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Development And Validation Of Analytical Method For The Estimation Of Magnesium Hydroxide, Aluminium Hydroxide By AAS And Simethicone By HPLC In Pharmaceutical Dosage Form

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Abstract

This study aimed to develop and validate reliable analytical methods for the simultaneous estimation of magnesium hydroxide, aluminium hydroxide, and simethicone in combined antacid suspensions. Owing to their distinct chemical properties, a dual-method approach was adopted using Atomic Absorption Spectrophotometry (AAS) for inorganic analytes and High-Performance Liquid Chromatography (HPLC) for simethicone. Method optimization focused on wavelength selection, flame conditions, mobile-phase composition, and chromatographic parameters. Both methods demonstrated excellent linearity ($r^2 > 0.999$), precision (%RSD < 2%), and accuracy, with recovery values ranging from 98% to 101%. Sensitivity parameters (LOD and LOQ) confirmed suitability for low-level detection. System suitability and robustness studies satisfied ICH Q2(R1) requirements, validating method reliability. The complementary nature of AAS and HPLC enabled accurate quantification of chemically diverse components within a single formulation. The validated framework is suitable for routine quality control, regulatory compliance, and broader application to similar multi-component pharmaceutical products.

Keywords: Analytical method validation, Atomic Absorption Spectrophotometry, High-Performance Liquid Chromatography, Combined antacid suspension, Simultaneous estimation, Magnesium Hydroxide, Aluminium Hydroxide, Simethicone.

Introduction

Combined antacid and antiflatulent formulations remain among the most frequently used over-the-counter remedies for the symptomatic management of gastric hyperacidity, acid reflux, and gas-related discomfort. Such preparations commonly contain magnesium hydroxide and aluminium hydroxide, two inorganic antacid agents that neutralize gastric acid through rapid and sustained buffering actions. In addition, simethicone—a chemically inert organosilicon polymer—is incorporated to relieve bloating and flatulence by decreasing surface tension and promoting the coalescence of gas bubbles. Owing to their complementary therapeutic roles, fixed-dose suspensions containing these three components have become a standard option for gastrointestinal relief across clinical and community settings [1-3].

Despite their widespread use, the simultaneous analytical quantification of magnesium hydroxide, aluminium hydroxide, and simethicone in a single formulation has posed persistent challenges. The primary difficulty arises from the stark chemical differences between the analytes: magnesium and aluminium hydroxides exist as inorganic metallic species, while simethicone is a hydrophobic, non-ionic polymer. This intrinsic diversity prevents the use of a single analytical technique for all three components. Moreover, formulation excipients, emulsifiers, and suspension vehicles further complicate accurate measurement by introducing matrix interferences. Ensuring simultaneous quantification is essential not only for batch-to-batch quality control but also for regulatory compliance, stability assessment, and therapeutic consistency [4, 5].

Traditional analytical practices have typically relied on separate methods for inorganic and organic constituents, increasing time, cost, and complexity. Atomic Absorption Spectrophotometry (AAS) remains the gold standard for metal ion quantification due to its sensitivity, specificity, and minimal interference in complex matrices. Conversely, High-Performance Liquid Chromatography (HPLC) is widely accepted for the detection of organic and polymeric compounds because of its versatility and precision. However, systematic development and validation of a dual-method framework tailored specifically for these antacid–antiflatulent combinations has been limited in the existing literature [4, 6, 7].

Recognizing this gap, the present study was undertaken to develop, optimize, and validate reliable analytical methods for the simultaneous estimation of magnesium hydroxide, aluminium hydroxide, and simethicone in combined pharmaceutical suspensions. The research integrated AAS for metal hydroxide determination and HPLC for simethicone analysis, aiming to create a comprehensive, accurate, and reproducible analytical platform. The methods were designed and validated in accordance with International Council for Harmonisation (ICH Q2(R1)) guidelines to ensure robustness and regulatory acceptability [5, 7, 8].

This work holds significance for the pharmaceutical industry, where multi-component formulations continue to expand and require sophisticated analytical strategies. By establishing a validated dual-method approach, the study provides a scientifically sound and practical framework that can be adopted for routine quality control, stability testing, and regulatory submissions. Additionally, the methodological principles demonstrated herein may be extended to other combination products containing both inorganic and organic actives, thereby contributing to the advancement of modern pharmaceutical analysis.

Materials and Methods

Materials

Analytical-grade reagents, certified reference standards, and pharmaceutical-grade excipients were used throughout the study to ensure accuracy, reproducibility, and compliance with standard analytical requirements. All analytical procedures were performed in accordance with the International Council for Harmonisation (ICH) guideline Q2(R1) for method validation. Reference standards of magnesium hydroxide and aluminium hydroxide (≥ 99.5% purity) were procured from Sigma-Aldrich, whereas simethicone (99% purity) was obtained from Loba Chemie. A commercially available antacid suspension containing magnesium hydroxide (400 mg/5 mL), aluminium hydroxide (400 mg/5 mL), and simethicone (30 mg/5 mL) was purchased from a local pharmacy and used as the test formulation after confirming its shelf-life suitability. HPLC-grade methanol and acetonitrile were sourced from Merck, while analytical-reagent grade nitric acid and hydrochloric acid were supplied by SRL Chemicals. Deionized water produced using a Milli-Q system was used for all solution preparations. All solvents and mobile phases were filtered through 0.45 µm membranes prior to use. Class-A borosilicate glassware was employed, each piece pre-treated with nitric acid and rinsed thoroughly to minimize contamination during elemental analysis. Stock solutions of each analyte were prepared from certified reference standards, stored in amber containers at 4 ± 1 °C, and used to prepare fresh working solutions on the day of analysis to avoid degradation. Laboratory temperature (25 ± 2 °C) and humidity (50 ± 5%) were controlled, and all analysts used appropriate personal protective equipment. Acids were handled inside a fume hood, and laboratory waste was segregated according to CPCB guidelines. To prevent trace-metal contamination during AAS analysis, acid-washed glassware, high-purity water, and separate pipettes for each metal solution were used.

Instruments and Equipment

The analytical work relied on calibrated and qualified instruments, each operated according to standard operating procedures.

Atomic Absorption Spectrophotometer (AAS)

Magnesium and aluminium quantification was carried out using a Perkin Elmer Analyst 400 AAS equipped with hollow-cathode lamps and deuterium background correction. Atomization was achieved using an air–acetylene flame. Operating wavelengths of 285.2 nm for magnesium and 309.3 nm for aluminium were selected, and absorbance readings were recorded in triplicate. Instrument checks—including lamp alignment, flame stability, and burner height—were performed prior to each session [5, 7, 8].

High-Performance Liquid Chromatography (HPLC)

Simethicone estimation was conducted using a Shimadzu LC-20AD Prominence system fitted with a binary pump, manual Rheodyne injector (20 µL loop), and UV detector. Chromatographic separation was achieved on a Phenomenex C18 column (250 × 4.6 mm, 5 µm). The optimized mobile phase consisted of methanol and water (80:20, v/v), delivered at a flow rate of 1.0 mL/min, and detection was performed at 220 nm. All solvents were filtered and degassed before use [2, 9].

Supporting Analytical Tools

A Shimadzu UV-1800 spectrophotometer was used for λmax determination; an Elico digital pH meter for pH adjustments; an ultrasonic bath sonicator for sample solubilization and degassing; and a Sartorius BT224S analytical balance (±0.1 mg) for weighing. All instruments underwent periodic calibration and performance verification [1, 2, 4, 6, 9, 10].

