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Original Article

Separation and Purification of Galegine using Column Chromatography followed by Molecularly Imprinted Polymer Technique

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Abstract

Galega officinalis L. (Papilionaceae) is a native to southeastern Europe that is used in the treatment of various diseases including diabetes. The extract of this plant contains many effective compounds such as Galegine that is a guanidine alkaloid. In this research, column chromatography with a molecularly imprinted polymer (MIP) technique was used to achieve this valuable material. The extracted components were separated by column chromatography using silica gel as stationary phase. For eluting of components from the Galegine extract, once mobile phase was completely non-polar hexane solvent, and then, the polarity of the solvent was increased until all the remaining components were removed. Finally, 11 fractions were achieved. Thin-layer chromatography (TLC) method was used to identify the isolated components. Then, the Galegine in the extraction fraction was purified using MIP technique. The results showed that the chromatographic separation method led to removing significantly the interfacial compounds. Interestingly, it was resulted that while the column chromatography was priority used from MIP adsorption, the purification and isolation efficiency of Galegine was enhanced up to 17 times compared to using MIP alone.

Keywords: Extracted Galegine, Column Chromatography, Molecularly Imprinted Polymer, Isolation

Introduction

Diabetes is one of the most common endocrine diseases that currently, affect 415 million people worldwide, and more than 90% of them having type 2 diabetes; it is estimated that about 179 million people with diabetes are not aware of their disease. About 462 million people are expected to develop this disease by 2040 [1]. In this metabolic disease, the function of other body systems is gradually impaired [2]. Diabetes is associated with a complete to partial lack of insulin, followed by an increase in blood sugar. The main role of insulin is to regulate blood sugar. Insulin dysfunction causes a chronic hyperglycemia, along with abnormalities in carbohydrate, fat, and protein metabolism following disease progression, tissue, vascular damage, and a variety of side effects including impaired vision, kidney, cardiovascular, nerve and it causes a variety of wounds and eventually, causes death [3]. Therefore, it can be said that diabetes causes a wide range of disorders, so, finding methods without side effects is one of the important goals of active researchers in this field. Currently, the main effective treatment for diabetes is the use of insulin and drugs to lower blood sugar. Unfortunately, many of these patients become insulin resistant due to prolonged illness and their injection has no beneficial effect on reducing blood sugar. But these compounds also exhibit several associated side effects. However, many hypoglycemic agents are artificially and nonspecifically able to prevent diabetes that can cause complications and poisonings. Some of these side effects include gastric, intestinal, diarrhea, and abdominal flatulence [4]. Before the discovery of insulin, as well as common anti-diabetic drugs, diabetic patients were treated with herbs and traditional treatments. So far, many herbs have been reported to have a positive effect on lowering blood sugar levels and reducing their side effects. Traditionally, various herbs have been used throughout history to lower blood sugar and improve diabetes, and more or less detailed information is available on traditional medicine

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in Iran and other countries around the world. Herbal medicines are widely prescribed and administered around the world because of their low side effects, availability, relatively low cost and effectiveness. There are several types of glucose-lowering drugs that exert their anti-diabetic effects through various mechanisms, including, Damask rose, fleawort, sweetbean, purslane, coriander, lettuce, sorrel, gum arabic and tabasheer [5, 6]. Despite the variety of medicinal herbs, *G. officinalis* L. has the potential to produce herbal drug used in the treatment of diabetic patients. Galega is an herbaceous, fluff-free, half to one meter tall belongs to Fabaceae. This plant grows in warm and humid regions and its origin is southeastern Europe [7]. The aerial part of the plant contains a set of hypoglycemic agents as an anti-diabetic drug in type 2 diabetes [8] and its other properties include anticoagulant properties [9], reducing body weight [10], and anti-inflammatory properties and antioxidant activities [11]. In this regard, it is important to note tha one of the derivatives of guanidine in this plant, called Galegine, is a substance prepared based on its chemical structure as Biguanide (metformin) and is used in the treatment of diabetes [12,13,14]. In several cases, the administration of Galegine and/or Galega lead to decrease daily insulin consumption dose (22-23 units), whereas insulin requirement returned to baseline levels after discontinuation. On the other hand, in recent years, there has been a growing interest in the use of medicinal plants worldwide due to the adverse effects of chemical drugs. It is necessary to carry out modern and complete research on the use of medicinal plants and their products, quality and safety. They should be considered precisely because unnecessary use of medicinal plants may have adverse effects and adversely affect human health [15]. Wide-ranging lists of active ingredients are found in plants such as phenolic compounds, chemicals, pigments, antioxida -nts, antimicrobial compounds, and perfume flavoring agents. Herbal extracts are widely used in the pharmaceutical, food and health industries [16]. Different methods of isolation have been applied to produce such valuable natural compounds from plants, and to commercialize them. Traditional solvent extraction methods are mainly based on proper solvent selection and the use of heat or stirring to improve mass transfer. These methods require longer extraction times, which can pose a serious risk for further thermal decomposition of plant components. The development of high-efficiency and rapid extraction methods is urgently needed due to the presence of several thousand secondary metabolites in plants. Therefore, to solve the abovementioned dilemmas, the present study was concerned on the new extraction methods with short extraction times, low solvent consumption and polymeric adsorbent of MIP for purifying of the mentioned compound.

Material and Methods

Galega officinalis L. was obtained from Zard Band Company which produces raw materials for pharmaceutical producers in September 2017. Leaves were dried at room temperature for 30 days prior to the experimental studies. The dried samples were stored inside a sealed plastic bag in a cold and dry place until use. Galega powder (5 g) was placed in 50 mL of distilled water for 24 hours at room temperature and was stirred on a shaker to extract. The extract was then filtered through Whatman Grade No. 41 Quantitative Filter Paper. The solvent was evaporated at 50-60 °C. To extract some compounds, ethanol 96% (25 mL) was used. The mixture was again filtered with a filter paper and was evaporated at 60-70 °C. The achieved extract was stored for loading on a column at 2 to 8 °C in a dark at closed glass container. Methacrylic acid (MAA), azobisisobutyron -itrile (AIBN), ethylene glycol dimethacrylate (EGDMA), acetonitrile, graphene oxide, and other solvents and employed chemicals including ethanol, methanol, acetic acid, acetonitrile, Chloroform, n-hexane with laboratory chemistry grade and ammonia 25%, graphene oxide, Silica gel 60 (63-230 mesh), TLC plates all were by Merck company, German, and Galegine Sulfate by Select Lab. Co. with Laboratory Chemistry grade. XRD apparatus (PHILIPS Company, Netherlands) was used to study the polymer particle size and crystalline properties of synthesized MIP and NIP. A Dualscope Atomic Force Microscope (AFM) by Dualscope Company, Denmark was used to investigating the internal structure and porosity of the polymer. FT-IR (AVATAR model, Thermo Company, U.S.) was used to study the functional groups of synthesized macropolecule GO-MIP, while the morphologies were studied by FE-SEM (MIRA3 model, TESCAN Co., Czech).

