

A Validated HPLC Assay Method for the Determination of Sodium Alginate in Pharmaceutical Formulations

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A high-performance liquid chromatography–diode array detector method was developed and validated for the quantification of sodium alginate in antacid oral suspension using a phenyl stationary phase and buffer solution at pH 7.0 as a mobile phase. The method was validated for specificity, linearity, range, accuracy, precision and robustness. The method was specific for the determination of sodium alginate in the bulk drug, pharmaceutical dosage form and under stress degradation. The method was linear over the range of 600 to 1,400 $\mu\text{g/mL}$ with $r^2 = 0.9999$, and accuracy and precision were acceptable with relative standard deviation $< 2.0\%$. The described method is simple, specific, precise, accurate, robust and stability–indicating, and can be successfully applied for the routine analysis of sodium alginate in bulk drug and pharmaceutical dosage form.

Introduction

Sodium alginate is a purified carbohydrate product extracted from brown seaweeds. It primarily consists of sodium salt of alginic acid, which is a mixture of polyuronic acids composed of residues of β -D-mannuronic acid (M unit) and α -L-guluronic acid (G unit) linked together through a $1 \rightarrow 4$ glycosidic linkage, as shown in Fig. 1 (1).

Alginates and alginic acid are widely used in the food industry, biotechnology and medicine because of their gel-forming capacity. The behavior of alginate is strongly dependent on its molecular size and intrinsic viscosity; because of the complexity and high molecular weight of alginate polymer, it is not easily measured. Nevertheless, the development of a simple and effective method for alginate quality control is important, especially for pharmaceutical use (2).

Different techniques have been developed for the quantification of alginates. In 2011, British Pharmacopeia (BP) and United States Pharmacopeia (USP) recommended a method for the assay of alginic acid and alginate as raw materials. The method was based on the stoichiometric reaction of the carboxyl groups in alginic acid or the carbon dioxide released from hydrolysis of alginate in a residual titration with standardized base (3, 4). Clearly, this method is not specific for alginic acid or alginate and inapplicable to pharmaceutical formulations.

The general methodology commonly used for quantification of alginate was based on a preliminary enzymatic depolymerization and/or chemical hydrolysis of alginate to release simple carbohydrates as uronic acids to be detected by colorimetric

methods (5, 6) or chromatographic methods using ion-exchange or size-exclusion chromatography (7, 8, 9). The disadvantage of this methodology is the indirect quantification of alginate content via detection of uronic acids; this technique requires sample pre-treatment and consumes time and reagents, in addition to the nonspecificity of colorimetric methods between variable uronic acids in the sample.

Few studies have been able to directly measure the total content of alginate without hydrolysis using capillary electrophoresis (CE). One study reported by Moore *et al.* (10) was based on sample pretreatment to separate the alginic acid from other ingredients in antacid formulation before quantification by CE. Another study by Oztekin *et al.* (2) developed a micelle electrokinetic chromatographic method to determine the total alginate content in antacid formulations by CE.

Currently, no high-performance liquid chromatography (HPLC) method has been reported for the direct determination of the total alginate content in pharmaceutical formulations without sample pretreatment. This work describes a simple HPLC method for the quantification of total alginate content in antacid suspension. The assay was validated and successfully applied for the quality control of sodium alginate in bulk drug and pharmaceutical dosage form.

Experimental

Materials

Sodium alginate with purity 99.8% was purchased from Qingdao Bright Moon Seaweed Group Company (China). Phosphoric acid and sodium hydroxide were purchased from Sigma-Aldrich (St. Louis, MO). Ultra-pure water was prepared using a Milli-Q Synthesis system (Millipore, Billerica, MA). Sodium alginate antacid suspension (Gaviqueen) produced by Sigma Pharmaceutical Industries (Egypt).

Instrumentation and chromatographic conditions

Chromatography was performed on a Shimadzu liquid chromatograph (Shimadzu Scientific Instrument Inc., Kyoto, Japan) consisting of a system controller CBM-20A, a solvent delivery module LC-20AD, an online degasser DGU-20A5, an autosampler SIL-20A, a diode array detector SPD-M20A and an LC workstation.