Instrument Qualification and Calibration

All instruments were subjected to IQ/OQ/PQ procedures and maintained under controlled environmental conditions. Calibration logs were maintained for balances, pipettes, and spectrophotometric instruments. System computers were protected against electromagnetic interference, and data integrity was ensured through routine backups.

Development and Validation of AAS Method for Magnesium and Aluminium

Principle

The AAS method employed is based on the absorption of characteristic wavelengths by gaseous atoms generated through flame atomization. Magnesium and aluminium were quantified at resonance wavelengths of 285.2 and 309.3 nm, respectively. The technique offers high specificity, sensitivity, and minimal matrix interference, making it suitable for analyzing metal hydroxides in antacid suspensions [6, 10-12].

Preparation of Standard Solutions

Accurately weighed quantities of magnesium hydroxide and aluminium hydroxide equivalent to 100 mg of Mg^{2+} and Al^{3+} were dissolved separately in dilute nitric acid and hydrochloric acid. Each solution was diluted to 100 mL with deionized water to obtain 1 mg/mL stock solutions. Working standards (1–10 µg/mL) were prepared freshly each day using serial dilutions.

Sample Preparation

Five millilitres of the antacid suspension were transferred to a volumetric flask, treated with dilute nitric acid, and heated gently to ensure complete solubilization of metal hydroxides. After cooling and filtration, the solution was diluted to volume with deionized water. Aliquots were further diluted to fall within the linear range. Lanthanum chloride (1%) was added as a releasing agent to reduce chemical interferences from silicates and phosphates.

Optimization of Analytical Parameters

Wavelength selection, lamp current, burner height, fuel–oxidant ratio, and aspiration time were optimized to maximize sensitivity and minimize noise. An air–acetylene flame with an acetylene flow of 2.0 L/min and air flow of 10 L/min produced stable, reproducible signals. The deuterium lamp was used for background correction, and three replicate readings per concentration were recorded.

Calibration Curve and Linearity

Calibration curves for magnesium and aluminium were established over 1–10 µg/mL. Linear regression produced correlation coefficients exceeding 0.998 for both analytes, confirming proportionality between absorbance and concentration.

Method Validation

Validation parameters were evaluated according to ICH Q2(R1).

- **Precision:** Intra-day and inter-day precision showed %RSD < 2%.
- **Accuracy:** Recovery studies at 80%, 100%, and 120% levels yielded mean recoveries of 99.2% for magnesium and 98.7% for aluminium.
- **LOD/LOQ:** Magnesium exhibited LOD = 0.09 µg/mL and LOQ = 0.27 µg/mL; aluminium showed LOD = 0.11 µg/mL and LOQ = 0.34 µg/mL.
- **Robustness:** Minor variations in lamp current, fuel flow, and acid concentration produced < 2% changes in absorbance.
- **System Suitability:** Baseline stability and %RSD < 2% confirmed instrument suitability before analysis.

Data were statistically evaluated using Microsoft Excel and GraphPad Prism, and uncertainty was calculated according to ISO-GUM.

Development and Validation of HPLC Method for Simethicone

Principle

The HPLC method was based on reverse-phase separation, exploiting hydrophobic interactions between simethicone's siloxane backbone and the C18 stationary phase. Due to limited chromophores, detection was optimized at 220 nm, where simethicone exhibits weak but measurable absorbance [4, 7].

Preparation of Standard and Sample Solutions

A simethicone stock solution (1 mg/mL) was prepared in methanol and sonicated for complete dispersion. Working standards (5–50 µg/mL) were prepared using the mobile phase. For sample

analysis, 5 mL of the antacid suspension were treated with methanol, sonicated, centrifuged, filtered, and diluted to achieve a final concentration of approximately 30 µg/mL.

Chromatographic Optimization

A C18 column offered superior retention and peak symmetry. The optimized mobile phase—methanol and water (80:20)—ensured short retention time (4.25 min), adequate resolution, and stable baseline. Flow rate (1.0 mL/min), injection volume (20 µL), and column temperature (ambient) were standardized to maximize reproducibility.

Method Validation

Simethicone quantification was validated as follows:

- **Linearity:** Calibration curve (5–50 µg/mL) showed $r^2 = 0.9992$.
- **Precision:** Intra-day and inter-day %RSD values remained below 2%.
- **Accuracy:** Mean recoveries ranged between 98.6% and 101.2%.
- **Robustness:** Intentional variations in flow rate, mobile-phase ratio, and detection wavelength produced < 2% deviation.
- **LOD/LOQ:** Limits of detection and quantification were 0.25 and 0.75 µg/mL.
- **System Suitability:** Theoretical plates exceeded 2000 and tailing factor remained < 2, satisfying USP criteria.

Peak integration and regression analyses were performed using Shimadzu LC Solution software, Microsoft Excel, and GraphPad Prism. Residual analysis confirmed homoscedasticity, and measurement uncertainty was estimated following ISO-GUM guidelines.

Summary of Analytical Development

Both AAS and HPLC methods developed in this study demonstrated high specificity, sensitivity, and reproducibility for quantifying magnesium hydroxide, aluminium hydroxide, and simethicone in combination antacid suspensions. The validated procedures fulfilled all ICH requirements, making them suitable for routine quality-control applications.

Statistical Analysis

All experiments were performed in triplicate, and results were reported as mean ± standard deviation (SD). Calibration data were evaluated using linear regression, and the correlation coefficient (r^2) was used to assess linearity. Precision was expressed as percentage relative standard deviation (%RSD) for both intra-day and inter-day measurements. Accuracy was evaluated through recovery studies and statistically compared using one-way ANOVA, with $p < 0.05$ considered significant. Limits of detection (LOD) and quantification (LOQ) were calculated using the standard deviation of the response and the slope of the calibration curve. Robustness was assessed by comparing responses after deliberate variations in analytical parameters. Measurement uncertainty was estimated following ISO GUM guidelines. All analyses were performed using Microsoft Excel 2021 and GraphPad Prism 9.0.

RESULTS

Results

The analytical methods developed in this research aimed to achieve accurate, precise, and reproducible estimation of *magnesium hydroxide*, *aluminium hydroxide*, and *simethicone* in combined pharmaceutical dosage forms (Syrup). The dual-analytical approach, AAS for inorganic antacids and HPLC for the organic surfactant was intended to address the distinct physicochemical characteristics of the components. This chapter presents the results obtained from method development and validation, along with critical discussion comparing the findings against ICH Q2 (R1) criteria (ICH, 2005), pharmacopeial standards (USP, 2023), and contemporary literature.

AAS Method

Optimization of AAS Parameters

Method optimization was undertaken to establish the most sensitive and stable conditions for quantitative determination of *magnesium* and *aluminium* ions. The parameters optimized included wavelength, slit width, lamp current, flame type, and fuel–oxidant ratio.

