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Research Article

Fenugreek galactomannan: High-Performance Thin-Layer Chromatography (HPTLC) method for identification and quantification of galactose and mannose

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Abstract

Fenugreek (*Trigonella foenum-graecum* L.) is a leguminous, medicinal plant that finds applications in traditional medicine and as an agent in pharmaceutical and nutraceutical products. Its seeds contain various compounds to which numerous beneficial effects are attributed, such as soluble polysaccharides, in particular galactomannan. There are limited data reporting the determination of Bulgarian fenugreek galactomannan composition by HPTLC. The analysis was successful in separating galactose and mannose, each demonstrating different Rf values 0.36, 0.48, respectively. After developing the method with the appropriate conditions, it was validated for linearity, accuracy and precision, range, limits of detection (LD) and quantification (LQ), and robustness. The LD of galactose and mannose was found at 91.34 ng/band and 85.26 ng/band, respectively. The method confirmed the composition of hydrolyzed galactomannan from fenugreek, revealing a ratio of galactose to mannose closed to 1:1, and this capability can be of significant value in both the pharmaceutical and food industries.

Keywords

fenugreek, galactose, HPTLC, mannose, polysaccharide

Introduction

High performance thin layer chromatography (HPTLC) is an instrumental automatized method which is simple, relatively fast, efficient and precise, and can be used for the analysis of polysaccharides. It offers several significant advantages, such as improved sample application, better and faster separation, greater safety, improved sensitivity, smaller volume of the mobile phase used, etc. It is a suitable technique for testing and investigating the stability of various active ingredients in pharmaceutical products, raw materials, essential oils and various plant extracts as well as the contained biologically active components such as polysaccharides. Galactomannans are water-soluble polysaccharides with a non-ionic structure. They are characterized by a high molecular weight and in water can form a highly viscous solution.

The application for quality demonstration of diverse medicinal preparations in the treatment of several disease conditions highlights its importance. In support of this statement, there is evidence in the literature of HPTLC methods developed to demonstrate trigonelline, administered as a cardiovascular agent, an alkaloid isolated from fenugreek (Yang et al. 2010; Shewiyo et al. 2012; Thombre and Gide 2015; Islam et al. 2020a; Alqarni et al. 2021; Petkov et al. 2021; Ivanova et al. 2022; Kozhuharov et al. 2023; Todorova et al. 2023). Fenugreek (*Trigonella foenum-graecum* L.) is an annual medicinal plant that belongs to the Leguminous family. It is commonly known as "Methi" and originates from the Mediterranean and West Africa. Nowadays, it is cultivated all over the world, including countries such as India, Egypt, and Canada. Fenugreek seeds are not only used as a spice in food preparation but also find applications in traditional medicine and as agents in pharmaceutical and nutraceutical products (Rashid et al. 2018; Salarbashi et al. 2019; Liu et al. 2022).

The seeds possess beneficial effects on diverse systems and can be applied to various ailments, such as tuberculosis, mild asthma, kidney disorders or favourable action on sexual function. These and a number of other benefits are attributed to the numerous components they contain, in particular secondary metabolites such as alkaloids, saponins, flavonoids and sterols (Yao et al. 2020; Kenda et al. 2021; Shahrajabian et al. 2021; Singh et al. 2022; Visuvanathan et al. 2022). Besides its anti-migraine, anti-hyperlipidemic, anti-hyperglycemic, anti-inflammatory properties and antitumor activity, Khalili et al. (2018) reported that a natural plant hormone – trigonelline, contained in fenugreek seeds showed a reduction in hippocampal oxidative stress and a reduction in lipopolysaccharide-induced cognitive decline in rats (Khalili et al. 2018; Laila et al. 2019). Furthermore, the addition of fenugreek seed flour in the preparation of biscuits can improve their physico-chemical properties and positively affect their fibre and protein content. Though increasing the amount of fenugreek added may negatively affect their color and taste, the inclusion of approximately 10% sprouted fenugreek seed flour markedly enhances their nutritional quality, and the taste remains pleasant (Negu et al. 2020). Something more, the incorporation of *Trigonella foenum-graecum* flour into beef burgers increases their amino acid content, including tryptophan and lysine, and its addition shows antibacterial and antioxidant properties that are retained even after freezing (Hegazy and Hegazy 2011). This once again highlights the advantages of its application in the food industry.

