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Original Paper

Determination of telmisartan in rat tissues by in-tube solid-phase microextraction coupled to high performance liquid chromatography

A poly(methacrylic acid-ethylene glycol dimethacrylate, MAA-EGDMA) monolithic capillary was used for the direct and on-line extraction of telmisartan from Sprague-Dawley rat tissue (heart, kidney, and liver) homogenates. Under optimized conditions, the tissue homogenates were simply diluted with a mixture of phosphate buffer (pH 2)/ACN (90 : 8 v/v), and then injected for extraction only after centrifugation and filtration. Coupled to HPLC with fluorescence detection, the method was linear over the range of 1.25–1500 ng/g for telmisartan in heart and kidney, 12.5–15 000 ng/g in liver with correlation coefficients over 0.9992. The detection limits were found to be in the range from 0.24 to 1.8 ng/g. RSDs for intra- and inter-day ranged from 1.2 to 8.1%. The determination of telmisartan in treated rat tissues was achieved by using the proposed method.

Keywords: Poly(methacrylic acid-ethylene glycol dimethacrylate) / Monolithic capillary / In-tube SPME LC / Telmisartan / Rat tissue

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1 Introduction

Telmisartan (Fig. 1), 4-[(2-*n*-propyl-4-methyl-6-(1-methyl-benzimidazol-2-yl)-benzimidazol-1-yl) methyl]-biphenyl-2-carboxylic acid, is a potent, long-lasting, nonpeptide antagonist of the angiotensin II type-1 (AT1) receptor that is indicated for the treatment of essential hypertension. It selectively and insurmountably inhibits stimulation of the AT1 receptor by angiotensin II without affecting other receptor systems involved in cardiovascular regulation. Very high lipophilicity, coupled with a high volume of distribution, indicates that the compound offers the clinically important advantage of good tissue penetration [1]. The plasma concentration-time profiles were characterized by fast absorption and a rapid biexponential decline after the peak plasma concentration, with a prolonged terminal elimination phase (>20 h) [2]. Telmisartan undergoes minimal biotransformation in the liver to form telmisartan 1-*O*-acylglucuronide [3], the major inactive metabolite in humans. A small amount of the dose is excreted in urine (<1%) with the rest of the parent compound (more than 98%) excreted in the feces [4].

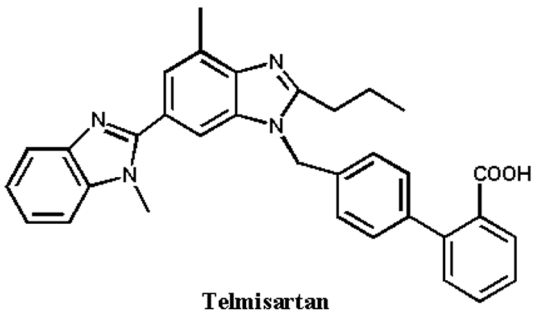

Figure 1. Structure of telmisartan.

The determination of telmisartan in biological samples such as plasma, urine and tissues has been reported using radio-labeled technique [5] and HPLC [6–9]. Because of the complexity of biological samples, pretreatment methods are necessary in these methods to concentrate the analytes and clean up the samples, such as protein precipitation [7], SPE [6] and liquid–liquid extraction [8, 9]. However, these methods require large volumes of samples and solvent, and are not easy to automate. In-tube solid-phase microextraction (in-tube SPME) coupled to HPLC was first introduced by Eisert and Pawliszyn in 1997 [10], which utilized an inner surface coated capil-

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Abbreviations: EGDMA, ethylene glycol dimethacrylate; **MAA**, methacrylic acid; **SPME**, solid-phase microextraction

lary as the extraction phase. And this technique has been applied successfully to determine various compounds in environmental waters, foods, biological samples, *etc.* [11 – 14]. As an ideal sample preparation technique, this on-line in-tube SPME technique integrates extraction, concentration, desorption, and injection into a single procedure, and can obtain better accuracy, precision and sensitivity than those of off-line methods. We previously introduced on-line in-tube methods using poly(methacrylic acid-ethylene glycol dimethacrylate, MAA-EGDMA) monolithic material as an extraction medium to determine some basic drugs in plasma [15], serum [16] and urine [17, 18] samples. This material was demonstrated to be biocompatible and no additional sample preparation (such as ultracentrifugation and other deproteinization steps) was needed to eliminate the protein component of the sample prior to extraction.

