

A Comparative In Vitro Assay of Drug Release Performance of Pyridostigmine Bromide Tablets

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ABSTRACT

Myasthenia gravis is an autoimmune disease that destroys key components of the neuromuscular system. The most common therapy uses reversible inhibitors of cholinesterase activity, such as pyridostigmine bromide (PB). The nature of this illness implies that we must be sure that all available PB immediate-release tablets produce the same therapeutic response.

The aim of this study was to analyze PB immediate-release formulations provided by pharmacies in MERCOSUR countries A, B, and C. The formulations, which were produced in different manufacturing plants of the same multinational company, were analyzed following USP 29 specifications.

The products fulfilled the assay, uniformity of dosage units, and dissolution test in S₂ stage. Dissolution profiles were carried out following EMEA and FDA regulations, and the similarity factor (f_2) was applied to A and C but not B, as this one did not fulfill the dissolution requirements. Pyridostigmine bromide tablets from countries A and C are considered to be similar and could be interchangeable. Formulation B exhibited such different dissolution behavior that its interchangeability is discouraged, as well as its introduction in countries A and C from the manufacturing country B.

INTRODUCTION

Myasthenia gravis is an autoimmune disease that destroys key components of the neuromuscular system. The most common therapy is the use of reversible inhibitors of cholinesterase activity, which block the degradation of acetylcholine in the neuromuscular junction, such as pyridostigmine bromide (PB).

The required dosage has to be determined individually according to different factors such as disease severity, poor and irregular absorption of PB from the gastrointestinal tract, its low liposolubility, and hydrolysis by cholinesterase and liver metabolism (1, 2). The dosage schedule could be adjusted for each patient and changed as needs arise and may vary according to remissions or exacerbations of the illness. In addition, the onset and duration of action varies with the physical and emotional stress situation of the patient (3).

The characteristics of this illness necessitate that all available PB immediate-release tablets produce the same therapeutic response. PB is considered a Class 3 drug (4, 5) in the Biopharmaceutics Classification System (6, 7) due to its high solubility and low permeability. The possibility of extending waivers of bioavailability–bioequivalence studies to this class of compounds has been suggested (8–10).

The difference in dissolution was a concern in light of the commercial need to manufacture the product in different manufacturing plants worldwide using excipients, equipment, and processes from local sources. In vitro dissolution testing plays an important role in detecting such differences. Therefore, an investigation was conducted to determine if there are differences in the dissolution profiles of PB (60 mg) immediate-release tablets from countries A, B, and C within MERCOSUR (South Common Market). The first purpose of this study was to assure that these products fulfill the same quality standards. But the most important objective was to evaluate the feasibility of product interchange from bordering countries A, B, and C, taking into account that they belong to the same Common Market where free trading is possible. It is important to emphasize that the formulation from country B has a much lower price than those from A or C, so importation is possible for economic reasons.

This type of study is not often performed because most products manufactured by the same company in different plants do not have as demanding a dosage as PB tablets, so their interchangeability would not be a topic of major concern.

MATERIALS AND METHODS

Chemicals

Analytical grade phosphoric acid, glacial acetic acid, and potassium chloride, and HPLC grade acetonitrile and

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sodium 1-heptanesulfonate were purchased from J. T. Baker (USA). Sodium hydroxide pellets analytical reagent grade was obtained from Mallinckrodt (USA); trihydrate sodium acetate analytical reagent grade from Merck (Germany); hydrochloric acid and monobasic potassium phosphate analytical reagent grade from Cicarelli (Argentina); and triethylamine HPLC grade from Fisher Scientific (USA). Distilled water was used for the preparation of dissolution media, and HPLC grade water was used for chromatographic determinations. PB was purchased from ICN Biochemicals and Reagents. PB immediate-release tablets were acquired from pharmacies of three countries:

1. Country A (manufactured in country D)
2. Country B (manufactured in the same country B)
3. Country C (manufactured in country E)

All evaluated products were manufactured by the same company but were produced in different manufacturing plants and had similar expiration dates. No other brand of PB was found in these countries.

Apparatus and Conditions

Dissolution tests were performed using USP Apparatus 2 (Vankel VK 7010). Dissolution samples were diluted with dissolution medium for UV analysis at 270 nm (Varian Cary 1E UV-Vis Spectrophotometer).