One of the primary soluble fibres found in *Trigonella foenum-graecum* seeds is galactomannan. It consists of a (1 → 4)-β-D-mannan backbone to which single α-D-galactopyranosyl groups are attached (Fig. 1). These are linked at the O-6 position of the D-mannopyranosyl residues with a galactose:mannose ratio of 1:1. Fenugreek seeds galactomannan exhibits a molecular weight, estimated at around 30,000 Da. These structural features of fenugreek galactomannan also determine its physicochemical properties. As an example, in galactomannan, which has a reduced numbers of galactose residues (Locust bean gum) so-called "smooth regions" are formed in the scaffold. This causes the formation of extensive interchain hydrogen bonds, making galactomannan less soluble in cold water. The relatively equable ratio between galactose and mannose in fenugreek gum results in its very good solubility in cold water and influences viscosity (Mathur and Mathur 2005).

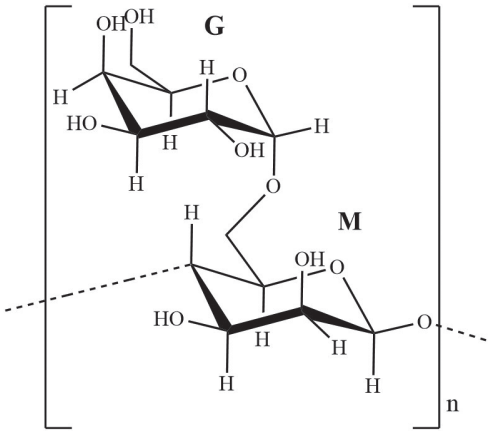

Figure 1. Structure of fenugreek seed gum (with M: Mannose unit and G: Galactose unit).

The dried galactomannan powder is almost colorless or has a subtle creamy tint and exhibits easy dispersibility in water at ambient temperatures. Its chemical composition plays a crucial role in shaping both its rheological properties and its diverse pharmacological effects and pharmaceutical applications (Gadkari et al. 2018; Shukla et al. 2017; Zhang et al. 2019; Liu et al. 2022). Beyond their role as remedies and drug delivery agents, fenugreek polysaccharides could be used as components in edible coating films to preserve foods. Rashid et al. demonstrated favourable outcomes in the improvement of apple quality and shelf life post-harvest when utilizing a packaging film composed of flaxseed and fenugreek polysaccharides (Rashid et al. 2020). The seeds, with their fibre content, may be mixed with flour to create a variety of baked goods, including bread, pizza, and pastries. They not only improve their texture and aesthetic appearance, but also have a positive impact on insulin and blood glucose levels when included in the diet. Moreover, the fiber produces a gel-like structure, leading to a sense of fullness and decreased appetite. This, in turn, might contribute to a lowering of obesity rates, a primary factor associated with the development of various diseases, such as type 2 diabetes (Kadam and Kharade 2016; Wani and Kumar 2018; Staynova and Yanachkova 2023). The emulsifying and suspending properties it possesses, as well as its stability, biodegradability and non-toxicity, make it a favourable excipient in the preparation of various dosage forms (Shukla et al. 2019; Dhull et al. 2023).

There is limited data in the literature regarding the separation of fenugreek galactomannan by high-performance thin-layer chromatography. The aim of the current study was to develop an HPTLC method for the identification and quantification of galactose and mannose in a polysaccharide isolated from Bulgarian fenugreek seeds.

