Evaluating the Effectiveness of Papain and Bromelain as Dissolution Tier 2 Enzymes for Cross-Linked Soft Gelatin Capsules
S. Tindal, K. Flood, J-E. Early, L. Covington, Z. Karjoo, E. Bailey
Catalent Pharma Solutions

Purpose
USP <711> Dissolution is under revision to update the Tier 2 testing parameters for gelatin capsules where cross-linking of the gelatin has been observed and is impacting the dissolution results of the gelatin capsule. The revision introduces two new enzymes for use in Tier 2 testing over the pH ranges where the current enzymes are not effective in cleaving the cross-linked gelatin to enable rupture and disintegration of the cross-linked capsule. The new enzymes, papain and bromelain, are included for use in dissolution media with pH values ranging from 4.0 – 6.8. The revision also changes the pH range where pepsin is used from 1 – 6.8 to 1 – 4, increases the amount of pancreatin from 1750 UPS units / L to 2000 UPS units / L and provides guidance for the use of an enzyme pre-soak for media where enzyme activity is inhibited (i.e., media containing some surfactants).

Work was reported in a Pharmacopeial Forum stimuli article establishing the appropriate levels of papain and bromelain as Tier 2 enzymes using hard gelatin capsules to over encapsulate acetaminophen tablets. The levels included in the proposed revision are NMT 550,000 units/L for papain or NMT 30 gelatin digestion units (GDU)/L for bromelain. The work reported here uses a similar approach to study the effectiveness of these enzymes on the dissolution rate of acetaminophen soft gelatin capsules. Cross-linking was achieved in the soft gelatin capsules in two different ways: 1) by exposing the capsules to formaldehyde vapors and 2) by adding various levels of formaldehyde to the fill solution and monitoring on stability.

Methods
Two studies were conducted to evaluate the performance of the new enzymes proposed in USP<711>. In one study, acetaminophen soft gelatin capsules were manufactured with 0, 25 and 75 ppm formaldehyde added to the gelatin fill solution. These capsules were packaged in tightly closed HDPE bottles and placed on ICH long term and accelerated stability. The capsules were tested for dissolution in 0.1N HCl for Tier 1 and 0.1N HCl containing pepsin, pH 4.5 acetate buffer containing papain or bromelain and pH 6.0 phosphate buffer containing papain or bromelain for Tier 2.

In the second study, acetaminophen soft gelatin capsules were exposed to various concentrations of formaldehyde vapor in vacuo to achieve cross-linked capsules that yielded adequate dissolution under Tier 2 testing in 0.1N HCl with pepsin and that failed Tier 2 testing with pepsin. The capsules were then also studied using papain and bromelain at various concentrations.

Results
The 0 ppm and 25 ppm formaldehyde-added capsules produced by Catalent met Tier 1 specifications for dissolution even after 26 weeks stored at the ICH accelerated condition of 40°C/75%RH. The 75 ppm formaldehyde-added capsules required Tier 2 testing during the stability study, failing Tier 2 with pepsin after 2 weeks stored at the ICH accelerated condition of 40°C/75%RH. Capsules that failed Tier 2 with pepsin were found to meet specification when tested using the USP recommended levels of papain or bromelain in pH 4.5 or pH 6.0 media. Reducing the enzyme concentrations by up to 25% of the recommended levels still yielded passing Tier 2 results using papain or bromelain.

A technique was developed to treat 30-42 capsules simultaneously in a vacuum desiccator with 100 µL of formaldehyde based on the work previously conducted in the stimuli article. Capsules meeting and failing Tier 2 dissolution testing using pepsin were produced by increasing the time of exposure. Tier 2 testing was then performed using various concentrations of papain and bromelain on capsules that failed Tier 2 testing with pepsin. As in the previous study, it was found that Tier 2 testing passed using papain and bromelain for samples that failed Tier 2 testing using pepsin. Reduction of up to 25% of papain and 33% of bromelain did not significantly impact the dissolution rate and passing results at Tier 2 were still obtained, except for the pH 4.5 acetate media with a 2.25 hour exposure (both enzymes). The condition for Tier 2 testing to be initiated is where Q=80 at 30 minutes was not met. It was also noted that the dissolution rate obtained using both papain and bromelain was greater at pH 6.0 versus 4.5, consistent with the pH dependency of the enzymes’ activities.

Conclusion
Catalent-produced acetaminophen soft gelatin capsules showed no evidence of cross-linking after 26 weeks at the ICH accelerated condition of 40°C/75%RH, even in the presence of up to 25 ppm formaldehyde-added to the fill solution. Capsules containing up to 75 ppm formaldehyde failed Tier 2 testing with pepsin after 2 weeks at 40°C/75% RH. A technique for producing multiple cross-linked capsules using formaldehyde was used to generate capsules that failed Tier 2 dissolution testing with pepsin. In both studies, it was found that passing Tier 2 results were obtained when papain and bromelain in pH 4.5 and 6.0 media were used even for samples that did not pass Tier 2 testing using pepsin in 0.1N HCl media. Reduction of up to 25% of papain and 33% of bromelain did not significantly impact the dissolution rate and passing results at Tier 2 were still obtained.

The results obtained in this study indicate that the levels of papain and bromelain should be considered in development of a product specific Tier 2 test. Further evaluation of the proposed changes to enzyme levels in USP <711> for gelatin capsules is encouraged.