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Journal of Chromatography B, 828 (2005) 62–69
JOURNAL OF CHROMATOGRAPHY B
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Biocompatible in-tube solid-phase microextraction coupled to HPLC for the determination of angiotensin II receptor antagonists in human plasma and urine

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Received 2 April 2005; accepted 7 September 2005
Available online 3 October 2005

Abstract

A poly (methacrylic acid-ethylene glycol dimethacrylate, MAA-EGDMA) monolithic capillary was used for the in-tube solid-phase microextraction (in-tube SPME) of several angiotensin II receptor antagonists (ARA-IIs) from human plasma and urine. Under the optimized extraction condition, the protein component of the biological sample was flushed through the monolithic capillary, while the analytes were successfully trapped. Coupled to HPLC with fluorescence detection, this on-line in-tube SPME method was successfully applied for the determination of candesartan, losartan, irbesartan, valsartan, telmisartan, and their detection limits were found to be 0.1–15.3 ng/mL and 0.1–15.2 ng/mL in human plasma and urine, respectively. The method was linear over the range of 0.5–200 ng/mL for telmisartan, 5–2000 ng/mL for candesartan and irbesartan, 10–2000 ng/mL for valsartan, and 50–5000 ng/mL for losartan with correlation coefficients being above 0.9985 in plasma sample and above 0.9994 in urine sample. The method reproducibility was evaluated at three concentration levels, resulting in the R.S.D. <7%. The poly (MAA-EGDMA) monolithic capillary was demonstrated to be robust and biocompatible by using direct injections of biological samples.
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Keywords: Poly (methacrylic acid-ethylene glycol dimethacrylate); Monolithic capillary; In-tube SPME; Liquid chromatography; Angiotensin II receptor antagonists; Candesartan; Losartan; Irbesartan; Valsartan; Telmisartan

1. Introduction

Angiotensin II receptor antagonists (ARA-IIs) are effective agents for the treatment of hypertension and heart failure, and have been considered as the alternative for the traditional inhibitors of angiotensin converting enzyme (ACE), because they can selectively block the angiotensin type 1 (AT1) receptor in the renin–angiotensin system [1]. It is recommended in the World Health Organisation (WHO) Guidelines that these drugs can be prescribed as the first-line treatment for essential hypertension [2]. ARA-IIs are rapidly absorbed after oral administration, and may reach their peak concentration in plasma 0.5–4 h later [3]. However, for some of these drugs a low level of concentration, normally in ng/mL occurs in human plasma after oral administration, posing problems for their detection and/or monitoring. A sensitive method should thus be established to detect these drugs in bodies of human beings.

Angiotensin II receptor antagonists are amphoteric compounds (pK_a 3.1–4.9), and share a same biphenyltetrazole moiety, except telmisartan which contains a structurally related biphenylcarboxylic acid moiety [4]. These structure similarities may imply that it is possible to analyze the concentration of these compounds simultaneously. Until now, high-performance liquid chromatography (HPLC) has been the major technique used for the determination of different ARA-IIs in biological samples with UV, fluorimetric, or MS detections [5–11]. Several sample pretreatment methods such as liquid–liquid extraction [8], solid-phase extraction [5–7,10,11] and protein precipitation [9] have been used for the cleanup of samples. However, these methods are time-consuming, and require a large volume of samples and solvents, and overall they are not easy to be automated at all.

Solid-phase microextraction (SPME) has gained increasingly wide acceptance as a simple, fast, solventless, reliable, and flexible sample preparation technique [12]. In-tube SPME is usually

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doi:10.1016/j.jchromb.2005.09.015

