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Carbidopa Capsules for Insulinoma Diagnostic: Compounding and Stability Study

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Abstract

Background: Carbidopa is a drug mainly used to treat Parkinson's disease. Associations with levodopa or with levodopa/entacapone are commercialized, but there is no oral formulation of carbidopa alone available in Europe. As carbidopa can also be used as premedication of adult patients for insulinoma diagnosis, it must be compounded as single dose mg capsules. The single dose administration of a magistral preparation implies the compounding of only one capsule, or the loss of consequent quantities of active pharmaceutical ingredient. As an alternative solution, carbidopa capsules could be compounded as batches of hospital preparation.

Method: With this objective, a stability-indicating dosing method for 200 mg carbidopa capsules was developed. Then, the compounding process was assessed according to the European Pharmacopeia requirements. Finally, the

stability of carbidopa capsules stored protected from light at room temperature was studied for one year.

Results: 200 mg carbidopa capsules compounding process was validated on three independent batches. The beyond use date was fixed at one year.

Conclusion: Our work confirms that carbidopa 200 mg capsules can be realized in hospital pharmacy and its stability allows the compounding of large batches.

Keywords: carbidopa, hospital preparation, HPLC, stability, radiopharmacy, diagnostic

Introduction

6- ^{18}F -fluoro-3,4-dihydroxyl-phenylalanin (^{18}F -DOPA) is a radiolabeled analogue of DOPA, an aromatic amino-acid that accumulates rapidly in target tissues and is transformed into dopamine. ^{18}F -DOPA positron emission tomography (PET) has a broad range of diagnostic indications not only in neurology but also in oncology for detecting pheochromocytomas, paragangliomas, gliomas, and various neuroendocrine tumors including insulinomas. In adult patients, premedication with a single *per os* administration of 200 mg carbidopa improves the sensitivity of ^{18}F -DOPA PET for insulinoma diagnosis [1, 2]. Carbidopa decreases physiological decarboxylation of ^{18}F -DOPA into ^{18}F fluoro-dopamine, and also decreases its renal clearance [3].

To date, only one oral formulation consisting in tablets containing 25 mg of carbidopa alone is commercialized in USA but is not available worldwide. In our hospital, the needs of the Nuclear Medicine department were met by the hospital pharmacy compounding a 200 mg carbidopa capsule as a time-consuming magistral formulation for each patient. Large-batch compounding of 200 mg carbidopa capsules would potentially decrease the time spent on formulation with subsequent production costs reduction. Carbidopa capsule hospital formulation also allow quality controls to secure the compounding process.

In this work, we experimentally performed the required quality controls and determine the stability of large homogeneous batches of 200 mg carbidopa capsules stored at room temperature for 12 months.

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Materials and methods

Materials

Carbidopa European Pharmacopoeia (EP) reference standard was used from the method validation and carbidopa Active Pharmaceutical Ingredient (API) of pharmaceutical grade (Inresa) was used for forced degradation study and compounding process.

Disintegration tests were performed on an Agilent® 100 automated disintegration apparatus. Dissolution tests were performed on an Agilent® 708-DS dissolution apparatus. The HPLC mobile phases were prepared using ultrapure water (HiPerSolv Chromanorm®, VWR International) and methanol (HiPerSolv Chromanorm®, VWR International) of HPLC grade. Phosphate buffered saline (PBS) was prepared from potassium phosphate monobasic (Prolabo), 14 g/L in ultrapure water. The mobile phases were filtered using 0.45 µm Millipore cellulose filters. Volumes were aliquoted with a precision pipette (Thermo Scientific Finn timer® F2 500 µL).

The chromatographic method was carried out on an automatic high-performance liquid chromatography Dionex Ultimate 3000® with a UV diode array detector. The apparatus was connected to an HP 1702 computer equipped with chromatographic data processing software (Chromeleon® Chromatography Management System, Version 6.80 SRH Biold 3161, 1994–2011 Dionex Corporation). Carbidopa separation was achieved by using a Kromasyl® C18 column with 5 µm particle size (250 mm x 4.6 mm).

