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Montmorillonite-based polymethacrylate composite monoliths as stationary phase materials for food and pharmaceutical analysis in capillary liquid and gas chromatography

Ahmad Aqel*, Ayman A. Ghfar, Kareem Yusuf, Khalid M. Alotaibi, Rayed M. Alafra'a, Mohamed A. Habila, Ahmed-Yacine Badjah-Hadj-Ahmed, Zeid A. ALOthman

Department of Chemistry, College of Science, King Saud University, Riyadh, 11451, Kingdom of Saudi Arabia

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ABSTRACT

This work relates to the preparation of novel and promising stationary phases containing inorganic-organic composites for capillary liquid and gas chromatography. A naturally occurring montmorillonite was introduced to polymethacrylate monoliths, then used under different conditions of GC and HPLC at the same time. The performance of the columns was evaluated for the separation of alkane and alkylbenzene series in GC and capillary HPLC, respectively. While the bare monoliths failed to separate the model analytes, montmorillonite-based polymethacrylate allowed a full separation of the mixtures with $R_s \geq 1.42$. The columns were applied for the determination of myrcene and limonene isomers in the peel extracts of some fruits using GC, and for the analysis of active ingredients including aspirin, vitamin-C, caffeine, and ibuprofen extracted from common drugs using capillary HPLC. In GC, fast separation was achieved in 1.0 min with R_s of 6.53. The columns exhibited the best efficiency for myrcene with 20,900 plates/m. Using the capillary HPLC columns, the active ingredients were resolved in 10 min with $R_s \geq 5.72$. The efficiency values located between 12,800–21,700 plates/m in all cases. The developed methods were found to be linear in the range of 0.10–10.0 and 0.20–180 μg/mL for GC and HPLC, respectively. In comparison with commercial columns, the results in GC methods reveal that, despite their much shorter length, the prepared columns proved a faster separation with higher efficiency and comparable detection limits and chromatographic resolution. The prepared HPLC capillaries exposed lower run times and detection limits with comparable efficiency and resolution, and consume fewer samples and mobile phase solvents. The results demonstrate that the montmorillonite-based polymethacrylate composites are applicable as stationary phases for routine analysis and quality control of important fields such as food and pharmaceutical samples.

1. Introduction

Separation and chromatographic techniques have developed in various divisions, and now, gas chromatography (GC) and high-performance liquid chromatography (HPLC) are the most attractive separation and analysis systems [1,2]. These techniques are widely used in the purification, isolation, and analysis of a wide range of chemicals not only restricted to food and pharmaceutical compounds [1–3]. However, many scientific fields are in continuous development and becoming more sophisticated, and facing new challenges. Therefore, the requirement to establish powerful separation methods and the improvement of the efficiency and the sensitivity of present techniques have increased accordingly to analyze more substances in sophisticated environments, taking into account the need to decrease the analysis costs, time, and waste.

In GC and HPLC techniques, the separation of the samples is taking place through columns containing suitable stationary phase materials. The development of productive GC and HPLC methods is very closely related to the preparation of powerful separation columns, which depend mainly on the development of high-performance stationary phases. The physical, chemical, and mechanical properties of the stationary phases are very important for successful separation and analysis processes. One of the key ad-

* Corresponding author: Ahmad Aqel, P.O. Box 2455 Riyadh, 11451, Saudi Arabia, Tel.: +966 11 4676009, Fax: +96614675992.

E-mail addresses: aifseisi@ksu.edu.sa (A. Aqel), aghafr@ksu.edu.sa (A.A. Ghfar), kmahmoud@ksu.edu.sa (K. Yusuf), khalid.m@ksu.edu.sa (K.M. Alotaibi), 437106628@student.ksu.edu.sa (R.M. Alafra'a), mhabila@ksu.edu.sa (M.A. Habila), ybadjah@ksu.edu.sa (A.-Y. Badjah-Hadj-Ahmed), zaothman@ksu.edu.sa (Z.A. ALOthman).

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vantages is that these techniques are extremely flexible and can be adapted to fulfill the analytical needs in the development process stages. Nevertheless, understanding modern column trends is very important since column technologies continue to evolve rapidly, resulting in new products with higher performance and more consistency [4,5].

This work is related to the synthesis of novel and promising stationary phases that could be used as column packing materials for both GC and HPLC applications at the same time. Monoliths represent a relatively recent and innovative type of separation media for rapid chromatographic analysis. The concept of monolithic columns has been identified as an alternative to particle-packed columns [6]. Monolithic materials are formed from a block of continuous single-piece materials made of porous rods with different sizes of pore structures, giving them good permeability and porosity, especially for medium to large range-sized compounds, typically biological samples such as peptides, proteins, and glycoproteins, as well as synthetic polymers and microorganisms [7–11]. According to the literature, several attempts were made to examine the performance of different monolithic materials for separation. In general, there are two main classes of monolith supports that have been developed for chromatographic applications; organic-based polymer monolithic columns produced by a simple molding process, and inorganic-based silica monolithic columns made by the sol-gel approach [12,13]. However, a hybrid structure consisting of both types (organic-inorganic) has been also reported by various methodologies since 2010 [14,15].

Monolithic phases are essentially synthesized from organic monomers such as styrene, acrylamide, acrylate, and methacrylate derivatives, or silica-based inorganic monoliths [16–20]. Practically, a polymerization mixture consisting of monomers and other additives can easily be introduced into empty columns. The monolithic structure is then formed *in-situ* using a thermal or another polymerization process [6–8], and then can be applied as the stationary phase. The typical organic or polymer-based monolithic structure consists of interconnected non-porous micro-globules and a lake of small mesopores that comes at the cost of surface area, which then reduces the number of sufficient interaction sites in the separation media and thus reducing the chance of retention and separation of small compounds [7–22]. However, several scenarios have been proposed to improve the adsorptive performance and enhance the retention properties of the bare monoliths [7–22].

In this work, we suggest the naturally occurring clay minerals, particularly montmorillonite (Mnt) as embedded material for the first time to improve the separation efficiency of the final stationary phase. Because of their considerable availability and versatility in nature, low cost, excellent thermal and mechanical strength, good solvents resistance, wide pH applicability, high specific surface area, low toxicity, and ease of modification, clay minerals such as kaolinite, chlorite, illite, and Mnt have been proved to have a wide variety of applications [23,24]. Mnt is one of the most inspected clay minerals, in which their particles are formed by stacking mineral layers. Silica is the dominant constituent of the Mnt clays, with alumina being essential. The chemical structure of Mnt is characterized by its sheet structure, every clay sheet consists of three layers with 9.6 Å thickness containing two tetrahedral silicate layers and one octahedral alumina layer [25,26].

The lack of sufficient interaction sites in the bare polymer monolithic structures may be overcome through the incorporation or introduction of small amounts of clay particles into the porous structure of the monolith channels. This hybrid structure is expected to have intermediate properties of organic and inorganic media based on the structure of the used polymer monolith and the incorporated material. The amazing physical, chemical, and mechanical properties of the natural clay minerals and organic polymers such as the high thermal and mechanical stability, sufficient surface area, and fast mass transfer between phases make them strong candidates to enter the world of chromatography as stationary phases for both GC and HPLC techniques.

2. Materials and methods

2.1. Chemicals and reagents

Mnt K 10 grade was acquired from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Glycidyl methacrylate, ethylene dimethacrylate, azobisisobutyronitrile, 3-(trimethoxysilyl)propyl methacrylate, aspirin, and ibuprofen were supplied from Sigma-Aldrich (St. Louis, MO, USA). Sodium hydroxide, hydrochloric acid, formic acid, acetic acid, alkane and alkylbenzene series, myrcene, limonene, caffeine, and vitamin-C used in this work were provided from Acros Organics (Morris County, NJ, USA) and BDH (Lutterworth, UK). HPLC grade solvents; methanol, acetonitrile, and ethanol were supplied from Fisher Scientific (Leicestershire, UK). Millipore system (Milli-Q, Millipore S.A.S. 67,120 Molsheim, France) was used for the preparation of Ultrapure water. Before use, the prepared water was always filtered with 0.22-μm nylon membrane filters from Whatman (Maidstone, UK).

