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A STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION OF LEDIPASVIR AND SOFOSBUVIR IN BULK BY UV SPECTROSCOPY AND RP-HPLC

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ABSTRACT

A new, simple, precise, accurate and reproducible RP-HPLC and UV method for stability indicating method development and validation of Ledipasvir and Sofosbuvir in bulk form. Separation of Ledipasvir and Sofosbuvir was successfully achieve Dona Zorbax C8, 250 X4.6, 5µm or equivalent in an isocratic mode utilizing 0.1% OPA: Methanol (45:55) at a flow rate of 1.0mL/min and eluate was monitored at 238nm, with a retention time of 3.296 and 7.257minutes for Ledipasvir and Sofosbuvir respectively. The method was validated and their response was found to be linear in the drug concentration range of 45µg/ml to135µg/ml for Ledipasvir and 200µg/ml to 600µg/ml for and Sofosbuvir. The values of the correlation coefficient were found to 0.999 for Ledipasvir and 1 for Sofosbuvir respectively. The LOD and LOQ for Ledipasvir were found to be 0.090 and 0.695 respectively. The LOD and LOQ for Sofosbuvir were found to be 0.6619 and 2.2063 respectively. This method was found to be good percentage recovery for Ledipasvir and Sofosbuvir were found to be 100 and 100 respectively indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard with the sample so, the method specifically determines the analyte in the sample without interference from excipients of tablet dosage forms. The method was extensively validated according to ICH guidelines for Linearity, Accuracy, Precession, Specificity and Robustness.

KEYWORDS

Ledipasvir, Sofosbuvir, High performance liquid chromatography and UV spectrometry.

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INTRODUCTION[1-21]

It is rare today that an HPLC-based method is developed that does not in same way relate (or) compare to existing, literature based approaches. Today HPLC (High performance liquid chromatography) is the method of choice used by the pharmaceutical industry to assay the intact drug

and degradation products. The appropriate selection and chromatographic conditions ensure that the HPLC method will have the desired specificity. UV spectroscopy is also a simple analytical tool widely used for routine assay of drugs. The assay of the selected drugs HPLC and UV spectroscopy has been chosen for these proposed methods. Pharmaceutical Analysis plays a major role today and it can be considered as an interdisciplinary subject. Pharmaceutical analysis derives its principles from various branches like chemistry, physics. And microbiology etc. pharmaceutical Analytical techniques are applied mainly in two areas, quantitative analysis and qualitative analysis, although there are several other applications. Drugs and pharmaceuticals are chemicals or like substances, which or of organic inorganic or other origin. Whatever may be the origin, we some property of the medicinal agent to measure them quantitatively or qualitatively.

In recent years, several analytical techniques have been evolved that combined two or more methods into one called "hyphenated" technique eg: GC/MS, LC/MS etc. The complete Analysis of a substance consists of four main steps.

Thereby it is advantageous than volumetric methods. Many HPLC methods has been developed and validated for the quantitative determination of various marketed drugs.

Analytical method development and validation places an important role in drug discovery and manufacture of pharmaceuticals. These methods are used to ensure the identity, purity, potency and performance of drug products majority of analytical development effort goes into validating a stability indicating method. So it is a quantitative analytical method based on the structure and chemical properties of each active ingredient of the drug formulation.

Most of the drugs can be analyzed by HPLC method because of several advantages like rapidity, specificity, accuracy, precision, reproducibility, ease of automation and eliminates tedious extraction and isolation procedures.

On the literature survey, it was found that most of the analytical method available for the above mentioned drug is applicable for quantification in plasma samples, the most widely used method being liquid chromatography-mass chromatography. So it is felt that there is a need to develop accurate, precise analytical methods for the estimation of the drug in solid dosage formulation.

METHODS VALIDATION

System suitability

Tailing factor for the peaks due to Ledipasvir and Sofosbuvir in standard solution should not be more than 2.0. Theoretical plates for the Ledipasvir and Sofosbuvir peaks in standard solution should not be less than 2000.

Specificity

Specificity studies were carried for both pure drugs and drug product by comparing with blank and placebo. These blank and placebo were compared with standard and sample shows that the analyte chromatographic peak is not attributable to more than one component as the impurities are not available.

Forced degradation studies

In the present investigation, as there was no interference of impurities with the analyte peaks, forced degradation studies were conducted with the same LC conditions developed to separate drug peaks of interest from their degradants which proves the stability indicating power of the method. Intentional degradation was attempted to various stress conditions such as acid hydrolysis (using 1.0N HCl), base hydrolysis (using 1.0 N NaOH), oxidative hydrolysis (using 3.0%v/v H2O2), thermal degradation (heated at 70^{o}C for 14 days) and photolytic degradation (to overall illumination of ≥210Wh/m2 at room temperature with UV light for 14 days), to evaluate the ability of the proposed method to separate Ledipasvir and Sofosbuvir from their degradation products. Acidic hydrolysis: Forced degradation in acidic media was performed by taking accurately weighed samples of 519.7mg of Ledipasvir and Sofosbuvir each in separate 5mL volumetric flasks. Then 2mL of 1N HCl was added,

made to dissolve and final volume was made up to the mark with 1N HCl to get mg/mL solutions and these were kept at 70ºC for 2 days and analyzed after suitable dilution.

Basic hydrolysis

Forced degradation in basic media was performed by dissolving separately an accurately weighed quantities (122.5mg each) of Ledipasvir and Sofosbuvir in 1N NaOH in 5mL volumetric flasks and final volume was made up to 5mL with the same to get mg/mL solutions and these solutions were kept at 70ºC for 2 days and analyzed after suitable dilution.

Oxidative degradation

Oxidative degradation studies were carried out in 3% (v/v) H2O2. Stock solutions of 122.5mg/mL of Ledipasvir and Sofosbuvir were prepared and kept at 70ºC for 2 days and analyzed after suitable dilution. Photo Degradation: For photolytic stress, samples of drug substances in solid state were irradiated with UV radiation (overall illumination of ≥210Wh/m2 at room temperature with UV radiation), for 14 days. Stock solutions of 1mg/mL was prepared in methanol from the exposed drug substances individually.

Thermal Degradation

For thermal stress, 122.5mg of samples of drug substances in solid state were packed in glass Vials and placed in a controlled temperature oven at 70c for 14 days. Stock solutions of 1mg/mL was prepared in methanol from the exposed drug substances individually. For HPLC analysis, all the stressed sample solutions were diluted with mobile phase to obtain final concentration of 60µg/mL of Ledipasvir and Sofosbuvir and 100µg/mL of Rabeprazole respectively. Similarly mixture of both drugs in a concentration of 60µg/mL of Ledipasvir and Sofosbuvir and 100µg/mL of Rabeprazole was prepared prior to analysis by HPLC. Besides, solutions containing 60µg/mL of Ledipasvir and Sofosbuvir and 100µg/mL of Rabeprazole for each drug separately were also prepared without performing the degradation of both the drugs. Then 20µL of above solutions were injected into the HPLC system and analyzed. Solution of standard, sample, blank and placebo were prepared as per test procedure and injected into the HPLC system.

Acceptance criteria

Chromatogram of standard and sample should be identical with near Retention time.

Blank interference

The interference of blank was conducted. Diluent was injected into HPLC system as per the test procedure.

Acceptance criteria

Chromatogram of blank should not show any peak at the retention time of analyte peak. There is no interference due to blank at the retention time of analyte. Hence the method is specific.

Linearity

Plot the graph of standard versus the actual concentration in µg/ml and determine the coefficient of correlation and basis for 100% response.

