*Journal of Chromatography*, 534 (1990) 87–100
*Biomedical Applications*
Elsevier Science Publishers B V , Amsterdam

CHROMBIO 5588

# Simultaneous high-performance liquid chromatographic analysis of carbidopa, levodopa and 3-O-methyldopa in plasma and carbidopa, levodopa and dopamine in urine using electrochemical detection

D C TITUS

*Merck Sharp & Dohme Research Laboratories, West Point, PA 19486 (U S A )*

T F AUGUST

*Hospital of the University of Pennsylvania, Philadelphia, PA 19104 (U.S.A.)*

K C. YEH*, R. EISENHANDLER and W. F BAYNE

*Merck Sharp & Dohme Research Laboratories, West Point, PA 19486 (U.S.A )*

and

D G MUSSON

*IOLAB Corporation, Claremont, CA 91711 (U S.A )*

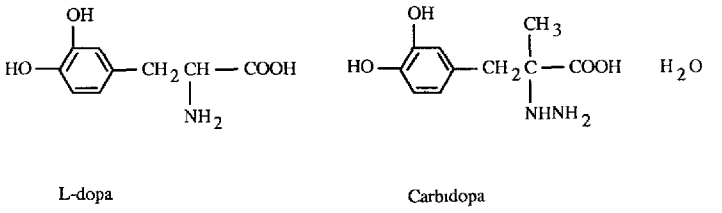
(First received May 7th, 1990, revised manuscript received August 20th, 1990)

## ABSTRACT

Two assay procedures are described for the analysis of levodopa, carbidopa and 3-O-methyldopa in plasma and levodopa, carbidopa and dopamine in urine. The methods are suitable for quantifying the analytes following therapeutic administration of levodopa and carbidopa. Both were based on reversed-phase high-performance liquid chromatography (HPLC) with electrochemical detection and with ethyldopa as the internal standard Plasma samples were prepared by perchloric acid precipitation followed by the direct injection of the supernatant. Urine was prepared by alumina adsorption, and the analytes were desorbed with perchloric acid solution containing disodium EDTA and sodium metabisulfite prior to injection into the HPLC system The methods have been utilized to evaluate the pharmacokinetics and bioavailability of oral dosage forms containing levodopa and carbidopa

## INTRODUCTION

Sinemet, a combination of levodopa [L-α-amino-β-(3,4-dihydroxybenzene)-propanoic acid] and carbidopa [L-α-hydrazino-α-methyl-β-(3,4-dihydroxybenzene)propionic acid monohydrate] (Fig. 1), is used therapeutically in the treatment of Parkinson's disease [1–3] Carbidopa inhibits the peripheral decarboxylation of levodopa and, thus, allows a greater amount of the levodopa dose to be transported into the central nervous system. Levodopa is a metabolic precursor of the neurotransmitter dopamine [4]. Dopamine has been postulated to be the




L-dopa Carbidopa

Fig. 1 Structures of levodopa and carbidopa

active agent in the treatment of Parkinson's disease [4]. In the presence of carbidopa, the primary metabolite of levodopa in the peripheral circulation is 3-O-methyldopa. This compound has been implicated in the dose-related side-effects of chronic levodopa therapy [5]. Dopamine, the decarboxylated metabolite of levodopa, is rapidly cleared from plasma but does not cross the blood–brain barrier [6].

Controlled-release formulations of Sinemet have been devised to provide an improved drug delivery system. To study the bioavailability and biopharmaceutic properties of these formulations, it became necessary to develop reliable methods for the simultaneous determination of levodopa and carbidopa in plasma and urine. In addition, it is also necessary to measure 3-O-methyldopa (3-OMD) in plasma and dopamine in urine in order to assess whether a modification of drug delivery mode would affect the rate of decarboxylation inhibition as manifested in the time profiles of these primary metabolites. A literature review of analytical methods shows that both radioenzymatic assays (REA) and high-performance liquid chromatography (HPLC)–electrochemical detection (ED) [7–9] are currently used. REAs are difficult to set up, expensive and require great care in maintaining satisfactory controls [10]. HPLC–ED has been the preferred method for the separation and detection of these compounds. However, there is no general agreement as to their extraction and clean-up procedures from biological fluids [11]. Furthermore, until now there has been no method available for the quantification of carbidopa in urine.

In this report, two reliable methods are described for the analysis of human plasma and urine. Methods for the simultaneous determination of these compounds in plasma have been previously reported [12–17]. However, our initial attempts at reproducing these extraction and isolation procedures were unacceptable because of low recoveries, inadequate sensitivities or poor reproducibilities. We have been unable to find any reports of methods for the simultaneous determination of levodopa, dopamine and carbidopa in human urine. The procedures reported here are reliable and have been used to process large numbers of clinical samples.

Stability problems can be encountered when working with catecholamines and catecholamine amino acids. With carbidopa, these problems are increased due to the presence of the hydrazino group. It has been shown that acidic solutions of

carbidopa may decompose in air to yield 3,4-dihydroxyphenylacetone [18]. We report here procedures to stabilize these compounds during sample preparation and subsequent analysis.