2.2. Capillary columns preparation

Empty untreated fused silica capillary tubings with 0.25 mm i.d. were purchased from Molex, Polymicro Technologies™ (Lisle, IL, USA). First, the capillaries were rinsed with ethanol and then washed with water. The capillaries were then flushed with a 0.20 mol/L NaOH solution for 1 h, then washed with water and dried in a stream of N_2 for 5 min. The capillaries were then flushed with a 0.20 mol/L HCl for 1 h and washed with water and dried in a stream of N_2 for 5 min. Then, the capillary tubings were rinsed with absolute ethanol for 5 min and flushed with 30% 3-(trimethoxysilyl)propyl methacrylate solution in ethanol for 1 h and stand with the same solution overnight, then rinsed with absolute ethanol for 5 min and dried with a stream of N_2 for 5 min. The activated tubings were then cut with a capillary cutter into about 32 cm long for GC columns and 22 cm long for HPLC columns. In total, 24 columns were prepared with different monomeric mixture compositions and Mnt contents; 12 columns for each GC and HPLC application, as described in Table 1.

The monomeric mixture compositions were optimized by varying the weight percentages of the monomer, crosslinker, and porogenic solvents. In all cases, 1% of azobisisobutyronitrile initiator, with respect to monomers, was added to the mixture. For GC applications, 14% glycidyl methacrylate, 8% ethylene dimethacrylate, and 77% porogenic solvents (propanol/1,4-butandiol; 50/50, v/v) were used. On the other hand, the composition of the mixture by weight percentages used for columns prepared for HPLC columns was 24% glycidyl methacrylate, 16% ethylene dimethacrylate, and 59% porogenic solvents (propanol/1,4-butandiol; 50/50, v/v). Different contents of Mnt particles ranging from 1.0 to 5.0 mg/mL were added to the monomeric mixtures. The same procedure was used for the preparation of the control columns without incorporating Mnt.

The monomeric mixtures were mixed into stable homogenous solutions with vortex, then sonicated and purged with N_2 for 5 min. The capillary columns were filled with the monomeric mixtures and both ends were plugged with pieces of GC septa. The polymerization was performed in the oven at 70 °C for 15 h. Prior to chromatographic evaluations, the prepared columns were washed with HPLC grade acetonitrile and water for 2 h. Then, 1.0 cm was cut from both column ends to get a total length of 30 cm for GC and 20 cm for HPLC columns.

Table 1
Composition of the polymerization mixtures by weight percentages used for preparation of the capillary columns. Azobisisobutyronitrile initiator has fixed at 1% (with respect to monomers in all cases) and the porogenic solvent comprises a mixture of 1,4-butandiol/1-propanol (50:50, v/v%) in all cases.

Column	Glycidyl methacrylate	Ethylene dimethacrylate	Porogenic solvent	Mnt (mg/mL)	
C_{G1}	14	6	79	0	GC columns
C_{G2}	14	8	77	0	
C_{G3}	14	10	75	0	
C_{G4}	14	12	73	0	
C_{G5}	12	8	79	0	
C_{G6}	16	8	75	0	
C_{G7}	18	8	73	0	
C_{G8}	14	8	77	1	
C_{G9}	14	8	77	2	
C_{G10}	14	8	77	3	
C_{G11}	14	8	77	4	
C_{G12}	14	8	77	5	
C_{L1}	24	14	61	0	HPLC columns
C_{L2}	24	16	59	0	
C_{L3}	24	18	57	0	
C_{L4}	24	20	55	0	
C_{L5}	20	16	63	0	
C_{L6}	22	16	61	0	
C_{L7}	26	16	57	0	
C_{L8}	24	16	59	1	
C_{L9}	24	16	59	2	
C_{L10}	24	16	59	3	
C_{L11}	24	16	59	4	
C_{L12}	24	16	59	5	

2.3. Characterization of the stationary phases

The prepared materials were evaluated by thermogravimetric analysis (TGA), scanning electron microscope (SEM), specific surface area, and Fourier transform infrared spectroscopy (FT-IR). The thermal stability of the synthesized materials was measured using TGA with a Mettler-Toledo TGA/DSC Stare system (Schwerzenbach, Switzerland). The samples were heated from 25 °C to 500 °C at a 10 °C/min ramp rate. The microscopic morphology of the prepared materials was acquired using a Jeol JSM-6380LA (Tokyo, Japan) analytical SEM at 20 kV. The Brunauer-Emmett-Teller (BET) technique was used to estimate the surface area of the synthesized monoliths using a Gemini VII 2390 Micromeritics surface area analyzer (Norcross, GA, USA) at 77 K. The materials were ground and degassed at 150 °C prior to the measurements. The FT-IR spectra of the synthesized stationary phases were obtained on a Thermo Nicolet 6700 FT-IR spectrophotometer (Madison, WI, USA).

2.4. Columns evaluation

The performance of the prepared columns was examined by GC and capillary HPLC. All GC analyses were performed using a Thermo Scientific - Trace GC Ultra system (Waltham, MA, USA). On the other side, the performance of the prepared columns was investigated in the capillary scale HPLC system. The analyses were carried out using Dionex Ultimate 3000 RSLC nanoLC (Sunnyvale, CA, USA) equipped with a 45 nL wavelength detection cell. Mixtures of standard alkane series; pentane, hexane, heptane, octane, and nonane and alkylbenzenes; toluene, ethylbenzene, n-propylbenzene, n-butylbenzene, n-pentylbenzene, and hexylbenzene were injected under various chromatographic conditions to test the performance of the prepared columns and the effect of Mnt incorporation.

The stability of the prepared column was assessed against different eluents, flow rate, and temperature. For this purpose, helium and acetonitrile were used in GC and HPLC, respectively, to estimate the pressure drop across the prepared columns at different flow rates and temperatures. While helium and acetonitrile passed through the columns, the permeability values were evaluated and measured according to Darcy's equation [27,28]. The porosity of the prepared columns was determined in a nanoLC system by injecting a small plug of acetonitrile. The first repeatable disturbance of the baseline was observed and used as the unretained marker.

2.5. Chromatography applications

The applicability of the prepared columns was evaluated for the analysis of real samples. For this purpose, GC columns were applied to the determination of myrcene and limonene in the peel extracts of some fruits including lemon, orange, grapefruit, and clementine. To determine the concentration of myrcene and limonene, 50 g of each fruit peel was dried in the air and blended into fine powders. The powders were subjected to Soxhlet extraction for 1 h using HPLC grade n-hexane at about 60 °C. The extracts were concentrated and stored in the refrigerator at 4 °C for GC analysis. The separation conditions were optimized in terms of the carrier gas flow rate, oven temperature, injection volume in split/splitless modes, and injector and detector temperatures. At the optimal conditions, the GC separation of myrcene and limonene was carried out in isothermal mode at 160 °C with a constant He flow of 1.0 mL/min. The injection port was held at 200 °C with the split mode at a ratio of 1:5. The FID temperature was also fixed at 200 °C and fed with 300:30 mL/min air/hydrogen gasses.

On the other hand, HPLC columns were subjected to the determination of the active ingredients extracted from two common drugs; Aspirin-C tablets labeled 400 mg aspirin and 240 mg vitamin-C (Bayer pharmaceutical company, Aktiengesellschaft AG, Germany), and Profinal-XP tablets labeled 400 mg ibuprofen and 65 mg caffeine (Julphar, Gulf Pharmaceutical Industries, Ras Al Khaimah, UAE). To estimate the concentration of each drug in the commercial tablets, three tablets of each drug were accurately weighed, ground, and extracted with the same composition as the mobile phase. The extracted solutions were filtered through a 0.45-µm nylon membrane, diluted, and 20 nL of the extracts along with the standard solutions were injected into the nanoLC system. All chromatographic conditions were validated in terms of the mo-

bile phase solvents and flow rate, column temperature, injection volume, and detection wavelength. At the optimal conditions, the separation of the active ingredients extracted from drugs was accomplished using a mixture of mobile phase composed of water/methanol (65:35, v/v) with 0.1% formic acid at a 24 μL/min flow rate and a detection wavelength of 230 nm. All real samples; fruits and drug tablets, were provided from local a market in Riyadh, KSA.