Acceptance criteria

The Linearity regression coefficient of average peak area response of replicate injections plotted against respective concentration should not be less than 0.999. The % y-intercept as obtained from the linearity data (without extrapolation through origin 0, 0) should be within ±2.0.

Statistical Evaluation

A graph between the concentration and the average area was plotted. Points for linearity were observed. Using the method of least squares, a line of best fit was taken and the correlation Coefficient, slope and, y-intercept were calculated.

RESULTS AND DISCUSSION

Result

Results of system suitability study are summarized in the above table. Six injections of the standard solution showed uniform retention time, theoretical plate count, tailing factor and resolution for both the drugs which indicate a good system for analysis.

Result

The forced degradation study showed the method was highly specific, the chromatographic peaks does not interfere with any other impurities. This proves that, excipients have no effect on the

analytical method. So the method is highly selective.

Result

A linear relationship between peak areas versus concentrations was observed for Ledipasvir and Sofosbuvir in the range of 50% to 150% of nominal concentration. Correlation coefficient was 0.999 for both Ledipasvir and Sofosbuvir which prove that the method is linear in the range of 50% to 150%.

System Suitability

Table No.1: System suitability data of Ledipasvir and Sofosbuvir

S.No	Parameter	Ledipasvir	Sofosbuvir	Acceptance criteria
1	Retention time	3.296	7.257	+-10
2	Theoretical plates	17053	10841	>2500
3	Tailing factor	1.10	1.07	<2.00
4	% RSD	0.7	0.8	<2.00

Table No.2: Standard Results of Ledipasvir

S.No	Sample name	RT	Area	USP plate count	USP tailing
1	Injection1	3.301	5154154	15844	1.12
2	Injection 2	3.308	5584697	16253	1.10
3	Injection 3	3.304	4622291	16360	1.09
4	Injection 4	3.301	3607569	15504	1.10
5	Injection 5	3.304	3336067	15520	1.09

Table No.3: Standard Results of Sofosbuvir

S.No	Sample Name	RT	Area	USP plate count	USP tailing
1	Injection 1	7.301	8518567	10496	1.08
2	Injection 2	7.334	9220560	10564	1.08
3	Injection 3	7.368	7640607	10781	1.07
4	Injection 4	7.392	5932037	10553	1.06
5	Injection 5	7.421	5512700	10416	1.06

Specificity

Table No.4: Specificity data for Ledipasvir and Sofosbuvir

S.No	Sample name	Ledipasvir Area	Rt	Sofosbuvir Area	Rt
1	Standard	4460355	3.301	7364894	7.301
2	Sample	4460496	3.296	7363003	7.257
3	Blank	-	-	-	-
4	Placebo	-	-	-	-

Table No.5: Results of forced degradation study for Ledipasvir

S.No	Type of stress	Degradation products/ Drug (D)	Retention time	% Area	Peak purity	Result
1	Acidic Hydrolysis (mg/mL in 1N HCl) at 70°C for 2 days	-	3.252	4236590	0.999	Passed
2	Basic Hydrolysis (mg/mL in 1N NaOH) at 70°C for 2 days	-	3.265	4227521	0.999	Passed
3	Oxidative Hydrolysis (mg/mL in 3% v/v H_2O_2) at 70°C for 2 days	-	3.277	4299323	0.999	Passed
4	Photo Degradation (to UV light) for 14 days	-	3.287	4261372	0.999	Passed
5	Thermal Degradation at 70°C for 14 days	-	3.287	4213277	0.999	Passed

Table No.6: Results of forced degradation study for Sofosbuvir

S.No	Type of stress	Degradation products/ Drug (D)	Retention time	% Area	Peak purity	Result
1	Acidic Hydrolysis (mg/mL in 1N HCl) at 70°C for 2 days	-	6.847	7039509	0.999	passed
2	Basic Hydrolysis (mg/mL in 1N NaOH) at 70°C for 2 days				0.999	passed
		-	6.977	7081666	0.999	passed
3	Oxidative Hydrolysis (mg/mL in 3% v/v) at 70°C for 2 days	-	7.056	7045562	0.999	passed
4	Photo Degradation (to UV light) for 14 days	-	7.138	7066831	0.999	passed
5	Thermal Degradation at 70°C for 14 days	-	7.113	7027361	0.999	passed

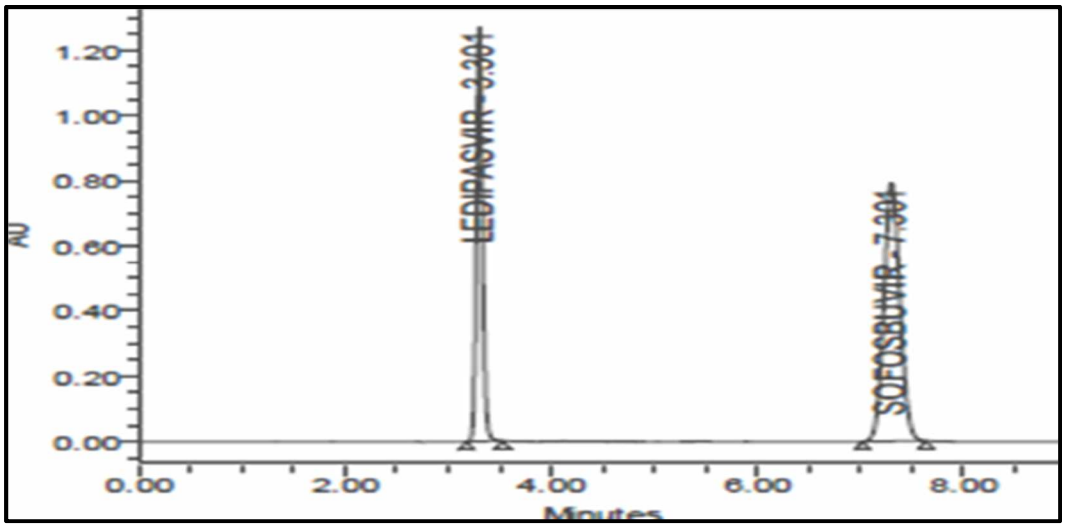
Linearity

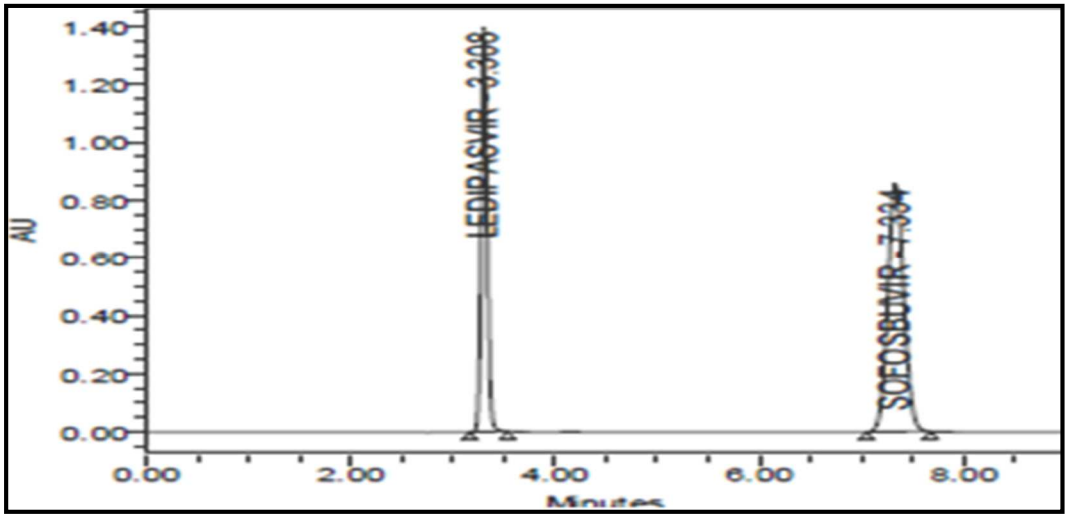
Table No.7: Linearity data for Ledipasvir