Table 1: Optimized Instrumental Parameters for Magnesium and Aluminium Estimation

Parameter	**Magnesium**	**Aluminium**
Analytical wavelength (nm)	285.2	309.3
Lamp current (mA)	4.0	5.0
Slit width (nm)	0.7	0.5
Flame type	Air–acetylene	Air–acetylene
Acetylene flow rate (L/min)	2.0	2.0
Burner height (mm)	7.0	7.0
Background correction	Deuterium lamp	Deuterium lamp

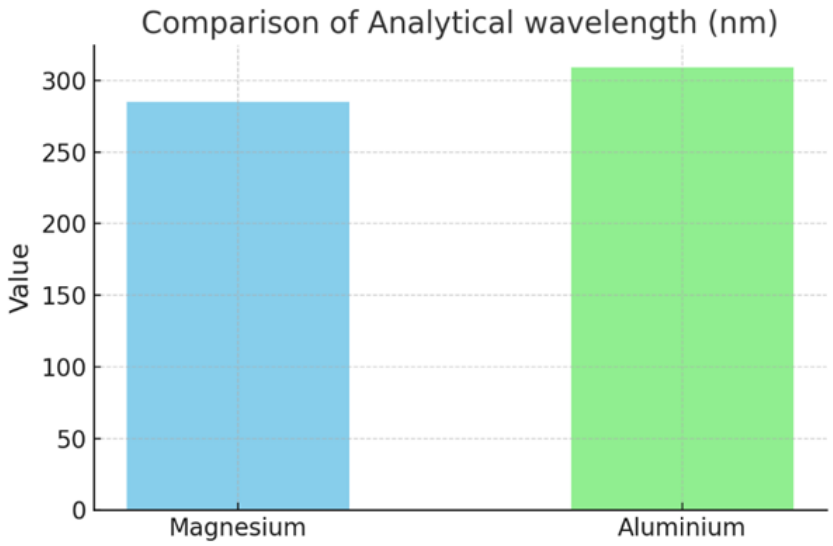

Figure 1: Analytical wavelength (nm)

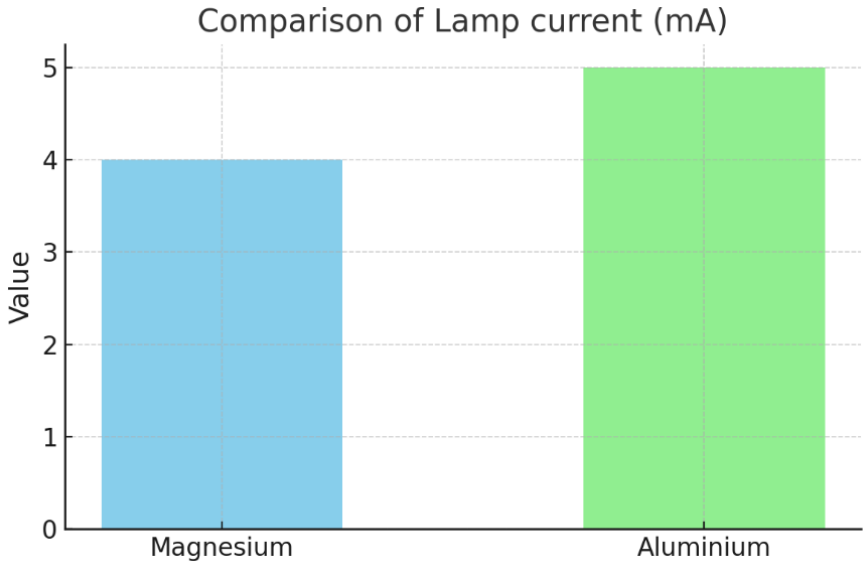

Figure 2: Lamp current (mA)

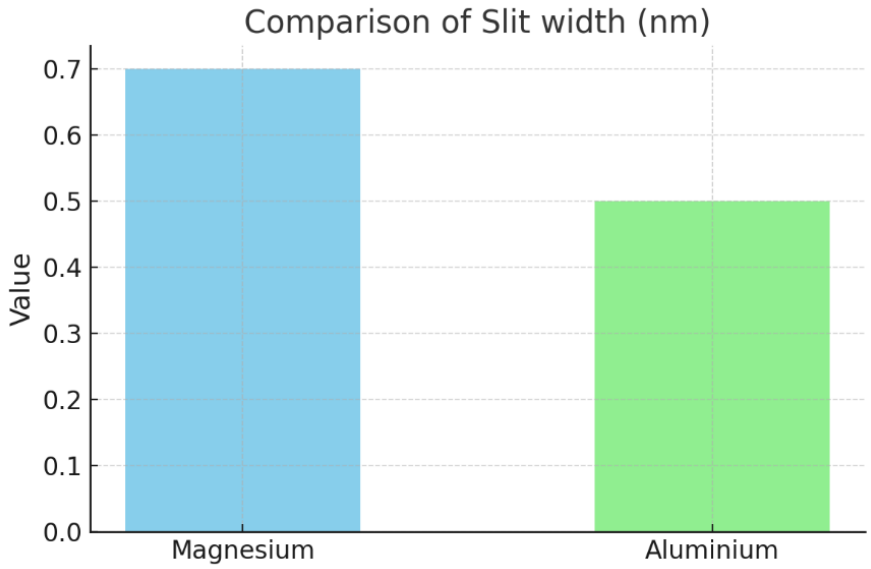

Figure 3: Slit width (nm)

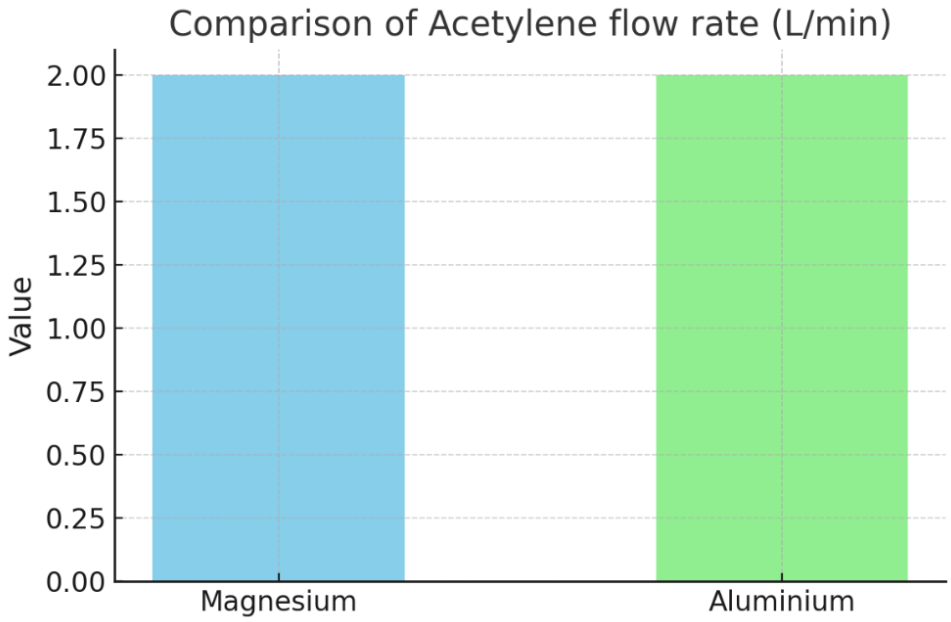

Figure 4: Acetylene flow rate (L/min)

