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Quantitative analysis of the opioid peptide DAMGO in rat plasma and microdialysis samples using liquid chromatography–tandem mass spectrometry

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ABSTRACT

A liquid chromatography–electrospray ionization-tandem mass spectrometry (LC–ESI-MS/MS) method for the quantification of the opioid peptide DAMGO in rat plasma, as well as DAMGO and the microdialysis recovery calibrator [$^{13}C_2$,^{15}N]-DAMGO in microdialysis samples, is described. The microdialysis samples consisted of 15 μL Ringer solution containing 0.5% bovine serum albumin. Pretreatment of the samples involved protein precipitation with acetonitrile followed by dilution with 0.01% formic acid. The lower limits of quantification were 0.52 ng/mL and 0.24 ng/mL for DAMGO and [$^{13}C_2$,^{15}N]-DAMGO respectively and the response was linear up to 5000 fold higher concentrations. The plasma samples (50 μL) were precipitated with acetonitrile containing the isotope labeled analog [$^{13}C_2$,^{15}N]-DAMGO as internal standard. The method was linear in the range of 11–110,000 ng/mL. The separations were conducted on a HyPurity C18 column, 50 × 4.6 mm, 3 μm particle size, with a mobile phase consisting of acetonitrile, water and formic acid to the proportions of 17.5:82.5:0.01. Low energy collision dissociation tandem mass spectrometric (CID-MS/MS) analysis was carried out in the positive ion mode using multiple reaction monitoring (MRM) of the following mass transitions: *m/z* 514.2 → 453.2 for DAMGO and *m/z* 517.2 → 456.2 for [$^{13}C_2$,^{15}N]-DAMGO. The intra-day precision and accuracy did not exceed 5.2% and 93–104% for both compounds and sample types described. The inter-day precision an accuracy were <6.8% and 95–105% respectively. The method described is simple, reproducible and suitable for the analysis of small sample volumes at low concentrations.

1. Introduction

DAMGO (H-Tyr-D-Ala-Gly-MePhe-Gly-ol) is a synthetic enkephalin analog, with high affinity and selectivity to the μ-opioid receptor [1–3]. It is widely used as a model drug in antinociceptive studies and binding assays. In spite of this extensively usage, little is known about the pharmacokinetic–pharmacodynamic relationship of the peptide. In order to understand the distribution of DAMGO in vivo, including the blood–brain barrier transport, it is necessary to develop a sensitive and selective analytical method for quantifying DAMGO in different biological matrixes. Methods for quantifying non-radiolabeled DAMGO are so far limited in the literature.

The pharmacokinetic profile in plasma is generally investigated by collecting plasma samples at a series of time points and quantifying the total concentration of the samples. Since DAMGO is active in the central nervous system, it is of interest to study the concentration profile of the active, unbound, drug in the in the extracellular fluid surrounding the μ-receptors in the brain, as well. Peptides are generally regarded to have a very limited brain distribution. The blood-brain barrier distribution of larger biological substances is, however, not adequately evaluated in vivo [4]. Microdialysis is a technique that makes it possible to study the unbound brain concentrations over time in the same animal [5]. By combining the information of free DAMGO in blood, also sampled by microdialysis, the blood–brain barrier distribution can be investigated. The quantification of microdialysis samples demands a very sensitive and selective method, since the drug concentrations in the microdialysate often are very low and the sample volumes are in the range of a few microliters. The method should also be able to handle high concentrations of salts from the dialysate matrix. To calibrate the recovery of the microdialysis technique, a stable isotope labeled analog of the compound should preferably be used and the analytical method needs to distinguish between these two compounds. In addition, a short analysis time and a simple sample work up procedure are desirable, due to a large number of samples. The quantification of DAMGO in plasma is more straightforward. The sample volumes are however small, due to the limited

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volume of blood that can be collected from a small animal without affecting the homeostasis. Liquid chromatography–tandem mass spectrometric (LC–MS/MS) can provide the detection limit needed.

Quantification of DAMGO in biological matrixes has so far usually been carried out by radioactivity labeling the peptide [6,7]. Radioimmunoassay (RIA) is another common method for analysis of peptides. RIA has high enough sensitivity, but cross reactivity with similar peptides could affect the results [8,9]. The last decade several methods for quantifying peptides with LC–MS/MS have been published. The enkephalin analogs [(d-Ser^2)Leu enkephalin-Thr^6), DADLE and DALDA has been quantified in plasma by using electrospray ionization or nanospray ion trap mass spectrometry [10–12]. Analytical methods for determination of the enkephalins Leu- and Met-enkephalin in microdialysis samples by LC–MS^2, LC–MS^3 or in CSF using capillary LC–MS/MS has also been described [9,13,14]. The quantification of peptides in microdialysate with LC–MS/MS has been reviewed by Lanckman et al. [15]. Desiderio and coworkers have developed several methods for analyzing peptides with mass spectrometry, including quantification of DAMGO in ovine plasma with matrix assisted laser desorption/ionization time-of-flight mass spectrometry [16–18].