S.No	Conc (µg/ml)	Area	RT
1	50	2230184	3.297
2	75	3347897	3.299
3	100	4462463	3.297
4	125	5577829	3.297
5	150	6694287	3.302
Correlation coefficient (r^2)		0.999	

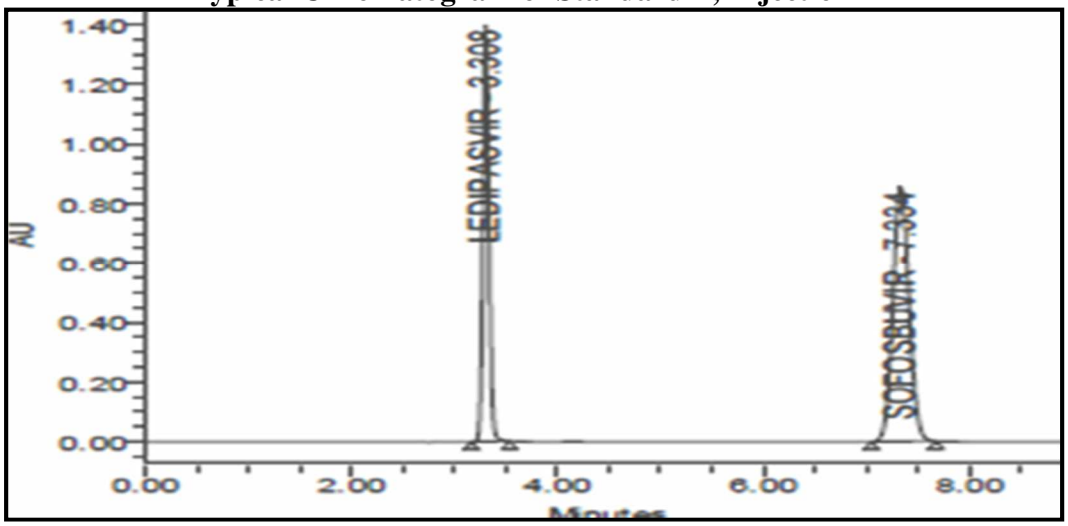
Table No.8: Linearity data for Sofosbuvir

S.No	Conc (µg/ml)	RT	Area
1	50	7.253	3680821
2	75	7.246	5524283
3	100	7.241	7363685
4	125	7.228	9204665
5	150	7.232	11026551
Correlation coefficient (r^2)			0.999

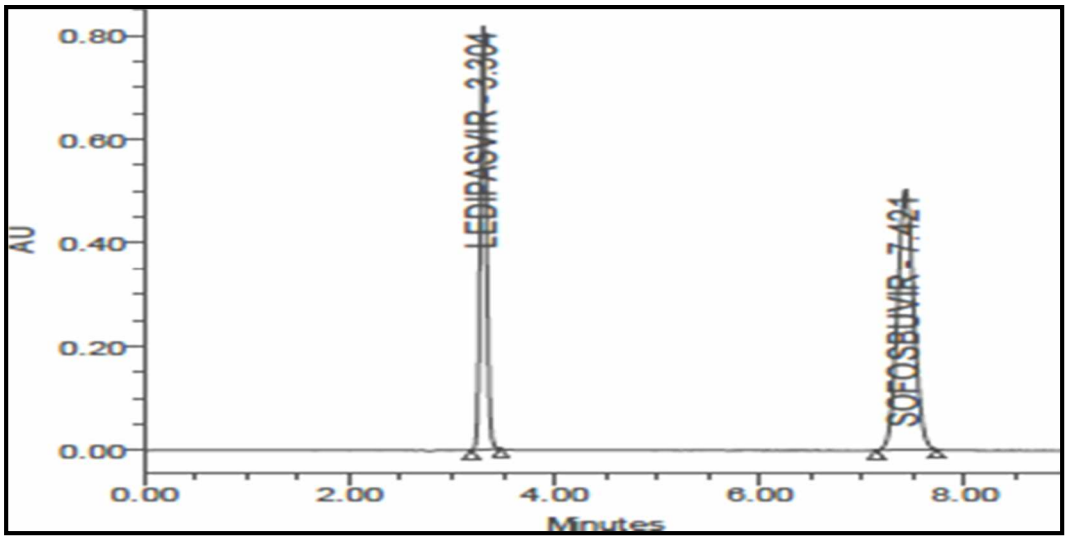




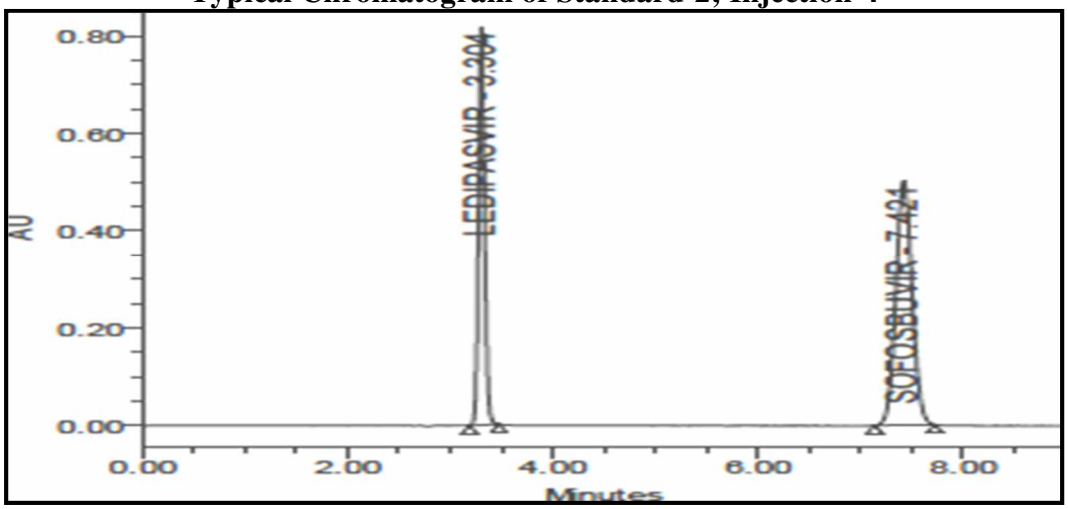
Typical Chromatogram of Standard-2; Injection-1



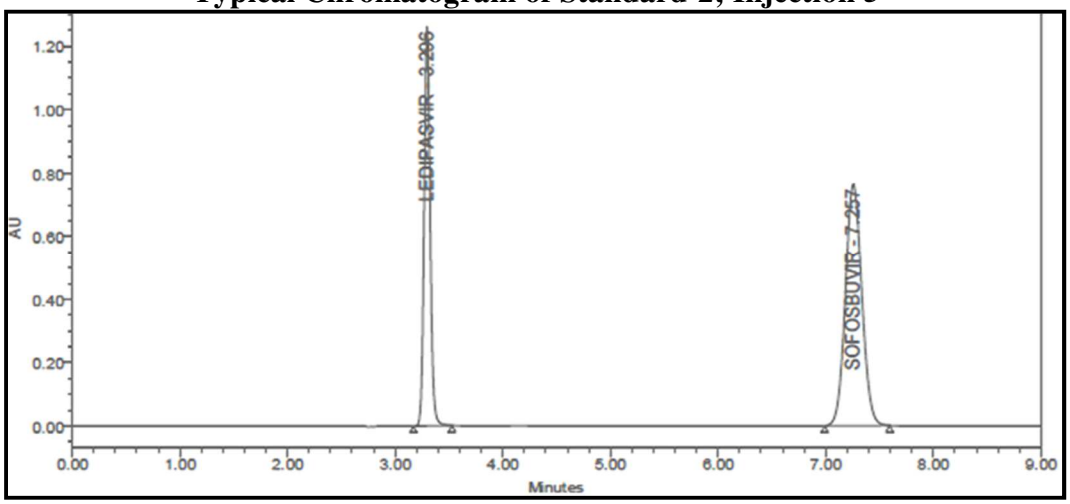
Typical Chromatogram of Standard-2; Injection-3