HPLC analytical conditions and method validation

Carbidopa quantification by HPLC was based on a methodology used for biological analysis [4]. The mobile phase (PBS: methanol, 98:2) was used in isocratic mode at a flow of 1 mL/min for 30 min. Wavelength for carbidopa detection was 282 nm and injection volumes were 20 µL. Carbidopa retention time was 15.6–15.7 min (Figure 1, chromatogram A).

Calibration curve was realized from an extemporaneously prepared solution of 10 mg/mL carbidopa in HCl 0.1N and was determined with seven dilutions: 3.75, 2.50, 1.25, 0.625, 0.3125, 0.156 and 0.078 mg/mL.

The following parameters were evaluated in the method validation: repeatability (within-day variation), intermediate precision (between-day variation), accuracy and uncertainty.

Within-day and between-day measurements were carried out at two concentrations: 1.25 and 2.50 mg/mL. For

within-day measurement, at least 15 samples for each concentration levels were analyzed on the same day. Between-day measurements were carried out at the same concentrations, on at least 25 samples on 3 consecutive days. Linearity was shown between 0.078 mg/mL and 3.75 mg/mL. The results are summarized in Table 1.

Capsule samples were treated as follow: 200 mg capsule of carbidopa were opened and solubilized in 20 mL of HCl 0.1N. The solution was vortexed (4 min) and centrifuged (2000 trs/min, 5 min). 1 mL was mixed with 7 mL of HCl 0.1N in order to obtain a solution of theoretically 1.25 mg/mL of carbidopa. Dissolution medias were only filtered and directly injected in HPLC apparatus.

Forced degradation studies

The forced degradation studies were performed for carbidopa under several experimental conditions: thermal, oxidation, light, alkaline and acidic conditions. Each condition was tested by a single experiment.

For thermal conditions, 1.25 mg/mL carbidopa solution was placed in an oven at 80 °C, and for light condition, it was put under sunlamp irradiation. For the oxidation conditions, 1.25 mg/mL carbidopa solution was realized in hydrogen peroxide (3 %). For the alkaline conditions, the same concentrations were realized in respectively sodium hydroxide 0.2 N and hydrochloric acid 0.5 N.

Formulation

First, 3 experimental batches of 30 capsules were realized by qualified personnel. Six grams of carbidopa (pharmaceutical quality) were weighted. Carbidopa was transferred to a 25 mL measuring cylinder, and microcrystalline cellulose was added qs 20.4 mL. A spatula tip of red carmine was added, and the mixture was transferred to a mortar to be gently mixed. This procedure allowed the compounding of a batch of 30 capsules (ivory, size 0). These batches were analyzed by quality control laboratory in accordance with European Pharmacopoeia specifications.

Stability study

Carbidopa capsules were stored protected from light at room temperature (20–25 °C) but the humidity was not controlled. Carbidopa capsules content was evaluated in triplicate at time zero, two weeks, one month, two months, three months, six months and one year after compounding. Disintegration

Figure 1: Forced degradation chromatograms. A: Standard solution; B: Heat (80 °C, 5 h); C: Light (Sunlamp, 5 h); D: Oxidation (H₂O₂ 3 %, 2 h); E: Acidic (HCl 0.5N, 1.5 h); F: Alkaline (NaOH 0.2 N, 1 h).

Table 1: Repeatability, intermediate precision and accuracy.

Samples	Repeatability (% RSD within-day (n = 15))	Intermediate precision (% RSD between-day (n = 25))	Accuracy (Bias in % (n = 25))
Carbidopa 1.25 mg/mL	0.44 %	2.27 %	3.33 %
Carbidopa 2.50 mg/mL	0.50 %	3.99 %	0.18 %

tests were also performed at time zero, one month, three months, six months and one year after compounding.