Synthesis and elution of molecularly imprinted polymer-graphene oxide (GO-MIP)

At the first, for the synthesis of polymer, 5 mmol of methacrylic acid (functional monomer) with 1 mmol of Galegine sulfate (template) and 9 mL of acetonitrile (solvent) were added and mixed. Then 30 mmol EGDMA as a crosslinker was added to the mixture and was mixed. The obtained mixture was transferred to a volumetric flask. Then, 0.12 gr 2, 2 Azobisisobutyro-nitrile (primer) and 0.05 g of graphene oxide were added. The mixture was denitrated for 20 min. The flask was placed at the water bath 70 °C for 24 hours to polymerize. The obtained polymer was put into an oven at 70-80 °C to dry. It was then powdered with a mortar.

Preparation of Plant Extract.

and passed through a sieve with 1000 to 2000 meshes. In order to remove the template molecules and also to remove any unreacted and disturbing from polymer texture, the polymer powder was stirred for 2-3 hours with methanol (1:10 g/v). This was repeated two times. Next, the polymer was washed with distilled water instead of methanol. The resulting polymer was then filtered through a filter paper and dried at 60-70 °C.

Synthesizing Non-NIP Polymer

Synthesizing non-NIP Polymer All polymer synthesis steps of non-NIP were exactly as mentioned for MIP preparation, but without the presence of the template molecule. This was perfomed in order to obtain surface adsorption by the synthesized polymers and also, to ensure that the holes created in the polymer are as large as the target molecule. The Galegine molecule does not exist in the resulting polymer and therefore, washing with methanol and water solvents was missied.

Optimization of the Factors Affecting Galegine Quantitative Measurement

After assuring the formation of detection sites in the MIP and response of polymer to adsorption on the target molecule polymer was evaluated that determined the best conditions for the variables affecting it. Factors affecting the quantitative extraction of Galegine were optimized. For this purpose, the following factors were studied to find optimal condition: the amount of MIP adsorbent, amount of graphene oxide nanoparticles, extraction time, and pH of the extraction medium, surfactant addition and ionic composition in each experiment. The factors affecting absorption are presented in Table (1). In this study, all electrochemical quantitative measurements were performed by a three-electrode system polarography. The dropping mercury electrode (DME) was selected as the working electrode, the saturated calomel electrod (SCE) as a reference electrode with a thin platinum rod as a counter electrode. The standard solution of Galegine (10 µl) was mixed with 20 mL of twice distilled water. Then, nitrogen gas was bubbled through the solution for 5 min to remove oxygen gas. Then, it was analyzed quantitatively using standard addition method and polarography pulse techniques. Each analyzing experiment was replicated 3 times at room temperature. For the analysis conditions, were set as follows: potential ranged from (0.1 to 0.7 mV) and scan rates at10 mV/s.

Detection of Galegine by Thin-layer Chromatography (TLC)

Thin-layer chromatography is one of the qualitative methods of measurement that is relatively accurate, fast, and inexpensive, and therefore, is recommended as pre-tests for high-performance liquid chromatography. Thin-layer chromatography is a solid-liquid adsorption chromatography method that the speed of materials movement on the chromatographic plates depends on their polarity and is described by the RF index. In this study, TLC paper was used as a stationary phase and solutions as mobile phase (table 2) [17]. In order to determine the Galegine in the extract and also, to select the most suitable solvent system for its isolation from other plant compounds in this study, more than 30 solvents system with different ratios was tested as the mobile phase, which some of these solvent systems is presented in Table (2). A suitable amount of solvent (4:3:0.5:0.5; Chloroform-methanol-ethyl acetate-acetic acid; mobile phase) was added to the beaker (25 mL) to perform the TLC test and it was capped off. Parallelly, the TLC sheets (2 × 5 cm) were spotted with two points using capillary tubes i) standard solution of Galegine and ii) diluted extract After the spot was dried, the TLC sheet was inserted into the TLC Tank and it was observed that the spotes were seprated and shifted to upwared, till about one centimeter at the end of the TLC sheet. It was removed from the TLC tank and after complete drying, the spot was appeared on the sheet with ultraviolet radiation.

Table 1 Investigating the variables affecting the measurement of Galegine

Factor	Scope of study	Optimal valye
MIP value	0.01-0.5 (g)	0.1 (g)
The amount of graphene oxide nanoparticles	0.001-.05 (g)	0.05 (g)
Extraction time	3-30 (s)	20 (s)
Ph	3.5-8.5	4.5-6.5
Triton-X100	0.01-0.2 (mmol/L)	-
Ionic composition of NaCl	0.01-1 (W/V %)	-

Table 2 Some solvent systems and selection of the best solvent to identify Galegine in Galega

No.	Solvent system	Volume ratio	TLC observations
1	n-hexane	-	Spot was not observed
2	n-butanol	-	Spot was not observed
3	n-Butanol-ammonium sulfate	4:1	Spot was not observed
4	n-Butanol-ammonium sulfate-methanol	4:1:1	Spot was not observed
5	n-Butanol-ammonium acetate-methanol	2:0.5:1	Spot was not observed
6	n-Butanol-ammonium acetate-ethanol	2:0.5:1	Spot was not observed
7	n-Butanol-ammonium nitrate-methanol	2:0.5:1	Spot was not observed
8	n-butanol-acetic acid-water	4:1:1	Spot was not observed
9	n-butanol-chloroform-water	2:0.5:1	Spot was not observed
10	Methanol - chloroform - water	2:2.5:0.5	Spot was observed
11	Ethanol-chloroform-water-ammonia	4.5:0.5:2.5:1	Spot was observed
12	Methanol, chloroform, water, ammonia	4.5:0.5:2:1	Spot was observed
13	Chloroform-methanol-ethyl acetate	2:0.5:0.5	Spot was observed
14	Chloroform-butanol-ethyl acetate	2:0.5:0.5	Spot was not observed
15	Chloroform-methanol-ethyl acetate-acetic acid	4:3:0.5:0.5	Spot was observed

Isolating active ingredient by column chromatography

The above operation was repeated for each spot on the TLC sheet and solvent systems. In thin-layer chromatography, if the elevation of the spot was equal to the standard spot, the material was present in the real sample. Galegine is known as a guanidine in the Galega extract. The initial isolation of the extract constituents was performed by column chromatography. The extract (0.1 g) was dissolved in a chloroform solvent (5 mL). Silicagel (1g) was added to convert the gum extract into powder, and its solvent was evaporated thoroughly on a rotary evaporator. The adsorbed extract on silica gel was prepared for loading on the chromatographic column after being completely powdered. The column used in this step was selected in terms of size and size proportional to the amount of sample injected into the column. The height and diameter of the column were 50 and 60 cm, respectively. Silicagel (100 g, 63-230 mesh) was slurried with n-hexane and filled into the column. To prepare the column for loading the extract, it was washed with the n-hexane solvent for 24 hours. During this time, the silica gel particles were pressed gently into the column. In order to further compression of the static phase and removal of the xcess of solvent, the valve of the column was alternately opened (Fig. 1).