Typical Chromatogram of Standard-2; Injection-4



Typical Chromatogram of Standard-2; Injection 5



Typical Chromatogram of Sample-1

Figure No.1: System suitability chromatography of Ledipasvir and Sofosbuvir

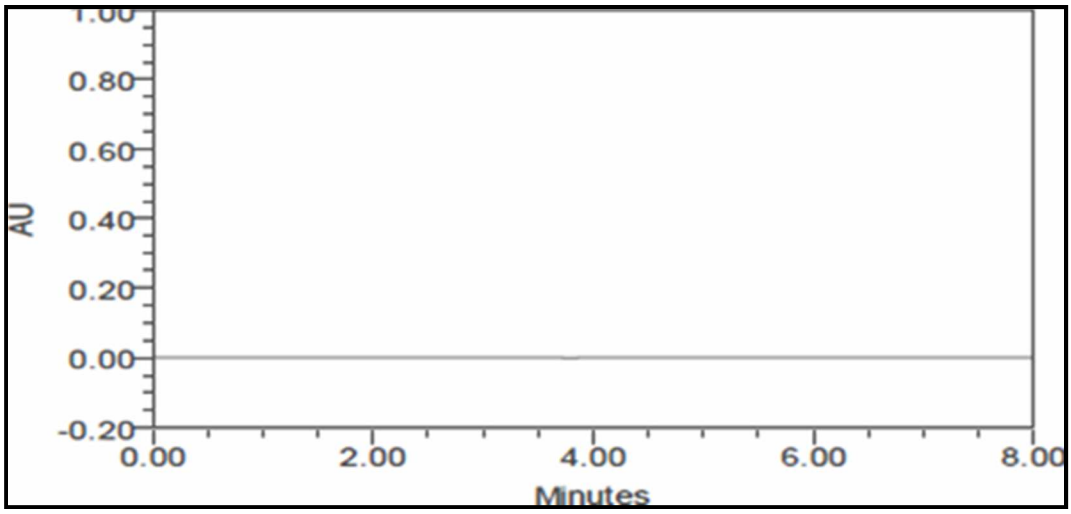


Figure No.2: Typical chromatogram of the blank

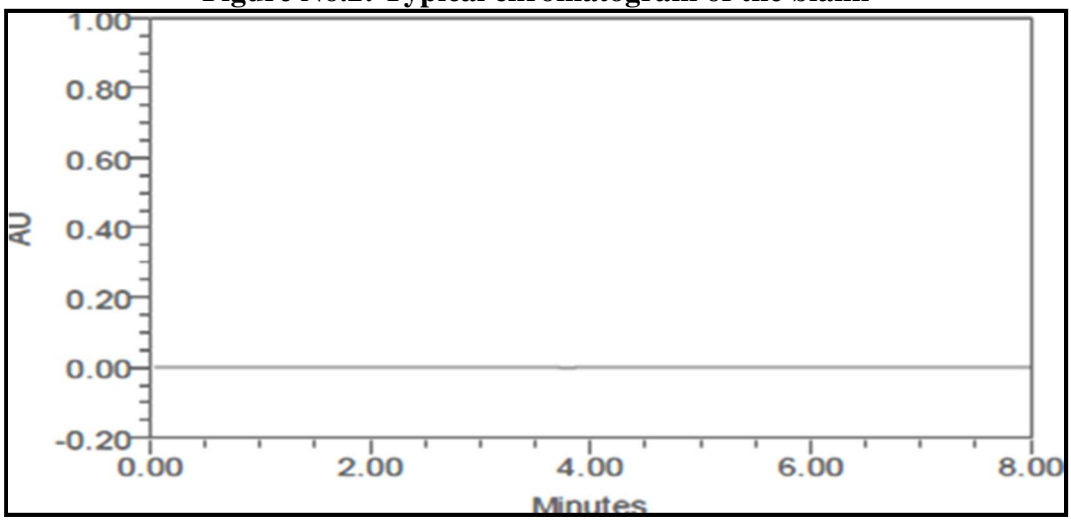


Figure No.3: Typical chromatogram of the Placebo

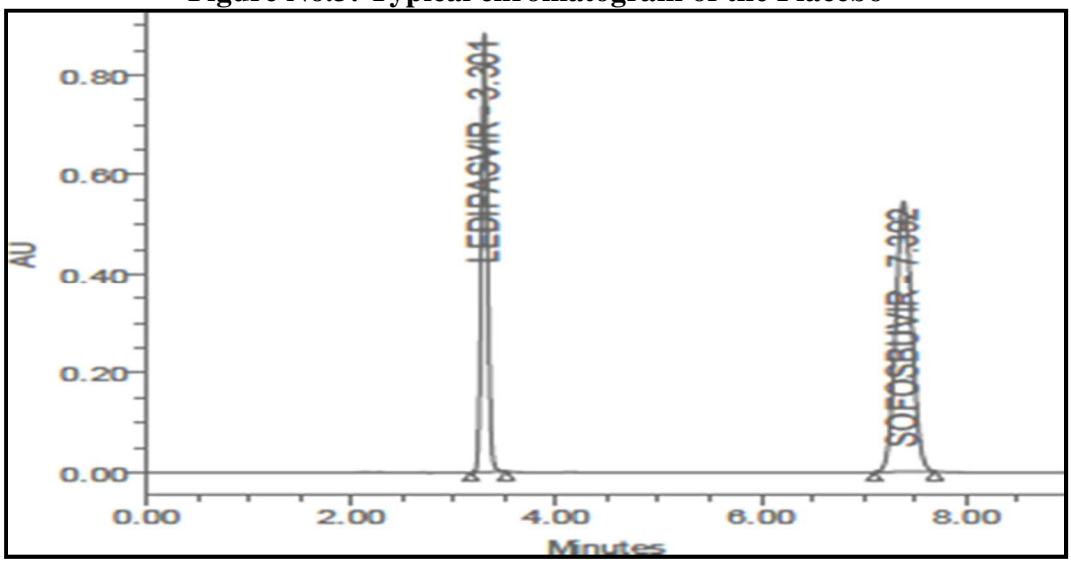


Figure No.4: chromatogram representing specificity of standard

