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Development and Validation of a Stability-Indicating HPLC Method for the Simultaneous Determination of Sodium Alginate and Parabens in Pharmaceutical Oral Suspensions

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Abstract

A stability-indicating high-performance liquid chromatographic (HPLC) method was developed and validated for simultaneous quantification of sodium alginate (Na-Alg), sodium methylparaben (Na-MP), and sodium propylparaben (Na-PP) in oral suspension formulations. This method addresses the need for reliable quality control of complex pharmaceutical matrices. Separation was achieved on an Ultisil XB-CN(Cyanopropyl) column at 40 °C, using an isocratic mobile phase composed of 0.05 M potassium dihydrogen phosphate buffer (pH 5.2) and acetonitrile (80:20, v/v) at a flow rate of 1.5 mL/min, with detection at 210 nm. The method was validated in accordance with the ICH Q2(R1) and USP guidelines to assess linearity, accuracy, precision, specificity, and robustness. The technique demonstrated excellent linearity ($R^2 > 0.9998$) over the concentration ranges of 10–1500, 1–150, and 0.16–24 μg/mL for Na-Alg, Na-MP, and Na-PP, respectively. The validation results showed high accuracy expressed as recovery (98.65–101.88%) across the tested concentration range of 50% to 150%, precision (%RSD < 1.9%), and sensitivity (LODs: 5.30, 0.038, and 0.062 μg/mL; LOQs: 17.65, 0.127, and 0.207 μg/mL) for Na-Alg, Na-MP, and Na-PP, respectively. The degradation products were successfully resolved from the analytes, thereby confirming the specificity of the method. The developed HPLC method used to analyze 34 commercial alginate suspension samples was highly precise (%RSD < 1.5%) and accurate (94.66%–105.20% recovery). It reliably quantified Na-Alg and preservatives (Na-MP and Na-PP), with results consistently matching the label claims. This robust method is suitable for routine quality control and stability testing, and effectively combines regulatory compliance with practical pharmaceutical analysis.

Article Info

Keywords:
Sodium Alginate , Parabens, Stability-indicating HPLC, Method Validation, ICH guidelines , Quality Control , Oral Suspensions

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1. INTRODUCTION

Sodium alginate is a natural anionic polysaccharide derived from brown seaweed (*Phaeophyceae*). It is a linear unbranched copolymer of two C-5 epimeric uronic acid monomers, β-D-mannuronic acid (M) and α-L-guluronic acid (G), linked predominantly via (1→4) glycosidic bonds [1–3]. It is widely used in the food, pharmaceutical, and cosmetic industries, including in wound dressings, drug delivery, tissue engineering, and antireflux formulations, owing to its unique physicochemical properties [4–10]. Through Ca^{2+}-mediated gelation, sodium alginate forms three-dimensional "egg-box" structures that provide exceptional rheological control in oral dosage forms. Its ability to create floating rafts in gastric acid aids in physically displacing the postprandial acid pocket, thereby relieving gastroesophageal reflux disease (GERD) symptoms. The excellent biocompatibility and safety profile of this polymer further enhance its pharmaceutical utility [11].

Preservatives, such as methylparaben and propylparaben, esters of p-hydroxybenzoic acid, are routinely

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included in these formulations to prevent microbial contamination and exhibit broad-spectrum antimicrobial activity [12]. Precise concentration control (90–110% of the label claim) is necessary to balance efficacy with toxicity concerns. Preservation stability is crucial, particularly under alkaline conditions, where hydrolysis forms inactive p-hydroxybenzoic acid [13]. Regulatory agencies require rigorous monitoring of preservative levels and degradation products throughout their shelf life [14–16].

Existing sodium alginate quantification methods often face challenges in complex suspensions. The USP titrimetric method for raw materials is unsuitable for suspensions because of interference from other components, which compromises accuracy [1]. Although it measures CO_2, it is complex, time-consuming, and susceptible to interference from carbonates/unkates, making it unsuitable for sodium alginate quantification in oral suspensions [17]. The existing sodium alginate analytical methods face significant challenges. Recent approaches [18, 19]involve complex pre-treatment and detection limitations, whereas HPLC methods either fail to quantify formulated products [20] or require impractical multi-step processes [21].

Sensitivity issues [22], labor-intensive preparation [23], and inconsistent hydrolysis [24] have historically limited the application of HPLC. Furthermore, many existing chromatographic methods are restricted to the analysis of raw materials [25] and are unsuitable for formulated products. Although more precise HPLC methods have been reported [26, 27], they are often hampered by insufficient validation or low throughput, which hinders their adoption for routine quality control. This lack of a suitable method exists alongside the stringent regulatory requirements. For instance, the British Pharmacopoeia mandates that the alginate content in suspensions must fall within 90–110% of the label claim [28], and general pharmaceutical quality standards are equally strict [29–31]. Consequently, there is an urgent need for a robust, universal quantification method that can meet these practical and regulatory demands.

This challenge is compounded by fundamental physicochemical differences between the analytes. Specifically, sodium alginate is a high-molecular-weight polymer (10,000–600,000 Da), while methyl and propyl parabens are low-molecular-weight compounds (152.15–180.20 g/mol). This disparity complicates the development of a unified analytical approach [1]. To date, no method has enabled simultaneous quantification of sodium alginate and preservatives in raft-forming pharmaceutical formulations.

Therefore, the primary goal of this study was to develop and validate a single, stability-indicating, High-Performance Liquid Chromatography (HPLC) method capable of the simultaneous and accurate quantification of both the high-molecular-weight polymer (sodium alginate) and low-molecular-weight preservatives (methylparaben and propylparaben) in complex oral suspension matrices.

To achieve this goal, we developed a stability-indicating HPLC method that provides baseline separation of all analytes using a suitable stationary phase, which was validated for accuracy and reliability. The degradation kinetics were studied under various stress conditions, confirming the stability-indicating capabilities of this method. The applicability of this method was demonstrated by successful analysis of commercial pharmaceutical products. This is the first unified protocol for the simultaneous quantification of polymeric and small-molecule components in complex pharmaceutical suspensions.

2. MATERIALS AND METHODS:

2.1. Standard materials, excipients, and chemicals:

The standard materials and excipients used in this study included sodium alginate (Na-Alg, 92.6%) from Nova Matrix (Norway), sodium methylparaben (Na-MP) and sodium propylparaben (Na-PP) (99.0%) from Ami Organic (India), carbomer (62.0%) from Gloria (India), 100% pure peppermint oil and strawberry liquid from Aromatech FSL (UAE), calcium carbonate (98%) from Magnesia (Germany), and sodium bicarbonate (100%) from TATA Chemical Europe Limited (UK).

All other chemicals and reagents were of analytical grade and obtained from CDH Corporation (India), including sodium hydroxide (97.0%), sodium dihydrogen phosphate (98.0%), potassium dihydrogen phosphate (99.0%), anhydrous sodium acetate (98.0%), glacial acetic acid (99.5%), phosphoric acid (85.0%), hydrochloric acid (37.0%), hydrogen peroxide (30.0%), and acetonitrile (99.9%). Deionized water was produced in-house using a Millipore treatment unit (USA).

2.2. Instrumentation:

Two HPLC systems were employed: a Jasco system (Japan) equipped with a Photo Diode Array Multiwavelength Detector (PDA MD-4010) and a pump (PU-4285) operated in isocratic mode, and a Waters system (USA) comprising an e2695 separation module, a 2489 UV/Vis Detector, and a column heater.

