ANALYTICAL METHOD VALIDATION/VERIFICATION PROTOCOL FOR AZITHROMYCIN CAPSULES USP 500 MG (ASSAY)

METHOD VERIFICATION

PROTOCOL FOR (ASSAY)

AZITHROMYCIN CAPSULE USP 500 MG

BY

HIGH PREFORMANCE LIQIUD CHROMATOGRAPHY

Protocol No.	
Supersedes	
Effective Date	
Document contains pages	

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ANALYTICAL METHOD VALIDATION/VERIFICATION PROTOCOL FOR AZITHROMYCIN CAPSULES USP 500 MG (ASSAY)

1.0 Protocol Approval:

This is a specific Protocol for Method Verification ofAzithromycin Capsules USP 500 mg.

1.1 **Initial Approval:** This Protocol has been approved by the following:

	Name	Designation	Signature	Date
Prepared by (QC)				
Checked by (QC)				
(Reviewed By) (QA)				

1.2 **Final Approval:** Final approval has been given by the following:

	Name	Designation	Signature	Date
Approved By (Head-Quality Assurance)				

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2.0 Objective:

This protocol shall be providing the documented evidence, that the Method Verification for Azithromycin Capsules USP 500 mg with the specified quality attributes in consistent manner.

3.0 Scope:

This protocol shall be used to provide the procedure for the Method Verification for Azithromycin Capsules USP 500 mg.

4.0 Responsibility:

To conduct the Method Verification for Azithromycin Capsules USP 500 mg. The Verification team is described through the following responsibility table.

S.No.	Department	Responsibility
1.	Quality Control	1) QC Chemist shall be responsible for conducting the verification carry out the verification analysis. 2) QC Executive or Designee shall be responsible for preparation of Verification Protocol, Reporting, Planning and Monitoring. 3) QC Manager shall be responsible for checking of Verification Protocol and Report. 4) QC Manager or Designee shall be responsible for provide the training for staff.
2.	Quality Assurance	QA Head or Designee shall be responsible for final approval of Testing Protocol.

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5.0 Specification:

S.No.	Parameters	Acceptance Criteria
1.	Specificity	No interference with blank or placebo.
2.	Precision	NMT - RSD < 2%.
	1)System Precision	Standard RSD shall be Not more than 2.0 %.
	2) Method Precision	Results RSD shall be Not more than 2.0 %.
	3) Intermediate Precision	Percentage RSD shall be Not more than 2.0 % for six results. Analyst 1 & analyst 2 results cumulative RSD shall be Not more than 2.0 %. Analyst 1 & analyst 2 results Coefficient of determination R^2 should be greater than 0.99
3.	Linearity	Coefficient of determination R^2should be greater than 0.995
4.	Range	Concentration where data can be reliably determined (98 to 102 % recovery)
5.	Accuracy	98 to 102 % (in range 80 to 120%)
6.	Robustness	Results RSD shall be not more than 2.0 %
7.	Solution stability	Results RSD shall be not more than 2.0 %

6.0 Analytical Method Verification Plan:

i) The experiment may be performed as sequential or parallel operation.

ii) Sample sequence for each experiment may run independently or together with necessary alteration of sample sequence.

iii) Same experiment may be use for more than one parameter.

7.0 Deviation: Any deviation for validation experiments and acceptance criteria (if observed) should be reported and justified**.**

8.0 Methodology for Verification:

8.1 Equipment:

S.No.	Instrument Name.	Manufactured By	Model No.	Calibration Date

8.2 Reagent:

S.No.	Name.	Manufactured By	Batch/Lot.No.	Mfg Date	Exp Date

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8.3 **Working Standard:**

S.No.	Name.	Manufactured By	Batch/AR.No.	Mfg Date	Exp Date

9.0 Experimental plan & Data evaluation:

9.1 **Specificity:** Specificity is the evidence of suitable separation of all solvents so that each solvent can specified & quantified.

9.1.1 **Chromatographic Conditions:**

Equipment : High Performance Liquid Chromatography

Column : 25-cm × 4.6-mm, 5- µm(L1)

Wavelength : 210 nm.

Flow Rate : 2 ml /min.

Injection volume : 100 µl.

Temperature : 50°C.

