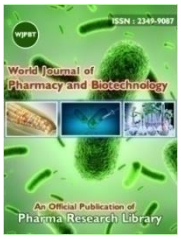

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Application of Quality by Design (QBD) to the Development and Valiadtion of Ensitrelvir Fumeric Acid in Tablet Dosage form by RP-HPLC

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A B S T R A C T

This study aimed to develop mouth-disintegrating tablets of Flurbiprofen through a straightforward and scalable direct compression method, accompanied by a reliable UV-Visible spectrophotometric assay for drug analysis. The optimized UV method, performed in 0.1N HCl, identified a maximum absorbance at 257nm, enabling accurate quantification of Flurbiprofen within tablet formulations. Tablets were formulated using common super disintegrants sodium starch glycolate, crospovidone, and croscarmellose sodium and evaluated for critical quality characteristics such as hardness, friability, weight variation, and drug content, all of which met pharmacopeial standards. In vitro dissolution studies revealed rapid drug release across all batches, with the crospovidone based formulation demonstrating superior performance by releasing nearly 99% of the drug within 30 minutes. Comparative analysis confirmed that crospovidone facilitated faster dissolution compared to other super disintegrants under the test conditions. The study successfully achieved the goal of formulating efficient, patient-friendly Flurbiprofen mouth-disintegrating tablets with minimal excipients and a simple manufacturing approach, supported by a practical and robust analytical method for quality assurance.

Keywords: Flurbiprofen, mouth-disintegrating tablets, super disintegrants, UV-Visible spectrophotometry, drug dissolution, crospovidone, direct compression

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

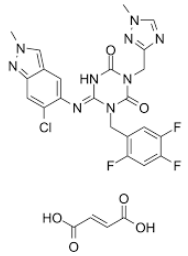

Fig.1: Ensitrelvir Fumeric Acid

Table.1: Drug profile Ensitrelvir Fumeric Acid

Molecular Formula	C22H19F2N5O4
Molecular Weight	455.42 g/mol
Generic Name	Ensitrelvir
Brand Names	Xocova
Drug category	Antiviral drug, specifically a 3CL protease inhibitor
Indications	Treatment of COVID-19
Pharmacology	Inhibits the 3CL protease

	enzyme, which is essential for viral replication
Potency	Highly potent against SARS-CoV-2
Tolerability	Generally well-tolerated, but may cause gastrointestinal and other adverse effects
Adverse Effects	Common adverse reactions include diarrhea, nausea, and others
Availability	Prescription-only medication, available in oral form
Mechanism of Action	Ensitrelvir inhibits the 3CL protease enzyme, blocking viral replication and reducing viral load

2. Materials and Methods

Table 2: List of Materials Used

S.No	Instrument	Model
1	HPLC	WATERS, software: Empower, 2695 separation module.2487 UV detector.
2	UV/VIS spectrophotometer	LABINDIA UV 3000$^+$
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

Table 3: Chemicals used

S.No	Chemical	Brand
1	Ensitrelvir	Supplied by MSN LAB
2	KH_2PO_4	FINAR chemical LTD
3	Water and Methanol for HPLC	Standard solutions Ltd
4	Acetonitrile for HPLC	Standard solutions Ltd
5	HCl, H_2O_2, NaOH	MERCK

HPLC Method Development:

Wave length selection:

UV spectrum of 10 µg / ml Ensitrelvir in diluent (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 228nm. At this wavelength both the drugs show good absorbance.

Optimized chromatographic conditions:

Instrument used: High performance liquid chromatography equipped with Auto Sampler and PDA detector

Temperature: Ambient

Column : Platsil C18-EP (4.6 x 250mm, 5□m)

Mobile phase : Acetonitrile: Triethylamine PH: 4 (60:40ml)

Flow rate : 1ml/min

Wavelength : 228 nm

Injection volume : 20 µl

Run time : 15 min.

Preparation of buffer and mobile phase:

Preparation of Triethylamine pH 4:

To prepare Trimethylamine buffer solution, by adding 3.5ml of Trimethylamine in 250ml water. Adjust this solution to pH 4 by using Acetic Acid.

Preparation of mobile phase:

Mix a mixture of above Acetonitrile 600ml (60%) and 400 ml Triethylamine PH: 4(40%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

Acetonitrile: Triethylamine PH: 4 (60:40) ratio.

