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Fast gradient high performance liquid chromatography method with UV detection for simultaneous determination of seven angiotensin converting enzyme inhibitors together with hydrochlorothiazide in pharmaceutical dosage forms and spiked human plasma and urine

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ABSTRACT

The development of a reversed phase liquid chromatographic method for the simultaneous determination of seven angiotensin converting enzyme (ACE) inhibitors; five drugs namely benazepril HCl (BZL), enalapril maleate (ENL), fosinopril sodium (FSP), lisinopril (LSP) and ramipril (RMP) and two metabolites captopril disulfide (CPD) and enalaprilat (ENT) together with hydrochlorothiazide (HCT) is described. The method can serve as a substitute for many published papers for the analysis of the targeted compounds with or without hydrochloothiazide in pharmaceutical formulations as well as in spiked human plasma and urine samples. The method utilizes a simple gradient procedure for the separation in a 11 min run time using acetonitrile aqueous ammonia buffer (pH 9) solution and an Extend RP-C18 (25 μm particle size, 4.6 mm × 250 mm, Agilent) HPLC column. The effluent was monitored on a UV detector at 215 nm. The effect of pH, solvent strength and analysis time on the peak shape and quantification were carefully studied in order to optimize the method. Adopting the proposed procedure, the analytes produce well-shaped peaks with good linear relationship over the investigated concentration ranges. The limits of detection (LOD) and limits of quantification (LOQ) from standard drug solutions lie in the range of 17–64 and 56–212 ng mL^{-1}, respectively. Correlation coefficient values (r) higher than 0.997 were obtained for all the studied drugs in spiked human plasma and urine samples. The intra-day and inter-day precision of the method was evaluated with relative standard deviation values being satisfactory for their purposed analysis. The method was validated with respect to specificity, recovery, accuracy, precision and linearity.

1. Introduction

Angiotensin converting enzyme (ACE) inhibitors have been available for more than two decades for clinical use to treat hypertension and congestive heart failure. They work by blocking of renin–angiotensin system, inhibiting the conversion of the inactive angiotensin I to the powerful vasoconstrictor and stimulator of aldosterone release angiotensin II and thereby reduce the blood pressure. They may also prevent the destruction of the powerful vasodilating kinins [1,4]. ACE inhibitors are effective for control of blood pressure, congestive heart failure, and prevention of stroke and hypertension, or diabetes-related kidney damage. They are especially important because they have been shown to prevent early death resulting from hypertension, heart failure or heart attacks [2,3]. With the exception of captopril and LSP, all other orally administrated ACE inhibitors are prodrugs [1,4], which require hepatic activation to form pharmacologically active metabolites. The metabolites are usually diacid structures being eluted in the first part of the chromatogram before their inherent prodrugs.

A wide range of methods was reported for analysis of ACE inhibitors in pharmaceutical formulations and biological fluids. Bioanalytical [5,6], potentiometric [7] and voltametric techniques [8,9] were reported. Nevertheless, these methods were either expensive, or not suitable for analysis of drug mixtures or metabolites. On the other hand, many developed HPLC assays with UV [10–13] or fluorescence [14,15] detection as well as capillary electrophoresis experiments for separate or simultaneous analysis of ACE inhibitors [16,17] were more specific. Most of them required a complicated sample pretreatment and time-consuming chromato-

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graphic separation as well as being not sufficiently sensitive for pharmacokinetic analysis of the drugs. GC and LC with mass spectrometric detection [18–20] were widely applied to ACE inhibitors determination in biological fluids. Being highly sensitive methods, nevertheless still require sophisticated equipments that were not available in many laboratories.

On the other hand, only few methods were reported for simultaneous determination of ACE Inhibitors [10,12,13,16–20], however they usually need a long time of separation increasing broadness of the peak and substantially worsen the limits of detection and quantification of the drugs or determining a few members of the drugs, like one drug and its metabolite. Only one CE method [16] was published in the year 2000 allowing analysis of 8 members of the group in more than 20 min using two separation buffers, as the separation was not possible in a single run, without dealing with the drug metabolites or separation in biological samples. Another HPLC UV method [13] allowing separation of 5 members of the group with relatively high quantification limits and more than 25 min run without dealing with their biological analysis.

The detection method most utilized for HPLC is UV detection due to super signal stability [21]. Lacking of selectivity could be always overcome by accompanying with separation media like HPLC as well as acceptable limits of sensitivity could be achieved by adopting some procedures of solid phase extraction or post-extraction sample evaporation.

