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Original Article

STRATEGIC DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING ANALYTICAL METHOD FOR S-ADENOSYLMETHIONINE

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ABSTRACT

S-Adenosylmethionine (SAMe) is a biologically essential compound involved in DNA methylation, liver detoxification, neurotransmitter synthesis, and joint health. Due to its instability and susceptibility to degradation, a validated analytical method is crucial for its accurate quantification and quality control. This research focuses on the development, validation, and stability assessment of a refined RP-HPLC method for the quantification of S-adenosylmethionine (SAMe) in both bulk material and pharmaceutical formulations. The method development involved selecting an appropriate mobile phase and wavelength, optimizing chromatographic conditions, and validating the method according to the standards outlined in International Conference on Harmonisation (ICH Q2R1). Forced degradation experiments were carried out under thermal, photolytic, acid, base, and oxidative stress conditions to assess stability. The optimized RP-HPLC method, with a mobile phase of ACN/0.1% OPA aqueous solution (20:80, v/v) and a detection wavelength of 259 nm, demonstrated high precision, linearity (R^2 = 0.99996), and accuracy (recovery between 98-102%). The method effectively detected degradation products, establishing the method's capability to indicate stability. The optimized RP-HPLC method demonstrated simplicity, accuracy, precision, and robustness for the quantitative analysis and stability evaluation of S-adenosylmethionine (SAMe). It is well-suited for quality control applications and aligns with regulatory standards, offering a reliable tool for routine analysis of SAMe in pharmaceutical formulations.

KEYWORDS: S-adenosylmethionine, method development, forced degradation, pharmaceutical analysis, RP-HPLC.

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1. Introduction

S-Adenosylmethionine (SAMe) is a naturally occurring molecule involved in various essential biological processes. SAMe contains key functional groups, including an amino group, thioether, carboxylic acid, and a positively charged sulfonium ion, along with a ribose sugar bearing a hydroxyl group and an adenine base (Figure 1). These structural features enable SAMe to act as a universal methyl donor, playing a vital role in DNA methylation, neurotransmitter synthesis, liver detoxification, and joint health [1]. Due to its crucial role in cellular function, SAMe acts broadly as a pharmaceutical and dietary additive to treat conditions such as depression, liver disorders, osteoarthritis, and neurological diseases [2]. To guarantee the quality, effectiveness, and safety of formulations containing SAMe, the development of a reliable and validated analytical method for its measurement and stability evaluation is essential. However, SAMe is highly unstable, making its analytical determination challenging. The presence of degradation products can

affect its potency, requiring a validated stability-indicating method for accurate analysis [3,4].

Also, understanding the pharmacokinetics and pharmacodynamics of SAMe is critical for designing an appropriate analytical method. As SAMe undergoes rapid metabolism and has low bioavailability, an advanced analytical method must be developed to detect its active form, degradation pathways, and metabolites in pharmaceutical formulations [5,6].

Therefore, the present study aims at developing and validating an analytical method for (SAMe), along with conducting stress degradation studies to assess its stability. The establishment of a precise and selective method will support the accurate estimation of SAMe in both bulk drug and formulated products, ensuring effective quality control and adherence to regulatory standards.

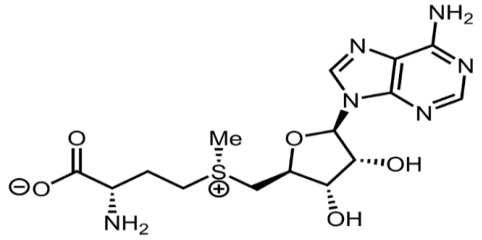


Fig. 1. Structure of SAMe.

2. Materials and Methods

2.1. Material

SAMe was obtained from Jodas Expoim Pvt. Ltd., Hyderabad. HPLC grade methanol and acetonitrile were supplied by Merck, while HPLC grade water was procured from Clairofilt India, Kasheli, Bhiwandi. Trifluoroacetic acid (TFA, 0.1% v/v aqueous solution, analytical grade) was sourced from Thermo Fisher Scientific, Nashik and used where specified for sample preparation or as a component in chromatographic trials. Zorilla Tablets containing S-adenosyl-L-methionine (SAMe) were procured from Wellness Forever, Satana. The product was manufactured by Zuventus Healthcare Ltd., Mumbai, India. Each tablet was labeled to contain 400 mg of S-adenosyl-L-methionine (SAMe).

The study utilized a Jasco UV-Visible spectrophotometer (Model: UV 550) and an Agilent HPLC Quaternary Gradient System (Model: 1260 Infinity II) equipped with a Phenomenex (250 × 4.6 mm, 5 µm) C18 column, and operated via OpenLab EZChrom software. Additional equipment included an Aczet precision balance (Model: CY 224C), a LabMan digital pH meter, a Bio-Technic Ultra Sonicator (13.5 L capacity), and 0.45 µm Nylon and PVDF membrane filters for sample filtration.

2.2. Method

2.2.1. Fourier Transform Infrared (FTIR) of Drug

FTIR analysis of SAMe was conducted using a Jasco 4600 IR spectrophotometer to evaluate the functional groups present in the active pharmaceutical ingredient (API). The spectra were recorded over a range of 4000–400 cm^{-1} to identify characteristic peaks corresponding to functional groups. This analysis confirmed the structural integrity of SAMe [7].

2.2.2. Selection of Analytical Wavelength

Preparation of Standard Solutions for UV Scanning

A stock solution was prepared by accurately weighing 10 mg of SAMe and transferring it into a 20 mL volumetric flask. About 15 mL of water was then added, and the solution was sonicated to ensure complete dissolution. The final volume was adjusted with distilled water to prepare a 500 ppm stock solution. From this, 0.8 mL was accurately measured and diluted to 20 mL with water to obtain a 20 ppm working solution. This solution was then scanned within the 200–400 nm range using a UV-visible spectrophotometer to identify the wavelength at which maximum absorbance occurred [8,9].

2.2.3. RP-HPLC Method Optimization

Standard Solution Preparation for Chromatographic Analysis

An accurately weighed 20 mg of SAMe was placed into a clean, dry 20 mL volumetric flask. Approximately 15 mL of distilled water was added, and the mixture was sonicated until complete dissolution occurred. The solution was then brought up to volume with water, resulting in a 1000 ppm stock solution. From this stock, 1 mL was transferred into a 10 mL volumetric flask and diluted with the mobile phase ACN/0.1% OPA aqueous solution (20:80, v/v) used in each chromatographic run to prepare a 100 ppm working solution. This solution was subsequently used for RP-HPLC method development [10,11].