System Suitability:

Tailing factor for the peaks due to Ensitrelvir in Standard solution should not be more than 2.0

Theoretical plates for the Ensitrelvir peaks in Standard solution should not be less than 2000

Calculation: (For Ensitrelvir)

$$\%\ Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

Where:

AT= average area counts of sample preparation. AS = average area counts of standard preparation.

WS= Weight of working standard taken in mg.

P= Percentage purity of working standard

LC= Label Claim mg/ml.

Results:

System Suitability Results:

1. Tailing factor Obtained from the standard injection is 1.16
2. Theoretical Plates Obtained from the standard injection is 3338

Validation parameters:

1. Assay:

Standard Solution Preparation:

Accurately weigh and transfer 50 mg of Ensitrelvir working standard into a 50 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.2ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. (120ppm)

Sample Solution Preparation:

Accurately weigh and transfer 50 mg of Ensitrelvir working standard into a 50 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.2ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure: Inject 10 µL of the standard, sample into the chromatographic system and measure the areas for the Ensitrelvir peaks and calculate the % Assay by using the formulae.

2. Linearity:

Preparation of stock solution:

Accurately weigh and transfer 50 mg of Ensitrelvir working standard into a 50 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make

volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (20ppm of Ensitrelvir):

0.2ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – II (40ppm of Ensitrelvir):

0.4ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – III (60ppm of Ensitrelvir):

0.6 ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – IV (80ppm of Ensitrelvir):

0.8ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – V (100ppm of Ensitrelvir):

1ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Procedure:

Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

3. Precision:

Preparation of stock Solution:

Accurately weigh and transfer 50 mg of Ensitrelvir working standard into a 50 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.2ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

4. Intermediate Precision/Ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day within the laboratory.

Preparation of stock solution:

Accurately weigh and transfer 50 mg of Ensitrelvir working standard into a 50 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.2ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

5. Accuracy: For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same.

Preparation of Standard stock solution:

Accurately weigh and transfer 50 mg of Ensitrelvir working standard into a 50 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.2ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration): Accurately weigh and transfer 50 mg of Ensitrelvir working standard into a 50 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.2ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

For preparation of 100% solution (With respect to target Assay concentration): Accurately weigh and transfer 50 mg of Ensitrelvir working standard into a 50 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 1.2ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

For preparation of 150% solution (With respect to target Assay concentration):

Accurately weigh and transfer 50 mg of Ensitrelvir working standard into a 50 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 1.2ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure: Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Ensitrelvir and calculate the individual recovery and mean recovery values.

6. Limit of Detection:

Preparation of Ensitrelvir solution:

Preparation of 1.83µg/ml solution:

Accurately weigh and transfer 50 mg of Ensitrelvir working standard into a 50 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette1.2 of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 1.1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 1.39 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

7. Limit of Quantification:

Preparation of Ensitrelvir solution:

Preparation of 6.20µg/ml solution: Accurately weigh and transfer 50 mg of Ensitrelvir working standard into a 50 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.2ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 4.1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Further pipette 1.26ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Robustness:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

- The flow rate was varied at 0.8 ml/min to 1.2 ml/min.
- Standard solution 120 µg/ml of Ensitrelvir prepared and analyzed using the varied flow rates along with method flow rate.
- The Organic composition in the Mobile phase was varied from 54% to 66%

Standard solution 120 µg/ml of Ensitrelvir was prepared and analyzed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

3. Results and Discussion

Optimized chromatographic conditions:

Instrument used: High performance liquid chromatography equipped with Auto Sampler and PDA detector
Temperature : Ambient
Column : PLATSIL C18-EP (4.6 x 250mm, 5µm)
Mobile phase : Acetonitrile: Triethylamine PH: 4 (60:40ml)
Flow rate : 1ml/min
Wavelength : 228 nm
Injection volume : 20 µl
Run time : 15 min.

