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ORIGINAL RESEARCH ARTICLE

A novel, validated, eco-friendly, and cost-effective rp-HPLC method for the determination of alginic acid content in seaweed, seaweed formulations, and other higher plant extracts

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ABSTRACT

Alginic acid products are widely used in all fields, and the main source of alginic acid is seaweed. Alginic acid varies in various varieties of seaweed, plant parts, season to season, and the temperature of the water in which it is grown. The content of alginic acid also varies from manufacturer to manufacturer, as it depends on the process of extraction. No full-proof method for quantification is available. As alginic acid became a point of concern for regulators, a robust method for quantifying alginic acid became a prime requirement. As seaweed is only considered a source of alginic acid, essential amino acids, and nonessential amino acids, other sources need to be identified to overcome future needs and maintain biodiversity. The author succeeds in identifying another source of alginic acid, nutrients, and amino acids, i.e., *Chromolaena odorata* leaf extract, which contains about 5 to 6% alginic acid and 30 to 40% amino acids and proteins. A novel RP-HPLC method was found to be simple, suitable, and robust for the determination of alginic acid content in seaweed extract, its formulations, and also in phytoextracts (*Chromolaena odorata* leaf extract). The alginic acid content in seaweed and the formulated product quantified shows 10.05 ± 0.1% and 1.0 ± 0.03%, respectively, and is in close agreement with the declared content. Various authors have used gravimetric, colorimetric, titrimetric, and HPLC methods, each with their own limitations. The novel method is simple in terms of sensitivity, eco-friendly mobile phase, regular HPLC column and detector, lowest retention time (rapid analysis), and no internal standard required.

Keywords: alginic acid; seaweed; *Chromolaena odorata*; novel RP-HPLC method; essential and nones-sential amino acids; process of extraction g.

1. Introduction

Alginate is a polysaccharide, an anionic polymer that occurs naturally in brown algae (Phaeophyceae). Alginate is a structural element of the seaweed, as is cellulose in terrestrial higher plants, which has gel located in the cell walls and intercellular matrix, providing the flexibility and mechanical strength necessary to withstand the force of the water in which the seaweed grows[1]. The compositional difference of alginates varies from species to species, season to season, and the temperature of the water in which seaweeds are grown.

Alginate is not a compound exclusively of brown algae; it is synthesized by bacteria, but commercially all alginate is extracted from algae biomass[2]. Industrial applications of alginate are linked to the gelation, viscosity, and stabilizer properties of alginate and the solutions and products to which it is added.

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Alginate is a matrix of alginic acid-bound cations, like calcium, sodium, or magnesium. The divalent cations give alginate a very rigid conformation and a stable structure, unlike alginate with monovalent cations.

Alginic acid was first discovered and patented (patent date: 12 January 1881) by the British chemical scientist E.C.C. Stanford, and he also continued the work on its discovery, and contributed to the chemical structure elucidation of alginic acid[2]. The patent explains how alginate can be extracted and its uses.

The alginate is an unbranched biopolymer of 1,4-β-D mannuronic acid (M) and 1,4 α-L-guluronic acid (G) monomers with a homogeneous (poly-G, poly-M) or heterogeneous (MG) block composition, as proven by partial acid hydrolysis. Each alginate-producing species shows different alginate compositions and differences in the ratio of mannuronic and guluronic acid blocks, varying in composition and sequence. The proportions of the two acids vary from species to species and from different parts of the same seaweed.

Mainly the brown seaweeds from the genera Macrocystis, Laminaria, Lessonia, Ascophyllum, Alaria, Ecklonia, Eisenia, Nereocystis, Sargassum, Cystoseira, and Fucus.

Out of these, *Macrocystis pyrifera* and *Ascophyllum nodosum* are used commercially for industrial applications.

Alginate present in the brown algae acts as a functional element of the traditional fertilizers, allowing water retention in the soils. So, the principal function of alginate in agricultural areas was as a soil conditioner, a superabsorbent (SAP), or water-retaining material that can absorb large amounts of water, as much as 100 to 200 times its mass. These alginates are generally known in agriculture as non-ionic or ionic moisture-holding hydrogels for increasing soil water retention, which is a basic soil property. Super-absorbents (SAP) in agricultural areas have been designed and developed for enrichment of the abiotic properties of soil by raising its water retention and water holding capacity, developing better water usage efficiency, enhancing soil permeability and infiltration rates, contributing to lower irrigation frequencies, preventing erosion and water drainage, enhancing plant performance, increasing soil aeration, lowering the dissolution of fertilizers, developing a better adsorption capacity, or enhancing the uptake of some nutrient elements by the plants responsible for increasing microbial activity. It is also responsible for suppressing soil-borne plant pathogens.

The alginate also acts as the principal emulsifier responsible for controlling the release of active ingredients in the agricultural field[3].

The conventional role of alginate in the biomedical area as a thickening, gel-forming, and stabilizing agent is that alginate can play a significant role in controlled-release drug products. But the main use of alginate in the biomedical area is actually in hydrogel form, used in wound healing, drug delivery, and tissue engineering applications. Alginate hydrogels are biocompatible and structurally identical to the macromolecular-based components in the human body and can regularly be convoyed into the body by minor invasive techniques of administration to the selected human body. Alginates are commonly used in the food industry as natural additives[4,5]. Alginates are also used for the removal of organic and metallic contaminants from wastewater[6].

Considering the application of alginic acid and alginates, various authors have characterised the seaweed extracts for their contents and applications using various techniques. Chemical characterization techniques, spectroscopic techniques, and studies of enzymatic activity have been employed for the evaluation of the efficacy of seaweed extract[7].

