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ARTICLES

Preparation and Characterization of Glycidyl Polymethacrylate Monolith Column and Its Application for Simultaneous Determination of Paracetamol and Chlorzoxazone in Their Combined Pharmaceutical Formulations

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Abstract—A home-made column (3.2 mm i.d., 100 mm length) was prepared in which glycidyl polymethacrylate was used. The optimum value of the initiator corresponded to 5 mg/mL. The column morphology was characterized by scanning electron microscopy. The permeability was evaluated using acetonitrile and water as a mobile phase and uracil as an unretained substrate. A simple and economical reversed-phase HPLC method has been developed for the simultaneous estimation of paracetamol and chlorzoxazone in their pharmaceutical formulations. Components were determined using a UV detector at 270 nm. The mobile phase was composed of 1% formic acid solution and acetonitrile (65/35, v/v); 0.7 mL/min flow rate and 5.0 μL injection volume were used. Peak resolution was 1.96. All findings allow concluding that the prepared stainless steel conventional HPLC column and the novel validated method are applicable for quality control and routine analysis.

Keywords: liquid chromatography, monolith, glycidyl methacrylate, paracetamol, chlorzoxazone

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Monolithic columns use separation media composed of one large particle that has no inter-particular voids. Consequently, the mobile phase flows through the stationary phase. Such flow causes a great acceleration of the mass transfer rate. Unlike diffusion, the major cause for mass transfer within the pores of the particles of the stationary phase during chromatographic separation, enhancement of the separation speed takes place by convective flow [1].

Monolithic phases have higher external porosity arising from the structure of the network of macropores. The networks of meso and macropores twist around each other and provide the intricate structure of the monolithic medium. These two structural characteristics allow the combination of low hydraulic resistance of the column to the stream of the mobile phase (low backpressure) and provide the surface area needed for analyte retention. Since monoliths possess no interstitial void volume, all the mobile phase has to flow through the pore channels of the support. This results in enhancing the rate of mass transfer of the analyte molecules through the beds of these continuous porous stationary phases. This effect achieves a positive impact on chromatographic efficiency. Monolithic HPLC columns consist of single rods of continuous porous materials; they, therefore, require no retaining frits. Due to the shrinkage during the synthesis of silica monolith, they can typically only be prepared in situ in capillary format. Consequently, the chromatographic behaviour of monolithic columns can differ markedly from that of the conventional columns packed with spherical particles [2].

Three types of monolithic supports are currently available, namely, inorganic polymers based on silica and more recent on carbon and zirconia, synthetic organic polymers such as polymethacrylates, polyacrylamide, polystyrenes, divinylbenzene and natural polymers such as agarose and cellulose [2]. A third approach for fast HPLC analysis is the use of monolithic silica stationary phases. The first generation reversed-phase monolith (Chromolith Performance® RP 18e) is composed of a continuous piece of porous

silica, produced using a sol-gel process leading to rod columns with a bimodal pore structure. The bimodal pore structure shows a special combination of macropores and mesopores; mesopores form a fine porous structure (average pore size of 13 nm) and create a large uniform specific surface area (300 m^2/g) on which adsorption occurs to enable separation. Macropores (average size of 2 μm) allow rapid flow of the mobile phase at low pressure. High permeability of the column allows for the use of a high flow rate without the development of high backpressure and thus enables fast analysis [3].

The first generation monolithic silica RP-18e columns have been successfully applied in method transfer from conventional particle packed columns or for the development of new fast methods [4–13]. Furthermore, application of the flow programming elution mode with monolithic silica RP-18e columns produced ultra-fast methods requiring just a few seconds with good precision [10, 14, 15]. In 2011, the second generation monolith (Chromolith High-Resolution® RP 18e) became commercially available. This new monolithic column differs from the first generation monolith in pore size, with smaller macropores (1.15 μm) to improve the peak shape [16, 17] and larger mesopores of 15 nm that provide 250 m^2/g specific surface area to enable even better separation. This type of monolith is characterized by a much more homogeneous porous silica structure than that of the first generation. Some successful applications of the second generation monolith silica RP-18e columns have been already published [3].