The extract was then loaded onto the desired column and the column was washed pure n-hexane non-polar solvent (100 mL). Subsequently, column was eluted in gradient (increasing polarity) by adding ethyl acetate to the mobile phase until the mobile phase polarity was reached to 100%. After this step, in order to increase the polarity, the chloroform and methanol was added to the solvent. Then, the column elution was finished in 100 % polarity. The 100 mL of solvents volume was added at each time and was used at different ratios 9:1 to 1:9. The column outlet fractions were collected in volumes of 15 mL and were analyzed by TLC compared to the Galegine standard solution for all samples. According to TLC data, similar fractions were mixied. The TLC results of the prepared extract showed that Galegine composition was present in Galega under UV light (254 nm).

Fig. 1 Chromatographic column loaded with the extract Galegine from Galega plant

Loading synthesized GO-MIP on chromatographic column outlet and measurement with HPLC (High-performance liquid chromatography) HPLC is an efficient method to measure the components of a material that depends on the correct choice of column type, mobile phase solvent, solvent passage rate, column temperature, and injection

volume. For further investigation, HPLC method was set as follows: column type was C_{18} column (250 × 4.6 mm, 300A, 5mm) which combined with pre-column and elution (mobile phase) was including water and methanol with 80:20 ratio Triethylamine and phosphoric acid were used for adjusting (pH=3) the mobile phase and with a current speed of 1 mL/min, column oven temperature 25 °C, injection volume 20 µL, and with detector UV-Vis spectrophotometer was used at a wavelength of 290 nm. To load the synthesized molecularly imprinted polymer onto the column outlet, GO-MIP (0.1 g) was added to 5 mL of sub-column fraction in which the presence of Galegine was previously determined and was placed in ultrasonic for 20 min and then, was filtered with a filter paper. Concentrations of solutions were measured before and after loading to HPLC.

Loading of Synthesized GO-MIP onto Galega Extract and Measurement with HPLC Method

The position of the Galegine peak was determined in the HPLC spectrum by preparing a standard solution of Galegine and injecting it into the HPLC column. If the extract also has a peak at the same retention time, it indicates the presence of Galegine in the extract, and the sample concentration can be measured based on the peak area. For this purpose, to measure Galegine adsorbed by molecularly template polymer was first added to the plant extract (5 mL) containing Galegine. Then, 0.05 g of synthesized GO-MIP was added and was ultrasonicated (20 min) to load. Then, the mixture was filtered. The sample (20 µl) was injected to the HPLC setup to the comparing concentration of Galegine before and after loading.

Results

Investigating XRD Diffraction Patterns

Figure 2 shows the XRD pattern of synthesized molecularly imprinted polymers with the Galegine pattern.

