Available online at www.sciencedirect.com
SCIENCE DIRECT
Analytica Chimica Acta 560 (2006) 41–49
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

# Determination of telmisartan in human blood plasma Part II: Liquid chromatography-tandem mass spectrometry method development, comparison to immunoassay and pharmacokinetic study

Christel Hempen [a], Liane Gläsle-Schwarz [b], Ulrich Kunz [b], Uwe Karst [a,*]

[a] *University of Twente, Chemical Analysis Group and MESA+ Institute for Nanotechnology, P.O. Box 217, 7500 AE Enschede, The Netherlands*
[b] *Boehringer Ingelheim Pharma GmbH & Co. KG, Drug Metabolism and Pharmacokinetics, 88397 Biberach an der Riss, Germany*

Received 24 October 2005; received in revised form 12 December 2005; accepted 12 December 2005
Available online 26 January 2006

**Abstract**

A new liquid chromatography/atmospheric pressure chemical ionization-tandem mass spectrometry (LC/APCI-MS/MS) method with on-line sample clean-up for the determination of telmisartan in human blood plasma is presented. This technique is compared to a previously introduced enzyme-linked immunosorbent assay (ELISA), where fluorescence is used as detection method. For the LC/MS method applying an internal calibration via a deuterated internal standard, the limit of detection was 0.3 ng/mL, the limit of quantification was 0.9 ng/mL and the linear range extended from 0.9 to 1000 ng/mL. Forty-eight plasma samples from four healthy volunteers were analyzed in a pharmacokinetic study to obtain data for the method comparison. As a result, these two new and independent analytical methods for the determination of telmisartan in human blood plasma proved to yield comparable results for the amount of analyte.


*Keywords:* Telmisartan; ELISA; LC/MS/MS; Turboflow chromatography; MNBDH

## 1. Introduction

Telmisartan, 4-[(2-*n*-propyl-4-methyl-6-(1-methylbenzimidazole-2-yl)-benzimidazole-1-yl)methyl]-biphenyl-2-carboxylic acid (Fig. 1), is a known drug against high blood pressure, which belongs to the group of angiotensin II receptor antagonists [1,2]. Other members of this group are, e.g. candesartan, eprosartan and valsartan. Telmisartan is highly selective for angiotensin II ($AT_1$) receptors. It inhibits the angiotensin II receptor in a way that the effect of angiotensin II is blocked resulting in a decrease of blood pressure [3]. As has already been described in Part I (immunoassay development), only few publications with respect to the investigation of telmisartan by analytical methods, such as chromatography, mass spectrometry or immunoassays have been published until now. These methods are mainly based on capillary zone electrophoresis (CZE) with subsequent UV/vis detection [4], HPLC measurements with UV/vis [4] or fluorescence detection [5] or polarographic methods [6,7].

Only two methods for the determination of angiotensin II receptor antagonists applying mass spectrometric techniques have been published up to now. Kondo et al. presented the first method in 1996 [8]. It is based on LC/electrospray-tandem MS investigations using an off-line sample extraction procedure for the characterization of candesartan cilexetil metabolites in rat blood plasma. Recently, Chen et al. published a paper on the determination of telmisartan in human blood plasma samples by a LC/ESI-MS method. They worked with an off-line procedure for sample preparation and used valsartan as internal standard [9].

In this paper, a new liquid chromatography/tandem mass spectrometry (LC/MS/MS) method with automated on-line sample clean-up using turbulent-flow chromatography for the determination of telmisartan in human blood plasma samples is presented. LC/MS/MS is currently the method of choice for the analysis of highly polar pharmaceuticals and offers the capability of simultaneous multianalyte determination. However,

DOI of original article:10.1016/j.aca.2005.12.036.
* Corresponding author. Present address: University of Münster, Institute for Inorganic and Analytical Chemistry, Corrensstr. 30, 48149 Münster, Germany. Fax: +49 251 833 6013.
*E-mail address:* uk@uni-muenster.de (U. Karst).

0003-2670/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2005.12.035

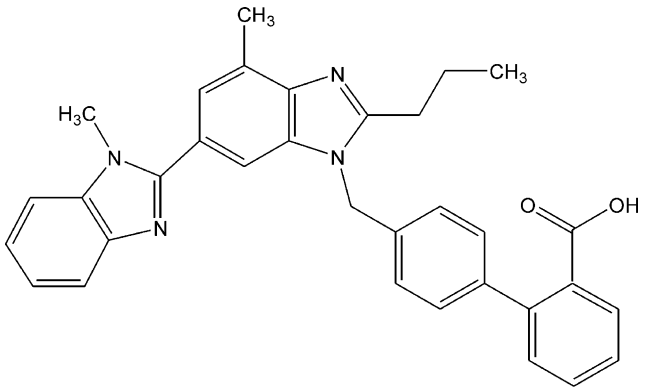


Fig. 1. Chemical structure of telmisartan.

the successful development of an LC/MS/MS method using the "soft" atmospheric pressure ionization (API) methods, such as electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) was not certain due to the low polarity of the analyte [10]. But as an immunoassay for the determination of telmisartan has already been developed successfully (Part I), the possibility of comparison by cross-validation of two independent high-sensitivity methods was given. To the best of our knowledge, both approaches are described in literature for the first time for the analysis of telmisartan or related compounds.

## 2. Experimental

### 2.1. Materials

Telmisartan reference substance (4-[(2-*n*-propyl-4-methyl-6-(1-methylbenzimidazole-2-yl)-benzimidazole-1-yl)methyl]-biphenyl-2-carboxylic acid), the hapten telmisartan-(2-amino acetic acid) (Tel-AAA), which is coupled to the enzyme glucose oxidase, the biotin-labeled polyclonal rabbit anti-telmisartan IgG, all plasma samples (12 individual plasma samples and pooled plasma from healthy volunteers) and the internal telmisartan standard (deuteration degree of the propyl group over seven hydrogen atoms: 78%) were kindly provided by Boehringer Ingelheim Pharma GmbH & Co. KG (Biberach, Germany). Acetonitrile and water for LC/MS measurements were purchased from Biosolve (Valkenswaard, The Netherlands). Bovine serum albumin (BSA) was purchased from Serva (Heidelberg, Germany). The enzymes glucose oxidase (GOD, E.C. 1.1.3.4) and horseradish peroxidase (POD, E.C. 1.11.1.7) as well as avidin were purchased from Sigma (Deisenhofen, Germany). The synthesis of 4-(*N*-methylhydrazino)-7-nitro-2,1,3-benzooxadiazole (MNBDH) was performed as described in literature [11]. All other chemicals were purchased from Aldrich (Steinheim, Germany), Merck (Darmstadt, Germany) and Fluka (Neu-Ulm, Germany) in the highest quality available.