The subject of this paper is the development of a sensitive and robust LC–MS/MS method for quantification of DAMGO in small volumes of rat plasma, as well as simultaneously quantifying DAMGO and stable isotope labeled DAMGO in microdialysis samples composed of Ringer solution containing BSA, which could be used for the determination of the in vivo pharmacokinetics and pharmacodynamics of DAMGO. The overall goal with the project is to learn more about the opioid system and the blood–brain barrier distribution of peptides, in order to design new drug candidates for improved pain therapy.

2. Experimental

2.1. Materials

DAMGO acetate salt (H-Tyr-D-Ala-Gly-MePhe-Gly-ol) was purchased from Bachem (Bubendorf, Switzerland). The stable isotope labeled [$^{13}C_2$,^{15}N]-DAMGO (H-Tyr-D-Ala-[$^{13}C_2$,^{15}N]Gly-MePhe-Gly-ol) was synthesized by Bachem UK Ltd. (Merseyside, UK). Acetonitrile (ACN) was of gradient grade and formic acid was of analytical grade (Merck, Darmstadt, Germany). The water was deionized in house and further purified with a Milli-Q Academic system (Millipore, Bedford, MA, USA). Rat plasma was obtained from male Sprague-Dawley rats (Taconic, Denmark). The Ringer solution consisted of NaCl (145 mM), KCl (0.6 mM), $CaCl_2$ (1.2 mM), $MgCl_2$ (1.0 mM) and ascorbic acid (0.1 mM) in 2.0 mM phosphate buffer pH 7.4. Bovine serum albumin (BSA) was purchased from Sigma Aldrich (Steinheim, Germany).

2.2. Instrumentation

2.2.1. LC system

Chromatographic separation was carried out using a LC-10ADvp pump and a SIL-HTc autosampler (Shimadzu, Kyoto, Japan) connected to a HyPurity C18 column (50 × 4.6 mm, particle size 3 μm) protected by a guard-column of the same material (10 × 4.0 mm) (Thermo Hypersil-Keystone, PA, USA). The mobile phase was prepared daily and consisted of ACN:water:formic acid (17.5:82.5:0.01; v/v/v). A constant flow rate of 0.8 mL/min was maintained, resulting in an operating pressure of 55 bar. The flow was split, allowing 0.3 mL/min to enter the detector.

2.2.2. MS system

A Quattro Ultima triple quadrupole mass spectrometer was used for detection (Waters, Milford, MA, USA). The spectra processing and mass control were carried out using MassLynx software, version 4.0 (Waters, Milford, MA, USA). The detector parameters were optimized to obtain the highest sensitivity possible, by direct infusion of 200 ng/mL DAMGO and [$^{13}C_2$,^{15}N]-DAMGO in mobile phase at a flow rate of 0.2 mL/min using a Harvard 22 syringe pump (Harvard Apparatus Inc. Holliston, MA, USA). The desolvation temperature was set to 450 °C and the source temperature to 130 °C. Cone gas (N_2) and desolvation gas (N_2) were maintained at 200 and 1000 L/h, respectively. The capillary voltage was 1.5 kV and the cone voltage was 50 V. The analysis was carried out in the positive ion mode using multiple reaction monitoring (MRM) of the following mass transitions: m/z 514.2 → 453.2 for DAMGO and m/z 517.2 → 456.2 for [$^{13}C_2$,^{15}N]-DAMGO. Collision-induced dissociation (CID) was carried out using argon adjusted to 3×10^{-3} mbar and the collision energy set to 16 eV. The resolution was adjusted to 1.5 u at half height for Q1 and Q3. The dwell time for both channels was set to 0.15 s.

2.3. Standard and quality control (QC) sample preparation

Stock solutions were prepared in water for DAMGO (1 mg/mL) and [$^{13}C_2$,^{15}N]-DAMGO (0.2 mg/mL). Separate stock solutions were used for the standard- and QC samples. The stock solutions were stored at −20 °C.

