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HPLC assays to simultaneously determine the angiotensin-AT_1 antagonist losartan as well as its main and active metabolite EXP 3174 in biological material of humans and rats[1]

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Abstract

Novel rapid and sensitive HPLC assays were developed to simultaneously determine losartan and its main active metabolite EXP 3174 in biological material of humans and rats following solid–phase or liquid–liquid extraction. The analytes were separated on a 3 μm particle-sized ULTREMEX™ CN column, which was preceeded by a 5 μm particle-sized guard column, using UV-detection at 245 nm. The assays provided high sensitivity with limits of quantification (LoQ) of 5 ng ml^{-1} for both compounds in human and rat plasma and 10 ng ml^{-1} in human and rat urine, respectively. In rat blood, bile and various tissues, limits of quantifications were achieved that ranged 10–15 ng per ml and per 100 mg tissue, respectively, for both analytes. © 1998 Elsevier Science B.V.

Keywords: Losartan; EXP 3174; Nonpeptide Angiotensin-AT_1 antagonists; Reversed-phase high-performance liquid chromatography; Solid–phase extraction; Plasma; Urine; Blood; Bile; Tissues; Rats

1. Introduction

Losartan, the potassium salt of 2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[(2-(1H-tetrazol-5-yl)-biphenyl-4-yl)methyl]imidazole is the prototype of the new generation of potent, orally active nonpeptide angiotensin-AT_1 antagonists (Fig. 1(a)) which have been developed in sequence to the ACE inhibitors as a further therapeutic approach to attenuate an overstimulated Renin–Angiotensin–Aldosterone System (RAAS) [1,2].

Following administration the angiotensin-II blocking activity of losartan is predominantly based on its major active metabolite EXP 3174 (Fig. 1(b)), a carboxylic acid, to which losartan is

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converted mainly by CYP 3A4 and 2C9 isoenzymes [3–5].

Losartan and—to a higher degree—EXP 3174 have proven to effectively lower elevated arterial blood pressure up to 24 h post-dose and, furthermore, to exert a beneficial effect on the regression of vascular and myocardial hypertrophy associated with hypertension [3]. Thus angiotensin-AT_1 antagonists such as losartan and EXP 3174 provide a powerful alternative to ACE inhibitors without their bradykinin-potentiating effects which seem to cause dry cough in patients who are treated with ACE inhibitors.

As already described for ACE inhibitors, the penetration and distribution of inhibitors of the Renin–Angiotensin–Aldosterone System into heart, lung, blood vessels and other tissues is crucial for their effect, since the cardiovascular homeostasis is not only regulated by a circulating (plasma)RAAS, but also by local RAAS that synthesize angiotensin II directly in the respective tissues [6].

A comprehensive analysis of nonpeptide angiotensin-AT_1 antagonists in biological material may thus also require analytical methods for tissue evaluations in addition to plasma and urine data as described in the following for losartan and EXP 3174.

2. Experimental

2.1. Materials

Losartan and EXP 3174 were kindly provided by Merck Sharp and Dohme Research Laboratories (Rahway, NJ, USA). The internal standard (L - 158.854, 2 - *n* - butyl - 4 - (2 - chloro - phenyl) - 5 - carboxyl - 1 - [(2 - (1H - tetrazol - 5 - yl)bi - phenyl-4-yl) methyl]imidazole) was a gift from the DuPont Merck Company (Wilmington, DE, USA).

HPLC-grade acetonitrile, methanol, tetrahydrofuran, methyl *tert*-butyl ether (MTBE) and acetone as well as extra pure ammonia solution 32%, sodium phosphate monobasic-dihydrate, sodium phosphate dibasic-dihydrate, potassium phosphate monobasic, 1-molar hydrochloric acid and 85% phosphoric acid were obtained from E. Merck (Darmstadt, Germany). Bidistilled water was always freshly prepared with a Destamat™ Bi 18E (Heraeus, Hanau, Germany).

Heparinized control human plasma was obtained from healthy volunteers. The animal studies were carried out using male Wistar rats weighing 250–350 g (Hoechst, Frankfurt/Main, Germany).

2.2. Standard solutions

The internal standard L-158.854 was dissolved in acetonitrile–methanol (1:1 v/v) to a primary concentration of 80 μg ml^{-1} which was kept at −30°C throughout the study; aliquots were further diluted to a working standard solution of 10 μg ml^{-1} in acetonitrile–methanol (1:1 v/v). Losartan which is readily dissolved in bidistilled water was prepared in 10% methanol to ensure stability, whereas EXP 3174 was dissolved in pure methanol for both compounds yielding concentrations of 200, 20 and 2 μg ml^{-1}. All standard solutions were stored at 4°C for about one month without any detectable degradation.

