ORIGINAL ARTICLE

Optimization and validation of UV-VIS spectrophotometry methods on the standardization of galactomannan levels inguar gum (*Cyamopsis tetragonoloba* L.)

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Abstract

Aim: Method validation is a step to ensure that the method used is in accordance with the desired goals. The Category I validation method determines the content of the main ingredient in the drug substance. Galactomannan is a water-soluble polysaccharide composed of D-galactose and D-mannose and is the main component in guar gum. **Materials and Methods:** To determine galactomannan content, concentrated phenol-sulfuric acid was used using the UV-Vis Spectrophotometry method. Hydrolysis of guar gum is required to react with phenolic reagents to form colored compounds. **Results and Discussion:** Optimization of the formation of colored derivative compounds was carried out by hydrolysis of guar gum with the addition of 1.0 mL of sulfuric acid, 30 min time, 100°C temperature, and color stability at 180 min. Method validation was successfully carried out by testing several parameters, including selectivity with the selected wavelength 492 nm. Linear regression equation y = 0.0255 × + 0.0420 (r: 0.9998) and Vxo = 0.76%. The precision expressed in the Coefficient of Variation (C.V) is 1.06%. Accuracy was obtained 100.35 ± 0.56 (%; w/w). **Conclusion**: In determining the galactomannan content in guar gum powder, it was 70.00 ± 0.24 (%; w/w).

Key words: D-galactose, D-mannose, galactomannan, guar gum, UV-Vis spectrophotometry method, validation

INTRODUCTION

The validation method is an assessment of certain parameters, based on laboratory experiments, to prove that these parameters meet the requirements for use.[1] Validation is a step to ensure that the method used is as intended. The purpose of validation is to ensure that the analytical method used is accurate, specific, repeatable, and within the range of the analyte to be tested.[2]

According to ISO 17025 (2017),[3] an analytical method must be validated to meet conditions such as non-standard methods. Other factors are the methods that textbooks obtain, and journals that have not been widely recognized, methods developed by laboratories, standard methods used outside their scope. The existence of the slightest change of the standard method, the combination of two or more standard methods, the combination of standard and non-standard methods also needs to be validated.

United State Pharmacopeia (2018)[4] has stated that parameters for evaluating method validation must not always be tested as a whole. Validation is divided into four categories as follows. Category I is to determine the content of the main ingredient in the drug substance in the finished drug product. Category II is for the determination of contaminants in medicinal ingredients or compounds due to degradation of pharmaceutical preparations. This procedure includes quantitative determination and boundary detection tests. Category III is for of the preparation (e.g., dissolution tests and drug release tests). Category IV is for analytical procedures in an identification (USP, 2018). The parameters that must be validated are listed in Table 1.

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Table 1: Characteristics of validation and types of analytical procedures*

Parameter	Category I	Category II		Category III	Category IV
		Quantitative	Test limits		
Accuracy	Yes	Yes	*	*	No
Precision	Yes	Yes	No	Yes	No
Specificity	Yes	Yes	Yes	*	Yes
Detection Limit	No	No	Yes	*	No
Quantification Limits	No	Yes	No	*	No
Linearity	Yes	Yes	No	*	No
Range	Yes	Yes	*	*	No

*USP (2018)

UV-Vis spectrophotometry is a method based on measuring the absorption of monochromatic light by solutions of active compounds. Compounds that can be analyzed by the UV-Vis spectrophotometry method must have conjugated double bonds.[5]

Guar gum powder from the seeds of *Cyamopsis tetragonoloba* L., Leguminosae family, which consists of 75–86% galactomannan which is soluble in water.[6-11] Galactomannan consists of D-manosa and D-galactose with a manose: galactose (M/G) ratio of 2:1.[12-14]

The galactomannan content in guar gum powder is used in various fields of life such as in the pharmaceutical field is widely used in drug delivery systems, drug additives, suspension agents, and thickening agents,[15] the food industry as a thickener, binder, to increase viscosity and as a stabilizer.[16] the cosmetic industry,[17] the paper industry,[18] the biomedical field,[19] and in the textile and printing industries.[20]

