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Determination of losartan, telmisartan, and valsartan by direct injection of human urine into a column-switching liquid chromatographic system with fluorescence detection

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ABSTRACT

Column-switching high-performance liquid chromatographic (HPLC) method has been developed and validated for quantification of losartan, telmisartan, and valsartan in human urine. Urine samples were diluted on the extraction mobile phase (1:4, v/v) and a volume of 20 μL of this mixture were directly injected onto the HPLC system. The analytes were extracted from the matrix using an on-line solid-phase extraction procedure involving a precolumn packed with 25 μm C_{18} alkyl-diol support (ADS), and a solution 2% methanol in 5 mM phosphate buffer (pH 3.8) at a flow-rate of 0.8 mL/min for isolation and preconcentration of losartan, telmisartan, and valsartan. The enriched analytes were back-flushed after, onto the analytical column with a mixture of 5 mM phosphate buffer (pH 3.8)–acetonitrile–methanol (65:20:15, v/v/v) at a flow-rate of 3.0 mL/min and detected by fluorescence at 259 and 399 nm as excitation and emission wavelength respectively. The separation of losartan, telmisartan, and valsartan was achieved on a Chromolith RP-18e monolithic column. The method provides extraction recoveries from spiked urine samples greater than 93%. Intra-day and inter-day precision were generally acceptable; the intra-day-assay C.V. was <3.5 for all compounds and the inter-day-assay C.V. was <3.7%. The estimated calibration range was 0.001–2.5 μg mL^{-1} with excellent coefficient of determination (>0.9981). The detection limits for losartan, telmisartan, and valsartan at a signal-to-noise ratio of 5:1 were 0.002, 0.0002 and 0.001 μg mL^{-1} when a sample volume of 20 μL was injected. The proposed method permitted the simultaneous determination of losartan, telmisartan, and valsartan in 8 min, with an adequate precision and sensitivity. However, the overlap of the sample cleanup step with the analysis increases the sampling frequency to 12 samples/h. The developed column-switching method was successfully applied for the determination of these analytes in human urine samples of patients submitted at ARA-IIs therapy.

1. Introduction

High blood pressure is quantitatively the largest single risk factor for premature death. Angiotensin II receptor antagonists (ARA-IIs) are effective agents for the treatment of hypertension and heart failure. They help to lower blood pressure by blocking angiotensin II, an hormone in the body that is an important component of hypertension. Among them, losartan, telmisartan, and valsartan are orally active and due to their specificity of action provide good conditions for patient compliance as well as high effectiveness [1,2].

Although the ARA-IIs share the same mechanism of action, their pharmacodynamic and pharmacokinetic profiles vary from compound to compound.

After oral administration, ARA-IIs are rapidly absorbed (time for peak plasma levels 0.5–4 h) and they have a range of bioavailability from 23% for valsartan, 33% for losartan, to 43% for telmisartan. Most of the orally administered dose of ARA-IIs is excreted via bile into the faeces and approximately 4%, 7–13%, <2% of the oral dose of losartan, valsartan, and telmisartan are excreted in the urine without change.

However, the particular information of this therapeutic group like pharmacokinetics on the antihypertensive effectiveness, potentiality to exert new actions and to reduce the cardiovascular and/or renal morbidity, adverse effects, as well as the information of its pharmacokinetic characteristics, is in continuous process of revision [1–3].

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Because of this, the development of analytical methods for the determination of ARA-IIs is crucial for the study of the antihypertensive efficacy of the dose of the drug during the dosage range since they would allow the monitorization of their biologic concentration levels and its elimination rate.

Several methods that are reported for ARA-IIs compounds estimation include: enzyme-linked immunosorbent assays (ELISA) for the determination of telmisartan in human blood plasma [4]; spetrofluorimetric for the determination of losartan and valsartan in human urine [5], colorimetric method [6], and UV-derivative spectrophotometric [7] for the determination of losartan potassium in bulk and in tablets. Tatar and Saglik [8] compared UV- and second derivative-spectrophotometric and high-performance liquid chromatographic methods for the determination of valsartan in pharmaceutical formulation. Also, capillary electrophoresis (CE), capillary electrochromatography (CEC), micellar electrokinetic capillary chromatography (MEKC) and capillary zone electrophoresis (CZE) methods have also been reported [9–13]. However, until now, high-performance liquid chromatography has been the major technique used in the determination of these compounds in different matrixes with UV, fluorimetric or mass spectrometry (MS) detections [14–16].

