Journal of Chromatography B, 902 (2012) 116–121

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

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

Simultaneous determination of oxymorphone and its active metabolite 6-OH-oxymorphone in human plasma by high performance liquid chromatography–tandem mass spectrometry

Wuyi Zha*, Linyee Shum

TDM Pharmaceutical Research, LLC, 100 Biddle Ave., Suite 202, Newark, DE 19702, USA

ARTICLE INFO

Article history:
Received 27 March 2012
Accepted 13 June 2012
Available online 1 July 2012

Keywords:
Oxymorphone
6-OH-oxymorphone
LC–MS/MS
SPE
Human plasma

ABSTRACT

A selective high performance liquid chromatography–tandem mass spectrometric (LC–MS/MS) method for the simultaneous determination of oxymorphone and its active metabolite 6-OH-oxymorphone in human plasma was developed and validated using oxymorphone-d_3 as the internal standard. Chromatographic conditions were optimized to separate oxymorphone from the other metabolite, oxymorphone-3-glucuronide, which may convert to oxymorphone in MS ion source, resulting in inaccurate quantitation of oxymorphone. Solid phase extraction (SPE) was used to extract oxymorphone and 6-OH-oxymorphone from plasma. SPE offered the advantage of being able to remove the unwanted metabolite, oxymorphone-3-glucuronide, through the wash step during the extraction. The developed method was precise and reproducible as shown by good linearity of calibration curves (correlation coefficients ≥0.9968 for oxymorphone and ≥0.9967 for 6-OH-oxymorphone) with high intraday assay and interday assay precision (CV% ≤11.0% for oxymorphone and ≤12.6% for 6-OH-oxymorphone) over a range of 35/25 – 5000/5000 pg/mL for oxymorphone/6-OH-oxymorphone. The method has been successfully applied to analyze oxymorphone and 6-OH-oxymorphone in plasma from 19 healthy volunteers in a bioequivalence study. A total of 1026 samples were analyzed. Good linearity (average correlation coefficient 0.9988 for oxymorphone and 0.9966 for 6-OH-oxymorphone) was achieved with calibration curves and high precision (CV% ≤5.9% for oxymorphone and ≤10.9% for 6-OH-oxymorphone) was obtained with QCs.

1. Introduction

Oxymorphone, 14-hydroxydihydromorphinone, is a semisynthetic μ-opioid agonist that is mainly used for the relief of moderate to severe pain [1]. It transfers faster across the blood–brain barrier when compared to other opioids such as oxycodone and morphine because it is more lipid soluble [2,3]. Therefore, it has a more rapid onset of action [4–6]. Studies also indicate that oxymorphone has a much higher analgesic potency than morphine and oxycodone [6,7]. The structure of oxymorphone is shown in Fig. 1(a).

Oxymorphone currently has various types of formulations available including suppository, parenteral injection, oral IR (immediate release) tablets and oral ER (extended release) tablets [6]. This provides patients more options for the drug administration. Oxymorphone IR has a duration of action for ca. 4–6 h while oxymorphone ER has been shown to be effective for over 12 h [6].

Oxymorphone is extensively metabolized to oxymorphone-3-glucuronide and analgesic active 6-OH-oxymorphone in the liver; only 2% is excreted unchanged in urine [6,8]. The structure of 6-OH-oxymorphone is shown in Fig. 1(b).

Different assays have been reported for the quantitation of oxymorphone together with other opioids in different matrices using LC–MS/MS [9–13], GC–MS [14] and HPLC with electrochemical detection [15]. The lower limits of quantifications (LLOQs) are usually higher than 0.5 ng/mL. To our knowledge, there is presently no LC–MS/MS method published for the simultaneous analysis of oxymorphone and its active metabolite 6-OH-oxymorphone. Here we describe a selective and sensitive LC–MS/MS method for the simultaneous determination of oxymorphone and 6-OH-oxymorphone in human plasma. The developed and validated method was successfully applied to the bioequivalence studies of oxymorphone tablets in healthy volunteers.

* Corresponding author. Tel.: +1 302 832 1008; fax: +1 302 832 1480.
E-mail addresses: Zha.Wuyi@tdmrxresearch.com, wuyizha@yahoo.com (W. Zha).

1570-0232/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jchromb.2012.06.035

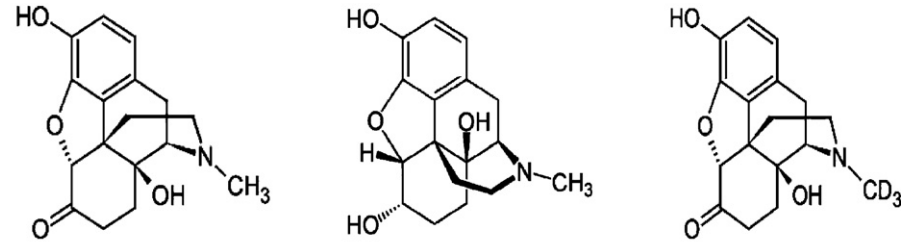


Fig. 1. Structure of oxymorphone (a), 6-OH-oxymorphone (b) and oxymorphone-d_3 (c).