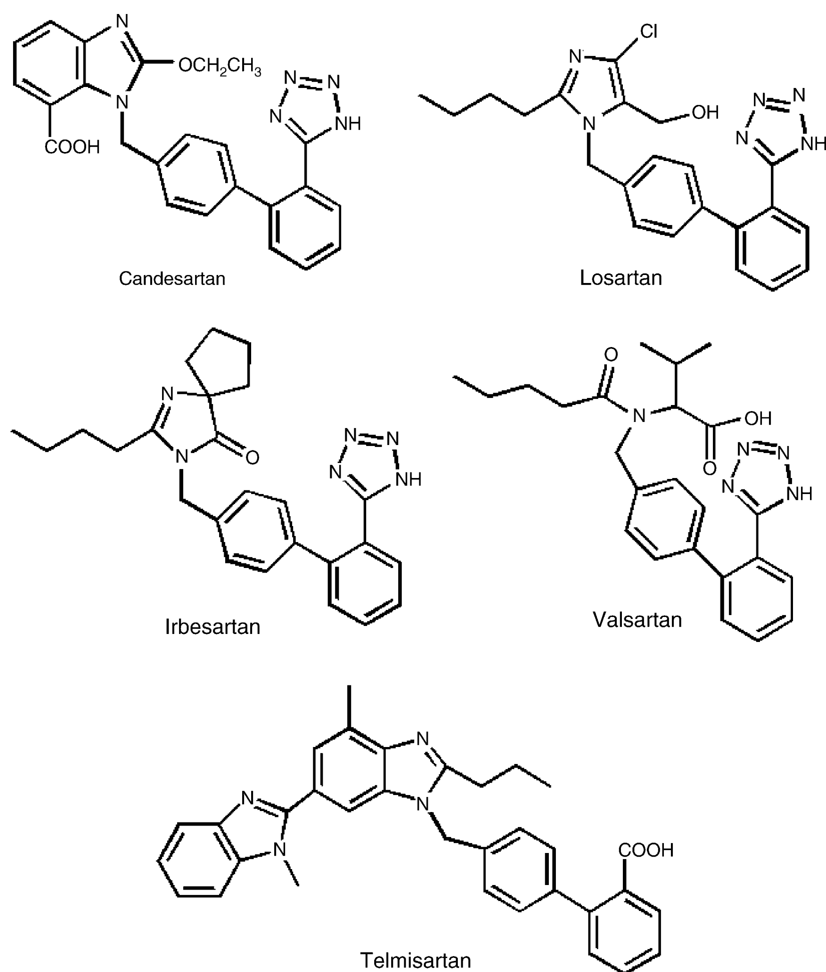

Fig. 1. Structures of ARA-IIs studied in the present study.

on-line coupled to high performance liquid chromatography, and this technique has been applied successfully to determine the content of various compounds such as pesticides, drugs, environmental pollutants, and food contaminants [13–16]. However, many SPME coatings lack biocompatibility. The direct SPME extraction of drugs from biological samples often requires additional sample preparation, such as ultra-centrifugation, in order to eliminate the protein component in samples [17]. One kind of biocompatible extraction phase is the restricted access materials (RAMs), which has been applied in the analysis of benzodiazepines in biological samples [18,19]. Recently, an in-tube SPME using poly (methacrylic acid-ethylene glycol dimethacrylate, MAA-EGDMA) monolithic material as a biocompatible extraction medium was developed, and was used successfully for the determination of some basic drugs in serum [20] and urine samples [21]. The polymer was demonstrated to be stable within the entire range of pH [22] and to be biocompatible when used for biological samples. Its hydrophobic main chain and acidic pendant groups make it a superior material for the extraction of basic analytes from aqueous matrix [20,21].

In the present study, we developed an on-line method based on poly (methacrylic acid-ethylene glycol dimethacrylate) monolithic in-tube SPME coupled to HPLC with fluorescence detection for determining the concentration of a class of ARA-IIs compounds in human plasma and urine samples.

The compounds studied were candesartan (2-ethoxy-1-{[2′-(1*H*-tetrazol-5-yl)-biphenyl-4-yl]methyl}-1*H*-benzimidazole-7 -carboxylic acid), losartan (2-*n*-butyl-4-chloro-5-hydroxymethyl-1-{[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}imidazole, irbesartan (2-butyl-3-[(2′-(1*H*-tetrazole-5-yl)-biphenyl-4-yl]methyl]-1,3-diazaspiro-[4,4]-non-1-en-4-one), valsartan ((*S*)-*N*-valeryl-*N*-{[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl} -valine), telmisartan (4-[(2-*n*-propyl-4-methyl-6-(1-methylbenzimidazol-2-yl)-benzimidazol-1-yl)methyl]-biphenyl-2-carboxylic acid). The formulae of these drugs are shown in Fig. 1.

2. Experimental

2.1. Chemicals and materials

The ethylene glycol dimethacrylate (EGDMA) was purchased from Acros (Sweden). The methacrylic acid (MAA), 2,2′-azobis (2-methylpropionitrile) (AIBN), dodecanol and toluene were obtained from Shanghai Chemical Co. Ltd. (Shanghai, China) and were of analytical reagent grade. Double distilled water was used for all experiments.

