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Method Development for Simultaneous Estimation of Antiviral Drugs Sofosbuvir and Ledipasvir

Sushant Srivastava[1], Ashwin Kumar Saxena[2], Aditya Sharma[3], Omprakash Goshain[4], Dheeraj Dubey[5], Abhishek Rajbhar[6], Abhishek Tiwari[7]

[1]School of Pharmaceutical Sciences, Shri Venkateshwara University, Gajraula, Uttar Pradesh, India

[2]School of Pharmacy, Shri Venkateshwara University, Gajraula, Uttar Pradesh, India

[3]School of Pharmaceutical Sciences, Shri Venkateshwara University, Gajraula, Uttar Pradesh, India

[4]School of Pharmaceutical Sciences, Shri Venkateshwara University, Gajraula, Uttar Pradesh, India

[5]College of Pharmacy, Shri Venkateshwara University, Gajraula, Uttar Pradesh, India

[6]School of Pharmaceutical Sciences, Shri Venkateshwara University, Gajraula, Uttar Pradesh, India

[7]School of Pharmaceutical Sciences, Shri Venkateshwara University, Gajraula, Uttar Pradesh, India

***Address for Correspondence**

Dr. Ashwin Kumar Saxena

Professor, School of Pharmacy, Shri Venkateshwara University, Gajraula, Uttar Pradesh, India

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KEYWORDS

Antiviral drugs, sofosbuvir, ledipasvir, UV spectroscopy, HPLC method

ABSTRACT:

The pharmaceutical industry is currently developing novel antiviral medications, available in either single or co-formulated tablet form. Economic and therapeutic policies are stimulating interest in the burgeoning market for antiviral medications. Consequently, there is an urgent need to validate analytical processes that monitor all parameters regarding quality, safety, and efficacy with optimal efficiency and precision while minimizing costs. High-performance liquid chromatography (HPLC) and ultraviolet (UV) spectroscopy provide an automated, rapid, and highly accurate method for detecting particular chemical constituents in a sample. This approach is used in many labs for chemical measurement and separation, including those focused on medicine, forensics, bioavailability, environmental studies, and industrial applications. Sofosbuvir, ledipasvir were antiviral medications that were quantitatively studied using HPLC methodology.

Introduction: Viruses are very small infectious agents. They're made up of a piece of genetic aterial, such as DNA or RNA, that's enclosed in a coat of protein. Viruses invade cells in your body and use components of those cells to help them multiply. This process often damages or destroys infected cells [1]. A viral disease is any illness or health condition caused by a virus. Viruses are probably the most common cause of infectious disease acquired within indoor environments and have considerable impact on human health, ranging from severe life–threatening illnesses to relatively mild and self–limiting or asymptomatic diseases. In particular, viruses causing gastrointestinal and respiratory

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diseases spread rapidly in the community and cause considerable morbidity. Increasing numbers of people who have impaired immunity, for whom the consequences of infection can be much more serious, are now cared for in 'out of hospitals settings [2]. Viruses are spread easily through closed environments such as the home, schools, workplaces, transport systems, etc. Although many of the respiratory and gastrointestinal infections caused by viruses can be asymptomatic or relatively mild and self–limiting (coughs and colds, etc.), they still represent a significant economic burden [3]. A virus is a small infectious agent that depends on host cells that they infect to reproduce. It is minimally constructed of two components, a genome consisting of either ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) and a protein coat also called as capsid which protects the genome [4]. Analytical methods development, identification, characterization of impurities and method validation play key role in the pharmaceutical's discovery, development and manufacturing. The new drugs introduced into the market are increasing every year in number. These drugs may be totally new or partial structural modification in existing molecules. Introduction of its better replacement, these new drugs may include later in official pharmacopoeias [5]. Therefore, due to this lag time, analytical procedures, and standards may not available in concerned pharmacopoeia, we cannot find these drugs and impurities even the drug substances and drug products introduced into the market increasing every year. These drug substances and drug products may be either partial structural modification of the existing drug substances or new entities [6]. The validation or verification of a method follows a standardized set of experimental tests which produce data related to validation parameter. Validation is an act of proving that any procedure, process, equipment, material, activity or system performs as expected under given set of conditions and also give the required accuracy, precision, sensitivity, ruggedness [7]. Chromatography is a non-destructive procedure for resolving a multi component mixture of trace, minor, or major constituents into its individual fractions. Chromatography is based on the principle where molecules in mixture applied onto the surface or into the solid, and stationary phase (stable phase) is separating from each other while moving with the aid of a mobile phase [8]. The factors effective on this separation process include molecular characteristics related to adsorption (liquid-solid), partition (liquid-solid), and affinity or differences among their molecular weights. The aim of this review is to provide a historical back-ground to the area, to describe the methods used to discover agents and to indicate promising antiviral drugs, both marketed and in development. Antivirals and Antiretroviral are specifically used to treat viral and emphasize contemporary and innovative methods to manufacture drugs and to combat evolution of drug-resistant viruses. It is an attempt has been made to develop an analytical method and validate the developed method as per the ICH guidelines for determination of Sofosbuvir and Ledipasvir, in pharmaceutical dosage form. The objectives of present study is to develop RP-HPLC method for Sofosbuvir and Ledipasvir (Antiviral) and validate the developed method. The study applies for development of new method for the