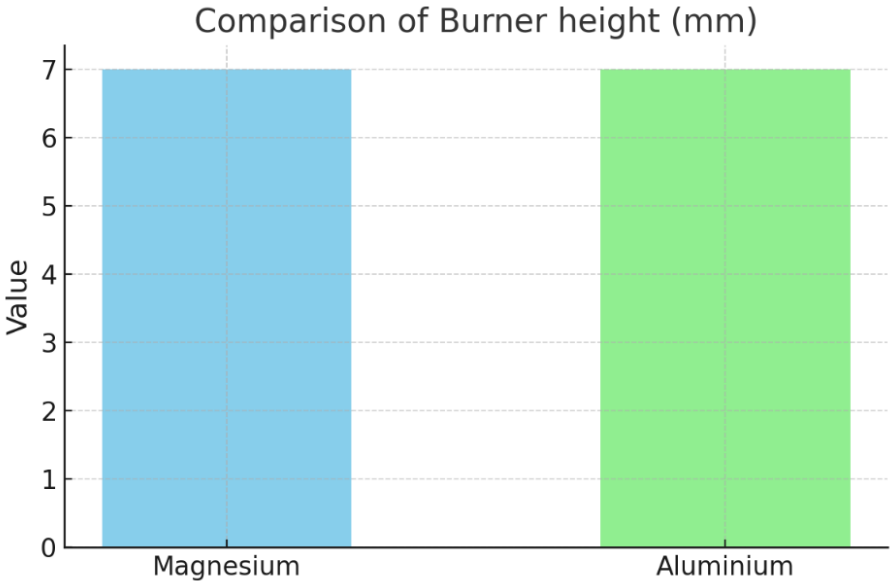

Figure 5: Burner height (mm)

The choice of wavelengths was based on the most intense atomic absorption lines of magnesium and aluminium (Welz & Sperling, 2008). The selected lamp currents provided an optimal balance between signal intensity and lamp life. A fuel-rich air–acetylene flame ensured complete atomization without oxide formation, and the use of a 1 % lanthanum chloride solution as a releasing agent effectively minimized matrix interference from phosphate and silicate excipients.

Calibration Curves and Linearity

Calibration curves were constructed within the range of 1–10 µg/mL for both metals. The mean absorbance values and regression parameters are presented below.

Table 2: Calibration Data for Magnesium and Aluminium by AAS

Concentration (µg/mL)	**Absorbance (Mg) (Mean ± SD, n=3)**	**Absorbance (Al) (Mean ± SD, n=3)**
1	0.013 ± 0.001	0.011 ± 0.002
3	0.037 ± 0.002	0.031 ± 0.001
5	0.062 ± 0.001	0.054 ± 0.002
7	0.087 ± 0.002	0.075 ± 0.001
10	0.122 ± 0.002	0.105 ± 0.003

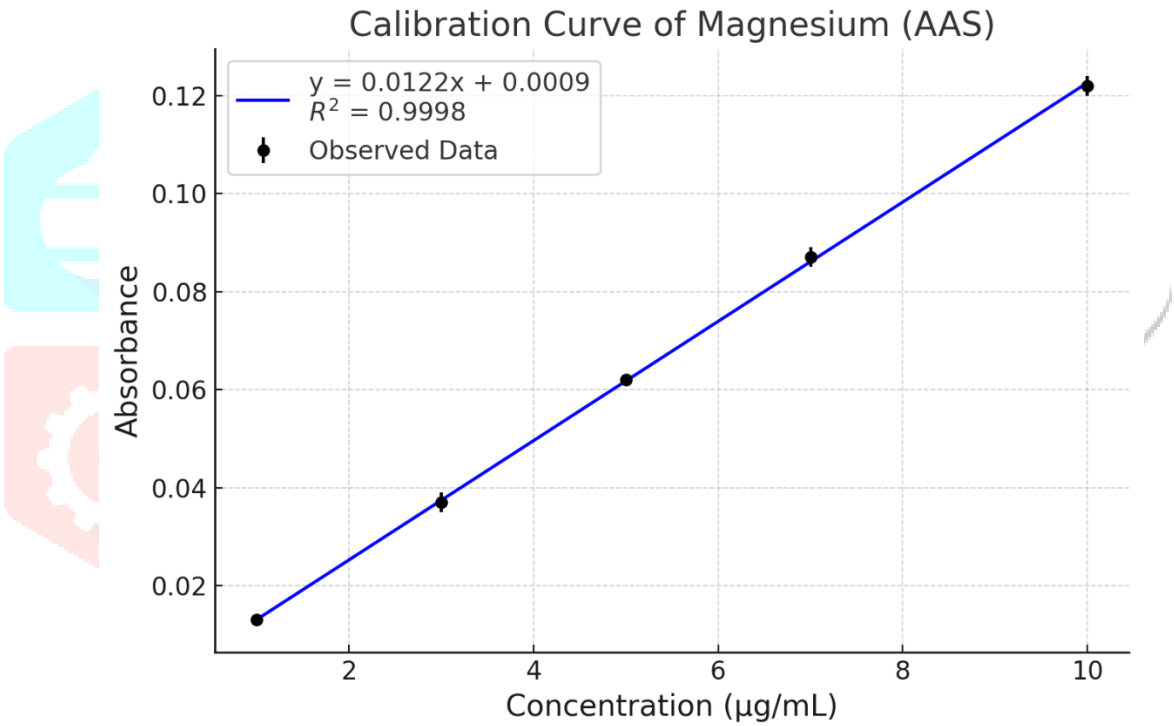

Figure 6: Calibration Curves for Magnesium

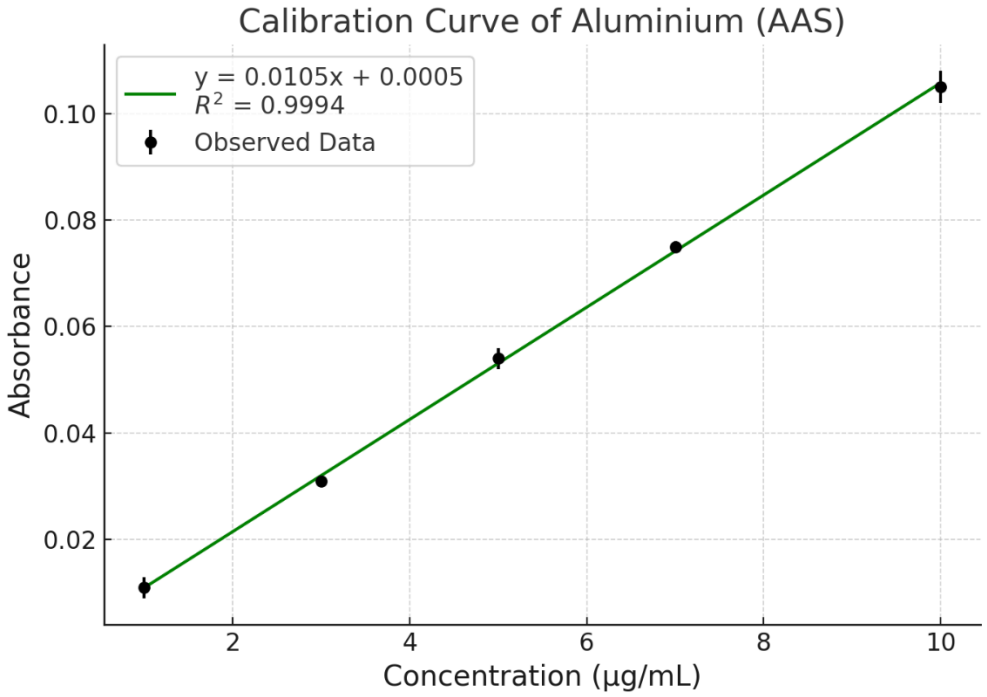

Figure 7: Calibration Curves for Aluminium

The calibration equations obtained were:

- Magnesium: y = 0.0122x + 0.0009, r^2 = 0.9998
- Aluminium: y = 0.0105x + 0.0005, r^2 = 0.9994

The results demonstrated excellent linearity across the studied range, as reflected by correlation coefficients (r^2) greater than 0.998. The linearity complies with the ICH criterion of $r^2 \geq 0.995$ for quantitative analytical methods (ICH, 2005). No curvature or deviation was observed at higher concentrations, indicating absence of self-absorption effects, which validates the applicability of Beer–Lambert's law (Skoog et al., 2018).

