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Analytical method development and validation for the estimation of Declatasvir in bulk and pharmaceutical dosage form using RP - HPLC

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ABSTRACT

A simple, precise, accurate and linear reverse phase isocratic HPLC was developed and validated for the determination of Daclatasvir in bulk and tablet dosage forms. Method development was carried out on Zorbax Eclipse XDB-C18 isocratic column, (250 mm × 4.6 mm, particle size 5 µ, maintained at ambient temperature). The mobile phase was a mixture of 0.01M Potassium dihydrogen orthophosphate and Acetonitrile (15:85), with apparent pH of 2.5 and the flow rate was set at 1.0 ml/min and UV detection at 284 nm. The statistical analysis shows that the method was found to be accurate, reliable, simple and reproducible. The accuracy of the method was proved. The proposed method was successfully applied for the quantitative determination of Daclatasvir in bulk form and could be used for routine analysis with phenomenal accuracy and precisions.

Keywords: Daclatasvir, RP-HPLC, reliable, validation, assay, hepatitis, isocratic.

INTRODUCTION

The word chromatography was first derived from Greek words – kromatos means colour & graphos means written (meaning colour writing). The study of chromatography was first started in eighteenth century by Runge. While studying the nature of inorganic compounds, he separated inorganic salts and observed that the inorganic salts travel to different extent producing attractive pattern. Later Tswett defined chromatography as a method in which the components of a mixture are separated on an adsorbent column in a mobile or flowing system. The adsorbent material, or stationary phase, may be a solid, thin layer of solids adhered to glass plates or packed in columns, a paper, immobilized liquids and gels. The flowing component or mobile phase is either a liquid or a gas. As adsorbent material has taken many forms over the years, there has been the development of methods more specific to particular classes of analytes. Further it is defined as a physical method of separation in which the components to be separated are distributed between two phases of which one is stationary while other moves across in a definite direction.

The amount of the sample mixture required is very less and complex samples can be separated easily compared to other physical and chemical methods. A typical HPLC unit consists of –

1. solvent reservoir and mixing system
2. high pressure pump
3. sample inlet pump
4. column
5. detector and recording unit

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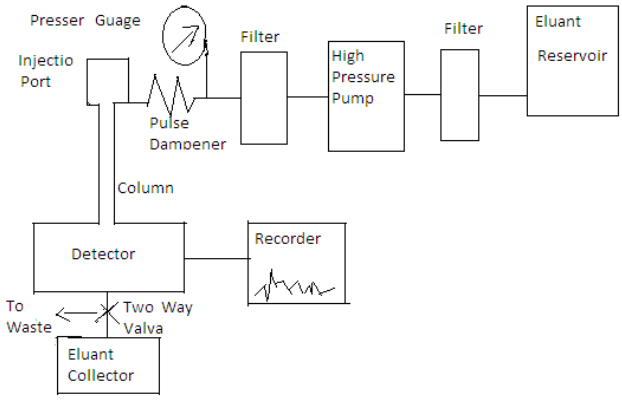


Figure 1.1: HPLC Instrumentation

The mobile phase (appropriate solvents) is allowed to enter from the reservoir to the mixing chamber where homogenous mixture is obtained. A pump capable of maintaining high pressures draws the solvent from the mixing chambers and pushes it through the column. The sample is injected through a port into the high pressure liquid carrier stream between the pump and the column. Typical flow rates are 1-2 ml/min with pressure up to several thousand psi. The column effluent passes through a non-destructive detector where a property such as ultraviolet absorbance, refractive index or molecular fluorescence is monitored, amplified and recorded as a typical detector response versus retention time chromatogram. The effluent may be discarded, recycled or saved for further studies in a fraction collector which is synchronized with the detector.

Drug profile

Declatasvir is a medication used in combination with other medications to treat hepatitis C. It is taken by mouth once a day. Declatasvir was approved for use in Europe in 2014 and in United States and India in 2015. It is on the world health organization's list of essential medicines, the most effective and safe medicines needed in a health system. Declatasvir is used in combination therapy for the treatment of hepatitis C genotype 1, 3, or 4 infections. The agents used in combination, which include Sofosbuvir, Ribavirin and Interferon varies based on the virus genotype and whether the person has cirrhosis.

