Simultaneous Capillary Liquid Chromatography Determination of Drugs in Pharmaceutical Preparations Using Tunable Platforms of Polymethacrylate Monolithic Columns Modified with Octadecylamine

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Simultaneous Capillary Liquid Chromatography Determination of Drugs in Pharmaceutical Preparations Using Tunable Platforms of Polymethacrylate Monolithic Columns Modified with Octadecylamine

Norah Albekairi[1] · Ahmad Aqel[2] · Zeid A. ALOthman[2]

Abstract

Nowadays, analytical techniques tend to develop cheap and green approaches to reduce the costs and minimize environmental impacts. In this study, poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) monoliths were prepared in capillary columns (0.25 mm i.d. × 20 cm length) as reactive and tunable platforms, and then simply functionalized based on a ring-opening reaction of epoxide with octadecylamine. The columns were successfully used as reversed-phase stationary phases for determination of ascorbic acid, paracetamol, caffeine, aspirin, and ibuprofen in pharmaceutical combinations. The synthesized monoliths have been estimated by FT-IR spectroscopy, SEM and specific surface area; also, the porous and hydrodynamic properties have verified the differences before and after modification. While the columns before modification were unable to separate the drugs, the columns after amination allowed for the complete separation of the five drugs in about 10 min at 23 $\mu L\ min^{-1}$ flow rate with chromatographic resolution more than 2.12. The developed assay showed excellent suitability (RSDs < 2.6%), wide linear ranges (0.07–300 $\mu g\ mL^{-1}$), sensitive detection limits (0.022–0.060 $\mu g\ mL^{-1}$), good repeatability (RSDs < 4.76%) and acceptable columns reproducibility (RSDs < 18.58%). The method was successfully applied for commercial pharmaceutical preparations; the average recovery values were found to be in the range of 98.03–104.54% (RSDs < 5.51%). The satisfactory recovery percentages along with the perfect correlation between the retentions of standard compounds and the active ingredients extracted from the commercial tablets indicate the accuracy and selectivity of the developed method. The prepared columns and validated method were finally compared with previous reports, particularly commercial C_{18} stationary phases. This is a very useful comparison due to the structural similarity between the prepared polymeric materials and C_{18} particulate stationary phases. The prepared capillary columns exhibited various advantages such as the fast separation time, wide linear ranges, low analysis costs, and trivial environmental wastes.

✉ Ahmad Aqel
    aifseisi@ksu.edu.sa

1   Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

2   Department of Chemistry, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

Graphical Abstract
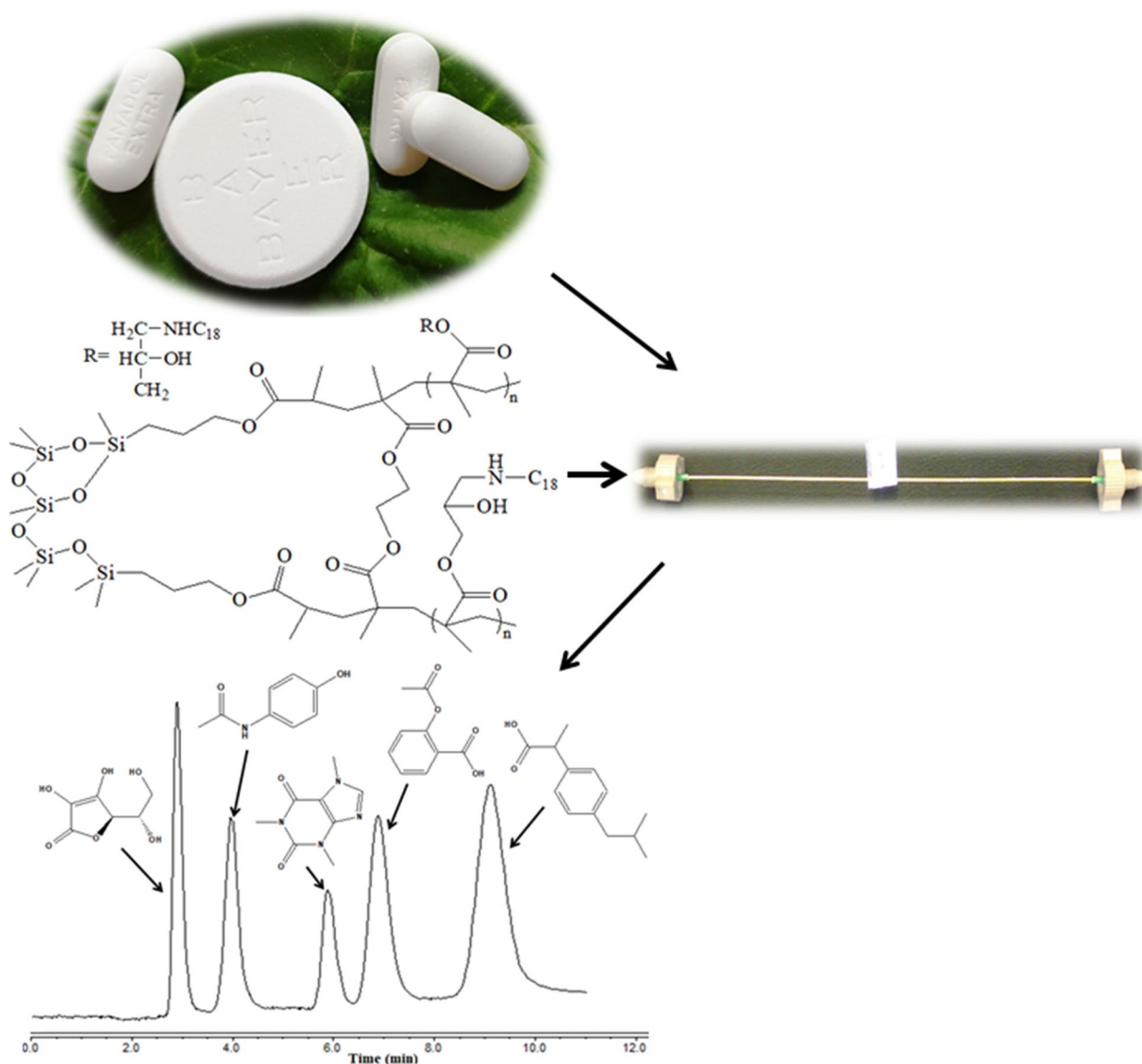
Introduction

"Single-piece, continuous block, porous structure", called monolith material, has become one of the most interesting and promising platforms in the chromatography world. Based on the chemistry of the structure, there are three types of monolithic materials; inorganic silica-based, organic polymer-based and hybrid organic–inorganic-based monoliths [1–3]. The structure of polymer-based monoliths comprises of a nonporous interconnected microglobules skeleton with large through-pores that allow high columns permeability to solvents flows with low back-pressures. Thereby, polymer-based monoliths are ideally suited for the rapid elution of large compounds such as proteins and nucleic acids [4, 5]. However, the lack of small pores in the structure of polymer-based monoliths means a lower surface area and fewer interaction sites, thus reducing the adsorptive performance for the small compounds [4, 5]. Since polymer science is extremely rich in options and reactions, polymer-based monoliths faced various developments and can be still improved; however, more experiments are highly desirable.