Additional equipment included an analytical balance GR-120 (A&D, Japan), ultrasonic bath (Sapeen, China), GM-05 vacuum pump (Germany), Jenway 3520 pH meter (UK), and Hettich EBA 20 centrifuge (Germany).

2.3. Chromatographic conditions:

Separation was achieved using an Ultisil XB-CN (250 x 4.6 mm, 5 μm); Welch, China) maintained at 40 ℃. The mobile phase was an isocratic mixture of 0.05 M

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potassium dihydrogen phosphate buffer and acetonitrile (80:20, v/v) (pH 5.2) at a 1.5 mL/min flow rate. Detection was performed at 210 nm with a 20 μL injection volume.

2.4. Solutions preparation:

Stock standard solutions were prepared by dissolving 100.0 mg Na-MP and 16.0 mg) in 100 mL of mobile phase. Working standard solutions were prepared by adding 5 mL of the stock solution to a mixture containing 50 mg of Na-Alg and diluting it to 50 mL with the mobile phase, followed by sonication for 10 min.

The sample preparation involved diluting 2 mL of the oral suspension to 100 mL with the mobile phase, followed by sonication, centrifugation, and filtration. Blank samples were prepared by dissolving the carbomer, calcium carbonate, sodium bicarbonate, and other excipients in deionized water, followed by sonication, centrifugation, and filtration before injection.

2.5. Development and optimization of the HPLC method:

A chromatographic method for the simultaneous analysis of Na-Alg, Na-MP, and Na-PP was developed by optimizing the following key parameters.

Mobile Phase/Buffer Systems: The method was optimized by testing different buffer systems (sodium acetate, NaH_2PO_4, KH_2PO_4), acetonitrile ratios (80:20 to 90:10, v/v), and pH values (5.0, 5.2, and 5.4). The final selection was 0.05 M KH_2PO_4 buffer and acetonitrile (80:20, (pH 5.2), v/v), which provided optimal peak shape and resolution.

· **Chromatographic Column:** Four stationary phases (C18, C8, silica, and cyano (CN); all 5 μm particle size) were screened. An Ultisil XB-CN column (25 × 0.46 cm, 5μm) was selected for best separation performance at an optimized column temperature of 40 ℃ (optimized between 35 ℃, 40 ℃, 45 ℃).

· **Instrumental Parameters:** The flow rate was optimized between 1.0 and 2.0 mL/min, with 1.5 mL/min selected. The detection wavelength was determined by scanning standard solutions from 200 to 650 nm, selecting 210 nm as the optimal wavelength for sensitivity and minimal interference. The injection volume used was 20 μL.

2.6. The Validation method:

The method was validated for specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), and robustness in accordance with ICH Q2(R1) [11] , USP guidelines [32], and previously reported studies [33–35].

2.7. Stress degradation studies:

Forced degradation studies were performed on the reference solutions of Na-Alg (1.0 mg/mL), Na-MP (0.10 mg/mL), and Na-PP (0.016 mg/mL) under various stress conditions. Acidic and alkaline hydrolysis were performed using 0.5 M HCl and 0.5 M NaOH, respectively, for 1 h in a 70 ℃ water bath. Oxidation was induced using 3% v/v H_2O_2 for 1 h. Thermal stress were storing solutions at 60 ℃ or 7 ℃ for 7 days. In addition, the solutions were subjected to photolytic stress via exposure to light.

2.8. Application:

The validated method was successfully applied to analyze 34 pharmaceutical sample suspensions collected from Yemen pharmacies. All samples were prepared and analyzed in triplicate according to established procedures.

3. RESULTS AND DISCUSSION:

3.1. Development and optimization of the HPLC method

High-performance liquid chromatography (HPLC) was optimized for the simultaneous analysis of Na-Alg, Na-MP, and Na-PP. The initial mobile phase was a mixture of "0.01 M KH_2 PO_4 and acetonitrile (90:10, v/v), which was selected based on the analyte polarity, detector compatibility, and buffering requirements for reversed-phase separation.

The optimal detection wavelength was determined empirically by scanning standard solutions from 200 nm to 650 nm using a photodiode array (PDA). The spectral data revealed that 210 nm offered the most suitable combined analytical signal for all the three target compounds. This choice aligns with the rationale, which recommends 210 nm to minimize the interference from common excipients in similar oral suspensions. Consequently, 210 nm was adopted as the final detection wavelength, meeting the requirements of both adequate sensitivity (Figure 1) and method specificity.

Column selection was critical for simultaneously separating the highly polar Na-Alg polymer and hydrophobic paraben preservatives. Initial trials with conventional reversed-phase columns (C18, C8) with low acetonitrile content produced broad, tailing Na-Alg peaks and poor preservative retention. Higher acetonitrile concentrations improved the paraben elution but failed to resolve the Na-Alg peak tailing.

The silica column also yielded inadequate separation owing to the peak overlap. In contrast, the cyanopropyl (CN) column produced sharp, well-defined peaks for all analytes under isocratic conditions (Figure 2A–E). This superior performance stems from the mixed-mode re-

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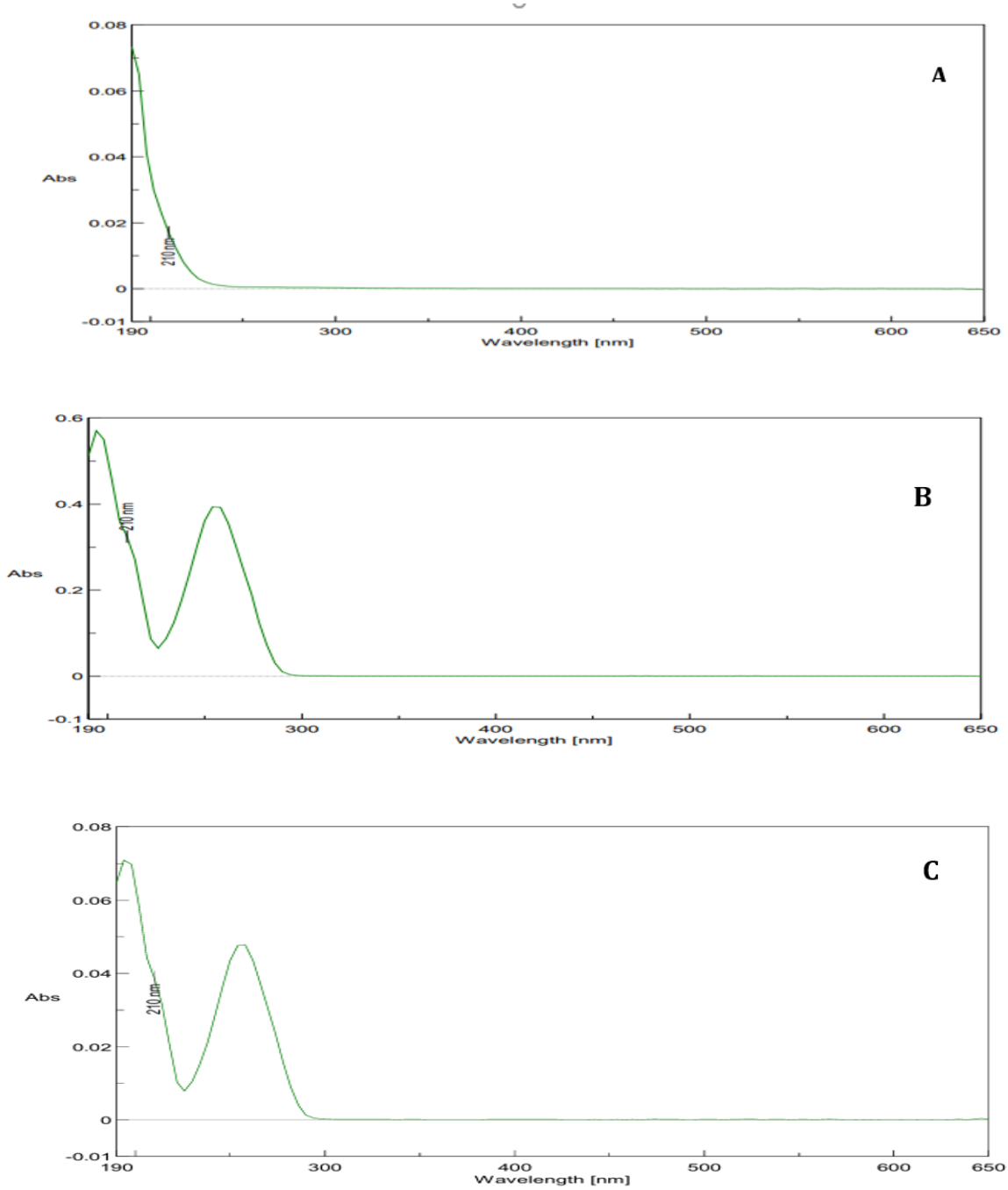


