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Method Development and Validation of RP-HPLC for Simultaneous Estimation of Sofosbuvir and Velpatasvir in Bulk and Film Coated Tablet Dosage Form

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ABSTRACT

A simple, rapid, accurate, and economical reverse phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for simultaneous estimation of sofosbuvir and velpatasvir in bulk and tablet dosage form. Chromatographic separation was achieved using Waters Symmetry C18 column (250 mm × 4.6 mm, 5 µm) with mobile phase consisting of acetonitrile and phosphate buffer pH 3.5 (55:45 v/v) at flow rate 1.0 mL/min and detection at 265 nm. Sofosbuvir and velpatasvir eluted at 3.2 and 5.8 minutes respectively with resolution 6.8. The method was validated according to ICH Q2(R1) guidelines demonstrating excellent linearity ($r > 0.999$), accuracy (recovery 99.95-100.00%), precision (%RSD < 1%), and sensitivity (LOQ: 0.203 and 0.058 µg/mL respectively). Forced degradation studies confirmed stability-indicating capability with complete separation of degradation products. The validated method was successfully applied for assay of commercial tablets showing 99.995% and 100.13%

label claim for sofosbuvir and velpatasvir respectively. The method is suitable for routine quality control analysis of sofosbuvir and velpatasvir combination products.

Keywords: Sofosbuvir, Velpatasvir, RP-HPLC, Method Validation, Simultaneous Estimation

1 INTRODUCTION

1.1 Overview of Analytical Method Development

Analytical chemistry plays a pivotal role in pharmaceutical research and development, particularly in the quality control and assurance of drug substances and drug products [1]. The development and validation of analytical methods are essential requirements for the pharmaceutical industry to ensure the safety, efficacy, and quality of medicinal products [2]. High-Performance Liquid Chromatography (HPLC) has emerged as one of the most versatile and widely used analytical techniques in pharmaceutical analysis due to its high sensitivity, selectivity, and reproducibility [3].

The pharmaceutical industry is governed by stringent regulatory guidelines established by various international regulatory bodies such as the United States Food and Drug Administration (FDA), European Medicines Agency (EMA), and International Council for Harmonisation (ICH) [4]. These guidelines mandate the development and validation of analytical methods that are capable of accurately determining the active pharmaceutical ingredients (APIs) in various dosage forms [5]. Method development involves the systematic optimization of various chromatographic parameters to achieve adequate separation, while validation ensures that the developed method is suitable for its intended purpose [6].

1.2 Importance of Simultaneous Estimation

The simultaneous estimation of multiple active pharmaceutical ingredients in fixed-dose combination (FDC) products has gained significant importance in recent years [10]. FDC products offer several advantages over single-component formulations, including improved patient compliance, reduced pill burden, simplified treatment regimens, and potential cost savings [11]. However, the analysis of FDC formulations presents unique challenges, as the

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method must be capable of resolving and quantifying each component with adequate precision and accuracy [12].

1.3 Hepatitis C: A Global Health Challenge

Hepatitis C virus (HCV) infection represents one of the most significant global health challenges, affecting approximately 58 million people worldwide [19]. The disease is characterized by chronic liver inflammation, which can progress to cirrhosis, hepatocellular carcinoma, and liver failure if left untreated [20]. According to the World Health Organization (WHO), approximately 1.5 million new infections occur each year, and an estimated 290,000 deaths are attributed to HCV-related complications annually [21].

1.4 Evolution of Hepatitis C Treatment

The treatment of chronic hepatitis C has undergone a remarkable transformation over the past three decades. The initial therapeutic approach relied on interferon-based regimens, which were associated with poor efficacy, long treatment durations, and significant adverse effects. The introduction of pegylated interferon-alpha in combination with ribavirin represented a major advancement, achieving sustained virological response (SVR) rates of 40-50% in patients infected with genotype 1 HCV.

2. DRUG PROFILE

2.1 Introduction

Velpatasvir is a next-generation, pangenotypic inhibitor of the hepatitis C virus (HCV) NS5A protein. It represents a significant advancement in hepatitis C therapy due to its potent activity against all major HCV genotypes and subtypes. Velpatasvir was specifically designed to overcome resistance-associated substitutions that limit the effectiveness of earlier NS5A inhibitors. The drug is available only in fixed-dose combinations with other direct-acting antivirals and is not marketed as a standalone product. It is most commonly formulated with sofosbuvir under the brand name Epclusa and with sofosbuvir plus voxilaprevir under the brand name Vosevi.