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estimation of its marketed preparation. The method was developed by using different types of columns, operating parameters, mobile phase composition, diluent and pH values. The analytical method developed was using column, mobile phase and buffer very common. It was done easily step by step and achieved through the current method.

Material And Methods:

Analytical study by UV spectrophotometer

Determination of absorption maxima (λ max): The absorption maxima (λ max) of drug were determined by scanning drug solution in double beam UV spectroscopy.

Preparation of phosphate buffer saline (pH 1.2): The solution of pH 1.2 0.1N Hcl dissolution medium was prepared as procedure given in Indian Pharmacopoeia 2007 as 85*X was dissolved in sufficient amount of water to produce 1000 ml and adjust the pH of final preparation and was check by digital pH meter.

Preparation of stock solution: 10 mg of mesalamine was accurately weighed and transformed to 100 ml clean and dry volumetric flask and 70 ml of solution (i.e. pH 1.2, 0.1 N Hcl and sonicate to dissolve the drug completely and make up the volume with same solvent.

Simultaneous estimation of Drug samples:

Preparation of Primary stock solution: The drug samples sofosbuvir and (10 mg) and ledipasvir (10 mg) dissolve in 100 ml of 0.1N Hcl pH 1.2 were used as standard stock solution.

Preparation of secondary standard solution: The Primary stock solution was again diluted upto 50 µg/ml and 10 µg/ml, respectively. These prepared solutions were scanned over the range of 200 nm and 400 nm in the spectrum manner the overlain spectra of the two were recorded. The overlain spectra exhibit major absorbance maxima at 274 nm and 317 nm for SOF and LED respectively. These spectra revealed that the peaks are well resolved, and satisfying the criteria for obtaining maximum precision based on absorbance.

Simultaneous estimation of sofosbuvir and ledipasvir: Simultaneous estimation of combination of drug samples sofosbuvir and ledipasvir were measured at λmax (λ1 and λ2) of both the drugs.

λ1=277 nm for sofosbuvir (SOF) and λ2 = 317 for ledipasvir (LED), the absorbance of the mixture is the sum of the individual absorbances of SOF and LED, two equations were constructed. The Simultaneous estimation method of combined drug sample was reported.

CSOF = A2ay1-A1ay2 / ax2ay1-ax1ay2

CLED = A1ax2-A2ax1 / ax2ay1-ax1ay2

Where, A1 and A2 are absorbance of mixture at λ1 and λ2, ax1 and ax2 are absorptivities of SOF at λ1 and λ2 respectively; ay1 and ay2 are absorptivities of LED at λ1 and λ2 respectively and CSOF and are LED concentrations of SOF and LED respectively.

Analytical study by RPHPLC chromatography

Optimized Chromatographic Condition:

The HPLC experimental conditions were optimized on the Cosmosil C18, (250mm x

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4.6mm, internal diameter, 5µm particle size) analytical column.

