# METHOD DEVELOPMENT AND VALIDATION FOR DIGESTANT ENZYME BY SPECTROPHOTOMETRIC METHOD

**Balaji Hari[1*], B. Nagesh[1], P. Sriramcharan[3], Aman Khandelwal[4]**

[1*]Department of Pharmacognosy, JSS Academy of Higher Education and Research, JSS College of Pharmacy, The Nilgiris, Ooty-643001, Tamilnadu, India.

[2]Department of Pharmaceutical Analysis, JSS Academy of Higher Education and Research, JSS College of Pharmacy, The Nilgiris, Ooty-643001, Tamilnadu, India.

[3]Department of Pharmaceutics, JSS Academy of Higher Education and Research, JSS College of Pharmacy, The Nilgiris, Ooty-643001, Tamilnadu, India

[4]Department of Pharmacy practice, JSS Academy of Higher Education and Research, JSS College of Pharmacy, The Nilgiris, Ooty-643001, Tamilnadu, India

**Corresponding Author**

**Mr. Balaji Hari**

PhD in Life Sciences
Department of Pharmacognosy,
JSS Academy of Higher Education and Research,
JSS College of Pharmacy,
The Nilgiris, Ooty-643001,
Tamilnadu, India.
Email Id: baluphd@jssuni.edu.in

## ABSTRACT

**Background**:

The method developed and validated by using Spectrophotometry for the estimation of pancreatic enzymes in a dosage form. **Method**: The pancreatic enzyme is dissolved in the Distilled $H_2O$ and its absorbance was estimated by using UV-Visible Spectrophotometry. Linearity, Regression equation, Accuracy, Precision, Standard deviation, etc., parameters were calculated and were validated as per ICH guidelines. **Results:** The maximum concentration of pancreatic enzyme in pure water was 259nm. In the range of

concentrations of 100-600g/ml, the drug exhibits linear behaviour, with a correlation coefficient of 0.9996. In order to verify the method's accuracy, three recovery experiments were carried out: one at 50 percent, one at 100 percent, and the third at 150 percent. It was determined that the percentage of recovery was between 99% and 101%. Precision, Accuracy, Repeatability, and Ruggedness were all shown by the method's low percentage of RSD. **Conclusion:** The above-validated method may be useful for routine analysis of Pancreatic enzyme in a pharmaceutical dosage form.

**Keywords:** Pancreatic enzyme, UV-Visible Spectrophotometry.

## INTRODUCTION

Amylase, lipase, and protease are commercial combinations of pancreatic enzymes known as Pancrelipase and Pancreatin. Since then at least in the 1800s, drugs derived from pancreatic enzymes were utilized to treat various ailments. They're used to treat pancreatic problems that cause malabsorption syndrome. Cystic fibrosis, surgical removal of the pancreas, long-term pancreatitis, or pancreatic cancer may be to blame for these pancreatic issues. Taken by mouth, the preparation is effective. Exocrine pancreatic deficiency in addition to some other digestive disorders are often treated with pancrelipases. Though it's neither a particular molecule nor an acid, pancreatin is occasionally referred to as "pancreatic acid." It aids in the digestion of food and treats a variety of disorders, such as pancreatic inflammation. A number of medications and items may interact with it, including the following precise, and vitamins are among the many over-the-counter and herbal remedies available. Using the strategy outlined in the literature study, we proceed to experiment on the drug category. 3-12

## MATERIAL AND METHODS

### Instruments used:

A Shimadzu 1800 UV/VIS double beam spectrophotometer with 1cm matched quartz cells was used for all spectral measurements processed by UV-probe. Single Pan Electronic balance (CONTECH, CA 223, India) were used for weighing purposes. Sonication of the solutions was carried out using an Ultrasonic Cleaning Bath (Spinco tech, India).

**Materials used:**

Distilled $H_2O$ was prepared using Milli-Q system in laboratory and Methanol make Sigma-Aldrich. Formulation of festal-N was purchased from a local pharmacy.

## METHOD DEVELOPMENT

**Selection of Diluents:**

Different Solvents like Water, 50% Ethanol, Methanol were employed for a recording of the UV spectrum and the optimization of the method. Solubility was found to be Distilled water.

