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Simultaneous Determination of Paracetamol and Chlorzoxazone in Their Combined Pharmaceutical Formulations by Reversed-phase Capillary Liquid Chromatography Using a Polymethacrylate Monolithic Column

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Abstract

Recently, analytical separation techniques have the potential toward green approaches to reduce the environmental impact. This study focuses on the development of an analytical method for the determination of paracetamol and chlorzoxazone in their pharmaceutical combination. The separation was achieved using a home-made capillary column (0.10 mm i.d. × 200 mm length) filled with porous cross-linked hexyl polymethacrylate as monolithic stationary phase. The method proved to be simple, fast, sensitive, efficient, cost-effective and green approach due to the combination of the amazing properties of a monolithic material and a miniaturized liquid chromatography, which would be considered as a step toward reducing the analytical costs and the environmental impact of chromatographic applications. Both components were detected using a 3-nL nano-UV cell fixed at 270 nm wavelength. The optimized mobile phase was composed of 1% aqueous formic acid solution and acetonitrile at 40:60 ratio, 1.0 μL/min flow rate, 4.0 nL injection volume and 50°C column temperature. Under the optimized conditions, paracetamol and chlorzoxazone have been separated in about 6.5 min with chromatographic resolution of 2.37. The prepared column and the analysis method was fully validated and compared with other reported works. All findings allow to conclude that the prepared column and proposed method are applicable for quality control and routine analysis of the two drugs.

Introduction

Paracetamol (PAR) is commonly used as analgesic, antipyretic drugs and it has weak anti-inflammatory effects (1, 2). The recommended dosage of PAR in adults is two 500 mg tablets (1.0 g) every 4–6 h, not exceeding eight tablets in any 24-h period. The onset of analgesia after oral administration of PAR is about 11 min, and its half-life

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is 1–4 h (1, 2). Chlorzoxazone (CZN) is used to reduce the muscle tone and tension and thus to relieve pain and spasm associated with musculoskeletal disorders and as histamine release (3). Possible side effects for the combination of CZN and PAR include dizziness, lightheadedness, malaise, nause, vomiting, liver dysfunction and added risk of hepatoxicity, which is why the combination is not recommended and the analysis is critical (1). Literature survey revealed that various methodologies such as spectrophotometric (4), spectrofluorimetric (5) electrochemical (6) and colourimetric (7) methods, high-performance liquid chromatography (HPLC) (8–10), gas chromatography (GC) (11) and high-performance thin-layer chromatography (12) have been reported for the estimation of PAR and CZN, individually or in combination with other drugs.

Although it was began in the late 1967 (13), miniaturization in column liquid chromatography (LC), including nano-, capillary and micro-LC, is one of the new and promising developments in separation science. Capillary LC technique uses smaller columns i.d. (less than 500 μm) and lower flow rates (less than 10 μL/min) compared to the standard HPLC; 3.2–4.6 mm column i.d. and 0.5–2.5 mL/min flow rate. Traditionally, capillary LC performed using fused silica tubings fabricated with several stationary phases; these materials are very promising in separating various compounds in different application fields (14–22).

The most important advantage of using smaller i.d. columns in HPLC analysis is the increased detection sensitivity that can be explained by the reduction of sample dilution (21–23). This is very useful to decrease the detection limits of the compounds present in limited volumes at low concentrations. Other advantages include the ability to analyze rare compounds of interest and lower samples and solvents consumption (21–23). Traditional HPLC systems consume large amounts of solvents, samples and even stationary phases material which consequently put a greater risk to the environment. Using capillary LC instead of conventional systems addresses the cost of solvents and chemicals and diminishes the environmental risks of the toxic chemicals. However, the successful development of these separation methods is closely related with the preparation of high-resolution analytical columns and validated methods.

Relative to the particulate stationary phases, monoliths are new structures and rapidly have become popular in separation science (24). In brief, three general classes of monolithic supports have been reported; organic polymers based on methacrylates, acrylates, styrenes or acrylamides and produced by a simple molding process (25–28), inorganic monoliths mostly based on silica and made by using a sol-gel approach (29) and organic–inorganic-based hybrid monoliths, which combine the properties of the two former types (30, 31). Although the three types of monolithic structures have some similar properties such as the presence of large macropores that enables high flow permeability, the morphological structures and pore distributions are totally different and hence lead for variations of their applications (32, 33). Due to their relatively simple preparation process and wide pH applicability, organic polymeric approach exhibited more interesting (33–35). Since polymer chemistry and materials science are highly rich in options, monoliths is always under innovations; however, this requires more experimental studies.

