Journal of Chromatography B 1087–1088 (2018) 29–35

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Validation of an LC-MS/MS method for simultaneous quantification of venlafaxine and its five metabolites in rat plasma and its application in a pharmacokinetic study

Guodong Gu[a,*], Michelle Black[a], Colt Cookson[a], Anna Fiorella[a], Yinghe Li[b], Steven H. Gorman[c], Ray Bakhtiar[c]

[a] Alliance Pharma, Inc., Malvern, PA, United States
[b] GlaxoSmithKline (GSK), Collegeville, PA, United States
[c] Teva Branded Pharmaceutical Products R&D, West Chester, PA, United States

ARTICLE INFO

Keywords:
Venlafaxine
LC-MS/MS
Method validation
Metabolites
Rat plasma

ABSTRACT

A sensitive, selective, and reliable LC-MS/MS method was developed and validated for simultaneous quantification of venlafaxine (VEN) and its 5 metabolites (ODV, NDV, NNDDV, OHV and NODDV) in rat plasma. The calibration ranges are 15.0 to 6000 ng/mL for VEN, 1.00 to 400 ng/mL for ODV, 5.00 to 2000 ng/mL for NDV, 1.00 to 400 ng/mL for NNDDV, 10.0 to 4000 ng/mL for OHV, and 0.200 to 20.0 ng/mL for NODDV. Briefly, 50 µL of rat plasma was extracted using liquid-liquid extraction (LLE) with methyl *tert*-butyl ether (MTBE). The analytes were separated on an Agilent SB-Phenyl (50 mm × 4.6 mm, 3.5 µm) column using a binary gradient of 0.1% formic acid in water *versus* 0.1% formic acid in acetonitrile at a flow rate of 0.8 mL/min. The method was validated following FDA guidance for bioanalytical method validation. Validated method was successfully applied to a pharmacokinetic study of VEN orally administered to rats.

1. Introduction

Venlafaxine (*DL*)-1-[2-dimethylamino-1-(4-methoxy-phenyl)-ethyl]-cyclohexanol hydrochloride, a dual serotonin-norepinephrine reuptake inhibitor, was initially launched by originator Wyeth in 1994 for the treatment of depression. In 2007, venlafaxine was the sixth most commonly prescribed antidepressant on the U.S. retail market, with about 17.2 million prescriptions. In addition to major depressive disorder (MDD) venlafaxine has also been marketed for treatment of generalized anxiety disorder, social anxiety disorder, and panic disorder. Venlafaxine (VEN), marketed as an extended release formulation (Effexor XR™), is available in dosage strengths of 37.5 mg, 75 mg, and 150 mg [1]. It is readily metabolized in the liver into its major active metabolite, O-desmthyl venlafaxine (ODV), as well as four other metabolites: rac-*N*-desmethyl venlafaxine (NDV), *D,L-N,N*-didesmethyl venlafaxine (NNDDV), 4-hydroxy venlafaxine (OHV), and rac-*N,O*-didesmethyl venlafaxine (NODDV). ODV is equipotent to VEN in *in vitro* inhibition of serotonin uptake [2]. The metabolism pathway scheme of VEN is presented in Fig. 1. Wyeth has developed the active ODV metabolite of venlafaxine. ODV received approval for major depression in 2008 and is marketed as Pristiq™.

VEN and its metabolites can be quantified using liquid chromatography (LC) for the separation of the analytes in conjunction with UV [3], fluorometric [4], and coulometric detection [5]. UV and coulometric detection methods generally have reduced sensitivity and a lack of selectivity. The direct fluorometric method using an excitation at 276 nm, is more sensitive compared to the other two techniques; however, only measurements of VEN and ODV have been reported [4].

Tandem mass spectrometry (MS/MS) is a more specific mean of detection and is highly utilized in pharmacokinetic (PK) studies. The lower limit of quantification (LLOQ) for VEN and ODV was reported to be as low as ~0.1 ng/mL in human plasma and whole blood samples [6]. Several LC-MS/MS methods were developed to quantify VEN in human tissue and plasma [7,8] and VEN and ODV in human plasma [9–12]. However, these methods were limited to only VEN and its major metabolite ODV. In one recent study, a method was developed for quantification of VEN, ODV, and two additional metabolites, NDV and NODDV, in human plasma using a solid phase extraction (SPE) method and LC-MS/MS analysis [13]. The purpose of that work was to stereoselectively quantify VEN and its metabolites. Consequently, the method had a lengthy 35-min run time to separate the enantiomers.