Materials and methods

Chemicals and pharmaceutical reference standards

All reagents utilized were of analytical grade. Hexane and 95% ethanol (used for the initial purification of the plant raw material), *n*-propanol, dichloromethane and methanol were purchased from Sigma Aldrich, Steinheim, Germany. Galactose and mannose standards were also purchased from Sigma Aldrich, Steinheim, Germany. Aniline, diphenylamine and *o*-phosphoric acid were purchased from Fillab.

Plant material and extraction of galactomannan

The plant raw material was purchased from an outdoor market in Bulgaria. The seeds were identified by the leading lecturers in the Department of Pharmacognosy and Pharmaceutical Chemistry. Fenugreek galactomannan was extracted according to the method described by Y. Brummer et. al 2003 with some modifications (Brummer et al. 2003). After initial cleaning, the seeds were defatted using a Soxhlet type apparatus and the lipid components were extracted using hexane (Merck KGaA Darmstadt, Germany). The plant substance was then further purified with 95% ethanol to eliminate the saponin fraction. The residue thus obtained was ground and extracted with demineralized water. The resulting supernatant was also precipitated with 95% ethanol. For purification, the extracted fenugreek galactomannan was solubilized twice in water and again subjected to precipitation with 95% ethanol. Finally, it was dried overnight at 50 °C and stored for further analysis.

Standard solutions and sample preparation

Solvent types were chosen based on pharmacopeial recommendations for galactose (water:methanol) (Pharmacopoeia 2016), for enhanced solubility and stability within the experimental parameters, a distillation water:methanol mixture (3:2, v/v) was used to dissolve both standard stock solutions (galactose and mannose) and the sample solution. Standard stock solutions were prepared at a concentration of 1 mg/mL, and ultrasound was used for better dissolution. The prepared solutions were stored in brown vials protected from light at -20 °C. Before use, from them were prepared working solutions at concentrations of 50 and 100 µg/mL and subsequently subjected to vortex for better dissolution. Fenugreek polysaccharides were hydrolyzed in sealed tubes with 2M Trifluoroacetic Acid (TFA) (Sigma Aldrich, Germany). The mixture was heated for 1 hour at 121 °C. TFA was removed by drying under vacuum at 50 °C. The waste was washed three times with distillation water and evaporated to dryness. Later, the fenugreek galactomannan was dissolved in distillation water:methanol (3:2, v/v) using an ultrasonic bath and vigorous vortexing. The prepared solutions were stored in brown bottles, protected from light at -20 °C before use.

Instrumentation

HPTLC analysis was established using a CAMAG HPTLC system (CAMAG, Muttenz, Switzerland) equipped with: CAMAG Limomat 5 (software-controlled applicator of CAMAG, Muttenz, Switzerland) used for application of the standard and sample solutions; CAMAG Automatic Developing Chamber 2 (CAMAG, Muttenz, Switzerland) for developing the plate, a dipping chamber (Biostep-Desaga, Burkhardsdorf, Germany) for staining the plate, CAMAG TLC plate heater III (CAMAG, Muttenz, Switzerland) was used for heating the plate and CAMAG TLC Visualizer 2 (CAMAG, Muttenz, Switzerland) for observation and imaging the plate. The software used was "VisionCATS" (version 3, CAMAG, Muttenz, Switzerland). Ultrasonic bath (Bandelin, Berlin, Germany) and vortex (Isolab Laborgerate GmbH, Eschau, Germany) were used for better dissolution of the stock and working standard solutions and the sample solutions.

Chromatography

The chromatography was established using a CAMAG HPTLC system. The analyses were carried out using silica gel 60 F254glass TLC plates, 10×20 cm, 200 µm layer thickness (E. Merck KGaA, Darmstadt, Germany). The mobile phase comprised n-propanol:dichloromethane:water in a ratio of 70:20:10 v/v/v. The volume of the mobile phase was 10 mL. Application type: band. Front: 70 mm. Time for development: 130 min and drying 5 min. The plate was immersed by dipping method for 1 min with aniline–diphenylamine–phosphoric acid solution and heated at 120 °C for 5 min on a plate heater. Detection was performed at White RT light using CAMAG TLC Visualizer 2.

