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Development and validation of RP-HPLC method for determination of Zinc Pyrithione in shampoo

Snehal Shinde[1*], Pratima Shinde[1], Ganesh Lamkhade[2], Vikas Kandekar[3], Rahul Dumbre[1], Minakshi Londhe[4], Gayatri Bhadange[4], Neha Parate[4]

[1]Siddhant College of Pharmacy, Sudumbre, Pune, Maharashtra 410501, India.

[2]Samarth Rural Educational Institute's Samarth Institute of Pharmacy, Belhe, Tal, Junnar, Maharashtra, 412410, India.

[3]Citron Life Sciences Pvt. Ltd. Sr. No.: 392 A/P: Nepti Tal: Nagar District: Ahmednagar Maharashtra 414004, India.

[4]Kasturi Shikshan sanstha college of pharmacy, P42P+8F6, Pratima Nagar Pabal Chowk, Pune-Nagar Road, Shikrapur, Shirur, Pune, Maharashtra 412208, India.

Abstract

Zinc Pyrithione functions as both an antifungal and antibacterial agent, disrupting membrane transport by inhibiting the proton pump responsible for energizing the transport mechanism. Its versatile applications encompass the treatment of various conditions, including psoriasis, eczema, ringworm, fungus, athlete's foot, dry skin, atopic dermatitis, tinea, and vitiligo. For the quantitative analysis of Zinc Pyrithione in commercial formulations, a rapid and sensitive reversed-phase high-performance liquid chromatographic gradient method was developed and validated. In this method, high-performance liquid chromatography utilized a Zobrax Extend C18 column (250mm x 4.6 mm, 5 µm, 80Å). The mobile phase comprised a phosphate buffer at pH 3.5 and Acetonitrile-Methanol. Detection was achieved using a UV-Visible detector at 254 nm, with a flow rate maintained at 1.0 ml/min. Rigorous validation of the method included assessments for linearity, precision, accuracy, specificity, robustness, and statistical analysis of the obtained data. The calibration curve exhibited linearity within a concentration range of 1–300 µg/mL, with a retention time for Zinc Pyrithione at 7.71 min and a regression coefficient value of 0.997. The limit of detection and limit of quantification were determined as 1.944 and 5.891 µg/mL, respectively. Precision assessment revealed a % RSD (Relative Standard Deviation) of less than 1%, demonstrating the method's precision. Additionally, a recovery study indicated a recovery range of 98 to 102%, with a % RSD less than 1%. This thorough evaluation not only underscores the accuracy and precision of the method but also highlights its sensitivity, solidifying its status as a reliable approach for the precise analysis of Zinc Pyrithione.

Keywords: Zinc Pyrithione, Method Development, Method Validation.

Full length article **Corresponding Author*, e-mail: snehals280699@gmail.com

1. Introduction

Zinc Pyrithione, known chemically as bis (2-pyridylthio) zinc 1,1'-dioxide, forms a coordination complex with zinc, showcasing remarkable properties as an antifungal and antibacterial agent. This colorless solid, featuring pyrithione ligands formally regarded as monoamines, coordinates with Zn^{2+} through oxygen and sulfur centers. In its crystalline state, Zinc Pyrithione adopts a centrosymmetric dimeric structure (Figure 1), where each zinc is bonded to two Sulfur and three oxygen centers. However, in solution, the dimers disassociate by breaking one Zn-O bond. Renowned for its efficacy in treating dandruff and seborrhea dermatitis. Zinc Pyrithione also exhibits potent antibacterial properties effective against various pathogens, including those from the streptococcus and staphylococcus classes. Its medical applications extend to treating psoriasis, eczema, ringworm, fungus, athlete's foot, dry skin, atopic dermatitis, tinea, and vitiligo. With low water solubility (8 ppm at neutral pH), Zinc Pyrithione finds utility in outdoor paints and other products providing mildew and algae protection.

Acting as an effective algaecide, it is incompatible with paints relying on metal carboxylate curing agents. Sequestering agents binding iron ions become necessary when used in latex paints with iron-rich water. Importantly, its slow decomposition by ultraviolet light ensures prolonged protection, even against direct sunlight. The antifungal activity of Zinc Pyrithione arises from its ability to disrupt membrane transport by blocking the proton pump that energizes the transport mechanism. In our literature review, we identified approximately 7 to 8 works related to our paper. Despite similarities, our work introduces distinct parameters such as the mobile phase, column, and temperature. These modifications were implemented to conduct an assay yielding superior results compared to existing studies. Therefore, we chose Reverse Phase High-Performance Liquid Chromatography to determine Zinc Pyrithione in a Marketed shampoo [1-10].

2. Materials and methods

2.1. Reagents and chemicals

The reference standard for Zinc Pyrithione was provided by Kumar Organic Products Limited. The pharmaceutical dosage form of Zinc Pyrithione, Scalpe+, boasting a 1%w/w label claim, was obtained from a local pharmacy and is manufactured by Glenmark Pharmaceutical Ltd, India. All chemicals utilized in the study were of HPLC grade, with Methanol, Acetonitrile, and water sourced from Merck Life Science. Additionally, Orthophosphoric Acid 85% was obtained from Merck Life Science, while Potassium Dihydrogen Phosphate and Disodium Edetate were acquired from Loba Chemie Pvt Ltd.

2.2. Instrument and chromatographic condition

The experimental setup employed the Hitachi L-2455 Elite LaChrom HPLC system, featuring a gradient pump with a flow range spanning from 0.001 to 9.999 mL/min. Chromatographic separation was carried out using a Zorbax Extend octa decyl silane C-18 column (250X4.6X5µ). The system was seamlessly integrated with EZChrom Software and included a double beam photometer illuminated by a deuterium lamp, offering a wavelength range of 190-600 nm along with an integrated degasser. The gradient system incorporated Mobile Phase A, a buffer with a pH of 3.5, and Mobile Phase B, consisting of Acetonitrile and Methanol. The flow rate was consistently maintained at 1 mL/minute, and the wavelength for chromatographic separation was set at 254 nm. The injection volume remained constant at 20 µl, and the column temperature was meticulously maintained at 30°C. Prior to use, the mobile phase underwent purification by passing through a 0.45 µm micron filter (Table 2).