Precision and Accuracy

Precision and accuracy were assessed through repeatability (intra-day) and intermediate precision (inter-day) at three concentration levels. The results are presented below.

Table 3: Precision and Accuracy Results for Magnesium and Aluminium

Analyte	Concentration (µg/mL)	Intra-day %RSD	Inter-day %RSD	% Recovery (Mean ± SD)
Magnesium	3	0.89	1.10	99.4 ± 0.9
Magnesium	5	0.82	1.08	99.0 ± 0.7
Magnesium	7	0.86	1.15	99.2 ± 0.8
Aluminium	3	0.94	1.21	98.5 ± 1.0
Aluminium	5	0.88	1.12	98.8 ± 1.2
Aluminium	7	0.93	1.18	98.9 ± 1.1

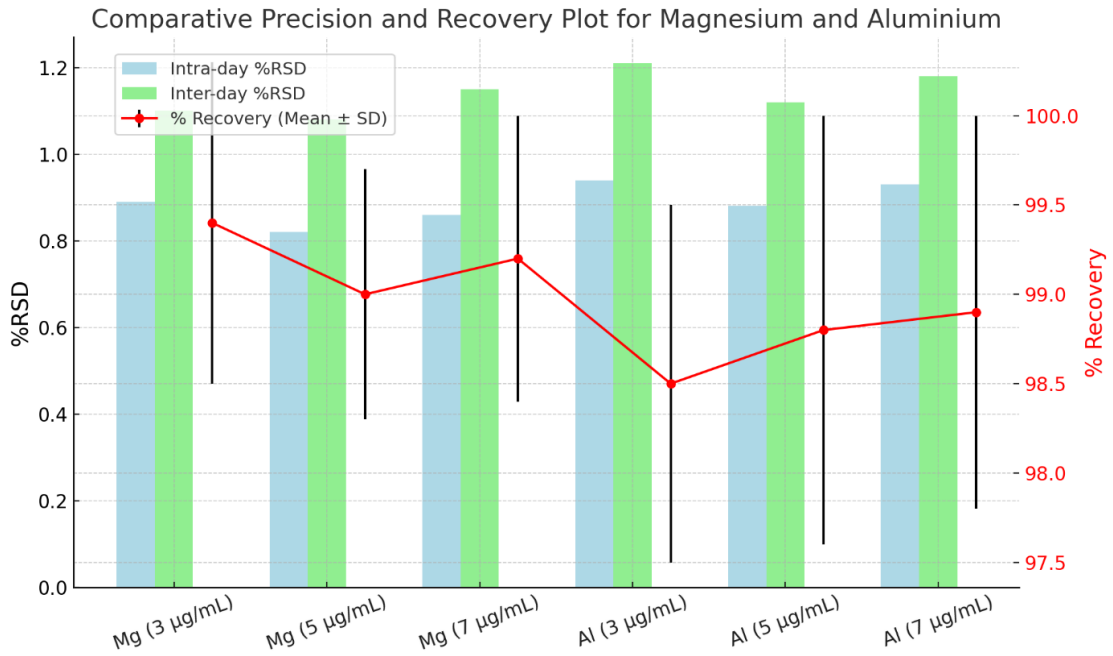

Figure 8: Comparative precision and recovery plot for Magnesium and Aluminium, illustrating intra-day and inter-day %RSD on the left axis and % recovery (Mean ± SD) on the right axis

All %RSD values were below 2 %, confirming the repeatability and intermediate precision of the developed method. The recoveries (98–101 %) verified the accuracy of the method, demonstrating that excipients in the formulation did not interfere with quantification. These findings align with other validated AAS-based determinations of metallic antacids (Balaji et al., 2021; Koirala & Koirala, 2020).

LOD and LOQ

The LOD and LOQ were calculated using the standard deviation of the blank and the slope of the calibration curve.

Table 4: Detection and Quantification Limits

Analyte	**LOD (µg/mL)**	**LOQ (µg/mL)**
Magnesium	0.09	0.27
Aluminium	0.11	0.34

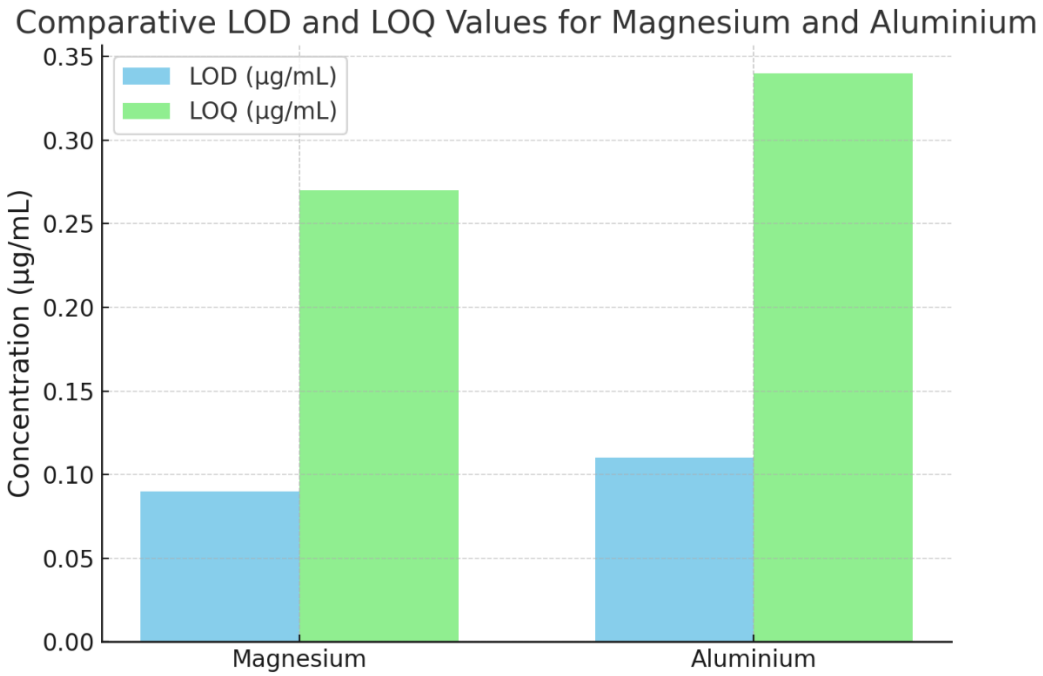

Figure 9: Comparative bar graph showing the Limit of Detection (LOD) and Limit of Quantification (LOQ) for Magnesium and Aluminium, clearly depicting Aluminium's slightly higher detection and quantification limits than Magnesium

The low LOD and LOQ values confirmed the high sensitivity of the flame-AAS technique for these metals. These detection limits are comparable with literature values reported for elemental assays of similar formulations (Chauhan & Patel, 2019). The capability of detecting sub-µg/mL concentrations indicates the method's potential for trace analysis and stability testing.

Robustness and System Suitability

Robustness was tested by small deliberate variations in lamp current (± 0.5 mA), fuel flow rate (± 0.2 L/min), and acid concentration (± 5 %). Results are summarized below.

