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Short communication

Rapid and sensitive method for determination of nimesulide in human plasma by high-performance liquid chromatography

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Abstract

A rapid and sensitive high-performance liquid chromatographic method for determination of nimesulide in human plasma has been developed. The chromatographic system uses a reversed-phase C_{18} column with UV–Vis detection at 230 nm. Mobile-phase consisted of phosphate buffer (pH 5.5)–methanol–acetonitrile (50:20:30, v/v) at a flow-rate of 1.4 ml/min. Nimesulide was extracted in a single step into dichloromethane.The overall mean extraction recoveries were above 98% for both inter- and intra-assay reproducibility,with CVs from 0.3 to 1%. The calibration curve was linear in the concentration range of 0.05–5 μg/ml, and the lower limit of detection was 30 ng/ml. This simple and sensitive method allows for determination of the range of plasma concentrations that is observed after administration of clinically relevant doses of nimesulide.

Keywords: Nimesulide

1. Introduction

Nimesulide (4-nitro-2-phenoxymethanesulfonanilide) is a compound with potent anti-inflammatory, antipyretic and analgesic properties that does not induce gastrointestinal ulceration [1–3]. The clinical pharmacodynamics and the therapeutic potential of nimesulide have been extensively studied [4]. The mechanism of action has been extensively investigated and involves interference with the production/action of mediators other than PGs such as enzymes, toxic oxygen derivatives, platelet-activating factor (PAF) and histamine [5].

Although high-performance liquid chromatographic methods have already been reported [6,7], a new method has been developed in order to obtain greater sensitivity. Since the pharmacologically effective dose of nimesulide for animal and therapeutic dose projected for humans are relatively low, a sensitive and specific plasma level method is needed to follow the pharmacokinetics of the drug. The objective of the present work was to develop a rapid and sensitive high-performance liquid chromatography (HPLC) method for determination of nimesulide in human plasma which can readily be utilized for routine pharmacokinetic studies.

2. Experimental

2.1. Chemicals and solvents

Sodium hydroxide and sodium dihydrogenortho-

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phosphate anhydrous purified were obtained from Rasayan Labs. (Mumbai, India). Methanol and acetonitrile of HPLC grade were purchased from Ranbaxy (Mumbai, India). Nimesulide was a generous gift sample from Recon (Bangalore, India). Cellulose nitrate filter (0.2 μm pore size) was purchased from Sartorius (Germany).

2.2. HPLC system

The HPLC system consisted of a delivery pump (IRICA, Model-871, Kyoto, Japan), a reversed-phase analytical column ODS, 5 μm, 250×4.6 mm I.D. (Zorbax, Tokyo, Japan) protected by a guard column ODS, 5 μm, 100×4.6 mm I.D. (Zorbax), an integrator (chromatocorder 11, System Instruments, Tokyo, Japan), a Rheodyne sample injector with a 100-μl loop volume (Model 7125,USA), and a variable-wavelength (UV–Vis) detector (Model ERC-8710, Tokyo, Japan).

2.3. Chromatographic conditions

The mobile phase consisted of phosphate buffer (pH 5.5)–methanol–acetonitrile (50:20:30, v/v). The solution was filtered through a (0.2-μm membrane filter, Sartorius). The eluent was monitored with a UV–Vis detector set at 230 nm with a flow-rate of 1.4 ml/min. Mobile phase was stirred on a magnetic stirrer during the HPLC run.

2.4. Extraction procedure

To a 1-ml volume of blank human plasma placed in a 5-ml centrifuge tube was added a known quantity of nimesulide working standard solution. The contents were mixed well. Dichloromethane (2 ml) was then added to each tube, and the tubes centrifuged for 10 min. Following centrifugation, 1.8 ml of organic phase was transferred to a clean tube and evaporated to dryness in a water bath at 50–60°C under a gentle stream of nitrogen. The residue was redissolved in 120 μl of mobile phase and a 100-μl aliquot was injected into the HPLC column.

2.5. Standard solution and calibration curve

A standard stock solution of nimesulide (1.0 mg/ml) was prepared in methanol. Subsequent dilutions were made in distilled water to give the following concentrations: 0.05, 0.1, 0.5, 1.5, 2.5 and 5 μg/ml. Blank human plasma samples were prepared to contain nimesulide at concentrations of 0.05, 0.1, 0.5, 1.5, 2.5 and 5 μg/ml. The samples were extracted in the same manner as described above (Section 2.4). The calibration curve was obtained by plotting the peak height versus nimesulide concentration.

