Stability of Levodopa/Carbidopa Rectal Suspensions

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ABSTRACT

Objective: The combination of levodopa and carbidopa (L/C) is used as an effective therapy for treating Parkinson's disease; however when oropharyngeal dysphagia develops or when insertion of an nasogastric tube is not possible, then rectal administration could be used. To reduce compounding workload and establish a beyond use date, this study was conducted to determine the stability of 2 L/C rectal suspension formulations when stored at either 22°C or 5°C.

Methods: Two formulations of L/C rectal suspension were compounded and then packaged in amber polypropylene bottles. Three bottles of each formulation were stored at either 22°C or 5°C and analyzed at 11 time periods. Physical parameters such as caking, ease of resuspending, and pH were also determined at each time period. A validated stability-indicating high-performance liquid chromatography (HPLC) method was used to analyze both active ingredients.

Results: All solutions were easy to resuspend, there were no signs of caking, and there was no significant change in pH over the 35 days of storage at either temperature. The glycerin-based formulation (formulation 1) was less stable at 22°C (10 days) than formulation 2 (24 days). Both rectal suspensions were stable for 35 days when stored at 5°C.

Conclusions: The physical compatibility and chemical stability of 2 formulations of L/C rectal suspension packaged in amber polypropylene bottles was determined to be either 10 or 24 days when stored at 22°C or 35 days at 5°C.

Key Words—carbidopa, drug stability, high-performance liquid chromatography, levodopa, suspension

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The combination of levodopa and carbidopa (L/C) has been used for years as effective therapy for treating Parkinson's disease (PD). Along with increasing the level of norepinephrine centrally, levodopa is metabolized to dopamine in the brain. The addition of carbidopa, an amino acid decarboxylase inhibitor, helps to protect the levodopa during peripheral circulation without inhibiting its central effects.

Oropharyngeal dysphagia is a common problem in Parkinsonian patients, thereby making administration of solid oral medications difficult. Stoppage of medications may cause worsening of the symptoms. When it is not possible to insert a nasogastric tube to administer L/C suspensions, administration of a rectal suspension could be of value.

There are sporadic reports in the literature of levodopa, with and without carbidopa, being administered rectally to patients in a coma or patients suffering from PD.^{1–6} When levodopa was given as an enema to a patient suffering from end-stage cirrhosis, normal mental status was reported 12 hours later.¹ In another report, levodopa 250 mg or Sinemet 100/10 was administered rectally as a tablet, capsules, insufflating a ground-up tablet, or as a cocoa butter suppository prepared from the same powder, however these met with little success.² However when a rectal suspension was instilled, positive results occurred in 3 patients.^{3–5} Based on the theory that alkaline conditions inhibit the absorption of levodopa in the rectum, a Sinemet suspension was created by adding citric acid to a 50% glycerin vehicle. The decrease

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in the pH of the final solution was thought to aid in the absorption of the levodopa leading to the positive results.⁵ Unfortunately there was no stability data for the resulting suspension, and a fresh batch had to be prepared daily.⁴

Recently a commercial L/C rectal suspension has been approved in the United States that is administered as an enteral infusion over a 16-hour period.⁷

The objective of our project was to determine the stability of 2 L/C rectal suspension formulations containing citric acid when stored in amber polypropylene bottles at either 22°C or 5°C.

METHODS

Materials

Levodopa USP and carbidopa USP, monohydrate used to compound the suspensions were purchased from Professional Compounding Centers of America, London, Ontario, Canada (Cat# 30-2139 and 30-2480, respectively). The amber polypropylene bottles were purchased from Richards Packaging Inc., Mississauga, Ontario (Cat# 061025019095). All other chemicals used were purchased from Fisher Scientific. The HPLC water used to prepare the mobile phase and diluting samples was purchased from Baxter Corporation.

Sample Preparation

L/C suspensions were compounded as outlined in the formulas found in **Appendices 1** and **2**. The first suspension was prepared using glycerin and adding citric acid to adjust the pH. The second suspension was prepared using a chelating agent, citric acid, and a preservative.

Each suspension was then divided into 6 separate amber polypropylene bottles. Containers were shaken by hand for 30 seconds, then 5 mL was collected from each bottle and frozen at -70°C for analysis at a later date. Three containers of each suspension were then stored at either 22°C or 5°C. Subsequently on days 3, 7, 10, 15, 17, 21, 24, 28, 31, and 35, containers were removed from storage and shaken by hand for 30 seconds, and then samples were collected and frozen.

