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Quantification of ketoprofen enantiomers in human plasma based on solid-phase extraction and enantioselective column chromatography

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Abstract

An HPLC method for the quantification of ketoprofen enantiomers in human plasma is described. Following extraction with a disposable C_{18} solid-phase extraction column, separation of ketoprofen enantiomers and I.S. (3,4-dimethoxy benzoic acid) was achieved using a chiral column [Chirex 3005; (*R*)-1-naphthylglycine 3,5-dinitrobenzoic acid] with the mobile phase, 0.02 *M* ammonium acetate in methanol, set at a flow-rate of 1.2 ml/min. Baseline separation of ketoprofen enantiomers and I.S., free from interferences, was achieved in less than 20 min. The calibration curves ($n=14$) were linear over the concentration range of 0.16 to 5.00 μg/ml per enantiomer [mean r^2 of 0.999 for both enantiomers, root mean square error were 0.015 for $R(-)$ and 0.013 for $S(+)$]. The inter-day coefficient of variation for duplicate analysis of spiked samples was less than 7% and the accuracy was more than 93% over the over the concentration range of 0.2 to 4.0 μg/ml for individual enantiomer using 1 ml of plasma sample. This method has been applied to a pharmacokinetic study from healthy human volunteers following the administration of a ketoprofen extended release product (200 mg). This method is simple, fast and should find wide application in monitoring pharmacokinetic studies of ketoprofen.

Keywords: Enantiomer separation; Ketoprofen

1. Introduction

Ketoprofen, a phenylpropionic acid derivative, is a non-steroidal anti-inflammatory drug (NSAID) that has analgesic, anti-inflammatory and antipyretic properties [1]. Pharmacological activity has been attributed mainly to the $S(+)$ isomer, while the $R(-)$ isomer is either inactive or has reduced activity [2–4]. Several methods are reported in the literature for the determination of ketoprofen by HPLC but most of them are based on non-stereoselective separation techniques [5–10]. For a racemic product such as ketoprofen, it is preferential to use a chiral method to differentiate the plasma levels of enantiomers. Several chiral methods have been reported in the literature in which diastereomeric derivatives were formed prior to HPLC separation and analysis [11–16]. These methods are generally time-consuming and are prone to interconversion of enantiomers [17–19], therefore, direct resolution of ketoprofen enantiomers using a chiral stationary phase (CSP) was preferred.

The direct separation of ketoprofen enantiomers has been successfully accomplished using various

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types of CSP [20–26]. Some columns are not available commercially, others may require prior derivatization to enhance sensitivity, or validation data for human pharmacokinetic studies is not provided. The method described is capable of separating ketoprofen enantiomers in human plasma, with direct resolution of enantiomers using a commercially available CSP that offers a simple, sensitive and fast alternative for ketoprofen determination and was applied to a pharmacokinetic study on healthy human volunteers following the administration of a ketoprofen extended-release product.

In addition to chromatographic separation, the described method utilized a sample clean-up procedure based on disposable solid-phase extraction columns. Sample clean-up methods reported in the literature, including the methodology previously utilized in our laboratory, are generally based on liquid–liquid extraction [11–16,20,24,25]. The reported liquid–liquid extraction procedure consists of a number of steps and various solvents, thus contributing to the cost and environmental concerns. The sample clean-up applied in this procedure is fast, avoids evaporation and reconstitution steps and is free of any interference.

2. Experimental

2.1. Materials

Ketoprofen [2-(3-benzoylphenyl)propionic acid] and the internal standard (I.S., 3,4-dimethoxy benzoic acid) were purchased from Sigma (St. Louis, MO, USA). Samples of individual *R*(−)- and *S*(+)-ketoprofen enantiomers were kindly supplied by Rhône-Poulenc Rorer (Montréal, Canada). All other chemicals and solvents were purchased from BDH (Toronto, Canada): ammonium acetate, ammonium sulfate, methanol, phosphoric acid, potassium dihydrogen orthophosphate monobasic, sodium hydroxide, sulfuric acid and were of analytical reagent grade. Human plasma was obtained from the Canadian Red Cross. All aqueous solutions were prepared using de-ionized and purified water (Milli-Q, Milford, MA, USA).

2.2. Instrumentation

The HPLC system consisted of a Waters (Milford, MA, USA) Model 600E pump, Model 717plus autosampler, Model 996 photodiode array detector. Data were collected and processed using a computer system equipped with Millennium 2.1 software (Waters). Peak-height response at 254 nm was used for quantitation.

Chromatographic separations were achieved using a 250×4.6 mm chiral column [Chirex 3005; (*R*)-1-naphthylglycine and 3,5-dinitrobenzoic acid] (Phenomenex, Torrance, CA, USA) with a C_{18} guard column (Supelco, Mississauga, Canada). The flow-rate of the mobile phase, 0.02 *M* ammonium acetate in methanol, was set at 1.2 ml/min at ambient temperature.

