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Research Article

Analytical Method Validation for Assay Method (By UV) of Trypsin-Chymotrypsin Tablets 50000 AU of Enzymatic Activity

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Abstract:

It is internationally recognized that validation is necessary in analytical laboratories. The use of validated methods is important for an analytical laboratory to show its qualification and competency. When analytical method is utilized to generate results about the characteristics of drug related samples it is essential that the results are trustworthy. They may be utilized as the basis for decisions relating to administering the drug to patients. Analytical method validation required during drug development and manufacturing and these analytical methods are fit for their intended purpose. The purpose of this validation is to show that processes involved in the analytical testing can be performed in an effective and reproducible manner. This article provides a good, complete, up-to-date collation of relevant information in the fields of analytical method validation of Trypsin-Chymotrypsin Tablets 50000 AU of Enzymatic Activity.

Keywords: Analytical method validation, Pharmaceutical analysis, Specificity, Precision, Accuracy.

Introduction

The purpose of preparation of Analytical Method Validation protocol[1-4] is to provide documented evidence that shows that data obtained during analysis complies with the minimum acceptance criteria and method utilized for the analysis is accurate, precise, linear and capable of producing and reproducing the reliable result for assay of Trypsin-Chymotrypsin Tablets 50000 AU of Enzymatic Activity. This article covers the scope of Analytical Method Validation study for Assay of Trypsin-Chymotrypsin Tablets 50000 AU of Enzymatic Activity by UV.

2. Materials and Methods

2.1 Selection of Analytical Performance Parameters:

As per cGMP/ICH guidelines the test methods, which are used for assessing the quality of pharmaceutical products with established specifications, must be validate for analytical performance parameters as mentioned below.

Name of the Finish product	**Trypsin-Chymotrypsin Tablets 50000 AU of Enzymatic Activity.**
Pharmacopoeial Status	**In-House**
Analytical Method to be Verified for Assay	• **Specificity** • **Precision (System Precision, Method Precision)** • **Linearity and Range** • **Accuracy** • **Solution stability** • **Filter paper study**

2.2 Trypsin-Chymotrypsin Tablets 50000 AU of Enzymatic Activity[5]

Trypsin-chymotrypsin tablets are indicated in post-operative wounds, oedema andhaematoma, prevention of inflammation of the surgical stitches, pelvic inflammatory disease, caesarean section, episiotomy, abdominal hysterectomy, tooth extraction, peri-apical abscess, maxillofacial surgery, post-traumatic oedema, soft tissue injury, fractures and dislocation, sports injuries, and sprains andstrains.

2.3 Methodology:

Description of Analytical Method

Reagent Preparation:

0.001 N Hydrochloric Acid:
Dilute 0.085 ml of conc. HCl diluted to 1000 ml with purified water.

0.5 N Sodium Hydroxide: (Freshly prepared):

5.25 gm of Sodium Hydroxide (NaOH) to 250 ml with purified water.

Folin-Cicaileau reagent:

10 ml of stock solution to be diluted to 30 ml with distilled water.

Standard Preparation: (10 mg/ml)

Weigh exactly about 200 mg working standard of Trypsin- Chymotrypsin 6:1 in a 20 ml volumetric flask. Add 0.001 N HCl and shake to dissolve. Make up the volume up to mark with 0.001 N HCl.

Sample Preparation: (10 mg/ml)

Weigh exactly eq. to about 200 mg of crushed test sample of Trypsin-Chymotrypsin 6:1 and transfer in a 20 ml volumetric flask. Add 0.001 N HCl, shake sonicate for 15 min. to dissolve. Make up the volume up to mark with 0.001 N HCl. Transfer the solution in a glass beaker then add about 0.500 gm activated charcoal make thin slurry and keep solution for 20 minutes to absorb color by activated charcoal then Filter the resulting solution with 0.45 micron Whatmann filter paper.

Placebo Preparation:

Proceed as per sample preparation except test sample weight shall be replaced with placebo.
Take 4 Stoppard clean and dried volumetric flask of 20 ml and marked as Standard, Sample placebo and blank.

Follow the below mention procedure for in sequential manner

Standard	**Sample**	**Blank**
5 ml of standard preparation + 5 ml of 0.5 N NaOH + 1 drop concentrate Folin-Cicaileau reagent and again 2 ml of prepared Folin-Cicaileau reagent**.** Filter it through Whatmann. No.42	5 ml of sample preparation + 5 ml of 0.5 N NaOH 1 drop concentrate Folin-Cicaileau reagent and again add 2 ml of prepared Folin-Cicaileau reagent**.** Filter it through Whatmann. No.42	5 ml of 0.001 M HCl + 5 ml of 0.5 N NaOH +1 drop concentrate Folin-Cicaileau reagent and again add 2 ml of prepared Folin-Cicaileau reagent**.** Filter it through Whatmann. No.42

Placebo Preparation: Weigh exactly eq. to about 200 mg of placebo sample as proceed as described under sample preparation.

After 15 min. take 5 ml of blank, Standard and Sample. Measure the absorbance of all solutions at 660 nm.