Monolithic columns have rapidly become highly popular and attracted increasing interest as separation media in all chromatographic methods. The unique structure of the monoliths in addition to their ease of preparation offers improved chromatographic performance and favourable properties for high efficiency [18]. The chemical and physical properties of the monolithic polymer depend, in addition to the preparation conditions such as the reaction time and temperature, on the type and concentrations of the monomer, crosslinker, porogenic solvent and initiator. Several monoliths, including methacrylate polymers, have been widely prepared and studied in the literature. Several advantages are associated with using methacrylate-based polymers as monolithic stationary phases such as high stability in a wide range of mobile phase pH 2–12, fast and simple preparation and easy functionalization. Methacrylate monolithic columns have also various selectivity values toward monomers with wide-ranging polarities [14].

Monolithic columns have rapidly become highly popular separation media in chromatography. Hjerten et al. [19] first introduced the use of monoliths with capillary liquid chromatography in 1989, and since that time, monolithic columns have been extensively studied for use in capillary HPLC [7–11]. Several different monolithic supports were described in the literature. In brief, two general categories of monolithic columns have been developed for chromatography: porous organic polymer-based monolithic columns produced by a simple moulding process and silica-based monolithic columns made by the sol-gel approach. Due to the simpler preparation process and easier adaptability of column selectivity, the organic polymeric approach exhibits more potential advantages compared with silica-based monoliths [20, 21].

Monolithic columns consist of one single piece of highly porous material with a bimodal pore size distribution, μm-sized through-pores (macropores) and nm-sized mesopores. The monolith structure does not contain interparticular voids. As a result, all the mobile phase must flow through the stationary phase. This unique structure exhibits many improvements including high porosity and surface area, fast mass transfer kinetics between the mobile and stationary phases and high binding capacity. On the other hand, the lower resistance to hydraulic flow reduces the backpressure drop across the column, while the absence of end frits, low consumption of chemicals and better exchange of the mobile phase (during gradient formation) are other advantages. In addition, the in situ preparation process of monolithic stationary phases from liquid precursors is relatively easy [20, 21].

Although they were evaluated as a polymeric chromatographic packing as early as 1978 by Svec et al. [22], methacrylate-based polymers were originally introduced for HPLC analysis in the early 1990s by Svec and Frechet. Methacrylates are now one of the most widely popular monoliths used as chromatographic separation media. There are several advantages associated with using methacrylate-based polymers as monolithic stationary phases, including high stability in a wide range of mobile phase pH 2–12, fast and simple preparation and easy functionalization. The methacrylate monolithic columns have also various selectivities toward monomers with wide-ranging polarities. However, although several solutions have been proposed by different research groups, most reported methacrylate-based monoliths were based on short alkyl chain monomers due to the limited solubility of long alkyl chain methacrylates. In this study, long-chain methacrylate monolithic columns were prepared by in situ copolymerization of either lauryl methacrylate or stearyl methacrylate with ethylene dimethacrylate in the presence of a suitable porogen using 200 mm length and 320 μm i.d. fused silica tubing. Characterization and physical properties of the prepared monolithic columns were thoroughly investigated. The columns were then evaluated and applied to the separation of different mixtures, including phenols, aromatics and drug samples [20, 21].

Fu et al. [23] presented a monolith prepared in a conventional stainless-steel column (7 cm long ×

4.6 mm i.d.) of methacrylic acid-co-ethylene dimethacrylate (**MAA-co-EDMA**) incorporated with UiO-66 for HPLC. The surface area of the UiO-66 MAA-co-EDMA composite column increased by approximately 67% compared with the control column without UiO-66, and a good efficiency of 28000 plates/m (for 2,6-dimethylphenol) was achieved. MIL-101(Cr) incorporated into a butyl methacrylate-co-EDMA monolith was introduced by Huang et al. [24] as the first capillary MOF-monolith composite column. The prepared column exhibited a satisfactory performance for capillary electrochromatography (52000 plates/m) and for nano-LC (24000 plates/m). The use of HKUST-1 nanoparticles to enhance the performance of a glycidyl methacrylate-co-EDMA monolith in a capillary column also led to a high efficiency ranging from 16300 to 44300 plates/m. Recently, the same research group examined the in situ grafting of HKUST-1 instead of incorporation. The technique of in situ synthesizing HKUST-1 in a MAA-co-EDMA monolith in a capillary column resulted in good performance and resolution, with the efficiency increasing with the HKUST-1 density [23].