Validated methods which allow the determination of a single drug [17–25] or combination of the ARA-IIs with hydrochlorothiazide or some of their metabolite [26–29] in urine, plasma and in pharmaceutical formulations [9,30] have also been published.

With few exceptions, HPLC determinations use conventional sample preparation procedures prior to injection, such as protein precipitation [14,26] liquid–liquid [25] or solid phase extraction in cartridges with sorbents of different nature [14,15,19,31]. However, direct injection technique is generally preferable, since the problems involved in off-line sample pre-treatment like, time consuming procedures, errors and the risk of low recoveries, can be readily avoided.

As an alternative to existing methods, a new column-switching high-performance liquid chromatographic method with fluorescence detection is presented here for the determination of ARA-IIs in human urine. The extraction of analytes was performed on a LiChrospher RP-18 ADS (alkyl-diol silica) belonging to the restricted access media (RAM) family which was developed as special packing material for precolumns used in LC-integrated sample processing systems of biological matrices and applied for the determination of different drugs in biological matrix [32–36].

The separation of ARA-IIs was achieved on a Chromolith RP-18e monolithic column. Monolithic columns, which are made by sol–gel technology and consist of a single rod of silica-based material, have attracted considerable attention in liquid chromatography as they allow achieving separation faster than the conventional columns. This kind of column could operate at a high flow-rate, thereby considerably reducing the analysis time and offering sharper peaks in an isocratic determination contributing to enhance *S*/*N*. Additionally, it has a very large active surface area for high efficiency separations [37].

With the emergence of commercial monolithic columns, the reports concerning practical applications of monolithic column in HPLC separations are increasing. In this sense, this column was also used by Zarghi [38] for the determination of losartan in human plasma with UV detection.

The originality of this work resides in a column-switching system integrating an ADS-precolumn for the extraction and preconcentration of the analytes and its later separation in a monolithic column and fluorescence detection which would provide a quick, selective and sensitive method for the determination of ARA-IIs compounds in human urine samples.

2. Experimental

2.1. Reagents and chemicals

Methanol and acetonitrile were HPLC grade and were purchased from Karl Fisher (Darmstadt, Germany). Orthophosphoric acid (Suprapur, 85%) and Natrium Hydroxide were analytical grade and purchased from J.T. Baker (Phillipsburg, NJ, USA). USP losartan, telmisartan and valsartan were kindly provided by Inversiones Dayamar CA (Venezuela). Pharmaceuticals tablets of ARA-IIs compounds were acquired in local pharmacies of the Mérida city (Venezuela).

All aqueous solutions were prepared with double de-ionized water of 18 MΩ cm^{-1} specific resistivity, obtained in a Milli-Q (Millipore, Bedford, MA, USA) ultrafiltration system.

Individual stock standard solutions of losartan, telmisartan, and valsartan (1000 μg mL^{-1}) were prepared in methanol and stored at −20 °C. Working solutions were prepared every week by an appropriate dilution of concentrated stock standard solutions in extraction mobile phase. These solutions were stored at 4 °C.

All standard solutions and samples for HPLC analysis were filtrated through 0.22 μm PVDF syringe filters (Millipore).

2.2. Instruments

The HPLC system was a PerkinElmer LC Series 200 (Norwalk, CT, USA) equipped with a quaternary Pump (P_2), UV/vis Detector, Fluorescence Detector (200a) and a Column Oven. A Digital Workstation with a TotalChrom Software Version 6.3 serves as both a controller and data manager for the overall system.

Injections were made with a Rheodyne type 7125 six-port valve equipped with 20 μL loop. For the column-switching purposes, a column-switching six-port valve (Supelco) (VS) controlled by the workstation was used, along with an additional Knauer 64 pump (Berlin, Germany) (P_1) to deliver the extraction mobile phase.

2.3. Sample preparation

Urine samples were obtained from volunteered healthy subjects and hypertensive patients in therapy with the studied drugs. Urine samples were collected in 50 mL polyethylene bottles and frozen immediately (−18 to −25 °C) until analysis. In preparation for the analysis, frozen urine samples were thawed, equilibrated to room temperature and then mixed to homogenize them thoroughly. Aliquots of 1 mL were transferred to 16 mm × 125 mm disposable glass tubes and 3 mL of extraction mobile phase (Solvent 1) was added to each tube and then vortex mixed to homogenize them thoroughly. Then, each sample was centrifuged at 2500 × *g* for 10 min. A total of 2.0 mL of the supernatant was transferred into glass screw-cap vials. The resulting urine samples were processed according to the on-line SPE procedure described below.