Measuring drug levels in tissues is important for the assessment of drug distribution in the body and the investigation of past exposure to drugs. To our best knowledge, direct determination of drug concentrations in tissues by in-tube SPME has not been reported. In the present study, a method was developed by using in-tube SPME coupled to HPLC with fluorescence detection for determining the concentration of telmisartan in rat tissues (heart, kidney, and liver). A poly MAA-EGDMA monolithic capillary was used as the extraction medium.

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 (Shanghai, China) and were of analytical reagent grade. Double distilled water was used for all experiments.

Telmisartan was kindly supplied by Jiangsu Institute for Drug Control (Nanjing, China). The stock solution of telmisartan at a concentration of 1 mg/mL was prepared in methanol and stored at 4°C in the dark. Working solutions were prepared daily by appropriate dilution of this stock solution with double distilled water.

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

The poly(MAA-EGDMA) monolithic capillary was synthesized inside a fused-silica capillary (20 cm × 0.25 mm, ID, Yongnian Fiber Plant, Hebei, China) by a polymerization method described previously by Fan [16]. Before each run, the capillary was first conditioned by ACN and then by the carrier solution.

2.3 Instrument and analytical conditions

The in-tube SPME-HPLC system (Fig. 2) consists of a pre-extraction segment, which includes a Rheodyne 7725i six-port valve (valve 1), a Shimadzu LC-10AT pump (pump A) (Shimadzu, Tokyo, Japan) and a PEEK tube (0.03″ ID, 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 microvacuum degasser, a Rheodyne 7725i 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. The extraction manipulation has been detailed in our previous work [19]. The extraction flow

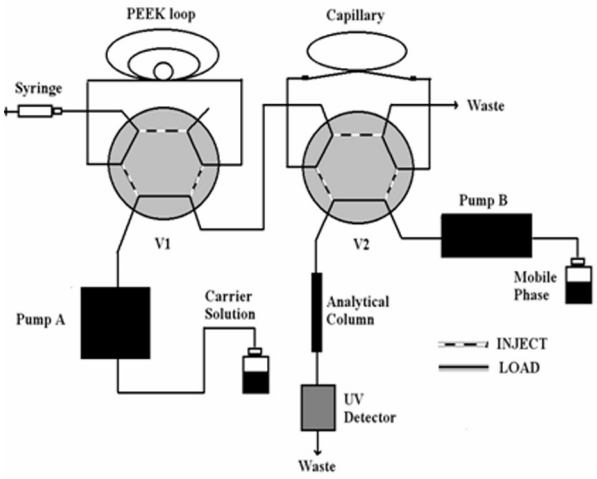

Figure 2. Construction of in-tube SPME-HPLC.

rate was set to 0.04 mL/min. After extraction, the carrier solution of 30 mM phosphate buffer solution (pH 2)/ACN (95 : 8 v/v) was kept to flow through the capillary for 8 min in the wash step. The desorption of the analyte was carried out by directing the mobile phase to flow through the capillary at 0.03 mL/min for 5 min.

The analytical column was a COSMOSIL packed column 5C18-MS-II (250 mm × 4.6 mm ID; 5 μm) (NACALAI TESQUE). The optimized mobile phase was ACN – 5 mM NaAc buffer solution at pH 6 (45:55 v/v), and the flow rate was kept at 0.6 mL/min. The FLD was set at an excitation wavelength of 300 nm and an emission wavelength of 370 nm.

2.4 Collection and preparation of samples

The heart, kidney, and liver were excised from the euthanized male SD rats. Immediately after the organ removal, 1 g of organ tissue was homogenized using a glass homogenizer in a mixture of phosphate buffer (pH 2)/ACN (90 : 8 v/v) to obtain final concentrations of 200 mg wet

tissue wt/mL. And then the homogenates were stored at −18°C until analysis.

The homogenized organ tissue of untreated rats was directly spiked with telmisartan, and then diluted five times (heart, kidney) or 50 times (liver) with a mixture of phosphate buffer (pH 2)/ACN (90 : 8 v/v). The obtained sample, in the concentration range of 0.05–60 ng/mL, was shaken vigorously for 10 min and centrifuged for 8 min at 13 000 rpm. After filtration (syringe microfilter, 0.45 μm), the supernatants were injected directly for extraction. The final concentration corresponded to 1.25–1500 ng telmisartan *per* gram heart or kidney, 12.5–15 000 ng telmisartan *per* gram liver.

The tissue homogenates of the treated rats were directly diluted five times (heart, kidney) or 50 times (liver) with a mixture of phosphate buffer (pH 2)/ACN (90:8 v/v). The obtained sample was shaken vigorously for 10 min and centrifuged for 8 min at 13 000 rpm. After filtration (syringe microfilter, 0.45 μm), the supernatants were injected directly for extraction.