2.6. Validation

All of the analytical parameters were validated in agreement with the criteria as per ICH guidelines [29]. The detection and quantitation limits were measured regarding the concentrations of the analytes that yielded S/N of 3:1 and 10:1, respectively. The peak areas were plotted versus the concentrations of the analytes to study the linearity of the method. The linearity limits were assessed by the linear regression analysis of the peak areas versus the respective concentrations of the analytes employing the least square method. All standard and real sample mixtures were filtered through 0.45-μm syringe filters and injected in three replicates for each solution. The recovery tests were performed by spiking method at a medium concentration level in triplicate. The recovery percentages were measured by means of the achieved linear regression slope between the found and added amounts of each analyte.

3. Results and discussion

3.1. Preparation capillary columns

In order to prepare high-performance chromatographic columns, various general and specific properties should be existing in the stationary phase. In this work, Mnt as a natural clay mineral was added to polymer monoliths to prepare new and innovative stationary phases for both GC and HPLC applications at the same time. For the synthesis of these composites, glycidyl methacrylate and ethylene dimethacrylate were selected as common monomers and crosslinkers. So, we and many other authors can easily track the changes in the morphology and porosity of monoliths and expect the influence of embedding Mnt in the polymerization mixture on the separation performance and selectivity of various applications. In this composite, Mnt act as a filler to the polymer monolith matrix that gives the bulk and body which holds the material together.

The properties of Mnt particles are expected to determine the internal structure of the final stationary phase and change its physical, chemical, and even mechanical properties. Mnt is a phyllosilicate mineral formed by stacking mineral layers. The chemical structure of Mnt is characterized by its sheet structure containing a tetrahedral silicate layer and an octahedral alumina layer [25,26]. Therefore, bare Mnt is intrinsically hydrophilic, then, the presence of its particles in the polymer monolith matrix is expected to enhance the retention of the moderately polar and high polar compounds.

Naturally occurring Mnt are aggregated to form a wide range of particle diameters; usually from millimeter to micrometer-scale particles. However, uniform particle size and a homogenous distribution are preferred to prepare highly efficient separation columns. For this reason, a sonic sifter separator was used to sieve the Mnt particles in the range of 5 to 10 µm. Then, the Mnt powders were dried at 100 °C for 1 hour to remove any moisture from their interlayers. To maintain a stable monomeric mixture inside the capillary columns, the resulting clay particles were mixed very well with the monomeric mixtures by vertex and sonication. Under the typical conditions, a stable suspension with no Mnt precipitates was observed for about one hour as shown in Fig. 1(A) corresponding to 3.0 mg/mL Mnt. The uniform mixture is then filled in the previously activated capillary columns as mentioned in the experimental section, and the polymerization process was accomplished in the oven at 70 °C for 15 h. The polymerization reactions were also carried out in a 1.5 mL vial under the same conditions. Fig. S1 (Supporting Information) reveals that there was no sedimentation of montmorillonite settled down the vial after the polymerization process. The same procedure was carried out to prepare the control columns, the columns consisting of the same monomeric mixtures but without incorporated Mnt particles.

3.2. Characterization of the columns

The characteristics of the prepared composites including their chemical and pore structure, stability, and surface properties are critical considerations regarding chromatographic conditions. The thermal stability of the separation phase is an essential prerequisite for GC columns. The TGA method was used to investigate the thermal stability of the control and composite monoliths corresponding to 3.0 mg/mL Mnt particles. The typical TGA plots illustrated in Fig. 1(B) exhibit that the degradation starts at about 185 °C for the bare monolith. On the other side, the Mnt-based glycidyl methacrylate-co-ethylene dimethacrylate composite exhibits better thermal stability and did not reveal any remarkable degradation below 250 °C. Moreover, the rate of the composite degradation is lower than the degradation rate of the control monolith due to the high thermal stability of the Mnt. These values of thermal stability enable higher operating temperatures for the prepared columns.

The morphology and pore properties of the synthesized monoliths were examined by SEM micrographs. The bulk region SEM image of the control column presented in Fig. 1 (C, left), demonstrates that the preparation of the monolith inside the column renders a permeable medium with a uniform structure. The bulk region SEM image of the Mnt monolith composite (Fig. 1 (C, right)) corresponding to 3.0 mg/mL Mnt exposed a significant change in the stationary phase morphology. Mnt particles are distributed on the polymer monolith to form a rough surface in the Mnt monolith composite column. The distribution of the Mnt on the bulk surface of the polymer monolith through pores led to reducing the average pore size of the resulting stationary phase.

The specific surface area of the control and composite monoliths was determined using BET and Langmuir methods. Based on the BET method, the specific surface area of the control and composite monolith corresponding to 3.0 mg/mL Mnt were 17.03 and 52.44 m^2/g, respectively. The addition of 3.0 mg/mL Mnt into the monomeric mixture increased the Langmuir surface area of the bare monolith from 31.95 to 82.26 m^2/g. These results confirmed that the embedding of very small amounts of Mnt into the monolithic material induced significant improvement in the specific surface area, which contributes to introducing more active sites to enhance the retention properties of the stationary phase.

FT-IR spectra of the polymethacrylate monolith, pure Mnt, and Mnt monolith composites have been investigated and provided respectively in the Supporting Information Figs S2 (A, B, & C). The three spectra confirm the presence of the characteristic frequencies of the control monolith and Mnt. Fig. S2 (A) shows the main absorption bands of the glycidyl methacrylate-co-ethylene dimethacrylate at 1144.96 cm^{-1}: ester C–O stretching, 1254.72 cm^{-1}: aromatic C–H bending, 1387.65 cm^{-1}: CH_3 symmetrical bending, 1455.12 cm^{-1}: aromatic C = C stretching, 1724.54 cm^{-1}: ester C = O stretching, and 2923.86 cm^{-1}: CH_3 asymmetric stretching. Fig. S2 (B) presents the primary frequencies of Mnt at 468.20 cm^{-1}: Si–O–Si bending vibrations, 525.01 cm^{-1}: Si–O–Al

