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Chiral liquid chromatography–tandem mass spectrometry assay to determine that dexpramipexole is not converted to pramipexole *in vivo* after administered in humans

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ABSTRACT

Dexpramipexole (DEX) was being investigated in clinical studies for the treatment of amyotrophic lateral sclerosis (ALS). To monitor the potential chiral interconversion of dexpramipexole to pramipexole (PPX) *in vivo*, a highly sensitive and selective chiral LC–MS/MS assay was developed and qualified for the detection of pramipexole in the presence of dexpramipexole in human plasma. In this assay, plasma samples were extracted by protein precipitation coupled with solid phase extraction (SPE). The analyte PPX was separated from its enantiomer DEX using a chiral HPLC method. The assay was qualified with a dynamic range of 0.150–1.00 ng/mL. The lower limit of quantitation (LLOQ) for PPX was 0.150 ng/mL in the presence of up to 1000 ng/mL of DEX. The qualified method was used to analyze plasma samples from a DEX clinical study. No PPX was detected in humans at pharmacologically significant levels after administration of dexpramipexole at single doses up to 600 mg per day.

1. Introduction

The enantiomers of chiral molecules often possess distinct pharmacological activities and potencies. In such cases, optically pure compounds are preferred for development as new therapeutic candidates. During drug development, it is critical to monitor the optical purity of the compounds being dosed and to assess exposure to alternate enantiomers *in vivo* after dosing. Dexpramipexole (6,7-tetrahydro-N6-propyl-2,6-benzothiazolediamine dihydrochloride monohydrate, Fig. 1, DEX) is a novel amino-benzothiazole that has shown neuroprotective properties *in vitro* and in *vivo* [1–8]. The molecule was an investigational compound that had been studied in clinical trials for the treatment of amyotrophic lateral sclerosis (ALS), or Lou Gehrig's disease but was recently found lacking of efficacy. DEX is the R-(+) enantiomer of pramipexole (PPX, Fig. 1), a non-ergoline dopamine agonist (Mirapex®) marketed for the treatment of Parkinson's disease and restless legs syndrome. Although DEX and PPX may share neuroprotective properties [9,10], DEX is pharmacologically distinct from PPX in that DEX has minimal affinity to dopaminergic receptors. For safety considerations, it is important to exclude the possibility of dopaminergic side effects such as hypotension and seizures due to exposure of PPX from *in vivo* enantiomer conversion. It is therefore critical to demonstrate that after oral administration of DEX in humans, there is no circulating PPX at a pharmacologically significant level. To monitor PPX exposure in humans, a highly sensitive and selective chiral assay is necessary to accurately quantify PPX in the presence of DEX in human plasma.

A variety of bioanalytical methods were developed and validated to measure pramipexole in human plasma and urine using HPLC-UV, HPLC-electrochemical detection [11,12], and LC–MS/MS instrumentation [13–16]. The lower limit of quantitation (LLOQ) of these methods ranged from 0.02–0.2 ng/mL for plasma, to 10 ng/mL for urine, respectively. LC–MS/MS assays were validated to measure dexpramipexole in human plasma and urine [5]. The lower limits of quantitation of these LC–MS/MS methods were 2–20 ng/mL in plasma and 150 ng/mL in urine, respectively. Chiral methods have been developed to separate PPX from DEX using chiral HPLC-UV or electrophoresis-UV instrumentation [17–19]. However these methods were designed to assess or remove the chiral impurities in drug material or tablets dissolved in solvents. The lower limit of quantitation for these chiral methods ranged from 300 to 3000 ng/mL. In this study, a novel LC–MS/MS method was developed and qualified to quantitate PPX in the presence of up to 1000 ng/mL of DEX in human plasma with a LLOQ 0.150 ng/mL.

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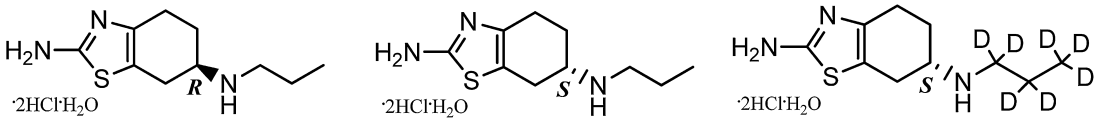

Fig. 1. Chemical structures of dexpramipexole, pramipexole and pramipexole-d7 (internal standard).

Pramipexole (Mirapex®) is given orally at doses of 0.125–1.5 mg per day. At a daily dose of 0.125 mg, a C_{max} of 0.30 ng/mL has been reported for PPX in human plasma [5]. Therefore the LLOQ of the assay is adequate to detect the pharmacologically active level of PPX.

