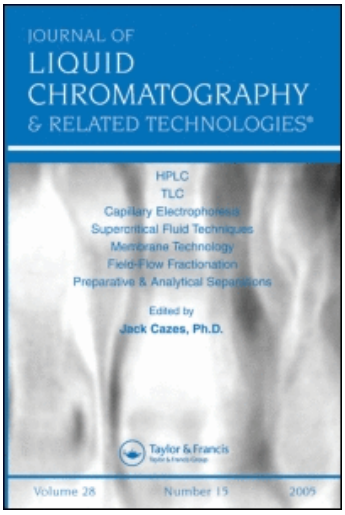

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A SIMPLE METHOD FOR DETERMINATION OF NIMESULIDE IN RAT BLOOD SAMPLES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A rapid and simple method for determination of nimesulide in blood samples, by high-performance liquid chromatography, was developed. This method includes a single extraction of acidified blood (1N hydrochloric acid) with diethyl ether. Blood extracts were analyzed on a reversed-phase column eluted with a mixture of methanol-0.05 M potassium dihydrogen phosphate solution (pH 5) and detected by absorbance at 290 nm.

Retention times for the internal standard and nimesulide were 4.2 and 8.2 min, respectively. The method was linear in the range of 0.2 to 5 μg/mL and the detection limit of the method was 40 ng/mL. This method is suitable for determination of nimesulide after oral administration, and could be used for pharmacokinetic studies of the drug in the rat using small volume samples.

INTRODUCTION

Nimesulide [4-nitro-2-(phenoxy)methanesulphonanylide] is a non-steroidal anti-inflammatory drug (NSAID) with analgesic, antipyretic, and anti-inflammatory activity.[1-4] This NSAID has been shown to be effective and well tolerated in adult patients with a variety of inflammatory and painful conditions.[2] Nimesulide is one of the newest NSAIDs which acts principally through the selective inhibition of cyclooxygenase 2 or COX-2.[5-6]

Because nimesulide doses are relatively low (50-100 mg, b. i. d.), it is necessary to have sensitive and selective methods for the determination of this drug. To our knowledge, there are several reported methods for the determination of nimesulide.[7-11] In one of them,[7] the sensitivity is not good enough. Moreover, the extraction procedure in those methods is complex and time-consuming. A double extraction with benzene or toluene,[8] or two double extractions with benzene and back-extraction are usually required.[9-11] Therefore, the purpose of this study was to develop a simpler method to detect nimesulide in rat blood samples by high-performance liquid chromatography.

EXPERIMENTAL

Reagents and Solutions

Nimesulide (Figure 1) was provided by Laboratorios Lakeside S. A. (Mexico City). Indomethacin (Figure 1) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Methanol was chromatographic grade (Merck, Darmstadt, Germany). Deionized water was obtained through a Milli-Q system (Continental Water Systems, El Paso, TX, USA). All other reagents were of analytical grade.

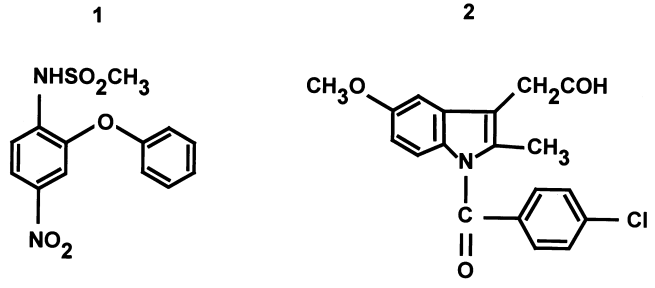

Figure 1. Chemical structure of nimesulide (1) and the internal standard, indomethacin, (2).

Stock solutions of nimesulide corresponding to 1 mg/mL were prepared by dissolving nimesulide or the internal standard (indomethacin) in methanol. Standard solutions were prepared by diluting the stock solution, ranging from 0.2 to 5 μg/mL in mobile phase. A standard solution of internal standard was prepared at a fixed concentration of 10 μg/mL in the mobile phase. The 0.05 M potassium dihydrogen phosphate solution, adjusted to pH 7.4 with 0.1 M sodium hydroxide, and 1 N hydrochloric acid were prepared in deionized water.