A phenyl stationary phase (250×4.6 mm i.d., $5 \mu\text{m}$) was used, operating at room temperature. Elution was performed

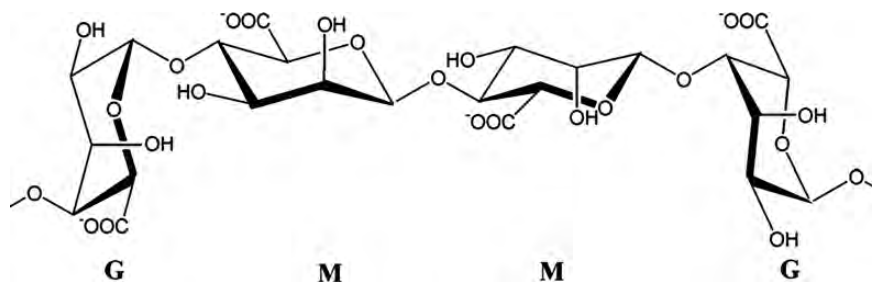


Figure 1. The chemical structure of alginate.

Table I

Accuracy and Precision Data of Sodium Alginate

Accuracy

Set	Concentration	Theoretical concentration ($\mu\text{g/mL}$)	Measured concentration ($\mu\text{g/mL}$)	Recovery (%)
I	80%	800	806.42	100.80
II	100%	1,000	1,011.79	101.18
III	120%	1,200	1,218.09	101.51
Mean recovery (%)				101.16
SD				0.353
RSD (%)				0.349
Precision				
Sample	Repeatability, recovery (%)		Reproducibility, recovery (%)	
1	102.86		101.31	
2	101.00		102.71	
3	101.52		101.26	
4	100.88		102.08	
5	100.97		101.65	
6	100.65		102.82	
Mean recovery (%)	101.31		101.97	
SD	0.810		0.682	
RSD (%)	0.799		0.669	

under isocratic conditions with a mobile phase of buffer solution consisting of 0.5 mL phosphoric acid in 1-L distilled water adjusted to pH 7.00 with sodium hydroxide, with a flow rate of 0.7 mL/min at ambient temperature (25°C), ultraviolet (UV) detection at 200 nm and injection volume of 20 μL ; the system was allowed to equilibrate with the mobile phase for 45 min before the first injection. Under these conditions, sodium alginate was eluted at 2.65 ± 0.1 min.

Preparation of standard solutions

Sodium alginate standard solution was prepared by transferring 50 mg of accurately weighed sodium alginate reference material into a 50-mL volumetric flask, which was then dissolved and completed to the volume with distilled water. The calibration standards were prepared by transferring different weights of sodium alginate reference material into 50-mL volumetric flasks to give the concentration range of 600 to 1,400 $\mu\text{g/mL}$ using distilled water as a solvent. The solutions were stored at 4°C until analysis.

Sample preparation

Sodium alginate test solution was prepared by accurately transferring 5 mL of Gaviqueen suspension equivalent to 250 mg of sodium alginate to a 250-mL volumetric flask, which was then

dissolved and completed to the volume with distilled water, then filtered by a 0.45- μm sample filter.

Method validation

The method was validated according to International Conference on Harmonization (ICH) guidelines and USP 34-NF 29 (4), in which the required validation criteria for the pharmaceutical assay method include specificity, linearity, range, accuracy, precision and robustness. Results were evaluated according to the stated validation limits for pharmaceutical assay methods based on comparison between USP, ICH and Food and Drug Administration (FDA) guidelines (11).

Linearity

Linearity was determined by a series of double injections of five concentrations, 600, 800, 1,000, 1,200 and 1,400 $\mu\text{g/mL}$. The calibration curve was constructed by plotting the concentrations of sodium alginate on the X-axis versus the chromatographic peak area of each concentration on the Y-axis. If there is a linear relationship, test results should be evaluated by appropriate statistical methods such as linear regression analysis to calculate the slope and intercept using the calibration curve data.