#### 2.1.1. Buffers

Buffer 1: 0.05 mol/L $Na_2HPO_4/KH_2PO_4$, 0.15 mol/L NaCl, pH 7.4;
Buffer 2: 0.05 mol/L $Na_2HPO_4/KH_2PO_4$, 0.15 mol/L NaCl, 5 g/L BSA, pH 7.4;
Buffer 3: 0.05 mol/L $Na_2HPO_4/KH_2PO_4$, 0.15 mol/L NaCl, 5 g/L BSA, 0.5 g/L $NaN_3$, pH 7.4.

#### 2.1.2. Unknown samples

Human plasma samples of four healthy male volunteers of a pharmacokinetic clinical Phase-I study with telmisartan were investigated by means of LC/MS and ELISA measurements. Blood was taken 0.5 h before as well as 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, 48 and 72 h after oral intake of 80 mg telmisartan and potassium EDTA was added. Aliquots of the subsequently generated plasma samples were stored at approximately −20 °C.

### 2.2. Instruments

For LC/MS investigations, an Agilent Technologies (Waldbronn, Germany) HP1100 liquid chromatograph for binary gradient elution was used: pump model G1312A, autosampler G1313A, diode-array detector G1315B. This system was coupled to an esquire 3000*plus* ion trap mass spectrometer (Bruker Daltonics, Bremen, Germany). The set-up of the LC/MS system is shown in Fig. 2. For HPLC separation (pumps 2 and 3), the following binary gradient consisting of buffer A ($HCOONH_4$/HCOOH, 20 mmol/L, pH 3) and acetonitrile (B) with a flow rate of 0.4 mL/min was used:

| Time (min) | $c_B$ (%) |
|---|---|
| 0 | 30 |
| 1.5 | 30 |
| 6.5 | 90 |
| 9.5 | 90 |
| 9.6 | 30 |
| 13 | 30 |

For sample clean-up, turbulent flow chromatography (TFC) using isocratic conditions were utilized ($H_2O$, pump 1). The flow rate was 1.2 mL/min in this case. The injection volume was 100 μL. For enrichment of telmisartan, a cyclone turboflow (TF) column (Cohesive Technologies Europe, Crownhill, United Kingdom) with the following parameters was used: particle size 60 μm, pore size 100 Å, column dimensions 50 mm × 0.5 mm. A Prontosil C18-ace-EPS column (Bischoff Chromatography, Leonberg, Germany) was used for HPLC separation (particle size 3.0 μm, pore size 120 Å; column dimensions 150 mm × 3.0 mm). From 0 to 1 min the valve was in the enrichment position. From 1.1 to 9.5 min, the valve switched to the separation position and at 9.6 min back into the initial position again.

All MS measurements were performed by means of atmospheric pressure chemical ionization (APCI) in the negative ion mode. Mass spectra were recorded in the full scan mode with a mass range from $m/z = 100$ to 1000. Tandem mass spectra of telmisartan were recorded in the multiple reaction monitoring (MRM) mode with a fragmentation amplitude of 1.0 V using the transition from $m/z = 513.2$ to 469.2 for quantification and additionally, the transitions from $m/z = 513.2$ to 302.2 and $m/z = 287.2$ for identification. The internal standard was determined by monitoring the transition from $m/z = 519.2$ to 475.2.

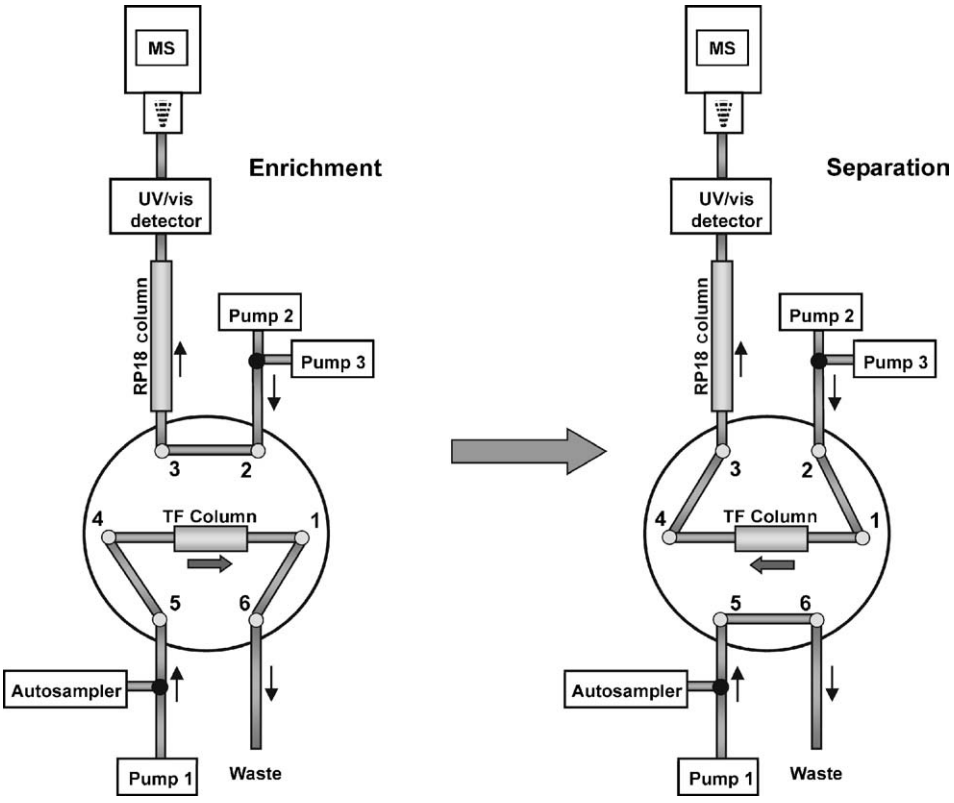


Fig. 2. Schematic set-up of the LC/MS system. The switching valve changes between the enrichment and the separation position. In the enrichment position, the TFC column is loaded with the plasma sample, whereas in the separation position, the TFC column is eluted (backflush) and the retained components are separated on the RP18 column and detected by means of UV/vis spectroscopy and APCI-MS.

The ion count cumulative target for the ion trap mass analyzer was 100,000, with a maximum accumulation time of 200 ms. Ions were generated with 10,000 nA corona current and guided towards the mass analyzer with 2582 V at the transfer capillary inlet, −205.7 V on the capillary exit and −49.8 V at the skimmer. Further ion source parameters were 50 psi nebulizer gas and 5.0 L/min of drying gas with a temperature of 350 °C.