2.3. Instrumentation

The HPLC system consisted of a Jasco pump PU-980, a Jasco autosampler 851-AS with a 100 μl filling loop and a Shimadzu SPD-6A UV detector. Data analysis was performed using a ChromJet Integrator SP4400 connected to LABNET/WINer 386 software (Thermo-Separation-Products). Solid–phase extraction was performed with a Varian Vac Elut SPS 24 by using Varian Bond Elut™ non-endcapped CN-cartridges (part. no. 1210-2007).

For centrifugation, a Heraeus Minifuge GL was employed. Evaporation of organic solvents was achieved with a Speed Vac™ Plus SC 210A, Savant Instruments. Rat tissues were homogenized with an Ultra Turrax T25 (Janke and Kunkel) as well as with a Potter S (B. Braun Biotech International).

2.4. Sample preparation

The internal standard (L-158.854), abbreviated as IS in the following, was in all cases used in a concentration of 300 ng ml^{-1} sample.

2.4.1. Solid–phase extraction of human and rat plasma

Solid–phase extraction was performed prior to HPLC using Varian Bond Elut™ non-endcapped CN-cartridges. These were activated with two column volumes (2 × 1 ml) of pure methanol and, subsequently, with two column volumes of bidistilled water. After the addition of IS to 500 μl of plasma, the mixture was acidified with 500 μl of 0.2 M HCl, thoroughly vortexed and applied to prepared solid–phase cartridges. The adsorbent which contained both analytes and internal standard was then washed with 500 μl of bidistilled water followed by the same amount of 10% methanol and, as last step, with 50 μl of pure methanol. The analytes and internal standard were eluted by gravity with 5 200 μl-portions of methanol adjusted to pH 8.0 (methanol:ammonia

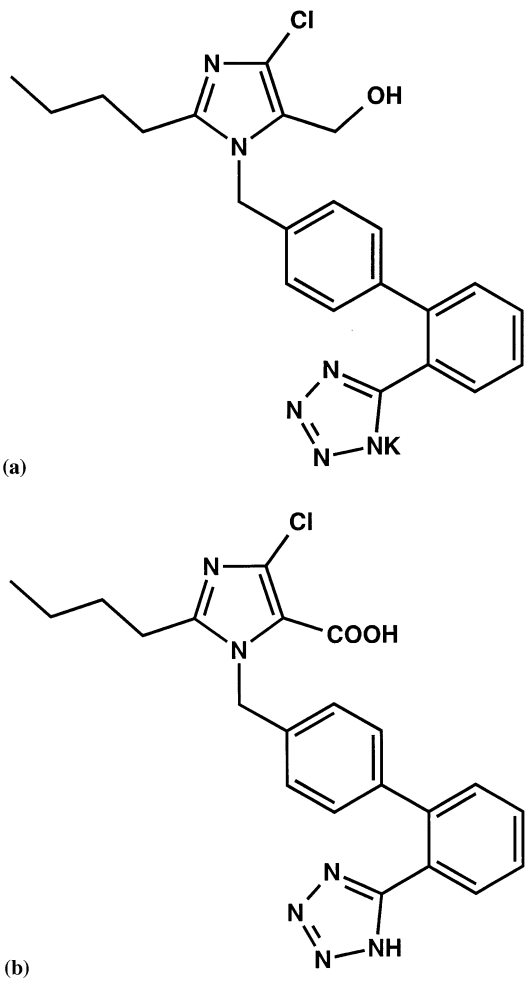

Fig. 1. Structures of compounds (a) Losartan; (b) EXP 3174.

Table 1
Chromatographic conditions for the analysis of losartan (L), EXP 3174 (E) and internal standard (IS): (**I**) following solid–phase extraction, (**II**) following liquid–liquid extraction (from rat blood and rat bile), (**III**) following liquid–liquid extraction (from rat tissues)

Column	ULTREMEX™ 3 CN (250 × 4.6 mm I.D.)
Guard column	ULTREMEX™ 5 CN (30 × 4.6 mm I.D.)
Flow-rate	0.6 ml min^{-1}
Column temperature	ambient temperature
Detection wavelength	254 nm
Components of mobile phase	A: 4.5 mM sodium dihydrogenphosphate buffer B: 7.5 mM sodium dihydrogenphosphate buffer C: acetonitrile D: tetrahydrofuran E: methanol F: phosphoric acid 85%
for **I**	A, C, D, E, F (69.9: 21: 4: 5: 0.1)
for **II**	B, C, D, F (68.9: 26: 5: 0.1)
for **III**	B, C, D, F (67.9: 29: 3: 0.1)
Average working pressure	
for **I**	18.0 MPa
for **II**	17.0 MPa
for **III**	16.5 MPa
Retention times	
for **I**	E: 15–16 min, L: 17–18 min, IS: 20–21 min
for **II**/**III**	E: 12–13 min, L: 14–15 min, IS: 18–19 min