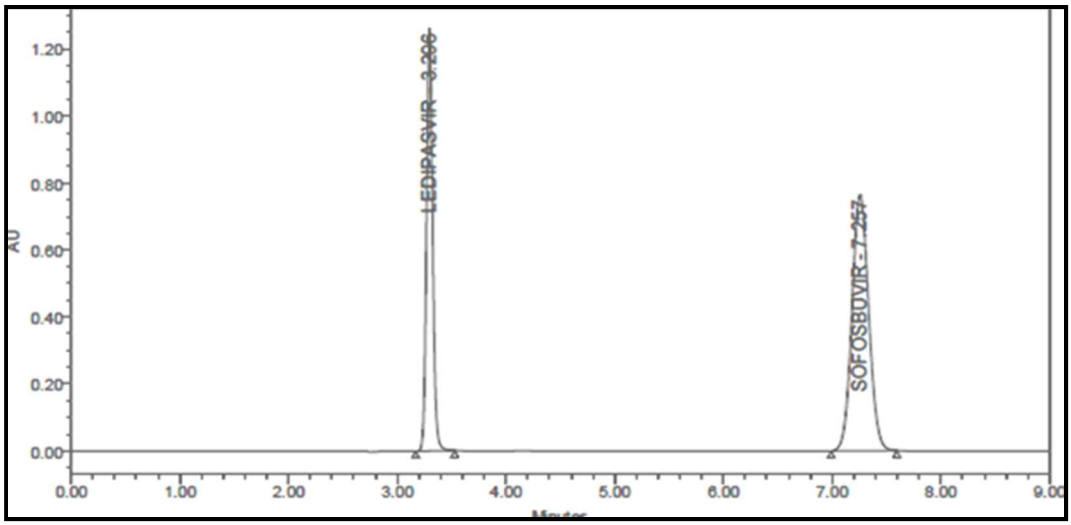


Figure No.5: Chromatogram representing specificity of sample

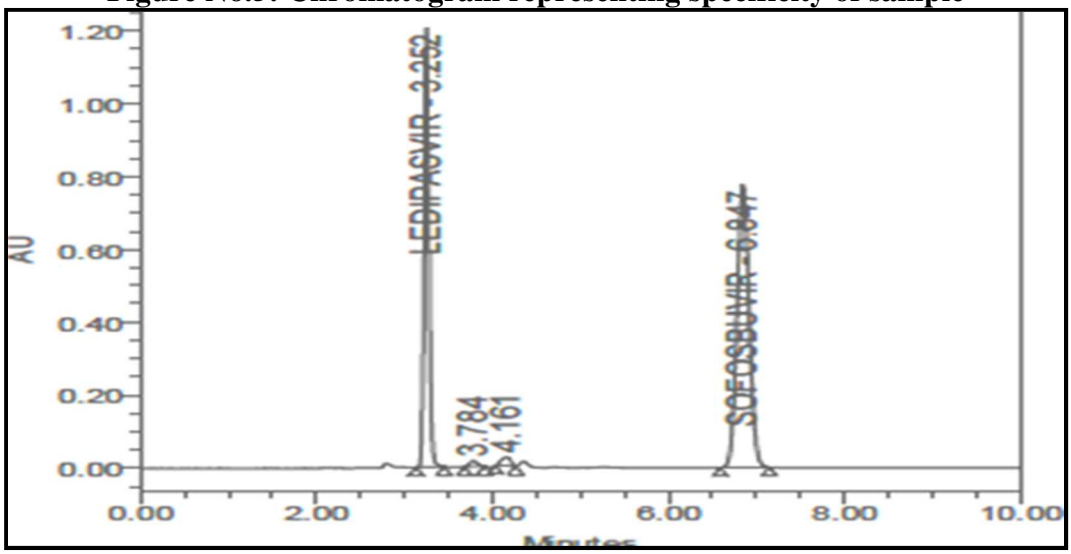


Figure No.6: Chromatograms of Acid stress treated Ledipasvir and Sofosbuvir mixture

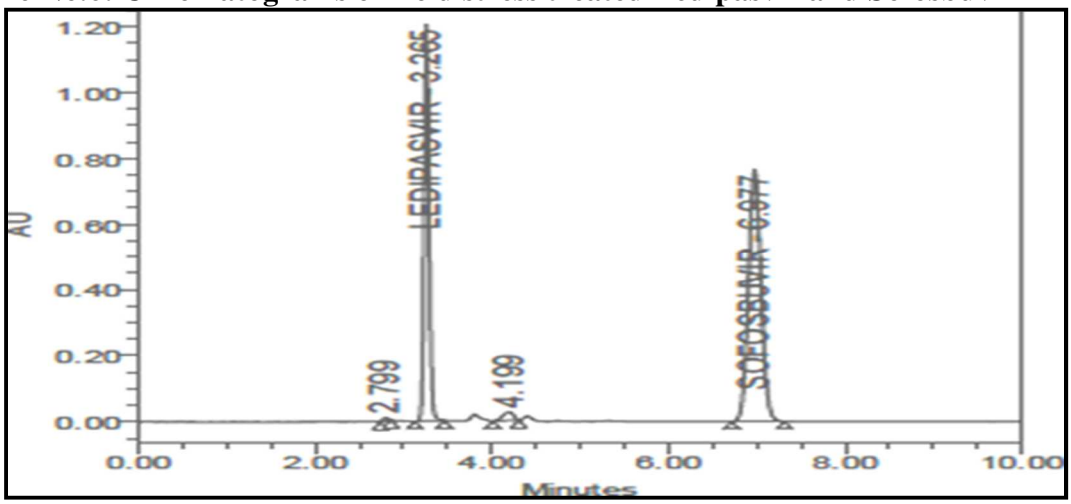


Figure No.7: Chromatograms of Base stress treated Ledipasvir and Sofosbuvir mixture

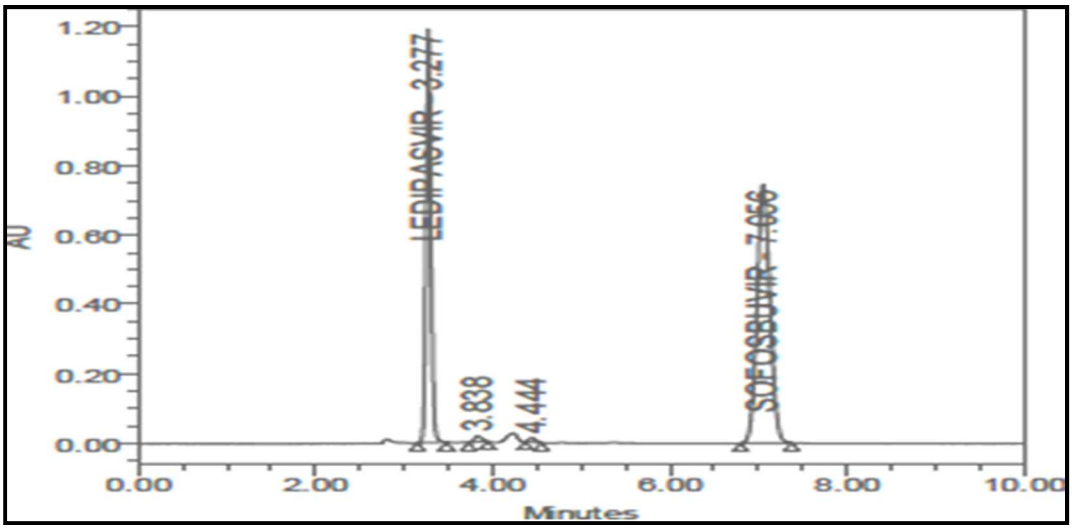


Figure No.8: Chromatograms of H2O2 stress treated Ledipasvir and Sofosbuvir mixture