Blank rat plasma was spiked with DAMGO to eleven standards in the range of 11–110,000 ng/mL, and four QC levels (37, 190, 3700, 74,000 ng/mL). All standards and QCs were stored at −80 °C. [$^{13}C_2$,^{15}N]-DAMGO was used as internal standard (IS) and a solution of 5 μg/mL in ACN was prepared daily and kept in dark on ice bath. For the microdialysate assay, blank Ringer solution containing 0.5%, w/w BSA was prepared. DAMGO and [$^{13}C_2$,^{15}N]-DAMGO were diluted to ten concentration levels in the ranges of 0.52–2600 and 0.24–1200 ng/mL respectively. QCs were prepared at three levels of 3.7, 74, and 1500 ng/mL of DAMGO, and 1.8, 37 and 730 ng/mL of [$^{13}C_2$,^{15}N]-DAMGO. Standards and QCs were aliquoted into polypropylene vials in volumes of 15 μL and stored at −80 °C.

2.4. Sample preparation

For the plasma assay, 50 μL plasma was precipitated with 150 μL ACN containing IS in a 1.5 mL polypropylene tube (Brand, Wertheim, Germany). The sample was vortex-mixed and centrifuged for 3 min at 10,000 rpm (7200 × g) using a Force 7 centrifuge (Denver Instrument Company, USA). Twenty-five μL of the supernatant was transferred to a new tube, containing 1000 μL of the mixture of ACN:water:formic acid (16:84:0.01; v/v/v), aiming to get the ACN content in the final sample to be similar to that in the mobile phase (approximately 17.5%). The sample was vortex-mixed and centrifuged before being transferred to an injector vial (polypropylene). The vial was placed in the autosampler and kept at 4 °C until injection. Ten μL was injected onto the LC/MS/MS system.

The microdialysis samples, containing 15 μl of Ringer solution with 0.5% BSA, were thawed and BSA was precipitated by adding 50 μL of ACN. The samples were vortex-mixed and centrifuged for 3 min using a Micro Centaur centrifuge (MSE, U.K.). Forty-five μL of the supernatant was added to 1.5 mL polypropylene tubes prefilled with 150 μL of 0.01% formic acid. After mixing, 85 μL was transferred to polypropylene vials and placed in the autosampler at 4 °C. A volume of 75 μL was injected onto the LC–MS/MS system.

2.5. Method validation

The method validation procedure was based on the recommendations in the FDA guidance on bioanalytical method validation [19]. The concentration of the unknown plasma samples and the

QCs were estimated from calibration curves generated by linear regression analysis of the peak area ratio of the test compounds and IS versus the nominal concentrations. For the microdialysis samples the calibration curves were generated by linear regression analysis of the peak area versus the nominal concentrations. The weighting factor $1/y^2$ was used and the standard curves were not forced through the origin. For both sample types, the intra-day precision and accuracy were determined by analyzing all standards, six replicates of each QC, six replicates of the lower limit of quantification (LLOQ) and blanks during one day. The precision, expressed as the coefficient of variation (CV), was calculated as the standard deviation as a percentage of the mean concentration. Accuracy was determined as the percent deviation of analyzed concentration from the nominal concentration. The LLOQ was defined as the lowest concentration that could be quantified with an intra-day CV <20% and an accuracy <±20%. Inter-day precision and accuracy were determined by analyzing each QC in duplicates together with samples at 5 (plasma) and 9 (microdialysis samples) occasions.

To evaluate assay specificity, six different lots of blank rat plasma were analyzed together with LLOQ samples to investigate the potential interferences at the peak region for DAMGO and IS. The extraction recovery of the plasma preparation method was determined by comparing the slope of four precipitated standard samples (57–57,000 ng/mL) with the slope of four standards of corresponding concentrations spiked in precipitated blank plasma. The level of ion suppression was evaluated by comparing the slope of spiked precipitated blanks to the slope of corresponding concentrations in mobile phase. The precipitation step and dilutions were prepared in triplicates. The relative matrix effect between plasma derived from four different rats was evaluated. Four standards in the range 57–57,000 ng/mL were prepared in each lot and the slopes constructed from peak area ratios of DAMGO/IS where compared. The variability is described as CV (%).

2.6. Stability

The stability of DAMGO in rat plasma was evaluated at different temperatures in vitro. For the determination of short time stability, blank plasma was spiked to the concentrations of 50 and 1000 ng/mL. The samples were incubated at 4 °C, 22 °C (light and dark) and 37 °C (water bath) for 6 h. Samples were withdrawn at 0, 1, 2, 4, and 6 h and immediately frozen until analysis. The long-time stability was evaluated at −20 °C and −80 °C (after 0.5 week, 1 week, 2 weeks, 1.5 months and 6 months) for the concentrations 50 ng/mL and 1000 ng/mL. Stored samples were compared to reference samples prepared in plasma at the day of analysis. All samples were analyzed in duplicates. The effect of freeze-thaw cycles on the stability of DAMGO in plasma were evaluated for three cycles. Samples of 1000 and 10,000 ng/mL were frozen at −20 °C for about 45 min, thawed in cold water for 5 min and thereafter stored at room temperature for 10 min before refrozen.