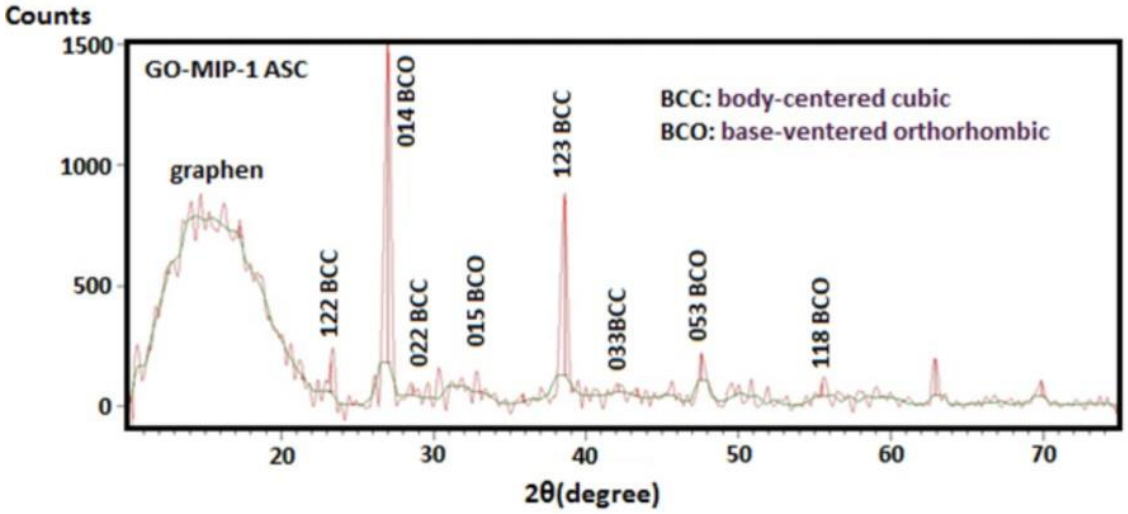


Fig. 2 XRD pattern for the prepared GO-MIP

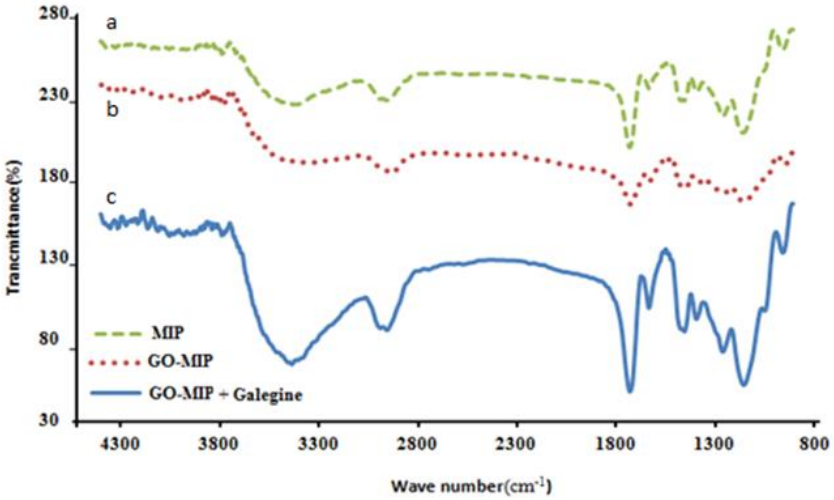
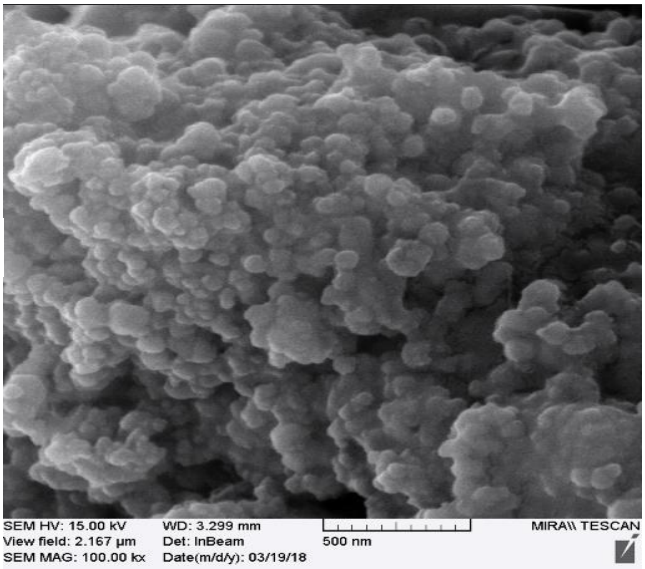


Fig. 3 Comparison of FT-IR spectra of spectra: A) MIP, B) GO-MIP and C) GO-MIP + Galegine polymers

a



b

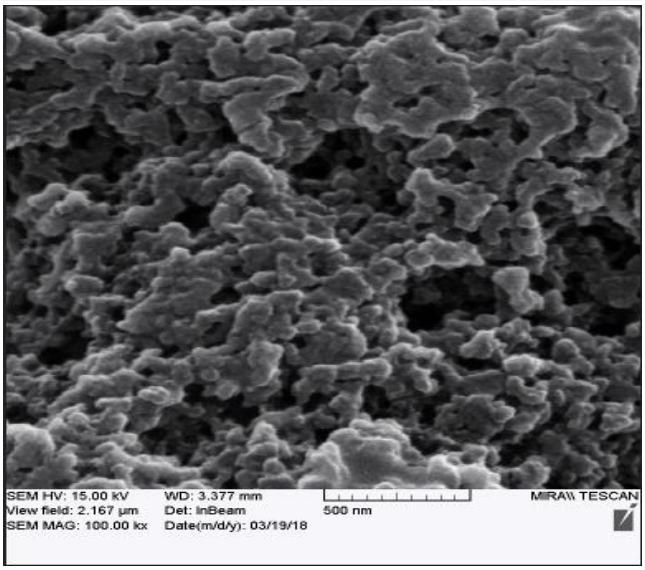


Fig. 4 FESEM images of (A) MIP and (B) modified GO-MIP

The morphology of the prepared molecularly imprinted polymers was investigated using scanning electron microscopy images. As shown in Figure (2), the prepared MIP and GO-MIP had dimensions in the range of 100 nm to 300 nm. According to the images, the formation of more homogeneous particles can be attributed to the presence of graphene oxide particles in GO-MIP. Atomic Force Microscopy (AFM) study According to the obtained AFM images Figure (5), by adding GO nano-layer to MIP, the roughness of the sample was promoted, in the other word, the surface uniformity was decreased for the polymer free GO. In these images, the highlighted areas shown as troughs belong to the areas enriched by MIP and the lighter-colored areas are the GO-containing sections and their placement in the polymer lamellae led to convert surface to discontinuous and agglomerated

Investigating MIP Structure by Field Scanning Electron Microscopy (FE-SEM)

As can be seen, the planes: 122, 022, 123, 033 at angles 2θ =24.66, 28.58, 38.58 and 43.2 for the BCC (body-centered cubic) crystal, while the planes: 133, 015, 014 and 118 at angles 2θ= 27.00, 33.00, 35.80 and 48.01 were related to the BCO (base-centered orthorhombic) crystals of the MIP structure. The broad peak below 2θ =20 was related to graphene oxide. The XRD diffraction peaks of the GO-MIP surface can be observed at an angle of 2θ equal to 24.66, 28.58, 38.58, 27.00, 33.00, 35.80, 48.01 and 55.51 which may indicate the lack of particle aggregation and the proper distribution of GO in the molecularly template polymer.

Fourier-transform Infrared Spectroscopy (FT-IR) study

The synthesized MIP enriched with graphene oxide nanoparticles (GO-MIP) and non-molecularly imprinted polymers (NIP) were studied by FT-IR technique and it was indicated that the graphene oxide nanoparticles were appropriately distributed in the polymer. The FT-IR spectrum in Fig. 3 shows that the appeared peak at about 3435/cm corresponds to the strong stretching vibrations of a hydroxyl group (–OH), which is related to the physical and morphology properties of methacrylic acid [18]. The peak at 1730/cm belongs to the C=O bond, also, vibrational peak related to C-O bonds were appeared at 1150/cm indicating that polymerization was carried out between Methacrylic acid (MAA) and EGDMA. On the other hand, FT-IR spectra of NIP, MIP, and GO-MIP were very similar to each other, which can be concluded that the molecularly imprinted polymer is well developed with the presence of graphene oxide nano-layer and the target molecule did not remain and the washing was well perfomed.

phase and its roughness and non-uniformity was increased with increasing nano-silver. Investigating Thin Layer Chromatography Experiments on Outlet Fractions of Chromatography Column Among the mentioned solvents in Table 2, finally, based on the resolution, speed of movement of the spot in the mobile phase and its resolution on TLC sheet, the solvent system of chloroform, methanol, ethyl acetate and acetic acid (4:3:0.5:0.5) was used as the best solvent system and the mobile phase to separate the Galegine present in the extract (Fig. 6). The calibration function was plotted over a wide range of concentrations. The outlet fractions from chromatography column were aligned with the standard spot (In TLC test) (Fig. 7), indicating the presence of Galegine in the extract (Fig. 8)

