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QUANTITATIVE ESTIMATION OF BETA GALACTOSIDASE BY UV SPECTROPHOTOMETRY

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Abstract

Quantitative estimation of beta galactosidase was carried out by UV spectrophotometry using distilled water as solvent. The procedure was performed at 280nm (λmax). Beer's law range was found to be 0.2-2 mg/ml. This method was validated as per ICH guidelines by using several parameters like accuracy, precision, linearity, LOD &LOQ. LOD & LOQ were found to be 0.192mg/ml & 0.581mg/ml respectively.

Keywords: Beta Galactosidase, Lactase, Proteins, UV Spectrophotometry.

1. Introduction

There are many methods available for determining protein concentration. Most of these methods rely either on colorimetric assays or the use of UV absorbance spectroscopy. The choice of method depends upon a variety of factors, including the amount of protein present, the specificity of the method, the presence of interfering substances, and the amino acid composition of the protein.[1] Protein, absorbs ultraviolet light quite strongly. Rather, it is the amino acids that make up the proteins that absorb the UV light. The strong absorbance of UV light by protein allows for rapid analysis of protein samples. Proteins in solution absorb ultraviolet light with absorbance maxima at 280 and 200 nm. Amino acids with aromatic rings are the primary reason for the absorbance peak at 280 nm. Many researchers[(1-6)] have shown the application of uv spectroscopy for the determination of protein content. Numbers of methods have been devised to measure protein concentration, which are based on UV-visible spectroscopy. These methods use either the natural ability of proteins to absorb (or scatter) light in the UV-visible region of the electromagnetic spectrum, or they chemically or physically modify proteins to make them absorb (or scatter) light in this region. An enzyme is a protein molecule that is a biological catalyst. The present paper shows the application of above principle for the determination of concentrationof enzyme in sample. Our main concern is

development and validation of UV spectrophotometric method as per ICH guideline. galactosidase,(commonly known as lactase) the enzyme present in the brush border of the proximal jejunum enterocytes, hydrolyses dietary lactose (from milk and dairy products) to glucose and fructose[7]. Lactose, the predominant carbohydrate in milk, is a disaccharide consisting of galactose bound to glucose. Intestinal absorption of lactose requires hydrolysis to its component monosaccharides by the brush-border enzyme lactase[8].

Absence of lactase enzyme in intestine leads to lactose intolerance whose approach for the treatment is enzyme replacement therapy which are commercially available in the form of pills, drops, tablets, capsules[11].

Lactase has been assayed by procedure given FOOD AND CHEMICAL CODEX. Activity test is based on hydrolysis of o-Nitrophenyl-β-D-Galactopyranoside (ONPG) substrate for 15 minutes at 37° and specified pH. 1 Lactase unit (LacU) is an amount of enzyme that extricates 1 μ mol of o-nitrophenol per 1 minute under the above conditions. The present paper will show the utilization of U.V.spectrophotometric method for the direct determination of quantity of lactase in a sample.

2. Materials and Method

Analytical grade chemicals and reagents were used throughout the work. Beta galactosidase (source Aspergillus Oryzae) was purchased from sigma laboratories. Commercial lactase preparation (Lacdigest^{TM} Tilattasi 2250 unita) was purchased from Milan Italy.

3. Instrument

UV visible Double Beam Spectrophotometer with 1cm matched glass cells were used for the analysis. (UV-1800 Shimadzu, Ltd)

4. Method

Primary stock solution of Lactase was prepared by using Distilled Water, from this different dilutions were prepared to determine λmax and beer's law range. Calibration curve was by using different concentrations of standard solution. Lactase in dosage form was estimated by calibration curve. Developed method was validated as per ICH guidelines with the help of several parameters like accuracy, precision, LOD, LOQ, and stability.

5. Preparation of standard Lactase solution.

The standard stock solution was prepared by dissolving lactase in distilled water to make final concentration of 2 mg/ml. Different aliquots were taken from stock solution and diluted with distilled water separately to prepare

series of concentrations from 0.2-2 mg/ml. The λ_{max} of lactase in distilled water was found in the range of 200-400 nm and it was found to be 280 nm. Absorbance was measured at 280 nm against distilled water as blank. The calibration curve was prepared by plotting absorbance versus concentration of lactase.