2.7. Application of method

The analytical method described herein was used for the quantification of DAMGO in plasma and microdialysis samples from brain and blood of rats. The rats received a 5 min loading infusion of DAMGO followed by a 180 min constant infusion (20 μg/min/kg). The microdialysis perfusion fluid consisted of Ringer solution containing 0.5%, w/w BSA to reduce sticking to the probe and tubings. [$^{13}C_2$,^{15}N]-DAMGO was used as a calibrator of the microdialysis recovery and was added to a concentration of 15 ng/mL. Blood was sampled during the infusion into heparinized 1.5 mL polypropylene tubes, and immediately centrifuged for 5 min (10,000 rpm, 7200 × *g*, Force 7 centrifuge, Denver Instrument Company, USA). The plasma was transferred to clean tubes. Microdialysis samples were collected in fractions of 15 μL in polypropylene vials (AgnThos, Lidingö, Sweden). All samples were stored at −80 °C until analysis.

3. Results and discussion

3.1. Analytical procedure

The amount of ACN in the mobile phase was chosen to optimize the chromatography; 17.5 units resulted in a retention time of 1.9 min, which was adequate to separate the analyte from the front, without broadening the peaks or cause unnecessarily long analysis time per sample. Formic acid has been used as additive in the mobile phases in previously described LC–MS methods for opioid peptides [11,12]. Trifluoroacetic acid (TFA) caused signal suppression for similar compounds (data not shown), a phenomenon previously described [20]. The optimal amount of formic acid was evaluated (0.1, 0.05, 0.03, 0.01%), resulting in higher response the lower the percentage. The selected concentration was 0.01%.

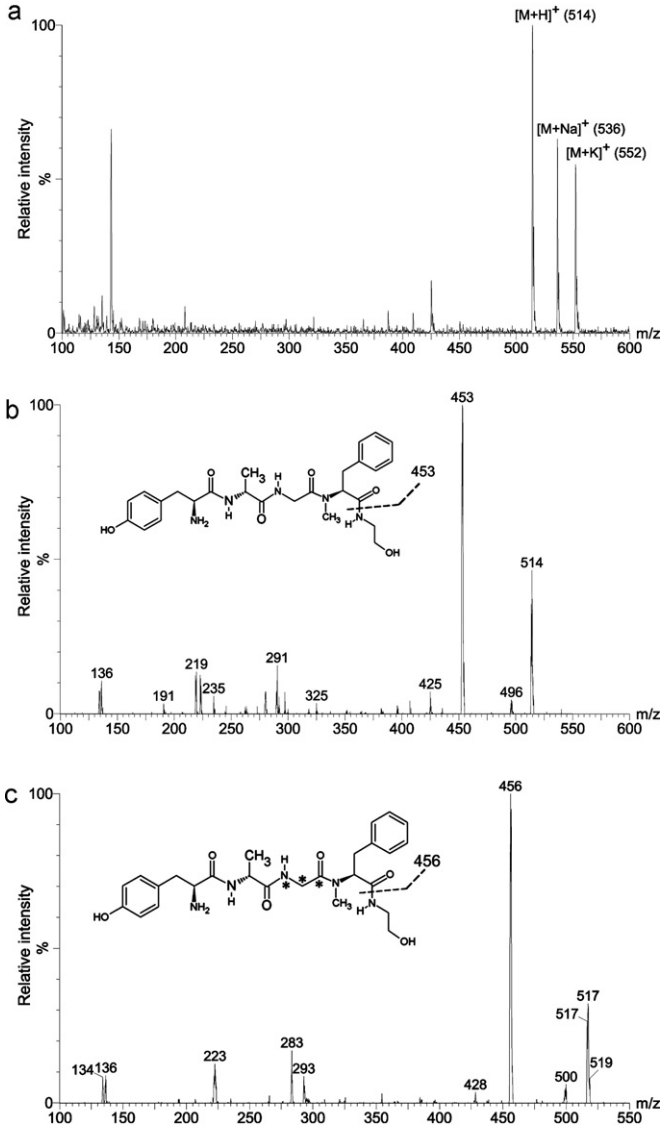

Fig. 1. (a) Single stage electrospray ionization mass spectrum (ESI-MS) of DAMGO recorded in the positive ion mode. (b) Product ion scan or CID-MS/MS of the precursor protonated molecule $[M+H]^+$ at *m/z* 514 extracted from DAMGO. (c) Product ion scan or CID-MS/MS of the precursor protonated molecule $[M+H]^+$ at *m/z* 517 extracted from [$^{13}C_2$,^{15}N]-DAMGO. (*) Indicates site of stable isotopic label.

