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ORIGINAL ARTICLE / ÖZGÜN MAKALE

GREEN PROCEDURE INDEX ASSESSMENT OF THE NOVEL STABILITY-INDICATING RP-HPLC METHOD FOR THE DETERMINATION OF CAPTOPRIL FROM PHARMACEUTICAL DOSAGE FORM

FARMASÖTİK DOZAJ FORMUNDAN KAPTOPRİL TAYİNİ İÇİN YENİ STABİLİTE-GÖSTERGELİ RP-HPLC YÖNTEMİNİN YEŞİL PROSEDÜR İNDEKSİ DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: *In this study, it was aimed to develop a novel reverse-phase liquid chromatography method for the ultra-sensitive determination of the antihypertensive drug captopril, using paracetamol, which is the common pain killer, as the internal standard. Optimization of all experimental conditions including composition of mobile phase, flow rate, and column temperature was carried out step by step, and the method validity of the developed method was examined according to international validation guidelines. Calibration range, linearity, the limit of determination, the limit of quantification, robustness, accuracy from commercial tablet samples, and method stability were examined in detail. In addition, the greenness profile for the developed method was assessed with the Green Analytical Procedure Index and Analytical Greenness Calculator techniques, which are frequently used in the literature.*

Material and Method: *The chromatographic method was conducted with an XBridge C18 column (25 cm x 4.6 mm ID; 5 µm) packed with fully porous silica materials. All analyses were performed isocratically with a mobile phase containing acetonitrile:5 mM, pH 7.0 ammonium acetate solution (50:50, v/v) at a flow rate of 1.5 ml min^{-1}. The injection volume was 5 µl, and the column was kept at 25°C in a column oven. The column eluate was monitored at 220 nm. Under optimized conditions, retention times of captopril, and paracetamol were approximately 1.59, and 2.0 min, respectively.*

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Result and Discussion: *This study described a fully validated, simple, sensitive, accurate, linear, precise, and reproducible reversed-phase liquid chromatography method for the determination of captopril in tablet samples. Under optimal experimental conditions, the linear range was found in the range of 0.5-200 µg ml^{-1} and the correlation coefficient was greater than 0.99. Method precision was acceptable, with coefficients of variation between 0.05% and 0.61%. In addition, as a result of the recovery studies carried out on the tablet samples, the accuracy was found to be within satisfactory limits between 99.45% and 102.55%. Moreover, the greenness profile of the developed method also showed that the method is environmentally friendly.*
Keywords: *Analytical greenness assessment, captopril, HPLC, optimization, validation*

ÖZ

Amaç: *Bu çalışmada, yaygın bir ağrı kesici olan parasetamol iç standart olarak kullanarak antihipertansif ilaç kaptopril'in ultra-hassas tayini için yeni bir ters fazlı sıvı kromatografi yönteminin geliştirilmesi amaçlanmıştır. Mobil faz bileşimi, akış hızı, kolon sıcaklığı gibi tüm deneysel koşulların optimizasyonu adım adım gerçekleştirilmiş ve geliştirilen yöntemin yöntem geçerliliği uluslararası validasyon kılavuzlarına göre incelenmiştir. Kalibrasyon aralığı, doğrusallık, tespit limiti, tayin limiti, sağlamlık, ticari tablet numunelerinden doğruluk ve metot stabilitesi detaylı olarak incelenmiştir. Ayrıca geliştirilen yöntemin yeşillik profili, literatürde sıklıkla kullanılan Yeşil Analitik Prosedür İndeksi ve Analitik Yeşillik Hesaplayıcı teknikleri ile değerlendirilmiştir.*
Gereç ve Yöntem: *Kromatografik yöntem, tamamen gözenekli silika materyaller ile doldurulmuş bir XBridge C18 kolonu (25 cm x 4.6 mm ID; 5 µm) ile gerçekleştirildi. Tüm analizler, 1.5 ml dk^{-1} akış hızında asetonitril:5 mM, pH 7.0 amonyum asetat çözeltisi (50:50, v/v) içeren bir mobil faz ile izokratik olarak yapıldı. Enjeksiyon hacmi 5 µl idi ve kolon, bir kolon fırınında 25°C'de sabit tutuldu. Kolon eluatı 220 nm'de izlendi. Optimize edilmiş koşullar altında, kaptopril ve parasetamolün alıkonma süreleri sırasıyla yaklaşık 1.59 ve 2.0 dakika olmuştur.*
Sonuç ve Tartışma: *Bu çalışma, tablet numunelerinde kaptopril tayini için tam olarak doğrulanmış, basit, hassas, doğru, doğrusal, kesin ve tekrar üretilebilir bir ters fazlı sıvı kromatografi yöntemini tanımlamıştır. Optimal deneysel koşullar altında lineer aralık 0.5-200 µg ml^{-1} aralığında bulundu ve korelasyon katsayısı 0,99'dan büyüktü. Yöntem kesinliği, %0.05 ile %0.61 arasındaki varyasyon katsayıları ile kabul edilebilir düzeydeydi. Ayrıca tablet numuneleri üzerinde yapılan geri kazanım çalışmaları sonucunda doğruluğun %99.45 ile %102.55 arasında tatmin edici sınırlar içinde olduğu görülmüştür. Ayrıca geliştirilen yöntemin yeşillik profili de yöntemin çevre dostu olduğunu göstermiştir.*
Anahtar Kelimeler: *Analitik yeşillik değerlendirmesi, HPLC, kaptopril, optimizasyon, validasyon*