Reversed-phase HPLC was performed on a system consisting of a dual-piston reciprocating Spectra Physics pump (model ISOChrom, USA), a Rheodyne injector (model 7125) with a 20- μ L loop, a UV-Vis Hewlett-Packard detector (model 1050, Japan), and a Hewlett-Packard integrator (series 3395, China). Mobile phase consisted of 1 g of sodium 1-heptanesulfonate, 5 mL of triethylamine, and 100 mL of acetonitrile diluted with HPLC grade water to 1000 mL, adjusted with phosphoric acid to pH 3.0, filtered through a 47-mm nylon membrane (0.45- μ m pore size, μ clar, Argentina), and vacuum-degassed before use (11). Separation was carried out using a Merck Lichrospher 100-RP18, 250 x 4.6 mm column, 5- μ m particle size, at room temperature. All analyses were performed under isocratic conditions at a 1.0-mL/min flow rate. The column was equilibrated for at least 45 minutes with mobile phase flowing through the chromatographic system before starting the assay. Standard and sample solutions were prepared on a weight basis using pH 7.0 buffer as diluent (11) and filtered through a 25-mm nylon membrane disposable filter (0.22- μ m pore size, μ clar, Argentina). They were injected in triplicate (RSD below 1.0 %) and the results averaged.

Uniformity of Weight and Dosage Units

Uniformity of weight and dosage units were performed using ten tablets of each sample following USP methodology (11).

Dissolution Test

Dissolution ($n = 6$) was conducted in USP Apparatus 2 at 50-rpm paddle speed, with 900 mL of deaerated water at

37 °C. Samples were taken at 60 minutes. The quantitation was done from ultraviolet absorbance at 270 nm using adequately filtered and diluted portions of the solution, in comparison with a reference solution having a known concentration of PB standard.

Dissolution Profiles

In order to assess similarity (12, 13), dissolution profiles were performed at pH 1.2, 4.5, and 6.8 under the same experimental conditions as the dissolution test. Hydrochloric acid buffer at pH 1.2, acetate buffer at pH 4.5, and phosphate buffer at pH 6.8 were prepared following USP 29 requirements (11).

Twelve tablets from each sample were analyzed in each condition. Samples (10-mL) were withdrawn at 8, 20, 40, and 60 minutes without replacement of medium, filtered through blue ribbon filter paper, and suitably diluted. Drug concentration was determined spectrophotometrically at 270 nm, in duplicate, in comparison with a reference solution having a known concentration of PB standard. Cumulative percentages of drug release were calculated.

The stability of PB solutions prepared with those buffers was previously evaluated.

RESULTS AND DISCUSSION

The percent relative standard deviation (% RSD) for weight variation was found to be between 0.9 and 1.5 (in compliance with USP 29 requirements), as shown in Table 1. Content uniformity analyses resulted in average values between 104.0 and 112.0% of labeled amount, with a relative standard deviation within 0.8–1.8%. These results satisfied the pharmacopoeial specifications for all products.

In the dissolution test, formulations A and C showed liberation of a coating film, while formulation B formed a small cone of aggregates at the bottom of the vessel. The mean values of dissolved drug for A, B, and C products were alike. As all samples tested failed to comply with S_1 criteria, another six tablets were analyzed. All products fulfilled S_2 dissolution criteria, and the results are shown in Table 2. Formulation B exhibited the highest variability (RSD 8.2%).

A significant difference in dissolution behavior between formulation B and formulations A and C is shown in Figures 1–3 and Table 3. Also, the greatest variability of

Table 1. Uniformity of Dosage Units.

Sample	Uniformity of Dosage Units			
	Weight (mg) ^a	RSD	Percent of Label Claim ^a	RSD
A	367.0 (361.5–374.4)	1.5	107.4 (104.2–109.6)	1.8
B	347.8 (339.7–356.3)	1.5	108.9 (107.4–112.0)	1.4
C	367.4 (361.2–371.3)	0.9	108.8 (106.8–110.3)	0.8

^a Average result for 10 dosage units followed by the range in parentheses.

Table 2. Dissolution Test Results.

Sample	Stage S ₁		Stage S ₂	
	Percent Label Claim Dissolved ^a	RSD	Percent Label Claim Dissolved ^a	RSD
A	83 (80–85)	2.2	83 (79–86)	2.7
B	80 (70–86)	9.1	80 (70–88)	8.2
C	81 (78–83)	3.5	81 (78–84)	2.7

^a Average result for twelve dosage units followed by the range in parentheses.

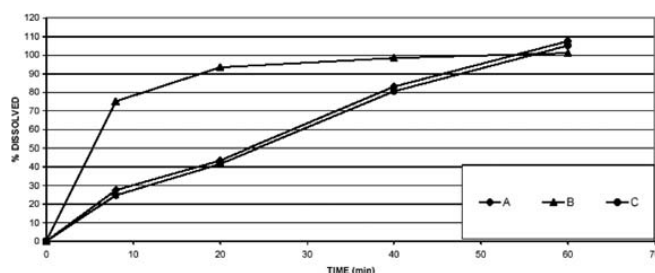


Figure 1. Dissolution profile in pH 1.2 buffer solution.

