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Determination of naftopidil enantiomers in rat plasma using chiral solid phases and pre-column derivatization high-performance liquid chromatography

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ABSTRACT

Two bioanalytical HPLC methods (chiral solid phases (CSPs) HPLC and pre-column derivatization HPLC) were developed and validated for the determination of naftopidil enantiomers in rat plasma. Analytes were extracted from biomaterials by liquid–liquid extraction. The pre-column derivatization HPLC method employed (+)-diacetyl-L-tartaric anhydride (DATAN) as the pre-column derivatization reagent, and subsequent separation of diastereomers was conducted on an Agilent Hypersil ODS column with a mixture of methanol–acetonitrile–phosphate buffer (pH 4.1; 20 mM) (40:30:30, v/v/v) flowing at 1 mL/min as the mobile phase. The CSPs HPLC method utilized a Chiralpak IA column with a mobile phase of methanol–acetonitrile–acetate buffer (pH 5.3; 5 mM) (50:25:25, v/v/v) flowing at 0.5 mL/min. In both methods, the analytes were monitored using a fluorescence detector with an excitation wavelength of 290 nm and an emission wavelength of 340 nm. Both methods were consistent (RSD < 15% by the derivatization method and < 10% by the CSPs method) and linear (r > 9950). Compared to the pre-column derivatization method, the CSPs method had lower quantification limits (10.6/9.6 ng/mL of (+)-/(−)-naftopidil by derivatization method and 1.1/1.8 ng/mL of (+)-/(−)-naftopidil by CSPs method), and was simpler to carry out. The validated CSPs method was successfully applied in a pharmacokinetic study of naftopidil enantiomers in rats, which showed that pharmacokinetic parameters of (+)- and (−)-NAF after intravenous administration of (±)-NAF were similar.

1. Introduction

Benign prostatic hyperplasia (BPH) occurs commonly in aging men and can lead to lower urinary tract symptoms (LUTSs). BPH is commonly treated with α-blockers, which reduce urethral resistance caused by smooth muscle overactivity [1,2]. Naftopidil (NAF), (±)-1-[4-(2-methoxyphenyl)-1-piperazinyl]-3-(1-naphthoxy)-2-proanol (1) (Fig. 1), a α-blocker, is utilized extensively for the treatment of benign prostatic hypertrophy (BPH) [3,4]. Kanda et al. [5] also reported the possibility of using naftopidil in the chemoprevention of prostate cancer and the intervention of hormone refractory prostate cancer. It was recently discovered that naftopidil has a high affinity for α_{1D}-adrenoceptor, which is mainly distributed in the central nervous system and bladder smooth muscle, and has a low affinity for α_{1A}- and α_{1B}-adrenoceptor [6]. The selectivity of naftopidil for inhibition of prostatic pressure and improvement of collecting disorders in BPH patients may be attributed to its high binding affinity for α_{1D}-adtrenoceptor subtypes [7,8].

Naftopidil is a chiral compound with one asymmetric carbon, but it is used as a racemic mixture. The FDA's policy for the development of chiral drugs stipulates that when stereoisomers are biologically distinguishable, they may appear to be different drugs, even it has been past practice to develop racemates [9]. However, very few studies have addressed the biological recognition of naftopidil enantiomers. All pharmacokinetic studies of naftopidil were performed on its racemic form [10–12], so the trait of each enantiomer and interactions between the enantiomers are not known. Enantioselective quantitative assays of *in vivo* samples are necessary to assess the potential for interconversion, absorption, distribution, biotransformation, and excretion profiles of individual enantiomers.

Stereoisomer separation of chemical pure naftopidil has been achieved only in the normal-phase mode using Chiralpak AD-H and Chiralcel OD [13,14]. However, none of the existing enantioselective methods have worked for the analysis of naftopidil in biological materials. Using Chiralcel OD, Aboul-Enein et al. (1995) separated chemical pure naftopidil but without validation, while Sun et al. (2009) achieved resolution of naftopidil enantiomers using Chiralpak AD-H but with high quantitation limits (780 ng/mL and

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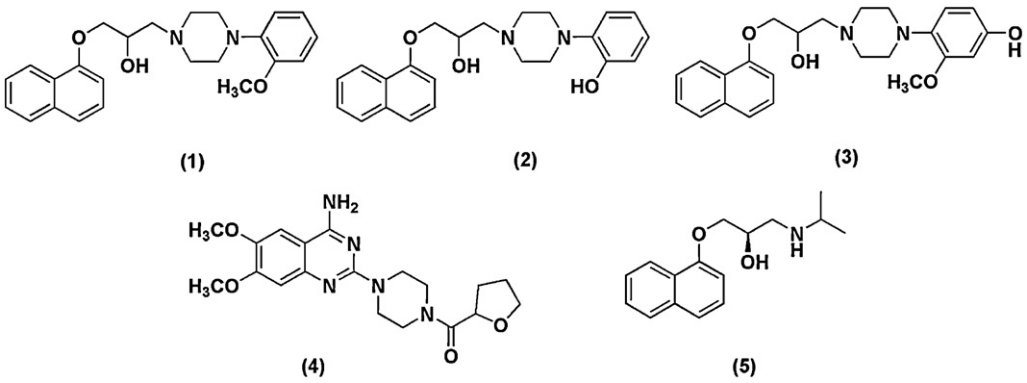

Fig. 1. Chemical structure of naftopidil (1), (±)-O-desmethyl naftopidil (2), (±)-hydroxy naftopidil (3), terazosin (4) and (+)-propranolol (5).