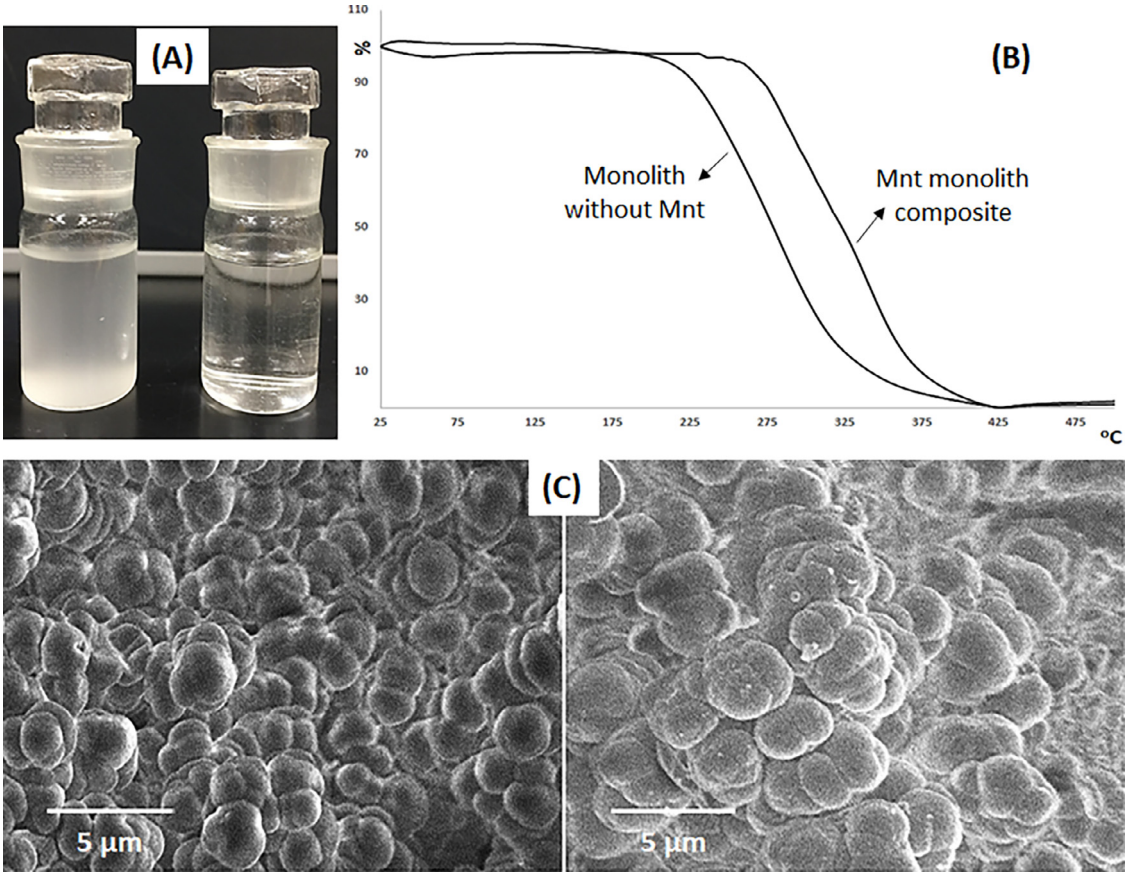

Fig. 1. (A) Porogenic solvents with 1.0 mg/mL Mnt nanoparticles (left) and without Mnt nanoparticles (right). (B) TGA behavior of the glycidyl methacrylate-co-ethylene dimethacrylate monolithic polymer before or after incorporation of Mnt. The samples were heated from 25 to 500 °C with a heating rate of 10 °C/min. (C) Bulk region SEM image of the monolith stationary phase without Mnt (left) and with Mnt nanoparticles (right).

(octahedral Al) group, 798.09 cm^{-1}: Si–O stretching of quartz and silica, 922.13 cm^{-1}: OH deformation of Al–Al–OH structural moiety, and 1048.83 cm^{-1}: Si–O stretching. Finally, Fig. S2 (C) clearly indicates the presence of Mnt in the final structure of the composite material corresponding to 3.0 mg/mL Mnt. The results also reveal that the signal strength of the Si–O groups becomes broader and more significant with an increase in the Mnt content from 1.0 to 5.0 mg/mL in the composite.

The stability of the prepared columns was also studied against different chromatographic conditions. For GC columns, Fig. 2(A) shows the relation between helium flow rate as a carrier gas and pressure drop at 180 °C isotherms for the control column C_{G2} and composite column C_{G10}. For HPLC columns, Fig. 2(B) displays the relation between acetonitrile and water flow rate as eluents and pressure drop at 30 °C for the control column C_{L2} and composite column C_{L10}. The columns prepared with Mnt clearly exposed higher backpressure in both GC and HPLC experiments. The composite columns prepared with Mnt exhibited backpressure values in the ranges of 80–993 kPa (12–144 psi) for GC columns and 340–19,760 kPa (49–2866 psi) for HPLC columns. As shown in Figs. 2 (A & B), the backpressures of the columns increased linearly over the studied flow rates; helium flow rates in GC: 0.23–2.31 mL/min, and acetonitrile and water flow rate in capillary HPLC: 1.0–50 μL/min. The elution of water through the columns exhibits higher backpressure values than acetonitrile because of its higher viscosity. The linear dependence between the eluents flow rates and the backpressure of the columns with correlation coefficient values between 0.9993 and 0.9998 indicates excellent permeability and mechanical stability of the prepared columns. As a whole, the measured backpressure values for the prepared columns are reasonable and suitable to extend their lifetime.

The permeability and total porosity of the prepared columns were also determined. For GC columns, these values were measured at 100 °C isothermal mode temperature and 0.930 mL/min helium flow rate. The values were measured for HPLC columns at 20 μL/min acetonitrile flow rate and 30 °C. The total porosity and permeability values of the prepared columns are summarized in Table 2. The results proved the decrease in the monolith average channels pore size. The decreasing of the monolithic channel pores caused lower values of permeability from 8.83×10^{-12} m^2 (89% total porosity) for the control column C_{G2} to 1.38×10^{-12} m^2 (79% total porosity) for the C_{G12} column corresponding to 5.0 mg/mL Mnt. The same observations were registered for capillary HPLC columns, the permeability values decreased from 6.93×10^{-14} m^2 (78% total porosity) for the C_{L2} control column to 1.10×10^{-14} m^2 (73% total porosity) for the C_{L12} column corresponding to 5.0 mg/mL Mnt. In summary, all of the stability experiments including eluent type and flow rate, columns backpressure and temperature, and porosity and permeability values indicate that there is no bleeding or degradation of the stationary phases under the applied conditions in GC and HPLC systems.

3.3. Chromatographic evaluation of the prepared columns

3.3.1. Separation of alkanes by gc

Before the real applications, the performance of prepared columns was evaluated for the separation of simple matrices. For this reason, the prepared columns were used to resolve five stan-

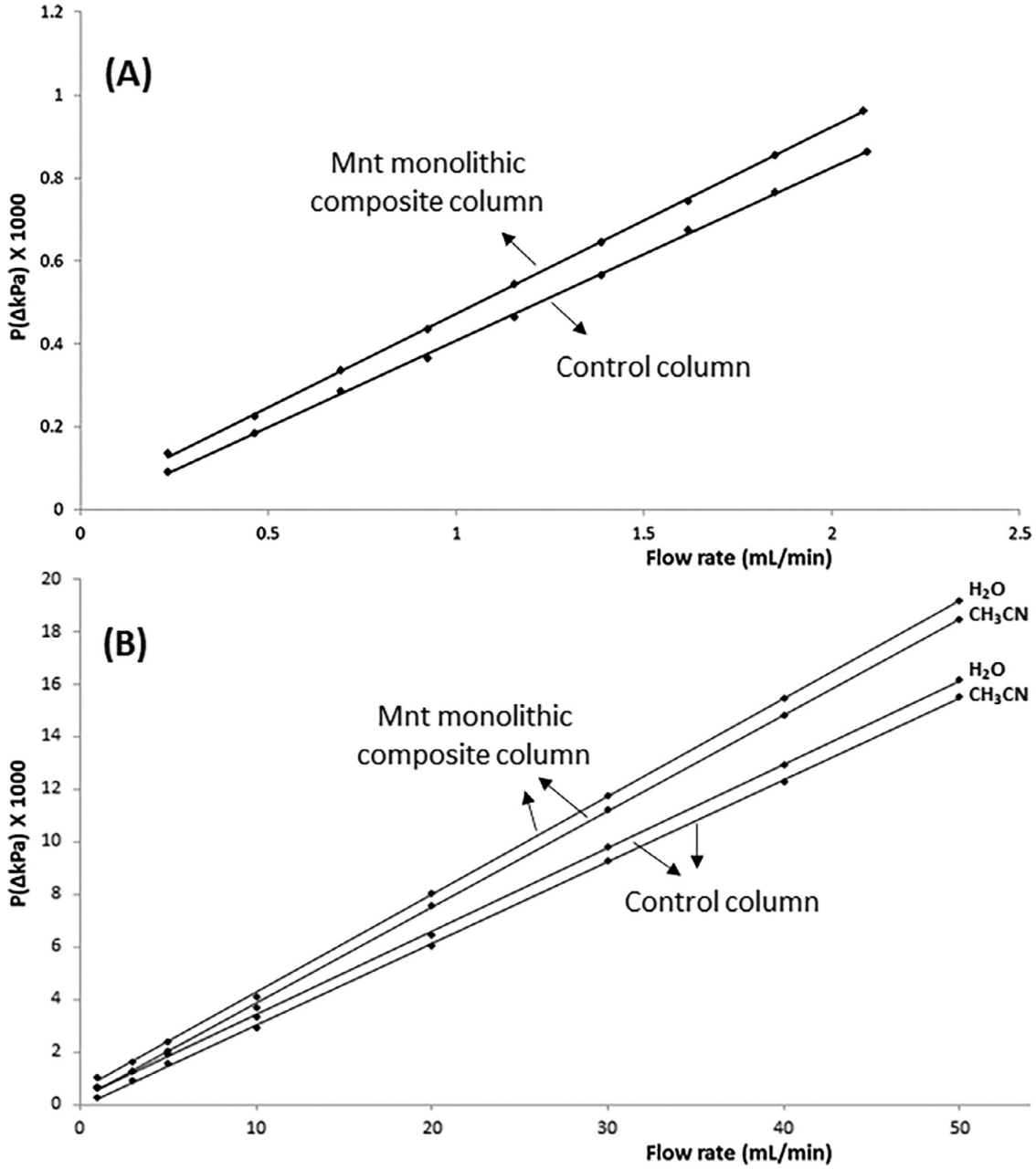

Fig. 2. (A) Graph illustrating plot of pressure drop vs. flow velocity using helium as a carrier gas in GC at 180 °C for monolithic columns with/without Mnt. (B) Graph illustrating plot of pressure drop vs. flow velocity using acetonitrile and water as mobile phases in HPLC at 30 °C for monolithic columns with/without Mnt.