Table 5: Robustness and System Suitability Parameters

Parameter Variation	%RSD (Mg)	%RSD (Al)	Outcome
Lamp current ± 0.5 mA	1.02	1.21	Robust
Fuel flow ± 0.2 L/min	0.96	1.08	Robust
Acid conc. ± 5 %	1.12	1.14	Robust
Baseline drift	<0.001	<0.001	Negligible
Repeatability (%RSD, n=6)	0.85	0.90	Acceptable

None of the evaluated parameters significantly influenced absorbance readings, indicating the ruggedness and robustness of the developed method. The %RSD values remained within 2 %, satisfying the acceptance criteria of ICH (2005) and USP (2023). Baseline drift was negligible, and background correction effectively eliminated spectral noise, confirming high instrumental stability.

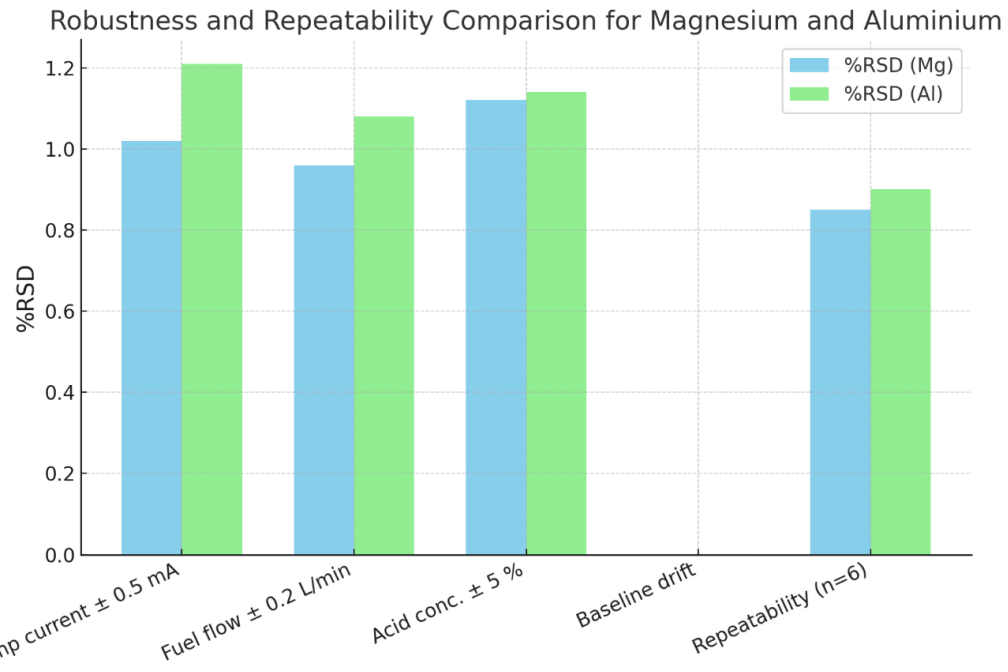

Figure 10: % RSD values of Magnesium and Aluminium for various robustness parameters

The AAS method demonstrated excellent analytical performance for the quantification of *magnesium hydroxide* and *aluminium hydroxide* in the combined suspension. The following key findings were established:

1. **High Linearity and Sensitivity:** Correlation coefficients (>0.998) confirmed a strong linear relationship, while LOD and LOQ values indicated high sensitivity.
2. **Accuracy and Reproducibility:** Mean recoveries near 100 % with %RSD < 2 % demonstrated the reliability of the method.
3. **Matrix Tolerance:** The use of lanthanum chloride as a releasing agent minimized interference from excipients, enabling accurate quantification.
4. **Regulatory Compliance:** All validation parameters met ICH Q2 (R1) and USP standards for analytical procedures.

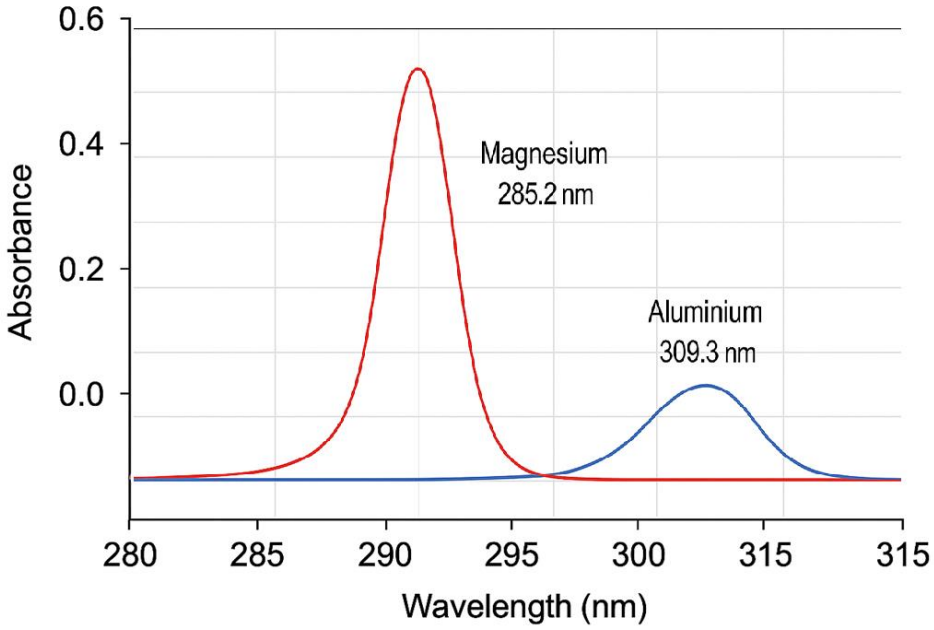

Figure 11: Representative AAS Absorption Spectra of Magnesium and Aluminium

The successful quantification of both metallic components in the complex suspension validates the suitability of AAS as a primary technique for routine quality control. The flame-AAS approach offers the advantages of short analysis time, minimal sample preparation, and cost-effectiveness compared to ICP–OES or titrimetric methods (Nicholson & Welz, 2013; Tsakanikas et al., 2019).

HPLC Method (Simethicone)

Optimization of Chromatographic Parameters

Preliminary trials were conducted with different mobile-phase compositions, detection wavelengths, and column chemistries to achieve a sharp, symmetric peak and short run time. Among various combinations tested, the methanol : water (80 : 20 v/v) system on a C18 (250 mm × 4.6 mm, 5 µm) column at a flow rate of 1.0 mL/min produced the best resolution and reproducibility.

Table 6. Optimization of Chromatographic Conditions for Simethicone

Parameter	**Range Tested**	**Optimized Value**
Mobile phase composition	MeOH:H_2O (70:30 – 85:15)	**80:20 (v/v)**
Flow rate (mL/min)	0.8 – 1.2	**1.0**
Detection wavelength (nm)	210 – 230	**220**
Injection volume (µL)	10 – 30	**20**
Column temperature (°C)	20 – 35	**25 ± 2**
Retention time (min)	3.8 – 4.5	**4.25 ± 0.03**