For chromatographic columns preparation, polymer-based monolithic stationary phases have been generally prepared by in situ co-polymerization of the functional monomer and cross-linker, in the presence of suitable porogenic solvents and a radical initiator. The reaction conditions such as the polymerization temperature and time also affect the prepared structure [5, 6]. Reactions of these types mostly lead to produce macroporous media with large through-pores. Several years ago, various attempts have been executed to prepare polymer-based monoliths possessing both large and small pores for fast and efficient separation of small molecules. In addition to the optimization of the monomeric mixture and the polymerization conditions [5, 6], some groups tried to add an additional hyper-crosslinking reaction [4], to terminate the polymerization at early stage [7], to use a single cross-linker [8], to initiate high-temperature polymerizations [9], or to introduce micro- or nanoparticles, within or attached to the monolith surface such as polymer latex [10], metal nanoparticles [11, 12], carbon nanomaterials [13, 14], metal–organic frameworks [15, 16], silica nanoparticles [17], and others [18–20].

In the last few years, miniaturization in HPLC holds great potential for achieving green, sensitive and cost-effective analysis. Miniaturized HPLC system is commonly divided into three categories according to the flow rate ranges and columns i.d.; nano, capillary, and micro scales. Capillary HPLC often refers to applications in flow rate range 0.5–10 µL min^{-1}, encompasses columns i.d. between 0.10 and 0.50 mm and sample injection volume 10–500 nL [21, 22]. Miniaturization in column liquid chromatography offers several advantages such as high detection sensitivity, low consumption of mobile and stationary phase, minute sample injection volumes, and easily hyphenating with MS detectors due to low solvents flow rate and thus enhancing the ionization efficiency and detection sensitivity [13, 21–23]. However, using capillary scale columns should be implemented with suitable HPLC systems and the extra-column volumes as well as the sample injection volume and the internal volume of the UV flow cell have to be minimized, otherwise, they would not contribute to the reduction of the separation efficiency and detection sensitivity. In conclusion, capillary HPLC technique can be competitive to the normal HPLC in several applications, including pharmaceutical compounds analysis.

This work presents preparation of poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) monoliths in capillary scale columns as a tunable stationary phase platform. A simple post-polymerization modification strategy based on a ring-opening reaction of epoxide with octadecylamine has been performed to enhance the chromatographic performance of the monolithic columns in the analysis of five drugs, namely ascorbic acid (ASC), paracetamol (PAR), caffeine (CAF), aspirin (ASP) and ibuprofen (IBU). Although

it has received great attention since the first attempt [24], using capillary monolithic columns for pharmaceutical compounds analysis is still very limited. This work focused on the development of a new analytical method for estimation of drugs in commercial pharmaceutical combinations. The prepared columns before and after modification in addition to the validated method have been extensively compared with other published studies, which were used for determination of the same compounds in pharmaceutical, food, and biological matrices.

Experimental

Chemicals

The analytical standards of ASC, PAR, CAF, ASP, and IBU were obtained from Sigma-Aldrich (St. Louis, USA) and Fluka (Buchs, Switzerland). As a real sample, Aspirin-C tablets labeled 400 mg ASP and 240 mg ASC (Bayer pharmaceutical company, Aktiengesellschaft AG, Germany), Panadol-Extra tablets labeled 500 mg PAR and 65 mg CAF (GlaxoSmithKline (gsk) Dungarvan Ltd, Ireland), and Profinal-XP tablets labeled 400 mg IBU and 65 mg CAF (Julphar, Gulf Pharmaceutical Industries, Ras Al Khaimah, UAE) were collected from local a market in Riyadh, KSA. Sodium hydroxide, acetic acid, hydrochloric acid, octadecylamine, and 1-propanol were supplied from BDH (Lutterworth, UK). Glycidyl methacrylate, ethylene dimethacrylate, azo-bis-isobutyronitrile, 3-(trimethoxysilyl)propyl methacrylate, octadecylamine, and 1,4-butanediol were purchased from Aldrich (Steinheim, Germany). The water was purified using a Millipore system (Milli-Q, Millipore S.A.S. 67120 Molsheim, France). HPLC-grade solvents acetonitrile, ethanol and acetone were acquired from Fisher Scientific (Leicestershire, UK).

### Columns Preparation and Post-polymerization Modification

The inner surface of the polyimide-coated fused silica capillary tubings (250 ± 6 µm i.d. × 360 ± 10 µm o.d., purchased from Polymicro Technologies, Molex, Phoenix, AZ, USA) were activated by successive washing with acetone, water, 0.20 mol L^{-1} sodium hydroxide, water, 0.20 mol L^{-1} hydrochloric acid and ethanol. The capillaries were then flushed with a solution of 3-(trimethoxysilyl)propyl methacrylate in ethanol 30% (v/v) adjusted to pH 5 using acetic acid at a flow rate of 0.5 µL min^{-1} for 4 h using a syringe pump (Fusion 200, Chemyx Inc., Stafford, TX, USA); the capillaries were then rinsed with ethanol and dried with a stream of pure nitrogen overnight.

