

Development and Validation of a Simple and Accurate RP-HPLC Method for Quantification of Zaltoprofen in Tablet Dosage Form

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Abstract—A simple, precise, accurate, and stability-indicating reverse-phase high performance liquid chromatography (RP-HPLC) method was developed and validated for the quantification of Zaltoprofen in tablet dosage form. Chromatographic separation was achieved on a C18 column (250 mm × 4.6 mm, 5 μm) using a gradient mobile phase of ammonium acetate buffer (pH adjusted with glacial acetic acid) and acetonitrile, at a flow rate of 1.0 mL/minute with UV detection at 240 nm. The method was validated as per ICH Q2(R1) guidelines for system suitability, precision, intermediate precision, specificity, accuracy, linearity, LOD, LOQ, and solution stability. The method showed excellent linearity ($r = 0.9943$) over the 80–120% concentration range, recovery values between 97.38% and 99.02%, and %RSD values within 2.0% for all precision parameters. Forced degradation studies under acidic, basic, neutral, oxidative, and photolytic conditions confirmed the stability-indicating capability of the method, with both S- and R-Zaltoprofen enantiomers well resolved from their degradation products; oxidative conditions produced the highest degradation. The developed method was found to be simple, accurate, sensitive, and suitable for routine quality control and stability analysis of Zaltoprofen in tablet formulations.

Index Terms—Zaltoprofen; RP-HPLC; Method validation; ICH Q2(R1); Stability indicating; Tablet dosage form.

I. INTRODUCTION

Zaltoprofen is a non-steroidal anti-inflammatory drug (NSAID) of the propionic acid derivative class, used in the management of osteoarthritis, rheumatoid arthritis, and postoperative pain. It acts as a preferential inhibitor of cyclooxygenase-2 (COX-2)

over COX-1¹, thereby reducing prostaglandin synthesis with a comparatively lower incidence of gastrointestinal adverse effects than non-selective NSAIDs. In addition to COX inhibition, Zaltoprofen has also been reported to suppress bradykinin-induced nociceptive signaling¹¹, contributing an additional analgesic mechanism distinct from other NSAIDs¹. Its effects on prostaglandin E2 production in synovial fibroblasts and proteoglycan biosynthesis in particular chondrocytes⁹, its pharmacokinetics in healthy volunteers¹⁰ and its effect on body-weight loss in rodent sickness-behaviour models¹² have also been investigated. Owing to its widespread clinical use, a simple, rapid and accurate analytical method is essential for the routine quality control estimation of Zaltoprofen in tablet dosage forms. Analytical method validation ensures reliable and reproducible results within a defined range, and according to ICH Q2(R1), the essential validation parameters include specificity, linearity, range, accuracy, precision, LOD, LOQ and robustness². Several analytical methods have previously been reported for the estimation of Zaltoprofen, including a UV-spectrophotometric method³, RPHPLC methods for Zaltoprofen alone and in combination with other drugs^{4,5,6,14,15}, a chiral HPLC method for enantioselective analysis⁷, and a bio-analytical RPHPLC method for estimation of Zaltoprofen in human plasma⁸. Most of the previously reported RP-HPLC methods for Zaltoprofen have employed isocratic elution with limited stress-degradation profiling^{5,13} or have been developed primarily for bio-analytical estimation in plasma rather than for routine tablet quality control⁸. In contrast, the present method employs a gradient

elution system optimized specifically for tablet dosage form analysis, combining routine assay estimation with a stability-indicating capability that resolves both S- and R-Zaltoprofen enantiomers from their degradation products, offering a more comprehensive and application-focused approach for pharmaceutical quality control. The present study describes the development and validation of a simple, accurate RP-HPLC method for the estimation of Zaltoprofen in tablet dosage form, in accordance with ICH Q2(R1) guidelines.

II. MATERIALS AND METHODS

Chemicals and Reagents

Zaltoprofen working standards were procured from local market. Acetonitrile and ammonium acetate (HPLC grade) and glacial acetic acid (AR grade) were obtained from E. Merck Pvt. Ltd. Purified water was used throughout the study. Marketed Zaltoprofen tablets (Zaltoken®, label claim: 80 mg/tablet) were procured from the local market.