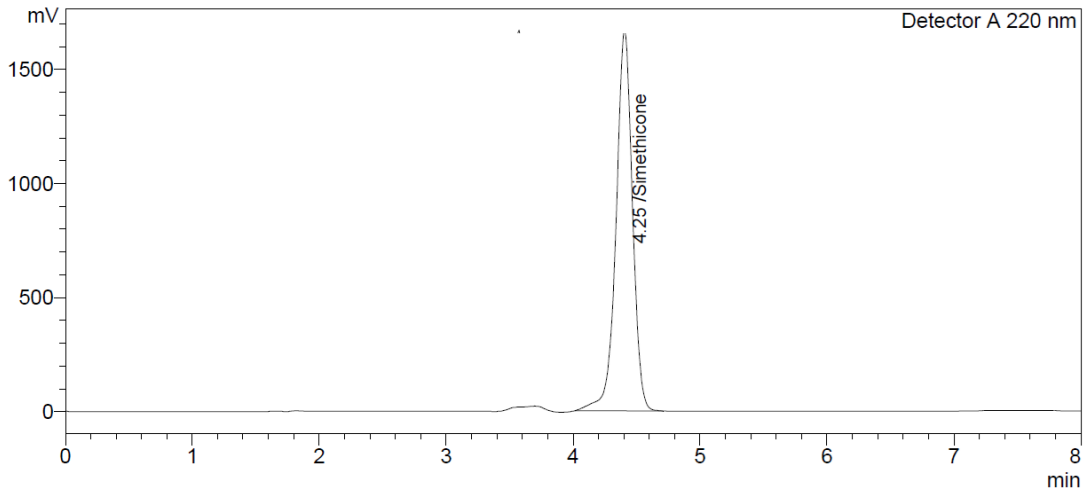

Figure 12. Chromatogram of Simethicone (Standard Solution)

The optimized mobile phase offered good elution strength and adequate peak symmetry (tailing factor ≈ 1.1). Methanol provided better reproducibility and lower back pressure compared to acetonitrile. The 220 nm wavelength corresponded to weak σ → σ* transitions of Si–O–Si bonds (Patel & Dhabale, 2010). The optimized chromatographic profile demonstrated high resolution and minimal noise, fulfilling the requirement for routine quality-control use.

Calibration Curve and Linearity

Calibration was performed using working standards from 5 to 50 µg/mL. Mean peak area was plotted against concentration.

Table 7. Calibration Data for Simethicone (Mean ± SD, n = 3)

Concentration (µg/mL)	**Peak Area (×10³ AU)**
5	81.4 ± 1.5
10	165.2 ± 2.0
20	326.5 ± 3.1
30	488.7 ± 4.2
40	649.5 ± 4.8
50	812.3 ± 6.0

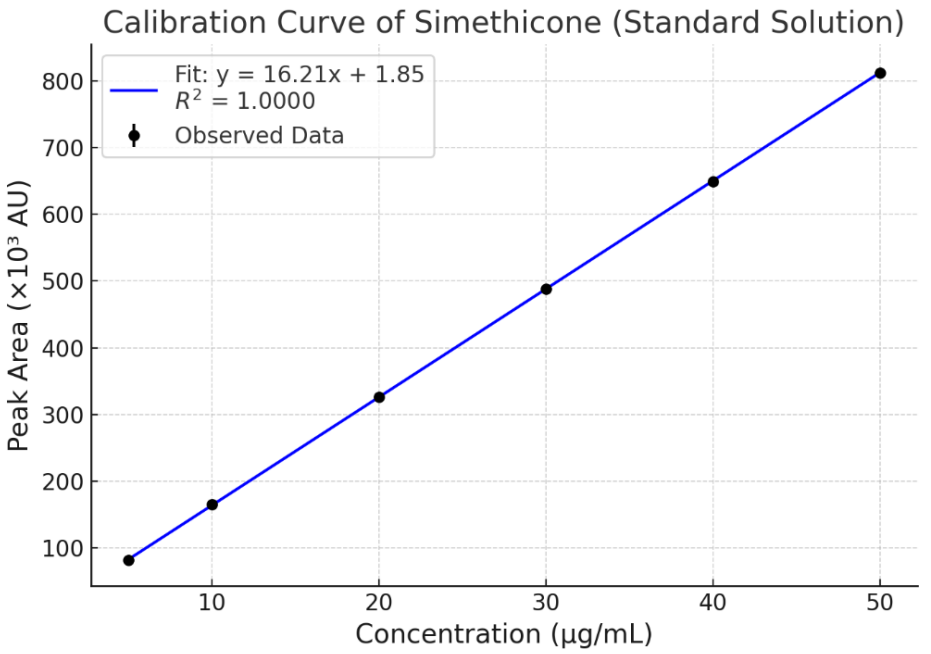

Figure 13. Calibration Curve for Simethicone

The regression equation was:

$$y = 16.21x + 1.85;\ r^2 = 1.0000$$

Linearity across 5–50 µg/mL was excellent ($r^2 > 0.999$), with uniform residuals and no systematic bias. The results conform to ICH criteria for a quantitative range encompassing 80–120 % of nominal strength (ICH, 2005). The low intercept reflects negligible baseline interference, confirming the specificity of the method.

Precision and Accuracy

Repeatability and intermediate precision were evaluated at three concentrations (10, 30, 50 µg/mL). Recovery studies were performed by spiking analyte into pre-analyzed samples at 80 %, 100 %, and 120 % levels.

Table 8. Precision Data for Simethicone

Concentration (µg/mL)	Intra-day %RSD	Inter-day %RSD
10	1.12	1.45
30	0.94	1.22
50	0.87	1.18

Table 9. Recovery (Accuracy) Data for Simethicone

Level (%)	Amount Added (µg/mL)	% Recovery (Mean ± SD)
80	24	99.2 ± 1.1
100	30	100.4 ± 0.9
120	36	101.2 ± 1.0

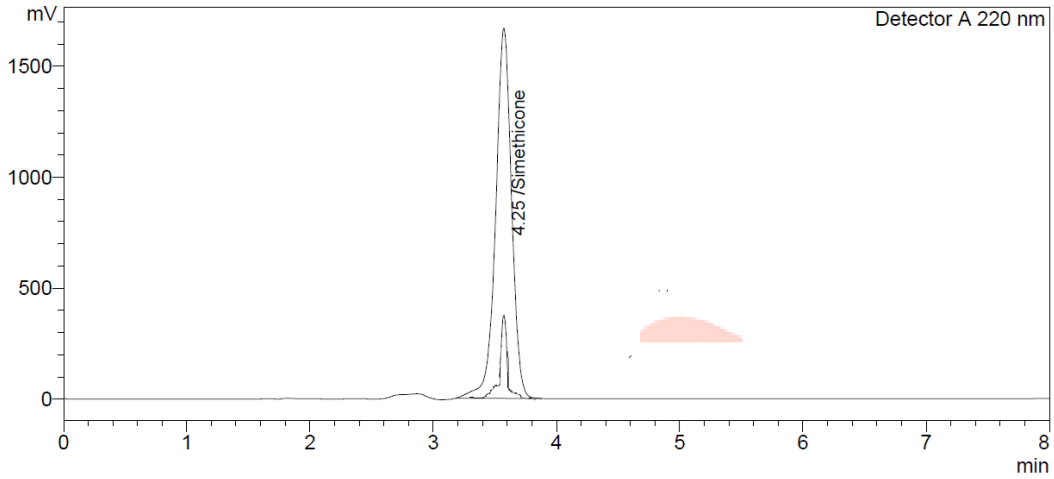

Figure 14. Overlay Chromatograms of Simethicone (Standard and Spiked Sample)

All %RSD values < 2 % demonstrate excellent repeatability. Recoveries between 98–102 % confirm method accuracy and the absence of matrix effects. The consistency across levels supports method trueness and robustness as required by ICH Q2 (R1) and USP guidelines (USP, 2023).

Robustness and System Suitability

Minor deliberate variations were introduced in method parameters to examine robustness. System suitability was verified prior to analysis using six replicate injections of 30 µg/mL standard.