Figure 1. Detection wavelength of (A) Na-Alg, (B) Na-MP, and (C) Na-PP.

tention of the CN column; its short alkyl chain provides hydrophobic interactions for parabens, while the polar nitrile group enables reversible dipole interactions with Na-Alg. This balance minimized silanol-related tailing, enabling robust separation, and establishing the CN column (Ultisil XB-CN) as optimal for further development. Table 1 summarizes the initial chromatographic parameters of the cyanopropyl (CN) column.

With the selected CN column, the mobile phase was refined by testing three 0.05 M buffer systems (sodium acetate, NaH_2PO_3, KH_2PO_3) in 10–20% acetonitrile. Sodium acetate yielded sharp peaks but significant baseline interference at 210 nm. The phosphate buffers performed well, with KH_2PO_3 providing the sharpest peaks and the best resolution at 80:20 (v/v, buffer: acetonitrile).

mobile phase pH (5.0, 5.2, 5.4) was evaluated at 40℃, no significant differences emerged in Na-Alg, Na-MP, or Na-PP elution or separation. Thus, the natural pH 5.2 of the KH_2PO_4-acetonitrile mixture was used for simplicity and robustness. A 40℃ column temperature was found to be optimal. This systematic optimization produced an efficient and reliable HPLC method for Na-Alg, Na-MP, and Na-PP quantification, enhancing the sensitivity, practicality, throughput, and cost-effectiveness.

This iterative and systematic approach is essential for the development of an efficient and reliable HPLC

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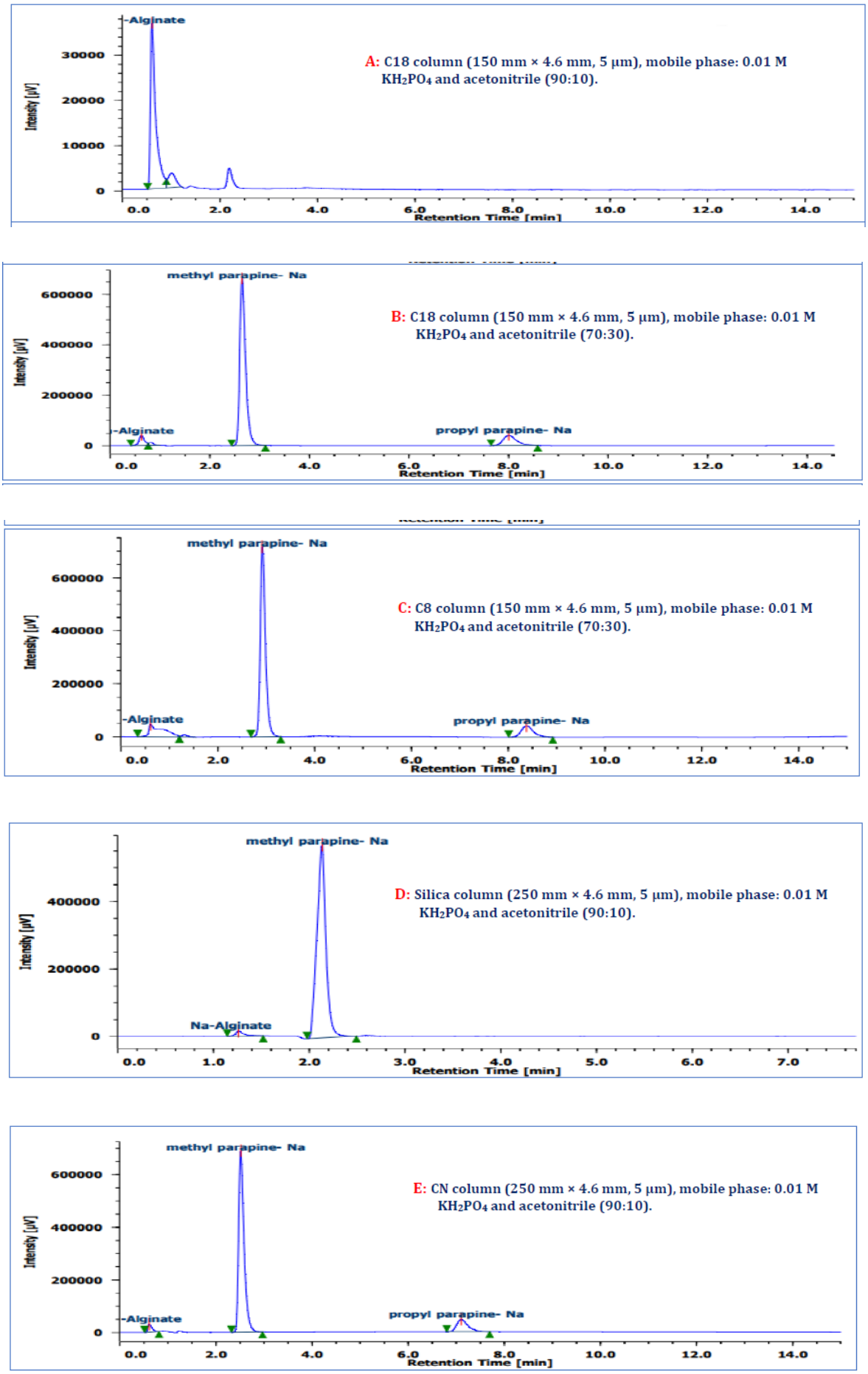


Figure 2. (A-E): Effect of different columns on Na-Alg, Na-MP, and Na-PP separation efficiency. in the initial method development.

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Table 1. Chromatographic parameters from initial method development on cyanopropyl (CN) column (pre-mobile phase optimization)

Peak Name	Retention Time (min)	Number of Theoretical Plates (NTP)	Resolution	Symmetry Factor (Tailing Factor)
Na-Alg	0.608	239	10.7	1.91
Na-MP	2.508	2540	14.971	1.55
Na-PP	7.083	4690	N/A	1.495
Figure Legend	X-axis: Time (minutes)		Y-axis: Intensity (μV), detector signal in microvolts	

Table 2. Linearity results of the HPLC for the determination of Na-Alg, Na-MP, and Na-PP

Parameter	Na-Alg	Na-MP	Na-PP
Correlation Coefficient (R^2)	0.999895	0.999977	0.999966
Slope	397.02	56695.59	48021.07
y- intercept	-4420.09	14612.55	1122.96
Regression line equation	y = 397.02x - 4420.09	y = 56695.6x + 14612.55	y = 48021.1x + 1122.96
The Range (μg/mL)	10 to 1500	1 to 150	0.16 to 24
%RSD	0.12 to 1.34%	0.07 to 1.58%	0.22 to 1.82%

method for the simultaneous quantification of Na-Alg, Na-MP, and Na-PP, leading to improved sensitivity, practicality, and cost-effectiveness.

