Comparison of high performance liquid chromatography and spectrophotometry in the determination of chitosan content in water-soluble fertilizers

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Abstract: Different analytical methods for the determination of chitosan content in water-soluble fertilizers by HPLC and spectrophotometry were established, and the hydrolysis conditions of chitosan were systematically studied. The results showed that the hydrolysis effect of 1+1 Hydrochloric acid solution at 100℃ for 24h was the best. Linear range of HPLC with 1-phenyl-3-methyl-5-pyrazolone (PMP) as derivatizing agent. The detection limit and spiked recovery are 1~200 mg/L, 0.07 mg/L and 95~101% respectively, and the corresponding indicators of spectrophotometry are respectively 0~100μg, 0.47μg and 94%. Through comparison, the two methods have good consistency in the determination results of water-soluble fertilizer samples, and can meet the analysis requirements of chitosan content in water-soluble fertilizer. Among them, HPLC has fewer interference factors and higher efficiency. In addition, methyl fluorene chloroformate (FMOC-Cl) was also used as a derivant to derive the hydrolysate of chitosan, and then detected by high performance liquid chromatography. It was found that the measured results were basically consistent with the above two methods, but the high performance liquid chromatography and spectrophotometry established in this study were not suitable for the determination of chitosan content in water-soluble fertilizer containing nitrate.

Keywords: Chitosan; High performance liquid chromatography; Spectrophotometry; Water soluble fertilizer; Determination method

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Received on: November 20, 2020; Date of employment: February 6, 2021

Fund Project: special fund for basic scientific research business expenses of central public welfare scientific research institutions (1610132016065); National key R & D plan (2016yff0201801).

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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 [1]. 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 [2-3]. The chemical name of chitosan (CTS) is β- 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 [4]. 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 [1].

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 [5] and has been used in biomedicine [6-8]. Food industry [9-10]. Water treatment [11-12] and agriculture [13-20] show broad development and application prospects. Because chitosan can promote the growth and development of crops. Improve crop quality. Improve crop stress resistance [14-16], so decompose with modern biotechnology. The fertilizer made of extracted chitosan and its research and application have gradually attracted people's attention [21-22].

At present, the method for the determination of chitosan is high performance liquid chromatography [23-26]. Spectrophotometry [27-28]. Capillary electrophoresis [29-30]. Infrared spectroscopy [31-32]. Gas chromatography [33]. Resonance Rayleigh scattering method [34], 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 [29, 31, 34]. Among the above methods, only spectrophotometry [27] 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 27mL of hydrochloric acid and dilute to 1 l with water.

Hydrochloric acid solution (8 mol/L): measure 680mLof 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 100mLvolumetric 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 0.45μM 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 500mLbeaker, dissolve it with water, and transfer it to a 1000mLvolumetric flask to fix the volume with water.

Acetylacetone solution: transfer 2.0mLof acetylacetone, dilute to 50mLwith 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 15mLof aldehyde free ethanol and 15mLof 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 100mLbeaker, dissolve it with water, and transfer it to a 100mLvolumetric flask to volume with water.

Glucosamine hydrochloride standard solution (20.0μG/mL): transfer 2.00mLof glucosamine hydrochloride standard solution (1.00 g/L) to a 100mLvolumetric 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); sevenmulti 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, 0.45μm).

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 100mLand place it in a clean place. In a dry container.

1.5.2 Preparation of sample solution

Weigh evenly mixed 0.5~1g solid sample or 5~10g liquid sample (accurate to 0.0001 g, preferably 200~400 mg chitosan), put it into a 100mLvolumetric flask, first add 1.0mLconcentrated hydrochloric acid, then add water to dissolve it, and then fix the volume. Transfer 1.00mLof solution to the hydrolysis tube, add 1.0mLof concentrated hydrochloric acid and 8.0mLof hydrochloric acid solution (1+1), and hydrolyze at 100℃ for 24 hours after sealing the tube. After hydrolysis, transfer it to a beaker, add 6mLof 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 100mLvolumetric flask, and fix the volume with water.

1.5.3 Derivatization (high performance liquid chromatography)

Suck 0.50mLof the sample solution into a test tube with a stopper, add 0.50mLof sodium hydroxide solution (0.3 mol/L) and 0.50mLof PMP methanol solution (0.5 mol/L), cover and mix well, put it into a constant temperature water bath at 70℃ for 70 minutes, take it out, cool it to room temperature, and add 0.50mLof hydrochloric acid solution (0.3 mol/L) for neutralization. Extract with 4mLof chloroform and discard the organic phase (lower layer). Repeat the extraction for 3 times, and pass the obtained upper solution for 0.45μm 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 5μm); 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℃; injection volume: 20μL; detection wavelength: 250 nm.

1.7 Drawing of standard curve

1.7.1 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

1.7.2 Spectrophotometry

Take glucosamine hydrochloride standard solution (20.0μg/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.

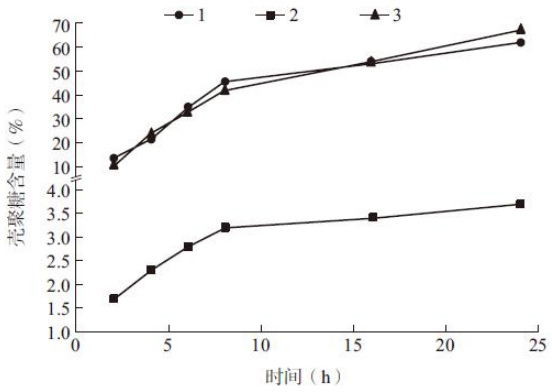
1.8 Determination of sample solution

1.8.1 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.