## EXPERIMENTAL

### *Chemicals and reagents*

Levodopa and 3-OMD were obtained form Sigma (St. Louis, MO, U.S.A.). Carbidopa, α-ethyldopa and dopamine were provided by Merck Chemical Division (Rahway, NJ, U.S.A.). Reagents used in preparing the mobile phase were Milli-Q water, prepared from the Millipore reagent water system (Bedford, MA, U.S.A.), methanol, purchased from Fisher Scientific (Springfield, NJ, U.S.A.), and sodium octanesulfonate, obtained from Aldrich (Milwaukee, WI, U.S.A.). Alumina N, activity grade I, obtained from ICN Biochemicals (Cleveland, OH, U.S.A.), was acid-washed using the method of Anton and Sayre [19]. Control pooled plasma was obtained from various commercial sources. All other reagents were ACS grade and were used without further purification.

### *Instrumentation*

The HPLC system for the plasma assay consisted of a Series 10 pump and an ISS-100 autosampler with a temperature-controlled sample tray, both from Perkin Elmer (Norwalk, CT, U S.A.), an electrochemical detector package containing two thin-layer transducers, connected in series to their respective LC-4B controllers (BAS, West Lafayette, IN, U.S A.), and a Model 4270 computing integrator from Spectra-Physics (San Jose, CA, U.S.A.).

The urine assay used a similar HPLC system except for the LC-4A controller and the solvent delivery system, which was a Model 6000A pump, and an autosampler WISP 710, from Waters Assoc. (Milford, MA, U.S.A.).

Plasma centrifugation was performed on a Microfuge 12 microcentrifuge from Beckman Instruments (Fullerton, CA, U.S.A.).

### *Chromatography*

The mobile phase for the plasma assay consisted of a 20 m*M* orthophosphoric acid–4 m*M* sodium octanesulfonate solution mixed with 25% (v/v) methanol and adjusted to pH 2.8 ± 0.05 with 50% (w/w) sodium hydroxide. This solution was vacuum-filtered through a 0.2-μm membrane filter and purged with nitrogen prior to use. The flow-rate was 1.0 ml/min. An analytical column and two guard columns were used for this separation. The analytical column was an Ultrasphere IP ($C_{18}$, 5 μm particle size, 250 mm × 4.6 mm I.D.) from Beckman Instruments, the guard columns were a pellicular-ODS (37–40 μm particle size) from Whatman coupled with a second Ultrasphere-IP guard column ($C_{18}$, 5 μm particle size, 45 mm × 4.6 mm I.D.) from Beckman. The columns were heated in a column oven at 40°C.

Mobile phase for the urine was a 22.5% (v/v) aqueous methanol solution containing 20 m*M* citric acid, 20 m*M* $Na_2HPO_4$, 4 m*M* sodium octanesulfonate and 0.05 m*M* disodium EDTA. After adjusting the pH to 2.74 ± 0.01 with 2 *M* citric acid, the solution was vacuum-filtered. The mobile phase was degassed with helium before use, sparged continuously and pumped at a flow-rate of 1.5 ml/min. The analytical column used was an Ultrasphere-IP ($C_{18}$, 5 μm particle size, 250 mm × 4.6 mm I.D., Beckman) with a Micro-Guard cartridge (Bio-Sil ODS-10, 40 mm × 4.6 mm I.D.) from Bio-Rad Labs. (Richmond, VA, U.S.A.).

*Detection*

All analytes were detected by amperometric electrochemical oxidation. For the plasma assay, the eluent from the analytical column flowed through two sequential electrochemical cells: one set at 20 nA/full scale for levodopa and 3-OMD and a second set at 5 nA full scale for cabidopa. The oxidation voltage was set at 0.75 V *vs.* Ag/AgCl reference for both cells.

The urine assay used a single cell set at a voltage of 0.54 V and a range of 50 nA full scale for levodopa, carbidopa and dopamine.

*Calibration standards and internal standard*

*Plasma.* Standard stock solutions of levodopa, carbidopa and 3-OMD were prepared at a concentration of 100 μg/ml in 0.1 *M* HCl. Serial dilutions of 1:10, 1:100 and 1:1000 were made of the stocks to obtain final working stock solutions. Standard solutions were prepared by mixing appropriate aliquots of the working stock solutions and the control plasma. The concentrations ranged from 25 to 1000 ng/ml for carbidopa and 25 to 5000 ng/ml for levodopa and 3-OMD.

The stock solution for the internal standard was prepared with 25 mg of α-ethyldopa in 100 ml of 0.1 *M* HCl. A final stock solution (5 μl/ml) was prepared in 0.1 *M* HCl and used in the sample preparation. The solutions were stored at 0–5°C.

*Urine.* The standard stock solutions were prepared as above for plasma using 10 m*M* HCl. The concentration ranges were 25–1000 ng/ml for dopamine and 0.25–10 μg/ml for levodopa and carbidopa.

The stock solution for the internal standard was 10 μg/ml in 10 m*M* HCl.