Paracetamol (**PAR**), chemically N-(4-hydroxyphenyl)-acetamide (acetaminophen), is commonly used as an analgesic (pain reliever) and antipyretic (fever reducer) drug. Its therapeutic properties are similar to salicylates, but it has weak anti-inflammatory effects [4, 5]. PAR helps to reduce menstrual pains, headaches and is a major ingredient in many cold and flu remedies. The recommended dosage of PAR in adults is two 500 mg tablets (i.e. 1.0 g of PAR) every 4 to 6 h, not exceeding 8 tablets (4.0 g) in any 24 h period. The onset of analgesia is approximately 11 min after oral administration of PAR, and its half-life is 1–4 h [4, 5]. On the other hand, chlorzoxazone (**CZN**), chemically 5-chloro-3H-benzoxazole-2-one, is used to decrease muscle tone and tension and thus to relieve spasm and pain associated with musculoskeletal disorders and as histamine release [6]. Possible side effects for combination of CZN and PAR include dizziness, lightheadedness, malaise, nause, vomiting and liver dysfunction. Used with PAR, it has added risk of hepatoxicity which is why the combination is not recommended and the analysis and estimation are critical [4]. Literature survey has revealed that various analytical methodologies such as spectrophotometric [7, 8], spectrofluorimetric [9, 10] electrochemical [11] and colorimetric methods [12], HPLC [13, 17] gas chromatography [17, 25, 26] and high performance thin-layer chromatography [27] have been reported for estimation of PAR and CZN individually or in combination with other drugs. The use of monolithic materials in chromatographic application of pharmaceutical compounds is still very limited in the literature. In this work, a new analytical method for the determination of PAR and CZN compounds in their pharmaceutical combination has been developed and validated. Due to their amazing properties, monolithic materials might provide a simple, fast, efficient and cost-effective analytical approach for quality control and routine analysis of drug samples.

EXPERIMENTAL

Chemicals. Working standards of PAR, CZN and excipients were supplied from Blue Nile Pharmaceuticals (Khartoum, Sudan). As a real sample, Relaxon tablets labelled to contain 300 mg of PAR and 250 mg of CZN were collected from a local market in Riyadh, KSA. Ethylene dimethacrylate, 3-(trimethoxysilyl) propyl methacrylate, azo-bis-isobutyronitrile (**AIBN**), glycidyl methacrylate and 1,4-butanediol were purchased from Aldrich (Steinheim, Germany). HPLC grade acetonitrile, acetone, ethanol and hexane were acquired from Fisher Scientific (Leicestershire, UK). The purified water was obtained using a Millipore system (Milli-Q Advantage Elix, Millipore S.A.S. 67120 Molsheim, France).

Monolithic column preparation. Empty stainless steel column (3.2 mm i.d. × 100 mm length) was purchased from Restek, Bellefonte, PA, USA). The empty column was washed by acetonitrile. 0.02 g of AIBN was weighed in a vial, 600 μL of each 1,4-butanediol and propanol were added to the vial. The mixture was mixed by a vortex mixer for 10 min. 480 μL of glycidyl methacrylate and 320 μL of ethylene dimethacrylate were added to the vial. The mixture was mixed and purged by nitrogen gas for 5 min and sonicated in an ultrasonic bath set at 50°C for 10 min. The stainless steel column was filled with the mixture after the removal of both frits and placed in an oven maintained at 70°C for 24 h. The unreacted materials were removed by washing the prepared column by acetonitrile for 24 h at 0.1 mL/min flow rate.

Characterization of monolithic column. Permeability and porosity for our column were evaluated by the flow method. The permeability (K_0) of the porous column was calculated according to Darcy's law [20].

The monolith rod in the column was dried and a scanning electron microscope (**SEM**) (Japan) was used to image the bulk of our column.

Instrumentation and chromatographic conditions. All chromatographic experiments were carried out using a Hitachi HPLC (Japan) equipped with a UV-Vis detector and an external injector with a fixed 5.0 μL loop. Microsoft Office Excel 2016 software was used for statistical parameters calculation. Simple isocratic elution was used with the mobile phase consisting of aqueous formic acid solution (1%, v/v)-acetonitrile (65 : 35) with the flow rate of 0.7 mL/min. 5.0 μL of each standard and sample were injected by an external injector and both active ingredients were detected at 270 nm. All analyses were performed at 25°C column temperature.