2.4. Chromatographic conditions

The extraction precolumn was a LiChrocart 25-4 LiChrospher RP-18 ADS 25 mm × 4 mm i.d., 25 μm particle diameter (Merck, Darmstadt, Germany) and the extraction mobile phase consisted of 2% methanol in 5 mmol L^{-1} phosphate buffer, pH 3.8 (Solvent 1), being pumped at a flow-rate of 0.8 mL min^{-1}.

A Merck Chromolith monolithic column, 25 mm × 4.6 mm i.d. was used as analytical column to provide further separation of analytes before detection. 5 mM phosphate buffer pH 3.8–acetonitrile–methanol (65:20:15, v/v/v) (Solvent 2) was used in isocratic elution mode as the mobile phase for the transfer and separation of losartan, telmisartan, and valsartan at a flow-rate of 3 mL min^{-1}. Before used, the mobile phases were vacuum filtered

through a 0.22 μm PVDF and Fluoropore FGLP membranes (Millipore) and degassed. All the chromatographic experiments were carried out at room temperature (22 ± 2 °C).

2.5. Column-switching procedure

2.5.1. Sample loading mode

Solvent 1 was delivered by P_1 at 0.8 mL min^{-1} for sample loading. The analytical column was flushed by P_2 at 3 mL min^{-1} with Solvent 2. After injection of a 20 μl sample volume, the extraction precolumn was washed for 4 min and the matrix compounds were directed into the waste.

2.5.2. Injection mode

By switching the valve (VS), the components retained on the extraction precolumn were back-flushed by P_2 delivering Solvent 2 for successive isocratic separation on analytical column and their later fluorescence detection. Fluorescence of the compounds was measured at an excitation wavelength of 259 nm and an emission wavelength of 399 nm. The next run was prepared by switching back the valve after 0.3 min with a re-equilibration period of 2 min.

2.6. Validation study

The method was validated prior to the analyses of human urine samples according to the guidance of bioanalytical method validation [39]. The limit of quantification, specificity, linearity, accuracy and precision of losartan, telmisartan, and valsartan in urine sample were assessed and evaluated.

2.6.1. Precision, accuracy and lower limit of quantification (LLOQ)

Replicate analysis ($n = 5$) of quality control samples at three concentration levels (0.6, 1.2 and 2.5 μg mL^{-1} for each drug) was used for determining the precision of the assay. Precision was calculated as the coefficient of variation (C.V., %) within a single run (intra-day), among different runs (inter-day). The accuracy was calculated as the deviation between nominal and measured concentrations. The LLOQs for losartan, telmisartan, and valsartan were experimentally chosen as the minimal quantitative concentration in urine samples. The FDA Guidance recommends that the response of the analyte should be at least five times that of the blank and the analyte peak (response), and that the analyte peak should be identifiable, discrete, and reproducible with precision of 20% and accuracy of 80–120%.

2.6.2. Absolute recovery

The absolute recovery was determined by comparing the peak areas of each compound after extraction with those obtained by direct injection of the same amount of analyte in aqueous solution.

2.6.3. Linearity

Linearity was demonstrated by running urine standards at six concentrations over the range of 0.006–2.5, 0.001–5.00 and 0.003–2.5 μg mL^{-1} of losartan, telmisartan, and valsartan respectively. Calibration curve was generated by plotting peak areas against drug concentrations. The coefficient of determination (r^2) was determined.

3. Results and discussion

3.1. Optimization of a column-switching system

The method development of a column-switching technique consisted of several experiments that were performed in order to determine the proper valve-switching times.

As a first step, a study employing a LiChrospher ADS precolumn (bonded alkyl chains C_{18}) was performed in order to determine its performance in decreasing matrix content. In this way, before HPLC analysis, macromolecular and other endogenous compounds have to be removed from the urine sample because of their precipitation by higher amounts of organic solvents and their binding on the surface of the packing material. LiChrospher ADS materials consist of a family of special reversed-phase sorbents [32–36] for LC-integrated sample preparation of biological fluids. These precolumn packing materials had been developed in a cooperation between Merck Eurolab and Boos [32,36]. At the outer surface of the spherical particles, hydrophylic, electroneutral diol groups are bound, preventing interactions with the protein matrix. The latter can be directly flushed into the waste. The inner surface, with the hydrophobic C-18 alkyl chains, is freely accessible for low-molecular analytes. The analytes are thus selectively extracted and enriched on the inner surface. Thus the packing material provides a direct on-line extraction-base, an on-column enrichment and subsequent analytical separation of low-molecular compounds from untreated urine samples.