The ELISA detection was performed as described in Part I: immunoassay development.

### 2.3. Optimization of LC/MS parameters

In order to find the best conditions for the LC/MS measurements, several parameters were optimized: telmisartan was on the one hand determined in initial measurements in the full scan mode and on the other hand in the tandem MS mode. The calibration curve of pooled plasma (10%) containing standards was compared with the calibration of standards without plasma and with the results of an internal calibration. Additionally, the breakthrough volume of the TFC column was determined by varying the injection volume between 10, 30, 50, 75 and 100 μL and comparing the peak areas of the respective mass traces or transitions.

### 2.4. LC/MS procedure

The telmisartan concentrations of the 12 unknown human plasma samples of every test person were determined by means of LC/APCI-MS measurements. In case of the external calibration, the standard solutions and the sample solutions were injected in triplicate into the system. Additionally, quality controls of 500 and 20 ng/mL (in pooled plasma; 10%) were analyzed two times in triplicate in between the determination of the real human plasma samples. In all cases, the solutions were diluted (buffer 2) to a final plasma concentration of 10%. Therefore, pooled plasma was added to the calibration standards, which were determined in the range of 0.01–1000 ng/mL.

For internal calibration, the response factor was initially determined. Two calibration curves of telmisartan/internal standard mixtures were recorded: on the one hand by mixing solutions of the analyte and the deuterated standard of the same concentration in the range from 0.3 to 1000 ng/mL and on the other hand by mixing solutions of this concentration range in opposite directions (starting for telmisartan at 0.3 ng/mL and for the standard at 1000 ng/mL). The response factor was calculated applying the following formula:

$$R = \frac{c_{\mathrm{inS}} A_{\mathrm{Telm}}}{c_{\mathrm{Telm}} A_{\mathrm{inS}}}$$

with internal standard (inS), area telmisartan ($A_{\mathrm{Telm}}$) and area internal standard ($A_{\mathrm{inS}}$).

This procedure was selected to exclude interferences from very high concentrations of one compound on very low concentrations of the other. Afterwards, the analysis of the plasma samples was carried out by adding the internal standard to each sample in a final concentration of 10.04 ng/mL. All sam-

ples, which were analyzed using an internal standard, were also diluted (buffer 2) to a final plasma concentration of 10%. For the two calibration measurements, pooled plasma was added to the solutions again. All measurements based on the use of the internal standard (calibration, real sample determination) were performed in triplicate as well. Furthermore, the telmisartan concentration of quality controls (500, 20 ng/mL) containing 10% pooled plasma was again determined two times in triplicate in between the human plasma samples. For all measurements, the detection was performed applying UV/vis absorbance spectroscopy and APCI-MS in the MRM mode.

### 2.5. ELISA procedure

The immunoassay was performed as described in Part I: immunoassay development.

## 3. Results and discussion

In order to decrease sample preparation time, thus allowing increased sample throughput, all tandem MS measurements were combined with an online TFC sample clean-up. In this enrichment step a relatively high flow rate is applied. Therefore, eddies are generated due to the large particles in the turbulent-flow column. This leads to a turbulent-flow profile instead of a laminar flow profile, which is observed in common HPLC columns. These eddies and the high flow rate increase the mass transfer and prohibit an interaction of large molecules with the particle pores inside the column so that large molecules as proteins are not retained on the column but directly flow into the waste [12,13].

### 3.1. LC/MS method

In Fig. 3, the APCI-MS (inserted spectrum) and APCI-MS/MS spectra of a telmisartan calibration standard (10 ng/mL; solved in buffer 2 including 10% pooled blood plasma) are shown. The peak at $m/z = 513.2$ is caused in both cases by the deprotonated telmisartan, whereas the ion at $m/z = 469.2$, which can also be observed in both measurements, is due to the loss of carbon dioxide from the carboxylic acid function. The fragment at $m/z = 302.2$ is caused by cleavage of the C–N bridge between the biphenyl part and the rest of the telmisartan molecule and the fragment at $m/z = 287.2$ by additional loss of a methyl group. The fragmentation scheme is shown in Fig. 4. The signal-to-noise ratio for the main MRM signal at $m/z = 469.2$ is approximately 200:1 at this telmisartan standard concentration. Tandem MS techniques have been selected for these investigations due to improved limits of detection in comparison to full scan APCI-MS. In case of the deuterated internal standard, the transition of the main signal at $m/z = 519.2$–$475.2$ was observed as expected due to the analogy to the native compound.

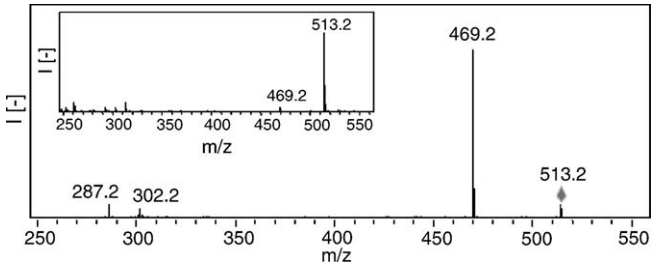


Fig. 3. APCI-MS (inserted spectrum) and APCI-MS/MS spectra of a telmisartan calibration standard (10 ng/mL; dissolved in buffer 2 including 10% pooled blood plasma). The peak at $m/z = 513.2$ is caused in both cases by telmisartan. The daughter ion at $m/z = 469.2$ in the tandem mass spectrum is caused by loss of $CO_2$ from the carboxylic acid group.

Fig. 5 shows the dependency of the signal intensity for the LC/APCI-MS (MRM) measurement on different injection volumes of the peak at $m/z = 469.2$ ($n = 3$). Different injection volumes between 10 and 100 μL have been selected for these investigations in order to determine the breakthrough volume of the TFC column. The results for higher injection volumes could not be compared due to the maximum volume of 100 μL of the injection loop. The coefficient of linear regression ($r^2$) is 0.9983 and the mean R.S.D. is 9.2%. As a wide range of telmisartan concentrations could be expected, a large linear range for the pharmacokinetic investigations was needed. Therefore, an injection volume of 100 μL was selected.