The polymerization mixture was prepared in the following percentage weights (all *w/w*): 25% glycidyl methacrylate, 15% ethylene dimethacrylate, 34% 1-propanol, 25% 1,4-butanediol and 1% azo-bis-isobutyronitrile. The mixture was mixed, vortexed for 1 min and sonicated for 3 min at 40 °C in the water bath. The homogenous solution was then filled inside the activated capillaries and both ends were closed with small pieces of GC septa. The polymerization reaction was completed in the same water bath for 20 h at 70 °C. After removing the seals, the prepared capillary columns were washed with acetonitrile at a flow rate of 1.0 μL min^{-1} using a nano-LC pumping system until a stable back-pressure was reached. Then, the columns were cut to the appropriate length of 200 mm. To functionalize the glycidyl reactive groups, 1.0 g of octadecylamine was dissolved in 100 mL acetonitrile, sonicated for 10 min and subjected to the prepared monolithic columns at a flow rate of 1.0 μL min^{-1} for 3 h using a nano-LC system, stand for 1 h at 60 °C, and finally washed with acetonitrile at 1.0 μL min^{-1} flow rate for another 3 h. Figure 1 represents the synthesis structures of the monolithic material and post-polymerization modification afforded in this study. For comparison, the repeatability of the developed method and the reproducibility of the prepared columns have investigated in terms of %RSD of selected compounds.

Instrumentation and Analysis Conditions

Analysis of drug samples was carried out using a Thermo Scientific Ultimate 3000 RSLC nano-LC system (Waltham, MA, USA), equipped with a 45 nL Ultimate 3000 variable wavelength detector cell. 20 nL of each standard and sample were injected. As shown in Table 1, the detector was set at different wavelengths according to the optimum conditions of each drug. Chromeleon 7.2 data package was used to control the nano-LC system and to interpret the results. Microsoft Office XLSTAT software 2010 package was used for statistical parameters calculation. Simple isocratic elution consisting of aqueous formic acid solution (1% v/v): acetonitrile (65:35) mobile phase was used at a constant flow rate of 23 μL min^{-1}. The column temperature was set at 50 °C. FT-IR spectra for the monolithic materials before and after polymerization modification were recorded on a Thermo Nicolet 6700 FT-IR spectrophotometer (Madison, WI, USA). SEM images of the synthesized monolith were estimated using a Jeol JSM-6380LA (Tokyo, Japan) analytical scanning electron microscope (SEM) at 5 kV. Adsorption–desorption isotherm of liquid N$_2$ was used for determination of the surface areas of the synthesized monoliths using a Gemini VII 2390 surface area analyzer (Micromeritics, Nor-cross, Georgia, USA) at − 196 °C.
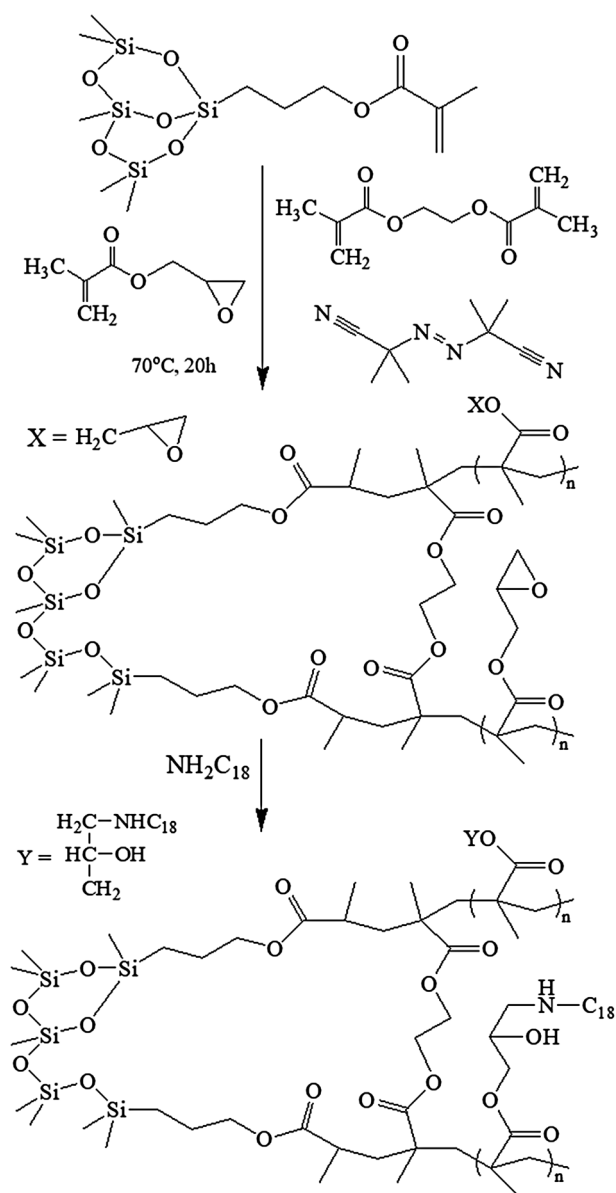
Fig. 1 Schematic representation of the synthesis and modification of the monolithic material

Samples Preparation and Validation

0.1 g of each drug standards were weighed and transferred to the same 100-mL volumetric flask. The flask was partially filled with the same composition of the mobile phase and sonicated for 5 min, cooled to room temperature; then, the volume was completed to the mark with the same solvent. Serial dilutions were made from the standard stock solution to prepare the calibration curves and to check the detection limit of each drug. 10 μg mL^{-1} of standard drugs mixture was injected six times for system suitability test. In the same way, all other standards were injected five times for calibrations and linearity studies.

Table 1 System suitability parameters (%RSD, $n=6$) for ASC, PAR, CAF, ASP and IBU drugs

	λ (nm)	t_R (min) $\pm$ %RSD[a]	Asymmetry $\pm$ %RSD[a]	Resolution $\pm$ %RSD[a]
ASC	264	2.82 ± 0.13	1.27 ± 1.24	3.38 ± 2.32
PAR	243	3.94 ± 0.17	1.35 ± 1.25	4.59 ± 1.16
CAF	274	5.85 ± 0.21	1.63 ± 0.82	2.12 ± 2.02
ASP	238	6.91 ± 0.34	1.57 ± 1.10	3.01 ± 1.38
IBU	264	9.12 ± 0.36	1.64 ± 1.06	

[a]For six consecutive injections

Five tablets of each of Aspirin-C, Panadol-Extra, and Profinal-XP drug were accurately weighed, transferred to a mortar and grinded. Average weights of the tablet were transferred to 100-mL volumetric flasks which were partially filled with the same composition of the mobile phase and sonicated for 5 min, cooled to room temperature and then the volume was completed to the mark. The resulting solutions were filtered through a 0.45-μm nylon membrane filters (Whatman, Maidstone, UK) and injected to the nano-LC system at optimum conditions to estimate the concentration of each drug in the commercial tablets. The prepared columns and developed method were validated as per International Conference on Harmonization (ICH) 2005 guidelines [25].

