Journal of Pharmaceutical and Biomedical Analysis 56 (2011) 1094–1100

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Short communication

Simultaneous quantitation of levodopa and 3-*O*-methyldopa in human plasma by HPLC–ESI-MS/MS: Application for a pharmacokinetic study with a levodopa/benserazide formulation

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ARTICLE INFO

Article history:
Received 17 June 2011
Received in revised form 20 July 2011
Accepted 26 July 2011
Available online 30 July 2011

Keywords:
Levodopa
3-*O*-Methyldopa
Benserazide
HPLC–ESI-MS/MS
Plasma
Pharmacokinetics

ABSTRACT

A sensitive and simple method was developed for the quantitation of levodopa and its metabolite 3-*O*-methyldopa, in human plasma, after oral administration of tablet formulations containing levodopa (200 mg) and benserazide (50 mg). The analytes were extracted by a protein precipitation procedure, using carbidopa as an internal standard. A mobile phase consisting of 0.2% formic acid and acetonitrile (94:6, v/v) was used and chromatographic separation was achieved using ACE C_{18} column (50 mm × 4.6 mm i.d.; 5 μm particle size). Selected reaction monitoring was performed using the fragmentation transitions m/z 198 → m/z 107, m/z 212 → m/z 166 and m/z 227 → m/z 181 for levodopa, 3-*O*-methyldopa and carbidopa, respectively. Calibration curves were constructed over the range 50.0–6000.0 ng/mL for levodopa and 25.0–4000.0 ng/mL for 3-*O*-methyldopa. The method shown to be specific, precise, accurate and provided recovery rates higher than 85% for all analytes. No matrix effect was detected in the samples. The validated method was applied in a pharmacokinetic study with a levodopa/benserazide tablet formulation in healthy volunteers.

1. Introduction

Parkinson disease is an age-related neurodegenerative disorder that affects around 1–2% of persons aged 60 years and older [1]. Current therapy is largely based on a dopamine replacement strategy, primarily using the dopamine precursor levodopa [2]. Combination therapy of levodopa with an inhibitor of extracerebral decarboxylase such as benserazide (Fig. 1) leads to a marked reduction in both the required levodopa dose and the incidence of undesired side effects [3,4].

Fixed dose combinations of levodopa (200 mg) and benserazide (50 mg) in tablet formulation have been developed [5,6]. For pharmacokinetic and bioequivalence studies of levodopa associated with benserazide, it is recommended to perform the quantitation of levodopa and its major metabolite, 3-*O*-methyldopa, in plasma, since the metabolism of levodopa is shifted towards this metabolite in the presence of a descarboxylase inhibitor [3].

Some methods have been reported for determining levodopa and its metabolite, 3-*O*-methyldopa, in plasma, by high performance liquid chromatography. However, the majority of levodopa and 3-*O*-methyldopa quantitation in plasma was performed by electrochemical detection [7–10], and few works detected the analytes by ultraviolet spectrophotometry [11–13]. In the last years, liquid chromatography combined with atmospheric pressure ionization mass spectrometric detection has almost completely replaced ultraviolet, electrochemical or fluorescence detection in the bioanalytical field, mainly due to the un-matched sensitivity and extraordinary selectivity of the detection [14,15]. The quantitation of levodopa in plasma using liquid chromatography coupled to mass spectrometric detection is described in some works [16–20].

The aim of this work was to develop and validate a rapid HPLC–ESI-MS/MS method for the simultaneous quantitation of levodopa and 3-*O*-methyldopa in human plasma. The method was applied to a pharmacokinetic study with a levodopa/benserazide tablet formulation in healthy volunteers.

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doi:10.1016/j.jpba.2011.07.040

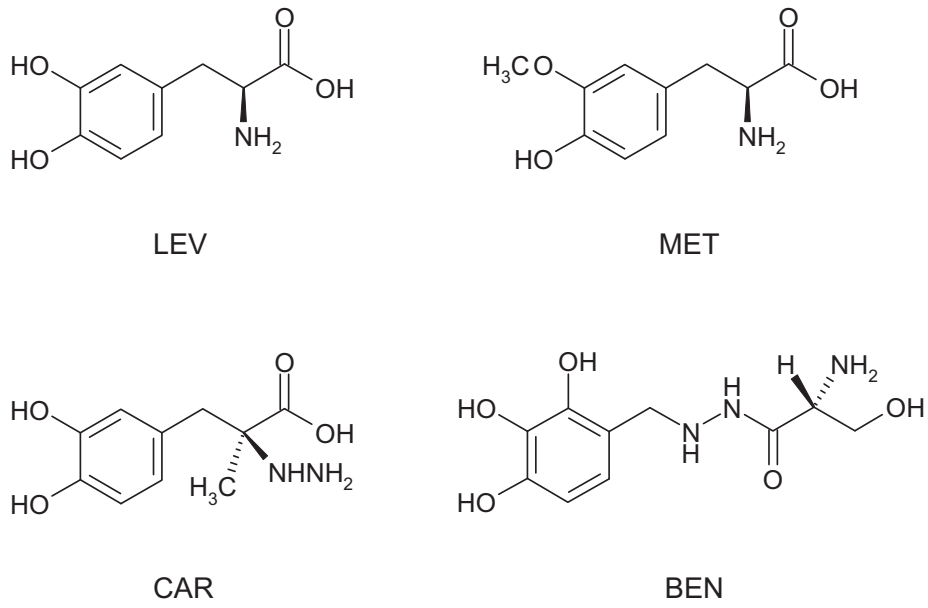

Fig. 1. Chemical structures of levodopa (LEV), 3-*O*-methyldopa (MET), carbidopa (CAR) and benserazide (BEN).