2.2.4. Analytical Method Validation

According to ICH Q2(R1) guidelines, the developed P-HPLC method was thoroughly validated. Key performance parameters such as system suitability, linearity, limit of detection (LOD), limit of quantification (LOQ), intraday and interday precision, accuracy, and robustness were systematically evaluated to confirm the reliability and consistency of the analytical method.

2.2.4.1. System Suitability

A stock solution of SAMe was prepared by accurately dissolving 20 mg of the compound in approximately 15 mL of distilled water, followed by sonication to ensure complete dissolution. The volume was then made up to 20 mL with water. From this stock, 1 mL was taken and diluted to 10 mL using the mobile phase ACN/0.1% OPA aqueous solution (20:80, v/v) to obtain a working solution with a concentration of 100 µg/mL. System suitability was evaluated by performing five replicate injections of this standard solution.

2.2.4.2. Specificity

Specificity of the method was demonstrated by injecting both a blank sample (mobile phase) and a placebo formulation. The placebo was laboratory-prepared using common excipients, including lactose (80 mg), starch (5 mg), magnesium stearate (5 mg), talc (5 mg), and crospovidone (5 mg). A portion of the placebo equivalent to 100 mg of SAMe (36.6 mg) was dissolved in distilled water, sonicated to ensure complete dissolution, filtered, and then suitably diluted with the mobile phase for analysis.

2.2.4.3. Linearity and Range

To evaluate the linearity of the method, a 500 ppm stock solution was prepared by dissolving 10 mg of the drug in 20 mL of water. From this stock, five different concentrations, representing 50%, 75%, 100%, 125%, and 150% levels were prepared by diluting 1.0, 1.5, 2.0, 2.5, and 3.0 mL of the stock solution to 10 mL using the mobile phase, ACN/0.1% OPA aqueous solution (20:80, v/v) resulting in concentrations ranging from 50.00 to 150.00 µg/mL. Each concentration was injected three times, and the average peak area was recorded. "A calibration curve was then constructed by plotting concentration against the corresponding mean area to assess the method's linearity. The linearity of the method was evaluated using the Simple Linear Regression Model, by plotting concentration versus mean peak area and determining the correlation coefficient (R^2), slope, and intercept".

2.2.4.4. Accuracy (% Recovery)

Accuracy of the analytical method was evaluated by assessing the closeness of agreement between observed and true values using SAMe spiked with placebo at 50%, 100%, and 150% of the working concentration. For each level, three replicates were prepared. At 50%, ~50 mg standard with ~36 mg placebo; at 100%, ~100 mg standard with ~36 mg placebo; and at 150%, ~150 mg standard with ~36 mg placebo. % Recovery, mean recovery, and % RSD were calculated for each level.

2.2.4.5. Precision

2.2.4.5.1. Preparation of Sample Solution

An accurately weighed portion of powdered material equivalent to 100 mg of S-adenosylmethionine (SAMe) was transferred into a clean and dry 100 mL volumetric flask. Around 70 mL of distilled water was added, and the mixture was sonicated for 15 minutes with occasional shaking to ensure complete dissolution. After sonication, the solution was allowed to cool to room temperature and then diluted to volume with water to obtain a stock solution containing 100 mg of SAMe. This solution was filtered through a 0.45 µm syringe filter, discarding the first 3–5 mL of filtrate to eliminate any potential particulates. A 1 mL aliquot of the filtered solution was then diluted to 10 mL with the mobile phase, ACN/0.1% OPA aqueous solution (20:80, v/v) and injected into the HPLC system for analysis, where chromatograms were recorded.

2.2.4.5.2. Repeatability

To assess the method's repeatability, six replicate samples of the powdered test material were prepared. Each sample, weighing between 136.2 mg and 136.9 mg, was initially diluted to 100 mL with distilled water. From each prepared solution, 1 mL was subsequently diluted to 10 mL using the mobile phase. The individual weights of the test samples were: Sample 1:136.2 mg, Sample 2:136.5 mg, Sample 3:136.4 mg, Sample 4:136.7 mg, Sample 5:136.9 mg, and Sample 6:136.2 mg. This uniform sample preparation and dilution procedure was carried out to evaluate the precision and repeatability of the developed analytical method.

2.2.4.5.3. Intermediate Precision

To evaluate the reproducibility of the method, the analysis was repeated on a different day under the same experimental conditions. The samples were prepared following the same procedure as used for the repeatability study, with six replicate preparations analyzed.

2.2.4.6. Limit of Detection (LOD) & Limit of Quantification (LOQ)

The LOD was determined according to the ICH Q2(R1) guidelines using the calibration curve approach. A series of standard solutions of SAMe at varying concentrations were prepared and analyzed under optimized chromatographic conditions.

2.2.4.6.1. LOD

A calibration curve was generated by plotting the concentration of the analyte against its corresponding peak area. Based on the regression analysis, the residual standard deviation (σ) of the y-intercepts and the slope (S) of the calibration line were determined.

The Limit of Detection (LOD) was calculated using the following equation:

$$LOD = 3.3 \times \sigma / S$$

Where:
- σ represents the residual standard deviation of the response;
- S is the slope of the calibration curve.

2.2.4.6.2. LOQ

The LOQ was also evaluated following the ICH Q2(R1) guidelines using the calibration curve approach. Using the same calibration data used for LOD, the residual standard deviation (σ) and slope (S) of the regression line were determined.

The LOQ was calculated using the formula:

$$LOQ = 10 \times \sigma / S$$

Where:
- σ represents the residual standard deviation of the response;
- S is the slope of the calibration curve.

2.2.4.7. Robustness

To assess the robustness of the developed RP-HPLC method, the standard solution of 10 ppm was injected under deliberately varied chromatographic conditions. The flow rate of the mobile phase was altered by ±10% (i.e., ±0.1 mL/min) from the optimized value to observe any impact on retention time and peak characteristics. The column oven temperature was modified by ±2 °C to evaluate the performance of the method under slight thermal fluctuations. Furthermore, the detection wavelength was varied by ±3 nm to determine the sensitivity of the method to minor changes in detection parameters. These intentional variations were performed to ensure the reliability, precision, and consistency of the method during routine analysis, as per ICH Q2(R1) guidelines [12,13].