Alginate was also extracted from seaweed using the formaldehyde alkali treatment method, precipitated with calcium chloride, washed in absolute ethanol, followed by acetone, dried at 40 ℃ to 60 ℃ and analysed

for carbohydrates, proteins, phenolic compounds, and minerals. The alginate was analysed by the gravimetric method and shows a content ranging from 20% to 40% in various species of seaweed[8].

Numerous analytical methods, including chemical, physicochemical, and enzymic procedures for the structural analysis of alginates, were also used for the investigation of other uronic acid-containing polysaccharides[9].

An analytical method for simultaneous determination of alginic acid, laminarin, and mannitol in seaweed extracts using ultra–ultra-high-performance liquid Chromatography (HPLC) coupled with a Refractive Index Detector (RID). A sample was extracted and diluted with ultrapure water, using glycerol as an Internal Standard (IS) and centrifuging the content. Chromatographic analysis was performed on a Bio-Rad Aminex HPX-87H column with 0.05% acetic acid as a mobile phase solution at a flow rate of 0.5 mL/min. The method was fully validated in terms of selectivity, Limits of Detection (LOD), Limits of Quantification (LOQ), linearity, recovery, precision, and robustness[10].

A high-performance liquid chromatography-diode array detector method was also used 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[11].

HPLC determination of alginate using a Resolve C18 column (3.9 × 150 mm, Waters, Milford, MA) and acetonitrile-water-butyl acetate (55: 42: 3) as the mobile phase at a flow rate of 0.6 ml/min and detection at 565 nm[12].

High-performance liquid chromatography (HPLC) has been used as an analytical tool for fingerprints and the quantification of marker compounds in seaweed extracts. HPLC analysis of the methanolic extract of seaweed is evidenced by the chromatogram obtained at various retention times (3.643, 3.819, and 6.463) at λ max 254 nm[13].

Various methods of analysis, like gravimetric, spectrophotometry, and HPLC with UV, diode arrays, and refractive index detectors, were found to be effective for analyzing the alginic acid in pharmaceutical products, foods, and biomedical products as they contain added pure alginates. The seaweed extract, its formulations, and other higher plant extracts are rich in polysaccharides, phenolic compounds, and amino acids, which interfere with the elution of alginic acid during HPLC, colorimetric, gravimetric, and spectrophotometric analysis. To remove background interference, matrix from alginate, mobile phase, stationary phase, flow, and wavelength, sample pretreatment before injecting into the liquid chromatograph was essential.

Looking at the exhaustive literature survey and proposed methods, none of the reported methods in the literature were fit for the purpose; hence, it was essential to develop a simple, rapid, inexpensive, user-friendly, and reliable analytical method for quantifying this analyte, which is present in variable concentrations in the source of origin and its formulations with variable dosage forms. The proposed method offers numerous advantages over the reported methods, such as using environmentally benign mobile phases (ethanol and water) with lower cost and low toxicity, high reproducibility, and a short analysis time. Additionally, it is less hazardous, time-saving, and cost-effective. After validation of the method for various parameters, the method proved to be successful and was applied to the analysis of alginic acid in seaweed, its formulations, phytoextracts of other higher plants, and other commercial products containing alginates.

2. Experimental

2.1. Chemicals and reagents

The chemicals and reagents utilized during the experimental work were of HPLC grade. HPLC-grade ethanol, water, orthophosphoric acid, and sodium hydroxide were purchased from Merck. The analytical standard is alginic acid (21.5%) from Sigma Aldrich and seaweed from Acadian, a liquid formulation containing seaweed and *Chromolaena odorata* leaf extract. The seaweed and its formulation samples were obtained from Indofil Industries Limited, Thane, India.

2.2. Instrumentation and chromatographic conditions

Alginic acid content in seaweed and its formulations were determined by the calibrated and validated HPLC instrument using the below optimized parameters.

Name of the instrument: High Performance Liquid Chromatography (HPLC)

Make and model: Shimadzu, LC-20AD with PDA detector

Wavelength: 205 nm

Pump mode: Isocratic

Column temperature: 30 ℃

Column: Phenomenex C8 (250 × 4.6 mm, 5 µm)

Mobile phase: 0.1% Orthophosphoric acid in water pH adjusted tom 7.0 with 1 N NaOH: Ethanol, ratio (90:10) *v/v,* (Premixed)

Flow rate: 0.5 mL/min

Injection volume: 20 µL

Retention time (approximately): 3.3 min

Total run time: 10.0 min

Diluent: 0.1% Orthophosphoric acid in water pH adjusted tom 7.0 with 1 N NaOH: Ethanol, ratio (90:10) *v/v,* (Premixed)

2.3. Preparation of mobile phase and diluent

Solvent A: 0.1% Orthophosphoric acid in HPLC water pH adjusted to 7.0 with 1 N NaOH 90 mL and Ethanol HPLC grade 10 mL v/v, (Premixed) 0.1% (v/v) mixed well and sonicated to degas for 15 min.

Diluent: 0.1% Orthophosphoric acid in HPLC water pH adjusted to 7.0 with 1 N NaOH 90 mL and Ethanol HPLC grade 10 mL v/v, (Premixed) 0.1% (v/v) mixed well and sonicated to degas for 15 min.

2.4. Preparation of standard solution

Weighed accurately about 40 mg of the Alginic acid reference standard and transferred into a clean and dry 100 mL volumetric flask. Added about 20 mL of diluent and sonicated to dissolve and diluted up to the mark with diluent and mixed well.

