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DEVELOPMENT OF A STABILITY-INDICATING UPLC METHOD FOR QUANTIFICATION OF MIRVETUXIMAB SORAVTANSINE-GYNX IN PHARMACEUTICAL FORMULATIONS USING QUALITY BY DESIGN (QBD) PRINCIPLES

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ABSTRACT
Background: This study intended to introduce a robust ultra-performance liquid chromatography (UPLC) method for quantifying Mirvetuximab soravtansine-gynx (MSG) in pharmaceutical dosage forms. A systematic approach incorporating the Design of Experiments (DoE) was employed to optimize reliable, sensitive, and efficient chromatographic conditions. **Methodology:** The finalized method utilized a Waters ACQUITY BEH Phenyl (50 mm) Column with a mobile phase comprising acetonitrile and 0.1% aqueous formic acid in 30:70 (v/v) at 0.2 mL/min and 271 nm and PDA wavelength. **Results and discussion:** The method validation demonstrates excellent linearity (R^2 = 0.991, $p < 0.05$) over 17.50–105 µg/mL. The Intraday and interday precision (%RSD) values were 0.568 and 0.544, respectively, confirming method reproducibility. Accuracy was validated through recovery studies, which produced results within the range of 100.1–100.5%, whereas the robustness test highlights the method's resilience to minor variations. This method detects MSG at a very low concentration of 0.42 µg/mL, confirming method sensitivity. The forced degradation studies were conducted under various stress conditions. The result suggests that moderate degradation of 10.3%, 14.5%, and 9.5 % was noticed in acidic, peroxide, and reduction (9.5%) conditions. **Conclusion:** The purity analyses confirm the absence of significant impurities in the stress degradation chromatogram, highlighting the stability of MSG and the reliability of the proposed method. In conclusion, the proposed method was rapid, sensitive, precise, robust, and stable for quantifying MSG in pharmaceutical formulations.

INTRODUCTION

The analytical AQbD is a systematic approach to developing analytical methods that ensure quality and reliability. It integrates risk assessment, method understanding, and control strategies to deliver robust methods with predefined performance criteria as per regulatory guidelines [1]. The QbD approach defines the Analytical Target Profile (ATP), which outlines the method's intended purpose and performance criteria.

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This approach utilizes risk assessment tools for identifying critical method parameters (CMPs) that influence the performance of the analytical method [2]. Then, the Design of Experiments (DoE) is utilized to evaluate the relationships between CMPs and critical quality attributes (CQAs) that enable the development of a method with an optimized design space. This approach ensures method flexibility, where changes within the design space do not require regulatory approval. Control strategies, such as robust system suitability criteria and continuous monitoring, are implemented to maintain method performance throughout its lifecycle. This approach aligns with the draft FDA method validation guidelines, and a three-stage approach was applied for method validation [3].

1. **Design of method**: This stage establishes the analytical method's specific requirements and operating conditions by identifying critical factors that influence the method's performance.
2. **Qualification of method**: This stage demonstrates the method's capability for its intended purpose and meets the predefined requirements.
3. **Verification of method**: This stage continuously monitors and evaluates the method during routine use to ensure it remains consistent, reliable, and within the desired control parameters.

Mirvetuximab soravtansine-gynx (MSG) is an antibody-drug conjugate (Figure 1) that is designed for targeted cancer therapy, especially for the treatment of fallopian tube cancer and epithelial ovarian cancer. It is a conjugate of cytotoxic payload (DM4) and a monoclonal antibody that specifically binds to folate receptor alpha (FRα), a protein overexpressed on the surface of ovarian cancer cells [4]. The antibody component of the drug provides selectivity by directing the conjugate to cancer cells expressing FRα with reduced off-target effect on healthy tissues [5]. In the cancer treatment process, it is internalized into the cancer cells, followed by cleavage of the link between the antibody and the cytotoxic drug. It releases DM4, which inhibits microtubule dynamics, leading to cell cycle arrest and apoptosis. This targeted approach enhances the therapeutic index by improving efficacy with reduced systemic toxicity compared to traditional chemotherapy [6]. The most common side effects identified during this treatment include ocular toxicity (keratopathy, blurred vision, and dry eyes), fatigue, gastrointestinal disturbances, hematological abnormalities, peripheral neuropathy, liver function impairment, and infusion-related reactions [7].