Although monolithic materials incorporated into capillary LC witnessed a great development since the first introduction (36), using of capillary monolithic columns for the determination of pharmaceutical and biomedical compounds and for quantitative analysis as a whole still very limited in the literature. This work focused on the development of a new analytical method for the estimation of PAR and CZN compounds in their pharmaceutical combination. The prepared column and the validated method have been extensively compared with other previously published works, which were used for the determination of PAR and CZN in different matrices. To the best of our knowledge, neither capillary scale LC nor monolithic type columns were used for the analysis of PAR and CZN in any type of samples. Due to their amazing properties, the combination of a monolithic material and a miniaturized LC system might provide fast, sensitive, efficient, cost-effective and green approach for drug analysis, which would be considered as a step toward reducing costs and environmental impact of the chromatographic tools.

Experimental

Chemicals

Working standards of PAR, CZN and excipients were supplied from Blue Nile Pharmaceuticals (Khartoum, Sudan). As a real sample, Relaxon tablets labeled 300 mg PAR and 250 mg CZN were collected from local a market in Riyadh, KSA. Acetic acid, sodium hydroxide, hydrochloric acid and 1-propanol were provided from BDH (Lutterworth, UK). Ethylene dimethacrylate, 3-(trimethoxysilyl)propyl methacrylate, azo-bis-isobutyronitrile, hexyl methacrylate and 1,4-butanediol were purchased from Aldrich (Steinheim, Germany). HPLC-grade solvents hexane, acetonitrile, ethanol and acetone were acquired from Fisher Scientific (Leicestershire, UK). The water was purified using Millipore system (Milli-Q, Millipore S.A.S. 67120 Molsheim, France).

Capillary monolithic column preparation

The inner wall of the fused silica capillary (0.10 mm i.d. × 0.365 mm o.d., purchased from Polymicro Technologies, Phoenix, AZ, USA) was activated by flushing with acetone, water, 0.20 mol/L sodium hydroxide, water, 0.20 mol/L hydrochloric acid and ethanol. The capillary was then flushed for 4 h with a solution of 3-(trimethoxysilyl)propyl methacrylate in ethanol 20% (v/v); the capillary was then rinsed with ethanol and dried with a highly pure nitrogen.

The polymerization mixture was prepared in the following weight percentages: 24% hexyl methacrylate as a monomer, 16% ethylene dimethacrylate as a cross-linker, 25% 1-propanol with 35% 1,4-butandiol as porogenic solvents and 1% (with respect to monomers) azo-bis-isobutyronitrile as a radical initiator. The reaction mixture was mixed into a homogeneous solution and then filled inside the activated column and both the ends were closed with small pieces of GC septa. The polymerization was performed in a water bath at 70°C for 20 h. Finally, both seals were removed, and the column was cut to 200 mm length and washed overnight with acetonitrile at 0.1 μL/min flow rate.

Instrumentation and chromatographic conditions

Chromatographic analysis were carried out using a Thermo Scientific Ultimate 3000 RSLC nanosystem (Waltham, MA, USA), equipped with an electric actuator external injector fixed with 4.0-nL inner sampling loop (Vici Valco, Houston, TX, USA) and a 3.0-nL Ultimate 3000 variable wavelength detection cell. Chromeleon 7.2 data package was used to control the nanosystem and to acquire 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 (40:60) mobile phase was used with flow rate of 1.0 μL/min. Four nanoliters of each standard and sample were injected by external injector and the active ingredients were detected at 270 nm. All analyses were

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performed at 50°C column temperature. The structural morphology of the synthesized monolith was evaluated using a Jeol (JSM-6380LA) analytical scanning electron microscope (SEM) at 5 kV.

Preparation and extraction methods

Standard solutions

PAR (0.300 g) and CZN (0.250 g) 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 10 min, cooled to room temperature; then the volume was completed to the mark with the same solvent. Series dilutions were made from the standard stock solution to give the concentrations of 90 µg/mL for PAR and 60 µg/mL for CZN. This solution was injected six times for system suitability test. 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 grinded. Average weight of tablet was transferred to a 100-mL volumetric flask which was then partially filled with the same composition of the mobile phase and sonicated for 10 min, cooled to room temperature and then the volume was completed to the mark. Subsequent dilutions were made with the same solvent similar to those made for standard preparation to achieve target concentration. The resulting solutions were filtered through a 0-45 µm nylon membrane filters. The recovered concentration was calculated by comparing the analyte response of the sample with that of the standard.