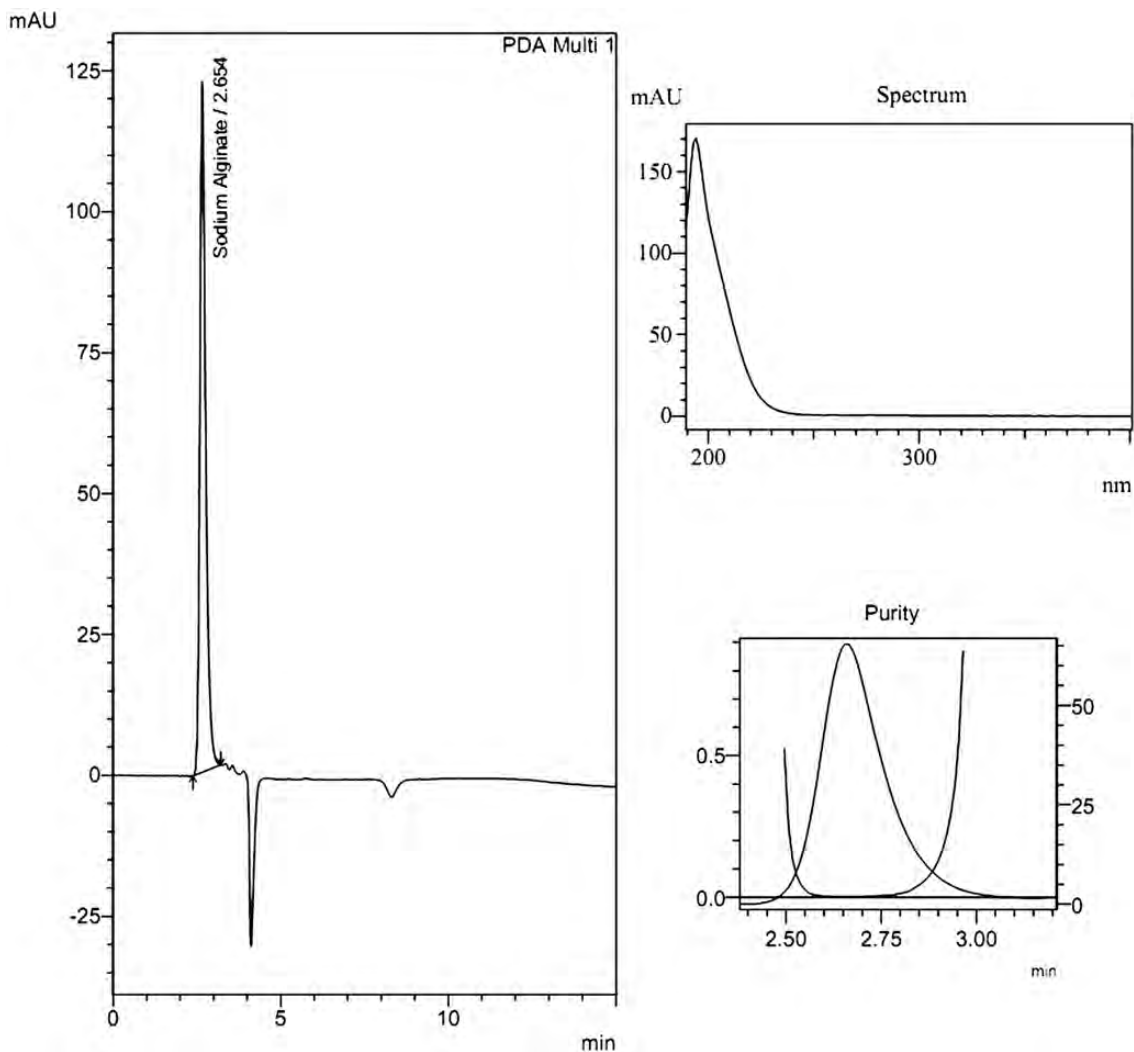


Figure 2. Chromatogram of sodium alginate standard solution (1,000 $\mu\text{g/mL}$) with its UV spectrum and purity.

Range

Method range was investigated by determining sodium alginate in samples with different concentrations ranging from 80 to 120% of sample preparation. This range is considered to be the minimum specified range that should be considered for the assay of a drug substance in a finished product.