Fig. 6 shows the APCI(-)-MS and UV/vis chromatograms of a telmisartan calibration solution (1000 ng/mL; dissolved in buffer 2 including 10% pooled plasma) after TF clean-up. The upper chromatogram shows the total ion current (TIC), whereas in the middle, the 513.2 → 469.2 transition in the MRM mode is observed. In the TIC chromatogram, the times of changing the switching valve position from enrichment into the separation (1 min) and back (9.5 min) are marked (dashed lines). Both chromatograms show only one peak at approximately 8.5 min, which can be assigned to telmisartan. Due to the low polarity of telmisartan, polar metabolites, such as the glucuronide are expected to elute earlier from a reversed-phase column than the compound itself. The third window shows the UV/vis chromatogram at the maximum absorption wavelength of 298 nm. Besides the pressure peak resulting from the injection (3.5 min), two very small and one larger peak can be seen. The retention time of the large peak corresponds to the telmisartan peaks of the other chromatograms. The two other peaks at approximately 6 and 7.5 min, respectively, can be observed in all other performed measurements, which includes also the determination of unknown samples and the measurement of the telmisartan calibration standard solutions without added plasma. This means that these peaks are probably caused by contaminations of the reagents and neither by the plasma itself nor by metabolism of telmisartan in the plasma. The comparison of the mass and UV traces shows the difference in sensitivity of the methods. Although the applied telmisartan concentration is very high, the UV peaks show only moderate peak intensities with a much lower signal-to-noise ratio than the MS traces. For the concentrations at the beginning of the linear range, no UV detection was possible. This confirms the argument for LC/MS measurements.

For the external calibration, 11 telmisartan standard solutions (concentration range: 0.01–1000 ng/mL) were injected in triplicate into the TFC/LC/MS system. In order to have the same conditions as for the determination of the unknown human

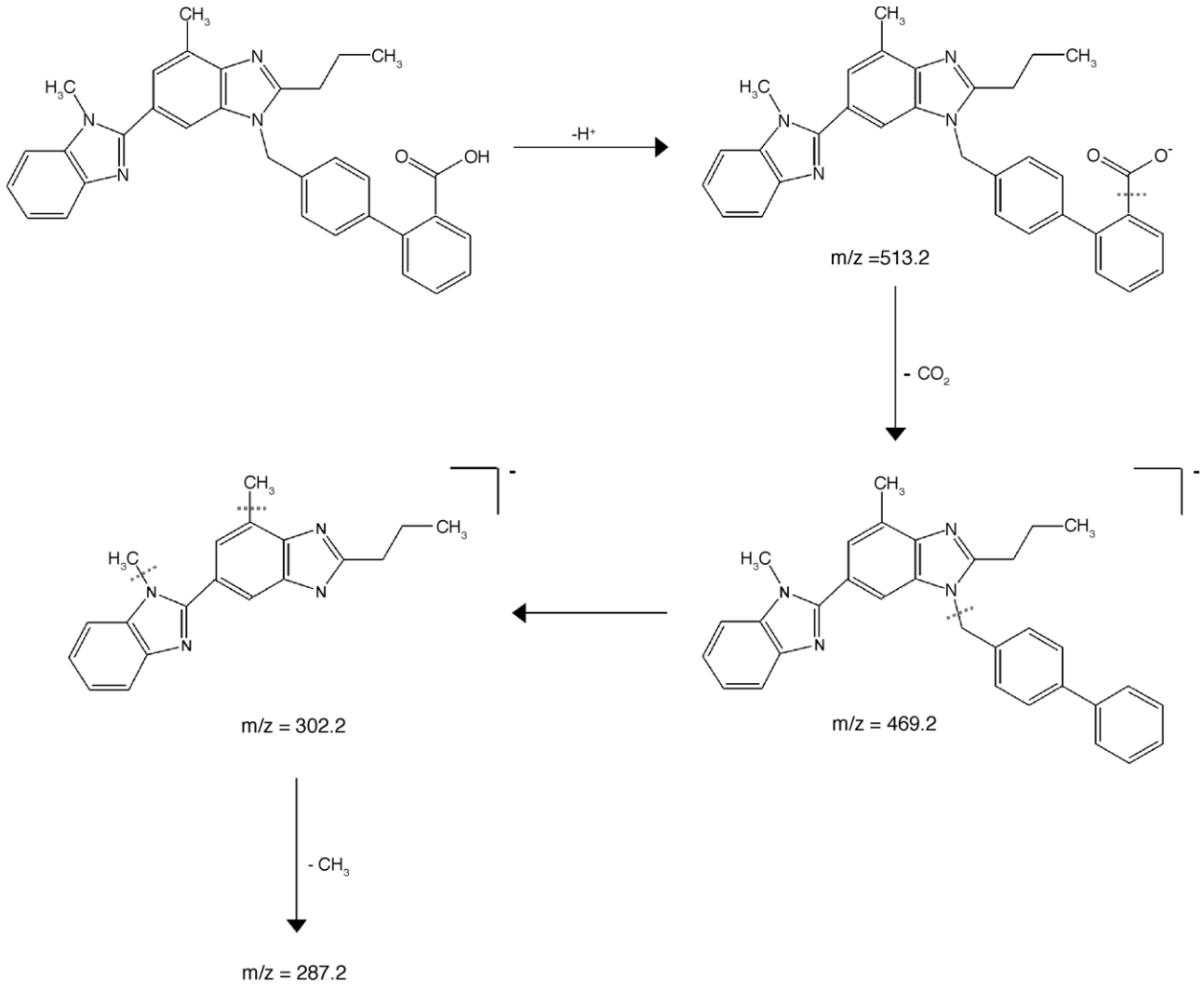


Fig. 4. Reaction scheme of the telmisartan fragmentation.

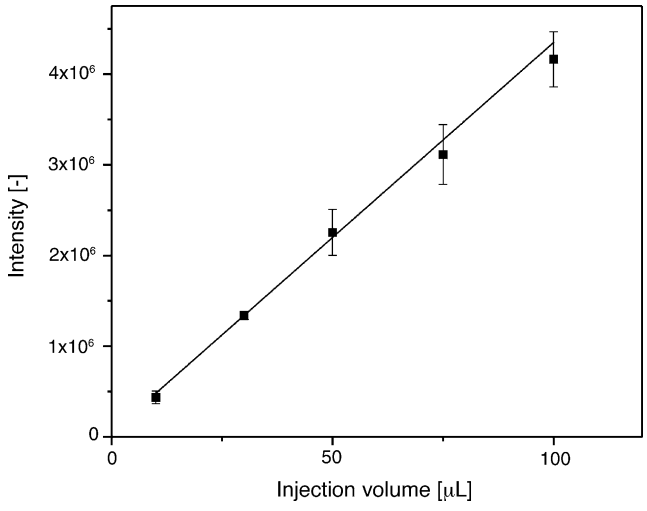


Fig. 5. LC/APCI-MS/MS (MRM) investigation regarding the dependency of different injection volumes on the signal intensity of the peak at $m/z = 469.2$ ($n = 3$).