2.2.4.8. Assay of Zorilla Tablet

2.2.4.8.1. Preparation of Standard Solutions

An accurately weighed 10 mg quantity of SAMe was transferred to a clean, dry 100 mL volumetric flask. The volume was then made up to the mark using a suitable diluent to prepare a stock solution with a concentration of 100 µg/mL. From this solution, 1.0 mL was taken and transferred into a 10 mL volumetric flask, followed by dilution up to the mark with distilled water to yield a final working solution of 10 µg/mL.

2.2.4.8.2. Sample Preparation

Ten Zorilla tablets were accurately weighed and powdered using a mortar and pestle. A quantity of tablet powder equivalent to 10 mg of SAMe was transferred into a 100 mL volumetric flask. About 70 mL of the mobile phase ACN/0.1% OPA aqueous solution-20:80 v/v was added. The mixture was sonicated for 15–20 minutes to ensure complete dissolution of the active ingredient. The solution was filtered through a 0.45 µm membrane filter and made up to volume with the same mobile phase. Further dilutions were made to achieve the required concentration for analysis. Subsequently, 1 mL of the filtered solution was further diluted to 10 mL with the diluent [14].

2.2.5. Forced Degradation Study on API

2.2.5.1. Thermal Degradation

To evaluate the thermal stability of SAMe, an accurately weighed amount of powdered API equivalent to 10 mg of S-adenosylmethionine (SAMe) was evenly spread in a clean, dry Petri dish and exposed to dry heat in a stability chamber at $60 \pm 2\,^{\circ}C$ for 48 hours. After the specified exposure period, the sample was removed, allowed to cool to room temperature in a desiccator, and then quantitatively transferred to a 100 mL volumetric flask containing approximately 50 mL of the mobile phase. The solution was sonicated for 20 minutes to facilitate complete dissolution of the drug, and the volume was then adjusted to 100 mL with the same mobile phase. The resulting solution was filtered using a 0.45 µm membrane filter to remove any undissolved particles. From the filtrate, 1.0 mL was taken and diluted to 10 mL using the mobile phase. This final solution was injected into the HPLC system to assess the extent of thermal degradation by comparing the assay and chromatographic profile with that of the untreated control sample.

2.2.5.2. Photolytic Degradation

SAMe was subjected to direct sunlight exposure for a duration of 72 hours. After exposure, the sample was allowed to cool to room temperature inside a desiccator. To prepare the test solution, 20.2 mg of the exposed active pharmaceutical ingredient was accurately weighed and transferred into a 20 mL volumetric flask containing 15 mL of distilled water. The mixture was sonicated for 15 minutes with occasional shaking to ensure complete dissolution, then the volume was adjusted to 20 mL with water. The resulting solution was filtered using a 0.45 µm syringe filter, discarding the initial 3–5 mL of the filtrate to remove impurities. From the clear filtrate, 1 mL was taken and diluted to 10 mL with the mobile phase before subjecting it to chromatographic analysis.

2.2.5.3. Acid Degradation

To evaluate the acid-induced degradation of SAMe, two experimental trials were performed. In the first trial, 20.2 mg of the active pharmaceutical ingredient was dissolved in water, sonicated to ensure complete dissolution, and treated with 2 mL of 5 N hydrochloric acid. The solution was kept at ambient temperature for 2 hours, after which it was neutralized using 5 N sodium hydroxide and diluted to the final volume. In the second trial, 20.4 mg of the API was subjected to the same procedure but allowed to react with the acid for 24 hours. In both trials, the resulting solutions were filtered using a 0.45 µm syringe filter, discarding the initial 3–5 mL of the filtrate. Then, 1 mL of the clear solution was further diluted to 10 mL with the mobile phase and analyzed using the chromatographic system [15].

2.2.5.4. Base/Alkaline Degradation

To assess the stability of SAMe under alkaline conditions, three separate trials were carried out. In the first trial, 20.1 mg of the API was treated with 2 mL of 5 N sodium hydroxide and kept at room temperature for 2 hours. After this period, the solution was neutralized using 5 N hydrochloric acid. In the second trial, 19.9 mg of SAMe was reacted with 0.2 mL of 0.5 N NaOH for 30 minutes and then neutralized with an equal volume (0.2 mL) of 0.5 N HCl. For the third trial, 20.4 mg of the drug substance was exposed to 0.1 mL of 0.5 N NaOH for 15 minutes, followed by neutralization with 0.1 mL of 0.5 N HCl. Each sample was sonicated, the volume was adjusted with distilled water, and the solution was filtered through a 0.45 µm syringe filter, discarding the initial 3–5 mL of the filtrate. Subsequently, 1 mL of the clear solution was diluted to 10 mL with the mobile phase and subjected to chromatographic analysis.

2.2.5.5. Peroxide Degradation

SAMe was subjected to oxidative stress through two trials. In the first trial, 20.2 mg of the active pharmaceutical ingredient was treated with 2 mL of 30% hydrogen peroxide and allowed to stand for 2 hours. In the second trial, 20.1 mg of the API underwent identical treatment but was exposed for 24 hours. Following the reaction, each solution was neutralized using 2 mL of a 30% sodium sulfite solution. The mixtures were then diluted to the final volume with distilled water, filtered through a 0.45 µm syringe filter (discarding the first 3–5 mL of the filtrate), and finally, 1 mL of the clear solution was diluted to 10 mL using the mobile phase for chromatographic analysis [16,17].

3. Results

3.1. FTIR Analysis of Drug

The FTIR spectrum of SAMe (Figure 2) reveals several characteristic absorption peaks that correspond to its functional groups. A broad absorption band at 3375.51 cm^{-1} indicates the presence of N–H or O–H stretching vibrations, suggesting the involvement of amino or hydroxyl groups possibly due to hydrogen bonding. A peak at 2930.65 cm^{-1} corresponds to aliphatic C–H stretching, commonly found in methyl or methylene groups. A strong peak observed at 1688.82 cm^{-1} signifies C=O stretching vibrations, confirming the presence of a carbonyl group, likely from a carboxylic acid or amide functionality. Additional peaks at 1611.95 cm^{-1} and

1509.86 cm^{-1} can be attributed to C=C or C=N stretching and aromatic or nitro group vibrations. The absorption at 1419.02 cm^{-1} is likely due to CH_2 bending or symmetric COO^- stretching. Peaks at 1325.50 cm^{-1} and 1294.60 cm^{-1} suggest C–N stretching and possibly C–O or S=O vibrations, indicating the presence of amine or sulfonium functionalities. Vibrations observed at 1142.09 cm^{-1} and 1025.95 cm^{-1} further confirm the presence of C–O, C–N, or C–S bonds. Lower frequency bands at 833.62 cm^{-1}, 719.55 cm^{-1}, and 610.26 cm^{-1} may be due to aromatic C–H bending and C–S or S–S stretching.