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2.2 Therapeutic Uses

Velpatasvir is indicated for the treatment of chronic hepatitis C virus infection in adults and pediatric patients (age varies by regulatory authority) across all major HCV genotypes (genotypes 1, 2, 3, 4, 5, and 6). The pangenotypic activity of velpatasvir eliminates the need for genotype testing before treatment initiation in most clinical scenarios, simplifying treatment algorithms.

Sofosbuvir/Velpatasvir (Epclusa): Used for treatment-naïve and treatment-experienced patients without cirrhosis or with compensated cirrhosis. Also used for patients with decompensated cirrhosis (Child-Pugh B or C) in combination with ribavirin. This regimen provides a simple, once-daily single-tablet option for pangenotypic treatment.

Sofosbuvir/Velpatasvir/Voxilaprevir (Vosevi): Reserved for patients who have previously failed treatment with other direct-acting antiviral regimens, including NS5A inhibitor-experienced patients. This triple-combination regimen addresses resistant viral variants that may emerge after treatment failure.

Clinical trial data have demonstrated sustained virologic response rates exceeding 95% across diverse patient populations including those with cirrhosis, prior treatment experience, and HIV coinfection. The high efficacy combined with excellent tolerability has established velpatasvir-based regimens as preferred options in clinical practice guidelines.

2.3 Dosage and Administration

Velpatasvir is administered as part of fixed-dose combination tablets and is not available as a standalone product. The standard dosing regimens are:

Sofosbuvir/Velpatasvir (400 mg/100 mg): One tablet taken orally once daily with food. Treatment duration is typically 12 weeks for most patient populations, including treatment-naïve and treatment-experienced patients with or without compensated cirrhosis. For patients with

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decompensated cirrhosis, treatment is administered for 12 weeks in combination with ribavirin or for 24 weeks without ribavirin.

Bradycardia with Amiodarone: Serious symptomatic bradycardia may occur when sofosbuvir/velpatasvir is used with amiodarone. Cardiac monitoring is recommended if coadministration cannot be avoided.

Hepatitis B Reactivation: All patients should be screened for current or prior hepatitis B virus infection before initiating treatment. HBV reactivation has been reported in HCV/HBV coinfected patients receiving direct-acting antivirals, sometimes resulting in fulminant hepatitis, hepatic failure, or death.

2.4 Drug Interactions

Velpatasvir is subject to several important drug interactions due to its involvement in drug transporter systems and metabolic pathways.

Interactions Decreasing Velpatasvir Levels:

P-glycoprotein Inducers and Moderate to Strong CYP Inducers:

- Rifampin, rifabutin, rifapentine: Contraindicated (significantly reduce velpatasvir levels)
- Carbamazepine, phenytoin, phenobarbital: Not recommended
- St. John's wort: Contraindicated
- Efavirenz: Avoid if possible; if necessary, may require increased monitoring

Interactions Increasing Velpatasvir Levels:

- Strong inhibitors of P-glycoprotein, CYP2B6, CYP2C8, or CYP3A4 may increase velpatasvir concentrations, but clinical experience suggests these interactions are not clinically significant

2.5 Storage and Stability

Fixed-dose combination tablets containing velpatasvir should be stored at room temperature between 20°C to 25°C (68°F to 77°F), with excursions permitted between 15°C to 30°C (59°F to 86°F). The medication must be stored in the original container to protect from moisture. The

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bottle contains silica gel desiccant which should remain in place and not be removed or discarded.

Tablets should be protected from light and kept away from excessive heat and humidity. The medication should not be stored in bathrooms or other areas with high moisture levels. The product shelf life when stored under recommended conditions is typically 24 to 36 months from the date of manufacture. The expiration date is printed on the product packaging, and expired medication should not be used. Once opened, the container should be tightly closed after each use to maintain stability and prevent moisture exposure.

3 REVIEW OF LITERATURE

3.1 Introduction

The development and validation of analytical methods for pharmaceutical analysis has been a subject of extensive research, particularly for newly approved drug combinations. The simultaneous estimation of multiple active pharmaceutical ingredients in fixed-dose combinations presents unique analytical challenges that have been addressed through various chromatographic techniques.

3.2 Analytical Methods for Sofosbuvir

Sofosbuvir, being approved earlier than velpatasvir, has been the subject of numerous analytical method development studies. Researchers have explored various analytical techniques to quantify sofosbuvir in bulk drugs, pharmaceutical formulations, and biological matrices.