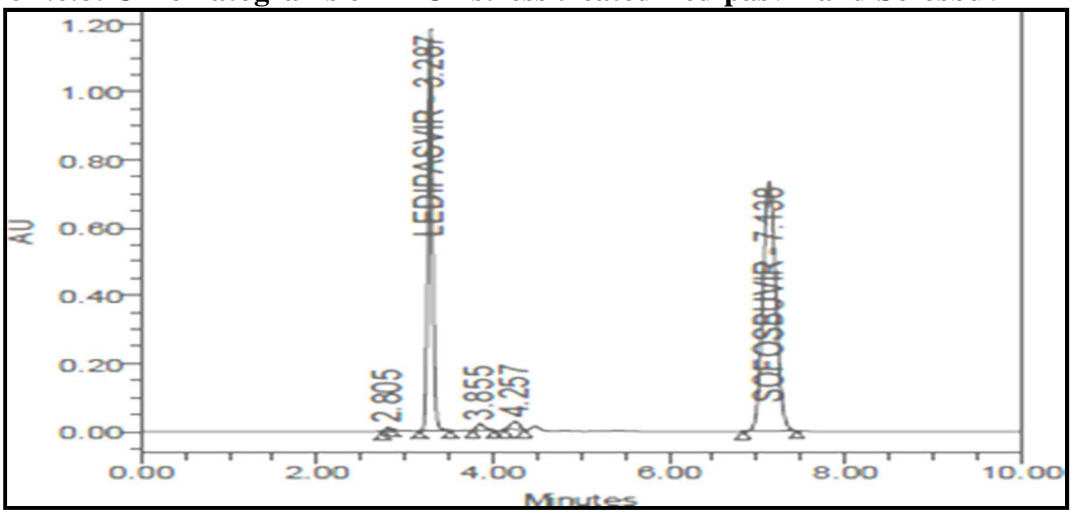


Figure No.9: Chromatograms of UV stress treated Ledipasvir and Sofosbuvir mixture

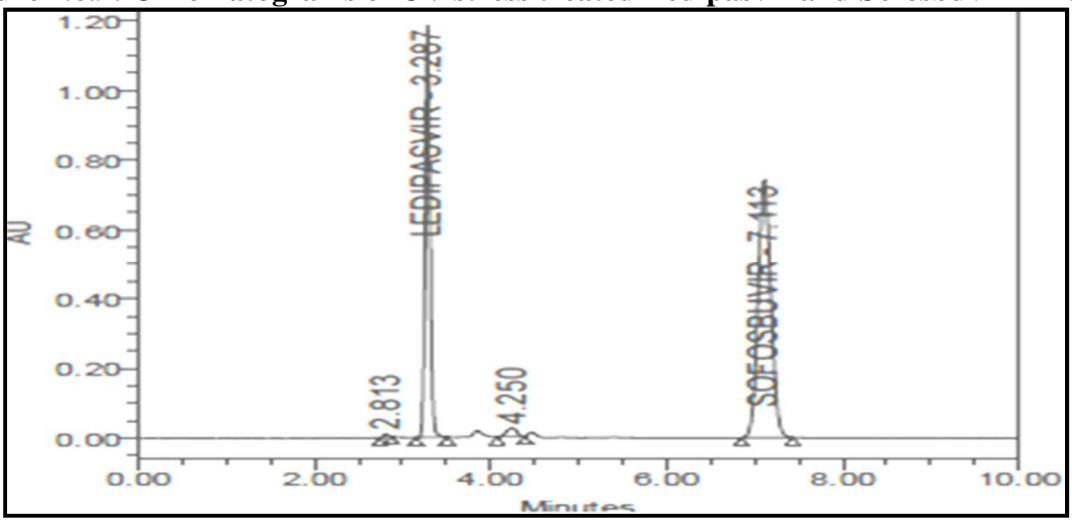


Figure No.10: Chromatograms of Thermal stress treated Ledipasvir and Sofosbuvir mixture

