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GREENNESS APPRAISAL AND BOX-BEHNKEN DESIGN ASSISTED STABILITY-INDICATING HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF MARAVIROC

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ABSTRACT

The goal of the current work was to use the QbD (Quality by Design) approach to develop as well as validate a stability-indicating RP-HPLC technique for the estimation of Maraviroc in pharmaceutical dosage form and in bulk, and to perform greenness assessment for the developed method. The optimization of drug-experimental conditions was accomplished through the application of Box-Behnken design (BBD). With the Inertsil ODS column (4.6×250mm, 5µm) at a detection wavelength of 223 nm, a successful separation was accomplished. The optimized chromatographic parameters determined using the Desirability functions approach included a mobile phase composed of Ortho phosphoric acid: Ethanol (70:30%v/v), pumped at a 1.1 mL/min flow rate. The finalized method underwent thorough validation as per ICH Q2(R2), followed by degradation studies conducted using ICH Q1A(R2) guidelines to confirm that the method was optimized, showing stability-indicating properties. The optimized method showed a linear response in a concentration range of 17.5-105 µg/mL. The limits for detection and quantification were found to be 0.42 and 1.4 µg/mL, respectively. All method validation parameters have been determined to be within the established limits. The degradation chromatograms of the sample exposed to various stress conditions displayed distinct peaks for the analyte and degradation products at different retention times. To evaluate the overall ecological sustainability of analytical procedures, including sample preparation, collection, and final estimation, Analytical Greenness (AGREE) and GAPI were used. The concordance between the outcomes of the AGREE evaluation method and the GAPI tool substantiates the environmentally friendly characteristics of the developed method.

Keywords: Maraviroc, QbD, Validation, BBD, AGREE, GAPI, Stability-indicating

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INTRODUCTION

Maraviroc (MVC), a drug belonging to the CCR5 antagonist class and sold under the brand name SELZENTRY (Tab 150 mg), was used for the treatment of HIV 1 infection. As an antagonist of CCR5, it binds to chemokine receptor CCR5 and CD4 cells and prevents CCR5-tropic HIV 1 from entering and infecting these cells, which results in inhibition of viral replication.[1-3] The molecular structure was shown in Fig.-1.

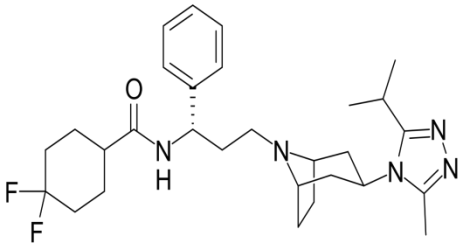

Fig.-1: Molecular Structure of Maraviroc

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An extensive literature survey revealed methods for the estimation of MVC using HPLC.,[4-5] UPLC,[6] LC-MS/MS[7-10] and phase studies were performed for the estimation of safety and efficacy. Till now, no competent HPLC methods for the estimation and validation of drugs using Design of Experiments, greenness assessment, and forced degradation studies have been reported. For developing an optimized method, critical method parameters (CMP) influencing responses or critical quality attributes (CQA) should be properly set. Therefore, to achieve the need, the strategy of Analytical Quality by Design (AQbD) was used. Response Surface Methodology (RSM) is an optimization design in which factors are analysed at more than 2 levels. The most frequently employed RSM designs are the Box-Behnken design (BBD) and the Central composite (CCD). ANOVA, multiple regression analysis, and the desirability functions approach were used for optimizing the chromatographic conditions.[11] Methanol and Acetonitrile are the most widely used organic solvents in RP-HPLC method development, but their use should be limited in terms of ecological impact and operator health. Both methanol and ACN are toxic, and their waste disposal is very difficult, generating about 1-1.5L per day from a conventional HPLC instrument. Hence, the concept of green analytical methods has gained importance by replacing hazardous chemicals with environment and operator-friendly solvents without compromising analytical method performance. Ethanol, Glycerol, Isopropyl alcohol, and HPLC-grade water are the most widely used green solvents in HPLC method development.[12-13] Thus, the objective of the current research was to develop and validate an RP-HPLC method for the determination of MVC using Ethanol as the mobile phase organic solvent, employing QbD principles, and perform stability studies. Additionally, software tools referred to as the AGREE and GAPI were used to evaluate the overall environmental compatibility of the developed analytical method.

