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Statistical Optimization of RP-HPLC for Sensitive Estimation of Nirmatrelvir by DOE Approach

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ABSTRACT:

Background:

The COVID-19 pandemic persists without a definitive treatment, necessitating supportive therapies. Nirmatrelvir, an antiviral drug targeting the SARS-CoV-2 major protease (Mpro), has shown promise in inhibiting viral replication. Combined with ritonavir, it forms Paxlovid, recommended for high-risk COVID-19 patients. Accurate estimation of nirmatrelvir concentration is crucial for ensuring its efficacy and safety. High-performance liquid chromatography (HPLC) is a reliable method for such analysis.

Method:

An RP-HPLC method was developed and optimized for nirmatrelvir estimation using a Design of Experiment (DOE) approach. Various experimental parameters, including injection volume, column temperature, flow rate, and mobile phase composition, were systematically adjusted. The final mobile phase consisted of methanol and formic acid (pH 4.0) in a 50:50 v/v ratio. The method's performance was evaluated through statistical analysis using ANOVA and Box-Behnken design to understand the effects and interactions of the chosen parameters.

Results:

The optimized HPLC method demonstrated excellent separation with consistent retention times and high theoretical plate counts. Statistical analysis confirmed the method's reliability and robustness, with significant effects observed for the key parameters. The Box-Behnken design provided insights into the interaction effects, enhancing the method's predictability and reproducibility.

Conclusion:

This study successfully developed a sensitive and accurate RP-HPLC method for nirmatrelvir estimation, contributing to the quality control of this antiviral drug. The application of DOE principles ensured a thorough exploration of the experimental space, resulting in a robust and reproducible method. This optimized HPLC method supports the therapeutic use of nirmatrelvir in the ongoing fight against COVID-19.

Keywords: COVID-19, Nirmatrelvir, RP-HPLC, Design of Experiment, Antiviral drug, Method optimization, Pharmaceutical analysis.

INTRODUCTION:

Although the COVID-19 pandemic is gradually abating, patients are left with no other option but to rely on supportive and general medicines in the absence of a definitive remedy. Antiviral medications may target the SARS-CoV-2 proteins major protease (Mpro) and 3C-like protease (3CLpro) (Chatterjee .S) (Weil T). Mpro is a very interesting therapeutic target because of its essential role in viral pathogenesis and protein processing. Nirmatrelvir, an antiviral medication, inhibits Mpro, preventing the replication of SARS-CoV-2 (Hashemian, S.M.R). The combination of nirmatrelvir and ritonavir, two HIV protease inhibitors, produced paxlovid, which boosts the medication's efficacy. Nirmatrelvir is an oral severe acute respiratory syndrome corona virus 2 major proteases (Mpro) inhibitor that exhibits high pan-human corona virus action in vitro (Saramago L.C).

The World Health Organization strongly advises nirmatrelvir in conjunction with low-dose Ritonavir for individuals with mild to severe COVID-19 who are most at risk of hospitalization (Carlin A.F., Hammond J., Singh.R.S.P).

Pharmaceutical research is constantly searching for new methods to enhance drug development, formulation, and analysis. A new powerful antiviral medication called nirmatrelvir has made a lot of promises about treating COVID-19. The precise and trustworthy calculation of this medication's concentration is essential to both its safety and effectiveness. High-performance liquid chromatography (HPLC) is a widely used method for evaluating drugs at various phases of manufacturing or confirming the caliber of pharmaceutical formulations and bulk pharmaceuticals. HPLC techniques frequently recommend the presence of a hydrophobic stationary phase and a polar mobile phase to generate a successful separation process.

DRUG PROFILE (https://pubchem.ncbi.nlm.nih.gov/compound/Nirmatrelvir)

Nirmatrelvir is a protease inhibitor specifically designed to target the SARS-CoV-2 virus, which causes COVID-19. Protease inhibitors work by binding to the active site of the viral protease enzyme, preventing it from cleaving viral polyproteins into functional components necessary for viral replication. By inhibiting this protease activity, nirmatrelvir effectively halts the replication

of the virus, reducing its ability to spread within the body and aiding in the recovery from COVID-19 infection.