As reported in several publications [5–10], a significant change in carbidopa content was defined as a “10 % change

in assay from its initial value”. Statistical significance was determined by simple linear regression (slope of the regression line is different from 0).

Results

Forced degradation study

Forced degradation study showed no interferences with dosing method, with the appearance of four peaks (RT 8.0 min, 14.0 min, 20.3 min and 22.7 min) corresponding to

degradation products. Results are summarized in Table 2 and chromatograms are represented in Figure 1.

Table 2: Carbidopa forced degradation study.

Experimental conditions	API degradation	Degradation products
Heat (80 °C, 5 h)	15 %	8.0 min, 14.0 min, 22.7 min
Light (Sunlamp, 5 h)	2 %	8.0 min
Oxidation (H ₂ O ₂ 3 %, 2 h)	13 %	20.3 min
Acidic (HCl 0.5N, 1.5 h)	16 %	8.0 min, 14.0 min, 20.3 min
Alkaline (NaOH 0.2 N, 1 h)	14 %	20.3 min, 22.7 min

Another peak (RT 10.4 min) detected both under all degradation conditions and in standard solution was suspected to be a native organic impurity of carbidopa API. According to the European Pharmacopoeia and the United States Pharmacopoeia, the main carbidopa API impurity is methylidopa. Moreover, methylidopa was also specified as the main impurity in the certificate of analysis of carbidopa API used for our study. To prove this, a methylidopa solution was realized from commercial drug (Aldomet® 250 mg, HAC Pharma). The retention time of its HPLC analysis allowed us to identify the peak detected at 10.4 min during forced degradation studies as methylidopa.

Content and content uniformity

Since carbidopa capsules contain more than 25 mg of active pharmaceutical ingredient and more than 25 % of capsule total mass, a mass variation test was performed [11, 12]. On 3 different batches, the net weight content of ten capsules was individually and accurately calculated by subtracting the weight of the shell from the respective gross weight, and the content of one capsule was quantified by HPLC analysis.

As reported in European pharmacopoeia (2.9.40.) the carbidopa content of each capsule, expressed as percentage of label claim, was calculated from the net weight of the individual capsule content and the result of HPLC analysis. The European Pharmacopoeia formulas were applied: % capsule estimated content of carbidopa = (weight content/mean weight content of the 10 capsules) X (carbidopa representative content (HPLC)/theoretical content) X 100. The acceptance values were also calculated. As carbidopa content was comprised between 98.5 % and 101.5 % for two of the three tested batches, the formula was:

acceptance value = 2.4 X standard deviation of estimated content for 10 capsules.” For the third one, with carbidopa content of 96.0 %, the acceptance value was: 98.5 – (mean percent of carbidopa estimated content) + 2.4 X standard deviation of estimated content for 10 capsules.” Results are summarized in Table 3.

Table 3: Content and content uniformity from experimental batches.

	Batch 1	Batch 2	Batch 3
Analyzed content (HPLC-UV)			
Capsule content	201 mg	202 mg	192 mg
Estimated content			
Capsule 1	200 mg	213 mg	190 mg
Capsule 2	196 mg	193 mg	190 mg
Capsule 3	208 mg	201 mg	209 mg
Capsule 4	199 mg	196 mg	188 mg
Capsule 5	195 mg	189 mg	194 mg
Capsule 6	196 mg	210 mg	194 mg
Capsule 7	205 mg	208 mg	203 mg
Capsule 8	204 mg	204 mg	182 mg
Capsule 9	206 mg	205 mg	192 mg
Capsule 10	201 mg	204 mg	177 mg
Mean estimated content (in percent of theoretical content)	100 %	101 %	96 %
Acceptance value	5.4 %	6.5 %	13.3 %

Disintegration and dissolution

Disintegration tests were performed on six capsules. As recommended in the European Pharmacopoeia 9th edition, all the capsules were disintegrated in less than 30 min. For dissolution, experimental conditions were taken on USP monography: “carbidopa and levodopa tablets” [13]. Dissolution media was 750 mL of HCl 0.1N. Apparatus 1 was used at 50 rpm. In USP, sampling time was defined as 30 min for carbidopa and levodopa tablets, as not less than 80 % of the labelled amount of carbidopa must be dissolved in 30 min. Results are summarized in Table 4.