INTRODUCTION

A serious medical disease called hypertension raises a person's risk of developing a number of problems that could impair the operation of their heart, brain, kidneys, and other vital organs. World Health Organization (WHO) data show that approximately 1.28 billion people between the ages of 30-79 have hypertension. Reports also show that 972 million people will be added to this prevalence in 2025. Hypertension treatment is challenging, particularly for people over 60. Because the pharmacological effects of initial conventional doses of antihypertensive drugs are sensitive to these patients [1,2]. Chemically known as ((2S)-1[(2S)-S-2-methyl-3-sulfanylpropanoyl]pyrrolidine-2-carboxylic acid), captopril (CAP) is a frequently used anti-hypertensive drug that is also used to treat different diseases such as rheumatoid arthritis, diabetic nephropathy, and vascular diseases. To keep the body's blood pressure stable, CAP is utilized as an angiotensin-converting enzyme inhibitor. However, agranulocytosis, teratogenicity, acute renal failure, proteinuria, angioedema, hyperkalemia, taste changes, proteinuria, postural hypotension, diarrhea, nephrotic syndrome, and skin rashes are some of the negative side effects of excessive CAP use in humans [3-6]. For this reason, CAP as an anti-hypertension drug should be analyzed carefully because it is frequently used in the treatment of diseases and because of its negative effects on the human body. Looking at the research in the literature, it is clear that several traditional approaches have been successfully used to identify the presence of CAP in pharmaceutical products and human fluid samples including spectrophotometric [7], Raman

spectroscopy [8], capillary electrophoresis [9], electrochemical [10-13], high-performance liquid chromatography (HPLC) [14-18]. HPLC is the most widely utilized method in the pharmaceutical industry for the manufacturing, development, and analysis of pharmaceuticals, particularly in quality control laboratories, as can be shown from literature studies. However, HPLC still uses a lot of organic solvents that can harm the environment and produce a lot of waste that needs to be disposed of, which poses a threat to worker safety and has an adverse effect on the environment. The purpose of this study is to present a novel chromatographic technique for CAP determination that can be viewed as a commonly employed, environmentally acceptable replacement using less organic solvent. This study focuses on how easily less harmful and environmentally friendly solvents can replace traditional mobile phases by enhancing technique performance.

MATERIAL AND METHOD

Reagents and Materials

The CAP and paracetamol (PAR), which was used as an internal standard (IS), were purchased from Sigma-Aldrich (St. Louis, MO). Ammonium acetate and potassium bromide were purchased from Sigma-Aldrich (St. Louis, MO). Other reagents, including sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2) and were provided from Sigma-Aldrich (St. Louis, MO). All chemicals were analytical reagent grade. Chromatography grade acetonitrile and methanol were purchased from Sigma-Aldrich (St. Louis, MO). Glacial acetic acid and hydrochloric acid (HCl) were purchased from Merck (Merck KGaA, Darmstadt, Germany). A Milli-Q® system (Millipore, Milford, MA, USA) was used to obtain chromatographic grade water to prepare the required solutions. Pharmaceutical dosage form known as Kaptopril® was provided local pharmacy.

Instruments

The reversed-phase (RP)-HPLC method was developed using the Agilent 1100 series LC system, which is furnished with a degasser, quaternary pump, autosampler, and diode array detector (DAD). As the stationary phase, an XBridge C18 (25 cm x 4.6 mm ID; 5 µm) column from Waters in Milford, Massachusetts, USA, was used for the analysis. pH measurements were taken using an Orion 3 Star Plus benchtop pH meter from Thermo Scientific (USA).

Chromatographic Conditions

For the RP-HPLC study, a stationary phase of XBridge C18 (25 cm x 4.6 mm ID; 5 µm) with a detection wavelength of 220 nm was used in conjunction with a mobile phase in isocratic mode (1.5 ml min^{-1} of flow rate) that contained a mixture of acetonitrile: 5 mM Ammonium acetate (50:50; v/v). Using 1 M NaOH, the mobile phase's pH was adjusted up to 7.0. The prepared mobile phase was first degassed using an ultrasonic bath, and then these solutions were filtered through a 0.45 µm filter in a vacuum. About 25 minutes prior to the injection, the column was prepared, and the injection volume was set at 5 µl. By injecting potassium bromide, which was predetermined for each mobile phase composition, the dead time (t_0) was calculated.