3 Results and discussion

3.1 Desorption and HPLC procedure

In manipulating the in-tube SPME-HPLC system, desorption of the analyte was simply accomplished by directing the mobile phase to flow through the capillary with a valve switch. The introduction of an additional desorption solvent was unnecessary. The desorption time was set at 5 min and the desorption flow rate was optimized in the range of 0.01–0.05 mL/min with no obvious peak broadening and tailing in the following chromatographic analysis. The flow resistance would increase with increasing desorption rate. It was showed that the analyte could be eluted completely from the capillary at the flow rate of 0.03 mL/min for 5 min. And a blank analysis was carried out to further confirm the successful desorption with no carryover found in the experiment. Under the optimized desorption condition, proper retention of telmisartan on the analytical column could also be obtained and matrix peaks were found to have no influence on the quantification of telmisartan.

3.2 Optimization of in-tube SPME conditions

The adsorption of the analyte on the monolithic capillary column was mainly based on hydrophobic interaction, hydrogen bonding and acid–base interaction with the carboxyl pendant groups of the polymer [17]. For in-tube SPME, it is important to increase the distribution factor of the analyte to obtain rapid and high extraction efficiency. The amount of analyte extracted onto the stationary phase of the capillary depends on the property of the extraction phase, extraction flow rate, extraction time and sample pH, *etc*. The optimization of extraction time has been reported in our previous study [19], and 10 min extraction time was selected for the subsequent analyses with satisfactory sensitivity obtained. The extraction flow rate of the sample solution was optimized in the range of 0.01–0.05 mL/min, and only a slight increase in the extraction efficiency was found as the flow rate decreased. Therefore, 0.04 mL/min was selected to offer both shorter extraction time and satisfying extraction efficiency.

The pH of the matrix relates closely to the interaction between analytes and the extraction phase and thus would influence the extraction efficiency. Optimization has been performed by using buffer solutions in the pH range of 2–7.5 [19]. The results showed that the highest extraction efficiency was achieved at pH 2 and the lowest at pH 5.5. Therefore, the tissue homogenates were adjusted to pH 2 by using 30 mM phosphate buffer solution in the following experiments. Moreover, the selected acidic medium could increase the solubility of telmisartan in the buffer solution and weaken the affinity between the analyte and proteins.

A small percentage of ACN was also employed in the preparation of sample solutions and the effect of its content on extraction performance has been investigated [19]. The advantages of employing ACN in sample solutions were to increase the solubility of telmisartan, to avoid the coextraction of the weak adsorption components, and most importantly, to effectively displace the bound drug from protein. Therefore, 8% ACN in samples was selected for the SPME of telmisartan from tissue homogenate samples. Under the optimized conditions, the extraction yield reached 96%, which was the percentage of extracted amounts of telmisartan over the total amounts injected.

3.3 Analysis of telmisartan in rat tissues

The tissue homogenates are more complicated biological samples than plasma and urine. The determination of target compounds in rat tissue by HPLC is not as easy as that in plasma and urine due to more endogenous compounds. Extraction of analyte by the poly(MAA-EGDMA) monolithic capillary is based on the adsorption mechanism [18]. Interferences can significantly affect the extraction of analyte. Interferences coextracted with the analyte of interest may reduce the amount of the analyte extracted and the linear range of the method [20]. The hepatic homogenates were diluted 2–50 times with a mixture of phosphate buffer (pH 2)/ACN (90:8 v/v). As shown in Fig. 3, with the increase of dilution factor for hepatic homogenates, the recovery of analyte was also increased. A sharp increase of recovery was observed when the dilution was increased from two to four times, and the recovery was almost 100% when the hepatic homogenate was diluted 50 times. The same results were

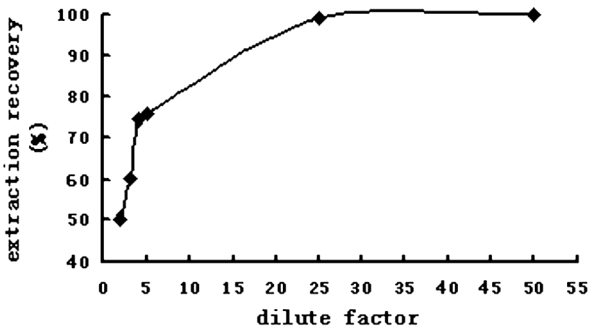

Figure 3. Effect of dilution factors on the recovery (the recovery of the analyte from spiked samples, calculated by comparing the obtained peak areas with those of spiked aqueous solutions) of telmisartan from hepatic homogenates. After dilution, the final concentrations of telmisartan in sample solutions were all 15 ng/mL. Extraction flow rate was 0.04 mL/min. Extraction time was 10 min. HPLC conditions were outlined in Section 2.

also obtained for cardiac and renal tissue homogenates. Because both sensitivity and precision had to meet the requirements for quantitative analysis, diluting five times was acceptable for cardiac and renal tissue homogenates in the following experiments, while hepatic tissue homogenate was diluted 50 times just because of the high drug concentrations in rat liver.

