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Research Article

Simple and rapid determination of ibuprofen without caffeine interference by HPLC-UV detection: application to pharmacokinetic studies in rats

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ABSTRACT

A simple and rapid method with HPLC-UV detection was developed for estimation of ibuprofen using only 50 µl of plasma samples. Analyses were performed on a Symmetry C_{18} column and UV detection at 196 nm. The method was validated according international criteria and it was used for quantification of ibuprofen plasma levels after p.o. administration of 17.8 mg/kg of the drug alone and combined with 17.8 mg/kg of caffeine to healthy male Wistar rats. Retention times for ibuprofen and mefenamic acid were < 5 min. No caffeine interference was found. Linearity was assessed with plasma solutions of 2.5–100 µg/ml. R^2 value was more than 0.999 ($p < 0.05$). The intra-day and inter-day precision, expressed as relative standard deviation, and accuracy, expressed as relative error, were < 15%. Stability was proved for four weeks at −20 °C. After concomitant caffeine administration no significant differences in ibuprofen plasma levels were found (p>0.05). The analytical assay is reliable and sufficiently sensitive for single-dose pharmacokinetic studies utilizing a small plasma sample. A large number of samples can be processed and run in a relatively short period of time.

Keywords: Animal models; Caffeine; HPLC; Ibuprofen; NSAIDs.

INTRODUCTION

Ibuprofen, [2-(4-Isobutylphenyl) propionic acid], is an important non-steroidal anti-inflammatory drug (NSAID) for the treatment of arthritis and for mild to moderate pain (Adams, 1992). Many published papers used for the quantification of ibuprofen in urine or plasma are often performed by high-performance liquid chromatography (HPLC) however, now modern, expensive and/or time-consuming analytical techniques are available for determination of this drug (Loudiki et al., 2016). In preclinical studies, e.g., quantification of drug plasma levels and the use of animal models are important tools for pharmacokinetic pharmacodynamic studies (Meibohm and Derendorf, 2002). So that, estimation of plasma concentration-time curves of ibuprofen in small laboratory animals, requires a fast and sensitive analytical method with small volumes of plasma. Previously, Litowitz et al. (1984) reported a simple ibuprofen extraction technique for pharmacokinetic studies, however, they employed 1ml of plasma sample and a mobile phase with 50% of acetonitrile at 3 ml/min. These are disadvantages because determination requires great volumes of plasma and a lot of a toxic dissolvent. Other authors, such as Lalande et al. (1986) and Blagbrough et al. (1992) reported methods without acetonitrile in the mobile phase, however, both employed 500 µl of plasma sample which is still a disadvantage for determination of ibuprofen in small animal models. Castillo and Smith (1993) reported a method with ibuprofen retention time of about 23 min. This is a very time-consuming method. Caffeine enhances the analgesic effect of NSAIDs and many drug products with this combination are available for relief or moderate pain. The purpose of this work is to validate and applicate a rapid and simple HPLC-UV method for estimation of ibuprofen using 50 µl of plasma sample, which can be used especially, to measure the drug in small laboratory animals without any impairment to its physiological state. Furthermore, the analytical procedure was used for the estimation of ibuprofen (with and without caffeine administration) in plasma samples arising from a pharmacokinetic study in rats.

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MATERIALS & METHODS

Reagents and chemicals

Ibuprofen and mefenamic acid used as internal standard (IS) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Acetonitrile and methanol were HPLC grade, 85% phosphoric acid was analytical grade and all reagents were purchased from J. T. Baker (Phillipsburg,

NJ, USA). Deionized water (18 Ω) was obtained from a Milli-Q filtration system (Millipore, Molsheim, France).