2.3. Preparation of standard solution

For the preparation of a Zinc Pyrithione stock solution with a concentration of 625 mg/mL, Zinc Pyrithione Reference Standard was dissolved in dimethyl sulfoxide. Following this, a subsequent dilution step was carried out by using dimethyl sulfoxide and water in a 70:30, v/v ratio which resulted in the production of a 250 ppm Zinc Pyrithione solution for the trial batches.

2.4. Preparation of sample solution

For the preparation of the test sample stock solution at a concentration of 2500 mg/mL, the shampoo test sample was dissolved in dimethyl sulfoxide. Subsequently, the sealed solution was thoroughly shaken and filtered through Whatman filter paper No.1. Following this, a subsequent dilution step was carried out by using dimethyl sulfoxide and water in a 70:30, v/v ratio resulting in a 250 ppm Zinc Pyrithione solution. Finally, 20 µL of this solution was injected into the HPLC column for analysis.

2.5. Analytical method development

Throughout the trial experiments, we explored various chromatographic columns, including Inertsil ODS 3 V (150 mm×4.6 mm, 5 µm), Inertsil ODS (250 mm×4.6 mm, 5 µm), and Sunniest C18 column (250 mm×4.6 mm, 5 µm). Isocratic elution was employed, utilizing solvent combinations such as ACN (acetonitrile) /MeOH (methanol) in a 60:40 ratio, 0.1% aqueous formic acid/MeOH (methanol) in a 40:60 ratio, and Buffer pH 3.5 and ACN (acetonitrile) in a 60:40 ratio. Additionally, a Zorbax Extend C18 column (250 mm×4.6 mm, 5 µm, 80Å) was tested with gradient elution using Buffer pH 3.5 /ACN (acetonitrile)-MeOH (methanol) as the mobile phase. For the proper extraction of the drug from the formulation, experimentation involved different diluents, such as ACN (acetonitrile)-Water in a 60:40 ratio, Dimethyl Sulphoxide-Water in a 50:50 ratio, and Dimethyl Sulphoxide-Water in a 70:30 ratio during the further dilution process. Throughout the trials, the sample volume for analysis, temperature, and flow rate were maintained at 20 µL, 30°C, and 1.0 mL/min, respectively. Ultimately, the Zorbax Extend C18 column (250 mm×4.6 mm, 5 µm, 80Å) with gradient elution using Buffer pH 3.5 (Phase I) /ACN (acetonitrile)-MeOH (methanol) (Phase II) combination, along with diluent Dimethyl Sulphoxide-Water (70:30 ratio), emerged as the optimal condition for the identification and estimation of Zinc Pyrithione. This decision was based on the achieved resolution, peak shape, and sensitivity values observed during the trials. The chosen gradient program was as follows: 0 min (75% Phase I and 25% Phase II), 6 min (75% Phase I and 25% Phase II), 15 min (25% Phase I and 75% Phase II), 20 min (25% Phase I and 75% Phase II), 22 min (75% Phase I and 25% Phase II), and 28 min (75% Phase I and 25% Phase II). Zinc Pyrithione was monitored at 254 nm, as this wavelength demonstrated the highest sensitivity for the compound [11-22].

2.6. Analytical method validation

2.6.1. Linearity

The standard stock solution of Zinc Pyrithione was created by dissolving the Zinc Pyrithione reference standard in 50 ml of dimethyl sulphoxide, yielding a concentration of 625 µg/ml. Linearity was demonstrated across the concentration range of 200 µg/ml to 300 µg/ml.

2.6.2. Precision studies

Precision studies were conducted through three distinct approaches: intraday precision, inter-day precision, and repeatability. Intraday Precision involved assessing precision for a single concentration at 250 µg/ml over the course of a single day at various time intervals (Morning, afternoon, evening). Interday Precision was evaluated over multiple days (day-1, day-2, and day-3) for a single concentration at 250 µg/ml.

Repeatability Studies were carried out by analyzing one concentration six times, specifically focusing on the concentration of Zinc Pyrithione at 250 µg/ml.

2.6.3. Recovery/accuracy studies

Recovery studies were performed by spiking the sample with the standard and then finding out the recovery at 80,100,120 percent. In this a chunk of standard drug was mixed to the placebo sample and find out recovered amount from sample preparation as % recovery.

2.6.4. Preparation of standard solution

For the preparation of a Zinc Pyrithione stock solution with a concentration of 625 mg/mL, Zinc Pyrithione Reference Standard was dissolved in dimethyl sulfoxide. Following this, a subsequent dilution step was carried out by using dimethyl sulfoxide and water in a 70:30, v/v ratio which resulted in the production of a 250 ppm Zinc Pyrithione solution for the trial batches.

2.6.5. Preparation of sample solution

Solutions of 80%, 100% and 120% were prepared by using the shampoo placebo sample and Zinc Pyrithione Reference Standard. Finally, 20 µL of each solution lotion injected into the HPLC column for analysis.

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2.6.6. Limit of detection (LOD)

For analytical methods characterized by baseline noise, the Limit of Detection (LOD) can be determined using a signal-to-noise ratio of 3:1. This ratio is typically expressed as the concentration (e.g., percentage) of the analyte in the sample. The LOD calculation involves injecting three repetitions of 200 µg/ml Zinc Pyrithione, followed by regression analysis. Subsequently, the standard deviation (SD)I is calculated and the slope is utilized to compute the LOD.

$$LOD = \frac{3.3 \times SD}{Slope}$$

2.6.7. Limit of quantitation (LOQ)

For analytical methods characterized by baseline noise, the Limit of Quantitation (LOQ) can be established using a signal-to-noise ratio of 10:1. This ratio is typically expressed as the concentration (e.g., percentage) of the analyte in the sample. The LOQ determination involves injecting three repetitions of 200 µg/ml Zinc Pyrithione, followed by regression analysis. Subsequently, the standard deviation (SD) is calculated, and the slope is utilized to determine the LOQ.

$$LOQ = \frac{10 \times SD}{Slope}$$

2.7. Assay studies

2.7.1. Reagents and chemicals

The Kumar Organic Products Limited provided the Zinc Pyrithione reference standard. The pharmaceutical dosage form of Zinc Pyrithione, known as Scalpe+, with a label claim of 1%w/w, was obtained from a local pharmacy. This product is manufactured by Glenmark Pharmaceutical Ltd in India. All the chemicals utilized, including HPLC grade Methanol, Acetonitrile, and water, were sourced from Merck Life Science. Orthophosphoric Acid 85% was also obtained from Merck Life Science, while Potassium Dihydrogen Phosphate and Disodium Edetate were procured from Loba Chemie Pvt Ltd.