Results and discussion

Method development

The initial stage of the HPTLC analysis was to select an appropriate solvent system. For the development of a suitable band and separation for the determination of galactose and mannose using HPTLC technique, various mobile phases such as tetrahydrofuran/water (85:15, v/v), n-propanol/chloroform/acetic acid/water (80:10:5:15, v/v/v/v), and various proportions of these mobile phases including dichloromethane/n-propanol/methanol (MeOH)/water (40:40:15:5, v/v/v/v), dichloromethane/n-propanol/MeOH/water (40:45:8:7, v/v/v/v), dichloromethane/n-propanol/MeOH/water (50:40:5:5, v/v/v/v), dichloromethane/n-propanol/MeOH/water (45:40:5:10, v/v/v/v), dichloromethane/n-propanol/MeOH/water (40:45:10:5, v/v/v/v), dichloromethane/n-propanol/MeOH/water (45:35:5:15, v/v/v/v), dichloromethane/n-propanol/MeOH/water (35:50:8:7, v/v/v/v) were investigated. Moreover, some of these mobile phases investigated were created under chamber saturation conditions. After development of the plates, after each of the mobile

phases, the plates were derivatized with the aniline-diphenylamine-phosphoric acid reagent (ADPAR) (Islam et al. 2020b) and observed with white RT, 254 nm and 366 nm lights. Each of the aforementioned mobile phases were unsuitable as galactose and mannose did not separate, or the resulting spots were tailing, with reduced color intensity and revealed weak chromatographic peaks. It was observed that within the examined solvent systems the best separation properties possess dichloromethane/n-propanol/water (20:70:10, v/v/v). Moreover, dipping of the plate in ADPAR for 1min and subsequently heating of the plate with 120 °C for 5 minutes and visualizing with white RT were the best conditions for derivatization and observing the resulting bands of fenugreek galactomannan. The HPTLC analysis successfully separated galactose and mannose with each of them showing different RF values (0.36, 0.48, respectively).

Method validation

After developing the method with appropriate conditions, because of its simplicity and good separation of the analytes, it was subsequently validated. The validation process was established following the guidelines provided by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) (Guy 2014). The parameters used for this purpose are linearity, accuracy and precision, range, limits of detection (LD) and quantification (LQ), and robustness.

Linearity

To evaluate the linearity a calibration curve consisting of six data points in concentration range 200 – 1000 ng/band were used. Concentrations and peak area of each sample were utilized to plot the calibration curves. The equation $y = ax \pm b$ was used to calculate the regression line, where x represents the concentration, y corresponds to the peak area of each sample. The y-intercept is denoted by b and a stand for the slope of the regression line. The regression line equation and the coefficient of determination (R^2) for galactose, were y = 1E-05x+ 0.0014 and R^2=0.9992 and for mannose were y = 2E-05x+ 0.0012 and R^2 = 0.9983. These findings indicated a substantial correlation, affirming the method's reliability in estimating galactomannans. In Fig. 2 is shown chromatogram of different level concentrations of fenugreek galactomannan and monosaccharides of fenugreek after derivatization, while in Fig. 3 is presented profiles of different level concentrations of galactose and mannose.

Detection limit and quantitation limit

The observed results established a significant correlation, validating the method's reliability for estimating these ones as monosaccharides. The parameters of HPTLC method such as linearity, limit of detection (LD), and limit of quantification (LQ) are presented in Table 1. Based on the good linearity of calibration curves LD and LQ for mannose and galactose were calculated using the following equations: LD=3.3 × σ / S and LQ=10 × σ / S, where σ is the standard deviation of the response and S is the slope of the calibration curve.