Extraction Procedure

Rat blood samples (100 μL) obtained from the caudal artery, by a cannula, were added to 400 μL of 0.05 M potassium phosphate buffer pH 7.4. Samples (0.5 mL) were pipetted into a conical glass tube and were mixed with 1 μg (100 μL of a solution of 10 μg/mL) of indomethacin (internal standard). After addition of 100 μL of 1 N hydrochloric acid, samples were agitated in a Vortex (Supermixer) and extracted with 5 mL of diethyl ether by agitation for 1 minute at maximal speed. The two phases were separated by centrifugation at 3000 g for 5 minutes.

The upper organic layer was transferred to another conical glass tube and evaporated to dryness at 50°C under a gentle nitrogen stream.

The dry residue was redissolved in 250 μL of mobile phase (see below) and 100 μL aliquots were injected into the chromatographic system.

Chromatographic System

The chromatographic system consisted of a Model 501 solvent delivery system (Waters Assoc., Milford, MA, USA), a Model 484 multi-wavelength detector (Waters), a Rheodyne 7725i injector (Cotati, CA) with a loop of 100 μL and a 120 Servogor register (Waters).

Elution of the compounds was performed on a 150 mm x 3.9 mm I.D. reverse-phase Symmetry^{R} C_8 column (Waters), using a mixture of methanol and 0.05 M potassium phosphate buffer pH 5 (57:43%) as the mobile phase at flow rate of 1.8 mL/min. Effluent from the column was detected by absorbance at 290 nm.

All analyses were carried out at room temperature. To prolong the life of the analytical column, a pre-column (25 mm x 3.9 mm ID) containing 37-50 μm Corasil C_{18} (Waters) was incorporated into the system.

Assay Calibration

Calibration of the method was carried out by addition of known amounts of nimesulide and the internal standard to drug-free blood samples that were extracted as described above.

Calibration curves were established in the range of 0.2 to 5 μg/mL. The internal standard was used at a fixed concentration of 10 μg/mL. The actual sample concentrations were calculated by determination of the peak-height ratios of nimesulide and the internal standard (indomethacin).

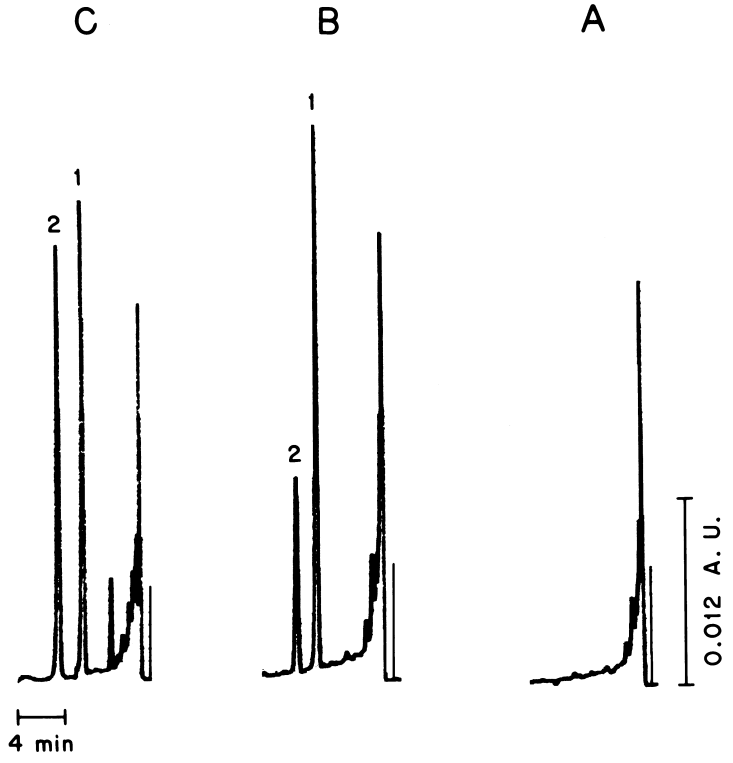

Figure 2. Typical chromatograms resulting of the injection of blood extracts into the chromatographic system. A) Drug-free blood, B) Blood spiked with 1 μg/mL of either internal standard (1) or nimesulide (2), and C) Blood obtained from a rat 12 h after oral administration of 10 mg/kg nimesulide.

RESULTS

Typical chromatograms, obtained after injection of blood extracts into the chromatographic system, are shown in Figure 2. Retention times for the internal standard and nimesulide were 4.2 and 8.2 minutes, respectively. No interfering peaks occurred at these times. Any endogenous contaminants remaining in the extracts were eluted before the internal standard, and samples could be injected immediately after elution of nimesulide.