2.7.2. Preparation of standard solution

For the preparation of a Zinc Pyrithione stock solution with a concentration of 625 mg/mL, Zinc Pyrithione Reference Standard was dissolved in dimethyl sulfoxide. Following this, a subsequent dilution step was carried out by using dimethyl sulfoxide and water in a 70:30, v/v ratio which resulted in the production of a 250 ppm Zinc Pyrithione solution for the trial batches.

2.7.3. Preparation of sample solution

For the preparation of the test sample stock solution at a concentration of 2500 mg/mL, the shampoo test sample was dissolved in dimethyl sulfoxide. Subsequently, the sealed solution was thoroughly shaken and filtered through Whatman filter paper No.1.Following this, a subsequent dilution step was carried out by using dimethyl sulfoxide and water in a 70:30, v/v ratio resulting in a 250 ppm Zinc Pyrithione solution. Finally, 20 µL of this solution was injected into the HPLC column for analysis. Finally, inject working standard preparation and working sample preparation for assay analysis.

2.7.4. Specificity

To analyze the specificity of the method different chromatograms was obtained for sample, placebo, blank and standard to ascertain that the method is specific or not or is there any interference from any impurities or excipients.

2.7.5. Robustness study

Robustness studies was performed by injecting working standard preparation for different wavelength, flow rate, different buffer pH and column temperature. Wavelength from +0.2units to -0.2units, Flow rate was changed from +0.2ml/minute to -0.2ml/minute, Buffer pH from+0.2 pH to -0.2pH and column temperature from +2°C to -2°C.

3. Results and discussion

3.1. Method development and optimization

Numerous experiments were conducted, exploring different columns, mobile phases, and pH levels in order to identify optimal chromatographic conditions with desirable peak characteristics. From the variety of columns tested, the Sunniest C18 column (250 mm × 4.6 mm, 5 µm), combined with a mobile phase composed of Acetonitrile-Water in a 60:40 ratio, and a flow rate of 1 mL/min, was determined to provide satisfactory peak symmetry for Zinc Pyrithione, as illustrated in Figure 2. By making a few adjustments to the chromatographic conditions, including switching to a Zorbax Extend C18 column (250 x 4.6 mm, 5 µm, 80Å), modifying the mobile phase to a composition of phosphate buffer (pH 3.5) and acetonitrile-methanol, maintaining a constant flow rate of 1.0 mL/min, and implementing a gradient program outlined in Table 1, we observed excellent resolution and peak separation, as depicted in Figure 3. Under optimized chromatographic conditions, a notable reduction in retention

time to 7.75 minutes was observed, a significant improvement compared to reported values in the literature.

This reduction has the potential to enhance efficiency and reduce both the run time and analysis cost for samples. All reagents utilized are readily available. While the mobile phase composition resembled that of various reported methods, featuring phosphate buffer and acetonitrile, the retention time for Zinc Pyrithione was notably decreased, shifting from 9.58 minutes to 7.75 minutes.This reduction in retention time could be attributed to the use of columns with different dimensions, such as 50 x 4.6 mm, 3.5 µm, 150 x 4.6 mm, 3.5 µm, and 250 × 4.6 mm, 5 µm. Specifically, analytical columns of 150 x 4.6 mm, 3 µm, reported a retention time of 6.9 minutes, while those of 75 x 4.6 mm, 3.5 µm, showed a retention time of 6.45 minutes. Notably, the investigation found that a mobile phase pH of 3.5 yielded optimal results, whereas higher pH values in reported methods led to peak splitting. A flow rate of 1.0 mL/min was identified as optimal for achieving proper retention time for Zinc Pyrithione. Higher flow rates were observed to contribute to further reduction in retention time, offering the potential to expedite analysis. Implementation of the gradient elution method resulted in improved resolution, enhanced detection, shorter analysis time, and increased overall efficiency.

3.2. Linearity

Figure 4 displays the linearity graph, while Table 3 presents the concentration versus area data for linearity. The method demonstrated linearity within the range of 200 µg/ml to 300 µg/ml.

3.3. Precision studies

Precision studies were conducted, revealing that the method exhibited precision in terms of intraday, inter-day, and repeatability determinations. In Table 4-6, the intraday precision figures are presented, showcasing % R.S.D within acceptable limits.

3.4. Intraday precision

Table 4-6 show the area, average area, standard deviation and %RSD at 250 µg/ml for intraday precision.

3.5. Inter-day precision

Table 7-9 show the area, average area, standard deviation and % RSD at 250 µg/ml for inter-day precision.

3.6. Repeatability studies

Table 10 show the area, average area, standard deviation and % RSD at 250 µg/ml. The method was found to be precise with the repeatability studies.

3.7. Recovery studies

Results obtained from recovery studies are given in Table 11 with average recovery found out to be 108.98, 109.95 and 102.52 respectively, standard deviation was 0.6109, 0.5543 and 0.7881 and % R.S.D was 0.561, 0.504 and 0.769. Percentage recovery was found to be within limits.

3.8. Limit of detection (D.L)

The limit of detection was found out to be 1.944.

3.9. Limit of quantitation (Q.L)

The limit of quantitation was found out to be 5.891.

3.10. Assay studies

Assay results are given in Table 12 which shows the area of standard, area of samples, % assay, average assay, standard deviation and % R.S.D of assay of Zinc Pyrithione.

3.11. Specificity

To assess the method's specificity, chromatograms were generated for the blank, standard, sample, and test, as depicted in Figures 5-8. The observation revealed the absence of any interference from impurities and excipients in the developed method.

