Journal of Chromatography B, 877 (2009) 2284–2289

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Identification and quantification of the atypical metabolite ornithine-lactam in human plasma by liquid chromatography–tandem mass spectrometry (LC–MS/MS)☆

Jens Martens-Lobenhoffer[a,*], Achim Becker[b], Horst Freude[b], Stefanie M. Bode-Böger[a]

[a] Institute of Clinical Pharmacology, Otto-von-Guericke University, 39120 Magdeburg, Germany
[b] Berlin-Chemie AG, Glienicker Weg 125, 12489 Berlin, Germany

ARTICLE INFO

Article history:
Received 19 December 2008
Accepted 26 January 2009
Available online 31 January 2009

Keywords:
Ornithine-lactam
Ornithine
HILIC
LC–MS/MS
Plasma

ABSTRACT

In the late 1970s the atypical metabolite of ornithine, ornithine-lactam, has been observed in urine samples of patients suffering from hyperornithinemia. However, not least due to insufficient analytical methods, until now there are no data available about ornithine-lactam in human plasma. Here, we describe a new method, which is, for the first time, suitable to identify and quantify ornithine-lactam in human EDTA-plasma. The method was validated according to the requirements of the FDA guidance for bioanalytical method validation. The analytes were extracted on mixed mode cation exchange SPE columns, separated on a silica analytical HPLC column working in the HILIC mode and detected on a tandem mass spectrometer equipped with an ESI ion source. As internal standard newly synthesized stable isotope labeled D_6-ornithine-lactam was used. The calibration function was linear in the range of 0.1–5 μM. Intra- and inter-day precision and accuracy was better than 14% at all concentration levels. In EDTA-plasma samples from 30 volunteers ornithine-lactam concentrations ranging from 0.136 to 0.653 μM were determined. These concentrations correlated significantly ($p < 0.001$, $R^2 = 0.784$) to those of ornithine in EDTA-plasma.

1. Introduction

The non-essential amino acid ornithine plays a central role in a couple of physiologically fundamental biochemical pathways. In the urea cycle it is synthesized from arginine by the enzyme arginase. In consecutive enzymatic reactions it is converted to citrullin, then to argininosuccinate and finally back to arginine. Keeping the homeostasis of the urea cycle is of vital importance for the organism [1]. Furthermore, ornithine is the biochemical source for the amino acids proline and glutamate and is the starting point of polyamine synthesis [2]. However, it is not incorporated in proteins. From a chemical point of view, ornithine is a α-δ-amino acid, which is capable to form by the abstraction of a water molecule a relatively stable δ-lactam, which features an unstrained six-membered ring (Fig. 1). First evidence that this conversion actually took place in humans stemmed from the observation of an additional ninhydrin-positive substance in the urine of patients suffering from hyperornithinemia. It could indeed be verified that the structure of this substance was 3-amino-piperidone, which is the chemical name of ornithine-lactam [3,4]. In the late 1970s, when this research was performed, the analytical techniques were incapable to detect ornithine-lactam in plasma, but it was suspected that it is also present in plasma. To test this hypothesis and to investigate correlations between ornithine-lactam concentrations and other biochemical parameters, a specific and highly sensitive (because of the expected low concentrations of ornithine-lactam in plasma) assay was required.

In the here presented study we developed and validated a method, which is for the first time suitable to identify and quantify ornithine-lactam in human plasma of apparently healthy human subjects. It featured hydrophilic interaction liquid chromatography (HILIC) with electrospray ionization (ESI) tandem mass spectrometric (MS/MS) detection. Newly synthesized isotopic labeled ornithine-lactam was used as I.S. to get reliable, precise and accurate results. The validation was carried out following the guidelines of the United States Federal Food and Drug Administration (FDA) for the validation of bioanalytical methods [5,6], which has become the current industrial standard.

☆ This paper is part of a special issue entitled "Method Validation, Comparison and Transfer", guest edited by Serge Rudaz and Philippe Hubert.