*Sample preparation prior to HPLC*

*Plasma.* A 1-ml volume of plasma (clinical sample or standard solution) was mixed with 100 μl of internal standard and 100 μl of 4 *M* perchloric acid. The sample was vortex-mixed and then centrifuged on a micro-centrifuge at 2000 *g* for 10 min. The supernatant was removed and 60 μl were injected into the HPLC system. The sample tray was kept at 6 ± 1°C.

*Urine.* A 100-μl aliquot of urine was placed in a Centrex microfilter tube (Schleicher and Schuell, Renee, NH, U.S.A.). A 100-μl volume of stabilizing ascorbic acid–EDTA solution (containing 55 m*M* ascorbic acid and 55 m*M* di-

sodium EDTA) and 25 $\mu$l of the internal standard solution were added and mixed; 25 mg of alumina and 1.0 ml of 2.0 *M* Tris–HCl buffer, pH 8.6, were added, mixed on a multi-tube vortex-mixer for 5 min and allowed to stand for 10 min. The tubes were attached to a vacuum manifold to remove the fluid from the alumina. The alumina was washed with 5 ml of Milli-Q water; the tubes were removed from the manifold and 5-ml receiving tubes were attached. An additional 5 ml of water were added to the tubes, the tubes were centrifuged (3000 *g*) and the filtrate was discarded. Eppendorf receiving tubes (1.5 ml) were then attached to the microfilters. The analytes were desorbed from the alumina by vortex-mixing with 400 $\mu$l of 0.2 *M* perchloric acid containing 11 m*M* disodium EDTA and 0.4 *M* sodium metabisulfite. The microfilter was centrifuged at 9000 *g* for 5 min. A 50-$\mu$l aliquot of the filtrate was injected into the HPLC system.

*Stabilization procedures during sample collection*

Approximately 5 ml of whole blood were collected in tubes containing EDTA. The plasma was separated promptly by centrifugation and transferred to a polypropylene tube containing 10 mg of sodium metabisulfite. The sample was immediately mixed by vortex, frozen on dry ice (−78°C) and stored at −70°C.

A 10-ml aliquot from each urine void was placed in a polypropylene scintillation vial containing 0.5 ml of 0.1 *M* HCl and 1.0 ml of the stabilizing ascorbic acid–EDTA solution. The urine solution was mixed and stored at −70°C.

## RESULTS AND DISCUSSION

*Sample preparation prior to HPLC analysis*

For assaying 3-OMD in plasma along with levodopa and carbidopa, various acid and organic deproteination schemes were evaluated. The perchloric acid precipitation preparation gave the best chromatographic results and was selected. The alumina extraction procedure [20] was used for assaying levodopa, carbidopa and dopamine in the urine since it was not necessary to measure 3-OMD. The alumina method allows for increased sensitivity by effectively eliminating endogenous non-catechol chromatographic interferences.

*HPLC–ED*

Typical chromatograms are shown in Fig. 2 for plasma and in Fig. 3 for urine. The structural similarity of ethyldopa to the analytes of interest, its electrochemical property and its elution time from the column make it amenable to both urine and plasma sample preparation procedures. As shown in the figures, ethyldopa is well resolved from all analytes of interest in 20 min.

The instrumentation and conditions of the two assays were developed differently as needed for optimal separation, analytes of interest (*e.g.* 3-OMD in plasma *versus* dopamine in urine), sensitivity, etc. In each case and for each chromatographic system, appropriate adjustments were made to the mobile phase. These

