HPLC-PDA METHOD FOR THE QUANTIFICATION OF PARACETAMOL IN PLASMA: APPLICATION TO PK/PD STUDIES WITH ARTHRITIC RATS

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Received: 15 Feb 2017 Revised and Accepted: 31 Mar 2017

ABSTRACT

Objective: To develop and validate an easy, rapid, sensitive and selective high-performance liquid chromatography with photodiode diode-array (HPLC-PDA) detection method for quantification of paracetamol and to demonstrate its application in a pharmacokinetic-pharmacodynamic study with arthritic rats.

Methods: Paracetamol was separated from plasma samples (50-100 µl) by a single protein precipitation step, prior to HPLC-PDA detection. The separation was performed on a Knauer Eurospher II, C18 column 5 µm, 150 × 4.6 mm. The mobile phase comprised a mixture of water: methanol (75:25) and the flow rate was 1.1 ml/min. The detection wavelength was set at 245 nm. All analyses were carried out at room temperature (25 °C). Pharmacokinetics data were obtained with a gout-type pain model in rats.

Results: The method was linear within a range of 0.2-200 µg/ml (R2=0.99). The intra-day and inter-day precision and accuracy expressed as coefficient of variation and relative error, respectively were below 10%. The lower limit of quantification was 0.2 µg/ml. Plasma samples were stable at least for 5 w at −20°C.

Conclusion: The validated method is sensitive, precise, accurate and specific as other more complex high-performance liquid chromatographic methods coupled to mass spectrometry (HPLC-MS), using small plasma samples (50-100 µl) and with a short time analysis (<5 min). The method was successfully applied to a pharmacokinetic-pharmacodynamic study of paracetamol in arthritic rats.

Keywords: Paracetamol, HPLC-PDA, Validation, Pharmacokinetic-pharmacodynamic, Arthritic rats

INTRODUCTION

Paracetamol is a non-steroidal anti-inflammatory drug (NSAID) with anti-inflammatory and analgesic properties. It is commonly used for the relief of mild to moderate pain. Paracetamol is metabolized by microsomal enzymes in the liver, with 85-90% of the drug undergoing glucuronidation and sulfation to inactive metabolites that are eliminated in the urine as paracetamol glucuronide, PG (55%) and paracetamol sulfa, PS (35%). A minor amount is conjugated with cysteine and mercapturic acid and only about 5% of the drug is eliminated unchanged in the urine. The total urinary recovery of paracetamol in 24 h has reported to be 71.5-95%, as free and/or conjugated [1]. Metabolism pathways of paracetamol are common in man and rats; however, rats excrete PS more than PG [2]. The elimination half-life of paracetamol is reported to be between 1.9 and 4.3 h, in both species [1, 3].

The pharmacokinetics and pharmacodynamics of NSAIWs alone or in combination with opioid drugs (morphine or tramadol), or additives (caffeine) have been previously studied in order to investigate the mechanism involved in the response of them under different conditions (i.e. chronic vs. acute administration). In such case, it is necessary to follow blood sample at the same time as the effect is measured. As repeated blood sampling is required in pharmacokinetic-pharmacodynamics 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 animals in order to avoid serious impairment to its physiological state.

The pharmacokinetics of paracetamol in arthritic rats after oral administration was reported [4] with a previously published high-performance liquid chromatography (HPLC) method [5]. However, this method utilizes 500 µl of plasma sample and 5 ml of ethyl acetate for drug extraction and evaporation of solvent before injection of the dissolved residue. HPLC with ultraviolet/visible (UV/Vis) and electrochemical detection or nuclear magnetic resonance-mass spectrometry, high-performance thin-layer chromatography, gas chromatography and capillary electrophoresis have been extensively used for the quantification of paracetamol and its metabolites in biological fluids [5, 7]. However, most of these methods use large plasma samples (0.2-1.0 ml), multiple liquid-liquid extraction and evaporation steps, a large volume of the extraction solvent [3, 5, 9, 10], short concentration intervals or long-time analysis [10, 11].

