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Use of mouse model in pharmacokinetic studies of poorly water soluble drugs: Application to fenofibrate

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ABSTRACT

Fenofibrate has recently been used as drug model in several studies with the objective of optimizing the development of some drug delivery systems to overcome the problem of poor aqueous solubility of the newly discovered API. The adequacy of the drug delivery systems to improve the oral bioavailability of encapsulated drug is generally evaluated by a pharmacokinetic study. The use of mouse as animal model for pharmacokinetic studies has become more important in the last decade because of many similarities with the human model in terms of the mechanisms of absorption, metabolism and elimination. Nevertheless, the mouse is often hampered by the very small volumes of blood that could be obtained during sampling. The aim of this work was to overcome the problem of lower volumes of plasma withdrawn by developing an appropriate protocol for sample preparation and a suitable HPLC method for drug quantification in mouse plasma. Linear calibration curve was obtained over the concentration range from 0,16 μg/mL to 32 μg/mL (r^2 = 0,9999) with LLOQ of 0,16 μg/mL The RSD in both intra-run and inter-run precision study was less than 11% and the extraction recoveries were above 91.9%. The reproducible method was successfully applied to the pharmacokinetic study of fénofibrate in mouse.

1. Introduction

Fenofibrate, is a well-studied lipid regulating agent used for the treatment of hypercholesterolemia and hyper-triglyceridemia. After absorption, the drug is completely hydrolyzed by esterases in its active metabolite, the fenofibric acid [1], which is eliminated with a half-life of 20 h in human after one daily dosage. Its maximal plasma concentration is obtained around 3–4 h post dosage.

Fenofibrate is practically insoluble in water and exhibits an extremely poor oral bioavailability [2]. Therefore, fenofibrate has recently been used as drug model in several studies with the objective of optimizing the development of some drug delivery systems to overcome the problem of poor aqueous solubility characterizing more than 50% of the newly discovered API. Among these systems: solid lipid nanoparticles [3] nanocrystals [4], nanosuspension [5], self-microemulsufying systems [6] that were developed using fenofibrate as a drug model. The adequacy of these drug delivery systems to improve the oral bioavailability of encapsulated drug is generally evaluated by a pharmacokinetic study which requires a suitable animal species that should closely resembles to human in terms of the mechanisms of absorption and elimination of the active ingredient.

The rat appears to be the primary species most commonly used in preclinical pharmacokinetic studies. However, it was shown that the oral bioavailability values and the metabolic enzymatic levels in intestine are distinct between humans and rats [7].

Moreover, the use of the mouse as an animal model for pharmacokinetic studies has become more important in predicting oral bioavailability due to several similarities in the absorption, metabolism and elimination processes between humans and mice [8]. Besides, the presence of gall bladder in mice led to the occurrence of an absorption mechanism, entitled collisional transfer, by which highly lipophilic drugs are diffused through the glycocalyx when they are intercalated in the bile acids (fasting state) or in the mixed micelles of bile acids (fasted state) [9]. As rats do not possess a gall bladder, we chose to perform our study in mice. Nevertheless, the mouse exhibits a particular challenge because of the very small volumes of blood that could be obtained during sampling and hence impediments for pharmacokinetics [8,10].

The aim of this work was at first to overcome the problem of lower volumes of plasma withdrawn by developing a one-step processing method for samples preparation that would be rapid in applying and

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ensuring at least 90% of drug extraction efficiency. Secondly, a fast and sensitive analytical method was developed for the dosage of the active metabolite of fenofibrate (fenofibric acid) by HPLC-UV.

This quantification method was subsequently validated according to the recommendations of the SFSTP [11] and the FDA guideline for validation of bioanalytical methods [12]. The feasibility of the animal model and the extraction protocol were evaluated by carrying out a pharmacokinetic application to fenofibrate in its commercialized form LIPANTHYL® with an oral dose corresponding to 5 mg/kg.

2. Materials and methods

2.1. Chemicals and reagents

Fenofibric acid (purity ≥98.0%) was provided from Sigma-Aldrich and the internal standard (I.S.), carbamazepine (≥99.0% purity) was purchased from Sigma-Aldrich (China). Methanol and deionized water of HPLC grade were bought from Carlo Erba (France).

Ammonium acetate was of analytical grade was purchased from Sigma-Aldrich (Netherlands) Hydrochloric acid and diethyl ether were acquired from Carlo Erba (France). Normal mouse plasma was brought from Sigma-Aldrich (USA) and stored at −20 °C.