The chemical structure of Zaltoprofen is shown in Fig. 1.

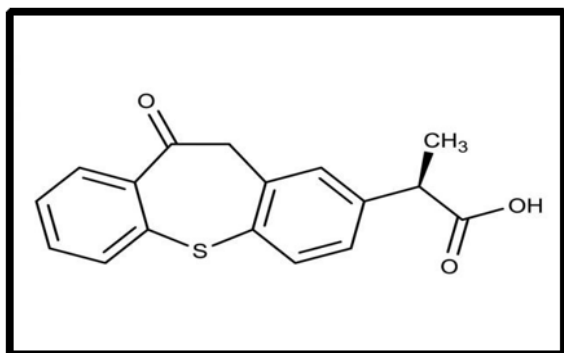


Fig:1 Structure of Zaltoprofen

Instrumentation

Chromatographic analysis was performed on a Shimadzu HPLC system equipped with LC Solutions software and a UV detector, using a Generic C18 column (250 mm × 4.6 mm, 5.0 µm particle size).

Chromatographic Conditions

The chromatographic separation was performed using a C18 column (250 mm × 4.6 mm i.d., 5 µm particle size). The mobile phase consisted of two components, mobile phase A, comprising ammonium acetate buffer prepared by dissolving 0.7791 g of

ammonium acetate in 1000 mL of water and adding 0.1 mL of glacial acetic acid, followed by mixing with acetonitrile in a ratio of 60:40 (v/v), and mobile phase B, consisting of acetonitrile. The separation was achieved using a gradient elution program as described below. The flow rate was maintained at 1.0 mL/min, with UV detection performed at 240 nm. An injection volume of 20 µL was used, and the total chromatographic run time was 35 minutes.

Optimized chromatographic conditions

The chromatographic conditions were optimized through systematic evaluation of different mobile phase compositions, including aqueous–organic combinations at varying ratios and pH conditions. The flow rate, detection wavelength, and injection volume were subsequently optimized to achieve a symmetrical Zaltoprofen peak, adequate separation from potential excipient interference, and satisfactory system suitability parameters. The optimized chromatographic conditions were selected based on the overall chromatographic performance and are presented above. The optimized gradient elution program was initiated with 100% mobile phase A and 0% mobile phase B from 0 to 7 min. The composition was then changed to 55% mobile phase A and 45% mobile phase B at 8 min and maintained until 24 min. Subsequently, the proportion of mobile phase A was reduced to 10%, while mobile phase B was increased to 90% at 25 min, and this composition was maintained until 29 min. At 29.1 min, the mobile phase composition was returned to the initial condition of 100% mobile phase A and 0% mobile phase B to allow column re-equilibration. The initial composition was maintained until the end of the 35-min run. This gradient program provided suitable chromatographic separation and was used for the subsequent method development and validation studies.

Preparation of Standard, Sample, Placebo and Blank Solutions

Standard solution: A weight of 12.5mg of Zaltoprofen was accurately measured and transferred into 100ml volumetric flask and dissolved in about 20 mL methanol, and made up to volume with the solvent mixture (ammonium acetate buffer: acetonitrile, 30:70 v/v). This stock solution was further diluted (5 mL to 25 mL) with the solvent mixture to obtain the working standard solution.

Sample solution: Twenty pills were ground into a fine powder after being weighed. A 100 mL volumetric flask was filled with an amount of powder equal to 25 mg of zaltoprofen, followed by the addition of 70 mL of a solvent combination, sonication for 15 minutes, volume adjustment, mixing, and centrifugation. Before injection, the solution was further diluted (5 mL to 25 mL) with the solvent combination after passing through a 0.45 μ m nylon filter.

Placebo solution: An amount of placebo blend equivalent to the excipient content of one tablet was accurately weighed and transferred into a 100 mL volumetric flask, dissolved/dispersed in the solvent mixture following the same procedure as the sample solution, mixed, and centrifuged. This solution was used for specificity studies.

Blank solution: The solvent mixture (ammonium acetate buffer: acetonitrile) used for sample and standard preparation was employed as the blank.