Table 2
Stability of losartan (L) and EXP 3174 (E) 0, 6 and 12 hours after reconstitution of the dried samples (from rat liver extraction) with mobile phase and storage in the auto-sampler at 4°C ($n = 6$)

h	Losartan/EXP 3174 Nominal concentration (ng/100 mg tissue)	Losartan/EXP 3174 Conc. Found (ng/100 mg tissue)	L/E RSD (%)	L/E RE (%)
0	200	184.9/188.0	1.5/1.4	−7.5/−6.0
6	200	202.6/194.8	5.1/4.7	1.3/−2.6
12	200	192.0/191.0	4.7/5.1	−4.0/−4.5

RSD (%) = Relative Standard Deviation
RE (%) = Difference between nominal and found concentration
h = Hours following reconstitution

solution, 99:1, v/v). The collected eluate was evaporated to dryness, reconstituted in 100 μl of mobile phase, which was adjusted to pH 1.9 with 85% phosphoric acid, and an aliquot of 80 μl was injected into the HPLC system.

2.4.2. Solid–phase extraction of human and rat urine

The sample preparation of urine prior to application to solid–phase extraction was slightly varied. After addition of IS and 500 μl of bidistilled water to 500 μl of urine, the mixture was acidified with 200 μl of 1.0 M HCl, thoroughly vortexed and subjected to prepared solid–phase cartridges. The urine samples were further treated as described for plasma.

2.4.3. Liquid–liquid extraction of rat blood, bile and tissues

Rat blood. After hemolysis by freezing at −30°C and removal of the erythrocytes by centrifugation (2000 g/−10°C/10 min), 500 μl of the supernatant were diluted with 500 μl of bidistilled water. Following addition of IS the mixture was acidified with 200 μl of 1.0 M HCl, thoroughly vortexed and extracted into 7 ml of methyl *tert*-butyl ether (MTBE) by shaking for 30 min at low speed. After centrifugation (2000 g/−10°C/15 min) the aqueous layer was frozen in a dry ice–acetone bath and discarded. The organic phase was evaporated to dryness, reconstituted in 120 μl mobile phase adjusted to pH 3.1 with 85% phosphoric acid and, if necessary, centrifuged (2000 g/0°C/5 min). An aliquot of 90 μl was injected into the HPLC system.

Rat bile. For removal of bile acids, 100 parts of bile were acidified with 20 parts of 1.0 M HCl, thoroughly vortexed and centrifuged (5000 g/15°C/15 min). An aliquot of 120 μl of supernatant (= 100 μl of bile) was diluted with 500 μl bidistilled water. Following addition of IS, the mixture was acidified with 100 μl of 1.0 M HCl, thoroughly vortexed and extracted into 7 ml of methyl *tert*-butyl ether (MTBE) as outlined for rat blood.

Rat tissues (except brain, perirenal fat and gluteal muscle), i.e. liver, kidneys, heart, lung, spleen and small intestine plus contents, were homogenized in phosphate buffer pH 7.4 (2-fold the weight of the resp. tissue) with an Ultra Turrax T25 at highest speed and, after filtration through cotton gaze, with a Potter S with 10–20 hubs at 450 g. All steps were carried out on ice.

After addition of IS to 1000 μl of homogenate (for liver: 300 μl), the mixture was acidified with 400 μl of 0.5 M HCl (for liver: 600 μl of 0.1 M HCl), thoroughly vortexed and extracted into 7 ml of MTBE by shaking for 30 min at low speed. After centrifugation (2000 g/4°C/15 min) the aqueous layer was frozen in a dry ice–acetone bath and discarded. The organic phase was evaporated to dryness, reconstituted in 120 μl mobile phase adjusted to pH 3.1 with 85% phosphoric acid and, if necessary, centrifuged (4000 g/15°C/30 min). An aliquot of 80 μl was injected into the HPLC system.

The analysis of small intestine contents required two subsequent centrifugation steps instead of homogenization (2000 g/−10°C/30 min; 5000 g/

4°C/15 min) prior to extraction. After addition of IS to 100 μl of the final supernatant, the mixture was further diluted with 300 μl of bidistilled water, acidified with 175 μl of 0.5 M HCl and extracted into 7 ml of MTBE as described above.

