Journal of Chromatography B, 901 (2012) 72–78

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

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

Quantitative determination of zolmitriptan in rat blood and cerebrospinal fluid by reversed phase HPLC–ESI-MS/MS analysis: Application to *in vivo* preclinical pharmacokinetic study

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ARTICLE INFO

Article history:
Received 25 November 2011
Accepted 3 June 2012
Available online 12 June 2012

Key words:
Zolmitriptan
HPLC–ESI-MS/MS
Rat blood samples
Rat cerebrospinal fluid samples
Pharmacokinetics

ABSTRACT

A fast HPLC–ESI-MS/MS method has been developed and validated for the quantification of the potent and selective antimigraine zolmitriptan in rat blood and cerebrospinal fluid (CSF). The assay has been then applied for *in vivo* preclinical studies. The analytical determination has been used to obtain pharmacokinetics of zolmitriptan in the two biological matrices after its intravenous or nasal administration. Liquid–liquid extraction of zolmitriptan was performed from 100 μL rat blood samples in the presence of N^6-cyclopentyladenosine (internal standard) with the employment of ethyl acetate. Calibration standards were prepared by using blood matrix and following the same liquid–liquid extraction procedure. CSF samples were analyzed without any pre-treatment steps and by using an external calibration method in pure water matrix. Chromatographic separation was performed under reversed phase and a gradient elution condition on a C18 packed column (100 × 2.0 mm, 2.5 μm particles diameter). The mobile phase was a mixture between acetonitrile, water and formic acid (0.1% v/v). The applied HPLC–MS/MS method allowed low limits of detection, as calculated from calibration curves, of 6.6 and 24.4 ng/mL for water matrix and rat blood extracts, respectively. Linearity of the calibration curves was established up to 5 μM (1.44 μg/mL), as well as good assay accuracy. The intravenous infusion of 20 μg zolmitriptan to male Sprague-Dawley rats produced blood concentrations ranging from 9.4 ± 0.7 to 1.24 ± 0.07 μg/mL within 10 h, with a terminal half-life of 3.4 ± 0.2 h. The nasal administration of a water suspension of 20 μg zolmitriptan produced blood concentrations ranging from 2.92 ± 0.21 to 0.85 ± 0.07 μg/mL within 6 h. One hour after zolmitriptan intravenous infusion or nasal administration, its CSF concentrations were 0.0539 ± 0.0016 and 0.0453 ± 0.0012 μg/mL, respectively. This study determined the suitability of the herein proposed method to investigate the pharmacokinetics of zolmitriptan after its administration by means of novel formulations and, hence, to evaluate the efficacy of innovative nose-to-brain drug delivery in preclinical studies.

1. Introduction

Antimigraine therapy includes, currently, potent serotonin 5-$HT_{1B/1D}$ receptor agonists, collectively known as triptan drug class. Among triptans, zolmitriptan (4S-4-({3-[2-(dimethylammino)ethyl]-1H-indol-5-yl}methyl-1,3-oxazolidin -2-one) is characterized, in humans, by a relatively high oral bioavailability (about 40%) and an *in vivo* plasma half-life of about 3 h [1]. It is currently available as conventional [2] or dispersible oral tablets [3], as well as nasal spray [4]. Clinical studies show that zolmitriptan half-life and bioavailability after nasal administration do not significantly differ from those obtained after oral intake of the drug [1]. However, nasal spray formulations appear to offer some advantages compared to oral ones [4]. For instance, nasal sprays can be used in patients with pretreatment nausea, for whom oral medication may not be the optimal choice. Additionally, nasal spray formulations have an earlier onset of action than the oral ones [5].

In the attempt to increase the therapeutic efficacy of the drug, new nasal formulations, able to enhance zolmitriptan

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http://dx.doi.org/10.1016/j.jchromb.2012.06.001

absorption and capable to directly drive it into the brain, are currently under investigation. New nasal formulations, based on mucoadesive microemulsions, [6], chitosan microparticles [7] or micellar nanocarriers [8] have been recently proposed as improved vectors for a more efficient nose-to-brain delivery. The ability of mucoadesive microemulsions and micellar nanocarriers to enhance the delivery of zolmitriptan to the brain has been preliminarily studied by using radiolabeled drugs, following their administration to rats [6,8]. On the other hand, the potential ability of chitosan microparticles [7] to deliver zolmitriptan to the brain can be hypothesized by considering their demonstrated efficacy in promoting the rat nose-to-brain transfer of anti-ischemic and antibiotic drugs [9,10]. Nevertheless, this hypothesis remains to be validated in preclinical studies and pharmacokinetic data of drug distribution in cerebrospinal fluid (CSF) are required. As additional point of interest, researches in this field are hampered by the lack of a suitable analytical method for the quantification of zolmitriptan after its extraction from small volumes of rat blood and/or CSF. In fact, only very sensitive high performance liquid chromatography (HPLC) methods for the quantification of zolmitriptan in samples extracted from at least 1 mL of blood, have actually been described [11,12]. In this study we propose an HPLC–MS/MS approach for the quantification of zolmitriptan in samples obtained by liquid–liquid extraction from a reduced volume of 100 μL of rat blood by means of internal calibration method in blood matrix. The method of analysis has been applied to evaluate the pharmacokinetics of zolmitriptan after its intravenous and nasal administration. Zolmitriptan assay has been validated through all the analytical parameters and in particular good linearity and satisfactory accuracy have been addressed. The terminal plasma elimination half-life ($t_{1/2}$) has been obtained by log-linear regression of the terminal portion of the blood concentration vs time curve. The drug quantification has also been performed in rat CSF with no sample treatment and an external calibration method. Zolmitriptan concentrations in CSF following these two administration routes have been compared.