* Corresponding author at: Insitut f. Klinische Pharmakologie, Leipziger Str. 44, 39120 Magdeburg, Germany. Tel.: +49 391 6713068; fax: +49 391 6713062.
E-mail address: jens.martens-lobenhoffer@med.ovgu.de (J. Martens-Lobenhoffer).

1570-0232/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2009.01.031

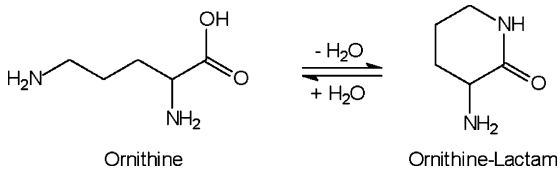

Fig. 1. Chemical structures and reaction pathway between ornithine and ornithine-lactam.

2. Experimental

2.1. Chemicals

Ornithine-hydrochloride (99% purity) was purchased from Sigma–Aldrich (Buchs, Switzerland). Stable isotope labeled ornithine-hydrochloride (3,3,4,4,5,5-D_6) was obtained from Cambridge Isotope Laboratory (Andover, MA, USA). All other chemicals were of analytical grade or better.

2.2. Synthesis of ornithine-lactam and D_6-ornithine-lactam

The synthesis of ornithine-lactam followed the procedure described by Blade-Font [7]. A solution of ornithine-hydrochloride (20 g, 118.6 mmol) in 50 ml water was cooled to 5 °C and a solution of NaOH (4.74 g, 118.6 mmol) in 20 ml water was added dropwise over a period of 30 min while stirring. The clear solution was frozen and then lyophilized. The remaining solid was suspended in toluene (700 ml) and aluminium oxide (36.3 g, 355.8 mmol) was added. The reaction mixture was boiled under reflux using a Dean-Stark trap to collect the water formed in the reaction. After a reaction time of 3.5 h the mixture was cooled to room temperature, filtered and the solid was repeatedly washed with a mixture of dichloromethane–methanol (9:1, v:v) to completely dissolve the lactam. The combined filtrates were evaporated to dryness in vacuo. The residue (ca. 12.5 g) was recrystallized from diisopropyl ether to yield ornithine-lactam as a colorless solid (11.3 g). After dissolving the lactam in water (40 ml), hydrochloric acid (1 M, 63.0 ml) was added. The solution was lyophilized and the residue was grinded to powder in ethanol. The final product, ornithine-lactam hydrochloride, was collected on a filter, washed with ethanol (5 ml) and dried under vacuum. The yield of racemic ornithine-lactam was 70.5% and the purity was 98.5%. The identity of the product was confirmed by ^{1}H NMR and mass spectrometry. The racemic nature of the product was detected by chiral chromatography on a Chiralpak AD column with a mobile phase consisting of 70% hexane and 30% ethanol containing 0.1% diethylamine.

D_6-ornithine-lactame hydrochloride was obtained in a similar manner starting with 250 mg D_6-ornithine hydrochloride. The synthesis resulted in 180 mg of the deuterated lactam (yield = 77.6%) in 99.7% purity.

2.3. Plasma samples

Pooled human EDTA-plasma and EDTA-plasma samples from 30 apparently healthy volunteers (15 male, 15 female, age 20–68 years, median 47 years) were obtained from In.Vent Diagnostica GmbH (Hennigsdorf, Germany). The volunteers gave written informed consent before enrolling in this study. The samples were stored at −20 °C until analysis.

2.4. Analytical equipment

The HPLC part of the analytical apparatus consisted of an Agilent 1100 system (Santa Clara, CA, USA) comprising a binary pump, an autosampler and a thermostated column compartment. The chromatographic separation took place on an Atlantis^{TM} HILIC silica 5 μm 150 mm × 2.1 mm column (Waters, Milford, MA, USA), protected by a SecurityGuard system (Phenomenex, Torrance, CA, USA) equipped with a 4 mm × 2 mm silica filter insert. The analytes were detected by a Thermo Electron TSQ Discovery Max triple quadrupole mass spectrometer equipped with a ESI ion source (San Jose, CA, USA). System control and data handling were carried out by the Thermo Electron Xcalibur software, version 1.2.

