

## ORIGINAL RESEARCH ARTICLE

# Comparing the use of High Performance Liquid Chromatography (HPLC) and Spectrophotometry to measure the amount of chitosan in water-soluble fertilizers

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### ABSTRACT

Several analytical techniques, including High Performance Liquid Chromatography (HPLC) and Spectrophotometry, were developed to quantify the chitosan content in water-soluble fertilizers, with a focus on optimizing the hydrolysis conditions for chitosan. The results showed that the optimal hydrolysis was found to occur using a 1+1 hydrochloric acid solution at 100°C for 24 hours. For HPLC, using 1-phenyl-3-methyl-5-pyrazolone (PMP) as the derivatizing agent, the method exhibited a linear range of 1-200 mg/L, a detection limit of 0.07 mg/L, and a spiked recovery rate of 95%-101%. Spectrophotometry, on the other hand, had a linear range of 0-100 µg, a detection limit of 0.47 µg, and a recovery rate of 94%. The two methods demonstrated good agreement in their determination of chitosan content in water-soluble fertilizer samples, satisfying the analytical requirements for chitosan in such fertilizers. HPLC proved to be more efficient with fewer interference factors. Additionally, the use of methyl fluorene chloroformate (FMOC-Cl) as a derivatizing agent for chitosan hydrolysate, followed by detection with HPLC, yielded results that were essentially consistent with the previous two methods. However, the HPLC and spectrophotometry methods established in this study were not suitable for determining chitosan content in water-soluble fertilizers that contain nitrates.

**Keywords:** chitosan; high performance liquid chromatography; spectrophotometry; water soluble fertilizer; determination method

Chitin is a natural organic compound second only to cellulose in quantity on the earth. It is the largest nitrogen-containing organic compound on the earth<sup>[1]</sup>. It mainly exists in arthropods, such as shrimp. Crab. The shell of insects, the inner shell and cartilage of mollusks such as squid are called animal cellulose<sup>[2,3]</sup>. The chemical name of chitosan (CTS) is  $\beta$ -1, 4-2-amino-2-deoxy-d-glucan, also known as deacetylated chitin, Chitosan, Chitosan, Soluble chitin, etc., are derivatives obtained after deacetylation of chitin<sup>[4]</sup>. Degree of deacetylation (DD) reflects the amino content on the linear molecular chain. Generally speaking, chitin with DD value greater than 55% can be called chitosan<sup>[1]</sup>.

Chitosan is the only alkaline amino polysaccharide abundant in natural polysaccharides, which has excellent biocompatibility. It is considered as one of the most promising functional biopolymers<sup>[5]</sup> and has been used in biomedicine<sup>[6-8]</sup>, food industry<sup>[9,10]</sup>, water treatment<sup>[11,12]</sup> and agriculture<sup>[13-20]</sup> show broad

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development and application prospects. Because chitosan can promote the growth and development of crops. Improve crop quality. Improve crop stress resistance<sup>[14-16]</sup>, so decompose with modern biotechnology. The fertilizer made of extracted chitosan and its research and application have gradually attracted people's attention<sup>[21,22]</sup>.

At present, the method for the determination of chitosan is high performance liquid chromatography<sup>[23-26]</sup>, spectrophotometry<sup>[27,28]</sup>, capillary electrophoresis<sup>[29,30]</sup>, infrared spectroscopy<sup>[31,32]</sup>, gas chromatography<sup>[33]</sup>, resonance rayleigh scattering method<sup>[34]</sup>, etc. Among them, high performance liquid chromatography is a mainstream analytical method for the determination of chitosan. This method usually first hydrolyzes chitosan to glucosamine with acid, then derivates it with derivative reagent, and finally analyzes the derivative products with liquid chromatography technology; although spectrophotometry can be used as a quantitative method of chitosan, there are many interference factors in the determination process, resulting in the low accuracy of the detection results. Poor stability; other methods usually only measure the degree of deacetylation of chitosan, do not conduct quantitative analysis of chitosan, or only apply to reagents. Simple sample detection of drugs and other substrates<sup>[29,31,34]</sup>. Among the above methods, only spectrophotometry<sup>[27]</sup> is reported to be applied to the determination of chitosan content in fertilizers, but this method does not study and analyze the hydrolysis conditions of chitosan. In this study, hydrochloric acid solution was used as the hydrolysis medium of chitosan, and different analytical methods for the determination of chitosan content in water-soluble fertilizers by HPLC and spectrophotometry were established.