Preparation of Solutions

To prepare a 1000 µg ml^{-1} stock solution of CAP and PAR, 5 mg of CAP or PAR was first weighed and dissolved in 5 ml of acetonitrile by the same procedure. For around 10 minutes, both solutions were allowed to dissolve in an ultrasonic bath. At 5°C ± 3°C, all solutions were stored in the refrigerator. Working solutions were arranged by diluting the stock solution with mobile phase in a range of 0.5 to 200.0 µg ml^{-1} while maintaining a constant PAR content of 100 µg ml^{-1}. To obtain the calibration plot, the linearity was established as the ratio of the peak area of the CAP against the peak area of the PAR. Related parameters and calculations were reported for this graph in the results and discussion section.

Pharmaceutical Dosage Form Analysis and Accuracy Study

Five Kaptopril® film-coated tablets (one tablet contains 25 mg of CAP) were precisely weighed and ground into a fine, homogenous powder in a mortar for the RP-HPLC analysis. This powder was

accurately weighted to represent one tablet's worth of substance, then deposited into a flask calibrated for 100 ml, volume completed using acetonitrile as a solvent, mixed for approximately 10 minutes, and then diluted to the desired volume using the acetonitrile. The clear solution was collected separately by filtration of this solution. A reasonable aliquot of the clear filtrate was taken, and the correct amount of PAR (100 μg ml^{-1}) was added before dilution with the mobile phase to provide the appropriate solutions. The associated regression equations were used to determine the CAP's content amount.

To demonstrate the applicability of the RP-HPLC method, by adding a specified amount of pure CAP in a pre-analyzed tablet, recovery studies were carried out. For this purpose, the dosage form of the tablet was supplemented with specified amounts of pure CAP (and at a 100 μg ml^{-1} PAR), and the mixtures were analyzed at optimal conditions. By comparing the concentration obtained from spiked samples with the additional concentration, the percent recovery was computed. In this way, to better understand how common excipients in tablet form affect chromatograms (such as tailing and broadening), the developed method was tested.

Preparation of Stressed Samples

According to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) recommendations, a forced degradation study on the bulk form of CAP was assessed in this study [19,20]. For this purpose, degradation experiments were applied for up to 6 hours to examine the effects of acidic hydrolysis (0.01 N and 0.1 N HCl), alkaline hydrolysis (0.01 N and 0.1 N NaOH), and oxidative degradation (3% and 30% H_2O_2). An air oven was set at 80°C for 6 hours to allow for the thermal degradation of a solid form of CAP. Additionally, for 6 hours at room temperature, the CAP solid form was exposed to 254 nm UV radiation. Prior to the examination of the forced degradation samples, a suitable blank was injected and 50 μg ml^{-1} of the constant concentration of solutions was used throughout.

RESULT AND DISCUSSION

Optimization of Chromatographic Conditions

In this study, it was aimed to determine CAP with lower analysis time and sharp peak shapes compared to other studies in the literature. To determine the optimal experimental conditions, the effects of wavelength, organic solvent type, mobile phase composition, pH of the buffer solution, temperature, and flow rate parameters were investigated. Moreover, the precision and accuracy of the developed method can be enhanced by using an appropriate IS. The use of IS also supports correcting fluctuations in the response of the detector. For this purpose, PAR was chosen as the most suitable IS. Initially, five different wavelengths were tested to obtain optimum high selectivity. When the peak heights and areas for CAP and PAR were evaluated, it was found that the most suitable wavelength for the analysis was 220 nm to obtain highly selective and sensitive results (Figure 1A). Organic solvents used as mobile phase components are directly related to the retention of substances in the column. For this purpose, experiments were carried out using methanol and acetonitrile. When methanol was utilized as the organic solvent, CAP was not retained because of its polar nature (logP: 0.34) (Figure 1B). By preparing various amounts of ammonium acetate concentration (5-40 mM), the concentration of the buffer solution was examined as a further parameter. Based on the shape and selectivity of the CAP peak, 5 mM was chosen for further investigation (Figure 1C). The ratio of organic solvent has a significant impact on the peak shape and retention in chromatographic investigations. Therefore, varying proportions of mobile phase compositions by buffer solution and acetonitrile in the range of 50-85% have been evaluated in studies. Finally, it was discovered that the ideal mobile phase composition consisted of 50% buffer solution and 50% acetonitrile (Figure 1D). According to the literature, CAP has pKa values of 3.7 (carboxyl group) and 9.8 (thiol group) [9], so different pH of the mobile phase was evaluated to achieve maximum retention. The buffer solution's pH of 7.0 was determined to be the optimal pH based on the resistance of the utilized stationary phase to pH (Figure 1E). As one of the important parameters, the column temperature was varied between 25°C and 45°C. Low temperatures resulted in better peak shape and shorter retention time. This is why 25°C was selected as the ideal column temperature (Figure 1F). Lastly, to obtain the best performance, the flow rate of the method was varied from 1.0 to 1.5 ml min^{-1}.