Five non-peptide angiotensin II receptor antagonists (ARA-IIs) were kindly offered by Jiangsu Institute for Drug Control (Nanjing, China). All analytes were prepared as 1 mg/mL standard solutions in methanol and stored at 4 °C. The sample solution was spiked with these standard solutions to the concentrations required for the experiments.

2.2. Preparation of poly (MAA-EGDMA) monolithic capillary

The poly (MAA-EGDMA) monolithic capillary was synthesized inside a fused silica capillary (20 cm × 0.25 mm, i.d., Yongnian Fiber Plant, Hebei, China) by a polymerization method described previously by Fan [20]. First, the activated capillary was derived by 3-(triethoxysilyl) propyl methacrylate methanolic solution (50%, v/v). Then, the pre-polymerization mixture, consisting of monomer MAA (48 mg), crosslinker EGDMA (420 mg), porogenic solvent toluene (110 mg), dodecanol (860 mg) and initiator AIBN (4.5 mg) was filled into the capillary to a length of 15 cm. The reaction was performed at 60 °C for 16 h. Finally, the capillary was washed with methanol to remove the unreacted components and porogenic solvent, prior to its first use. Before each run, the capillary was conditioned initially by acetonitrile and then by the carrier solution.

2.3. Instrument and analytical conditions

The configuration of the in-tube SPME-HPLC device used for the study is shown in Fig. 2 [21]. The whole system consists of a pre-extraction segment, which includes a six-port valve (valve 1), a Shimadzu LC-10 AT pump (pump A) (Shimadzu, Tokyo, Japan) and a PEEK tube (0.03″ i.d., 0.7 mL total volume), and an analytical segment, which utilizes an Agilent 1100 series HPLC system (Agilent Technologies, Palo Alto, CA, USA). The Agilent 1100 series HPLC system includes a quaternary pump (pump B), a micro-vacuum degasser, a six-port valve (valve 2), a photodiode array detector (DAD) and a fluorescence detector (FLD). Valves 1 and 2 are connected with a PEEK tube.

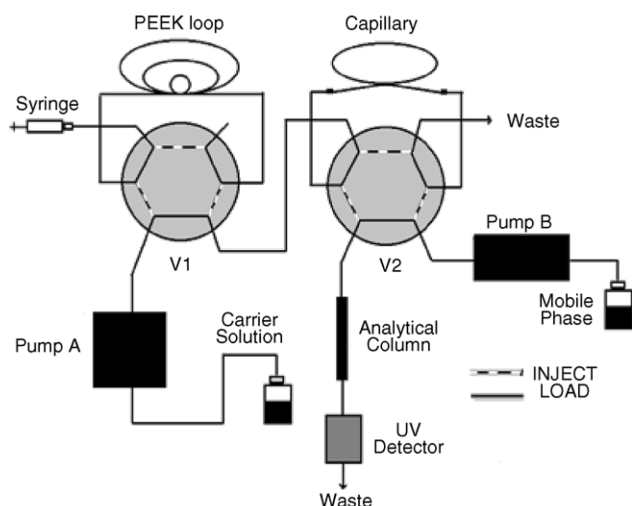

Fig. 2. Construction of in-tube SPME-HPLC.

The analytical column was a Betasil C18 column (250 mm × 4.6 mm i.d.; 5 μm) (Elite, Dalian, China). The optimized mobile phase was acetonitrile–5 mM NaAc buffer solution at pH 3.5 (40:60; v/v), and the flow rate was kept at 1.0 mL/min. The UV detection was 250 nm, and the fluorescence detector was set at an excitation wavelength of 250 nm and an emission wavelength of 380 nm.

2.4. In-tube SPME procedure

As shown in Fig. 2, the monolithic capillary was placed on valve 2 in the position where the injection loop was originally positioned. Valve 2 was set initially at LOAD position, and the mobile phase was driven by pump B directly through the analytical column to obtain a stable baseline in preparation for chromatographic separation. Before extraction, valve 1 was switched to LOAD position and the carrier solution was driven by pump A to flow through the capillary for conditioning at 0.04 mL/min. At the same time, the PEEK tube was filled with the sample solution using a syringe. When extraction began, valve 1 was switched to INJECT position for a given time interval (extraction time) and returned to LOAD position immediately thereafter extraction. The carrier solution, 30 mM phosphate buffer solution (pH 2):acetonitrile (95:5, v/v), was kept to flow through the capillary for 3 min after valve 1 switched back to the LOAD position in order to eliminate the residual sample solution and remove residual proteins in the capillary. Then the extracted analytes were desorbed from the monolithic capillary to the analytical column with the mobile phase at a flow rate of 0.02 mL/min for 5 min by switching valve 2 to INJECT position, followed by adjusting the flow rate of mobile phase to 1.0 mL/min for chromatographic separation.