EXPERIMENTAL

Materials and Methods

HPLC-grade Ethanol, Water, and Ortho phosphoric acid (OPA) were procured from Sigma-Aldrich and used in the current study. A gift sample of MVC was supplied by Shree ICON Pharmaceutical laboratories, Vijayawada. WATERS HPLC 2695 was provided with a 2996 PDA detector, and data acquisition and processing were accomplished using Empower 2 software. Design Expert® (13.1.0.1) modelling software was utilized for RSM. HPLC grade Ethanol and 0.1% Ortho phosphoric acid in 30:70%V/V was filtered via a 0.45µ membrane filter paper and used as a mobile phase. 1N HCL, 1N NaOH, 20% H_2O_2 were used to perform stress studies. 70µg/mL of MVC was prepared in the mobile phase and used for further studies.

General Procedure

Box-Behnken design under RSM was used for method optimization**.** In compliance with ICH Q2(R2) guidelines, the optimized technique was validated regarding specificity, precision, accuracy, linearity, robustness, limit of detection, and quantitation.[14] After method development and validation, degradation studies have been conducted in accordance with ICH Q1A(R2) to establish the stability-indicating nature of the optimized method. A range of stress conditions, including hydrolysis (acidic, basic, and neutral), oxidative, thermal, and photolytic, was applied. The pure API solution underwent exposure to various stress conditions: acidic stress with 1 mL of 1 N HCl, basic stress using 1 mL of 1 N NaOH solution, neutral stress using 1 mL of drug solution in 10 mL of water, and oxidative stress with a 3% v/v hydrogen peroxide solution. The API solution underwent thermal stress by being maintained at a temperature of 105°C for a duration of 180 min. The standard solution underwent exposure to UV light in a UV chamber for a duration of 24 h to induce photolytic stress. The temperature regulation for samples subjected to hydrolysis and oxidation, as well as their corresponding control blank solutions, was maintained at 60°C.

Detection Method

Preliminary trials were needed to develop the final method. Chromatographic separation was achieved on Inertsil ODS (250x4.6mm, 5µm) column at 300 °C. A mixture of 0.1% ortho-phosphoric acid (pH 2.5) and Ethanol was used as a mobile phase. The UV detection was done at 223 nm.

RESULTS AND DISCUSSION

Optimization Design

17 trial runs were conducted using the three-factor, three-level BBD design, and software was used to analyse the data, which are displayed in Table-1.

Table-1: BBD Study with Responses

Run	Factor A: Flow rate (mL/min)	Factor B: %Organic content (%v/v)	Factor C: Buffer pH	Response 1RT (min)	Response 2 TP	Response 3 TF
1	1	20	3	3.501	7689	0.71
2	1.1	30	3	3.139	8071	0.76
3	1	20	3	3.504	7692	0.73
4	1	30	3.5	3.324	7842	0.79
5	1	20	3	3.505	7685	0.72
6	0.9	20	2.5	3.583	7655	0.71
7	1	10	3.5	3.821	7501	0.79
8	0.9	10	3	3.952	7443	0.71
9	1.1	20	3.5	3.472	7738	0.82
10	1	10	2.5	3.796	7560	0.68
11	1	20	3	3.502	7682	0.74
12	1	20	3	3.507	7693	0.75
13	1.1	10	3	3.735	7584	0.77
14	1	30	2.5	3.236	7989	0.69
15	0.9	20	3.5	3.628	7621	0.78
16	0.9	30	3	3.389	7819	0.76
17	1.1	20	2.5	3.409	7765	0.7

RT: Retention time; TP: Theoretical plates; TF: Tailing factor

Statistical Analysis of BBD Results Employing Design-Expert Software

Analyzing the outcomes of the BBD experimental data allowed for the development of complex statistical models and the determination of their relationships. Table-2 shows the results of the ANOVA used to establish the model significance for all 3 responses. Using ANOVA, it can be decided whether to include or exclude coefficients of linear, interaction, or quadratic terms in the regression model, and it can be included if the *p*-value for each coefficient regression term is less than 0.05. If the *p*-value > 0.05, it indicates that the response is not influenced by varying the levels of the input factor, and that term should be eliminated from the regression model. To investigate the influence of key factors on responses, 2D Contour plots were drawn, shown in Fig.-2, where dark red denotes greater values and dark blue denotes lower values, and remaining areas reflect intermediate values.

Based on Contour plots in Fig.-2, increased organic composition, flow rate, and lower buffer pH were associated with shorter retention time. Higher theoretical plate count was observed at higher organic content, flow rate, and lower buffer pH. It was observed that a lower pH was associated with a decreased tailing factor. The flow rate and organic content were found to have no substantial impact on the tailing factor.