2. Experimental

2.1. Material and reagents

The analyte, pramipexole (purity 99.8%), was purchased from US Pharmacopeia (Rockville, MD). Dexpramipexole (purity 100%, chiral purity >99.95%) and isotopically labeled internal standard pramipexole-d7 (purity 98.2%, PPX-d7 or IS) were manufactured by Biogen Idec. Human plasma with K_2EDTA as the anticoagulant was purchased from Bioreclamation (Hicksville, NY). HPLC-grade hexane, heptane and methanol were purchased from Fisher Scientific (Fair Lawn, NJ). HPLC grade ethanol, diethylamine (DEA), and ammonium hydroxide (33% NH_3) were purchased from Sigma–Aldrich (St. Louis, MO). Formic acid (purity 99.8%) was obtained from ACROS Organics (Fair Lawn, NJ). Deionized water was obtained through a Milli-Q water purification device (Millipore, Billerica, MA).

2.2. Preparation of standards and quality control samples

A summary of calibration standards (STDs) and quality control samples (QCs) used for the assay is provided in Table 1. The calibration range of the method was pramipexole concentrations of 0.150–1.00 ng/mL in the presence of 1000 ng/mL dexpramipexole in human plasma. The lower limit of quantitation (LLOQ) for this assay was 0.150 ng/mL and the upper limit of quantitation (ULOQ) was 1.00 ng/mL, both in the presence of DEX up to 1000 ng/mL. Stock solutions of PPX (analyte), PPX-d7 (internal standard), and DEX were prepared by accurately weighing the compounds and then dissolving in HPLC-grade water to the concentration of 1.00 mg/mL corrected by salt, water content and total purity (including chiral purity). Calibration standard and quality control samples were manually prepared *via* serial dilution using pooled blank human plasma. The calibration standards were prepared by spiking both PPX (concentrations of 0.00, 0.150, 0.200, 0.300, 0.500. 0.750, and 1.00 ng/mL) and DEX (1000 ng/mL). Three sets of QC samples were prepared. The spiked PPX concentrations in these QC samples were 0.00, 0.150, 0.300, and 0.800 ng/mL, while the spiked DEX concentrations were 0, 500, or 1000 ng/mL. A dilution QC sample was prepared containing spiked PPX and DEX concentrations of 1.60 ng/mL and 2000 ng/mL, respectively. The QC samples were prepared in a large pool, aliquoted into cryovials, and stored at $-70 \pm 10\,^{\circ}C$ until use. Calibration standards were prepared fresh daily.

Table 1
Calibration standards and spiked quality control samples in human plasma.

Quality controls	Dexpramipexole concentration (ng/mL)	Pramipexole concentration (ng/mL)
Standards		
1	1000	1.00
2	1000	0.750
3	1000	0.500
4	1000	0.300
5	1000	0.200
6	1000	0.150
Quality controls		
HQC-1	1000	0.800
MQC-1	1000	0.300
LQC-1	1000	0.150
Blank-1	1000	0.000
HQC-2	500	0.800
MQC-2	500	0.300
LQC-2	500	0.150
Blank-2	500	0.000
LQC-3	0	0.150
QCD	2000	0.800

2.3. Sample preparation

Plasma samples were extracted in two steps: protein precipitation extraction (PPE) by acetonitrile followed by solid phase extraction (SPE). A volume of 500 μL of plasma samples was used for sample extraction. Calibration standards, QCs, blanks and double blanks were aliquoted (500 μL) into corresponding clean polypropylene Eppendorf tubes (2.2 mL size, Fisher Scientific). A volume of 30 μL internal standard working solution (10.0 ng/mL of PPX-d7 in water) was added into each tube, except for the double blank, in which 30 μL of water was added. Then a volume of 1500 μL of acetonitrile with 0.1% formic acid was added. The tubes were capped and vortexed for 5 min. A centrifugation step was applied for 10 min at 14,000 rpm. The supernatant was transferred into a clean 15 mL test tube using a transfer pipette. HPLC grade water was added into each tube to a total volume of approximately 4 mL. The tubes were centrifuged for 10 min at 3400 rpm. The supernatants were transferred to a clean set of tubes for solid phase extraction.

A cation exchange solid phase extraction 96-well plate, Oasis MCX μElution (Waters, Milford, MA) was used for the SPE procedure. The SPE plate was placed on the vacuum manifold. The SPE plate was first conditioned with 600 μL methanol, then equilibrated with 600 μL of water. Solvents were drawn through the wells using a vacuum at a minimum of 10″ Hg height. The sample supernatants from the previous PPE step were loaded into the SPE wells, up to 600 μL each time, until all the volume was loaded. The SPE wells were washed with 600 μL HPLC-grade water containing 2% formic acid v/v, followed by 600 μL of 50% methanol in water. The analyte was eluted twice with 100 μL of acetonitrile containing 20% ammonium hydroxide. The eluent was collected in an injection plate, dried down under a nitrogen flow, and reconstituted with 100 μL heptane/ethanol (80:20, 0.1% DEA). The plate was covered, mixed well, and submitted to a high performance liquid chromatography (HPLC) tandem mass spectrometric system (LC–MS/MS). A volume of 40 μL was injected.

2.4. Liquid chromatography–mass spectrometry

The HPLC tandem mass spectrometric system consisted of an AB Sciex API 5500 Triple Quadruple mass spectrometer (AB Sciex, Foster City, CA); and a Shimadzu LC20-ADXR Prominence UFLCXR