3.12. Robustness studies

The robustness study was conducted, affirming the method's robust nature. Flow rate data presented in Table 13 & Table 14 highlight variations in flow rate by increments or decrements of 0.2 at a concentration of 250 µg/ml. Additionally, Tables 15 & 16 illustrate the impact of a temperature change of 2℃ at the same concentration, while Tables 17 & 18 detail the effect of altering pH by increasing or decreasing by 0.2. Lastly, Tables 19 & 20 outline the consequences of a change in wavelength by increasing or decreasing by 2 units at a concentration of 250 µg/ml.

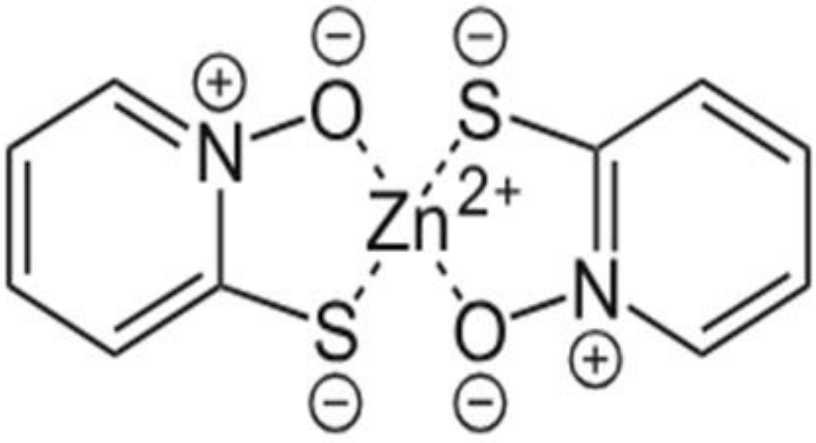

Figure 1: Chemical Structure of Zinc Pyrithione.

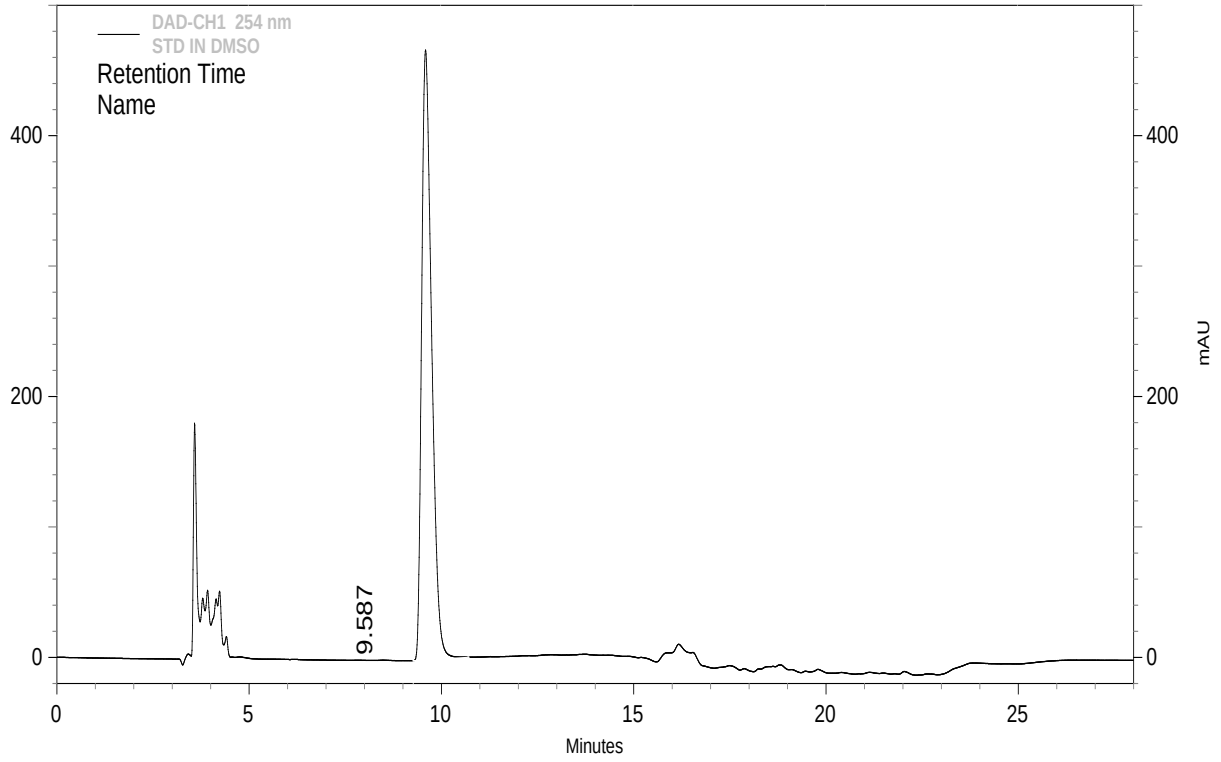

Figure 2: This trial was performed in mobile phase combination of Acetonitrile -Water in a 60:40 ratio.