Most methods utilize liquid-liquid extraction for sample preparation, removing plasma proteins simultaneously. Protein precipitation has also been carried out by the addition of acetonitrile, methanol, perchloric acid, trichloroacetic acid, ethyl acetate or diethyl ether [9, 10, 12]. Solid-phase extraction methods (SPE), including C8 Sep-Pak cartridges for the separation of paracetamol, acetylsalicylic and caffeine [11] and the simultaneous determination of paracetamol and dextropropoxyphene from human plasma have also been reported [3]. However, most of these methods involve elution, evaporation and reconstitution steps and are time-consuming procedures. Table 1 includes the main characteristics of some reported HPLC methods for quantification of paracetamol in small plasma samples.

Up to this moment, none of the previously published methods fulfills the requirements to develop a pharmacokinetic-pharmacodynamics study of paracetamol in rats; so the aim of the present research was to develop and validate a rapid, easy, selective and reliable HPLC isocratic method with photo-diode-array (PDA) detection, using a single protein precipitation step, for the quantification of paracetamol in a small volume of plasma (50-100 µl). The potential importance of the assay was demonstrated by the application of this method to a pharmacokinetic-pharmacodynamics study after oral administration of paracetamol to arthritic rats, allowing simultaneous blood sampling and measurement of antinociceptive effect in the same animal.
Table 1: Published chromatographic methods for paracetamol quantification in small plasma samples

| Plasma sample preparation | Method | Mobile phase | Run time (min) | Concentration range | Recovery, Precision & Accuracy | Reference |
|---------------------------|--------|--------------|----------------|---------------------|------------------------------|-----------|
| 200 µl (SPE) Elution      | HPLC-UV| Gradient Elution: 1 to 85% ACN: Water | 14 | Range: 0.1-1 µg/ml | Recovery: 73.7 % CV: NR; RE: NR | [11]      |
| Solvent Evaporation       |        |              |                |                     |                              |           |
| 100 µl (PP) + 100 µl H₂O + 10 µl HClO₄ (30%) | HPLC-UV| Isocratic Elution: KH₂PO₄ 0.1 M: Isopropanol: THF (100: 1.5: 0.1) | 22 | Range: 0.06-50 µg/ml | Recovery >95 % CV<29.7 % RE:7.5 % LLOQ: 0.06 µg/ml | [20]      |
| + 50 µl MeOH/ H₂O (1:1) + 3 ml Ethyl acetate Solvent evaporation | LCE-MS-MS| Isocratic Elution: MeOH: ACN: 1% Formic acid (60: 20: 20) | 500 µl (LLE) | 2.5 | Range: 0.02-10 µg/ml | Recovery <70 % CV<14.5 % RE:7.3 % LLOQ: 0.02 µg/ml | [21]      |
| + 100 µl ACN Centrifugation (twice) | HPLC-UV| Isocratic Elution: ACN: H₂O (12: 88) | 100 µl (PP) | 8 | Range: 0.25-200 µg/ml | Recovery >99 % CV<5 % RE:6 % LLOQ: 0.43 µg/ml | [13]      |
| + MeOH/EtOH (1:1) Solvent evaporation | UHPLC-MS/MS| Gradient Elution: 5 mM Ammonium Acetate buffer (pH 3.75) | 50 µl (PP) | 18 | Range: 0.02-10 µg/ml | Recovery: 90.3-109 % CV<12 % RE:12 % LLOQ: 0.02 µg/ml | [18]      |
| 100 µl (PP) + 300 µl ACN: propylene glycol (9:1) Solvent evaporation | HPLC-MS/MS| Isocratic Elution: ACN: MeOH: Aq. 0.0875 % Formic acid [4:4: 92] | 300 µl (LLE) | 6.5 | Range: 0.01-30 µg/ml | Recovery: 99 % CV<15 % RE:15 % LLOQ: 0.01 µg/ml | [10]      |
| + 5 ml Ethyl acetate Solvent evaporation | HPLC-UV| Isocratic Elution: 10 mM phosphate buffer (pH 7.5): ACN (70: 30) | 25 µl (PP) + 400 µl MeOH. Centrifugation Solvent evaporation | 12 | Range: 0.1-40 µg/ml | Recovery: 94 % CV<7 % RE:6 % LLOQ: 0.04 µg/ml | [19]      |
| 100 µl (LLE) + 3 ml N-butyl-tert ether: dichlorometane:hexane Solvent evaporation | UPLC-MS/MS| Isocratic Elution: Aq. 1% Formic acid: MeOH (80: 20) | 100 µl (LLE) | 10 | Range: 0.25-20 µg/ml | Recovery: 72.4-105.9 % CV<12 % RE<5 % LLOQ: 0.25 µg/ml | [22]      |
| 50 µl (PP) + 950 µl ACN Dilution of supernatant with mobile phase (1:5) 100 µl (PP) + 150 µl MeOH/EtOH (1:1) | HPLC-UV| Gradient elution: 90 to 10% H₂O (0.1% Formic acid): ACN | 15 | Range: 0.05-100 µg/ml | Recovery: 92 % CV<10 % RE:8 % LLOQ: 44.8 ng/ml | [17]      |