3.3 High-Performance Liquid Chromatography Methods

Several HPLC methods have been developed for the determination of sofosbuvir in tablet dosage forms. Kumar et al. developed and validated a simple RP-HPLC method for the estimation of sofosbuvir in bulk and tablet formulation [31]. The method employed a C18 column with a mobile phase consisting of acetonitrile and phosphate buffer in the ratio of 60:40 v/v. Detection was performed at 261 nm using a UV detector. The method demonstrated good linearity in the concentration range of 10-60 µg/mL with a correlation coefficient of 0.9998. The retention time

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of sofosbuvir was approximately 3.2 minutes, and the method was validated according to ICH guidelines for parameters including linearity, accuracy, precision, specificity, and robustness.

3.4 Ultra-Performance Liquid Chromatography Methods

The application of UPLC technology for sofosbuvir analysis has been explored by several research groups.Rezk et al. developed a UPLC method coupled with tandem mass spectrometry for the determination of sofosbuvir and its major metabolite GS-331007 in human plasma [35]. The method utilized a BEH C18 column with gradient elution and positive electrospray ionization. The chromatographic run time was reduced to less than 3 minutes, demonstrating the speed advantage of UPLC over conventional HPLC. The method was validated according to FDA bioanalytical method validation guidelines and successfully applied to pharmacokinetic studies.

3.5 Spectrophotometric Methods

In addition to chromatographic techniques, several spectrophotometric methods have been developed for sofosbuvir quantification. Srinivasarao et al. developed UV spectrophotometric methods for the estimation of sofosbuvir in bulk and pharmaceutical formulations [37]. Three different methods were established based on measurement at wavelength maximum (261 nm), area under the curve between 255-267 nm, and first-order derivative spectroscopy. The methods showed linearity in the concentration range of 5-30 μg/mL and were validated for parameters including accuracy, precision, and specificity.

3.6 HPLC Methods for Velpatasvir

Nagajyothi et al. developed a novel RP-HPLC method for the determination of velpatasvir in bulk and tablet dosage form [39]. The method utilized a C18 column with a mobile phase consisting of acetonitrile and phosphate buffer (pH 6.8) in the ratio of 60:40 v/v. Detection was performed at 270 nm, and the retention time was approximately 3.8 minutes. The method demonstrated linearity in the range of 25-150 μg/mL with a correlation coefficient of 0.9996.

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Validation parameters including precision (% RSD less than 2%), accuracy (recovery 98-102%), and specificity were found to be within acceptable limits.

3.7 RP-HPLC Methods for Simultaneous Estimation

Chunduru et al. reported a validated RP-HPLC method for the simultaneous estimation of sofosbuvir and velpatasvir in tablet dosage form [42]. The chromatographic separation was achieved on a C18 column using a mobile phase consisting of acetonitrile and phosphate buffer (pH 3.0) in the ratio of 55:45 v/v. The flow rate was maintained at 1.0 mL/min, and detection was performed at 265 nm. Sofosbuvir eluted at 2.8 minutes and velpatasvir at 4.3 minutes with good resolution between the peaks. The method was linear in the concentration range of 10-60 μg/mL for both drugs with correlation coefficients greater than 0.999. Recovery studies yielded values between 99.2% and 100.8%, confirming the accuracy of the method.

4 AIM AND OBJECTIVE

4.1 Introduction

The development and validation of analytical methods for pharmaceutical products is a fundamental requirement in the pharmaceutical industry to ensure drug quality, safety, and efficacy. Fixed-dose combination products containing multiple active pharmaceutical ingredients require robust analytical methods capable of simultaneously determining each component with adequate precision, accuracy, and specificity. The combination of sofosbuvir and velpatasvir represents an important therapeutic advancement in hepatitis C treatment, providing a single-tablet, once-daily, pangenotypic regimen that has transformed patient care.

4.2 Aim of the Present Work

The primary aim of the present research work is to develop and validate a simple, rapid, accurate, precise, and economical Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of sofosbuvir and velpatasvir in bulk drug and film-coated tablet dosage form. The method should be suitable for routine quality control applications in pharmaceutical industries and testing laboratories, meeting all regulatory requirements for analytical method validation.