2.5. Preparation of sample solution

Weighed accurately about 65 mg of seaweed, 550 mg of seaweed formulation, and 120 mg of solid from *Chromolaena odorata* leaf extract into a clean and dry 100 mL volumetric flask. Add about 20 mL of diluent, sonicate it to extract, dilute it up to the mark with diluent, and mix well.

2.5 mL of sample solution was added to a 5 mL screw-cap vial, then 50 mg of activated charcoal was added, and the vial was capped and sonicated for 10 min with continuous hand swirling to remove the matrix from other organic components in the sample. Then the content was transferred to a syringe and filtered through a 0.45-micron syringe filter.

$$\%\ \text{Alginic acid content (w/w)} = \frac{A_1 \times W_1 \times P}{A_2 \times W_2} \quad (1)$$

where A_1 = Mean peak area of Alginic acid in sample chromatogram, A_2 = Mean peak area of Alginic acid in standard chromatogram, W_1 = Weight of standard, in mg, for standard solution preparation, W_2 = Weight of sample taken, in mg, for the test solution preparation, P = Percent purity of Alginic acid reference standard.

3. Result and discussion

3.1. Method development and optimization

Due to similarity in the physical and chemical properties of alginic acid and other components of seaweed extract, its formulation, and phytoextract (matrix), several proportions of mobile phases (like acetonitrile, methanol, ethanol, and water with or without buffer in different combinations) and columns were trailed initially to get resolution of alginic acid from the matrix on HPLC. The suitability of the column and the mobile phase used in the optimized method has been decided on the basis of selectivity and sensitivity, as well as acceptable chromatographic parameters of the produced peak in terms of peak sharpness, peak symmetry, tailing factor, good resolution, best reproducibility of the results, and short run time, which were considered crucial parameters during method development. The mobile phase was utilised as a diluent for standard and samples to resolve the matrix of the analyte and minimise noise from unwanted peaks.

Columns applied in our initial trials

- Phenomenex, C18 (250 mm × 4.6 mm × 5 µm).
- Chromasil, Phenyl (250 mm × 4.6 mm × 5 µm).
- Waters, Symmetry C18 (250 × 4.6 mm, 3.5 µm).
- Phenomenex, C8 (250 mm × 4.6 mm × 5 µm).

Examples of buffers trailed with water, based on previous literature

- Phosphate buffers, different pH values (5–7)
- Acetate buffer pH 7.
- Triethylamine 0.1% pH 7.0 (with 10 % OPA).

0.1 % Orthophosphoric acid in HPLC water pH adjusted to 7.0 with 1 N NaOH (For optimized method).

3.2. Method validation parameters

The optimized chromatographic conditions were validated by evaluating specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness, and system suitability parameters by the SANCO and ICH guidelines[14–16].

3.2.1. System suitability test

System-suitability being an integral part of method development, was used to ensure the adequate performance of the chromatographic system. Retention time, tailing factor, peak separation (resolution), and percent relative standard deviation (% RSD) of the area of seven replicate injections of the standard solution

were evaluated. The system suitability test was performed using seven replicate injections of reference standard before analysis of the sample. The observed system suitability parameters of the present method were within the acceptable limits and summarised in **Table 1**.

Table 1. System suitability test results for Alginic acid.

Sr. No.	Parameters	Dodine	Acceptance criteria[15,16]
1	Retention time (min)	3.38	-
2	Tailing factor	1.13	≤2.0
3	% RSD of Peak areas (n = 7)	1.09	≤2.0

3.2.2. Specificity

Specificity is the is the first and crucial step in analytical method development and validation, and it refers to the ability of the analytical method to discriminate between the between the interference of the analyte with the matrix and vice versa. Specificity was evaluated by comparing the chromatograms of mobile phase blank, formulation blank solution, standard solution, and sample solutions. For this purpose, 20 µL of the above solutions were injected into the HPLC system separately, and the chromatograms of individual standards and samples and their specificity were evaluated. (**Figure 1**). From the chromatograms, it was observed that there were no co-eluting peaks at the retention time of alginic acid, and hence no interference was observed. This was also verified by the result of the peak purity of the analyte, i.e., (⩾0.99), which confirmed the specificity of the method (**Figures 2–5**).