When the results are evaluated, the flow rate was set constant at 1.5 ml min^{-1} (Figure 1G).

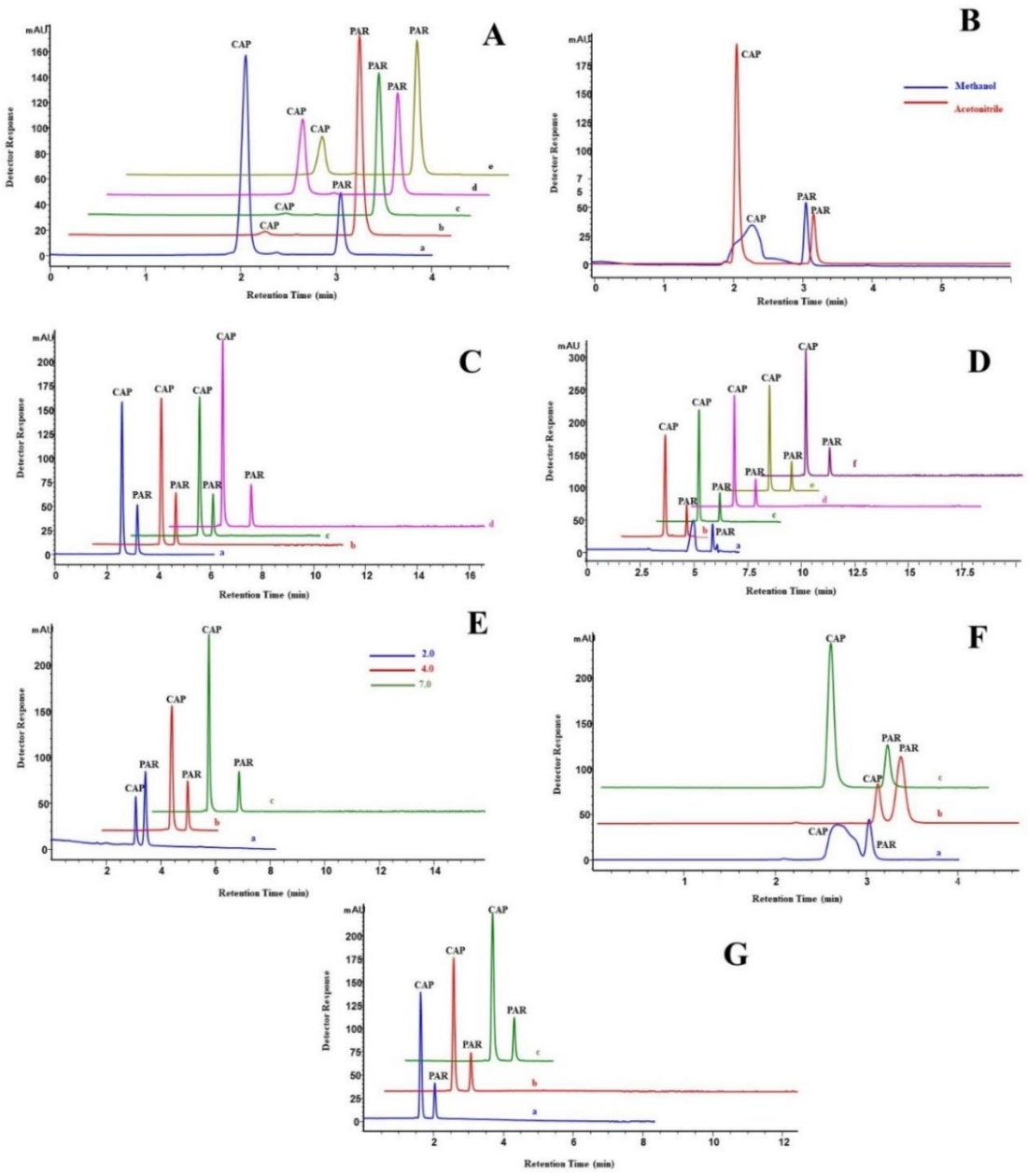

Figure 1. A) Effect of wavelength; 220 nm(a), 250 nm (b), 260 nm (c), 230 nm (d), 210 nm (e), **B)** Effect of organic solvent, **C)** Effect of buffer solution concentration; 10 mM Ammonium acetate (a), 20 mM Ammonium acetate (b), 40 mM Ammonium acetate (c), 5 mM Ammonium acetate (d), **D)** Effect of mobile phase composition; Acetonitrile: Ammonium acetate; (85/15; v/v) (a), (80/20; v/v) (b), (75/25; v/v) (c), (70/30; v/v) (d), (60/40; v/v) (e), (50/50; v/v) (f), **E)** Effect of pH of buffer solution, **F)** Effect of column temperature; 45°C (a), 35°C (b), 25°C (c); **G)** Effect of flow rate; 1.5 ml min^{-1} (a), 1.25 ml min^{-1} (b), 1.0 ml min^{-1} (c)

After the optimum conditions were determined, the system suitability test (SUT) parameters were evaluated based on the ICH guidelines for the determination and quantification of CAP. Under optimized conditions, the retention time of CAP was found as 1.59 min, and other parameters were presented in Table 1.