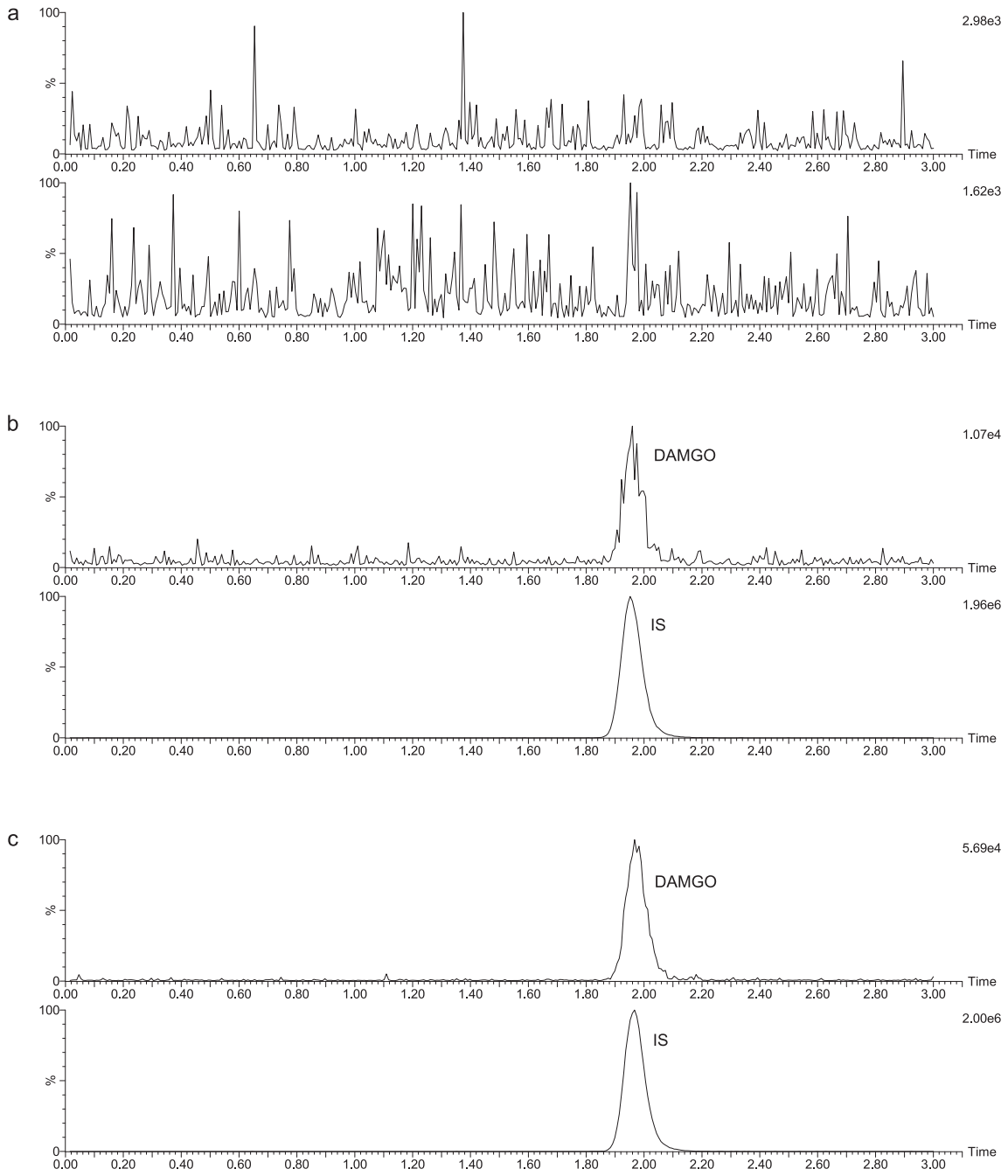

Fig. 2. Chromatograms of (a) blank rat plasma, (b) a spiked standard sample containing 57 ng/mL DAMGO and the internal standard [$^{13}C_2$,^{15}N]-DAMGO, and (c) a plasma sample derived from a rat 45 min after a 5 min loading dose of DAMGO followed by a constant infusion of 20 μg/min/kg, where the found concentration of DAMGO was 405 ng/mL. The peak highs are described in arbitrary units in the upper right corners of the chromatograms.

Electrospray ionization mass spectrometry (ESI-MS) was used for the characterization of DAMGO and [$^{13}C_2$,^{15}N]-DAMGO. ESI-MS has already been established as a suitable technique for the ionization of peptides. It is a mild method that can easily ionize polar, high molecular weight and thermo labile substances [8,19]. The single-stage ESI-MS is shown in Fig. 1a.