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4.3 Application to Pharmaceutical Formulations

To apply the validated method for:

- Quantitative determination of sofosbuvir and velpatasvir content in commercially available film-coated tablet formulations
- Assay of multiple batches or brands of tablets to demonstrate the method's practical applicability
- Comparison of label claims with actual drug content determined by the developed method
- Statistical evaluation of results to assess method performance in real-world applications

4.4 Forced Degradation Studies

To establish the stability-indicating capability of the developed method through:

- Subjecting sofosbuvir and velpatasvir standards and tablet formulations to various stress conditions as recommended by ICH guidelines, including:
  - Acid hydrolysis using hydrochloric acid at specified concentration and temperature
  - Base hydrolysis using sodium hydroxide at specified concentration and temperature
  - Oxidative degradation using hydrogen peroxide
  - Thermal degradation by exposure to elevated temperature
  - Photolytic degradation by exposure to UV and visible light
- Quantification of the extent of degradation under each stress condition
- Demonstration that the method can separate and resolve degradation products from the parent drug peaks
- Assessment of peak purity using photodiode array detection or other appropriate techniques to confirm that drug peaks are free from co-eluting degradation products

5 PLAN OF WORK

5.1 Overview

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The present research work has been planned systematically to achieve the stated objectives in a logical and sequential manner. The plan encompasses all stages from initial literature review through method development, optimization, validation, and application to pharmaceutical formulations. The work has been organized into distinct phases to ensure comprehensive coverage of all aspects of analytical method development and validation.

5.2 Expected Timeline

The entire research work is planned to be completed in approximately 24 weeks (6 months), with appropriate time allocated for each phase. This timeline allows for systematic execution of all experimental work, adequate replication of studies, troubleshooting if required, and comprehensive documentation of results. The plan remains flexible to accommodate any unforeseen challenges or additional experiments that may be identified during the course of the work.

6 RESULTS

6.1 Chromatographic Conditions

After systematic optimization, the following chromatographic conditions were finalized:

Parameter	Specification
Column	Waters Symmetry C18, 250 mm × 4.6 mm, 5 µm
Mobile Phase	Acetonitrile : Phosphate Buffer (pH 3.5) = 55:45 (v/v)
Flow Rate	1.0 mL/min
Column Temperature	30°C
Detection Wavelength	265 nm
Injection Volume	20 µL
Run Time	10 minutes
Diluent	Mobile Phase

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Buffer Preparation: Phosphate buffer (pH 3.5) was prepared by dissolving 1.36 g of potassium dihydrogen phosphate in 1000 mL of HPLC grade water and adjusting the pH to 3.5 with orthophosphoric acid. The buffer was filtered through 0.45 µm membrane filter and degassed by sonication for 15 minutes.

Mobile Phase Preparation: The mobile phase was prepared by mixing acetonitrile and phosphate buffer (pH 3.5) in the ratio of 55:45 (v/v). The mixture was filtered through 0.45 µm membrane filter and degassed by sonication for 15 minutes before use.

6.2 Method Optimization

6.2.1 Effect of Mobile Phase Composition

The ratio of acetonitrile to buffer was varied from 50:50 to 65:35 (v/v) while keeping other parameters constant.

Table 6.1: Effect of Mobile Phase Composition on Chromatographic Parameters

ACN:Buffer Ratio	SOF Rt (min)	VEL Rt (min)	Resolution	SOF Tailing	VEL Tailing
50:50	4.2	8.5	8.2	1.18	1.35
52:48	3.8	7.2	7.5	1.15	1.28
55:45	3.2	5.8	6.8	1.12	1.18
58:42	2.8	4.5	5.2	1.10	1.15
60:40	2.4	3.8	4.5	1.08	1.12
65:35	1.9	2.8	3.2	1.06	1.10

Observation: As the proportion of acetonitrile increased, retention times decreased but resolution also decreased. The ratio of 55:45 (v/v) provided optimal balance between resolution, retention time, and peak symmetry.

6.3 Effect of Buffer pH

The pH of the phosphate buffer was varied from 2.5 to 5.0 while keeping the mobile phase ratio constant at 55:45 (v/v).

Table 6.2: Effect of pH on Chromatographic Parameters

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pH	SOF Rt (min)	VEL Rt (min)	Resolution	SOF Tailing	VEL Tailing
2.5	3.0	5.2	5.8	1.25	1.42
3.0	3.1	5.6	6.2	1.18	1.28
3.5	3.2	5.8	6.8	1.12	1.18
4.0	3.3	6.2	6.5	1.15	1.22
4.5	3.4	6.8	6.2	1.18	1.35
5.0	3.5	7.2	5.8	1.22	1.48

Observation: pH 3.5 provided the best peak symmetry and resolution between the two analytes.