Table 1. Characteristics of the HPTLC method.

Parameter	Galactose	Mannose
R_f	0.36	0.48
Range	200–1000 ng/band	200–1000 ng/band
Calibration equation	y = 1E-05x + 0.0014	y = 2E-05x + 0.0012
R^2	0.9992	0.9983
LD	91.34 ng/band	85.26 ng/band
LQ	276.79 ng/band	258.37 ng/band

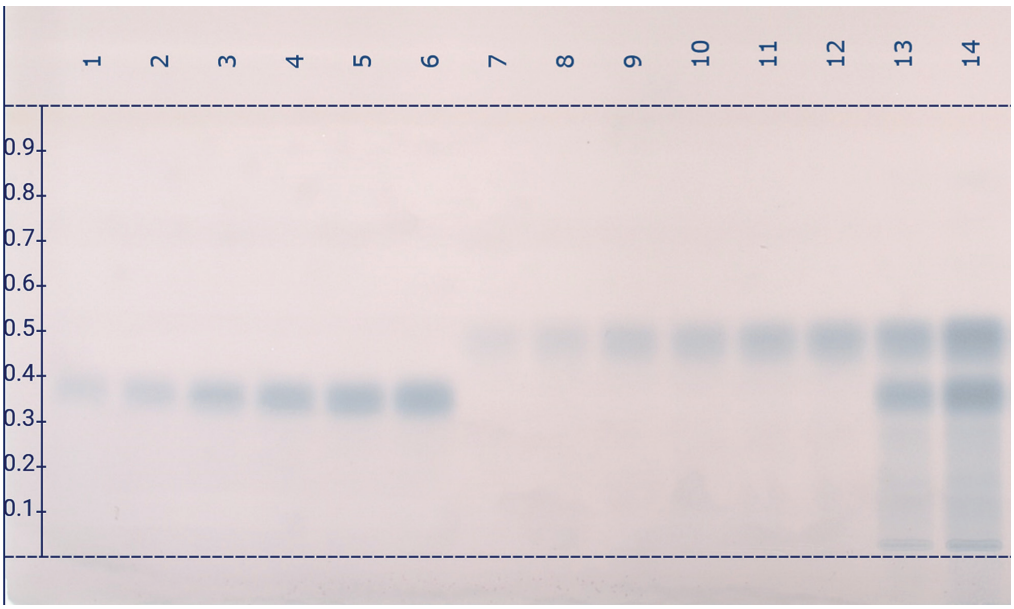

Figure 2. HPTLC image taken after derivatization of galactose, mannose and fenugreek galactomannan, where tracks. **1.** Galactose 200 ng/band; **2.** Galactose 300 ng/band; **3.** Galactose 500 ng/band; **4.** Galactose 650 ng/band; **5.** Galactose 800 ng/band; **6.** Galactose 1000 ng/band; **7.** Mannose 200 ng/band; **8.** Mannose 300 ng/band; **9.** Mannose 500 ng/band; **10.** Mannose 600 ng/band; **11.** Mannose 800 ng/band; **12.** Mannose 1000 ng/band; **13.** Hydrolyzed Fenugreek PS (diluted ×32); **14.** Hydrolyzed Fenugreek PS (diluted ×16).