Results and Discussion

Columns Preparation and Evaluation

As a reactive material, glycidyl methacrylate has been used as a tunable platform for various purposes [26–34]. In the present study, organic monolith has been thermally prepared by in situ copolymerization of glycidyl methacrylate and ethylene dimethacrylate in the presence of azo-bis-isobutyronitrile as radical initiator. Although it is poor as a stationary phase, glycidyl methacrylate is used in this work to provide epoxy functionalization with octadecylamine to accomplish a suitable reversed-phase medium for drugs analysis. The main preparation steps, polymerization and post-polymerization modification are demonstrated in Fig. 1.

To assess the reaction and post-polymerization modification, the monoliths were examined by FT-IR spectroscopy to identify the organic functional groups of the polymeric phase. Figure 2a exhibits the FT-IR spectra for the synthesized monoliths before and after modification; both spectrums show the presence of the main peaks corresponding to the ester of the methacrylate groups at 1730 cm^{-1}, corresponding to ester C=O stretching and at 1147 cm^{-1}, corresponding to ester C–O stretching group. The ring-opening amination of epoxide was confirmed by the enhancement of the absorption peaks at 2922 and 2851 cm^{-1} which corresponding to the C–H stretching vibration in methylene of octadecyl group, disappearance of C–O epoxy group at

915 cm^{-1} and the formation of C–N secondary amine group at 1056 cm^{-1} as can be seen in the FT-IR spectrum Fig. 2a.

Figure 2b illustrates the SEM micrographs of the bulk region of the synthesized monoliths before and after amination. These pictures indicate that the morphology was continuous and permeable with a uniform structure. On the other hand, there is no clear difference between the monoliths bulk region before and after modification. FT-IR spectra and SEM images demonstrated that the proposed reaction and the post-polymerization amination were suitable to prepare homogenous and continuous organic monoliths resulting from the reaction of glycidyl methacrylate and ethylene dimethacrylate co-monomers, and then modified with octadecylamine to prepare a tunable reversed-phase stationary medium inside the capillary columns. For further investigation, the specific surface area of the monolithic materials before and after the modification was measured using liquid N$_2$ physisorption according to the BET method. The measured values were 16.04 m^2 g^{-1} for the control monolithic material and 24.61 m^2 g^{-1} for the polymeric material after modification. These values confirmed that the post-polymerization amination led to a slight increase in surface area, which adds more beneficial to the interaction with drugs compounds.