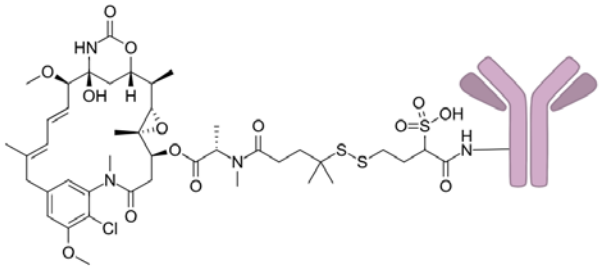


Figure 1: Molecular structure of MSG

Analytical methods for the characterization and quantifying MSG are crucial for ensuring its quality, efficacy, and safety. A comprehensive review suggests that Beck *et al*. 2019 discussed the advanced analytical and structural characterization techniques for various antibody-drug conjugates, including MSG [8]. Additionally, Tu *et al*. 2024 conducted a population pharmacokinetic analysis of MSG to understand the impact of patient characteristics on the pharmacokinetic parameters of the antibody-drug conjugates, their DM4 payload, and S-methyl-DM4 metabolite in patients with FRα-positive ovarian cancer [9]. These studies are unsuitable for quantifying MSG in bulk drugs and formulations and underscore the importance of proposing a sophisticated analytical method. Hence, this study proposed a simple and stable analytical UPLC method for quantifying MSG in pure drugs and formulations. This study preferred UPLC over HPLC due to its superior resolution and time efficiency. Using smaller particle-sized columns (typically <2 µm) in UPLC enhances chromatographic separation, leading to sharper peaks and improved resolution compared to HPLC. UPLC enabled the quantification of MSG with high sensitivity and precision while minimizing solvent consumption and overall analysis time.

MATERIALS AND METHODS

Materials

The active pharmaceutical ingredient of MSG, with a purity of 99.9 %, was generously provided as a gift sample by Zydus Lifesciences Ltd., Ahmedabad, India. The HPLC grade analytical solvents and other chemicals used in this study were procured from Merck, Mumbai. The mobile phase was filtered through a 0.22 µm membrane filter (Merck, USA) and degassed using an ultrasonic bath (Model PS-10A, RoHS, Chaina) before use to ensure consistency, minimize baseline noise, and prevent potential interference during analysis. The commercially available injection formulation, having the ELAHERE brand with 200 mg/20 mL, was purchased from Biocordis Pharmaceuticals Pvt. Ltd., Chandigarh, India.

Instrumentation

The chromatographic analysis of MSG was performed on an ACQUITY UPLC system (Waters, Massachusetts, USA) equipped with a Photo Diode Array (PDA) detector. The UPLC system comprises a Quaternary Solvent Manager (QSM) pump for precise solvent delivery and a Sample Manager with injector for efficient and accurate sample introduction. A column heater (CH-A) was employed to maintain the desired column temperature and ensure consistent separation performance. The Empower 2 (Waters) software was used for data acquisition and processing, enabling advanced method development, integration, and reporting.

Chromatographic conditions

Waters ACQUITY BEH Phenyl Column 50 mm x 2.1 mm, 1.7 µ was utilized for the analysis, and the column was equilibrated with acetonitrile and 0.1 % aqueous formic acid in a 70:30 (v/v). The flow rate was maintained at 0.2 mL/min, and the column was operated at ambient temperature. Detection of eluents was performed using a PDA detector set at a wavelength of 271 nm based on the maximum absorbance (λmax) of MSG. This wavelength ensures optimal sensitivity and specificity with minimal interference from excipients and mobile phase components. These chromatographic conditions provided satisfactory separation and peak symmetry for MSG.

Preparation of standard and calibration solutions

An accurately weighed 25 mg of MSG was dissolved in 25 mL of mobile phase using an analytical balance. This process results in a 1000 µg/mL stock solution, and from this stock solution, a sub-stock solution with 700 µg/mL was prepared by appropriate dilution. From this stock solution, aliquots of 0.25 to 1.5 mL were separately taken in 10 mL flasks and were made to 10 mL with mobile phase to obtain linear dilutions in the range of 17.5 µg/mL to 105 µg/mL. All these solutions were prepared carefully to ensure precision and accuracy for further analysis.

Preparation of assay solution

The injection formulation of MSG with the ELAHERE brand at 200 mg/20 mL was utilized to prepare the assay solution. The injection powder equivalent to 25 mg of MSG was dissolved in the mobile phase and sonicated for around 10 minutes. The solution was then diluted, filtered through a 0.2 µm membrane filter, and diluted to 70 µg/mL. This solution was analysed using the proposed UPLC method.

QBD APPROACH IN UPLC METHOD DEVELOPMENT

Selection of Quality Target Product Profile (QTPP) and identification of CQAs

The selection of QTPPs is a foundational step in the Analytical QbD method, which helps identify the factors influencing the desired analytical conditions. This study selected parameters such as retention time, theoretical plates, and peak asymmetry as the key QTPP attributes. These parameters define method performance and ensure reliable and reproducible results. CQAs are the method variables that directly impact the QTPPs. In this study, the composition and flow rate of the mobile phase were treated as critical method parameters. These variables must be carefully controlled to ensure that the QTPP parameters remain within an acceptable range with consistent and accurate analytical performance.

Experimental design for method optimization

A central composite experimental design with the identified QTPP and CQAs was employed to optimize the UPLC method. This statistical approach allows the systematic evaluation of the interactions and quadratic effects of the critical factors on the QTPPs, such as retention time, theoretical plates, and peak asymmetry. A 2-factor design that incorporates mobile phase composition and flow rate at different levels was implemented through Design Expert® software (Version 11.0, Stat-Ease Inc.). This design facilitates the exploration of second-order polynomial models and quadratic response surfaces that identify the method's optimal conditions. The approach ensures a robust, reliable method to deliver consistent results under varied conditions.