On the day of analysis, the samples were removed from the freezer and allowed to warm to room temperature for 1 hour. After hand shaking each bottle for 30 seconds, an accurate amount of sample (≈ 0.5 g) was weighed into a 10 mL volumetric flask. The sample was then made to volume with HPLC water and sonicated for 2 minutes. An aliquot was then centrifuged for 20 minutes at 14,000 rpm. Clear

supernatant (50 μ L) was combined with 100 μ L of internal standard and 850 μ L of 4% methanol and mixed well before analysis.

Physical Compatibility

Physical parameters such as caking and ease of resuspending were determined by visual inspection with the aid of a 4x illuminated magnifying glass. pH of the solutions were determined using a calibrated pH meter^a.

The specific gravity of the 2 suspensions was determined using a 10 mL serialized pycnometer^b at 22°C.

Chromatographic Conditions

Samples were monitored using an HPLC system. The column was packed with a stationary phase containing C₈ reverse phase material^c. Samples were analyzed using an isocratic solvent pump^d, an autoinjector^e, and a photodiode array detector^f.

The mobile phase was prepared by mixing 0.05 M ammonium acetate buffer containing heptanesulfonic acid sodium (1 mg/mL) with methanol in the ratio of 96:4. The pH of this buffer was adjusted to 4.1 ± 0.1 using glacial acetic acid. The flow was set at 1 mL/min and the injection volume was 20 μ L. Chromatograms were monitored at 280 nm. Methyldopa HCl, 100 μ L of 1.25 mg/mL solution, was added to each sample as an internal standard.

Assay Validation

Forcibly degraded samples were used to confirm the stability-indicating nature of the HPLC method. A stock solution of L/C (5/1.25 mg/mL) was prepared and the pH of an aliquot of the stock solution was adjusted to 1.6 using concentrated hydrochloric acid. An alkaline sample was prepared by adjusting the pH of a second aliquot of the stock solution to 8.1 with 5N sodium hydroxide. To a third aliquot of stock solution, 200 μ L of 30% hydrogen peroxide was added. A fourth sample was heated to 40°C. A Time 0 sample was assayed to determine the initial concentrations of the stock solution. The degradation samples were then followed for a total of 8 time periods over a total of 459 hours. Samples were diluted (1:10) with mobile phase before analysis. Multi-wavelength (280 and 230 nm) and ultraviolet (UV) spectral analysis (200-350 nm) were used to determine peak purity.

Intraday variation was measured by analysis of 5 replicate injections at 0, 10, and 30 hours. Interday variation was determined from results collected

for the slopes, r^2 , and a standard solution on 5 separate days. Analysis of a recovery solution of known concentration on 5 separate days was used to determine the method accuracy. The limit of quantification (LOQ) was determined by analysis of increasingly more dilute standard solutions.

To calculate the linearity of a concentration versus response curve and LOQ, least squares linear regression was used. The percent remaining values are reported as the mean $\pm$ standard deviation of 6 samples.

RESULTS

All degradation conditions produced similar results but to different degrees (Figure 1). There were 2 faster eluting peaks that formed and 2 peaks that eluted between the levodopa and carbidopa peaks. Oxidation conditions produced the greatest amounts of the faster running peaks (Panel C) while the acidic (Panel A) and alkaline conditions (Panel B) produced the most between peaks. Heating the sample (Panel D) produced the least amount of between peaks. None of the degradation peaks compromised either of the parent peaks or internal standard peak.

Purity of all parent peaks was confirmed by multi-wavelength and UV spectral analysis (Purity Index >0.99). Comparison of the UV spectra of levodopa and carbidopa peaks from a reference sample with the UV spectra from the parent peaks in all the degradation samples gave a correlation coefficient of 0.9953 or greater.

The correlation coefficient for the intraday analysis was 0.25% for the levodopa and 0.78% for the carbidopa. Interday variation was 0.94%, 0.15%, and 1.35% for the levodopa and 1.59%, 0.10%, and 1.05% for the carbidopa when comparing the slopes, r^2 , and area of a standard solution, respectively. The average recovery result was $100.1\% \pm 0.9\%$ for the levodopa and $99.7\% \pm 1.9$ for the carbidopa. The standard curve was linear over the range of 0.125 to 0.500 mg/mL of levodopa ($r^2 = 0.9997$) and 0.03 to 0.125 mg/mL of carbidopa ($r^2 = 0.9998$). The LOQ was calculated to be 3.11 $\mu\text{g/mL}$ for the levodopa and 0.78 $\mu\text{g/mL}$ for the carbidopa.