2.6. Validation of the assay

The precision of the method was determined by replicate analysis ($n=5$) of human drug-free plasma spiked with nimesulide standard concentrations of 0.05, 0.1, 0.5, 1.5, 2.5 and 5 μg/ml. The linearity of the standard curve was confirmed by plotting the peak height versus nimesulide concentration, prepared over a range of 0.05–5 μg/ml. Linear regression analysis was performed to calculate the slope, the intercept and the correlation coefficient (r) of the calibration curve. The extraction efficiency (recovery) was calculated as follows: we compared the peak height of drug-free plasma spiked with a known amount of drug submitted to the sample extraction procedures with the peak height of directly injected standards in water.

2.7. Stability and robustness

The stability of nimesulide in plasma and sample extracts were studied. Spiked plasma samples stored at −10°C were stable for a period of three months. The concentration of storage sample compared with the initial concentration showed no change when stored at −10°C. All stock solutions of standards were stable at 2–8°C during the three-month period. Sample extracts reconstituted in mobile phase and stored at room temperature (up to 16 h) showed excellent stability. Slight variation in ambient temperature did not significantly affect the column efficiency.

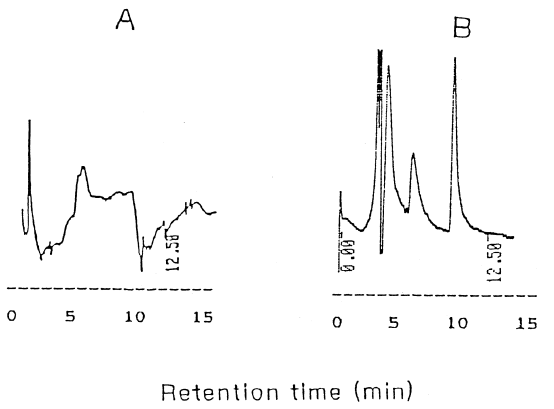

Fig. 1. HPLC profiles of (A) blank human plasma and (B) human plasma spiked with 0.1 μg/ml nimesulide.

3. Results and discussion

Fig. 1 shows typical chromatograms of extracted samples of human plasma (A), plasma spiked with nimesulide equivalent to 0.1 μg/ml (B). The retention time of nimesulide was approximately 12 min. No interfering peaks from endogenous materials in the plasma were found at the retention time. A sharp peak corresponding to nimesulide was clear on the chromatogram with stable retention time, therefore, we did not use an internal standard. The overall extraction recovery ±SD (n=5) from human drug-free plasma spiked with known amounts of the drug was found to be above 98% which was determined by comparing the peak height of directly injected standards in water with peak height of the respective drug after extraction from spiked plasma samples. The calibration curve was linear in the range tested, 0.05–5 μg/ml with correlation coefficient >0.997. Plasma samples were stable for a period of three months when stored at −10°C. Under the described experimental conditions, the lower limit of detection was 30 ng/ml.

Percentage recovery and the precision of the method were determined by processing spiked plasma samples. Five replicate samples were run for each concentration. The results are given in Table 1. Although analytical methods for the quantitation of nimesulide in human plasma by HPLC have been briefly described in some clinical reports [6,7], these methods either involve extensive sample preparation with large amounts of extraction solvents, or give minimal validation data. The lower limit of detection in our assay was 30 ng/ml which is more sensitive than previously reported methods [6,7]. The extraction was carried out in a single step with extraction recoveries above 98%, with minimal reagent volumes without affecting the validation parameters.

In conclusion, our assay is rapid, sensitive, reproducible and well suited to routine measurements.

4. Application

This method can be applied to the determination of nimesulide in animal and human plasma after administration of the drug in different dosage forms. The rapidity of the extraction procedure makes it possible to analyze large numbers of clinical samples within a short period of time.

Table 1
Intra- and inter-assay reproducibility of replicates at two nimesulide concentrations (μg/ml) in human plasma by HPLC

Assay	Amount added (μg/ml)	Amount found[a] (μg/ml)	Recovery[a] (%)	CV (%)
Intra-day	0.10	0.098	98.8±1.09	1.02
	2.50	2.51	100.6±0.45	0.43
Inter-day[b]	0.10	0.099	99.4±0.89	0.7
	2.50	2.53	101.4±0.21	0.3

[a] Mean±SD (n=5).
[b] Over a period of six days.

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