Disease

Hepatitis is defined as the inflammation of the liver caused by virus or bacterial infections or continuous exposure to alcohol, drugs or toxic chemicals. In viral hepatitis the presence of the virus in the liver cells causes the immune system to attack the liver, resulting in inflammation & impaired function. It is caused by a type of virus known as Hepatitis A, B OR C.

The infection caused by Hepatitis C virus (HCV) is a slowly programming infection that is spread by the intravenous drug users. In other terms it can be defined as a blood born infectious disease that is caused by Hepatitis C virus, affecting the liver. The infection is often asymptomatic, but once established, chronic infection can cause inflammation of the liver.

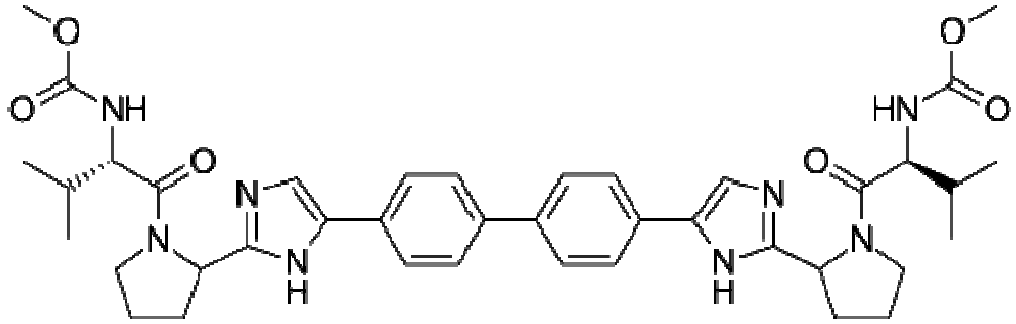


Figure 1.2: Chemical structure of Declatasvir

IUPAC name: Dimethyl N,N'-([1,1'-biphenyl]-4,4'-diylbis{1H-imidazole-5,2-diyl-[(2S)-pyrrolidine-2,1-diyl][(2S)-3-methyl-1-oxobutane-1,2-diyl]})dicarbamate

The primary objectives of the proposed work are -

1. To review the literature for Declatasvir regarding its physical and chemical properties and various analytical methods those were conducted for quantitative estimation.
2. To develop a suitable, simple, precise and accurate RP-HPLC method for routine analysis of Declatasvir in formulations.
3. To validate the developed method for both framing and documenting the capabilities.

MATERIALS AND METHOD

There are few analytical methods that have been reported for the estimation of Declatasvir in bulk and in pharmaceutical formulations at the time of commencement of research work.

Table 3.1: List of chemicals & drugs

S.no	Chemicals/drugs	Type	Brand
1	Acetonitrile	HPLC Grade	Qualigens
2	Water	HPLC Grade	Milli-Q
3	Potassium dihydrogen phosphate	HPLC Grade	Rankem
4	Ortho-phosphoric acid	HPLC Grade	Rankem
5	Declatasvir tablets	-	Declahep®

Table 3.2: List of instruments

S. No	Instruments	Model
1	HPLC	WATERS HPLC 2695 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2
2	UV/VIS spectrophotometer	PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was used for measuring absorbance of solutions
3	pH meter	Thermo scientific
4	Micro balance	Sartorius
5	Sonicator	Ultrasonic sonicator

Composition of mobile phase

The contents of the mobile phase were 3.48gms of Potassium dihydrogen phosphate (0.03M) in 1000 ml of water and by adjusting the pH to 2.5 with dilute ortho-phosphoric acid (mobile phase solvent-A) and acetonitrile (mobile phase solvent-B) in a isocratic mode in the ratio of 15: 85 (v/v). They were filtered before use through a 0.45 µm membrane filter and degassed by sonication.

Validation of developed rp-hplc method

The following parameters were considered for the analytical method validation of Declatasvir.