Chromatographic conditions

The chromatographic equipment was a Perkin-Elmer HPLC Series 200 (Norwalk, CT, USA) with a binary pump, a manual injector (20-µl loop) and an UV 785 Model Detector (Applied Biosystems). The separation was performed on a C_{18} Symmetry stainless steel column (Waters Assoc., Milford, MA, USA) (3.5-µm particle size; 4.6 x 75 mm). Security Guard Phenomenex C_{18} packed with ODS, octadecyl material was used (4.0 mm L. x 3.0 mm I.D.). Perkin-Elmer software was used for data processing (Perkin-Elmer, Norwalk, CT, USA). The mobile phase was a mixture of acetonitrile:water:methanol:phosphoric acid (58:37:5:0.05, v/v/v/v) pumped at 1.8 ml/min. The mobile phase was filtered through 0.45 µm filters (Sartorius, Gottingen, Germany) and degassed with an ultrasonic bath (Branson Ultrasonic Corporation, Eagle Road, Danbury CT, USA). Samples were chromatographed at 25°C. Ibuprofen was determined at 196 nm.

Standard solutions and sample preparation

Stock solutions of ibuprofen (3 mg/ml) and IS (1 mg/ml) were dissolved in acetonitrile and stored at 4 °C. Ibuprofen solutions were prepared with rat blank plasma at 2.5, 5, 25, 50, and 100 µg/ml.

The assay was modified and validated from that reported by Shah and Jung (1985). Briefly, to an aliquot of 50 µl of plasma sample, 100 µl of acetonitrile containing IS (40 µg/ml) were added. Then, plasma and acetonitrile were mixed for 10 s and centrifuged by 10 min at 3000 rpm. Finally, 20 µl of supernatant was injected onto the HPLC system.

Method validation

To evaluate the selectivity of the proposed assay, drug-free rat plasma and plasma solutions with known concentrations of ibuprofen and IS were analyzed. Drugs were extracted as previously was described and injected onto the HPLC system.

The absolute recovery of ibuprofen from plasma was evaluated by extracting quality control (QC) samples (n = 3) at 2.5, 25, and 100 µg/ml. The resulting peak-areas were compared with resulting peak-areas of non-extracted acetonitrile solutions, at the same concentrations. At each level, percentage of recovery and relative standard deviation (RSD) were calculated.

Ibuprofen standard calibration curves of 2.5–100 µg/ml were prepared with drug-free rat plasma and analyzed (n = 3). Curves were determined by plotting peak-area ratios of ibuprofen/IS vs ibuprofen concentrations. A linear regression analysis was carried out to know slope, intercept, $CI_{95\%}$ for the intercept and R^2 values. Linear regression analysis of variance (ANOVA) was determined and a $p < 0.05$ was considered statistically significant.

The intra-day precision was evaluated on the same day with a set of QC samples (n = 3), at three concentration levels (2.5, 25, and 100 µg/ml). A standard calibration curve was analyzed. The inter-day precision, was evaluated on three different days, by performing the assay of QC samples (n = 3) at 2.5, 25, and 100 µg/ml. A standard calibration curve was analyzed. The value of RSD was taken as a precision measure. The RSD should be <15%, except at limit of quantification (LOQ) where it should be < 20% (FDA, 2001).

The accuracy of the method was evaluated by comparing the average of the found ibuprofen concentrations respect to nominal values. The deviation from the nominal values, relative error (RE%), was a measure of accuracy. The mean value of RE should be ± 15% of the nominal concentration, except at the LOQ where it should be <20% (FDA, 2001).

The limit of quantification (LOQ) and limit of detection (LOD) were calculated using the equations: $10 \times \sigma/s$ and $3.3 \times \sigma/s$, respectively. The parameter σ is the SD of the analytical response and s is the slope of the least-square linear regression analysis (FDA, 1996).

QC samples prepared at 2.5, 25, and 100 µg/ml of ibuprofen (n = 3) were stored at −20 °C for four weeks. Ibuprofen concentrations were determined by the above method the day that they were prepared and after four weeks. The difference between the values at zero time and after four weeks was expressed as RE. The RE value should be ± 15% of the concentration at initial time.