Standard solutions. PAR (0.300 g) and CZN (0.250 g) were weighed accurately and transferred

quantitatively to the same 100 mL volumetric flask. The flask was half-filled with the mobile phase and sonicated for 10 min, cooled to room temperature, and the volume was completed to the mark with the same solvent.

Subsequent dilutions were made from the standard stock solution with the mobile phase to give the concentrations of 90 μg/mL for PAR and 60 μg/mL for CZN. In the same way, all other concentrations were prepared with appropriate dilution of the stock solution.

Assay preparation. Twenty tablets were weighed, transferred to a mortar and ground. Average weight of tablet was transferred to a 100 mL volumetric flask which was half-filled with the mobile phase and sonicated for 10 min, and the volume was completed to the mark with the same solvent. Subsequent dilutions were made with the mobile phase similar to those made for standard preparation to achieve target concentration. The resulting solution was filtered through a 0.45 μm membrane nylon filter prior to injection.

Method validation. *System suitability.* For system suitability study, subsequent dilutions were made from the stock solution with the mobile phase to give concentrations of 90 μg/mL for PAR and 60 μg/mL for CZN. System suitability solution was injected six times under optimum conditions; parameters such as relative standard deviation (**RSD**, %) for the peak area, theoretical plate number, resolution and asymmetry factor of the peaks were calculated for each component.

Linearity. The method linearity was examined for PAR and CZN within the concentration ranges of 36–144 and 30–120 μg/mL, respectively. Each mixed standard solution was injected in triplicate. The developed method was found to be linear in the proposed concentration range. The calibration graphs were obtained using Excel 2016 for PAR and CZN.

Selectivity. The selectivity of the developed method was evaluated by the analysis of standard, sample and placebo solutions under optimum chromatographic conditions.

Accuracy. Accuracy of the method was evaluated by spiking the placebo with known amounts of PAR and CZN standards. The recovery of the method was studied at 40, 60, 80, 100, 120, 140 and 160%. Each solution was injected in triplicate under optimum conditions, and the obtained results were compared with the calculated results and percent recoveries of the added drugs as well as RSD values (%) were calculated.

Precision. In order to demonstrate the suitability of the optimized method using the monolithic prepared column, intraday and interday variability studies were carried out by measurement of three different concentration levels (80, 100 and 120%) for PAR and CZN. The intraday study was performed by the measurement of the three concentrations five times in the same day, while the interday precision was checked by repeating the assay for the same solutions on three consecutive days.

Robustness. In order to check the method robustness, various parameters including the mobile phase composition, flow rate, column temperature and detection wavelength have been varied within a realistic range. The optimum conditions were altered, and an assay was performed with each change. The influences were expressed in terms of percentage recovery of each drug.

Determination of paracetamol and chlorzoxazone in tablets. As mentioned in the experimental section, the labelled content in each Relaxon tablet was 300 mg of PAR and 250 mg of CZN. The contents of PAR and CZN in tablets were estimated by the proposed method. For this purpose, 20 tablets were ground; the active ingredients were extracted and injected under optimum chromatographic conditions. The average amounts of active ingredients were found to be 298.0 ± 1.1 and 253.2 ± 1.3 mg for PAR and CZN, respectively.

RESULTS AND DISCUSSION

Characterization of the monolithic column. The permeability and stability of the stationary phase inside the column were evaluated using both water and acetonitrile. Pressure drops across the column have been evaluated at different flow rates ranging from 0.1 to 2.0 mL/min. The column shows stable permeability and perfect mechanical stability over the investigated flow range with regression factor (R^2) of 0.9998 and 0.9997 for water and acetonitrile, respectively. The permeability values of the prepared column were determined at 25°C, while pure acetonitrile and water eluents passed through the column at a volumetric flow rate of 1.0 mL/min. The permeability values of the prepared column were 2.71×10^{-12} and 2.36×10^{-12} m^2 corresponding to the measured pressure drop of 435 (30 bar) and 993 psi (68.5 bar) for acetonitrile and water, respectively. Figure 1 shows a directly proportional relationship between acetonitrile flow rate and column backpressure at 25°C. The total porosity value was found to be 0.78; it was calculated using uracil as unretained solute, which is in accordance with the results obtained from SEM images (Fig. 2). Both permeability and total porosity values are very close to the values previously published for related methacrylate monoliths [18, 21, 28].

