Journal of Pharmaceutical and Biomedical Analysis 118 (2016) 81–88

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Development and validation of an enantioselective LC–MS/MS method for the analysis of the anthelmintic drug praziquantel and its main metabolite in human plasma, blood and dried blood spots

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ARTICLE INFO

Article history:
Received 13 August 2015
Received in revised form 6 October 2015
Accepted 7 October 2015
Available online 22 October 2015

Keywords:
Praziquantel
R-*trans*-4-OH-PZQ
Enantiomer
Dried blood spot
LC–MS/MS
Pharmacokinetics

ABSTRACT

Praziquantel (PZQ) is the treatment of choice against various trematode and cestode infections. To study the pharmacokinetics of PZQ in patients infected with the liver fluke *Opisthorchis viverrini*, we developed and validated an enantioselective liquid chromatography coupled to tandem mass spectrometry method for the analysis of *R* - and *S* -PZQ and its *R* -*trans*-4-OH-PZQ metabolite in human plasma, blood and dried blood spots (DBS). The analytes were detected in the positive mode using selected reaction monitoring (*R*- and *S*-PZQ: m/z 312.2 → 202.2; *R*-*trans* -4-OH-PZQ: m/z 328.0 → 202.0). Prior to the chiral separation with a cellulose tris(3-chloro-4-methylphenylcarbamate) column, the analytes were purified from matrix contaminants and concentrated on a C-18 trapping column. The analytical range for each PZQ enantiomer was 0.01–2.5 μg/mL, and 0.1–25 μg/mL for the metabolite. The method met the requirements regarding precision (±15%, ±20% at the lower limit of quantification-LLOQ), intra- and inter-assay accuracy (85–115%, 80–120% at LLOQ), and linearity ($R^2 \geq 0.998$). The analytes were stable in stock solutions as well as in plasma, blood and DBS. For DBS, the influences of hematocrit and blood spot size were considered as minor. Our validation results show that the method presented here is precise, accurate and selective, and can be used for pharmacokinetic studies. Moreover, the enantioselective separation was achieved with a run time of 11.5 min and a simple sample processing method.

1. Introduction

Praziquantel (PZQ), a pyrazino-isoquinoline available as a racemic mixture of *R*- and *S*-enantiomers, is the drug of choice for the treatment of most trematode and cestode infections, including the liver fluke *Opisthorchis viverrini*, a parasite affecting more than 8 million people in Southeast Asia. PZQ undergoes an important enantioselective first-pass metabolism in the liver, mainly through CYP450 3A4 [1]. *R*-PZQ is mostly metabolised to *R*-*trans*-4-OH-PZQ in humans or *R*-*cis*-4-OH-PZQ in mice, while *S*-PZQ breaks down into various mono- and di-hydroxy metabolites additionally to *S*-*trans*- and *S*-*cis*-4-OH-PZQ [2–4]. PZQ disposition is heavily dependent on the fasting state or the co-administered food type, as well as on the liver function level [5,6]. Therefore, it is of primordial importance to evaluate the pharmacokinetics of PZQ in patients suffering from worm infections affecting the liver, such as opisthorchiasis and schistosomiasis, and relate treatment efficacy with dosage regimens and plasma concentrations.

The dried blood spotting (DBS) technique is an useful alternative for the collection of pharmacokinetic (PK) blood or plasma samples. In short, blood drops are collected by pricking a patient's finger, deposited on a filter paper and kept dried until analysis. This method provides many advantages over alternative sampling methods: no cold chain is required, handling and storage of samples are simple, and minimal blood withdrawal volumes are needed [7]. The ease of sample collection allows handling a much larger group of patients and performing population PK studies in rural areas without clinical settings. However, DBS concentrations are not necessarily equal to blood or plasma concentrations. For example, the drug concentrations in capillary blood might be different to

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http://dx.doi.org/10.1016/j.jpba.2015.10.011
0731-7085/

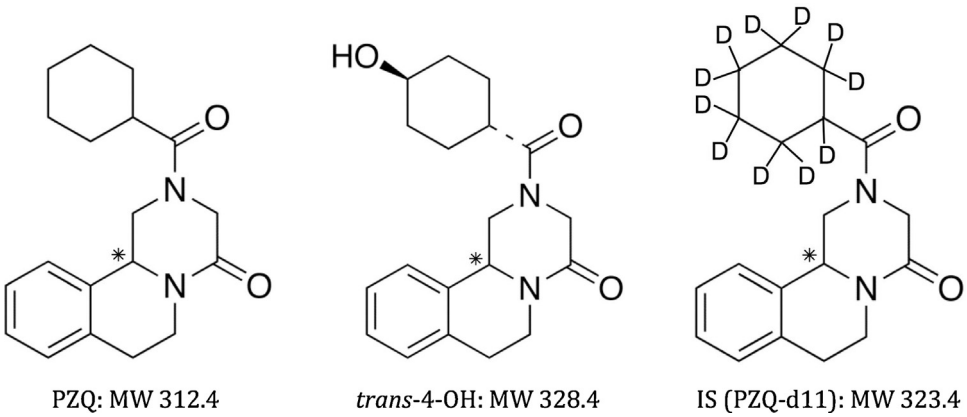

Fig. 1. Chemical structures of PZQ (A), the main metabolite *trans*-4-OH (B) and the internal standard (IS) PZQ-d11 (C), with the chiral centre designated with *.

that in venous blood, or the analyte might display a strong plasma protein binding [8,9]. To date different high-pressure liquid chromatography (HPLC) and liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) methods were published for chiral separation of PZQ enantiomers [10–14], but they focused on the analysis of plasma samples only. The objective of this study was to develop a rapid, precise and accurate LC–MS/MS method to analyze *R*-PZQ, *S*-PZQ and *R-trans*-4-OH-PZQ metabolite in human plasma, blood and DBS. This method can be applied to future studies comparing PZQ concentrations in different bio-fluids and to support PK studies in patients in disease-endemic countries.

