

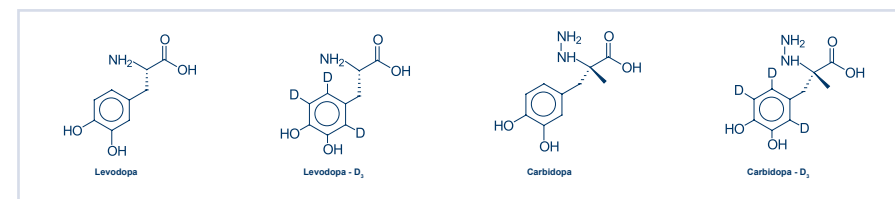
Determination of Levodopa and Carbidopa in Human Plasma by Solid-Phase Extraction and LC/MS/MS

Authors:

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Introduction:

Levodopa is a prodrug used in clinical management of Parkinson's disease and syndrome. In the central nervous system it converts to the naturally occurring neurotransmitter dopamine, which is critical to proper motor function and cognitive processes. Levodopa is typically co-administered with carbidopa, a structural analog which slows metabolic deactivation of levodopa outside the central nervous system by inhibition of decarboxylase activity. An efficient bioanalytical method for determination of levodopa and carbidopa in human plasma that is suitable for monitoring clinical trials is described herein.



Objective:

- To devise an efficient and rugged procedure for the accurate determination of levodopa and its analog carbidopa in K_2 -EDTA human plasma by LC/MS/MS.
- To validate analysis of levodopa and carbidopa over the ranges 10.0 – 2000 ng/mL and 2.00 – 400 ng/mL, respectively.
- To quantify levodopa and carbidopa in plasma samples collected from dosed human subjects.

Methodology:

Chemicals

Reference materials (levodopa and carbidopa) and internal standards (levodopa- D_3 and carbidopa- D_3) were obtained from commercial sources. All solvents were HPLC grade and additional reagents were ACS Reagent grade or better.

Sample Preparation

Individual stock solutions of levodopa and carbidopa were prepared in 0.1% phosphoric acid/water (v/v), and these stocks were used to prepare a combined intermediate solution at a known concentration of each analyte. These compounds decompose rapidly in plasma, so blank matrix was stabilized by the addition of sodium metabisulfite and hydrazine dihydrochloride solutions (20% wt/vol) at a proportion of 25 μ L per mL of K_2 -EDTA human plasma. Calibration standards covering the required ranges were then prepared in this stabilized blank matrix and stored in 0.200 mL aliquots at -70°C . Stock solutions of levodopa- D_3 and carbidopa- D_3 were prepared in 0.1% phosphoric acid/water (v/v), and a combined internal standard working solution in the same solvent was prepared from these stocks.

For validation, Quality Control samples were prepared in stabilized K_2 -EDTA human plasma at three different concentration levels (QC Low = 30.0 ng/mL levodopa, 6.00 ng/mL carbidopa; QC Medium = 500 ng/mL levodopa, 100 ng/mL carbidopa; QC High = 1500 ng/mL levodopa, 300 ng/mL carbidopa). These QC samples were stored in 0.200 mL aliquots at -70°C .

Plasma samples were extracted as indicated in the scheme below. To further minimize decomposition of the analytes samples were kept cold before loading on SPE cartridges.

Extraction

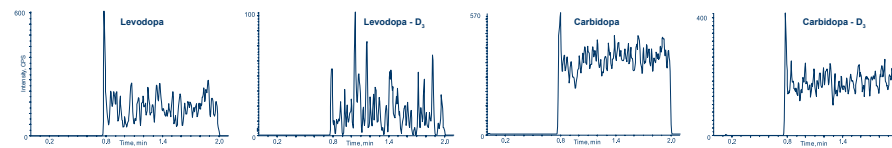
- Aliquot 0.200 mL stabilized K_2 -EDTA human plasma in an ice-water bath
- Add 25.0 μ L of working internal standard solution
- Add 200 μ L of cold ammonium phosphate buffer (20 mM, pH 10)
- Centrifuge at 4°C
- Condition Strata Screen A SPE cartridges (Phenomenex, 100 mg/1 mL) with 1 mL of methanol followed by 1 mL of water
- Load cold samples onto SPE cartridges
- Wash cartridges with 1 mL of water
- Elute with 0.800 mL of 3% formic acid in water
- Add 20 μ L of sodium metabisulfite solution (20% wt/vol)
- Analyze by LC-MS-MS