2.5. Analyte solutions, calibration – and quality control samples

Stock solutions were prepared by dissolving 50 μmol ornithine-lactam in 100 ml methanol and 25 μmol of the I.S. D_6-ornithine-lactame in 50 ml methanol, respectively. The I.S. working solution was freshly prepared on each analysis day by diluting 100 μl of the I.S. stock solution with 900 μl water. From the stock solution of ornithine-lactam, fresh working solutions in the concentrations of 250, 25 and 2.5 μM were prepared by adding proportionate amounts of water. Calibration samples were prepared by spiking human EDTA-plasma or water, respectively, to concentrations of 0, 0.1, 0.2, 0.5, 1, 2 and 5 μM. Quality control (QC) samples were prepared in a similar manner from human EDTA-plasma with spike-concentrations of 0, 0.5 and 4 μM. The QC-samples were stored at −20 °C until analysis.

2.6. Sample preparation

Calibration-, QC- and unknown samples were prepared by solid phase extraction (SPE) on mixed mode cation exchange columns (Oasis MCX, Waters, Milford, CA, USA). The SPE-columns were activated with both 1 ml of methanol and 1 ml of water. Plasma samples (500 μl) were mixed with 20 μl of the I.S. working solution and 100 μl 1 M HCl and subsequently passed through the SPE-columns. The columns were rinsed with 1 ml 1 M acetic acid and 1 ml methanol and sucked dry for 5 min under vacuum. The analytes were eluted from the columns by 1 ml of methanol containing 4% (v:v) concentrated ammonia. The eluate was evaporated in vacuo and the residue was reconstituted with a mixture of 100 μl 0.1% (v:v) formic acid and 1 ml acetonitrile. About 200 μl of this mixture was transferred into autosampler vials with microliter inserts.

2.7. Chromatography and mass spectrometric detection

The chromatographic separation of ornithine-lactam took place in the hydrophilic interaction liquid chromatography (HILIC) mode with a mobile phase consisting of 0.1% (v:v) formic acid (A) and acetonitrile (B). A step gradient starting at 15% A and 85% B, then instantly going up to 45% A and holding constant until 10 min was employed. A re-equilibration time of 7 min after each run was necessary. The flow rate was 0.25 ml/min, the column temperature was set to 30 °C and the injection volume was 10 μl. Under these conditions, ornithine-lactam and the isotopic labeled I.S. eluted synchronously as sharp and symmetric peaks at about 7.0 min.

The analytes were ionized in an ESI ion source working in the "positive" mode and were detected by tandem mass spectrometry (MS/MS). The spray voltage was set to 3500 V, the capillary temperature was 200 °C and the sheath gas and auxiliary gas flows were set to 25 and 2 units, respectively. These parameters were optimized for maximum response by semi-automatic procedures of the mass spectrometer. Under these conditions, ornithine-lactam and the I.S. formed quasimolecular ions $[M+H]^+$ with mass- to charge-ratios of m/z 115 and m/z 121, respectively. No dimers or solvent adducts were observed. These precursor ions were fragmented to product ions with m/z 70 and m/z 75 for ornithine-lactam and the I.S., respectively. Argon was used as collision gas at a pressure of

1.2 mTorr and the collision energies were 14 V for both analytes. These ions were observed for mass spectrometric quantification.

2.8. Validation

The validation of the method was performed according to the requirements of guidelines of the Federal Food and Drug Administration (FDA). Extraction yields were determined at three different concentrations (0.1, 1 and 5 μM, $n = 5$) by comparing peak areas of extracted samples with peak areas of blank samples spiked after the extraction with amounts equal to 100% extraction yield. Matrix effects on the mass spectrometric response were investigated by comparing peak areas of pure solutions equivalent to 5 μM D_6-ornithine-lactame with peak areas of six plasma samples from different individuals spiked with the same concentrations after extraction. In both experiments only the I.S. was used because it is, contrary to ornithine-lactam, no endogenous substance. Specificity of the method was evaluated by analyzing plasma samples from six different individuals with and without the addition of the I.S. To evaluate intra- and inter-day precision and accuracy, QC-samples of all three levels were analyzed 6-fold on 1 day and additionally on 5 different working days. Additionally, the intra-day precision and accuracy of the lowest calibration level (0.1 μM) was evaluated ($n = 6$). The accuracies (in %) of the QC-samples were calculated by the formula

$$\left(\left(\frac{C_M}{(C_{QC-1} + C_S)}\right) - 1\right) 100 \quad (1)$$

C_M, mean of measured concentrations; C_{QC-1}, mean concentration of QC level 1; C_S, spike concentration.