Preparation of Solutions:

A. Orthophosphoric acid in water (0.1% V/V):

Pipette out 0.5 mL of orthophosphoric acid into measuring cylinder (500 mL capacity) containing 250 mL HPLC grade water and made up the volume up to 500mL with HPLC grade water. Transferred into a reagent bottle and mixed the contents thoroughly. Stored at ambient temperature. This solution was used within 3 days from the date of preparation. B. **B. Mobile phase**:

Acetonitrile: 0.1% OPA (55:45 % V/V) In measuring cylinder 550 mL of Acetonitrile and 450 mL of 0.1% OPA was taken, then transferred into a reagent bottle and mixed the contents thoroughly. Stored at ambient temperature. This solution was used within 3 days from the date of preparation. The same was used as the diluent.

C. Auto sampler: Rinsing Solution In measuring cylinder 500 mL of Methanol and 500 mL of water was taken, then transferred into a reagent bottle and mixed the contents thoroughly. Stored at ambient temperature. This solution was used within 3 days from the date of preparation.

Sofosbuvir: Stock Solution, 4000 µg/ mL Accurately weighed 40 mg of standard Sofosbuvir was transferred to a 10 mL volumetric flask and appropriate volume of Methanol was added to make final concentration of Sofosbuvir equivalent to 4000 µg /mL. The solution was stored in refrigerator at 5±3°C and used within solution within 7 days from date of preparation.

Ledipasvir: Stock Solution, 900 µg/ mL Accurately weighed 9 mg of standard Ledipasvir was transferred to a 10 mL volumetric flask and appropriate volume of Methanol was added to make final concentration of Ledipasvir equivalent to 900 µg/ mL. The solution was stored in refrigerator at 5±3°C and used within solution within 7 days from date of preparation.

Mix Stock solution, (Sofosbuvir 40 µg/mL and Ledipasvir 9 µg/mL): 0.1 mL of Stock Solution of Sofosbuvir 4000 µg/ mL and Ledipasvir 900µg/ mL was transferred in 10.0 mL volumetric flask. The solution was made up to the mark using diluent to obtain a solution containing concentration of Sofosbuvir 40 µg/mL and Ledipasvir 9 µg/mL. The solution was stored in refrigerator at 5±3°C and used within solution within 7 days from date of preparation.

System Suitability: This was carried out to verify that the chromatographic system was suitable for intended application. Some of the parameters which can be checked using system suitability are Precision Requirement, Theoretical Plates and Tailing Factor.

Acceptance criteria: Relative standard deviation for peak area less than 2%. Theoretical plates more than 2000. Tailing Factor between 0.85 to 2.0

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Results And Discussion

Analytical study by UV spectrophotometer:

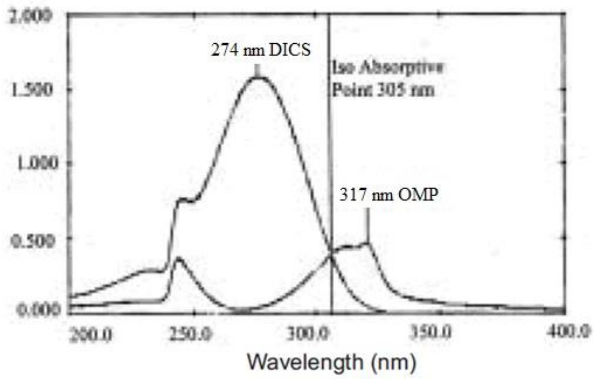

Figure 1: Overlain spectra exhibit major absorbance maxima for sofosbuvir and ledipasvir

Simultaneous estimation of combination of drug sample sofosbuvir and ledipasvir were measured at λmax (λ1 and λ2) of both the drugs.

λ1=277 nm for sofosbuvir (SOF) and λ2 = 317 for and Ledipasvir (LED), the absorbance of the mixture is the sum of the individual absorbances of SOF and LED, two equations were constructed. The Simultaneous estimation method of combined drug sample was reported.