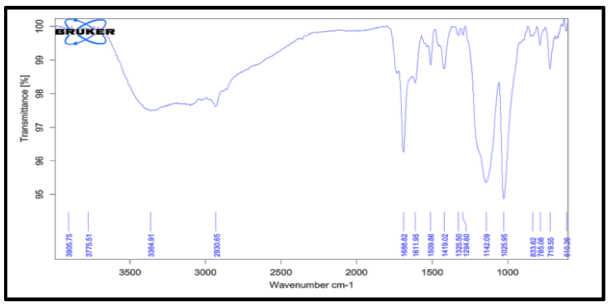


Fig. 2. FTIR Spectrum of SAMe.

3.2. Selection of Analytical Wavelength

The λmax of SAMe was identified by scanning its standard solution across the wavelength range of 200–400 nm using a UV-visible spectrophotometer. The compound showed its highest absorbance at 259 nm (Figure 3), which was chosen as the detection wavelength for all future analyses. During the method development phase, several chromatographic conditions were assessed. Of these, the eighth trial provided the most favorable results in terms of peak symmetry, retention time, and minimal tailing. As a result, the parameters from this trial were finalized as the optimized chromatographic conditions and used in the validation process.

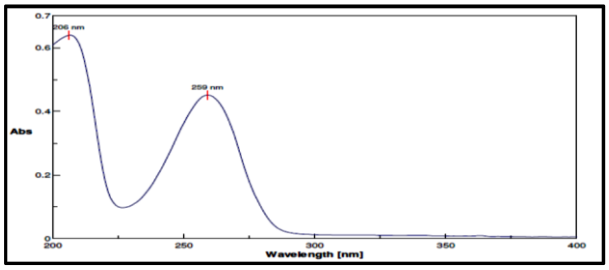


Fig. 3. UV Spectrum of SAMe.

3.3. Method Development by RP-HPLC

SAMe eluted at 2.85 minutes with good chromatography as shown in Figure 4, (asymmetry: 1.39 and theoretical plates: 6439), as shown in Table 1.

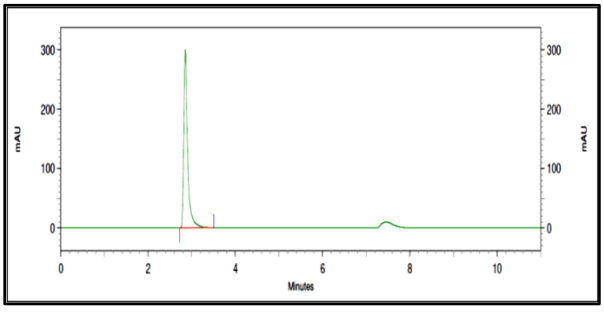


Fig. 4. Representative Chromatogram Obtained from Trial 8.

Table 1. Finalized Parameters for Optimized Chromatographic Method.

Parameter	Description
Mode	Isocratic
Column Name	Kromasil C18, 250 mm X 4.6mm ID, 5 µm
Detector	UV Detector
Injection Volume	20 µl
Wavelength	259 nm
Column Oven temp	40°C
Mobile Phase	ACN/0.1% OPA aqueous solution (20:80, v/v)
Flow Rate	1.0 ml/min
Run time	13 Minutes

3.4. Method Validation

3.4.1. System Suitability Parameters

System suitability for the developed HPLC method targeting SAMe was assessed by performing five consecutive injections of the standard solution. The average peak area obtained was 31,235,574, with a relative standard deviation (%RSD) of 0.02%, reflecting excellent consistency and reproducibility of the method. The asymmetry factor remained consistent at 1.38–1.39, and the mean theoretical plate count was 6450, suggesting good column efficiency. These values are summarized in Table 2.

These results indicate that the method is precise, as reflected by the low %RSD of the area, well within the acceptable limit of ≤2.0%. The consistent asymmetry factor confirms symmetrical peak shapes, which are crucial for reliable quantification. Additionally, the high theoretical plate count supports adequate separation efficiency of the column. Therefore, the method demonstrates satisfactory system suitability and is deemed reliable for routine analysis of SAMe.

Table 2. Evaluation of System Suitability Parameters for SAMe.

Sr. No.	Standard solution	Area	Asymmetry	Theoretical plates
1.	Standard1	31238524	1.39	6452
2.	Standard 2	31232449	1.39	6438
3.	Standard 3	31229856	1.39	6479
4.	Standard 4	31230452	1.39	6429
5.	Standard 5	31246588	1.38	6452
Mean		31235574	1.39	6450
STD Dev		7048.169		
% RSD		0.02		

3.4.2. Specificity

The chromatographic technique used for SAMe analysis demonstrated specificity, as evidenced by the purity peaks in both standard and test solutions, which fell within acceptable limits (Figure 5 and 6). This indicates the absence of interfering responses in both blank and placebo chromatograms components. Therefore, the method is capable of accurately detecting and quantifying SAMe without interference from other substances present in the sample matrix.

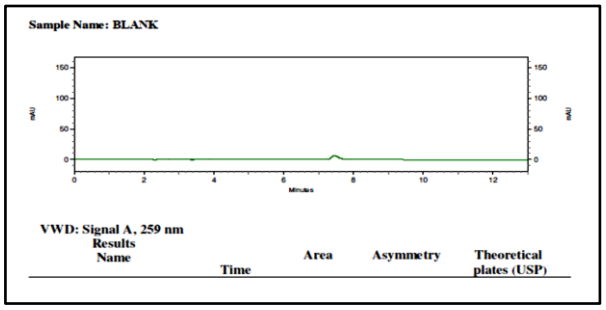


Fig. 5. Representative Chromatogram of the Blank Sample.

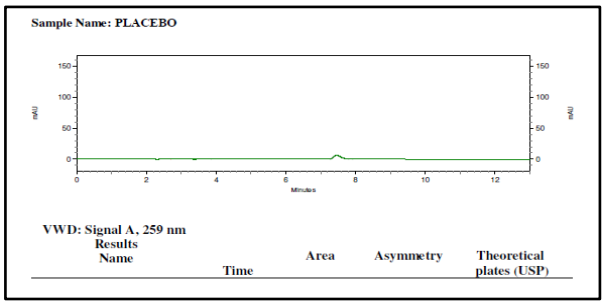


Fig. 6. Representative Chromatogram of the Placebo Sample.