2. Materials and methods

2.1. Chemicals and solvents

Zolmitriptan (batch number 070701) was purchased from Haorui Pharma-Chem Inc. (New Jersey, USA). N^6-cyclopentyladenosine (CPA), ethyl acetate and acetonitrile (LC–MS grade) were purchased from Sigma Aldrich (St. Louis, MO, USA). Formic Acid (LC–MS grade) was purchased from Fluka (St. Louis, MO, USA). Ultra-pure grade water was obtained by Millipore filtration system (Billerica, MA, USA). Male Sprague-Dawley rats were purchased from Harlan SRC (Milan, Italy).

2.2. Instruments and experimental conditions

Liquid chromatographic–mass spectrometric analysis was undertaken with a micro HPLC Surveyor and a LTQ XL mass spectrometer both from Thermo Scientific (Waltham, MA, USA). The HPLC separation was performed with a Luna HST C18 packed column (100 × 2.0 mm, 2.5 μm particles diameter) manufactured by Phenomenex (Torrance, CA, USA). Mobile phases were a mixture between water:acetonitrile:formic acid operated at a flow rate of 150 μL/min. The required relative amount (% v/v) of the three mobile phase components was obtained by a quaternary pump mixing two solvent channels (A: 0.1% v/v, formic acid in water; B: 0.1% v/v, formic acid in acetonitrile). The gradient profile was programmed as following: 1 min isocratic elution at 5% of channel B; 6 min linear gradient elution from 5% to 40% of channel B; 1 min isocratically at 40% of channel B. After each cycle the column was conditioned back to the initial conditions for at least ten column volumes. Each sample was analyzed in triplicate with an injection volume of 20 μL.

The MS detector was equipped with the electrospray ionization (ESI) interface and operated in positive polarity. Operational ESI parameters were: capillary temperature 275 °C; spray voltage 4 kV; capillary voltage 49 V; tube lens 105 V. MS/MS analysis was performed by using the collision induced dissociation (CID) mode at 30% of maximum energy. Positive ion electrospray MS and MS/MS spectra of zolmitriptan and IS are shown in Fig. 1a–d. The observed ions for both compounds were $[M+H]^+$ at $m/z = 288$ for zolmitriptan (Fig. 1a) and $m/z = 336$ for CPA (Fig. 1b). MS/MS spectra report single transitions for both ions, that are experimentally observed at $m/z = 288 \rightarrow m/z = 243$ (Fig. 1c) and $m/z = 336 \rightarrow m/z = 204$ (Fig. 1d), respectively.

2.3. In vivo zolmitriptan administration

The concentrations of Zolmitriptan after its intravenous or nasal administration to male Sprague-Dawley rats (200–250 g) were measured. A total of 16 rats were randomly assigned to 4 groups (4 rats/group): (1) intravenous (i.v.) control group, receiving a femoral i.v. infusion of zolmitriptan-vehicle (20% DMSO and 80% physiologic solution); (2) zolmitriptan group, receiving a femoral i.v. infusion of zolmitriptan (20 μg/mL dissolved in a medium constituted by 20% DMSO and 80% physiologic solution); (3) nasal control group, receiving 10 μL of water in each nostril using a semiautomatic pipette; (4) nasal zolmitriptan group, receiving 10 μL of an aqueous suspension of the drug (1.0 mg/mL) in each nostril using a semiautomatic pipette.

Blood and CSF samples were collected from each rat. No drug was detected in the blood samples collected from i.v and nasal control groups. Moreover, a blood sample was collected from each zolmitriptan-treated rat before infusion or nasal administration of the drug. No drug was detected in these samples. Finally, zolmitriptan was not detected from CSF samples obtained from i.v. and nasal control groups.

For the intravenous zolmitriptan administration, the animals were slightly anesthetized and received a femoral intravenous infusion of zolmitriptan (20 μg/mL) with a rate of 0.2 mL/min for 5 min. Blood samples (100 μL/sample) were then collected at the end of the infusion and after 30, 60 and 90 min, 2, 3, 4, 5, 6, 7, 9 and 10 h. A single CSF sample (100 μL) was withdrawn 1 h after the drug infusion by a cisternal puncture and a successive gentle suction through a needle attached to polyethylene tubing connected to a syringe. A sample of blood was collected from each rat before infusion of the drug. No medications were given to rats other than zolmitriptan.

Nasal administration of zolmitriptan was performed on anesthetized rats laid on their backs, by the introduction of 10 mL of an aqueous suspension of the drug (1.0 mg/mL) in each nostril of rats using a semiautomatic pipette. After the administration, blood samples and a single CSF sample were withdrawn as specified above. Also in this case, a sample of blood was collected from each rat before infusion of the drug.

All experiments were performed in accordance with the guidelines issued by the Italian Ministry of Health (D.L. 116/92) and (D.L. 111/94-B), the Declaration of Helsinki, and the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institute of Health (Bethesda, MD).