**Preparation of standard stock solution:**

Standard Pancreatic enzyme100mg was weighed and transferred on 100ml volumetric flask and dissolved in the solvent mixture and the volume was made up to the mark with the solvent mixture in 100ml volumetric flask as it is a 1000µg/ml concentration. The optimized solution was prepared from the stock solution with a solvent mixture, which was used as a working standard.

**Selection of Wavelength**

At 259 nanometres, the spectrum was seen in a range of wavelengths from 200 to 400 nanometres. The standard stock solution, which is 1 mg/ml, was used to make 100 g/ml. The blank and reference materials were made using a solvent combination.

**Preparation of sample solution:**

This sample solution was made by weighing and dissolving 100mg of analyte in 100 ml of diluent and then filling the volumetric flask up to mark with diluent.

**Calibration curve for Pancreatic Enzyme**

From the standard stock solution of Pancreatic enzyme sufficient aliquots were pipetted into 10 ml volumetric flasks then dilutions were made using diluents to generate working standard solutions at concentrations from 100-600 µg/ml and also the overlay spectrum is shown in **figure**-2.

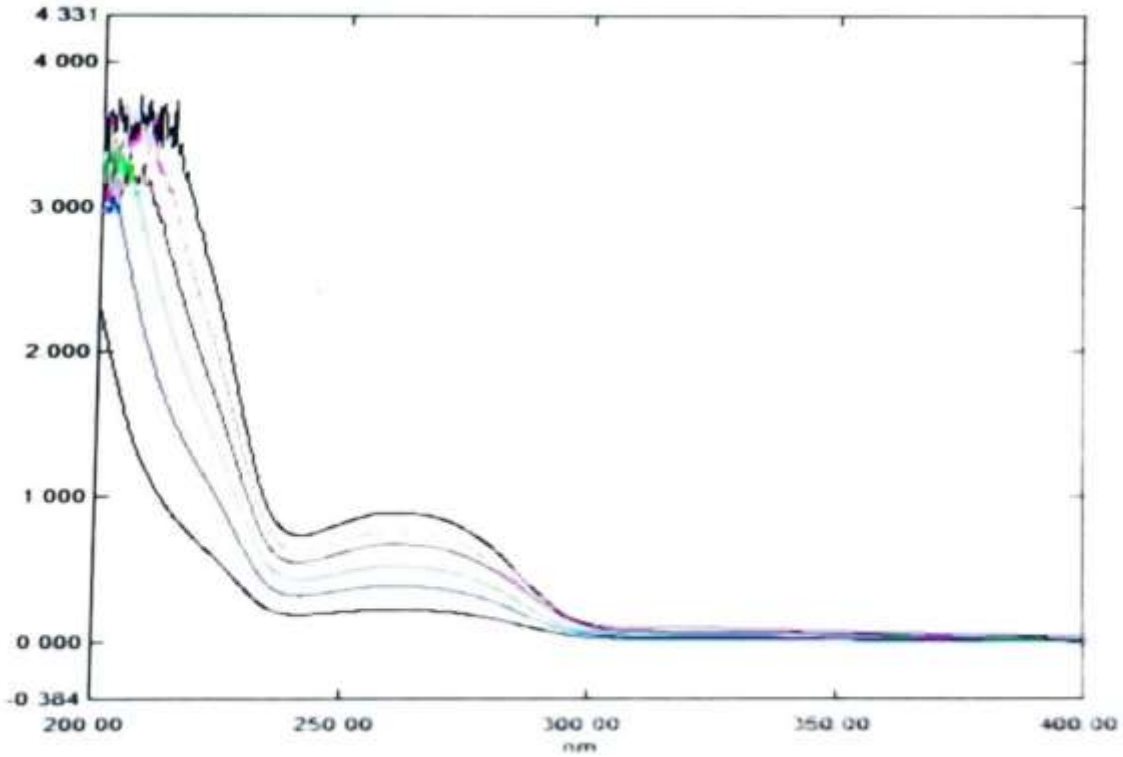


**Figure-2 Overlay Spectrum**

## RESULTS AND DISCUSSION

In the Zero-order spectroscopy method, the pancreatic enzyme attains maximal absorption at 259nm; it showed a good linearity range in the concentrations of 100-600µg/ml. This linearity range obeys beer-lamberts' law and statistical data of quantitative results obtained for this method is shown in the table-1. These results were subjected to statistical analysis to find out standard deviation and standard error values and the obtained results are below the precision of the methodology hence the assay and accuracy were performed at three different levels it confirms the method having repeatability and reproducibility which has been validated as per ICH guidelines [Q2 (R1)].