The protonated molecules (abundance 100%) were chosen for the quantification of DAMGO and [$^{13}C_2$,^{15}N]-DAMGO, respectively. The product ion scan of the selected $[M+H]^+$ precursor ion for both peptides is shown in Fig. 1b and c. The formation of the most intensive product ions was derived by the loss of the reduced amino acid glycine.

Two analytical columns were evaluated, a narrow bore column, Zorbax SB-C18 (50 × 2.1 mm I.D., 5 μm particle size), used for quantification of similar peptides by others [11], and the HyPurity C18 column. Both columns were suitable for analysis of DAMGO in plasma. For the microdialysis samples, high sensitivity was needed. The limited injection volume (20 μL) for the narrow bore column resulted in lower response, compared to injection of a larger volume (75 μL) on the HyPurity column. Finally, the HyPurity C18 column was chosen due to its robustness, good peak performance and due to the possibility of injecting larger volumes, which is required for the microdialysis samples. Representative chromatograms are shown in Figs. 2 and 3.

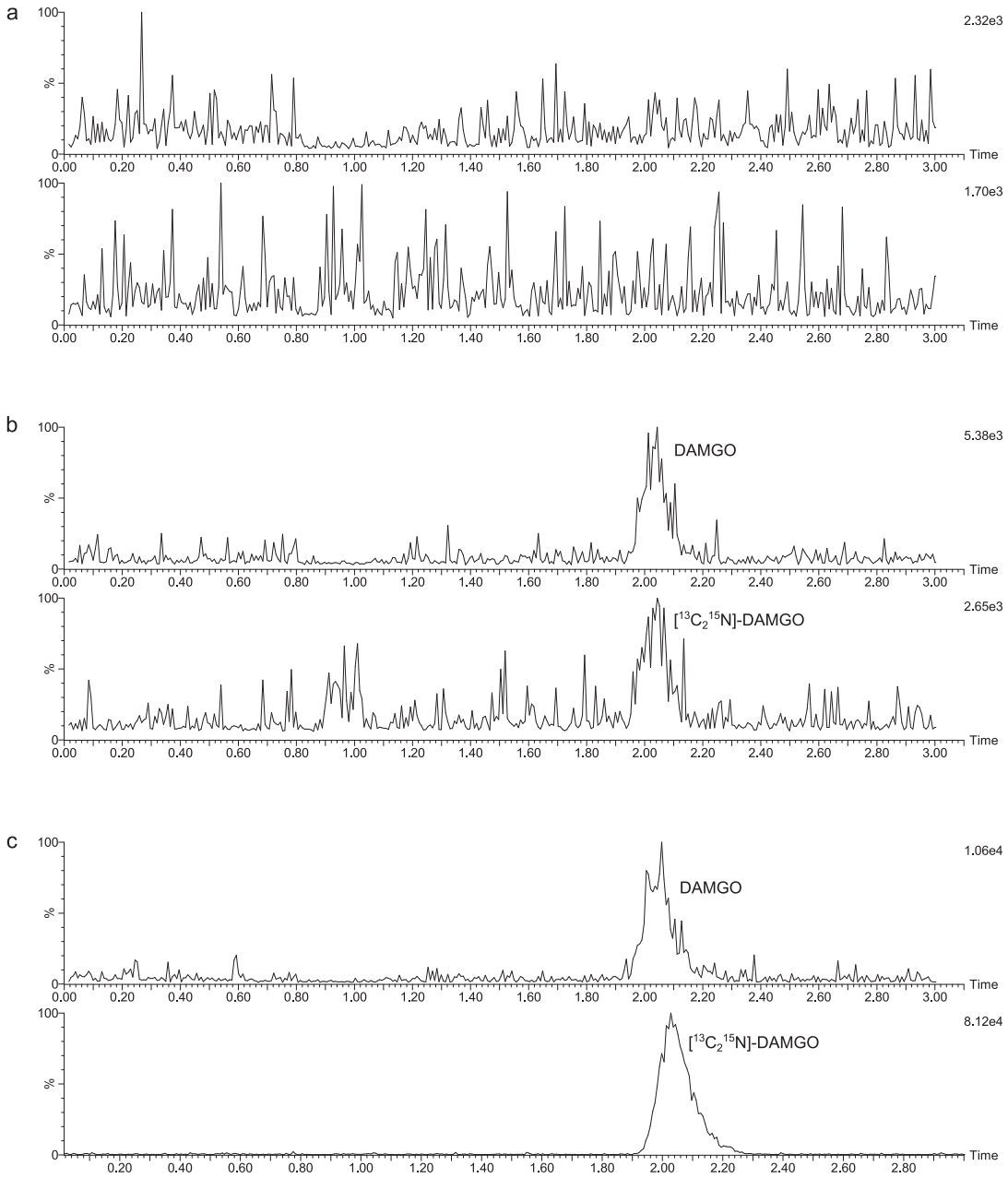

Fig. 3. Chromatograms of (a) blank microdialysis sample consisting of Ringer solution with BSA, (b) microdialysis sample spiked with 0.52 ng/mL DAMGO and 0.24 ng/mL [$^{13}C_2$,^{15}N]-DAMGO, and (c) microdialysis sample from rat brain 60–90 min after a 5 min loading dose followed by a constant infusion of 20 μg/min/kg. The found concentration of DAMGO was 1.2 ng/mL and the concentration of the calibrator, [$^{13}C_2$,^{15}N]-DAMGO, was 13.0 ng/mL. The peak highs are described in arbitrary units in the upper right corners of the chromatograms.

Precipitation of proteins was conducted by adding ACN to the plasma and the microdialysis samples. Addition of 0.1% TFA or 0.1% formic acid to the ACN was also evaluated, but did not affect the recovery. The method was very sensitive to the ACN content in the sample. More ACN, compared to the mobile phase, resulted in broadening of the peak. The microdialysis samples were therefore diluted with 0.01% formic acid after the precipitation step to a final concentration of 17.4% ACN. For plasma samples the supernatant were extensively diluted with ACN:water:formic acid (16:84:0.01) to avoid overloading of the mass spectrometer, and to reach the same ACN content as the mobile phase. [$^{13}C_2$,^{15}N]-DAMGO was selected as an IS for the plasma samples. The stable isotope labeled analog was chosen in order to provide similar interactions in chromatography as well as in the pretreatments of samples [21].

3.2. Validation