## 1. Materials and methods

### 1.1. Method and principle

#### 1.1.1. High performance liquid chromatography

Chitosan is hydrolyzed with hydrochloric acid to produce glucosamine, which is derived with 1-phenyl-3-methyl-5-pyrazolone (PMP). After extracting and removing excess PMP, the derivatives are separated by high performance liquid chromatography, detected by UV detector and determined by external standard method.

#### 1.1.2. Spectrophotometry

Chitosan is hydrolyzed by hydrochloric acid to produce glucosamine, which condenses with acetylacetone under alkaline conditions to form chromogen 2-methyl-3-diacetylpyrrole derivatives. The chromogen reacts with p-Dimethylaminobenzaldehyde under acidic conditions to form a purplish red compound. Its absorbance is measured at the wavelength of 525 nm. Within a certain concentration range, the absorbance of the compound is directly proportional to the concentration of glucosamine, so it can be measured by spectrophotometry.

### 1.2. Reagents

The preparation of reagents and solutions used shall be carried out in accordance with hg/t 3696 if the specification and preparation method are not indicated. The water used for high performance liquid chromatography is ultra pure water, and the water used for spectrophotometry is tertiary deionized water.

Acetonitrile (chromatographic purity); methanol (chromatographically pure); chloroform; concentrated hydrochloric acid; glucosamine hydrochloride standard; aldehyde free ethanol (more than 95%); acetylacetone; chitosan 1 (degree of deacetylation 80%–95%); chitosan 2 (degree of deacetylation >95%).

Hydrochloric acid solution (1 + 1): the volume ratio of hydrochloric acid to water is 1:1. Hydrochloric acid solution (0.3 mol/L): measure 27 mL of hydrochloric acid and dilute to 1: 1 with water.

Hydrochloric acid solution (8 mol/L): measure 680 mL of hydrochloric acid, and then add water to dilute to 1 L.

Sodium hydroxide solution (10 mol/L): weigh 400 g of sodium hydroxide, dissolve it with a small amount of water, and then add water to dilute it to 1 L.

Sodium hydroxide solution (1 mol/L): weigh 40 g of sodium hydroxide, dissolve it with a small amount of water, and then add water to dilute it to 1 L.

Sodium hydroxide solution (0.3 mol/L): weigh 12 g of sodium hydroxide, dissolve it with a small amount of water, and then add water to dilute it to 1 L.

PMP methanol solution (0.5 mol/L): weigh 8.71 g of PMP, dissolve it with methanol, transfer it to a 100 mL volumetric flask and dilute it with methanol.

Potassium dihydrogen phosphate solution (0.1 mol/L): prepare with ultra pure water, adjust the pH to 6.7, over 0.45  $\mu$ M water system microporous filter membrane for standby (now used and now equipped).

Sodium carbonate solution (0.5 mol/L): weigh 53 g of sodium carbonate, place it in a 500 mL beaker, dissolve it with water, and transfer it to a 1000 mL volumetric flask to fix the volume with water.

Acetylacetone solution: transfer 2.0 mL of acetylacetone, dilute to 50 mL with sodium carbonate solution (0.5 mol/L), and place in the refrigerator overnight.

P-Dimethylaminobenzaldehyde solution: weigh 0.8 g of p-Dimethylaminobenzaldehyde, dissolve it in 15 mL of aldehyde free ethanol and 15 mL of concentrated hydrochloric acid, and mix well (now used and now prepared).

Glucosamine hydrochloride standard solution (1.00 g/L): weigh 0.100 g of glucosamine hydrochloride, place it in a 100 mL beaker, dissolve it with water, and transfer it to a 100 mL volumetric flask to volume with water.

Glucosamine hydrochloride standard solution (20.0  $\mu$ G/mL): transfer 2.00 mL of glucosamine hydrochloride standard solution (1.00 g/L) to a 100 mL volumetric flask, and fix the volume with water (used and prepared now).

### **1.3. Instrument**

2695 high performance liquid chromatograph with UV detector (waters company of the United States); TU-1901 ultraviolet visible spectrophotometer (Beijing puxie General Instrument Co., Ltd.), equipped with 1 cm cuvette; AE 100s electronic analytical balance (Swiss Mettler company, accuracy 0.0001 g); seven multi pH meter [METTLER TOLEDO instrument (Shanghai) Co., Ltd]; ph-240a electric blast drying oven (Shanghai Yiheng Scientific Instruments Co., Ltd.); swb3d digital temperature control water bath (Bibby scientific Co., Ltd.); sk8200hp ultrasonic cleaner (Shanghai Kedao Ultrasonic Instrument Co., Ltd.); microporous filter membrane (water system and organic system, 0.45  $\mu$ m).

