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High-performance liquid chromatographic assay for morphine in small plasma samples: Application to pharmacokinetic studies in rats

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Abstract

In order to perform a reliable pharmacokinetic study of morphine during subchronic treatment in rats, an easy, rapid, sensitive and selective analytical method was proposed and validated. The analyte and internal standard (naloxone) were extracted from plasma samples (100 μL) by a single solid-phase extraction method prior to reverse-phase high performance liquid chromatography (HPLC) along with electrochemical detection (ED). Standard calibration graphs were linear within a range of 3.5–1000 ng/mL ($r = 0.999$). The intra-day coefficients of variation (CV) were in the range of 5.8–8.5% at eight concentration levels (7–1000 ng/mL) and the inter-day coefficient of variation ranged from 7.4 to 8.8%. The intra-day assay accuracy was in the range of −5–10% and the inter-day assay accuracy ranged from 3.0 to 9.3% of relative error (RE). The limit of quantification was 3.5 ng/mL using a plasma sample of 100 μL (15.8% of CV and 12.8% of RE). Plasma samples were stable for 2 months at −20 °C. This method was found to be suitable for pharmacokinetic studies in rats, after subcutaneous administration of morphine (5.6 mg/kg per day) in subchronic treatment for 6 and 12 days.
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1. Introduction

Morphine is widely used as an analgesic drug for both post-operative and cancer pain. One inconvenience of long-term morphine therapy is the development of tolerance to its analgesic effect. The rat has been used in several studies as a model for morphine tolerance development evaluation. Adequate pharmacokinetics–pharmacodynamic (PK–PD) relationships after chronic administration of morphine have been established using tail-flick test for the evaluation of the antinociceptive response [1–4]. In such studies, the simultaneous determination of the effect and the concentrations of the drug in the same animal, has been preferred.

A pronounced tolerance development to the antinociceptive effect of morphine in rats, after the subchronic administration of a daily subcutaneous dose of morphine (5.6 mg/kg) for 6 and 12 days was previously demonstrated, using the pain-induced functional impairment model in the rat (PIFIR) [5]. It would, therefore, be desirable to investigate the pharmacokinetics of morphine in rats following the schedule treatment applied to develop tolerance in that study. As repeated sampling of blood is required in pharmacokinetic studies in small species (rats), it is necessary to utilize a sensitive and selective method and reduce the total volume of plasma extracted from the rat in order to avoid serious impairment to its physiological state.

Several methods for the quantification of morphine in biological fluids and/or tissues, either in humans or in animals (rat, rabbit, dog, monkey, etc.) have been published. Radioimmunoassay (RIA) is a widely used sensitive method, however, antibody cross-reactivity with glucuronide metabolites and other opioids limit their use [6]; gas–liquid chromatographic meth-

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ods (GC) [7–9] and GC coupled to mass spectometric detection (GC–MS) [10–12] have been reported, but they commonly include derivatization, multiple extraction steps, solvent evaporation, large sample volumes [13] and expensive equipment. Reverse-phase liquid chromatography after solid phase extraction or liquid–liquid extraction and electrochemical detection [14,15], UV detection [16–18] or fluorometric detection [19,20], have been also used. A number of methods employing HPLC combined with electrospray ionization–tandem mass spectrometry (HPLC–ESI–MS–MS) for low level quantitation of morphine and its glucuronides have recently been described [21–23], but the sample preparation is complicated and time consuming, as the technique being relatively expensive.

High-performance liquid chromatographic analysis (HPLC) with electrochemical detection (ED) of the parent drug has proved to be a sensitive and reasonably selective method for the detection and quantification of morphine in blood [24–27]. The electrochemical detector (amperometric) has the advantage of being relatively inexpensive, simple to use and reliable in operation. This method of detection is sufficiently selective, as the main metabolite of morphine in rats, morphine-3-glucuronide (M3G) is not detected [25,28].