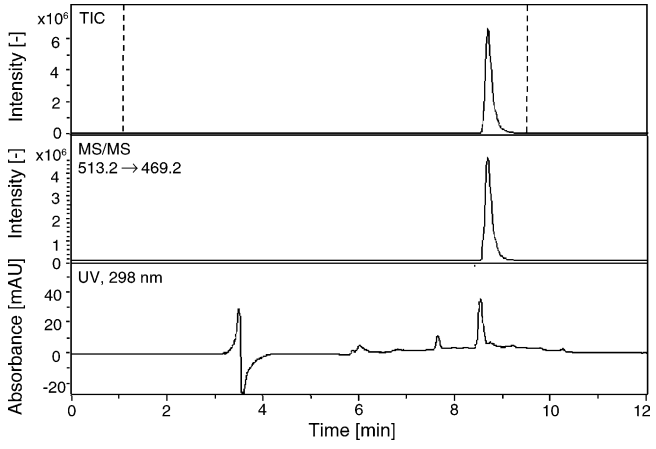


Fig. 6. APCI(-)-MS and UV/vis chromatogram of a telmisartan calibration solution (1000 ng/mL; dissolved in buffer 2 including 10% pooled plasma) after TFC clean-up and LC separation recorded in the scan mode ($m/z = 50$–$500$), in the MRM mode ($m/z = 469.2$) and at the respective absorption wavelength of 298 nm.

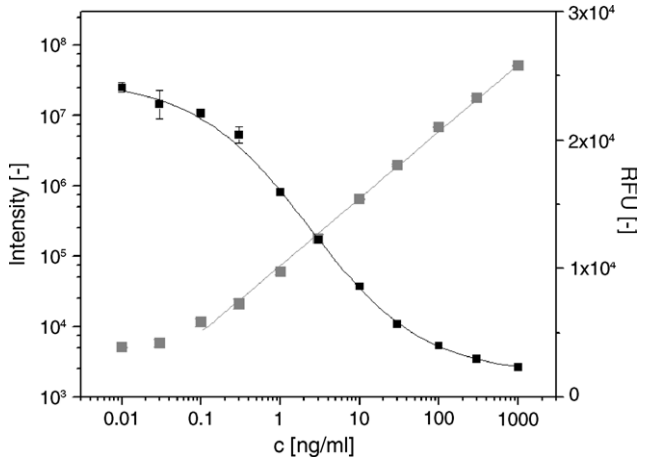


Fig. 7. Comparison of the two different types of calibration curves: APCI-MS/MS-calibration (gray) and ELISA-calibration (black).

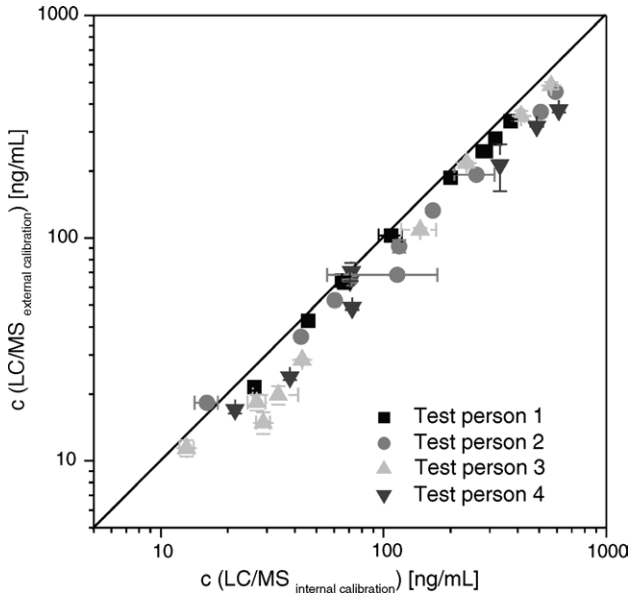


Fig. 8. Correlation plot between the determined telmisartan concentrations by means of the LC/MS method with external calibration and with internal calibration.

plasma samples, all telmisartan standards were diluted in such a way that the final pooled plasma concentration was 10% in buffer 2. The calibration curve is shown in Fig. 7. The limit of detection (LOD), determined according to the concentration giving a signal 3 standard deviations (S.D.) above the mean for the blank, is 0.3 ng/mL. The limit of quantification (LOQ), determined according to the concentration giving a signal 10 S.D. above the mean for the blank, is 0.9 ng/mL and the linear range is between 0.9 and 1000 ng/mL. The coefficient of the linear regression ($r^2$) is 0.992 and the mean R.S.D. = 7.7% (R.S.D. between 1.0 and 9.6%).

For the determination of the response factor of the internal calibration, two calibration curves of telmisartan/internal standard mixtures were measured: on the one hand, by mixing solutions of the native compound and the deuterated standard of the same concentrations (parallel calibration) and on the other hand by mixing different concentrations (cross calibration) of these solutions of this range. The response factor was calculated as 1.77 (S.D. = 0.20, R.S.D. = 11.3%) as described above by applying the formula $R = (c_{\mathrm{inS}}A_{\mathrm{Telm}})/(c_{\mathrm{Telm}}A_{\mathrm{inS}})$.

In Table 1 (a–d), the results for the LC/MS determination with internal and external calibration of several pharmacokinetic human plasma samples from four healthy volunteers are presented. Plasma samples were taken at 12 different time points: before and after administration of telmisartan tablets. The telmisartan concentration was determined in triplicate after diluting all human plasma samples by factor 10 with buffer 2 (and adding the deuterated standard, in case of the internal calibration). The highest drug concentrations in the plasma samples of each test person were determined in agreement with the results of the external calibration measurements.

By comparing the results of these measurements, two observations are most prominent: first, both series of measurements deliver comparable pharmacokinetic time–concentration profiles of the drug in the plasma samples. This becomes obvious from Fig. 8. However, the second observation, which can be made by comparing the LC/MS results in Table 1 and in Fig. 8, is that the measured telmisartan concentrations are usually higher in case of using the deuterated standard. This result is consistent with the very high degree of protein binding of telmisartan. The consequence is that not all telmisartan is retained by means of the TFC column but that it is partly transferred to the waste. Therefore, no quantitative recovery is observed for external calibration. The use of a stable-isotope-labeled internal standard, which shows the same degree of protein binding, is the best means to avoid respective problems.

