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Validated UHPLC–MS/MS method for the simultaneous determination of pramipexole and ropinirole in plasma of patients with Parkinson's disease

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ABSTRACT

A simple and validated ultra high pressure liquid chromatography–tandem mass spectrometry method was developed for the simultaneous determination of the dopaminergic agents pramipexole and ropinirole in plasma of patients with Parkinson's disease. Following liquid–liquid extraction with *tert*-butyl methyl ether from 250 μL plasma, the separation of the analytes was achieved on a Gemini NX3 column using 10 mM pH 6.0 ammonium formate and 10 mM ammonium formate in methanol as binary gradient mobile phase at a flow rate of 0.3 mL/min. The MS/MS ion transitions were 212.10 → 153.03 for pramipexole, 261.2 → 114.2 for ropinirole and 256.1 → 211 for the internal standard (lamotrigine). The lower limit of quantitation (LLOQ) for both analytes was 80 pg/mL and the linearity was determined from 80 to 4000 pg/mL for pramipexole and from 200 to 10000 pg/mL for ropinirole. Mean recoveries were 94% for PRA and 73% for ROP. Both intra- and inter-assay precision and accuracy were ≤20% at LLOQ concentration and ≤15% at other concentrations. The proposed validated method was successfully applied to measure plasma concentrations of pramipexole and ropinirole in a series of patients with Parkinson's disease on chronic treatment. By grouping the two dopaminergic agents in the same assay, the method allows a large series of patient samples to be processed in a single analytical session.

1. Introduction

Pramipexole [(6*S*)-6-*N*-propyl-4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-diamine] (PRA) and ropinirole {4-[2-(dipropylamino) ethyl]-1,3-dihydroindol-2-one} (ROP) are non-ergoline oral dopaminergic agonists (DA) used as first line drugs to treat Parkinson's disease (PD) and restless legs syndrome (RLS) [1]. Post-marketing experience has disclosed a series of central and peripheral possibly dose-related adverse reactions, including impulse control disorders (compulsive gambling, buying, and sexual behavior) [2] and risk of heart failure with PRA [3], which can hamper the use of these agents. Clinical pharmacokinetics of both compounds is poorly characterized in patients with PD [4]; as a result, optimal drug dosage is still tailored to patients on a trial-and–error basis. Data from healthy volunteers showed that renal clearance accounts for approximately 80% of oral PRA clearance and correlates with creatinine clearance [4]. Factors such as renal function, sex and age can significantly affect PRA oral clearance [1–4]. In vitro metabolic studies have shown that ROP undergoes extensive (>90%) oxidative metabolism in the liver with the primary mediation of the CYP1A2 isoenzyme [4]. ROP bioavailability is approximately 50%, with an important interindividual variability [5]. Few methods for the determination of PRA [6–8] and ROP [9–11] concentrations in human plasma have been published so far, mostly based on LC–MS/MS coupled with liquid–liquid extraction (LLE) (6,8), solid phase extraction (7,9,11) or protein precipitation (10) and applied to pharmacokinetic studies in pre-clinical trials.

Here we describe a UHPLC–MS/MS method with LLE sample pretreatment for the simultaneous determination of both PRA and ROP in human plasma, developed and validated for application to clinical pharmacological investigations in patients with PD and addressed to a therapeutic drug monitoring (TDM) setting.

Abbreviations: PRA, pramipexole; ROP, ropinirole; IS, internal standard; DA, dopaminergic agonists; PD, Parkinson's disease; UHPLC–MS/MS, ultra high performance liquid chromatography-tandem mass spectrometry; LLE, liquid–liquid extraction; TDM, therapeutic drug monitoring; LLOD, lower limit of detection; LLOQ, lower limit of quantification; QC, quality control; SRM, selected reaction monitoring.