Some of the extraction procedures of morphine from plasma samples have included liquid–liquid extraction (LLE). Most of them required a long-term time for the preparation of the sample with at least two-step extraction and evaporation of the solvent [29]. Other authors have proposed simple and easy solvent extraction methods, as the one-step extraction method with isoamilic alcohol–hexane (1:1, v/v) at pH 8.9, evaporation of the organic phase and reconstitution in mobile phase prior to reverse-phase HPLC [30]. Plasma samples have also been deproteinated by addition of acetonitrile and removing the sovent by evaporation prior to HPLC–ESI–MS–MS) analysis [31]. However, they often use large plasma samples or short concentration intervals.

The extraction method reported by Svensson [14], with some modifications proposed by Joel et al. [15] has been widely used in pharmacokinetic studies of morphine and its metabolites, both in humans [32,33] and in animals [2,4,25]. The method involved two solid-phase extraction (SPE) steps to isolate morphine and its glucuronides from plasma. The compounds were collected from the second cartridge in 3 mL of eluate (buffer solution) and up to 1 mL of this eluate was injected into the HPLC system, to achieve the required sensitivity. Other authors included the elution of the drug from the second cartridge with 3 mL of methanol and the evaporation and reconstitution of the compounds in a smaller volume of mobile phase [1,4].

In this study, we propose an easy and reliable chromatographic method for the quantification of morphine from a small volume of rat plasma (100 μL). We describe the improvements to the sample extraction and the chromatographic system to previous HPLC methods, while retaining the use the amperometric detection for the quantification of morphine. The potential clinical importance of the assay was demonstrated by the application of this method to a pharmacokinetic study of morphine in rats, after the subchronic administration of a daily subcutaneous dose of 5.6 mg/kg, over two separate periods of 6 and 12 days.

2. Experimental

2.1. Chemicals and reagents

Morphine hydrochloride and naloxone hydrochloride were kindly supplied by the Mexican Secretary of Health, Mexico City, Mexico. Methanol for the mobile phase was chromatographic grade (Baker, Mexico). All other reagents were analytical grade (E. Merck Kga, Darmstadt, Germany). HPLC grade water (18 Ω) was obtained by purifying distilled water in a Milli-Q filtration system (Millipore, Bedford, MA, USA). Mobile phase was filtered through 0.45 μm pore size membranes (Millipore) and degassed in an ultrasonic bath (Branson Ultrasonic Corp., Eagle Road, Danbury, CT, USA).

2.2. Preparation of calibration standards and quality control samples

Primary stock solutions of morphine (100 μg/mL) and the internal standard, naloxone (300 μg/mL), were prepared in methanol and stored at −4 °C. Working solution of morphine and naloxone (standards) were daily prepared by diluting primary stock solutions with deionised water. Rat plasma calibration standards of morphine were prepared by spiking appropriate aliquots of the working standard solution of morphine to drug-free rat plasma to give final concentrations from 3.5 to 1000 ng/mL. Quality control (QC) samples at concentrations of 7.5, 62.5 and 500 ng/mL were prepared by adding the appropriate working standard solution to drug-free rat plasma. The QC samples were aliquoted (100 μL) into propylene tubes and stored at −20 °C until analysis.

2.3. Sample preparation

Extractions were done by passing samples through a preconditionated Sep-Pack C18 cartridge, (Waters Milford, MA, USA), with the aid of a vacuum device (Vac-Elute, Speed Mate 10, Applied Separations). Cartridges were preconditioned by flushing with 3 mL of methanol and 3 mL of distilled water. One milliliter of borate buffer (pH 9.0; 0.2 M) and 100 μL of the internal standard solution (naloxone 3 μg/mL), were added to 100 μL of blank plasma, calibration standards or QC samples. After vortex mixing, the sample was passed through the column for 2–3 min and then washed with 20 mL of water and dried under vacuum. The analyte and the internal standard were eluted with 2 mL of methanol, at a flow rate of 1 mL/min. The eluate was evaporated to dryness in a water bath at 45 °C under a gentle stream of nitrogen. The residue was reconstituted in 100 μL of mobile phase and 20 μL were injected into the HPLC system.