3.2. The Validation method

3.2.1. Specificity

The specificity of the method was demonstrated by comparing the HPLC chromatograms of Na-Alg, Na-MP, and Na-PP standards against a blank placebo suspension prepared without the active analytes. As shown in Figure 3, chromatographic comparison revealed no interfering peaks from the excipients at the retention times of the active ingredients, confirming a clean, matrix-free baseline. This high selectivity was further corroborated by an accuracy study (Section 3.2.3, which demonstrated recovery results within the accepted range, thereby confirming the ability of the method to accurately quantify Na-Alg and parabens in the presence of all other formulation components.

3.2.2. Linearity

The linearity was evaluated by constructing triplicate calibration curves for each analyte by plotting the average peak area against ten distinct concentrations. The concentration ranges studied were 10–1500 μg/mL for Na-Alg, 1–150 μg/mL for Na-MP, and 0.16–24 μg/mL for Na-PP. Least-squares regression analysis demonstrated a strong linear relationship across the respective concentration ranges for all three compounds, as confirmed by the high correlation coefficients ($R^2 > 0.999$) detailed in Table 2 2. Furthermore, the low % Relative Standard Deviation (%RSD) values (maximum 1.82%) demonstrate the high precision and reliability of the analytical measurements throughout the working range.

3.2.3. Accuracy

The homogenized blank samples were spiked with Na-Alg, Na-MP, and Na-PP at five concentration levels ranging from 50% to 150% of the target concentrations (500–1500 μg/mL) for Na-Alg, (50–150 μg/mL) for Na-MP, and (8–24 μg/mL) for Na-PP. The samples were analyzed using the proposed method. The results showed excellent accuracy, with percentage recoveries (%R) between 98.65% and 101.88% and a relative standard deviation (%RSD) of not more than 0.47% (Table 3 The consistent peak shapes and conformance shown in Figure 4 further validate the reliability of the method at all the tested levels.

3.2.4. Repeatability and Ruggedness

The precision of the analytical method was evaluated using repeatability (intraday precision) and ruggedness (intermediate precision). For repeatability, six spiked samples prepared at 100% of the target concentration were each injected three times, demonstrating excellent results with mean Relative Standard Deviation (%RSD) values of 0.35% for Na-Alg, 0.22% for Na-MP, and 0.43% for Na-PP (Table 4). The consistent peak shapes observed in Figure 5 further confirmed the precision and stability of the system.

Furthermore, intermediate precision was achieved by analyzing the same samples using different analysts and different instruments on three consecutive days. The results of this evaluation (Table 4) showed that the %RSD values for all three analytes remained below 2.0%. These findings confirm the high repeatability, ruggedness, and reliability of the method under various analytical condi-

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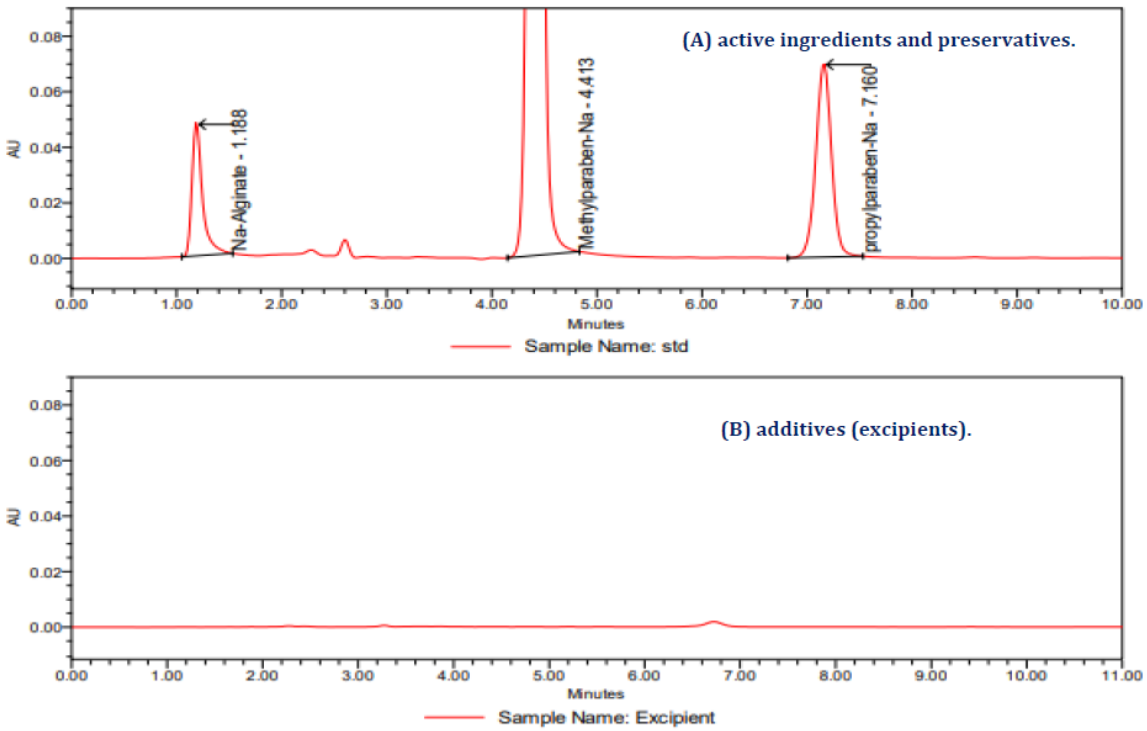


Figure 3. Method selectivity demonstrating the differentiation between (A) active ingredients and preservatives, (B) additives (excipients).

Table 3. Accuracy results for Na-Alg, Na-MP, and Na-PP

Conc., %	Na-Alg		Na-MP		Na-PP	
	% R	RSD %	% R	RSD %	% R	RSD %
50.00	101.88	0.09	100.15	0.13	98.65	0.16
75.00	101.72	0.12	99.27	0.13	99.21	0.09
100.00	100.05	0.44	99.99	0.48	100.54	0.47
125.00	100.15	0.07	99.08	0.12	100.01	0.23
150.00	99.99	0.26	99.49	0.26	101.36	0.30

tions.

3.2.5. Limit of detection (LOD) and limit of quantitation (LOQ)

The limits of detection (LOD) and quantification (LOQ) were determined using a solution containing Na-Alg (20 μg/mL), Na-MP (0.25 μg/mL), and Na-PP (0.04 μg/mL). The signal-to-noise (S/N) ratios of 3:1 and 10:1 were used to define the LOD and LOQ, respectively. The LOD values were 5.30 μg/mL for Na-Alg, 0.038 μg/mL, and 0.062 μg/mL, respectively. The LOQ values were established at 17.65 μg/mL for Na-Alg, 0.127 μg/mL, and 0.207 μg/mL, respectively. These results confirmed the sensitivity of the method for reliable quantification at low analyte concentrations.

3.2.6. Robustness

The robustness of the HPLC method was rigorously evaluated by introducing deliberate variations in key analytical parameters, including mobile phase pH, flow rate, detection wavelength, column temperature, buffer salt concentration (±10%), mobile phase composition (±10% acetonitrile), and changes in the column supplier, analyst, and instrumentation.