3.4.3. Linearity

The correlation coefficient (R2) and calibration curve for SAMe within the range of 10 to 150 mg/ml were determined to be 0.99998 and 0.99994, respectively. This indicates a high degree of linearity between the concentration of the drug and its corresponding response in the specified range. The calibration curve shown in Figure 7 revealed a linear response within the 10-150 µg/ml range, with a regression value within the limit. From the calibration curve, it was concluded that SAMe shows a linear response in the range of 50-150 µg/ml. The regression value was found to be well within the limit, as shown in Table 3 and 4.

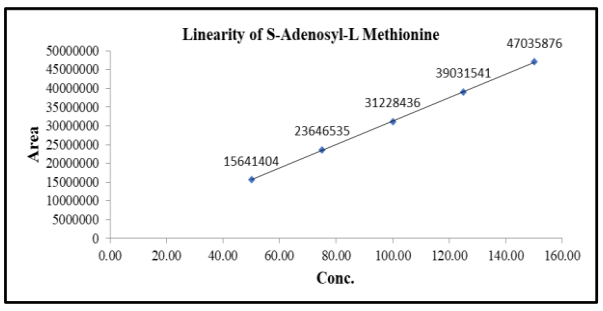


Fig. 7. Calibration Curve of SAMe.

Table 3. Calibration Curve Data Depicting Linearity of SAMe.

Level	Conc. (µg/mL)	Area	Mean	% RSD
50%	50	15649582	15641404	0.047
		15635210		
		15639421		
75%	75	23669561	23646535	0.104
		23620459		
		23649586		
100%	100	31232597	31228436	0.012
		31226854		
		31225857		
125%	125	39025416	39031541	0.014
		39032681		
		39036527		
150%	150	47025468	47035876	0.019
		47039567		
		47042593		

Table 4. Linearity Summary of SAMe.

Sr. No.	Parameter	Result Value	Acceptance Criteria
1.	Beer's linearity range	50.00-150.00µg/mL	NA
2.	Correlation coefficient (R2)	0.99996	NLT 0.98
3.	Intercept	47178.40	To be reported
4.	Slope	312695.80	To be reported
5.	% RSD for area at each level	NA	NMT 2.0

3.4.4. Accuracy

The analytical procedure demonstrated satisfactory recovery at all three levels, with no impact from changes in analyte concentration. The overall recovery rate was high, achieving 99.94%, which shows the efficiency and reliability of the process. The % RSD for overall recovery was remarkably low at 0.831%, indicating minimal variability and confirming the consistency and robustness of the method in delivering precise outcomes. This exceptional performance highlights the suitability of the method for reliable and accurate analysis.

Table 5. Result of Accuracy

Level (%)	Area	Recovered Conc. (µg/mL)	Added Conc. (µg/mL)	% Recovery	Mean Recovery	% RSD
50	15460146	49.50	50.20	98.61	99.11	0.532
	15440596	49.43	49.90	99.06		
	15596268	49.93	50.10	99.66		
100	31242593	100.02	100.10	99.92	100.01	0.286
	31305965	100.23	99.90	100.33		
	31259458	100.08	100.30	99.78		
150	47026965	150.56	150.20	100.24	99.76	0.825
	46932680	150.25	149.90	100.23		
	46296853	148.22	150.00	98.81		

This method is reliable because, as indicated in Table 5, each sample's recovery percentage, mean recovery, and overall recovery should fall between 98-102%.

3.4.5. Precision

The precision was evaluated using %RSD and average recovery rate as shown in Table 6. The acceptance criteria were set as less than the average recovery rate of 100 ± 5 %. The method was found to be precise and reproducible, as the %assay and %RSD values were found to be within the acceptance limits.

Table 6: Summary of Precision Evaluation Results.

Repeatability	Sample	Test Sample (mg)	Area	% Assay
	Sample 1	136.2	31096524	99.81
	Sample 2	136.5	31102532	99.61
	Sample 3	136.4	30958465	99.22
	Sample 4	136.7	30859581	98.69
	Sample 5	136.9	30652967	97.88
	Sample 6	136.2	31056221	99.68
		Mean		99.15
		STD DEV		0.7426
		%RSD		0.749
Intermediate Precision (Inter-Day)	Sample 1	136.4	30659882	98.26
	Sample 2	136.5	30969534	99.18
	Sample 3	136.9	30520162	97.46
	Sample 4	136.7	30956854	99.00
	Sample 5	136.8	30625941	97.87
	Sample 6	136.4	30965310	99.24
		Mean		98.50
		STD DEV		0.7478
		%RSD		0.759
Repeatability and Intermediate Precision		Mean		98.825
		STD DEV		0.7867
		%RSD		0.796

3.4.6. LOD and LOD

σ = 110392.76 (Residual standard deviation of a regression line)

s = 312695.80 (Slope)

Limit of Detection (LOD)

LOD = 3.3 x σ / S

LOD = 3.3 x 110392.76 / 312695.80

LOD = 1.17 µg/mL

Limit of Quantitation (LOQ)

LOQ = 10 x σ / S

LOQ = 10 x 110392.76 / 312695.80

LOQ = 3.53 µg/mL

The limit of detection (LOD) and limit of quantification (LOQ) were estimated using the standard deviation of the response (σ) and the slope (S) obtained from the calibration curve. The residual standard deviation (σ) was determined to be 110,392.76, while the slope (S) of the linear regression was calculated as 312,695.80. Applying the formula LOD = 3.3 x σ/S, the LOD was found to be 1.17 µg/mL, indicating the smallest concentration that can be detected but not accurately quantified. Similarly, the LOQ, calculated using LOQ = 10 x σ/S, was 3.53 µg/mL, indicating the lowest level at which the analyte can be measured with acceptable accuracy and precision. These findings demonstrate the method's high sensitivity and its effectiveness in detecting and quantifying SAMe at trace levels.

3.4.7. Robustness

Table 7: Result of Robustness Study of SAMe.