### 3.2. Method comparison

The telmisartan concentration data obtained by the two independent analytical methods for human plasma samples were compared. Calibration curves for both methods are provided in Fig. 7. While the LC/MS/MS method provides a linear calibration curve, the immunoassay calibration typically results in a sigmoidal function.

The concentration–time profile for each subject is shown in Fig. 9. Here, the results of the immunoassay are directly compared to the results of the LC/MS/MS method applying internal calibration. It can be seen that the telmisartan concentration–time profile differs considerably from healthy volunteer to healthy volunteer, but that both analytical methods provide similar profiles, with the largest deviations being observed close to the limit of quantification. In the last two columns of Table 1, the ratio between concentrations obtained by ELISA and LC/MS (internal or external calibration) are calculated for any of the individual samples. By comparing these results, it can be observed that the deviation between ELISA and LC/MS with internal calibration is usually higher than for ELISA and LC/MS with external calibration.

Fig. 10 describes the correlation between the results of the immunoassay and the LC/MS/MS technique (internal standard) for all four subjects in a scatter plot. The diagonal of the diagram represents the line of 100% agreement of both methods.

Table 1
Telmisartan concentration in the human plasma samples for four test persons (a–d)

| Time (h) | APCI-MS external (ext.) calibration | | | APCI-MS internal (int.) calibration | | | Immunoassay | | | *c*(ELISA) compared to *c*(LC/MS) ext. calibration (%) | *c*(ELISA) compared to *c*(LC/MS) int. calibration (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Average (ng/mL) | S.D. ($n=3$) | R.S.D. (%) | Average (ng/mL) | S.D. ($n=3$) | R.S.D. (%) | Average (ng/mL) | S.D. ($n=3$) | R.S.D. (%) | | |
| (a) Test person 1 | | | | | | | | | | | |
| −0.5 | <LOQ | / | / | <LOQ | / | / | <LOQ | / | / | / | / |
| 0.5 | 186.7 | 7.2 | 3.9 | 199.9 | 11.9 | 6.0 | 115.8 | 9.2 | 7.9 | 62.0 | 57.9 |
| 1.0 | 334.6 | 7.2 | 2.2 | 372.5 | 17.3 | 4.6 | 244.4 | 21.1 | 8.6 | 73.0 | 65.6 |
| 1.5 | 279.7 | 2.0 | 0.7 | 318.0 | 17.5 | 5.5 | 227.9 | 37.5 | 16.4 | 81.5 | 71.7 |
| 2.0 | 245.0 | 5.7 | 2.3 | 284.1 | 22.8 | 8.0 | 208.8 | 25.3 | 12.1 | 85.2 | 73.5 |
| 4.0 | 102.5 | 2.4 | 2.3 | 108.0 | 2.5 | 2.3 | 85.8 | 2.3 | 2.7 | 83.8 | 79.4 |
| 6.0 | 63.0 | 3.9 | 6.2 | 66.8 | 0.6 | 0.9 | 52.4 | 3.7 | 7.0 | 83.2 | 78.4 |
| 8.0 | 64.4 | 2.1 | 3.3 | 65.2 | 0.8 | 1.2 | 64.7 | 8.6 | 13.4 | 100.5 | 99.3 |
| 12.0 | 42.7 | 0.5 | 1.2 | 45.8 | 1.8 | 3.9 | 40.0 | 3.8 | 9.5 | 93.8 | 87.4 |
| 24.0 | 21.5 | 0.8 | 3.6 | 26.3 | 0.9 | 3.6 | 22.9 | 3.1 | 13.4 | 106.5 | 87.1 |
| 48.0 | <LOQ | / | / | <LOQ | / | / | 9.4 | 0.6 | 6.3 | / | / |
| 72.0 | <LOQ | / | / | <LOQ | / | / | <LOQ | / | / | / | / |
| (b) Test person 2 | | | | | | | | | | | |
| −0.5 | <LOQ | / | / | <LOQ | / | / | <LOQ | / | / | / | / |
| 0.5 | 69.3 | 0.8 | 1.2 | 115.1 | 1.0 | 0.9 | 90.0 | 1.8 | 2.0 | 131.6 | 78.2 |
| 1.0 | 192.3 | 4.5 | 2.3 | 261.0 | 9.0 | 3.5 | 198.8 | 12.7 | 6.4 | 103.4 | 76.2 |
| 1.5 | 454.9 | 4.2 | 0.9 | 591.1 | 14.6 | 2.5 | 545.0 | 26.9 | 4.9 | 119.8 | 92.2 |
| 2.0 | 368.4 | 3.4 | 0.9 | 506.8 | 20.0 | 3.9 | 466.5 | 33.8 | 7.3 | 126.6 | 92.1 |
| 4.0 | 132.9 | 4.1 | 3.1 | 166.2 | 12.9 | 7.8 | 132.1 | 13.4 | 10.2 | 99.4 | 79.5 |
| 6.0 | 91.7 | 5.9 | 6.5 | 117.3 | 2.7 | 2.3 | 90.4 | 5.3 | 5.8 | 98.6 | 77.1 |
| 8.0 | 65.1 | 2.2 | 3.4 | 70.7 | 1.8 | 2.5 | 52.3 | 5.9 | 11.2 | 80.3 | 74.0 |
| 12.0 | 52.4 | 0.5 | 0.9 | 60.4 | 1.3 | 2.1 | 61.0 | 10.3 | 16.9 | 116.3 | 100.9 |
| 24.0 | 36.1 | 1.1 | 3.1 | 42.6 | 1.2 | 2.8 | 39.1 | 4.4 | 11.2 | 108.3 | 91.8 |
| 48.0 | 18.3 | 0.5 | 2.7 | 16.1 | 0.1 | 0.9 | 22.5 | 0.5 | 2.0 | 123.1 | 139.6 |
| 72.0 | <LOQ | / | / | <LOQ | / | / | 5.8 | 0.4 | 6.5 | / | / |
| (c) Test person 3 | | | | | | | | | | | |
| −0.5 | <LOQ | / | / | <LOQ | / | / | <LOQ | / | / | / | / |
| 0.5 | 483.4 | 19.4 | 4.0 | 564.4 | 59.3 | 10.5 | 439.7 | 22.1 | 5.0 | 91.0 | 77.9 |
| 1.0 | 353.9 | 17.9 | 5.0 | 414.1 | 53.6 | 12.9 | 352.0 | 42.4 | 12.0 | 99.5 | 85.0 |
| 1.5 | 217.1 | 4.4 | 2.0 | 235.8 | 4.4 | 1.9 | 265.2 | 16.2 | 6.1 | 122.2 | 112.5 |
| 2.0 | 108.7 | 2.2 | 2.0 | 146.0 | 17.7 | 12.1 | 139.2 | 2.3 | 1.7 | 128.1 | 95.3 |
| 4.0 | 28.5 | 0.4 | 1.3 | 43.2 | 3.7 | 8.5 | 36.3 | 4.8 | 13.2 | 127.4 | 84.1 |
| 6.0 | 19.8 | 1.9 | 9.4 | 33.7 | 0.3 | 0.8 | 27.1 | 1.3 | 4.7 | 137.0 | 80.5 |
| 8.0 | 18.3 | 1.5 | 8.3 | 27.0 | 1.1 | 4.2 | 26.0 | 3.2 | 12.1 | 142.1 | 96.3 |
| 12.0 | 14.8 | 1.7 | 11.2 | 28.8 | 2.4 | 8.2 | 25.1 | 0.9 | 3.6 | 169.4 | 87.1 |
| 24.0 | 11.4 | 1.0 | 8.5 | 13.1 | 0.7 | 5.5 | 13.5 | 2.5 | 18.6 | 118.1 | 103.1 |
| 48.0 | <LOQ | / | / | 11.0 | 1.9 | 17.5 | 6.7 | 2.0 | 30.1 | / | 60.9 |
| 72.0 | <LOQ | / | / | <LOQ | / | / | <LOQ | / | / | / | / |
| (d) Test person 4 | | | | | | | | | | | |
| −0.5 | <LOQ | / | / | <LOQ | / | / | <LOQ | / | / | / | / |
| 0.5 | 70.5 | 6.7 | 9.5 | 71.7 | 2.3 | 3.3 | 60.8 | 6.2 | 10.2 | 86.2 | 84.7 |
| 1.0 | 375.6 | 9.0 | 2.4 | 612.0 | 14.5 | 2.4 | 425.4 | 96.2 | 22.6 | 113.3 | 69.5 |
| 1.5 | 317.6 | 1.3 | 0.4 | 486.6 | 1.9 | 0.4 | 369.5 | 31.2 | 8.4 | 116.3 | 75.9 |
| 2.0 | 212.9 | 50.7 | 23.8 | 332.5 | 26.1 | 7.9 | 264.8 | 2.3 | 0.9 | 124.4 | 79.6 |
| 4.0 | 64.1 | 0.1 | 0.2 | 70.8 | 0.4 | 0.5 | 61.4 | 9.2 | 15.0 | 95.7 | 86.7 |
| 6.0 | 48.6 | 0.9 | 1.8 | 72.3 | 7.8 | 10.7 | 52.4 | 5.3 | 10.1 | 107.8 | 72.5 |
| 8.0 | 23.7 | 0.7 | 2.8 | 38.0 | 2.5 | 6.5 | 26.7 | 2.7 | 10.1 | 112.5 | 70.3 |
| 12.0 | 17.0 | 0.6 | 3.5 | 21.6 | 2.1 | 9.7 | 19.6 | 1.6 | 8.3 | 115.5 | 90.9 |
| 24.0 | <LOQ | / | / | 10.4 | 1.0 | 9.2 | 8.3 | 1.5 | 17.7 | / | 80.1 |
| 48.0 | <LOQ | / | / | <LOQ | / | / | 5.0 | 0.4 | 7.4 | / | / |
| 72.0 | <LOQ | / | / | <LOQ | / | / | <LOQ | / | / | / | / |