Stability of ornithine-lactam against three freeze–thaw cycles ($n = 5$), stability at room temperature for 16 h ($n = 5$), stability at −20 °C for 15 days ($n = 5$) and stability of prepared samples left in the autosampler for 12 h ($n = 6$) was tested within all three levels of the QC-samples. Stability of the stock solutions of ornithine-lactame and the I.S. was tested by comparing solutions stored at 8 °C for 11 days with freshly prepared ones.

3. Results and discussion

3.1. Sample preparation

In previous work our group has shown that with HILIC chromatography and mass spectrometric detection amino acids such as arginine, ornithine and related compounds can be readily quantified from biological samples, requiring no sample preparation other than protein precipitation [8,9]. Unfortunately, with ornithine-lactam being less hydrophilic in comparison to ornithine, such a simple sample preparation leads to extreme broadening of the chromatographic peaks when samples are prepared from EDTA-plasma. To remove endogenous substances responsible for the chromatographic disturbances, SPE with mixed mode cation exchange columns was necessary. Due to the high retention strength of this column type for basic compounds at acidic pH, rinsing with methanol to remove all non-polar and all acidic compounds without loss of analyte was possible. The analytes were subsequently eluted from the column with basified methanol with nearly quantitative yield and in acceptable purity. The extraction yield could only be quantified for the I.S. D_6-ornithine-lactam since the unlabeled ornithine-lactam is an endogenous substance with unknown concentrations in plasma. The complicated procedure described in Section 2.8 was necessary because of the potential matrix dependency of the response (see next section) of the mass spectrometric detector, which prevents comparison of extracted matrix samples with pure solutions of the analytes. The yield was found to be 113.97 ± 16.06% at 0.1 μM, 94.50 ± 2.84% at 1 μM and 89.15 ± 4.75% at 5 μM, without significant differences between the different concentration levels. It was assumed that no difference in extraction recovery exists between ornithine-lactam and its stable isotope labeled I.S.

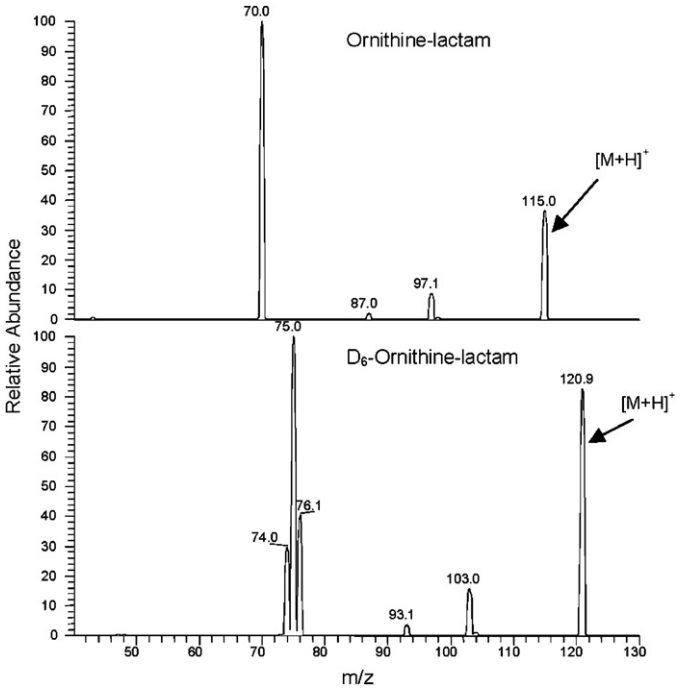

Fig. 2. Tandem mass spectra of ornithine-lactam (upper panel) and the I.S. D_6-ornithine-lactam (lower panel). The quasimolecular ions $[M+H]^+$ are marked.