<SPE= solid phase extraction; PP = protein precipitation; LLE = liquid-liquid extraction; NR = not reported>

**MATERIALS AND METHODS**

**Materials**

Paracetamol, PG and p-aminophenol (PAP) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Methanol and acetonitrile were chromatographic grade (J. T. Baker, Mexico). All other reagents were analytical grade (E. Merck KGaA, Darmstadt, Germany). Chromatographic grade water (18°) was obtained by purifying distilled water in a Milli-Q filtration system (Millipore, Bedford, MA, USA). The mobile phase was filtered through 0.45 µm pore size nylon filters (Millipore, Bedford MA, USA) and degassed in an ultrasonic bath (Branson Ultrasonic Corp., Danbury CT, USA).

**Experimental equipment**

The chromatographic system consisted of a Knauer high-performance liquid chromatograph (Berlin, Germany), equipped with a Smartline pump 100, a Smartline PDA detector 2800 and a Smartline autosampler 3950. The chromatographic station Clarity Chrom V2.6. xx software was used for acquisition and processing of data. The separation was performed on a Knauer Eurospher II, C18 column 5 µm, 150 × 4.6 mm (Berlin, Germany). The mobile phase consisted of a mixture of water: methanol (75:25) degassed before use, and flow rate of 1.1 ml/min, the detection wavelength was set at 245 nm. All analyses were carried out at room temperature (25 °C).

**Animals**

Male Wistar rats [Crl: (WI) BR] weighing 200-250 g from our own breeding (Universidad Autonoma Metropolitana-Xochimilco, Mexico), were used in this study. Rats were maintained under controlled environmental conditions at 22 °C, under a 12 h light/dark cycle and provided with standard chow (Purina Laboratory Rodent Diet 5001) and water ad libitum. Twelve hours before the experiments food was withheld, but animals had free access to water. Experiments were performed during the light phase and animals were used only once. All experimental procedures were approved by the local Institutional Animal Care and Use Committee, in accordance with the Mexican federal regulations for the care and use of laboratory animals, NOM-062-ZOO-1999 (Mexican Ministry of Health) and with adherence to the Guide for Care and Use of Laboratory Animals, Washington, D. C. [15]. Protocol No. 16, January 29th, 2014.
Separation of plasma from rat blood

Rat blood was collected by cardiac puncture from rats under isoflurane anesthesia, which was fasted overnight and had free access to water. Blood was transferred into heparinized glass tubes and centrifuged at 6000 rpm. Plasma was separated and stored at −20 °C until further use.