Risk Assessment

The method was finalized based on its ability to meet predefined method attributes and ensure it performs efficiently throughout the product's lifecycle. The QbD-based risk assessment was conducted for this finalized method based on principles outlined in ICH Q8 and Q9 guidelines. This approach assesses the robustness and ruggedness of the technique by focusing on its stability and performance under various conditions. Robustness was tested by examining the method's performance with small variations in optimized parameters, whereas ruggedness was evaluated by replicating the method across different analysts over multiple days. These evaluations proved the consistency and reliability of the proposed method under diverse operational conditions [10].

Method Validation

It is a documented process that provides strong evidence to ensure the reliability and suitability of the proposed analytical method for its intended purpose. The UPLC method proposed for the quantification of MSG was validated as per ICH Q2 (R1) guidelines [11]. The linearity was established by analysing various concentrations with different concentration ranges. The linear curve within the analysed concentration range was plotted as concentration vs area response. The proposed method considered the calibration range that exhibits strong correlation coefficient values as the range of MSG. Precision was assessed through the repeatability of six replicate samples at 70 µg/mL of MSG and evaluated as intra- and interday precision. The intraday precision was assessed by analysing the solution six times with 2h intervals on the same day. In contrast, the same level solution was assessed six times on two days for interday precision. The area response's relative standard deviation (%RSD) was determined, and a result of less than 2% was treated as acceptable. Accuracy was determined through recovery studies, which were performed over a concentration range of 35, 70, and 105 µg/mL in the linearity range. The % recovery within the 98–102% ICH guideline range was considered accurate. The LOD and LOQ represent the method's sensitivity for detection and quantification of MSG, which was calculated by utilizing the standard deviation of the y-intercept (σ) and slope (SD) from the calibration curve by substituting the formulas LOD=3.3×σ/SD and LOD = 10×σ/SD. Robustness was evaluated by minor changes to the proposed method parameters, like organic modifier composition and flow rate. In contrast, the system suitability was confirmed by analyzing parameters like retention time, theoretical plates, and peak asymmetry through six replicate analyses of MSG at a precision level concentration.

Forced degradation studies

The forced degradation studies [12] were conducted on MSG to evaluate its stability under various stress conditions. In acid degradation, 0.14 mL of the sample was mixed with 1 mL of 1N HCl, 1N NaOH, 10% H_2O_2, and 10% sodium bisulfite separately for acid, base, peroxide, and reduction degradation studies. These solutions were incubated for 15min, neutralized with appropriate diluent, and filtered. 50mg of MSG was exposed to sunlight and dry heat at 105°C for 6 hours separately for photolytic and thermal degradation, respectively. The standard solution of MSG was treated with 1 mL of HPLC water for 15 min in hydrolysis degradation. All these stress solutions were filtered, diluted to a precision 70 µg/mL concentration, and then transferred to vials for subsequent analysis in the proposed method.

Assay Method

The prepared assay solution at a 70 µg/mL concentration of MSG was analysed under the same chromatographic conditions used for linearity, and the mean of three replicates was used to calculate the % assay of MSG. The % assay results suggest the applicability of the proposed method for routine analysis of MSG.

RESULTS AND DISCUSSION

The most suitable wavelength for detecting MSG was determined by scanning 400 to 200 nm through a PDA detector. Based on the results, it was found that 271 nm provided the highest sensitivity and clarity for detection. This wavelength was therefore selected and consistently used during the subsequent method optimization process. In the initial trials, the Phenomenex C18 (50 mm) column was utilized as a stationary phase using 0.2 mL/min of Acetonitrile and 0.1% trifluoroacetic acid in 30:70 (v/v) as a mobile phase. This condition produces a broad peak with an improper baseline (Figure 2A). The same mobile phase conditions were tested on the Phenyl column, which produced a very asymmetric peak with a very low plate count (Figure 2B). Then, an equal volume of acetonitrile and pH 7.0 phosphate buffer at 0.2 mL/min was tested on a Waters ACQUITY BEH Phenyl Column (50 mm) UPLC column. It produces a broad peak with less plate count and a long elution time (Figure 2C). Further, 0.1 % aqueous formic acid and acetonitrile in 80:20 (v/v) were tested on the Phenyl (50 mm) column, and it produced a symmetric peak with less intensity and less area response (Figure 2D). Further method optimization of various parameters was conducted within the design space using a central composite design.

QbD approach for method validation

In the QbD approach for validation of analytical UPLC method, the peak asymmetry, theoretical plates, and retention time were selected as QTPPs. In contrast, the composition of acetonitrile and mobile phase flow rate are treated as CQAs. Keep this consideration, the quadric design, the central composite design, and the response surface study were conducted with 13 runs. The finalization of mobile phase proportion and flow was evaluated through central composite design, as tabulated in Table 1, and results related to CQAs in these runs were tabulated.

