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RESEARCH PAPERS

DEGRADATION OF BISOPROLOL FUMARATE IN TABLETS FORMULATED WITH DICALCIUM PHOSPHATE

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ABSTRACT

Degradation of Bisoprolol Fumarate in tablet dosage forms was accelerated when granular Dicalcium Phosphate Anhydrous USP was substituted for milled Dicalcium Phosphate Anhydrous USP. Studies demonstrate that instability with the granular material is due to an acidic microenvironment created by this material in the presence of moisture. Bisoprolol is less stable at this low pH compared to a neutral microenvironmental pH which is created with milled Dicalcium Phosphate Anhydrous.

BACKGROUND

Calcium Phosphates are popular excipients for the formulation of tablet and capsule dosage forms. They are compatible with most active ingredients, insoluble in water, and the granular forms are excellent direct compression vehicles. The three types of Calcium

Phosphate used in solid dosage forms are: Dicalcium Phosphate Dihydrate (DCPD, $CaHPO_4 \cdot 2H_2O$), Dicalcium Phosphate Anhydrous (DCPA, $CaHPO_4$) and Tricalcium Phosphate (Hydroxyapatite, $Ca_5(OH)(PO_4)_3$). These are available in both powdered and granular forms.

The Calcium Phosphate salts are unaltered by storage. However, Dicalcium Phosphate Dihydrate tends to irreversibly give off moisture above 40-45°C. This may produce misleading results in accelerated stability testing with this excipient. Water-sensitive drugs may react with released water at high temperatures, giving an apparent incompatibility. An extrapolation is not appropriate since DCPD does not lose water at room temperature (1). To avoid the presence of water that may lead to instability at room or accelerated temperature, Dicalcium Phosphate Anhydrous could be used. A-Tab®, a commercial granular form of DCPA, and powdered DCPA are both USP grade and are the same with the following exceptions (2):

	A-Tab®	powdered DCPA
particle size	granular	powder
density (tapped)	~50 lb/ft^3	~83 lb/ft^3
pH 20% slurry	5.0-5.6	6.6-7.4
surface area	20-30 m^2/g	0.5-2 m^2/g

Bisoprolol Fumarate is a white, odorless, crystalline powder having the structure shown in Figure 1. It is the hemifumarate salt of a weak base (pK_a 9.5)

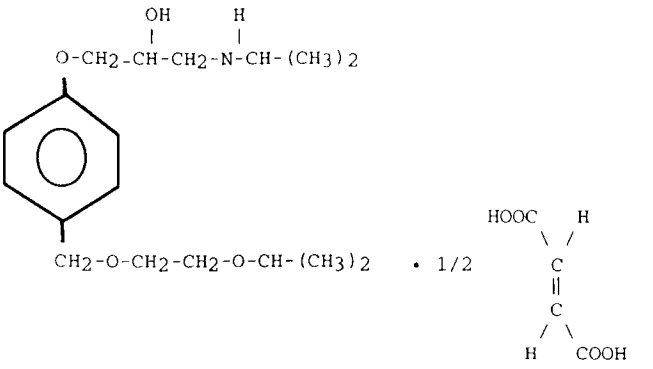

FIGURE 1

and the dose is given as the hemifumarate salt. It is highly water soluble (0.6 g/ml) and has excellent solid stability. Degradation of Bisoprolol occurs primarily by cleavage of the benzyl ether followed by oxidation to produce an aldehyde. Direct oxidation of Bisoprolol at the benzyl carbon also yields an ester product. Besides these two primary degradation compounds, a number of minor degradation products have also been found.

A combination film-coated tablet of Bisoprolol Fumarate and Hydrochlorothiazide was developed using powdered DCPA as the filler. These tablets had a good stability profile. Switching from powdered to granular DCPA would require fewer manufacturing steps and give significant cost savings. Therefore, a small batch of these tablets was prepared and placed in polyethylene bottles for stability evaluation. The results are given **in table 1.**

TABLE 1

Condition	% Bisoprolol remaining
Initial	98.8
42°C 1 month	94.6
42°C 2 months	81.8
56°C 2 weeks	89.4
56°C 1 month	78.8
56°C 2 months	78.4
40°C/75%RH 1 month	84.2
40°C/75%RH 2 months	82.4

Peaks in the chromatograms indicated the formation of degradation products concomitant with loss of drug. Significant loss of Bisoprolol was also seen at room temperature after 18 months. These results were unexpected since the formulation was stable and DCPA USP was substituted for DCPA USP.