Galactomannans do not have conjugated double bonds, so they cannot be detected by UV-Vis Spectrophotometry, therefore, to be determined by the UV-Vis spectrophotometry method, a derivative compound with phenol reagents – concentrated sulfuric acid is made. Galactomannans with concentrated phenol-sulfuric acid become 5-hydroxy methyl furfural by dehydration reaction, then 5-hydroxymethyl furfural with two phenol molecules form a compound 4-[{5 (-(hydroxymethyl) furan-2-il) (4-hydroxyphenyl)methylene} cyclohexa-2,5-dien-1-yliden]oxoniumyellow-orange with a wavelength of 490 nm.[21-25]

The UV-Vis spectrophotometry method with concentrated phenol-sulphuric acid reagents has the advantage of being easy, sensitive, reproducible, and specific enough to determine carbohydrate levels compared to other methods such as gas chromatography and high-performance liquid chromatography.[10,26,27] Concentrated phenol-sulfuric acid reagents are easy-to-use, inexpensive, and sensitive reagents.[21,27] Because it is not possible to make an artificial sample matrix and the sample already contains the active substance to be determined, standardization is determined by conventional addition.

MATERIALS AND METHODS

Instrument

The tools used are the UV-Vis *Single Beam* Spectrophotometer (Agilent Cary 60), analytical scales, *Memmert* water batch, Hermle Z 207 A *Centrifuge*, and glassware commonly used in the laboratory.

Chemicals

Guar gum powder *(C. tetragonoloba L.)*; Galactose; Phenol p.a (Merck CAS108-95-2); Sulfuric acid p.a.(Merck CAS 7664-93-9; 95–98%); and aquades.

Procedure

Pre-treatment of guar gum samples

Weighing guar gum powder as much as 50.0 mg using analytical scales, then add 10 mL of water to the mortar, brushing to form a thick liquid. Put into a 50.0 mL measuring flask and add 1.0 mL of sulfuric acid 2 M. Heat the measuring flask to a temperature of 100°C for 30 min. Cool to room temperature and add distilled water up to the mark, shake the solution in a 50.0 mL pumpkin gauge until homogeneous. Then, centrifuged the solution for 40 min at 4000 rpm, supernatant for testing.

Hydrolysis Optimization

Sulfuric acid addition

Optimization of sulfuric acid addition was carried out by absorbance reading in various acid addition series to guar

gum samples, namely 0.5 mL; 1.0 mL; 1.5 mL; 2.0 mL; and 2.5 mL. with 2M sulfuric acid.

Hydrolysis time

Guar gum hydrolysis time by reading the absorbance at 15, 30, 45, 60, and 90 min.

Hydrolysis temperature

Optimization of guar gum powder hydrolysis temperature with absorbance readings at 20, 40, 75, and 100°C.

Color stability test

Color stability test with absorbance readings at 180, 240, and 300 min at the selected wavelength.

Validation Method

Select the selected wavelength

The selectivity test for determining galactomannan content in guar gum powder was by looking at the spectral profile of the sample, D-galactose, and samples added with D-galactose at a wavelength of 400–550 nm.

Linearity

Some series of curves of D-galactose standard solution 5, 10, 15, 20, 25, and 30 ppm, added 1.0 mL phenol 5% and 5.0 mL concentrated sulfuric acid, and aquades to the exact mark in 10.0 mL flask.

Precision

Standard D-galactose levels of 8 ppm, added 1.0 mL of 5% phenol and 5.0 mL of concentrated sulfuric acid, and aquades to the exact mark in a 10.0 mL flask.

Accuracy

Accuracy test performed at 80%, 100%, and 120% or the addition of D-galactose 80 mg; 100 mg; 120 mg then added guar gum solution added successively by 0.0 mL; 0.5 mL; 1.0 mL; 1.5 mL; and 2.0 mL. Add 1.0 mL of 5% phenol solution (w/v); 5.0 mL of concentrated sulphuric acid; and aquades up to 10.0 mL volume.

Standardization Galactomannan in Guar Gum Powder

The guar gum powder added of D-galactose solution added successively by 0.0 mL; 0.5 mL; 1.0 mL; 1.5 mL; and 2.0 mL. Add 1.0 mL of 5% phenol solution (w/v); 5.0 ml of 2M sulphuric acid; and aquades up to 10.0 mL volume.