CSOF = A2ay1-A1ay2 / ax2ay1-ax1ay2

CLED = A1ax2-A2ax1 / ax2ay1-ax1ay2

Where, A1 and A2 are absorbance of mixture at λ1 and λ2, ax1 and ax2 are absorptivities of SOF at λ1 and λ2 respectively; ay1 and ay2 are absorptivities of LED at λ1 and λ2 respectively and CSOF and are CLED concentrations of SOF and LED respectively.

Analytical study and Method Development by RPHPLC:

Selection of stationary phase: Both the drugs Sofosbuvir and Ledipasvir are polar, hence carried out using C18 column.

Selection of wavelength: Selectivity of HPLC method depends on the wavelength selected hence a selection of the wavelength was done in such a way that both the drugs gave good response, 283 nm was selected as a detection wavelength for Sofosbuvir and Ledipasvir.

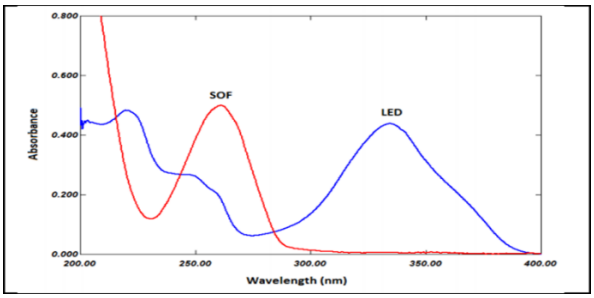

Figure 2. Wavelength Selection - Overlay UV spectra of SOF (20 µg/mL) and LED (4.5 µg/mL)

Selection of mobile phase: Based on the literature survey, a mixture of Acetonitrile and 0.1% Ortho phosphoric acid in different ratio was selected as the mobile phase and trials were carried out. From the trials carried, finally a mixture of Acetonitrile: 0.1% OPA in the ratio of 55:45 %V/V was selected and further trials were carried out to get optimized chromatograms by changing the other parameters.

Trials taken during the selection of mobile phase and other parameters.

Column: C18 COSMOSIL, (250mm x 4.6mm, 5microns)

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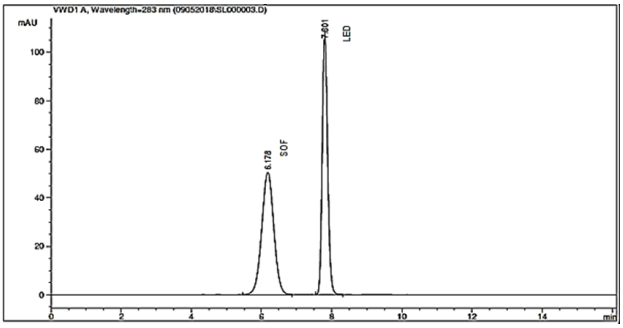
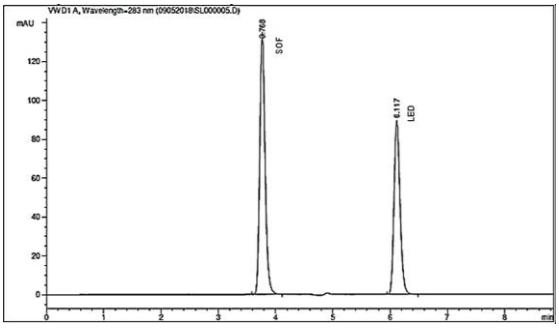
Table 1: Trials taken for the selection of mobile phase

Sr. No.	Mobile phase	Flow rate mL/min	Observation
1	MeOH: 0.1 % OPA (80:20%V/V)	0.7 mL	Poor Chromatogram was observed
2	MeOH: 0.1 % OPA (70:30%V/V)	0.7 mL	Poor Chromatogram and less resolution were observed
3	ACN: 0.1 % OPA (80:20 %V/V)	0.7 mL	Better resolution and slight tailing were observed
4	ACN: 0.1 % OPA (75:25 %V/V)	0.7 mL	Better resolution and slight tailing were observed
5	ACN: 0.1 % OPA (50:50 %V/V)	1.0 mL	Chromatogram was Good but still slight resolution modification was required for better result
6	ACN: 0.1 % OPA (50:50 %V/V)	0.7 mL	Better resolution and chromatography were observed