The aim of the present method is to simultaneously separate and accurately determine 7 drugs belonging to the family of ACE inhibitors together with HCT in a short run time with UV detection adopting one procedure and the same chromatographic conditions for their analysis in tablets, spiked human urine and plasma with acceptable limits of sensitivity. The developed method can serve well as a substitute for many published methods for analysis of the drugs with or without HCT saving time, reagents, tools and elaboration.

2. Experimental

2.1. Reagents and materials

All drug standards were purchased from China's National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). L-Aspartyl-L-phenylalanine methyl ester (internal standard, IS) was obtained from Acros Organics (NJ, USA). HPLC-grade acetonitrile was obtained from Tedia Company (Fairfield, USA). Other reagents were of analytical reagent grade. Ammonia solution obtained from Jinsha Chemicals (Shantou, China). Hydrochloric acid was delivered from Jinlu Chemicals (Shanghai, China). Ultra-pure water was prepared by the Millipore Milli-Q Academic water-purification system (Molsheim, France). Drug-free human plasma was supplied by the Blood Bank of Zhejiang Province. 10-fold fresh human urine samples were used for the analysis.

2.2. Chromatographic system

The chromatographic system was Dionex Ultimate 3000 series (USA) equipped with a quaternary pump, a temperature controlled auto injector. The Ultimate 3000 quad-wavelength ultraviolet detector was set at 215 nm for all drugs. The separation was performed at ambient temperature on a 4.6 mm × 250 mm, 5 μm Zorbax extend C18 analytical column (Agilent Technologies, USA). The data collection and analysis were run with Chromeleon client software (Dionex, USA). Yamei pH meter (Yamei electrical instruments, Hangzhou, China) was used to measure the pH. C18 SPE cartridges (300 mg/1 mL 40 μm particle diameter packing) were purchased from Tianjin Fuji Science and Technology Co., Ltd., China.

Table 1
Optimum gradient elution profile for the simultaneous separation of studied drugs.

Step	Time (min)	Solvent A[a]	Solvent B[b]
Analysis[c]	0	91	9
	2.5	91	9
	2.5	80	20
	8.5	40	60
Re-equilibration	8.5	91	9
	14	91	9

[a] Solvent A, 25 mM ammonia buffer (pH 9).
[b] Solvent B, acetonitrile.
[c] UV acquisition at 215 nm in the time 0–11 min.

The mobile phase was 25 mM ammonia buffer (pH 9) acetonitrile mixture. The mobile phase was used in a gradient mode according to the profile shown in Table 1. Ammonia buffer was prepared as 25 mM by diluting 1.7 mL concentrated ammonia solution in 1 L container vessel to about 900 mL, adjusting the pH to 9 with 0.5 M HCl and completing to volume with distilled water. The flow rate was 1.0 mL/min.

2.3. Preparation of stock solutions, calibration standards

Standard stock solutions of each analyte and the IS were prepared separately as 20 mg% in distilled water (except RMP and CPD being first dissolved in about 10% of the final volume acetonitrile or methanol, respectively, before dilution to mark with water) and stored at 4 °C for a maximum of two weeks. Working solutions were prepared by appropriate dilution of stock solutions using distilled water and stored at 4 °C. Calibration standards for each drug in the concentration range from 0.1 to 4 μg mL^{-1} were prepared by spiking 0.45 mL of blank plasma or urine with 50 μL of the appropriate working solution. QC samples were prepared in bulk at the concentration of 0.2, 1 and 4 μg/mL and stored at −20 °C.

2.4. Sample preparation

2.4.1. Drug tablets

A powdered sample of each drug tablets was extracted for 15 min under ultrasonication using the same solvent used for preparation of stock standards, then completed to volume. Working solution containing concentrations lying in the linear range of the regression equations were prepared by dilution with water and 30 μL were injected to the system. The content of the drug samples was calculated by referring to its linear regression equation.

2.5. Analysis of biological samples

2.5.1. Using acetonitrile for sample pretreatment

Aliquots of 500 μL plasma or urine samples were diluted with 250 μL water in a 4 mL polypropylene tube. 50 μL IS solution (10 μg mL^{-1}) were then added followed by 1200 μL acetonitrile. The mixtures were vortex mixed for about 30 s then centrifuged at 16,000 × *g* for about 30 min. 1800 μL of the clear supernatant were pipetted and was evaporated under vacuum in a stream of nitrogen at about 40 °C to near dryness and the volume was completed to 225 μL with water. The sample was filtered through a 0.45 μm syringe filter if necessary then 30 μL were directly injected to the system.

Table 2
Collective calibration data of the studied drugs standard mixture by the proposed method.

