Journal of Pharmaceutical and Biomedical Analysis 149 (2018) 475–481

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Bioanalytical methods for the quantification of hydromorphone, fentanyl, norfentanyl, morphine, morphine-3ß-glucuronide and morphine-6ß-glucuronide in human plasma

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ARTICLE INFO

Article history:
Received 6 September 2017
Received in revised form
10 November 2017
Accepted 11 November 2017
Available online 17 November 2017

Keywords:
Opioids
Morphine glucuronides
UPLC–MS/MS
Human plasma

ABSTRACT

The aim of this study was to develop an assay for the quantification of hydromorphone, morphine, fentanyl and the metabolites norfentanyl, morphine-3ß-glucuronide and morphine-6ß-glucuronide in human plasma to support pharmacokinetic studies investigating the large interpatient variability in response to opioid treatment.

For the quantitation of hydromorphone, morphine, fentanyl and its metabolite norfentanyl aliquots of 200 μL human potassium EDTA plasma were deproteinized with deuterated internal standards in a mixture of acetonitrile and acetone, followed by a liquid-liquid extraction with 4% ammonium hydroxide and ethyl acetate. Morphine-3ß-glucuronide and morphine-6ß-glucuronide were extracted by a solid phase extraction using 10 mM ammonium carbonate pH 8.8 and a deuterated internal standards solution. Morphine, hydromorphone, fentanyl and norfentanyl were separated on an Aquity UPLC® BEH C18 column 1.7 μm, 100 mm × 2.1 mm at 50 °C. Separation, was achieved on a gradient of methanol with an overall run time of 6 min. The compounds were quantified by triple-quadrupole mass spectrometry in the positive ion electrospray ionization mode. Morphine-3ß-glucuronide and morphine-6ß-glucuronide were separated on a VisionHT C18-P; 3 μm 2.1 × 50 mm, column at 40 °C on a gradient of acetonitrile, with an overall run time of 10 min. Both methods were precise and accurate, with within-run and between-run precisions within acceptable limits and accuracy ranging from 84.0 to 105.5%. The methods were successfully applied to support clinical pharmacological studies in patients treated with opioids for the treatment of moderate to severe cancer-related pain.

1. Introduction

Many patients with cancer at all stages of disease suffer from mild to severe pain requiring treatment with opioids [1]. Unfortunately, the response to opioid treatment is highly variable among patients. Some patients suffer from severe side effects and other patients have inadequate pain relief despite increasing opioid doses [2,3].

An important factor responsible for the variation in response might be the (largely unexplained) variability in pharmacokinetics [4,5]. To reduce the incidence of severe opioid related side effects and inadequate pain relief, factors explaining pharmacokinetic variability need further investigation. This requires a validated method to measure opioids in plasma. In this study, the opioids morphine, fentanyl and hydromorphone are investigated.

Morphine undergoes extensive glucuronidation to the two main morphine metabolites, morphine-3ß-glucuronide (M3G) and morphine-6ß-glucuronide (M6G) [6,7]. M3G is thought to be inactive, since administration of M3G to healthy volunteers did not result in any clinical effect [8]. M6G however, is able to contribute to the analgesic effects. This is especially relevant in patients with renal impairment which may result in M6G accumulation [9,10].

Fentanyl is a synthetic opioid approximately 75–100 times more potent than morphine and is mainly metabolized to the inactive metabolite norfentanyl [11]. Advantages that promote its use are the various patient friendly administration routes, the relatively

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https://doi.org/10.1016/j.jpba.2017.11.035
0731-7085/

low incidence of gastrointestinal related side effects and its recommended use in patients with renal impairment [12].

Last, but not least, hydromorphone is a derivate of morphine and approximately 5 times more potent compared to morphine [13]. If inadequate pain relief is reached with the more common opioids fentanyl, morphine and oxycodone, hydromorphone can be considered.