The stability of the prepared columns against mobile phase flow rate, operation days and temperature was also evaluated. HPLC-grade acetonitrile has been passed through the columns for the measurement of the pressure drop at different flow rates ranging from 1.0 to 50 μL min^{-1}. Figure 3a shows the relationship between the acetonitrile flow rate and columns back-pressure at 30 °C for the control capillary column and the post-polymerization-modified column. A linear dependence with regression factors better than 0.9997 indicates perfect mechanical stability and permeability for both columns. The back-pressure values showed that the post-polymerization amination induced a significant increase in column back-pressure, about 1.37 ± 0.12 times at all studied flow rates. This means that the monolith pore channels radius decreased due to the amination reaction. The permeability values were determined using acetonitrile as mobile phase through the columns at a volumetric flow rate of 10 μL min^{-1} and 30 °C column temperature. The calculated values were 1.49×10^{-13} and 1.03×10^{-13} m^2
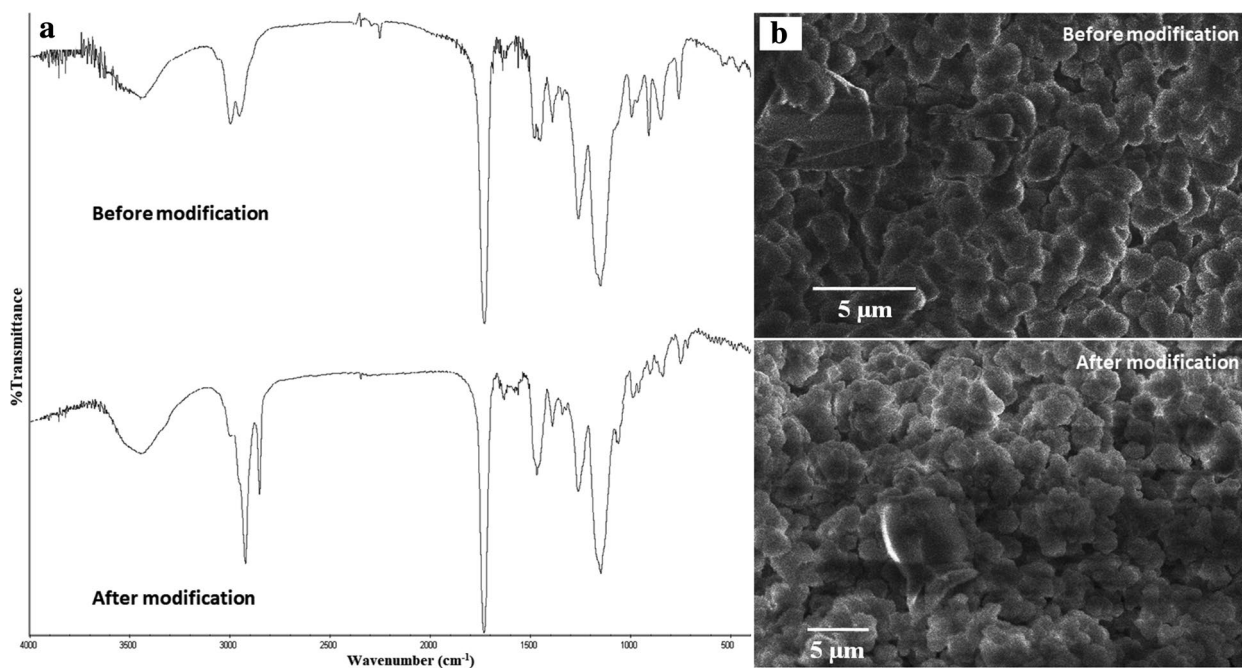
Fig. 2 **a** Infrared spectra of the synthesized monolith before and after modification with octadecylamine. **b** Bulk region SEM images of the synthesized monolith before and after modification with octadecylamine
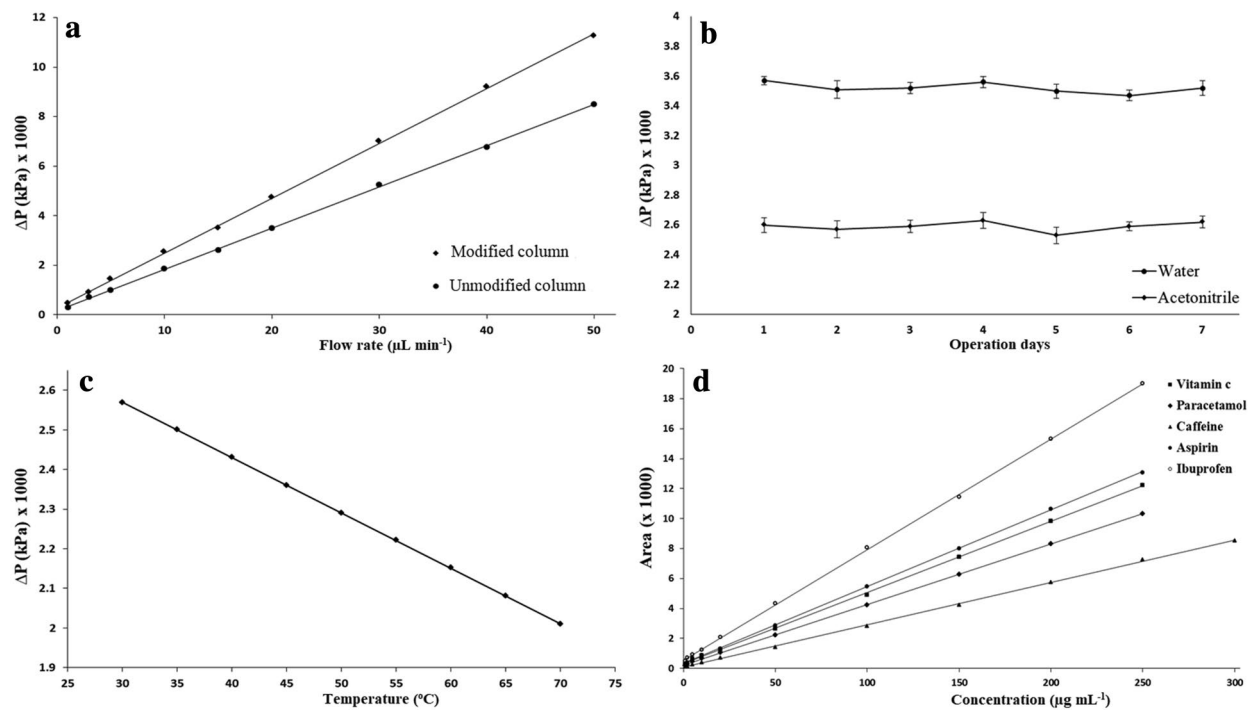
Fig. 3 **a** Columns back-pressure versus acetonitrile flow rate curves of a monolithic poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) capillary column before and after modification with octadecylamine. **b** Back-pressure versus operation days for poly(glycidyl methacrylate-co-ethylene dimethacrylate) modified column at 30 °C and constant flow rate (10 μL min^{-1}) of water and acetonitrile as elu-ents. **c** Back-pressure versus column temperature at a constant flow rate of acetonitrile (10 μL min^{-1}) as a measure of the modified column stability. **d** External calibration curves of the five drugs using a poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) modified column at optimum conditions

corresponding to the measured pressure drop of 1850 and 2530 kPa for control column and post-polymerization-modified column, respectively. The total porosity, on the other side, was calculated using uracil as an unretained solute. The measured values were 0.82 and 0.76 for the control column and post-polymerization-modified column, respectively. The post-polymerization modification to the control monolith exhibited lower permeability and porosity values and higher column back-pressure, which could be explained by the reducing of the monolith pore structure radius due to the two-step reaction approach instead of one-step synthesis in the control column, which would increase the surface density of the stationary phase.

Stability of the columns was also performed on a monolithic modified capillary column to investigate its repeatability and durability over 7 successive days. As shown in Fig. 3b, the prepared column also displays very good stability in the back-pressure over the operating days at a fixed flow rate of 10 μL min^{-1} using pure acetonitrile and water as eluents at 30 °C. The curves exhibited higher pressure while pure water passed through the column against pure acetonitrile by about 1.36 ± 0.02 times due to the difference in the viscosity of the solvent. As revealed in Fig. 3c for the monolithic capillary column after amination with octadecylamine, an inversely proportional linear relationship was obtained for the temperature versus column back-pressure curve, using acetonitrile as mobile phase at a constant flow rate of μL min^{-1} over a temperature range from 30 to 70 °C. The column back-pressure decreased by about 71 ± 2.11 kPa for each 5 °C temperature increase. The reduction in back-pressure with the increasing column temperature is due to the reduction of the acetonitrile viscosity.

Validation and Chromatographic Evaluation

After these estimations, the control column and the modified capillary column were subjected for separation of drug standards. Figure 4a, b shows the separation chromatograms for the mixed standard solution of the five compounds at optimum chromatographic conditions for each column. While the control column was unable to separate the drugs sample at all examined conditions (Fig. 4a), the five active ingredients were completely separated in 10 min at 23 μL min^{-1} flow rate using the same capillary monolithic column after modification with octadecylamine (Fig. 4b). In addition to the changing of the functionality of the stationary phase, the two-step reaction approach provides more interaction sites of the desired functional groups, and increases the surface density of stationary phase, which leads to enhance the retention properties and the efficiency of the separation process. The modified column was evaluated as a part of system suitability studies in terms of various parameters and 10 μg mL^{-1} of the five standard drugs mixture was injected

six times to evaluate the precision of the developed method in terms of %RSD of each parameter. As summarized in Table 1, the tailing factor for the five drugs ranged from 1.27 for ASC to 1.64 for IBU, while the chromatographic resolution located between 2.12 and 4.59. The measured values of %RSD for all tested parameters were found to be less than 2.6%. All system suitability test parameters are in agreement with the criteria as per ICH guidelines [25].

