RESEARCH PAPER

DEVELOPMENT AND VALIDATION OF A UPLC-MS METHOD FOR QUANTITATIVE DETERMINATION OF SULFASALAZINE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Sulfasalazine is a disease-modifying antirheumatic drug commonly used for the treatment of rheumatoid arthritis and inflammatory bowel disease. It is essential to develop a reliable analytical method as quality, safety and effectiveness of pharmaceutical products depend on precise drug measurement. This study presents a rapid, sensitive, and dependable UPLC-MS method for quantification of Sulfasalazine in pharmaceutical dosage forms. The analysis was performed on Waters Acquity H-Class Plus UPLC system coupled with SQ Detector 2 with an Acquity UPLC BEH C18 column (2.1 × 50 mm, 1.7 µm). The 5mM ammonium acetate and acetonitrile in gradient mode at a flow rate of 0.5 mL/min was used. Sulfasalazine gave a sharp, symmetrical peak at 1.64 min with a 6 minute total run time. The method was validated as per ICH guidelines for system suitability, linearity, accuracy, precision, robustness, LOD and LOQ. It showed excellent linearity from 60-140 µg/mL (R^2 = 0.9996). The recovery values were found to be between 98.24% and 102.34%. Intraday and interday precision gave %RSD of 0.065% and 0.055% respectively. LOD and LOQ were 0.18 µg/mL and 0.54 µg/mL. Robustness study confirmed that small, deliberate changes in chromatographic conditions did not compromise performance. The method yielded an average assay of 99.67% when applied to a marketed Sulfasalazine tablet. Overall, the developed method is accurate, precise, sensitive, and robust, making it well-suited for routine analysis of Sulfasalazine in pharmaceutical dosage forms.

KEYWORDS Sulfasalazine, UPLC-MS, Method Development, Method Validation, ICH Guidelines, Pharmaceutical Formulations

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INTRODUCTION

Rheumatoid Arthritis is a disease that is inflammatory and autoimmune in nature that results in the progressive damage of the joints due to the inflammation of synovium and production of autoantibodies like RF and Anti-Cyclic Citrullinated Peptide (Anti-CCP).[1]However, rheumatoid arthritis mainly targets the synovial joints where it causes pain, swelling, stiffness, and immobility. Apart from targeting the joints, rheumatoid arthritis can also cause damage to other organs including the heart, lungs, skin, and nervous system.[2]

In the treatment of RA, there are various types of medicines used to control the disease's progress due to their mechanisms of action. The medications are primarily grouped into three categories namely csDMARDS, bDMARDS, and tsDMARDS. Biosimilar DMARDS are again classified into biologic originator DMARDS and biosimilar DMARDS.[3]

Sulfasalazine (SSZ) is a disease-modifying antirheumatic drug that is approved by the United states Food and Drug Administration (USFDA) for the management of rheumatoid arthritis and ulcerative colitis. This medication is also prescribed in an off-label manner for many dermatological diseases owing to its anti-inflammatory nature.[4]It is made up of two pharmacologically active substances; sulfapyridine and 5-aminosalicylic acid (5-ASA). It is connected through an azo linkage. The compound is hydrolyzed into its active forms by the bacterial enzymes present in the intestines, thus exhibiting systemic and local pharmacological actions. Sulfapyridine exhibits its pharmacological activity systemically through the modulation of inflammatory mediator levels and cytokine secretion, whereas 5-ASA works locally in the intestinal walls through reduction of oxidative stress and maintenance of the mucosa integrity.[5]

Methods involving hyphenation technique are often employed to ascertain the presence and quantification of such compounds in drugs and living systems.[6]UPLC-MS is one of the most widely used hyphenated analytical techniques for pharmaceutical analysis.UPLC is a form of analysis which provides many advantages including fast analysis, sensitivity, and resolution.[7]

Although there are many chromatographic techniques available for Sulfasalazine, information about a fast UPLC–MS technique for its analysis is not abundant in literature. Hence, the current study was designed with an objective of developing and validating a UPLC-MS method for estimating Sulfasalazine.