Accuracy

Testing the method accuracy was performed by the addition of sodium alginate reference material to three sets of solutions containing the inactive materials of Gaviqueen suspension. Accuracy was expressed by percent recovery of samples with known concentrations by comparison of the observed concentration values with the expected values. Three determinations at each of three concentrations across the range of 80 to 120% of sample preparation were used to investigate the accuracy.

Precision

Precision is a measure of the degree of repeatability and reproducibility of the HPLC method under normal operating

conditions, which is usually expressed as the standard deviation (SD) or relative standard deviation (RSD) of a series of measurements. Precision in this method was determined at two levels, repeatability and reproducibility. Repeatability was performed by analyzing six sample preparations of 100% test concentration within the same laboratory over a short period of time using the same analyst and the same equipment, while reproducibility was performed in another laboratory, on another day by another analyst.

Specificity and stress degradation studies

Specificity is the ability to assess the analyte in the presence of components that may be expected to be present. This typically might include impurities, excipients or degradation products. Specificity was investigated by comparing the chromatograms of sodium alginate reference material solution, Gaviqueen suspension test solution and the dissolved inactive materials of Gaviqueen suspension.

The stress degradation studies were performed by using sodium alginate reference solution with a concentration of 1,000 $\mu\text{g/mL}$. Sodium alginate solution was exposed to stress