2. Experimental

2.1. Chemicals and reagents

Levodopa and carbidopa (internal standard) reference standards were purchased from the United States Pharmacopoeia (Rockville, MD, USA). 3-*O*-Methyldopa reference standard was purchased from Purity Grade Standards (Carrboro, NC, USA). Ultra-pure water was obtained from a Millipore system (Bedford, MA, USA). Acetonitrile and methanol (HPLC grade) were purchased from Tedia (Fairfield, OH, USA) and formic acid, perchloric acid, hydrochloric acid and sodium metabisulfite (analytical grade) were obtained from J.T. Baker (Phillipsburg, NJ, USA).

2.2. Instrumentation and analytical conditions

The HPLC–ESI-MS/MS analyses were carried out on an Agilent 1200 system (Santa Clara, CA, USA), composed of a quaternary pump, an autosampler, a column oven and an API 5000 triple quadrupole mass spectrometer (MDS-SCIEX, Concord, Ontario, Canada), equipped with an electrospray ion source. Analyst v.1.4.2 software was used for data acquisition and analysis. LC separation was performed on an ACE C_{18} column (50 mm × 4.6 mm i.d.; 5 μm particle size) from ACT (Aberdeen, Scotland), at 18 °C. The mobile phase consisted of 0.2% formic acid and acetonitrile (94:6, v/v), at a flow rate of 0.2 mL/min. The run time was 7.5 min and the injection volume was 10 μL.

Mass spectrometric detection was performed using electrospray ion source in positive ionization mode. The turbo-gas temperature was 500 °C, with an ion spray voltage of 4500 V and declustering potential of 96 for levodopa, 91 for 3-*O*-methyldopa and 116 for carbidopa. Nitrogen was used as nebulizer gas. Curtain gas setting was 10 and collision gas setting was 6. The collision energies were optimized at 35 V for levodopa, 21 V for 3-*O*-methyldopa and 17 V for carbidopa. Selected reaction monitoring (SRM) was employed for data acquisition. The SRM fragmentation transitions were m/z 198 → m/z 107, m/z 212 → m/z 166 and m/z 227 → m/z 181 for levodopa, 3-*O*-methyldopa and carbidopa, respectively. The scan dwell time was set at 0.5 s for each channel.

2.3. Preparation of standard solutions

Stock solution of levodopa was prepared by dissolving the accurately weighed reference substance in methanol and water (7:3) containing 0.5% hydrochloric acid and 0.1% sodium metabisulfite. Stock solution of 3-*O*-methyldopa was prepared by dissolving the accurately weighed reference substance in methanol and water (7:3) containing 0.1% sodium metabisulfite. Stock solution of carbidopa was prepared by dissolving the accurately weighed reference substance in methanol and water (7:3). The working solution of carbidopa (IS) was prepared by diluting the stock solution with methanol and water (1:1) to a final concentration of 1000 ng/mL.

2.4. Preparation of calibration and QC samples

Six calibration samples were prepared by spiking appropriate amounts of the stock solutions of levodopa and 3-*O*-methyldopa in blank plasma. The volume of the stock solutions added did not exceed 1% of the total plasma volume. Serial dilutions were performed to obtain the concentrations of the calibration samples in plasma: 50, 100, 500, 1500, 3000 and 6000 ng/mL for levodopa and 25, 100, 500, 1000, 2000 and 4000 ng/mL for 3-*O*-methyldopa. Quality control (QC) samples in plasma were prepared in a similar way, at low, middle and high concentrations: 150, 2400 and 4800 ng/mL for levodopa and 75, 1600 and 3200 ng/mL for 3-*O*-methyldopa.

2.5. Sample preparation

A 50 μL aliquot of the IS solution (1000 ng/mL of carbidopa) and a 50 μL aliquot of 2 M perchloric acid were added to 250 μL of plasma sample. The sample was vortex mixed for 20 s and centrifuged at 17,500 × *g* for 10 min, at 5 °C. Then, 100 μL of the supernatant were transferred to a vial containing 500 μL of methanol and water (1:1). After vortex mixed for 20 s, a 10 μL aliquot was injected into the chromatographic system.

2.6. Method validation

The validation process was carried out according to Guidance for Industry – Bioanalytical Method Validation, recommended by US Food and Drug Administration [21].