Change in Parameter	R.T. [min]	Standard Area	Asymmetry	Theoretical Plates
Wavelength by +3 nm (262 NM)	2.85	30625964	1.38	6405
Wavelength by -3 NM (256 nm)	2.85	30029651	1.37	6385
Flow rate by +10% (1.1mL/min)	2.59	28349851	1.34	6635
Flow rate by -10% (0.9mL/min)	3.16	34772581	1.36	6753
Column oven temp by +2°C (42 °C)	2.84	31239568	1.37	6496
Column oven temp by -2°C (38 °C)	2.86	31209459	1.39	6250

The robustness study (Table 7) indicates that minor deliberate changes in chromatographic conditions, such as wavelength variation (±3nm), flow rate adjustment (±10%), and column oven temperature adjustment (±2°C), did not significantly affect the performance of the method. The retention time varied slightly between 2.69 and 3.26 minutes, but remained consistent and acceptable. Peak asymmetry values were within the range of 1.14 to 1.20, demonstrating good peak shape under all conditions. Theoretical plate counts were consistently above 8000, indicating that column efficiency was maintained. Overall, the results confirm that the developed method is robust and reliable under small variations in analytical conditions.

3.4.8. Assay Performance on Marketed Pharmaceutical Products

The assay of the marketed Zorilla 400 mg tablet was performed using a validated HPLC method. The mean assay value of the test sample was found to be 99.65%, with individual sample results of 99.94% and 99.36% respectively, as presented in Table 8.

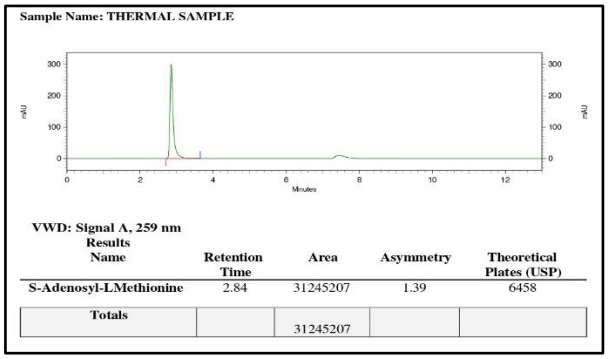
Table 8: Assay Results of Zorilla Tablet (400 mg).

Sample	Area	%Assay	Mean Assay
Sample 1	31182429	99.94	99.65%
Sample 2	30956421	99.36	

3.5. Forced Degradation Study

3.5.1. Thermal Degradation

The chromatogram of the thermal degradation sample showed a clear peak for SAMe with acceptable asymmetry and theoretical plate count, indicating stability under the applied thermal stress conditions. No significant degradation peaks were observed, as shown in Figure 8.

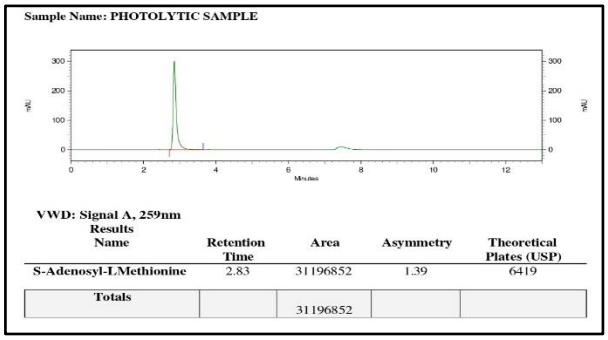


Name	Retention Time	Area	Asymmetry	Theoretical Plates (USP)
S-Adenosyl-LMethionine	2.84	31245207	1.39	6458
Totals		31245207		

Fig. 8. Typical Chromatogram of Thermal Sample.

3.5.2. Photolytic Degradation

The chromatogram of the photolytic degradation sample (Figure 9) showed a distinct peak for SAMe with acceptable asymmetry and theoretical plate count. No major degradation peaks were observed, indicating the compound's stability under photolytic stress conditions.

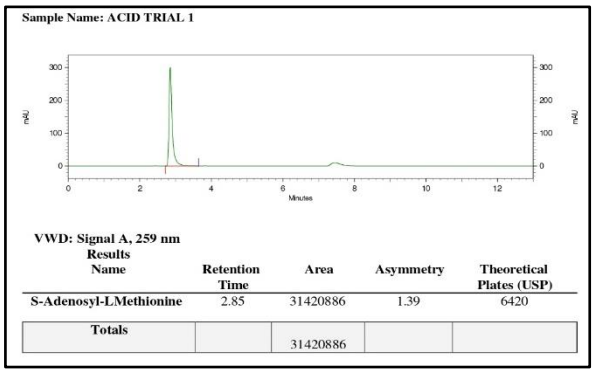


Name	Retention Time	Area	Asymmetry	Theoretical Plates (USP)
S-Adenosyl-LMethionine	2.83	31196852	1.39	6419
Totals		31196852		

Fig. 9. Typical Chromatogram of Photolytic Sample.

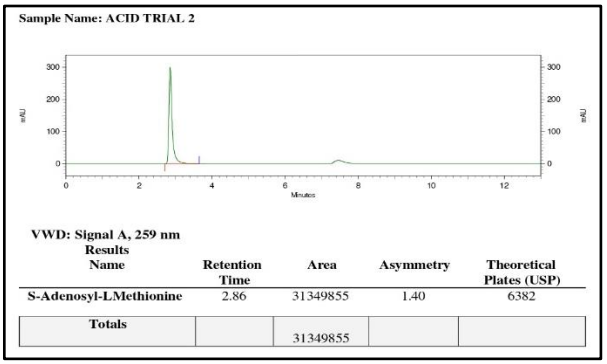
3.5.3. Acid Degradation

Chromatograms of both trials of acid-degraded samples (Figure 10 and 11) showed a well-defined peak for SAMe with acceptable asymmetry and theoretical plate count. No significant degradation peaks were observed, suggesting minimal degradation under acidic conditions.



Name	Retention Time	Area	Asymmetry	Theoretical Plates (USP)
S-Adenosyl-LMethionine	2.85	31420886	1.39	6420
Totals		31420886		

Figure 10: Typical Chromatogram of Sample Exposed Under Acid Trial 1.