Table-2: ANOVA for the Three Responses

ANOVA for Response Surface Linear Model of RT						
Source	SS	df	MS	F value	*p*-value	Inference
Model	3.903E+05	3	1.301E+05	95.91	< 0.0001	Significant
Factor A	48050.00	1	48050.00	35.42	< 0.0001	Significant
Factor B	3.333E+05	1	3.333E+05	245.73	< 0.0001	Significant
Factor C	8911.13	1	8911.13	6.57	0.0236	Significant
ANOVA for Response Surface Linear Model of TP						
Model	0.0213	3	0.0071	21.38	< 0.0001	Significant
Factor A	0.0010	1	0.0010	3.05	0.1045	Insignificant
Factor B	0.0003	1	0.0003	0.9399	0.3500	Insignificant

Factor C	0.0200	1	0.0200	60.16	< 0.0001	Significant
ANOVA for Response Surface Linear Model of TF						
Model	0.0213	3	0.0071	21.38	< 0.0001	Significant
Factor A	0.0010	1	0.0010	3.05	0.1045	Insignificant
Factor B	0.0003	1	0.0003	0.9399	0.3500	Insignificant
Factor C	0.0200	1	0.0200	60.16	< 0.0001	Significant

Df: Degrees of freedom; *p*-value: Probability value; SS: Sum of squares; MS: Mean square

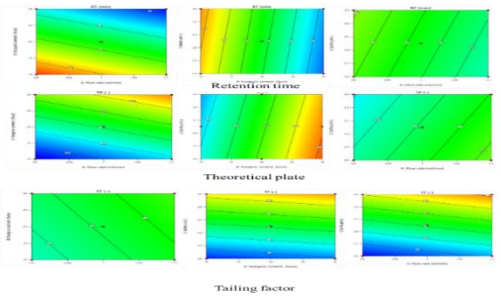


Fig.-2: 2D Contour Plots of all Three Responses of MVC

Method Optimization using the Desirability Functions Approach

Successful separation was done on an Inertsil ODS (250*4.6mm, 5µm) column. The mobile phase, consisting of 30:70 %v/v of Ethanol and ortho-phosphoric acid, pH 2.5, pumped at a flow rate of 1.1 mL/min, produced the greatest desirability of 0.900. Three replicates containing 70 µg/mL MVC was injected into the HPLC system, and the final optimized chromatogram was represented in Fig.-3, where the drug eluted at RT of 3.087min, having a TP count of 8110 and a TF value of 0.734.

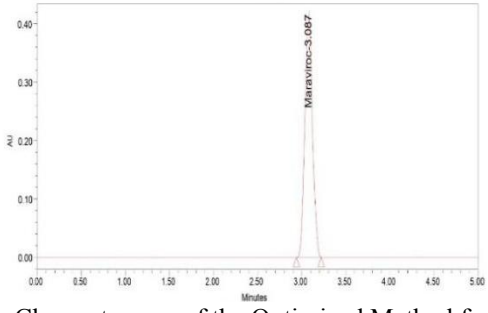


Fig.-3: Chromatogram of the Optimized Method for MVC

Method Validation

The concise validation parameters were shown in Table-7.

Table-7: Results for Method Validation

S. No.	Parameter		Result
1	Linearity	Linearity (µg/mL) Correlation Coefficient	17.5-105 0.999
2	Accuracy	50%, 100%,150% levels	99.9-100.3
3	Precision (% RSD)	Intermediate precision Repeatability	0.525 0.424
4	Sensitivity	LOD (µg/mL) LOQ (µg/mL)	0.42 1.4
5	Robustness (% RSD)	Flow rate (±0.1 mL/min) Organic content (±5%)	0.35 0.38
6	System suitability	RT (min) TF TP	3.087 0.734 8110

Forced Degradation Studies

The samples exposed to various stress settings displayed distinct peaks for the analyte and degradation at varying retention durations. Under some conditions, distinct degradation peaks are not observed; rather, a reduction in size and height of the analyte peak was noted, with corresponding data displayed in Table-8 and respective chromatograms in Fig.-9.

Table-8: Degradation studies under stress conditions for MVC

Degradation condition	% Drug degraded	Purity angle	Purity threshold	Pass/Fail
Control	0	1.427	2.132	Pass
Acidic (1N HCl, 60ºC, 1 hour)	10.1	1.444	2.124	Pass
Alkali (1N NaOH,60ºC, 1 hour)	12.7	1.416	2.165	Pass
Peroxide (3% H_2O_2, 60ºC, 1 hour)	14.9	1.482	2.154	Pass
Thermal (105ºC, 3 hours)	7.9	1.485	2.119	Pass
Photolytic (U.V Light, 3 hours)	0.6	1.497	2.105	Pass
Hydrolysis (H_2O, 60ºC, 1 hour)	0.8	1.458	2.172	Pass