### **1.4. Test samples**

The samples are from the fertilizer products of fertilizer production enterprises.

### **1.5. Sample pretreatment**

#### **1.5.1. Preparation of samples**

Reduce the solid sample to about 100 g by quartering method, grind it quickly until it passes the 0.50 mm aperture test sieve (if the sample is wet, it can pass the 1.00 mm aperture test sieve), mix it evenly, and place

it in a clean place. In a dry container; after shaking the liquid sample evenly, quickly take out about 100 mL and place it in a clean place. In a dry container.

### **1.5.2. Preparation of sample solution**

Weigh evenly mixed 0.5~1 g solid sample or 5~10 g liquid sample (accurate to 0.0001 g, preferably 200~400 mg chitosan), put it into a 100 mL volumetric flask, first add 1.0 mL concentrated hydrochloric acid, then add water to dissolve it, and then fix the volume. Transfer 1.00 mL of solution to the hydrolysis tube, add 1.0 mL of concentrated hydrochloric acid and 8.0 mL of hydrochloric acid solution (1 + 1), and hydrolyze at 100 °C for 24 h after sealing the tube. After hydrolysis, transfer it to a beaker, add 6 mL of sodium hydroxide solution (10 mol/L), adjust the pH to 7.0 with hydrochloric acid solution (0.3 mol/L) and sodium hydroxide solution (0.3 mol/L), and then transfer it to a 100 mL volumetric flask, and fix the volume with water.

### **1.5.3. Derivatization (high performance liquid chromatography)**

Suck 0.50 mL of the sample solution into a test tube with a stopper, add 0.50 mL of sodium hydroxide solution (0.3 mol/L) and 0.50 mL of PMP methanol solution (0.5 mol/L), cover and mix well, put it into a constant temperature water bath at 70 °C for 70 min, take it out, cool it to room temperature, and add 0.50 mL of hydrochloric acid solution (0.3 mol/L) for neutralization. Extract with 4 mL of chloroform and discard the organic phase (lower layer). Repeat the extraction for 3 times, and pass the obtained upper solution for 0.45 µm organic microporous filter membrane to be tested. 1.6 instrument conditions (high performance liquid chromatography) chromatographic column: Sunfire C18 (150 mm × 4.6 mm, particle size 5 µm); mobile phase: acetonitrile + potassium dihydrogen phosphate solution (0.1 mol/L, pH 6.7) = 18 + 82; flow rate: 1.0 mL/min; column temperature: 25 °C; injection volume: 20 µL; detection wavelength: 250 nm.

## **1.6. Drawing of standard curve**

### **1.6.1. High performance liquid chromatography**

Pipette 0, 0.50, 1.00, 2.00, 3.00, 4.00 and 5.00 mL of glucosamine hydrochloride standard solution (1.00 g/L) into seven 100 mL volumetric flasks, and dilute to volume with water. The mass concentrations of glucosamine hydrochloride in this standard series of solutions were 0, 5.0, 10.0, 20.0, 30.0, 40.0 and 50.0 mg/L. Pipette 0.50 mL of each of the above standard solutions into 7 stoppered test tubes for derivatization reaction, and inject samples according to the concentration from low to high. Take the mass concentration (mg/L) of the standard series solution of glucosamine hydrochloride as the abscissa, the corresponding peak area is the ordinate, and the standard curve is drawn

### **1.6.2. Spectrophotometry**

Take glucosamine hydrochloride standard solution (20.0 µg/mL) 0, 0.50, 1.00, 2.00, 3.00, 4.00 and 5.00 mL into 7 tubes with plugs and add water to 5.00 mL. The standard series of solutions contained glucosamine hydrochloride in amounts of 0, 10.0, 20.0, 40.0, 60.0, 80.0, 100.0 µg, respectively. Add 1.00 mL of acetylacetone solution respectively, mix well, place in a boiling water bath (covered after 1 min) for 25 min, take out, quickly cool to room temperature with ice water, add 3.00 mL of aldehyde-free ethanol, and then add p-dimethylamino 1.00 mL of benzaldehyde solution, mixed well, placed in a 60 °C water bath (covered after 5 min) for 1 h, taken out and immediately cooled to room temperature with cold water, at a wavelength of 525 nm, use a 1 cm cuvette for colorimetry, with the standard solution containing 0 µg of glucosamine hydrochloride was zeroed, and the absorbance was read. Draw the standard curve with the mass (µg) of glucosamine hydrochloride in the standard series solution as the abscissa and the corresponding absorbance as the ordinate.