To successfully apply the ADS precolumn for the fractionation of complex samples, the elution profile of the sample matrix, as well as elution profile of the analytes should be recorded, and according to them, the duration of the fractionation event optimized. To record the matrix elution profile, urine sample was injected into the ADS precolumn, which was directly coupled to a UV detection system and flushed with 2% methanol in 5 mM phosphate buffer pH 3.8 at a flow-rate of 0.8 mL min^{-1}. In this way, matrix elution was achieved within 4 min. Once the fractionation step is terminated, the valve is switched and the analytes are transferred to the analytical HPLC column with a mobile phase with the stronger elution power in order to allow a so-called peak compression and narrow profile.

Thus, analytes transfer from ADS precolumn to the analytical column, and subsequent chromatographic separation, was performed with mobile phase containing 65% 5 mM phosphate buffer pH 3.8:20% acetonitrile, 15% methanol (65:20:15, v/v/v) at a flow-rate of 3 mL min^{-1} during 0.3 min.

3.2. LC separation and fluorescence detection

The analytes isolated from ADS precolumn by valve-switching step were focused on the analytical column for its separation.

Separation was performed on a reversed-phase monolithic column, which has lower separation impedance comparing to the particulate packings, and therefore it allows easy optimizing chromatographic conditions to obtain desirable resolution in a short time.

The most unique feature of these columns is their high permeability, which is nearly twice as high as that of packed columns. Therefore, monolithic silica columns can be operated at high flow-rates, thus allowing fast separations of various mixtures. They represent an approach that provides high rates of mass transfer at lower pressure drops as well as high efficiencies even at elevated flow-rates. By this means, much faster separations are possible and the productivity of chromatographic processes can be increased by at least one order of magnitude as compared to traditional chromatographic columns packed with porous particles. This enhances the speed of the separation process and reduces backpressure and unspecific binding without sacrificing resolution [37,40,41].

To achieve better separation and peak shapes, the mobile phase containing varying pH and acetonitrile and methanol content were tested in Chromolith column. Mobile phases containing phosphate buffer of various pH values and different percentages of acetonitrile and methanol as organic modifier were investigated for elution from the separation column. As we mentioned it previously, the suitable condition was obtained with an isocratic analytical mobile

Table 1
Operating conditions of the column-switching HPLC system.

Step	Component system	Condition
Sample injection (t = 0 min)	Rheodyne 7125 valve: inject position Switching valve: load position	Injection volume: 20 μL
Sample extraction (t = 4 min)	Precolumn: LiChrospher RP-18 ADS 25 mm × 4 mm i.d., dp: 25 μm	Extraction mobile phase: 2% de methanol in 5 mmol L^{-1} phosphate buffer, pH 3.8 Flow-rate: 0.8 mL min^{-1}
Analytes transfer (t = 0.3 min)	Switching valve: inject position	Analytical mobile phase: 5 mM phosphate buffer pH 3.8–acetonitrile–methanol (65:20:15, v/v/v) Flow-rate: 3 mL min^{-1}
Analytes separation (t = 3.5 min)	Switching valve: load position	Analytical mobile phase: 5 mM phosphate buffer pH 3.8–acetonitrile–methanol (65:20:15, v/v/v)
	Analytical column: Chromolith monolithic column 25 mm × 4.6 mm i.d.	Flow-rate: 3 mL min^{-1} Fluorescence detection λ_{ex}: 259; λ_{em}: 399 nm
Next injection (t = 5 min)		

phase composed of 65% acetonitrile, 20% of methanol in 5 mM phosphate buffer pH 3.8 (65:20:15, v/v/v) at a flow-rate of 3 mL min^{-1}.

For the sensitive detection of all analytes, the excitation and emission wavelengths of the fluorescence detector were investigated.