2.4. Sample preparation

The blood samples were hemolysed immediately after their collection with 500 μL of ice cold water (HPLC grade), then 50 μL of 3 N NaOH and 100 μL of 100 nM CPA stock solution (as internal standard, IS) were added. The samples were

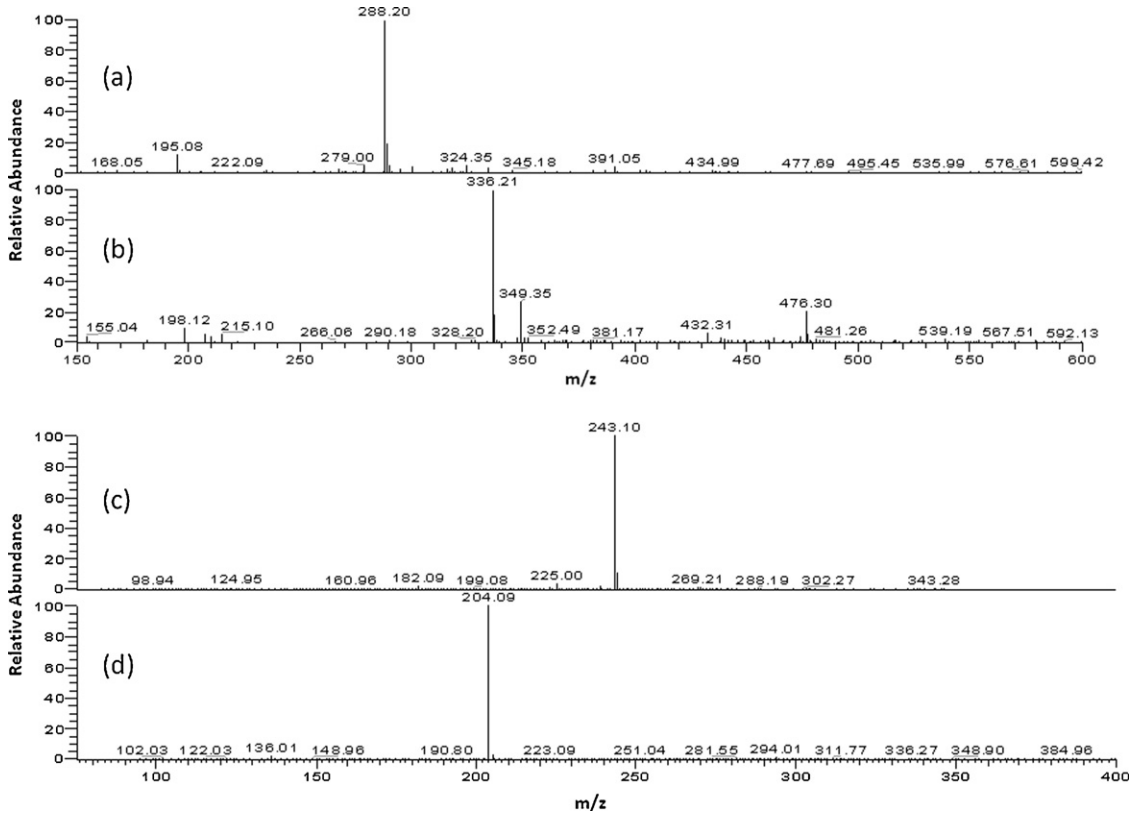

Fig. 1. Positive ESI mass spectra of (a) zolmitriptan and (b) CPA. Positive ESI-MS/MS transitions for (c) zolmitriptan and (d) CPA.

extracted twice with 900 μL of water-saturated ethyl acetate. After centrifugation (10 min at 12 000 × *g*, 4 °C), the organic layer was evaporated by drying in nitrogen flow. 200 μL of mobile phase (0.1% v/v, formic acid in water:acetonitrile 95:5) were added and, after centrifugation, 20 μL were directly injected or adequately diluted (2- to 10-fold) according to the sensitivity and linearity of the assay for the HPLC–ESI-MS/MS analysis. CSF samples were not treated before HPLC–ESI-MS/MS analysis and 20 μL aliquots were directly injected into the HPLC system.

2.5. Preparation of calibration standards and blank samples

Collected rat blood samples were spiked at different concentration of zolmitriptan ranging from 25 nM to 5 μM and then processed as described in Section 2.4. In this work, external calibration in pure water was used for quantitative analysis of zolmitriptan in rat CSF and collected CSF samples were injected without treatment procedure. Analogous experimental approaches have been reported in the literature for calibration and determination of target analyte in CSF [13,14]. This seems to be justified by the observation that CSF only contains approximately 0.3% of plasma proteins [15] and that a diluted salt solution has been used to mimic CSF matrix [16]. In addition, the low volume of collected rat CSF can cause important drawbacks when samples are spiked with standard drug solution at different concentration levels for the calibration procedure.

For *in vitro* blood analysis zolmitriptan was dissolved in DMSO at the concentration of 10^{-2} M; then, this stock solution was diluted in water or directly in the blood. DMSO or water diluted solutions were inserted in blood samples with a ratio of 1:100 (v/v). The internal standard for *in vitro* and *in vivo* measurements was dissolved in DMSO at the concentration of 10^{-2} M; then the stock solution was diluted in water to the concentration 100 nM. The experimental set-up comprises blank rat blood extracts prepared before the pharmacokinetic study to verify the absence of target drug from rat blood (see below).