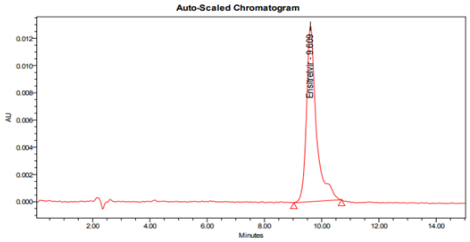

Fig.2: Chromatogram for system suitability

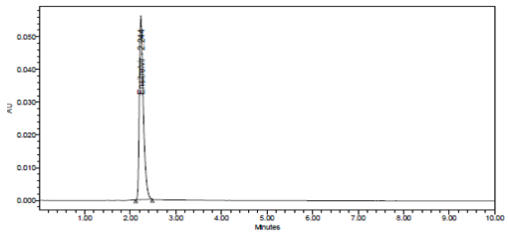

Fig.3: Chromatogram for Sample

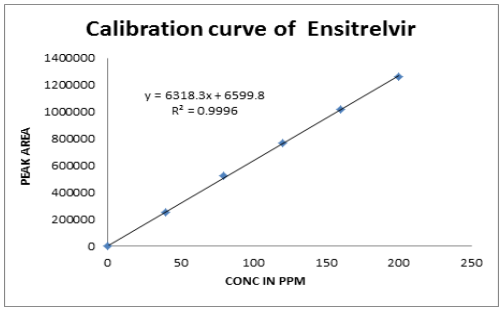

Fig.4: Calibration graph for Ensitrelvir

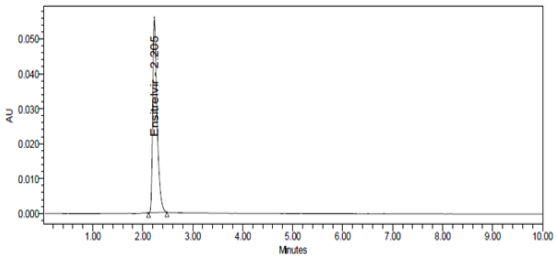

Fig.5: Chromatogram for ID Precision -6

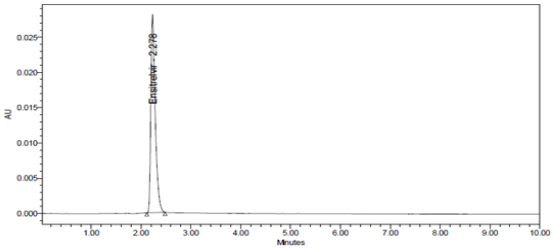

Fig.6: Chromatogram for Accuracy 50%-3

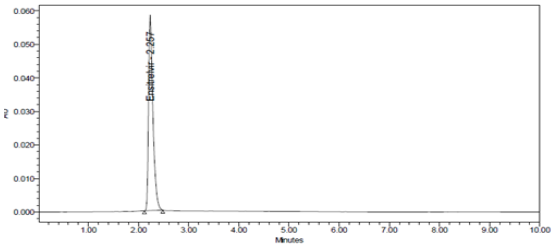

Fig.7: Chromatogram for Accuracy 100%-3

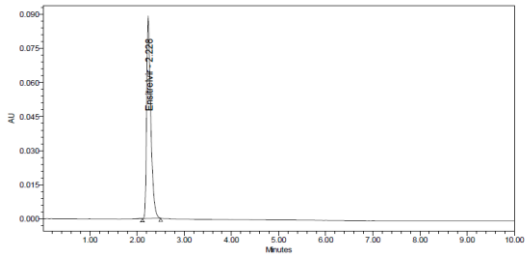

Fig.8: Chromatogram for Accuracy 150%-3

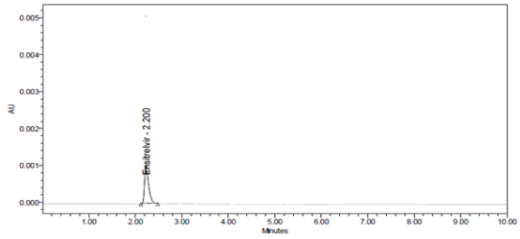

Fig.9: Chromatogram of Ensitrelvir showing LOD

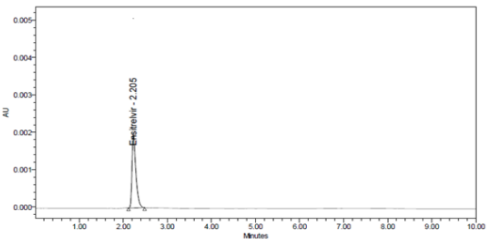

Fig.10: Chromatogram of Ensitrelvir showing LOQ

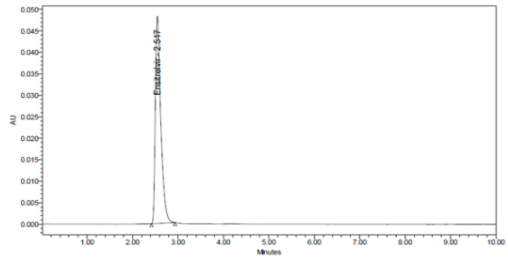

Fig.11: Chromatogram showing less flow

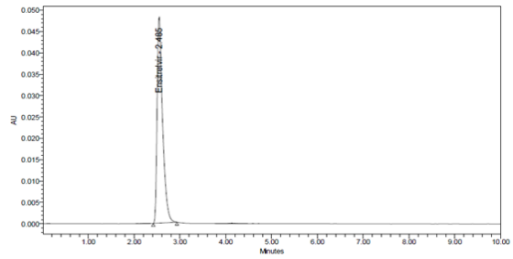

Fig.13: Chromatogram showing less organic composition

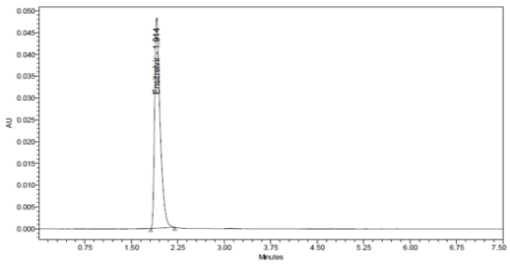

Fig.12: Chromatogram showing more flow

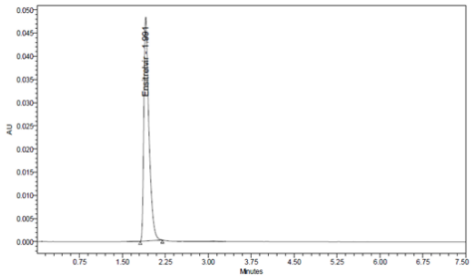

Fig.14: Chromatogram showing more organic composition

Table.4: Accuracy (recovery) data for Ensitrelvir

%Concentration (at specification Level)	Area*	Amount Added(mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	383650	25	24.91	99.6	99.4
100%	758759	50	49.26	98.5.	
150%	1154796	75	74.98	100.0	

Table.5: Results for variation in flow for Ensitrelvir

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	5473	1.3
2	1	5496	1.08
3	1.2	5473	1.2

Table.6: Results for variation in mobile phase composition for Ensitrelvir

S.No	Change in OrganicComposition in theMobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less(54ml)	5473	1.3
2	*Actual(60ml)	5496	1.08
3	10% more(66ml)	5473	1.2

Table.7: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained(µV)	S/N ratio	Conc.
Ensitrelvir	67	197	2.94	1.83µg/ml

Table.8: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio	CONC.
