon SC and Zuckerman E: Grazoprevir and Elbasvir plus ribavirin for chronic HCV genotype-1 infection after failure of combination therapy containing a direct-acting antiviral agent. J Hepatol. 2015; 63: 564-72.
5. Howe AY, Black S and Curry S: Virologic resistance analysis from a phase 2 study of MK-5172 combined with pegylated interferon/ribavirin in treatment-naive patients with hepatitis C virus genotype 1 infection. Clin Infect Dis. 2014; 59: 1657-65.
6. Sulejmani N, Jafri SM and Gordon SC: Pharmaco-dynamics and pharmacokinetics of Elbasvir and Grazoprevir in the treatment of hepatitis C. Expert Opin Drug MetabToxicol. 2016; 12: 353-61.
7. Vancha AR, Naresh D, Sowjanya P and Kumar GV: Analytical Method Development and Validation for Elbasvir and Grazoprevir in Combine Phaarmaceutical Dosage forms by RP-HPLC. Pharma research library.
8. Akram NMD, Umamahesh M and Ramachari T: A new validated RP-HPLC method for the determination of Elbasvir and Grazoprevir in its bulk and pharmaceutical dosage forms. International Journal of Chemical and Pharmaceutical Analysis 2017; 4(3): 1-11.
9. Potluri H, Battula SR and Yeturu S: Picogram Level Quantification of Grazoprevir and Elbasvir with Deuterated Internal Standards in Human Plasma Samples by LC–ESI-MS/MS. Indian Journal of Pharmaceutical Education and Research 2016; 50(4): 612-619.
10. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH harmonised tripartite guideline: validation of analytical procedures: text and methodology Q2(R1). ICH 2005; 1-13.

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