2.6. Method validation

The validation of analytical assay of zolmitriptan from blood extracts was performed according to current guidelines [17]. Calibration curves were prepared as described in Section 2.5 on three different days and then compared. Quantitative determination of zolmitriptan in blood extracts was based on the internal standard calibration method within a 25–5000 nM (0.007–1.44 μg/mL) working range by using a fixed CPA concentration of 50 nM. 5–5000 nM (0.0014–1.44 μg/mL) concentration range of zolmitriptan was instead applied for external calibration in pure water. The Hubaux–Vos method [18] at the 95% confidence level has been employed for the estimation of the limit of detection (LOD) and the limit of quantification (LOQ) from the calibration curves. Precision and accuracy for zolmitriptan assay in blood extract were determined from triplicate runs of Quality Control (QC) samples at three concentration levels (100, 500 and 5000 nM) by means of the relative standard deviation (RSD) and relative error (RE), respectively. Recovery of zolmitriptan from blood and its stability during the extraction procedure were discussed. The zolmitriptan extraction recovery, determined with respect to its initial spiking level in rat blood, was calculated by means of the peak area as obtained from extracted sample runs. Validation for the assay in CSF with calibration standard solutions prepared in pure water was partially addressed by triplicate injections of three QC samples (10, 100 and 200 nM) and their back-calculated concentrations. Linearity, precision, accuracy, and limits of detection and quantification will be discussed in the following section.

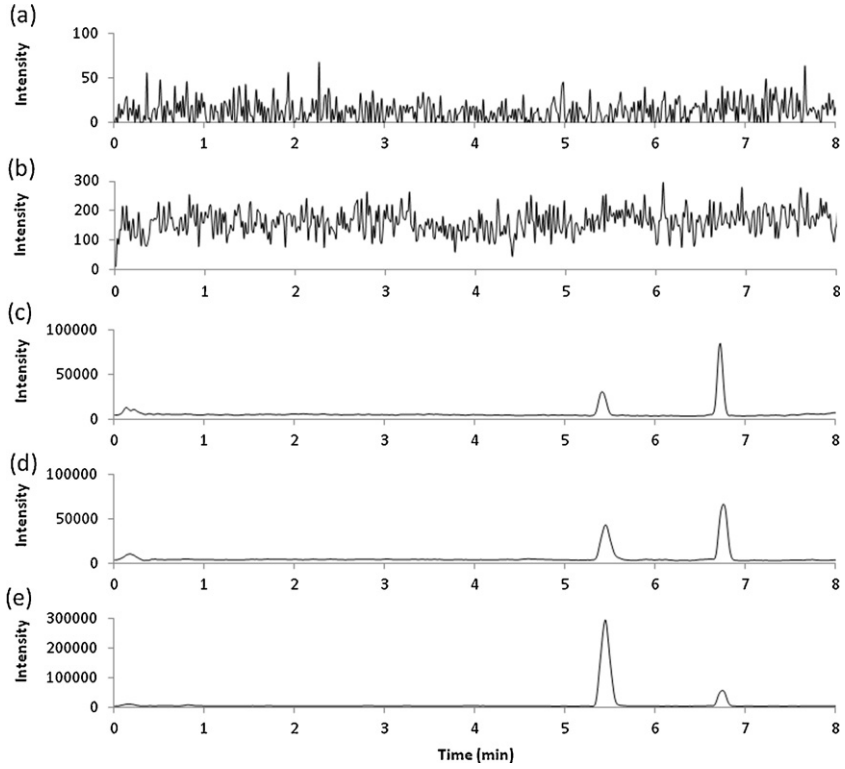

Fig. 2. (a and b) Filtered HPLC–ESI-MS/MS chromatograms for a blank blood sample: (a) zolmitriptan and (b) CPA. (c–e) Filtered HPLC–ESI-MS chromatograms for three calibration points: (c) 50 nM, (d) 100 nM (close to LOQ) and (e) 5000 nM of spiked zolmitriptan. CPA spiked concentration was 50 nM.

3. Results and discussion

3.1. Separation and specificity

It is well known that the most critical points of quantitative analytical determination in biological samples are interferences between target compounds and other matrix components or additives employed during sample treatment [19–21]. The most important interference in HPLC–MS determinations is the matrix effect: it usually produces MS signal suppression of target compounds, due to the presence of other co-eluting molecules that reach the electrospray interface at the same time than those of target compound. Interferents can be better ionized than the target analyte, producing a significant variation of the signal intensity and negatively affecting several analytical parameters (such as limits of detection and quantification, precision and accuracy of the assay). The employment of appropriate gradient elution conditions is pivotal to achieve a fast separation combined with a reliable quantitative determination of zolmitriptan and internal standard (CPA) in biological matrices. The target compound and the internal standard were chromatographically well resolved with respect to each other (Fig. 2c–e). Their peak shapes were characterized by high symmetry and an average bandwidth of 7 s at half height, regardless of whether the injected samples were blood extracts, CSFs or pure water standard solutions. No interfering compounds or loss of chromatographic efficiency were observed for both zolmitriptan and CPA peaks with the developed gradient program. The MS spectra did not reveal the presence of co-eluting compounds with zolmitriptan and CPA (Fig. 1a and b) and MS/MS transitions were always checked during method development and employed for confirmation/quantification step (Fig. 1c and d). This provided for reliable HPLC–ESI-MS/MS analysis from biological samples without severe interferences, although the sample matrix was rather complex (rat blood) due to the minimal sample treatment (hemolysis and liquid–liquid extraction, see Section 2.3).