2.3. Standard solutions

2.3.1. Ketoprofen

Ketoprofen (1 mg/ml) stock solution was prepared in aqueous 0.06 *M* phosphate buffer (pH 6.8). The standard solutions were prepared by spiking 100 μl of working solution (3.12 to 100 μg/ml of racemic ketoprofen) into 1 ml human plasma. Final concentrations of ketoprofen enantiomers were 0.16, 0.31, 0.63, 1.25, 2.50 and 5.00 μg/ml in plasma. The solutions were subjected to analysis according to the method described for samples (Section 2.4).

2.3.2. Internal standard (I.S.)

A methanolic working solution of 3,4-dimethoxybenzoic acid was prepared to give a concentration of 50 μg/ml. A 100-μl aliquot of this solution was used for spiking each 1 ml plasma sample giving a final concentration of 5 μg/ml.

2.4. Sample preparation

To blank human plasma, 100 μl of ketoprofen working solution and 100 μl of I.S. solution was added, while for study samples, only 100 μl of I.S. was added. These were vortexed with 0.36 g of ammonium sulphate to deproteinate the plasma. After 30 min, the samples were centrifuged at 4°C for 30 min (8000 *g*). The supernatant was acidified by mixing 3 ml of aqueous sulfuric acid (0.1 *M*) and passed through a C_{18} solid-phase extraction column (100 mg/1 ml) (Varian, Mississauga, Canada), previously conditioned with 1 ml of methanol followed by 1 ml of 0.10 *M* phosphate buffer (pH 2.5). The column was washed with 2×750 μl of methanol in 0.10 *M* aqueous phosphate buffer (pH 2.5) (20:80) and ketoprofen enantiomers and I.S. were eluted with 2×500 μl of 75% methanol in 0.05 *M* aqueous phosphate buffer (pH 7.4). A 40-μl aliquot was injected in a partial loop mode using a 200-μl loop.

3. Results and discussion

3.1. Chromatographic separation

Analytical separation of the ketoprofen enantiomers and I.S. was achieved and chromatograms from human plasma were free from interference. A representative chromatogram of spiked plasma samples is shown in Fig. 1. The retention times of *R*(−), *S*(+) and I.S. were 14.2, 16.1 and 18.1 min, respectively. Chromatograms, obtained by spiking the blank plasma sample with individual enantiomers and processed through the sample preparation procedure, showed no interconversion of enantiomers (Fig. 1). The chromatographic run was completed in less than 20 min and conditioning of the HPLC column was not necessary prior to the next injection.

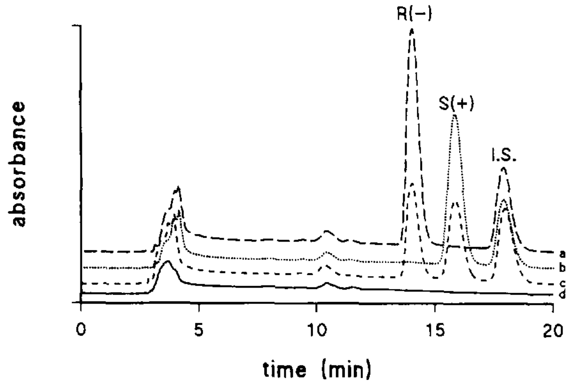

Fig. 1. Chromatograms of extracts from human plasma samples spiked with (a) 10 μg/ml *R*(−)-ketoprofen, (b) 10 μg/ml *S*(+)-ketoprofen, (c) 5 μg/ml of each ketoprofen enantiomers and (d) blank plasma sample.

3.2. Extraction recovery

The absolute extraction recovery was determined by comparing the peak height of individual enantiomers plasma extracts ($n=6$) with those obtained by directly injected solutions ($n=2$) of the analyte made in the same injection solvents. The absolute recovery values (mean±S.D.) of ketoprofen enantiomers at 0.20 μg/ml were 84.9±6.7 for *R*(−), 86.3±4.9 for *S*(+) and 84.0±4.3 for *R*(−) and 85.2±3.0 for *S*(+) at 4.00 μg/ml. The absolute recovery of the internal standard ($n=18$) was 87.5±3.8.

3.3. Assay validation

Best-fit calibration lines of peak-height ratios (enantiomer/I.S.) vs. concentrations were determined by weighted (1/peak-height ratio) least square linear regression analysis using SAS software (SAS Institute, Cary, NC, USA). The calibration curves ($n=14$) were linear over the concentration range of 0.16 to 5.00 μg/ml for individual enantiomer (mean r^2 of 0.999 for both enantiomers). The slope values (mean±S.D.) were 0.256±0.009 for *R*(−) and 0.221±0.012 for *S*(+) with minimal intercept values of −0.004 and −0.005 for *R*(−) and *S*(+), respectively. The mean intra-day accuracy defined as mean per cent deviation from nominal value and the mean R.S.D. for precision are listed in Table 1. The inter-day accuracy and mean R.S.D. values obtained over a one month period are presented in Table 2.

