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A new HILIC-MS/MS method for the simultaneous analysis of carbidopa, levodopa, and its metabolites in human plasma

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ABSTRACT

Monitoring of the plasmatic levels of levodopa (LEV) and carbidopa (CAR) is necessary to adjust the dose of these drugs according to the individual needs of Parkinson's disease patients. To support drug therapeutic monitoring, a method using HILIC mode and LC–MS/MS was developed for the simultaneous determination of carbidopa, levodopa, and its metabolites (3-*o*-methyldopa (3-OMD) and dopamine (DOPA)) in human plasma. A triple quadrupole mass spectrometry was operated under the multiple reaction-monitoring mode (MRM) using the electrospray ionization technique. After straightforward sample preparation via protein precipitation, an Atlantis HILIC (150 × 2.1 mm, 3 μm, Waters, USA) column were used for separation under the isocratic condition of acetonitrile/water (79:21, *v/v*) containing 0.05% formic acid and 3 mmol/L ammonium formate and the total run time was 7 min. Deuterated LEV was used as internal standard for quantification. The developed method was validated in human plasma with a lower limit of quantitation of 75 ng/mL for LEV, 65 ng/mL for CAR and 3-OMD, and 20 ng/mL for DOPA. The calibration curve was linear within the concentration range of 75–800 ng/mL for LEV, 65–800 ng/mL for CAR and 3-OMD, and 20–400 ng/mL for DOPA ($r > 0.99$). The assay was accurate and precise, with inter-assay and intra-assay accuracies within ±13.44% of nominal and inter-assay and intra-assay precision ≤ 13.99%. All results were within the acceptance criteria of the US FDA and ANVISA guidelines for method validation. LEV, CAR, 3-OMD and DOPA were stable in the battery of stability studies, long-term, bench-top, autosampler, and freeze/thaw cycles. Samples from patients undergoing treatment were analyzed, and the results indicated that this new method is suitable for therapeutic drug monitoring in Parkinson's disease patients.

1. Introduction

New drugs have become available in recent years for the treatment of Parkinson's disease. However, since the introduction of dopamine (DOPA) supplementation, levodopa (LEV) has been considered the gold standard treatment for motor symptoms [1–3].

Human plasma contains several catechols, including LEV and DOPA (plasmatic levels at 1.75 and 0.01 ng/mL, respectively) [4]. LEV is a natural precursor of DOPA, and it is used as a prodrug because, in contrast to DOPA, LEV can cross the brain-blood barrier (Fig. 1). Due to its extensive metabolization in extracerebral tissues, LEV is usually associated with enzymatic inhibitor drugs such as carbidopa (CAR) or benserazide (aromatic L-amino acid decarboxylase inhibitors) and tolcapone or entacapone (catechol-*o*-methyltransferase inhibitors) [5].

Prolonged use of LEV leads to fluctuations and motor complications such as the "wearing-off" phenomenon characterized by moments without the benefits provided by therapy and moments with its benefits but with added dyskinesias. Studies have shown that high doses of LEV are also related to dyskinesias presented by patients [6]. Therefore, it is recommended that LEV doses should be adjusted according to the individual needs of patients based on clinical response and the profile of adverse events [1].

Many analytical methods have been described in the literature for the determination of LEV and its metabolites in biological matrices using high-performance liquid chromatography (HPLC)

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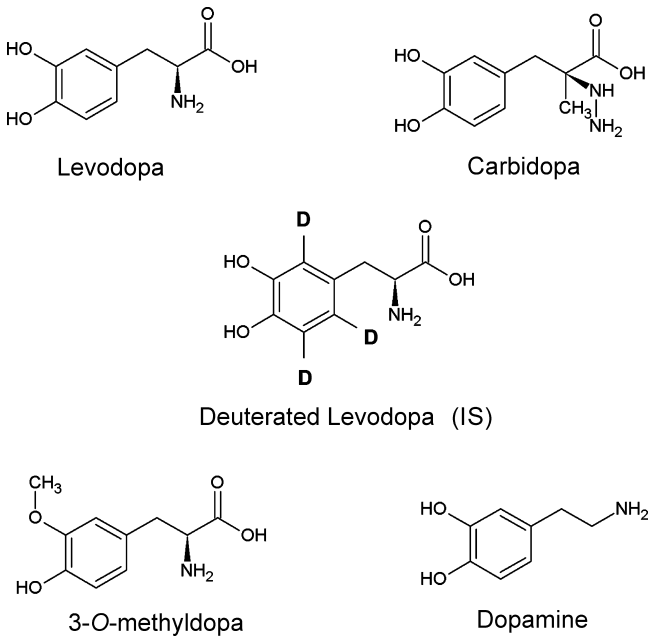

Fig. 1. Chemical structures of levodopa, carbidopa, 3-*o*-methyldopa, dopamine, and deuterated levodopa as the internal standard (IS).

and various detection techniques such as electrochemical detection [7–15], tandem mass spectrometry (MS/MS) [16–21], and fluorescence [22,23]. However, these methods use reversed-phase liquid chromatography (RPLC). Given the hydrophilic nature of these compounds, RPLC methods can result in low retention and usually necessitate the use of ion pair agents or derivative reagents that are incompatible with MS detection. For the most part, RPLC methods need high concentrations of aqueous phase (>90%) to obtain an acceptable retention [16,17,19]. Other problems include peak tailing following the presence of ionized silanol groups on the stationary phase and phase dewetting caused by the low concentrations of organic modifier that RPLC methods require for adequate retention of very polar analytes [24,25].