Method validation

The developed method was validated as per International Conference on Harmonization (ICH) guidelines which include system suitability, linearity, specificity, accuracy, inter and intraday precision and robustness tests (37).

Results

Capillary column preparation and evaluation

HPLC-grade acetonitrile was used to check the mechanical stability and permeability of the prepared column. Pressure drops across the column have been evaluated at flow rates ranging from 0.10 to 3.0 µL/min. The column shows perfect mechanical stability and permeability over the investigated flow range with regression factor R^2 0.9994. The permeability value of the prepared column was determined at 24°C, while acetonitrile eluent was passed through the column at a 500 nL/min volumetric flow rate. The prepared column permeability value was 5.44×10^{-14} m^2 corresponding to the measured pressure drop of 189 psi (13 bar). The total porosity value was 0.79; it was calculated using uracil as an unretained solute.

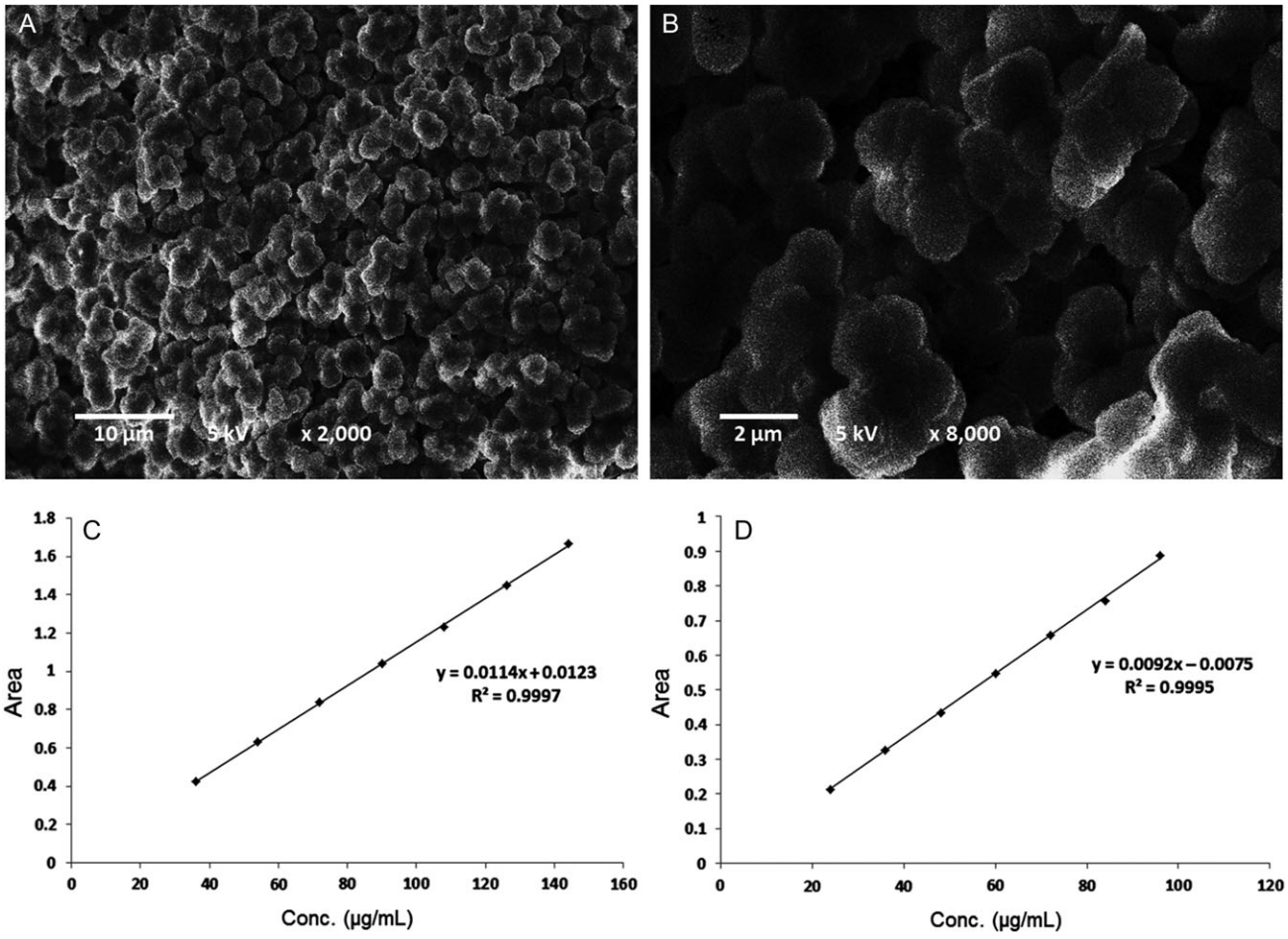

Figure 1. SEM images of the synthesized monolith bulk region at (A) x 2000 and (B) x 8000 magnification powers. Peak area vs. concentration plots of (C) PAR and (D) CZN.