Trial No 1



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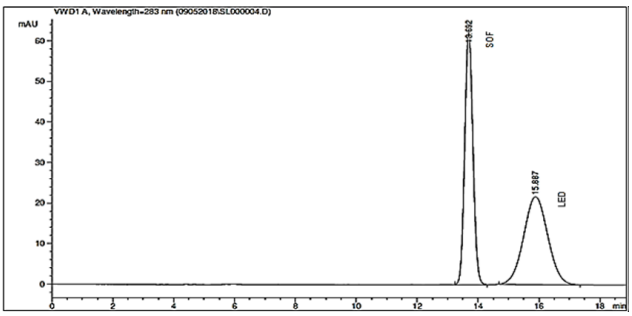

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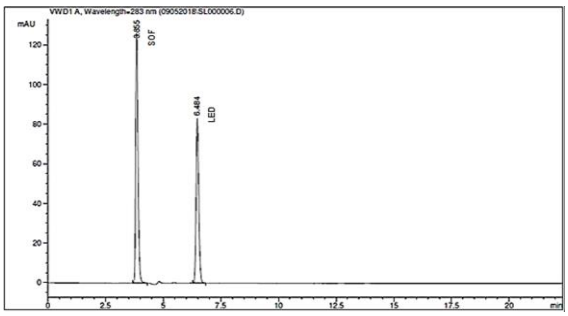

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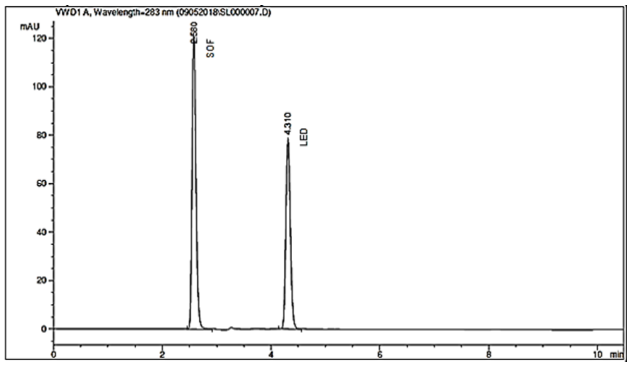

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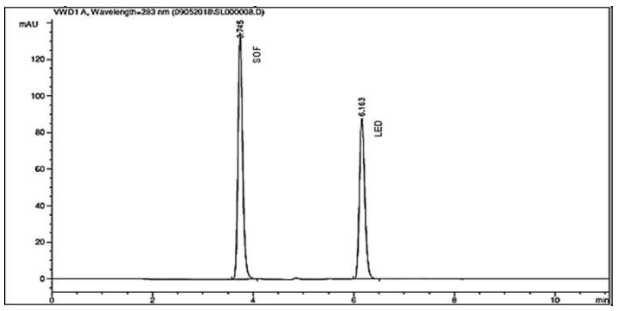

Trial No 6

Figure 2: Trial Chromatogram of Sofosbuvir and Ledipasvir

Establishment of the Retention time of individual drugs: The diluted mixed standard of the drugs was injected into the system to check for the separation. Slight changes were done in flow rate so as to achieve greater resolution and higher number of theoretical plates and finally the following conditions were established to give an optimized chromatogram.

- HPLC make: Agilent Technologies 1100 gradient system
- Particle size packing: 5µm
- Stationary phase: C_{18} COSMOSIL, (250mm x 4.6mm, 5microns)
- Detection wavelength: 283 nm
- Flow rate: 0.7 mL/min
- Mobile Phase: Acetonitrile: 0.1% OPA, in the ratio of 55:45 %V/V
- Temperature: Ambient
- Size: 20 µL

Standard drug mixture separation: The retention time, Theoretical Plates of the two drugs is shown in table 7.5 and the chromatogram is shown in Figure 7.6.