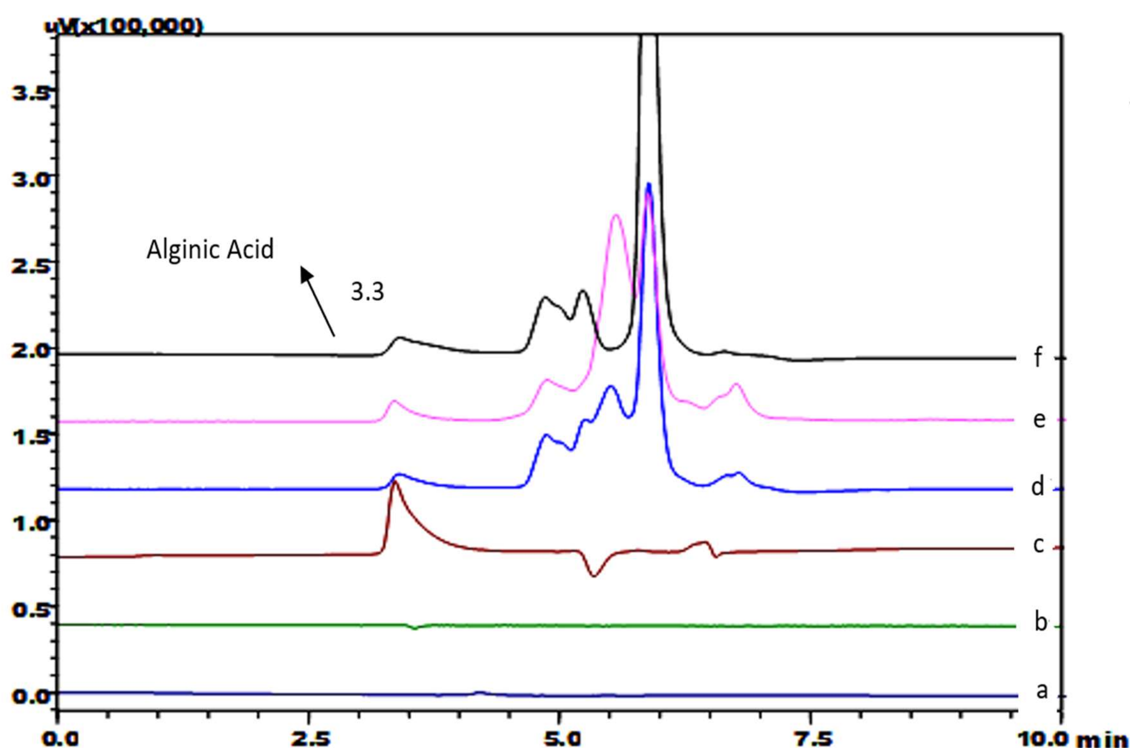

Figure 1. (a) Mobile phase blank; **(b)** Formulation blank; **(c)** Alginic acid standard; **(d)** Maxilizer liquid; **(e)** Seaweed extract; **(f)** *Chromolaena odorata* phytoextract.

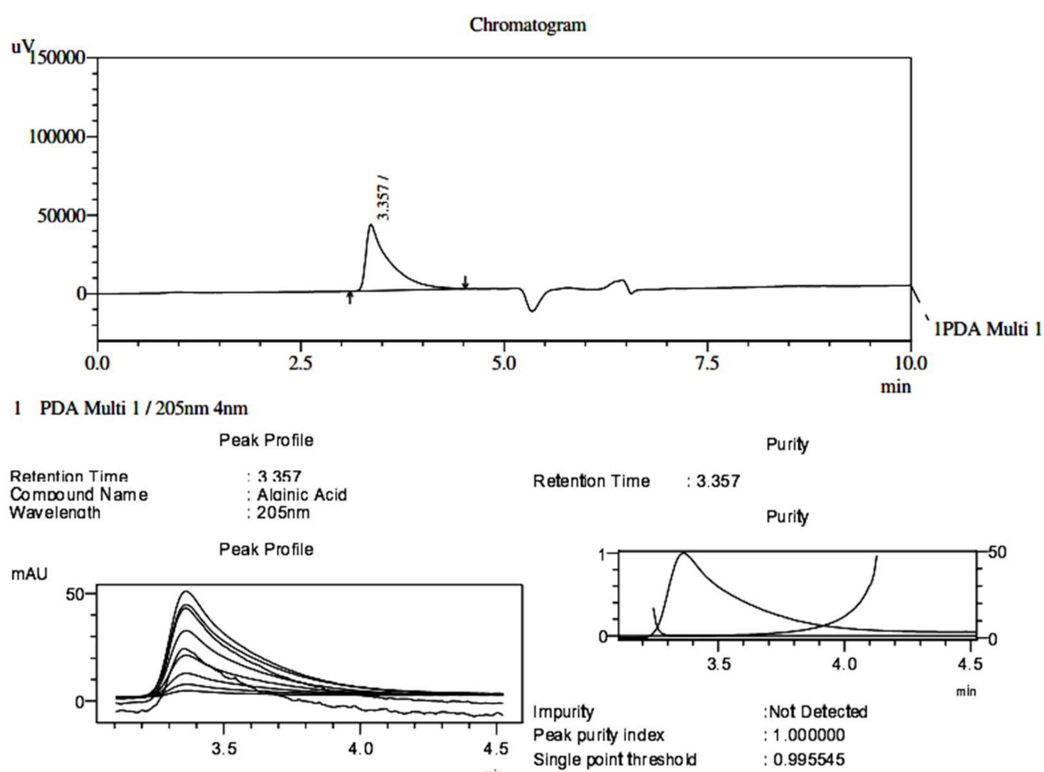

Figure 2. Alginic acid standard.