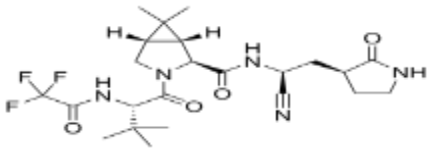

Figure No -1: Structure of Nirmatrelvir

This study presents a novel approach for estimating nirmatrelvir utilizing a Design of Experiment (DOE) methodology. DOE, a statistically based approach, allows researchers to systematically adjust experimental parameters to develop more reliable and effective analytical techniques.

Objectives:

1. Develop a reverse-phase high-performance liquid chromatography (RP-HPLC) method with enhanced sensitivity and accuracy for nirmatrelvir estimation.
2. Validate the optimized method in accordance with regulatory requirements, ensuring its repeatability and reliability for routine analysis.
3. Employ DOE to systematically evaluate and optimize key variables impacting HPLC analysis, such as injection volume, column temperature, flow rate, and mobile phase composition.

This research aims to provide an improved and more accurate HPLC method for estimating nirmatrelvir, thereby enhancing the quality control of this crucial antiviral medication. The

application of DOE principles ensures a systematic and thorough exploration of the experimental space, leading to reliable and applicable analytical techniques.

The methodology, findings, and analysis of the experimental results will be discussed in subsequent sections of this manuscript. Additionally, a comparison of the optimized RP-HPLC method with other existing approaches will be provided, along with the validation and implementation of the method for estimating nirmatrelvir in actual pharmaceutical samples. This study seeks to make a significant contribution to pharmaceutical analysis and analytical chemistry by offering a refined and validated HPLC method for nirmatrelvir estimation. The systematic application of DOE principles in method development underscores the importance of a methodical approach in achieving reliable and accurate analytical results.

Experimental:

Chemicals and reagents: Pfizer manufactured the antiviral drug nirmatrelvir, which is marketed under the brand name Paxlovid. Methanol and formic acid are utilized as the mobile phases.

Instrumentation: Waters alliance liquid chromatography (model 2695) monitored with empower 2.0 data handling system and a detector of PDA was used for this study.

Method optimization: Initially, different pH combinations and quantities of methanol, ammonium acetate buffer, and methanol, phosphate buffer were explored as the mobile phase. Ultimately, methanol and formic acid (pH 4.0) in a 50:50 v/v ratio were optimized for the mobile phase. Nirmatrelvir was detected by scanning in the 200–400 nm region in methanol diluents. from the chosen wavelength of 240 nm in the UV spectrum. This wavelength exhibits good absorption for both medications. The dose form of nimatrelvir was estimated using the established HPLC method.

Preparation of buffer and mobile phase:

Preparation of 0.1% Formic acid buffer: In a 1000 ml volumetric flask, 1 ml of orthophosphoric acid was pipetted out, dissolved, and diluted with 1000 ml of HPLC water. The volume was then adjusted to pH 4.0 using triethylamine, yielding 0.1 % formic acid and methanol (50:50) %v/v as a mobile phase.

Preparation of Nirmatrelvir sample solution:

Measure the exact weight of ten Nirmatrelvir powder tablets, crush them in a mortar and pestle, and transfer the resulting 380 mg, or 300 mg, of Nirmatrelvir. A 50 mg sample was collected from this and diluted in 10 ml of methanol. It was then ultrasonically sonicated for 10 minutes and filtered using microfiltration (Stock –A). A workable solution is obtained by extracting 1 milliliter of the stock and dissolving it in 10 milliliters of methanol. A 0.44 micron injection filter is then used to filter it. (Nirmatrelvir Sample Stock Solution)

Design of Experiment: **https://asq.org/quality-resources/design-ofexperiments**)

The design of experiment procedure was applied to the Nirmatrelvir medication sample. The fundamental facts of the impacts of factors and their interactions on certain technique responses were found using the Box-Behnken response surface design. There were seventeen runs carried out.

Statistical analysis:

❖ By using ANOVA, the statistical calculations were processed for variables screening and optimization of the method the statistical tools provide the numerical verification of variables and its effect on responses.

Method operable design region: The different amalgamation and reciprocity of input factors produces the space referred as Design space. The establishment of design space was made by utilizing the contour graphs of Sigma tech software.

Method Verification: The optimized method conditions were proposed by the software in order to reach the desired method goals. The method was verified to check the predictability of the proposed model.