The method showed consistently reliable performance across most variations with several informative effects. The use of a CN column from a different supplier (Teknokroma Tracer Excel 120 CN) improved the theoretical plate counts for all analytes [Na-Alg: 784-913; Na-MP: 7881-8654; Na-PP: 10570-10205] while maintaining a high paraben resolution (11.64 vs 11.4 initially). The resolution was pH-sensitive, decreasing from 11.4 to 7.9 when the pH was adjusted from 5.2 to 5.0. Furthermore, the use of an alternative HPLC instrument increased the Na-Alg tailing factor to 2.0.

A notable decrease in the Na-Alg recovery (96.2%) was observed at an elevated flow rate of 2.0 mL/min. This effect is attributed to the polymeric nature of Na-Alg,

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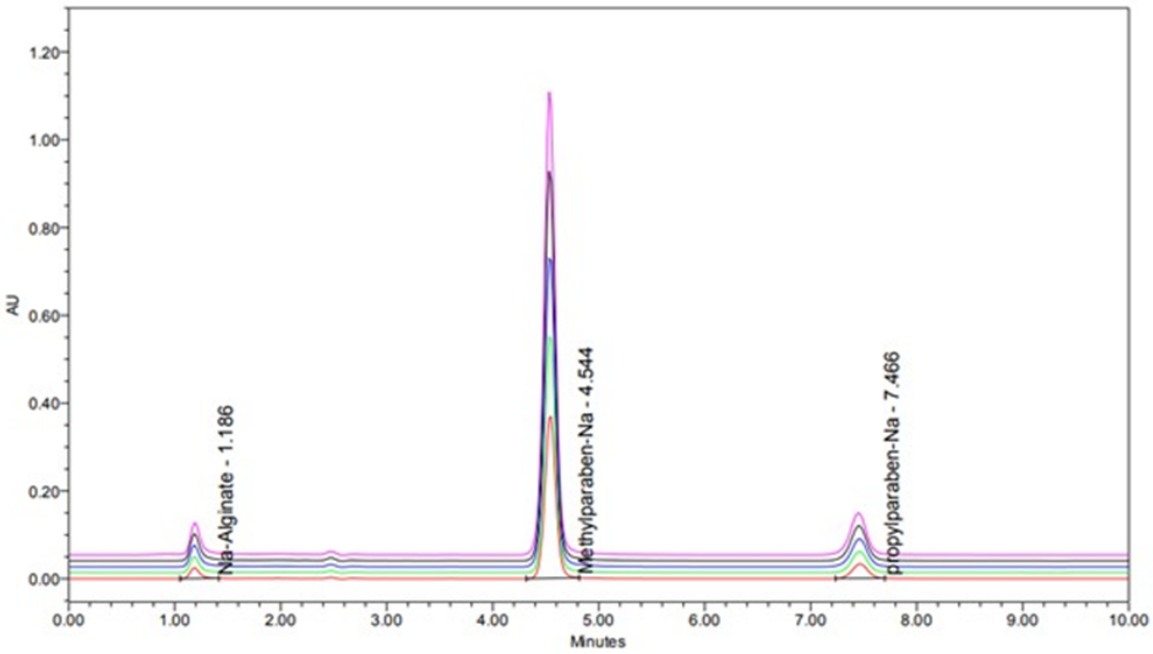


Figure 4. Overlay chromatograms of Na-Alg, Na-MP, and Na-PP at different levels

Table 4. Intra-day (Repeatability)-and intermediate precision results for Na-Alg, Na-MP, and Na-PP.

Sample	A- Repeatability (Intra-Precision)						B- Intermediate Precision (Ruggedness)*											
							1- Analyst Change						2- Instrument Change					
	Na-Alg		Na-MP		Na-PP		Na-Alg		Na-MP		Na-PP		Na-Alg		Na-MP		Na-PP	
Code	Conc*. (μg/ml) (n=3)	%RSD	Conc*. (μg/ml) (n=3)	%RSD	Conc*. (μg/ml) (n=3)	%RSD	Conc*. (μg/ml) (n=3) ※	%RSD	Conc*. (μg/ml) (n=3) ※	%RSD	Conc*. (μg/ml) (n=3) ※	%RSD	Conc*. (μg/ml) (n=3) ※	%RSD	Conc*. (μg/ml) (n=3) ※	%RSD	Conc*. (μg/ml) (n=3) ※	%RSD
S1	1025.6	0.14	101.6	0.07	16.29	0.11	979.4	0.03	100.2	0.05	16.21	0.06	1018.9	0.51	100.2	0.05	16.03	0.29
S2	1024.4	0.26	101.5	0.18	16.27	0.20	995.1	0.26	100.2	0.19	16.20	0.18	1019.7	1.04	100.0	0.11	16.00	0.16
S3	1023.4	0.18	101.5	0.12	16.29	0.10	996.7	0.20	99.9	0.10	16.18	0.13	1016.8	0.20	100.2	0.11	15.99	0.07
S4	1029.1	0.07	102.0	0.14	16.36	0.11	978.0	0.38	100.3	0.07	16.19	0.49	1020.2	0.37	100.4	0.36	16.12	0.83
S5	1018.0	0.09	101.8	0.08	16.34	0.12	966.6	0.09	101.1	0.15	16.22	0.15	1017.8	1.28	100.7	0.35	16.19	0.86
S6	1025.0	0.38	101.4	0.06	16.16	0.62	1002.4	0.21	100.1	0.12	16.04	0.11	970.0	1.46	101.1	0.12	16.33	0.20
Mean	1024.3		101.65		16.29		986.38		100.30		16.17		1010.55		100.44		16.11	
SD	3.63		0.22		0.070		13.80		0.43		0.069		19.90		0.42		0.133	
%RSD	0.35		0.22		0.43		1.40		0.43		0.43		1.97		0.42		0.83	

*The concentration indicated in the table is for the diluted sample measured (1:50).

※ The analysis was performed across three consecutive days.

where higher flow rates reduce the stationary-phase interaction time and can limit the mass-transfer efficiency of high-molecular-weight species [36]. In contrast, paraben recoveries remained within ICH Q2(R1) assay limits and reflected a deliberate extreme variation outside the normal operating range.

Across all robustness conditions, the method performed reliably, with mean recoveries of 100.7% (Na-Alg), 101.1% (Na-MP), and 100.7% (Na-PP) and low RSD values of 2.24%, 2.02%, and 1.04%, respectively (Table 5). These results confirmed the robustness and suitability of this method for routine laboratory quality control.