2.4. HPLC apparatus and conditions

The chromatographic system consisted of a LC-250 pump (Perkin-Elmer, Norwalk, USA), a LC-4B electrochemical (amperometric) detector, with CC-5 cell (Bioanalytical Systems, West Laffayette, Inc.) and an injection valve with a 20 μL sam-

pling loop. The separation was performed on an Alltech Adsorbosphere catecholamine column (3 μm, 100 mm × 4.6 mm), using a mixture of sodium dihydrogen phosphate buffer (pH 3.6; 0.1 M) containing 2.4 mM of sodium octyl sulfate (SOS) and 1 mM of disodium etilendiaminetetracetate and methanol (75:25, v/v) at a flow rate of 0.8 mL/min. This mobile phase was a modification of those used in previously published analytical methods for morphine, and was finally selected after testing different pHs, ion-pair compounds and concentrations of the ion-pair. The working electrode was maintained at an applied potential of +0.8 V, at a sensitivity of 10 nA, which gave the optimum response for both, the analyte and the internal standard detection. All analysis were carried at room temperature (25 °C).

2.5. Method validation

2.5.1. Selectivity

To determine the selectivity of this method, blank plasma obtained from rats, alone and spiked with known amounts of morphine, naloxone (internal standard) and/or other drugs including metamizol, paracetamol, dextrometorphan and naltrexone, were analyzed. An amount of 2 μg of the above drugs, was added into 1 mL of plasma, extracted and injected into the HPLC system to test their potential interference with the assay.

2.5.2. Calibration curves and linearity

Three calibration curves in a concentration range of 3.5–1000 ng/mL (3.5, 7, 15, 30, 60, 125, 250, 500 and 1000 ng/mL) were determined. Peak-height ratios of morphine to the internal standard were used to generate standard calibration curves by plotting peak-height ratio of morphine/naloxone versus morphine concentration in plasma samples. A least-squares linear regression analysis was performed to determine slope, intercept and coefficient of correlation.

2.5.3. Intra-day and inter-day precision and accuracy and lower limit of quantification (LLQ)

Intra- and inter-assay precision were evaluated by analyzing batches of calibration standards at each concentration. For the intra-day variation, sets of five replicates were analyzed on the same day at nine concentration levels (3.5–1000 ng/mL). For the inter-day validation, five replicates of three concentration levels (7.5 ng/mL or lower quality control sample, LQC; 62.5 or middle quality control sample, MQC and 500 ng/mL or upper quality control sample, UQC), were analyzed along with a standard curve, on three different days. The coefficient of variation (CV) served as a measure of precision. The CV should be less than 15%, except at the LLQ where it should not exceed 20% [34].

The accuracy of the assay was determined on the above samples, by comparing the means of the measured morphine concentrations with the specified concentrations either in standard samples (intra-day accuracy) or in QC samples (inter-day accuracy). The percentage deviation of the mean from true values, expressed as relative error (RE) served as a measure of accuracy. The mean value of RE should be within ±15% of the nominal value, except at the lower limit of quantification (LLQ) where it should not exceed 20% [34].

2.5.4. Recovery

The absolute recovery of morphine was determined by extracting standard solutions of the drug by the proposed method, at three different concentrations (7.5, 62.5 and 500 ng/mL; $n = 5$) and the peak heights obtained were compared to those obtained after direct injection of non-extracted standard solutions, at the same concentrations.

2.5.5. Stability

Plasma samples spiked with 500 ng/mL of morphine, were stored at −20 °C and analyzed in five replicates at time 0, 2, 4 and 8 weeks of storage, and morphine concentration was determined by the above method. Data were compared by one-way ANOVA in order to evaluate the stability of the drug in plasma.