Table 7.5: Retention time of SOF and LED

Parameters	Sofosbuvir	Ledipasvir
Retention time (min)	3.669	5.926
Theoretical plates	5535	17825
Tailing factor	0.925	0.81
Resolution	4.6	11.8

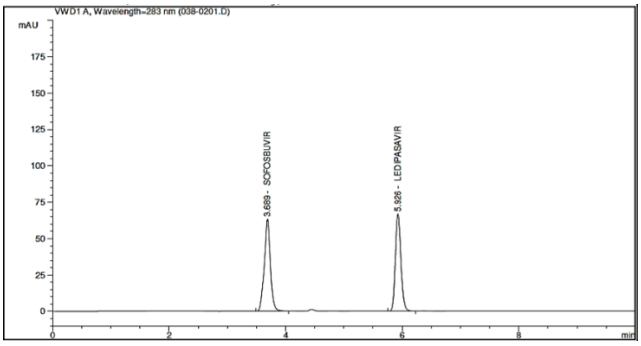

Figure 3: Chromatogram for separation of Sofosbuvir and Ledipasvir

System Suitability

The column efficiency, resolution and tailing factor were calculated for the standard solutions. The values obtained demonstrated the suitability of the system for the analysis of the selected drug combinations. System

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suitability parameters may fall within 2% relative standard deviation range during routine performance of the method. The chromatogram is in figure 7.6 and comparative results of system suitability parameter are in table 7.6-7.8.

Table 2: System Suitability Results for SOF

n =6	Peak area	Retention time	Tailing factor	Theoretical plates
1	461.385	3.783	0.93	5531
2	461.812	3.776	0.92	5513
3	462.082	3.763	0.91	5474
4	462.58	3.74	0.93	5556
5	463.86	3.739	0.91	5552
6	464.06	3.728	0.93	5520
AVG	462.63	3.75	0.92	5524.33
SD	1.1	0.02	0.01	27.37
RSD	0.24	0.54	0.97	0.5

Table 3: System Suitability Results for LED

n =6	Peak area	Retention time	Tailing factor	Theoretical plates
1	452.568	5.992	0.8	17849
2	453.035	6	0.81	17925
3	452.296	5.99	0.81	17855
4	450.56	5.98	0.81	17750
5	451.25	5.99	0.8	17827
6	451.87	5.97	0.81	17739
AVG	451.93	5.99	0.81	17824.17
SD	0.91	0.01	0.004	63.896
RSD	0.2	0.179	0.584	0.358

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Table 3: Comparative results of both the drugs

Parameters	Sofosbuvir	Ledipasvir
Retention time (min)	3.75	5.99
Theoretical plate	5524.33	17824.17
Tailing factor	0.92	0.81
Resolution	11.8	

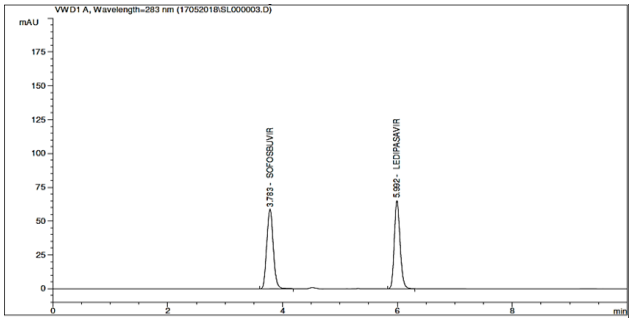

Figure 4: All system suitability variables of the developed method complied with its standard.

Summary And Conclusion: The developed UV and RP-HPLC method was used for the estimation of Sofosbuvir and Ledipasvir. The developed method was successfully validated as per ICH Q2 (R1), and from the results, it was concluded that the present method might be used for the routine estimation of the raw materials and in the pharmaceutical formulations. From the successful completion of the validation study and the results found, it was concluded that the proposed method was linear, sensitive, precise, robust and accurate for the simultaneous estimation of Sofosbuvir and Ledipasvir in raw materials and pharmaceutical formulation. From the successful completion of the validation study and the results found, it was concluded that the proposed method was linear, sensitive, precise, robust and accurate for the simultaneous estimation of Sofosbuvir and Ledipasvir in raw materials and pharmaceutical formulation.

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