A linear relationship was obtained when height ratios of nimesulide to internal standard were plotted against nimesulide concentration ranging between 0.2 and 5 μg/mL (r = 0.9995), as shown in Figure 3.

The recovery of nimesulide and the internal standard from blood samples were similar and ranged between 90 and 100%, by comparison of peak heights from blood extracts with those from standard solutions. The intra- and inter-day accuracy and precision was evaluated by sextuplicate analysis of drug-free blood samples to which nimesulide had been added at concentrations of 0.45, 1.5, and 4 μg/mL (Table 1). In both cases, coefficient of variation was between 2.9 and 11.9%. Detection limit of the method was 40 ng/mL.

To evaluate the usefulness of the method for determination of nimesulide after oral administration, a group of 6 rats received 10 mg/kg of nimesulide and blood samples were obtained at selected times. Time course of nimesulide concentrations is shown in Figure 4. Pharmacokinetic parameters

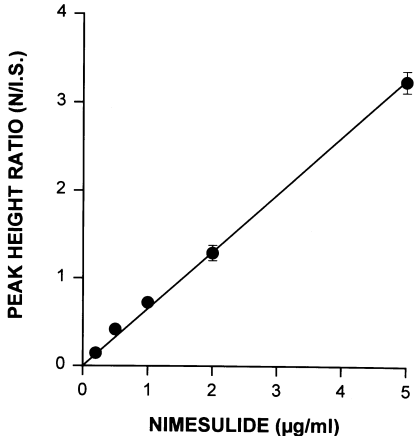

Figure 3. Calibration curves of nimesulide in whole-blood samples established in the range of 0.2 to 5 μg/mL. Data are expressed as mean of 6 determinations ± s. e. m.

Table 1

Accuracy and Precision of the Determination of Nimesulide in Rat Whole Blood Samples*

Added Concentration (μg/mL)	Found Concentration (μg/mL)	Accuracy (%)	Coefficient of Variation (%)
Intra-Day			
0.45	0.42 ± 0.005	94.8	2.9
1.5	1.67 ± 0.051	111.7	6.8
4.0	4.36 ± 0.216	109.2	11.0
Inter-Day			
0.45	0.44 ± 0.021	98.7	9.8
1.5	1.64 ± 0.056	109.4	6.9
4.0	4.07 ± 0.243	101.9	11.9

* Data are presented as the mean (n = 6) ± s. e. m.

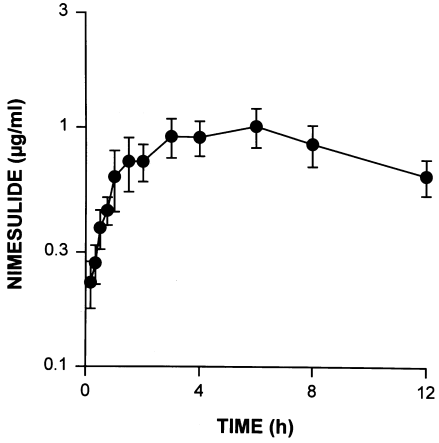

Figure 4. Time course of nimesulide blood levels in rats that received a single oral dose of 10 mg/kg of nimesulide. Data are presented as the mean of six rats ± s. e. m.

obtained in those rats were maximal concentration (C_{max}) of 1.2 ± 0.2 μg/mL, time to reach C_{max} (t_{max}) 5.2 ± 1 h and area under the blood level against-time curve (AUC) of 8.6 ± 1.2 μg.h/mL.

DISCUSSION

A new method for determination of nimesulide in blood samples has been developed. This method is more simple and rapid than those previously reported by HPLC or thin-layer chromatography,[7-11] since a single extraction with diethyl ether is used. In contrast to previous procedures, this method uses small volume samples (100 μL). Additionally, it is more sensitive than those reported previously, allowing us to detect 40 ng/mL. As the method is rapid and simple, over 40 samples can be analyzed by one analyst in a working day. Results of this study show that the method reported here is sensitive and selective for nimesulide determination in whole-blood samples. It is economical, as only one extraction is needed. Moreover, the method was sensitive enough and useful for determination of nimesulide in whole blood rat micro-samples.

In conclusion, the method reported here is suitable to carry out pharmacokinetic studies in rats with the use of small volume samples.

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