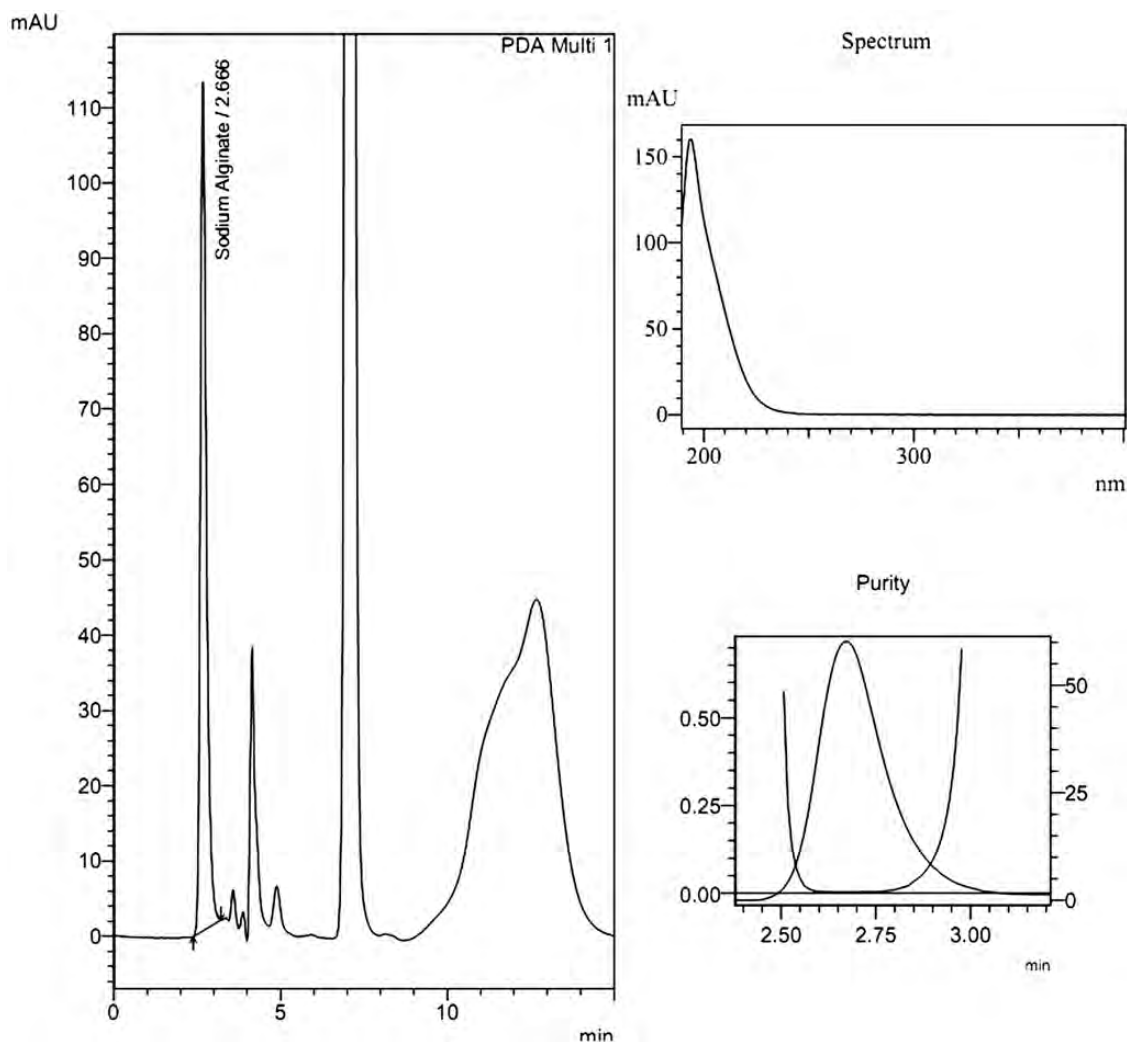


Figure 3. Chromatogram of test solution of sodium alginate antacid suspension.

conditions (alkaline condition using 5 N sodium hydroxide with reflux for 4 h, acidic condition using 5 N hydrochloric acid with reflux for 4 h, oxidation using 30% H₂O₂ for 48 h and photodegradation by exposure to light for 5 h).

Robustness

Robustness refers to the measure of the method's capacity to remain unaffected by small but deliberate variations in method parameters to provide an indication of the method's reliability during normal use. Robustness was demonstrated by small changes (temperature, pH of mobile phase and column batch) to investigate the effects on the quantification of sodium alginate.

Results and Discussion

Method development and optimization

Sodium alginate is a purified carbohydrate product that dissolves slowly in water, forming a viscous solution. The use of

water as a solvent and buffer solution as a mobile phase was critical in the method development to maintain the high molecular weight of alginate polymer in a soluble, less viscous form to avoid blocking of the column or HPLC tubes. Adjusting the pH of mobile phase was based on several trials to improve the sodium alginate peak shape, resulting in a good symmetric peak at pH 7.0. Using a flow rate of 0.7 mL/min resulted in the elution of a highly pure peak of sodium alginate at 2.65 ± 0.1 min during assay and stability studies. Decreasing the flow rate to 0.5 mL/min was applicable without affecting the method resolution, but it was time-consuming, whereas a slight increase in the flow rate to 0.8 mL/min resulted in a less pure peak with poor resolution. The method was developed at ambient temperature (25°C), giving a satisfactory peak shape with good resolution and purity. The optimization of the HPLC method chromatographic conditions was important for the development of a simple, cost-effective and less time-consuming method to be easily used during the routine analysis of sodium alginate samples in pharmaceutical quality control.