Table 6.3 Chromatographic Conditions

After systematic optimization, the following chromatographic conditions were finalized:

Parameter	Specification
Column	Waters Symmetry C18, 250 mm × 4.6 mm, 5 µm
Mobile Phase	Acetonitrile : Phosphate Buffer (pH 3.5) = 55:45 (v/v)
Flow Rate	1.0 mL/min
Column Temperature	30°C
Detection Wavelength	265 nm
Injection Volume	20 µL
Run Time	10 minutes
Diluent	Mobile Phase

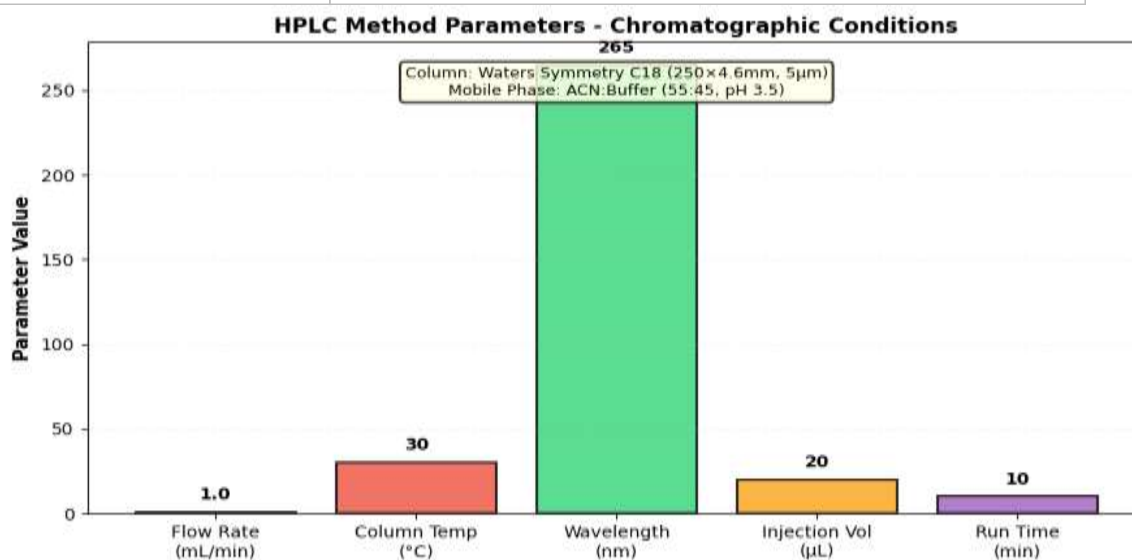

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Figure 1: Chromatographic Conditions

Buffer Preparation: Phosphate buffer (pH 3.5) was prepared by dissolving 1.36 g of potassium dihydrogen phosphate in 1000 mL of HPLC grade water and adjusting the pH to 3.5 with orthophosphoric acid. The buffer was filtered through 0.45 µm membrane filter and degassed by sonication for 15 minutes.

Mobile Phase Preparation: The mobile phase was prepared by mixing acetonitrile and phosphate buffer (pH 3.5) in the ratio of 55:45 (v/v). The mixture was filtered through 0.45 µm membrane filter and degassed by sonication for 15 minutes before use.

6.4 Preliminary Studies

6.4.1 Determination of Wavelength Maxima

Standard solutions of sofosbuvir (10 µg/mL) and velpatasvir (10 µg/mL) were prepared separately in methanol and scanned in the UV-Visible spectrophotometer over the range of 200-400 nm.

Results:

- Sofosbuvir showed λmax at 261 nm
- Velpatasvir showed λmax at 270 nm
- Overlapping spectra showed an isoabsorptive point near 265 nm

Based on these observations, 265 nm was selected as the detection wavelength for simultaneous determination of both drugs, providing adequate sensitivity for both analytes.

6.4.2 Selection of Diluent and Mobile Phase

Various solvent systems were evaluated for their ability to dissolve both drugs and provide good peak shapes:

Diluents Tested:

- Methanol: Both drugs showed good solubility
- Acetonitrile: Both drugs showed good solubility
- Water: Poor solubility, especially for velpatasvir
- Mobile phase: Both drugs showed excellent solubility and stability

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Result: Mobile phase was selected as the diluent for preparation of standard and sample solutions.

Table 6.4 Mobile Phase Optimization: Various combinations of organic modifiers and buffer systems were evaluated:

Trial	Composition	Observation
1	Methanol : Water (60:40)	Poor resolution, broad peaks
2	Acetonitrile : Water (50:50)	Good peak shape but long retention time
3	Acetonitrile : Phosphate buffer pH 4.5 (60:40)	Good separation but peak tailing for velpatasvir
4	Acetonitrile : Phosphate buffer pH 3.5 (55:45)	Excellent separation, symmetric peaks, optimum retention
5	Acetonitrile : Phosphate buffer pH 3.0 (50:50)	Good separation but slightly longer run time

Result: Acetonitrile and phosphate buffer (pH 3.5) in the ratio of 55:45 (v/v) was selected as the optimum mobile phase composition.