3.2. Chromatography and mass spectrometric detection

Because of the less polar nature of ornithine-lactam in comparison to ornithine, attempts were made to separate it on reversed phase columns or porous graphitic carbon columns. However, it turned out that on these columns it could be retained only with mobile phases comprising very high water contents, leading to poor ionization efficiency in the ESI ion source. In contrast, chromatography in the HILIC mode on a silica column resulted in good retention with a mobile phase rich in acetonitrile. Pure solutions of ornithine-lactam showed symmetric and sharp peaks under isocratic conditions, but in samples prepared from plasma occasionally peak broadening and distortion was observed, despite the sophisticated sample preparation. To address this problem, a step gradient elution was applied to refocus the analyte peaks at the start of the chromatographic separation. Under these conditions, sharp and symmetrical peaks were observed in EDTA-plasma samples.

In the ESI ion source of the mass spectrometer, the quasimolecular ions $[M+H]^+$ of ornithine-lactam and its I.S. with the masses m/z 115 and 121, respectively, were produced. No adduct ions or dimers of the analytes were observed. To achieve optimal selectivity, tandem mass spectrometry was applied with argon as collision gas (see Fig. 2). In the case of ornithine-lactam, only one major fragment ion with the mass of m/z 70 was observed. The I.S., however, fragmented to three major fragment ions with the masses m/z 74, 75 and 76 with intensity ratios of 1:2:1. This effect seems to stem from the probability of the different pathways leading to fragment ions via the loss of two, one or no deuterium atoms. Obviously, no such effect could be observed in unlabeled ornithine-lactam. For quantification, the fragment ion traces of m/z 70 and m/z 75

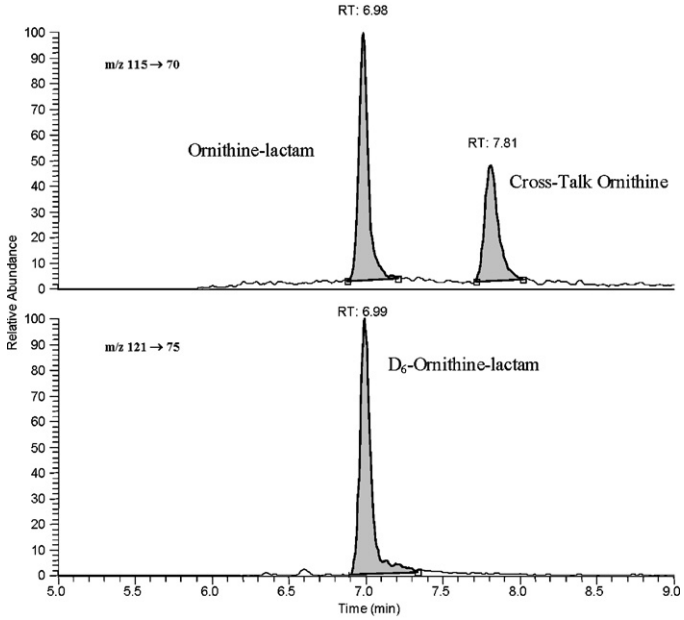

Fig. 3. Representative chromatogram of ornithine-lactam (upper trace) and the I.S. D_6-ornithine-lactam (lower trace) from EDTA-plasma. The additional peak at 7.8 min corresponds to the mass spectrometric crosstalk of ornithine. The ornithine-lactam concentration of this plasma sample was 0.393 μM.