Drug	Regression equations[a]		Calibration coefficient	Linear range ($\mu g\,mL^{-1}$)	LOD[b] ($ng\,mL^{-1}$)	LOQ[c] ($ng\,mL^{-1}$)
	b	*a*				
BZP	1.1570	−0.0204	0.9999	0.09–8.00	25	82
CPD	0.2281	0.0044	0.9990	0.25–8.00	64	212
ENL	0.4537	−0.0041	0.9997	0.14–8.00	42	139
ENT	0.5661	−0.0354	0.9998	0.12–8.00	34	114
FSP	0.6602	−0.0552	0.9991	0.15–8.00	44	145
LSP	0.4679	−0.0036	0.9999	0.12–8.00	36	120
RMP	0.5253	0.0072	0.9993	0.16–8.00	48	159
HCT	2.1327	−0.0080	0.9999	0.60–8.00	17	56

[a] With respect to $A = bC + a$, where C is the concentration in ($\mu g\,mL^{-1}$), A is the peak area of the drug, a is the intercept and b is the slope.
[b] Lower limit of detection.
[c] Lower limit of quantification.

2.5.2. Using solid phase extraction

To each 500 μL plasma or urine samples, 50 μL IS solution ($10\,\mu g\,mL^{-1}$) and 550 μL 50 mM HCl were added in a 4 mL polypropylene tube. The mixtures were vortex-mixed for 30 s, and then loaded to the SPE cartridge. The cartridge was preconditioned by washing with 3 mL water, 3 mL acetonitrile then another 2 mL water and finally 2 mL 50 mM HCl. After loading the sample, the cartridge was washed by 2 mL HCl 50 mM the dried by drawing air through under vacuum. The sample was then eluted using 1.2 mL of 5% 25 mM ammonia buffer pH 9 in acetonitrile. The collected eluent was evaporated under vacuum in a stream of nitrogen at about 40 °C to near dryness and the volume was completed to 250 μL with water. The sample was filtered through a 0.45 μm syringe filter if necessary then 30 μL were directly injected to the system.

2.6. Assay validation

The extraction recoveries of each drug and I.S. were determined by comparing the peak area of spiked extracted samples to those of standard solutions at same concentration. Calibration curves were constructed with plasma and urine standards spiked with 0.1, 0.2, 0.5, 1, 2, 4 μg/mL of each drug. During the method validation, calibration standards were independently prepared and measured on 3 consecutive days. The intra-day and inter-day precision and accuracy were determined based on pentuplicate measurements of QC samples at low, middle and high concentrations (0.2, 1 and $4\,\mu g\,mL^{-1}$). All samples were spiked with the drug standards on day 1, and then analyzed on five different days. The drugs concentrations in QC samples were calculated from the linear regression equation obtained on the same day.

3. Results and discussion

3.1. Development of the RP-HPLC method

On the way of optimizing the method, different experimental conditions like the pH, ionic strength, organic modifier, ion pairing reagents, temperature that could affect the results were carefully studied. Reversed-phase chromatographic conditions were found to be suitable to modulate the retention of all of the selected drugs. In analytical liquid chromatography, gradient elution is widely applied to improve the separation of mixtures by varying the solvent strength during the elution process. In the present work, the optimum gradient was selected through a large number of empirical attempts. Applications of the gradient with a growing elution force allowed to separate compounds with lower polarity and to reduce analysis time [22]. In order to shorten the analysis time, acetonitrile as HPLC solvent was used as an organic modifier due to its relatively high elution strength of strongly non-polar compounds like FSP. The gradient program was selected to achieve the maximum separation and sensitivity for an optimum elution profile and is presented in Table 1.

Another important factor was the pH of the used buffer of the mobile phase. In fact, the Extend-C18 columns incorporate a unique patented bidentate silane, combined with a double-endcapping process that protects the silica from dissolution at high pH—up to pH 11.5. Columns are best applied for separations of compounds that are either (1) basic and have little or no retention at low or intermediate pHs, (2) more stable or more soluble at high pH, or (3) basic and show poor peak shape at low or intermediate pH [24,25]. Therefore, different pH extending to the basic side including 2.5, 4, 7, 8, 9, 10 and 11 were feasibly tried. In acidic buffers, the studied drugs were strongly retained with peaks being broader and more or less overlapped, making the elution of the drug mixture not feasible in a single run with reasonable retention time. Moreover, using acetonitrile as an organic modifier, the peak splitting and broadening characteristic for proline peptide bond based compounds are strongly prominent at acidic pH. This peak deformation is especially noticed in case of ENL that gives two equally height characteristic non-base separated distinct peaks [23]. At intermediate pH; phosphate buffer pH 7 and tris buffer pH 8, an enhanced improvement of peak shape, drug resolution with shortening of the peak width and the total analysis time was gained, however, the maximum outcome was obtained at pH 9 using ammonia buffer. At this pH, ENL was eluted as a well-shaped peak that was well separated from RMP and BZP. Only RMP peak suffered a little tailing with no serious effect on its analytical estimation. No much improvement was obtained at higher pH up to 11; instead, the peak shapes and resolution were being slightly affected. Therefore, ammonia buffer pH 9 was selected for further investigations. 10, 25, 50 and 100 mM ammonia buffer concentrations were tried, 25 mM was chosen over the others as being the least concentration giving the desired effect. On the other hand, the column showed excellent durability during the analysis. It has been used by our laboratory for more than about 1000 runs at pH 9 during method optimization and actual analysis without any significant drop in its performance regarding separation and peak shape.