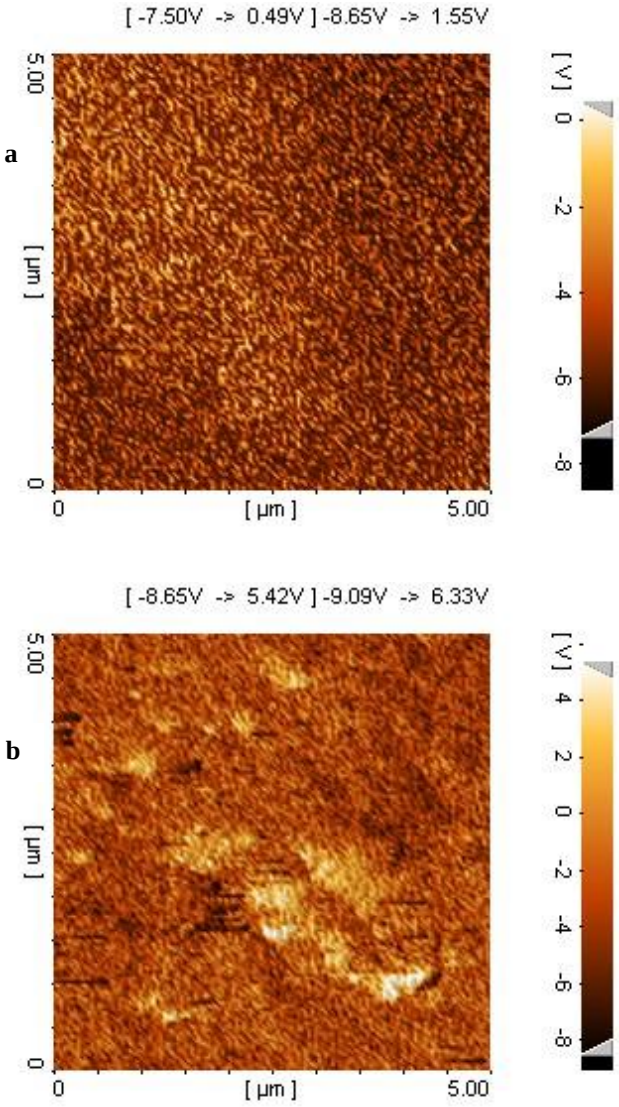


Fig. 5 AFM images: A**)** molecularly imprinted polymer, B**)** molecularly imprinted polymer with graphene oxide.

Calibration Curve and Linear Concentration Range

To achieve the linear curve in the calibration technique, the comparison between peaks area changes and the amount of analyte on the chromatogram plate is shown in Figure 9. To achieve the calibration function and linear concentration range, solutions with concentrations of 5, 15, 25, 50 and 150 mg/L was prepared from the standard metformin solution (metformin was used according to the literature review and structure similarity with Galegine) and was injected into the HPLC apparatus (Fig. 10).

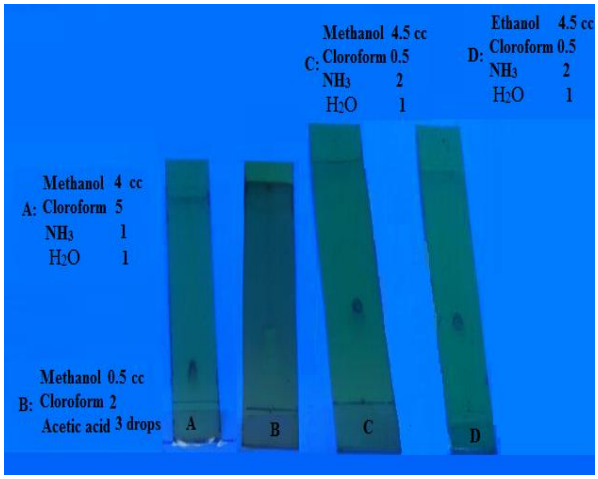


Fig. 6 Samples of standard TLC with different solvent systems

The values of the peak area according to different standard concentrations are presented in Table 3. The high value of regression coefficient (R^2=0.9994) indicates the linearity of the relationship between the apparatus response and the concentration of Galegine in the concentration range 5-150 mg/L. In all experiments, double-distilled water was filtered using 0.45 µm filter paper for the preparation of the mobile phase, and it was perged via the vacum. The mobile phase velocity was adjusted to 1 mL/min. In all experiments, a sample of 20 µm was injected into the HPLC apparatus.

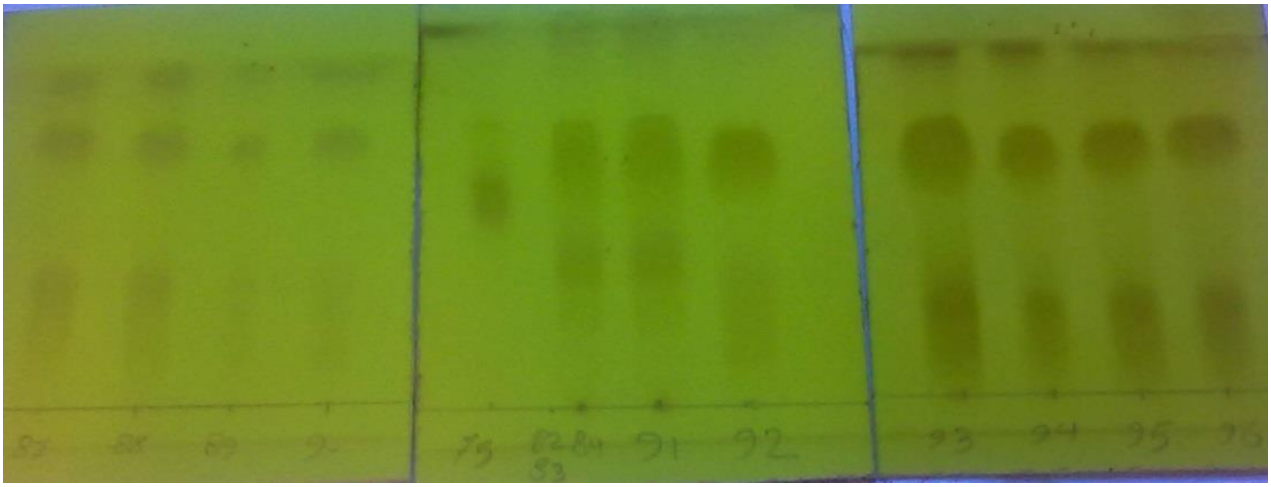


Fig. 7 Investigating the effect of solvent system on chromatographic column outlet