All solutions were easy to resuspend, and there were no signs of caking. The pH of formulation 1 increased by 0.2 units when stored at 22°C and 0.1 units when stored at 5°C over the course of the study. Formulation 2 pH started slightly lower, 1.88 versus 2.24, but remained at that pH under both storage conditions for the 35 days.

The specific gravity of the 2 suspensions was determined to be 1.183 g/mL for formulation 1 and 1.073 g/mL for formulation 2. These values were used to calculate the concentrations at Time 0 since the samples were weighed out. The samples were weighed, instead of volumetrically measured, to increase the accuracy of the quantities due to bubble formation during the 30-second shaking process.

The results from the chemical analysis of the samples are summarized in Tables 1 through 4. Formulation 1 was stable for 10 days at 22°C and 35 days when stored at 5°C . Formulation 2 was slightly more stable at 22°C (24 days) and 35 days at 5°C . The carbidopa was the least stable of the drugs at 22°C while both were stable at 5°C .

DISCUSSION

The stability of L/C oral suspensions has been reported previously when prepared using a commercially available suspending vehicle.⁸⁻¹⁰ Some suspensions were prepared using pharmaceutical grade powder^{8,10} while the other used commercially available tablets.⁹ When powder was used, the beyond use date was 7⁸ and 30 days¹⁰ or 90¹⁰ days when stored under refrigeration. Suspensions prepared using the commercial tablets were stable for 28 days at room temperature and 42 days when refrigerated.⁹ Ascorbic acid (2 mg/mL) was added to the second tablet formulation,⁹ which caused the suspensions to be slightly less stability. The ascorbic acid caused only a slight decrease in the pH (≈ 0.8 units) most likely due to the buffering capacity of the OraSweet/OraPlus product.

Due to the fact that these suspensions are going to be administered rectally, the formulations were modified to reduce the number of ingredients found in the final product. Both formulations contained citric acid that reduced the pH to approximately 2, which did not change over the course of the study. This was done to address the theory that a lower pH would aid in the absorption of the levodopa leading to positive results.^{2,4}

There seems to be a theoretical advantage of administering L/C rectally due to a lower level of decarboxylase activity leading to a lower systemic conversion of levodopa to dopamine.⁵ Another factor that could lead to clinical success is the administration in a liquid form instead of a solid dosage form. The liquid form would lead to a more uniform dose, exposure to a larger surface area, and possibly enhanced absorption.

Figure 1. Sample chromatograms of levodopa, carbidopa, and degradation products. Panel A represents acid degraded levodopa/carbidopa after 191 hours. Panel B represents alkali-degraded levodopa/carbidopa after 22 hours. Panel C represents oxidized sample after 94 hours. Panel D represents heated sample after 264 hours. Peak 1 indicates levodopa and peak 2 represents carbidopa. mAu = milli-absorbance units.

Table 1. Stability of levodopa/carbidopa (50/12.5 mg/mL) rectal suspension stored in plastic amber bottles at 22°C (formulation 1)

Drug	Initial conc. ^a (mg/mL)	% of initial concentration remaining ^b													
		Day 3	Day 7	Day 10	Day 15	Day 17	Day 21	Day 24	Day 28	Day 31	Day 35				
Levodopa	41.7±0.83	100.9±1.5	100.0±1.7	102.0±1.3	101.5±3.3	100.0±0.2	100.5±1.1	98.4±4.4	100.7±1.1	100.4±0.9	99.4±1.1				
Carbidopa	11.8 ±0.20	97.4±0.8	93.4±0.9	92.3±1.2	88.7±2.0	86.4±1.2	83.9±1.8	80.3±4.0	77.7±2.2	76.1±1.2	73.4±1.3				

Table 2. Stability of levodopa/carbidopa (50/12.5 mg/mL) rectal suspension stored in plastic amber bottles at 5°C (formulation 1)

Drug	Initial conc. ^a (mg/mL)	% of initial concentration remaining ^b													
		Day 3	Day 7	Day 10	Day 15	Day 17	Day 21	Day 24	Day 28	Day 31	Day 35				
Levodopa	43.5±0.23	99.2±1.0	97.7±0.7	100.3±2.0	97.7±1.0	97.6±1.2	99.3±1.6	101.1±0.9	98.9±0.3	97.7±0.9	96.0±2.0				
Carbidopa	12.2±0.07	100.3±1.2	100.4±1.9	103.2±0.6	101.4±0.8	98.8±1.0	95.8±1.0	95.5±0.7	94.0±1.1	92.5±1.3	95.9±1.1				

Table 3. Stability of levodopa/carbidopa (5/1.25 mg/mL) rectal suspension stored in plastic amber bottles at 22°C (formulation 2)