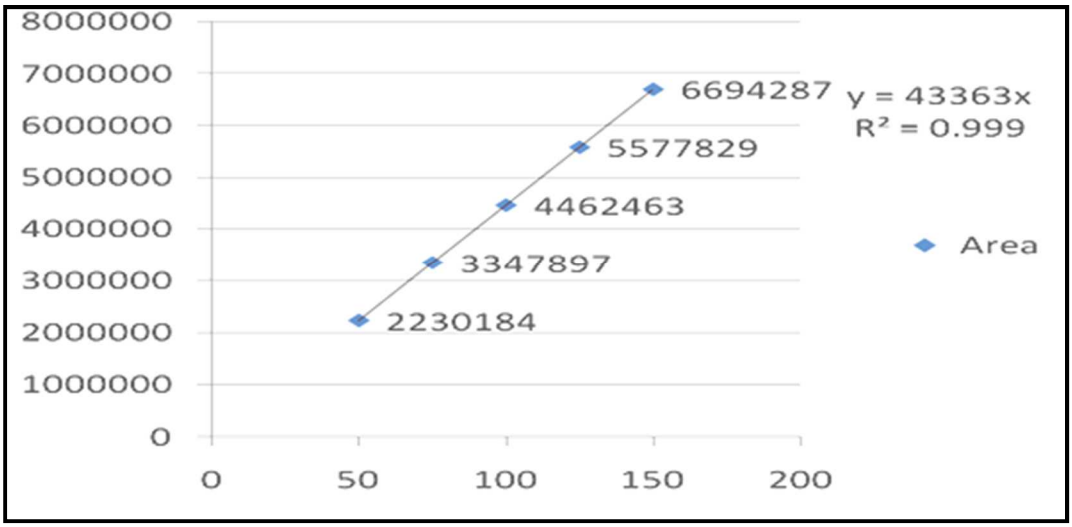


Figure No.11: Linearity plot of Ledipasvir

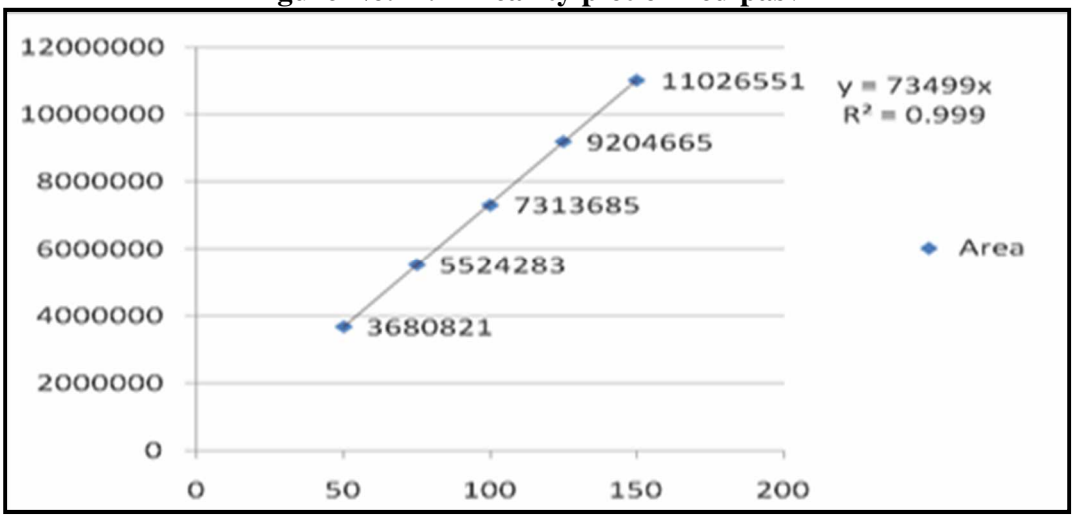


Figure No.12: Linearity plot of Sofosbuvir

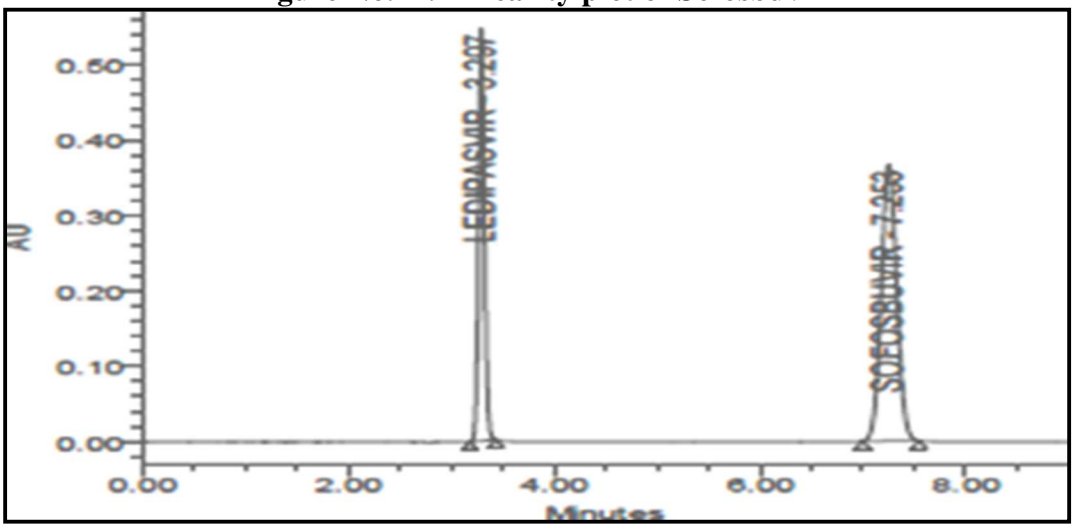


Figure No.13: Chromatogram representing linearity 1

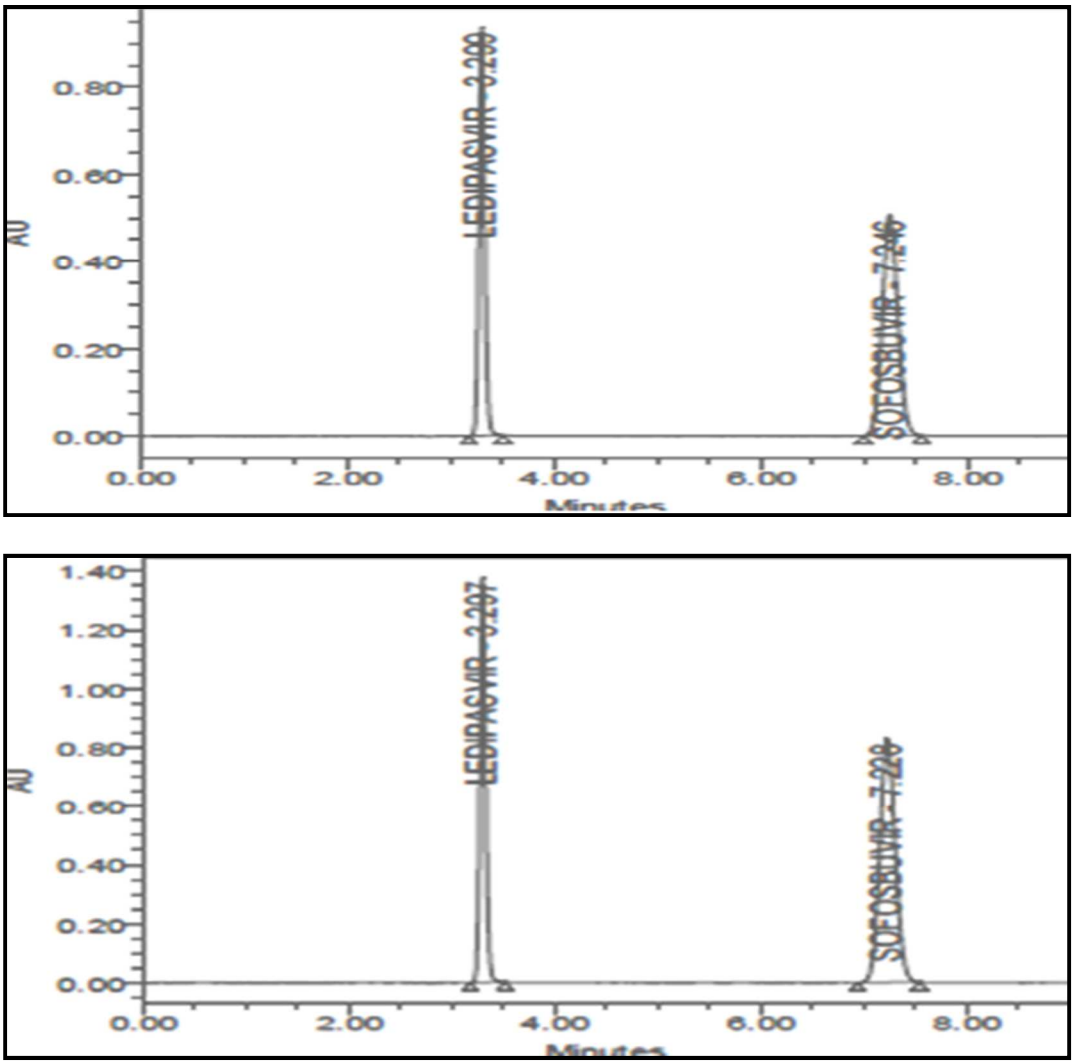


Figure No.14: Chromatogram representing linearity 2

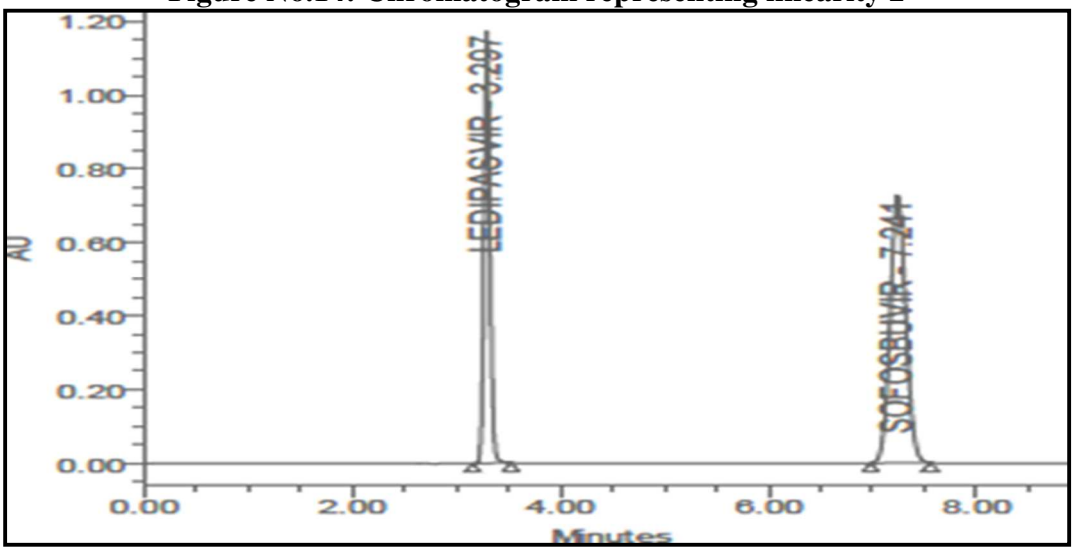


Figure No.15: Chromatogram representing linearity 3

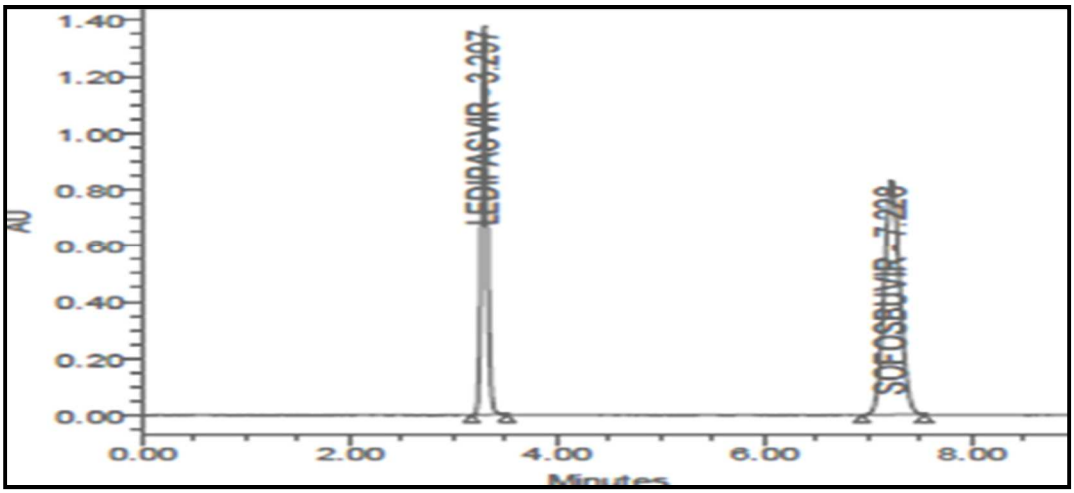


Figure No.16: Chromatogram representing linearity 4

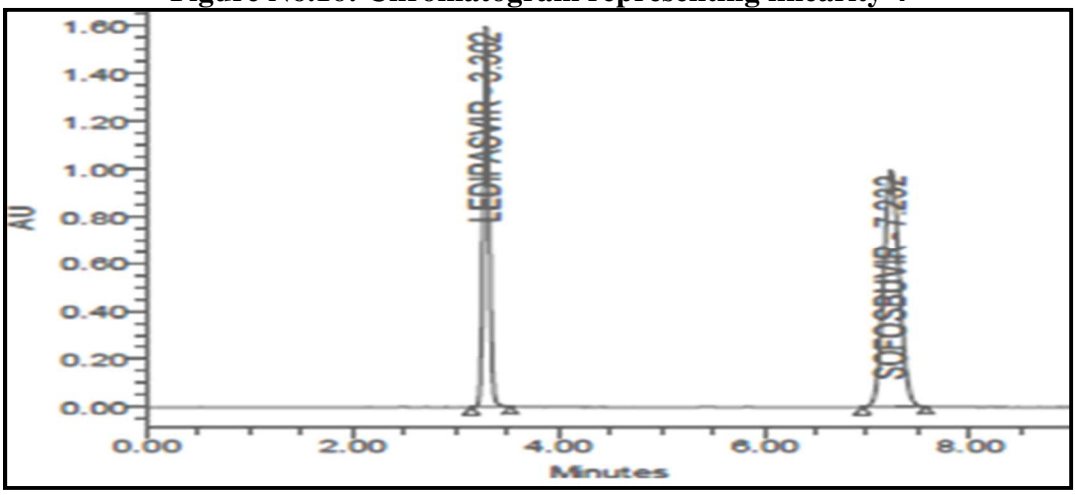


Figure No.17: Chromatogram representing linearity 5

SUMMARY AND CONCLUSION

It was concluded that there was no stability indicating method reported for the above selected multi component dosage form, which promote to pursue the present work. The scope and objective of the present work is to develop and validate a new simple Stability Indicating UV and RP-HPLC method for Ledipasvir and Sofosbuvir in bulk form.

In simultaneous RP-HPLC method development, Waters HPLC with UV detector and column used is Zorbax C8 (250 X 4.6mm) column with 5-micron particle size. Injection volume of 10 µL is injected and eluted with the mobile phase selected after optimization was 0.1% OPA and Methanol in the ratio of 45:55 was found to be ideal. The flow rate was found to be optimized at 1.0mL/min. Detection was carried out at 236nm. Quantitation was done by external standard method with the above mentioned optimized chromatographic condition. This system produced symmetric peak shape, good resolution and reasonable retention times of Ledipasvir and Sofosbuvir were found to be 3.296 and 7.257 minutes respectively.

The Ledipasvir and Sofosbuvir showed linearity in the range of 45-135µg/mL and 200-600µg/mL respectively. The slope and correlation coefficient values for Ledipasvir were found to be 43363 and 0.999 respectively and 73499 and 0.999 respectively for Sofosbuvir which indicates excellent correlation between response factor Vs concentration of standard solutions.

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CONFLICT OF INTEREST

There is no conflict of interest.

BIBLIOGRAPHY

1. Sharma B K. Instrumental methods of chemical analysis, Introduction to analytical chemistry, *Go*
2. Willard H H, Merritt L L, Dean J A, Settle F A. Instrumental methods of analysis, *CBS Publishers and Distributors, New Delhi,* 7th Edition, 1986, 518-521, 580-610.