Perirenal fat and gluteal muscle tissues necessitated organic solvents during the homogenization process in order to obtain reasonable recovery. The only differing step in the preparation of perirenal fat and gluteal muscle samples was the homogenization of the tissues with a mixture of phosphate buffer pH 7.4 and acetone (1:1 v/v) instead of buffer alone (2-fold the weight of the resp. tissue).

Brain tissue was homogenized with phosphate buffer pH 7.4 (2-fold the weight of the resp. tissue) using the Ultra Turrax. After the addition of IS to 1000 μl of homogenate, the mixture was acidified with 400 μl of 1.0 M HCl and precipitated with acetonitrile/methanol (1:2 v/v), while incubated for 1 h on ice. After subsequent centrifugation (2000 g/0°C/15 min) the aqueous layer was frozen in a dry ice–acetone bath and discarded. The organic phase was evaporated to dryness, reconstituted in 120 μl of mobile phase adjusted to pH 3.1 and, if necessary, centrifuged (2000 g/0°C/5 min). An aliquot of 90 μl was injected into the HPLC system.

2.5. Chromatographic conditions

The chromatographic separation of losartan, EXP 3174 and the internal standard was achieved on a 3 μm particle-sized ULTREMEX™ CN column. The respective mobile phases for HPLC following solid–phase or liquid–liquid extraction, flow-rates and working pressures are listed in Table 1. The UV-absorbance of the analytes was measured at 254 nm with EXP 3174 exhibiting a distinctly higher extinction coefficient than losartan. The corresponding molar absorptivities were 25000 (losartan), 44000 (EXP 3174) and 31000 (internal standard).

2.6. Assay validation

The validation was performed in accordance to the summary report of the conference on 'Analytical Methods Validation: Bioavailability, Bioequivalence and Pharmacokinetic Studies' which has provided guidelines for pharmacokinetic studies not only in humans, but also in animals [7].

Calibration data were generated by spiking blank samples with losartan and EXP 3174 yielding concentrations of 5–3200 ng ml^{-1} in rat and human urine, 12.5–1600 ng ml^{-1} in rat bile and 10–6400 ng per 100 mg in rat tissues (i.e. per 0.3 ml homogenate). Calibration curves were generated by plotting the ratios of the peak areas (LOS/IS; EXP/IS) versus concentration and calculating the linear regression ($y = a + bx$; weighing $y = 1$). The limit of quantification was defined as the lowest concentration with a coefficient of variation of $\leq 10\%$ that yielded a 3-fold higher signal than background levels.

Intraday precision and accuracy were determined using six samples of two to four different concentrations at the limit of quantification as well as at medium and high concentrations, which were prepared and analyzed on the same day.

Interday variability was assessed using six samples of two to four different concentrations (similar range as outlined above) analyzed on three different days.

Intraday and interday variabilities were characterized by the relative standard deviation (RSD%) and by the difference between nominal and measured concentrations (RE%). For both, the limits of acceptable variance were set to 15% for medium and high concentrations and to 20% for concentrations in the range of the limit of quantification, respectively.

The mean absolute recovery of losartan and EXP 3174 was evaluated by comparing peak areas from unextracted standards with those of extracted standards from all biological materials analyzed within the range of the respective standard curves.

2.7. Stability studies

The stability of losartan and EXP 3174 [200 ng ml^{-1} tissue homogenate] was investigated during one sample run in the autosampler at 4°C after several hours of reconstitution following extraction from rat liver. Mean found concentrations were 92.5–101.3% of nominal (Table 2).