1. System suitability
2. Specificity
3. Precision
4. Linearity
5. Accuracy
6. Limit of detection
7. Limit of quantification
8. Robustness

RESULTS & DISCUSSION

Figure: Chromatogram for trail 3

Run time : 15minutes

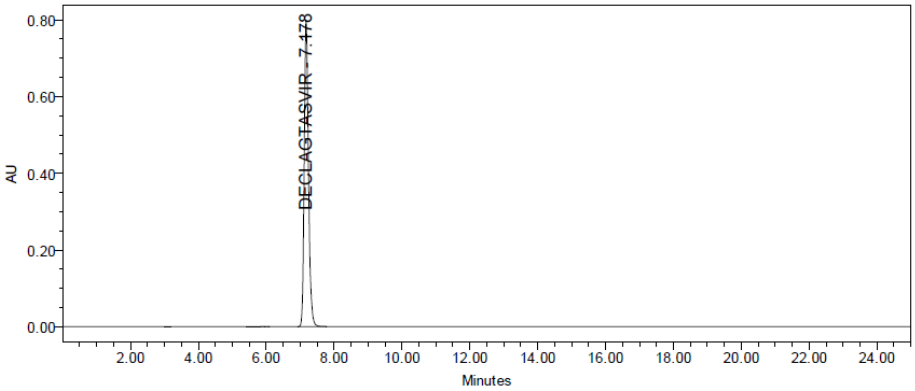


Figure: Optimized chromatogram (standard)

Table 4.4: Peak results for optimized chromatogram (standard)

S. No	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Daclatasvir	7.178	692871	42596	1.15	16421

Optimized chromatogram (sample)

Column : Zorbax Eclipse XDB-C18
Column temperature : Ambient
Wavelength : 285nm
Mobile phase ratio : Solvent A & solvent B (15:85 v/v)
Flow rate : 1ml/min
Injection volume : 10µl
Run time : 15minutes

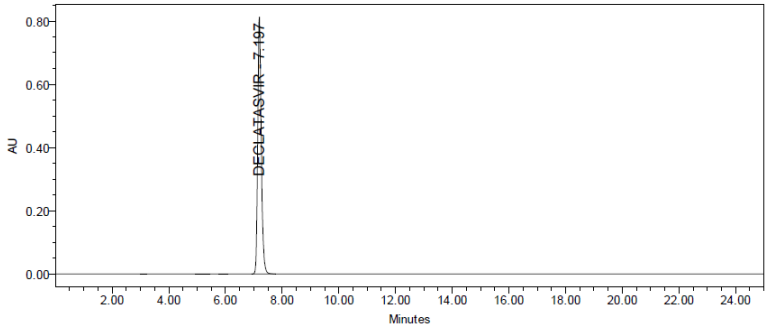


Figure 4.5: Optimized chromatogram (sample)

Table 4.5: Peak results for optimized chromatogram (sample)

S. No	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Declatasvir	7.197	692896	42598	1.12	16592

Observation

Theoretical plates from the above chromatogram are more than 2000 and tailing factor is less 2.0.

Method Validation

The following parameters were used to validate the method for the estimation of Declatasvir in bulk sample and in Tablets.

System suitability

The system suitability tests were carried out on freshly prepared standard stock solution of Declatasvir. The system was suitable for use, the tailing factors for Declatasvir were 1.23 and USP theoretical plates were found to be significantly high around 16305.

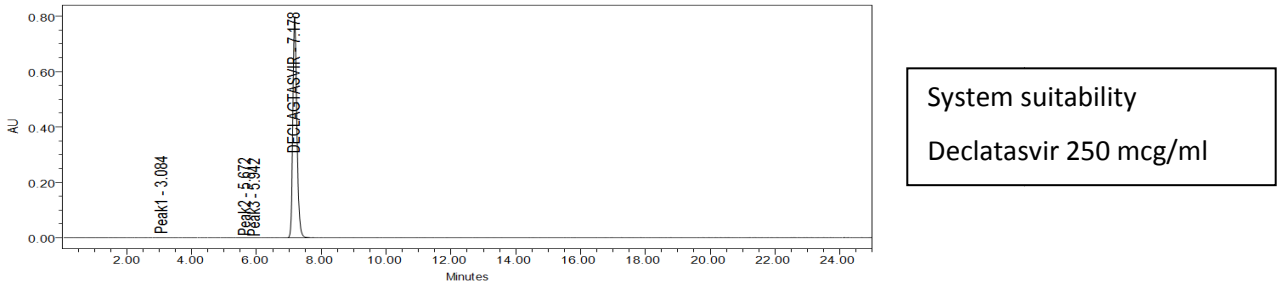


Figure 4.6: Typical System suitability Chromatogram of Declatasvir Working standard solution