were observed for ornithine-lactam and the I.S., respectively. In the course of the method development it was observed that in the ion trace of ornithine-lactam in plasma samples always an additional peak showed up. Subsequently, this signal could be associated with ornithine, which is present in all plasma samples in relatively high amounts. Beside its quasimolecular ion with a mass of m/z 133, it is converted by in-source collision-induced dissociation (CID) in a small percentage to ornithine-lactam with m/z 115. It was therefore necessary to separate the two substances chromatographically to avoid wrong quantitative results for ornithine-lactam caused by mass spectrometric crosstalk from ornithine. In Fig. 3 a typical chromatogram from human EDTA-plasma is depicted. As it can be seen, in the ion trace for ornithine-lactam (retention time 7.0 min) an additional peak, relating to ornithine, showed up at 7.8 min. No other endogenous peaks were observed in the ion traces for ornithine-lactam or the I.S. Matrix effects on the mass spectrometric responses were tested with six different EDTA-plasma samples. As it was the case in the evaluation of the extraction yield, only the I.S. could be used for the investigation because of the unknown endogenous amounts of ornithine-lactam in the plasma samples. Peak areas of pure solutions with concentrations of 5 μM were compared to blank EDTA-plasma extracts, spiked after extraction with 5 μM. The matrix samples showed higher responses of 110.0 ± 5.2% in comparison to pure solutions, resulting in a positive matrix effect of 10%. However, since the matrix effects act in the same way on ornithine-lactam as on the I.S., no matrix effect on the quantitative results would occur (see next section).

A method utilizing LC–MS/MS technology provides a high inherent specificity due to its ability to distinguish the analytes not only by their chromatographic retention times, but also by their molecular masses and fragmentation patterns. On the other hand, it is difficult to ensure specificity for endogenous substances as ornithine-lactam because there is no analyte free matrix available. To evaluate the specificity of our method, we analyzed plasma samples with and without the addition of the I.S. In the samples prepared without I.S., no peaks in the ion traces of the I.S. could be observed. In the samples with I.S., the peaks for ornithine-lactam showed the same shapes and retention times as the peaks for the I.S., so we concluded that the ornithine-lactam peaks were uncompromised by other endogenous substances.

3.3. Calibration

The calibration is always a major problem in the development of a method for the quantitative determination of endogenous substances because no authentic matrix free of the analyte is available [10]. One common method for calibration is the addition of calibration solution to matrix samples. This results in a calibration function not intercepting at the origin, but at an offset equivalent to the unknown amount of analyte in the matrix before addition of the calibration solution. The only way to avoid such an offset calibration function is to calibrate from non-matrix (i.e. water) samples. In this case it has to be verified that both calibration functions from matrix samples and from water samples result in identical slopes for the calibration functions, thus confirming matrix independence of the method. In the case of the here described method, this precondition for matrix independent calibration was fulfilled. A linear weighed ($1/x$) least square calibration from water samples resulted in the parameters slope = 1.081 ± 0.059, an insignificant ($p = 0.196$) intercept of 0.025 ± 0.039 and a correlation coefficient of $R = 0.999$, while the respective parameters of the calibration from plasma samples were slope = 1.062 ± 0.061, intercept = 0.315 ± 0.042 and $R = 0.999$. The differences in the slopes between the calibration functions of the two matrices were less than 2% and therefore negligible. In consequence, the calibration from water samples is usable to quantify ornithine-lactam in unknown plasma samples. A weighed least square regression was selected, because the standard deviations of the measurements were nearly linearly increasing with concentration and therefore the higher calibration levels were dominating the regression if no weighing was introduced.

The lower limit of quantification (LOQ) was set to the lowest concentration of the calibration range, i.e. 0.1 μM. Accuracy and precision was acceptable at this level in accordance with the FDA validation guidelines (see next section). The limit of detection was found to be 0.02 μM at a signal-to-noise ratio of 3.

Table 1
Intra- ($n = 6$) and inter-assay ($n = 5$) precision and accuracy.

Sample	Matrix	Spike concentration (μM)	Measured concentration (μM)	R.S.D. (%)	Accuracy (%)
Intra-assay					
Calib. level 1	Water	0.1	0.117	14.3	16.9
QC-level 1	Plasma	0.0	0.269	12.5	N/A[a]
QC-level 2	Plasma	0.5	0.754	4.2	−1.9
QC-level 3	Plasma	4.0	4.173	3.0	−2.3
Inter-assay					
QC-level 1	Plasma	0.0	0.249	14.1	−7.4
QC-level 2	Plasma	0.5	0.711	4.1	−5.8
QC-level 3	Plasma	4.0	4.322	2.5	3.6

[a] Not applicable.