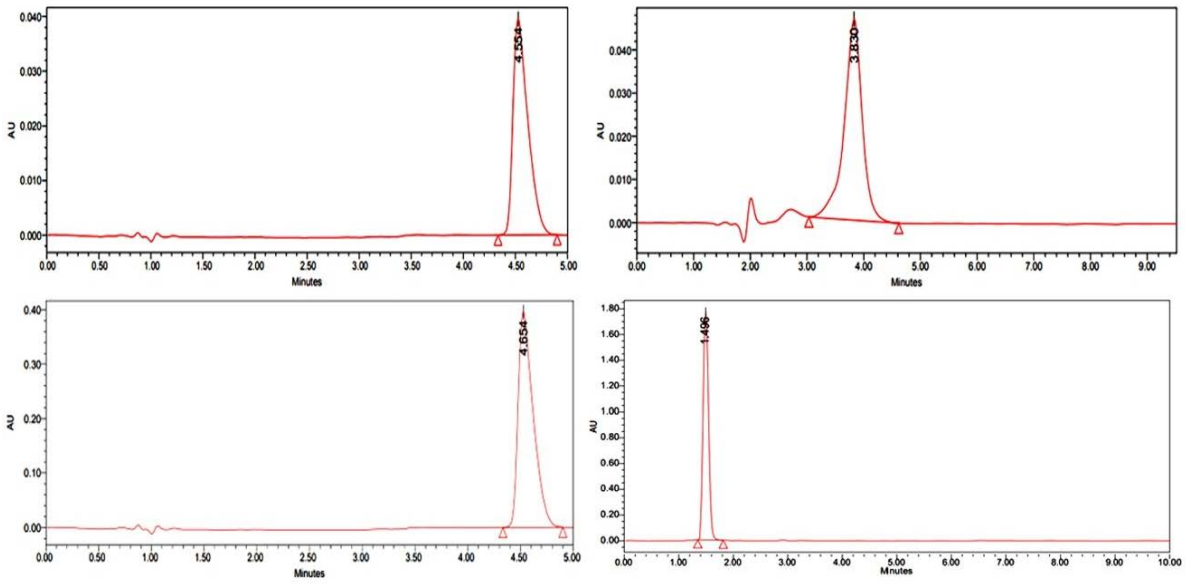


Figure 2: Chromatograms observed during optimization of a method for quantification of MSG

QbD approach for method validation:

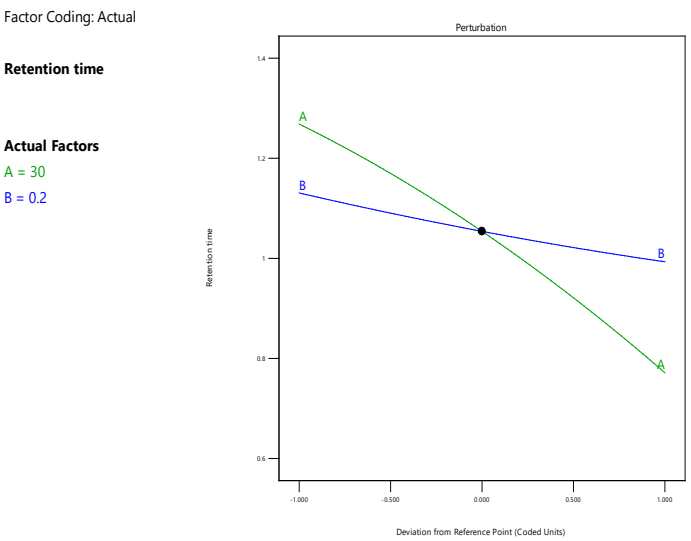
In the QbD approach for validation of analytical UPLC method, the peak asymmetry, theoretical plates, and retention time were selected as QTPPs. In contrast, the composition of acetonitrile and mobile phase flow rate are treated as CQAs. Considering this, the quadric design, central composite design, and response surface study were conducted with 13 runs. The finalization of mobile phase proportion and flow was evaluated through central composite design, as tabulated in Table 1, and results related to CQAs in these runs were tabulated.

Table 1: Parameters optimized for the analysis of MSG using a central composite design

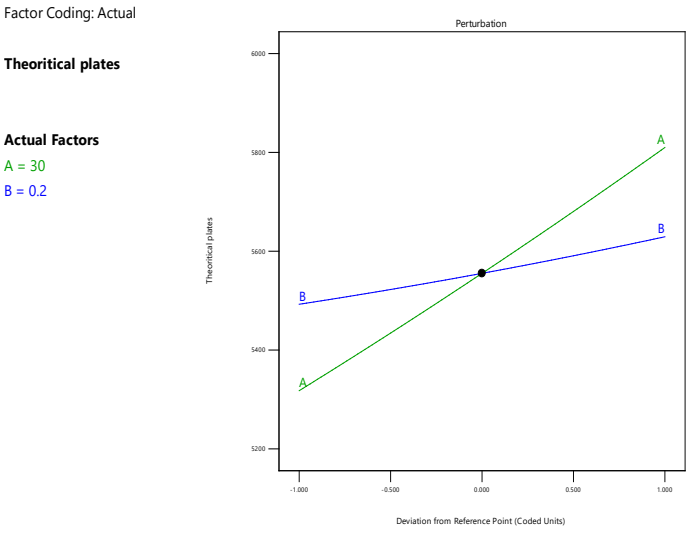
Run	Aceto-nitrile	Flow rate	Retention time	Theoretical plates	Tailing factor
1	30	0.2	1.052	5542	1.12
2	22.93	0.13	1.325	5284	1.19
3	30	0.1	1.167	5463	1.15
4	30	0.25	1.056	5561	1.11
5	30	0.15	1.053	5546	1.11
6	30	0.35	0.973	5648	1.1
7	30	0.2	1.055	5558	1.12
8	22.93	0.27	1.228	5369	1.14
9	37.07	0.13	0.867	5737	1.09
10	30	0.25	1.054	5569	1.12
11	20	0.2	1.337	5225	1.18
12	37.07	0.27	0.691	5934	1.08
13	40	0.2	0.635	5897	1.07