The overall reproducibility and ruggedness of the developed method based on the precision (%R.S.D.) ranging from 1.97 to 6.06% and an accuracy of more than 93% (0.2 to 4.0 μg/ml) are acceptable levels.

3.4. Application of method to pharmacokinetics studies

The method has been applied successfully to the evaluation of pharmacokinetics of ketoprofen enantiomers in healthy human volunteers. Two human volunteers, who gave their informed consent, received a single dose of a 200-mg sustained-release

Table 1
Intra-day precision and accuracy for ketoprofen enantiomers in plasma (n=6)

Actual concentration (μg/ml)	Precision				Accuracy, mean deviation from nominal (%)	
	Mean calculated concentration (μg/ml)		R.S.D. (%)			
	R(−)	S(+)	R(−)	S(+)	R(−)	S(+)
0.20	0.20	0.20	2.83	1.06	4.00	1.50
2.00	2.04	2.07	4.18	1.09	1.85	3.42
4.00	4.12	4.14	2.31	0.69	3.12	3.54

Table 2
Inter-day precision and accuracy for ketoprofen enantiomers in plasma (n=14) over a one month period

Actual concentration (μg/ml)	Precision				Accuracy, mean deviation from nominal (%)	
	Mean calculated concentration (μg/ml)		R.S.D. (%)			
	R(−)	S(+)	R(−)	S(+)	R(−)	S(+)
0.20	0.20	0.20	3.95	6.06	1.25	1.36
2.00	1.88	1.87	2.94	2.97	6.11	6.35
4.00	3.76	3.78	2.69	1.97	6.05	5.46

ketoprofen tablet. Blood samples were collected from an antecubital vein at various times following the administration of the drug. The plasma samples were analyzed as described above (Section 2.4). Fig. 2 demonstrates chromatograms of a human plasma sample spiked with 2.50 μg/ml of each ketoprofen enantiomers and a volunteer plasma sample containing 1.40 μg/ml R(−) and 1.36 μg/ml S(+) of ketoprofen enantiomers. The mean plasma concentration profiles for the volunteers are shown in Fig. 3.

The applicability of the method was established by monitoring the plasma ketoprofen levels from a pharmacokinetic study in healthy humans. The method was found free from interference and ketoprofen plasma concentration profiles were in the range reported in the literature [27–31].

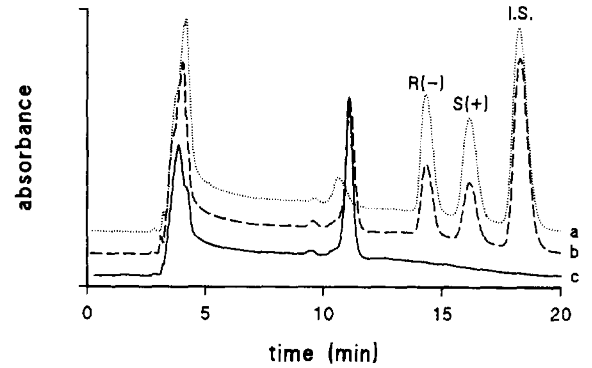

Fig. 2. Chromatograms of extracts from (a) human plasma spiked with 2.5 μg/ml of each ketoprofen enantiomers, (b) volunteer plasma sample [1.40, 1.36 μg/ml for R(−) and S(+) ketoprofen] following the administration of a 200-mg oral dose of ketoprofen sustained-release, (c) volunteer blank plasma sample.

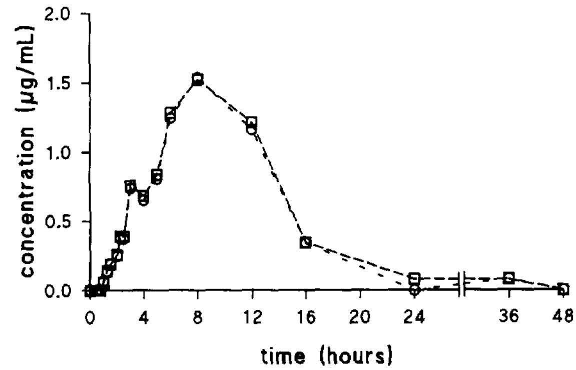

Fig. 3. Mean (n=2) plasma concentration vs. time profile of (□) R(−) and (○) S(+) enantiomers after administration of a 200-mg sustained-release ketoprofen tablet in healthy human volunteers.

4. Conclusion

This new method which has been successfully applied to ketoprofen quantitation in human plasma, allows rapid extraction, baseline separation of enantiomers, eliminates derivatization and reconstitution steps and avoids interconversion. In summary, the method described is simple, reproducible and efficient for determining ketoprofen enantiomers in human plasma samples with good precision and accuracy.

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