## 1.7. Determination of sample solution

### 1.7.1. High performance liquid chromatography

Measure the solution to be tested under the same conditions as the standard series solution, and find out the mass concentration (mg/L) of glucosamine hydrochloride in the corresponding sample solution on the standard curve.

### 1.7.2. Spectrophotometry

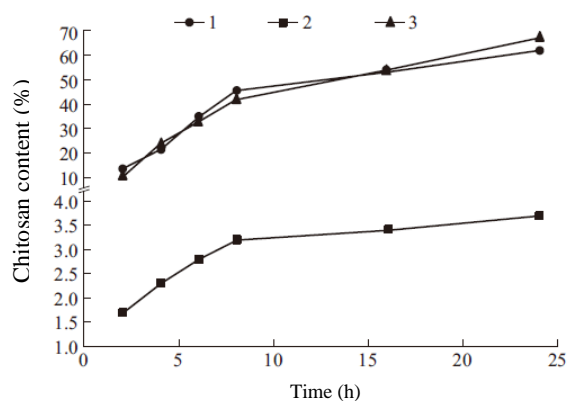
Accurately pipette 1.00–5.00 mL of the sample solution (containing 10–100  $\mu\text{g}$  of glucosamine hydrochloride) and place it in a stoppered test tube, add water to 5.00 mL, and develop color under the same conditions as the standard series of solutions. For colorimetry, zero-adjust the blank experimental solution, read the absorbance, and find out the mass ( $\mu\text{g}$ ) of the corresponding glucosamine hydrochloride on the standard curve. During the measurement, if the sample solution has color before adding the color-developing agent, add the same volume of the sample solution to another stopper test tube, except for the color development stage, use 1.00 mL of hydrochloric acid solution (1 + 1) except for replacing the p-dimethylaminobenzaldehyde solution, the rest of the operations are the same, the absorbance is measured, and the absorbance is deducted during the calculation.

## 2. Results and analysis

### 2.1. Determination of experimental conditions

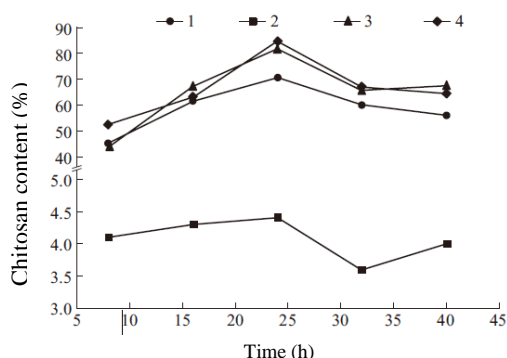
#### 2.1.1. Hydrolysis conditions of Chitosan

Chitosan is generally hydrolyzed by hydrochloric acid. The higher the concentration of hydrochloric acid, the higher the hydrolysis temperature and the faster the hydrolysis rate, but the more serious the damage to the hydrolyzed glucosamine<sup>[23]</sup>. Therefore, a milder hydrolysis condition (1 + 1 hydrochloric acid solution, 100 °C) was selected for the optimal hydrolysis time experiment. The specific operation was as follows: Weigh about 1 g of sample 1 (containing about 70% of chitosan) respectively. Sample 2 (containing about 4% chitosan) is about 10 g. Sample 3 (containing about 80% chitosan) is about 1 g, and the volume is adjusted to 100 mL. Pipette 1 mL of solution (containing 4–8 mg of chitosan) into a hydrolysis tube, add 1.0 mL of concentrated hydrochloric acid and 8.0 mL of 1 + 1 hydrochloric acid solution, seal the tube and hydrolyze at 100 °C for different times. The content of chitosan was determined according to the regulations, and the results are shown in **Figure 1**.