The method was linear in the investigated ranges for both sample types; 11–110,000 ng/mL for DAMGO in the plasma matrix and 0.52–2600 and 0.24–1200 ng/mL for DAMGO and [$^{13}C_2$,^{15}N]-DAMGO in the Ringer-BSA microdialysis matrix. The weighting factor of $1/y^2$ resulted in an even distribution of the residuals and a coefficient of determination greater than 0.995 at all occasions. The intra-day precision and accuracy were <5.2% and 93–104% and the inter-day precision and accuracy became <6.8% and 95–105% (Table 1).

The LLOQ for the plasma assay was 11 ng/mL with precision and accuracy of 8.2% and 100%, respectively. The LLOQ for microdialysate assay were 0.52 and 0.24 ng/mL for DAMGO and

Table 1
Intra- and inter day precision and accuracy for DAMGO in plasma and DAMGO and [$^{13}C_2$,^{15}N]-DAMGO in microdialysis perfusion fluid. For intra-day variability, six replicates of each concentration level were analyzed according to the method at the same day. For inter-day variability quality controls were analyzed in duplicate together with study samples at 5 (plasma) and 9 (microdialysate) different occasions.

Compound	Nominal conc. (ng/mL)	Intra-day				Inter-day			
		Found conc. (ng/mL)	CV (%)	Accuracy (%)	*n*	Found conc. (ng/mL)	CV (%)	Accuracy (%)	*n*
Plasma									
DAMGO	37.13	36.31	5.2	98	6	37.32	3.2	101	4[a]
	185.7	192.8	2.4	104	6	189.8	5.2	102	10
	3713	3850	0.9	104	6	3810	6.8	103	10
	74,270	74,150	1.3	100	6	70,460	3.8	95	10
Microdialysate matrix									
DAMGO	3.713	3.470	4.2	93	6	3.898	5.5	105	18
	74.27	73.20	3.6	99	6	71.73	5.0	97	18
	1485	1470	2.7	99	6	1507	3.6	102	18
[$^{13}C_2$,^{15}N]-DAMGO	1.834	1.758	4.9	96	6	1.891	5.0	103	18
	36.68	37.22	3.7	102	6	36.29	4.3	99	18
	733.7	744.6	2.4	102	6	744.2	3.0	101	18

[a] The lowest QC was added as a complement to the study at a later time point and was only included in the analysis of plasma samples at two occasions.

[$^{13}C_2$,^{15}N]-DAMGO, with the precision of 7.0% and 11.4% and the accuracy of 96% and 99% respectively. The precision and accuracy were thus with good margins below 20% and within 90–120%. The sensitivity of the plasma method could be increased further by reducing dilution during the sample pretreatment and/or by increasing the injection volume, but this was not necessary for the samples at hand. No interfering peaks at the retention times of DAMGO or IS were observed in any of the six different lots of blank plasma.

The recovery of the plasma sample work up procedure was determined by comparing the average slope from three standard curves in plasma (CV = 4.7%) with the average slope from three standard curves in precipitated blank plasma (CV = 0.6%) and was determined to be 95.4%. The ion effect of the plasma matrix was determined by dividing the average slope derived from the precipitated blank plasma with the average slope from three curves in mobile phase (CV = 1.8%). The response in the plasma matrix was increased to 123% compared to the mobile phase. The precision value for relative matrix effect between four different lots of plasma, expressed as CV of the calibration curve slopes, was 0.72%.