All the ARAs-II compounds studied showed maximum fluorescence response in a narrow wavelength range, 250–270 nm for excitation and 370–390 nm for emission [15]. The acidic characteristics of these antihypertensive drugs (pK_a values from 3 to 6) confirmed a high dependence of fluorescent properties on pH, though all the ARAs-II compounds did not exhibit the same fluorescence at acidic pH values.

A good separation for all the ARAs-II compounds was achieved at pH 3.8. This is not the optimal pH value for fluorescence of losartan as reflected in some of the references for its single determination which use lower pH values [42,43]. Taking into account that the aim of this work is the simultaneous determination of these three ARAs-II compounds, loss of some sensitivity was preferred to provide a good performance for the majority of these compounds. However, to improve the sensitivity of the method, a program of sensitivities in the detector was standardized and optimal wavelengths for their simultaneous determination were set at 259 and 399 nm for excitation and emission respectively.

Under the optimized chromatographic conditions (Table 1), losartan, telmisartan, and valsartan peaks were well resolved. Endogenous urine compounds did not give any interfering peaks. Fig. 1 shows typical chromatograms of blank urine in comparison to spiked urine samples analyzed. The average retention times of losartan, valsartan, and telmisartan were 5.2, 5.8 and 7.3 min respectively. None of the endogenous compounds interfered with analytes peaks as well.

3.3. Features of the method

The precision expressed by coefficient variation (C.V.) was < 3.5% for all tested concentrations, showing that the method provided good reproducibility. The inter-day-assay precision was also determined and C.V. did not exceed 3.7% in all concentrations, demonstrating the good stability and repeatability of this described system.

Recovery values for all cases were between 92.9 and 102.5 with a C.V. < 4.0%. These values are quantitative and demonstrate the extraction efficiency of the ADS precolumn.

Calibration curves over the studied range were constructed for aqueous standards and for human urine samples spiked with all analytes. The curves were prepared using six concentrations of each compound and a blank and injected in triplicate into the column-switching HPLC system. The regression equations of the peak area as a function of losartan, telmisartan, and valsartan concentration were obtained from aqueous standard and from spiked human urine samples. Each calibration set included seven data points and each point was run at least three times. Table 2 shows the linear regression analysis of the calibration curve data. For all regression lines, the coefficient of determination (r^2) was greater than 0.9981. On the other hand, the slope of the calibration using aqueous standard solutions was not statically different (P < 0.05) from those for losartan, telmisartan, and valsartan additions to real urine samples, hence, the standard calibration technique with aqueous standards

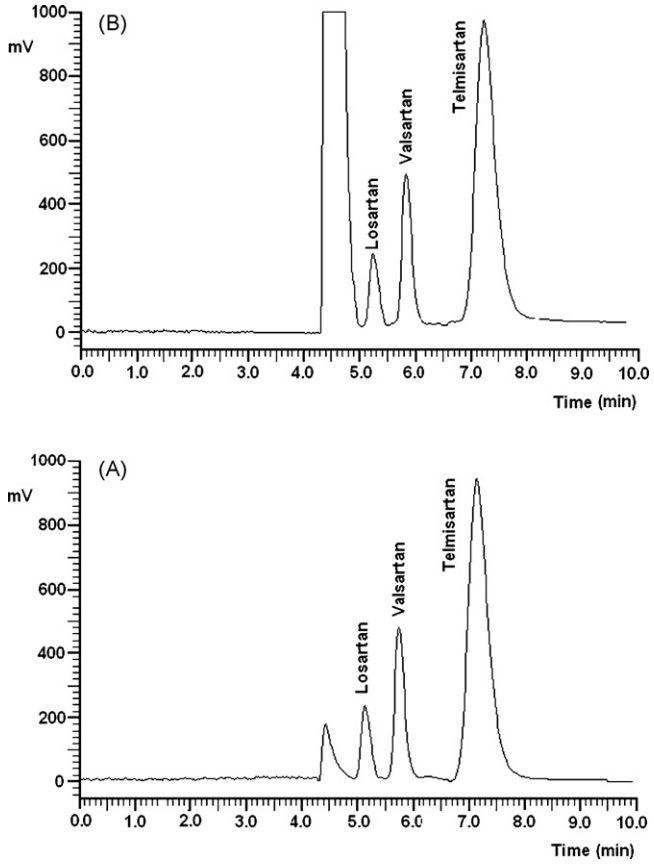

Fig. 1. Chromatograms of losartan, telmisartan, and valsartan obtained after 20 μL direct injection of (A) standard solution at 1.00 μg mL^{-1} and (B) urine sample spiked with 1.00 μg mL^{-1} of analytes. Chromatographic conditions in text.