RESULTS AND DISCUSSION:

Table: 1 Box - Behnken design experimental runs

S.No	Std	Run	Factor 1 A:buffer	Factor 2 B:flow rate	Factor 3 C:ph	Response 1 R1 Retention Time	Response 2 R2 Theoretical plate
1.	1	1	35.00	1.20	3.50	2.147	2030.5
2.	5	2	35.00	1.30	3.00	2.132	2012.61
3.	16	3	40.00	1.30	3.50	2.146	2118.81
4.	2	4	45.00	1.20	3.50	2.147	2030.5
5.	17	5	40.00	1.30	3.50	2.146	2118.81
6.	12	6	40.00	1.40	4.00	2.149	2180.5
7.	3	7	35.00	1.40	3.50	2.147	2170.65
8.	6	8	45.00	1.30	3.00	2.132	2012.61
9.	13	9	40.00	1.30	3.50	2.146	2118.81
10.	8	10	45.00	1.30	4.00	2.148	2181
11.	15	11	40.00	1.30	3.50	2.146	2118.81
12.	9	12	40.00	1.20	3.00	2.121	2010.15
13.	7	13	35.00	1.30	4.00	2.148	2181
14.	11	14	40.00	1.20	4.00	2.147	2117.76
15.	14	15	40.00	1.30	3.50	2.146	2118.81
16.	10	16	40.00	1.40	3.00	2.135	2014.51
17.	4	17	45.00	1.40	3.50	2.147	2170.65

Retention Time:

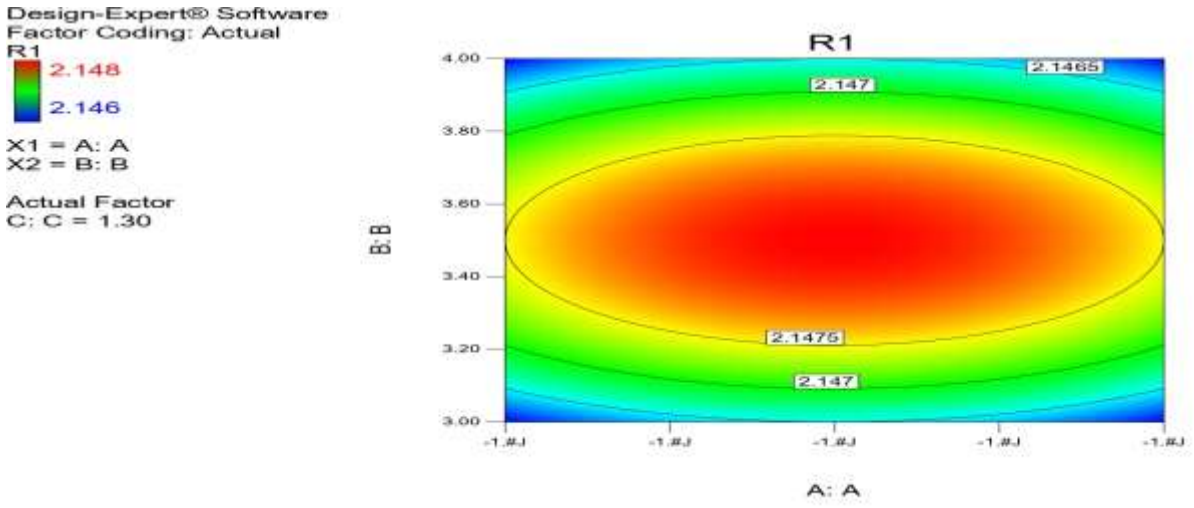

Figure No -2: Retention Time

Theoretical Plate count:

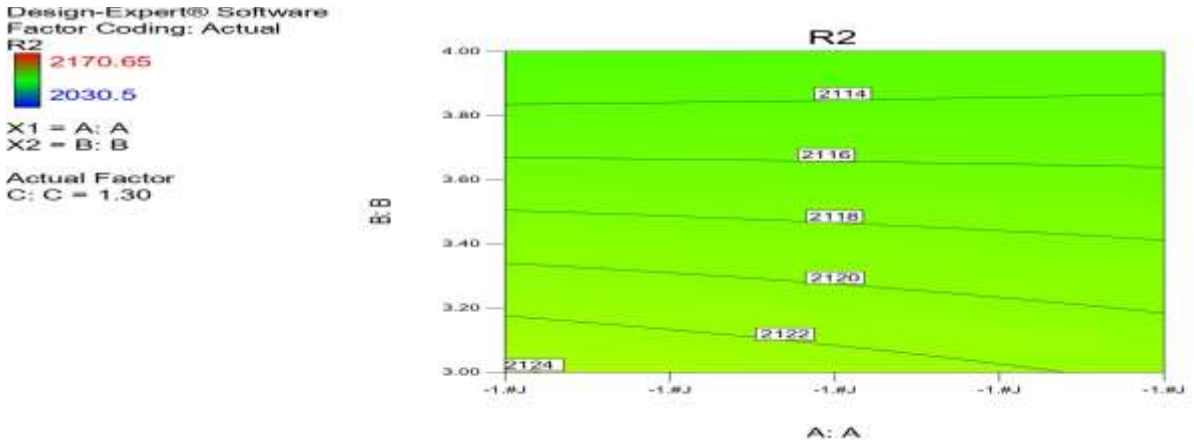

Figure No -3: Theoretical plate count

Method Optimization Peak:

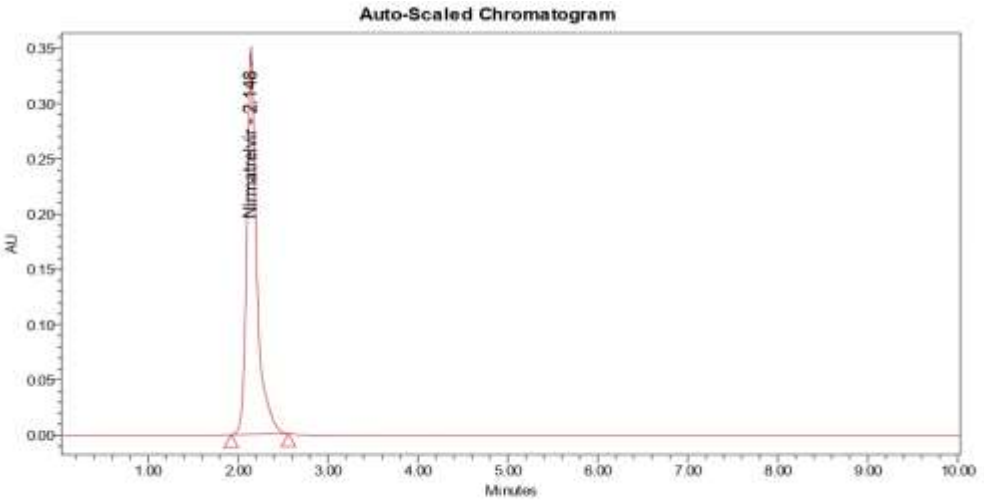

Figure No -4: Method optimization peak

CONCLUSION:

This study successfully developed and optimized an RP-HPLC method for the estimation of Nirmatrelvir, a potent antiviral drug used in the treatment of COVID-19. Using a Design of Experiment (DOE) approach, key parameters such as the injection volume, column temperature, flow rate, and mobile phase composition were systematically adjusted to achieve a method that is both sensitive and accurate.

The optimized HPLC method utilized a mobile phase consisting of methanol and formic acid (pH 4.0) in a 50:50 v/v ratio. This method demonstrated excellent separation, as indicated by consistent retention times and high theoretical plate counts across multiple runs.

Statistical analysis via ANOVA confirmed the reliability and robustness of the method, highlighting the significant effects of the chosen parameters on the method's responses. The Box-Behnken design provided a comprehensive understanding of the interaction effects between variables, further enhancing the method's predictability and reproducibility.

The application of DOE principles ensured a thorough exploration of the experimental space, resulting in an optimized method that can be reliably used for routine analysis of Nirmatrelvir in pharmaceutical formulations. This method not only improves the quality control of this crucial antiviral medication but also contributes significantly to pharmaceutical analysis and analytical chemistry.

Overall, the developed RP-HPLC method offers an efficient, accurate, and reproducible means for the estimation of Nirmatrelvir, supporting its therapeutic use in the ongoing fight against COVID-19.

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