Especially opioid rotations are moments at risk for severe opioid toxicity or inadequate pain relief [14]. The opioid dose conversions schemes give some direction for dose adjustments but the optimal time interval between stopping the prior opioid and starting a new opioid is unknown and needs further pharmacokinetic evaluation.

Several multi-analyte assays for the simultaneous detection of opioids and metabolites in human plasma or serum are described [15,16]. A disadvantage of these methods is the use of laborious and time-consuming solid phase extraction. The method described by Eckart et al. includes several opiods and metabolites, but the detection limit for fentanyl and norfentanyl was validated at a concentration of 1 ng/mL which is not sensitive enough for use in pharmacokinetic studies, especially when fentanyl is administered sublingually or change in opioid regimen with subcutaneous and transdermal fentanyl in cancer patients [17]. Musshoff et al. described also a method for the detection of several opioids by using a solid-phase extraction, but they use a large sample size of 1-mL and also the detection limit for fentanyl and norfentanyl is 1 ng/mL.

The aim of this study was to develop sensitive bioanalytical assays to measure hydromorphone, fentanyl, norfentanyl, morphine, morphine-3ß-glucoronide and morphine-6ß-glucuronide in human plasma.

2. Experimental

2.1. Chemicals

Hydromorphone, fentanyl, norfentanyl oxalate, morphine, morphine-3ß-glucuronide, morphine-6ß-glucuronide and the deuterated internal standards hydromorphone-d6, fentanyl-d5, norfentanyl-d5 oxalate, morphine-d6, morphine-3ß-glucuronide-d3 and morphine-6ß-glucuronide-d3, were obtained from Cerilliant (Round Rock, TX, USA). Acetonitrile, methanol, water and ethyl acetate were purchased from Biosolve BV (Valkenswaard, The Netherlands). Dimethylsulphoxide and ammonium formate were from Sigma-Aldrich (Zwijndrecht, The Netherlands). Formic acid and ammonium hydroxide were obtained from J.T. Baker (Deventer, The Netherlands) and 2-propanol, acetone and ammonium carbonate from Merck (Darmstadt, Germany). Blank potassium EDTA plasma was purchased from Biological Specialty Corporation (Colmar, PA, USA). All chemicals were of analytical grade or higher.

2.2. Preparation of stock solutions, calibration standards and quality control samples

Hydromorphone, morphine, fentanyl and norfentanyl oxalate stock solutions were provided as ready to use solutions with a concentration of 1 mg/mL free base in methanol. Morphine-6ß-glucuronide was provided as ready to use solution with a concentration of 0.1 mg/mL free base in methanol/water (1:1, v/v) and morphine-3ß-glucuronide was provided as ready to use solution with a concentration of 1 mg/mL free base in methanol/0.05% NaOH (w/v). The deuterated internal standard stock solutions were provided as ready to use solutions with a concentration of 0.1 mg/mL for hydromorphone-d6, fentanyl-d5, norfentanyl-d5 oxalate in methanol, while morphine-d6 was provided as ready to use solution with a concentration of 1 mg/mL free base in methanol. Morphine-3ß-glucuronide-d6 and morphine-6ß-glucuronide-d6 were provided as ready to use solutions with a concentration of 0.1 mg/mL in methanol/0.05% NaOH (w/v) and methanol/water (1:1, v/v) respectively. All stock solutions were stored at T < –70 °C. Internal standard stock solutions of morphine-d6, hydromorphone-d6, fentanyl-d5 and norfentanyl-d5 oxalate were diluted in acetonitrile, resulting in an internal standard solution containing 125 ng/mL morphine-d6, 12.5 ng/mL hydromorphone-d6, 0.5 ng/mL fentanyl-d5 and 10 ng/mL norfentanyl-d5 oxalate respectively and stored at T < 8 °C for a maximum of 3 months. Internal standard stock solutions of morphine-3ß-glucuronide-d3 and morphine-6ß-glucuronide-d3 were prepared by dilution of stock solutions in acetonitrile/water (1:1, v/v) resulting in an internal standard solution containing 100 ng/mL and stored at T < 8 °C for a maximum of 3 months.