NDV, NNDDV, OHV, and NODDV are minor VEN *in vivo* metabolites

* Corresponding author.
E-mail address: ggu@alliancepharmaco.com (G. Gu).

https://doi.org/10.1016/j.jchromb.2018.04.033
Received 6 February 2018; Received in revised form 6 April 2018; Accepted 21 April 2018
Available online 23 April 2018
1570-0232/ © 2018 Elsevier B.V. All rights reserved.

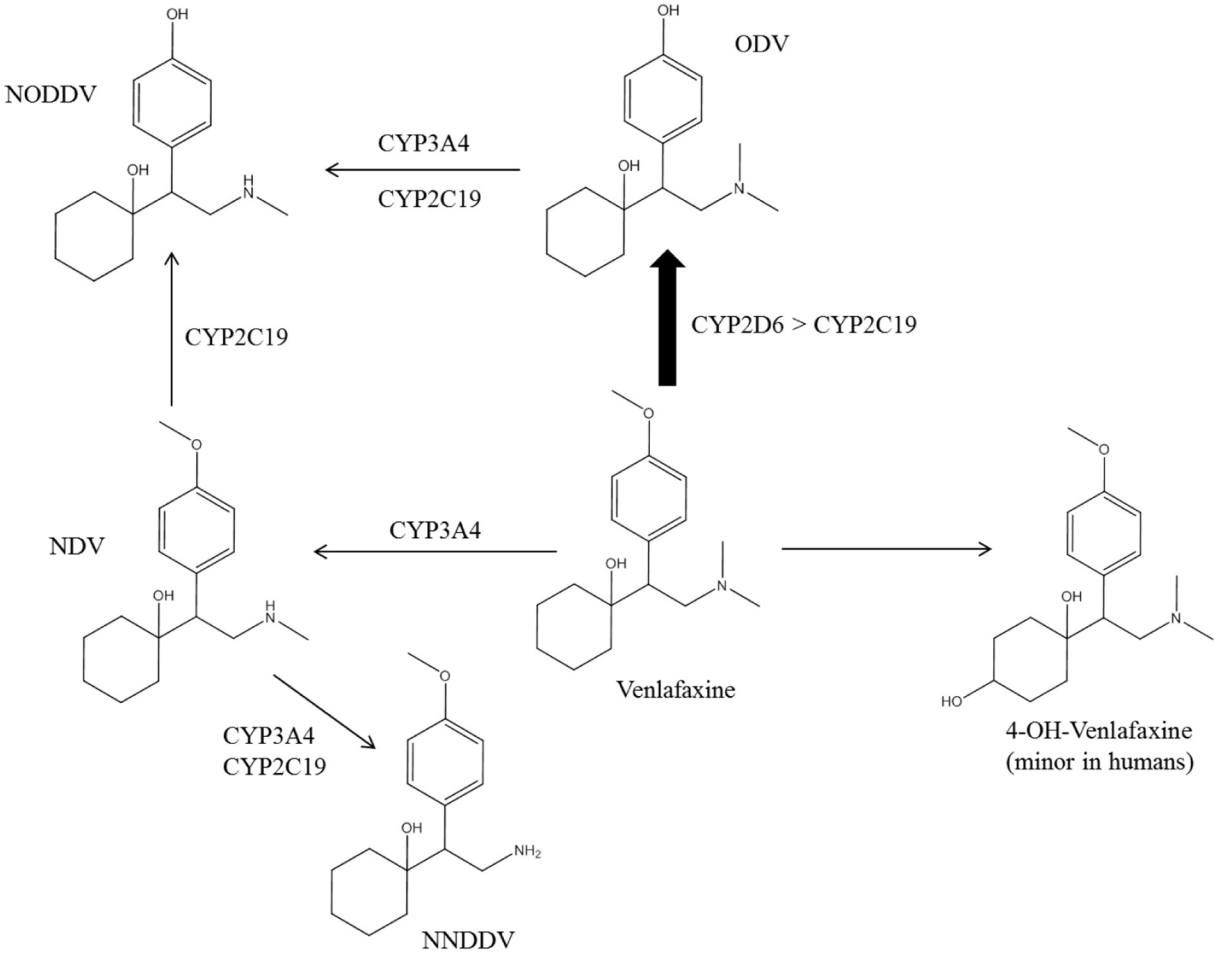

Fig. 1. Venlafaxine metabolic pathway in human.