Note: Deduct the placebo absorbance with sample absorbance.

Calculation:

$$\text{Assay (Enzyme activity)} = \frac{Au \times W_1 \times 20 \times P \times \text{Average Weight}}{As \times 20 \times W_2}$$

$$\%\ \text{Assay} = \frac{\text{Armor Unit}}{\text{Label Claim}} \times 100$$

Where,

Au	Absorbance at 660 nm obtained from Sample solution
As	Absorbance at 660 nm obtained from Standard solution
W_1	Taken weight of Trypsin-Chymotrypsin 6:1 WS in mg
W_2	Taken weight of Trypsin-Chymotrypsin 6:1 test sample in mg
P	Potency of Trypsin-Chymotrypsin 6:1 WS in unit

Specification Limit for Assay: NLT 90.0 %

3. Result and Discussion:

3.1 Validation Parameters: [5-10]

Parameter used for Analytical method Verification for Assay:

The analytical method needs to be verified for below mentioned validation parameters:

Specificity

Placebo Preparation

Weigh exactly eq. to about 200 mg of placebo sample as proceed as described under sample preparation.

Standard Preparation: (10 mg/ml)

Weigh exactly about 200 mg working standard of Trypsin- Chymotrypsin 6:1 in a 20 ml volumetric flask. Add 0.001 N HCl and shake to dissolve. Make up the volume up to mark with 0.001 N HCl.

Sample Preparation: (10 mg/ml)

Weigh exactly eq. to about 200 mg of crushed test sample of Trypsin-Chymotrypsin 6:1 and transfer in a 20 ml volumetric flask. Add 0.001 N HCl, shake sonicate for 15 min. to dissolve. Make up the volume up to mark with 0.001 N HCl. Transfer the solution in a glass beaker then add about 0.500 gm activated charcoal make thin slurry and keep solution for 20 minutes to absorb color by activated charcoal then Filter the resulting solution with 0.45 micron Whatmann filter paper.

Preparation (Final Dilution):

Standard	**Sample**	**Blank**
5 ml of standard preparation + 5 ml of 0.5 N NaOH + 1 drop concentrate Folin-Cicaileau reagent and again 2 ml of prepared Folin-Cicaileau reagent**.**	5 ml of sample preparation + 5 ml of 0.5 N NaOH 1 drop concentrate Folin-Cicaileau reagent and again add 2 ml of prepared Folin-Cicaileau reagent**.** Filter it through Whatmann. No. 42	5 ml of 0.001 M HCl + 5 ml of 0.5 N NaOH +1 drop concentrate Folin-Cicaileau reagent and again add 2 ml of prepared Folin-Cicaileau reagent**.** Filter it through Whatmann. No. 42

Method:

Observe the colour develops after the addition of Folin-Cicaileau reagent in standard, sample and blank solution, and record. Measure the absorbance of prepared solution at 660 nm

Sample Table for Specificity

Sr. No.	Sample Identification	No. of samples
1.	Blank	1
2.	Placebo	1
2.	Standard Solution	1
3.	Sample Solution	1

Acceptance criteria:

After the addition of Folin-Cicaileau reagent standard and sample solution should develop blue colour, whereas blank should be colourless.

- Interference of blank should be not more than 2.0 % to that of standard solution.

Linearity and Range

Linearity Standard stock Solution Preparation: (10 mg/ml)

Weigh exactly about 500 mg working standard of Trypsin- Chymotrypsin 6:1 in a 50 ml volumetric flask. Add 0.001 N HCl and shake to dissolve. Make up the volume up to mark with 0.001 N HCl.

Sr. No.	Linearity Solution Level.	Linearity stock solution in ml	Volume of 0.5 M NaOH added in ml	Folin-Cicaileau Reagent added in ml	Final volume in ml	Conc. in IU
1	50%	2.5	7.5	2	12	5927083
2	80%	4	6	2	12	9483333
3	100%	5	5	2	12	11854167
4	120%	6	4	2	12	14225000
5	150%	7.5	2.5	2	12	17781250

Sample Table:

Sample	Parameter	Wavelength
Linearity Solution-01 to Linearity Solution-05	Absorbance	**660 nm**

Acceptance Criteria:

Parameter	Limits
Correlation Coefficient	≥ 0.950

Accuracy

Preparation

Placebo of **Trypsin-Chymotrypsin** Tablet (As per formulation) shall be spiked with drug Substance (Trypsin-Chymotrypsin API) at three different levels, 50%, 100%, and 150% and analyzed in triplicate as described under Method, Accuracy will be expressed in terms of % Recovery and Relative standard deviation calculated .

Prepare a standard solution of 100 % concentration.

For Method, Refer Point No. 7 (Methodology)

Standard Stock Solution: (10 mg/ml)

Weigh exactly about 500 mg working standard of Trypsin- Chymotrypsin 6:1 in a 50 ml volumetric flask. Add 0.001 N HCl and shake to dissolve. Make up the volume up to mark with 0.001 N HCl.