Calibration standards were prepared freshly on the day of analysis, in duplicate, by addition of 10-μL aliquots of appropriate dilutions of hydromorphone, morphine, fentanyl and norfentanyl stock solution in acetonitrile/DMSO (1:1, v/v) to 190-μL aliquots of human potassium EDTA plasma at the following concentrations: 1.00, 2.50, 5.00, 10.0, 25.0, 50.0, 90.0, and 100 ng/mL as free base for hydromorphone and morphine, 0.200, 0.500, 1.00, 2.00, 5.00, 10.0, 18.0 and 20.0 ng/mL as free base for norfentanyl and 0.100, 0.250, 0.500, 1.00, 2.50, 5.00, 9.00 and 10.0 ng/mL as free base for fentanyl. Calibration standards for morphine-3ß-glucuronide and morphine-6ß-glucuronide were prepared freshly on the day of analysis, in duplicate, by addition of 10 μL aliquots of appropriate dilutions of stock solution in acetonitrile/DMSO (1:1, v/v) to 190-μL aliquots of human potassium EDTA plasma at the following concentrations: 10.0, 25.0, 50.0, 100, 250, 500, 900, and 1000 ng/mL as free base for morphine-3ß-glucuronide and 2.00, 5.00, 10.0, 20.0, 50.0, 100, 180 and 200 ng/mL as free base for morphine-6ß-glucuronide.

Five pools of quality control (QC) samples were prepared in human potassium EDTA plasma at concentrations of 1.00 ng/mL (lower limit of quantitation, LLQ), 3.00 ng/mL (QC-Low), 40.0 ng/mL (QC-Middle), 80.0 ng/mL (QC-High) and 400 ng/mL (QC-diluted) for hydromorphone and morphine, 0.200 ng/mL (LLQ), 0.600 ng/mL (QC-Low), 8.00 ng/mL (QC-Middle), 16.0 ng/mL (QC-High) and 80.0 ng/mL (QC-diluted) for norfentanyl, and 0.100 ng/mL (LLQ), 0.300 ng/mL (QC-Low), 4.00 ng/mL (QC-Middle), 8.00 ng/mL (QC-High) and 40.0 ng/mL (QC-diluted) for fentanyl. QC-diluted was processed after a 20-fold dilution in blank human potassium EDTA plasma. For morphine-3ß-glucuronide and morphine-6ß-glucuronide five pools of QC samples were prepared in human potassium EDTA plasma at concentrations of 10.0 ng/mL (LLQ), 30.0 ng/mL (QC-Low), 400 ng/mL (QC-Middle), 800 ng/mL (QC-High) and 4000 ng/mL (QC-diluted) for morphine-3ß-glucuronide and 2.00 ng/mL (LLQ), 6.00 ng/mL (QC-Low), 80.0 ng/mL (QC-Middle), 160 ng/mL (QC-High) and 400 ng/mL (QC-diluted) for morphine-6ß-glucuronide. Pools of QC samples were aliquotted and stored at T < –70 °C upon processing.

2.3. Plasma sample preparation for hydromorphone, morphine, fentanyl and norfentanyl

Aliquots of 200 μL of plasma samples were transferred into 1.5-mL microcentrifuge tubes, and 100 μL of internal standard solution and 100-μL aliquots of acetone were added.

Hereafter, the samples were vigorously mixed for 5 min and then centrifuged at 18,000 × *g* at ambient temperature for 10 min. The supernatant was transferred into 2-mL microcentrifuge tubes after which 100 μL of 4% ammonium hydroxide solution and 1-mL ethyl acetate was added. Hereafter, the samples were vigorously mixed for 5 min and then centrifuged at 18,000 × *g* at ambient temperature for 10 min. The organic phases were transferred into 4.5-mL glass tubes and evaporated under a stream of nitrogen at T = 70 °C. The residues were resuspended in 100-μL aliquots of