DRUG PROFILE

The physicochemical properties of Sulfasalzine are summarized in Table 1.

Table 1: Drug Profile of Sulfasalazine

Attributes	Description
Name	Sulfasalazine
Category	Disease Modifying Anti-rheumatic Drug; Anti-inflammatory Drug
Molecular Formula	$C_{18}H_{14}N_4O_5S$
IUPAC Name	2-hydroxy-5-[[4-(pyridin-2-ylsulfamoyl)phenyl]diazenyl]benzoic acid
Molecular Weight	398.4 g/mol
Melting Point	240–245 °C
Solubility	Practically insoluble in water; Slightly soluble in ethanol; Soluble in THF, DMSO; Soluble in alkaline solutions
Route of Administration	Oral
Storage	Store at controlled room temperature (20-25°C), Protect from light

Figure 1: Chemical Structure of Sulfasalazine[8]

MATERIALS AND METHODS

Chemical and Reagents:

Sulfasalazine API was supplied as a gift sample. The various chemicals and solvents utilized in the analysis such as acetonitrile, DMSO, ammonium acetate, and Milli-Q water were all of LC-MS grade.

Instruments:

The analysis was done using the Waters Acquity H-Class Plus UPLC coupled with a mass spectrometer (SQ Detector 2). The other instruments utilized were analytical balance, sonicator, and membrane filtration setup.

Solubility Determination:

The solubility of Sulfasalazine was evaluated using various solvents. Sulfasalazine was found to be soluble in DMSO and methanol, and showed adequate solubility in an ammonium acetate solvent system. Based on the solubility properties and compatibility with mass spectrometric detection, a solvent mixture of ammonium acetate buffer/acetonitrile was chosen for method development.

Optimized Chromatographic Conditions:

Various mobile phase compositions, flow rates, and column types were tested. After several trials,

optimized chromatographic conditions were achieved, which are presented in the following table 2.

Table 2: Optimized Chromatographic conditions

Sr No	Specifications	Description
1	Equipment	Waters Acquity H- Class Plus UPLC SQ Detector 2
2	Software	MassLynx
3	Detection	Diode Array (190 nm to 500 nm)
4	Column	Acquity UPLC BEH C18
5	Dimensions	1.7 µm(2.1×50 mm)
6	Mobile phase	Line A: 5mM Ammonium acetate Line B: Acetonitrile
7	Elution method	Gradient
8	Injection Volume	1 µL
9	Flow rate	0.5 mL/min
10	Autosampler Temperature	5°C
11	Column Temperature	30°C
12	Run time	6 Minutes

Preparation of Solutions:

Preparation of Diluent:

Diluent was prepared in the ratio of 5 mM ammonium acetate buffer and acetonitrile with the same ratio (50:50v/v). Fresh diluent was prepared for the preparation of standards.

Preparation of Standard Stock Solution:

The standard stock solution of Sulfasalazine (1000 µg/ml) was prepared by accurately weighing 100 mg of Sulfasalazine API in a 100 mL volumetric flask. Initially, 1 mL of DMSO was taken in the volumetric flask to dissolve the compound. When the compound was completely dissolved, then the solution was further diluted with diluent and shaken properly to form a homogenous solution.

Preparation of Working Standard Solutions:

Further dilution was done from the stock solution (1000 µg/ml) with the diluent to prepare different concentrations of sulfasalazine.

Preparation of Tablet Powder Solution:

The sulfasalazine tablets were accurately weighed and powdered in a mortar and pestle. A quantity of the powdered material that represented 100 mg of sulfasalazine was carefully measured out and placed in a 100 mL volumetric flask. Initially, 1 mL of DMSO was used to dissolve the sulfasalazine. The prepared solvent was then added after which the mixture was sonicated for about 10 to 15 minutes. This helped in dissolving all the sulfasalazine from the tablet powder. The resultant solution was filtered using a 0.45 µm filter and diluted further with the solvent.