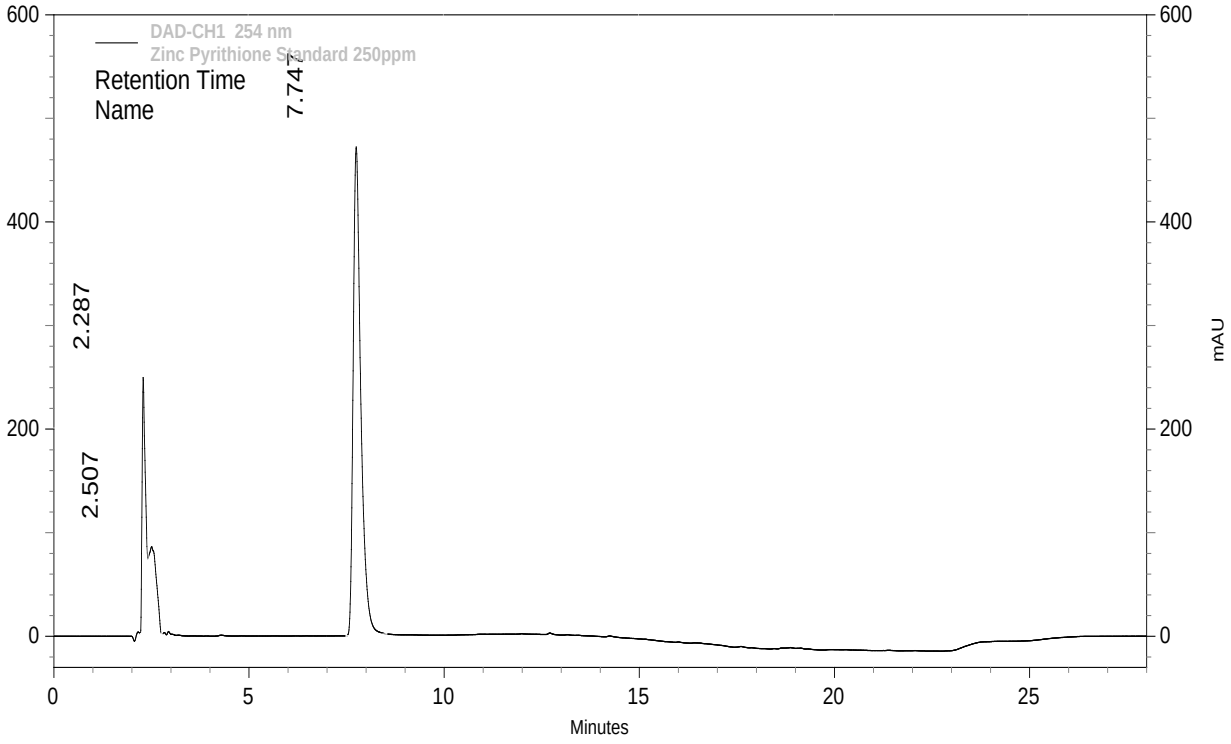

Figure 3: This trial was performed in mobile phase combination of phosphate buffer (pH 3.5) and acetonitrile-methanol.

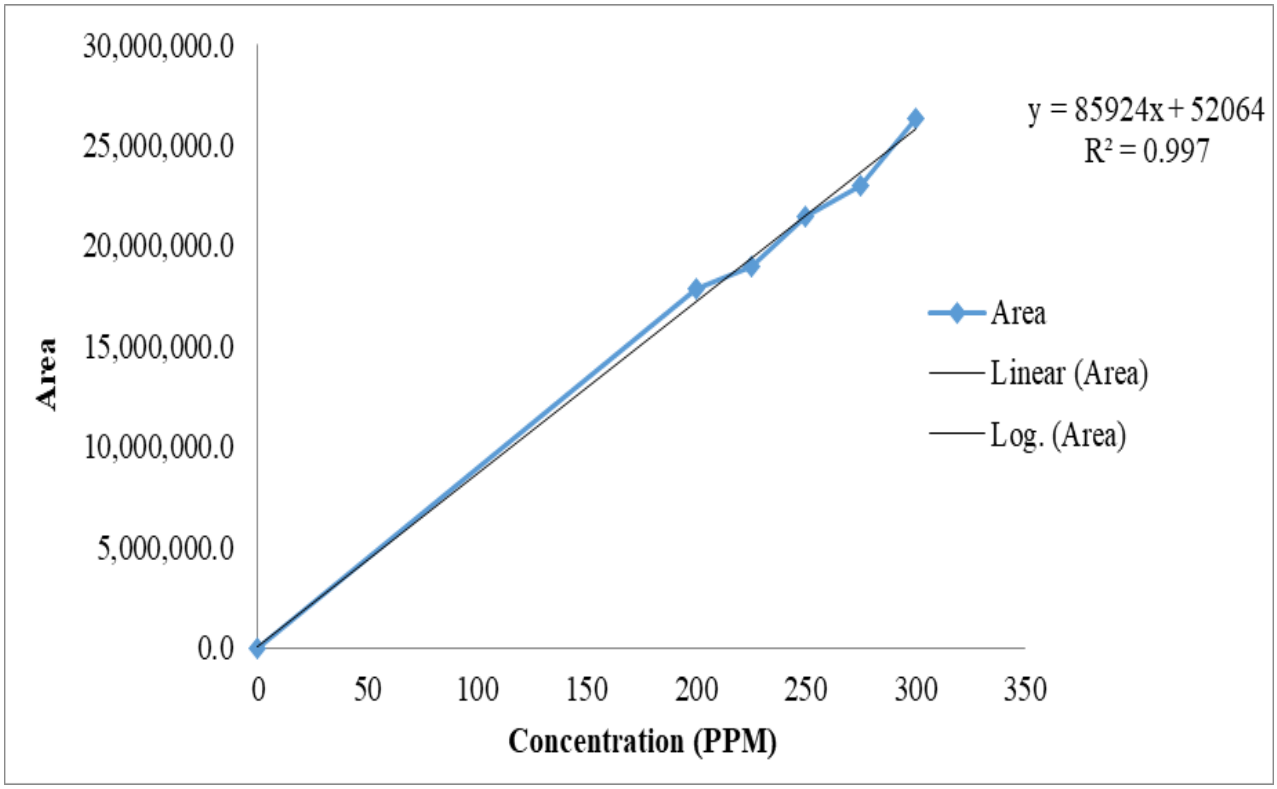

Figure 4: Linearity graph of Zinc Pyrithione.

