Talanta 134 (2015) 373–378

Contents lists available at ScienceDirect

Talanta

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

Chiral on-line solid phase extraction coupled to liquid chromatography–tandem mass spectrometry assay for quantification of (R) and (S) enantiomers of methadone and its main metabolite in plasma

Régis Bouquié [a,*], Hélène Hernando [b], Guillaume Deslandes [c], Amina Ben Mostefa Daho [d], Christian Renaud [e], Marie Grall-Bronnec [f], Eric Dailly [g], Pascale Jolliet [h]

[a] Assistant Professor, Clinical Pharmacology Department, and EA 4275 Biostatistique, Pharmacoépidémiologie et Mesures Subjectives en Santé, Laboratoire de Pharmacologie clinique, Hôtel Dieu, 9 Quai Moncousu, 44093 Nantes Cedex, France
[b] Practitioner, Clinical Pharmacology Department, Nantes University Hospital, France
[c] Practitioner, Clinical Pharmacology Department, Nantes University Hospital, France
[d] Technician, Clinical Pharmacology Department, Nantes University Hospital, France
[e] Engineer, Clinical Pharmacology Department, Nantes University Hospital, France
[f] Practitioner, Department of Addictology and Psychiatry, Nantes University Hospital, France, and EA 4275 Biostatistique, Pharmacoépidémiologie et Mesures Subjectives en Santé, Nantes, France
[g] Professor in Pharmacology, Clinical Pharmacology Department, and EA 4275 Biostatistique, Pharmacoépidémiologie et Mesures Subjectives en Santé, Nantes University Hospital, France
[h] Professor in Clinical Pharmacology, Clinical Pharmacology Department, and EA 4275 Biostatistique, Pharmacoépidémiologie et Mesures Subjectives en Santé, Nantes University Hospital, France

ARTICLE INFO

Article history:
Received 24 September 2014
Received in revised form
17 November 2014
Accepted 22 November 2014
Available online 29 November 2014

Keywords:
EDDP
Methadone enantiomers
Mass spectrometry
Method validation
Therapeutic drug monitoring

ABSTRACT

The authors aimed at developing a liquid chromatography tandem mass spectrometry (LC-MS/MS) method with online extraction to determine (R)- and (S)- methadone enantiomers and its main metabolite 2-ethylidine-1,5-dimethyl-3,3 diphenylpyrrolidine (EDDP) in plasma.

The analysis combined straightforward sample preparation, consisting of protein precipitation with acetonitrile, and an online enrichment by a flush/back-flush cycle before the second dimension chromatography.

Using D_3-deuterated internal standards allows overcoming significant relative matrix effect. Our method was linear up to 2000 ng/mL. This simple sample preparation provides sensitive (the limit of quantitation is 25 ng/mL for (R,S)-methadone and EDDP and 12.5 ng/mL for (R)- and (S)- methadone), accurate and precise (the intra-day and inter-day imprecision and inaccuracy are lower than 15%) quantification of the plasma concentration of these drugs.

We have developed a reliable LC-MS/MS method for both routine therapeutic drug monitoring and pharmacokinetics studies and for toxicology analysis in the setting of methadone treatment or intoxication

1. Introduction

Methadone (MTD) is a synthetic opioid sold in France as a racemic (50/50) mixture of (R,S)–MTD. MTD is prescribed for opiate dependence, in accordance with guidelines of the health authorities used for opiate substitution treatment or maintenance treatment. Following administration, MTD is extensively metabolized. EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine), an inactive metabolite, is the main product of this process. The main P450 cytochromes involved are: 1A2, 2B6, 3A4/5, 2C19, 2C9, 2E1 and 2D6 [1–6]. Some of these cytochromes display stereospecificity for one of the enantiomers. For example, CYP450 2B6

Abbreviations: EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; MTD, methadone; LC-MS/MS, Liquid Chromatography tandem mass spectrometry; SPE, Solid Phase Extraction; ACN, Acetonitrile; QC, Quality Control; MRM, Multiple Reaction Monitoring; LLOQ, Lower Limit Of Quantification; CV, Coefficient of Variation; EMEA, European Medicines Agency; FDA, Food and Drug Administration; IS, Internal Sandard

* Corresponding author. Tel.: +33 2 40 08 40 12; fax: +33 2 40 08 39 96.
E-mail address: regis.bouquie@chu-nantes.fr (R. Bouquié).

http://dx.doi.org/10.1016/j.talanta.2014.11.052
0039-9140/

primarily metabolizes the (S)- form, CYP450 2C19 the (R)- form, while CYP450 3A4 not stereoselective metabolizes both enantiomers. The (R)- enantiomer is the active form of MTD, whose affinity is 10 times greater than that of the (S)- enantiomer for the μ1 and μ2 opioid receptors. The (S)- form is responsible for the poor cardiac tolerance to MTD (heart rhythm disorders such as QTc prolongation…) [7–9].

MTD is a molecule with a narrow therapeutic index that displays broad inter-individual variability, both in terms of pharmacokinetics and pharmacodynamics, which leads to considerable variability in terms of response to and tolerance of treatment [6–8]. Lot of studies purposed to measure plasma concentration of MTD by chromatography [10–24]. The majority of these methods used liquid-liquid extraction [7,10,15–19,24]. Several assays were proposed to measure plasma concentrations of (R)- and (S)- enantiomers of MTD and EDDP by liquid chromatography–tandem mass spectrometry assay (LC-MS/MS) [16,17]. We present the first liquid chromatography–tandem mass spectrometry method with a simple sample pre-treatment, which can be used for the simultaneous quantification of (R)- and (S)- enantiomers of MTD and its main metabolite EDDP.