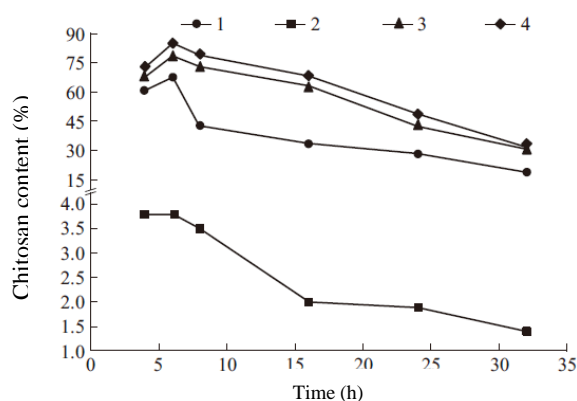
**Figure 1.** 1 + 1 experimental results of hydrolysis time of hydrochloric acid solution.

According to the determination results, it is preliminarily judged that the sample is not hydrolyzed completely. Therefore, the optimal hydrolysis time experiment is carried out again after reducing the weighing amount. The specific operation is: weigh about 0.5 g of sample 1 respectively. Sample 2 is about 5 g. Sample 3 and sample 4 (containing about 80% chitosan) are about 0.5 g, and the volume is fixed to 100 mL. Suck 1 mL of solution (containing 2~4 mg of chitosan) into the hydrolysis tube, add 1.0 mL of concentrated hydrochloric acid and 8.0 mL of 1 + 1 hydrochloric acid solution, and hydrolyze at 100 °C for different times after sealing the tube. Determine the content of chitosan according to the regulations, and the results are shown in **Figure 2**.



**Figure 2.** Experimental results of hydrolysis time of 1 + 1 hydrochloric acid solution after reducing the sample weight.

The results showed that the effect of hydrolysis of 2~4 mg chitosan with 1 + 1 hydrochloric acid solution at 100 °C for 24 h was the best. In order to verify the hydrolysis effect of 1 + 1 hydrochloric acid solution, the experiment of hydrolysis with 8 mol/L hydrochloric acid solution was carried out. The specific operation is: weigh about 0.5 g of sample 1 respectively. Sample 2 is about 5 g. Sample 3 and sample 4 are about 0.5 g, and the volume is fixed to 100 mL. Suck 1 mL of solution (containing 2~4 mg of chitosan) into the hydrolysis tube, add 2.0 mL of concentrated hydrochloric acid and 7.0 mL of 8 mol/L hydrochloric acid solution, and hydrolyze at 100 °C for different times after sealing the tube. Determine the content of chitosan according to the regulations, and the results are shown in **Figure 3**.



**Figure 3.** 8 experimental results of hydrolysis time of mol/L hydrochloric acid solution.

The results showed that the effect of hydrolysis of 2~4 mg chitosan with 8 mol/L hydrochloric acid solution at 100 °C for 6 h was the best, but the result was slightly lower than that of hydrolysis of 1 + 1 hydrochloric acid solution for 24 h. Therefore, the final choice was to use 1 + 1 hydrochloric acid solution for 24 h.

### 2.1.2. Determination wavelength and derivation conditions (high performance liquid chromatography)

The full wavelength scanning of glucosamine derivative solution was carried out in the wavelength range of 210~400 nm. The results showed that glucosamine derivative had large UV absorption at 250 and 320 nm, of which 250 nm was its maximum UV absorption wavelength, and the solvent. The interference of matrix is small, so 250 nm is selected as the determination wavelength.

In addition, according to literature<sup>[25]</sup> and experimental results, the derivation condition of glucosamine, the hydrolysate of chitosan, was determined as:  $c(\text{NaOH}) = 0.3 \text{ mol/L}$ .  $c(\text{PMP}) = 0.5 \text{ mol/L}$ . The derivation temperature is 70 °C. The derivation time is 70 min.

### 2.1.3. Color development conditions (spectrophotometry)

Combined with previous research reports<sup>[27]</sup>, the effects of acetylacetone sodium carbonate solution concentration and preparation time on the experiment were investigated, and the 4% acetylacetone sodium carbonate solution prepared the previous day was compared. According to the three standard curves of the current 4% acetylacetone sodium carbonate solution and the current 7% acetylacetone sodium carbonate solution, it is found that the highest absorbance value of the standard curve using 7% acetylacetone sodium carbonate solution is only 0.27, while the highest absorbance value of the standard curve using 4% acetylacetone sodium carbonate solution can reach 0.45, of which, the correlation coefficient of the standard curve using the 4% acetylacetone sodium carbonate solution prepared now is 0.9987, and the correlation coefficient of the standard curve using the 4% acetylacetone sodium carbonate solution prepared the day before is 0.9996. Therefore, it is determined to use 4% acetylacetone sodium carbonate solution for color development, and it should be prepared the day before use.