Table 2
Porosity and permeability values of some of the prepared columns.

Column	Permeability (m^2)		Porosity (%)	
	CH_3CN[a]	He[b]		
C_{G2}	4.95×10^{-13}	8.83×10^{-12}	89	GC
C_{G8}	3.90×10^{-13}	3.84×10^{-12}	86	
C_{G10}	1.44×10^{-13}	2.76×10^{-12}	83	
C_{G12}	9.02×10^{-14}	1.38×10^{-12}	79	
C_{L2}	6.93×10^{-14}	5.89×10^{-13}	78	HPLC
C_{L8}	6.50×10^{-14}	4.95×10^{-13}	77	
C_{L10}	3.43×10^{-14}	>1000 kPa[c]	75	
C_{L12}	1.10×10^{-14}	>1000 kPa[c]	73	

[a] Measured by nanoLC at 20 μL/min CH_3CN flow rate and 30 °C column temperature.
[b] Measured by GC at 1.0 mL/min He flow rate and 100 °C column temperature.
[c] The pressure values are exceeded 1000 kPa (maximum operating pressure in the normal GC system).

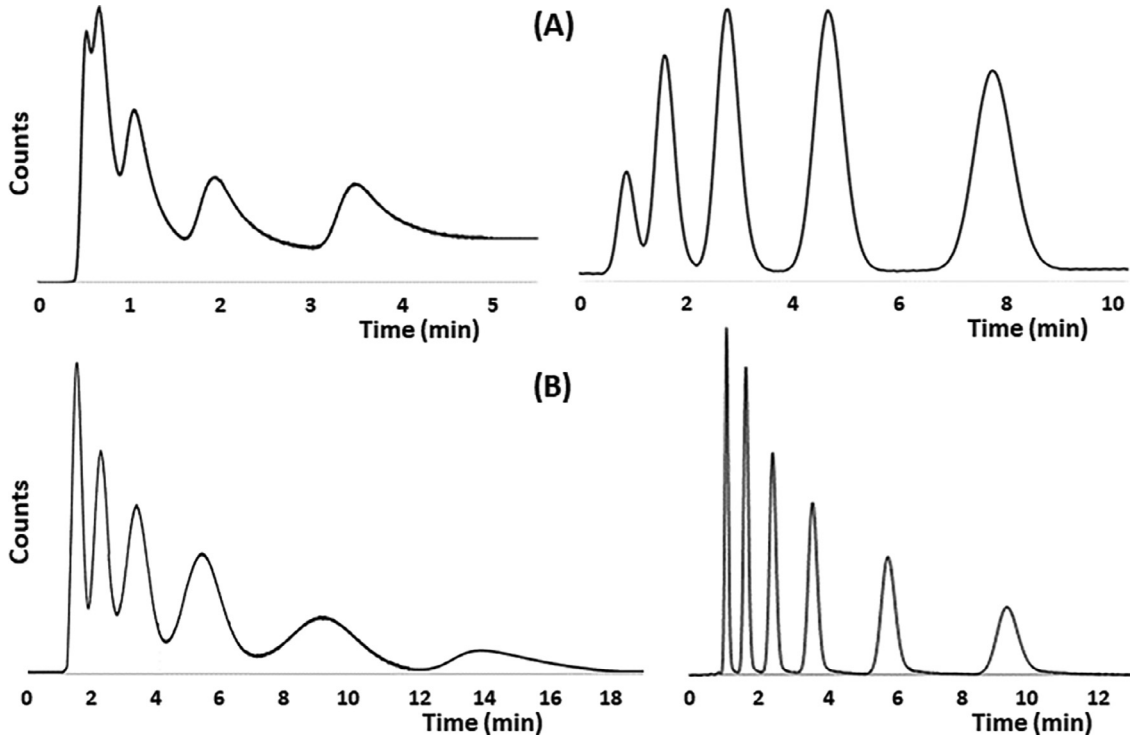

Fig. 3. (A) GC separation of standard alkane series at the optimal conditions using control column (left) and Mnt monolithic composite column (right). Compounds by order of elution: pentane, hexane, heptane, octane, and nonane. (B) Capillary LC separation standard alkylbenzene series at the optimal conditions using control column (left) and Mnt monolithic composite column (right). Compounds by order of elution: toluene, ethylbenzene, n-propylbenzene, n-butylbenzene, n-pentylbenzene, and hexylbenzene.

dard linear alkanes, from pentane to nonane using different chromatographic GC conditions. Fig. 3(A) shows the separation of the compounds under the same conditions (He carrier gas flow rate of 1.2 mL/min with a temperature program 80–150 °C, 30 °C/min) using glycidyl methacrylate-co-ethylene dimethacrylate and Mnt monolithic composite columns. Under all studied conditions, the control column showed incomplete separation with chromatographic resolution (R_s) ≤1.14 as shown in Fig. 3 (A, left). On the other hand, the Mnt monolithic composite column allowed a complete separation of all analytes with R_s ≥1.42 as demonstrated in Fig. 3 (A, right).

The presence of Mnt exhibited a clear improvement of the monolith efficiency by increasing the plate number (N) by a factor between 2.8 to 5.5 times for all injected solutes. As an example, the measured N value for heptane injected on the Mnt monolithic composite column was 31,100 plates/m, compared with 6800 plates/m for the control column using the same conditions. By varying the carrier gas flow rate from 0.10 to 2.0 mL/min, the height equivalent to a theoretical plate (H) fluctuates from 0.031 to 0.087 mm for all alkanes. Fig. S3 (A) (Supporting Information) presents the van Deemter plots for some of the studied alkanes using the Mnt monolithic composite column.

At the same chromatographic conditions, the incorporated monoliths revealed slightly higher run time in comparison with the control column. Since Mnt is intrinsically hydrophilic and alkanes are quite nonpolar compounds, this could be explained by the reduction of the porosity and permeability of the incorporated columns as illustrated in Table 2. The peak shape, width, and asymmetry were also improved by the presence of Mnt. Low asymmetry factors were registered at 10% of the peak heights for the five alkanes located between 1.07–1.16.

3.3.2. Separation of alkylbenzenes by capillary hplc

The evaluation of the columns prepared for capillary HPLC application was investigated for the separation of six homologous alkylbenzene compounds including toluene, ethylbenzene, n-propylbenzene, n-butylbenzene, n-pentylbenzene, and hexylbenzene. The effect of the incorporated Mnt onto the monoliths was very clear. As shown in Fig. 3 (B, left), the bare monoliths failed to separate the model analytes. On the other hand, Mnt-based polymethacrylate monoliths allowed a full separation of the compounds in about 10 min with R_s ≥2.86 using a binary water/acetonitrile (60:40, v/v) as a mobile phase at a flow rate of 20 µL/min as displayed in Fig. 3 (B, right). The mixture was detected at 254 nm while the sample injection volume was fixed at 10 nL under optimal conditions.

While the mobile phase flow rate fluctuated from 1.0 to 50 μL/min, the van Deemter curves of alkylbenzenes were plotted by using Mnt monolithic composite column prepared for capillary LC applications (Supporting Information Fig. S4 (A)). The H values vary from 0.019 to 0.079 mm in all cases. The best efficiency at the optimum conditions was measured for n-propylbenzene at a 10 μL/min flow rate which corresponded to 52,300 theoretical plates/m. The efficiency of the control monolithic column, on the other hand, did not exceed 10,700 theoretical plates/m in all cases. Under all of the studied conditions, the presence of Mnt improved the monolith efficiency by increasing the measured N values by a factor between 1.2 to 7.3 times for alkylbenzenes.