Pharmacokinetic study

Male Wistar rats [Crl (WI)fBR] of 180–200 g were used. All experimental procedures followed the guidelines of Committee for Research and Ethical Issues of the International Association for the Study of Pain (Covino et al., 1980) and of the Ethical Issues of the International Association of Pain (Zimmermann, 1983). Rats were used under experimental conditions (22°C, 12h light/dark cycle) and provided with standard chow (Purina Laboratory Rodent Diet 5001) and water ad libitum. Groups of rats (n = 6) were used in the pharmacokinetic study. Rats in Group A received 17.8 mg/kg of ibuprofen and animals in Group B received the same dose of ibuprofen but combined with 17.8 mg/kg of caffeine as analgesic adjuvant. Rats were slightly anesthetized with isofluorane and the caudal artery was cannulated with a PE-10 cannula connected to a PE-50 cannula (Clay Adams, Parsippany, NJ). Before p.o. administration, a 100 µl of blood sample was drawn of each rat to serve as control. At zero time, rats were p.o. administered with ibuprofen suspended in 0.5% carboximethyl cellulose in a single-dose or combined with caffeine. Then, 100 µl of blood sample was taken at 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 h and collected in heparinized polypropylene tubes. Animals were used only once. Finally, to avoid suffering, animals were euthanized with CO_2. The total volume of blood taken

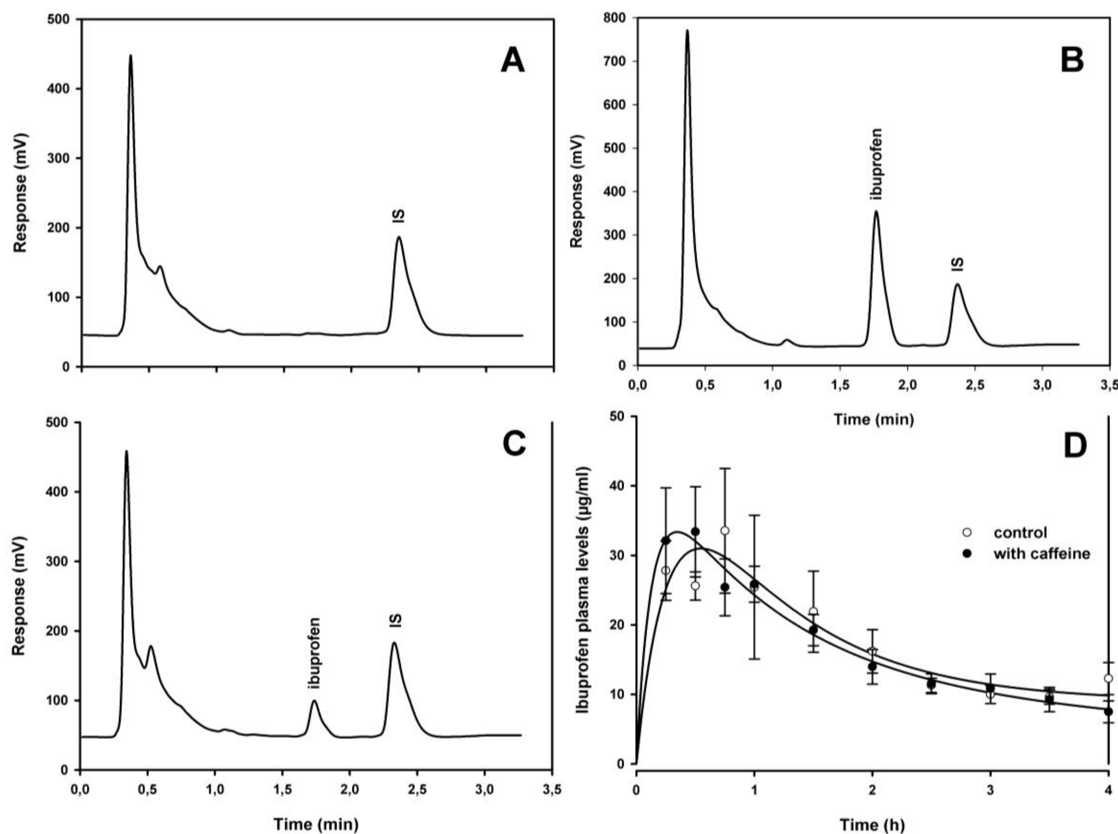

Figure 1: Typical chromatograms of ibuprofen and internal standard (A-C). Ibuprofen pharmacokinetics with and without caffeine administration (D)