Table 2
Calibration curves.

Compound	Matrix	Dynamic range (μg mL^{-1})	Equation[a]	r^2 [b]	C.V. of slope[c] (%)
Losartan	Aqueous standard	0.006–2.5	$A = 5269.6C$	0.9995	1.1
	Urine		$A = 5.52 + 5234.9C$	0.9990	0.9
Valsartan	Aqueous standard	0.003–2.5	$A = 18320C$	0.9999	0.4
	Urine		$A = 563 + 18331.3C$	0.9997	1.4
Telmisartan	Aqueous standard	0.001–2.5	$A = 85248C$	0.9981	1.3
	Urine		$A = 3603 + 85865C$	0.9982	1.3

[a] A: peak area; C: concentration of each compound.
[b] Coefficient of determination.
[c] Coefficients of variation of the slope ($n = 3$).

could be used for the determination of these compounds in human urine samples.

The lowest concentrations that can be quantified with acceptable accuracy and precision for losartan, telmisartan, and valsartan were 0.006, 0.001 and 0.003 μg mL^{-1} which allow their determination at expected urine concentration levels. Furthermore, the limits of detection for losartan, telmisartan, and valsartan, defined as signal-to-noise (S/N) ratio of 5:1 were 0.002, 0.0002 and 0.001 μg mL^{-1} when a sample volume of 20 μl was injected. However, it was possible to enhance the sensitivity further by injecting larger volumes, up to 200 μL. As can be seen, the method proved to be sufficiently sensitive for the determination of losartan, telmisartan, and valsartan in urine according to the pharmacodynamic and pharmacokinetic profiles of these compounds reported by some researchers [44,45].

Ruggedness tests were performed on HPLC assays of losartan potassium, telmisartan, and valsartan reference standard solutions and pharmaceuticals tablets.

The effects of different chromatographic columns were evaluated on the responses of peak areas. Results presented in Table 3 show relative errors no more than 4.9% indicating good ruggedness of the developed HPLC method.

3.4. Analysis of real samples

The validated method was applied satisfactorily for the determination of losartan and valsartan in urine samples of eight hypertensive patients without complications.

Table 3
Ruggedness study of the HPLC method.

Samples	Column 1[a]	Column 2[b]	e_r, %	Column 3[c]	e_r, %
Losartan potassium (50 mg)	48,307	47,515	1.2	45,083	4.9
Genven (50 mg)	49,235	47,778	2.1	47,166	3.0
Presartan (50 mg)	47,566	46,119	2.2	45,912	2.5
Calox (50 mg)	47,899	46,808	1.6	45,946	2.9
Elter (50 mg)	49,249	46,973	3.3	47,362	2.8
Valsartan (40 mg)	28,731	28,719	0.03	26,870	4.7
Diovan (40 mg)	28,080	29,404	3.3	29,433	3.3
Valsartan (80 mg)	56,818	56,836	0.03	56,860	0.1
Genven (80 mg)	55,963	57,805	2.3	56,740	1.0
RX (80 mg)	56,111	57,376	1.6	55,856	0.3
Valsartan (160 mg)	111,658	113,675	1.3	112,195	0.3
Calox (160 mg)	115696	113,633	1.3	112,004	2.3
Telmisartan (40 mg)	52,320	51,460	1.6	53,518	2.3
Micardis (40 mg)	50,272	49,641	1.3	51,368	2.2
Telmisartan 80 mg	114,196	111,792	2.2	117,051	2.5
Pritor (80 mg)	102,550	100,010	2.5	105,196	2.6

[a] Chromolith C_{18} 25 mm × 4.6 mm (Merck).
[b] X-Terra C_{18} 250 mm × 3.9 mm; dp: 3.5 μm (Waters).
[c] X-Bridge C_{18} 150 mm × 4.6 mm; dp: 3 μm (Waters).

Table 4
Concentrations (μg mL^{-1}) obtained in urine from hypertensive patients under therapy with Cozaar (Losartan 100 mg) and Diovan (Valsartan 80, 160 and 320 mg)[a].