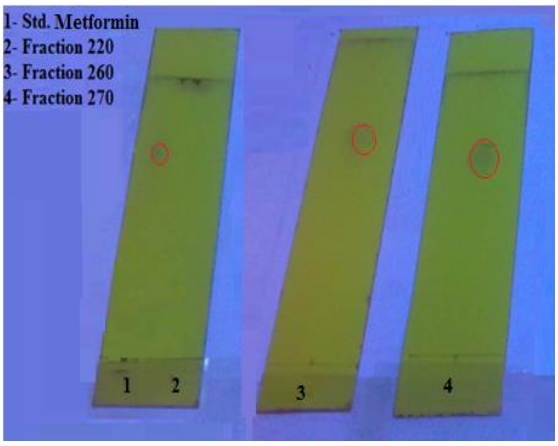
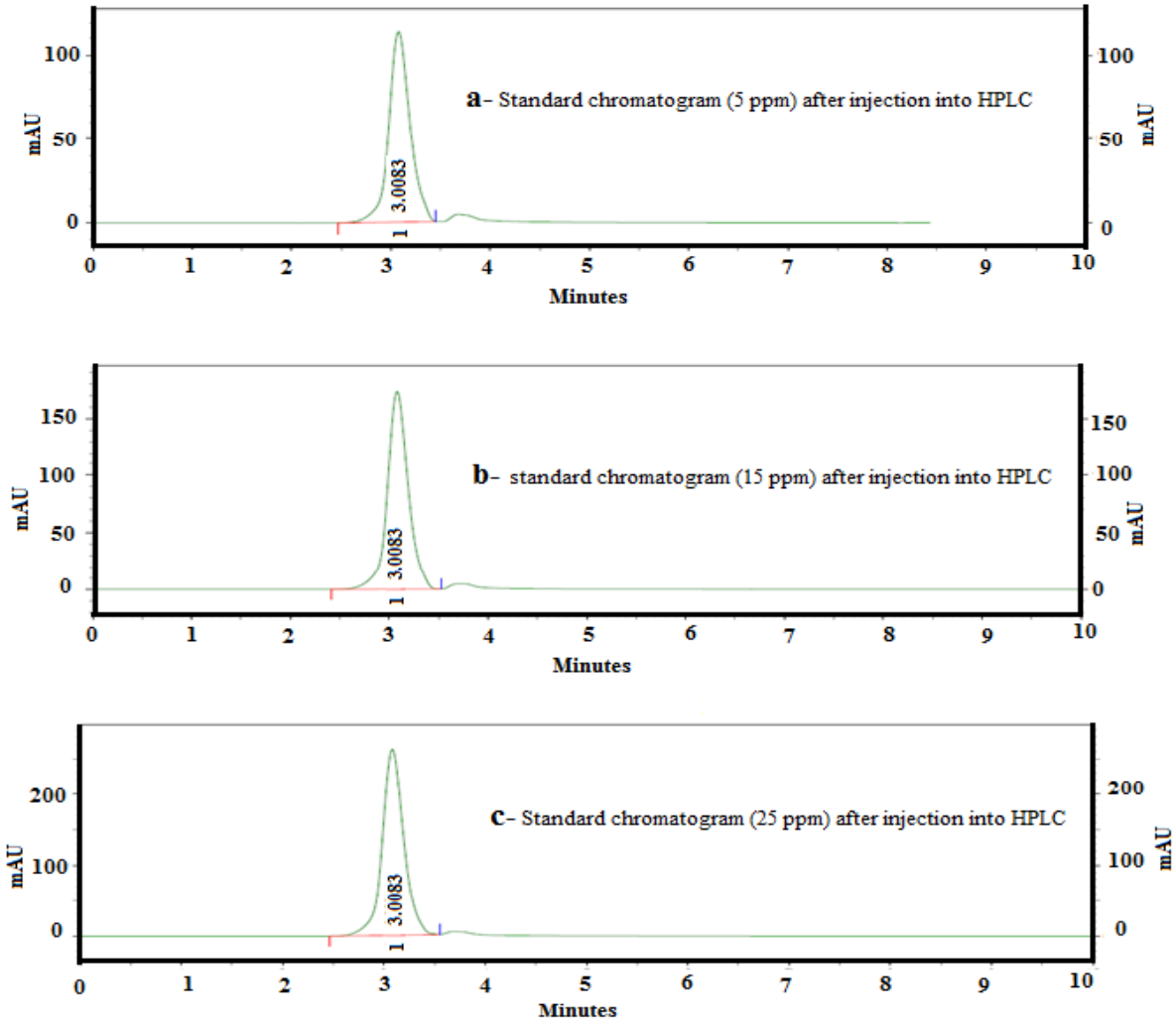


Fig. 8 TLC comparison of standard and column outlet fractions

Determining Adsorbance of GO-MIP on Galega Extract by HPLC

The GO-MIP polymer based was used as an adsorbent to extract the Galegine from the alcoholic extract of Galega. synthesized molecularly imprinted polymer (0.1 g) was loaded to the extract solution (10 mL) After that, it was placed in an ultrasonic for 20 min and then, it was filtered with a Whatman filter paper No. 40. Concentrations of solutions were measured before and after loading to the HPLC (Fig. 1).



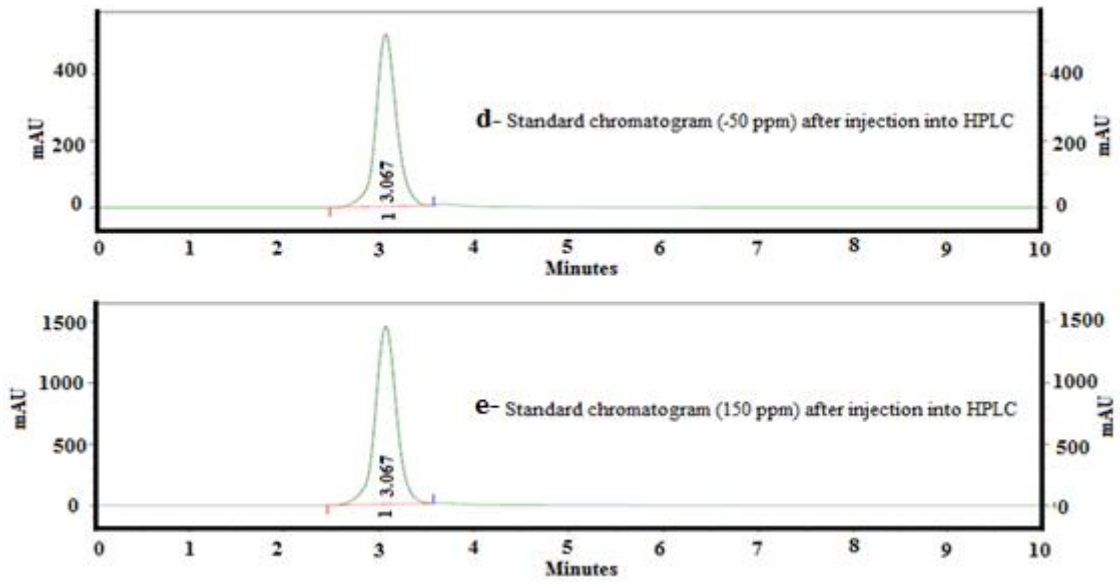


Fig. 9 Standard chromatogram: a- (5 ppm), b-(15ppm), c-(25ppm), d-(50ppm) and e-(150ppm) after injection into HPLC