Method Development

A UPLC-MS method for the determination of sulfasalazine was developed and optimized based on its physicochemical and solubility properties. Different mobile phase compositions and chromatographic conditions were evaluated to get good peak shape, sensitivity and reproducibility. From among all the conditions examined, the ammonium acetate buffer with acetonitrile and a BEH C18 column showed satisfactory results in the study of chromatographic method. The optimized method was subsequently used for validation studies.

Method Validation

The developed UPLC–MS method for the estimation of Sulfasalazine was validated according to ICH Q2(R2) guidelines for analytical method validation.

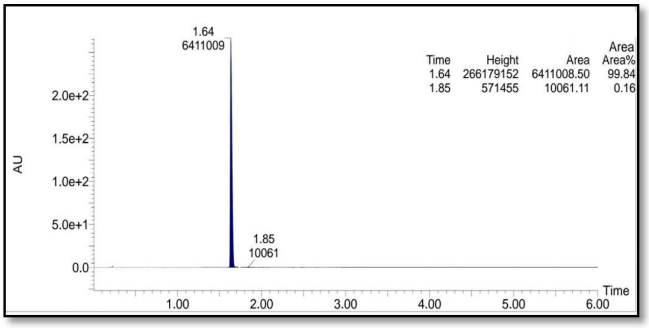
RESULTS AND DISCUSSION

Method Development

The developed UPLC–MS technique showed a satisfactory result in terms of chromatographic efficiency in the determination of Sulfasalazine with optimized analytical parameters. A clear, symmetric, and well-resolved peak with good sensitivity and reproducibility was achieved, suggesting the applicability of the developed method.

The optimized chromatogram of standard Sulfasalazine is presented in Figure 2. The corresponding mass spectrum confirmed the characteristic molecular ion peak and m/z values of Sulfasalazine, as illustrated in Figure 3.

Figure 2: Standard Chromatogram of Sulfasalazine



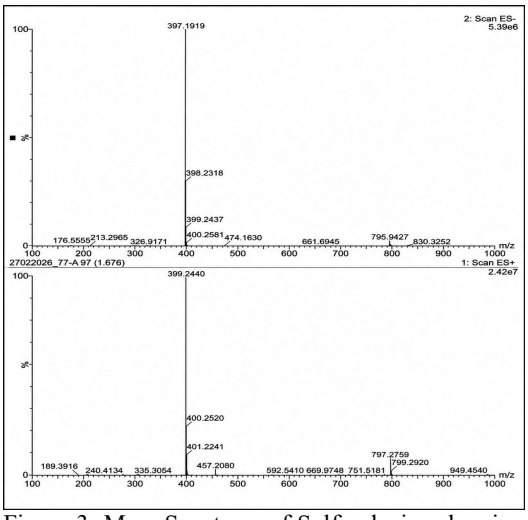


Figure 3: Mass Spectrum of Sulfasalazine showing characteristic m/z peaks in both positive (ES+) and negative (ES-) scan modes.

Method Validation

The UPLC-MS technique was validated as per the ICH Q2(R2) recommendations for various validation parameters. The validation data confirmed the reliability of the technique and its suitability for the analysis and quantification of Sulfasalazine. The results for various validation parameters have been tabulated below.

I) System Suitability

System suitability test for Sulfasalazine was conducted on four replicates of the drug solutions. The various factors considered included peak area, theoretical plates, and retention time.

Table 3: System Suitability Parameters of Sulfasalazine

Sr No	Concentration (µg/mL)	Peak Area	Retention Time	Number of Theoretical Plates
1	100	1362509.50	1.66	6554.4
2	100	1368986.25	1.65	6698.24
3	100	1368205.13	1.66	6724
4	100	1368113.63	1.66	6500.8
Mean		1366953.62	1.6575	
SD		2988.51	0.005	
%RSD		0.2187	0.3	

Acceptance Criteria: %RSD ≤ 2.0; Theoretical Plates>2000

II) Linearity

The developed method showed good linearity in the concentration range of 60–140 µg/mL. The calibration curve of concentration versus peak area exhibited a strong linear relationship with the regression equation y = 12804x + 34813 and correlation coefficient (R^2) of 0.9996 (Figure 4).