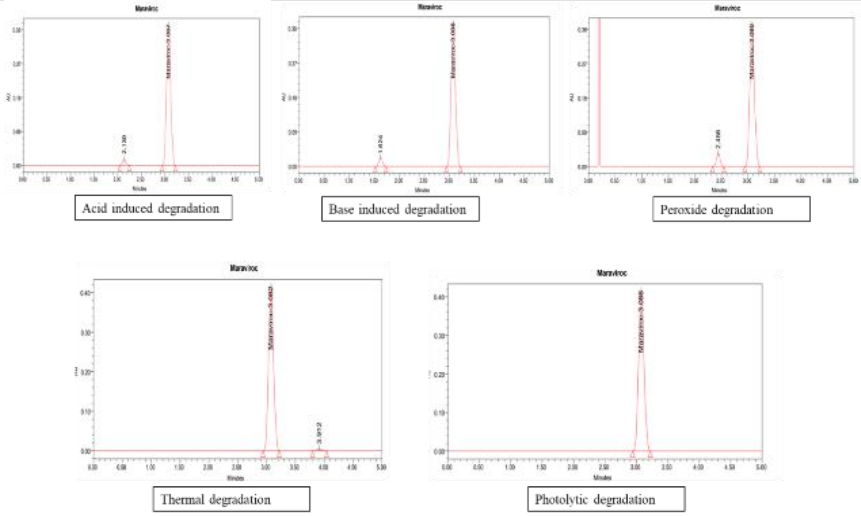


Fig.-9: Various Degradation Studies Chromatograms of MVC

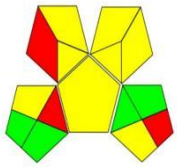
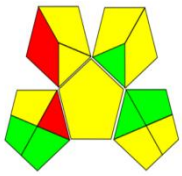
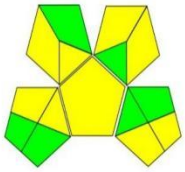
Method Greenness Assessment

Table-9 presents evaluation of the Proposed HPLC Method with Previously Published Methodologies. While, Table-10 demonstrates that the AGREE and GAPI pictograms effectively convey the green concept, with a central score of 0.78 and different shades of green representing the degree of environmental sustainability.

Table-9: Evaluation of the Proposed HPLC Method with Previously Published Methodologies

Parameter	Reported Method[4]	Reported Method[5]	Developed HPLC Method
Linearity range (µg/mL)	0.5-4.5	80-240	17.5-105
Mobile Phase	0.01M KH_2PO_4 pH 3 adjusted with OPA: Methanol (70:30%v/v)	0.02 M K_2HPO_4 pH 2.5 adjusted with OPA: Acetonitrile (60:40%v/v)	Ortho phosphoric acid buffer pH 2.5: Ethanol (70:30%v/v)
Stationary phase	Waters X-Bridge C18(250x4.6mm 5µ)	Inertsil ODS-3V C-18	Inertsil ODS (250*4.6mm, 5µ)
Run time (min)	70	10	5
Flow Rate (mL/min)	0.8	0.8	1.1
Solvent waste (mL Per Run)	56	8	5.5
RT (min)	37.7	4.33	3.087

Table-10: Evaluation of the Greenness Profile of the Proposed HPLC Method in Comparison to Existing Methods

Green Assessment Tool	1st Reported Method[4]	2nd Reported Method[5]	Proposed HPLC Method
Green Analytical Procedure Index(GAPI)			
Analytical Greenness(AGREE)			

CONCLUSION

RSM was implemented to develop and subsequently validate a novel stability-indicating analytical method for the determination of MVC. Chromatographic separation was performed using an Inertsil ODS column (250x4.6 mm, 5µ) at 223 nm wavelength. Optimization was conducted utilizing BBD under RSM with factors examined at three distinct levels. 3.087 min was the RT of MVC. The optimized method has undergone validation in accordance with ICH Q2(R2). Degradation studies were performed following ICH Q1A(R2) guidelines, exhibiting stability across different stress conditions. RSM was regarded as a method development tool that allows for early testing of robustness, rather than during validation, with statistical analysis of results conducted by ANOVA. The developed method may be used for performing pharmacokinetic studies and can be extended to bioanalytical applications in different biological matrices by modifying sample preparation and analytical techniques. The proposed method, based on AGREE and GAPI greenness metrics, has the potential to reduce the environmental footprint of various organic mobile phase solvents meeting SDG-3, while simultaneously improving safety for analysts conducting regular quality assessments.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. All the authors contributed significantly to the manuscript, were involved in reviewing/editing, and approved the final draft for publication. The research profile of authors can be verified from their ORCID ids given below:

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