2.5. Preparation of plasma and urine samples

Plasma and urine samples were collected from drug-free healthy volunteers. The plasma sample was centrifuged at 16,000 rpm for 5 min and the urine sample was centrifuged at 5000 rpm for 5 min, respectively, to remove any precipitated material. The five ARA-IIs were directly spiked into the supernatant of the biological samples. The plasma samples were diluted 10 times with a mixture of phosphate buffer (pH 2)–acetonitrile (90:5, v/v), and urine samples diluted with an equal volume of phosphate buffer (pH 2)–acetonitrile (90:10, v/v). The obtained samples in the concentration range of 0.5–5000 ng/mL were used directly for the analysis.

3. Results and discussion

3.1. Optimization of in-tube SPME

3.1.1. Effect of extraction time

In order to evaluate the capacity of the poly (MAA-EGDMA) monolithic capillary in the extraction of ARA-IIs, in-tube SPME of water sample were performed and the results were compared with those obtained through direct injection (Fig. 3). A great rise in the peak height of the analytes was observed after in-tube

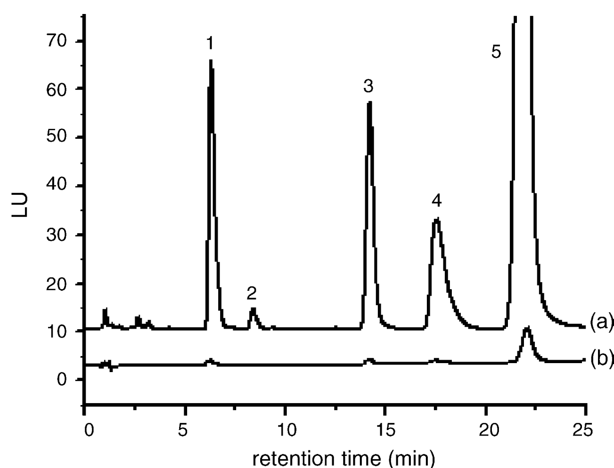

Fig. 3. Chromatograms obtained by (a) in-tube SPME of five ARA-IIs from aqueous sample and (b) direct injection of 7 μL of aqueous sample. Sample solution consisted of five ARA-IIs spiked at 200 ng/mL. The extraction flow rate was 0.04 mL/min. The extraction time was 10 min. HPLC conditions were outlined in Section 2. The fluorescence detector was set at an excitation wavelength of 250 nm and an emission wavelength of 380 nm; (1) candesartan; (2) losartan; (3) irbesartan; (4) valsartan and (5) telmisartan.

SPME (Fig. 3), indicating that a remarkable increase in sensitivity was achieved by in-tube SPME. The calculated enrichment factor for the analysis of the standard solution containing five ARA-IIs is listed in Table 1. The amount of analytes extracted by SPME was also calculated with the following equation [23]:

$$n_{\mathrm{A}} = FA = \left(\frac{m}{A_{\mathrm{d}}}\right) A \quad (1)$$

where n_{A} is the amount (mass) of analytes extracted by SPME, F is the detector response factor which is equal to the ratio of the amount of injected analyte (m) to the area count (A_{d}) obtained through direct injection ($F = m/A_{\mathrm{d}}$), A is the response (area count) obtained by SPME. The corresponding extraction yields were in the range of 89–96% (Table 1). The poly (MAA-EGDMA) monolithic capillary is thus of high extraction efficiency for these ARA-IIs compounds, which may be attributed to its better loading capability and faster mass transfer than conventional open-tubular liquid phase coated capillaries [20].