The plasma samples were taken at 12 different times and were diluted with buffer 2 (10%).

As all points are concentrated around this line and no strong outliers can be observed, the two methods show in average and within the scope of the measurements a good correlation. The equation for the linear regression through all points ($n=40$) is $y=0.955x+0.0074$ with the linear regression coefficient ($r^2$) 0.987.

The observation, which can be made in Fig. 10 and in Table 1, is that the determined telmisartan concentrations are

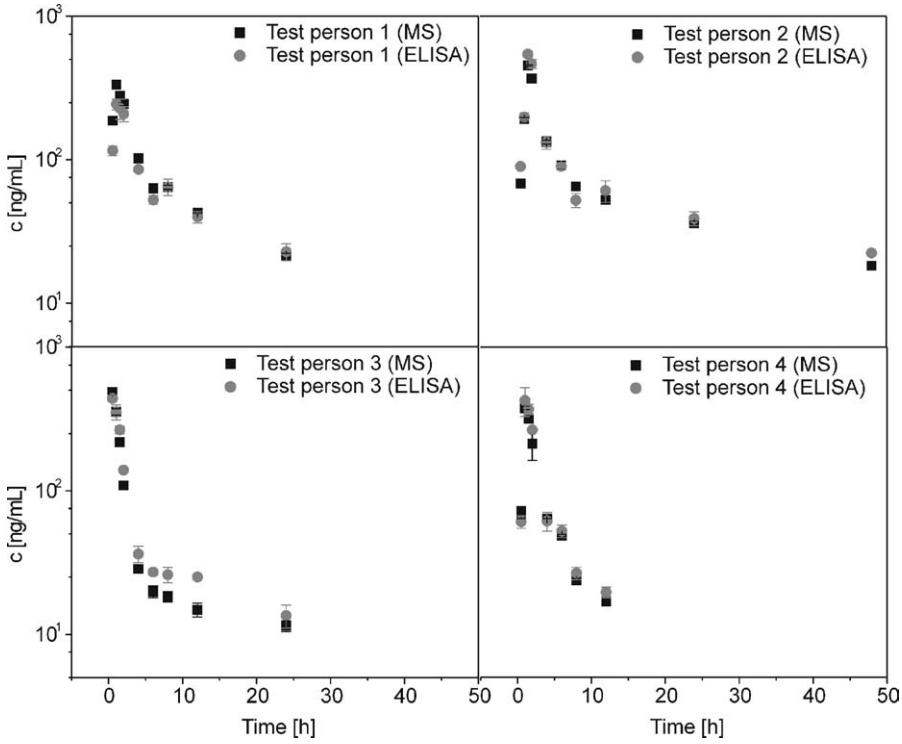


Fig. 9. Comparison of the ELISA and LC/MS results regarding the time-dependent development of the telmisartan concentrations in blood plasma of four different healthy volunteers.

in general slightly lower for the immunoassay than for the LC/MS/MS measurements (averaged: 84.1%). One explanation for this could be the influence of the metabolite telmisartan acylglucuronide on the immunoassay. This would mean that the metabolite somehow inhibits the ELISA. But as the structure of telmisartan acylglucuronide is very similar to the structure of telmisartan, the metabolite would compete with the enzyme tracer for the antibody binding sides as well as telmisartan itself. Therefore, this would cause the determination of higher telmisartan concentrations instead of lower.