840 ng/mL). Since absolute bioavailability of racemic naftopidil has been shown to be very low (9%) [15], increasing the quantitation limits is the most critical requirement for bioanalysis of naftopidil enantiomers. Furthermore, reversed phase mode is more suitable compared to normal phase mode for complicated bioanalysis for two major reasons. One is that the reversed phase mode can quickly flush interferences with strong polarity existing in biomaterials. The other is that the reversed phase method employs more flexible mobile phase, which can be adjusted to meet complicated requirements of bioanalysis by changing the type, concentration and pH value of aqueous phase. Therefore, a sensitive reversed phase chiral method would be suitable for bioanalysis of naftopidil enantiomers.

The aim of this study was to develop methods for the simultaneous detection of naftopidil enantiomers in plasma samples by HPLC. There are two commonly used chiral HPLC methods for separating stereoisomers. One is the chiral solid phases (CSPs) HPLC method, which is simple, stable and based on a chiral solid phase, while the other involves a pre-column derivatization based on a chiral derivatization agent and ordinary achiral column. Here, we developed and validated both of these RP-HPLC methods to analyze naftopidil enantiomers in rat plasma, and chose one with simpler process and better validation parameters to apply in an enantioselective pharmacokinetic study of naftopidil in rats.

2. Materials and methods

2.1. Chemicals

(±)-NAF, (+)-NAF (ee purity > 99.5%), (−)-NAF (ee purity > 99.5%), (±)-DMN and (±)-PHN were obtained from Boehringer Mannheim (Ingelheim, Germany). Terazosin (I.S. for the CSPs method) (4) and (+)-propranolol (I.S. for the derivatization method) (5) (Fig. 1) were purchased from the Guangzhou Institute for Drug Control. Methanol (MeOH), acetonitrile (ACN), dichloromethane (CH_2Cl_2), ethyl acetate (AcOEt), acetic acid (AcOH) and trichloroacetic acid (TCA) of HPLC grade were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Mobile phases were filtered through a 0.45 μm PALL-GHP filter (Washington, NY, USA). Stock solutions of (±)-NAF, (+)-NAF, (−)-NAF, (±)DMN, (±)-PHN and I.S. were prepared by dissolving 10 mg compounds in 10.0 mL of methanol and were stored at −20 °C. (+)-Diacetyl-L-tartaric anhydride (DATAN, 97% purity) was purchased from J&K Scientific (Guangzhou, China). The working solution was prepared daily by dissolving DATAN in an 8:2 mixture of methylene dichloride and acetic acid. Drug-free rat plasma were collected from healthy Sprague-Dawley rats of both sexes and stored at −20 °C. Ultrapure water (18.2 MΩ cm) was prepared using a Millipore water-purification system (Millipore, MA, USA).

2.2. Animals

Sprague-Dawley rats, weighing 220–250 g, were obtained from the Guangdong Medical Laboratory Animal Center (Guangzhou, China). Prior to the administration of the drug, animals were fasted overnight with water available *ad libitum*. The handling and treatments of all animals used in this study were in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC). The animal use and care protocol was reviewed and approved by the ethics committee of the Guangzhou Medical College.

2.3. Drug administration and sampling

Six rats (three females and three males) were intravenously administered a single dose of racemate naftopidil (10 mg/kg). The (±)-NAF powder was dissolved in saline solution containing 10% PEG (polyethylene glycol) 400. Serial blood samples (approximately 0.3 mL) were collected from the jugular veins of the subjects at 0, 5, 10 and 30 min and 1, 4, and 8 h post-dosing while still anaesthetized. The subjects were anaesthetized by injecting a combination of pentobarbital sodium (30 mg/kg) and 0.002% buprenorphine hydrochloride (0.05 mg/kg), an analgesic. We then centrifuged the blood at 13,000 × *g* for 3 min and isolated the plasma. All samples were stored at −20 °C until they were analyzed.

2.4. Plasma sample treatment

After spiking with the internal standard solution (10 μL), 100 μL rat plasma samples were alkalinized with 100 μL of 0.1 M aqueous sodium carbonate solution, then extracted with 300 μL ethyl acetate by vortex-mixing for 5 min. After centrifugation at 13,000 × *g* for 5 min and separation of the organic phase, the combined extracts were evaporated to dryness under nitrogen flow. For the CSPs method, the residues were dissolved directly in 200 μL MeOH and analyzed immediately following preparation.

2.5. Derivatization procedures

The residue obtained from the extraction procedure was dissolved in 200 μL of CH_2Cl_2, then 100 μL TCA (0.01 M) and 100 μL derivatization agents (DATAN dissolved in a mixture of methylene dichloride–acetic acid (8:2, v/v) to achieve a final concentration of 0.1 M) were added. The derivatization reaction was carried out for 3.5 h at 45 °C. Following that, the mixture was evaporated under nitrogen and reconstituted in 200 μL methanol. Samples were analyzed immediately following preparation.