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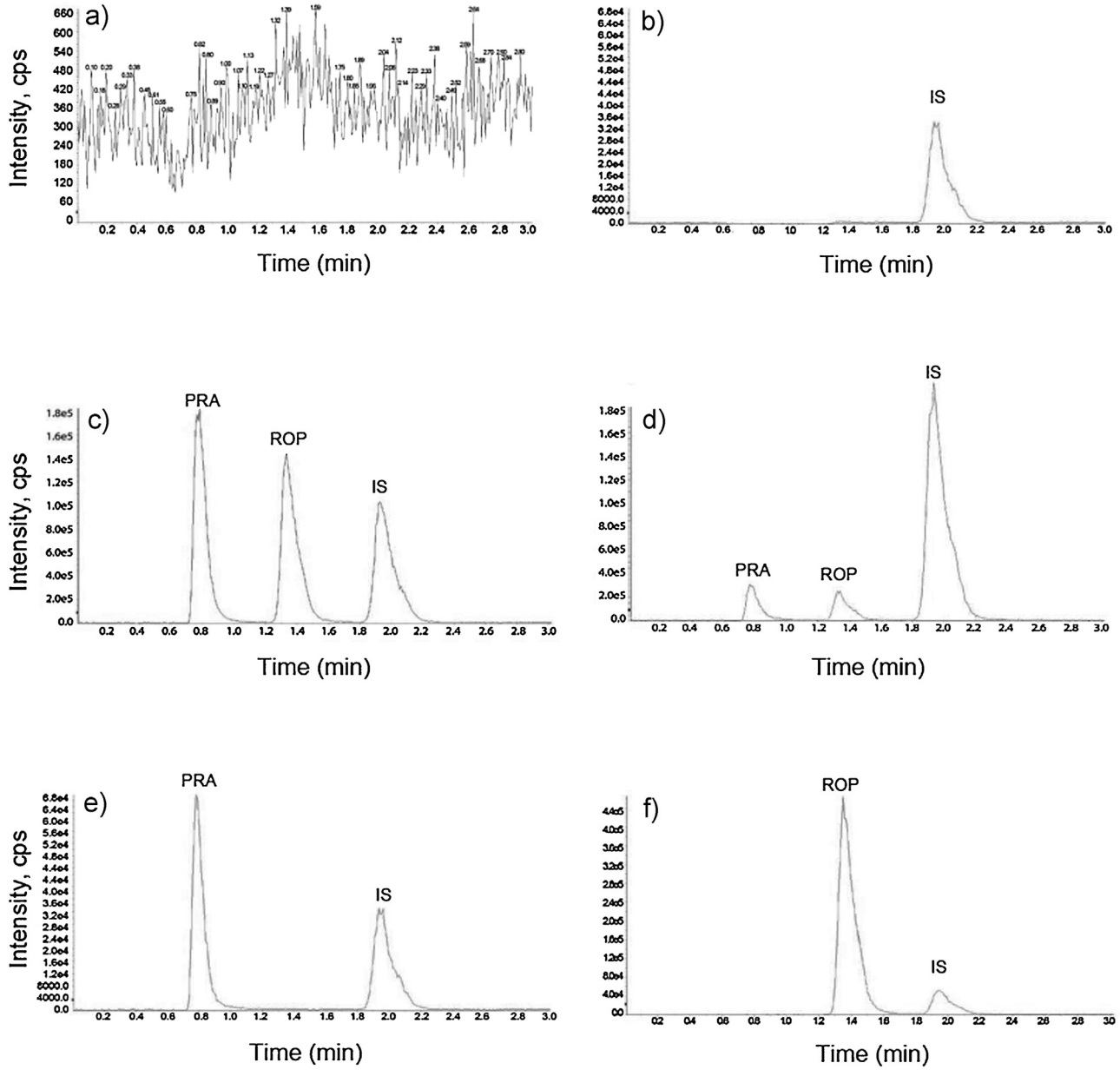

Fig. 1. Chromatograms obtained by injecting 5 μL of (a) extracted blank plasma; (b) extracted blank plasma spiked with I.S. (50 ng/mL); (c) mix standard solution in 10 mM, pH 6.0 ammonium formate of PRA, 1000 pg/mL, ROP, 1000 pg/mL and I.S., 50 ng/mL; (d) extracted blank plasma spiked with PRA and ROP at LLOQ (80 pg/mL) and I.S., (50 ng/mL); (e) extracted plasma specimen of a PD patient treated with PRA (1.05 mg/day): PRA, 1526.7 pg/mL; (f) extracted plasma specimen of a PD patient treated with ROP (16 mg/day): 8481.5 pg/mL. PRA, pramipexole; ROP, ropinirole; I.S., internal standard.

2. Experimental

2.1. Reagents and standards

Pramipexole dihydrochloride, ropinirole hydrochloride, internal standard (I.S.) lamotrigine, ammonium formate, *tert*-butyl methyl ether and sodium hydroxide (NaOH) were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade acetonitrile and methanol were purchased from Merck Millipore (Darmstadt, Germany). Ultrapure water was obtained from a MilliQ Gradient A10 apparatus (Merck Millipore).

Frozen, drug-free plasma (blank plasma) for the preparation of calibrators and the assessment of method selectivity was obtained from the blood bank of the Maggiore Hospital of Bologna, stored at −20 °C and thawed at room temperature before use.

Stock solutions (1 mg/mL) and subsequent dilutions (500 ng/mL, 100 ng/mL, 10 ng/mL working solutions) of PRA and ROP were prepared by dissolving pure standards in methanol. Internal standard stock solution (1 mg/mL) was prepared by dissolving 10 mg lamotrigine in acetonitrile. All solutions were prepared monthly and stored at 4 °C.

Calibrators at 80, 400, 1000, 2000, 4000 pg/mL for PRA and 200, 1000, 2000, 4000, 10000 pg/mL for ROP were prepared by evaporating to dryness (40 °C), in disposable glass tubes (16 × 100 mm), appropriate volumes of working solutions of the analytes (10 ng/mL and 100 ng/mL) using a RapidVap vacuum evaporator (Labconco, Kansas City, Missouri, USA). Quality controls (QCs) for method validation were similarly prepared using suitable volumes of the same PRA and ROP working solutions, to yield three concentrations (i.e. 80, 1000 and 4000 pg/mL), corresponding to the low, middle and high QCs. Low QCs coincided with the lower limit of quantitation