Table 3 The peak area of the standard samples

Concentration (mg/l)	Peak area
5	1615175
15	2701270
25	4177924
50	8046751
150	23751129

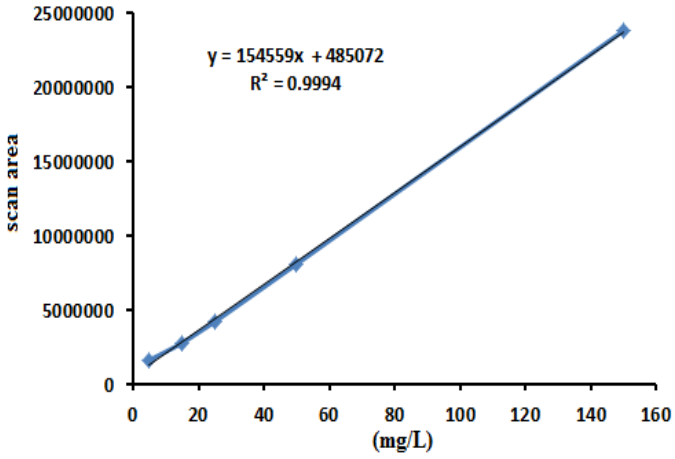


Fig. 10 Calibration curve based on a standard absorbance and concentration

Quantifying of the Adsorbed Galegine on the GO-MIP Using HPLC Technique

Another aim of this study was to use chromatographic column to isolate the raw extracted from plant and determine the adsorbed amount of Galegine (from outlet fraction of column) on the GO-MIP. Therfore, the synthesized molecularly imprinted polymer was loaded to the column outlet containing Galegine. Then, it was filtered through a filter paper. Concentrations of solutions were measured before and after injection to the HPLC (Fig. 12 a, b) and (Table 4).

Table 4 The loading results of synthesized polymer on plant extract and chromatographic column outlet

	Solution	(Mau**)** Peak area
Plant extract	Solution before loading GO-MIP	18212451
	Solution before loading GO-MIP	2910156
Column outlet	Solution before loading GO-MIP	299484
	Solution before loading GO-MIP	204927

The best results of this method are achieved when the column was combined with this polymer.