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Figure 1A and B illustrates the SEM micrographs of bulk region of the hexyl methacrylate-co-ethylene dimethacrylate. The SEM images show that the morphology of the synthesized monolith was permeable with a homogeneous structure. The approximate diameter of the continuous monolith microglobules that appear in the figures ranged from 1 to 2 µm.

After this preliminary investigation, the prepared capillary column was also used for the separation of PAR and CZN standards. Figure 2 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 6.5 min at 1.0 µL/min flow rate. In order to evaluate the column efficiency, plate numbers, height equivalent to a theoretical plate, band broadening, peaks asymmetry and chromatographic resolution have been measured for each standard at different mobile phase flow rates. The height equivalent to a theoretical plate fluctuates from 0.0046 to 0.047 mm over the whole examined mobile phase flow rate range (0.1–3.0 µL/min). At 1.0 µL/min flow rate, the column exhibited an efficiency of 18,800 plates/m for PAR and 6400 plates/m for CZN, while the best performance was obtained at 0.1 µL/min for both compounds which corresponded to a column efficiency of 43,600 and 10,500 plates/m for PAR and CZN, respectively. Asymmetry, theoretical plates and resolution values under optimum conditions are summarized in Table I.

Validation of the developed method

System suitability

For system suitability studies, series dilutions were made from the stock solution with the same composition of mobile phase to give the concentrations of 90 µg/mL for PAR and 60 µg/mL for CZN. The solution was injected six times under optimum conditions; parameters such as %RSD for the retention time, peak area, theoretical plates, resolution and asymmetry factor of the peaks were calculated for both components and are summarized in Table I. The average tailing factor for PAR and CZN was 1.60 and 1.17, respectively, while the average chromatographic resolution for the six replicates was 2.37. The measured values of %RSD for all system suitability test parameters were found to be less than 2.32%, which are in agreement with the criteria as per ICH guidelines.

Linearity, limit of detection and limit of quantitation

The linearity of method for PAR and CZN was tested from 40% to 160% of the targeted level of the assay concentration for both compounds. All of the standard solutions containing 36–144 µg/mL of PAR and 24–96 µg/mL CZN in each linearity level were injected in triplicate. The developed method was found to be linear in the proposed concentration range when peak areas were used for signal evaluation. The calibration graphs were acquired using XL-STAT 2015 for PAR and CZN as shown in Figure 1C and D. The regression coefficient factors R^2 were found to be 0.9997 and 0.9995, respectively, indicating excellent values of method linearity. The typical regression equations of calibration curves were $y = 0.0114x + 0.0123$ for PAR and $y = 0.0092x - 0.0075$ for CZN; where y is the peak area and x is the concentration of the corresponding standard.

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated from linearity data according to ICH. LOD and LOQ represent the concentrations of the solutes that would yield signal-to-noise ratios of 3 and 10 for LOD and LOQ, respectively. A series of dilutions for the standard stock solution were made to determine the LOD and LOQ. The respective values of LOD and LOQ were 0.09 and 0.25 µg/mL for PAR and 0.2 and 0.7 µg/mL for CZN.

Selectivity

In this test, samples and placebo solutions were injected under optimum chromatographic conditions. As shown in Figure 3, the placebo solution showed no peaks at the PAR and CZN peaks' retention times. This indicates that the used excipients did not interfere with the determination of the active ingredients in the drug tablets. Also, based on Figure 2, the system suitability parameters in the respective chromatogram were almost typical to those of the standard one indicating that excipients in the formulation did not affect separation and analysis of PAR and CZN. On the other hand, there is perfect correlation between the retentions of the standards PAR and CZN (Figure 2) and active ingredients extracted from Relaxon tablets (Figure 3), which indicates that the validated method is specific.