Ensitrelvir	67	666	9.94	6.20µg/ml

4. Conclusion

A simple, accurate, and robust RP-HPLC method was developed and validated for the estimation of Ensitrelvir in pharmaceutical dosage forms in accordance with ICH Q2(R1) guidelines. Chromatographic separation was achieved on a PLATSIL C18-EP column (4.6×250 mm, 5 µm) using a mobile phase of acetonitrile and triethylamine buffer (pH 4) in the ratio of 60:40 v/v, at a flow rate of 1.0 mL/min, with detection at 228 nm. The method demonstrated excellent system suitability parameters, with USP plate count above 2000 and tailing factor well below 2. Linearity was observed in the range of 40–200 µg/mL with a correlation coefficient of 0.9996. Precision studies

showed %RSD values of 0.4% for repeatability and 0.3% for intermediate precision, confirming high reproducibility. Accuracy results were within the acceptable recovery range of 98–102%, with a mean recovery of 99.4%. The LOD and LOQ were determined to be 1.83µg/mL and 6.20µg/mL, respectively. Robustness testing indicated that small deliberate variations in flow rate and mobile phase composition did not significantly affect the chromatographic performance. The validated RP-HPLC method meets all acceptance criteria for specificity, linearity, precision, accuracy, detection limits, and robustness as per ICH Q2(R1) guidelines. The method offers high sensitivity, reproducibility, and reliability for routine quality control of Ensitrelvir in bulk and dosage forms. Its ability to maintain performance under slight changes in chromatographic conditions ensures its suitability for application across different laboratory environments. The simplicity, cost-effectiveness, and accuracy of this method make it an ideal choice for pharmaceutical industries to ensure consistent product quality and regulatory compliance.

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