S.No.	Peak name	RT	Area	% Area	RT Ratio	USP Tailing	USP Plate count	USP Resolution
1	Peak 1	3.084	3047	0.04	0.430	1.38	6197.56	
2	Peak 2	5.672	4133	0.06	0.790	0.81	4407.18	7.21
3	Peak 3	5.942	3284	0.05	0.828	1.15	16866.78	1.16
4	Declatasvir	7.178	6928714	99.85		1.23	16656.66	6.01

Specificity

The common excipients present in the pharmaceutical dosage form did not interfere with the elution or quantification of the method. Each Declahep®-60 mg Film coated tablet, Hetero Health Care, contains equivalent to Declatasvir 60 mg and the Tablet include the following inactive ingredients: Tablet Core: Declahep 60 mg tablets contain 60 mg Declatasvir (equivalent to 66 mg Declatasvir dihydrochloride) and the inactive ingredients anhydrous lactose (116 mg), microcrystalline cellulose, croscarmellose sodium, silicon dioxide, magnesium stearate, and Opadry green. Acceptence criteria for specificity, RSD should be less than 2%.

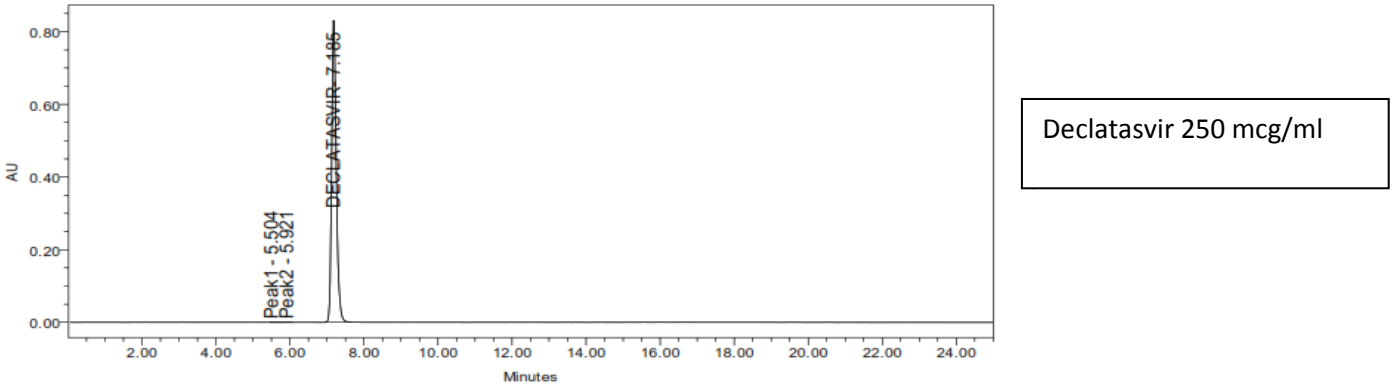


Fig 4.8: Typical specificity chromatogram of Declatasvir Working standard solution

Table 4.7: Specificity studies (Declatasvir Working standard solution)

S.No.	Peak name	RT	Area	% Area	RT Ratio	USP Tailing	USP Plate count	USP Resolution
1	Peak 1	5.504	4550	0.06	0.766	3.45	60163.08	
2	Peak 2	5.921	4868	0.07	0.824	0.92	581.35	0.88
3	Declatasvir	7.185	7149527	99.87		1.23	16632.23	3.46