PURPOSE

The purpose of this study is to determine the reasons for observed losses of Bisoprolol Fumarate in tablet dosage forms with granular Anhydrous Dicalcium Phosphate.

MATERIALS AND METHODS

Bisoprolol Fumarate was used as received (purity 99-100%). Dicalcium Phosphate Anhydrous USP, granular, was A-Tab® (Rhône-Poulenc). All other chemicals (e.g., buffering agents) were reagent or USP grade. pH measurements were performed on an Orion model EA940 or

290A pH meter. Vortexing was performed with a SP Vortex Jr.

HPLC Method

The method of analysis used for Bisoprolol in these studies was:

Column:	Waters µBondapak Phenyl or equivalent USPL11, 15.0 cm x 4.6 mm ID 10µ particle size
Mobile Phase:	Acetonitrile:Aqueous phase, 20:80; Aqueous Phase: 0.2% Triethylamine adjusted to pH 3 with 85% Phosphoric acid
Flow Rate:	1.5 ml/min
Detector:	UV absorbance at 227 nm
Injection Vol.:	20 µl
Retention time:	Approx. 5 min.
Total run time;	Approx. 9 min.

Testing was carried out on a Waters HPLC system consisting of a 991 Photodiode Array, 600E System Controller, and 715 UltraWISP sample processor.

Bisoprolol pH Stability Study

The following buffers were used: pH 1.5, 2.5 - HCl; 4,5,6 - Acetic Acid/Sodium Acetate; 7,8 - Sod. Phosphate Monobasic/Dibasic; 9, 11 - NaOH. Ionic strength was maintained constant at 0.3 by addition of KCl. Bisoprolol Fumarate solution was prepared in these

buffers at 100 µg/ml, sealed in 5 ml ampules, and stored in a 56°C oven. Samples were taken at selected intervals and assayed for Bisoprolol Fumarate concentration. An early HPLC method of analysis was used for this study, different from that described above: Perkin Elmer Integral 4000C LC was used with the following: LC8, 5µ column, 50 µl injection volume, 220 nm UV detection, 15 min run time, mobile phase 80/20 buffer/acetonitrile - 0.03M KH_2PO_4 adjusted to pH 2.5 with H_3PO_4.

The solution stability of Bisoprolol Fumarate was also studied in 0.1M citrate buffer. Stability was determined at 5 pH levels (3.2, 4.0, 4.5, 5.0, and 5.5) and 3 temperatures (42°, 56°, and 70°C). Citric Acid and Trisodium Citrate were used to prepare the buffer solutions. Bisoprolol was dissolved in the buffer to make 100 µg/ml and the solutions were sealed in 5 ml ampules. Samples were taken periodically and tested for concentration of Bisoprolol Fumarate by HPLC.

pH of Dicalcium Phosphate Slurries

Dicalcium Phosphate slurries at 1% to 40% (w/w) in Purified Water were prepared and measured for pH. The pH was also measured after the addition of 75 µl saturated KCl solution per 25 ml to determine if there is a salt effect.

A-Tab® Washing Study

Approximately 25 g of A-Tab® was weighed and 100 ml Pur. Water or 0.001N NaOH was added in a beaker with

mixing. This mixture was poured onto a Whatman #40 filter on a vacuum filtration funnel. After pulling a vacuum for a few minutes, the A-Tab® was recovered and the procedure was repeated 2 more times with Pur. Water. An additional 100 ml Pur. Water was used to rinse the remaining A-Tab® from the beaker and again to wash the A-Tab® on the filter. The A-Tab® was collected from the filter and dried overnight in a vacuum oven at 50°C.

After preparing the washed samples, mixtures of Bisoprolol were prepared with A-Tab®, water-washed A-Tab®, and NaOH-washed A-Tab®. These mixtures were blended for 15 minutes in a Turbula mixer at a Bisoprolol:A-Tab® ratio of 1:45 and 1:90.

Compatibility Studies

Bisoprolol compatibility studies with A-Tab® were performed in 5 ml ampules by weighing 15 mg of Bisoprolol Fumarate directly into an ampule and then weighing either 675 mg (1:45) or 1.35 g (1:90) of A-Tab® into the ampule. The contents were blended with the aid of a vortexing device. Just before flame sealing, 10% water (69 µl or 136 µl) was pipetted into the ampule. The ampules were stored in a 70°C oven and sampled after 3 and 6 weeks for HPLC analysis of Bisoprolol. Samples were prepared for assay by breaking the ampule and transferring the contents of the ampule into a 200 ml volumetric flask using Purified Water. The samples were sonicated for 10 minutes, cooled, diluted to volume, filtered and assayed.