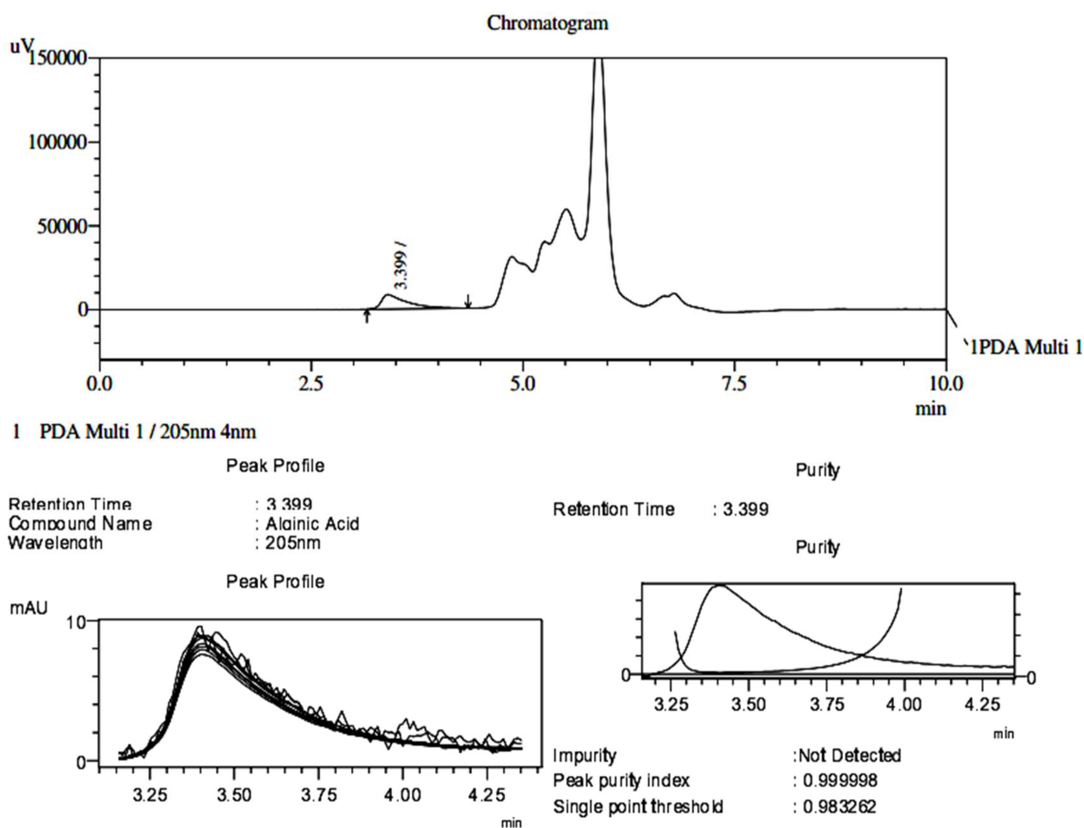

Figure 3. Maxilizer liquid.

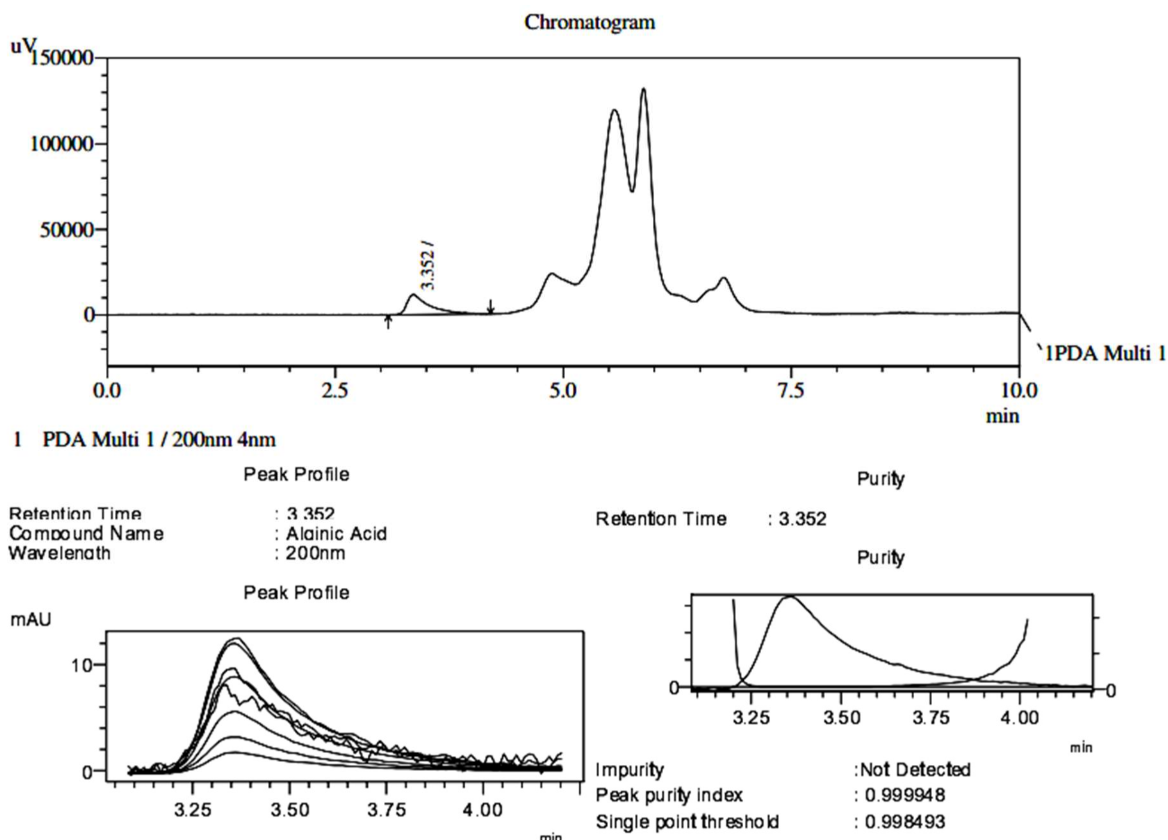

Figure 4. Seaweed extract.