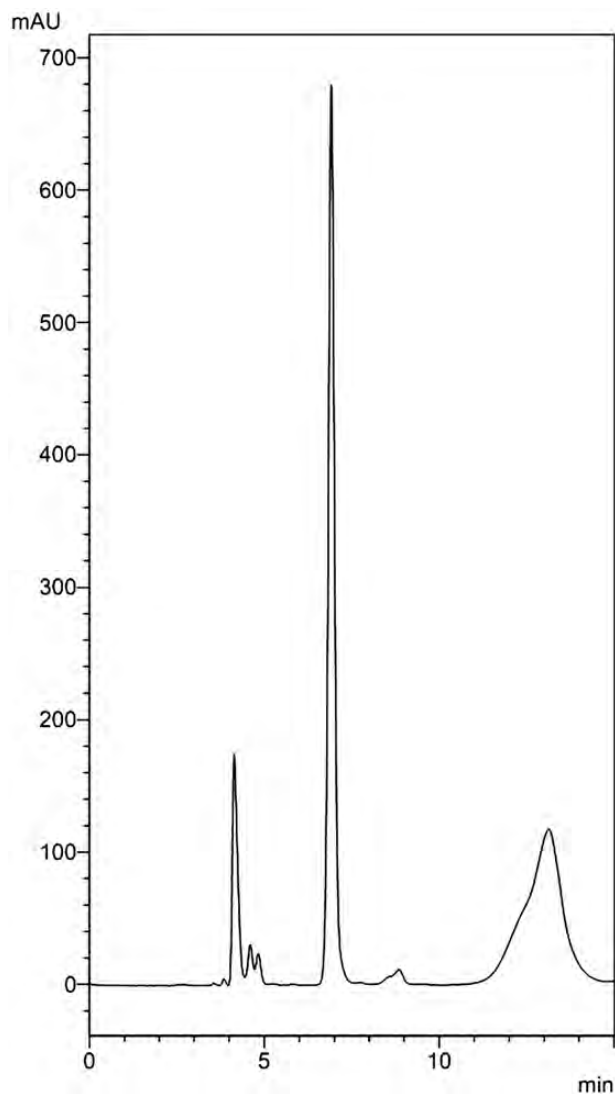


Figure 4. Chromatogram of the placebo of sodium alginate antacid suspension.

Method validation

Linearity and range

A linear relationship exists between the peak area and the concentration of sodium alginate over the range of 600 to 1,400 $\mu\text{g}/\text{mL}$ with correlation coefficient (r^2) = 0.9999, which meets the acceptance criterion of linearity for pharmaceutical assay methods ($r^2 \geq 0.999$) with regression equation (peak area = $1,467.154 \times \text{concentration} + 10,759.9$). The range of the method was established as 80 to 1,200 $\mu\text{g}/\text{mL}$ (representing 80 to 120% of the working concentration of the method), where RSD between sample results over that range was 0.42%, which is less than 2.0%. This proves that the accepted range for this method is 80 to 120% and the calibration curve is linear over that range.

Accuracy and precision

The results for accuracy and precision are presented in Table I. In accuracy, the mean percent recovery for samples of

Sets I–III (presenting concentrations ranging from 80 to 120% of method concentration) is 101.16% ($100 \pm 2\%$) and RSD is 0.349% ($<2\%$), indicating that the proposed method is accurate for the quantification of sodium alginate. In precision, the RSD for the repeatability samples is 0.799%, and the RSD for the reproducibility samples is 0.669%, which is $<2\%$, thus proving that the proposed method is precise for the quantification of sodium alginate and has acceptable repeatability and reproducibility.

Specificity

Specificity was confirmed by comparing the chromatogram of sodium alginate standard solution (Figure 2) with test (Figure 3), placebo (Figure 4) and stressed solutions. By investigating the peak purity of sodium alginate in each chromatogram by a photodiode array (PDA) detector, it was found to be 0.9999, indicating that no additional peaks were co-eluting with sodium alginate. This also proves the ability of the method to unequivocally assess sodium alginate in the presence of potential interferences.

Stress degradation tests

Alkaline condition

An alkaline stress test of sodium alginate reference standard solution (1,000 $\mu\text{g}/\text{mL}$) was performed by using 5 N sodium hydroxide with reflux for 4 h; this condition showed no significant change in the peak area of sodium alginate with peak purity 0.9999. This indicates that sodium alginate is stable under alkaline conditions.

Acidic condition

The addition of acid to sodium alginate solution leads to the isolation and precipitation of insoluble alginic acid. In this study, an acidic stress test of sodium alginate reference standard solution (1,000 $\mu\text{g}/\text{mL}$) was performed by using 5 N hydrochloric acid with reflux for 4 h, followed by neutralization with 5 N sodium hydroxide to restore the solubility of sodium alginate. This condition showed no significant change in the peak area of sodium alginate after neutralization with peak purity 0.9999 (Figure 5). This indicates that alginic acid is stable under this acidic stress conditions.

However, strong acid hydrolysis causes a breaking down of the 1 \rightarrow 4 linkage between uronic acids of alginate (M and G units shown in Figure 1).

Oxidative condition

Sodium alginate was completely stable to oxidation. No degradation was observed in sodium alginate peak after using 30% H_2O_2 for stress degradation at room temperature up to 48 h.

Photolytic condition

Sodium alginate reference standard solution (1,000 $\mu\text{g}/\text{mL}$) showed no significant change in the peak area of sodium alginate after exposure to visible and UV light for 5 h at room temperature.