Table 1. SUT parameters in optimized conditions

Parameters	CAP	PAR
Retention time (min)	1.59	2.00
Capacity factor (k')	0.13	0.43
Resolution (R_s)	4.40	-
Theoretical plates (N)	3476	6004
Selectivity factor (α)	3.31	-
Tailing factor	0.81	0.82

Analytical Performance and Validation

In this study, the developed RP-HPLC method was validated in accordance with standard validation guidelines [19-21]. A series of standard solutions (eleven different concentrations) containing CAP (0.5-200 µg ml^{-1}) and PAR (100 µg ml^{-1}) were analyzed with the optimized method (Figure 2). The linearity of the developed RP-HPLC method was evaluated by a correlation coefficient ($R^2 > 0.999$) of linear in the stated ranges. The limit of detection (LOD) and limit of quantification (LOQ) were evaluated to demonstrate the sensitivity of the developed RP-HPLC method based on the ICH guideline.

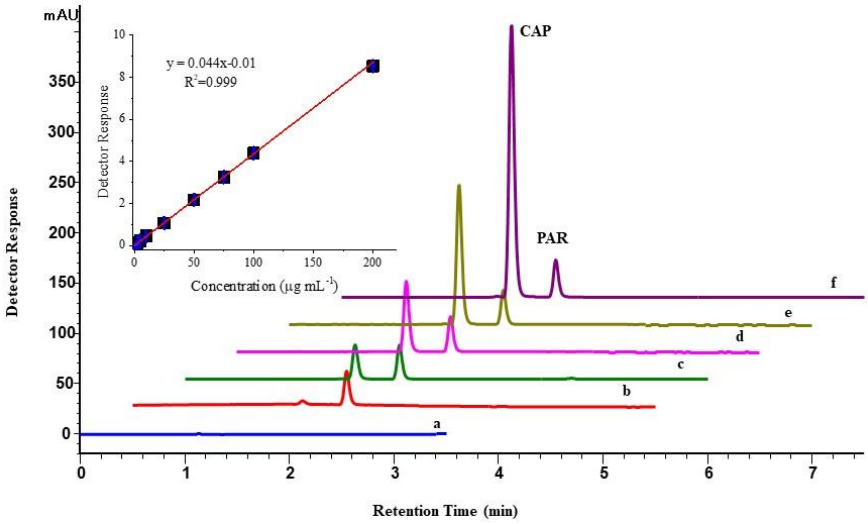

Figure 2. Calibration chromatograms of CAP mobile phase as a blank (a), 2.5 µg ml^{-1} (b), 10 µg ml^{-1} (c), 50 µg ml^{-1} (d), 100 µg ml^{-1} (e), 200 µg ml^{-1} (f) in optimized conditions (Inset: Calibration curve for CAP analysis)

The 3 s/m and 10 s/m criteria, where 's' was taken as the standard deviation of the peak area of the lowest concentration and, 'm' was taken as the slope of the associated calibration curve, were used to determine LOD and LOQ, respectively. Based on the calculations, LOD was determined to be 0.51 ng ml^{-1} and LOQ was determined to be 1.56 ng ml^{-1}. Furthermore, the accuracy of the developed method was also tested intraday and between days by injecting three different calibration solution levels on the same day and three days in a row, respectively. The relative standard deviation (RSD%) was determined as a percentage obtained to be acceptable and less than 2%. Table 2 provides a summary of the calibration results' characteristics and the associated validation parameters. Moreover, the analysis of CAP from tablet samples was also achieved and reported in terms of labeled and found amount in table 2.

Table 2. Validation parameters for the determination of CAP by the developed RP-HPLC method

Parameters	CAP
Linear concentration range (µg ml^{-1})	0.5-200
Slope of calibration curve	0.044
Intercept of calibration curve	-0.01
Correlation Coefficient	0.999
Standard error of slope	5.41×10^{-4}
Standard error of intercept	2.72×10^{-4}
LOD (µg ml^{-1})	5.10×10^{-4}
LOQ (µg ml^{-1})	1.56×10^{-3}
Within-day Precision (RSD %)*	0.05
Between-day Precision (RSD %)*	0.61
Labeled amount (µg ml^{-1})	20.00
Amount found (µg ml^{-1})	19.68
RSD (%)*	0.63
Bias (%)	1.59

* Each result was obtained by means of five experiments

The method accuracy was examined according to recovery experiments from the tablet dosage form of CAP. After analyzing the known amount of CAP in tablet form, these samples were spiked with the bulk CAP solution by rates ranging from 25–150% (Figure 3). The recovery data were shown together with the RSD% and Bias% after each measurement was carried out 5 times. The recoveries were found in the range between 99.45%–102.55% for the commercial dosage form, and all results were tabulated in Table 3.