	0–2 h	2–4 h	4–8 h
Losartan			
Patient 1 (Cozaar 100 mg)	2.57 ± 0.09	7.62 ± 0.27	6.01 ± 0.24
Patient 2 (Cozaar 100 mg)	3.36 ± 0.14	11.92 ± 0.41	8.97 ± 0.27
Valsartan			
Patient 3 (Diovan 80 mg)	1.49 ± 0.06	2.48 ± 0.10	3.59 ± 0.07
Patient 4 (Diovan 80 mg)	1.30 ± 0.05	2.14 ± 0.09	3.30 ± 0.09
Patient 5 (Diovan 80 mg)	1.69 ± 0.06	2.72 ± 0.09	2.91 ± 0.10
Patient 6 (Diovan 160 mg)	3.55 ± 0.13	5.89 ± 0.21	6.89 ± 0.21
Patient 7 (Diovan 160 mg)	3.25 ± 0.13	6.49 ± 0.27	8.45 ± 0.31
Patient 8 (Diovan 320 mg)	4.35 ± 0.17	10.18 ± 0.41	11.53 ± 0.46

[a] Expressed as mean ± standard deviations (SD) ($n = 3$).

The patients in therapy were administered orally: two of them with 100 mg of losartan and the others; three with 80 mg, two with 160 mg and one with 320 mg of valsartan. Urine samples were taken from each of the subjects during the 0–8 h time interval after administration. The results are shown in Table 4. The amounts excreted over the whole period studied are in good agreement with results previously reported in literature [5,44,45].

4. Conclusions

The newly developed procedure allows efficient extraction of losartan, telmisartan, and valsartan from urine samples and provides high sensitivity for their fluorescence detection. It allows measuring the analytes in 20 μL of human urine sample. The method permits simultaneous trace analysis of ARA-IIs, in less than 4 min., which is a significant improvement in comparison to the methods reported previously. The use of a dual column LC system with restricted access precolumn packing allows efficient and rapid clean-up of urine sample, thus reducing the concentration of interferences and minimizing matrix-induced effects. On the other hand it is important to indicate that in this method, the overlap of sample cleanup, analysis and recondition of the precolumn increases the sample throughput to 12 samples/h, increasing the usefulness of the method for routine analysis.

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References

[1] B. Porta Oltra, C. Borrás Almenar, N.V. Jiménez Torres, Farm. Hosp. 29 (2005) 104–112.
[2] B. Schmidt, B. Schieffer, J. Med. Chem. 46 (2003) 2261–2270.
[3] M.D. Helmy Siragy, Am. J. Cardiol. 84 (1999) 3–8.
[4] Ch. Hempen, L. Gläsle-Schwarz, U. Kunz, U. Karst, Anal. Chim. Acta 560 (2006) 35–40.
[5] E. Cagigal, L. González, R.M. Alonso, R.M. Jiménez, Talanta 54 (2001) 1121–1133.