Preparation of calibration standards and quality control samples

Stock solution of paracetamol (1 mg/ml) was prepared in methanol and stored at 4 °C. Rat plasma calibration standards were prepared by spiking appropriate aliquots of the stock solution to drug-free rat plasma to give final concentrations of 0.2 to 200 µg/ml. Quality control (QC) samples were prepared by adding the appropriate aliquots of the stock solutions to drug-free rat plasma. The QC samples were aliquoted (50-100 µl) into polypropylene tubes and stored at −20 °C until analysis.

Sample preparation procedure

Plasma sample (50-100 µl) was aliquoted into a microcentrifuge tube and spiked with 50 µl of 10% zinc sulfate aqueous solution and 150 µl of methanol for protein precipitation. The sample was vortexed for 1 min, stored at 4 °C for 15 min and centrifuged at 14,000 rpm for 10 min. Supernatant layer was separated out of which 30 µl were injected onto the column and peak areas were recorded.

Method validation

The method was validated according to the FDA guidelines for validation of bioanalytical methods [14].

Selectivity

To determine the selectivity of the method, drug-free rat plasma alone and spiked with known amounts of paracetamol, PG and PAP, were analyzed. In addition, plasma samples of rats administered with paracetamol in a single dose (316.2 mg/kg, p.o.) were analyzed to test the potential interference of endogenous compounds and other paracetamol metabolites.

Calibration curves and linearity

Three calibration curves from 0.2 to 200 µl were determined. Standard calibration curves were generated by plotting paracetamol peak-area vs. drug plasma concentration. A least-squares linear regression analysis was performed to determine slope, intercept, determination coefficient (R²) and linear regression analysis of variance. A *P<0.05 was considered significant.

Intra-day and inter-day precision and accuracy

For determination of intra-day variation sets of five replicates of QC samples of paracetamol at 1, 10 and 100 µg/ml and lower limit of quantification (LLOQ) at 0.2 µg/ml along with a standard calibration curve were analyzed on the same day. For the inter-day precision, three replicates of each concentration level were analyzed along with a standard calibration curve in plasma on three different days. The coefficient of variation (CV) served as a precision measure. The mean value of AD should be within ±15% of the nominal value, except for the LLOQ where it should not exceed 20% [14].

The accuracy of the assay was determined on the above samples, by comparing the means of the measured concentrations with the nominal concentrations. The percentage deviation of the mean from true values expressed as a relative error (RE %) served as a measure of accuracy. RE% was calculated as follows:

\[
RE\% = \frac{\text{added concentration} - \text{recovered concentration}}{\text{added concentration}} \times 100
\]

The mean value of RE% should be within ±15% of the nominal value, except for the LLOQ where it should not exceed 20% [14].

Absolute recovery and LLOQ

The absolute recovery of the method was determined by extracting five replicates of QC samples at 1, 10, and 100 µg/ml. The peak areas obtained were compared to those obtained after direct injection of non-extracted standard solutions in the mobile phase, at the same concentration levels.

The lower standard concentration of the calibration curve with a signal (peak-area) at least five times greater than the baseline noise was taken as the LLOQ. Additionally, it was calculated using the equation: \[10^\sigma (\text{µ}g/ml)\]. Where \(\sigma\) is the standard deviation of the magnitude of the analytical response, and “s” is the slope of the regression equation. The limit of detection (LOD) was calculated using 3.3*σ/s, where “s” and “σ” were already defined.

Stability

QC samples containing paracetamol at concentrations of 0.2 and 10 µg/ml were prepared and analyzed by the previously described method in five replicates, at zero time and after stored at −20 °C for five weeks. The absolute difference (AD %) between mean values of concentrations obtained at two times was calculated as follows:

\[
\text{AD \%} = \frac{\text{initial concentration} - \text{concentration at 5th week at } -20 \text{ °C}}{\text{initial concentration}} \times 100
\]

The mean value of AD should be within ±15% of the concentration at zero time [14].