The linearity of the method for the five drugs was tested using the standard concentration of each drug. The developed method showed wide linear ranges, from LOQ value of each drug up to 300 μg mL^{-1} for CAF, and 250 μg mL^{-1} for the rest of the compounds in the proposed concentration range when peak areas were used for signal evaluation. The calibration graphs (at ten concentration levels) were acquired using XL-STAT 2015 as shown in Fig. 3d. The regression coefficient factors R^2 were found to be higher than 0.9996 in all curves, indicating excellent values of method linearity. The typical regression equations of the five drugs are presented in Table 2. The LOD and LOQ were measured from linearity data based on the ICH guidelines. LOD and LOQ represent the concentrations of the analytes that would yield S/N ratios of 3/1 and 10/1 for LOD and LOQ, respectively. Consecutive dilutions for the standard mixture of the five drugs were made to determine the LOD and LOQ. The estimated values of LOD for the drugs ranged from 0.022 μg mL^{-1} for ASC to 0.060 μg mL^{-1} for CAF. On the other hand, the measured values of LOQ are the lowest concentrations in the linear ranges. The results of quality parameters including linear equations, R^2 values, dynamic ranges, and LODs are presented in Table 2.

Determination of Drugs in Real Tablets

The applicability of the prepared columns and the validated method was evaluated for simultaneous determination of the five drugs in commercial tablets. As claimed by the manufacturers, the labeled contents of each of Aspirin-C, Panadol-Extra and Profinal-XP tablet are reported in Table 3. The contents of ASC, PAR, CAF, ASP and IBU drugs in tablets were estimated by the optimized method using external calibration curves. For this purpose, five tablets from each drug were separately grinded and the active ingredients were extracted, diluted and injected into the prepared column at optimum conditions. The average amounts of active ingredients are given in Table 3. The achieved experimental values and the amount claimed by the manufacturers of the commercial tablets are in good concurrence. In all cases, the percentage recovery ranged from 98.03 to 104.54 and the %RSD values were less than 5.51% at the investigated concentrations. The results presented in Table 3 indicate that the accuracy (in terms of percentage recovery) and precision (in terms of %RSD

Fig. 4 Typical separation chromatograms of the five drugs using a monolithic poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) capillary column before modification (**a**) and after amination with octadecylamine (**b**). The separations were performed using isocratic elution consisting of aqueous formic acid solution (1% v/v): acetonitrile (65:35) mobile phase at a flow rate of 23 μL min^{-1}. Column temperature was set at 50 °C while the sample injection volume was 20 nL
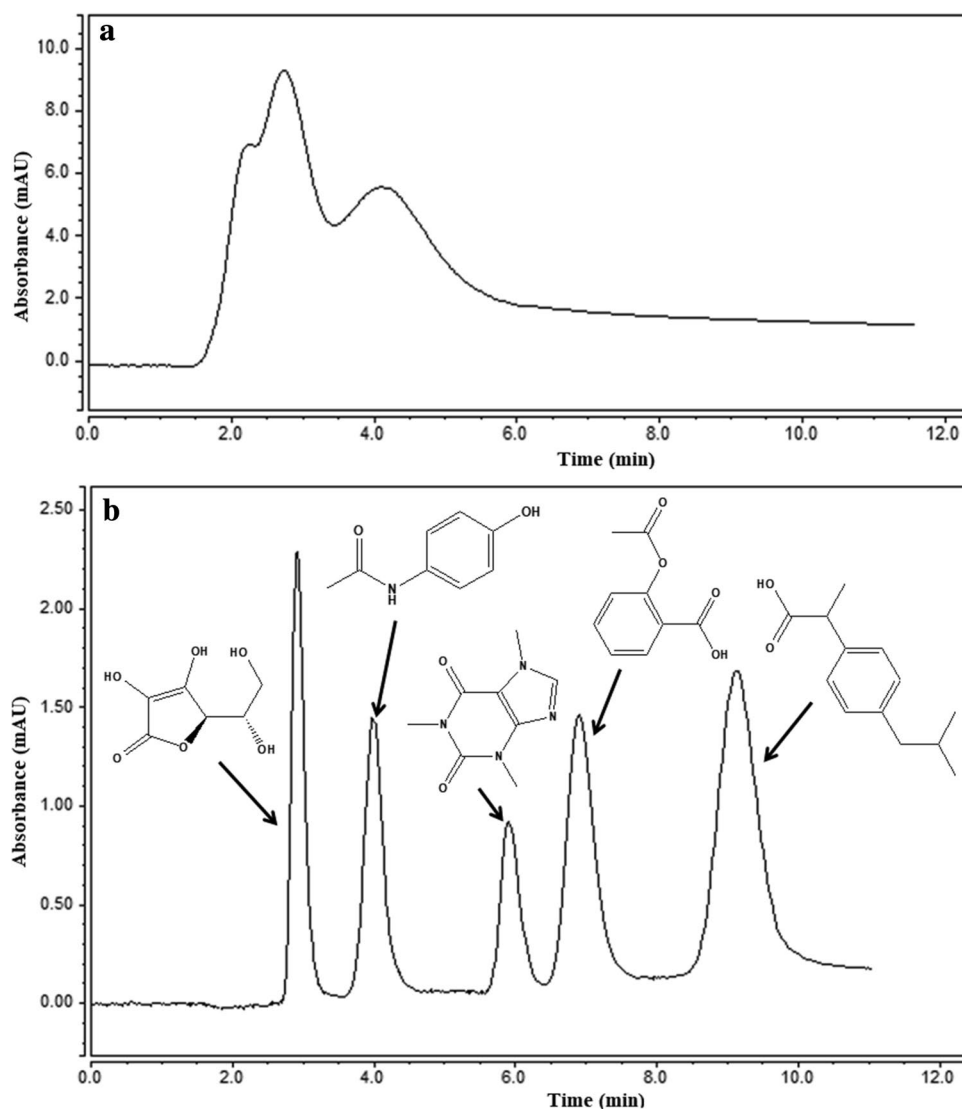
Table 2 Quality parameters of the proposed method