2.6. Pharmacokinetic studies

Female Wistar rats [Crl:(WI)BR] weighing 180–220 g, were used in this study (from our own breeding, CINVESTAV, Mexico). All experimental procedures followed the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain [35], and of the Ethical Issues of the International Association of Pain [36], and were carried out according to a protocol approved by the local Animal Ethics Committee. Animals were housed in groups of six per cage in a room with controlled temperature (22–24 °C) and with a 12-h light:12-h dark cycle, and provided with standard rat chow and water ad libitum. Two groups of six rats were used in pharmacokinetic study, after subchronic treatments of morphine for 6 and 12 days (A and B). Rats in Group A received a daily subcutaneous dose of 5.6 mg/kg of morphine hydrochloride dissolved in saline solution over 6 days. Group B received the same daily dose of drug over 12 days. The day of the study, rats were lightly anaesthetized with diethyl ether and the caudal artery was cannulated with PE-10 cannula (Clay Adams, Parsippany, NJ, USA) connected to a PE-50 cannula. The cannula was kept patent with heparinized saline solution and stoppered with a needle. Rats were allowed to recover from anaesthesia and a dose of 5.6 mg/kg of morphine hydrochloride, dissolved in saline solution, was subcutaneously administered. Blood samples were withdrawn from the caudal artery at 0 h (before the administration of the drug) and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5, 3, 4, 6 and 8 h after the administration of the drug, and transferred to heparinized polypropylene tubes. The total volume of blood taken from each animal did not exceed 1.8 mL. Plasma was separated by centrifugation at 3000 rpm for 10 min at 25 °C and stored at −20 °C until analysis.

Plasma samples from pharmacokinetic studies and a duplicate of three quality control samples (LQC, MQC and UQC) were analyzed together with a standard curve in plasma prepared the day of the analysis. Assays were acceptable if the accuracy of QC samples were within ±15%.

3. Results and discussion

3.1. Chromatography and extraction procedure

High performance chromatographic method with electrochemical detection (HPLC–ED) is a common method for the analysis of morphine in biological samples. However, many published papers describe the need for multiple extraction and complex chromatographic systems to ensure reproducibility and resolution. Sample preparation in this study included a modification of the extraction methods proposed for the separation of morphine from plasma by other authors [14,15] including a single step extraction in solid-phase. Morphine is an alkaloid that contains a tertiary amine and a phenolic group of pK_a values of 7.9 and 9.9, respectively; therefore, morphine has the lowest net charge and the best solubility in organic solvents at pH about 9, consequently this pH was chosen for the solid-phase extraction (C18 cartridges). A highly automated procedure has also recently been developed for the SPE of morphine from a 0.25 mL of the plasma sample, prior to HPLC analysis, but a robotic liquid handler is required for the preparation and transfer of samples during the extraction procedure [21]. The extraction method used by us allowed the analysis of a large number of samples, handling about 40 samples in 1.5 h.

Good sensitivity and short retention times were obtained with the HPLC–ED system proposed. Morphine and naloxone (internal standard) gave well resolved, sharp peaks, with retention times of 7.6 and 16.5 min, respectively, under previously described conditions. No interfering peaks were observed around the retention times of these compounds with only one-step extraction when every drug-free plasma sample was treated. The chromatographic background after extraction was clean so that low concentrations of morphine can be detected. The detection limit based on a signal:noise ratio of 3:1, was 1 ng/mL. Typical chromatogram of morphine after extraction from plasma is shown in Fig. 1.

Separations with an adsorbosphere C18 (3 μm) column gave good resolution of the compounds. Most of the HPLC methods previously reported have used C18 (5 μm) columns [1,4,23,28,32]. The column and the chromatographic system (2.4 mM SOS in mobile phase), employed in the present study, improved the resolution between morphine and naloxone and other tested compounds. In order to protect the column and improve its lifetime, a guard column packed with the same material was used. Adopting these precautions, the column was maintained in good conditions for long periods of time. Additionally, a good reproducibility among different columns was observed.