3. John Adamovies. Chromatographic analysis of pharmaceutical, *Marcel Dekker Inc, New York,* 2nd Edition, 74, 1997, 5-15.
4. Gurdeep Chatwal, Sahm K. Anand. Instrumental methods of chemical analysis, *Himalaya Publishing House, New Delhi,* 5th Edition, 2002, 1.1-1.8, 2.566-2.570.
5. Skoog D A. Holler J, Nieman T A. Principle of instrumental analysis, *Saunders College Publishing,* 1, 5th Edition, 1998, 778-787.
6. Skoog, Holler, Nieman. Principals of instrumental analysis, *Harcourt Publishers International Company,* 5th Edition, 2001, 543-554.
7. William Kemp. Organic spectroscopy, *Palgrave, New York,* 2005, 7-10, 328-330.
8. Sethi P D. HPLC: Quantitative analysis pharmaceutical formulations, *CBS Publishers and Distributors, New Delhi,* 2001, 3-137.
9. Michael E, Schartz I S, Krull. Analytical method development and validation, 2004, 25-46.
10. Snyder R, Kirkland J, Glajch L. Practical HPLC method development, *A Wiley International Publication,* 2nd Edition, 1997, 235, 266-268, 351-353, 653-600, 686-695.
11. Basic education in analytical chemistry, *Analytical Science,* 17(1), 2001.
12. Method validation guidelines International conference on harmonization, *Geneva,* 1996.
13. Berry R I, Nash A R. Pharmaceutical process validation, Analytical method validation, *Marcel Dekker Inc, New York,* 57, 1993, 411-428.
14. Anthony C Moffat, M David Osselton, Brian Widdop. Clarke's analysis of drugs and poisons, *Pharmaceutical Press, London,* 2004, 109-110, 1601-1602.
15. Klaus Florey. Analysis profile of drugs substances, *Academic Press, New York,* 2005, 406-435.
16. Arora P N, Malhan P K. Biostatistics, *Himalaya Publishers House, India,* 3rd Edition, 113, 2018, 139-140, 154.
17. Doserge. Wilson and Gisvold's text book of organic medicinal and pharmaceutical chemistry, *Lippincott Company,* 8th Edition, 1982, 183-197.
18. http//en.wikipedia.org/wiki/Chromatography.
19. German P, Yang J, West S, Han L, Sajwani K, Mathias A. Effect of food and acid reducing agents on the relative bioavailability and pharmacokinetics of ledipasvir/sofosbuvir fixed dose combination tablet, *15th International Workshop on Clinical Pharmacology of HIV and Hepatitis Therapy, Washington, DC, Abstract,* 2014, 15.
20. Afdhal N, Zeuzem S, Kwo P *et al*. Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection, *N Engl J Med,* 370(20), 2014, 1889-1898.
21. Afdhal N, Reddy K R, Nelson D R *et al*. Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection, *N Engl J Med,* 370(16), 2014, 1483-1493.

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