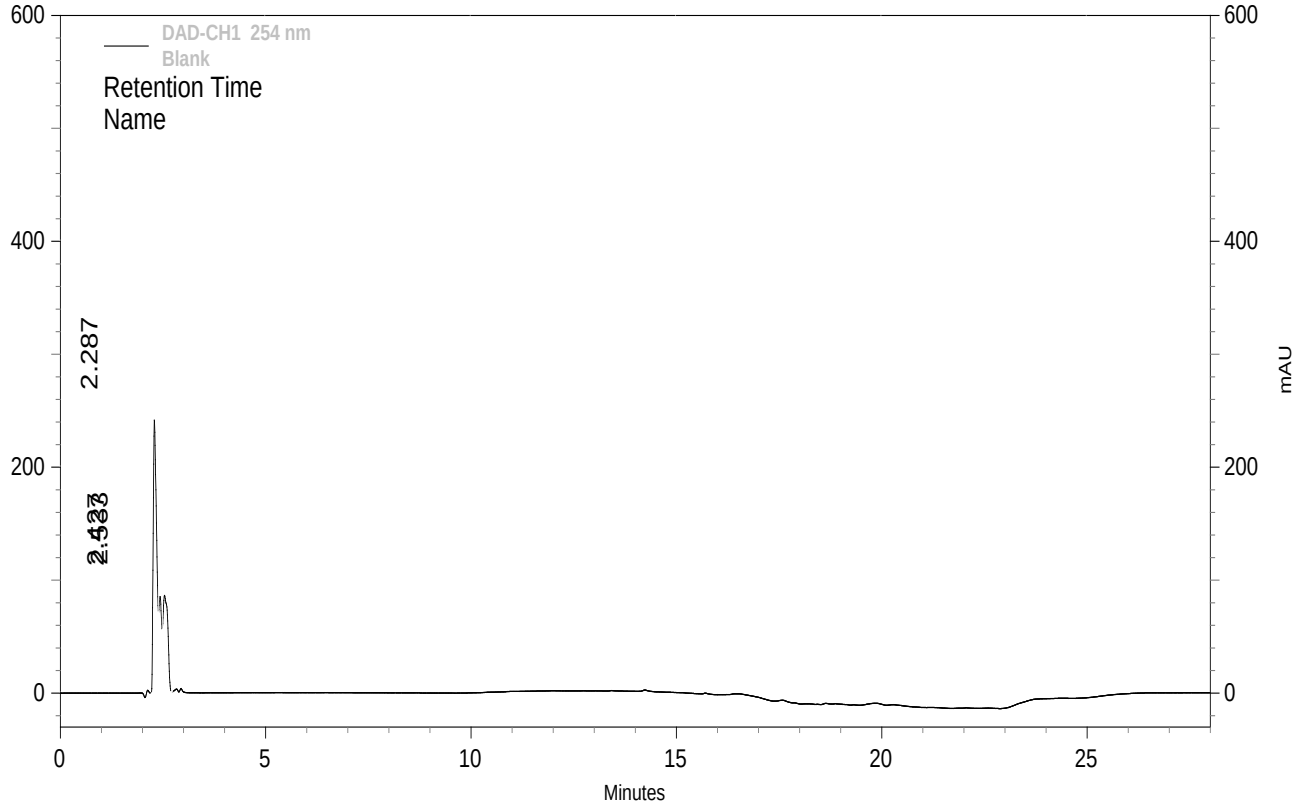

Figure 5: Method specificity chromatogram obtained for blank.

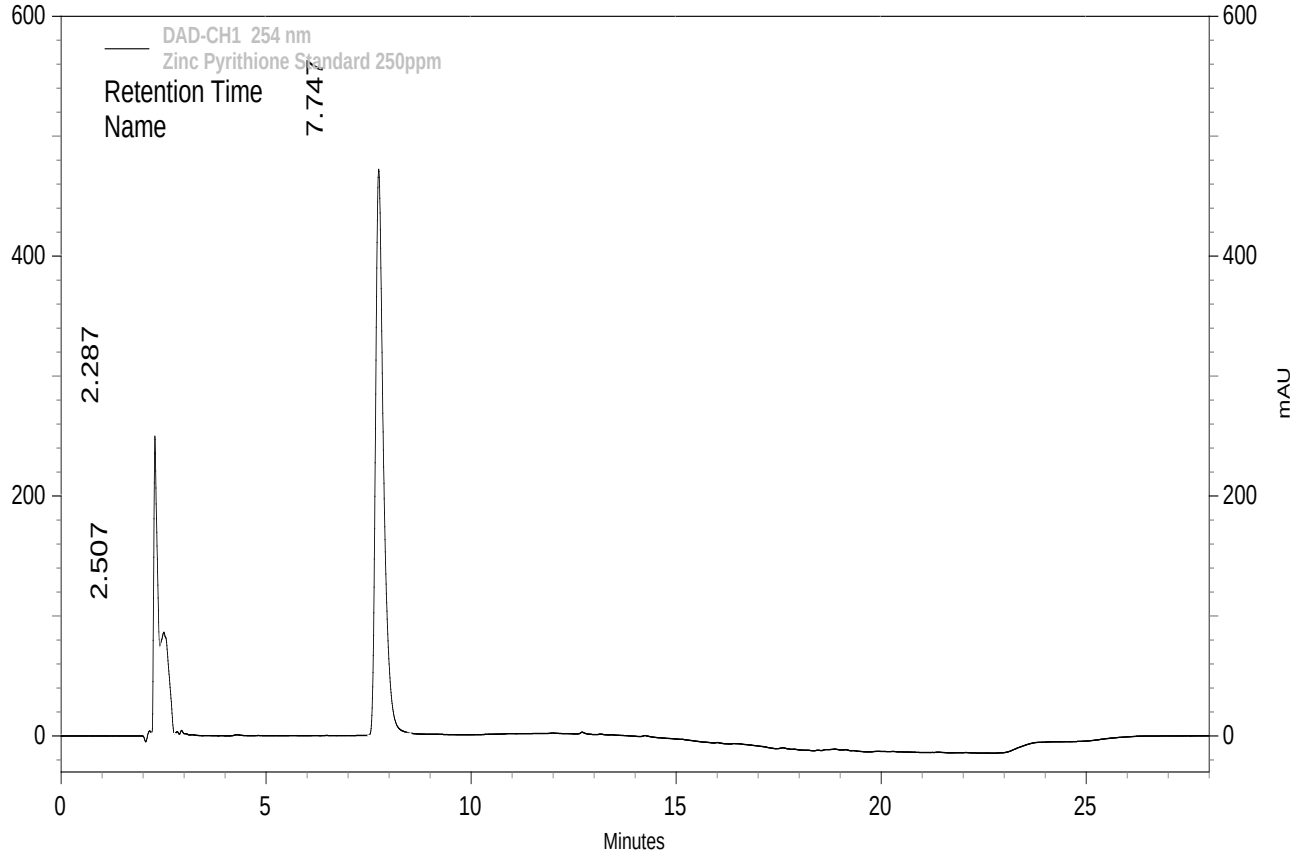

Figure 6: Method specificity chromatogram obtained for standard.