RESULTS AND DISCUSSION

Hydrolysis Optimization

Sulfuric acid addition

Hydrolysis is a way to convert galactomannans into D-galactose and D-mannose.[28] Hydrolyzed galactomannans can react with concentrated phenol-sulfuric acid color reagents to form a compound 4,4 '-((5-hydroxymethyl)furan-2-yl)methylene) diphenol. The compound then forms 4-[{5 (-(hydroxymethyl) furan-2-il) (4-hydroxyphenyl)methylene} cyclohexa-2,5-dien-1-yliden]oxonium which will then form a yellow-orange compound with a maximum wavelength of 490 nm.[28-31]

Based on Figure 1 above, hydrolysis optimization of guar gum powder on acid addition parameters is known that in the addition of 1.0, 1.5, and 2.0 ml of 2M of sulfuric acid the absorbance value of the sample is constant or stable. Then, three replications were made, so that with ANOVA one-way obtained $P = 0.184$ ($P > 0.05$). There was no significant difference in absorption value, so 1.0 ml of 2 M sulfuric acid was selected to hydrolyze guar gum.

Hydrolysis time

Based on Figure 2 above, at a hydrolysis optimization of guar gum powder on the parameters of hydrolysis time, known

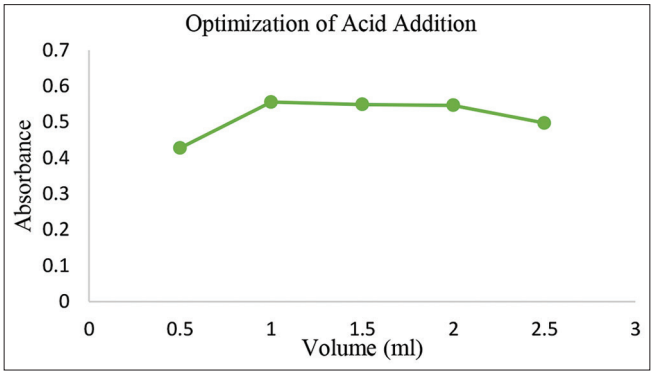

Figure 1: Addition of 2M sulphuric acid versus absorbance

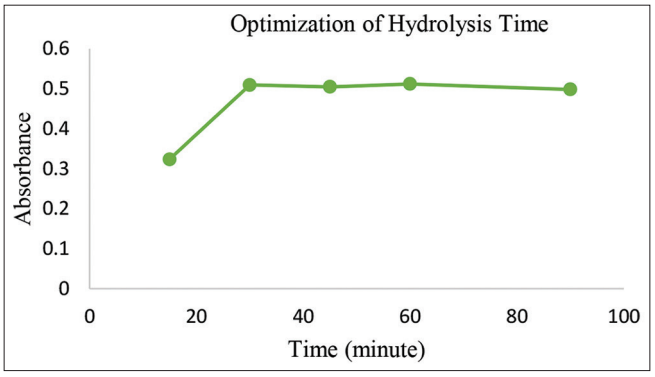

Figure 2: Hydrolysis versus absorbance time

hydrolysis time of 30, 45, and 60 min of constant or stable sample absorbance value. Then, three replications were made, so that with ANOVA one-way obtained $P = 0.238$ ($P > 0.05$), and there was no significant difference, so the guar gum hydrolysis time was chosen for 30 min.

Hydrolysis temperature

Based on Figure 3 above, it is known that the maximum absorption to hydrolyze guar gum is at 100°C, so the temperature is selected to hydrolyze guar gum. This result is in line with the hydrolysis temperature used to hydrolyze polysaccharides with a temperature of 100°C.[25,27]