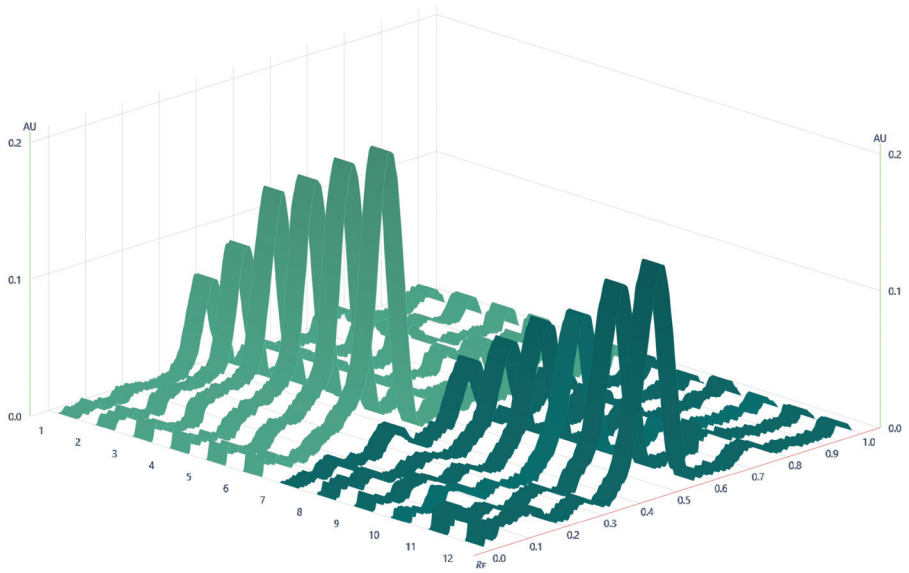

Figure 3. HPTLC profiles observed after derivatization of galactose and mannose, where tracks. **1.** Galactose 200 ng/band; **2.** Galactose 300 ng/band; **3.** Galactose 500 ng/band; **4.** Galactose 650 ng/band; **5.** Galactose 800 ng/band; **6.** Galactose 1000 ng/band; **7.** Mannose 200 ng/band; **8.** Mannose 300 ng/band; **9.** Mannose 500 ng/band; **10.** Mannose 600 ng/band; **11.** Mannose 800 ng/band; **12.** Mannose 1000 ng/band.

Accuracy and precision

The recovery percentage was used as a measure to assess accuracy and according to the ICH-Q2 (R1) guidelines it was performed with three different level concentrations (400, 500 and 800 ng/band) in five repetitions (n=5) of each concentration. The accuracy of galactose, 104.00%, 103.60% and 101.10% at 400, 500 and 800 ng/band quality control, respectively, and for mannose 100.50%, 98.14% and 97.28% at concentrations 400, 500 and 800 ng/band, respectively. The results of the accuracy assessment were expressed as the difference between the mean and the accepted true value and were presented as % of recovery and % of coefficient of variation (%CV) of the presented method are shown in Table 2.

Table 2. Evaluation of accuracy of galactose and mannose (mean ± SD, n = 5).

Concentration (ng/band)	Conc. (ng/Band) ± SD	% Recovery	%CV
Galactose			
400	416±8.94	104.00	2.15
500	518±2.11	103.60	1.78
800	808.8±10.26	101.10	1.27
Mannose			
400	402±2.85	100.50	0.71
500	490.7±8.74	98.14	1.78
800	778.2±5.02	97.28	0.65

The accuracy of the method was further investigated by percentage recovery using the technique of spiking the sample with known concentrations of galactose and mannose. The analysis involved the examination of samples both before and after the addition of predetermined amounts. The obtained results from evaluation of recovery from spiking technique are show in Table 3.

Table 3. Evaluation of recovery analysis of galactose and mannose spiked in the sample (n = 3).

Compound	Spiked concentration (ng/mL)	% Recovery	%CV
Galactose	500	102.70	1.48
Mannose	500	97.56	1.96