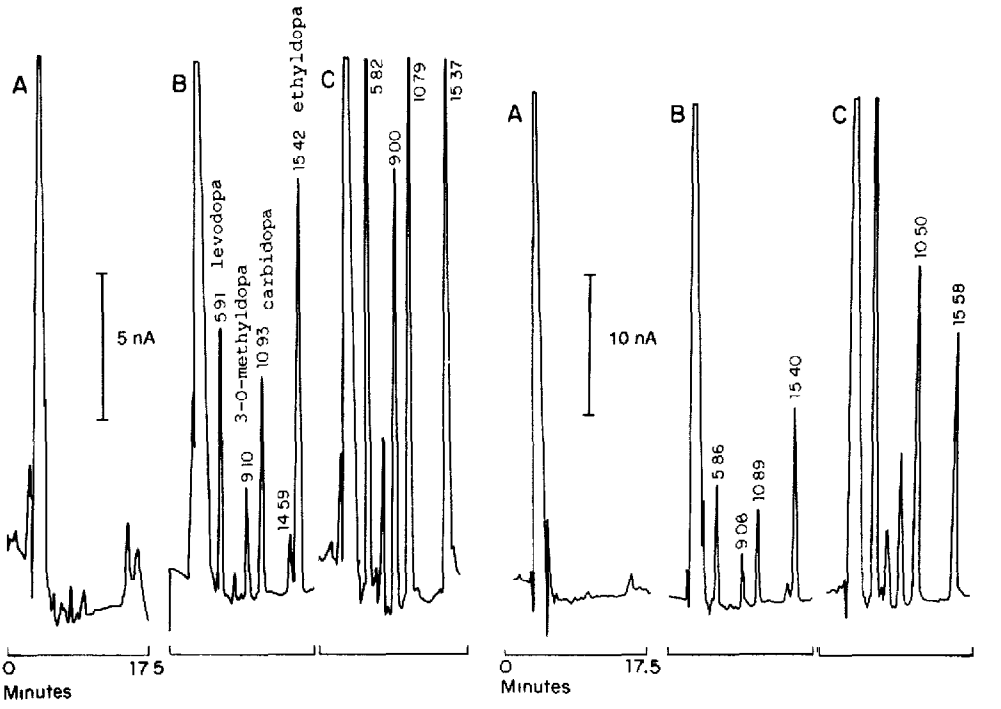



Fig. 2 Representative chromatograms of (A) plasma blank, (B) standard (100 ng/ml) of levodopa, carbidopa and 3-O-methyldopa with ethyldopa as internal standard in control plasma and (C) clinical sample Detector settings at 5 nA full scale (left panel) and at 20 nA full scale (right panel).

include the selection of the buffer system, pH, methanol content, column temperature and the concentration of ion-pairing reagent Guard columns were used to protect the analytical column head from degradation.

As shown in Fig. 4, the oxidation potentials for the compounds of interest range from +0.7 to +0.9 V. While the voltage across the electrochemical cell was set at 0.75 V for the plasma assay, a lower potential of 0.54 V was adapted for the urine assay to reduce the background noise.

*Stability*

*Plasma.* The stability of levodopa and cabidopa in drug-free control plasma at room temperature is shown in Fig. 5. Levodopa and 3-OMD are stable for up to 6 h at room temperature in normal laboratory conditions (light and atmosphere). Carbidopa is highly susceptible to degradation. 3,4-Dihydroxyphenylacetone has been identified as the major decomposition product of carbidopa via oxidation [18]. Heat and oxygen catalyzed this reaction. The rate of this decomposition can be greatly reduced by adding an antioxidant (sodium metabisulfite) and a heavy metal chelator (EDTA) (Fig. 5). Long-term storage of clinical samples is ac-

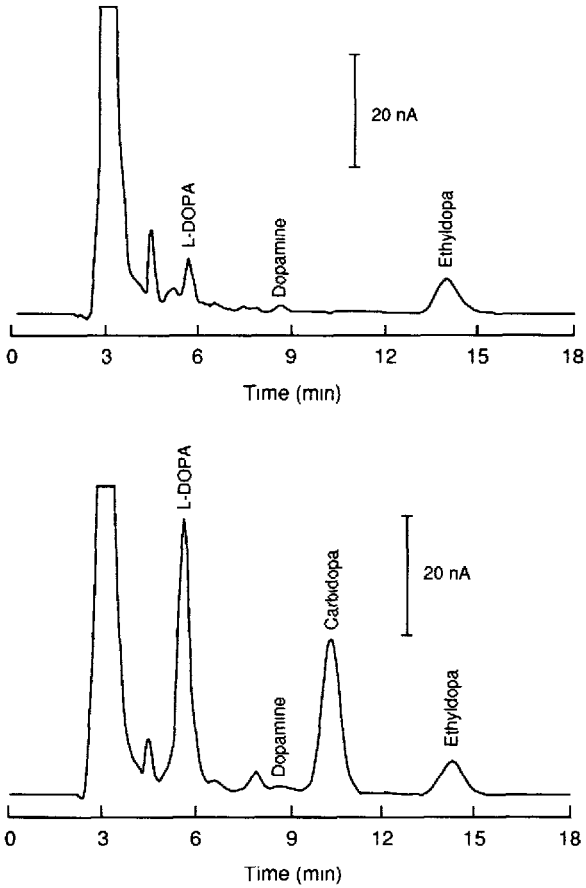



Fig. 3. Representative chromatograms of urine (spiked with ethyldopa) from a volunteer before (upper panel) and 2 h after (lower panel) intake of carbidopa and levodopa

complished by the addition of these stabilizers, rapidly freezing the samples as collected and storing them at −70°C.

Thawing the clinical samples in the refrigerator (1–2 h) prior to analysis and storing the prepared samples on a sample tray at 6°C was found to greatly improve the stability of carbidopa for up to 60 h.

*Urine.* Initial reproducibility studies of replicate urine standards containing only HCl for stabilization gave coefficients of variation (C.V.) of 10–20% over the concentration range 0.25–10 μg/ml for carbidopa. Levodopa and dopamine gave acceptable C.V.s of $< 10\%$. The above results and the inherently greater instability of carbidopa over levodopa, ethyldopa and dopamine to oxidation resulted in further stability experiments with carbidopa in urine. The degradation of carbidopa in acidified urine and acidified urine with sodium metabisulfite–EDTA at ambient temperature showed significant losses of the intact drug in 2 h.