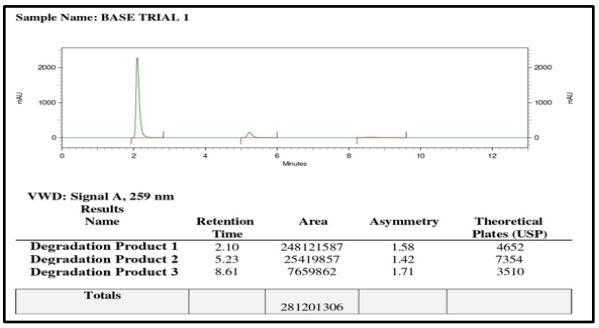


Name	Retention Time	Area	Asymmetry	Theoretical Plates (USP)
S-Adenosyl-LMethionine	2.86	31349855	1.40	6382
Totals		31349855		

Fig. 11. Representative Chromatogram of SAMe Sample Subjected to Acid Degradation (Trial 2).

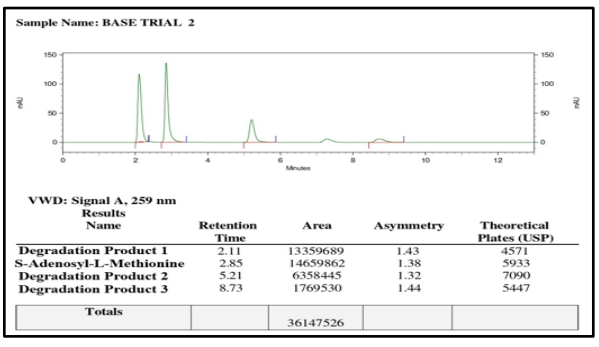
3.5.4. Base Degradation

In the base degradation trials (Figure 12, 13 and 14), SAMe exhibited a retention time of 2.85 minutes with an area of 14,659,862, an asymmetry factor of 1.38, and a theoretical plate count of 5,933. Additionally, three degradation products were identified: degradation product 1 appeared at 2.11 minutes with an area of 13,359,689, asymmetry of 1.43, and 4,571 theoretical plates; degradation product 2 was observed at 5.21 minutes with an area of 6,358,445, asymmetry of 1.32, and 7,090 theoretical plates; and degradation product 3 eluted at 8.73 minutes with an area of 1,769,530, asymmetry of 1.44, and 5,447 theoretical plates. The total chromatographic area recorded was 36,147,526.



Name	Retention Time	Area	Asymmetry	Theoretical Plates (USP)
Degradation Product 1	2.10	248121587	1.58	4652
Degradation Product 2	5.23	25419857	1.42	7354
Degradation Product 3	8.61	7659862	1.71	3510
Totals		281201306		

Fig. 12. Typical Chromatogram of Sample Exposed Under Base Trial 1.



Name	Retention Time	Area	Asymmetry	Theoretical Plates (USP)
Degradation Product 1	2.11	13359689	1.43	4571
S-Adenosyl-L-Methionine	2.85	14659862	1.38	5933
Degradation Product 2	5.21	6358445	1.32	7090
Degradation Product 3	8.73	1769530	1.44	5447
Totals		36147526		

Fig. 13. Typical Chromatogram of Sample Exposed Under Base Trial 2.

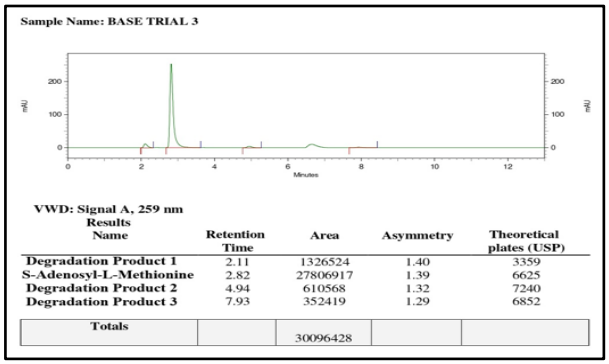


Fig. 14. Typical Chromatogram of Sample Exposed Under Base Trial 3.

3.5.5. Peroxide Degradation

The oxidative degradation of SAMe was evaluated under forced degradation conditions using 30% hydrogen peroxide. In both trials, no additional degradation peaks were observed in the chromatograms, indicating the oxidative stability of the drug under the tested conditions. In trial 1 (2-hour exposure) (Figure 15), SAMe exhibited a retention time of 2.84 minutes, with a peak area of 31,196,859, an asymmetry factor of 1.40, and 6,316 theoretical plates, demonstrating acceptable peak shape and column efficiency.

In trial 2 (24-hour exposure) (Figure 16) no additional peaks were observed, confirming the absence of significant peroxide-induced degradation products. These results suggest that SAMe is stable under oxidative stress conditions over both short and prolonged exposure periods.

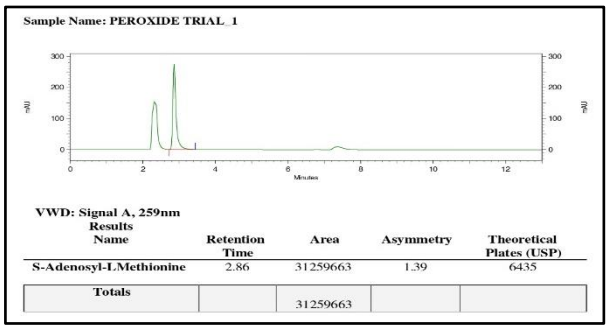


Fig. 15. Typical Chromatogram of Sample Exposed Under Peroxide Trial 1.

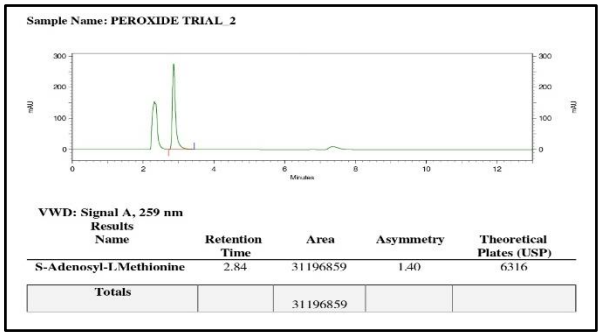


Fig. 16. Typical Chromatogram of Sample Exposed Under Peroxide Trial 2.

Table 9: Result Summary of Force Degradation of SAMe API.