After this preliminary investigation, the prepared monolith inside the conventional stainless steel column was used for the separation of PAR and CZN standards. Figure 3 shows the separation chromatogram for targeted concentrations of the mixed standard solution under optimum chromatographic conditions. The two active ingredients were completely separated in about 9.0 min at 0.7 mL/min flow rate. In order to evaluate the column efficiency, various chromatographic factors such as the number of theoretical

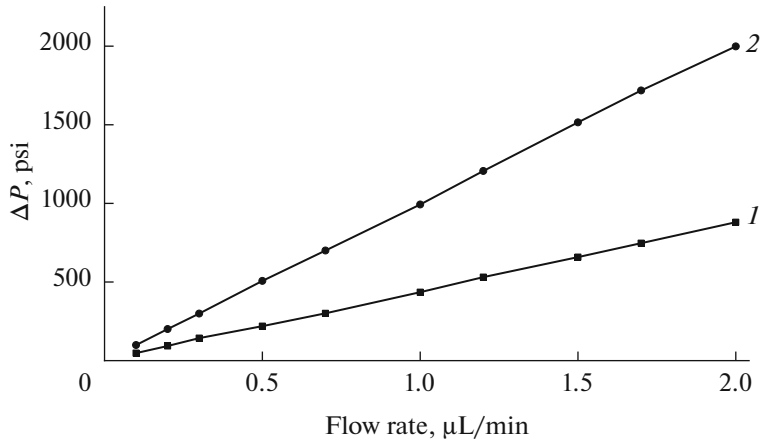

Fig. 1. Mechanical stability plots of the prepared column: plots of backpressure vs. acetonitrile (*1*) and water (*2*) flow rates.

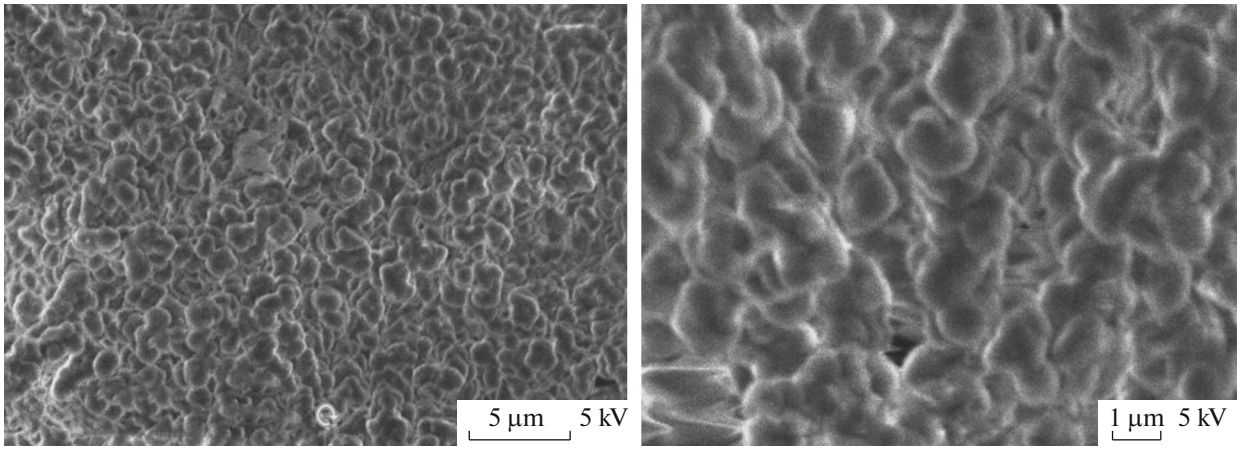

Fig. 2. SEM images of bulk region glycidyl polymethacrylate monolith column.

plates, peaks asymmetry and resolution have been measured for each standard at different mobile phase flow rates. At 0.7 mL/min flow rate, the column exhibited an average efficiency of 2500 plates/m for PAR and 5900 plates/m for CZN, while the average chromatographic resolution for the 6 replicates was about 2.0. Asymmetry, theoretical plate number and resolution values under optimum conditions are summarized in Table 1.