The use of mobile phase additives like triethylamine and 1-octanesulfonate (25 mM) at pH 2.5 and 7 were tried but no improvement in the peak shape was observed; instead a more pronounced bimodal peak shape with increased retention time was obtained. Tetrabutylammonium hydroxide (25 mM) was also tried at pH 7 with no improvement in the peak shape being attained. These findings were in accordance with the literature [23].

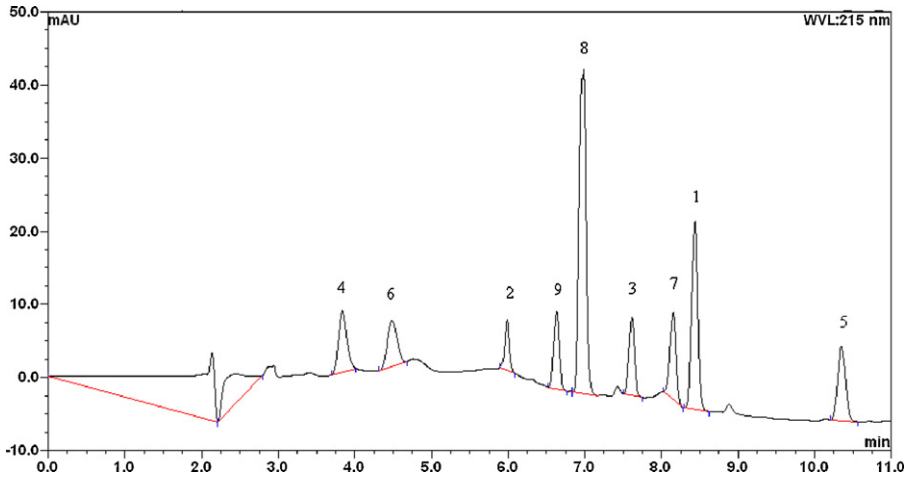

Fig. 1. (A) Representative typical HPLC–UV chromatogram of a combined standard drugs solution, 2 μg mL^{-1} each. (Chromatographic conditions as described in the text) BZP, 1; CPD, 2; ENL, 3; ENT, 4; FSP, 5; LSP, 6; RMP, 7; HCT, 8; IS, 9.

At temperatures higher than 40 °C up to 55 °C, ACE inhibitors were eluted as single peaks with broad baseline and relatively high peak width; this was reported in many previous studies [23]. Still, 60 °C, which is the maximum operating temperature for most HPLC columns, is not enough to get the same outcome of pH 9-elution reported in this work regarding peak shape and sharpness. Therefore, fast separation, efficient resolution and better peak shapes were achieved applying the proposed gradient pH 9-elution profile while ambient temperature was chosen in that work for more practical conditions.

Detection wavelength was set at 215 nm in order to have a good sensitivity for ACE inhibitors having low molar absorptivity values at higher wavelengths. A representative chromatogram of a combined mixture of the standard analytes is shown in Fig. 1.

3.2. Applications

The HPLC method was applied for the determination of the studied drugs in their commercial tablets and results were found in good agreement with the claimed content. Concentrations of the drugs were calculated by referring to the calibration equations obtained for pure standards shown in Table 2. Results for the analysis of different tablet dosage forms are shown in Table 3.

Analysis of spiked human plasma and urine samples were also successfully conducted. Calibration graphs were constructed for the studied drugs in spiked human plasma and urine samples according to the proposed procedures detailed in Section 2 using both protein precipitation and C18 solid phase extraction procedures (Table 4). Lower concentrations were also tried and better sensitivity and detection limits for the drugs in plasma and urine samples could be achieved by reconstituting drug residues after extraction and evaporation in lower injection volumes such as 100 μL but for more convenience and better baseline 250 μL was chosen. The assay could be more convenient for pharmacokinetic studies if however more sensitive detectors like MS were hyphenated with the procedure.