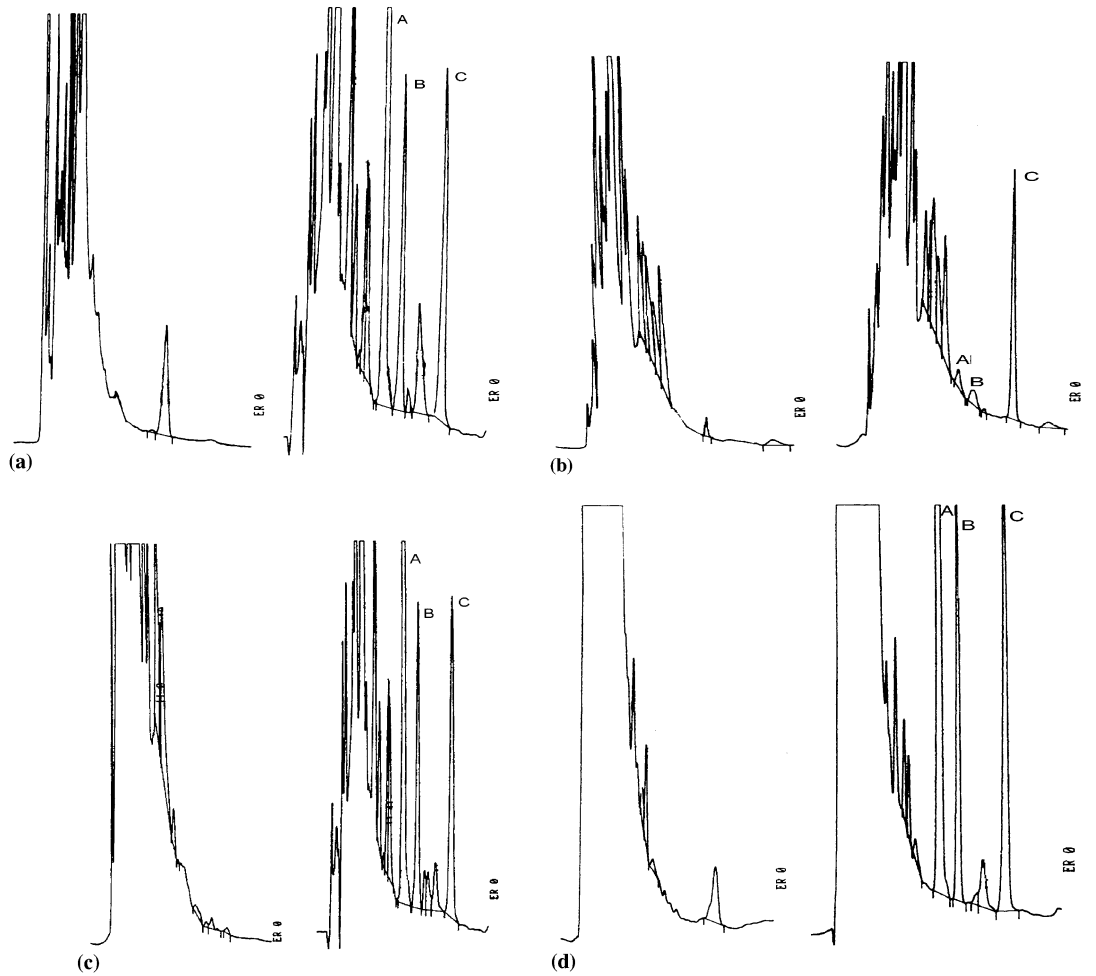

Fig. 2. (A) Representative chromatogram of **A** (EXP 3174), **B** (losartan) and **C** (internal standard) in rat plasma. (Left) Control plasma (Right) 12 h sample following multiple p.o. dosing of losartan (10 mg kg^{-1} BW): 287 ng ml^{-1} A and 147 ng ml^{-1} B. (B) Representative chromatogram of **A** (EXP 3174), **B** (losartan) and **C** (internal standard) in rat urine. (Left) Control urine (Right) 8 h sample following single p.o. dosing of losartan (10 mg kg^{-1} BW): 10 ng ml^{-1} A and 14 ng ml^{-1} B. (C) Representative chromatogram of **A** (EXP 3174), **B** (losartan) and **C** (internal standard) in human plasma. (Left) Control plasma (Right) 4 h sample following a single peroral 50-mg dose of losartan: 266 ng ml^{-1} A and 139 ng ml^{-1} B. (D) Representative chromatogram of **A** (EXP 3174), **B** (losartan) and **C** (internal standard) in human urine. (Left) Control urine (Right) Control urine spiked with 200 ng ml^{-1} A and 200 ng ml^{-1} B.

The stability of losartan and EXP 3174 during a sample run was verified by analysis of quality control samples distributed over the whole run. The limits of acceptable difference between the nominal and found concentrations were set to 15% throughout all studies of biological samples (see below).

2.8. Biological samples

The methods described have been successfully applied to the quantification of losartan and EXP 3174 in rat tissues and body fluids (see Section 2.4 for comprehensive list) following single p.o. and i.p. (10 mg kg^{-1} and 3 mg kg^{-1} body weight,

BW) as well as chronic p.o. administration of losartan (10 mg kg^{-1} BW). Furthermore, the assays have proven their suitability in the analysis of some clinical plasma and urine samples following a single peroral dose of 50 mg of losartan.

3. Results and discussion

Specific and sensitive HPLC methods were established for the determination of losartan and its main active metabolite EXP 3174 in biological material from humans and rats. Sample processing involved either solid–phase extraction using CN-cartridges or liquid–liquid extraction with methyl *tert*-butyl ether.

Representative chromatograms obtained following oral administration of losartan are shown in Fig. 2 (from rat plasma, rat urine, human plasma and human urine, after solid–phase extraction) and Fig. 3 (from rat liver and rat bile, after liquid–liquid extraction) in comparison to the respective blank samples.