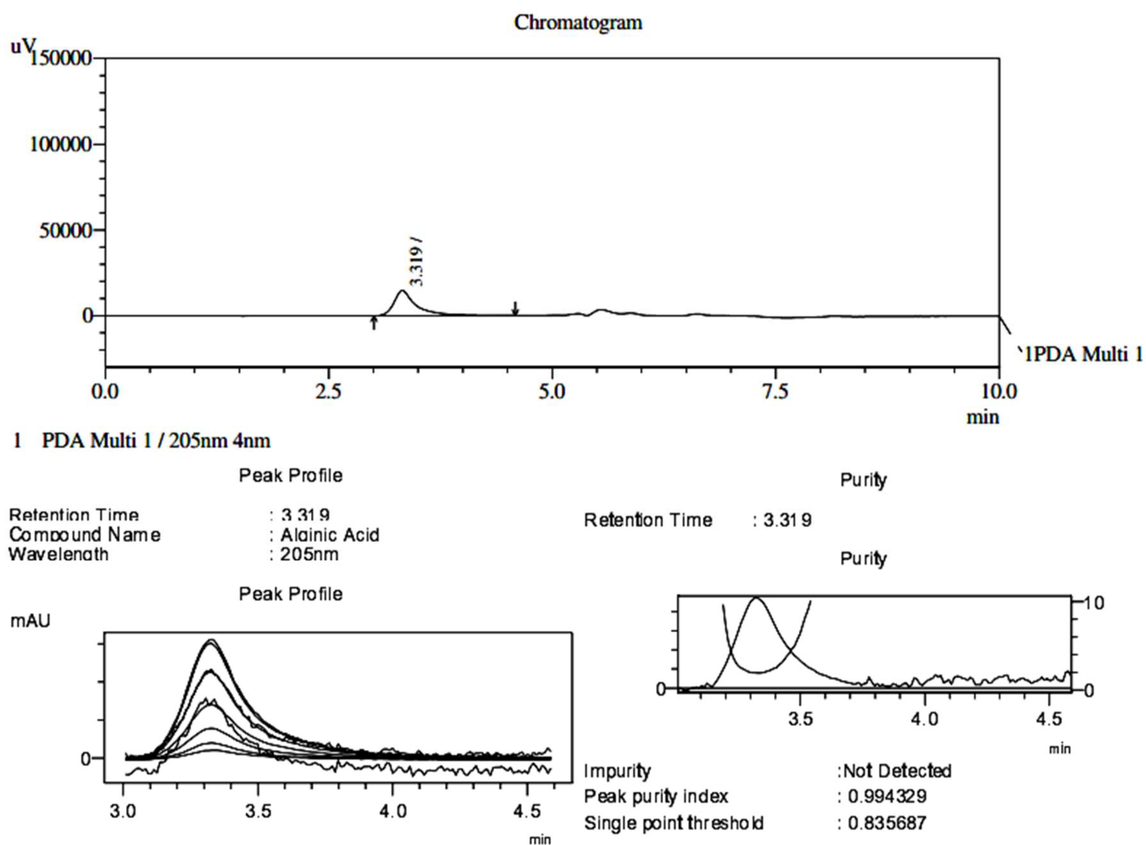

Figure 5. *Chromolaena odorata* phytoextract.

3.2.3. Linearity

The linearity of the analytical method is defined as the ability of the method to produce test results that are directly proportional to the concentration of the analyte within a specific range. The mean peak area of the alginic acid standard peak obtained from the HPLC chromatogram was plotted against corresponding concentrations of the alginic acid standard to obtain the calibration curve (**Table 2** and **Figure 6**). From the figure, it was observed that a linear relationship has been established over the concentration range of 25 to 700 mg/L for alginic acid. From the regression analysis, the correlation coefficient of the regression was found to be 0.9995, indicating a linear relationship between the concentration of analytes and the area under the peaks, and the method is fit for the purpose.

Table 2. Linearity test results for Alginic acid standard.

Levels	Concentration (mg/L)	Mean peak area
Level-1	50.40	56238
Level-2	100.80	109043
Level-3	201.60	217953
Level-4	302.40	326429
Level-5	403.20	443490
Level-6	504.0	543512
Level-7	604.80	654160
Level-8	705.60	745820
Level-9	806.40	874301
	Linear regression equation	y = 1073.45x + 2610.77
	Intercept	2610.77
	Slope	1073.45
	Correlation coefficient	0.9995

Acceptance criteria: The correlation coefficient should be greater than or equal to 0.99[15,16].

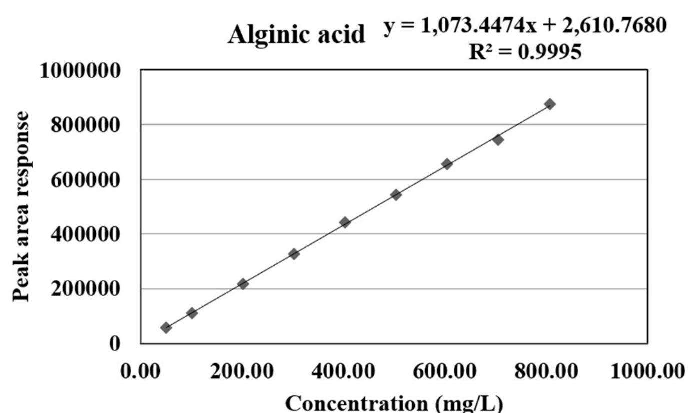

Figure 6. Linearity of Alginic acid standard.

3.2.4. Precision

In the precision study, intra-day precision was determined by seven different weights and solution preparations of a single batch of seaweed and its formulation against a reference standard solution on the same day. Inter-day precision was repeated using different analysts on different days with the same system. The percent relative standard deviation (% RSD) for active ingredient content between the two sets of data was

calculated as per the modified Horwitz equation (**Table 3**). From the result of precision, it was observed that the method was found to be precise for the quantification of alginic acid in seaweed's formulations and phytoextracts of *Chromolaena odorata* (Equation (1)).

Table 3. Precision test results for alginic acid in seaweed extract and it's formulation.