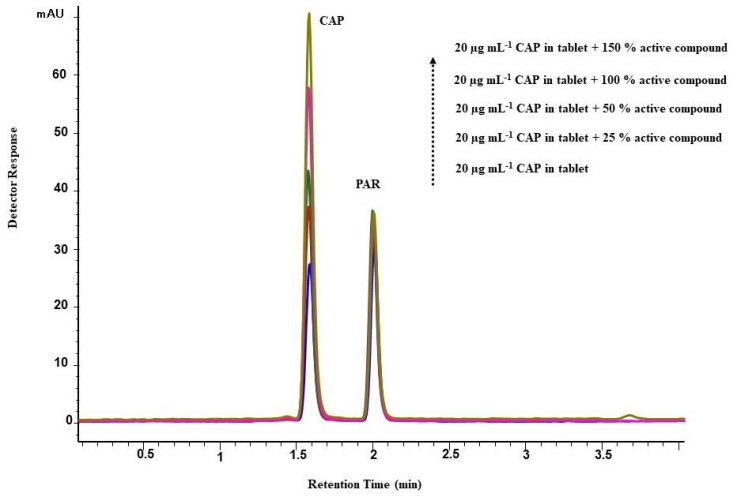

Figure 3. Recovery chromatograms in optimized conditions

Table 3. The accuracy results of the CAP determination in tablet dosage form by the developed RP-HPLC method

Parameters	Accuracy Levels			
Added (µg ml^{-1})	5 *(for 25%)*	10 *(for 50%)*	20 *(for 100%)*	30 *(for 150%)*
Found (µg ml^{-1})	5.64	10.14	20.32	28.77
Recovery (%)	102.55	100.45	100.80	99.45
RSD% of recovery*	1.16	0.86	0.64	0.38
Bias (%)	-2.55	-0.45	-0.80	0.55

* Each result was obtained by means of five experiments

The robustness of the developed method was examined as part of validation studies by assessing the bulk form of CAP under the deliberate modifications of the optimal conditions. For this assay, 50 µg ml^{-1} of standard CAP was used. The resulting RSD% values (<2) were used to evaluate the deliberate changes in the wavelength (±2 nm), the flow rate of the developed method (±0.1 ml min^{-1}), and the temperature of the column (±2°C). The robustness study's findings show that minor adjustments to the optimal conditions had little impact on the developed chromatographic method that had been adjusted (Table 4).

Table 4. Robustness results for CAP determination

Changed Parameters	R_t (min)	Response (concentration µg ml^{-1})
Wavelength: 220 nm	1.590	49.79
SD	0.002	0.21
RSD%	0.13	0.42
Wavelength: 218 nm	1.590	52.54
SD	0.003	0.36
RSD%	0.21	0.69
Wavelength: 222 nm	1.589	48.17
SD	0.001	0.31
RSD%	0.08	0.64
Flow rate: 1.50 ml min^{-1}	1.590	49.79
SD	0.002	0.21
RSD%	0.13	0.42
Flow rate: 1.40 ml min^{-1}	1.690	52.74
SD	0.008	0.53
RSD%	0.48	1.00
Flow rate: 1.60 ml min^{-1}	1.485	53.20
SD	0.004	0.45
RSD%	0.27	0.85
Temperature: 25°C	1.590	49.79
SD	0.002	0.21
RSD%	0.13	0.42
Temperature: 23°C	1.594	51.80
SD	0.004	0.54
RSD%	0.24	1.03
Temperature: 27°C	1.589	50.43
SD	0.006	0.25
RSD%	0.39	0.49

Finally, forced degradation studies were carried out to produce possible degradation products, and the specificity of the developed RP-HPLC method was investigated among the possible interferences. Table 5 and Figure 4 include the findings of the degradation studies. Under drastic conditions including 0.1 N HCl, 0.1 N NaOH, and 0.3% H_2O_2 within 5 min. CAP completely degraded. When the CAP was exposed to 0.01 N HCl for 6 h at room temperature, the acidic hydrolysis was carried out, and degradation of CAP was found 28% at the end of this period. In basic hydrolysis with 0.01 N NaOH, only 7% of CAP was degraded. Under oxidative degradation using 0.03% H_2O_2, almost total CAP degraded gradually over the end of 6 hours. Moreover, when photodegradation and thermal degradation were investigated, it can be said that the CAP did not degrade for 6 hours.

Moreover, there are many research studies for the determination of CAP using HPLC. From the reported methods, CAP was detected in human serum, human plasma, and tablets. This novel RP-HPLC method is compared with the reported methods, as shown in Table 6. Consequently, it can be said that the more sensitive RP-HPLC method, which was less time-consuming and has good recovery values, was developed.