[6] A.H. Prabhakar, R. Giridhar, J. Pharm. Biomed. Anal. 27 (2002) 861–866.
[7] O.C. Lastra, I.G. Lemus, H.J. Sánchez, R.F. Pérez, J. Pharm. Biomed. Anal. 33 (2003) 175–180.
[8] S. Tatar, S. Saglik, J. Pharm. Biomed. Anal. 30 (2002) 371–375.
[9] M.G. Quaglia, E. Donati, G. Carlucci, P. Mazzeo, S. Fanali, J. Pharm. Biomed. Anal. 29 (2002) 981–987.
[10] M. Zhang, F. Wei, Y.-F. Zhang, J. Nie, Y.-Q. Feng, J. Chromatogr. A 1102 (2006) 294–301.
[11] S. Hillaert, T.R.M. De Beer, J.O. De Beer, W. Van den Bossche, J. Chromatogr. A 984 (2003) 135–146.
[12] R.C. Williams, M.S. Alasandro, V.L. Fasone, R.J. Boucher, J.F. Edwards, J. Pharm. Biomed. Anal. 14 (1996) 1539–1546.
[13] S. Hillaert, W. Van den Bosshe, J. Pharm. Biomed. Anal. 31 (2003) 329–339.
[14] L. Kristoffersen, E. Leere Oiestad, M. Stokke Opdal, M. Krogh, E. Lundanes, A. Solberg Christophersen, J. Chromatogr. B 850 (2007) 147–160.
[15] L. González, J.A. López, R.M. Alonso, R.M. Jiménez, J. Chromatogr. A 949 (2002) 49–60.
[16] J. Nie, M. Zhang, Y. Fan, Y. Wen, B. Xiang, Y.-Q. Feng, J. Chromatogr. B 828 (2005) 62–69.
[17] N. Ferreirós, G. Iriarte, R.M. Alonso, R.M. Jiménez, Talanta 73 (2007) 748–756.
[18] N. Koseki, H. Kawashita, H. Hara, M. Niina, M. Tanaka, R. Hawai, Y. Nagae, N. Masuda, J. Pharm. Biomed. Anal. 43 (2007) 1769–1774.
[19] N. Torrealday, L. González, R.M. Alonso, R.M. Jiménez, E. Ortiz Lastra, J. Pharm. Biomed. Anal. 32 (2003) 847–857.
[20] A.K. Shakya, Y.M. Al-Hiari, O.M.O. Alhamami, J. Chromatogr. B 848 (2007) 245–250.
[21] P. Li, Y. Wang, Y. Wang, Y. Tang, J.P. Fawcett, Y. Cui, J. Gu, J. Chromatogr. B 828 (2005) 126–129.
[22] J. Macek, J. Klima, P. Ptácek, J. Chromatogr. B 832 (2006) 169–172.
[23] N. Ferreirós, G. Iriarte, R.M. Alonso, R.M. Jiménez, E. Ortiz, J. Chromatogr. A 1119 (2006) 309–314.
[24] D. Liu, P. Hu, N. Matsushima, X. Li, L. Li, J. Jiang, J. Chromatogr. B 856 (2007) 190–197.
[25] N. Daneshtalab, R.Z. Lewanczuk, F. Jamali, J. Chromatogr. B 766 (2002) 345–349.
[26] N. Erk, J. Chromatogr. B 784 (2003) 195–201.
[27] H. Li, Y. Wang, Y. Jiang, Y. Tang, J. Wang, L. Zhao, J. Gu, J. Chromatogr. B 852 (2007) 436–442.
[28] M. Polinko, K. Rifle, H. Song, M.-W. Lo, J. Pharm. Biomed. Anal. 33 (2003) 73–84.
[29] L. González, R.M. Alonso, R.M. Jiménez, Chromatographia 52 (2000) 735–740.
[30] D.L. Hertzog, J. Finnegan McCafferty, X. Fang, R. Jeffrey Tyrrell, R.A. Reed, J. Pharm. Biomed. Anal. 30 (2002) 747–760.
[31] P.K.F. Yeung, A. Jamieson, G.J. Smith, D. Fice, P.T. Pollak, J. Pharm. Biomed. Anal. 204 (2000) 17–22.
[32] K.-S. Boos, A. Rudolphi, LC–GC Int. 11 (1998) 84–95.
[33] K.-S. Boos, A. Rudolphi, S. Vielhauer, A. Walfort, D. Lubda, F. Eisenbeiβ, Fresen. J. Anal. Chem. 352 (1995) 684–690.
[34] Ch. Schäfer, D. Lubda, J. Chromatogr. A 909 (2001) 73–78.
[35] K.-S. Boos, C.-H. Grimm, Trends Anal. Chem. 18 (1999) 175–179.
[36] S. Vielhauer, A. Rudolphi, K.-S. Boos, D. Seidel, J. Chromatogr. B 666 (1995) 315–322.
[37] K. Cabrera, J. Sep. Sci. 27 (2004) 843–852.
[38] Food and Drug Administration, Guidance for Industry, Bioanalytical Method Validation, 2001.
[39] A. Zarghi, S.M. Foroutan, A. Shafaati, A. Khoddam, Arzneimittel Forsch. 55 (2005) 569–572.
[40] K. Miyabe, G. Guiochon, J. Sep. Sci. 27 (2004) 853–873.
[41] K. Cabrera, LC GC North Am. 26 (2008) 32–35.
[42] D. Farthing, D. Sica, I. Fakhry, A. Pedro, T.W.B. Gehr, J. Chromatogr. B 704 (1997) 374–378.
[43] M.A. Ritter, C.I. Furtek, M.W. Lo, J. Pharm. Biomed. Anal. 15 (1997) 1021–1029.
[44] Z.H. Israili, J. Hum. Hypertens. 14 (2000) S73–S86.
[45] R. Hernández-Hernández, M.C. Armas-Padilla, M.J. Armas-Hernández, M. Velasco, J. Hum. Hypertens. 14 (2000) S2–S5.