Pharmacokinetic-pharmacodynamic study of paracetamol

One group of six rats was used in this study. The day of the study, under isoflurane anesthesia, rats were injected with 50 µl of uric acid (30%) into the right knee joint to induce nociception, and the caudal artery was cannulated with a PE-10 cannula (Clay Adams, Parsippany, NJ, USA) connected to a PE-50 cannula. The cannula was kept patent with a heparinized saline solution and stoppered with a needle. Rats were allowed to recover from anesthesia and a dose of 316.2 mg/kg of paracetamol (suspended in a 0.5% aqueous solution of carbamoylmethyl cellulose) was orally administered. Blood samples were withdrawn from the caudal artery at 0 h (before the administration of the drug) and at 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 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 2.0 ml. The cannula was withdrawn and the animal was sacrificed after the last blood sample was taken. Plasma was separated by centrifugation at 3500 rpm for 10 min at 4 °C and stored at −20 °C until analysis. Plasma samples and a duplicate of QC samples at three concentration levels were analyzed together with a standard calibration curve prepared the day of the analysis. Assays were acceptable if the accuracy of QC samples were within ±15% of the nominal value.

Antinociception was determined before blood sampling times, using the "pain-induced functional impairment in rat" (PIFIR) model [16]. This model was specially designed for evaluation of arthritic gout-type pain in rats. Pharmacokinetic parameters: maximum plasma concentration (Cmax), time to reach Cmax (tm), area under the curve from zero time to 4 h (AUC0−4), area under the curve from zero time to infinite (AUC0−∞) and elimination constant rate (K) were calculated by non-compartmental analysis using WinNonlin v. 4.1 (Pharsight, Mountain View, CA, USA). Pharmacodynamic parameters: maximum effect (Emax), time to reach Emax (tmax) and cumulative antinociceptive effect during the whole observation period expressed as area under the effect-time curve (AUCt) were directly obtained from anti-nociceptive data. All AUC values were calculated by trapezoidal rule.

RESULTS AND DISCUSSION

Chromatography and extraction procedure

HPLC method with UV detection is a common method for the analysis of paracetamol in biological samples. However, many published papers describe the need for multiple extraction steps and/or complex chromatographic systems to ensure reproducibility and improve the analyte recovery (table 1). In this study, sample preparation is a modification of the proposed method of Soysa and Kolambage [13] for the analysis of paracetamol and its metabolites.
In our proposed method, paracetamol is separated after precipitation of plasma proteins with 10% zinc sulphate aqueous solution and methanol with no previous sample preparation. Other precipitating solvents were initially tested as acetonitrile, diethyl ether, perchloric acid, trichloroacetic acid and methanol alone. The selected procedure gave cleaner supernatant and the highest extraction efficiency (99.7 to 108.1%), which is comparable or higher than other published methods. Good sensitivity and adequate retention times were obtained with the isocratic system, water: methanol (75:25), and the reproducibility expressed as %CV on the retention time and peak area (n=5) was 0.2%. Paracetamol, PG and PAP metabolites were eluted in a total run time of 5 min.

Method validation

Selectivity

Chromatograms of drug-free rat plasma, plasma sample spiked with paracetamol, PG and PAP as well as plasma samples were taken after 1.5 and 4 h after a single dose of paracetamol (316.2 mg/kg, p. o.) to Wistar rats are shown in fig. 1. Drug-free plasma was found to be free of interfering peaks. The proposed method allowed the adequate separation of paracetamol in a low plasma volume (50 to 100 µl) with a clean chromatogram, without the interference of endogenous compounds.

Separations of spiked plasma with PG and PAP, gave good resolution for the compounds peaks with good reproducibility and accuracy. No interfering signals were observed around the retention times of these compounds with only one-step extraction. The chromatographic system employed gave satisfactory resolution for PG, PAP and paracetamol with retention times of 1.2, 2.9 and 3.98 min, respectively. Resolution between PG and PAP and paracetamol with retention times of 1.2, 2.9 and 3.98 min, respectively. Resolution between PG and PAP and paracetamol was 3.03. Plasma samples collected during the pharmacokinetic study did not show a signal of interference that could be attributed to endogenous compounds or other paracetamol biotransformation products.