**Table 1: Statistical data of Zero-order Spectroscopic method for Pancreatic Enzyme:**

| Parameters | Zero-order spectroscopy |
|---|---|
| | Pancreatic Enzyme |
| λmax (nm) | 259 |
| Linearity range(µg/ml) | 100-600 |
| Regression equation (Y*) | 0.0013x+0.1133 |
| Slope (m) | 0.0013 |
| Intercept (c) | 0.1133 |
| Correlation coefficient ($r^2$) | 0.9956 |
| Precision Interday (%RSD) | 0.45 |
| Precision Intraday (%RSD) | 0.86 |
| LOD(µg/ml) | 7.120 |
| LOQ(µg/ml) | 21.575 |

## METHOD VALIDATION

### LINEARITY:

Plotting concentration vs analyte absorbance yielded the study's recommended linearity range, which reveals a strong correlation between concentration and absorbance. Shows on Figure 3.

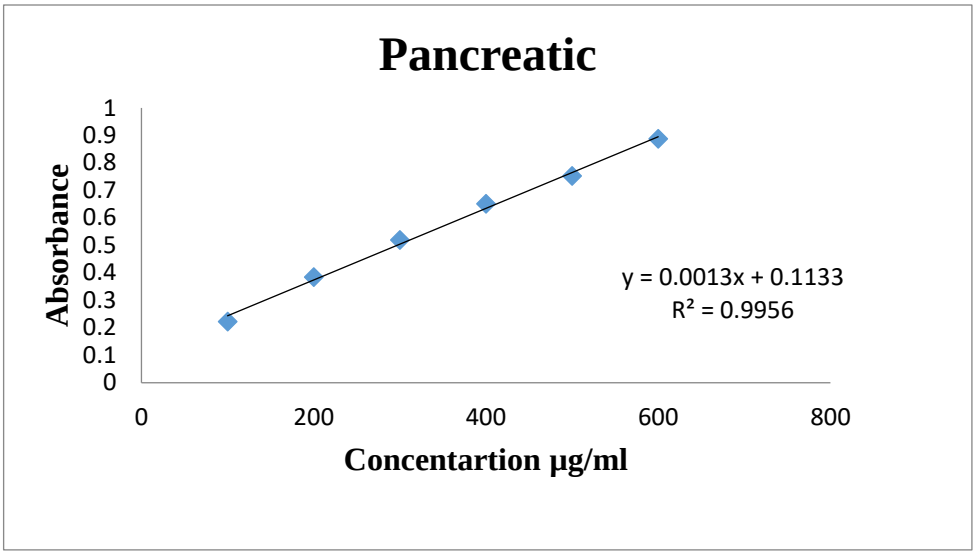


**Figure**.3. Linearity graph for Pancreatic

**ACCURACY:**

Recovery experiments of the triplet standard addition technique at varying concentration levels of 50%, 100%, and 150% were used to test the method's accuracy. Pre-analyzed samples with known concentrations of 50.0, 100.00, and 150.03 g/ml were used in the proposed approach. A breakdown of recovery studies may be seen in table 2.

**Table 2: Accuracy data**

| Pancreatic Enzyme | | | |
|---|---|---|---|
| **Spiked con** | **Amount added (µg/n** | **Amount found (µg/m** | **%Mean recove** |
| 200 | 50.01 | 49.837 | 101.09 |
| | | 50.814 | |
| | | 50.977 | |
| 400 | 100.00 | 99.672 | 100.99 |
| | | 100.164 | |
| | | 100.657 | |
| 600 | 150.03 | 148.526 | 99.45 |
| | | 151.965 | |
| | | 147.052 | |

**PRECISION:**

The estimated sample analysis, carried out by six duplicates of a constant concentration from the formulation, demonstrates the method's repeatability and reproducibility. The outcomes of the intraday and inter day were likewise done at a confidence interval. Because the RSD was less than 2%, these findings were considered to be of excellent accuracy by the method's developers.