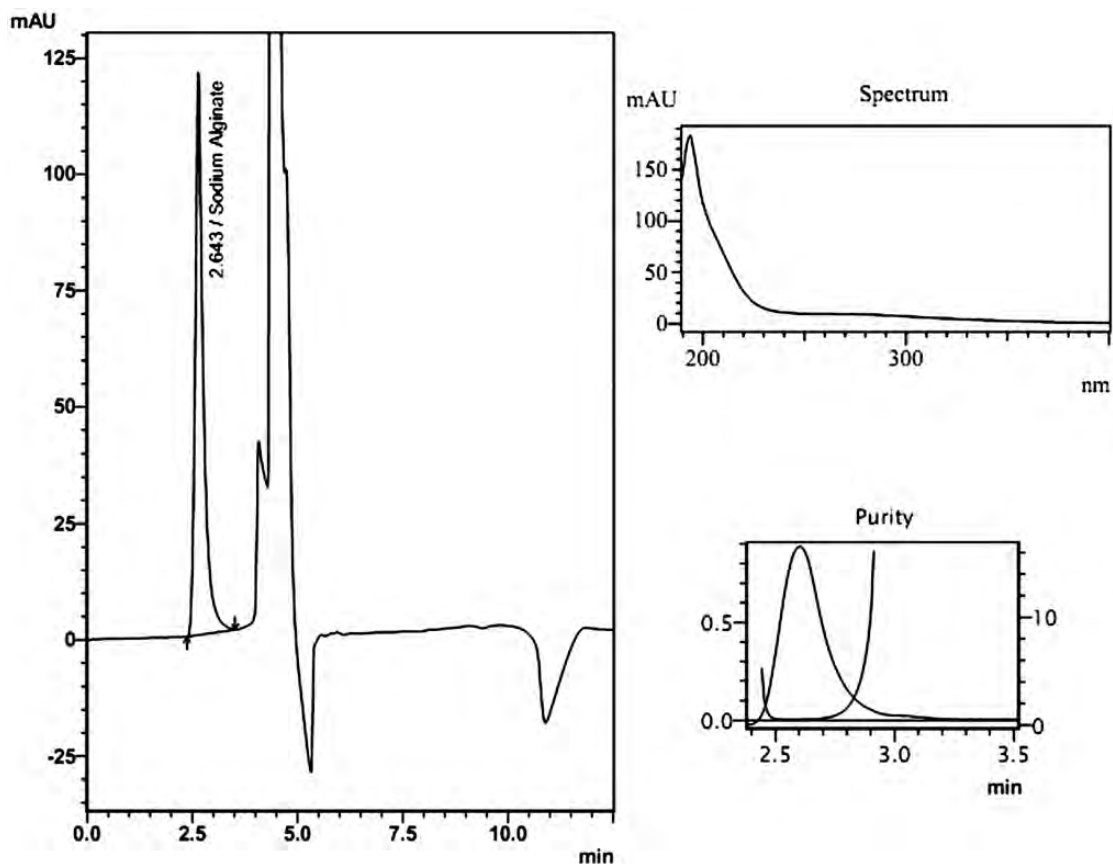


Figure 5. Chromatogram of the acidic stress test of sodium alginate standard solution after neutralization.

Table II
Robustness Data of Sodium Alginate

Parameter	Variations	Recovery (%)
pH of mobile phase	6.8	100.2%
	7.0	100.8%
	7.2	100.5%
Temperature (°C)	23°C	99.7%
	25°C	100.8%
	27°C	100.7%
Column batch	Batch I	100.8%
	Batch II	101.2%

Robustness

Slight changes in chromatographic parameters (pH of mobile phase, temperature and column batch) showed no significant change in percent recovery of sodium alginate (<2.0%) compared to the optimized chromatographic parameters, as shown in Table II, indicating that the proposed method is robust for the quantification of sodium alginate.

Conclusions

A simple, stability-indicating HPLC method was developed and validated for the quantitation of sodium alginate in sodium alginate antacid oral suspension using a phenyl stationary phase. The proposed method is specific, precise, accurate and robust,

and can be successfully applied for the routine analysis of sodium alginate in bulk drug and pharmaceutical dosage formulations.

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