Accuracy

The accuracy of the analytical method was evaluated by the addition of known concentrations of PAR and CZN standards to a fixed amount of preanalyzed drug sample. The recoveries of the method were performed at 40, 60, 80, 100, 120, 140 and 160 of the drug labeled 300 mg PAR and 250 mg CZN per tablet. The resulting solutions were injected in a triplicate under optimum conditions, and the obtained results were compared with the calculated results and the %recoveries of the added drug as well as the %RSD were measured. Table II presents the accuracy data expressed in recovery percentage obtained for each concentration level. Satisfactory recovery

Table I. System suitability parameters for PAR and CZN

STD	PAR			CZN			Resolution PAR–CZN
	Area	Theoretical plates*	Asymmetry factor	Area	Theoretical plates*	Asymmetry factor	
STD 1	10,494	18,958	1.57	5,446	6,437	1.20	2.35
STD 2	10,452	18,654	1.60	5,369	6,529	1.12	2.42
STD 3	10,482	18,594	1.62	5,378	6,382	1.18	2.35
STD 4	10,409	18,772	1.59	5,394	6,473	1.17	2.39
STD 5	10,468	18,954	1.61	5,437	6,360	1.16	2.37
STD 6	10,451	18,746	1.63	5,479	6,433	1.18	2.35
Average	10,459	18,780	1.60	5,417	6,436	1.17	2.37
%RSD	0.29	0.81	1.35	0.80	0.95	2.32	1.20

*Theoretical plates (plates/m).

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values for PAR and CZN in the ranges 98.32–101.27% and 98.61–102.28%, respectively, were obtained using the prepared column and the proposed method. The average recovery of seven levels for PAR and CZN were 99.83 and 100.57%, respectively.

Precision

In order to demonstrate the suitability of the optimized method using the monolithic prepared column, intra and interday variability studies were carried out by the analysis of three different concentration levels (80, 100 and 120) for PAR and CZN. The intraday study was performed using five replicates of the same concentration, while as the interday precision was checked by repeating the injections on 5 consecutive days. The results presented in Table III indicate that the precision of the proposed method is reliable and reproducible. In both cases, the percentage recovery ranged from 98.42 to 102.51 and the %RSD values were less than 1.9% at the investigated concentrations.

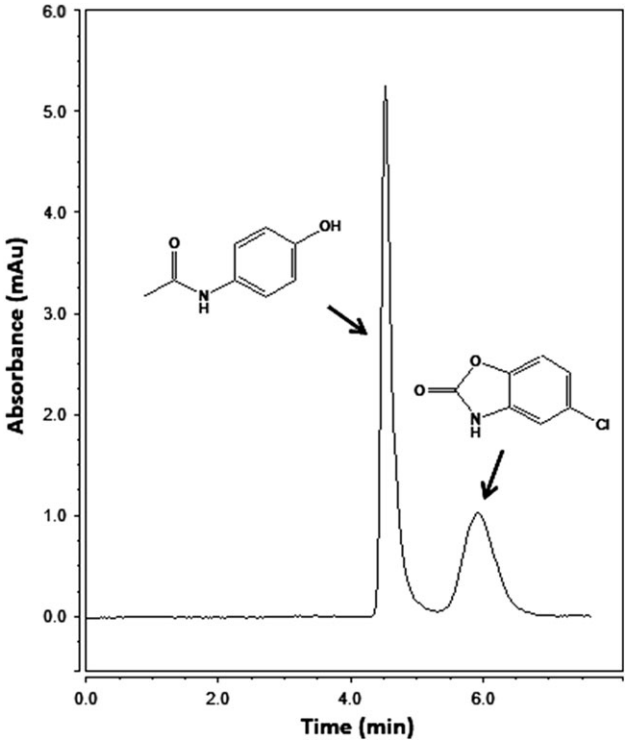

Figure 2. Chemical structures of PAR and CZN and chromatogram of the mixed standard solution; 90 μg/mL PAR and 60 μg/mL CZN (100% concentration) under the optimized conditions.

Robustness

In order to check the method robustness, various parameters including mobile phase composition and flow rate, column temperature and detection wavelength have been varied within a realistic range. Each solution was injected three times and the influences were expressed in terms of percentage recovery of each drug. The complete results are summarized in Table IV. It was observed that there were no significant variations in the %recovery values calculated for each parameter and that estimated under optimum conditions.

Determination of PAR and CZN in tablets

As mentioned in the experimental section, the labeled content of each Relaxon tablet was 300 mg PAR and 250 mg CZN. The contents of PAR and CZN drugs in tablets were estimated by the validated method using calibration curves. For this purpose, seven tablets were separately ground; then the active ingredients were extracted and injected under optimum chromatographic conditions. The average amount of active ingredients was found to be 298.0 mg ± 1.10 and 253.2 mg ± 1.25 for PAR and CZN, respectively.