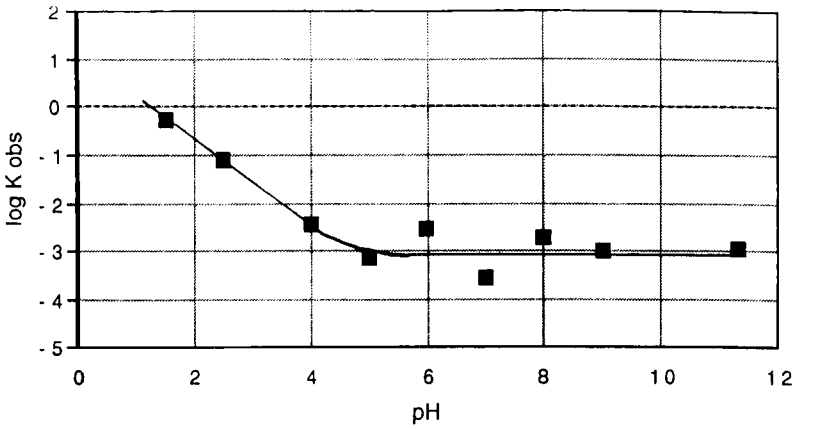

FIGURE 2
pH Stability Profile for Bisoprolol at 56 C

RESULTS AND DISCUSSION

Bisoprolol pH Stability Study

Bisoprolol is very stable in solution above pH 5. The pH stability profile at 56°C is shown in Figure 2. Degradation follows pseudo-first order kinetics. There is scatter in the data above pH 5 because degradation is very slow and essentially not measurable under the conditions and time of the study.

The results for the pH stability study in citrate buffer at 56° and 70°C are given in Figure 3. Degradation at 42°C was slow and the stability profiles at 56° and 70°C are plotted in Figure 4. As in the pH stability profile in Figure 2, the degradation of Bisoprolol begins to accelerate at pH values below 5.

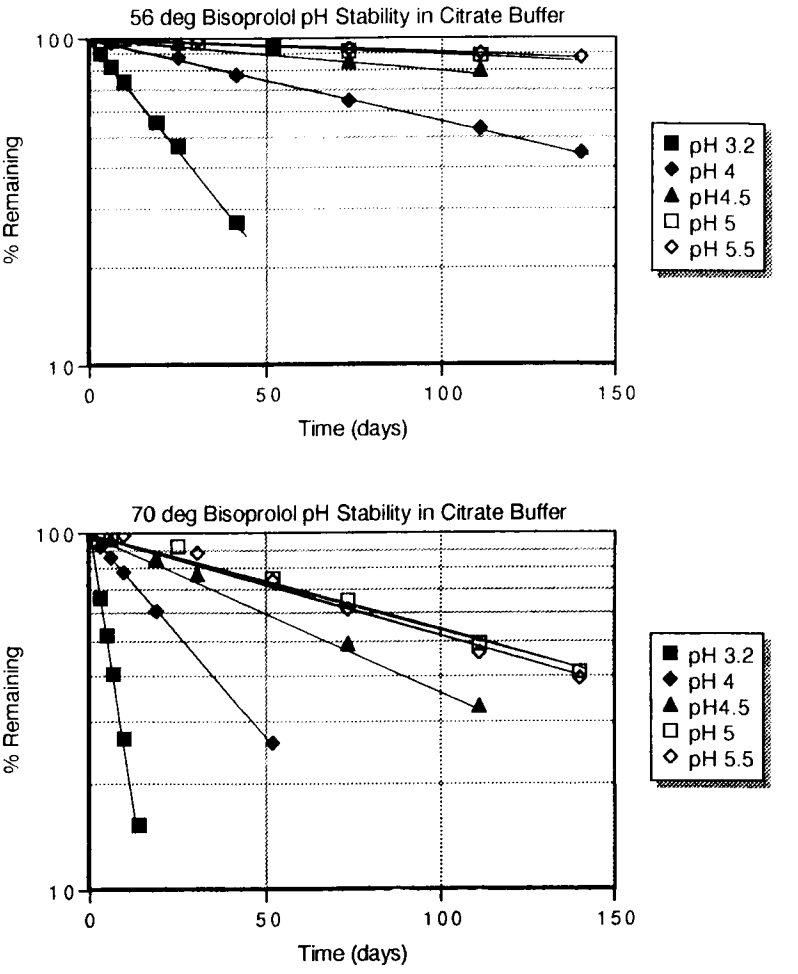

FIGURE 3

pH of Dicalcium Phosphate Slurries

Since one of the differences between A-Tab® and milled DCPA is the pH of a 20% slurry, this study was carried out to look at the pH at various slurry concentrations. Results for A-Tab® slurries are given in Table 2 and plotted in Figure 5. A 20% slurry of