Table 2
Stability of ornithine-lactam in plasma samples under various conditions.

Sample	Spike (μM)		Type of experiment				
			Plasma at RT[a] for 16 h (n = 5)	Three freeze–thaw cycles (n = 5)	Processed samples at RT for 12 h (n = 6)	Storage at −20 °C for 8 days (n = 5)	Storage at −20 °C for 15 days (n = 5)
QC-level 1	0.0	Average (μM)	0.282	0.262	0.299	0.248	0.276
		R.S.D. (%)	7.1	7.6	6.6	7.1	10.4
		Stability (%)	104.7	97.6	111.3	92.1	102.8
QC-level 2	0.5	Average (μM)	0.767	0.841	0.767	0.752	0.701
		R.S.D. (%)	9.0	4.9	8.5	8.9	1.7
		Stability (%)	101.7	108.0	101.7	99.7	92.9
QC-level 3	4.0	Average (μM)	4.363	4.179	4.006	4.268	3.915
		R.S.D. (%)	2.3	3.2	5.5	1.1	4.5
		Stability (%)	104.6	100.2	96.0	102.3	93.8

[a] RT, room temperature (approximately 23 °C).

3.4. Accuracy and precision

To evaluate the precision and accuracy of the method, quality control samples with the spike concentrations 0 (i.e. endogenous amount), 0.5 and 4 μM were prepared and analyzed. Since there were no plasma samples with negligible low amounts of ornithine-lactam available, aqueous test solutions (sample solvent purified water) containing ornithine-lactam in a concentration of 0.1 μM were also analyzed. The results are summarized in Table 1. The R.S.D.'s for the intra-assay as well as for the inter-assay precision are in the range of ± 15%, which is acceptable according to the FDA guidelines for bioanalytical method validation. The accuracy for QC-level 1 could not be calculated because the amount of endogenous ornithine-lactam was unknown in principle. For QC-levels 2 and 3, the accuracies were in the acceptable range of ± 15%. At the LOQ, the accuracy was found to be 16.9%, which is also in the acceptable range of ±20% for this level.

3.5. Stability of ornithine-lactam

During the method development, there were concerns that ornithine-lactam could be of limited stability in aqueous solvents due to hydrolysis to ornithine [11]. Therefore, various experiments investigating the stability were performed as requested by the FDA guideline. The results are summarized in Table 2. In all experiments, no clear trends to higher or lower concentrations of ornithine-lactame could be observed. Thus, the stability of ornithine-lactame in plasma is given for more than 2 weeks and during sample processing and measurement. The stability of ornithine-lactam and the I.S. in the stock solutions was tested by comparison of old solutions stored for 11 days at 8 °C with freshly prepared ones. The differences between the old and new stock solutions were less than 5% (n = 5).

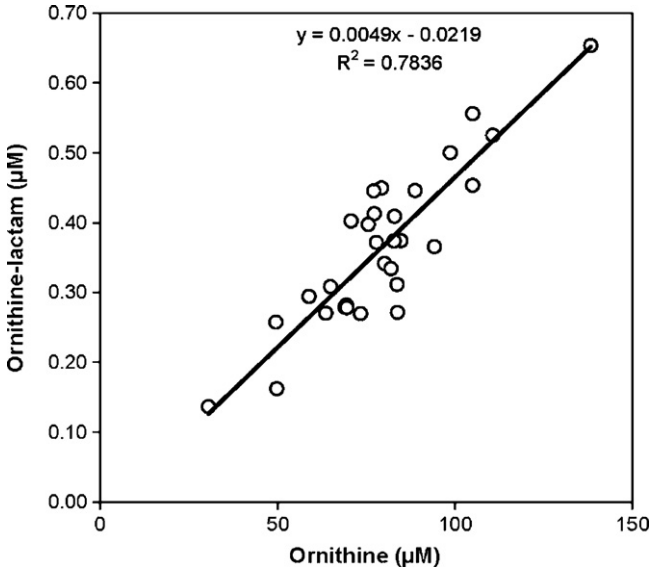

Fig. 4. Correlation between EDTA-plasma concentrations of ornithine-lactam and ornithine in 30 healthy volunteers.