Results	Seaweed extract		Maxilizer liquid	
	Intra-day	Inter-day	Intra-day	Inter-day
Mean A.I. content (%, w/w)	10.09	10.14	0.93	0.93
% RSD	0.84	1.23	1.78	2.03
% Acceptance criteria[15,16]	2.83		3.64	

3.2.5. Accuracy

The accuracy of an analytical procedure expresses the closeness of the results obtained by the method to their true value. In the present study, consecutive analysis (n = 3) for three different concentrations of the standard (50%, 100%, and 150% of the nominal concentration of analyte) was fortified in seaweed and its formulation (Maxilizer liquid), respectively, and the mean recovery was determined to verify the accuracy of the proposed method. The accuracy of the developed method was determined by the method of standard addition (**Table 4**).

Table 4. Accuracy test results for Alginic acid in seaweed extract and its formulation.

Results of seaweed extract and its formulation (maxilizer liquid)						
Level	Claimed amount (mg)	Amount added (mg) n = 3	Amount recovered (mg)	% Recovery	% RSD	% Acceptance criteria[15,16]
% Recovery of seaweed extract						
Level-1 (50%)	10.0	3.30	3.28	99.74	0.38	97 to 103
		3.30	3.25	99.54		
		3.30	3.33	100.26		
Level-2 (100%)	10.0	6.60	6.61	100.10	0.04	
		6.60	6.60	100.05		
		6.60	6.60	100.00		
Level-3 (150%)	10.0	9.90	9.89	99.92	0.12	
		9.90	9.93	100.16		
		9.90	9.91	100.09		
% Recovery of seaweed extract formulation (maxilizer liquid)						
Level-1 (50%)	1.0	3.30	3.30	100.00	0.57	97 to 103
		3.30	3.26	99.35		
		3.30	3.36	100.65		
Level-2 (100%)	1.0	6.60	6.57	99.53	0.49	
		6.60	6.64	100.00		
		6.60	6.59	100.00		
Level-3 (150%)	1.0	9.90	9.91	100.00	0.22	
		9.90	9.84	99.63		
		9.90	9.89	99.91		

3.2.6. Robustness

To measure the ability of the proposed method to remain unaffected by small, but deliberate, changes to the method parameters, evaluation has been done by making slight changes in the chromatographic conditions, such as variations in the wavelength (± 2 nm), flow rate (± 0.1 mL/min), and column oven temperature (± 0.2 ℃). In this study, the chromatographic responses were monitored by the peak area variation of the analyte (**Table 5**). From the result, it was observed, that the % RSD values in all varied parameters were less than 2.0, which indicates the good robustness of the developed analytical method.

Table 5. Robustness test results for Alginic acid in seaweed extract and its formulation.

Parameters	Mean peak area (n = 5)	Alginic acid	
		Peak area	% RSD
Wavelength (λ max)			
203 nm	Mean	413475	0.71
205 nm	Mean	423111	0.43
207 nm	Mean	323696	1.13
Flow rate (mL/min)			
0.4 mL/min	Mean	485309	1.04
0.5 mL/min	Mean	456342	0.41
0.6 mL/min	Mean	425726	0.45
Temperature (℃)			
28 ℃	Mean	424305	0.59
30 ℃	Mean	423111	0.43
32 ℃	Mean	422738	0.61

Limit of detection and limit of quantification (LOD & LOQ):

The sensitivity of the method was evaluated by determining the limit of detection (LOD) and the limit of quantification (LOQ). The limit of detection (LOD) is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated, while the limit of quantification (LOQ) is the lowest amount of analyte in a sample that can be quantitatively determined with the signal-to-noise ratio method. The LOD and LOQ values of the compound were determined with a signal-to-noise ratio of 3:1 and 10:1, respectively (**Table 6**).

Table 6. Limit of detection and limit of quantification test results for Alginic acid.

Sample	LOD (mg/L)	LOQ (mg/L)
Alginic acid	1.21	2.83

3.3. Performance of the batch analysis results

Three different batches of seaweed extract and its formulation were analysed validated method. The analysis was carried out in triplicate for each batch. The mean peak area of the alginic acid in the sample and standard was calculated, and the percentage of alginic acid in the sample was determined (Equation (1)). Similarly, the residue obtained by drying the *Chromolaena odorata* leaf extract is also analysed for the determination of alginic acid in three replicates (Equation (1)). The result was found to be comparable with the declared nominal value **(Table 7).**

Table 7. Alginic acid content in seaweed extract, its formulation and other higher plant extract.

Batch No.	Alginic acid content (%, w/w) in seaweed extract			Mean content. (%, w/w)	STD. DEV.	% RSD
	W1	W2	W3			
Results of seaweed extract and its formulation (maxilizer liquid)						
# 1	10.17	9.85	10.03	10.02	0.1595	1.59
# 2	10.01	10.05	10.08	10.05	0.0346	0.34
# 3	10.07	10.07	10.06	10.07	0.0048	0.05
Alginic acid content (%, w/w) in seaweed extract formulation						
# 1	0.98	0.97	0.99	0.98	0.0084	0.85
# 2	0.99	0.98	1.01	0.99	0.0168	1.69
# 3	1.00	0.99	0.98	1.00	0.0094	0.95
Alginic acid content (%, w/w) in *Chromolaena odorata* phyto extract residue						
# 1	5.44	5.41	5.45	5.43	0.0247	0.45