**Table 3: Precision Data**

| S.No | Inter Day | Intra Day |
|---|---|---|
| 1 | 0.622 | 0.610 |
| 2 | 0.624 | 0.614 |
| 3 | 0.625 | 0.616 |
| 4 | 0.620 | 0.622 |
| 5 | 0.628 | 0.622 |
| 6 | 0.623 | 0.623 |
| Mear | 0.623666667 | 0.617833333 |
| Std. D | 0.00273 | 0.00530 |
| %RSI | 0.44 | 0.86 |

**DETECTION OF LIMITS (LOD&LOQ):**

The smallest quantity of analyte in a sample that could be detected and not necessarily quantified is known as the detection limit of a certain analytical process. Analyte detection limit may be computed as LOD=3.3 * standard deviation ()/ s... The LOQ (limit of quantification) is defined as 10* standard deviation/second. Table 1 shows the findings of LOD and LOQ.

**ROBUSTNESS:**

While tiny modifications are made in the analytical technique, purposeful adjustments in method parameters show the analytical method that will be unaffected and offers an indicator of its dependability during regular use. Table 4 displays the robustness data.

**Table 4: Robustness data**

| S. No. | Robust Condition | Parameter | %RSD |
|---|---|---|---|
| 1. | Wavelength ± 3 nm | 222nm | 0.63 |
| 2. | | 225nm | 0.49 |
| 3. | | 228nm | 0.47 |

**CONCLUSION:**

This method has been successfully developed and validated and comply with regulatory standards for linearity, accuracy, precision, reproducible, specific, robust, and economical. This method has be used for the routine quality control analysis of an analyte in dosage form and simultaneous estimation in digestant enzyme by spectrophotometric method and recovery studies.

**ACKNOWLEDGEMENT**

We acknowledge the research infrastructure and generous support rendered by the, JSS Academy of Higher Education & Research (JSS AHER), JSS College of Pharmacy, Ooty, Tamilnadu, India. **Mr. Balaji Hari** acknowledges to JSS AHER.

**CONFLICTS OF INTEREST**

The authors declare no conflict of interest

## REFERENCES


1. https://newdrugapprovals.org/2018/04/18/cadexomer-iodine
2. Low LL. Wound Care. The Singapore Family Physician. 2015:27-34.
3. Schultz GS, Sibbald RG, Falanga V, Ayello EA, Dowsett C, Harding K, Romanelli M, Stacey MC, Teot L, Vanscheidt W. Wound bed preparation: a systematic approach to wound management. Wound repair and regeneration. 2003 Mar;11:S1-28.
4. https://en.wikipedia.org/wiki/Cadexomer_iodine.
5. Shivani, Ajay Kumar Kpilesh, Megha Sharma, *Validation and Analytical Method Development for Determination of Ornidazole in Ointment Formulation by U.V Spectrophotometric Method.*International Journal of Pharmaceutical Technology and Biotechnology2014; 1(1):01-10.
6. Noda Y, Fujii K, Fujii S. Critical evaluation of cadexomer-iodine ointment and povidone-iodine sugar ointment. International journal of pharmaceutics. 2009 May 8;372(1-2):85-90.
7. Parmar AP, Maheshwari D. Simultaneous Estimation of Mupirocin and Mometasone Furoate in Pharmaceutical Dosage Form by Q-Absorption Ratio Method. International Research Journal of Pharmaceutical and Applied Sciences. 2015 Apr 30;5(2):1-7.
8. Kumar BK, Rajan VT, Begum NT. Analytical method development and validation of lidocaine in ointment formulation by U. V spectrophotometric method. Int J Pharm Pharm Sci. 2012;4(2):610-4.
9. Shabir GA, Bradshaw TK. Determination of 1, 7, 7-trimethyl-bicyclo (2, 2, 1) heptan-2-one in a cream pharmaceutical formulation by reversed-phase liquid chromatography. Indian journal of pharmaceutical sciences. 2010 Nov;72(6):809.
10. Lohar RJ, Patil VM, Gaikwad RG, Patil SS. Development and validation of uvvisible spectrophotometric method for estimation of selected antiseptic drug in bulk and pharmaceutical dosage form. World journal of pharmacy and pharmaceutical sciences. 2016 Jun 21:1197-205.
11. Ghurghure S, Sawant K, Deokar SS. UV Spectrophotometric Method Development and Validation of Mesalazine in Bulk and Solid Dosage Form.
12. Manoharan G, Al-Bratty M. Development and validation of ultra violet spectrophotometric and reversed-phase high performance liquid chromatography

techniques for simultaneous estimation of brinzolamide and brimonidine tartrate in ophthalmic suspension formulation. Orient. J. Chem. 2016 Apr 1;32(2):1111-20.