3.3. Assay validation

3.3.1. Specificity and recovery

Representative chromatograms of blank plasma and urine samples as well as their spiked parallels were shown in Figs. 2–5. It is clearly noticed that only ENT was strongly interfered by biological endogenous components as well as HCT in urine samples and cannot be determined by protein precipitation procedure. Other drug peaks were not interfered under optimized assay conditions. ENT and HCT can be determined by adopting the C18 SPE procedure with good accuracy as indicated from the results shown in Table 4. The SPE procedure could provide good interpretation for all drugs, however the sensitivity for LSP and ENT, being highly polar, was found to be affected due to low recovery of about 60.7 and 65.4 for ENT and 62.8 and 70.3 for LSP from plasma and urine samples, respectively. This can be clearly noticed from their LOQ and LOD being found much higher than in protein precipitation procedure due to significant loss during C18 SPE (Table 4). Other drugs have a good recovery from C18 SPE of more than 90%.

Table 3
Results for the analysis of the studied drugs in their commercial tablets.

Drug	Tablets	
	(%) Recovery[a]	(%) RSD
BZP[b]	99.30	1.31
ENL[c]	97.52	1.98
FSP[d]	101.20[d]	2.8
LSP[e]	100.09[e]	1.05
RMP[f]	97.60[f]	3.21
HCT[g]	97.20[g]	1.32

[a] The data are expressed as percentage of the claimed content and are average of 6 determinations.
[b] Beijing Nuohua Pharmaceutical Ltd. Co. under license from Novartis, Switzerland. (Batch no. X1338.)
[c] Packed by Hangzhou Moshadong Pharmaceutical corporation for Merck Sharp & Dohme, Australia. (Batch no. K3438.)
[d] Sino-American Shanghai Squib Pharmaceuticals Ltd., China. (Batch no. 0909086.)
[e] Livzon Group New Beijiang Pharmaceutical Co., Ltd., China. (Batch no. 0908009.)
[f] Sino-American Shanghai Squib Pharmaceuticals Ltd., China. (Batch no. K1269.)
[g] Changzhou Pharmaceutical limited corporation, China. (Batch no. 09032311.)

3.3.2. Calibration graphs

Regression parameters for pure drug standard mixtures including the slopes intercepts and regression coefficients are summarized for all the analyzed drugs in Table 2. These regression parameters were used for calculation of drugs concentrations in their pharmaceutical tablets.

Table 4
Results for the analysis of the studied drugs in spiked human plasma and urine samples.

Drug	Matrix	Protein precipitation procedure					C18 SPE extraction procedure				
		Accuracy (%)[b]	Precision R.S.D. (%)		LOQ[e]	LOD[f]	Accuracy (%)	Precision R.S.D. (%)		LOQ	LOD
			Intra-day[c]	Inter-day[d]				Intra-day	Inter-day		
BZP [a]	Plasma	97.74	3.40	4.82	50	20	99.66	2.54	5.24	50	20
CPD		98.99	3.87	5.59	125	50	98.22	2.95	5.21	125	50
ENL		100.55	3.65	3.60	75	30	97.14	2.97	4.64	75	30
ENT							103.55	3.66	3.70	120	40
FSP		99.01	2.52	3.58	75	30	101.55	2.81	2.89	75	30
LSP		100.47	3.55	3.70	75	30	97.08	5.30	9.11	120	40
RMP		101.06	3.84	4.91	100	35	102.77	3.36	4.24	100	35
HCT		99.15	1.93	2.30	40	15	100.57	1.44	2.18	40	15
BZP	Urine	100.14	2.44	5.67	50	20	97.98	2.43	3.33	50	20
CPD		96.99	4.80	5.02	125	50	100.55	4.73	5.43	125	50
ENL		102.28	3.61	4.74	75	30	102.61	3.59	4.23	75	30
ENT							98.74	3.16	5.02	120	40
FSP		99.59	1.98	3.47	75	30	99.66	2.91	3.78	75	30
LSP		102.23	3.72	5.09	75	30	98.79	1.98	2.22	120	40
RMP		99.33	4.01	6.18	100	35	98.39	2.78	3.66	100	35
HCT							99.31	2.85	3.62	40	15

[a] Results shown for a selected quality control concentration of 1 μg mL for all the studied drugs.
[b] Calculated as mean% recovery ($n = 5$).
[c] Expressed as relative standard deviation (RSD) ($n = 5$) on the same day and five different days, respectively.
[d] Expressed as relative standard deviation (RSD) ($n = 5$) on the same day and five different days, respectively.
[e] Calculated on the basis of a signal-to-noise ratio of 10.
[f] Calculated on the basis of a signal-to-noise ratio of 3.