3.3. Stability

DAMGO was stable in rat plasma for at least 6 months when stored at −20 °C, as well as at −80 °C (data not shown). Profiles of the stability of DAMGO in plasma during 4 h incubation at 4 °C, 23 °C and 37 °C are shown in Table 2. No light sensitivity was observed. No degradation of DAMGO in plasma could be observed after up to three freeze-thaw cycles. Worked-up samples could be stored during at least 24 h at 4 °C without degradation (data not shown). The metabolic stability of DAMGO in mouse plasma has previously been evaluated by Van Dorpe et al., resulting in a half-life of more than 2 h [7].

3.4. Application of method

The described method was used to determine the DAMGO concentration in plasma and microdialysis samples during an intravenous infusion of 20 μg/min/kg to rats. No interfering peaks were detected in the chromatograms (Fig. 2c and 3c). The concentration–time profile for DAMGO in one individual is depicted in Fig. 4. Total DAMGO levels in plasma, as well as unbound drug

Table 2
Stability of DAMGO during 4 h incubation in rat plasma at 4 °C, 23 °C in dark, 23 °C in sun light and 37 °C in water bath. Added concentrations were 1000 ng/mL and 50 ng/mL.

Incubation properties	Time (h)	Found conc. of DAMGO described as % of C_0[a]		$T½$[b] (h)
		50 ng/mL	1000 ng/mL	
4 °C	0	100.0	100.0	
	0.5	97.8	100.3	
	1	97.4	99.9	
	2	99.3	97.4	
	4	95.0	92.9	>24
23 °C dark	0	100.0	100.0	
	0.5	95.2	96.8	
	1	92.9	91.4	
	2	83.2	80.5	
	4	62.7	61.7	5.5
23 °C light	0	100.0	100.0	
	0.5	95.0	96.6	
	1	92.0	89.7	
	2	82.9	80.8	
	4	61.8	59.6	5.4
37 °C	0	100.0	100.0	
	0.5	86.6	88.1	
	1	71.9	70.2	
	2	49.4	48.7	
	4	21.9	19.7	1.7

[a] Results are described as percentage of the found concentration at time 0.
[b] Half life of DAMGO calculated from the average degradation of the low and high concentration samples.

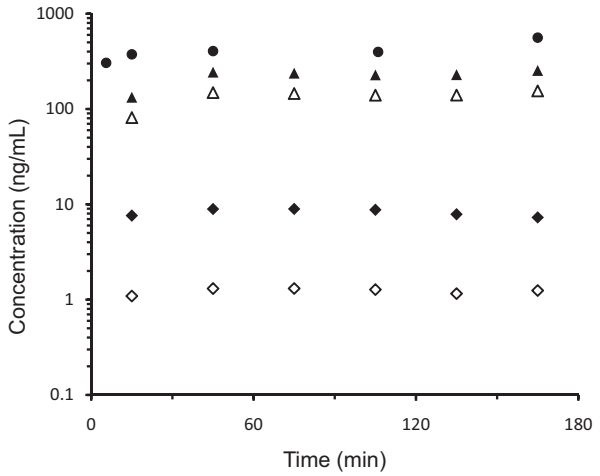

Fig. 4. Representative concentration–time profile from one rat after a 5 min bolus infusion followed by a 20 μg/min/kg constant infusion of DAMGO. Total plasma concentrations are represented by filled circles, unbound concentrations in blood by filled triangles, and unbound concentrations in brain by filled diamonds. The unbound concentrations are derived from microdialysis samples after adjustment for probe recovery. The concentrations in the microdialysate before recovery adjustment are represented by open triangles (blood) and open diamonds (brain).

concentrations in blood and brain derived from the microdialysis samples are shown. Steady state was reached rapidly in both blood and brain, suitable for determination of the net transport across the blood–brain barrier.

4. Conclusions

A LC–ESI-MS/MS method for the determination of the opioid peptide DAMGO in microdialysis samples and rat plasma have been successfully developed and validated. The method provided high selectivity in different biological matrixes without the need of radiolabeling. Owing to the good sensitivity of the method in both sample types, only very small sample volumes are required and it is possible to quantify the low concentrations in brain despite low recovery in the microdialysis technique. Linearity over a wide range of concentrations as well as a low variability was reported. In addition, the simple sample pretreatment followed by the rapid analysis with retention times of 2 min, makes the method highly suitable for the analysis of a large number of samples derived from preclinical studies in small laboratory animals.

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