Validation of the developed method. To develop an effective method for the determination of the drugs, preliminary tests were performed in order to select adequate and optimum conditions. Parameters such as detection wavelength, ideal mobile phase and its combination, optimum pH and concentrations of the standard solutions were studied. HPLC method was found to be simple, accurate, economic and rapid for routine simultaneous estimation of PAR and CZN in tablet dosage forms. The conditions were optimized to obtain an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried to separate drugs components. Mobile phase and flow rate selection were based on peak parameters particularly run time and resolution. Since the monolithic material is not affected by pH, the system with formic acid (1%, v/v): acetonitrile in the ratio of 65 : 35 with 0.7 mL/min flow rate is quite robust. The optimum detection wavelength was 270 nm at which a better detector response for drugs was obtained. The average retention times for PAR and CZN were found to be 1.675 and 5.995 min, respectively. According to the United States Pharmacopeia (**USP**) [29], system suitability tests are an integral part of the chromatographic method. They are used to verify the reproducibility of the chromatographic system. From this test, RSD values for the two active ingredients were found to be less than 2.0%; low RSD values indicate that the method is precise and accurate.

The developed assay was found to be linear in the proposed concentration range when the peak area was used for signal evaluation. The calibration graphs were obtained using Excel 2016 for PAR and CZN. The

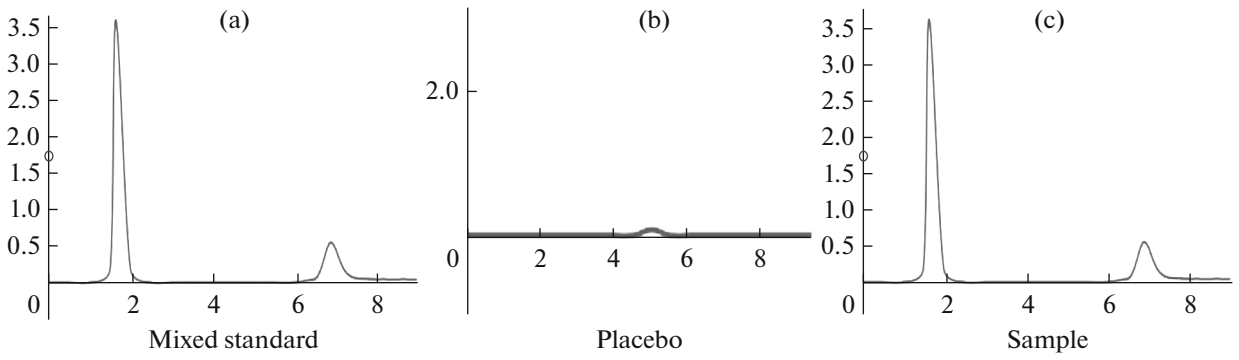

Fig. 3. Mixed standard (a), placebo (b) and sample chromatograms (c) obtained in the selectivity test of the proposed method using the home-made column.

regression coefficient values R^2 were found to be 0.9990 and 0.9993, respectively, indicating an excellent degree of method linearity [30]. The typical regression equations of calibration curve were $A = 14552c + 14431$ for PAR and $A = 11103c - 1464$ for CZN, where A is the peak area and c is the concentration of the corresponding standard.

Detection (**LOD**) and quantification (**LOQ**) limits were calculated from linearity data according to the International Conference on Harmonisation (**ICH**) guidelines [30]. LOD and LOQ represent the concentrations of the solutes that would yield signal-to-noise ratios of 3 and 10, respectively. A series of dilutions for the standard stock solution were made to determine LOD and LOQ values. The respective values of LOD and LOQ were 2.2 and 14 μg/mL for PAR, and 2.9 and 10 μg/mL for CZN. In the selectivity test, the placebo solution showed no peaks at the retention times of PAR and CZN peaks. This indicates that the excipients used in the formulation did not interfere with the estimation of the active ingredients in the tablets(30). Also, based on Fig. 3, the system suitability parameters in the respective chromatogram were almost typical to those of the standard chromatogram indicating that excipients in the sample did not affect separation. On the other hand, there is perfect correlation between the retentions of PAR and CZN standards (Fig. 3) and active ingredients extracted from Relaxon tablets (Fig. 3), which indicates that the validated method is specific [30]. The accuracy of the method was proven by the recovery test. The method has shown good and consistent recoveries for PAR and CZN close to 100% as shown in Table 2. The results of the precision study listed in Table 3 indicate that the proposed method is reliable and reproducible [30]. In both cases, the percent recovery ranged from 97 to 102% and RSD values were less than 1.9% for the investigated concentrations.