Sample Name	Treatment	Exposure condition	% Assay	% Degradation
API	Sample	NA	100%	NA
	Thermal	60°C for 48 Hours	99.53	Nil
	Photolytic	Direct sunlight for 72 hours	98.88	Nil
	Acid	2 mL of 5 N HCl for 2 Hours at R.T.	99.59	Nil
		2 mL of 5 N HCl for 24 Hours at R.T.	98.39	Nil
	Base	2 mL of 5 N NaOH for 2 Hours at R.T.	0	100
		0.2 mL of 0.5 N NaOH for 30 minutes at R.T.	47.17	52.83
		0.1 mL of 0.5 N NaOH for 15 minutes at R.T.	87.28	12.72
	Peroxide	2 mL of 30% H2O2 for 2 Hours at R.T.	99.08	Nil
		2 mL of 30% H2O2 for 24 Hours at R.T.	99.38	Nil

4. Discussion

The FTIR spectrum confirmed the functional groups present in SAMe, aligning with its known chemical structure. A broad absorption near 3375 cm^{-1} indicated N–H and O–H stretching, suggesting amino or hydroxyl functionalities. Peaks at 2930.65 cm^{-1} and 1688.82 cm^{-1} corresponded to aliphatic C–H and C=O stretching, confirming the presence of methyl/methylene and carbonyl groups. Signals at 1611.95 cm^{-1} and 1509.86 cm^{-1} suggested aromatic or imine functionalities. Additional peaks from 1419.02 cm^{-1} to 1025.95 cm^{-1} indicated CH_2 bending, C–N, C–O, and S=O vibrations, characteristic of sulfonium structure. Low-frequency peaks below 900 cm^{-1} were due to C–S or S–S bonds. No significant spectral shifts were observed, indicating chemical compatibility and structural integrity [18].

UV spectrophotometric analysis showed a maximum absorbance at 259 nm in distilled water, indicating $\pi \rightarrow \pi^*$ transitions common in conjugated systems. The selected wavelength was ideal for HPLC detection due to its sensitivity and minimal interference, supporting accurate quantification.

A robust and efficient RP-HPLC method was developed using a Kromasil C18 column with an isocratic mobile phase of acetonitrile and 0.1% OPA (20:80 v/v). Detection was set at 259 nm, as determined by UV analysis. The method yielded a sharp peak with a retention time of 2.85 min, an asymmetry factor of 1.39, and high column efficiency (6439 theoretical plates), indicating suitability for routine analysis [19].

System suitability parameters were within acceptable pharmacopeial limits. The %RSD of peak areas was only 0.02%, showing excellent reproducibility. Theoretical plates remained high, and the asymmetry factor was near 1.39, confirming the reliability and suitability of the system for SAMe quantification. Specificity testing confirmed that the method could selectively detect SAMe

in the presence of common excipients. No interfering peaks were observed in the chromatograms of blank and placebo solutions at the retention time of the drug, indicating high specificity. The method demonstrated excellent linearity over the concentration range of 50–150 µg/mL, with a correlation coefficient (R^2) of 0.99996. The calibration curve showed a consistent response proportional to concentration, and %RSD values at each level were below 2%, confirming the method's quantitative reliability. Recovery studies conducted at three concentration levels (50%, 100%, and 150%) showed mean recoveries between 99.11% and 100.01%, with %RSD values all below 1%. These results confirm the method's accuracy and its capacity to yield true values in the presence of excipients. Both repeatability (intra-day) and intermediate precision (inter-day) demonstrated high consistency. % Assay values ranged from 97.46% to 99.81%, with %RSD values under 0.8%. This confirms that the method produces reliable and reproducible results across different time points. The method showed high sensitivity with a limit of detection (LOD) of 1.17 µg/mL and a limit of quantification (LOQ) of 3.53 µg/mL. These values reflect the method's capability for low-level detection and quantification, suitable for trace analysis and stability studies. Robustness testing confirmed that minor variations in flow rate, detection wavelength, and column temperature did not significantly affect chromatographic performance. Retention time, peak shape, and efficiency remained consistent, proving the method's reliability under typical operational changes [20].

The obtained assay values fell within the pharmacopeial acceptance criteria of 98.0% to 102.0%, indicating that the marketed formulation contains the labeled amount of the API within acceptable limits. The close agreement between the individual sample results and the mean value suggests a high degree of uniformity and precision in the tablet formulation. These results reflect the robustness of the manufacturing process and the stability of the active ingredient in the final dosage form. Furthermore, the use of HPLC as an analytical technique ensured high specificity and accuracy in quantifying the API content, confirming the suitability of the method for routine quality control of Zorilla 400 mg tablets [21,22].

Forced degradation studies (Table 9) indicated that SAMe is stable under acidic, thermal, oxidative, and photolytic stress, with minimal degradation ranging from 0.41% to 1.12%. However, the compound showed high instability under alkaline conditions, exhibiting significant to complete degradation. These results highlight the need to avoid alkaline exposure during formulation and storage. The method effectively separated degradation products from the parent drug, confirming its stability-indicating nature.

5. Conclusion

The study successfully developed and validated a robust, reliable, and stability-indicating RP-HPLC method for the quantification and stability assessment of SAMe. The optimized chromatographic conditions comprising a mobile phase of acetonitrile: 0.1% ortho-phosphoric acid (20:80, v/v) and a detection wavelength of 259 nm yielded a well-resolved peak with a retention time of 2.85 minutes. Forced degradation studies revealed that SAMe is stable under thermal, photolytic, acidic, and oxidative conditions but undergoes significant degradation (52.83%) under alkaline conditions, indicating its sensitivity to high pH environments. The method validation complied with ICH Q2(R1) guidelines, demonstrating excellent linearity (R^2 = 0.99996), high sensitivity (LOD: 1.17 µg/mL, LOQ: 3.53 µg/mL), accuracy (99.63% recovery), and precision (RSD < 2%). The validated method was successfully applied to the assay of zorilla 400 mg tablets, showing a drug content of 99.65%, thus confirming its suitability for routine pharmaceutical quality control. Overall, the developed RP-HPLC method is suitable for the analysis of SAMe in both bulk and dosage forms, with the capability to serve as a key tool for regulatory compliance, stability assessment, and impurity profiling.

Author Contributions: Mayur S. Bhamare conceptualized and designed the study, performed experimental work, analyzed the data, and prepared the original manuscript draft. Ganesh B. Sonawane and Vijayraj N. Sonawane assisted in methodology development, experimentation, and data interpretation. Rushikesh L. Bachhav contributed to experimental execution and validation of results. Chandrashekar D. Patil provided critical guidance, supervision, and manuscript review. Sunil K. Mahajan supported data analysis and revised the manuscript for intellectual content. Dipak D. Sonawane contributed to resources, technical support, and final manuscript approval. All authors have read and approved the final version of the manuscript.

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