The effect of individual factors on a variable response was visualized through perturbation charts. These charts help to understand the sensitivity of the response to changes in the levels of each factor while keeping all other factors constant at a specific reference point. The perturbation charts related to retention time (Figure 3A), theoretical plates (Figure 3B), and tail factor (Figure 3C) were depicted in Figure 3. The 2D contour plots and 3D surface plots correspond to MSG's theoretical plates and tailing factor in DoE studies, which are presented in Figures 4 and 5, respectively.

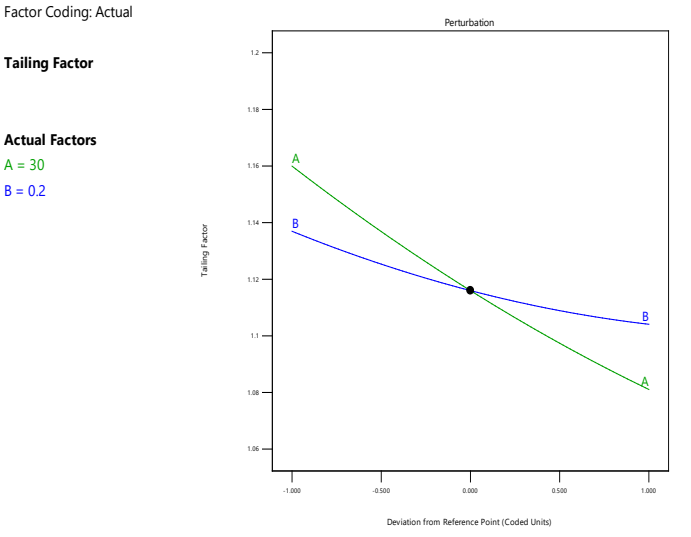
The study utilizes the DoE tool to systematically analyse all the responses under various experimental conditions. The optimal HPLC conditions were identified based on the analysis, and the predicted responses were recorded. These predictions were verified, and the actual response values were evaluated by running the UPLC chromatogram using the specified conditions. The results achieved during this test run were correlated with the predicted responses to assess the accuracy and reliability of the optimization process. Based on the results it was proved that the 30:70 (v/v) composition of acetonitrile and formic acid (0.1 %) at 0.2 mL/min flow was finalized as mobile phase, Phenyl column (2.1 mm X 50mm, 1.7 µ), PDA detection at 271 nm with 5 µL sample volume was proved to be ideal for the resolution and analysis of MSG. The study was completed within a very short run time of 2 min. This brief time with a significantly lower flow rate of 0.2 mL/min facilitates minimum consumption of the mobile phase and allows for more analysis runs to be completed quickly. The chromatogram noticed in the optimized conditions is presented in Figure 6.



A

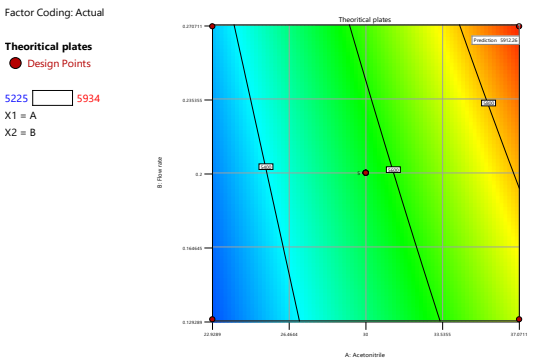


B

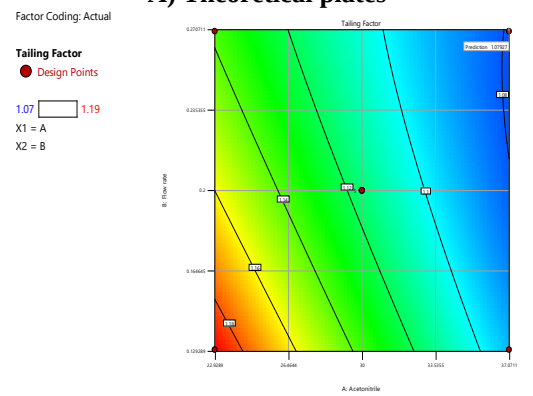


C

Figure 3: Perturbation charts noticed during optimization of analytical method for quantification of MSG