Table 1: Pharmacokinetic parameters of ibuprofen. Mean ± SEM, n = 6

Parameter	Control	With caffeine
C_{max} (µg/ml)	40.86 ± 8.33	40.62 ± 6.48
T_{max} (h)	0.54 ± 0.14	0.58 ± 0.14
AUC_{0-4} (µgh/ml)	68.81 ± 10.52	66.68 ± 8.65
λ_z (h^{-1})	0.32 ± 0.10	0.47 ± 0.07

from each rat did not exceed of 1.5 ml. Plasma was collected (3000 rpm, 10 min) and stored at −20 °C until analysis. The ibuprofen plasma data were subject to interactive nonlinear analysis by WinNonlin version 2.1 program (Parsight Corp. Palo Alto, CA, USA). Samples of pharmacokinetic studies and QC samples (n = 2) were analyzed as well as a plasma calibration curve. Results were acceptable if QC samples were ± 15% of the nominal value.

RESULTS AND DISCUSSION

Analytical method validation

The extraction procedure allowed good separation of ibuprofen and IS. Adequate sensitivity and short retention times for both drugs were observed with the proposed assay. The ibuprofen and IS gave well resolved, sharp peaks. Retention times of ibuprofen and IS were 1.7 and 2.4 min, respectively. No interfering peaks were found close to the retention times of ibuprofen and IS. Chromatogram of drug-free rat plasma + IS is shown in Fig. 1A. Plasma spiked with 100 µg/ml of ibuprofen and IS is shown in Fig. 1B and chromatogram of sample taken 1.5 h after p.o. administration of 17.8 mg/kg of ibuprofen combined with 17.8 mg/kg of caffeine is shown in Fig. 1C. Along the analysis, caffeine response was not found.

Absolute recoveries were: 108.85, 105.74, and 102.49%, with a good precision: 10.02, 8.30, and 2.69% as RSD, respectively. These results essentially showed a complete recovery of ibuprofen.

When ratio of ibuprofen/IS peak-areas vs ibuprofen plasma concentrations (2.5−100 µg/ml) were plotted a linear relationship was found ($R^2 > 0.999$). Linear regression equation was significant ($y = 0.0148x–0.0026$; $p<0.05$) with a $CI_{95\%}$ of – 0.0159 to 0.0107 for intercept.

The intra-day RSD values for QC samples were 12.95, 5.24, and 3.27%, respectively. The inter-day RSD values were 10.94, 5.63, and 5.59%. These data confirmed the precision of the method (RSD < 15%).

The intra-day RE values were –2.90, 2.24, and 2.21%. The inter-day RE values were –0.32, 3.22 and 0.54%. These data demonstrated the accuracy of the proposed assay (RE ± 15%).

The estimated LOQ and LOD of 0.50 and 0.15 µg/ml respectively, were proved by assaying QC samples (n = 3) added with ibuprofen at these concentration levels (data not shown).

Plasma samples containing ibuprofen at 2.5, 25, and 100 µg/ml were stable after four weeks at −20°C (p>0.05). RE values at each concentration level were–0.70, 4.57, and 4.83%, respectively. Additionally, RSD data at each level were calculated. Results were 4.02, 5.83, and 5.40%, respectively. Good stability was found with RE and RSD values <15%.

Pharmacokinetic study

The observed ibuprofen pharmacokinetics after p.o. administration of 17.8 mg/kg of ibuprofen alone and combined with 17.8 mg/kg of caffeine are shown in Fig. 1D (Mean ± SEM, n = 6). Mean ibuprofen pharmacokinetic parameters computed with data adjusted with non-compartment analysis are shown in Table 1. C_{max}, T_{max}, and AUC_{0-4} values after administration of ibuprofen alone and combined with caffeine were statistically similar (p > 0.05).

NSAIDs combined with caffeine are a common drug mixture for relief or moderate pain. In this study, caffeine does not modify the ibuprofen pharmacokinetics. Results agree with those found by other authors where caffeine was administered with paracetamol or acetylsalicylic acid to Wistar rats and plasma levels were similar to drug administration alone (Granados-Soto et al., 1993; Castañeda-Hernández et al., 1994). Ibuprofen plasma concentration-time curves found in this work were similar than those reported by Satterwhite and Boudinot (1989).