2. Material and methods

2.1. Samples

All samples tested in this work derived from an ongoing drug-monitoring program and were reported in accordance with ethical guidelines. Informed consent was not required.

2.2. Chemicals, reagents and standard solutions

All solvents and reagents were HPLC-grade and were purchased from VWR International (Fontenay-sous-Bois, France). Racemic MTD, racemic MTD-D_3, EDDP and EDDP-D_3 solutions were purchased from LGC standards (Molsheim, France) (Fig. 1). Purified (S)–MTD, purchased from Alsachim (Illkirch, France), was only used to identify the (S)–MTD enantiomer peak. Racemic MTD-D_3 and EDDP-D_3 were used as internal standard (IS). In-house prepared multilevel plasma calibrator containing both MTD and EDDP were used for all LC-MS/MS assays. In house prepared quality control (QC) (two levels) and one level of commercial QC provided by Medichem Diagnostica (Steinenbronn, Germany) were used for all LC-MS/MS assays. Protein precipitation solution was a mixture of ACN containing IS (1 μg/mL) and stored at −20 °C. The different lots of drug-free plasma samples originated from our laboratory.

2.3. LC-MS/MS assay

2.3.1. Standard, quality controls and sample preparation

Working solutions were prepared as follows: (R, S)–MTD, EDDP, MTD-D_3 and EDDP-D_3 at a concentration of 10 μg/mL (methanol). A seven-point calibration curve and homemade quality controls were prepared for each analyte by diluting know volumes of the working solution in drug-free human plasma (collected with K_3EDTA as anticoagulant). For (R,S)–MTD and EDDP the calibration curve points were: 0, 100, 250, 500, 1000 and 2000 ng/mL, and the homemade quality control values were 200 and 800 ng/mL. The commercial QC value was 100 ng/ml for (R,S)–MTD and 28 ng/mL for EDDP.

Each sample was further treated as described. Protein precipitation was carried out in 1.5 mL polypropylene tubes (Eppendorf, Le Pecq, France). A volume of 100 μL of calibrator, QC or patient sample was mixed with 200 μL of precipitation solution. The mixture was vortex-mixed for 5 min, and centrifuged 10 min at 15300 g at 4 °C. Subsequently the supernatant was transferred in a polypropylene tube with pierceable membrane screw caps, and 20μl was injected in the chromatographic system.

2.3.2. Instrumentation setup

Instrument setup is shown in Fig. 2. The chromatographic system consists of Agilent 1200 Series components (Palo Alto, USA) including two binary pumps, column oven, and autosampler. The hardware configuration included a triple quadrupole mass spectrometer ABSciex API 3200 QTrap™ (Toronto, Canada) equipped with a turboionSpray ionization source. ESI voltage was set to 5000 V with positive ionization mode. Nitrogen was used both as nebulizing gas and drying gas. The source temperature was 600 °C. Positive ion electrospray, schedule MRM mode was used for analytes and IS (Table 1).

2.3.3. Two dimension chromatographic separation

The chromatographic conditions are presented in the Fig. 2. First dimension chromatography is an on-line enrichment performed by a perfusion column (Oasis HLB™ 2.1 × 20 mm, 5 μm, Waters, Saint-Quentin En Yvelines, France). The binary pump 1 A supplied water/ACN (95:5, v/v) delivered at a flow rate of 4 mL/min. After 1.2 min of enrichment of analytes and IS on the Oasis column, the valve was switched. The back-flush elution was performing by the mobile phase constituted of water/ACN (50:50, v/v) / triethylamine (0.04%)

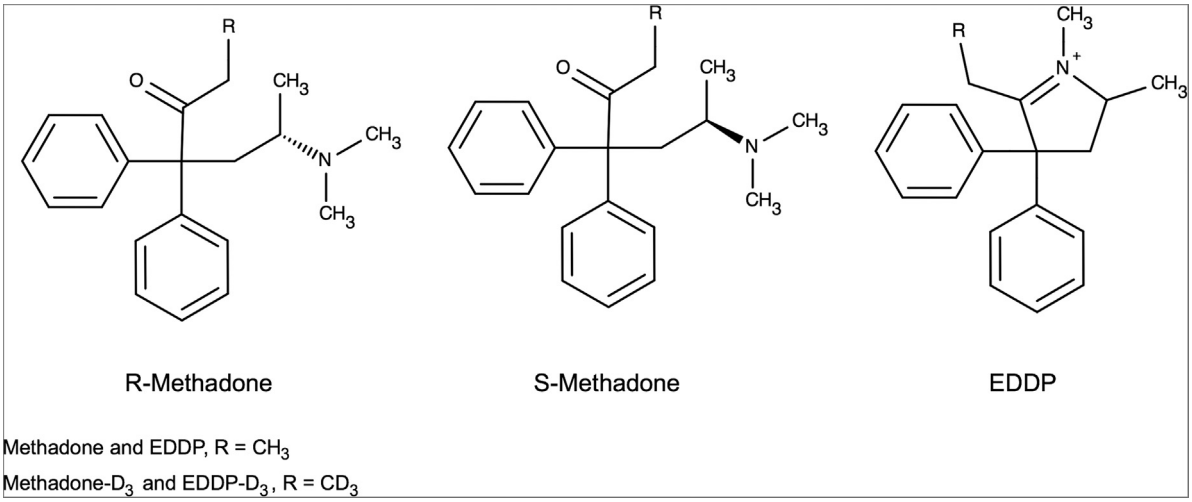

Fig. 1. Chemical structure of methadone, EDDP and internal standards.