6.5 Preparation of Standard and Sample Solutions

6.5.1 Preparation of Standard Stock Solutions

Sofosbuvir Standard Stock Solution (1000 µg/mL): Accurately weighed 25 mg of sofosbuvir working standard and transferred into a 25 mL volumetric flask. Added about 15 mL of diluent, sonicated for 10 minutes to dissolve, cooled to room temperature, and made up to volume with diluent. Mixed well.

Velpatasvir Standard Stock Solution (250 µg/mL): Accurately weighed 6.25 mg of velpatasvir working standard and transferred into a 25 mL volumetric flask. Added about 15 mL of diluent, sonicated for 10 minutes to dissolve, cooled to room temperature, and made up to volume with diluent. Mixed well.

6.5.2 Preparation of Standard Working Solution

From the standard stock solutions, a working standard solution was prepared containing sofosbuvir 400 µg/mL and velpatasvir 100 µg/mL by appropriate dilution with mobile phase.

Procedure: Pipetted 10 mL of sofosbuvir stock solution and 10 mL of velpatasvir stock solution into a 25 mL volumetric flask and diluted to volume with mobile phase.

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6.5.3 Preparation of Sample Solution

Weight of 20 tablets: 10.254 g Average weight per tablet: 512.7 mg

Twenty tablets were weighed and finely powdered. Tablet powder equivalent to one tablet (containing sofosbuvir 400 mg and velpatasvir 100 mg) was accurately weighed and transferred into a 100 mL volumetric flask. Added about 70 mL of diluent and sonicated for 30 minutes with intermittent shaking to ensure complete extraction of drugs. The flask was cooled to room temperature, diluted to volume with diluent, and mixed well. The solution was filtered through 0.45 µm syringe filter, discarding the first few mL of filtrate. From this solution, 10 mL was pipetted into a 25 mL volumetric flask and diluted to volume with mobile phase to obtain a sample solution containing sofosbuvir 400 µg/mL and velpatasvir 100 µg/mL.

6.6 Method Optimization

6.6.1 Effect of Mobile Phase Composition

The ratio of acetonitrile to buffer was varied from 50:50 to 65:35 (v/v) while keeping other parameters constant.

Table 6.5: Effect of Mobile Phase Composition on Chromatographic Parameters

ACN:Buffer Ratio	SOF Rt (min)	VEL Rt (min)	Resolution	SOF Tailing	VEL Tailing
50:50	4.2	8.5	8.2	1.18	1.35
52:48	3.8	7.2	7.5	1.15	1.28
55:45	3.2	5.8	6.8	1.12	1.18
58:42	2.8	4.5	5.2	1.10	1.15
60:40	2.4	3.8	4.5	1.08	1.12
65:35	1.9	2.8	3.2	1.06	1.10

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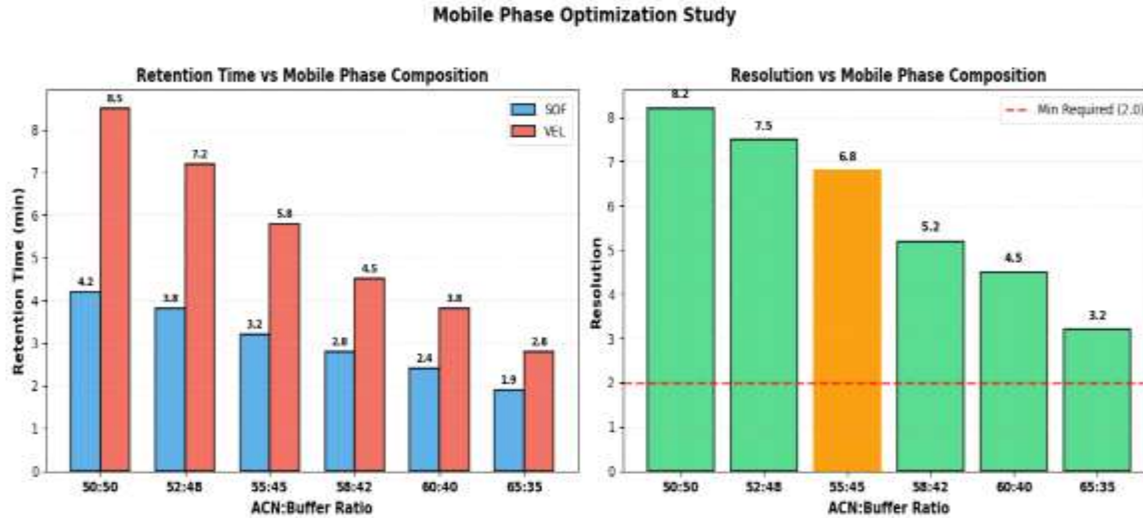

Figure 2: Effect of Mobile Phase Composition on Chromatographic Parameters

Observation: As the proportion of acetonitrile increased, retention times decreased but resolution also decreased. The ratio of 55:45 (v/v) provided optimal balance between resolution, retention time, and peak symmetry.