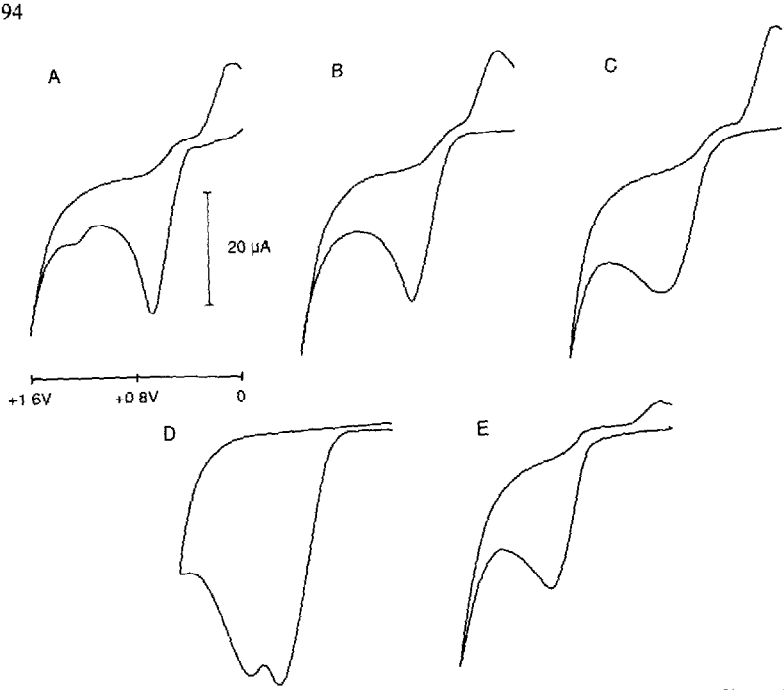



Fig 4 Cyclic voltamograms for levodopa (A), ethyldopa (B), dopamine (C), carbidopa (D) and 3-O-methyldopa (E) at 2 mg/ml in 22 5% (v/v) aqueous methanol solution containing 50 m*M* citric acid, 5 m*M* $Na_2HPO_4$, 3 7 m*M* sodium octane sulfate and 0 05 m*M* EDTA, pH 2.7

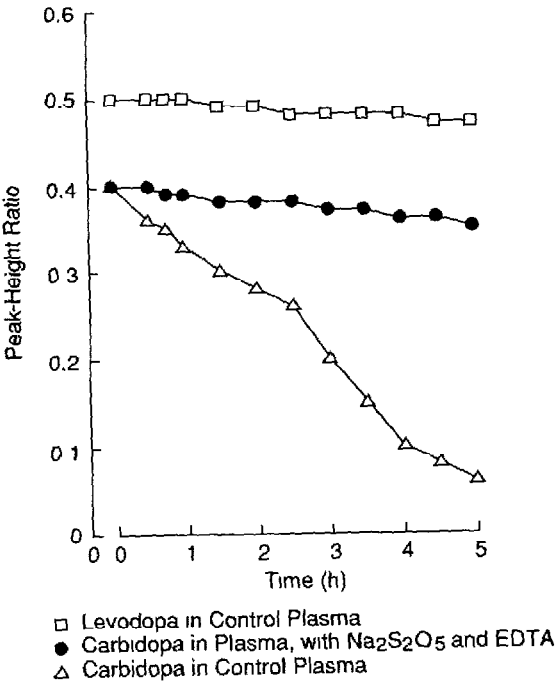



Fig 5 Stability of levodopa and carbidopa in control plasma at 23°C. Both levodopa and carbidopa v prepared at 100 ng/ml and stored in glass vials used for the autosampler

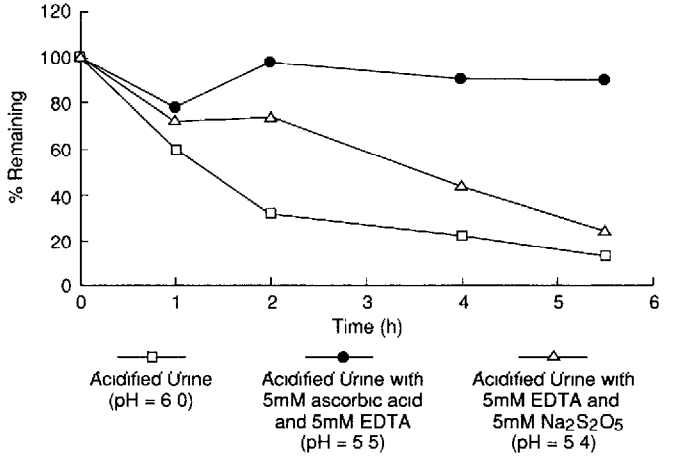



Fig 6 Comparison of carbidopa (1 μg/ml) stability in acidified urine (5 m*M* HCl) with and without additional stabilizers

However, degradation was minimal for the combination ascorbic acid–EDTA (Fig. 6).