3.8.1 **Buffer pH 7.6± 0.05:** Dissolve 4.6 gm of monobasic potassium phosphate anhydrous in 1000 ml of water and adjust pH 7.5 ± 0.05 with 1N sodium hydroxide.

3.8.2 **Mobile Phase Preparation:** Buffer: Acetonitrile (35:65).

9.1.2 **Blank and diluent solution:** Mobile phase.

9.1.3 **Placebo Solution:** Transfer and weight a quantity of the powder about 8mg of Placebo in 100 ml volumetric flask add75 ml of the mobile phase dissolve and shake and sonicate and make up with mobile phase.

3.8.3 **Standard preparation:** Dissolve and transfer about equivalent to 100 mgAzithromycin working standard in 100 ml volumetric flask add 75 ml mobile phase shake and sonicate and make up the volume with mobile phase.

9.1.4 **Test preparation:** Weigh and transfer of powdered tablet Eq. to 100 mg of Azithromycin in to 100 ml volumetric flask add 75 ml of the mobile phase dissolve, shake and sonicate and make up with mobile phase.

9.1.5 **Procedure:** Separately inject 100 µl one injection of blank placebo, five injections of standard preparation followed by two injections of sample preparation.

9.1.6 **Observation:**

Components	Acceptance Criteria
Diluent	There shall be no interference in Sample response due to blank and placebo.
Placebo	
Standard	
Sample	

9.2 Precision:

9.2.1 **System Precision:** Precision is the agreement between a set of replicate measurements.

9.2.2 **Blank&Chromatographic Conditions:** As per specificity test.

9.2.2.1 **Standard preparation:** Dissolve and transfer about equivalent to 100 mgAzithromycin working standard USP in 100 ml volumetric flask add 75 ml mobile phase shake and sonicate and make up the volume with mobile phase.

9.2.2.2 **Procedure:** Separately inject 100 µl one injection of blank, six injections of standard preparation.

9.2.2.3 **Acceptance criteria:** Percentage RSD shall be not more than 2.0 % for replicate standardand tailing factor not more than 2.

9.2.3 **Method Precision:** Repeatability evaluates the variation experienced by a single analyst on a single instrument Repeatability is performed by analyzing multiple replicates of an assay composite sample using the analytical method. The recovery value is calculated and reported for each value.

9.2.3.1 **Blank:** As per specificity test.

9.2.3.2 **Standard preparation:** Dissolve and transfer about equivalent to 100 mgAzithromycin working standard in 100 ml volumetric flask add 75 ml mobile phase shake and sonicate and make up the volume with mobile phase.

9.2.3.3 **Test preparation:** Weigh and transfer of powdered tablet Eq. to 100 mg of Azithromycin in to 100 ml volumetric flask add 75 ml of the mobile phase dissolve, shake and sonicate and make up with mobile phase. Prepare the six separate samples.

9.2.3.4 **Procedure:** Separately inject 100 µl one injection of blank, five injections of standard preparation followed by two injections of each sample preparation.

9.2.3.5 **SystemSuitability&Acceptance criteria:**

- Percentage RSD shall be not more than 2.0 % for replicate standardand tailing factor not more than 2.
- Percentage RSD shall be not more than 2.0 % for six results.

9.2.3.6 **Calculation:** Calculate the content of Azithromycin (in mg): -

$$\frac{At}{As} \times \frac{Ws}{100} \times \frac{100}{P} \times \frac{748.98}{785.02} \times \text{Average fill Wt.}$$

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Where

A_T : Average area of Azithromycin peak from injections of test preparation.

A_S : Average area of Azithromycin peak from five injections of standard Preparation.

W_S : Weight of Azithromycin working standard in mg.

W_t : Weight of test in mg.

P : Potency of Azithromycin working standard used in percent.

9.2.4 **Intermediate Precision:** Intermediate precision was formally known as ruggedness. A second analyst repeats the repeatability analysis on a different day using different conditions and different instruments. The recovery values are calculated and reported. A statistical comparison is made to the first analyst's results.

9.2.5 **BlankSolution&Chromatographic Conditions:** As per specificity test.

9.2.5.1 **Standard preparation:**Dissolve and transfer about equivalent to 100 mgAzithromycin working standard in 100 ml volumetric flask add 75 ml mobile phase shake and sonicate and make up the volume with mobile phase.