	Equation	R^2	Linear range (μg mL^{-1})	LOD (μg mL^{-1})
ASC	$y = 47.51\,x + 305.49$	0.9997	0.07–250	0.022
PAR	$y = 40.46\,x + 214.86$	0.9999	0.12–250	0.037
CAF	$y = 28.24\,x + 82.73$	0.9996	0.20–300	0.060
ASP	$y = 51.21\,x + 331.29$	0.9998	0.12–250	0.038
IBU	$y = 73.82\,x + 547.64$	0.9998	0.11–250	0.033

Table 3 Level and recovery (%) of ASC, PAR, CAF, ASP and IBU obtained in drug tablets (%RSD, $n = 5$)

	Labeled claim (mg per tablet)	Found concentration (mg per tablet ± %RSD[a])	Recovery (%)
ASC	240[b]	236.82 ± 2.57	98.68
PAR	500[c]	505.49 ± 4.75	101.10
CAF	65[c]	67.95 ± 5.51	104.54
CAF	65[d]	63.72 ± 0.83	98.03
ASP	400[b]	396.41 ± 3.06	99.15
IBU	400[d]	395.03 ± 1.60	98.76

[a]For five tablets of each drug

[b]Amount claimed by the manufacturer (Bayer) in Aspirin-C tablet

[c]Amount claimed by the manufacturer (GlaxoSmithKline) in Panadol-Extra tablet

[d]Amount claimed by the manufacturer (Julphar) in Profinal-XP tablet

of five estimated tablets) of the prepared column and proposed method is reliable and reproducible. On the other hand, 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 developed method.

Columns Repeatability and Reproducibility

To check the method repeatability and prepared columns reproducibility, three parameters including retention time (t_R), height equivalent to a theoretical plate (H) and chromatographic resolution (R_s) have been studied and estimated in terms of %RSD for PAR and CAF. To accomplish these tests, run-to-run and day-to-day repeatability was evaluated for the proposed method at optimum conditions, whereas column-to-column and batch-to-batch reproducibility was examined for columns prepared in the same batch and in three different batches. For the three studied parameters and the two selected drugs, the repeatability levels based on run-to-run injections was less than 2.6% ($n = 6$) and based on day-to-day injections were less than 4.76% for 6 consecutive days. Injection of PAR and CAF into six columns prepared from three separate polymerization and post-modification mixtures shows lower reproducibility; the calculated values of %RSD for height equivalent of theoretical plate were 8.84% for PAR and 18.58% for CAF, while the %RSD for the peaks resolution was 2.07%. The results revealed very good repeatability on a single column and a little bit lower reproducibility on columns prepared in the same and different batches. This could be explained by the presence of various variables in three different preparation steps; empty column inner wall activation, polymerization of the monolith material and post-polymerization modification of the monolithic surface with octadecylamine. The complete results are listed in Table 4.

Comparison Study

The aim of this section is to compare the characteristics of the proposed method and the prepared columns with other reported works for the determination of drugs in different matrices. This type of comparison is useful but very limited in the literature. To the best of our knowledge, most of the previous studies used narrowbore to conventional-scale columns (2.1–4.6 mm internal diameter), packed with commercial C_{18} particulate stationary phases possessing 3–5 μm particle size for normal HPLC and sub-2 μm for UHPLC systems. Table 5 shows that direct comparison of the developed method with different works, published in 2007 and beyond [35–57], used HPLC for analysis of drugs in terms of mobile phase flow rate, injection volume, run time, mobile phase consumption and percentage recovery. This comparison is very useful since the structure of the prepared polymeric material is similar to the particulate C_{18} used in packed columns.

Figure 4b shows that the developed method could effectively separate the five drugs in about 10 min at a flow rate of 23 μL min^{-1}, when 20 nL of the sample is injected into the prepared capillary monolithic column. The main advantages of the described method is the lower solvents and samples consumption; this is very clear in the mobile phase consumption which is particularly lower with capillary scale (1.38 mL h^{-1}) than with conventional scale columns (from 15 to 90 mL h^{-1}). The prepared capillary columns only require several nano-liters of injected samples in comparison with 5–20 μL for conventional columns. Moreover, the capillary monolithic columns need very small amounts of chemicals and reagents for all in situ preparation steps. As a result, the cost of the columns preparation and the analysis process is significantly lower than that of the commercial conventional columns, and the amount of toxic wastes generated using this method is negligible relative to other works.

Looking to the number of the analyzed compounds, the prepared columns exhibited another advantage in terms of separation time. In general, monolithic materials have significantly higher total porosity compared to the particulate

Table 4 Repeatability and reproducibility study for the developed method and prepared columns expressed for PAR and CAF as %RSD in terms of t_R, H and R_s parameters

Run-to-run						Day-to-day						Column-to-column and batch-to-batch					
Run	PAR		CAF			Day	PAR		CAF			Batch	PAR		CAF		
	t_R^a	H^b	t_R	H	R_s		t_R	H	t_R	H	R_s		t_R	H	t_R	H	R_s
1	3.93	0.019	5.85	0.012	4.64	1	3.94	0.019	5.85	0.012	4.59	1	3.94	0.019	5.87	0.012	4.59
2	3.95	0.019	5.84	0.012	4.66	2	3.95	0.020	5.89	0.011	4.64	–	4.17	0.015	6.02	0.010	4.66
3	3.95	0.019	5.83	0.012	4.51	3	3.97	0.021	5.92	0.011	4.61	2	3.55	0.023	5.60	0.018	4.62
4	3.94	0.019	5.85	0.012	4.60	4	3.92	0.020	5.83	0.011	4.55	–	3.51	0.025	5.49	0.020	4.47
5	3.94	0.019	5.87	0.012	4.55	5	3.94	0.018	5.88	0.012	4.60	3	3.87	0.019	5.91	0.014	4.64
6	3.94	0.019	5.85	0.012	4.55	6	3.93	0.018	5.87	0.011	4.57	–	4.02	0.016	6.22	0.011	4.76
Avg.	3.94	0.019	5.85	0.012	4.59	Avg.	3.94	0.019	5.87	0.012	4.59	Avg.	3.84	0.019	5.81	0.014	4.62
%RSD	0.17	1.47	0.21	2.60	1.16	%RSD	1.44	3.72	1.20	4.76	1.68	%RSD	8.84	13.93	4.60	18.58	2.07

[a]t_R: retention time (min), rounded to two decimals

[b]H: height equivalent of theoretical plate (mm), rounded to three decimals

Table 5 Comparison of the prepared columns and developed method with other reported HPLC methods (published in 2007 and beyond) for the determination of drugs in pharmaceutical, food and biological samples