The presented assays provided the desired sensitivity for the determination of losartan and EXP 3174 in the course of an extended rat study following single and multiple p.o. administration of

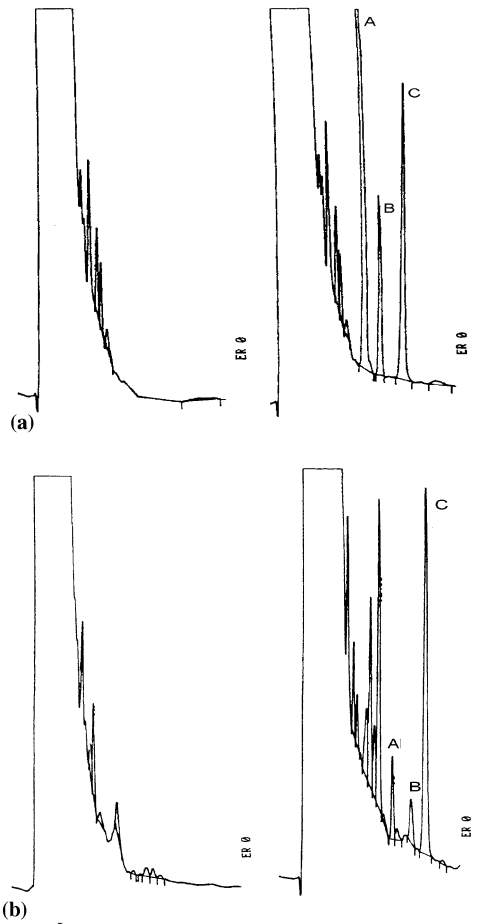

Fig. 3. (A) Representative chromatogram of **A** (EXP 3174), **B** (losartan) and **C** (internal standard) in rat liver. (Left) Control liver homogenate (Right) 12 h sample following single p.o. dosing of losartan (10 mg kg^{-1} BW): 255 ng per 100 mg tissue A and 72 ng per 100 mg tissue B. (B) Representative chromatogram of **A** (EXP 3174), **B** (losartan) and **C** (internal standard) in rat bile. (Left) Control bile (Right) Control bile spiked with 20 ng ml^{-1} A and 20 ng ml^{-1} B.

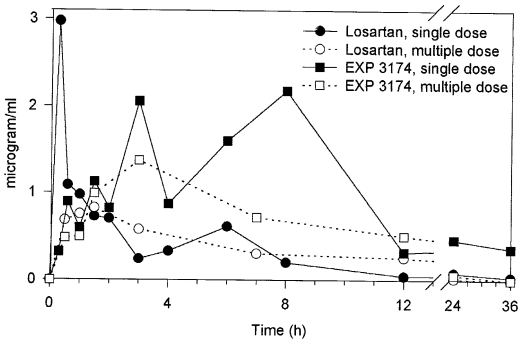

Fig. 4. Rat plasma concentration–time profiles of losartan and EXP 3174 following single and multiple oral dosing of losartan (10 mg kg^{-1} BW). Each point represents the mean values of $n = 4$ rats.

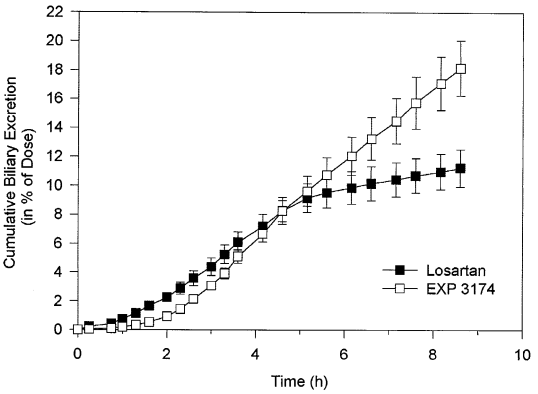

Fig. 5. Cumulative biliary excretion of losartan and EXP 3174 in percent of dose in rats (mean values for $n = 5$ rats)

Table 3
Recovery (%) of losartan, EXP 3174 and internal standard (IS) following solid–phase extraction (mean values for $n = 6$)

Biological Material	Losartan/EXP 3174 nominal concentration (ng ml^{-1})	Losartan/EXP 3174 recovery (%)	IS (300 ng ml^{-1}) recovery (%)
Rat plasma	20/20	75.5/72.8	
	200/200	81.0/76.0	
	1600/1600	85.0/78.7	81.7
Human plasma	20/20	81.5/70.2	
	200/200	93.3/74.6	
	1600/1600	93.5/77.6	87.1
Rat urine	20/20	81.5/80.2	
	200/200	93.3/84.0	
	1600/1600	93.5/87.6	86.0
Human urine	20/20	83.0/74.0	
	200/200	84.0/77.6	
	1600/1600	93.0/78.8	88.9

losartan (10 mg kg^{-1} BW) and single i.p. administration of losartan (3 mg kg^{-1} BW).