For the analysis of the studied drugs in plasma and urine samples, an internal standard was necessary to compensate for any variability of the extraction recoveries during sample preparation. The choice of a suitable internal standard under optimal conditions was a real problem due to large number of peaks and short analysis time. After many investigations during which over about 50 compounds have been tried, only L-aspartyl-L-phenylalanine methyl ester ($t_R = 6.6$ min) was found suitable for that purpose. On the other hand, one drug analyte can work as an IS if the drugs are not simultaneously determined. Calibration equations were obtained by plotting each drug peak area to IS ratio vs. the concentration of each drug. In all cases, the intercepts were not significantly different from zero and correlation coefficients were greater than 0.997. Lower limits of detection (LOD) and quantification (LOQ) were also calculated from the obtained chromatograms for a signal-to-noise ratio (S/N) of 3 and 10 ($n = 8$), respectively. Results are shown in Table 4.

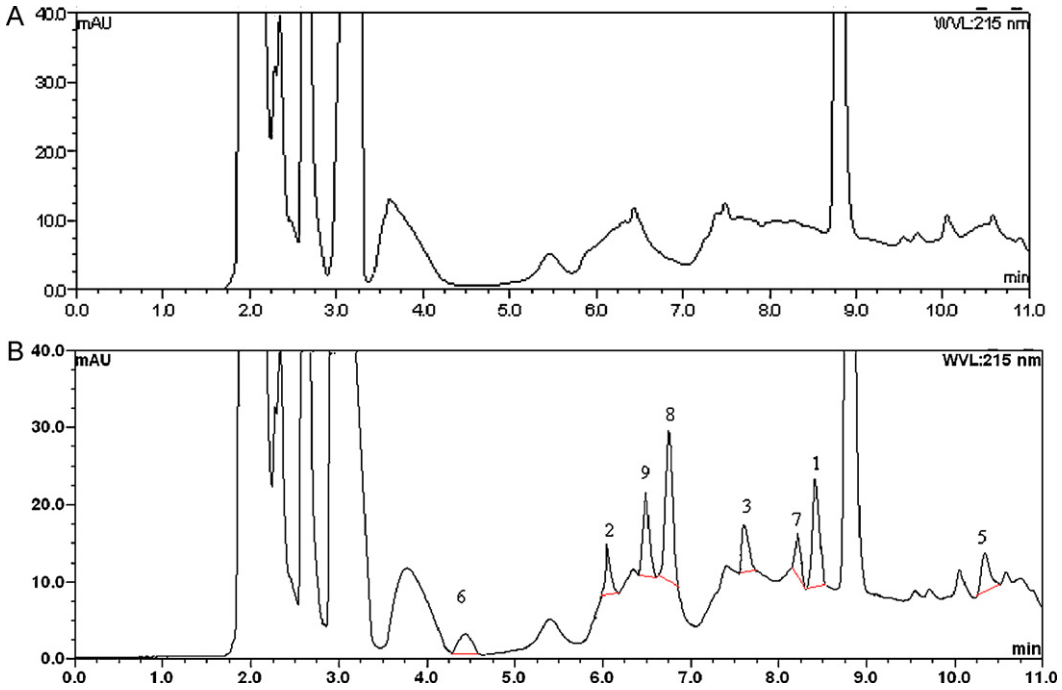

Fig. 2. (A) Representative typical HPLC–UV chromatograms of a blank human plasma sample from a healthy human volunteer (A) and that spiked with a combined standard drugs solution, 0.5 μg mL^{-1} each, IS 1 μg mL^{-1} (B); prepared according to the protein precipitation procedure. (Chromatographic conditions as described in the text) BZP, 1; CPD, 2; ENL, 3; ENT, 4; FSP, 5; LSP, 6; RMP, 7; HCT, 8; IS, 9.