3.3. Stress degradation studies:

Forced degradation studies confirmed the stability-indicating properties of this method. Reference solutions of Na-Alg (1.0 mg/mL), Na-MP (0.10 mg/mL), and Na-PP (0.016 mg/mL) were subjected to acidic hydrolysis (0.5 N HCl, 1 h), alkaline hydrolysis (0.5 N NaOH, 1 h), oxidation (3% H_2O_2, 1 h), photolytic stress (one-week light

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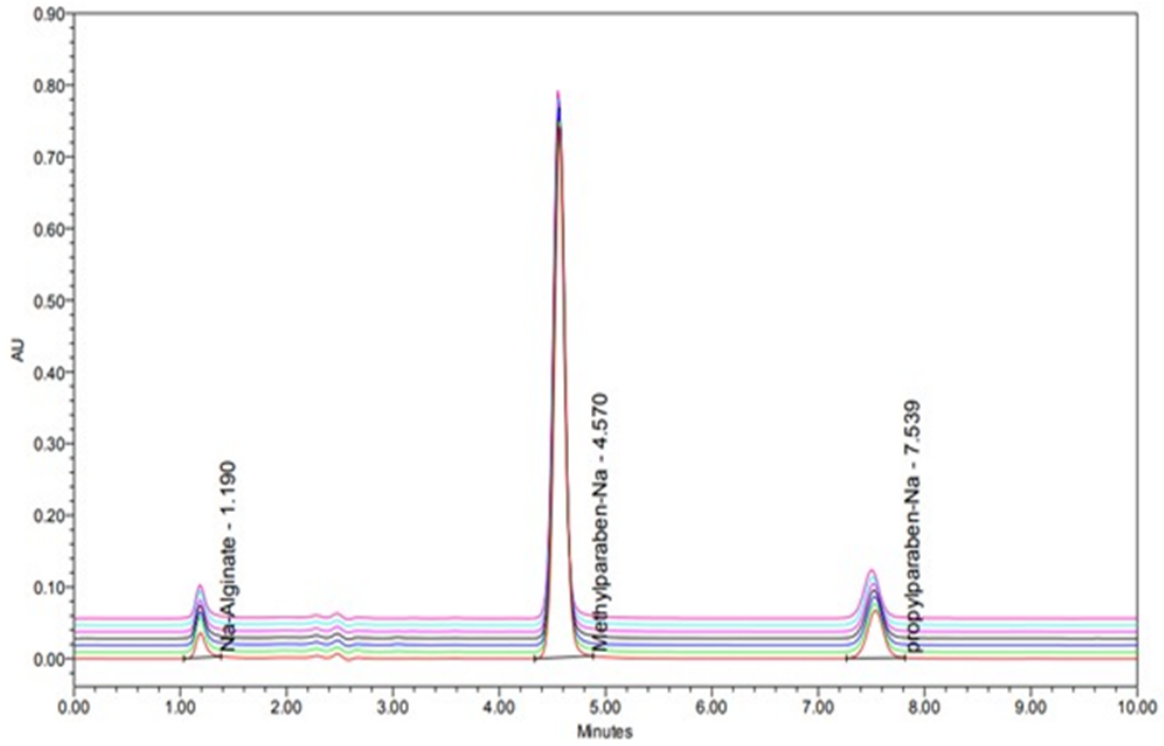


Figure 5. Overlay of HPLC chromatograms from the precision study, illustrating consistent retention times and peak shapes for Na-Alg, Na-MP, and Na-PP at 100% concentration across six samples and the standard.

exposure), and thermal stress (60 ℃ and 7 ℃, 7 days).

The results in Table 7 show the Na-Alg stability under most conditions, with minimal degradation under acidic (18%) and alkaline (7%) stresses. Conversely, Na-MP (100%) and Na-PP (90%) were degraded substantially under alkaline conditions via hydrolysis to p-hydroxybenzoic acid. Critically, the degradation products did not interfere with parent peak quantification, confirming the method selectivity (Figure 6 A–C). Standard solutions remained stable ≥ 1 week under photolytic, thermal (60 ℃), room temperature, and refrigerated conditions (Figure 7 A–D).

3.4. Application

Statistical Analysis of Commercial Oral Suspension Samples

The validated HPLC method was applied to quantify Na-Alg, Na-MP, and Na-PP in eight commercial oral suspension batches (OS1–OS34), using triplicate analyses (n=3 runs/sample), except for single determinations for batches 5–7. Table 6 presents comprehensive results, including mean concentrations (1:50 diluted samples), standard deviation (SD), relative standard deviation (RSD%), 95% confidence intervals (CI), and standard error (SE).

Method Precision: Excellent precision was achieved for all analytes and batches. Na-Alg %RSD ranged 0.13–1.45% (959–1047 μg/mL), Na-MP 0.06–1.43%, and Na-PP 0.09–6.1%. The elevated Na-PP %RSD (6.1%, Batch 1 at $\tilde{1}1$ μg/mL) reflects the inherent variability at low concentrations, yet all values remained within the ICH/USP acceptance limits (<2%).

Statistical Reliability: Narrow 95% CIs and low SE values in multi-replicate batches confirmed high certainty (e.g., Na-Alg Batch 8 [n=7]: 979.02–981.30 μg/mL; Na-PP Batch 3 SE: 0.012 μg/mL). Single-replicate batches 5–7 showed wider CIs (e.g., Na-Alg Batch 5:1007.97–1112.33 μg/mL; SE: 15.18 μg/mL) and higher %RSDs (0.28–1.45%), emphasizing the need for ≥ 3 replicates in regulatory QC.

Batch Variability: Significant inter-batch differences were detected: Na-Alg 959–1047 μg/mL (Batch 7 vs 5), Na-MP 51.8–101.7 μg/mL (Batch 1 vs 2), and Na-PP 11.0–16.6 μg/mL. These variations indicate formulation differences, manufacturing inconsistencies, or degradation, demonstrating the utility of this method for batch-release testing and quality surveillance.

Table 6 confirms the precision, accuracy, and robustness of the HPLC method for the routine analysis of commercial oral suspensions. Its sensitivity to batch variability supports the adoption of QC laboratories to ensure product consistency, safety and regulatory compliance.

4. CONCLUSION

A reliable and well-optimized HPLC method was developed and validated for the simultaneous determination of Na-Alg, Na-MP, and Na-PP in oral suspensions. The systematic optimization strategy enabled the effective res-

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Table 5. Robustness results under initial and different method parameters.