The intra-day reproducibility of carbidopa in the presence of the ascorbic acid–EDTA stabilizer was further tested. Again, C.V.s for carbidopa were high (>10%) but not as great as before and a trend was evident in the peak-height ratios with time. Stability studies were repeated in various alumina extracting solutions (Fig. 7): 0.2 *M* perchloric acid ($HClO_4$), 0.2 M $HClO_4$ with 0.4 *M* sodium metabisulfite, 0.2 *M* trichloroacetic acid (TCA) and 0.2 *M* $HClO_4$ neutralized. The results showed loss of carbidopa over 25 h with $HClO_4$ and neutral-

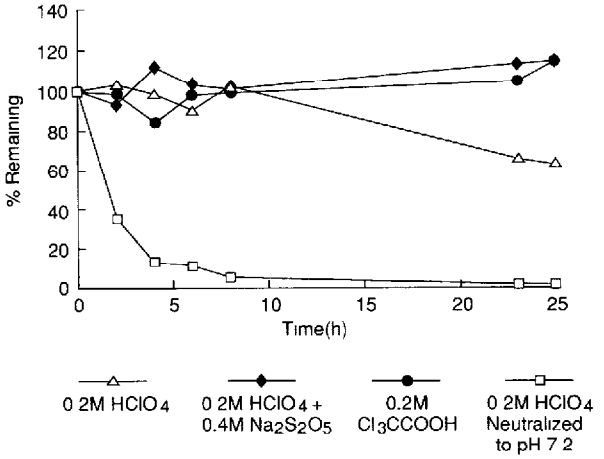



Fig. 7 Carbidopa (1 μg/ml) stability in various alumina extracts from acidified urine. Carbidopa was desorbed with 0 2 *M* $HClO_4$ or 0 2 *M* $Cl_3CCOOH$. The $HClO_4$ extracts were treated with $Na_2S_2O_5$ or neutralized to pH 7 2 with 1 *M* NaOH The samples were allowed to incubate at 23°C in glass vials used for the autosampler and analyzed over 25 h

ized $HClO_4$ (pH 7.2). Initial work with TCA over time resulted in an accelerated degradation of the column as measured by loss of retention and separation of dopamine and carbidopa. Reproducibility studies with $HClO_4$ and sodium metabisulfite were acceptable with C.V.s $< 10\%$. The desorbing solution used for the clinical studies was $HClO_4$ plus sodium metabisulfite and EDTA.

*Assay parameters*

*Linearity.* Linearity was established for each assay in working standard calibration ranges (Table I). In urine, the standard curves were linear from 0.25 to 10 μg/ml for levodopa and carbidopa and 25 to 1000 ng/ml for dopamine. In plasma, they were linear from 25 to 5000 ng/ml for levodopa and 3-OMD and 25 to 1000 ng/ml for carbidopa. Sample concentrations that were above the linear calibration range were diluted to within the range.

*Specificity.* The assays are specific with respect to levodopa, dopamine and 3-OMD which are products of normal metabolism and were present in the control blanks and 0-h collections of plasma and urine. No endogenous interfering peaks were noted for carbidopa, and baseline separation of levodopa, 3-OMD, dopamine and carbidopa was achieved.

*Sensitivity.* The minimum detection limit (Table I) of each assay for each analyte was determined on the basis of reproducibility and linearity criteria. Nor-

TABLE I

INTRA-DAY VARIABILITY OF PLASMA AND URINE ASSAY OF REPLICATE ANALYSIS ($n = 6$)

| Concentration (ng/ml) | Coefficient of variation (%) | | | | | |
|---|---|---|---|---|---|---|
| | Plasma | | | Urine | | |
| | Levodopa | Carbidopa | 3-OMD | Levodopa | Carbidopa | Dopamine |
| 25 | 2.5[a] | 5.1[a] | 1 1[a] | – | – | 5 6[a] |
| 50 | 3.3 | 5 1 | 5 6 | – | – | 1 6 |
| 100 | 5.3 | 3 7 | 3 0 | – | – | 8 3 |
| 250 | 2.1 | – | – | 4 6[a] | 10.3[a] | 2.6 |
| 500 | 1.9 | 7 4 | 4 7 | 1 5 | 4.5 | 6 5 |
| 1000 | 5 2 | 5 6 | 2 5 | 5 7 | 2.9 | 5 0 |
| 2000 | 2 0 | – | – | – | – | – |
| 2500 | 1 6 | – | 3 3 | 6 4 | 6.6 | – |
| 5000 | 1.8 | – | 2 6 | 4 0 | 1.9 | – |
| 10000 | – | – | – | 10.5 | 1.3 | – |

[a] Minimum concentration quantified on the calibration curve.

mal endogenous plasma levodopa levels range from 1 to 2 ng/ml, but are elevated to 6–8 ng/ml in the presence of carbidopa [21]. As a result, the presence of levodopa peak was detected in the drug-free plasma samples causing large C V.s (greater than 10%) below the quantitation limit of 25 ng/ml. Similar results were observed for the urine assay with respect to endogenous levodopa and dopamine

*Reproducibility*. Intra-day C.V s were calculated from the analysis of replicate standards ($n = 6$) at each concentration on the calibration curve. The C.V.s for the three analytes in plasma show variations of 1.6 to 5.3% for levodopa, 1.1 to 5 6% for 3-OMD and 3.7 to 7.4% for carbidopa; for urine, the variation is 1.5 to 10.5% for levodopa, 1.6 to 8.3% for dopamine and 1.3 to 10 3% for carbidopa.