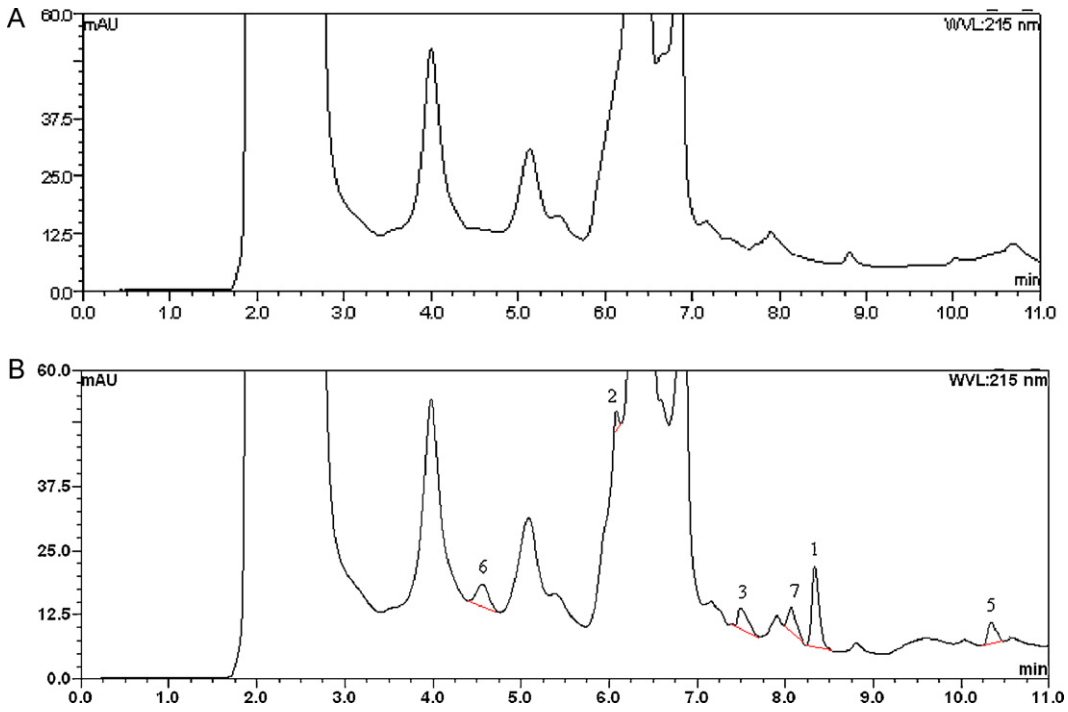

Fig. 3. (A) Representative typical HPLC–UV chromatograms of a blank human urine sample from a healthy human volunteer (A) and that spiked with a combined standard drugs solution, 0.5 μg mL^{-1} each, IS 1 μg mL^{-1} (B); prepared according to the protein precipitation procedure. (Chromatographic conditions as described in the text) BZP, 1; CPD, 2; ENL, 3; ENT, 4; FSP, 5; LSP, 6; RMP, 7; HCT, 8; IS, 9.

3.3.3. *Precision and accuracy*

The intra- and inter-day precision and accuracy of the developed method were evaluated with five replicates of samples at concentration of 0.2, 1, 4 μg/mL, and on five different days. The precisions ranged from 1.35 to 12.41% RSD for all the studied drugs at the three concentration levels which should be satisfactory to determine the drugs in a sample matrix. The method proved to be sufficiently accurate with mean% recovery in the range of 93.21–104.53 at the three concentration levels for all the studied drugs. Results for a chosen concentration of 1 μg/mL for all the studied drugs are shown in Table 4. The drugs concentrations in QC samples were calculated from the linear regression equation obtained on the same day.