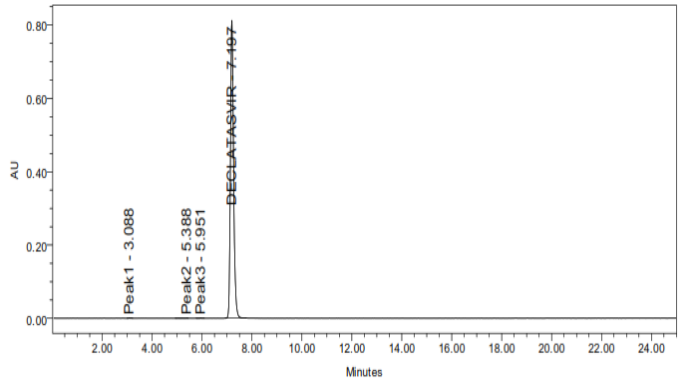


Fig 4.9: Typical specificity chromatogram of Declatasvir sample (Declahep®-60 mg tablets)

Table 4.8: Specificity studies (Declatasvir sample)

S.No.	Peak name	RT	Area	% Area	RT Ratio	USP Tailing	USP Plate count	USP Resolution
1	Peak 1	3.088	4065	0.06	0.429	1.23	12191.27	
2	Peak 2	5.388	7374	0.10	0.749	0.57	810.11	7.17
3	Peak 3	5.951	3999	0.06	0.827	0.86	160.91	1.44
4	Declatasvir	7.197	7036920	99.78		1.23	16304.73	5.13

Precision

The precision of the method was ascertained separately from the peak area obtained by actual determination of 6 replicas of a fixed amount of drug and formulation. The HPLC systems was set up the described Chromatographic conditions, mentioned as above and follow the system to equilibrate, and then injected the 250 µg/ml concentration of Declatasvir standard 6 times and recorded the response (peak area). The proposed method was extended to the pharmaceutical dosage forms by injecting the 250 µg/ml of Declatasvir sample with the formulated sample from (Declahep®-60mg, Hetero Health Cae, Tablets) contains Declatasvir of same concentration 6 times and recorded the response (peak area). The percent relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated.

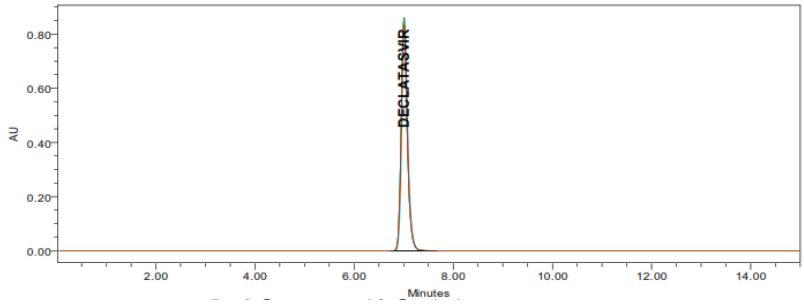
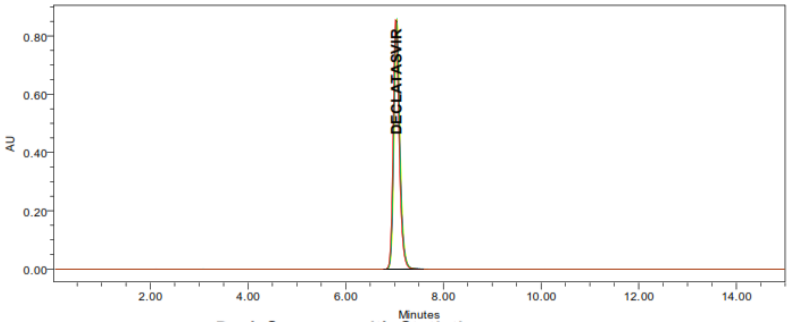


Fig 4.10: Chromatogram to illustrate Precision of Standard Declatasvir drug

Table 4.9: Precision of Declatasvir Standard drug with statistics

Injection No.	Name of the drug & conc. (250 µg/ml)	Retention time in min.	Peak Area
1	Declatasvir injection-1	7.008	7239863
2	Declatasvir injection-2	7.002	7365271
3	Declatasvir injection-3	7.001	7313593
4	Declatasvir injection-4	7.011	7217132
5	Declatasvir injection-5	7.013	7396742
6	Declatasvir injection-6	7.022	7225713
Mean			7293052.2
Std. Deviation			76833.0
% RSD			1.1