Comparison study

The characteristics of the prepared column and the developed method have been compared with other reported works. In order to enable the direct comparison, the prepared column and the validated method were compared with other methods which were used only HPLC for the determination of PAR and CZN particularly in pharmaceutical and biomedical. The analytical parameters of the comparison studies are presented in Table V. With very few exceptions, all previous studies used C_{18} particulate stationary phases at

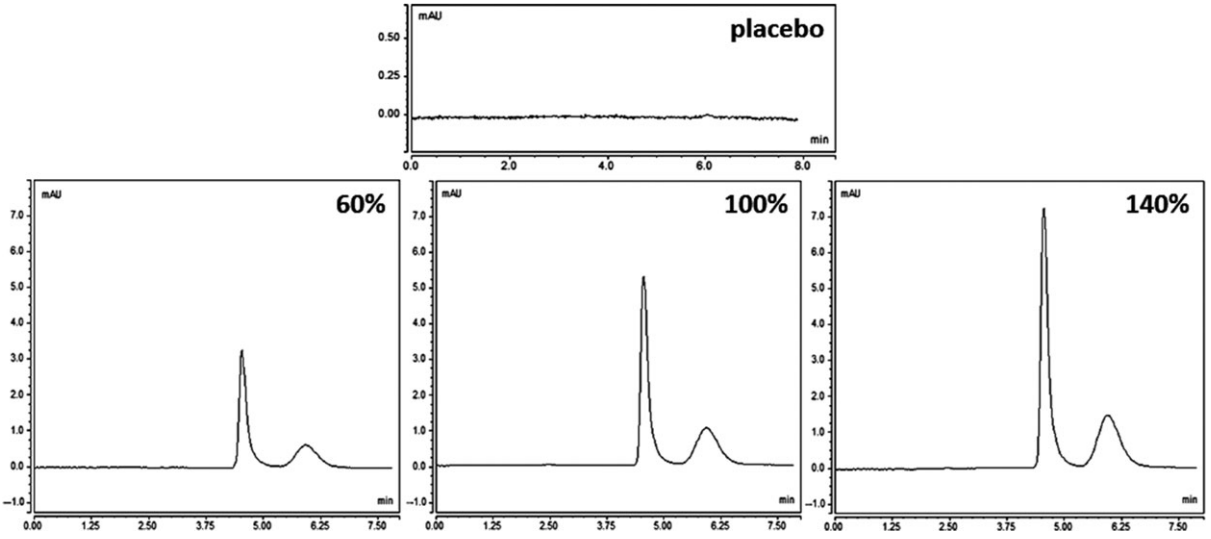

Figure 3. Chromatograms of placebo, 60, 100 and 140 solutions concentration under optimum conditions.

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Table II. Accuracy results for PAR and CZN in terms of %recovery of each drug

Amount added (%)	Recovery (%)	
	PAR	CZN
40	101.27	98.61
60	100.89	100.50
80	100.38	100.36
100	99.33	101.29
120	98.32	101.11
140	98.91	99.82
160	99.71	102.28
Average	99.83	100.57
%RSD	1.07	1.16

Table III. Intra and interday precision results for PAR and CZN in terms of %recovery of each drug

	PAR			CZN		
	80%	100%	120%	80%	100%	120%
Intraday						
Assay 1	99.37	99.91	98.56	103.60	100.42	99.91
Assay 2	99.96	99.80	98.42	100.76	100.86	99.80
Assay 3	98.19	101.80	101.68	101.84	100.62	101.80
Assay 4	102.01	101.12	102.42	102.51	100.36	101.12
Assay 5	102.13	100.29	101.69	99.98	99.76	100.28
Average	100.33	100.58	100.55	101.74	100.40	100.58
%RSD	1.70	0.85	1.89	1.40	0.41	0.85
Interday						
Day 1	99.96	100.65	101.68	100.76	100.63	101.80
Day 2	98.72	99.72	98.87	98.97	100.32	100.36
Day 3	100.20	99.16	100.39	100.75	100.50	100.21
Day 4	99.74	100.04	99.52	99.32	99.94	99.73
Day 5	98.92	99.24	99.84	99.64	101.04	100.09
Average	99.51	99.76	100.06	99.89	100.49	100.44
%RSD	0.66	0.61	1.06	0.83	0.41	0.79