HPLC column (length×i.d. mm, particle size μm)	Common compounds	Flow rate (mL min^{-1})	Injection volume (μL)	Retention time (min)[a]	Mobile phase consumption (mL h^{-1})[b]	Recovery (%)	References
Zorbax SB C$_{18}$ (250×4.6, 5)	PAR	1.0	10	8.56	60	100.4–101.4	[35]
BEH C$_{18}$ (100×2.1, 1.7)	ASC and ASP	0.5	5	1.66	30	99.0–99.9	[36]
Zorbax SB CN (150×3.9, 3.5)	PAR, CAF and ASP	1.0	5	5.91	60	–	[37]
XDB C$_{18}$ (250×4.5, 5)	PAR and CAF	1.0	–	3.83	60	98.9–99.0	[38]
Promosil C$_{18}$ (250×4.6, 5)	ASC and PAR	1.0	20	4.70	60	97.5–101.8	[39]
Poly(hexyl methacrylate) (150×0.53)	CAF	0.041	1	1.16	2.46	89.0–116.7	[40]
Betasil C$_{18}$ (150×4.6, 3)	CAF	0.5	10	15.41	30	–	[40]
Chromolith SpeedRod (50×4.6)	CAF	3.0	20	0.68	180	97.7–103.0	[41]
Kinetex C$_{18}$ (150×4.6, 5) core–shell	PAR and CAF	1.0	20	16.34	60	101.1–101.4	[42]
Nova-Pak C$_{18}$ (150×3.9, 5)	ASC and CAF	1.0	20	20.94	60	95.4–101.3	[43]
BEH C$_{18}$ (100×2.1, 1.7)	ASC and CAF	0.25	1	3.5	15	88.1–105.1	[44]
Poly(stearyl methacrylate) (200×0.32)	ASC and ASP	0.003	0.5	6.74	0.18	–	[45]
C$_8$ (250×4.6, 4.6)	PAR	1.0	20	11.49	60	–	[46]
Bondapak C$_{18}$ (300×3.9)	ASC, PAR and ASP	1.8	10	13.36	108	99.4–100.0	[47]
Kromasil C$_{18}$ (250×4.6, 5)	PAR, ASP and IBU	1.1	–	25.36	66	98.7–101.9	[48]
Betasil C$_{18}$ (150×4.6, 3)	ASC and ASP	1.0	5	4.89	60	98.4–99.3	[49]
Phenomenex C$_{18}$ (250×4.6, 5)	CAF	1.0	20	10.6	60	–	[50]
C$_{18}$ (250×4.6, 5)	PAR, CAF and IBU	1.0	10	9.57	60	92–107	[51]
Inertsil C$_{18}$ (250×4.6, 5)	PAR	1.0	–	6.4	60	98.5–100.5	[52]
Phenomenex ODS C$_{18}$ (250×4.6, 3-5)	PAR	1.0	20	5.51	60	97.3–102.0	[53]
Zorbax SB C$_{18}$ (150×4.6, 3.5)	PAR and ASP	1.5	20	7.65	90	95.8–103.0	[54]
Luna C$_{18}$ (150×4.6, 3)	PAR and ASP	1.5	20	7.41	90	–	[55]
Hypersil Gold C$_{18}$ (250×4.6, 5)	PAR	1.0	20	13.39	60	98.9–100.9	[55]
UPLC HSS C$_{18}$ (150×2.1, 1.8)	PAR, CAF and IBU	0.4	10	16.05	24	49–106	[56]
Atlantis C$_{18}$ (150×4.6, 5)	CAF	1.0	100	8.8	60	85–96	[57]
Poly(glycidyl methacrylate) modified with octadecylamine (200×0.25)	ASC, PAR, CAF, ASP and IBU	0.023	0.02	9.12	1.38	98.0–104.5	This work

[a]Retention time of the most retained drug referred to in the study

[b]Calculated from the mobile phase flow rate

stationary phases, which contributes to reduce the separation time and to decrease the columns back-pressure [13, 14, 16, 17, 40, 45]. However, the decrease in retention times would be desirable for pharmaceutical analysis to save significant time and to minimize the waste generation. Furthermore, polymer-based stationary phases are highly stable against pH of the mobile phase; this can be explained by the ability to apply 1% formic acid to the composition of mobile phase which is not possible in most silica-based materials. The data show some other advantages such as wide linear dynamic ranges. On the other side, any significant variations in the percentage recovery values calculated for the

present method and that estimated from other studies were not observed. According to all these features, the prepared poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) capillary monolithic columns functionalized with octadecylamine have shown to be very useful, and acceptable to alternate the commercial analytical scale C_{18} packed columns for an accurate, cost-effective, green and fast determination of drugs in their pharmaceutical formulations. All of the comparison data are given in Table 5.

Conclusions

The final properties of the fabricated columns are highly affected by the preparation conditions and type and percentages of the each of the stationary phase contents. In this work, polymer-based monoliths stationary phase were prepared and functionalized inside fused silica capillary tubings in three consecutive steps: empty capillary inner wall activation to enhance the silanol groups content and create vinyl functionalities, polymerization of the monomeric mixture to prepare naked and reactive monolithic material from poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) cross-linked polymer and post-polymerization amination of the active monolithic surface with octadecylamine to prepare a stable and efficient reversed-phase HPLC capillary column. The prepared columns have been characterized and used for simple, green, efficient and reliable isocratic elution capillary liquid chromatography procedure to assess ASC, PAR, CAF, ASP and IBU drugs in their pharmaceutical combinations. When compared to the standard HPLC approaches, the most important advantages of the proposed method are its low cost and green analytical approach with a mobile phase consumption of only 1.38 mL h^{-1}. According to the acceptance criteria as per ICH guidelines, all validation parameters proved the suitability of the prepared columns and the optimized method for quality control and routine analysis of the drugs in their combined pharmaceutical preparations. As a reactive platform, it would be desirable to functionalize glycidyl methacrylate with hydroxyl or amine compounds through esterification or amination processes, to impart a variety of stationary phase properties suitable for wide range of applications.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest. The authors of this publication have research support by King Saud University through Research Project no. R-17-02-27. The terms of this arrangement have been reviewed and approved by King Saud University at Riyadh, Saudi Arabia in accordance with its policy on objectivity in research.

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