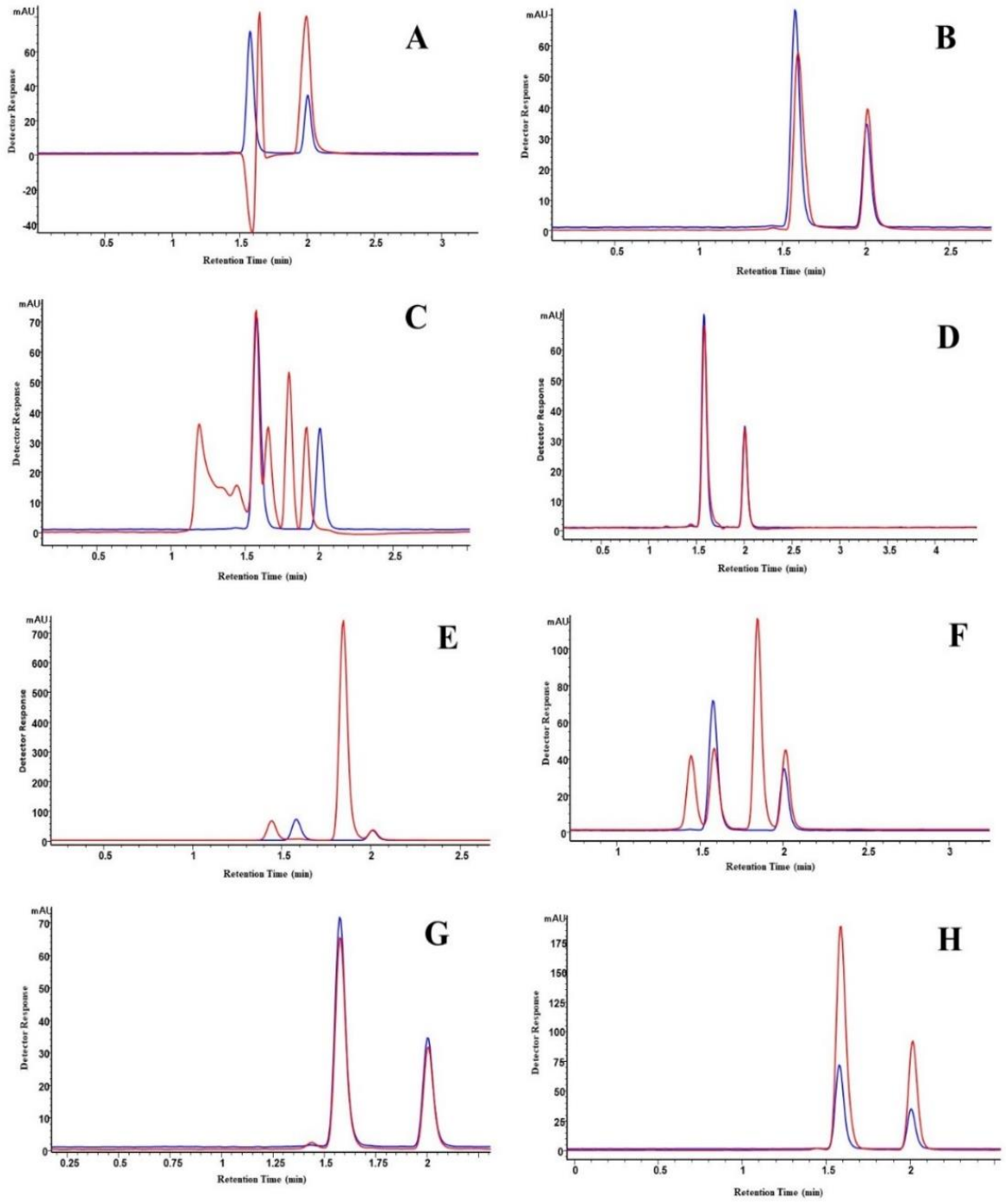

Figure 4. Chromatograms for acid degradation after 12 h; A: 0.1 N HCl, B: 0.01 N HCl, alkaline degradation; C: 0.1 N NaOH, D: 0.01 N NaOH, oxidative degradation; E: 0.3% H_2O_2, F: 0.03% H_2O_2, G: photodegradation, H: thermal degradation (blue line: original chromatograms, red line degradation chromatograms)

Table 5. % Degradation results for CAP

Degradation type	Degradation condition	% Degradation
Acid degradation	HCl (*0.1 N*), after 5 min	100 %
	HCl (*0.01 N*), after 5 min	22 %
	HCl (*0.01 N*), after 1 h	22 %
	HCl (*0.01 N*), after 3 h	26 %
	HCl (*0.01 N*), after 6 h	28 %
Alkaline degradation	NaOH (*0.1 N*), after 5 min	100 %
	NaOH (*0.01 N*), after 5 min	4 %
	NaOH (*0.01 N*), after 1 h	4 %
	NaOH (*0.01 N*), after 3 h	7 %
	NaOH (*0.01 N*), after 6 h	7 %
Oxidative degradation	H_2O_2 (*0.3%*), after 5 min	100 %
	H_2O_2 (*0.03%*), after 5 min	8 %
	H_2O_2 (*0.03%*), after 1 h	33 %
	H_2O_2 (*0.03%*), after 3 h	76 %
	H_2O_2 (*0.03%*), after 6 h	95 %
Photodegradation	After 5 min	1 %
	After 1 h	6 %
	After 3 h	7 %
	After 6 h	7 %
Thermal degradation	After 5 min	2 %
	After 1 h	3 %
	After 3 h	4 %
	After 6 h	4 %