Three chromatographic runs, respectively, of blank blood extract, standard water solution of zolmitriptan and blood extract sample spiked at the same concentration level as water solution are compared in Fig. 2. For each run, MS and MS/MS detection were examined. The background intensities for the three samples decreased of about one order of magnitude when MS/MS detection is used instead of MS. This simultaneously influences the signal decrement for zolmitriptan and CPA when MS/MS method is employed. Thus, the MS/MS ion transitions allow the development of a specific detection/quantification method, even if with a slightly lower sensitivity than the MS quantification. No ion suppression effects were observed for extracted blood samples under the employed chromatographic conditions, as demonstrated by comparable intensities of LC–MS baselines for extracted blood samples and blank blood samples ($3–4 \times 10^3$ ion count). The same conclusion can be derived for CSF samples.

3.2. Stability

Several drugs can be degraded by products employed during the hemolysis processes, as demonstrated for artemether and artemisine, two potent antimalarial agents [22,23]. For this reason the stability of zolmitriptan has been tested for its extraction under the employed experimental conditions. In particular the extracted zolmitriptan amount following protein precipitation in acid environments (by adding 50 μL of 10% sulphosalicilic acid) has been compared with that obtained following protein precipitation in basic environments. Under these two experimental conditions, no significant differences in zolmitriptan extraction were observed, thus suggesting the absence of chemical processes potentially degrading the drug in hemolysed samples. Taking into account that the degradation rate of artemether and artemisine induced by blood hemolysis was temperature dependent [22,23], zolmitriptan-containing blood samples were hemolysed and centrifuged at 4 °C, to avoid enzymatic processes potentially able to

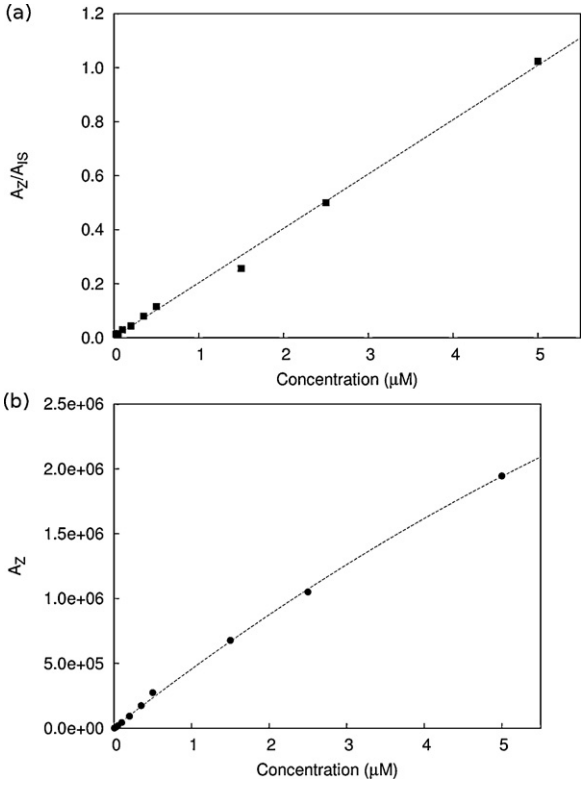

Fig. 3. Calibration curves for Zolmitriptan in (a) blood extract (■) and (b) water (•). Reported experimental data in the plots refer to HPLC–MS/MS detection.

induce drug degradation. Under the tested conditions, zolmitriptan was not degraded during its incubation in hemolysed samples. This is also confirmed by the literature data [24]: the investigated stress conditions showed that only base hydrolysis and oxidation (0.01% H_2O_2) can produce very small amount of degradation by-products at room temperature, while acid hydrolysis, heat (60 °C) and light did not show remarkable degradation.

3.3. Method validation

Fig. 2a and b reports a LC–MS/MS chromatogram for a blank blood sample showing no endogenous levels of zolmitriptan or internal standard. HPLC–ESI/MS and HPLC–ESI/MS/MS chromatograms of blank CSF samples were also characterized by the absence of significant endogenous compounds during the gradient elution. Two very intense, not interfering signals were detected at significantly low retention times (roughly 1 and 1.5 min), suggesting the presence of very hydrophilic substances. These findings support the applicability of the external calibration procedure in water matrix for CSF samples as well as their direct injection into the chromatograph without treatments (see Section 2.6). Validation parameters will be discussed separately in the following subsections.

3.3.1. Linearity

Linearity of the two calibration curves is provided by the regression results (dashed lines in Fig. 3a and b). Calibration curve in blood extract matrix (Fig. 3a) reports the ratio between the peak areas of zolmitriptan (A_Z) and CPA (A_{IS}) vs the nominal concentration of zolmitriptan before extraction. Calibration curve in water (Fig. 3b) simply reports A_Z vs its nominal concentration in standard water solutions. In the former case, a straight line function is fitted to the standard calibration points over the entire concentration range ($r = 0.9986$), while in case of calibration in water matrix the experimental data are better fitted by using a quadratic function ($r = 0.9993$) over the same concentration range. Good linearity towards straight line function for calibration data in water has been reached up to 500 nM ($r = 0.9992$): this is the effectively working range used for zolmitriptan determination in CSF and calculation of calibration limits (see below).

Table 1
Linearity and inter-day precision in the linear regression parameters for calibration curve of zolmitriptan in blood extract samples.

Days	Slope	Intercept	Correlation (r)
1	0.000216	0.00592	0.9986
2	0.000211	0.00283	0.9782
3	0.000221	0.00741	0.9807
Mean ± SD	$0.000216 \pm 5 \times 10^{-6}$	0.0054 ± 0.002	0.9858 ± 0.01
RSD (%)	2.31	43.37	1.03

The comparison of three different calibration curves for zolmitriptan assay in blood extract, obtained in different experiment sets, is summarized in Table 1.