Table 4: Linearity Data of Sulfasalazine

Sr No	Concentration (µg/ml)	Peak Area
1	60	801395.69
2	80	1051614.63
3	100	1328419.88
4	120	1574016.63
5	140	1820589

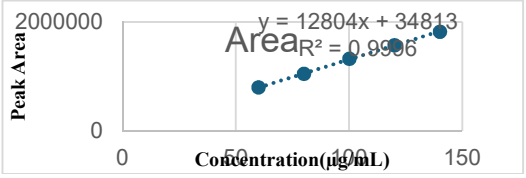


Figure 4: Linearity Calibration Curve of Sulfasalazine

III) Range

The linear working range of the method was identified as 60 – 140 µg/mL, where the method displayed good linearity, precision, and accuracy. Good linearity of the response vs concentration was achieved over the tested range due to the high value of the correlation coefficient (R^2 = 0.9996).

IV) Accuracy

The developed UPLC–MS method exhibited satisfactory accuracy, with recovery values ranging from 98.24% to 102.34%, indicating that the method is reliable for the quantitative estimation of sulfasalazine.

Table 5: Accuracy Results of Sulfasalazine by Recovery Studies

Amount of Drug Taken (mg)	Amount of Sample Added (mg)	Amount Found	Peak Area	% Recovery	Mean Recovery	SD	%RSD
80	20	19.81	288482.7033	99.05	98.70	0.35	0.36
80	20	19.73	287533.7033	98.68			
80	20	19.67	286681.5833	98.35			
80	40	39.01	534333.83	97.53	98.24	1.2	1.25

			33			3	
80	40	39.01	534414.9533	97.54			
80	40	39.87	545414.8333	99.67			
80	60	61.68	824615.2033	102.80	102.34	0.48	0.47
80	60	61.10	817254.5833	101.84			
80	60	61.42	821297.7033	102.37			

The overall mean recovery was 99.76 %, which meets ICH acceptance criteria (98–102% recovery, %RSD ≤ 2.0).

V)Precision

The developed analytical method showed consistent reults in both intraday and interday precision studies.

The developed analytical method produced consistent results in both intraday and interday precision studies. The %RSD values obtained of 0.065% and 0.055% were indicative of high precision and reproducibility of the method.

Repeatability (Intraday)

The intraday precision was evaluated by performing three replicate injections of Sulfasalazine at 100 µg/mL on the same day.

Table 6: Intraday Precision Results of Sulfasalazine

Sr No	Concentration (µg/ml)	Peak Area	Peak Height
1	100	1370325.25	84560232
2	100	1371611.3	84567680
3	100	1369867	84555520
Mean		1370601.21	
SD		904.33	
%RSD		0.065	

Acceptance Criteria: % RSD ≤ 2.0

Interday

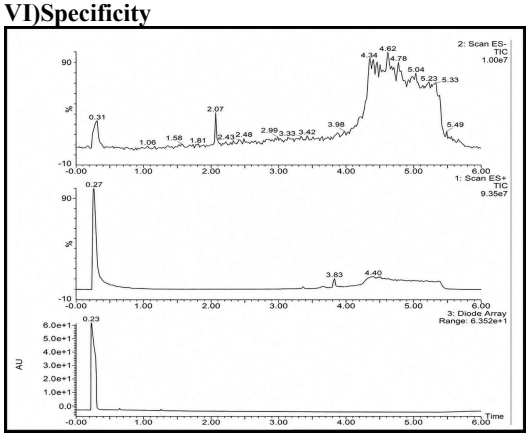
The interday precision was evaluated by performing three replicate injections of Sulfasalazine at 100 µg/mL on three different days.

Table 7: Interday Precision Results of Sulfasalazine

Sr No	Concentration (µg/ml)	Peak Area	Peak Height
1	100	1371611.38	84567680
2	100	1370079.38	84563888
3	100	1370775.25	84566176
Mean		1370822	
SD		767.06	
%RSD		0.055	

Acceptance Criteria: % RSD ≤ 2.0

VI)Specificity



The blank chromatogram showed no interference at the retention time of Sulfasalazine (1.64 min). A sharp and well-defined peak for Sulfasalazine was noted at 1.64 min in the standard chromatogram, proving that the UPLC-MS method developed is highly specific.