1.8.2 Spectrophotometry

Accurately pipette 1.00-5.00 mL of the sample solution (containing 10-100 μg 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 (μg) 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.



Chitosan content
Time

Figure 1 1 + 1 Experimental results of hydrolysis time of hydrochloric acid solution

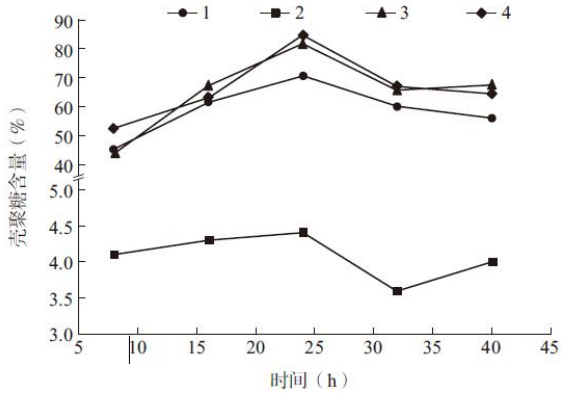
2 Results and analysis

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 [23]. 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.

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 0.5g of sample 1 respectively. Sample 2 is about 5 g. Sample 3 and sample 4 (containing about 80% chitosan) are about 0.5g, and the volume is fixed to 100 mL. Suck 1mLof solution (containing 2~4 mg of chitosan) into the hydrolysis tube, add 1.0mLof concentrated hydrochloric acid and 8.0mLof 1 + 1 hydrochloric acid solution, and hydrolyze at 100℃ for different times after sealing the tube. Determine the content of chitosan according to the regulations, and the results are shown in Figure 2.



Chitosan content
Time

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℃ for 24 hours 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 0.5g of sample 1 respectively. Sample 2 is about 5 g. Sample 3 and sample 4 are about 0.5g, and the volume is fixed to 100 mL. Suck 1mLof solution (containing 2~4 mg of chitosan) into the hydrolysis tube, add 2.0mLof concentrated hydrochloric acid and 7.0mLof 8 mol/L hydrochloric acid solution, and hydrolyze at 100℃ for different times after sealing the tube. Determine the content of chitosan according to the regulations, and the results are shown in Figure 3.

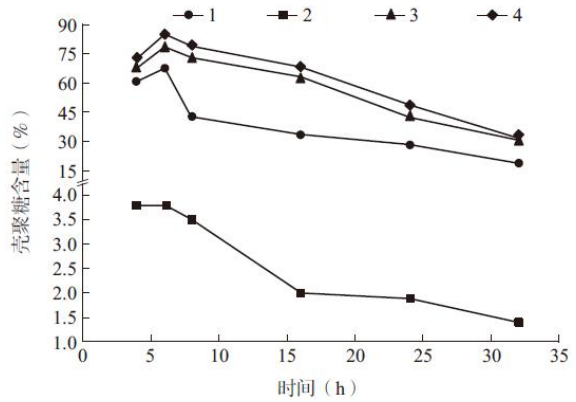


Figure 3 8 Experimental results of hydrolysis time of mol/L hydrochloric acid solution

Chitosan content
Time

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

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 [25] and experimental results, the derivation condition of glucosamine, the hydrolysate of chitosan, was determined as: *c*(NaOH)=0.3mol/L. *c*(PMP)=0.5 mol/L. The derivation temperature is 70℃. The derivation time is 70 min.

2.1.3 Color development conditions (spectrophotometry)

Combined with previous research reports [27], 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

$m_{l/2}$: $m_{nitrate}$	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

$m_{l/2}$: $m_{corn\ flour}$	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(μg) 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 [35]. 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 oefficient (R)	Detection limit
High performance liquid chromatography	$y = 3.48\times10^{4}x - 7.62\times10^{3}$	1~200 mg/L	0.9998	0.07 mg/L
Spectrophotometry	$y = 0.004x - 0.0017$	0~100 Mg	0.9995	0.47μg

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

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 1mLsolution 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 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

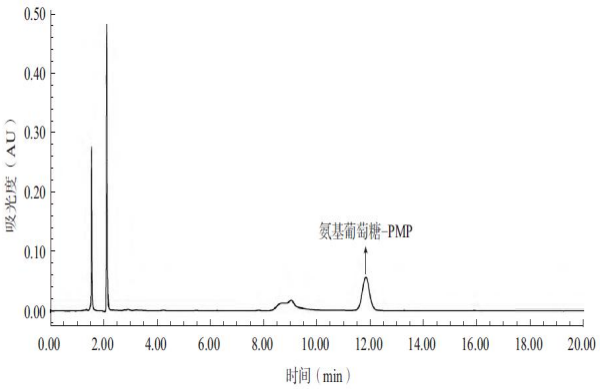
The content of chitosan in 10 water-soluble fertilizer samples was determined by high-performance liquid chromatography and spectrophotometry to investigate whether the results of the two methods were consistent. In addition, these samples were also detected by high performance liquid chromatography with methyl fluorene chloroformate (Fmoc CL) as the derivant [36], 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

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 [26, 36], which will have a certain impact on the quantitative detection of chitosan, and the related problems need to be further studied.



Aminoglucose
Time
Absorbance

Figure 4 Chromatogram of glucosamine PMP derivatives

Note: the concentration of glucosamine hydrochloride is 30.0 Mg/L.

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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