Again, the results indicate that the addition of Mnt into the monoliths enhances the retention of alkylbenzenes at the same separation conditions. Due to the hydrophilic structure of the Mnt, this is mainly attributed to the reduction of the porosity and permeability of the columns. As a whole, the embedding of very small amounts of Mnt into the polymer monoliths increased the retention of the studied mixture and improved the efficiency of the prepared columns. Using the incorporated column, the peak tailings were significantly reduced with asymmetry factors measured between 1.04–1.21 for all compounds compared with 1.26–1.53 using the control column.

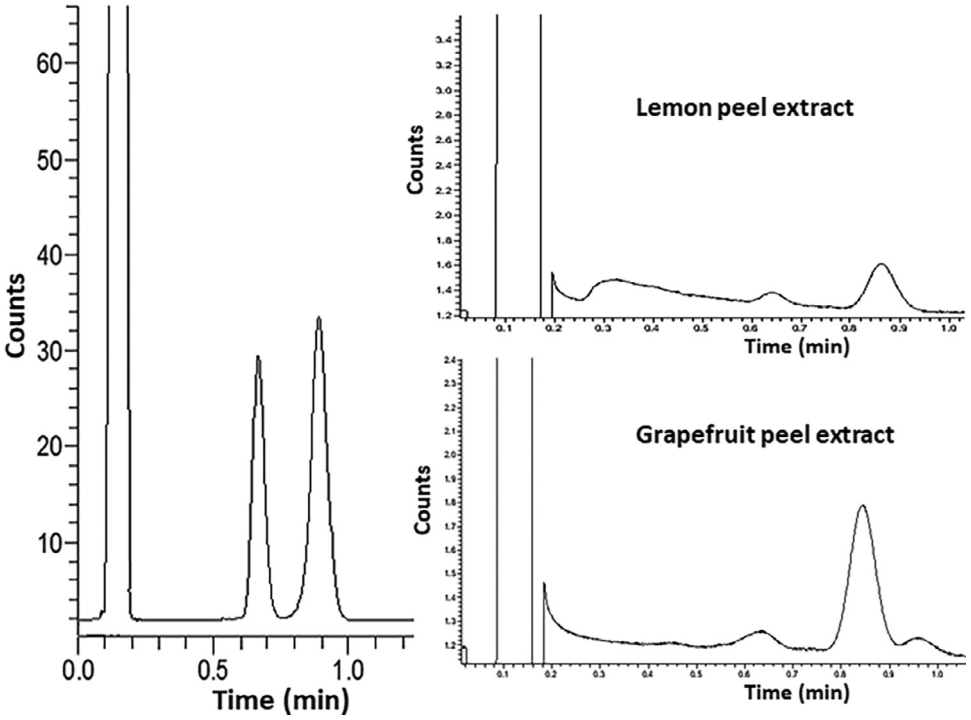

Fig. 4. GC separation of standard myrcene and limonene mixture (1.0 μg/mL of each analyte) at the optimal conditions using Mnt monolithic composite column (left). Compounds by order of elution: hexane, myrcene, and limonene. GC separation of the peel extracts of some samples at the optimal conditions using Mnt monolithic composite column (right).

3.4. Real samples analysis

3.4.1. Determination of myrcene and limonene in some fruits peel

The applicability of the prepared columns was investigated in real applications. For this reason, the prepared GC columns were applied for the determination of myrcene and limonene isomers in the peel extracts of some fruits. Myrcene is a recognizable monoterpenic hydrocarbon found in cannabis, thyme, mangoes, bay, hops, and some fruit peels [30]. Limonene, on the other hand, is the most abundant terpene found in the oil of fruit rinds, particularly citrus fruits, and prevalent in some herbs like peppermint and rosemary [31]. For the analysis of myrcene and limonene in the peel extracts, a simple extraction procedure was performed using HPLC-grade hexane solvent.

Under the optimized conditions, fast and full separation of the standards myrcene and limonene was achieved in about 1.0 min with a chromatographic resolution of 6.53 using Mnt-based glycidyl methacrylate-co-ethylene dimethacrylate column as shown in Fig. 4. Again, the control column was unable to separate the myrcene and limonene isomers at all examined conditions as demonstrated in Fig. S5 (Supporting Information). At the proposed conditions for the Mnt-based monolithic column, the performance in terms of the height equivalent to a theoretical plate was 0.048 and 0.055 mm for myrcene and limonene, respectively. Fig. S3 (B) (Supporting Information) displayed the van Deemter plots for myrcene and limonene using Mnt monolithic composite column. The peaks were almost symmetric with tailing factors close to unity for both compounds. The chromatographic parameter values are summarized in Table 3.

The findings revealed that the calibration curves were linear over the solute concentrations range of 0.10–10.0 μg/mL with regression coefficients of more than 0.9994. The detection limits of myrcene and limonene were 20 and 16 ng/mL, respectively. The analytical development parameters are presented in Table 3. Myrcene and limonene were extracted from a peel of some fruits including lemon, orange, grapefruit, and clementine. While limonene was detected in all extracts, myrcene was only detected in lemon and grapefruit extracts. Fig. 4 displays the chromatogram of lemon and grapefruit peel extract using the Mnt monolithic composite column under the optimized conditions.

The content and recovery of myrcene and limonene in the real samples are reported in Table 4. The highest content of limonene was found in the extract of clementine followed by grapefruit and then lemon peel corresponding to 727.04±3.85, 572.55±3.06, and 185.16±4.58 µg/g. On the other hand, the content of myrcene found in grapefruit was the highest with 108.17±4.66 µg/g of the fruit peel. The results showed that the prepared columns could be used for fast and sensitive qualitative and quantitative analysis of various volatile compounds in real samples. The determination of the solute was investigated by spiking the sample extracts with myrcene and limonene at different concentration levels. The recovery percentages ranged between 80.37% and 88.46% with%RSD values less than 11.36 ($n = 3$). The measured recovery values demonstrated good applicability of the extraction procedure. The recovery percentages and%RSD values are listed in Table 4.

3.4.2. Determination of drug active ingredients

The applicability and reliability of the prepared columns were used for the quality control of some active ingredients in common drug tablets. For this purpose, two commonly used drugs; aspirin-C tablets (labeled 240 mg vitamin-C and 400 mg aspirin) and profinal-XP tablets (labeled 400 mg ibuprofen and 65 mg caffeine) were selected for this investigation as real samples.

While the bare monolith failed to separate the drugs sample under all examined conditions as revealed in Fig. S6 (Supporting Information), the four active ingredients were separated with complete resolution and acceptable run time under the optimized chromatographic conditions using Mnt monolithic composite column. Figs. 5 (A & B) show the typical chromatographic curves for

Table 3
Peak and analytical parameters for the separation of the samples and the developed methods.

Analyte	t_R (min)	Equation	Conc. range (μg/mL)	R^2	LOD (ng/mL)	N (plates/m)	A_s	R_s	
Myrcene	0.63	$y = 17{,}548\ x + 896$	0.10–10.0	0.9996	20	20,900	1.06	–	GC
Limonene	0.89	$y = 19{,}425\ x + 855$	0.10–10.0	0.9994	16	18,300	0.95	6.53	
Vitamin-C	1.26	$y = 5374\ x + 458$	0.20–170	0.9993	20	16,100	1.06	–	HPLC
Caffeine	3.15	$y = 4862\ x + 364$	0.20–180	0.9988	49	21,700	1.10	13.53	
Aspirin	4.77	$y = 8405\ x + 584$	0.20–150	0.9990	35	12,800	1.13	5.72	
Ibuprofen	7.67	$y = 8658\ x + 670$	0.20–150	0.9991	47	13,900	1.19	6.06	

Table 4
The content and recovery of the analytes in the real samples.