and have not been studied extensively, but the concentration data of these metabolites will help to better understand the *in vivo* pharmacodynamic (PD) profile of VEN [14]. In order to concurrently quantify VEN, ODV, NDV, NNDDV, OHV, and NODDV in rat plasma, we developed a sensitive, selective, and robust LC-MS/MS method. The method was validated following FDA guidance for bioanalytical method validation. Satisfactory method sensitivity, selectivity, precision, and accuracy were confirmed by the validation results.

2. Materials and method

2.1. Materials and reagents

Reference standards *D,L*-venlafaxine hydrochloride (VEN), *D,L-O*-desmethyl venlafaxine (ODV), rac-*N*-desmethyl venlafaxine (NDV), *D,L-N,N*-didesmethyl venlafaxine (NNDDV), and rac-*N,O*-didesmethyl venlafaxine (NODDV) were supplied by Toronto Research Chemicals (Toronto, Ontario, Canada). Internal standards d_9-venlafaxine hydrochloride (d_9-VEN), d_6-ODV, d_6-NDV, d_3-NNDDV, and d_3-NODDV were supplied by Irvine Pharmaceutical Services (Irvine California). The reference standard for 4-hydroxy-venflafaxine (OHV) and the internal standard d_9–4-hydroxy-venlafaxine (d_9-OHV) were provided by Pharmaron (Beijing, China). Sodium hydroxide (NaOH, ACS grade), acetonitrile (ACN, HPLC grade), methyl *tert*-butyl ether (MTBE, HPLC grade), ethyl acetate (HPLC grade), and dimethyl sulfoxide (DMSO, HPLC grade) were obtained from Thermo Fisher Scientific (Fair Lawn, New Jersey). Formic acid (FA, HPLC grade) was obtained from Sigma-Aldrich Corporation (St. Louis, Missouri). K_2EDTA rat plasma and K_2EDTA rat blood were obtained from BioreclamationIVT (Westbury, New York). Ultrapure water was from a Milli-Q water purification system (Bedford, Massachusetts).

2.2. Preparation of standards and quality control samples

Stock standard solutions (1 mg/mL) of VEN, ODV, NDV, NNDDV, OHV, and NODDV were prepared by dissolving the dry compounds in DMSO. Stock standard solutions were stored at 4 °C. Working standard solutions were prepared by serial dilution of stock standard solution with ACN:water 1:1 (v:v). Working standard solutions were stored at 4 °C. Stock and working solutions for calibration standard (CS) and quality control (QC) samples were prepared separately.

The CS and QC sample were prepared as pools in rat plasma from working standard solutions at the concentrations listed in Table 1 and stored at −80 °C. The CS samples that were used for matrix stability assessments were freshly prepared on the day of the experiment.

Individual stock internal standard (IS) solutions (1 mg/mL) of d_9-VEN, d_6-ODV, d_6-NDV, d_3-NNDDV, d_9-OHV, and d_3-NODDV were prepared by dissolving the dry compounds in DMSO. The individual stock IS solutions were diluted with ACN:water 1:1 (v:v) to prepare a combined working IS solution (at a concentration of 50 ng/mL of each). The individual stock IS solutions and working IS solutions were stored at 4 °C.

2.3. Sample preparation

VEN and its metabolites were extracted using liquid-liquid extraction (LLE) with MTBE. Generally, 50.0 µL of rat plasma was spiked with 50.0 µL of combined internal standard working solution in a 96-well

Download English Version:

https://daneshyari.com/en/article/7615036

Download Persian Version:

https://daneshyari.com/article/7615036

Daneshyari.com