Standard Solution:

5 ml of standard preparation + 5 ml of 0.5 N NaOH + 1 drop concentrate Folin-Cicaileau reagent and again 2 ml of prepared Folin-Cicaileau reagent**.** Filter it through Whatmann. No. 42

Standard Placebo Solution:

Weigh exactly about 8000 mg placebo in a 100.0 ml volumetric flask. Add 0.001 N HCl and shake to dissolve. Make up the volume up to mark with 0.001 N HCl.

Sample Solution:

Accuracy level 50%:

2.5 ml of standard preparation + 5 ml of placebo + 7.5 ml of 0.5 N NaOH + 1 drop concentrate Folin-Cicaileau reagent and again 2 ml of prepared Folin-Cicaileau reagent**.** Filter it through Whatmann. No. 42.

Accuracy level 100%:

5 ml of standard preparation + 5 ml of placebo + 5 ml of 0.5 N NaOH + 1 drop concentrate Folin-Cicaileau reagent and again 2 ml of prepared Folin-Cicaileau reagent**.** Filter it through Whatmann. No. 42

Accuracy level 150%:
7.5 ml of standard preparation + 5 ml of placebo + 2.5 ml of 0.5 N NaOH + 1 drop concentrate Folin-Cicaileau reagent and again 2 ml of prepared Folin-Cicaileau reagent**.** Filter it through Whatmann. No. 42**.**

Sample Table

Sr. No.	Solution	No. of samples	Wavelength
1	Blank solution	1	660 nm
2	Placebo	1	
3	Standard solution	1	
4	Accuracy level_50 %_1	1	
5	Accuracy level_50 %_2	1	
6	Accuracy level_50 %_3	1	
7	Accuracy level 100 % 1	1	
8	Accuracy level_100 %_2	1	
9	Accuracy level_100 %_3	1	
10	Accuracy level 150% 1	1	
11	Accuracy level 150% 2	1	
12	Accuracy level 150% 3	1	

Acceptance Criteria:

Sr. No.	Parameter	Acceptance criteria
1	Recovery at 50% level to 150% level,	NLT 95.0 %. and NMT 105.0 %
2	% RSD of mean % recovery at 80% to 150 %	NMT 2.0 %

Precision
Precision parameter shall be carried out as**:**

- System Precision
- Method Precision
- Intermediate Precision

System Precision:

For Method and solution preparation, Refer (Methodology)
Prepare a standard solution of 100 % concentration. (10 mg/ml)
Six replicate measurement of standard solution shall be carried out and result shall be expressed in terms of Relative Standard Deviation.

Sample Table:

Sr. No.	Solution	No. of Reading	Wavelength
1.	Blank solution	1	660 nm
2.	Standard Preparation	6	

Acceptance Criteria:

Sr. No.	Parameter	Acceptance criteria
1	% RSD of all the six absorbance	Not more than 2.0 %

Method Precision: For Method and solution preparation, Refer (Methodology), Prepare a standard solution of 100 % concentration. (10 mg/ml).
Six independent samples along with 1 placebo preparation shall be prepared and assayed separately and % Assay will be calculated for each. Method Precision will be expressed in term of relative standard deviation of six results.

Sample Table:

Sr. No.	Solution	No. of Reading	Wavelength
1.	Blank solution	1	660 nm
2.	Placebo Preparation	1	
3.	Standard Preparation	1	
4.	Sample – 1	1	
5.	Sample – 1	1	
6.	Sample – 1	1	
7.	Sample – 1	1	
8.	Sample – 1	1	
9.	Sample – 1	1	

Acceptance Criteria:

Sr. No.	Parameter	Acceptance criteria
1	Relative Standard Deviation of individual Assay test	Not more than 2.0 %

Solution Stability

Preparation

Note: Refer methodology for preparation

Sequence Table

Sr. No	Solution	No. of Reading
1	Blank solution	1
2	Placebo	1
3	Standard solution	1
4	Test solution	1

Note: Study to be performed at 0 hr. 1 hr., 2 hrs.

Acceptance Criteria

Difference in absorbance from the initial value should not be more than 3.0 %.

Filter Paper Study

Note: Refer methodology for preparation

Filter the solution through different types of filter (Whatman filter paper, 0.45µ Nylon filter etc.)

Sample Table:

Sr. No.	Solution	No. of Reading
1	Blank solution	1
2	Placebo	1
3	Standard solution	1
4	Test solution Unfiltered (centrifuge)	1
5	Test solution Filtered through Whatman	1
6	Test solution Filtered through 0.45 µ Nylon filter	1
7	Standard Solution (Bracketing)	1

Acceptance Criteria

% Difference of assay with filtered, unfiltered and centrifuge sample from standard and sample should not be greater than 2.0.

Conclusion

This article provides an idea how to perform validation process to prove that the method is apt for its intended purpose and to assure the capabilities of the test method. The definitions of method validation parameters are well explained. Although the requirements of validation have been clearly documented by regulatory authorities, the approach to validation is varied and opened to interpretation, and validation requirements differ during the development process of pharmaceuticals. Validation is an important procedure in the pharmaceutical industry and it is utilized to ensure that quality is built in to the processes supporting drug development and manufacture.

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