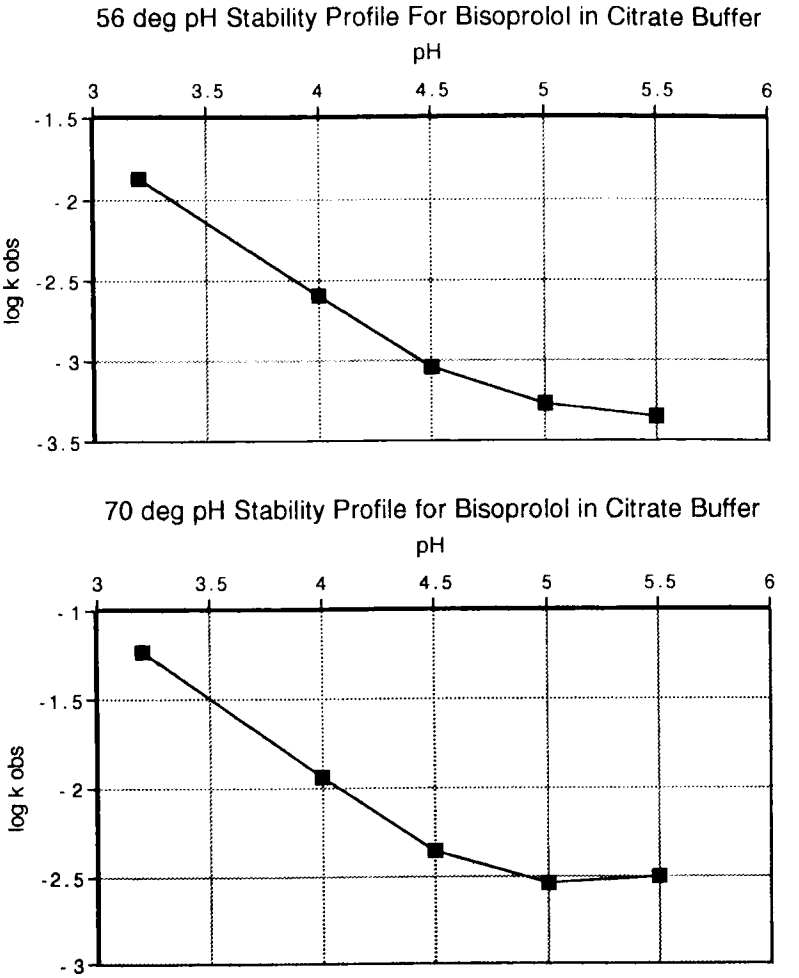

FIGURE 4

TABLE 2
pH of A-Tab® Slurries

% w/w	pH
1	6.3
5	6.1
10	5.8
20	5.6
30	5.4
40	5.3

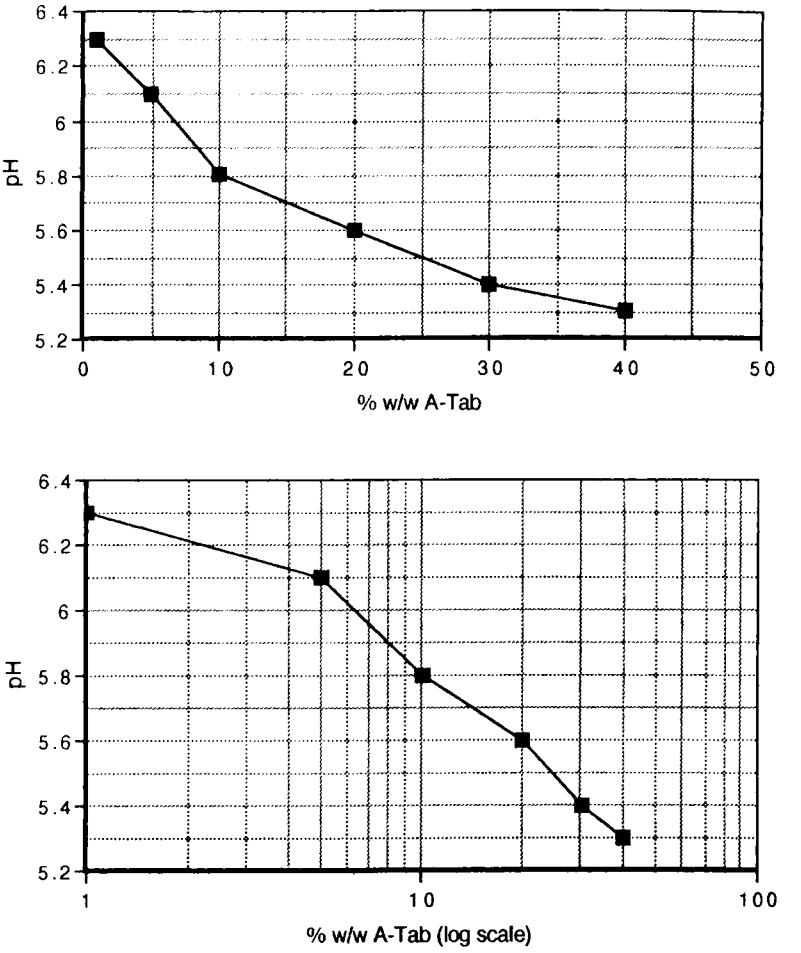

FIGURE 5
pH of A-Tab slurries as a function of concentration

milled DCPA had a pH of 7.7. There was no difference in pH when KCl was added, and the pH of water, measured by the USP method with KCl, was 5.9. In Figure 5, pH decreases as the concentration of A-Tab® increases. If a monovalent substance is present in A-Tab® that produces a concentration dependent change in pH, then a linear correlation would be expected between pH and the

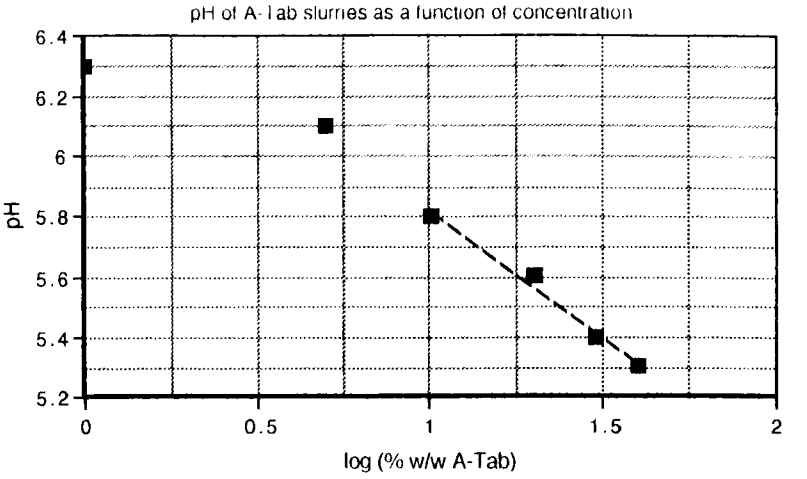

FIGURE 6

Regression Output:			
Constant		6.659785774	
Std Err of Y Est		0.031176895	
R Squared		0.986820356	
No. of Observations		4	
Degrees of Freedom		2	
X Coefficient(s)		-0.84367377	
Std Err of Coef.		0.068943344	
X	logX	Y	
99	1.9956	4.9761	<= extrapolated pH for 1% moisture

log of the concentration of that substance. A plot of A-Tab® concentration on a log scale (Fig. 5) vs. pH shows a linear region at a pH of 6.1 and below. In Figure 6 a regression analysis is done for the portion of the line below pH 5.9. This line is extrapolated to a theoretical A-Tab® concentration of 99%. This could be taken to represent a tablet having 1% moisture. The pH at this concentration is approximately 5.

TABLE 3

Sample		% Theory
A-Tab®	1:45	96.8
A-Tab®	1:45	98.2
A-Tab®	1:45	91.8
A-Tab®	1:90	95.1
Water-washed	1:45	87.4
Water-washed	1:90	88.5
NaOH-washed	1:45	88.8
NaOH-washed	1:90	90.4

A-Tab® Washing Study

To determine the effect of pH on stability of Bisoprolol in the solid state, A-Tab® was washed with water and with dilute NaOH solution to remove or reduce the surface acidity.