3.2. Method validation

3.2.1. Selectivity

The extraction method also allowed the adequate separation of morphine from other possible endogenous compounds. Neither morphine-3-glucuronide (M3G) nor morphine-6-glucuronide (M6G) were detectable in the present study. While M3G lacking the phenolic hydroxyl is "per se" not oxidizable at the used potential [28], M6G is not formed in rats [37]. Additionally, when plasma samples containing different compounds were analyzed by the proposed method, no interference was found. The relative retention times ($t_{R\,compound}/t_{R\,morphine}$) for other analyzed drugs were: 0.4 for paracetamol, 1.6 for metamizol, 2.2 for naloxone, 2.9 for dextrometorphan and 4.2 for naltrexone.

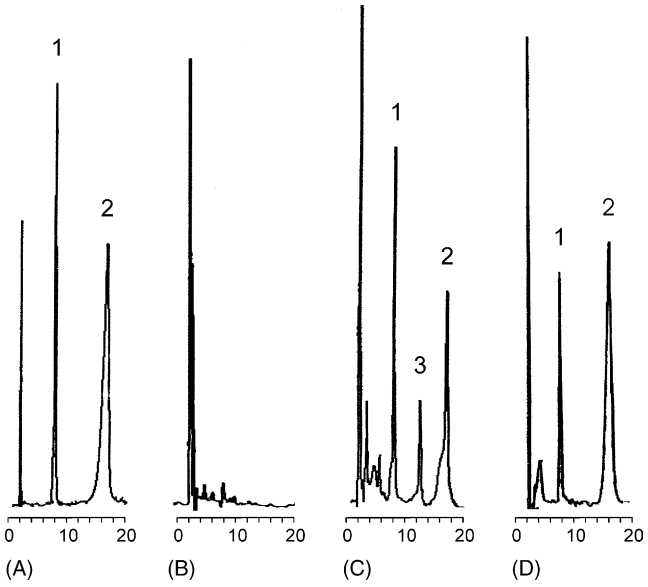

Fig. 1. Chromatograms of: (A) standards of morphine (1) and internal standard, naloxone (2); (B) blank plasma; (C) spiked plasma with morphine (1), naloxone (2) and metamizol (3) and (D) plasma sample of a rat administered with morphine. Time in minutes.

3.2.2. Calibration curves and linearity

A linear relationship ($r = 0.999$) was found when the ratio of peak height of morphine and peak height of the internal standard was plotted against morphine plasma concentration ranging from 3.5 to 1000 ng/mL. Linear regression of the data was significant in the range of concentrations studied ($p < 0.001$) with an intercept equal to zero (CI 95%: −0.019 to 0.067). The variations between the back-calculated values for plasma standards and the theoretical concentrations were well within the acceptance criterion of <15% for RE and CV (Table 1). The low CV for the slopes of the regression lines (<5%) also indicates the repeatability of the method.

3.2.3. Precision, accuracy and lower limit of quantification (LLQ)

Table 2 shows a summary of intra-day and inter-day precision and accuracy of the method. Intra-assay and inter-assay CV values ranged from 5.8 to 8.5% and from 7.4 to 8.8%, respectively, at the eight concentration levels (7–1000 ng/mL), showing the good precision of the method. Precision of the method is comparable to recent published methods that use a higher volume of plasma sample [23]. Plasma concentrations of morphine can be accurately quantified up to 3.5 ng/mL (lower limit of quantification or LLQ) with a coefficient of variation less than 20%. The sensitivity of our method is equivalent to other methods previously employed in other pharmacokinetic studies in rats

Table 1
Calculated concentration of morphine in calibration standards prepared in rat plasma ($n = 3$)

	Nominal concentration (ng/mL)								
	3.5	7	15	30	60	125	250	500	1000
Mean (ng/mL)	3.8	7.6	15.6	31.6	58.9	118	248	497	1001.4
CV (%)	8.2	8.3	3.0	4.2	3.2	6.8	2.2	3.4	2.0
RE (%)[a]	8.6	8.6	4.0	5.3	−1.8	−5.6	−0.8	−0.6	0.1

[a] RE (%) = [(nominal concentration − mean concentration found)/nominal concentration] × 100.