Linearity

A linear relationship was found when paracetamol peak areas were plotted against drug plasma concentrations (y=39.32x+1.84; R²=0.997). Linear regression was significant (*P<0.05) for the concentration range studied (0.2 to 200 µg/ml). For our purposes, this range was considered as the concentrations expected after the
administration of doses needed to relieve arthritic pain in rats [10 to 316 mg/kg] [4, 17].

**Precision and accuracy**

Table 2 shows a summary of intra-day and inter-day precision of the method. CV values were below 1.0 and 10.0% respectively, demonstrating the good precision of the method. The precision of the method is comparable or even better than other published methods that use a higher volume of plasma sample and/or more complex sample preparation and/or more complex chromatographic systems [3, 9, 17-21]. The intra-day RE values assessed by quintuplicate analysis of QC samples ranged from 2.97 to 9.92%. Inter-day accuracy, assessed by the triplicate analysis of QC samples in three different days, gave RE values from 5.01 to 8.78%. These results demonstrate the accuracy of the method.

### Table 2: Intra-and inter-day precision and accuracy for paracetamol analysis of QC samples by HPLC-PDA method

| Added (µg/ml) | Recovered (µg/ml) | Intra-day (n = 5) | Added (µg/ml) | Recovered (µg/ml) | Inter-day (3 d, n = 9) |
|--------------|------------------|------------------|--------------|------------------|-----------------------|
| 0.202        | 0.196            | 1.07             | 2.97         | 0.205            | 0.221                 |
| 1.120        | 1.160            | 0.90             | 3.57         | 1.002            | 0.914                 |
| 10.10        | 9.780            | 1.20             | 3.17         | 10.015           | 9.400                 |
| 100.25       | 90.310           | 0.94             | 9.92         | 100.150          | 95.130                |

**Recovery and LLQ**

The best recovery after using different precipitating solvents was obtained with the addition of 10% zinc sulphate aqueous solution together with methanol.

Moreover, the cleanest supernatant was obtained with this method. Absolute recoveries, calculated by comparing peak areas from extracted samples with peak areas of non-extracted standards, were between 99.7 and 108.1% with a good precision (CV<4%), independently of the studied concentration (data not shown). Additionally, a SPE method was also proved, but the drug recovery was only about 84% (data not shown), which is near to other methods that utilize SPE [11].

In addition, the recovery obtained with the selected method was higher than others that use shorter concentration intervals [1, 18, 22]; UPLC-MS/MS methods with simple protein precipitation with acetonitrile [23] or larger volumes of plasma samples [11, 19, 21, 22]; or at least two-step liquid-liquid extraction and evaporation [10, 18, 22, 24]. Other of the advantages of this method is that it allows a high recovery of the analyte from the biological matrix (>99%) and allows multiple samples to be processed in a short period of time.

Plasma concentrations of paracetamol can be accurately quantified up to 0.2 µg/ml, with a CV and RE<15%, this was the reason by it was considered as the LLQ. The LOD was 0.06 µg/ml.

**Stability**

From the stability study, it was found that plasma samples containing 0.2 and 10 µg/ml of paracetamol were stable for at least five weeks at −20 °C, with an AD of 4.4 and 10.3%, respectively. Furthermore, stock solutions of paracetamol in methanol, stored at 4 °C, were stable for at least two weeks. Samples in mobile phase were stable for 24 h at room temperature.

**Pharmacokinetic-pharmacodynamic study**

The validated HPLC-PDA method was used to analyze paracetamol pharmacokinetics in arthritic rats, after single oral administration of 316.2 mg/kg of the drug. QC samples in each analytical run were within 15% of the nominal value. No interfering peaks were found during the analysis of the samples. PG and PAP metabolites were not detected in plasma samples. The proposed method was validated only for paracetamol as it is known that only the parent drug shows antinociceptive activity. All concentrations were above the LLQ. Mean plasma paracetamol concentration vs. time data are shown in fig. 2.