3.6. Application of the method

In the course of our study, EDTA-plasma samples from 30 apparently healthy individuals were tested for the concentration of ornithine-lactam. In addition to each batch of samples, two sets of QC-samples were measured. All quality control samples show deviations from the expected values of less than 15%, thus satisfying the requirements of the FDA guidelines for routine drug analysis. Volunteer characteristics were age (20–68 years, median 47 years), body mass index (20.1–31.2 kg/m^2, median = 24.8 kg/m^2), plasma creatinine (0.7–1.3 mg/dl, average = 1.0 mg/dl) and gender (15 male, 15 female). All individuals showed clearly detectable ornithine-lactame signals, with concentrations ranging from 0.136 to 0.653 μM with an average of 0.364 μM. To compare these concentrations with the ornithine plasma values, ornithine was determined independently according to a previously published method [9]. Ornithine concentrations were 30.5–138.2 μM with an average of 79.2 μM. As it turns out (see Fig. 4), a highly significant correlation between the concentrations of ornithine-lactam and ornithine in plasma exits, with a correlation coefficient of R^2 = 0.784 (p < 0.001). No further significant correlations between ornithine-lactam and the other recorded parameters were observed. The correlation between ornithine and ornithine-lactam may indicate that both compounds are in a dynamic equilibrium, as the ornithine-lactame values are always about 0.49% of the ornithine concentrations. This equilibrium may be of purely chemical nature [11] or mediated by enzymatic processes [12]. The determination of the mechanism and the rate constant leading to the equilibrium of the two substances and the renal clearance of ornithine-lactam are tasks for future studies.

4. Conclusion

The described method is suitable to identify and quantify ornithine-lactam in human EDTA-plasma. It is selective and sensitive, especially the potentially interfering compound ornithine is clearly separated and does not compromise the quantification of ornithine-lactam. Its quantitative results are independent from the matrix used, thus it seems to be possible to apply the method without major changes for the determination of ornithine-lactam

in urine. This method enables therefore to investigate the role of ornithine-lactam in crucial biochemical pathways.

Acknowledgement

This study was financially supported by the pharmaceutical company Berlin Chemie AG, Berlin, Germany.

References

[1] J.L. Deignan, J.C. Livesay, P.K. Yoo, S.I. Goodman, W.E. O'Brien, R.K. Iyer, S.D. Cederbaum, W.W. Grody, Mol. Genet. Metab. 89 (2006) 87.
[2] G. Wu, S.M. Morris Jr., Biochem. J. 336 (1998) 1.
[3] V. Fell, R.J. Pollitt, Clin. Chim. Acta 87 (1978) 405.
[4] V.G. Oberholzer, A. Briddon, Clin. Chim. Acta 87 (1978) 411.
[5] V.P. Shah, K.K. Midha, J.W. Findlay, H.M. Hill, J.D. Hulse, I.J. McGilveray, G. McKay, K.J. Miller, R.N. Patnaik, M.L. Powell, A. Tonelli, C.T. Viswanathan, A. Yacobi, Pharm. Res. 17 (2000) 1551.
[6] Federal Food and Drug Administration. Guidance for Industry, Bioanalytical Method Validation, 2001. http://www.fda.gov/CDER/GUIDANCE/4252fnl.pdf.
[7] A. Blade-Font, Tetrahedron Lett. 21 (1980) 2443.
[8] J. Martens-Lobenhoffer, S.M. Bode-Böger, Clin. Chem. 52 (2006) 488.
[9] J. Martens-Lobenhoffer, S. Postel, U. Tröger, S.M. Bode-Böger, J. Chromatogr. B 855 (2007) 271.
[10] J. Martens-Lobenhoffer, S.M. Bode-Böger, J. Chromatogr. B 851 (2007) 30.
[11] K. Golankiewicz, M. Dezor, M. Wiewiorowski, Acta Biochim. Pol. 13 (1966) 1.
[12] H. Jakubowski, Biochemistry 38 (1999) 8088.