The robustness of the method was checked by deliberately varying the mobile phase composition, flow rate, detection wavelength and column temperature, which shows that the small changes of the method parameter do not affect the performance of our method. All the results presented in Table 4 were

Table 1. System suitability parameters for paracetamol and chlorzoxazone

Standard	Paracetamol			Chlorzoxazone			Resolution
	area × 10^6	number of theoretical plates[a]	asymmetry factor	area × 10^4	number of theoretical plates[a]	asymmetry factor	
STD 1	1.317	2300	1.67	8.371	5000	1.45	1.97
STD 2	1.323	1700	2.40	8.457	7100	1.17	2.01
STD 3	1.321	2100	1.50	8.349	6400	1.20	2.04
STD 4	1.320	3500	1.75	8.397	5300	1.27	1.86
STD 5	1.322	2100	2.75	8.435	6400	1.20	2.03
STD 6	1.330	3500	1.40	8.392	5300	1.19	1.86
AVG	1.322	2500	1.91	8.400	5900	1.25	1.96
STDV	4629			4014			
RSD, %	0.4			0.5			

[a]Plates/m.

Table 2. Accuracy results for paracetamol and chlorzoxazone in terms of recovery of each drug

Amount added, %	Recovery, %	
	paracetamol	chlorzoxazone
40	103	99
60	100	100
80	102	99
100	100	98
120	100	100
140	98	98
160	101	100
Average	101	99
RSD, %	1.5	0.9

Table 3. Intra- and inter-day precision results for paracetamol and chlorzoxazone in terms of recovery (in %) of each drug

Assay number	Paracetamol			Chlorzoxazone		
	80%	100%	120%	80%	100%	120%
Intra-day						
Assay 1	102	100	101	99	99	101
Assay 2	99	99	100	98	99	101
Assay 3	103	101	99	98	101	97
Assay 4	102	99	99	100	98	98
Assay 5	102	102	100	100	101	97
Average	101	100	100	99	100	99
RSD, %	1.6	1.4	1.0	0.8	1.3	1.9
Inter-day						
Day 1	102	100	101	99	99	101
Day 2	102	101	100	100	101	101
Day 3	101	102	100	98	100	98
Average	102	101	101	99	100	100
RSD, %	0.6	0.9	0.3	1.1	1.1	1.7

in accordance with the results for original conditions. The RSD value obtained for the assay in the changed conditions was less than 2%, which indicates the robustness of the proposed method [30]. In conclusion, all validation parameters permit to conclude that the prepared column and proposed method are applicable for quality control and routine determination of PAR and CZN in their combined pharmaceutical formulations.

Determination of paracetamol and chlorzoxazone in tablets. The labelled contents of PAR and CZN in Relaxon tablet are 300 and 250 mg, respectively. The contents of PAR and CZN drugs in tablets were estimated by the proposed method using a calibration curve. For this purpose, 20 tablets were separately ground. Then, the active ingredients were extracted and injected under optimum chromatographic conditions. The average amounts of active ingredients in each tablet were found to be 301.3 ± 1.5 and 247.4 ± 0.9 mg for PAR and CZN, respectively.

Table 4. Robustness values of the method for paracetamol and chlorzoxazone in terms of recovery (in %) of each drug

Condition	Value	Recovery, %	
		paracetamol	chlorzoxazone
Optimum	–	100	99
Flow rate, mL/min	0.73	98	98
	0.67	100	100
Column temperature, °C	30	100	100
	28	100	102
Detector wavelength, nm	272	100	102
	268	99	97
Formic acid aqueous solution–acetonitrile ratio	63 : 37	100	101
	67 : 33	99	98
RSD, %	–	0.8	1.7

CONCLUSIONS

In this work, glycidyl methacrylate-co-ethylene dimethacrylate monolithic has been synthesized in stainless steel HPLC column and used for simple, efficient and reliable isocratic elution by reversed-phase HPLC-UV technique to assess PAR and CZN in their pharmaceutical combination. The method was validated and showed good accuracy and precision. Therefore, this methodology based on application of conventional HPLC available in almost all laboratories around the world is highly recommended and might be suitable for routine determination of the drugs as well as for research purposes.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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