Figure 5: Chromotagramshowing Blank Preparation

Table 8: Specificity Results of Developed UPLC-MS Method

Parameter	Observation
Retention Time of Sulfasalazine	1.64 min(Figure 2)
Blank interference at analyte RT	Not observed

VII)Robustness

The robustness of the UPLC–MS method was determined through the alteration of parameters like flow rate and column temperature. Flow rate was varied from 0.45 to 0.55 ml/min while column temperature was varied from 28°C to 32°C. The %RSD values obtained in all cases were less than 2%, which indicated the stability of the method.

Table 9: Robustness Results of Sulfasalazine under Varied Analytical Conditions

Sr No	Parameter	Variations	Peak Area	SD	%RSD
1	Flow Rate (mL/min)	0.45	13616111.27	2081.64	0.15
		0.50	13410204.04	10661.26	0.79
		0.55	13344807.09	13100.40	0.98
2	Column Tempera	28	13404111.00	530.55	0.04

	ture (°C)	30	1341024.04	10661.26	0.79
		32	1350498.85	1454.41	0.10

Acceptance Criteria: % RSD ≤ 2.0

VIII)LOD and LOQ

The limit of detection (LOD) and limit of quantitation (LOQ) have been determined following ICH guidelines by using standard deviation of response and the slope of calibration curve. The LOD and LOQ have been calculated using the formulae LOD = 3.3σ/S and LOQ = 10σ/S. The obtained values of 0.18 µg/mL and 0.54 µg/mL show that the method of UPLC-MS developed is highly sensitive to detect and quantify Sulfasalazine.

Table 10: LOD and LOQ Results

Parameter	Value (µg/mL)	Acceptance Criteria
LOD	0.18	Low detectable concentration
LOQ	0.54	Quantifiable concentration

Analysis of Marketed Formulation

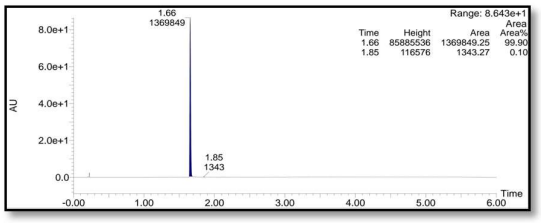


Figure 6: Chromatogram of Sulfasalazine Marketed Formulation

The optimized UPLC-MS method was also used to quantitatively determine the amount of Sulfasalazine present in commercially available Sulfasalazine tablets (Saaz 500 mg, IPCA Laboratories Ltd.). The assay values were found to be 99.35%, 99.87%, and 99.80%, with the mean assay value being 99.67% and a %RSD of 0.29%.

CONCLUSION

A novel rapid, simple, and highly sensitive UPLC-MS technique was developed and validated for the quantitative determination of Sulfasalazine in dosage forms. The developed method provided excellent linear response in the concentration range of 60-140 µg/mL with a correlation coefficient (R^2) of 0.9996. The validation studies revealed that the proposed method is reliable and provides high accuracy with a mean recovery of 99.76% and acceptable precision indicated by %RSD of intraday and interday analysis equal to 0.065% and 0.055%, respectively. Additionally, the robustness of the method was proved due to the absence of effect of the deliberate changes in the chromatographic conditions on the analytical results. The sensitivity of the method was confirmed by very low LOD (0.18 µg/mL) and LOQ (0.54 µg/mL) values. In order to prove the practical applicability of the method, the average assay value of 99.67% was obtained when the proposed method was used for the analysis of Sulfasalazine tablets from the market. Overall, the validated UPLC-MS method was found to be accurate, precise, robust, and sensitive, making it a suitable tool for the routine quality control and quantitative determination of Sulfasalazine in pharmaceutical formulations.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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