Condition	Name	Conc.*	%RSD	%R	#NTP	RS	TF
Initial normal method condition	Na-Alg	1024.8	0.14	102.5	784.0	–	1.4
	Na-MP	101.6	0.07	101.6	7881.0	17.6	0.9
	Na-PP	16.3	0.11	101.9	10570.0	11.4	0.9
Change in pH = 5.4	Na-Alg	1039.5	0.50	104.0	907.0	–	1.2
	Na-MP	103.9	0.50	103.9	6822.0	15.7	0.9
	Na-PP	16.3	0.50	102.1	8700.0	8.8	0.9
Change in pH = 5.0	Na-Alg	1031.1	0.50	103.1	850.0		1.3
	Na-MP	103.9	0.60	103.9	6845.0	15.2	1.0
	Na-PP	16.3	0.60	101.9	7571.0	7.9	1.0
Change in flow rate to 2 ml/min.	Na-Alg	961.7	0.40	96.2	538.0		1.4
	Na-MP	97.2	0.40	97.2	5552.0	14.5	0.8
	Na-PP	15.9	0.50	99.1	8135.0	9.8	0.8
Change in flow rate to 1 ml/min.	Na-Alg	977.6	1.30	97.8	800.0		1.4
	Na-MP	98.0	0.40	98.0	8104.0	17.8	1.0
	Na-PP	15.9	0.30	99.5	10676.0	11.5	0.9
Change in wavelength to 212 nm	Na-Alg	994.9	0.80	99.5	889.0		1.5
	Na-MP	100.2	0.30	100.2	9406.0	19.0	1.0
	Na-PP	16.0	0.20	100.0	11835.0	12.4	1.0
Change in wavelength to 208 nm	Na-Alg	992.0	0.60	99.2	870.0		1.5
	Na-MP	100.4	0.40	100.4	9454.0	19.2	1.0
	Na-PP	16.1	0.10	100.7	11846.0	12.5	1.0
Change in column temp. 45 °C	Na-Alg	987.7	0.30	98.8	813.0		1.4
	Na-MP	99.5	0.30	99.5	7885.0	17.4	0.6
	Na-PP	15.8	0.10	99.0	1011.0	11.0	0.9
Change in column temp. 35 °C	Na-Alg	995.9	0.30	99.6	783.0		1.3
	Na-MP	99.3	0.20	99.3	8477.0	18.8	0.9
	Na-PP	16.0	0.20	99.7	11282.0	12.6	0.9
Change in Column another supplier, teknokroma, Tracer Excel 120CN; 4.6-mm × 25-cm; 5 μm.	Na-Alg	999.3	0.50	99.93	913	-	2.03
	Na-MP	102.4	0.10	102.36	8654	18.87	0.87
	Na-PP	16.1	0.30	100.5	10205	11.64	0.79
Change in analyzer-2	Na-Alg	995.1	0.40	99.5	918.0		1.3
	Na-MP	100.2	0.10	100.2	8695.0	18.4	1.0
	Na-PP	16.2	0.04	101.3	10185.0	12.3	1.0
Change in instrument (Jasco HPLC system) UV/Vis. Detector model 2075 and Pump PU-2080	Na-Alg	1018.9	0.90	101.9	913.0		2.0
	Na-MP	100.2	0.50	100.2	8654.0	18.8	0.8
	Na-PP	16.0	0.30	100.2	10205.0	11.6	0.8
Concentration of salts in the buffer +10%	Na-Alg	1018.6	1.30	101.9	840.0		1.3
	Na-MP	102.9	0.03	102.9	7890.0	17.7	0.9
	Na-PP	16.3	0.08	101.7	10371.0	11.2	0.9
Concentration of salts in the buffer -10%	Na-Alg	1015.9	0.50	101.6	838.0		1.3
	Na-MP	102.9	0.10	102.9	7967.0	17.8	0.9
	Na-PP	16.2	0.03	101.4	10504.0	11.3	0.9
influence of acetonitrile in mobile phase +10%	Na-Alg	1033.9	0.12	103.4	862.0		1.2
	Na-MP	102.1	0.03	102.1	7612.0	16.9	1.0
	Na-PP	16.2	0.09	101.1	10069.0	9.9	0.9
influence of acetonitrile in mobile phase -10%	Na-Alg	1032.1	1.01	103.2	855.0		1.2
	Na-MP	103.2	0.17	103.2	7874.0	18.4	0.9
	Na-PP	16.3	0.14	101.8	10362.0	11.9	0.8
(Na-Alg)	Mean Recovery (%)			100.7	RSD%		2.24
(Na-MP)	Mean Recovery (%)			101.1	RSD%		2.02
(Na-PP)	Mean Recovery (%)			100.7	RSD%		1.04

*The concentration (μg/ml) indicated in the table is for the diluted sample measured (1:50), (n=3).

#NTP: Number of Theoretical Plates; RS: Resolution; TF: Tailing Factor.

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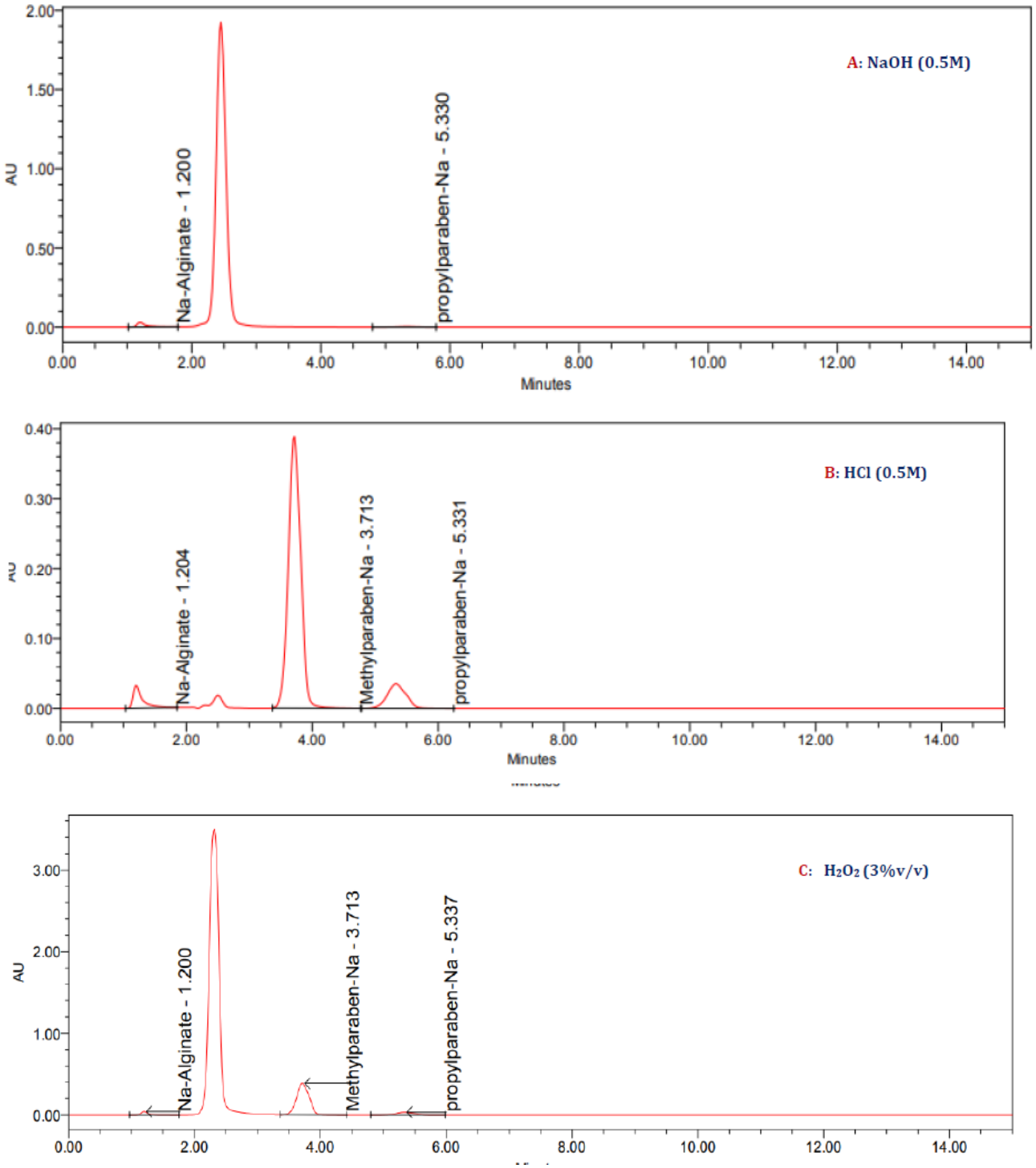


Figure 6. (A-C): HPLC chromatograms of samples subjected to forced degradation (A: acidic, B: basic, and C: oxidative stress).

olution of chemically diverse analytes, and the method demonstrated excellent linearity, precision, accuracy, and robustness in compliance with ICH guidelines. Its application to multiple commercial batches further confirmed its consistency and suitability for routine quality control. This method provides a practical and scientifically sound tool for analyzing these components in complex pharmaceutical matrices. For further research, the suggested analytical approach can be used for the simultaneous determination of active ingredients and preservatives in pharmaceutical preparations.