**Pharmacokinetic parameters** were calculated by non-compartmental analysis from paracetamol concentration data. Results are summarized in table 3. Antinociceptive effect expressed as functionality index was plotted vs. time to construct the temporary course of the effect (data not shown). Pharmacodynamic parameters calculated from these data are also shown in table 3.

**Pharmacokinetic and pharmacodynamic parameters** values determined in the present study are comparable to those previously reported, under similar conditions [4].

This method might be applied to determine paracetamol plasma levels in pharmacokinetics-pharmacodynamics studies.
CONFLICT OF INTERESTS

All authors have none to declare.

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Table 3: Pharmacokinetic and pharmacodynamic parameters obtained after oral administration of 316.2 mg/kg of paracetamol to arthritic rats

| Parameter                  | Value*       |
|----------------------------|-------------|
| Cmax (µg/ml)               | 139.11±20.16|
| tmax (h)                  | 0.38±0.13   |
| C0 (µg/ml)                | 21.5±4.21   |
| AUC[0-4h] (µg*h/ml)       | 239.15±42.68|
| AUC[0-∞] (µg*h/ml)        | 276.63±30.55|
| K (1/h)                   | 0.81±0.26   |
| t½ (h)                    | 1.17±0.22   |
| CI/F (l/kg/h)             | 1.21±0.12   |
| Vd/F (l/kg)               | 2.05±0.40   |
| Pharmacokinetic Emax (%)  | 74.75±4.67  |
| t250 (h)                  | 0.75±0.02   |
| E40 (%)                   | 32.26±10.37 |
| AUC (%)                   | 163.30±27.53|

*mean±SEM, n=6.

CONCLUSION

A reliable, simple and fast reversed-phase HPLC-PDA method for the quantification of paracetamol in small rat plasma samples was developed and validated. The method is simpler than many other published methods used for the quantification of paracetamol. It uses isocratic elution and avoiding salts buffers-organic solvent mixtures as the mobile phase. Excellent results for recoveries by simple protein precipitation were obtained, as well as intra-and inter-day precision along the entire concentration range studied. The resolution of paracetamol from endogenous plasma components and paracetamol metabolites was satisfactory attained, as no other signals that could interfere with the quantification of the drug were observed. The present method proved to be useful for the determination of paracetamol plasma levels, in a small sample volume. So, a sufficient number of samples can be obtained from the same animal immediately after measuring the antinociceptive effect of the drug, in order to support pharmacokinetic-pharmacodynamic studies, without any impairment to its physiological state.

ACKNOWLEDGEMENT

We wish to thank J. Gutiérrez, K. de la Cruz, L. Oliva and J. Sabino for technical assistance.

AUTHORS CONTRIBUTION

A. M. Domínguez-Ramírez proposed the research protocol, supervised and directed the development and validation of the analytical method and the pharmacokinetic study, analysis of data and the writing of the paper; F. J. López-Muñoz had the direction of the pharmacodynamic study and revision of the article; J. R. Medina participated in the analysis of data and discussion of results, writing, organization and revision of the paper; M. Hurtado and G. Alarcón-Angéles contributed with ideas on the modification of the analytical method and revision of the paper; L. A. Moreno-Rocha participated in the development of the pharmacodynamic-pharmacokinetic study, the statistical analysis of data and the elaboration of fig. and tables; A. D. Pineda assisted in the development of the analytical method.
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How to cite this article
• Adriana Miriam Domínguez-Ramírez, Francisco Javier López-Muñoz, José Raúl Medina, Marcela Hurtado, Georgina Alarcón Angeles, Adriana Desiree Pineda, Luis Alfonso Moreno-Rocha. HPLC-PDA method for the quantification of paracetamol in plasma: application to PK/PD studies with arthritic rats. Int J Pharm Pharm Sci 2017;9(5):233-239.