## 2.2. Interference experiment

### 2.2.1. Effect of nitrate on the determination of Chitosan

In the study of interference factors, it was found that nitrate would interfere with the hydrolysis of chitosan to glucosamine by hydrochloric acid. In order to investigate the influence of nitrate on the determination of chitosan, different qualities of nitrate were added to the two samples for hydrolysis and determination. The results are shown in **Table 1**.

**Table 1.** Effect of nitrate on determination of Chitosan.

$m_{\text{1}}/2:m_{\text{nitrate}}$	Recovery rate of sample 1 (%)	Recovery rate of sample 2 (%)
200:1	104	99
50:1	91	98
1:1	One	14
1:4	One	1

Note: “-” indicates that the experiment has not been carried out.

The results showed that a very small amount of nitrate had no effect on the determination of chitosan, but with the increasing content of nitrate, the determination results of chitosan will be lower and lower. Therefore, the high performance liquid chromatography and spectrophotometry in this study are not suitable for the determination of chitosan content in water-soluble fertilizer containing nitrate.

### 2.2.2. Effect of protein on determination of Chitosan

According to the production process of chitosan, chitosan products may contain undeleted protein, which will be hydrolyzed by hydrochloric acid to amino acids with a structure similar to glucosamine. Therefore, in

order to investigate the effect of protein on the determination of chitosan, two samples (both containing about 80% chitosan) were hydrolyzed by adding different qualities of corn flour (protein content of about 8%). The results are shown in **Table 2**.

**Table 2.** Effect of protein on determination of Chitosan.

$m/2:m_{\text{corn flour}}$	Recovery rate of sample 1 (%)	Recovery rate of sample 2 (%)
1:2	99	100
1:6	97	101

The results showed that when the mass ratio of the sample to corn flour was 1:2 and 1:6, that is, the protein content in the sample was 20% and 60% of the chitosan content, the recovery of the two samples reached 97%–101%. Therefore, a small amount of protein that may remain in the actual sample has no effect on the determination of chitosan.

## 2.3. Method evaluation

### 2.3.1. Linear correlation and detection limit

High performance liquid chromatography: determine a series of glucosamine hydrochloride standard solutions with different concentrations, taking the mass concentration  $x$  (mg/L) of glucosamine hydrochloride as the abscissa. Take the peak area  $y$  as the ordinate, draw the standard curve, and estimate the detection limit with 3 times the signal-to-noise ratio (S/N). See **Table 3** for the results.

Spectrophotometry: measure a series of standard solutions of glucosamine hydrochloride with different concentrations, take the mass  $x$  ( $\mu\text{g}$ ) of glucosamine hydrochloride in the standard series solution as the abscissa, and the corresponding absorbance  $y$  as the ordinate, draw the standard curve. The standard deviation was calculated using the measurement results of 20 blank samples, and the detection limit was estimated by 3 times the standard deviation of the blank value<sup>[35]</sup>. The results are shown in **Table 3**.

**Table 3.** Comparison of linear range and detection limit between HPLC and spectrophotometry.

Analytical method	Regression equation	Linear range	Correlation coefficient ( $R$ )	Detection limit
High performance liquid chromatography	$y = 3.48 \times 10^4 x - 7.62 \times 10^3$	1~200 mg/L	0.9998	0.07 mg/L
Spectrophotometry	$y = 0.004x - 0.0017$	0~100 Mg	0.9995	0.47 $\mu\text{g}$

### 2.3.2. Accuracy and precision

The accuracy of high performance liquid chromatography and spectrophotometry was evaluated by standard addition and recovery experiments. After two parallel determinations, the recoveries of the two methods were counted. The results showed that the chitosan recovery rates of these two methods were 95%~101% and 94% respectively. The results are shown in **Table 4**.

In order to investigate the precision of high performance liquid chromatography and spectrophotometry, weigh 6 samples each, fix the volume to 100 mL, suck 1 mL solution into the hydrolysis tube, and determine the content of chitosan after hydrolysis according to the regulations. The results show that the relative standard deviations of the measured values are 1.2% and 3.6% respectively. See **Table 5** for the results.