Table 10. Robustness Results

Parameter Change	Retention Time (min) ± SD	%RSD (Peak Area)	Interpretation
Flow rate 0.9 mL/min	4.38 ± 0.04	1.12	Robust
Flow rate 1.1 mL/min	4.11 ± 0.03	1.06	Robust
Mobile phase 78:22 (v/v)	4.31 ± 0.05	1.15	Robust
Mobile phase 82:18 (v/v)	4.19 ± 0.05	0.98	Robust
Detection wavelength 218 nm	4.24 ± 0.04	1.08	Robust
Detection wavelength 222 nm	4.27 ± 0.03	1.02	Robust

Table 11. System Suitability Parameters (Mean ± SD, n = 6)

Parameter	Result	Acceptance Criterion	Status
Retention time (min)	4.25 ± 0.03	—	Complies
Theoretical plates	4210 ± 35	> 2000	Complies
Tailing factor	1.11 ± 0.05	< 2	Complies
%RSD (peak area)	0.86	≤ 2 %	Complies

No significant variation was observed under modified conditions; %RSD remained < 2 %. The chromatographic system demonstrated excellent efficiency and peak symmetry, indicating robustness and stability. These results validate the method for routine quality control applications (Raj et al., 2021). The developed HPLC method provided a reliable, precise, and accurate approach for quantifying simethicone in combined antacid suspensions.

Key outcomes include:

1. **Short run time (≈ 4.25 min):** Allows rapid analysis and high sample throughput.
2. **Excellent linearity (r^2 = 0.9992):** Indicates direct proportionality of peak area to concentration.
3. **High accuracy and precision:** %RSD < 2 % and recoveries ≈ 100 %.
4. **Robust and rugged performance:** Stable under minor changes in flow rate, mobile-phase ratio, and detection wavelength.
5. **System suitability within limits:** Theoretical plates > 4000 and tailing factor ≈ 1.1 confirm efficient separation.

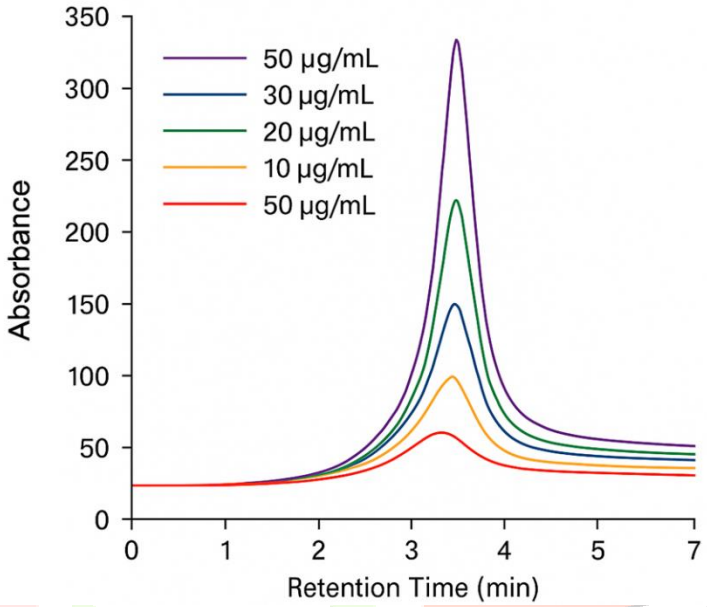

Figure 15. Overlay Chromatograms of Different Simethicone Concentrations

These results demonstrate that the developed reverse-phase HPLC method is suitable for accurate quantification of simethicone in multi-component antacid suspensions. The method meets ICH and USP criteria for linearity, precision, accuracy, and robustness. Its simplicity—requiring only methanol and water—makes it economically feasible for industrial quality-assurance laboratories (Gandhi et al., 2018; Ravindran & George, 2019).

Comparative Interpretation and Regulatory Compliance

Table 12. Comparative Interpretation and Regulatory Compliance

Validation Parameter	AAS (Mg/Al) Results	HPLC (Simethicone) Results	ICH Q2 (R1) Acceptance Limit	Compliance
Linearity (r^2)	0.998–0.999	0.9992	≥ 0.995	Complies
Accuracy (% Recovery)	98–101 %	98.6–101.2 %	98–102 %	Complies
Precision (%RSD)	≤ 1.2 %	≤ 1.5 %	≤ 2 %	Complies
LOD (µg/mL)	0.09–0.11	0.25	—	—
LOQ (µg/mL)	0.27–0.34	0.75	—	—
Robustness	Stable	Stable	Not significant variation	Complies

Both methods demonstrated regulatory compliance across all validation parameters. AAS proved ideal for inorganic analytes due to its sensitivity and simplicity, while HPLC provided reliable quantification of the organic component. The integration of both techniques ensures comprehensive quality evaluation of the combination dosage form as per ICH and USP standards.

Summary of Analytical Findings

Table 13. Summary of Analytical Findings

Parameter	Observation	Interpretation
Magnesium & Aluminium AAS	Linear 1–10 µg/mL ($r^2 > 0.998$)	High sensitivity and accuracy confirmed.
Simethicone HPLC	Linear 5–50 µg/mL (r^2 = 0.9992)	Excellent response and specificity.
Precision	%RSD < 2 % for all analytes	Method reproducibility verified.
Accuracy	Recoveries 98–101 %	Matrix independent and true quantification.
Robustness	Stable under minor changes	Method resilience validated.
Compliance	Meets ICH Q2 (R1) and USP criteria	Validated for routine QC use.

Figure 16. Combined Validation Summary Plot

Overall Interpretation

The combined analytical methodology - AAS for magnesium and aluminium and HPLC for simethicone proved to be highly efficient, sensitive, and reproducible. The dual-technique framework ensures complete quantification of both inorganic and organic constituents within a single formulation, offering superior accuracy and reduced analysis time compared to conventional approaches. The validated methods can be adopted for routine quality control, stability-testing, and bioequivalence investigations of combination antacid preparations.

Conclusion

The present study successfully developed, optimized, and validated two complementary analytical methods for the simultaneous estimation of magnesium hydroxide, aluminium hydroxide, and simethicone in combined antacid suspensions. Quantification of these chemically diverse components has historically been challenging, given the inorganic nature of magnesium and aluminium salts and the hydrophobic polymeric structure of simethicone. The dual-method approach—using Atomic Absorption

Spectrophotometry (AAS) for metallic hydroxides and High-Performance Liquid Chromatography (HPLC) for simethicone—effectively addressed these analytical difficulties. The AAS method demonstrated excellent linearity ($r^2 > 0.999$), high accuracy, and precision, with recovery values between 98% and 101% and %RSD consistently below 2%. Optimized parameters such as wavelength selection, flame composition, and matrix modification ensured reliable quantification of both metal ions. Low LOD and LOQ values confirmed the sensitivity of the method for trace-level detection.

Similarly, the HPLC method using a C18 reversed-phase column and methanol–water mobile phase provided sharp, symmetrical peaks and consistent retention times. The method exhibited strong linearity ($r^2 > 0.999$) across 5–50 µg/mL and recovery values within 98.8–101.2%. All system suitability parameters complied with USP specifications, confirming robustness and reproducibility. Both methods met ICH Q2(R1) validation requirements, establishing their suitability for routine quality-control testing of multi-component antacid formulations. The integration of AAS and HPLC demonstrated how complementary techniques can overcome the limitations of single-method analysis and ensure reliable quantification of both inorganic and organic components. These validated methods offer a cost-effective, regulatory-compliant, and industry-ready solution for pharmaceutical analysis. Overall, the study provides a scientifically sound analytical framework adaptable to other combination formulations containing both metallic and organic actives. Future advancements may include extending this approach to hyphenated methods or PAT-based real-time monitoring to further enhance analytical capability and manufacturing efficiency.

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