The extraction capacity of the capillary could be clearly determined by constructing extraction time profile. Higher extraction efficiency was achieved for analytes with higher partition coefficient, but a longer time duration was needed to reach equilibrium compared with analytes of lower partition coefficient. The extraction time profile for ARA-IIs was obtained by

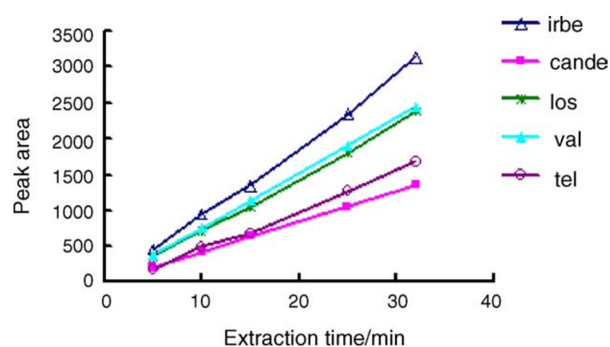

Fig. 4. In-tube SPME-HPLC extraction time profile of five ARA-IIs. Sample solution consisted of five ARA-IIs spiked at 1 μg/mL. The extraction flow rate was 0.04 mL/min. HPLC conditions were outlined in Section 2. UV detection was set at 250 nm.

extracting the sample solution of 1 μg/mL for extended duration of sampling time. The flow rate of the carrier solution was kept at 0.04 mL/min, and the valve switching time interval was increased from 5 to 30 min. As shown in Fig. 4, the extraction equilibrium was not achieved even after 30 min of sampling time. The increase in the extracted amount (peak area) of these compounds with the increase of sampling time indicated a great enrichment capacity of the poly (MAA-EGDMA) monolithic capillary for these analytes. Although longer extraction time may provide higher sensitivity, it prolongs the analysis time, and it is considered not suitable for the practical analyses of these compounds. The 10 min extraction time was selected for the subsequent analysis.

3.2. *Effect of sample solution*

In general, it is possible to increase extraction efficiency by changing pH and salt level of the sample solution. The buffer concentration of the sample matrix (10–100 mM) showed no significant influence on the extraction efficiency, being consistent with the previous report [20].

ARA-IIs studied here have functional groups with acid–basic properties such as the tetrazole group and the carboxyl group [24]. Losartan and irbesartan have tetrazole groups; valsartan and candesartan have both tetrazole and carboxyl groups; while telmisartan has a biphenylcarboxylic acid moiety instead of a biphenyltetrazole group. The pH of the sample matrix influenced the molecule form of analytes, resulting in different extraction efficiency. Optimization was performed by using buffer solutions within the pH range from 2 to 7.5. As shown in Fig. 5, similar effects of pH on the extraction could be observed between

Table 1
The enrichment factors[a] and extraction yields[b] for five ARA-IIs from aqueous samples extracted by the poly (MAA-EGDMA) monolithic capillary column

Compound	Candesartan	Losartan	Irbesartan	Valsartan	Telmisartan
Enrichment factors	52.9	53.1	54.3	51.1	55.1
Extraction yields (%)	91.7	96.5	94.7	89.4	96.4

[a] Calculated on the basis of peak areas obtained with in-tube SPME and those without preconcentration, i.e. the sample volume of direct injection was the same as the void volume (7 μL) of the extraction capillary.
[b] The percentage of extracted amounts of ARA-IIs over the total amounts injected.

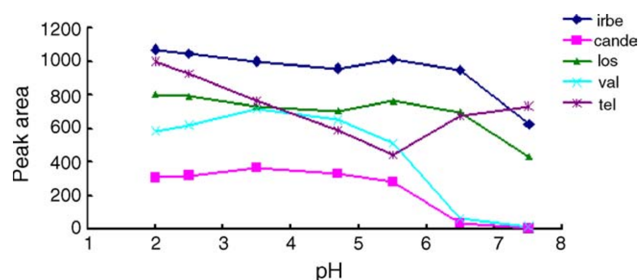

Fig. 5. Effect of pH on the extraction efficiency of ARA-IIs. Five ARA-IIs were spiked in 0.03 mol/L phosphate buffer solution at different pH levels at 1 μg/mL. The extraction flow rate was 0.04 mL/min. The extraction time was 10 min. HPLC conditions were outlined in Section 2. UV detection was set at 250 nm.

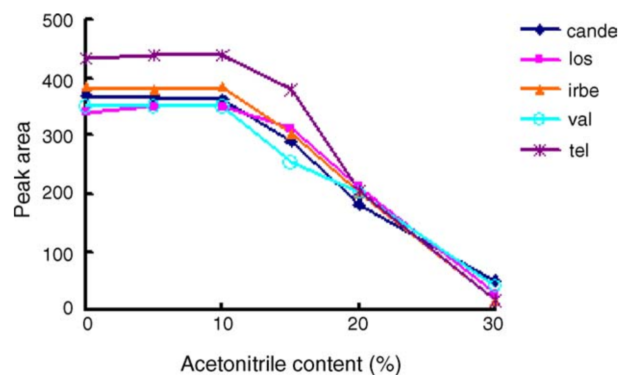

Fig. 6. Effect of acetonitrile contents in sample solutions on the extraction efficiency for ARA-IIs. Sample solutions consisted of five ARA-IIs spiked at 0.5 μg/mL. The extraction flow rate was 0.04 mL/min. The extraction time was 10 min. HPLC conditions were outlined in Section 2. UV detection was set at 250 nm.