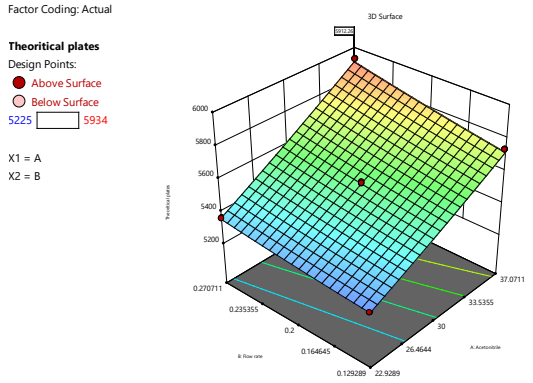


A) Theoretical plates

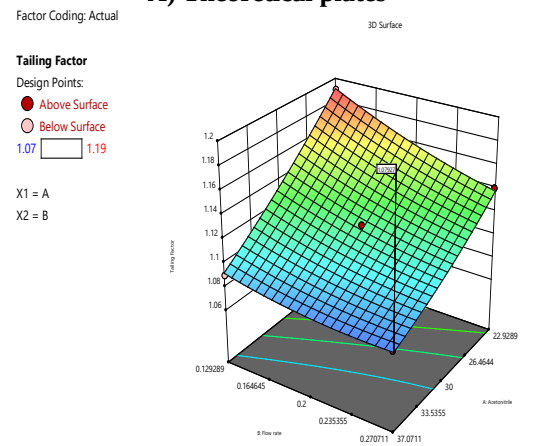


B) Tailing factor

Figure 4: 2D Contour plot noticed during optimization of analytical method for quantification of MSG



A) Theoretical plates



B) Tailing factor

Figure 5: 3D Surface plots noticed during optimization of analytical method for quantification of MSG

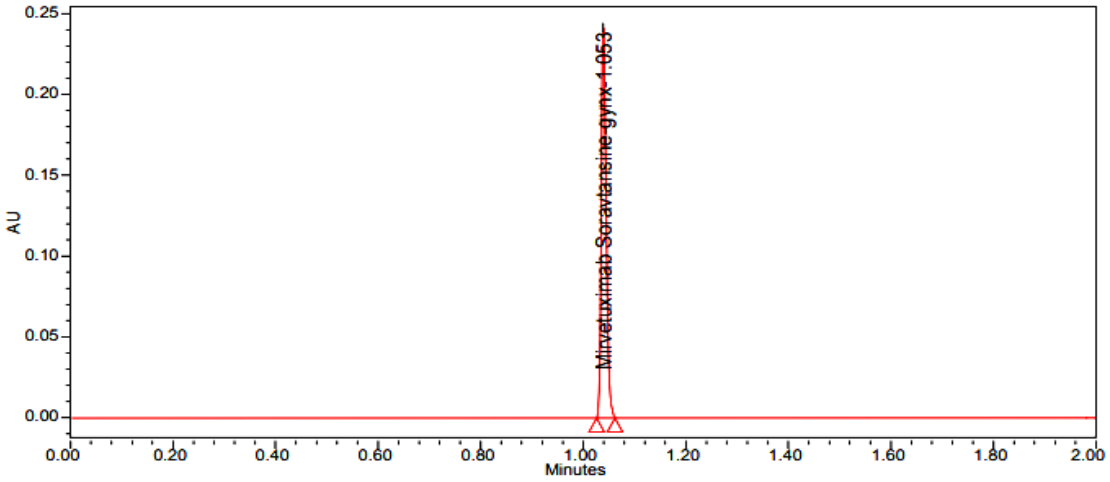


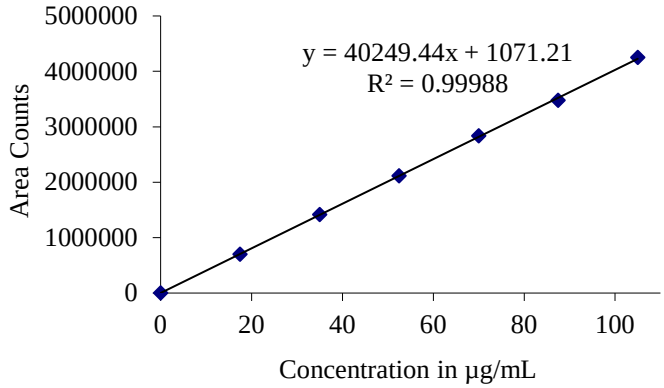
Figure 6: System suitability chromatogram observed in the method conditions optimized for the quantification of MSG

Method validation

The system suitability test was performed on a representative chromatogram to evaluate critical parameters and ensure the system's performance. The retention time of MSG was recorded as 1.053 min, with the theoretical plate count calculated at 5546, indicating good column efficiency. The peak asymmetry of 1.12 was noticed to reflect an acceptable peak shape. Additionally, the relative standard deviation (% RSD) for six replicates was determined to be 0.568, confirming method reproducibility. The constructed concentration vs. area response calibration curve demonstrates linearity across the 17.50 to 105 µg/mL range, as depicted in Table 2. The regression equation for the calibration curve was determined to be y = 40249.44x + 1071.21, and the coefficient of determination (R^2) was calculated as 0.9999, indicating a strong linear relationship between peak area and concentration.