2. Material and methods

2.1. Chemicals and reagents

Racemic (rac) PZQ was purchased from Sigma-Aldrich (Buchs, Switzerland). Enantiomers of PZQ and *cis*- and *trans*-4-OH-PZQ (*trans*-4-OH) were obtained from Merck Serono (Darmstadt, Germany). As internal standard (IS), eleven-fold deuterized PZQ (PZQ-d11) was purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). Chemical structures of PZQ, PZQ-d11 and *trans*-4-OH are displayed in Fig. 1. Acetonitrile, ethanol and methanol of MS grade were obtained from Carl Roth GmBH (Allschwil, Switzerland). Ammonium formate, ammonium acetate and formic acid of MS grade were purchased from Sigma-Aldrich (Buchs, Switzerland). Ultrapure water was supplied with a Millipore Milli-Q water purification system (Merck Millipore, Darmstadt, Germany). Blank human plasma and blood were obtained in lithium heparin-coated vacutainer tubes (BD, Allschwil) from the local blood donation centre (Basel).

2.2. LC–MS/MS equipment and conditions

The HPLC system (Shimadzu, Kyoto, Japan) consisted of four LC-20AD pumps, a CTC HTS PAL autosampler (CTC Analytics, Zwingen, Switzerland), and an online DG-3310 degasser (Sanwa Tsusho, Tokyo, Japan), coupled to an API 3000 triple quadrupole mass spectrometer (MS) (AB Sciex, Framingham, MA, USA) with a Turbo Ionspray interface. A first chromatographic separation was achieved at 25 °C through a column trapping system (HALO C-18, 4.6 × 5 mm, Optimize Technologies, Oregon City, OR, USA) using 10 mM ammonium acetate and 0.15% formic acid in ultrapure water as mobile phase at a flow rate of 0.3 mL/min. The sample injection volume was set at 20 μL. After 1 min, the analytes were eluted via a ten-port switching valve (VICI Valco Intruments, Schenkon, Switzerland) from the trapping to the main column, a Lux Cellulose-2 column [cellulose tris(3-chloro-4-methylphenylcarbamate) phase, 150 × 4.6 mm, 3 μm, Phenomenex, Torrance, CA, USA], for enantioselective separation. The elution gradient was defined as follows: 1-2 min, B 70-90%; 2-9.5 min, B 90%; 9.5–10 min, B 90–0%; 10–10.5 min, B 0–70%; 11–11.5 min, B 70% with mobile phase A consisting of 20 mM ammonium formate in ultrapure water and mobile phase B of pure acetonitrile. The flow rate was 1.5 ml/min. Carry-over problems were controlled by rinsing the syringe and the loop twice with pure water and acetonitrile - isopropanol (1:1, v/v) after each injection. To avoid contamination of the MS, a second ten-port switching valve (VICI Valco Instruments) was used to divert the effluent of the analytical column from the MS during 0–3 and 9.5–11.5 min of each run. Due to the high flow rate of 1.5 mL/min, 50% of the flow was diverted through a splitter.

The analytes were detected by selected reaction monitoring (SRM) in the positive mode. Parameters were optimized by direct infusion of 1 μg/ml of PZQ or the *trans*-4-OH metabolite in acetonitrile at a rate of 10 μL/min (Harvard apparatus infusion pump 11, Holliston, MA, USA). Spectrometer parameters were as follows: nebulizer gas (N_2) flow was 12 L/min, curtain gas (N_2) flow was 12 L/min, source temperature was 400 °C, ion spray voltage was 5500 V, collision gas (N_2) flow was 4 L/min, entrance potential 10 V and dwell time was 300 ms. Analyst 1.5 software package (AB Sciex) was used for instrument control and data collection.

2.3. Standard, quality control and internal standard preparation

Stock solutions (racemic PZQ, *R*-PZQ and *S*-PZQ: 1 mg/mL; *cis*- and *trans*-4-OH in racemic or enantiomeric forms: 10 mg/mL; and IS: 1.25 mg/mL) were prepared in methanol and kept at -20 °C. For the preparation of calibration and quality control (QC) samples, PZQ and *R-trans*-4-OH stock solutions were mixed to obtain a stock mix solution of 60 μg/mL of *R*- and *S*-PZQ and 600 μg/mL of *R-trans*-4-OH. Working solutions of *R*-PZQ, *S*-PZQ and *R-trans*-4-OH were obtained by serial dilution of the stock mix solution with pure water – acetonitrile (1:2, v/v) to concentrations ranging from 0.6 to 60 and 6 to 600 μg/mL, respectively. The extraction solution for blood and plasma consisted of 0.5 μg/ml IS diluted in acetonitrile. For DBS, the extraction solution containing IS was further diluted with ultrapure water [IS solution-water (4:1, v/v)].

2.4. Plasma, blood and DBS sample preparation

Calibration samples were freshly prepared and included in each analytical run by spiking blank samples with the working solutions to reach final concentrations of 2.5 down to 0.01 (lower limit of quantification-LLOQ) μg/mL for *R*- and *S*-PZQ, and of 25 to 0.1 (LLOQ) μg/ml for *R-trans*-4-OH. QC samples were simi-