6.6.2 Effect of Buffer pH

The pH of the phosphate buffer was varied from 2.5 to 5.0 while keeping the mobile phase ratio constant at 55:45 (v/v).

Table 6.6: Effect of pH on Chromatographic Parameters

pH	SOF Rt (min)	VEL Rt (min)	Resolution	SOF Tailing	VEL Tailing
2.5	3.0	5.2	5.8	1.25	1.42
3.0	3.1	5.6	6.2	1.18	1.28
3.5	3.2	5.8	6.8	1.12	1.18
4.0	3.3	6.2	6.5	1.15	1.22
4.5	3.4	6.8	6.2	1.18	1.35
5.0	3.5	7.2	5.8	1.22	1.48

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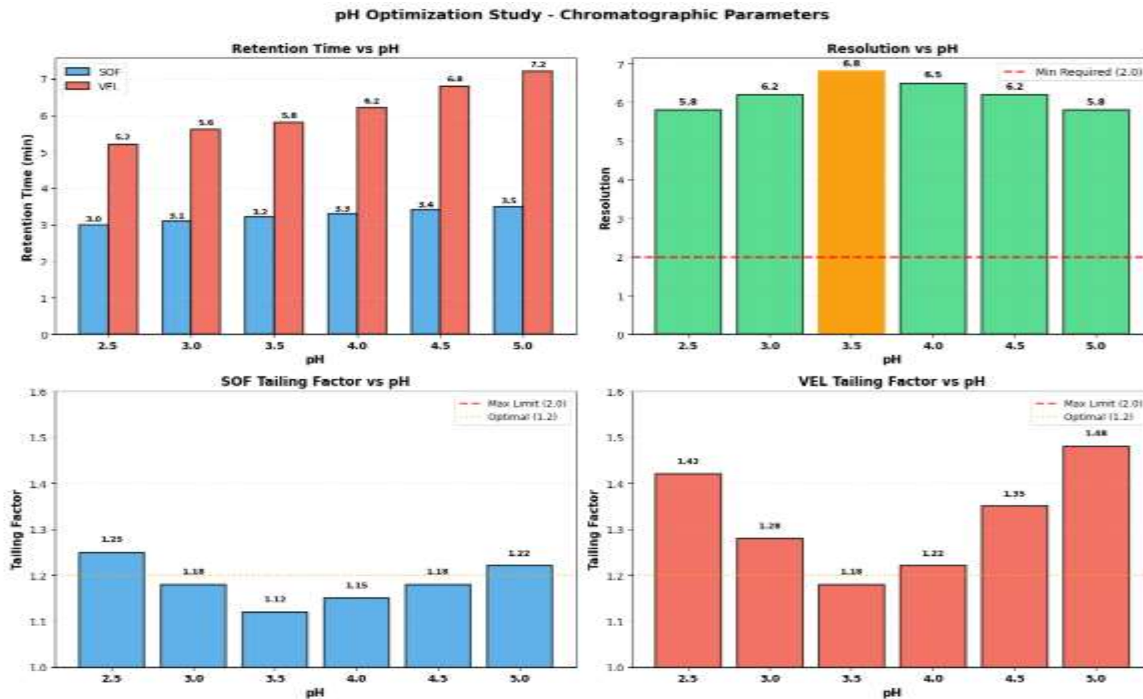

Figure 3: Effect of pH on Chromatographic Parameters

Observation: pH 3.5 provided the best peak symmetry and resolution between the two analytes.

6.6.3 Effect of Flow Rate

The flow rate was varied from 0.8 to 1.4 mL/min while keeping other parameters constant.