Drug	Initial conc. ^a (mg/mL)	% of initial concentration remaining ^b													
		Day 3	Day 7	Day 10	Day 15	Day 17	Day 21	Day 24	Day 28	Day 31	Day 35				
Levodopa	4.9±0.11	95.1±1.2	98.1±0.4	97.3±2.6	98.2±1.1	93.2±0.8	96.0±1.1	96.7±2.8	96.9±0.6	96.1±2.1	94.3±0.5				
Carbidopa	1.2±0.03	98.0±0.9	95.8±1.1	96.0±0.7	94.7±1.1	94.1±0.4	91.7±0.5	92.3±1.6	90.5±0.6	88.8±0.8	87.8±1.0				

Table 4. Stability of levodopa/carbidopa (5/1.25 mg/mL) rectal suspension stored in plastic amber bottles at 5°C (formulation 2)

Drug	Initial conc. ^a (mg/mL)	% of initial concentration remaining ^b													
		Day 3	Day 7	Day 10	Day 15	Day 17	Day 21	Day 24	Day 28	Day 31	Day 35				
Levodopa	6.5±0.35	96.1±1.7	98.3±2.3	96.7±2.1	96.9±2.7	94.1±2.1	98.3±3.4	96.2±1.8	95.0±1.8	94.3±3.2	94.2±2.3				
Carbidopa	1.5±0.01	100.1±0.8	99.7±1.0	97.7±1.0	98.1±0.9	99.0±0.8	97.1±1.3	96.7±0.4	97.6±1.6	99.7±0.6	100.0±0.9				

^a Initial concentration determined on Day 0 (100%).^b Values are mean ± SD (*n* = 6).

CONCLUSION

The stability of 2 formulations of L/C rectal suspensions packaged in amber polypropylene bottles was determined when stored at either 5°C or 22°C. The suspensions were stable for either 10 or 24 days at 22°C or 35 days at 5°C.

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^a Fisher Scientific Model Accumet 25 pH meter. Fisher Scientific Company, Nepean, Canada.

^b Kimble Glass Inc., (Article No. 15123R-10) Specific gravity bottle. Serialized pycnometer, 10 mL

^c Phenomenex Luna, 5µm, C-8 250mm x 4.6mm column. Phenomenex Inc., Torrance, CA, Serial: 294056-1.

^d Shimadzu Model LC-10AT[VP] Delivery Module. Shimadzu Corporation, Kyoto, Japan.

^e Shimadzu Model SIL-20AC Autosampler. Shimadzu Corporation.

^f Shimadzu Model SPD M20A Photodiode Array Detector. Shimadzu Corporation.

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APPENDIX 1

Formulation 1

Levodopa/Carbidopa Rectal Suspension (50 mg/12.5 mg per mL)

Ingredients	Formula amount
Levodopa/carbidopa 100/25 tablets	10
Glycerin	10 mL
Distilled water	10 mL
Citric acid, monohydrate	1 g

Directions:

- Crush tablets and grind into a fine powder using a mortar and pestle.
- Dissolve citric acid in the distilled water.
- Measure out glycerin then add citrated water. Mix well with stir rod.
- Add enough of glycerin citrate mixture to powders to make a paste.
- Add half of remaining glycerin citrate mixture and mix well.
- Quantitatively transfer suspension to graduated cylinder and make to volume with remaining glycerin citrate mixture. Mix well with stir rod.
- Transfer final liquid to amber polypropylene bottle and then shake well.

Label:

Note Dosage Strength

Stability: 35 days in frig or 10 days at room temperature

APPENDIX 2**Formulation 2**

Levodopa/Carbidopa Rectal Suspension (5 mg/1.25 mg per mL)

Ingredients	Formula amount
Levodopa/carbidopa 100/25 tablets	20
Sodium benzoate	400 mg
Aspartame	200 mg
EDTA disodium	40 mg
Citric acid, monohydrate	84 g
Distilled water	400 mL

Directions:

- Crush tablets and grind into a fine powder using a mortar and pestle.
- Weigh out aspartame, sodium benzoate, and EDTA and add to mortar.
- Dissolve citric acid in the distilled water.
- Add enough of citric acid solution to powders to make a paste.
- Add half of citric acid solution and mix well.
- Quantitatively transfer suspension to graduated cylinder and make to volume with remaining citric acid solution. Mix well with stir rod.
- Transfer final liquid to amber polypropylene bottle and then shake well.

Label:**Note Dosage Strength**

Stability: 35 days in frig or 24 days at room temperature