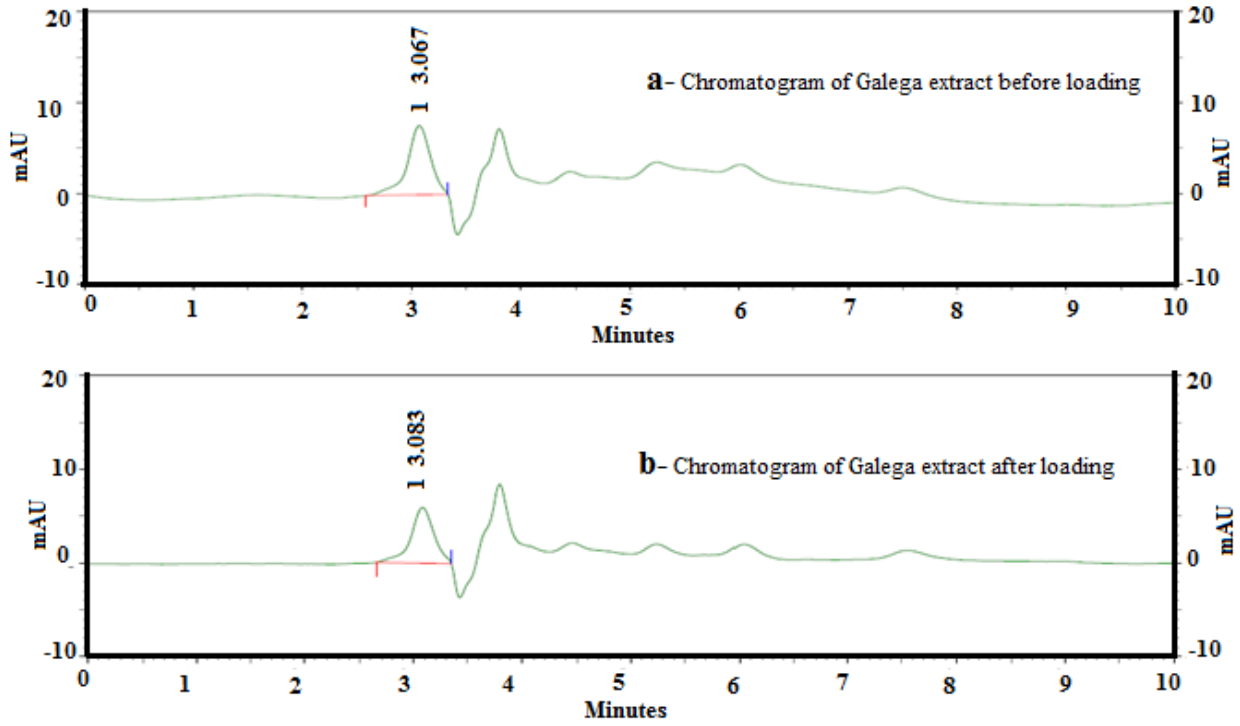


Fig. 11 Chromatogram of Galega extract: a- before and b- after loading

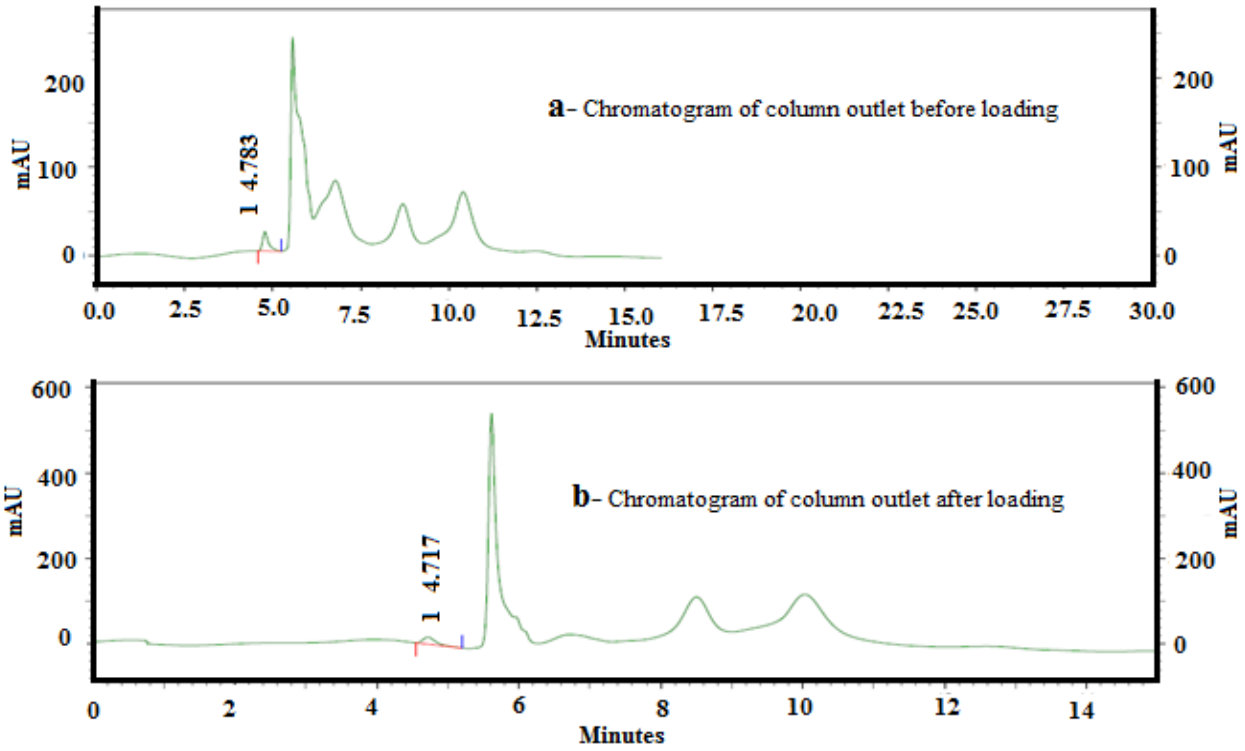


Fig. 12 Chromatogram of column outlet: a- before and b- after loading

Discussion

Solid phase extraction-based molecularly imprinted polymers are one of the most sensitive, selective methods of molecularly separation. Despite the known composition of Galegine reported in this paper by scientific studies, as far as our best knowledge, it was found out that there is no report in this approach (Galegine by combined column chromatography and GO-MIP). In this study, Galegine was subjected to molecularly ionization by preparative methods. The extracted Galegine from the extract of Galega plant using carbon nanotube-reinforced molecularly imprinted polymer was finally assayed by HPLC. As shown in

Figure 16, GO-MIP has been able to purify and extract Galega extract. In Figure 11-a, the hydroalcoholic extract of Galega was injected into the chromatograph and it was removed at retention time about 3.06 min. To prove this, the standard solution of metformin was injected into the chromatographic apparatus at the same concentrations and the same conditions and only one chromatogram peak was emerged at the retention time (Figs. 9-a-b-c-d-e). Fig. 11-b shows the chromatogram obtained from injection of the extracted Galega solution by molecularly imprinted solid phase shows selective adsorbent attitude for the Galegine. The importance of this method in the purification has been widely welcomed as main reason in the pharmaceutical and food industries. Regarding, Galega is currently used as a blood sugar lowering drug and on the other hand, the usual extraction methods are often based on solvent extraction, it seems that this new method may give a favorable perspective to the related consumer industries of this product. Noteworthy, it was found out that the used method was assisted to the green chemistry due to the lack of organic solvents and less environmentally polluting than other competing methods. Therefore, the extractor developed in this study based on the solid phase and molecularly imprnted technique enriched with carbon nanotubes has the appropriate potential to purify Galegine as a glucose-lowering drug in the Galega medicinal plant. Based on the comparing peak area of the Extracted galegine by GO-MIP and standard injected solutions to the HPLC, the concentration of extracted Galegine was calculated 193 µg/g. On the other hand, the obtained amount of Galegine absorbed by GO-MIP was 330 µg/g. Interestingly, the results show that the column can increase the efficiency of the MIP technique up to 17 times in addition to reducing the annoying compounds. Given that Galega is currently used as a blood sugar lowering drug and the usual extraction methods are often based on solvent extraction, it seems that this new method may give a new perspective to the related consumer industries of this product. Benn *et al*. (1996) isolated two types of alkaloids from Galega plant using alumina column and chloroform solvents [19]. They have shown that through various experiments, the amount of alkaloid is very low (about 0.2%) in the leaf of this type of Galega and this amount can not be risky to livestock. In 2003, Atanasov *et al*. [20] separated the active ingredient (2.5 g) from 1000 g of lyophilized aqueous extract of Galega plant. The fraction of aqueous extract of *G. officinalis* L was isolated by chromatography column. The Sephadex column was used for this experiment. Also, in 2015 Hande *et al*. [21] have reported the extract diphenyl amine by combining molecularly imprinted polymers and Flash column chromatography. Flash chromateography is a type of rapid chromatography in which the solvent is pumped at flow velocity. This results in many separations to take selectively Galegine among of other compounds in place in less than 20 minutes. Davoodi *et al*. (2016) have studied the extraction conditions of Galegine from the Galega plant in shorter time than the other methods [22].

Conclusion

The current study was conducted to optimize the new process of Galegine extraction from Galega plant as a very valuable compound in modern pharmaceutical industry based on column chromatography separation and molecularly imprinted polymer purification. The solid phase extraction through molecularly imprinted polymer technique was used in Galegine extraction for the first time and no scientific report has been published to date. This study showed that the extraction and preconcentration of Galegine through molecularly imprinted polymer-based solid phase extraction is easily possible. The results also showed that the preparation of sample with column chromatography yields better results amount of the absorbed Galegine by GO-MIP was 330 µg/gfrom the felesased fraction of column albeit, the isolated amount of Galegine by GO-MIP was 193 µg/gfrom the raw extracted. Therefore, the combination of GO-MIP with column chromatography can be directly used for extraction and separation of Galegine from the plant extract. The results of the proposed method in this study from Galega extract showed that the obtained amount of Galegine was comparable to expected amount in the plant and reported in scientific reports. This method can be suggested to use for many compounds of herbal products by further developement and improvement of this method.

Conflict of Interests

The authors have not declared any conflict of interests.

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