3.3.2. Precision and accuracy

The precision for measurement of zolmitriptan extracted from rat blood shows intra-day RSD ranging from 0.98% to 2.29% and inter-day RSD between 2.12% and 13.54% (higher values at lower QC sample concentration). The assay accuracy is described by relative errors comprised between −3.58% and 0.11% for intra-day and between 0.74% and −4.17% for inter-day. In spite of this, all pharmacokinetic studies were performed within 24 h with freshly generated calibration curves.

Parameters for calibration curve in pure water matrix obtained by external calibration were also calculated. RSD were 7.2% (QC sample 100 nM) and 9.1% (QC sample 200 nM), while it exceeded 50% for the lowest concentration (QC sample 10 nM). RE ranged between −3.6% and 7% for the three QC samples.

3.3.3. Recovery

The efficacy of zolmitriptan extraction from blood spiked above 100 nM shows an average recovery of 7.8 ± 1.5% (RSD < 20%). This can possibly occur as a consequence of the reduced blood sample employed and the simplified procedure of sample treatment. All the quantitative zolmitriptan determinations have been performed in the 100–5000 nM range where the drug recovery from rat blood by liquid–liquid extraction is low but reproducible. Under the same experimental conditions, the average IS recovery was 70.8 ± 2.1% (RSD < 5%). Despite these differences between target analyte and internal standard, the *in-blood* calibration procedure employed in this study – areas ratio in blood extract vs spiked concentration in whole blood – enables correct quantification of zolmitriptan.

3.3.4. Limit of detection and limit of quantification

The calculation method (see Section 2.6) was applied in the lower concentration range of both calibration curves (blood extracts – Fig. 3a – and water solutions – Fig. 3b) by linear fit of experimental data to straight line functions and calculation of the confidence bands for linear regression (see Fig. 4a and b). LOD was estimated as 6 times the standard deviation of the detector response, that is determined by the standard deviation of y-intercept of regression lines. LOQ is then calculated as 10 times the standard deviation of the detector response.

A comparison between MS and MS/MS detection is provided. In pure water matrix, zolmitriptan LOD was 16.5 nM (4.8 ng/mL) for HPLC–MS method and 23.0 nM (6.6 ng/mL) in case of HPLC–MS/MS analysis. When blood extracts were injected, the LOD was 84.8 nM (24.4 ng/mL) for HPLC–MS determination and 92 nM (26.5 ng/mL) for HPLC–MS/MS. These results evidence that the MS/MS detection allows still a sensitive determination of zolmitriptan that is

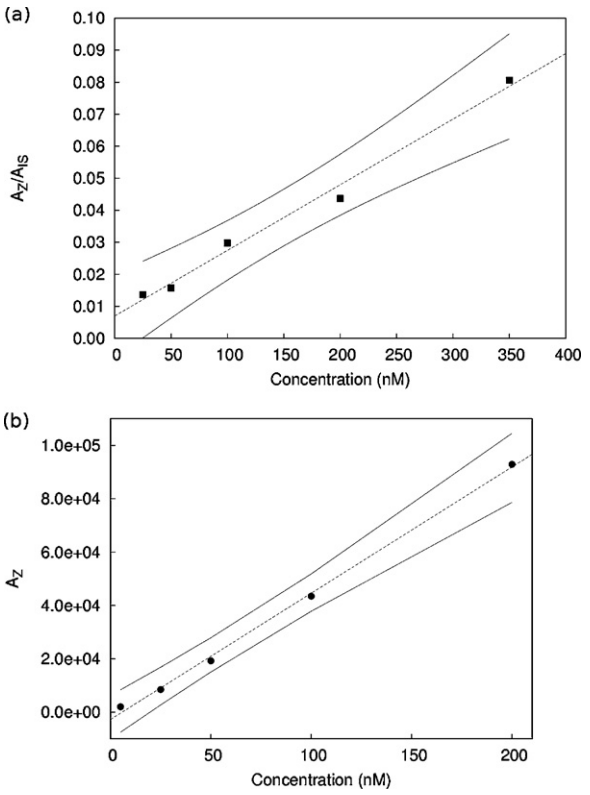

Fig. 4. Calibration curves in the lower concentration range (dashed lines) and confidence bands at 95% (solid curves) (a) in blood extract and (b) in water matrix. Typical equations and correlation coefficients of fitted straight lines are: (a) $y = 2.16 \times 10^{-4}$ ($\pm 0.098 \times 10^{-4}$)$x + 5.9 \times 10^{-3}$ ($\pm 2.4 \times 10^{-3}$), $r = 0.9938$; (b) $y = 473(\pm 12)x - 2734$ (± 1300), $r = 0.9979$. Numbers in parentheses are the standard errors for the slope and the intercept, respectively. Reported experimental data in the plots refer to HPLC–MS/MS detection.

comparable to the single stage MS detection. Calculated LOQs that can be obtained with HPLC–MS/MS method were 38.3 nM (11 ng/mL) and 153.6 nM (44.2 ng/mL) for water and blood extract samples, respectively. However, Hubaux–Vos method provided for low critical concentrations for HPLC–MS/MS analysis of 11 nM (3.2 ng/mL) and 46 nM (13.2 ng/mL) for water and blood extract, respectively. This means that determination of zolmitriptan can still be performed (even if at a lower precision level) when its concentration range is between 3.2 and 11.0 ng/mL in water and 13.2 and 44.2 ng/mL in blood extract.