Table IV. Robustness values of the method for PAR and CZN in terms of %recovery of each drug

Condition	Recovery (%)	
	PAR	CZN
Optimum	99.33	101.29
0.95 µL/min flow rate	99.97	99.82
1.05 µL/min flow rate	100.03	99.80
53°C column temperature	99.35	100.28
47°C column temperature	99.52	99.51
273 nm detector λ	101.82	100.77
267 nm detector λ	101.82	100.33
H_2O:ACN (35:65, *v/v*)	100.55	100.55
H_2O:ACN (45:55, *v/v*)	99.55	99.98
%RSD	0.99	0.56

conventional scale columns with internal diameter ranged between 2.1 mm for ultra-high performance liquid chromatography (UHPLC) and 4.6 mm for normal HPLC instruments, all these columns were commercial and packed with 3–5 µm particle size for HPLC and sub-2 µm for UHPLC technology.

The prepared hexyl polymethacrylate monolithic-based capillary column along with the proposed method showed advantages in terms of lower solvents and samples consumption; this is very clear in the extremely small mobile phase flow rate and minute sample injection volume as shown in Table V. The solvent consumption is notably lower with capillary columns (0.06 mL/h) than with commercial columns (from 24 to 120 mL/h) and requires smaller samples' injection volume; 4 nL in comparison with 2–20 µL for conventional columns. The cost of a home-made cross-linked polymethacrylate monolithic column is significantly lower than that of the conventional C_{18} columns. Additionally, polymer-based stationary phases are highly stable under a wide range of mobile phase pH, this can be inferred from the ability to add 1% of formic acid to the mobile phase composition which is not possible with most silica-based stationary phases.

Discussion

Many previous works showed that in addition to the polymerization conditions, type and percentages of the monomeric mixture play an important role in the final characteristics and chromatographic performance of the fabricated column. In this work, a monolithic stationary phase was prepared by polymerization of hexyl methacrylate with ethylene dimethacrylate and chemically attached to the inner surface of the capillary tube.

The prepared capillary column was evaluated by investigating the porosity, permeability and mechanical stability. Both porosity and permeability values of the prepared capillary column are in a good convergence with that previously published for hexyl methacrylate and other polymethacrylate monolithic columns (38–41). The column was used for the separation of PAR and CZN standards. Plate numbers, height equivalent to a theoretical plate, band broadening, peaks asymmetry and chromatographic resolution have been measured for each standard at different mobile phase flow rates. According with the acceptance criteria as per ICH guidelines (37), all parameters proved the suitability of the prepared column and the optimized method for the analysis of PAR and CZN.

Under the optimum conditions, PAR and CZN have been separated in about 6.5 min with chromatographic resolution of 2.37. The mobile phase was composed of acetonitrile and 1% aqueous formic acid solution at 60:40 ratio flows at 1.0 µL/min (0.06 mL/h solvent consumption) which minimizes the environmental impact. Furthermore, because of the requirements of much smaller amount of samples (4 nL sample injection volume) and stationary phase materials, preparation of monolith inside 0.1 mm i.d. scale column is also more cost efficient.

The chromatographic separation was optimized and the analytical method was completely validated in terms of system suitability, linearity, LOD and LOQ, specificity, accuracy, inter and intraday precision and robustness tests. The developed assay exhibited good linearity in the proposed concentration range of each compound (36–144 µg/mL of PAR and 24–96 µg/mL CZN). The regression coefficient values indicating excellent degree of method linearity when peak area was used for signal evaluation. A series of dilutions for the standard stock solution were made to determine the detection and quantification limits based on the signal-to-noise ratios. Samples and placebo solutions were injected under the optimized conditions. The excipients did not affect the estimation of the PAR and CZN, since no peaks were detected at their retention times. On the other hand, the perfect correlation between the retentions of PAR and CZN standards and active ingredients extracted from

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Table V. Comparison of the developed method with other methods for the determination of PAR and CZN in pharmaceutical and biological samples