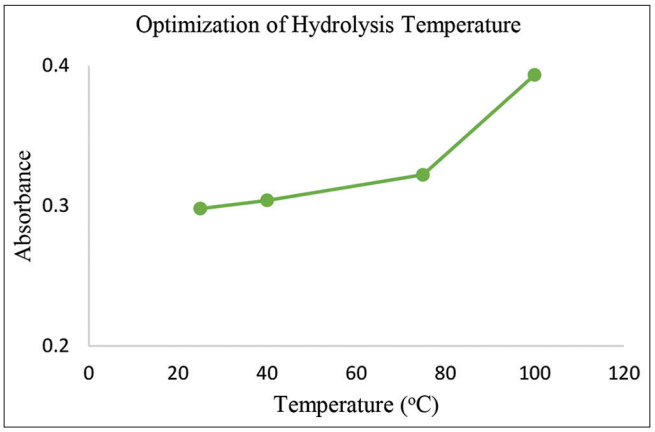

Figure 3: Hydrolysis temperature optimization of guar gum

Color stability test

The color stability test, Figure 4 was obtained on the 180th, 240th, and 300th min of absorbance measurements, the value was stable or constant. One-way ANOVA test obtained $P = 0.226$ ($P > 0.05$), absorbance does not differ. The absorbance measurement of the sample was carried out after the180th min was calculated starting the beginning of the color formation reaction.

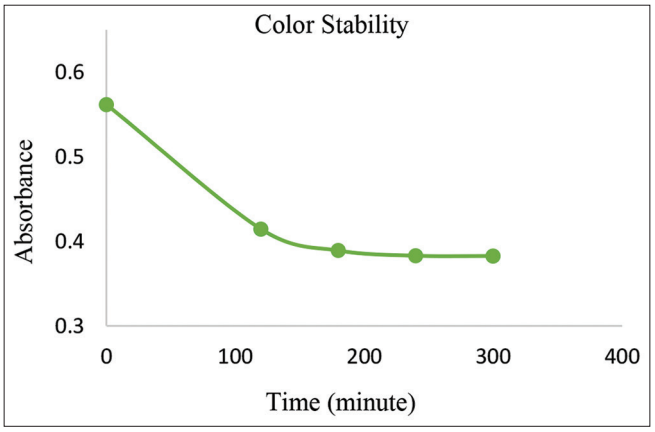

Figure 4: Time versuscolor stability absorbance

Validation Method

Selectivity select the selected wavelength

Because it is not possible to make an artificial sample matrix and the sample already contains the active ingredient compound to be determined, standardization is carried out using conventional addition techniques. Based on Figure 5, the three solutions, namely, galactose 10.0 ppm + guar gum, guar gum and galactose 10.0 ppm standard solution provide maximum 492 nm. Selectivity or specificity is the ability to measure certain substances in the sample matrix such as impurities, degradation products, and matrix components.[2]

In this study, it is known that the wavelength that provides maximum absorbance is 492 nm. In the previous article, it was known that hexose sugars provide maximum absorption 490 nm.[13,30-32] Wavelength differences of no more than four nanometers to measure the absorbance value of the sample can be considered the same.[33]

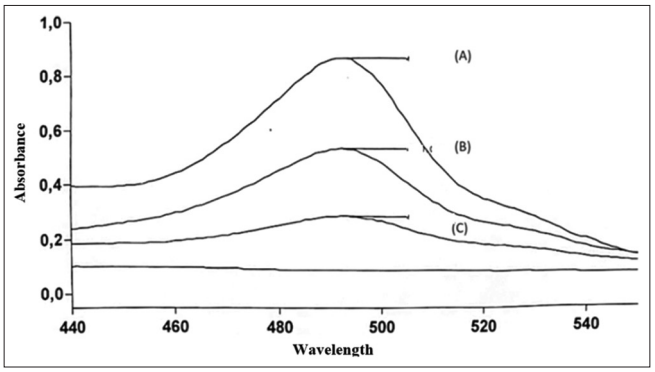

Figure 5: Selectivity test of A. galactose 10.0 + guar gum; B. Guar gum; C. Galactose 10.0 ppm

Linearity

Linearity is the relationship between absorption and concentration, Figure 6 was y = 0.0255 x + 0.0420 (r: 0.9998; $P < 0.01$). There is a significant effect between galactoseconcentration with galactose standard absorbance levels. The result of Vxo calculation is 0.76% (<5%). Based on these data, it can be explained that there is a relationship between absorbance and galactose concentration according to the Lambert-Beer law.

Precision

Precision is a measure that shows the degree of conformity between individual test results, measured through the distribution of individual results from the average if the procedure is applied repeatedly to samples taken from homogeneous mixtures. Precision is measured as a Standard Deviation or Coefficient of Variation (CV). Precision is calculated through the value of the CV as based on data presented in Table 2, the average absorbance value is 0.2742

with a CV: 1.06% (CV ≤2%), so that the precision parameters have been qualified.