GC method						
	Concentration and recovery% (μg/g ±%RSD, $n = 3$)					
Analyte Sample	Myrcene	Added conc. (μg/mL)	Recovery (%)	Limonene	Added conc. (μg/mL)	Recovery (%)
Lemon	53.02±6.28	0.50	81.20±11.36	185.16±4.58	1.00	83.61±7.35
Orange	not detected	–	–	96.27±4.82	0.50	80.37±11.04
Grapefruit	108.17±4.66	0.50	86.94±9.55	572.55±3.06	1.00	83.01±5.24
Clementine	not detected	–	–	727.04±3.85	1.00	88.46±5.93
HPLC method						
	Concentration and recovery% (mg/tablet ±%RSD, $n = 3$)					
Sample Analyte	Aspirin-C	Added conc. (μg/mL)	Recovery (%)	Profinal-XP	Added conc. (μg/mL)	Recovery (%)
Vitamin-C	232.90±3.15	50	97.04±5.17	–	–	–
Caffeine	–	–	–	61.80±5.16	20	95.08±4.27
Aspirin	381.08±1.57	50	95.27±4.19	–	–	–
Ibuprofen	–	–	–	393.32±1.08	50	98.33±4.74

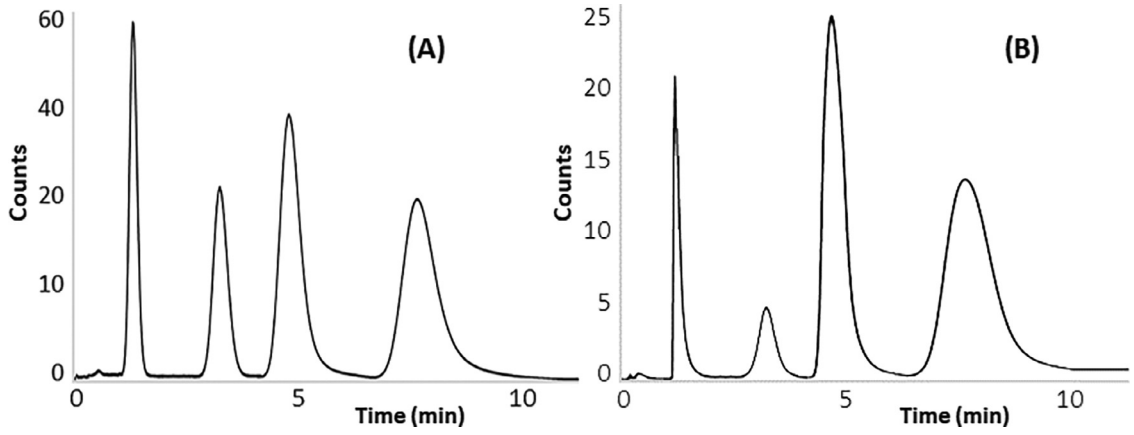

Fig. 5. (A) HPLC separation of standard drugs mixture (10.0 μg/mL of each analyte) at the optimal conditions using Mnt monolithic composite column. (B) HPLC separation of the active ingredients extracted from drugs at the optimal conditions using Mnt monolithic composite column. Compounds by order of elution: vitamin-C, caffeine, aspirin, and ibuprofen.

standard drug mixture and the active ingredients extracted from drugs, respectively.

Under the developed conditions, the active compounds were totally separated in about 10 min with a chromatographic resolution ≥5.72 using Mnt monolithic composite column. The average asymmetry factors ranged from 1.06 to 1.19. The influence of mobile phase flow rate was examined in the range of 1.0 to 50 μL/min. The van Deemter curves for the drugs using Mnt monolithic composite column are demonstrated in Fig. S4 (B) (Supporting Information). The calculated efficiency values of the column in terms of the number of theoretical plates located between 12,800 plates/m for aspirin and 21,700 plates/m for caffeine; however, much higher plates number were measured at smaller flow rate values. In comparison to the previous mixtures, the retention of the drugs and the efficiency of the prepared column are more affected by the presence of Mnt particles. This is not only attributed to the reduction of the porosity and permeability of the columns, but also the presence of more interaction sites due to the hydrophilic character of the bare Mnt and the compounds. All of the peak parameters for the separation of the four analytes are summarized in Table 3.

The linearity of the method was established using the standard concentration of each compound. The developed method exhibited wide linear concentration ranges, from 0.20 to 180 μg/mL when peak areas were used for signal evaluation. The correlation coefficient factors were found to be ≥0.9988 in all methods, indicating good curves linearity. Consecutive dilutions for the standard mixtures were made to determine the detection limits of the drugs. The estimated values of LOD fluctuated from 20 ng/mL for vitamin-C to 49 ng/mL for caffeine. The typical linear equations and quality parameters of the four drugs are given in Table 3.

The amounts of the four active ingredient compounds in tablets were examined by the developed methods. The average contents

of active compounds in drugs are presented in Table 4. The determined values and the amounts claimed by the pharmaceutical manufacturers are in good correspondence. The recovery (%) ranged from 95.08 to 98.33 and the%RSD values were less than 5.17% at the studied concentrations. The results indicate that the accuracy in terms of percentage recovery, and the precision in terms of%RSD of the prepared column and the proposed method is reliable and reproducible. The perfect interrelated relationship between the retention times of the active ingredients extracted from the tablets and that of the standard compounds indicates the selectivity of the proposed method.

3.5. Reproducibility of the prepared columns

The reproducibility of the prepared stationary phases was accomplished by estimating five columns prepared in the same batch (column-to-column reproducibility) and three columns prepared in different batches (batch-to-batch reproducibility). The columns prepared by the same procedure corresponding to the same composite stationary phases for C_{G10} and C_{L10} were selected for this test. The reproducibility of the columns was estimated in terms of%RSD of t_R, N, and R_s parameters. The%RSD values based on column-to-column reproducibility were ≤8.94% for the C_{G10} columns and ≤14.82% for the C_{L10} columns. In batch-to-batch reproducibility tests, the injection of the studied analytes into the three columns of each of C_{G10} and C_{L10} showed lower reproducibility values. In all cases, the measured values of%RSD are located between 4.86% for R_s values and 18.16% for t_R values. The results exhibited good reproducibility for the same batch columns and a little bit lower reproducibility for the columns prepared in different batches. This could be clarified by the presence of several variables and various preparation steps. The complete results are given in Table 5.

3.6. Comparison study

In this step, the prepared capillary columns were compared with those of commonly used commercial columns in GC and HPLC applications. For this purpose, the performance of the prepared columns was compared with a polymethylphenyl siloxane (95% methyl, 5% phenyl) open-tubular capillary column (30 m long × 0.25 mm i.d. × 0.25 µm film thickness) for GC, and a particulate-packed C_{18} column (15 cm long × 4.6 mm i.d., 3 µm particle size) for HPLC comparisons. HPLC analyses were performed using a Shimadzu LC-20AD liquid chromatograph equipped with a standard flow cell for the SPD-M20A UV detector with a cell path length of 10 mm and volume of 10 µL (Kyoto, Japan). Each column was run under the optimum conditions for separating the myrcene and limonene mixture in GC, and the mixture of the drug-active ingredients in HPLC. The results were compared with those of the prepared capillary monolithic composite columns in terms of some of the important parameters including solute retentions, detection limits, plate numbers, chromatographic resolutions, and peak symmetry. Table 6 provides the chromatographic parameters for the separation of the standard mixtures using commercial columns.

Under the optimized isothermal conditions for each column, the results in GC methods show that the composite monolithic columns allowed to separate the isomeric mixture much faster than the commercial polymethylphenyl siloxane open-tubular column. The typical separation chromatogram of standard myrcene and limonene mixture using a commercial GC column at the optimal conditions is shown in Fig. S7 (Supporting Information). Regarding the peak and analytical parameters, the two columns were successfully used to resolve the isomeric mixture with a good resolution and comparable detection limits and tailing factors. However, higher efficiency is in favor of the prepared composite mono-

Table 5
Reproducibility study for the C_{G10} and C_{L10} columns measured as%RSD in terms of t_R, N, and R_s parameters.