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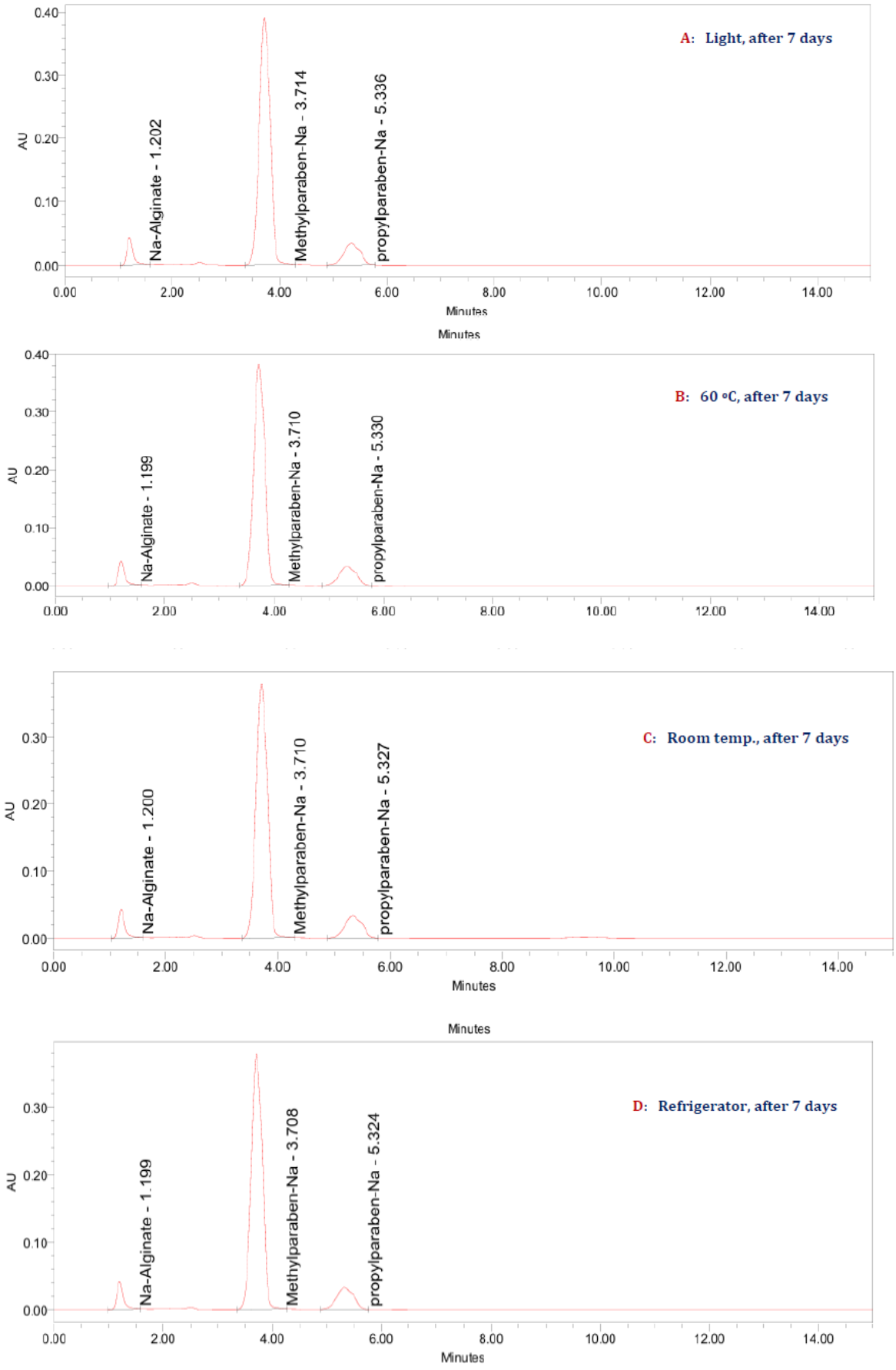


Figure 7. (A–D): Chromatograms of Samples Following One-Week Forced Degradation Under Thermal Stress Conditions

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Table 6. Statistical Summary of Analytical Results for Commercial Batches (n=3 runs per sample)

Analyte	Batch Code	Sample ID	Mean Range (n=3 runs) (μg/mL) *	Batch Mean (μg/mL) *	Batch SD	Batch %RSD	95% CI (μg/mL)	SE
Na-Alg	1	OS1-OS6	1038 - 1052	1044.6	5.60	0.50	1038.72-1050.48	2.29
	2	OS7-OS12	1018-1029	1024.3	3.60	0.40	1020.52-1028.08	1.47
	3	OS13-OS18	938-1002	976.4	1.93	0.20	974.35-978.41	0.79
	4	OS19-OS24	970-1020	1010.6	8.14	0.81	1002.01-1019.09	3.32
	5	OS25	1047±15.2	1047.0	15.18	1.45	1007.97-1112.33	15.18
	6	OS26	968±3.7	968.0	3.68	0.38	958.54-983.83	3.68
	7	OS27	959±2.7	959.0	2.69	0.28	952.10-970.55	2.69
	8	OS28-OS34	970-986	980.2	1.23	0.13	979.02-981.30	0.46
Na-MP	1	OS1-OS6	51-53	51.8	0.70	1.43	51.07-52.53	0.29
	2	OS7-OS12	101-102	101.7	0.20	0.20	101.49-101.91	0.08
	3	OS13-OS18	100-101	100.3	0.11	0.11	100.18-100.42	0.04
	4	OS19-OS24	100-101	100.4	0.18	0.18	100.25-100.63	0.07
	5	OS25	101.8±0.33	101.8	0.33	0.32	100.96-103.20	0.33
	6	OS26	96.05±0.06	96.0	0.06	0.06	95.90-96.30	0.06
	7	OS27	96.8±0.23	96.8	0.23	0.24	96.20-97.80	0.23
	8	OS28-OS34	90-91	90.5	0.14	0.15	90.38-90.64	0.05
Na-PP	1	OS1-OS6	10-11.5	11.0	0.70	6.10	10.27-11.73	0.29
	2	OS7-OS12	16.3-16.4	16.3	0.10	0.40	16.20-16.40	0.04
	3	OS13-OS18	16.03-16.22	16.2	0.03	0.19	16.14-16.20	0.01
	4	OS19-OS24	15.99-16.33	16.1	0.06	0.40	16.05-16.17	0.02
	5	OS25	16.6±0.07	16.6	0.07	0.40	16.43-16.89	0.07
	6	OS26	16.3±0.02	16.3	0.01	0.09	16.22-16.32	0.01
	7	OS27	16.4±0.05	16.4	0.05	0.28	16.28-16.60	0.05
	8	OS28-OS34	13.27 – 13.5	13.4	0.02	0.12	13.37-13.41	0.01

Used column: Ultisil XB-CN, 25 $\times$ 0.46 cm, 5 μm, OS: Oral Suspension.
*The concentration indicated in the table is for the diluted sample measured (1:50).
CI: Confidence Interval at 95% confidence level, SE: Standard Error.

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Table 7. Results for forced degradation study and stability assessment of Na-Alg, Na-MP, and Na-PP under various stress conditions

Stress Conditions	Na-Alg		Na-MP		Na-PP	
	Assay, %	%RSD	Assay, %	%RSD	Assay, %	%RSD
Base Hydrolysis	82.43	0.21	0.00	0.00	9.83	0.36
Acid Hydrolysis	93.65	0.46	98.24	0.27	101.12	0.25
Oxidation	98.23	0.07	98.65	0.13	99.85	0.07
Light for 1 Week	99.81	1.45	101.98	1.14	99.56	1.06
60 °C for 1 Week	98.36	0.63	100.98	0.03	98.05	0.24
Room Temperature for 1 Week	99.40	0.36	100.71	0.24	98.33	0.17
Refrigerator at 7 °C for 1 Week	100.94	0.49	101.08	0.06	98.17	0.08

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