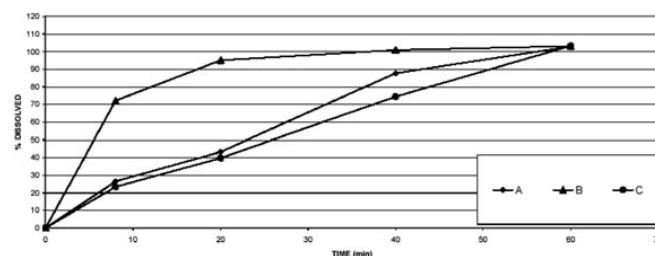


Figure 2. Dissolution profile in pH 4.5 buffer solution.

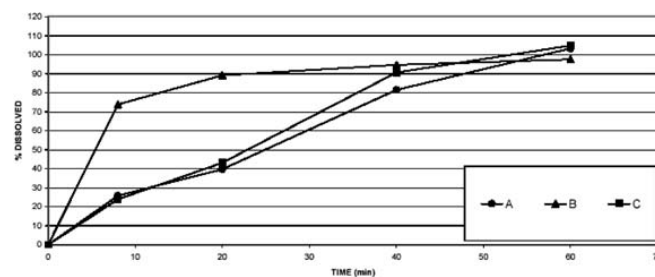


Figure 3. Dissolution profile in pH 6.8 buffer solution.

dissolution values corresponds to formulation B, as shown in Table 3.

In order to assess similarity and interchangeability of analyzed products, the similarity factor (f_2) was calculated. Brand B did not fulfill the requirements because the relative standard deviation at the first sampling point was

higher than 20.0% in two dissolution media (pH 1.2 and 4.5) and higher than 10.0% at the second sampling point in buffer pH 6.8, and in the three conditions the amount of drug dissolved was higher than 85% at the second time point (7, 12, 13). Therefore, only formulations A and C were considered in calculating f_2 , where A was chosen as

Table 3. Cumulative Percentage Dissolved.

Medium	Time (min)	Sample A		Sample B		Sample C	
		% Label Claim Dissolved ^a	RSD	% Label Claim Dissolved ^a	RSD	% Label Claim Dissolved ^a	RSD
pH 1.2	8	28 (23–32)	9.3	75 (43–97)	23.3	25 (21–27)	7.1
	20	44 (36–48)	7.3	93 (76–106)	9.4	42 (35–45)	7.6
	40	83 (69–95)	7.7	98 (89–106)	5.0	81 (76–87)	5.0
	60	108 (104–110)	2.0	101 (95–106)	3.1	105 (101–108)	1.8
pH 4.5	8	27 (22–28)	6.8	72 (37–94)	26.7	23 (20–26)	9.5
	20	43 (39–45)	4.3	95 (77–105)	9.1	40 (34–45)	9.4
	40	88 (79–96)	6.8	101 (90–106)	4.5	74 (67–86)	6.9
	60	103 (100–105)	1.6	103 (96–106)	2.6	103 (101–105)	1.2
pH 6.8	8	26 (23–29)	6.0	74 (46–93)	19.6	24 (22–26)	5.7
	20	40 (35–43)	5.0	89 (64–107)	13.4	43 (37–49)	8.2
	40	82 (74–96)	8.5	95 (77–109)	9.6	91 (83–102)	5.8
	60	103 (100–107)	2.0	98 (84–110)	7.2	105 (102–108)	1.5

^a Average result for twelve dosage units followed by the range in parentheses.

reference (R) and C as test (T) product. The similarity factors obtained were 78 (for pH 1.2), 53 (for pH 4.5), and 64 (for pH 6.8).

CONCLUSIONS

All analyzed formulations fulfilled the requirements of the assay, uniformity of dosage units, and dissolution test in S₂ stage. However, marked differences were recorded regarding dissolution profiles and similarity. Formulation B could not be included in the similarity study. The values of f₂ for A and C over 50 suggest that both formulations are similar and may be interchangeable. It is clearly shown that the dissolution behavior of formulation B is significantly different from the others, so its interchangeability may produce a severe impact on the therapeutic response of myasthenic patients. Despite the fact that it could not be included in the similarity study, it is clearly demonstrated that formulation B is not interchangeable with the other two formulations. Therefore, importation of this product in countries A and C is discouraged.

Nevertheless, further in vivo bioavailability studies should be conducted in order to confirm any correlation with the in vitro performance of pyridostigmine bromide products.

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