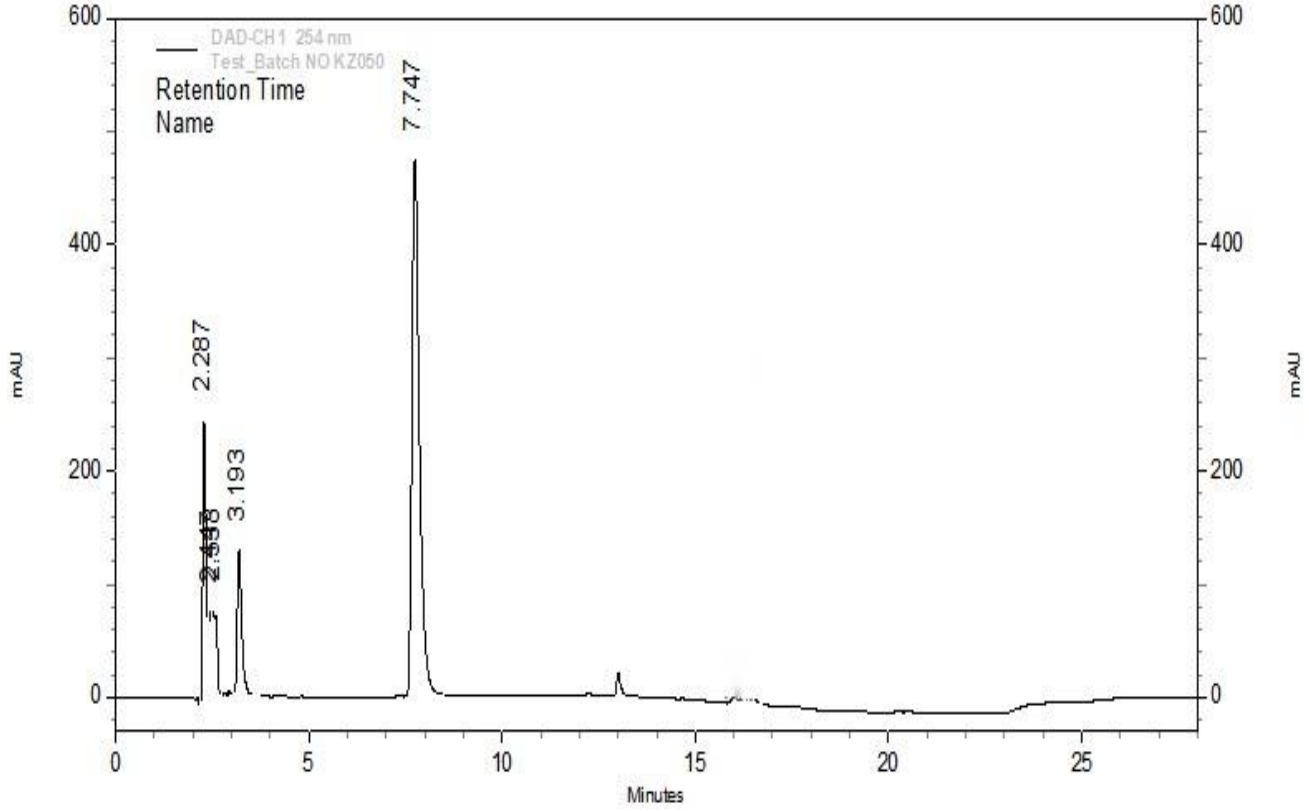

Figure 7: Method specificity chromatogram obtained for sample.

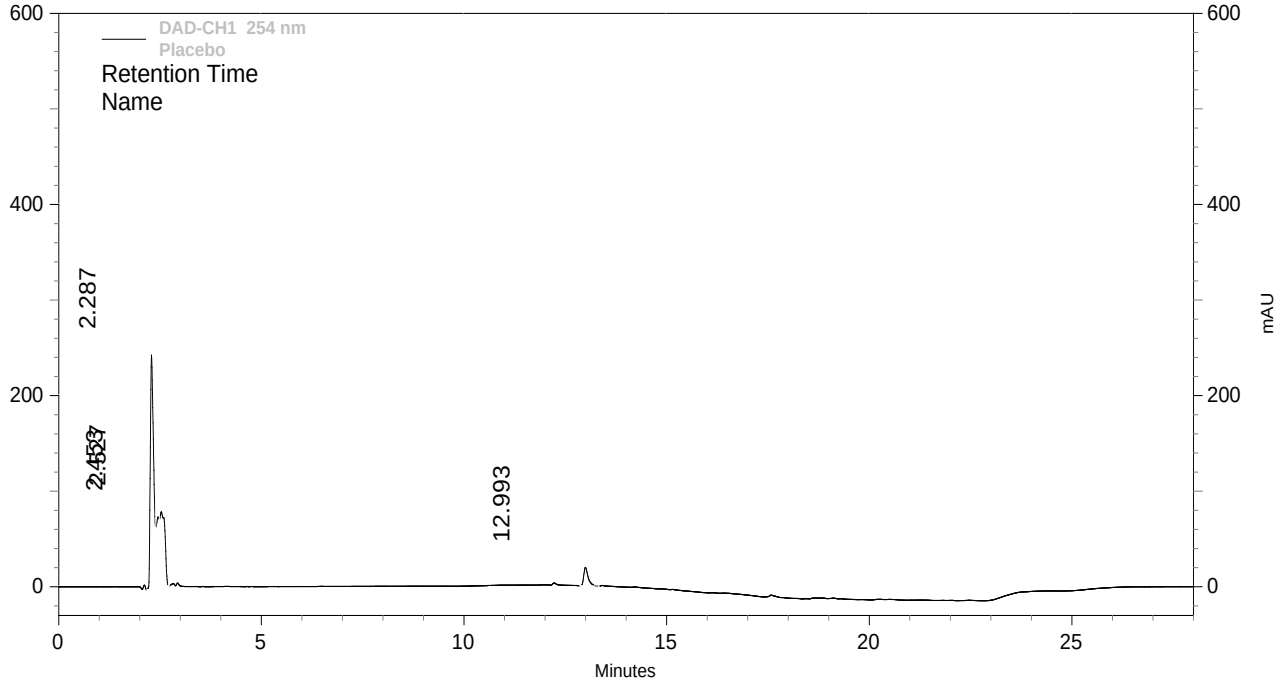

Figure 8: Method specificity chromatogram obtained for placebo.