2. Experimental

2.1. Chemicals

Oxymorphone (1 mg/mL in methanol, 100%), 6-OH-oxymorphone (powder, 98%) and oxymorphone-d_3 (1 mg/mL in methanol, 99.9%) were all purchased from Cerilliant (Round Rock, TX, USA). Sodium phosphate, dibasic, heptahydrate (100%) was supplied by Mallinckrodt Baker (Phillipsburg, NJ, USA). Methanol and acetic acid were both HPLC grade from EMD Chemicals Inc. (Gibbstown, NJ, USA). Deionized water was produced in-house using a Millipore ultrapure water purification system (Billerica, MA, USA). Bond Elut C18 (3 mL) cartridges were bought from Agilent (Santa Clara, CA, USA).

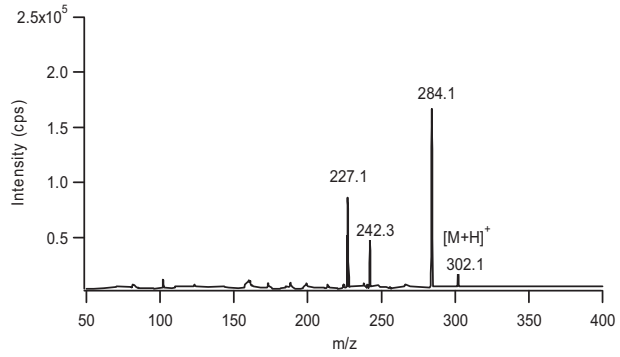
2.2. High performance liquid chromatography and mass spectrometry

Chromatographic separation was obtained under isocratic conditions using a Shimadzu LC-10AD *vp* LC system (Columbia, MD, USA) with a Phenomonex (Torrance, CA, USA) Synergi Polar-RP column (75 mm × 2.0 mm, 4 μm). The mobile phase consisted of 62% A (water with 0.1% acetic acid) and 38% B (methanol) by volume. The flow rate for the mobile phase was set at 0.18 mL/min.

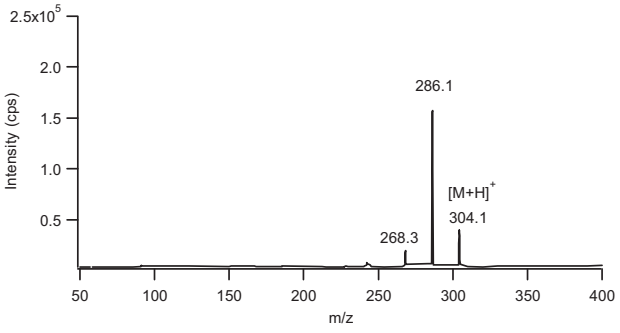
Detection was performed by an AB/Sciex (Concord, ON, Canada) API 4000 triple quadrupole mass spectrometer with a turbo-ion spray interface in the positive ion mode. Oxymorphone-d_3 was used as the internal standard (IS). The structure of oxymorphone-d_3 is shown in Fig. 1(c). The MS/MS transitions (m/z) used for quantification were: oxymorphone, 302.1 → 227.1; 6-OH-oxymorphone, 304.1 → 286.1; and oxymorphone-d_3 (IS), 305.1 → 230.1 (shown in Fig. 2). The ion source was heated to 700 °C and the spray voltage was set at 4000 V. The nebulizer gas, auxiliary gas, collision-activated dissociation (CAD) gas and curtain gas were all ultra-high purity nitrogen gas and applied at 60, 60, 8 and 20 psi (413,685, 413,685, 55,158 and 137,895 Pa), respectively. The collision energy was set at 37 V for oxymorphone, 29 V for 6-OH-oxymorphone and 37 V for oxymorphone-d_3. The Software Analyst 1.4.2 (Sciex, Concord, ON, Canada) was used to control the LC–MS/MS system and acquire the data.

2.3. Preparation of stock solution and standards

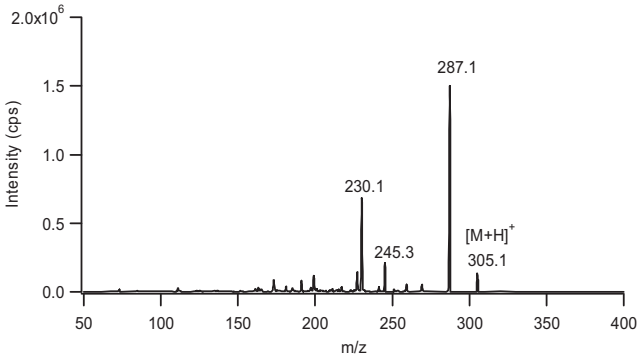
Oxymorphone (1.00 mg/mL in methanol) and IS (1.00 mg/mL in methanol) purchased from Cerilliant were used as stock solutions. 6-OH-oxymorphone stock solution, 1.00 mg/mL, was prepared in methanol. All stock solutions were stored at −20 °C. Two independent stock solutions for each analyte were used for the standard and QC preparation (one for standards and the other one for QCs). Two-in-one working solutions of oxymorphone/6-OH-oxymorphone (200/200 ng/mL, 20/20 ng/mL and 7.0/5.0 ng/mL) were prepared



(a) Product ion mass spectra of $[M+H]^+$ of oxymorphone



(b) Product ion mass spectra of $[M+H]^+$ of 6-OH-oxymorphone



(c) Product ion mass spectra of $[M+H]^+$ of oxymorphone-d_3

Fig. 2. Product ion mass spectra of $[M+H]^+$ of oxymorphone (a), 6-OH-oxymorphone (b) and oxymorphone-d_3 (c).