The decrease of the matrix interference could be realized by increasing the wash time. The wash step was achieved by allowing the carrier solution, 30 mM phosphate buffer solution (pH 2)/ACN (95 : 8 v/v), to flow through the capillary for 8 min. It was demonstrated that the extraction and preconcentration of analyte were uninfluenced by the wash step.

Under the optimized conditions, the poly(MAA-EGDMA) monolithic capillary was applied for the determination of telmisartan in tissue homogenates. The blank tissue samples (heart, kidney, and liver) were also extracted respectively, and no interfering peak was observed in influencing the quantification of telmisartan. After extraction and wash steps, telmisartan was desorbed and transferred directly to the analytical column without any loss, so no internal standard was needed actually. And the results showed that the reproducibility and accuracy were satisfactory without the internal standard.

As shown in Table 1, the linear range for telmisartan in the spiked tissue homogenates was 1.25 – 1500 ng *per* gram in heart or kidney and 12.5 – 15 000 ng *per* gram in liver. The results demonstrated good fit to the weighted regression lines (the weight = $1/y^2$). The weighted regression was employed only because of the wide range of linearity (reach to three orders of magnitude). The regression coefficients were better than 0.9992 and the detection and quantification limits were also calculated

Table 1. Calibration curves for in-tube SPME of telmisartan from tissue homogenate samples

Tissue	Linear range, ng/g	Calibration curves ($y = bx + a$)			LOD ng/g	LOQ ng/g
		Slope	Intercept	R		
Heart	1.25 – 1500	2.385 ± 0.042	14.90 ± 30.26	0.9992	0.3	0.9
Kidney	1.25 – 1500	2.588 ± 0.034	14.14 ± 22.95	0.9996	0.2	0.8
Liver	12.5 – 15 000	0.346 ± 0.002	1.13 ± 14.35	0.9992	1.8	6.2

Number of data points for calibration curves was six. Weight of regression lines was $1/y^2$. Extraction flow rate was 0.04 mL/min. Extraction time was 10 min. HPLC conditions were outlined in Section 2.

Table 2. Reproducibility and accuracy for in-tube SPME of telmisartan from tissue homogenate samples

Tissue	Nominal concentration, ng/g	Precision (RSD%)		Mean found concentration, ng/g	Mean relative error,%[a)]
		Intraday (n = 5)	Interday (n = 5)		
Heart	5	1.8	8.1	5.14	2.7
	125	1.9	5.9	126	0.5
	1250	2.7	3.0	1.20×10^3	4.0
Liver	50	2.5	5.2	49.2	1.5
	1250	1.2	4.3	1.31×10^3	4.6
	12500	3.5	3.7	1.20×10^4	3.8
Kidney	5	2.5	6.8	5.02	0.4
	125	1.6	4.8	124	0.8
	1250	2.3	5.8	1.28×10^3	2.6

a) Mean relative error (RE) = |mean measured concentration – nominal concentration| × 100/nominal concentration.

Intra-day precisions were calculated by performing five extractions of independently prepared tissue homogenate samples with telmisartan spiked at different concentrations over a day. Inter-day precisions were assessed by performing five extractions of independently prepared tissue homogenate samples with telmisartan spiked at different concentrations for continuous 5 days. Extraction flow rate was 0.04 mL/min. Extraction time was 10 min. HPLC conditions were outlined in Section 2.

experimentally with the S/N set at 3 and 10, respectively. The application of this SPME-HPLC-fluorimetric method to the quantitation of telmisartan in tissue homogenates of the treated rats confirmed the appropriate concentration range of the calibration curve.

The reproducibility of the developed method was determined by the interday and intraday precisions. As shown in Table 2, the relative standard deviations for intra- and inter-day precisions were found to be < 8.1% RSD at different concentration levels. Accuracy values were studied at three concentration levels and satisfactory for all the concentrations tested.