Fig. 4 shows the plasma concentration–time profiles of both losartan (○, ●) and EXP 3174 (□, ■) following single and multiple dosing of losartan (10 mg kg^{-1} BW). Remarkably, it was found that following multiple dosing (once daily during 14 days) the area under the curve (AUC) of the metabolite in comparison to the AUC of the parent compound decreased from about 4.2-fold (after single dose) to only 2-fold.

Fig. 5 illustrates the cumulative biliary excretion of losartan and EXP 3174 in percent of dose (following single oral administration of losartan 10 mg kg^{-1} BW) yielding a biliary excretion rate of 0.218 h^{-1} and 0.31 h^{-1} for losartan and EXP 3174, respectively.

Compared to a previously published liquid–liquid method [8] for human plasma and urine measurement, the herein described liquid–liquid extraction procedure for the determination in rat bile, blood and tissues accomplished to considerably shorten and simplify analysis. In addition, the solid–phase extraction method provided a fast and reliable procedure to quantify losartan and EXP 3174 in human and rat plasma and urine yielding a more sensitive limit of quantification than the approach depicted in [8].

Following solid–phase extraction, the mean absolute recoveries were more than 70% for both analytes and internal standard. The internal standard was characterized for its usually applied concentration of 300 ng ml^{-1} sample with a recovery of 81–88.9% (Table 3). Liquid–liquid extraction yielded a recovery of more than 60% for losartan as well as EXP 3174 and 52% for the internal standard (Table 4).

The methods proved to be linear over the concentration range investigated with average correlation coefficients of six calibration curves being > 0.999 and reasonable percentage differences between nominal and found concentrations of the standards in the assessment of interday and intraday precision and accuracy. Tables 5 and 6 show the data following liquid–liquid as well as solid–phase extraction. In all cases, the coefficients of variation were below 10% for both compounds and for each concentration analyzed.

Under the conditions described, for both losartan and EXP 3174 the limit of quantification was between 10 and 15 ng per 100 mg tissue for all tissues analyzed, 12.5 ng ml^{-1} for rat bile and blood, 5 ng ml^{-1} for human and rat plasma as well as 10 ng ml^{-1} for human and rat urine.

Table 4
Recovery (%) of losartan, EXP 3174 and internal standard (IS) following liquid–liquid extraction (mean values for $n = 6$)

Biological Material	Losartan/EXP 3174 nominal concentration (ng/100 mg tissue)	Losartan/EXP 3174 recovery (%)	IS (300 ng ml^{-1}) recovery (%)
Liver	25/25	92.2/61.1	
	200/200	72.7/62.3	
	800/800	71.0/65.4	
	6400/6400	85.0/87.6	75.0
Kidney	25/25	88.6/66.2	
	200/200	90.3/63.2	87.0
Heart	25/25	75.0/92.0	
	200/200	79.0/70.0	82.0
Lung	25/25	80.0/74.0	
	200/200	85.0/66.0	85.0
Gut	25/25	89.5/68.0	
	200/200	81.8/76.4	
	800/800	80.8/77.3	79.3
Gut contents	25/25	84.3/80.2	
	200/200	90.6/74.6	
	800/800	81.2/77.6	81.8
Spleen	25/25	88.0/69.4	
	200/200	82.2/74.0	72.1
Muscle	10/10	64.0/69.0	
	25/25	83.0/68.0	62.0
Fat	10/10	62.0/65.0	
	25/25	70.0/70.0	58.0
Brain	10/10	67.5/67.0	67.0

4. Conclusion

Specific, sensitive and precise HPLC assays (liquid–liquid and solid–phase extraction) are reported for the determination of the angiotensin-II-antagonist losartan and its main active metabolite EXP 3174 in biological material from rats and humans. The limits of quantification have proven to be appropriate for the evaluation of the tissue distribution of losartan and EXP 3174 in rats.

The specificity of both assays indicates their potentially wider suitability for the determination of other angiotensin-II antagonists in humans and animal species.