Table 2: Linear results including the linearity graph noticed for MSG in the optimised method

S No	Concentration in µg/mL	Area response
1	17.50	701865
2	35.00	1417730
3	52.50	2116595
4	70.00	2835461
5	87.50	3474326
6	105.00	4253191
Correlation coefficient		0.99988
Slope		40249.44
Intercept		1071.21



ICH guidelines validated the proposed UPLC method for MSG, and the results are tabulated in Table 3. The intraday precision, which was evaluated through six replicate measurements within the same day, shows a % RSD of 0.568. In contrast, the interday precision assessed over multiple days produces a % RSD of 0.544, indicating excellent repeatability and consistency. The method accuracy was confirmed through recovery studies at three concentration levels of 35 µg/mL, 70 µg/mL, and 105 µg/mL in the calibration curve, and results produced a recovery value of 100.5% ± 0.719, 100.1% ± 0.360, and 100.5% ± 0.525, respectively. The % recoveries are within the acceptable range of 98–102%, demonstrating method accuracy. Robustness testing involved deliberate variations in flow rate and organic modifier composition, and results produced a minimal % change observed, showing the method's resilience to minor alterations. The method sensitivity test reveals an LOD of 0.42 µg/mL and an LOQ of 1.40 µg/mL, confirming the capability of the method to detect and quantify low concentrations of MSG. The % assay of MSG in the formulation analysis exhibits a % assay of 99.99, proving the method's suitability for accurate quantification of

MSG in pharmaceutical preparations. The validation results demonstrated that the proposed UPLC method was reliable and robust for the quantification of MSG.

Table 3: Summary of method validation results noticed for MSG in the optimised method

S No	Parameter	Results
1	Intraday precision (% RSD, n = 6)	0.568
2	Interday precision (% RSD, n = 6)	0.544
3	% accuracy at 35 µg/mL level (n= 3)	100.5 ± 0.71
4	% accuracy at 70 µg/mL level (n= 3)	100.1 ± 0.36
5	% accuracy at 105 µg/mL level(n=3)	100.5 ± 0.52
6	Robustness (% change; n=3)	
	Positive change in flow rate	0.518
	Negative change in flow rate	0.661
	Positive change in organic modifier	0.242
	Negative change in organic modifier	0.118
7	LOD in µg/mL	0.42
8	LOQ in µg/mL	1.40
9	% formulation assay	99.99

A forced degradation study of MSG under various stress conditions was performed using the proposed UPLC method to evaluate the stability and integrity of the MSG. The test results are tabulated in Table 5. In acidic conditions, MSG exhibits a % degradation of 10.3% with one degradation product retained at 0.239 min (Figure 7A). The peroxide degradation exhibits a high % degradation of 14.5 %, and the chromatogram retained an additional degradation product peak at 1.201 min (Figure 7B). The peroxide degradation produced a significantly higher degradation percentage due to the strong oxidative nature of hydrogen peroxide, which likely induced oxidative cleavage of MSG. The presence of susceptible functional groups such as aromatic rings, amides, or sulfide linkages may facilitate oxidation that leads to forming degradation products. Similarly, the reduction degradation conditions exhibit a % degradation of 99.5 % with one additional degradation product retained at 1.332 min (Figure 7C). A significantly lower % degradation of 1.8 % was noticed in base degradation, 0.9 % in photolytic, 0.5 % in hydrolytic, and 2.1 % in thermal conditions. These findings collectively underscore that MSG exhibits a low percentage of degradation across various stress conditions, reflecting its remarkable stability profile. These stability results were further supported by the purity results of the MSG peak. The purity angle in all stress conditions was consistently lower than the purity threshold, confirming the absence of significant impurities or co-eluting degradation products. The forced degradation studies' chromatograms confirm that the main peak remains well-resolved with no co-elution of degradation products, ensuring accurate quantification of MSG. The observed stability results ensure the reliability and robustness of the developed UPLC method for analysing MSG in various pharmaceutical preparations.

CONCLUSION

In this study, a stability-indicating UPLC method was successfully optimized and validated as per ICH guidelines. The DoE studies employed a systematic and scientific approach in the method optimization process to ensure method reliability and accuracy. The method utilizes a Phenyl (50 mm) column with a mobile phase of acetonitrile and 0.1% aqueous formic acid in 30:70 (v/v) at a 0.2 mL/min flow rate. The method achieves a short retention time of 1.053 min, suggesting the high efficiency of the method with minimal mobile phase consumption. The method validation results confirm linearity over 17.5 – 105 µg/mL (R^2 = 0.991) with intraday and interday %RSD values of less than 0.6. The forced degradation studies reveal the moderate degradation of MSG under acidic (10.3%), peroxide (14.5%), and reduction (9.5%) conditions.