Inter-day C.V.s for the assays were determined from quality control standards (QCs) that were analyzed before and during a study. The QCs were prepared at high and low concentrations within the standard calibration curve in plasma or urine. A standard was divided into a number of aliquots and frozen at −70°C for long-term storage. Representative QCs for the plasma and urine assays for each analyte are shown in Table II.

*Recovery for plasma assay*. The absolute recovery of plasma assay for levodopa, 3-OMD and carbidopa was calculated by comparing peak heights obtained from the injection of standard solutions with the peak heights measured by injecting deproteinized blank plasma containing known quantities of the compounds. Recovery values were found ranging from 95 to 99% of the compounds of interest over the entire calibration curve range, which are similar to those reported earlier [15].

TABLE II

INTER-DAY REPRODUCIBILITY FOR THE PLASMA AND URINE ASSAYS AS REPRESENTED BY QUALITY CONTROL (QC) STANDARDS AT TWO CONCENTRATIONS

| Analyte | Low-concentration QC | | | High-concentration QC | | |
|---|---|---|---|---|---|---|
| | Concentration (ng/ml) | *n* | C V. (%) | Concentration (ng/ml) | *n* | C.V (%) |
| *Plasma*[a] | | | | | | |
| Levodopa | 100 | 24 | 8.0 | 1000 | 24 | 7.1 |
| 3-O-Methyldopa | 100 | 24 | 13 7 | 1000 | 24 | 6 3 |
| Carbidopa | 100 | 24 | 9 4 | 1000 | 24 | 7 4 |
| *Urine*[b] | | | | | | |
| Levodopa | 500 | 26 | 10 0 | 5000 | 26 | 8 6 |
| Dopamine | 50 | 26 | 9 3 | 500 | 26 | 6 7 |
| Carbidopa | 500 | 26 | 9 0 | 5000 | 26 | 6.7 |

[a] Over a period of one month

[b] Over a period of two months

TABLE III

RECOVERY OF ANALYTES FROM URINE BY ALUMINA ADSORPTION PROCEDURE ($n$ = 3)

| Concentration ($\mu$g/ml) | Recovery (%) | | |
|---|---|---|---|
| | Levodopa | Carbidopa | Dopamine |
| 1.25 | 98 4 | 67.5 | 93 3 |
| 2 50 | 91.3 | 69.9 | 93 5 |
| 3 13 | 84 2 | 65 1 | 89.9 |

*Recovery for urine assay.* Recoveries of known amounts of levodopa, dopamine and carbidopa in urine carried through the alumina adsorption procedure were compared to the corresponding direct standards. Results, shown in Table III, indicate high recoveries. Using a similar procedure, lower recoveries from plasma have been reported earlier [16].

*Analysis of clinical samples.* The above assay methods have been employed for the routine analysis of 1500 plasma and 800 urine samples generated from a multiple-dose pharmacokinetic study of standard and controlled-release formulations of levodopa and carbidopa combinations, results of which have been published elsewhere [22]. Typical plasma level profiles for levodopa, carbidopa and 3-OMD are shown in Fig. 8.

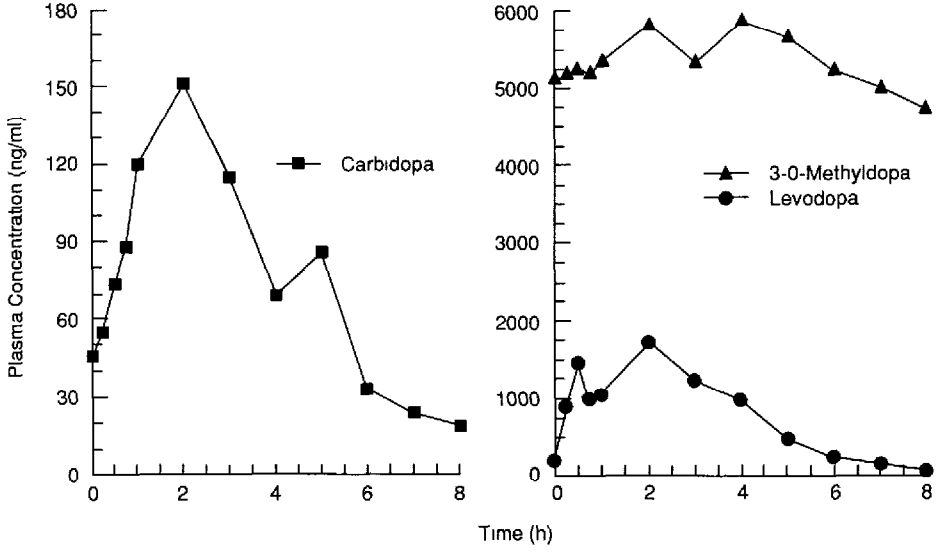



Fig. 8 Plasma level profiles of carbidopa, 3-O-methyldopa and levodopa in a healthy volunteer who received repeated every 8 h administration of one controlled-release formulation of SINEMET CR (50/200 mg) for ten doses