Accuracy

Accuracy is a measure that shows the degree of proximity of the analyst's results to the actual level of the analyte.

Table 2: Precision

Observation	Absorbance (λ 492 nm)
1	0.2774
2	0.2750
3	0.2762
4	0.2786
5	0.2754
6	0.2714
7	0.2693
8	0.2750
9	0.2717
10	0.2725
Mean±SD	0.2742±0.0029
C.V	1.06%

SD: Standard deviation, CV: Coeffcient of variation

Accuracy is expressed as a percentage of recovery by addition technique. The difference between the two results is compared to the actual rate (expected result). The accuracy test is expressed in (%) recovery, carried out with three types of concentrations and each concentration level is repeated 3 times as shown in Table 3. In the guar gum powder accuracy test, (%) recovery was obtained at 100.35 ± 0.56 (%; w/w). The results of the guar gum powder accuracy test have met the accuracy requirements of 98–102%.[34]

Standardization Galactomannan in Guar Gum Powder

Calculation of galactomannan content in guar gam powder is presented in Table 4 as follows.

The galactomannan content in guar gum powder using phenol-sulfuric acid reagent with UV-Vis Spectrophotometry that has been validated is 70.00 ± 0.24 (%; w/w). In several other studies, it is known that galactomannan levels are 75–85%.[6,12,14,35] In a study conducted the galactomannan content in guar gum powder was 81.50 ± 1.73 (%; w/w).[32] Varied levels can be caused by plant age, plant varieties, climatic and weather conditions, time and manner of harvest, location or soil

Table 3: Accuracy

Concentration (%)	Replication	Added weight (g)	The weight gained (g)	(%) Recovery
80	1	0.0803	0.0807	100.49
	2	0.0804	0.0808	99.50
	3	0.0810	0.0820	101.23
100	1	0.1021	0.1026	100.49
	2	0.0991	0.1002	101.11
	3	0.1040	0.1039	99.90
120	1	0.1216	0.1224	100.66
	2	0.1211	0.1210	99.92
	3	0.1205	0.1203	99.83
Mean±SD				100.35±0.5645
CV				0.56

SD: Standard deviation, CV: Coefficient of variation

Table 4: Determination of galactomannan content in guar gum

Replication	Weighed Guar gum (g)	Regained D-galactose and D-mannose (g)	Regained (% b/b)
1	0.0504	0.0351	69.64
2	0.0487	0.0342	70.23
3	0.0500	0.0351	70.20
4	0.0495	0.0346	69.89
5	0.0511	0.0358	70.06
Mean±S.D			70.00±0.2440
C.V			0.35%

SD: Standard deviation, CV: Coefficient of variation

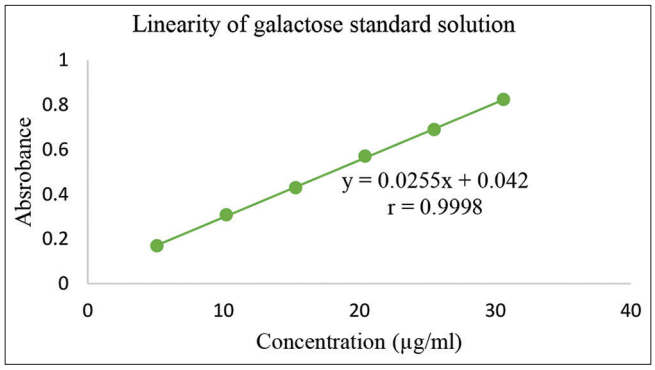

Figure 6: Linearity of galactose solution

structure, treatments given before being produced, tools, places, and methods used.[11]

CONCLUSION

The UV-Vis spectrophotometry method can determine the galactomannan content in guar gum powder (*C. tetragonoloba* L.) which is calculated as the total D-galactose and D-mannose. Galactomannan in guar gum powder are 70.00 ± 0.2440(%;w/w).

Suggestions

Validated UV-Vis spectrophotometry method with the phenol-sulfuric acid concentrated is an alternative for the determination of galactomannan powder with the advantages of cheap, easy, reproducible, sensitive, selective, accurate, and precision.

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