Stability study

Quantification of 200 mg carbidopa capsules stored at room temperature and protected from light showed no significant variation up to one year. Moreover, disintegration tests were compliant with the recommendation of the European Pharmacopoeia and showed no difference over time. Results are summarized in Figure 2.

Table 4: Dissolved carbidopa (% of labelled amount) after 30 min dissolution of capsules.

Capsule	Amount dissolved (%)
1	91,5
2	85,5
3	85,0
4	87,0
5	90,3
6	91,8
7	92,3
8	89,7
9	88,3
10	94,7
11	83,0
12	86,6
Mean value	88,8

Discussion

In this work, forced degradation study was performed to ensure the stability-indicating character of our method. Forced degradation studies of carbidopa were previously described in the literature. However, it is difficult to compare our results with the degradation profiles reported in these studies since they were all performed on carbidopa combined with levodopa [14, 15]. During forced degradation study, we identified methyl-dopa as the main carbidopa API impurity. Peak area of methyl-dopa did not increase during forced degradation study and during stability study.

A compounding process validation was also realized. Reference values for the carbidopa content were taken on USP monography: “carbidopa and levodopa tablets” [13]. For the 3 tested batches, carbidopa capsules contained not less than 90.00 % and not more than 110.00 % of the labeled amount of carbidopa. Moreover, content uniformity results were also compliant to the European Pharmacopeia standard as the acceptance values were lower than 15.00 %.

Dissolution test results were consistent with USP monographies, but also with European Medicines Agency for oral immediate release products [16] which specify that immediate release is identified as at least 75 % of the active substance is dissolved within 45 minutes. 200 mg Carbidopa capsules we compounded can be administered to patient, as our formulation allows the liberation of the requested amount.

In this work, we validated the formulation process and secured the delivery of 200 mg carbidopa capsules compounded by a hospital pharmacy. The stability of carbidopa capsules was not surprisingly found to be higher than the previously reported stability of liquid formulations of carbidopa. Rectal suspension of levodopa/carbidopa was stable for 28 days at 22 °C and for 35 days at 5 °C [15]. Oral suspension of levodopa/carbidopa (5.00/1.25 mg/mL) in Ora Plus/Ora Sweet was found to be stable for 30 days at room temperature and for 90 days at 2/8 °C [14]. Oral suspension of levodopa/carbidopa (5.00/1.25 mg/mL) in Syrspond SF pH4 was stable for 90 days at 2/8 °C and 30 days at ambient temperature [17]. From our knowledge, the feasibility and the stability of a solid formulation of carbidopa alone was never assessed.

Figure 2: Quantification of carbidopa content.

During the whole stability study, carbidopa API used for capsule compounding remained under the expiration date specified by the manufacturer. As this point could interfere with the stability, carbidopa API used for compounding must have an expiration date of more than one year to ensure the stability of 200 mg carbidopa capsules.

Since 2017, 12 batches were compounded in our hospital. Quality control for batch releasing involves a mass variation test. We dispensed these capsules to many patients, without any report of undesirable effect.

Conclusion

Compounding of batches of 200 mg carbidopa capsules can be realized in hospital pharmacies to be dispensed safely to hospital Nuclear Medicine department for premedication of patients before insulinoma diagnosis. These formulations of carbidopa can be stored protected from light at room temperature for one year. Hospital pharmacists can compound large batches of 200 mg carbidopa capsules for [^{18}F]F-DOPA PET premedication in adult patients, enabling rationalization of production time and volume, and the resulting reduction of hospital costs.

Conflict of interest statement: The authors state no conflict of interest. The authors have read the journal's Publication ethics and publication malpractice statement available at the journal's website and hereby confirm that they comply with all its parts applicable to the present scientific work.

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