Optimization of Methods to Analyze Enzymatic Mixture in Oral Gavage Formulation

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1 Purpose

A stable mixture of three pancreatic enzymes including lipase, protease and amylase was formulated to be administered as an oral gavage. The purpose of this study was to transfer the currently available USP assays for each enzyme and to optimize the assays to analyze the enzymes individually in an oral gavage formulation containing sodium acetate buffer. Freeze-thaw stability as well as long-term frozen (-20°C) stability following 0.5, 1.0 and 2.0 months was assessed to determine the shelf-life of these individual/enzyme formulations in sodium acetate buffer.

2 Method

The principle of analysis of lipase is based on hydrolysis of olive oil (1:1:1) into mono- and diglycerides and fatty acids. Each bond hydrolyzed produces one fatty acid and a molecule of glycerol. The pH of the solution is maintained at 7.5. The rate of enzymatic hydrolysis is measured by the decrease in absorbance at 505 nm over time. A spectrophotometer is used to measure the rate of hydrolysis. The lipase activity is measured by comparing the absorbance value of the test sample with the reference standards. The lipase assay is performed using the following equation:

\[ \text{Lipase Activity} = \frac{A_{	ext{sample}} - A_{	ext{blank}}}{A_{	ext{standard}} - A_{	ext{blank}}} \times \text{Standard Activity} \]

3 Results

- The USP methods were transferred successfully for analyzing the enzymes in an oral gavage formulation containing sodium acetate buffer.
- The lipase method was optimized in terms of techniques used to formulate the olive oil substrate formulation as well as for the use of a manual titrator compared to an autotitrator used typically.
- The olive oil substrate was homogenized followed by sonication in order to obtain the desired particle size necessary for the assay.
- Important points to be considered for lipase assay were substrate preparation, concentration of substrate, size of lipid droplets, consistency of temperature, stirring rate, uniform enzyme preparation time and concentration of olive oil.
- The important factors for the amylose assay was to ensure uniform reference standard stock formulations.
- The protease assay, the important factors were constant temperature and the preparation of casein substrate.
- It was reported that the minimum volume of formulation needed for each enzyme to be prepared for analyses was 50 mL in sodium acetate buffer. Any volume lower than that would result in a non-homogenous suspension.

4 Conclusion

- Long-term stability following 0.5, 1.0 and 2.0 months of frozen (-20°C) storage was also established for all three individual enzymes.

5 Reference & Acknowledgements

- www.USP.org
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