Table 6.7: Effect of Flow Rate on Chromatographic Parameters

Flow Rate (mL/min)	SOF Rt (min)	VEL Rt (min)	Resolution	Backpressure (psi)
0.8	4.0	7.2	7.5	1850
0.9	3.6	6.4	7.0	2020
1.0	3.2	5.8	6.8	2180
1.1	2.9	5.3	6.5	2350
1.2	2.7	4.9	6.2	2520
1.4	2.3	4.2	5.5	2850

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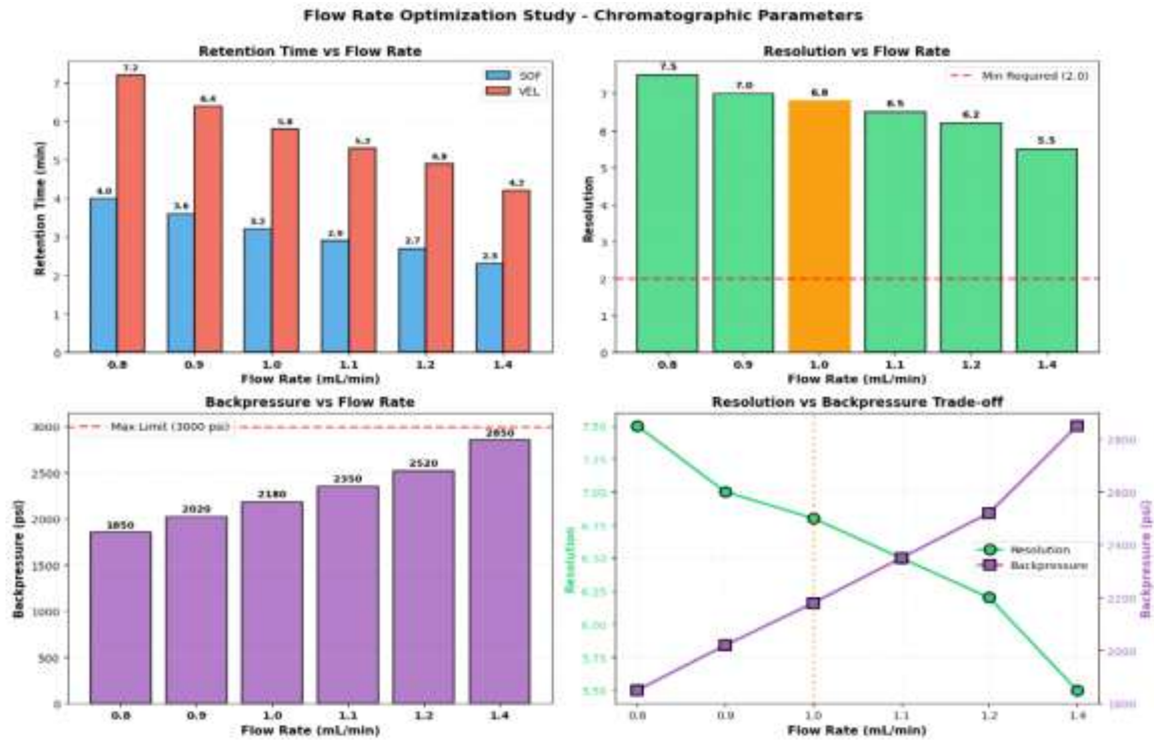

Figure 4: Effect of Flow Rate on Chromatographic Parameters

Observation: A flow rate of 1.0 mL/min provided optimal resolution with acceptable analysis time and backpressure.

6.6.4 Precision

6.6.4.1 System Precision

Six replicate injections of standard working solution were analyzed.

Table 6.8: System Precision Data

Injection No.	Sofosbuvir Peak Area	Velpatasvir Peak Area
1	2,485,320	3,702,450
2	2,491,850	3,715,820
3	2,478,920	3,695,350
4	2,489,560	3,708,920
5	2,482,350	3,698,580
6	2,487,940	3,705,280
Mean	**2,485,990**	**3,704,400**
SD	**4,521**	**7,185**

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% RSD	**0.18%**	**0.19%**

Result: % RSD values were less than 2.0%, indicating excellent system precision.

Intermediate Precision

Intermediate precision was evaluated by analyzing samples on two different days and by two different analysts.

7 CONCLUSION

The present research work successfully accomplished its objective of developing and validating a simple, rapid, accurate, precise, and economical RP-HPLC method for the simultaneous estimation of sofosbuvir and velpatasvir in bulk drug and film-coated tablet dosage form. The method was developed following a systematic approach that included careful selection and optimization of chromatographic conditions to achieve optimal separation and detection of both analytes.

The optimized method utilized a Waters Symmetry C18 column (250 mm × 4.6 mm, 5 µm) with a mobile phase consisting of acetonitrile and phosphate buffer (pH 3.5) in the ratio of 55:45 (v/v), delivered at a flow rate of 1.0 mL/min. Detection was performed at 265 nm using a photodiode array detector. Under these conditions, sofosbuvir eluted at approximately 3.2 minutes and velpatasvir at 5.8 minutes with excellent resolution (Rs = 6.8) and peak symmetry.

8 References

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