III. RESULTS AND DISCUSSION

Method Development and Optimization

Preliminary trials were carried out with different mobile phase combinations of acetonitrile, methanol and water, with and without buffer/pH adjustment, to identify a system capable of resolving the Zaltoprofen peak with acceptable symmetry and retention. An ammonium acetate buffer–acetonitrile system was found to give the best peak shape and was therefore selected. To achieve adequate resolution of the analyte peak from formulation excipients within a reasonable analysis time, the mobile phase composition was optimized using gradient elution rather than an isocratic mode. The flow rate, detection wavelength, and injection volume were likewise optimized to 1.0 mL/minute, 240 nm and 20 μ L respectively, based on their effect on peak shape, sensitivity and system backpressure.

IV. METHOD VALIDATION

System Suitability

System suitability parameters were evaluated on the standard solution prior to validation. The method gave

a tailing factor of 1.053 and column efficiency of 4271 theoretical plates, both of which complied with the acceptance criteria (tailing factor NMT 2.0; theoretical plates NLT 2000), confirming the suitability of the chromatographic system for the intended analysis.

Precision

System precision was assessed by five replicate injections of the standard solution, and method precision was evaluated using six independent sample preparations. Intermediate precision was studied by a second analyst on a different day and column.

Table 1. Precision and intermediate precision results

Precision parameter	Acceptance criteria	Result (%RSD)
System precision	NMT 2.0%	0.25%
Method precision	NMT 2.0%	0.65%
Intermediate precision – System precision	NMT 2.0%	0.21%
Intermediate precision – Method precision	NMT 2.0%	1.62%

As shown in Table 1, all %RSD values were well within the acceptance limit of 2.0%, demonstrating that the method is repeatable and reproducible under normal and varied analytical conditions.

Specificity

The retention time of Zaltoprofen in the sample preparation was comparable to that in the standard preparation, and the blank/placebo solution showed no interfering peak at the retention time of Zaltoprofen. These results confirm that the method is specific for the estimation of Zaltoprofen in the presence of tablet excipients. The specificity results are presented in Table 2.

Table 2. Specificity Result

S. No	Name of the test solution	Retention time (min)	Peak area (Inj. 1)	Peak area (Inj. 2)	Mean peak area	Calculated result (%)	Remarks
1	Blank	28.1	ND	ND	NA	NA	Complies
2	Placebo	28.1	ND	ND	NA	NA	
3	Standard solution	28.1	478968	479222	479095	NA	
4	Sample solution	28.1	498469	495304	496887	100.40	

Accuracy

Accuracy was assessed by recovery studies at 80%, 90%, 100%, 110% and 120% of the target concentration, performed in triplicate at each level by spiking placebo with known amounts of Zaltoprofen.

Table 3. Recovery results

Spike level	% Recovery (mean)
80%	98.08%
90%	97.38%
100%	97.93%
110%	98.56%
120%	99.02%

As shown in Table 3, recovery at all levels was within the acceptance criterion of 95.0–105.0%, confirming that the method is accurate and unaffected by the presence of formulation excipients.

Linearity

Linearity was established over the 80–120% concentration range (equivalent to approximately

19.94–29.90 µg/mL), using the peak area data compiled from the accuracy study.(As shown in table 4)

Table 4. Linearity data

Concentration level	Concentration (µg/mL, approx.)	Mean peak area
80%	19.94	403713
90%	22.43	460179
100%	24.92	501825
110%	27.41	530625
120%	29.90	588521

A linear relationship was observed between concentration and peak area over the studied range, with a regression equation of $y = 17659x + 56910$ and a correlation coefficient (r) of 0.9943, complying with the acceptance criterion of $r \geq 0.99$. The linearity plot is shown in Fig. 2.