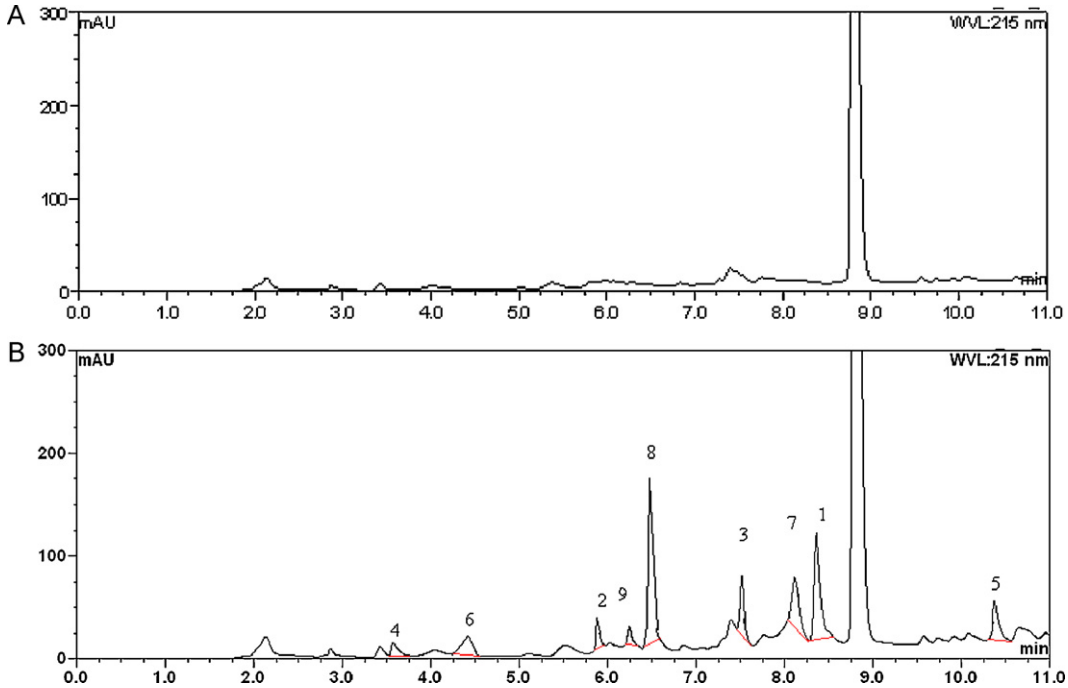

Fig. 4. (A) Representative typical HPLC–UV chromatograms of a blank human plasma sample from a healthy human volunteer (A) and that spiked with a combined standards solution, 4 μg mL^{-1} each, IS 1 μg mL^{-1} (B); prepared according to the C18 SPE extraction procedure. (Chromatographic conditions as described in the text) BZP, 1; CPD, 2; ENL, 3; ENT, 4; FSP, 5; LSP, 6; RMP, 7; HCT, 8; IS, 9.

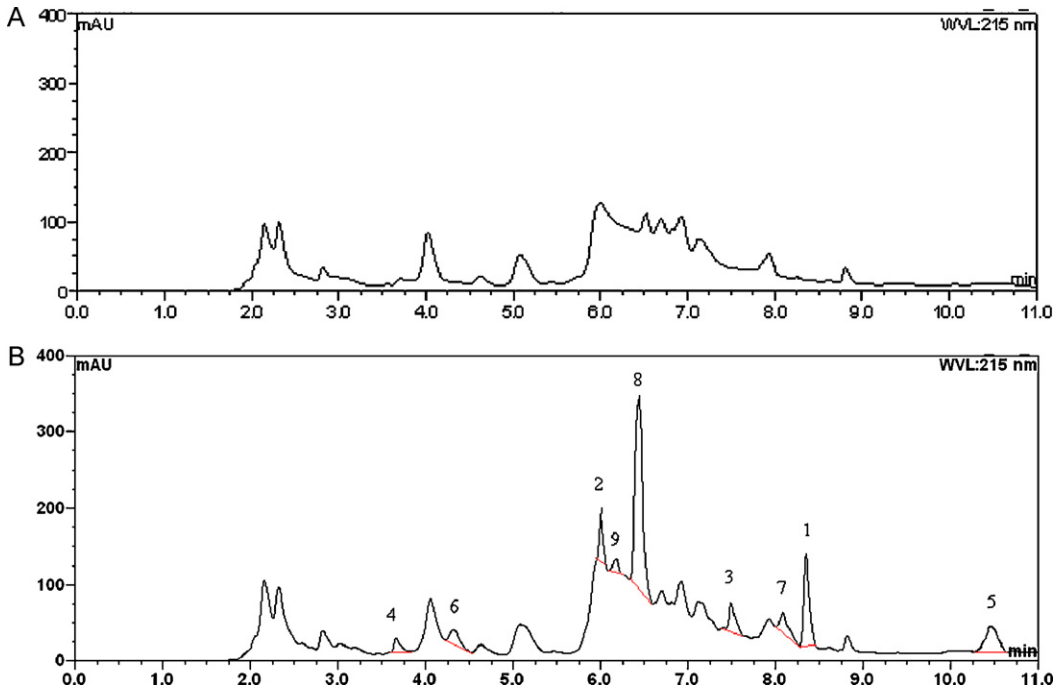

Fig. 5. (A) Representative typical HPLC–UV chromatograms of a blank human urine sample from a healthy human volunteer (A) and that spiked with a combined standards solution, 4 μg mL^{-1} each, IS 1 μg mL^{-1} (B); prepared according to the C18 SPE extraction procedure. (Chromatographic conditions as described in the text) BZP, 1; CPD, 2; ENL, 3; ENT, 4; FSP, 5; LSP, 6; RMP, 7; HCT, 8; IS, 9.

4. Conclusion

The method proposed in this work permits accurate measurements of 7 ACE inhibitors together with the mostly concurrent administrated drug hydrochlorothiazide in pharmaceutical formulations as well as spiked human plasma and urine with an acceptable sensitivity. The fundamental advantage for the procedure lies in its high simplicity, convenience, fast and effective sample preparation for the simultaneous one-procedure analysis of an important class of drugs either simultaneously or as single analytes making it more practical substitute for many published procedures saving reagents, tools, time and effort and making it an easy decision about applying the method in large pharmaceutical companies or medical laboratories. The suitability of the proposed procedure for analysis of the studied drugs in biological fluids with simple technique and equipment is a great outcome. Still the door is open for more promising approach if hyphenated with more sensitive detectors like MS to permit more convenient pharmacokinetics profile studies of sub-50 ng mL^{-1} drug plasma concentrations.

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