2.2. Chromatographic conditions

Analyses were performed on a Shimadzu HPLC Class VP series (Shimadzu, Kyoto, Japan) which was composed of a LC-10ADvp quadratic pump, an auto-sampler (model SIL- 10Avp Shimadzu), a variable UV–Visible wavelength detector (model SPD 10Avp; Shimadzu) and the data were analyzed by Class-VP series software, version 5.03 (Shimadzu, Japan). The analytical column used was LiChrospher 100 RP8 (125 mm × 4.6 mm I.D, 5 μm particle size). The detection wavelength was fixed at 287 nm and the column oven temperature was set at 35 °C.Elution was obtained by applying the gradient steps summarized in Table 1 and corresponding to solvents A (Methanol) and B (5 mM ammonium acetate buffer (pH 3.3)). In all cases the flow rate was 1.5 ml/mn and the UV detection was achieved at a wavelength of 287 nm.

2.3. Preparation of standard and stock solutions

The Stock solution of fenofibric acid was prepared in methanol at a concentration of 1600 μg/ml.

In the other hand, the preparation of the internal standard stock solution was done at a concentration of 4.7 μg/ml by dissolving 2.35 mg carbamazepine in 500 ml HCl (1 M).

Then, the standard solutions of fenofibric acid with concentrations of 1600, 800, 500, 160, 80, 16and 8 μg/ml were prepared after serial volumetric dilution of fenofibric acid stock solution in methanol.

These solutions were prepared and stored in appropriate conditions (at −20 °C) until analysis.

Table 1
Gradient elution system for separation of fenofibric acid and internal standard.

Cumulative time of acquisition (min)	Gradient	
	% Solvent A Methanol	% Solvent B 5 mM ammonium acetate buffer (pH 3.3)
5	56	44
10.5	66	34
12.5	76	24
13	66	34

2.4. Extraction methods and sample preparation procedure

25 μL aliquot of mouse plasma was mixed with 12.5 μL of the internal standard solution in 1.5 ml Eppendorff tube.

Precipitation of the plasma proteins has been carried out by adding 250 μL of methanol then by vortexing the mixture for 30 min using a vortex mixer (Eppendorf, Hamburg, Germany). Afterward the samples were placed in an ice bath for 60 min, followed by centrifugation for 10 min at 12.000 rpm (4° C). The top layer was injected (20 μL) into the HPLC system for analysis.

2.5. Calibration curves and quality control plasma samples preparation

Calibration curves of fenofibric acid were prepared with concentration levels of 32, 16, 3.2, 1.6, 0.32and 0.16 μg/ml by spiking 0.5 μL of the standard solution in 24.5 μL of mouse plasma. This ratio of 2:100 was chosen in order to avoid plasma alteration with the standard solution [13].

Three control samples (QC) were prepared in blank mouse plasma at different concentrations of fenofibric acid (0.64, 16 and 25.6) μg/ml. All samples were conserved at −80 °C until analysis.

A single set of quality controls and standards were analyzed each day of the overall work using the same procedure described above for plasma samples.

2.6. Method validation

A complete validation of the used method for the determination of fenofibric acid in mouse plasma was done according to the FDA guideline for validation of bioanalytical methods [12]and to the SFSTP recommendations [11].

The method has been validated for selectivity, linearity, lower limit of quantification (LLOQ), precision and accuracy, stability and recovery.

2.6.1. Selectivity

Chromatograms of drug-free mouse plasma were compared (n = 6). All samples were processed using the protein precipitation procedure in order to ensure the absence of endogenous peaks co-eluted with fenofibric acid or the internal standard.

2.6.2. Precision and accuracy

To assess the intra- and inter-day accuracy and precision, repetitive measurements of the three QC sample concentrations (0.64, 16 and 25.6 μg/ml) were realized. Intra-day accuracy was assessed through the QC samples analyzed in replicates of five per day. The inter-day precision was determined by performing the same procedure once a day during three consecutive days. Precision and accuracy were expressed as relative standard deviation (RSD) and relative error (RE) respectively. The precision and accuracy acceptance criterion for each QC sample concentration was within ± 15%.

2.6.3. Linearity and limit of quantification

To assess the linearity, six-point standard curves had been run within the concentration range 0.16–32 μg/ml on three different days with replicates of four for each concentration per day (n = 4).

Standard curves were fitted by plotting peak area ratio of fenofibric acid to internal standard versus the corresponding concentration. Calibration curves were obtained by least-squares linear regression method using weight scheme as x (x = concentration).

The acceptance criterion of the coefficient of correlation for the calibration curves was 0.999 or greater and every back-determined standard concentration had to be within 15% deviation with the exception of the lower limit of quantification (LLOQ) that was within 20%. The LLOQ is corresponding to the lowest concentration on the standard curve which could be evaluated with adequate precision and