Sample Analysis by LC-MS-MS

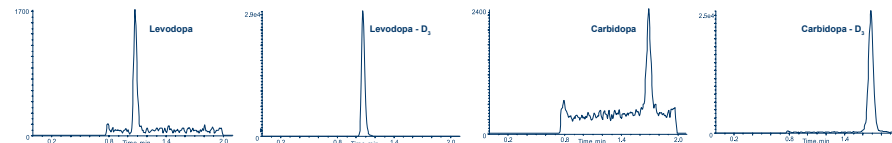
- Instrumentation: Perkin-Elmer 200 Series Autosampler
Shimadzu LC-10AT HPLC Pump
Applied Biosystems/MDS Sciex API 5000 mass spectrometer
- Column: XBridge Phenyl, 3.5 micron 4.6 x 50 mm
- Mobile phase: acetonitrile/water/formic acid (35/965/1.0, v/v/v)
- Flow rate: 0.6 mL/min
- Ionization mode: ESI+
- Ions monitored: m/z 198 \rightarrow 152 (levodopa)
m/z 201 \rightarrow 154 (levodopa- D_3)
m/z 227 \rightarrow 181 (carbidopa)
m/z 230 \rightarrow 184 (carbidopa- D_3)

Representative Chromatograms:

Blank



LLOQ



Results:

Standard Precision and Accuracy

Levodopa: Mean of Three Validation Runs

Amount Added, ng/mL	10.0	20.0	40.0	100	300	600	1800	2000
Mean Found, ng/mL	9.83	21.3	37.2	102	307	585	1770	2040
CV (%)	4.1	8.0	6.4	1.5	6.8	6.1	2.8	0.8
% Bias	-1.7	6.5	-7.0	2.0	2.3	-2.5	-1.7	2.0

Carbidopa: Mean of Three Validation Runs

Amount Added, ng/mL	2.00	4.00	8.00	20.0	60.0	120	360	400
Mean Found, ng/mL	1.96	4.16	8.00	20.0	60.0	121	356	395
CV (%)	3.4	6.6	1.2	6.1	4.1	2.1	5.0	4.3
% Bias	-2.0	4.0	0.0	0.0	0.0	0.8	-1.1	-1.3

LLOQ and QC Precision and Accuracy

Levodopa: Mean of Three Validation Runs (n=18)

Amount Added, ng/mL	10.0	Low	Med	High
Mean Found, ng/mL	10.4	31.5	516	1570
CV (%)	11.1	6.1	3.8	4.8
% Bias	4.0	5.0	3.2	4.7

Carbidopa: Mean of Three Validation Runs (n=18)

Amount Added, ng/mL	2.00	6.00	100	300
Mean Found, ng/mL	2.11	6.11	98.5	288
CV (%)	15.1	6.7	5.3	5.2
% Bias	5.5	1.8	-1.5	-4.0

Stability

Levodopa

Concentration, ng/mL	30.0	1500
Mean % Change		
BTS, 24 hrs @ 1°C	4.3	5.1
FTS, 5 cycles	6.6	4.2
XTS, 21 hrs @ 22°C	14.2	7.6
LTS, 22 days @ -70°C	5.6	-6.0

Carbidopa

Concentration, ng/mL	6.00	300
Mean % Change		
BTS, 24 hrs @ 1°C	-7.6	-9.9
FTS, 5 cycles	6.8	-9.3
XTS, 21 hrs @ 22°C	-3.9	-3.2
LTS, 22 days @ -70°C	-9.4	-8.8

Levodopa and carbidopa decompose rapidly in unstabilized plasma but show acceptable stability when blank matrix is treated with the preservatives sodium metabisulfite and hydrazine dihydrochloride, and when samples are kept cold prior to extraction. Solid phase extraction on an anion exchange support provides recovery of over 70% for both analytes. Eluted samples were analyzed without evaporation/reconstitution, although an aliquot of sodium metabisulfite in water (20% wt/vol) was added prior to injection to improve room-temperature stability. Representative chromatograms from control blank and LLOQ samples are shown at left. Three validation runs performed on different days demonstrated good linearity (mean correlation coefficients ≥ 0.995) over a quantitative range of 10.0 to 2000 ng/mL for levodopa and 2.00 to 400 ng/mL for carbidopa. Intra- and interday accuracy and precision met acceptance criteria for both analytes ($< 15\%$ bias and CV, $< 20\%$ at the LLOQ). No chromatographic interferences or matrix effects were observed for plasma samples from six individuals. Stability for both analytes was established in matrix (for short-term storage in an ice bath and through multiple freeze/thaw cycles), in the final extract, and for long-term storage at -70°C .

Conclusion:

An LC-MS-MS method for quantitation of levodopa and carbidopa in K_2 -EDTA human plasma stabilized with sodium metabisulfite and hydrazine dihydrochloride has been developed. This method was shown to be specific, accurate, and robust, and has been validated for use in pharmacokinetic studies. Approximately 1400 clinical study samples have been successfully analyzed by this procedure.