losartan and irbesartan, and between valsartan and candesartan. But telmisartan showed quite different extraction behavior. Their adsorption on the monolithic capillary column was based on hydrophobic interaction, hydrogen bonding and acid–base interaction with the carboxyl pendant groups of the polymer [21]. The mix-mode mechanism involved in above extraction procedure was the cause of different extraction performance for these analytes under different pH values. The different influence of pH on telmisartan may suggest that other extraction mechanisms are also likely involved. A sharp decrease in the extraction efficiency was observed when pH level was above 6 (except telmisartan), so the pH below 6 was desirable for the subsequent experiments. When pH 4.5 was chosen for plasma samples, an obvious increase in the back pressure of the HPLC column was observed, indicating the presence of protein precipitation on the analytical column. But, when pH was adjusted below 2.5 no increase was detected in the back pressure of the analytical column. As ARA-IIs can bind with proteins in plasma samples [3], part of the analytes extracted by the monolithic capillary would be combined with proteins. And the co-elution of proteins with the analytes would lead to the contamination of the analytical column. Thus pH 2 was selected for the biological sample matrix in the following experiments to increase electrostatic repulsions between the drugs and proteins [25].

A small percentage of an organic solvent has been reported to be able to displace bound drugs since most drugs are reversibly bound to serum proteins such as albumin via several lower affinity sites [25]. Furthermore, organic solvents were able to avoid the co-extraction of weakly adsorbed components, and thus the matrix interference would be reduced. Acetonitrile was employed in the preparation of sample solutions and the effect of its content on extraction performance was also investigated. As shown in Fig. 6, the extraction efficiency was almost uninfluenced when 10% (v/v) or less acetonitrile was used. However, the extraction efficiency of ARA-IIs would decrease when acetonitrile content exceeded 10%. In addition, too much organic solvent in sample solutions would lead to irreversible protein precipitation during the extraction and result in the block of the monolithic capillary. Therefore, 5% acetonitrile in samples was selected for the SPME of ARA-IIs from the plasma and urine samples.

3.3. *In-tube SPME desorption and HPLC separation of ARA-IIs*

After extraction of the analytes, on-line elution was simply accomplished by directing the mobile phase through the capillary as shown in Fig. 2. However, it was important to ensure that the selected mobile phase would not only provide quantitative transfer of the extracted analytes from the capillary into the analytical column, but also allow their separation on the analytical column. After the analytes were eluted from the capillary with the mobile phase of acetonitrile–5 mM NaAc buffer solution at pH 3.5 (40:60; v/v) at a flow rate of 0.02 mL/min for 5 min, a blank analysis following separation procedure was carried out to confirm the successful desorption, with no carryover found in the experiment. Under the desorption condition, adequate chromatographic resolution by the analytical column could be obtained and matrix peaks were found to have no influence on the quantification of ARA-IIs with fluorescence detection.

3.4. *Analysis of five ARA-IIs in human plasma and urine*

For highly protein bound-drugs, changes of pH or addition of organic solvent or combination of such approaches [25] may be required to achieve the release of these drugs. Under the previously determined extraction and elution conditions, the plasma and urine samples were successfully extracted. For biological samples it was important to apply a wash step immediately after the extraction to remove any residual proteins from the extraction capillary, which ensured the reduction of matrix interference and the prevention of proteins from polluting the analytical column. The carrier solution of 30 mM phosphate buffer solution (pH 2):acetonitrile (95:5, v/v) was kept to flow through the capillary for 3 min in the wash step. It was demonstrated that the extraction and preconcentration of analytes were uninfluenced by the wash step.