4. Conclusion

A novel RP-HPLC-DAD method for the determination of alginic acid content in seaweed extract, its formulations, and also in phytoextracts (*Chromolaena odorata* leaf extract) has been developed and validated effectively. As per the review of literature, various authors have used gravimetric, colorimetric, titrimetric, HPLC with fluorescence detector, and refractive index detector, but each method has its own limitations, and hence the content of alginic acid in seaweed varies from method to method, techniques to techniques, and results are not consistent. As seaweed and alginic acid are widely used in the agroindustry, pharmaceuticals, and medicines, and government authorities have made it mandatory to declare the content of alginic acid in the product, it is very essential to have a simple, eco-friendly, and robust method for determining alginic acid. The novel method developed has tremendous scope in all fields of interest. The method is simple in terms of sensitivity, use of an eco-friendly mobile phase, regular HPLC column and detector, lowest retention time (rapid analysis), and no internal standard. All these factors make this method superior for routine analysis of alginic acid in various formulations. The method was designed to be adequate and advantageous, presenting simplicity, being sensitive, robust, reproducible, accurate, inexpensive, easy to perform, and specific as the matrix present in the dosage forms has no interference in the determination of the active constituents. Moreover, the lower solvent consumption along with the short analytical run time leads to a user-friendly and cost-effective chromatographic method.

Conflict of interest

The author declares no conflict of interest.

Abbreviations

RP-HPLC: Reverse-phase high performance liquid chromatography

PDA: Photodiode array detector

OPA: Orthophosphoric acid

% RSD: Percentage relative standard deviation

LOD: Limit of detection

LOQ: Limit of quantification

W: Number of weights

RT: Retention time

A. I.: Active ingredient

SANCO: South African national civic organization

ICH: International conference on harmonization

References

1. Pereira L, Cotas J. Introductory chapter: Alginates—A general overview. In: Pereira L (editor). *Alginates - Recent Uses of This Natural Polymer*. IntechOpen; 2020. doi: 10.5772/intechopen.88381
2. Rashedy SH, Abd El Hafez MSM, Dar MA, et al. Evaluation and characterization of alginate extracted from brown seaweed collected in the red sea. *Applied Sciences* 2021; 11(14): 6290. doi: 10.3390/app11146290
3. de Castro VA, Duarte VGO, Nobre DAC, et al. Plant growth regulation by seed coating with films of alginate and auxin-intercalated layered double hydroxides. *Beilstein Journal of Nanotechnology* 2020; 11: 1082–1091. doi: 10.3762/bjnano.11.93
4. Cotas J, Pacheco D, Gonçalves AMM, et al. Seaweeds' nutraceutical and biomedical potential in cancer therapy: A concise review. *Journal of Cancer Metastasis and Treatment* 2021; 7: 13. doi: 10.20517/2394-4722.2020.134
5. Lagopati N, Pavlatou EA. Advanced applications of biomaterials based on alginic acid. *American Journal of Biomedical Science & Research* 2020; 9(1): 47–53. doi: 10.34297/ajbsr.2020.09.001350
6. Vijayaraghavan G, Shanthakumar S. Efficacy of alginate extracted from marine brown algae (*Sargassum* sp.) as a coagulant for removal of direct blue2 dye from aqueous solution. *Global NEST Journal* 2015; 17(4): 716–726. doi: 10.30955/gnj.001735
7. Ertani A, Francioso O, Tinti A, et al. Evaluation of seaweed extracts from *Laminaria* and *Ascophyllum nodosum* spp. as biostimulants in *Zea mays* L. Using a combination of chemical, biochemical and morphological approaches. *Frontiers in Plant Science* 2018; 9. doi: 10.3389/fpls.2018.00428
8. Kumar S, Sahoo D. A comprehensive analysis of alginate content and biochemical composition of leftover pulp from brown seaweed *Sargassum wightii*. *Algal Research* 2017; 23: 233–239. doi: 10.1016/j.algal.2017.02.003
9. Usov AI. Alginic acids and alginates: Analytical methods used for their estimation and characterisation of composition and primary structure. *Russian Chemical Reviews* 1999; 68(11): 957–966. doi: 10.1070/rc1999v068n11abeh000532
10. Valverde S, Williams PL, Mayans B, et al. Comparative study of the chemical composition and antifungal activity of commercial brown seaweed extracts. *Frontiers in Plant Science* 2022; 13. doi: 10.3389/fpls.2022.1017925
11. Awad H, Aboul-Enein HY. A validated HPLC assay method for the determination of sodium alginate in pharmaceutical formulations. *Journal of Chromatographic Science* 2012; 51(3): 208–214. doi: 10.1093/chromsci/bms129
12. Owlia P, Souri E, Behzadian-Nejad Q. Novel high-performance liquid chromatography method for detection of alginate in pseudomonas aeruginosa. *Iranian Journal of Pathology* 2007; 2(3): 105–108.
13. Jayabarath J, Jeyaprakash J. HPLC profiling of brown seaweeds (*Turbinaria conoides)*. *International Journal of Latest Engineering Management Research* 2017; 2(9): 75–78.
14. Center for Drug Evaluation and Research, U.S. Food and Drug Administration. *Reviewer Guidance, Validation of Chromatographic Methods*. Center for Drug Evaluation and Research, U.S. Food and Drug Administration; 1994.
15. European Commission Directorate General Health and Consumer Protection. Technical active substance and plant protection products: Guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex (Section 4) of Regulation (EU) No 283/2013 and Annex (Section 5) of Regulation (EU) No 284/2013. Available online: https://food.ec.europa.eu/system/files/2019-03/pesticides_ppp_app-proc_guide_phys-chem-ana_3030.pdf (accessed on 2 March 2023).
16. ICH Expert Working Group. ICH Harmonised tripartite guideline: Validation of analytical procedures: Text and methodology Q2(R1). Available online: https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf (accessed on 2 March 2023).