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Table 5
Intraday variability following liquid–liquid extraction and solid–phase extraction (mean values for $n = 6$)

Biological Material	Losartan/EXP 3174 nominal concentration	Losartan/EXP 3174 Conc. found	Los/EXP RSD (%)	Los/EXP RE (%)
Liquid–liquid extraction				
	(ng/100 mg tissue)	(ng/100 mg tissue)		
Liver	25/25	26.9/27.0	3.2/4.0	7.6/8.0
	200/200	194.3/198.2	8.2/3.7	−2.8/−0.9
	800/800	761.2/810.9	2.3/2.4	−4.8/1.4
	6400/6400	6171.7/6205.6	1.7/1.6	−3.6/−3.0
Kidney	25/25	26.3/24.3	5.4/6.0	5.1/−2.8
	200/200	207.9/200.4	4.8/3.1	3.9/0.2
Heart	25/25	23.0/24.0	7.2/7.7	−9.2/−4.0
	200/200	187.4/179.8	4.2/6.2	−6.3/−9.0
Lung	25/25	23.8/23.6	9.1/7.5	−4.8/−5.6
	200/200	211.4/193.8	1.5/3.2	5.7/−3.1
Spleen	25/25	25.3/23.3	7.2/6.1	1.2/−6.8
	200/200	204.9/197.2	3.2/1.5	2.5/−1.4
Gut wall	25/25	23.5/22.9	7.2/4.6	−6.0/−8.4
	200/200	204.9/191.1	4.5/5.3	−5.2/−4.5
Gut contents	25/25	22.6/25.7	6.7/4.8	−9.6/2.8
	200/200	197.6/193.8	5.4/4.4	−1.2/−3.1
Muscle	25/25	23.3/23.3	7.4/6.1	−6.8/−6.8
	200/200	214.6/187.2	1.7/8.3	7.3/−6.4
Fat	25/25	23.6/23.7	1.4/4.0	−5.6/−5.2
	200/200	216.0/192.3	1.4/1.5	8.0/−3.8
Solid–phase extraction				
	(ng ml^{-1})	(ng ml^{-1})		
Human plasma	20/20	21.8/21.3	3.4/6.7	9.0/6.5
	200/200	210.6/189.0	5.7/6.0	5.3/−5.5
Human urine	20/20	20.6/21.7	4.4/9.0	3.0/8.5
	200/200	206.2/195.3	7.4/5.9	3.1/−2.3
Rat plasma	20/20	19.7/18.9	7.0/6.0	−1.5/−5.5
	200/200	204.0/189.9	8.7/2.0	2.0/−5.1
Rat urine	20/20	18.9/20.7	5.4/8.9	−5.5/3.5
	200/200	215.4/195.5	2.2/1.1	7.7/−2.3

RSD% = Relative Standard Deviation
RE% = Difference between nominal and found concentration

Table 6
Interday variability following liquid–liquid extraction and solid–phase extraction (mean values for n = 6)

Biological Material	Losartan/EXP 3174 nominal concentration	Losartan/EXP 3174 Conc. found	Los/EXP RSD (%)	Los/EXP RE (%)
Liquid–liquid extraction				
	(ng/100 mg tissue)	(ng/100 mg tissue)		
Liver	50/50	50.7/47.0	2.4/3.4	1.4/−6.0
	400/400	414.4/411.4	5.4/2.5	3.5/2.8
	1600/1600	1566.2/1597.4	4.4/3.4	−2.1/−0.2
	6400/6400	6312.5/6025.1	6.2/3.5	−1.4/−5.8
Kidney	50/50	52.8/51.2	5.8/6.6	5.6/2.3
	200/200	209.2/196.7	2.8/1.8	−4.6/−1.7
Heart	25/25	24.5/22.7	8.5/7.6	−2.0/−9.2
	200/200	202.6/189.8	5.8/8.7	1.3/−5.1
Lung	25/25	23.7/25.3	5.7/4.4	−5.2/1.2
	200/200	213.0/199.1	9.8/1.8	6.5/−0.4
Gut contents	25/25	27.0/26.9	4.3/3.4	8.0/7.6
	400/400	431.6/375.7	8.8/7.3	7.9/−6.1
Solid–phase extraction				
	(ng ml^{-1})	(ng ml^{-1})		
Human plasma	20/20	18.7/20.3	4.4/6.4	−6.5/1.5
	200/200	203.9/199.1	6.0/3.1	2.0/−0.5
Human urine	20/20	18.2/21.9	1.3/7.4	−9.0/9.5
	200/200	211.6/182.7	9.8/4.3	5.8/−8.6
Rat plasma	20/20	18.7/17.3	5.5/9.4	−6.5/−13.5
	200/200	213.0/190.1	9.0/3.8	6.5/−5.0
Rat urine	20/20	22.0/18.9	4.0/3.0	10.0/5.5
	200/200	201.9/188.8	4.8/7.0	1.0/−5.6

RSD% = Relative Standard Deviation
RE% = Difference between nominal and found concentration

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