Generally, when in-tube SPME was performed with devices constructed as in Fig. 2, the extraction segment and the analytical segment could be manipulated separately by controlling valve 1. When chromatographic separation was performed on the ana-

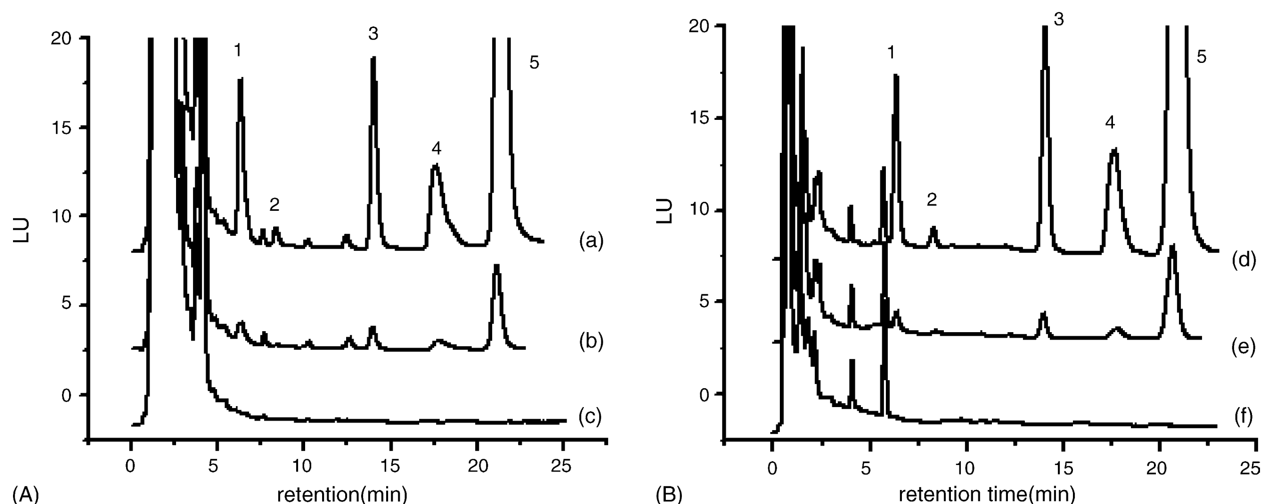

Fig. 7. Chromatograms obtained by in-tube SPME of five ARA-IIs from spiked plasma and urine samples: (a) urine sample spiked at 50 ng/mL; (b) urine sample spiked at 5 ng/mL; (c) blank urine sample; (d) plasma sample spiked at 50 ng/mL; (e) plasma sample spiked at 5 ng/mL and (f) blank plasma sample. The extraction flow rate was 0.04 mL/min. The extraction time was 10 min. HPLC conditions were outlined in Section 2. The fluorescence detector was set at an excitation wavelength of 250 nm and an emission wavelength of 380 nm; (1) candesartan; (2) losartan; (3) irbesartan; (4) valsartan and (5) telmisartan.

lytical column, the extraction segment was ready for another extraction. Therefore, the total time for a whole analysis was the separation time plus the desorption time (around 25 min in the present experiment). Moreover, the sample consumption for each run of extraction was low, being 0.4 mL, which was especially suitable for the analysis of biological samples.

Selectivity study was performed by evaluating several plasma samples from different sources and several urine samples from healthy volunteers. No interfering peak was observed in influencing the quantification of five analytes. In Fig. 7, typical chromatograms obtained from blank and ARA-IIs spiked human plasma and urine samples (spiked at 5 and 50 ng/mL, respectively) were shown. Fluorescence detection was chosen for the further increase of sensitivity. Baseline separation of all compounds was observed under optimized conditions.

Plasma and urine samples were spiked over a range of concentrations (0.5–5000 ng/mL) with five ARA-IIs compounds. Calibration curves for the five ARA-IIs were constructed with the satisfactory regression coefficient ($R > 0.9985$) as shown in Tables 2 and 3, respectively. The average recoveries of the analytes from spiked samples, calculated by comparing the obtained peak areas with those of spiked aqueous solutions, were found to be in the range from 84% to 99%, and no significant differ-

Table 2
Calibration curves for in-tube SPME of ARA-IIs from plasma samples

Compound	Linear range (ng/mL)	Calibration curves			LOD (ng/mL)	LOQ (ng/mL)
		Slope	Intercept	*r*		
Candesartan	5–2000	3.71	45.69	0.9985	0.8	2.7
Losartan	50–5000	0.41	−3.64	0.9994	15.3	51.0
Irbesartan	5–2000	5.16	140.63	0.9986	1.2	3.7
Valsartan	10–2000	5.55	56.85	0.9997	2.9	9.7
Telmisartan	0.5–200	53.61	−109.54	0.9993	0.1	0.4

Number of data points for calibration curves was 6. The extraction flow rate was 0.04 mL/min. The extraction time was 10 min. HPLC conditions were outlined in Section 2. The fluorescence detector was set at an excitation wavelength of 250 nm and an emission wavelength of 380 nm.