To validate a method, it is necessary to assess intra-day and inter-day precision, crucial indicators of the analytical method's dependability and uniformity. Intra-day precision refers to the precision under the same operating conditions on a single day or over a short interval of time. Inter-day precision includes the variability of results from multiple measurements of the same sample across different days. The precision of the developed method was assessed for both intra- and inter-day analyses, utilizing linear regression data from the calibration curves. The intra-day assay involved the quantification of newly prepared galactose and mannose solutions at three different concentration levels (400, 500 and 800 ng/band) on the same day. Each concentration was measured in five replicates (n=5). The inter-assay precision involved the quantification of freshly prepared galactose and mannose solutions at three different concentration levels (400, 500 and 800 ng/band) over three consecutive days. Each concentration was measured in five replicates (n=5). For both intra- and inter-day precision the results were evaluated as coefficient of variation. The obtained %CV values for intra-day precision were found to be in a range of 1.26% and 4.08% for galactose and in a range of 0.76% and 2.14% for mannose. Respectively, for inter-day precision the obtained %CV values were found to be from 1.39% to 4.83% for galactose and from 0.53% to 1.91% for mannose. These results suggest that the method exhibits a good degree of precision. In Table 4 are presented the intra- and inter-day precision for galactose and mannose in the three level concentrations.

Table 4. Evaluation of the intra- and inter-day precision for galactose and mannose in the three different level concentrations in five replicates.

Concentration (ng/band)	Intra-day precision			Inter-day precision		
	Mean	SD	%CV	Mean	SD	%CV
Galactose						
400	434.0	5.48	1.26	439.2	6.14	1.39
500	534.6	17.7	3.31	527.6	10.06	1.91
800	768.8	31.39	4.08	759.7	36.67	4.83
Mannose						
400	405.0	8.66	2.14	402.2	2.59	0.64
500	484.7	9.05	1.87	484.8	9.26	1.91
800	777.1	5.92	0.76	776.8	4.09	0.53

Robustness

Robustness is the quality that evaluates the ability of a method to cope with slight and deliberate changes in its parameters and provides insights into its reliability under typical operational conditions. To evaluate the robustness of the devised method, intentional changes were introduced in the compositions of the mobile phase and in the total run length. The solvent ratio of dichloromethane/propanol/water (20:70:10, v/v/v) was adjusted to a range of 1%, and the HPTLC response was recorded for each set of conditions. The total distance of the solvent was changed from the original 70 mm to 72 mm and 68 mm, and the HPTLC response was observed. The observed changes in the Rf values were within the range of 0.02, which indicates the robustness of the method.

To assess the stability of the standard solution, they were stored at 2–8 °C for a week, and the visual examination confirmed the clarity of the solution. Afterwards, the chromatograms of freshly prepared solutions were compared to those of stored solutions. Comparative analysis revealed that the samples maintained stability throughout storage.

The proposed method is innovative, sensitive, and cost-effective. The method does not require changes in humidity or saturation of the chamber, which would complicate and prolong the process. It is characterized by easy sample preparation, near one-time correlation factors, the appropriate percentage of accuracy, satisfactory coefficients of variation values and lower limit of detection and limit of quantification. According to these characteristics the proposed method is linear, accurate and reliable to determine and quantify galactose and mannose.

This validated method has been used to determine and confirm the composition of the hydrolyzed galactomannan from fenugreek, revealing quantity was found to be relatively 1:1 in the ratio of galactose to mannose. This contributes to the developed method being the first HPTLC method to identify and quantify galactose and mannose in galactomannan isolated from fenugreek.

Due to the increasing use of monosaccharides, particularly mannose and galactose, in the pharmaceutical and food industry (Silveira and Bresolin 2011), it would be beneficial to employ a sensitive HPTLC method for the quality and quantity analysis of them. Fenugreek serves as one of the primary sources of galactomannans (Rashid et al. 2018). However, there is insufficient literature data regarding the analysis of the presence of galactose and mannose in it, as evidenced by Table 5, which presents data from previously developed HPTLC methods for the identification of galactomannans isolated from other plant species. The type of linkage of galactomannan isolated from fenugreek differs from *Cordyceps*, Locoweed, *Caesalpinia pulcherrima*, etc. (Olennikov and Rokhin 2010; Xie et al. 2012; Thombre and Gide 2013; Guo et al. 2014; Wu et al. 2014). Therefore, a new HPTLC method that offers high sensitivity, efficiency, and reproducibility has been developed and validated. The established precision, accuracy, reliability, and practicality of the proposed method ensure its use for routine quality and quantity control in the pharmaceutical and food industries. The method is suitable for the analysis of galactose and mannose separately or simultaneously.