[4,37], using 100 μL of plasma sample. A rapid and sensitive HPLC–ED assay for the quantification of morphine in small blood samples (50–200 μL), using a single-step liquid–liquid extraction (LLE), was recently published by Groenendaal et al. [38], however, the limit of quantification reported for this method was 25 ng/mL. In the present method, using a single-step solid-phase extraction, sensitivity was improved to 3.5 ng/mL, using 100 μL of plasma. It is known that SPE provides higher recoveries of the drug from the biological matrix than LLE, and consequently improves the sensitivity of the method. With the choice of suitable solvents for the conditioning, washing, and elution steps, it can be shown that the method here proposed was more sensitive and efficient than the one-step LLE previously reported [38].

The accuracy of the method, determined by comparison of the concentrations of morphine recovered with those concentrations added to the spiked samples, was assessed by the percentage deviation of the mean from the true values (RE). The intra-day RE values ranged from −5 to 10%. Accuracy was confirmed by plotting the intra-day recovered amounts versus added amounts of morphine per mL of blank plasma samples. Linear regression of these data gave a slope of 1.003 (CI 95%: 0.986–1.029) and an intercept of −0.75 (CI 95%: −2.19–1.69) and $r = 0.999$. Inter-day accuracy, assessed by the analysis of quality control samples at three different concentrations and five replicates, in three different days, gave a RE from 3.0 to 9.3% (<15%), demonstrating the accuracy of the method (Table 2). This method is as sensitive, accurate and precise as other recently reported in the literature [27,31], while others have used higher volumes of plasma sample [17,21,30], or more laborious plasma extraction procedures [26], with similar results.

Table 2
Intra- and inter-day precision and accuracy for analysis of morphine in rat plasma

Added (ng/mL)	Found (ng/mL)	Recovered (%)	CV (%)	RE (%)
Intra-day ($n = 5$)				
3.5[a]	3.8	111.4	15.8	12.8
7	7.5	107.1	8.5	7.1
15	16.5	110.0	7.9	10.0
30	30.6	98.6	6.3	2.0
60	61.4	102.3	6.6	2.3
125	118.7	94.9	7.9	−5.0
250	260.5	104.2	5.9	4.2
500	494.0	98.8	6.1	−1.2
1000	1013.5	101.4	5.8	1.35
Inter-day ($n = 5$)				
7.5	8.2	108.8	8.8	9.3
62.5	65.5	104.8	7.4	4.8
500	515	103	7.8	3.0

[a] LLQ.

3.2.4. Recovery

The best recovery after the one-step SPE was obtained when samples were buffered at pH 9 and washed with water, previously to the elution with methanol. The final solvent volume used to elute the compounds was reduced to 2 mL, without sacrificing the recovery of the drug. Absolute recoveries, calculated by comparing height peaks from extracted samples with height peaks of unextracted standards, were between 82 and 85%, with a good precision (CV < 10%), independently of the concentration studied. The recovery of the method is comparable to other methods that use larger plasma samples and at least two-step LLE [29]. One of the advantages of SPE is that it allows a better recovery of the drug, from the biological matrix, than LLE. This can be confirmed by comparing the recovery of the current method, to that obtained by Groenendaal et al. [38] after a one step liquid–liquid extraction of about 62 ± 4%, for a concentration of 250 ng/mL of morphine in small blood samples (50–250 μL). However, these authors determined the extraction yields comparing the peak ratios after extraction from blood with the peak ratios of non-extracted standards. In our case, when the extraction yields were calculated by comparing the peak height ratios after extraction from QC samples with the peak ratios of non-extracted standards, the recovery was practically 100%.

3.2.5. Stability

From the stability study, it was found that plasma samples containing 500 ng/mL of morphine were stable for at least 8 weeks at −20 °C (Table 3). Stock solutions of morphine in methanol, stored at −4 °C, were stable for at least 2 weeks.

Table 3
Stability of morphine in plasma samples stored at −20 °C

Sample	Concentration (ng/mL)			
	Initial	Second week	Fourth week	Eighth week
1	504	490	509	492
2	510	501	500	501
3	496	495	492	491
4	499	492	493	502
5	501	505	506	493
Mean (ng/mL)	502	497	500	496
CV (%)	1.1	1.3	1.5	1.0
RE (%)	0.4	−0.7	0.0	−0.8

$F_{cal} = 1.005$; $p = 0.376$.