Precision

Daclahep 250 mcg/ml

Fig 4.11: Chromatogram to illustrate Precision of Declatasvir Sample solution

Table 4.10: Precision study of Sample Solution (Declahep® 60 mg, Tablets) with statistics

Injection No.	Name of the drug & conc. (250 µg/ml)	Retention time in min.	Peak Area
1	Declahep® injection-1	7.023	7338308
2	Declahep® injection-2	7.037	7325813
3	Declahep® injection-3	7.041	7326778
4	Declahep® injection-4	7.053	7371503
5	Declahep® injection-5	7.040	7305680
6	Declahep® injection-6	7.047	7362341
Mean		7.023	7338404.0
Std. Deviation			24629.7
% RSD			0.3

Observation: %RSD is within limits. Hence the method is precise.

Table 4.16: Standard calibration values of Declatasvir

Concentration of drug (µg/ml)	Peak Area
100	2951349
150	4256089
200	5667685
250	7170168
300	8698745

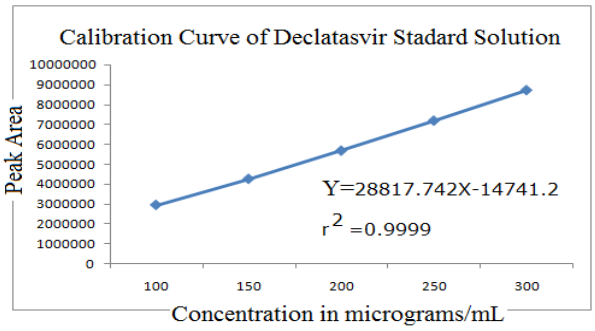


Figure 4.22: Standard Calibration Curve of Declatasvir

Table 4.36: Optical & Regression Characteristics of HPLC method

Parameter	Results of HPLC Method
Detection wavelength (nm)	284
Linearity range (µg/ml)	100-300
Regression Equation (y=mx + c)	Y=28817.742X-14741.2
Slope (m)	28817.742
Intercept (c)	-147412
Correlation coefficient	0.9999
Relative Standard deviation*	1.1

% error in bulk samples	0.234

* Average of six determinations

Table 4.37: Performance & Detection Characteristics of HPLC method

Parameter	Results of the proposed HPLC method	
	Declatasvir Standard solution	Declatasvir Sample (Declahep®-60 mg tablets) Solution
Retention time (min)	7.185	7.197
Theoretical plates (n)	16633.23	16304.73
Plates per meter (N)	66532.8	65218.92
HETP	1.5030×10^{-5}	1.5333×10^{-5}
Peak asymmetry (T)	1.23	1.23
Linearity range (µg/ml)	100-300	
Limit of Detection (µg/ml)	0.05	
Limit of Quantification (µg/ml)	0.15	

CONCLUSION

A sensitive, accurate and precise RP - HPLC method was developed for the estimation of Declatasvir in bulk drug and in dosage form. Reverse phase HPLC is one of the most sophisticated analytical method in recent years.

From the typical chromatogram of Declatasvir as shown in fig, it was it found that the retention time was 7.185 min. The contents of the mobile phase were Buffer: Acetonitrile 15: 85 (v/v). Solvent-A (Buffer) is 3.48gms of Di Potassium hydrogen orthophosphate (0.03M) in 1000 ml of water and by adjusting the pH to 2.5 with dilute orthophosphoric acid and Solvent-B is Acetonitrile in an isocratic mode of separation was used to resolute the Declatasvir at a flow rate of 1.0 ml/min and eluents were monitored at 284 nm, was found to be most suitable to obtain a peak well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (r^2=0.9999) was observed between the concentration range of 100-300µg/ml. The assay of Declatasvir in bulk was found to be 99.85%. From the recovery studies it was found that about 191.10 % on average of Declatasvir was recovered which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the Tablets. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of bulk and pharmaceutical dosage form of Declatasvir within a short analysis time.

It can be seen from the results presented that the proposed procedure has good precision and accuracy. Results of the analysis of pharmaceutical formulation revealed that proposed method is suitable for the analysis with virtually no interference of the usual additives present in the pharmaceutical formulations.

The above proposed method obviates the need for any preliminary treatment and is the method that could be of use for process development as well as quality assurance of Declatasvir in bulk drugs.