6. Result and Discussion

In this study colorless clear solution was developed by dissolving the sample in distilled water and that was scanned for λmax against blank solution and λmax was found to be 280 nm. Calibration curve was prepared by using the standard Lactase solution at different concentrations. The beer's law range was 0.2 – 2 mg/ml (Table 1) (Fig 1). The experiment preparation of calibration curve was repeated six times for inter day. The average % RSD of inter day measurements were recorded (Table 2). The values of LOD and LOQ for beta galactosidase at selected wavelength was noted (Table 3). Accuracy of the proposed method was determined by performing recovery studies (Table 4). In formulations Lactase was estimated by making the solution to beer's law range and recording the absorbance at 280 nm against blank solution.

Table 1: Calibration plot of Lactase at 280 nm

S.No	Concentration in mg/ml	Absorbance
1	0.1	0.051
2	0.4	0.099
3	0.6	0.147
4	0.8	0.197
5	1	0.25
6	1.2	0.307
7	1.4	0.357
8	1.6	0.41
9	1.8	0.462
10	2	0.51

Fig-1: Calibration curve of Lactase.

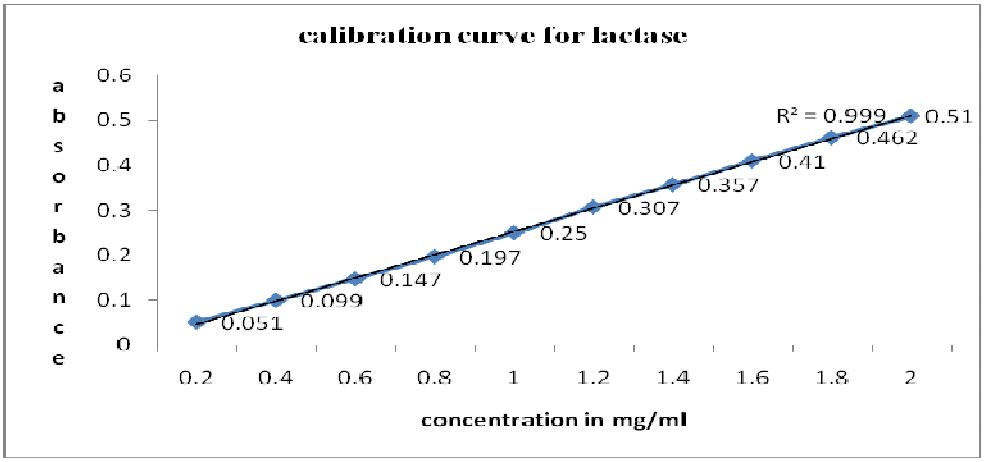

Table-2: Inter day precision.

Conc (mg/ml)	Mean absorbance ± SD		Relative standard deviation (RSD%)	
	Day 1	Day 2	Day 1	Day 2
0.2	0.051 ± 0.002	0.048 ± 0.001	3.9	2
0.4	0.101 ± 0.001	0.102 ± 0.003	0.99	2.9
0.6	0.15 ± 0.001	0.152 ± 0.003	0.66	1.9

Average of 6 readings

Table-3: Limit of detection and quantitation.

Parameters	In Distilled Water
Absorbance maximum (λ_{max}) in nm	280
Beer's law limit (mg/ml)	0.2-2
Slope	0.051
Intercept	-0.005
Correlation coefficient	0.9997
LOD (mg/ml)	0.192
LOQ (mg/ml)	0.581

Table-4: Recovery studies.

Amount of Drug (mg/ml)	Amount Found (mg/ml)	% Recovery	Precision (interday) RSD%	Precision (intraday) RSD%
0.8	0.75	94.1	0.8	1.2
1	1.	100.98	1	0.83
1.2	1.18	98.6	0.51	0.19

7. Conclusion

This method was found to be suitable for the estimation of Beta galactosidase in dosage forms. By results, % recovery with + 5% indicates better accuracy, % RSD was always less than + 2 % it indicates higher Precession. This method is easy to perform and it can be applied for routine analysis and it is low at cost.

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