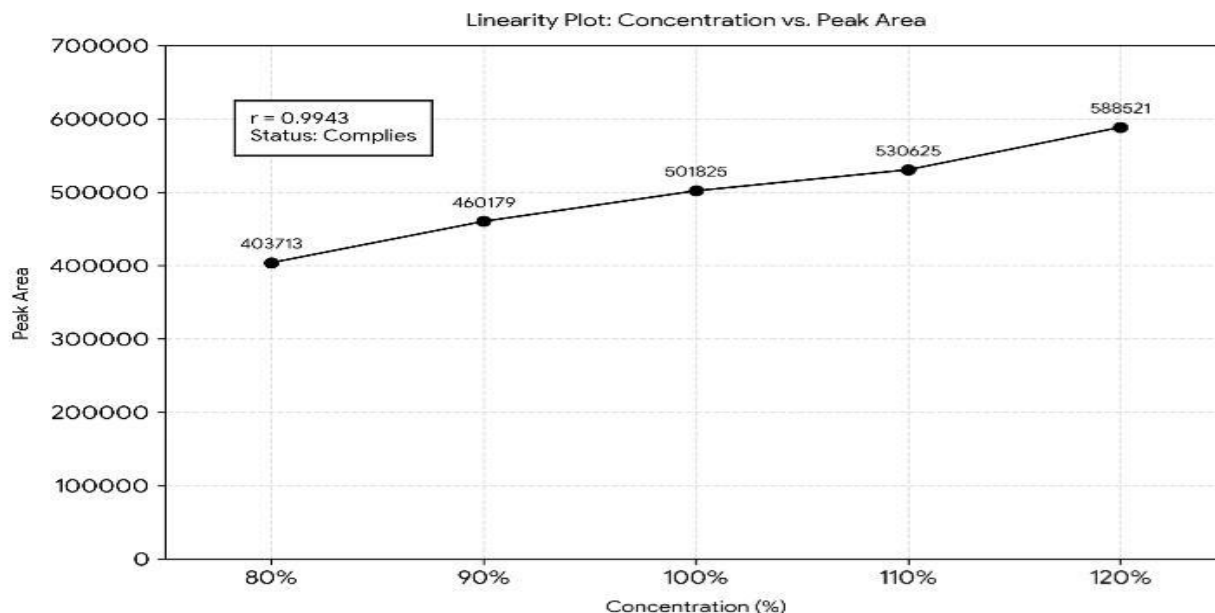


Fig:2 Linearity Plot of Zaltoprofen

Limits of Detection (LOD) and Quantification (LOQ) LOD and LOQ were calculated using the response's standard deviation and the calibration curve's slope, as per ICH Q2(R1): $LOD = 3.3\sigma/S$ and $LOQ = 10\sigma/S$, where σ is the residuals' standard deviation and S is the calibration curve's slope obtained from the linearity data (Table 4).

The residual standard deviation (σ) was derived from the standard deviation of the residuals of the linear regression line (peak area versus concentration, $n = 5$), calculated using $n-2$ degrees of freedom. The calculated LOD and LOQ values are presented in Table 5.

Table 5. LOD and LOQ (regression method)

Parameter	Value
Slope (S)	17659
Residual SD (σ)	8617.9
LOD	1.61 $\mu\text{g/mL}$
LOQ	4.88 $\mu\text{g/mL}$

Note: The linearity data were used to estimate LOD and LOQ using the regression approach.

Solution Stability

Solution stability was assessed by comparing duplicate injections of the standard solution at defined time intervals against freshly prepared standard. The difference in response between the freshly prepared and stored standard solution was 0.39%, 0.41% and -0.13% on Day 2, Day 3 and Day 6, respectively, all complying with the acceptance criteria, indicating that the standard solution remains stable for up to 6 days when stored under the prescribed conditions.

STABILITY STUDIES FOR ZALTOPROFEN

Forced degradation studies were carried out on Zaltoprofen standard solution (100 $\mu\text{g/mL}$) under acidic (0.1 N HCl), basic (0.1 N NaOH), neutral (water), oxidative (3% H_2O_2) and photolytic (sunlight; powder and solution form) stress conditions. Samples were withdrawn at intervals up to 24 h and analyzed under the optimized chromatographic conditions, and the percentage degradation of S- and R-Zaltoprofen was calculated from the peak area response. The optimized conditions provided satisfactory separation of S- and R-Zaltoprofen from their degradation products.

The results are summarized in Table 6. Oxidative stress produced the highest degradation (36.11% for S- and 49.93% for R-Zaltoprofen), followed by basic (14.53% and 13.69%) and acidic (7.75% and 8.45%)

conditions. Under neutral conditions, degradation was low for S-Zaltoprofen (1.49%) but comparatively higher for R-Zaltoprofen (6.14%). Photolytic degradation was minimal in the powder form (0.21% and 0.24%) and slightly higher in the solution form (0.98% and 1.61%). The degradation products were adequately separated from the intact enantiomeric peaks, confirming the stability-indicating capability of the developed method.