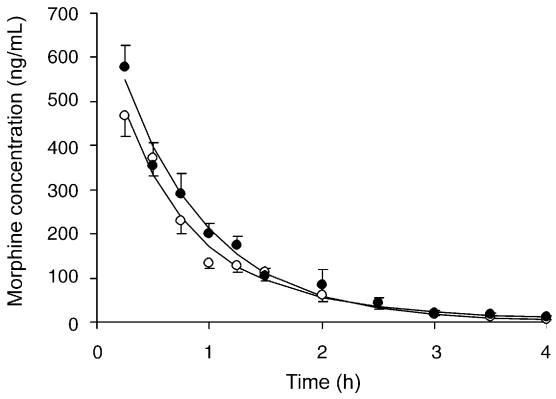

Fig. 2. Plasma concentration–time curves of morphine after subchronic treatment of a daily s.c. dose of morphine (5.6 mg/kg) to Wistar rats ($n = 6$), for 6 (empty symbol) and 12 days (full symbol). Each point represents the mean ± S.E.M. Continuous lines represents data adjusted to two-compartmental model.

In addition, enough information about the stability of morphine in plasma samples exists in the literature. Other authors have shown that morphine and its metabolites are stable in plasma for up to 2 years, when stored at −20 °C [17].

3.3. Pharmacokinetic studies

The validated HPLC–ED method was used to analyse plasma morphine pharmacokinetics in rats after the subchronic administration of a daily subcutaneous dose of 5.6 mg/kg of the drug over 6 and 12 days. Quality control samples in each analytical run were within 15% of the nominal value. No interference peak was found during the analysis of the samples obtained for the pharmacokinetic study sample analysis.

The observed plasma concentration–time curves for morphine in rats after both subchronic schedules of morphine for both treatments are shown in Fig. 2. The data were successfully fitted to a two-compartment model with one distribution phase and one elimination phase.

The pharmacokinetic parameters calculated are summarized in Table 4. No difference in the pharmacokinetic parameters between 6 and 12-days schedules was found by Student's *t*-test ($p > 0.05$).

The present method proved to be useful for the determination of plasma levels of the parent drug (morphine) in rats, in a small sample volume (100 μL). So, a sufficient number of samples can be obtained in the same animal in order to define the pharmacokinetics of morphine, without any impairment to its physiological state [1,3].

The selectivity, sensitivity, precision and accuracy obtained with this method make it suitable for the purpose of the present study. In conclusion, the method used in the present study is easy and fast to perform; it is also characterized with an adequate accuracy, precision, selectivity and stability, using a small sample volume (100 μL).

The method was successfully applied to a pharmacokinetic study of morphine in rats, after subchronic treatment of a daily dose of 5.6 mg/kg during 6 and 12 days.

Table 4
Pharmacokinetic parameters of morphine obtained after administration of a daily subcutaneous dose (5.6 mg/kg) in rats, for 6 and 12 days ($n = 6$, mean ± S.E.M.)

Parameter	6 days	12 days
$ABC_{0-\infty}$ (ng h/mL)	537.25 ± 23.97	712.83 ± 66.45
T_{max} (h)	0.25 ± 0.00	0.25 ± 0.00
C_{max} (ng/mL)	467.09 ± 44.76	578.27 ± 48.73
C_{4h} (ng/mL)	4.97 ± 0.86	5.11 ± 1.70
β (h^{-1})	1.19 ± 0.06	1.06 ± 0.31
$t_{1/2}\beta$ (h)	0.58 ± 0.03	0.66 ± 0.19
MRT (h)	0.76 ± 0.06	0.78 ± 0.03
$V\beta/F$ (L/kg)	8.79 ± 1.62	7.45 ± 0.74
Cl/*F* (L/(h kg))	10.42 ± 2.07	7.86 ± 0.78

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