Table 1: Gradient program for the mobile phase during analytical method development.

Time (min)	Mobile phase-A Buffer (%)	Mobile Phase-B ACN: MeOH (%)
0	75	25
6	75	25
15	25	75
20	25	75
22	75	25
28	75	25

Table 2: Optimized chromatographic conditions.

Equipment	HPLC with DAD detector- Hitachi L-2455 Elite LaChrom
Mobile Phase	Mobile Phase A: Phosphate Buffer pH 3.5
	Mobile Phase B: Acetonitrile: Methanol
Column	Zorbax Extend octa decyl silane (C-18, 250X4.6X5µ) column
Column Temperature	30°C
Injection Volume	20 µL
Flow Rate	1.0 ml/min
Wavelength	254 nm
Diluent	Dimethyl Sulphoxide: Water (70:30)

Table 3: Linearity data- concentration versus area.

Conc (ppm)	Area
200	17878608
225	18998387
250	21483956
275	22997293
300	26359354

Table 4: Intraday precision at Morning.

Standard	Area
1	21032272
2	21182782
3	21322954
4	21296205
5	21239594
6	21380576
Average	21242397
Standard Deviation	104111
% RSD	0.490

Table 5: Intraday precision at Afternoon.

Standard	Area
1	21196145
2	21222704
3	21359531
4	21268995
5	21427690
6	21060284
Average	21255892
Standard Deviation	55520
% RSD	0.261

Table 6: Intraday precision at Evening.

Standard	Area
1	22288984
2	22392045
3	22578624
4	22534655
5	22574666
6	22527631
Average	22482767
Standard Deviation	104853
% RSD	0.466

Table 7: Inter-day precision at Day-1.

Standard	Area
1	21255477
2	21534548
3	21629280
4	21543812
5	21506677
6	21549935
Average	21503288
Standard Deviation	128134
% RSD	0.596

Table 8: Inter-day precision at Day-2.

Standard	Area
1	21134756
2	21162457
3	21278716
4	21158646
5	21418245
6	21066666
Average	21203248
Standard Deviation	125633
% RSD	0.593

Table 9: Inter-day precision at Day-3.

Standard	Area
1	21518118
2	21512954
3	21617982
4	21415137
5	21667696
6	21538726
Average	21545102
Standard Deviation	88356
% RSD	0.410

Table 10: Repeatability study at 250 µg/ml.

Standard	Area
1	21513014
2	21435926
3	21715800
4	21436716
5	21753239
6	21569462
Average	21570693
Standard Deviation	136981
% RSD	0.635

Table 11: Area of sample and standard, Percentage recovery, standard deviation and % R.S.D.

Standard Amount Added (mcg/ml)	Sample amount (mcg/ml)	Test Area	% Recovery	Average	Standard Deviation	RSD
200	200	22111051	108.78	108.98	0.6109	0.561
200	200	22291158	109.67			
200	200	22054481	108.50			
250	250	27661575	110.44	109.95	0.5543	0.504
250	250	27569373	110.07			
250	250	27389099	109.35			
300	300	30855519	102.82	102.52	0.7881	0.769
300	300	30499334	101.63			
300	300	30945692	103.12			

Table 12: Area of standard, area of samples average assay, standard deviation and % R.S.D of assay.

S. No.	Area of Standard	
1	26912319	
S. No.	Area of Test	% Assay
1	26899254	99.83
2	26751947	99.37
3	26914217	99.92
Average		99.71
Standard Deviation		0.2950
% RSD of Assay		0.296

Table 13: Effect of increase in flow rate.

Standard	**Area**
1	18889493
2	18735697
3	18888775
4	19055489
5	19039469
6	19147107
Average	18959338
Standard Deviation	148860
% RSD	0.785

Table 14: Effect of decrease in flow rate.

Standard	**Area**
1	27548061
2	28162254
3	27986895
4	27957213
5	27927684
6	28252356
Average	27972411
Standard Deviation	243690
% RSD	0.871

Table 15: Effect of increase in temperature by 2℃.

Standard	Area
1	22418246
2	22748853
3	22630617
4	22648670
5	22534648
6	22718728
Average	22616627
Standard Deviation	122643
% RSD	0.542

Table 16: Effect of decrease in temperature by 2℃.

Standard	Area
1	22701692
2	22888808
3	23092769
4	23029226
5	22686978
6	22963219
Average	22893782
Standard Deviation	168792
% RSD	0.737

Table 17: Effect of increase in pH by 0.2.

Standard	Area
1	23428691
2	23457915
3	23562706
4	23499125
5	23644832
6	23652456
Average	23540954
Standard Deviation	94810

% RSD	0.403

Table 18: Effect of decrease in pH by 0.2.

Standard	Area
1	21047485
2	21055625
3	21045691
4	21250758
5	21197884
6	21172140
Average	21128264
Standard Deviation	89885
% RSD	0.425

Table 19: Effect of increase in wavelength by 2 units.

Standard	Area
1	26007552
2	25882050
3	25916974
4	25941885
5	26244144
6	26147339
Average	**26023324**
Standard Deviation	**143032**
% RSD	**0.550**

Table 20: Effect of decrease in wavelength by 2 units.

Standard	Area
1	20201251
2	20104672
3	20134468
4	20147995
5	20164071
6	20086974
Average	20139905
Standard Deviation	41196

% RSD	0.205

4. Conclusions

An efficient, time-saving method was developed for analyzing Zinc Pyrithione in a semi-solid dosage form, specifically a shampoo. The method exhibited precision, specificity, accuracy, and reproducibility. Its innovative nature not only saves time but also deems it suitable for application in pharmaceutical industries. System suitability criteria were successfully met with a combination of pH 3.5 buffer and acetonitrile: methanol. Extensive trials involving different solvent combinations were conducted to achieve these criteria. The method demonstrated specificity, showing no interference from excipients or degradation products, and % R.S.D remained within acceptable limits. Minor variations in mobile phase composition, pH, and flow rate did not significantly impact method performance, confirming its robustness.

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