CONCLUSION

The chromatographic analysis here described has proven to be adequate and sufficiently sensitive for single-dose pharmacokinetic studies utilizing only 50 µl of plasma sample. A large number of samples can be processed and run in a relatively short period of time because samples require one-step deproteinization and chromatographic separation takes < 5 min. The application of this analytical procedure allowed us to obtain reliable data about ibuprofen plasma concentration-time curves without caffeine interference. The proposed analytical method is rapid and simple without the need of high cost investment.

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REFERENCES

Adams SS., 1992. The propionic acids: a personal perspective. Journal of Clinical Pharmacology, 32, 317–323.

Blagbrough IS, Daykin MM, Doherty M, Pattrick M, Shaw PN., 1992. High-performance liquid chromatographic determination of naproxen, ibuprofen and diclofenac in plasma and synovial fluid in man. Journal of Chromatography B: Biomedical Applications, 578, 251–257.

Castañeda- Hernández G, Castillo-Méndez MS, López-Muñoz FJ, Granados-Soto V, Flores-Murrieta FJ., 1994. Potentiation by caffeine of the analgesic effect of aspirin in the pain-induced functional impairment model in the rat. Canadian Journal of Physiology and Pharmacology, 72, 1127–1131.

Castillo M. and Smith PC., 1993. Direct determination of ibuprofen and ibuprofen acyl glucuronide in plasma by high-performance liquid chromatography using solid-phase extraction. Journal of Chromatography B: Biomedical Applications, 614, 109–116.

Covino BG, Dubner R, Gybels J., 1980. Ethical standards for investigation of experimental pain in animals. Pain, 9, 141–143.

Food and Drug Administration, 1996. Guidance for industry, Q2B validation of analytical procedures: metodology. Center for Drug Evaluation and Research (CDER), U.S. Department of Health and Human Ser-vices.

Food and Drug Administration, 2001. Guidance for industry bioanalytical method validation. Center for Drug Evaluation and Research (CDER), U.S. Department of Health and Human Services.

Granados-Soto V, López-Muñoz FJ, Castañeda Hernández G, Salazar LA, Villarreal JE, Flores-Murrieta FJ., 1993. Characterization of the analgesic effect of paracetamol and caffeine combinations in the pain-induced functional impairment model in the rat. Journal of Pharmacy and Pharmacology, 45, 627–631.

Lalande M, Wilson DM, McGilveray IJ., 1986. Rapid high-performance liquid chromatographic determination of ibuprofen in human plasma. Journal of Chromatography B: Biomedical Applications, 377, 410–414.

Litowitz H, Olanoff L, Hoppel CL., 1984. Determination of ibuprofen in human plasma by high-performance liquid chromatography. Journal of Chromatography B: Biomedical Applications, 311, 443–448.

Loudiki A, Boumya W, Hammani H, Nasrellah H, El-Boua-bi Y, Zeroual M, Farahi A, LAhrich S, Hnini K, Achak M, Bakasse M, Mhammedi E., 2016. Ibuprofen analysis in blood samples by palladium particles-impregnated sodium montmorillonite electrodes: validation using high performance liquid chromatography. Materials Science and Engineering: C, 69, 616–624.

Meibohm B, Derendorf H., 2002. Pharmacokinetic/pharmacodynamic studies in drug product development. Journal of Pharmaceutical Sciences, 91, 18–31.

Satterwhite JH. and Boudinot FD., 1989. High-performance liquid chromatographic determination of ibuprofen in rat and human plasma. Journal of Chromatography B: Biomedical Applications, 497, 330–335.

Shah A. and Jung D., 1985. Improved high-performance liquid chromatographic assay of ibuprofen in plasma. Journal of Chromatography B: Biomedical Applications, 344, 408–411.

Zimmermann, M., 1983. Ethical guidelines for investgation of experimental pain in conscious animals. Pain, 16, 109–110.