LODs and LOQs obtained in this study are not as low as reported by recent literature in different types of biological matrices (i.e., rat or human plasma) [11,12,25–27]. However, we strongly believe that these data are meaningful for several practical reasons. On the one hand, very reduced volumes of rat blood (100 μL) were employed, instead of ten times (or even more) larger volumes usually handled for human studies. On the other hand, the quantitative method of analysis described is characterized by sufficiently good accuracy and precision with respect to the concentration values involved in this pharmacokinetic study (see below).

3.4. In vivo preclinical pharmacokinetics of zolmitriptan

Fig. 5 reports the blood zolmitriptan concentrations detected in rat following the intravenous infusion and the nasal administration of a 20 μg dose of the drug. No drug was detected in the blood samples collected before zolmitriptan administration or collected from rats that did not receive the drug (i.e. control groups). The peak concentration obtained at the end of the infusion process was 9.4 ± 0.7 μg/mL, then the values decreased with a terminal

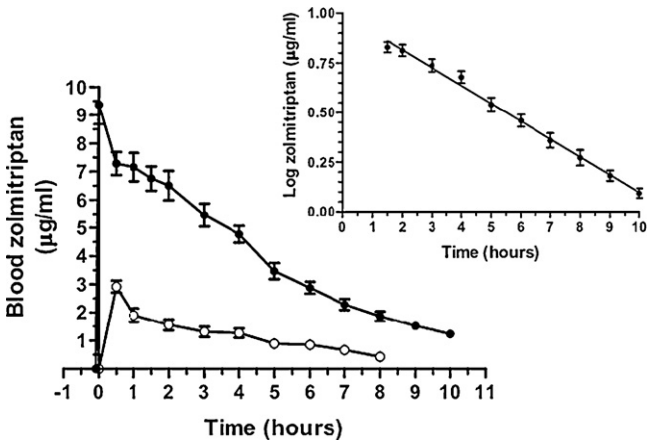

Fig. 5. Pharmacokinetic profiles of zolmitriptan in blood after the administration of 20 μg by intravenous infusion (•) or nasal administration (○) to rats. Data are expressed as the mean ± SD of four independent experiments. The half-life related to intravenous administration was calculated to be 3.4 ± 0.2 h by the terminal portion of the semilogaritmic plot reported in the inset ($n = 10$, $r = 0.984$).

half-life of 3.4 ± 0.2 h, as calculated by the terminal portion of the semilogaritmic plot reported in inset of Fig. 5 ($n = 10$, $r = 0.984$). Ten hours after the intravenous infusion, the blood concentration of zolmitriptan was 1.24 ± 0.07 μg/mL, about 1.5 orders of magnitude higher than its LOD and one order of magnitude larger than its LOQ in blood. The drug concentration in CSF, 1 h after its intravenous administration, was 0.0539 ± 0.0016 μg/mL, about eight times larger than its detection limit and two times larger than its quantification limit in water. Zolmitriptan was not detected from CSF samples of rats that did not receive the drug (i.e. control groups). After nasal administration of zolmitriptan water suspension, a peak blood concentration of 2.92 ± 0.21 mg/mL was reached in 30 min; thereafter the blood drug concentrations decreased up to 0.85 ± 0.070 mg/mL within 6 h after the administration. The CSF drug concentration, measured 1 h after zolmitriptan nasal administration was 0.0453 ± 0.0012 mg/mL, leading to a blood/CSF ratio of 42. The blood/CSF ratio obtained after the intravenous administration of the same dose of zolmitriptan was 133. The 20 μg dose of zolmitriptan administered to rats in the present study corresponds to a human dose of about 5 mg (ca. 70 μg/kg). It has been reported that such an intravenous dose can induce, in human blood, a zolmitriptan peak concentration of about 0.1 μg/mL [28], that is two orders of magnitude lower than the value found in rats. These data may suggest a different pattern of drug distribution in the rats with respect to humans, even if the half-life values of zolmitriptan appear to be very similar (about 3 h) in both species [1]. The relatively high concentrations of the drug registered in rat blood induced its low extraction efficacy (about 8%, see Section 3.1), but allowed also its detection more than 10 h after its administration, by liquid–liquid extraction from 100 μL blood samples according to our experimental approach. Additionally, zolmitriptan is well detectable also in CSF samples after the intravenous or nasal administration of the drug. Thus, the analytical method described in the present study is also suitable for the evaluation of the efficacy of new formulations aimed to improve the drug delivery into the brain. In particular, the opportunity to determine zolmitriptan concentration in animal models (blood and CSF) after its intravenous or nasal administration can allow identification of the pharmacokinetic and bioavailability parameters related to new formulations, whose neuro-efficacy can be related to the ratio of the drug concentrations between CSF and blood.

4. Conclusions

An HPLC–ESI-MS/MS method for the quantification of zolmitriptan in samples obtained by liquid–liquid extraction from 100 μL of rat blood has been successfully applied to determine zolmitriptan in blood extracts and evaluate its pharmacokinetics after intravenous or nasal administration to rats. The herein proposed method takes the advantage of reduced blood sample processed with a simplified and rapid sample treatment without negatively affecting its use for preclinical pharmacokinetic studies. Satisfactory sensitivity and specificity of the assay are addressed, together with a fully reliable HPLC–ESI-MS/MS method of analysis characterized by good linearity, accuracy and precision. This assay also allowed the drug quantification in CSF samples and was suitable to study the pharmacokinetic profile of zolmitriptan in such biological samples. This HPLC–ESI-MS/MS method showed its potential use to evaluate effectiveness of novel formulations in implementing the drug delivery in preclinical studies.