Table 6. Forced Degradation Study

Stress Condition	Degradation of S-Zaltoprofen (%)	Degradation of R-Zaltoprofen (%)
Acidic (0.1 N HCl, 24 h)	7.75	8.45
Basic (0.1 N NaOH, 24 h)	14.53	13.69
Neutral (Water, 24 h)	1.49	6.14

Oxidative (3% H ₂ O ₂ , 24 h)	36.11	49.93
Photolytic – Powder (Sunlight, 24 h)	0.21	0.24
Photolytic – Solution (Sunlight, 24 h)	0.98	1.61

The chromatograms presented in Fig. 3–10 demonstrate the separation of S- and R-Zaltoprofen from the degradation products under acidic, basic, neutral, oxidative and photolytic stress conditions. The results confirm that the developed method is capable of effectively monitoring Zaltoprofen degradation and demonstrating its stability-indicating capability.

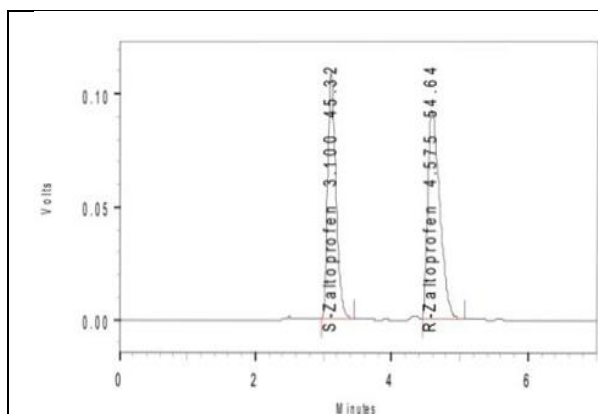


Fig 3

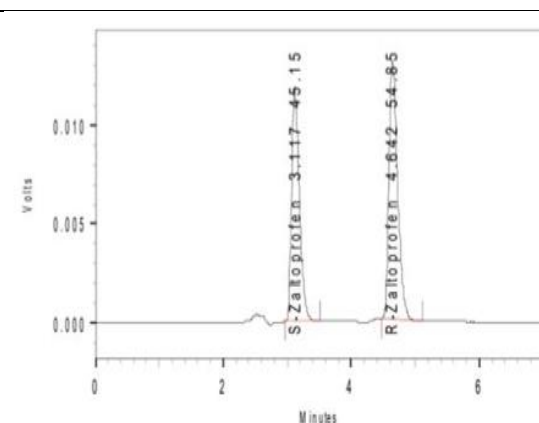


Fig 4

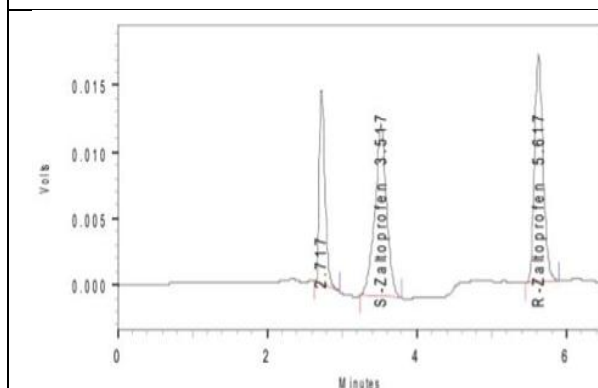


Fig 5

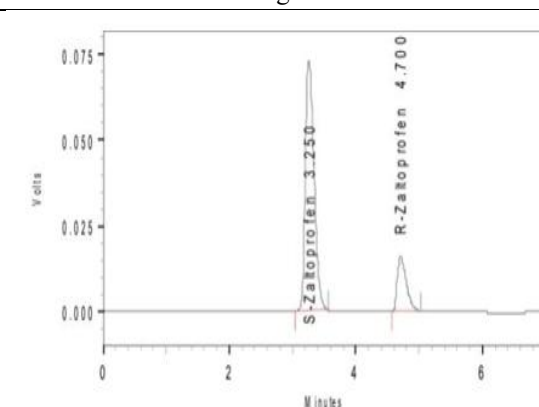


Fig 6

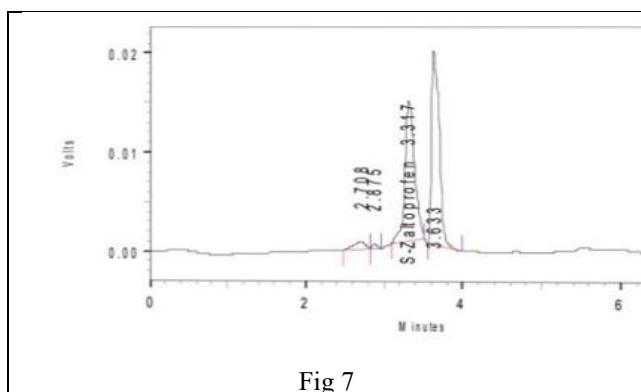


Fig 7

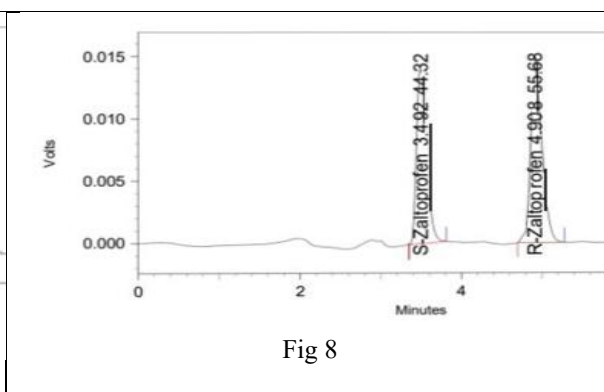


Fig 8

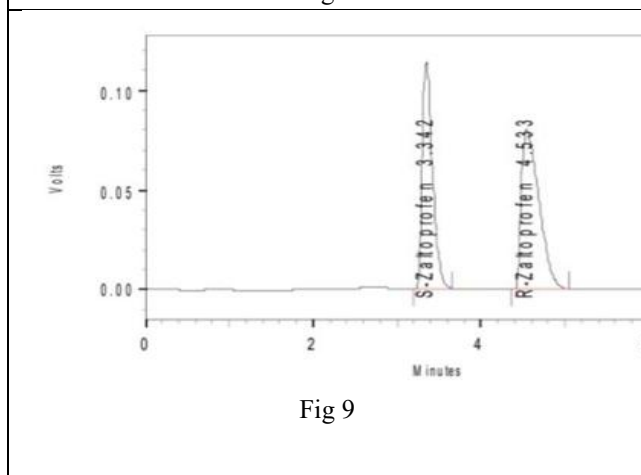
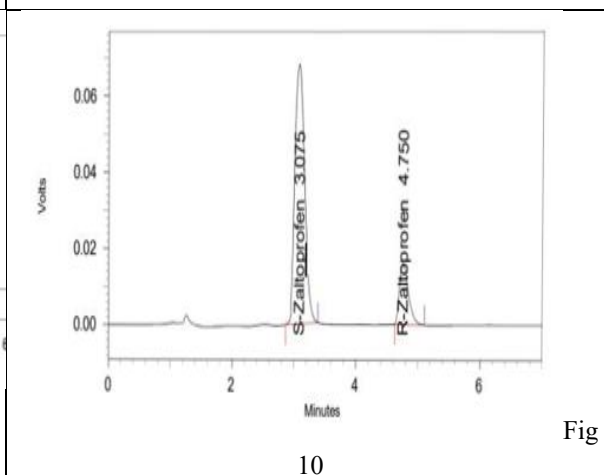