The extraction recovery was calculated by comparing the obtained peak area of tissue samples with that of aqueous solutions at the same concentration. The extraction recovery in kidney and heart was approximately 75%, and was almost 100% in liver. The difference in extrac-

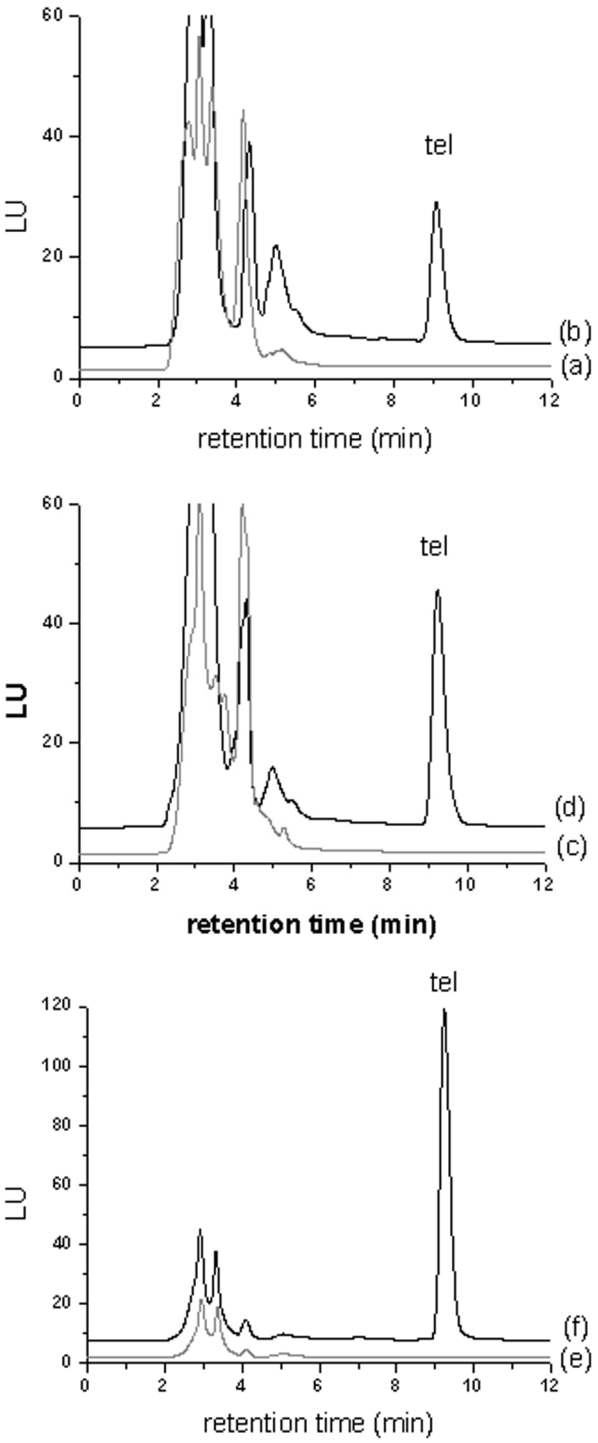

Figure 4. Chromatograms obtained by in-tube SPME of telmisartan from tissue homogenate samples taken 2 h after oral administration. (a) A blank cardiac tissue homogenate sample; (b) a cardiac tissue homogenate sample from a treated rat; (c) a blank renal tissue homogenate sample; (d) a renal tissue homogenate sample from a treated rat; (e) a blank hepatic tissue homogenate sample; and (f) a hepatic tissue homogenate sample from a treated rat. Extraction flow rate was 0.04 mL/min. Extraction time was 10 min. HPLC conditions were outlined in Section 2.

tion recovery was due to the different concentrations of interferences in the matrix resulting from different dilution factors.

3.4 Application to real samples

The developed method was applied to the determination of telmisartan in tissues of the treated rats. Figure 4 showed the chromatograms of rat tissues removed 2 h after a single oral administration of telmisartan (16.5 mg/kg). Concentration values were interpolated from the daily calibration curves of tissue homogenates. Concentration values were 5.8 μg/g in liver, 206.2 ng/g in heart and 348.5 ng/g in kidney. These results were in accordance to the previously published report that high levels of radioactivity were observed in the liver after oral administration of ^{14}C-telmisartan to rats [5].

4 Concluding remarks

An in-tube SPME coupled to HPLC method using (MAA-EGDMA) polymer monolithic capillary as the extraction medium was developed for the direct determination of telmisartan in rat tissues (heart, kidney, and liver). The validation of the assay is adequate in terms of generally accepted guidelines for linearity, LOQ, precision, and accuracy in the bioanalytical laboratory. In comparison with existing extraction procedures for the drug determination in tissues [21], the proposed method is simple and accurate with small consumption of organic solvents. Future investigations would apply this method to different tissue samples to further verify this method.

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