TABLE IV

INCREMENTAL URINARY EXCRETION OF LEVODOPA, DOPAMINE AND CARBIDOPA IN ONE SUBJECT FOLLOWING REPEATED EVERY 8 h ADMINISTRATION OF ONE CONTROLLED-RELEASE FORMULATION OF SINEMET CR FOR TEN DOSES

Values in parentheses indicate recoveries in % of dose Each SINEMET CR dose contains 50 mg carbidopa and 200 mg levodopa

| Analyte | Excretion (mg) | | | |
|---|---|---|---|---|
| | 0–2 h | 2–4 h | 4–6 h | 6–8 h |
| Levodopa | 3 19 (1.6%) | 2.82 (1 4%) | 0.12 (0.1%) | 0[a] |
| Dopamine | 0 43 | 0.74 | 0 26 | 0 15 |
| Carbidopa | 1 04 (2.1%) | 1.31 (2 6%) | 0 27 (0 5%) | 0 11 (0.2%) |

[a] Concentration below detection limit

These data indicate a very low accumulation of both levodopa and carbidopa following repeated administration of the drugs, which was consistent with their relatively short half-lives (less than 2 h). In contrast, the half-life for 3-OMD is in the order of 15 h [22]. As a result, there was an extensive accumulation of 3-OMD and its levels were significantly higher than that of levodopa upon repeated administration.

Table IV lists the urinary recoveries of the three analytes over one 8-h dosing interval It is apparent that the majority of the excreted levodopa and carbidopa was recovered during the first 4 h, and there is a proportionally greater excretion of the carbidopa dose than the levodopa dose. The observed low dopamine excretion is attributable to the decarboxylation inhibition effect of carbidopa.

## CONCLUSION

HPLC assays are reported for levodopa, carbidopa and 3-OMD in plasma and levodopa, carbidopa and dopamine in urine. The methods have been applied to evaluate the pharmacokinetics and bioavailability of new Sinemet CR formulations.

## REFERENCES

1 S. Shimomura, *J Pharm Sci*, 12 (1972) 29
2 J G Nutt and J H. Fellman, *J. Neuropharm*, 7 (1984) 35
3 J R Bianchine and G M Shaw, *Clin Pharmacol*, 1 (1976) 313
4 C C Porter, L S. Watson, D C. Titus, J A Totaro and S. S. Byer, *Biochem. Pharmacol.*, 11 (1962) 1067
5 R Rahn, *Neurology*, 24 (1974) 431
6 I. Merits, D J Anderson and R C Sonders, *Drug Metab Dispos*, 1 (1973) 691

7 J M. Holly and H. L Makın, *Anal. Bıochem.*, 128 (1983) 257
8 A. M Krstulovic, *Adv. Chromatogr* , 17 (1979) 279.
9 G. M. Baker and R T Coutts (Editors), *Evaluation of Analytical Methods in Biological Systems, Part A, Analysis of Biogenic Amines*, Elsevier, Amsterdam, 1972.
10 L. Rentzhog, *Acta Physiol Scand.*, 377 (1972) 1.
11 E. Nıssınen and J. Taskınen, *J. Chromatogr.*, 231 (1982) 459.
12 M. P. Beers, M Stern, H. Hurtz and A. Scarpa, *J Chromatogr* , 336 (1984) 380.
13 P. Y Lin, M C Bulawa, P. Wong, L Lin, J Scott and C L. Blank, *J. Liq. Chromatogr* , 7 (1984) 509
14 J. M. Cedarbaum, R Williamson and H Kutt, *J Chromatogr.*, 415 (1987) 393
15 Y. Mıchotte, M. Moors, D Deleu, D Herregodts and G. Ebinger, *J Pharm Biomed. Anal* , 5 (1987) 659
16 C Lucarellı, P Betto, M Gıambenedettı, G. Rıcciarello, S. Ruggerı and F. Stocchı, *Ital Chim Clin* , 12 (1987) 224.
17 J G. Nutt, W R Woodward and J H. Carter, *Neurology*, 36 (1986) 1206
18 S Vickers, E Stuart and H Hucker, *J Med. Chem* , 18 (1975) 134
19 A. H Anton and D F Sayre, *J Pharmacol.*, 138 (1962) 360
20 A. H Anton and D F Sayre, *J Pharmacol. Exp. Ther.*, 145 (1964) 326.
21 M DaPrada, R. Kettler, G. Zurcher, R Schaffner and W E Heafely, *Eur Neurol.*, 27 (Suppl. 1) (1987) 9
22 K C Yeh, T F August, D F. Busch, K C Lasseter, D. G. Musson, S Schwartz, B. Smith and D C. Titus, *Neurology*, 39 (Suppl 2) (1989) 25.