Table 3
Calibration curves for in-tube SPME of ARA-IIs from urine samples

Compound	Linear range (ng/mL)	Calibration curves			LOD (ng/mL)	LOQ (ng/mL)
		Slope	Intercept	*r*		
Candesartan	5–2000	4.33	42.07	0.9995	0.7	2.3
Losartan	50–5000	0.40	−3.25	0.9998	15.2	50.7
Irbesartan	5–2000	5.12	103.46	0.9994	0.9	3.0
Valsartan	10–2000	5.83	−13.25	0.9999	2.9	9.6
Telmisartan	0.5–200	45.76	−63.80	0.9998	0.1	0.4

Number of data points for calibration curves was 6. The extraction flow rate was 0.04 mL/min. The extraction time was 10 min. HPLC conditions were outlined in Section 2. The fluorescence detector was set at an excitation wavelength of 250 nm and an emission wavelength of 380 nm.

Table 4
Intra- and inter-day precision for in-tube SPME of ARA-IIs from plasma and urine samples

Compound	Concentration (ng/mL)	Precision (R.S.D., %)			
		Plasma sample		Urine sample	
		Intra-day ($n = 5$)	Inter-day ($n = 5$)	Intra-day ($n = 5$)	Inter-day ($n = 5$)
Candesartan	10	1.2	3.2	1.4	4.5
	200	5.0	6.6	2.9	4.3
	1000	2.6	4.5	1.6	5.8
Losartan	50	0.3	1.5	2.5	2.3
	500	0.9	2.7	2.1	2.4
	2000	0.1	1.5	4.0	3.8
Irbesartan	10	3.1	2.8	2.6	3.5
	200	2.0	2.1	0.6	1.6
	1000	2.4	3.9	3.5	4.2
Valsartan	10	1.7	3.2	1.7	2.5
	200	3.7	3.9	3.0	4.9
	1000	3.6	3.3	3.9	3.2
Telmisartan	1	0.2	4.5	1.5	5.8
	50	0.1	3.9	1.7	2.6
	200	0.7	3.7	0.9	2.5

Intra-day precisions were calculated by performing five extractions of independently prepared plasma and urine samples with ARA-IIs spiked at different concentrations over a day. Inter-day precision was accessed by performing five extractions of independently prepared plasma and urine samples with ARA-IIs spiked at different concentrations for continuous 5 days. The extraction flow rate was 0.04 mL/min. The extraction time was 10 min. HPLC conditions were outlined in Section 2. The fluorescence detector was set at an excitation wavelength of 250 nm and an emission wavelength of 380 nm.

ence in the recovery for all analytes was observed between the plasma and urine samples.

The limit of detection (LOD) and limit of quantification (LOQ) for each compound in plasma and urine were determined at concentrations where the signal/noise ratios were equal to 3 and 10, respectively, and the results are also shown in Tables 2 and 3.

The reproducibility of the developed method was determined by inter- and intra-day precision. As shown in Table 4, the intra- and inter-day precision did not exceed 7% R.S.D. at different concentration levels. Several analysts have confirmed these results over a span of many weeks, indicating the ruggedness of the proposed method. Our previous study has proved that the monolithic capillary can be used for more than 200 times without any decrease in extraction efficiency [20]. In the present study, no decrease in the extraction efficiency of the monolithic capillary was observed over 2-month period for the analysis of ARA-IIs, providing further support for the high stability of this monolithic capillary. Moreover, the reproducibility of the monolithic capillary has been confirmed in our previous study [21].

4. Conclusions

An in-tube SPME coupled to HPLC method using a (MAA-EGDMA) polymer monolithic capillary as the extraction medium was developed for the direct determination of ARA-IIs in biological samples. In comparison with existing extraction procedures for the determination of the ARA-IIs in the biological samples, the method established in the present study is simple in preparation procedure, and is relatively easy and accurate with also characters of on-line analysis. Using fluorescence detection, the method has higher sensitivity for the determination of the ARA-IIs with the limit of detection (LOD) ranging from 0.1 to 15.3 ng/mL.

Acknowledgements

The authors gratefully acknowledge the National Nature Science Foundation of China (Grant: 20275029), the Excellent Young Teachers Program of MOE, China.

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