Hydrophilic interaction liquid chromatography (HILIC) has been reported as an alternative to RPLC for the analysis of polar compounds [26,27]. In HILIC, a hydrophilic stationary phase (bare silica or polar bonded silica) and a mobile phase consisting of >60% organic content, commonly acetonitrile, with a minimum of 2% of water are used [28]. The term HILIC was first used by Alpert, who considered the main retention mechanism to be the partitioning between the mobile phase and a mobile phase layer enriched with water partially immobilized on the stationary phase [29]. On the contrary, the retention mechanism in HILIC mode appears to be complex, involving also secondary electrostatic, hydrophobic, and hydrogen-bonding interactions dependent on the conditions used—for example, mobile phase additives [26,30].

Despite its complex mechanism, HILIC has the following advantages over RPLC: good peak shapes for bases, good retention of polar compounds, higher flow rates owing to the high organic content, and enhanced mass spectrometer sensitivity due to the high organic content in the mobile phase and the high efficiency of desolvation in electrospray (ESI) techniques [31]. In recent years, HILIC has been used to separate catecholamines [24] and neurotransmitters such as biogenic amines and amino acid precursors [28], but no technique has been developed for the simultaneous monitoring of LEV treatment with MS/MS detection. Considering the characteristics of these compounds and the advantages of HILIC, the aim of this study was to develop and validate a sensitive and selective HILIC-MS/MS method to quantify LEV, CAR, 3-*o*-methyldopa (3-OMD), and DOPA simultaneously in human plasma.

2. Materials and methods

2.1. Chemicals, reagents, and samples

LEV (98.0%), CAR (98.0%), dopamine hydrochloride (98.0%), and internal standard (IS) deuterated LEV (98.0%) were purchased from Sigma-Aldrich (St. Louis, MO, USA); 3-OMD (92.0%) was obtained from United States Pharmacopoeia (Rockville, MD, USA). Acetonitrile and methanol (HPLC grade) were obtained from Tedia (Fairfield, CA, USA). Formic acid (88%) was obtained from J.T. Baker (Phillipsburg, NJ, USA). Ammonium formate (≥97%) was obtained from Acros Organics (Fair Lawn, NJ, USA). Hydrochloric acid (36–38.0%) was purchased from Mallinckrodt (Edo. de Mexico, Mexico). Ultrapure water was obtained using a Milli-Q purification system from Millipore (Milford, MA, USA). Blank plasma (including lipemic and hemolyzed plasmas) were obtained from Centro de Hematologia e Hemoterapia do Paraná (HEMEPAR, Curitiba, Brazil) and EDTA was used as anticoagulant in these samples.

2.2. Standard solution preparation

2.2.1. Standard stock solutions

Stock solutions of LEV, CAR, 3-OMD, DOPA, and the IS were prepared separately at a concentration of 1 mg/mL each in methanol containing hydrochloric acid 0.5% (*v*/*v*) and stored at −40 °C. IS and combined working solutions of all compounds were freshly prepared as needed for each experiment via direct dilution of the stock standard solutions in methanol for intermediate working solutions and in acetonitrile containing 0.05% formic acid and 3 mmol/L ammonium formate to obtain the final desired concentration. The solutions were then filtered through a polyvinylidene fluoride (PVDF) syringe filter (13 mm, 0.22 μm; Millipore Millex, Billerica, MA, USA) before injection.

2.2.2. Calibration curve and quality control (QC) sample preparation

Calibration curves and QC samples were prepared by spiking 400 μL blank plasma with 50 μL of the appropriate work standard solution and 50 μL of the IS solution. Then, 1500 μL of acetonitrile containing 0.05% formic acid and 3 mmol/L ammonium formate was added, vortexed for 3 min, and centrifuged (Eppendorf 5810-R, Hamburg, Germany) at 36,000 × *g* and 5 °C for 20 min. An aliquot of supernatant was filtered through a PVDF syringe filter before injection. To protect analytes of interest from degradation, all samples were prepared under low light exposure [32].

Calibration curves were prepared in the range of 75–800 ng/mL for LEV, 65–800 ng/mL for CAR and 3-OMD, and 20–400 ng/mL for DOPA. All values were defined by lower and upper limits of quantification (LLOQ/ULOQ), respectively. QC samples were prepared at low, medium, and high concentrations, respectively, as follows: 225, 430, and 640 ng/mL for LEV; 195, 430, and 640 ng/mL for CAR and 3-OMD; and 60, 210, and 320 ng/mL DOPA. The IS concentration was 2500 ng/mL for all samples.

2.3. Sample preparation

Samples were thawed at room temperature. Then, 400 μL of plasma sample was pipetted into a 2-mL polypropylene tube, 50 μL of IS solution was added to each sample, and the mixture was vortexed for 1 min. Then, 1550 μL of acetonitrile containing 0.05% formic acid and 3 mmol/L ammonium formate was added, vortexed for 3 min, and centrifuged at 36,000 × *g* and 5 °C for 20 min. An aliquot of supernatant was filtered through a PVDF syringe filter