HPLC column (length × i.d. mm, particle size μm)	Target	Flow rate (mL/min)	Injection volume (μL)	Retention time (min)[a]	Mobile phase consumption (mL/h)[b]	Recovery (%)	Ref.
Luna C18 (250 × 4.6, 5)	PAR and CZN	1.5	20	1.8 and 2.6	90	—	(3)
Zorbax SB C18 (250 × 4.6, 5)	PAR and CZN	1.0	10	2.63 and 6.16	60	100.5–101.4	(8)
Intersil C18 (250 × 4.6, 5)	PAR and CZN	1.0	10	3.4 and 11.25	60	98.93–101.36	(9)
Inertsil C18 (250 × 4.6, 5)	PAR and CZN	1.0	—	2.8 and 4.2	60	99.59–100.48	(10)
C8 (250 × 4.6, 4.6)	PAR	1.0	20	7.11	60	—	(12)
Eclipse plus C_{18} (50 × 4.6, 1.8)	PAR and CZN	0.5	—	0.94 and 1.92	30	99.39–99.95	(42)
Phenomenex ODS C_{18} (250 × 4.6, 3-5)	PAR and CZN	1.0	20	2.17 and 5.51	60	97.27–102.0	(43)
Purospher C_{18}e (125 × 3.0, 5)	PAR	0.75	20	4.81	45	97.08–103.63	(44)
C_{18} (150 × 4.6, 5)	PAR	1.3	20	2.6	78	99.96–100.05	(45)
Hypersil GOLD C_{18} (250 × 4.6, 5)	PAR and CZN	1.2	20	2.69 and 4.61	72	99.12–100.78	(46)
Zorbax SB CN (150 × 3.9, 3.5)	PAR	1.0	5	4.18	60	—	(47)
Promosil C_{18} (250 × 4.6, 5)	PAR	1.0	20	3.8	60	100.46–101.8	(48)
Bio SiL HL C_{18} (250 × 4.6, 5)	PAR	2.0	—	2.97	120	99.93–102.11	(49)
Kinetex C_{18} (150 × 4.5, 5) core shell	PAR	1.0	20	9.85	60	101.07–101.4	(50)
μ-Bondapack C_8 (250 × 4.6, 5)	PAR	1.0	20	4.88	60	95.42–106.25	(51)
Bondapak C_{18} (300 × 3.9)	PAR	1.8	10	2.66	108	99.28–100.72	(52)
Kromasil C_{18} (250 × 4.6, 5)	PAR	1.1	—	3.34	66	99.93–100.15	(53)
C_{18} (250 × 4.6, 5)	PAR	1.0	10	3.9	60	96.0	(54)
Hypersil ODS (150 × 4.6, 5)	PAR	1.5	20	2.32	90	—	(55)
Luna C_{18} (150 × 4.6, 3)	PAR	1.5	20	3.49	90	—	(55)
Zorbax SB C_{18} (150 × 4.6, 3.5)	PAR	1.5	20	2.49	90	—	(55)
Hypersil Gold C_{18} (250 × 4.6, 5)	PAR	1.0	20	3.11	60	99.36–100.85	(56)
Acquity HSS C_{18} (150 × 2.1, 1.8)	PAR and CZN	0.4	10	2.08 and 10.9	24	—	(57)
XBridge C_{18} (250 × 4.6, 5)	PAR and CZN	1.0	10	6.4 and 15.8	60	—	(58)
Acquity BEH C_{18} (50 × 2.1, 1.7)	PAR and CZN	0.6	2	1.2	36	—	(58)
Poly(hexyl methacrylate) (200 × 0.10)	PAR and CZN	0.001	0.004	4.5 and 5.9	0.06	98.32–102.28	This work

[a]Retention times for PAR and CZN, respectively.
[b]Calculated from mobile phase flow rate.

Relaxon tablets indicating the selectivity of the method. Satisfactory recovery values were obtained at different concentration levels indicating that the proposed method is accurate and reproducible for simultaneous determination of PAR and CZN.

In comparison with other reported works used the particulate stationary phases for the determination of PAR and CZN, the prepared capillary monolithic column exhibits various characteristics as packing materials for chromatographic analysis such as the lower solvents and samples consumption which leads to reduce both the analysis costs and the environmental impact. In conclusion, all method validation parameters permit to conclude that the prepared column and proposed method are applicable for quality control and routine analysis of PAR and CZN in their combined pharmaceutical formulations.

Conclusions

In this work, hexyl methacrylate-*co*-ethylene dimethacrylate monolithic capillary column has been prepared and used for simple, green, efficient and reliable isocratic elution nano-LC–UV procedure to assess PAR and CZN in their pharmaceutical combination. When compared to the standard LC procedures, the most important advantages of the proposed method are that its high recovery rates, low cost and green analytical approach with a mobile phase consumption of only 0.06 mL/h. The method was validated and showed good accuracy and precision. Therefore, this methodology based on application of capillary columns in nano-LC is highly recommended and might be suitable to be used for routine analysis of drugs as well as for research purposes.

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