Table 4: Forced degradation study results noticed for MSG in the optimised method

S No.	Degradation condition	Area Counts	% Label Claim	% Degradation	Mass Balance	Pass / Fail
1	Control	2833553	100	0	--	Pass
2	Acid	2542874	89.7	10.3	99.94	Pass
3	Alkali	2782451	98.2	1.8	98.20	Pass
4	Peroxide	2422428	85.5	14.5	100.01	Pass
5	Reduction	2566465	90.5	9.5	100.75	Pass
6	Photolytic	2809070	99.1	0.9	99.10	Pass
7	Hydrolysis	2820149	99.5	0.5	99.50	Pass
8	Thermal	2775165	97.9	2.1	97.90	Pass

Acid (A), peroxide (B), and reduction (C) degradation chromatograms and the peak purity chromatograms are inserted in all stress study chromatograms

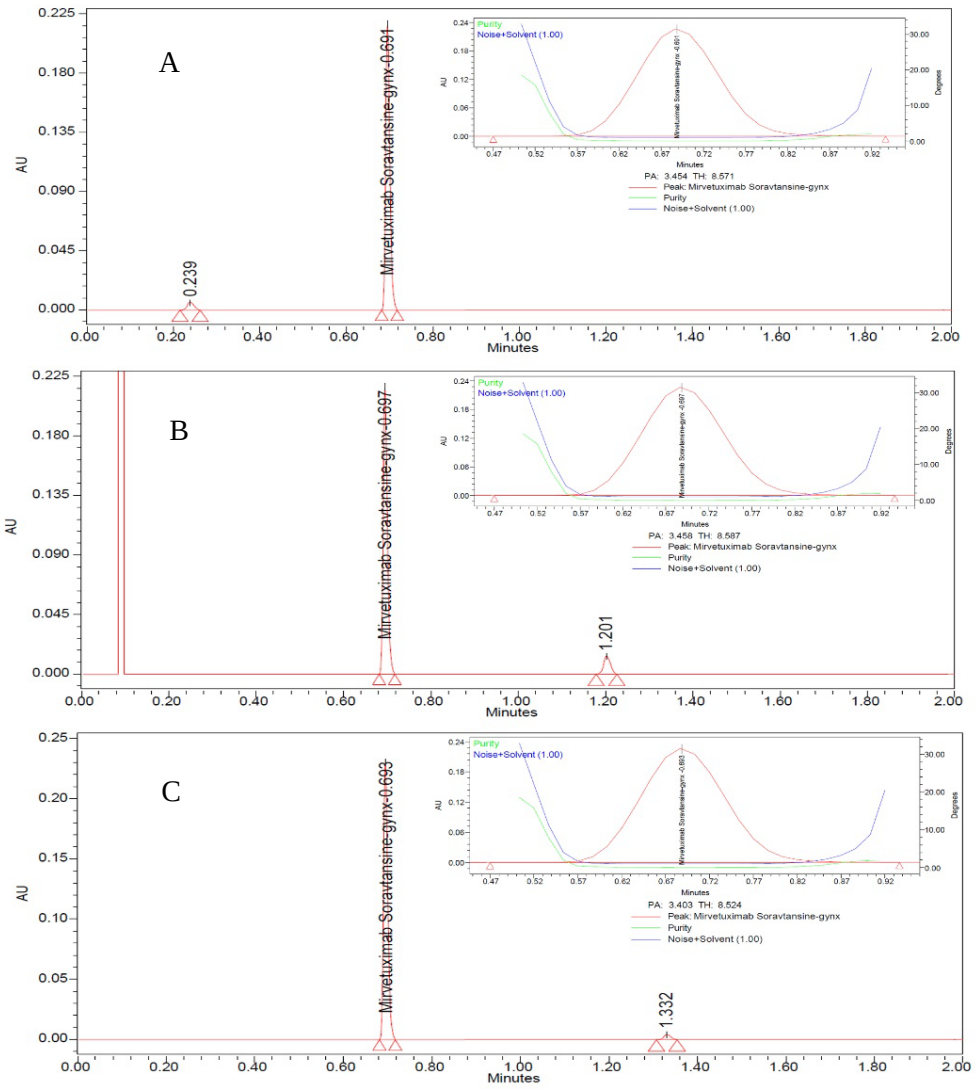


Figure 7: Forced degradation chromatograms noticed for MSG in the proposed method

Purity analysis further confirmed MSG's stability. The results suggest that the purity angle was consistently below the purity threshold in all stress conditions, indicating no significant co-eluting impurities or degradation products. This study primarily focused on the quantification of MSG in pharmaceutical dosage forms, which limits its applicability to biological matrices and does not include comprehensive degradation pathways. The developed UPLC method aligns with ICH guidelines that ensure robust quality control measures in pharmaceutical industries for maintaining batch-to-batch consistency of MSG formulations.

FINANCIAL ASSISTANCE
Nil

CONFLICT OF INTEREST
The authors declare no conflict of interest

AUTHOR CONTRIBUTION
K.V. Ummaheswara Rao and Neetu Shorgar collaborated on the literature survey and analysis. K.V. Ummaheswara Rao prepared the initial draft of the manuscript, which was thoroughly reviewed and revised by all authors. Neetu Shorgar provided consistent guidance throughout the finalisation of the manuscript.

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