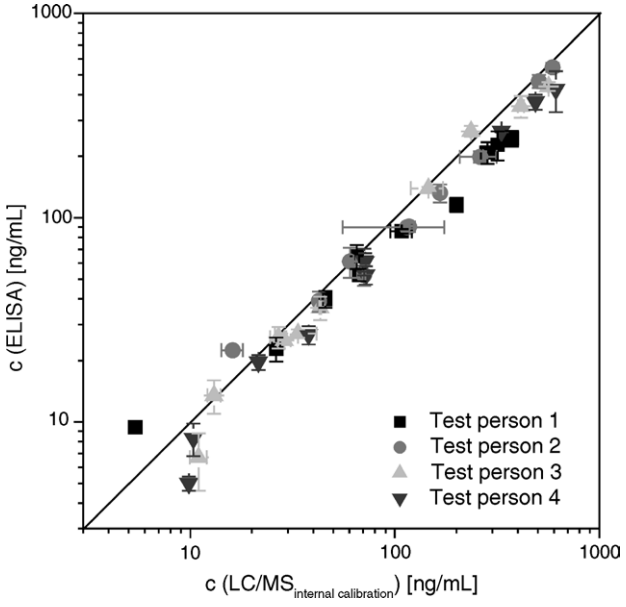


Fig. 10. Correlation plot between the telmisartan concentrations as determined by means of immunoassay and LC/MS method.

Another reason for the observations in Table 1 and in Fig. 10 might be the external calibration of the immunoassay. For this calibration, pooled human plasma is taken, which probably has other properties than the human plasma of the real samples. Therefore, effects, which appear in case of the real samples and which influence the performance and the result of the ELISA might be suppressed by mixing different plasmas. This explanation is confirmed by the results of Table 2. Here, the determined telmisartan concentrations of two quality control samples are presented for each method. In this case all samples and all external calibration solutions were dissolved in pooled plasma. First, it can be observed that the results for ELISA and LC/MS measurements with internal calibration differ only slightly from the concentrations of the quality controls. The deviation in case of the external LC/MS method may be higher due to protein adsorption effects. Second, it can also be seen that the differences between the determined concentrations for ELISA and LC/MS with internal standard are negligible.

However, it was shown that both methods are suitable for the determination of telmisartan in human blood plasma samples: a cross-validation of the obtained results is possible. In case that a high sample throughput for the determination of the telmisartan concentration of a large number of samples is necessary, the ELISA would be advantageous because of short analysis times and low operating costs. Generally, an immunoassay could be favored in comparison to LC/MS measurements in case that a known analyte has to be determined routinely in many samples.

Table 2
Telmisartan concentrations for quality control samples (500 and 20 ng/mL) in pooled plasma and diluted with buffer 2 (10%)

| $c$ (ng/mL) | APCI-MS external (ext.) calibration | | | | APCI-MS internal (int.) calibration | | | | Immunoassay | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Average (ng/mL) | S.D. ($n = 6$) | R.S.D. (%) | Recovery (%) | Average (ng/mL) | S.D. ($n = 6$) | R.S.D. (%) | Recovery (%) | Average (ng/mL) | S.D. ($n = 12$) | R.S.D. (%) | Recovery (%) |
| 500.0 | 408.3 | 24.4 | 6.0 | 81.7 | 524.6 | 7.4 | 1.4 | 104.9 | 492.8 | 43.3 | 8.8 | 98.5 |
| 20.0 | 13.3 | 0.6 | 4.5 | 66.6 | 17.8 | 0.9 | 5.0 | 89.0 | 18.2 | 0.1 | 0.4 | 91.1 |

The use of the LC/MS measurements could be preferred in the case that metabolites or additives show influence on the ELISA or shall be investigated. A method development would be faster for LC/MS than for an immunoassay. In the latter case, the search for the right antibody/antigen system and the necessary syntheses and investigations are very time-consuming. Therefore, LC/MS is the method of choice for new analytes and non-routine analyses.

## 4. Conclusions

A new and independent LC/APCI-MS/MS method for the determination of telmisartan in human blood plasma has been developed. By using turbulent flow chromatography as on-line sample clean-up procedure, a short analysis-time is guaranteed. The results of our investigations show that the comparative pharmacokinetic study on telmisartan in human blood plasma by means of the two new introduced methods, which are based on completely different principles, was successful. The immunoassay and the on-line TFC/LC/UV/APCI-MS/MS method allow the cross-validation of the obtained results. Both techniques enable a fast, sensitive and selective determination of telmisartan. For future work, further investigations regarding the determination of drugs in blood plasma samples using these measurement principles are foreseen.

## Acknowledgement

Financial support of the Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO, The Hague, The Netherlands) is gratefully acknowledged.

## References

[1] B. Pitt, M.A. Konstam, Am. J. Cardiol. 82 (1998) 47S–49S.
[2] T. Unger, Am. J. Cardiol. 84 (1999) 9S–15S.
[3] R. Willenheimer, B. Dahlöf, E. Rydberg, L. Erhardt, Eur. Heart J. 20 (1999) 997–1008.
[4] L. Gonzalez, U. Akesolo, R.M. Jimenez, R.M. Alonso, Electrophoresis 23 (2002) 223–229.
[5] N. Torrealday, L. Gonzalez, R.M. Alonso, R.M. Jimenez, E.O. Lastra, J. Pharm. Biomed. Anal. 32 (2003) 847–857.
[6] T.X. Maotian, J.F. Song, N. Li, Anal. Bioanal. Chem. 377 (2003) 1184–1189.
[7] T.X. Maotian, J. Song, Y. Liang, J. Pharm. Biomed. Anal. 34 (2004) 681–687.
[8] T. Kondo, K. Yoshida, Y. Yoshimura, M. Motohashi, S. Tanayama, J. Mass. Spec. 31 (1996) 873–878.
[9] B. Chen, Y. Liang, Y. Wang, F.L. Deng, P. Zhou, F. Guo, L. Huang, Anal. Chim. Acta 540 (2005) 367–373.
[10] H. Hayen, U. Karst, J. Chromatogr. A 1000 (2003) 549–565.
[11] A. Büldt, U. Karst, Anal. Chem. 71 (1999) 1893–1898.
[12] G. Hopfgartner, E. Bourgogne, Mass. Spect. Rev. 22 (2003) 195–214.
[13] M. Jemal, Biomed. Chromatogr. 14 (2000) 422–429.