Column	C_{G10}				C_{L10}											
Solute	Myrcene		Limonene			Vitamin-C (a)		Caffeine (b)		Aspirin (c)		Ibuprofen (d)				
	t_R (min)	N	t_R (min)	N	R_s	t_R (min)	N	t_R (min)	N	t_R (min)	N	t_R (min)	N	R_s (a-b)	R_s (b-c)	R_s (c-d)	
1	0.63	20,900	0.89	18,300	6.53	1.26	16,100	3.15	21,700	4.77	12,800	7.67	13,900	13.53	5.72	6.06	Column-to-column
2	0.58	20,200	0.82	18,200	6.49	1.11	15,300	2.86	21,400	4.48	11,300	6.59	11,400	13.76	5.89	4.55	
3	0.56	21,400	0.83	18,500	7.47	1.34	18,200	3.32	23,700	4.94	14,700	7.84	15,300	14.09	5.83	6.24	
4	0.69	21,800	0.96	19,600	6.43	1.17	16,000	3.05	21,300	4.57	11,100	6.65	13,200	13.98	5.30	4.61	
5	0.67	21,100	0.93	18,800	6.27	1.20	16,600	3.11	22,000	4.62	12,300	7.28	13,600	14.14	5.41	5.73	
Avg.	0.63	21,100	0.89	18,700	6.64	1.22	16,400	3.10	22,000	4.68	12,400	7.21	13,500	13.90	5.63	5.44	
%RSD	8.94	2.83	6.89	3.01	7.16	7.23	6.62	5.38	4.44	3.87	11.62	7.95	10.43	1.83	4.62	14.82	
1	0.63	20,900	0.89	18,300	6.53	1.26	16,100	3.15	21,700	4.77	12,800	7.67	13,900	13.53	5.72	6.06	Batch-to-batch
2	0.52	18,600	0.76	17,400	6.88	1.48	19,700	3.94	23,600	5.62	14,500	8.40	15,100	15.24	5.21	5.42	
3	0.75	21,800	0.99	19,900	5.45	1.12	14,600	2.86	19,400	4.35	10,700	7.03	12,000	13.09	5.34	5.66	
Avg.	0.63	20,400	0.88	18,500	6.29	1.29	16,800	3.32	21,600	4.91	12,700	7.70	13,700	13.95	5.42	5.71	
%RSD	18.16	8.08	13.11	6.83	11.89	14.10	15.60	16.85	9.75	13.17	15.03	8.90	11.44	8.13	4.86	5.72	

Table 6
Peak and analytical parameters for the separation of the standard mixtures using commercial separation columns.

Analyte	t_R (min)	LOD (ng/mL)	N (plates/m)	R_s	A_s	
Myrcene	5.37	25	10,500	–	1.11	GC
Limonene	5.83	25	11,200	11.74	1.14	
Vitamin-C	1.75	172	15,900	–	1.09	HPLC
Caffeine	4.67	434	17,800	11.59	0.96	
Aspirin	5.88	203	15,200	2.84	1.17	
Ibuprofen	9.31	268	15,600	5.45	1.22	

lithic column based on the plate number values measured at the optimal conditions of each column.

Nevertheless, the most important drawback for the prepared composite monolithic columns in GC applications could arise from the limitations of the temperatures that can be applied to these types of composites (250 °C compared to 300 °C for the commercial polymethylphenyl siloxane column), as well as the limitations of the permissible pressures in the normal GC systems. Normal GC instruments are candidates to work with open-tubular columns under a maximum operating pressure of about 145 psi (1000 kPa). However, the wide variety of polymeric-based materials and their properties can offer many innovative solutions to these limitations. Moreover, controlling the monomeric mixture contents, as proposed in this work, along with the polymerization conditions can also contribute to overcoming these obstacles. In summary, the results reveal that the prepared columns allow a faster separation with higher efficiency, and lower consumption of carrier gas, despite their much shorter length; 100 times shorter, and a lower flow rate (1.0 mL/min compared to 1.5 mL/min for the commercial open-tubular column) performed at the optimum conditions.

Regarding the separation of the drug-active ingredients in HPLC, the prepared composite capillary monolithic column was compared with the most commonly used conventional particulate C_{18} silica-based column packed with particles size of 3 μm. Each column was performed at its optimum conditions to compromise the run time and resolution for the separation of the drug-active compounds. Fig. S8 (Supporting Information) represents a typical separation chromatogram of standard drug mixture at the optimal conditions using a particulate-packed C_{18} commercial column. Under the optimum separation achieved in an isocratic mode for both columns, the prepared column demonstrated the best performance for caffeine corresponding to 21,700 plates/m compared to 17,800 plates/m for the particulate-packed column. The comparison results in HPLC methods exposed lower run time for the prepared column, and comparable chromatographic resolution and tailing factors.

Based on the chromatographic profiles, the elution of the drug compounds injected into the prepared columns was in the same order as the commercial C_{18} column, which indicates that the main retention mechanism is the reversed-phase mode. As a whole, the prepared columns proved high resolution, good separation efficiency, and low asymmetry factors for the neutral (alkylbenzenes), weakly acidic (vitamin-C, aspirin, and ibuprofen), and weak basic (caffeine) compounds. On the other side, the polar structure of the stationary phase might not be suitable for compounds with a basic character [32–34]. However, the column application could be extended to other types of chemicals since glycidyl methacrylate could be replaced with relatively less polar monomers such as hexyl, lauryl, stearyl, and benzyl methacrylate. Further, the surface of Mnt could be functionalized or modified to be more hydrophobic to open up many opportunities for a wider range of compounds.

The prepared capillary column noticeably exhibited lower detection limits (about 10-times lower) compared to the conventional column for all compounds. This influence is expected since the smaller i.d. reduces the solute dilution through the column during the elution process. The other significant advantages of the prepared capillary column and its proposed method are the lower samples, mobile phase solvents, and stationary phase materials consumption. This was very clear in the sample injection volumes (20 nL compared to 10 µL for the commercial C_{18} column) and mobile phase flow rate (24 μL/min compared to 1.0 mL/min for the commercial C_{18} column). The capillary columns also require very small amounts of chemicals for their preparation in comparison with the conventional scale columns. On the other hand, capillary columns are more affected by factors such as the selected tubings, fittings, connections, and dead and extra-column volumes. Thus, more precautions are needed to control the sample injection volume, mobile phase flow rate, and detector cell volume in order to successfully use the capillary columns. Nevertheless, all advantages provided by the prepared monolithic capillary columns are contributing to drastically reducing the cost of analysis and the amount of generated waste.

4. Conclusions

In the present work, a novel and promising stationary phase, Mnt-based polymethacrylate composites were successfully prepared, evaluated, and utilized for food and pharmaceutical analysis in capillary liquid and gas chromatography. Utilization of the same stationary phase for GC and HPLC columns at the same time is highly innovative in the chromatography arena. Mixtures of alkane and alkylbenzene series were used as model chemicals to evaluate the separation performance of the prepared columns. The prepared capillary columns showed fast, efficient, and reproducible separation of studied compounds under several chromatographic conditions. Then, the applicability and suitability of the columns were investigated in real applications. GC columns were applied for the determination of myrcene and limonene isomers in the peel extracts of some fruits, while the prepared capillary HPLC columns were used for the quality control of some active ingredients in common commercial drug tablets. This work presents a novel separation phase that may open up hopeful perceptions for food and pharmaceutical analysis. However, the applications could be expanded to many other fields since the polymers and materials are rich in options and functionalization, and since the surface of Mnt could be modified to be more hydrophobic to open up many opportunities for different applications.

Declaration of Competing Interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

CRediT authorship contribution statement

Ahmad Aqel: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft. **Ayman A. Ghfar:** Data curation, Resources, Software, Visualization, Writing – review & editing. **Kareem Yusuf:** Conceptualization, Investigation, Methodology, Validation, Writing – review & editing. **Khalid M. Alotaibi:** Data curation, Formal analysis, Validation, Visualization, Writing – review & editing. **Rayed M. Alafra'a:** Data curation, Formal analysis, Software, Validation, Visualization. **Mohamed A. Habila:** Formal analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing – review & editing. **Ahmed-Yacine Badjah-Hadj-Ahmed:** Conceptualization, Investigation, Methodology, Software, Validation, Writing – review & edit-

ing. **Zeid A. ALOthman:** Conceptualization, Resources, Validation, Writing – review & editing.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2022.463695.

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