Fig 9



Fig

10

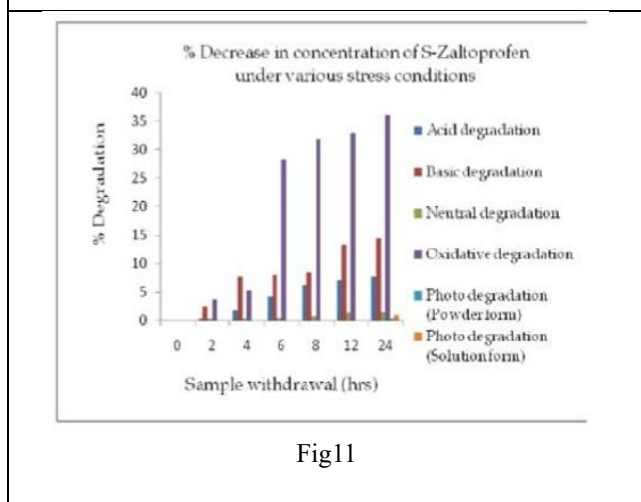


Fig11

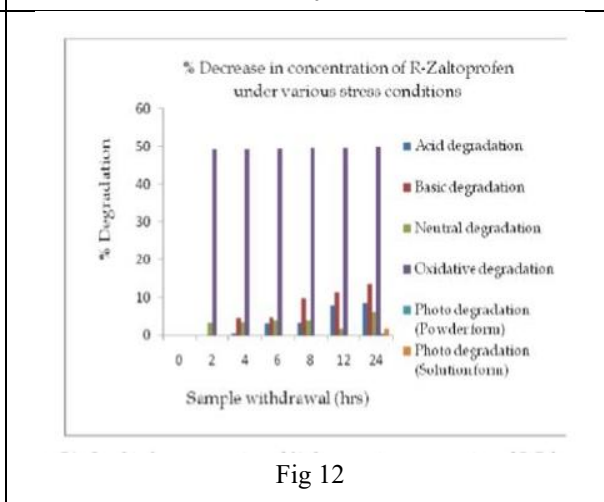


Fig 12

Fig. 3: Zaltoprofen Standard Solution. Fig. 4: Chromatogram of Sample Containing Zaltoprofen Fig. 5: Acidic Degradation of Zaltoprofen (R&S) at 24 Hours Fig. 6: Oxidative Degradation of Zaltoprofen (R&S) with 3% H₂O₂ at 24 Hours Fig. 7: Basic Degradation of Zaltoprofen (R&S) with 0.1 N NaOH at 24 Hours Fig. 8: Neutral Degradation of Zaltoprofen (R&S) with Water at 24 Hours Fig. 9: Photo Degradation of Zaltoprofen (R&S) at 24 Hours

(Powder Form) Fig 10: Photo Degradation of Zaltoprofen (R&S) with Sunlight at 24 Hours (Solution Form) Fig. 11: Percentage Decrease in Concentration of S-Zaltoprofen Under Various Stress Conditions Fig. 12: Percentage Decrease in Concentration of R-Zaltoprofen Under Various Stress Conditions

The results presented in Fig. 11 & 12 demonstrate the chromatographic performance of Zaltoprofen, supporting the suitability of the developed method for its analysis.

V. DISCUSSION

The developed RP-HPLC method demonstrated satisfactory analytical performance for the quantitative determination of Zaltoprofen in tablet dosage form. The system suitability parameters, including tailing factor and theoretical plate count, confirmed adequate peak symmetry and chromatographic efficiency under the optimized conditions. The low %RSD values obtained during system precision, method precision, and intermediate precision studies demonstrated good repeatability and reproducibility, supporting the reliability of the method for routine analysis. Specificity studies confirmed the absence of significant interference from blank and placebo components at the retention time of Zaltoprofen, thereby establishing the selectivity of the method. The accuracy studies showed acceptable recoveries across the evaluated concentration range, indicating minimal influence of the formulation matrix on drug estimation. The method exhibited satisfactory linearity over the studied range, with an r value of 0.9943, along with adequate sensitivity based on the calculated LOD and LOQ values. Solution stability studies further confirmed the suitability of the standard solution for laboratory applications. Importantly, forced degradation studies established the stability-indicating capability of the method, with effective separation of Zaltoprofen from its degradation products under different stress conditions. The comparatively higher degradation observed under oxidative conditions suggests increased susceptibility of Zaltoprofen to oxidative degradation. Overall, the validated method provides a reliable, precise, accurate, sensitive, and stability-indicating analytical procedure for routine quality control and stability assessment of Zaltoprofen tablet formulations.

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