**Table 4.** Experimental results of chitosan recovery (%).

Analytical method	Sample No.	Background value	Scalar addition	Measured value	Rate of recovery
High performance liquid chromatography	1	1.44	4.05	5.34	96
	2	2.45	3.90	6.14	95
	3	3.32	3.70	7.04	101
Spectrophotometry	4	3.59	2.29	5.75	94

**Table 5.** Precision inspection test results of the method (%).

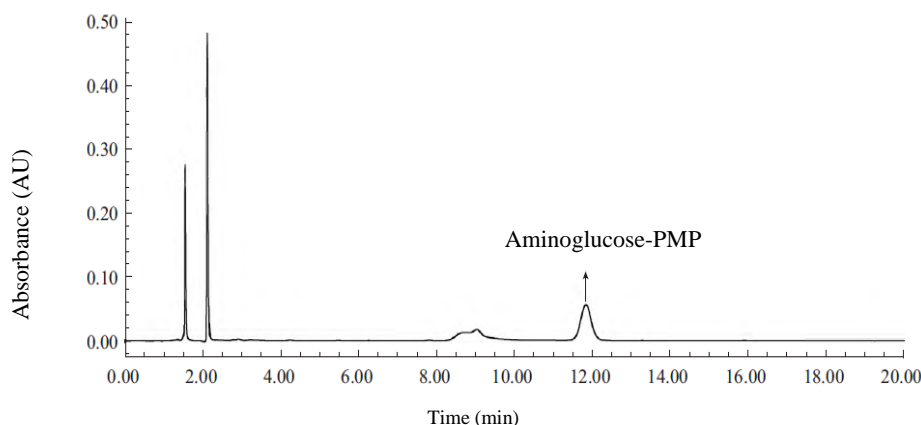
Analytical method	Sample No.	Measured value						Average value	Relative standard deviation
		1	2	3	4	5	6		
High performance liquid chromatography	1	2.65	2.61	2.68	2.68	2.61	2.63	2.64	1.2
Spectrophotometry	2	1.11	1.14	1.10	1.14	1.20	1.19	1.15	3.6

## 2.4. Method comparison

The content of chitosan in 10 water-soluble fertilizer samples was determined by high-performance liquid chromatography and spectrophotometry to investigate whether the results of the two methods were consistent. In addition, these samples were also detected by high performance liquid chromatography with methyl fluorene chloroformate (Fmoc CL) as the derivant<sup>[36]</sup>, and the method of this study was used for pretreatment. The results are shown in **Table 6**. The chromatogram of glucosamine PMP derivatives is shown in **Figure 4**.

**Table 6.** Comparison of chitosan content determined by different analytical methods (%).

Sample No.	High performance liquid chromatography (PMP as derivatizing agent)	Spectrophotometry	High performance liquid chromatography (Using Fmoc CL as derivatizing agent)
1	4.2	4.3	4.0
2	4.4	4.3	4.2
3	1.4	1.6	1.6
4	2.4	2.0	2.0
5	1.2	1.2	1.1
6	3.4	3.5	3.5
7	0.3	0.2	0.1
8	0.1	0.1	0.2
9	2.5	3.3	3.1
10	1.8	1.8	1.7



**Figure 4.** Chromatogram of glucosamine PMP derivatives. Note: the concentration of glucosamine hydrochloride is 30.0 Mg/L.

The results show that the three methods have good consistency. In addition, this study also found that the high-performance liquid chromatography with methyl fluorene chloroformate (Fmoc CL) as the derivatizing agent, due to the two different configurations of -OH at the 1 position of glucosamine, produces two isomers after derivatization, resulting in double peaks in the chromatogram, which is consistent with the previous relevant research reports<sup>[26,36]</sup>, which will have a certain impact on the quantitative detection of chitosan, and the related problems need to be further studied.

### 3. Conclusion

High performance liquid chromatography and spectrophotometry were established to determine the content of chitosan in water-soluble fertilizers, and the results of the two methods were compared. The results show that these two methods can meet the analysis requirements of chitosan content in water-soluble fertilizer, which provides a technical guarantee for the standardized production of the product and a technical basis for administrative management and market supervision. In contrast, the experimental instruments required for spectrophotometry are simple. It has wide applicability, while high performance liquid chromatography is operable. Sensitivity. It has more advantages in stability and reliability, and the inspectors can choose the corresponding detection method according to the actual situation.

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### Conflict of interest

The authors declare no conflict of interest.

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