The determination of carbohydrates plays a vital role in the food industry as well as in pharmacy (Guille and Martı 2004). Galactomannans exhibit numerous properties such as thickening, emulsifying, suspending, etc. For that reason, they can be used to prepare edible coatings film for food packaging or as an agent for various drug delivery systems such as colon tablets or ophthalmic medicaments etc. (Cerqueira et al. 2011; Yana and Margarita 2022; Dhull et al. 2023). Ristivojevic et al. (2017) revealed the efficacy of HPTLC in combination with other detection techniques to determine the composition of different thickeners. Nowadays, carbohydrates are very widely used worldwide and in various fields as excipients precisely

Table 5. Separation and detection of polysaccharides by previously proposed HPTLC methods.

№	Samples	Hydrolysis	Mobile phase	Stationary phase	References
1.	Polysaccharides from seven species of *Cordyceps*	TFA (0.5 mol/L)	1-Butanol/isopropanol/acetic acid/water, 7:5:1:2 (v/v/v/v)	Silica gel plates 20×10	(Wu et al. 2014)
2.	Galactomannan of the Locoweed (*Oxytropis lanata*)	TFA (2 mol/L)	p-Propanol/ethanol/water, 7:1:2 (v/v/v)	Chromatographic plates	(Olennikov and Rokhin 2010)
3.	Galactomannan of *Caesalpinia pulcherrima*	2 N HCl (2 mol/L)	Acetone/ Water, 9:1 (v/v)	HPTLC plates precoated with 200 µm layer of Si-gel Si60F254	(Thombre and Gide 2013)
4.	Polysaccharides from *G. lucidum* and *G. sinense*	TFA (5 mol/L)	Chloroform/n-butanol/methanol/acetic acid/water, 5.5:11.0:5.0:1.5: 2.0 (v/v/v/v)	0.2 mm Nano-silica gel 60 HPTLC plates	(Xie et al. 2012)
5.	Galactomannan from the coral endophytic fungus *Aspergillus ochraceus*	HCl (1 mol/L)	Triethylamine/n-butanol/water, 0.7:60:30, (v/v/v)	HPTLC Silica gel plate (2 cm × 4.5 cm)	(Guo et al. 2014)

because of their many rheological characteristics. For this reason, it is necessary to continuously develop new, efficient, fast and reliable methods for their chemical composition recognition as well as for their quantification (Ristivojević and Morlock 2017).

Furthermore, galactomannans are one of the primary choices for thickeners in textile printing pastes (Özen et al. 2023). Due to biodegradability, non-toxicity and biocompatibility, the nature of galactomannans combined with their rheological properties, make them effective as flocculating agents in wastewater treatment (Sharma et al. 2020) such as the HPTLC methodology introduced in this study. The proposed method could be applicable for determining galactose and mannose, not only of galactomannan from Fenugreek, but also for a number of other natural biopolymers such as gum guar, locust gum, etc.

Conclusion

Polysaccharide from *Trigonella foenum-graecum* grown in Bulgaria was isolated for the first time. The residues of galactose and mannose, components of the obtained galactomannan, were confirmed through HPTLC. It is an automatized analytical method that is easy, sensitive, cost-efficient and environmentally friendly. In addition, the method was developed and validated according to ICH for linearity, accuracy, precision, range, limits of detection (LD), quantification (LQ), and robustness. The HPTLC validated method was applied to determine and confirm the composition of hydrolyzed galactomannan from Fenugreek, revealing a ratio of galactose to mannose close to 1:1, and this capability can be of significant value in both the pharmaceutical and food industries.

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