Acknowledgement

This work was supported in part by Grants 20098SJX4F_004 and 2009ZSC5K2_004 of the Italian Ministry of Universities and Scientific Research, and in part by Grant POR-FESR 2007-2013 Priority 1 (Regional Operational Programme of the European Regional Development Fund, Industrial Research and Technological Transfer). The authors acknowledge Dr. F. Dondi (University of Ferrara) for fruitful discussions. The authors thank also the ECO.RA.V. SPA Company (Longarone, Belluno, Italy) for supporting our work and are grateful to L. Minella of the same company for helpful comments and discussion.

References

[1] P.J. Goadsby, R. Yates, Headache 46 (2006) 138–149.
[2] G.D. Solomon, R.K. Cady, J.A. Klapper, N.L. Earl, J.R. Saper, N.M. Ramadan, Neurology 49 (1997) 1219–1225.
[3] A.J. Dowson, E.A. MacGregor, R.A. Purdy, W.J. Becker, J. Green, S.L. Levy, Cephalalgia 22 (2002) 101–106.
[4] C. Dahlöf, M. Linde, E. Kerekes, J. Headache Pain 5 (2004) 237–242.
[5] B.R. Charlesworth, A.J. Dowson, A. Purdy, W.J. Becker, S. Boes-Hansen, M. Farkkila, CNS Drugs 17 (2003) 653–667.
[6] T.K. Vyas, A.K. Babbar, R.K. Sharma, A. Misra, J. Drug Target 13 (2005) 317–324.
[7] A. Alhalaweh, S. Andersson, S.T. Velaga, Eur. J. Pharm. Sci. 38 (2009) 206–214.
[8] R. Jain, S. Nabar, P. Dandekar, V. Patravale, Pharm. Res. 27 (2010) 655–664.
[9] A. Dalpiaz, E. Gavini, G. Colombo, P. Russo, F. Bortolotti, L. Ferraro, S. Tanganelli, A. Scatturin, E. Menegatti, P. Giunchedi, J. Pharm. Sci. 97 (2008) 4889–4903.
[10] E. Gavini, G. Rassu, L. Ferraro, A. Generosi, J.V. Rau, A. Brunetti, P. Giunchedi, A. Dalpiaz, J. Pharm. Sci. 100 (2011) 1488–1502.
[11] Z. Zhang, F. Xu, Y. Tian, W. Li, G. Mao, J. Chromatogr. B 813 (2004) 227–233.
[12] X. Chen, D. Liu, Y. Luan, F. Jin, D. Zhong, J. Chromatogr. B 832 (2006) 30–35.
[13] M. van der Ham, T.J. De Koning, D. Lefeber, A. Fleer, B.H.C.M.T. Prinsen, M.G.M. de Sain-van der Velden, J. Chromatogr. B 878 (2010) 1098–1102.
[14] Z. Shi, Q. Zhang, X. Jiang, Life Sciences 77 (2005) 2574–2583.
[15] K. Felgenhauer, Klin. Wochenschr. 52 (1974) 1158–1164.
[16] A. Madu, C. Cioffe, U. Mian, M. Burroughs, E. Tuomanen, M. Mayers, E. Schwartz, M. Miller, Antimicrob. Agents Chemother. 38 (1994) 2111–2115.
[17] M. Rambla-Alegre, J. Esteve-Romero, S. Carda-Broch, J. Chromatogr. A 1232 (2012) 101–109.
[18] A. Hubaux, G. Vos, Anal. Chem. 42 (1970) 849–855.
[19] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019–3030.
[20] E. Chambers, D.M. Wagrowski-Diehl, Z. Lu, J.R. Mazzeo, J. Chromatogr. B 852 (2007) 22–34.
[21] A. Van Eeckhaut, K. Lanckmans, S. Sarre, I. Smolders, Y. Michotte, J. Chromatogr. B 877 (2009) 2198–2207.
[22] D.K. Muhia, C.G. Thomas, S.A. Ward, G. Edwards, E.K. Mberu, W.M. Watkins, Biochem. Pharmacol. 48 (1994) 889–895.
[23] N. LindegardhW. Hanpithakpong, B. Kamanikom, P. Singhasivanon, D. Socheat, P. Yi, A.M. Dondorp, R. McGready, F. Nostend, N.J. White, N.P.J. Daya, J. Chromatogr. B 876 (2008) 54–60.
[24] B. Mallikarjuna Rao, M.K. Srinivasu, G. Sridhar, P. Rajender Kumar, K.B. Chandrasekhar, A. Islam, J. Pharm. Biomed. Anal. 39 (2005) 503–509.
[25] J.-S. Ding, R.-H. Zhu, Y.-G. Zhu, H.-D. Li, Chin. Pharmaceut. J. 41 (2006) 1488–1490.
[26] K. Vishwanathan, M.G. Bartlett, J.T. Stewart, Rapid Commun. Mass Spectrom. 14 (2000) 168–172.
[27] B. Kilic, T. Özden, S. Toptan, S. Özilhan, Chromatographia 66 (2007) 129–133.
[28] E.J. Seaber, R.W. Peck, D.A. Smith, J. Allanson, N.R. Hefting, J.J. van Lier, F.A.E. Sollie, J. Wermer, J.H.G. Jonkman, Br. J. Clin. Pharmacol. 46 (1998) 433–439.