Table 6. Comparison of studies for determination of CAP

Method	IS	Linear range (µg ml^{-1})	LOD/LOQ (µg ml^{-1})	Applications	R_t	References
HPLC-DAD	-	500-1200 100-240	-	Tablet	~ 2 min	[14]
HPLC-DAD	Thiol	30-130	LOD: 0.018 LOQ: 0.05	Tablet	7.21 min	[15]
HPLC–UV	2-propene-1-thiol	0.003-2	LOQ: 0.003	Human plasma	8.4 min	[16]
HPLC-PDA	-	5-35	LOD: 0.4763 LOQ: 1.4434	Bulk form	1.589 min	[17]
HPLC–UV	-	5.05-50.5	LOD: 1.13 LOQ: 3.394	Bulk form	~ 12 min	[18]
HPLC–UV	-	2.5-250	LOD:0.145 LOQ: 0.441	Tablet and human serum	4.78 min	[22]
HPLC–UV	-	30-300	LOD:0.204 LOQ: 0.620	Tablet and human serum	1.09 min	[23]
LC–UV	-	0.25-25	LOD:0.0013 LOQ: 0.0042	Human serum	2.80 min	[24]
HPLC–UV	-	0.25-200	LOD:0.08 LOQ: 0.25	Tablet	~ 2 min	[25]
RP-HPLC-DAD	PAR	0.5-200	LOD: 0.00051 LOQ: 0.00156	Tablet	1.60 min	This study

Green Assessment Using Analytical Greenness Calculator (AGREE) and Green Analytical Procedure Index (GAPI)

In the literature, the greenness of the developed analytical methods is evaluated using a variety of tools. In this work, the greenness of the RP-HPLC method for CAP was assessed using two different online software. For this purpose, covering the entire procedure from sample preparation to the end of the analysis, the Green Analytical Procedure Index (GAPI) based on the 12 principles of green analytical chemistry (SIGNIFICANCE) and the newly suggested Analytical Greenness Calculator (AGREE) techniques were used. These evaluations have the advantages of being quick, easy, efficient, and producing clear results. The AGREE and GAPI approaches display the evaluation as a graph that resembles a clock, with the final score and color representation in the centre, which are the outcomes of the evaluation of the different criteria. While the fifteen pentagrams that make up GAPI each represent a different stage of the analysis process and are color-coded according to their influence on the environment, AGREE gives a numeric number from 0 to 1 for evaluation within the core pictogram [26-28]. As shown in Figure 5, AGREE has an overall score of 0.7 but has 8 yellow and 6 green pentagrams and a single red zone in all criteria in the GAPI assessment. These results showed the green effect of the developed new method on both GAPI and AGREE metrics for CAP analysis. In addition, when the developed RP-HPLC method was compared with the reported methods in the literature, it stands out with its shorter analysis time and is environmentally friendly [17,29,30].

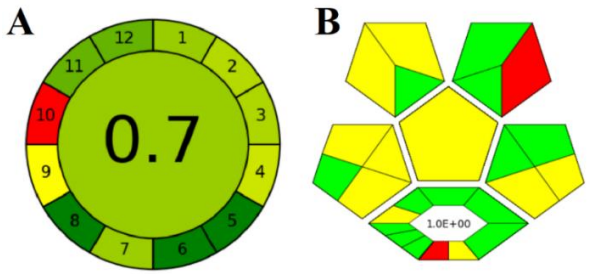

Figure 5. Greenness assessment of the developed HPLC method by AGREE (A) and GAPI (B) approaches

In conclusion, the correct use of drugs in recommended doses has important effects on the quality of the treatment of diseases and on human health. Therefore, it is necessary to monitor the active ingredient content in commercial dosage forms. In this research, a novel RP-HPLC method is introduced for the determination of the antihypertensive drug CAP. The method, which was validated by optimizing the parameters required for the analysis, was successfully applied to the tablet dosage form of CAP. Analyzing CAP in a short time like 1.59 minutes without any interference effect and with higher sensitivity has outperformed the methods in the literature. Also, looking at the greenness profile of the method, it can be said that the developed HPLC method was cheaper and environmentally friendly.

AUTHOR CONTRIBUTIONS

Concept: C.E., B.U.; Design: C.E., B.U.; Control: B.U.; Sources: B.U.; Materials: B.U.; Data Collection and/or Processing: C.E.; Analysis and/or Interpretation: C.E.; Literature Review: C.E.; Manuscript Writing: C.E., B.U.; Critical Review: C.E., B.U.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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