Content Uniformity: To determine the adequacy of mixing in the Turbula mixer, samples were taken from each mixture and prepared for HPLC assay. Also, one of the mixtures was sampled three times to determine sample-to-sample variability from the same mixture. The results are given in table 3.

These results indicate poor recovery and uniformity from these powder blends. They were not suitable for compatibility stability studies.

pH of Washed A-Tab® Slurries: The 1:90 samples above were used to prepare slurries to determine pH in Pur. Water. The results are given in table 4.

TABLE 4

Sample	Conc. (%w/w)	pH
A-Tab®	10	5.93
	20	5.58
	40	5.20
Water-washed	10	6.41
	20	6.10
	40	5.86
NaOH-washed	10	6.40
	20	6.07
	40	5.80

There is no apparent difference in pH between water-washed and NaOH-washed samples. If the same data treatment is applied here as previously with A-Tab® slurries, the following pH values are calculated for a theoretical A-Tab® concentration of 99%:

TABLE 5

	pH
A-Tab®	4.73
Water-washed	5.49
NaOH-washed	5.40

It is interesting that this A-Tab® sample is lower than the pH calculated in the previous slurry (4.98). This may indicate variability in the within-batch surface acidity of A-Tab®. There is a small amount of

Bisoprolol present in the above samples, but if anything it would be expected to increase the pH of the slurry slightly. The pH values of the washed samples are notably higher than untreated A-Tab®, creating a microenvironmental pH that is more stable for Bisoprolol.

Compatibility Studies

To verify that microenvironmental pH determines the stability of Bisoprolol in the presence of A-Tab®, studies were set up to monitor the stability of Bisoprolol with A-Tab®, water-washed A-Tab®, and NaOH-washed A-Tab®. First, five samples were prepared to determine the variability when the samples are made by weighing Bisoprolol and A-Tab® directly into the ampule. The results are given below. These samples were prepared with 1:45 Bisoprolol:A-Tab® (untreated) and 10% moisture.

TABLE 6

Sample	% Theory
1	97.2
2	98.3
3	98.6
4	93.9
5	95.7

Mean = 96.8
RSD = 1.8

A 97% recovery was achieved and variability was relatively low, making this sample preparation method suitable for compatibility studies. Samples were prepared in quadruplicate for a compatibility study with each of the three types of A-Tab®. More samples would provide greater confidence, four was selected due to practical limitations.

Table 7 shows the HPLC results after 3 and 6 weeks storage at 70°C. The results are given as the mean of four ampule assays with the RSD in parentheses.

The reason for the high variability in the 1:90 A-Tab® samples is unknown. There is little difference between the 1:45 and 1:90 samples for the treated (washed) A-Tab® samples and a difference is apparent for the untreated A-Tab® samples.

Much greater losses of Bisoprolol were observed in the untreated A-Tab® samples. Water-washed and NaOH-washed samples had similar slurry pH results and similar compatibility results. Given the difference in slurry pH of untreated and treated A-Tab® and the pH-stability profile for Bisoprolol, this difference can be attributed to the microenvironmental pH produced as a result of surface acidity of A-Tab®.

CONCLUSIONS

1. Differences in Bisoprolol Fumarate solid stability with granular or milled Anhydrous Dicalcium Phosphate have been shown to be due to the microenvironmental pH in the presence of moisture. Granular DCPA produces a low-pH environment (4.7 - 5.0)

TABLE 7

Sample	Ratio	3 weeks	6 weeks
A-Tab®	1:45	56.0 (0.88)	42.7 (3.03)
A-Tab®	1:90	48.2 (11.8)	32.6 (16.4)
Water-washed	1:45	74.8 (0.59)	69.1 (1.11)
Water-washed	1:90	73.2 (4.77)	66.1 (1.21)
NaOH-washed	1:45	75.2 (1.26)	69.9 (1.96)
NaOH-washed	1:90	73.6 (2.12)	67.6 (3.47)

whereas milled DCPA is near neutral. Bisoprolol shows little degradation at neutral pH but degradation accelerates below 5.

2. Stability and/or excipient compatibility studies are required when switching suppliers of a raw material, even though these materials may both meet USP requirements. Caution should be exercised especially when switching from milled to granular DCPA for a drug which is sensitive to low pH.

ACKNOWLEDGEMENTS

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