The primary purpose of this research project was to develop and to validate a simple, precise and accurate HPLC method for determination of Declatasvir in the bulk and in finished product. Clearly, it is highly important to accurately measure its concentration alone or in combination with other compounds. A high speed method was sought to measure the concentration of this compound within a short span of time. This is beneficial in any pharmaceutical analysis/clinical environment where the concentration of Declatasvir is needed to understand any patient issues along with the pharmaceutical industry to prepare the multiple steps that may be needed to prepare the raw material for production. The high speed method will eliminate/reduce any waste or costs that are required with the preparation of the raw material

REFERENCES

1. Hanaa S., Gamal H. and Othman A., 2016, “Stability indicating HPLC method development and validation for determination of Declatasvir in pure and tablet dosage forms”, *Indoamerican journal of pharmaceutical sciences*, 3(12), PP 1565-1572.
2. Chakravarthy V. and Sailaja B.B.V., 2016, “method development and validation of assay and dissolution methods for the estimation of Declatasvir in tablet dosage forms by RP-HPLC”, *Europian journal of pharmaceutical and medical research*, 3(7), PP 356-364.
3. Sreekanth N. and Jane T., 2016. “Quantitative estimation of Declatasvir in drug substances and formulated drug product by UPLC”, *Scholars research library*, 8(13), PP 280-284.
4. Kader, Mondal, Rahman, Parvin, Rafiquzzaman and kumar, 2017, " development and validation of a new RP-HPLC method for the determination of Declatasvir dihydrochloride in bulk and pharmaceutical dosage form", *International journal of pharmacy*, 7(4), PP 7-13.
5. Sreenivasa C., Sridhar C. and Chandra S., 2016, “Analytical methods for the recently approved FDA new molecular entities”, *Journal of comprehensive pharmacy*, 3(3), PP 70-82.

6. Jyothi B. and Padmaja G., 2016, "UV……". *International research journal of pharmacy*, **7**(9), PP 1-3..
7. Chakravarthy V., Sailaja B.B.V. and Kumar A 2016, "Method development and validation of UV-viscible spectroscopic method for the estimation of hepatitis – C drugs - Declatasvir and Sofosbuvir in active pharmaceutical ingredient form", *Asian journal of pharmaceutical and clinical research*, 9(3), PP 61-66.
8. Sumathi K., Thamizhvan and Vijayraj S., 2016, "Development and validation of stability indicating RP-HPLC method for the estimation of Declatasvir in bulk and formulation". *Scholars research library*, 8(15), PP 107-113..
9. ICH Q2A, "Validation of analytical methods, definitions and terminology", ICH harmonized tripartite guideline, (1999).
10. Alavian and Moayed, 2016, "Recommendations for the Clinical Management of Hepatitis C in Iran: A Consensus-Based National Guideline".
11. Institute for quality and efficiency in health care, 2015, "Declatasvir (Dalkinza) for hepatitis C: overview" PubMed Health.
12. Bell J. and Thomas W., 2010, "Drugs for hepatitis C: unlocking a new mechanism of action". MedChem. **5**(10), PP 1663–1665.
13. Sulkowski, Mark S.; et al. 2014, "Declatasvir plus Sofosbuvir for previously treated or untreated chronic HCV infection", *New England Journal of Medicine*.
14. Edwards, Danny J., Coppens, Delphi GM., Prasad, Tara L., Rook, Laurien A., Iyer and Jayasree K., 2015, "Access to hepatitis C medicines". *Bulletin of the World Health Organization*.
15. Belema, Makonen, Meanwell and Nicholas A., 2014, "Discovery of Declatasvir, a pan-genotypic hepatitis C virus NS5A replication complex inhibitor with potent clinical effect", *Journal of Medicinal Chemistry*, **57**(12), PP 5057–5071.
16. Snyder LR., Kirkland JJ. and Glajch JL., "Practical HPLC method development" 2nd edition, published by John Wiley & sons, New Jersy, 2011.
17. Yugandharudu T., Surendra M. and Viswasanthi T., 2012, "A review on analytical method development and method validation", *International journal of pharmaceutical research and analysis*, 2(1), PP 32-48.