9.2.5.2 **Test preparation:**Weigh and transfer of powdered tablet Eq. to 100 mg of Azithromycin in to 100 ml volumetric flask add 75 ml of the mobile phase dissolve, shake and sonicate and make up with mobile phase. Prepare the six separate samples. Prepare the six separate samples.

9.2.5.3 **Procedure:** Separately inject 100 µl one injection of blank,five injections of standard preparation followed by two injections of each sample preparation.

9.2.5.4 **SystemSuitability:**

- Percentage RSD shall be not more than 2.0 % for replicate standardand tailing factor not more than 2.

9.2.5.5 **Acceptance criteria:**

- Percentage RSD shall be Not more than 2.0 % for six results.
- Analyst 1 & analyst 2 results cumulativeRSD shall be Not more than 2.0 %.
- Coefficient of determination (r2) should be greater than 0.99

9.2.5.6 **Calculation:** Calculate the content of Azithromycin (in mg): -

$$\frac{At}{As} \times \frac{Ws}{100} \times \frac{100}{Wt} \times \frac{P}{100} \times \frac{748.98}{785.02} \times \text{Average fill Wt.}$$

Where

A_T : Average area of Azithromycin peak from injections of test preparation.

A_S : Average area of Azithromycin peak from five injections of standard Preparation.

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W_S : Weight of Azithromycin working standard in mg

W_t : Fill weight of test in mg.

P : Potency of Azithromycin working standard used in percent.

9.3 Linearity: Linearity evaluates the analytical procedure's ability (within a given range) to obtain a response that is directly proportional to the concentration (amount) of analyte standard.

9.3.1 **Blank Solution&Chromatographic Conditions:**As per specificity test.

9.3.1.1 **Standard solution stock:** Dissolve and transfer about equivalent to 1000 mg Azithromycin working standard USP in 100 ml volumetric flask add 75 ml mobile phase shake and sonicate and make up the volume with mobile phase.

9.3.1.2 **Standard Solution:** Further transfer 10 ml of this solution in to a 100 ml volumetric flak, dilute with a mixture of mobile phase to volume, and mix.

9.3.2 Prepare five standard solutions of the analyte at 80%,90 %,100%,110%, and 120% of the method concentration using serial dilutions from a Standard stock solution.

9.3.2.1 **Standard Preparation for 80 %:** To 8 ml of the Standard stock resulting solution ina 100 ml volumetric flak, dilute with a mixture of mobile phase to volume, and mix.

9.3.2.2 **Standard Preparation for 90 %:** To 9 ml of the Standard stock resulting solution ina 100 ml volumetric flak, dilute with a mixture of mobile phase to volume, and mix.

9.3.2.3 **Standard Preparation for 100 %:** To 10 ml of the Standard stock resulting solution ina 100 ml volumetric flak, dilute with a mixture of mobile phase to volume, and mix.

9.3.2.4 **Standard Preparation for 110 %:** To 11 ml of the Standard stock resulting solution ina 100 ml volumetric flak, dilute with a mixture of mobile phase to volume, and mix.

9.3.2.5 **Standard Preparation for 120 %: %:** To 12 ml of the Standard stock resulting solution in a 100 ml volumetric flak, dilute with a mixture of mobile phase to volume, and mix.

9.3.3 **Procedure:** Separately inject 100 µl one injection of blank, five injections of standard preparation followed by one injections of each concentration samplesolution.

9.3.4 **SystemSuitability:** Percentage RSD is not more than 2.0 % for replicate standard and tailing factor not more than 2.

9.3.5 **Acceptance criteria:**

9.3.5.1 Percentage RSD shall be Not more than 2.0 %.

9.3.5.2 Coefficient of determination (r2) should be greater than 0.995

9.4 Range: Range is the interval between the upper and lower concentrations (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity.

9.4.1 **Low and High concentration Preparation:** Prepare standard solutions of the analyte at ~80%, and 120% of the method concentration using serial dilutions from a Standard stock solution as per Linearity test.

9.4.2 **Procedure:**Separately inject 100 µl one injection of blank, five injections of standard preparation followed by six replicate injections of each lower and higher concentration sample solution.

9.4.3 **Acceptance criteria:**

9.4.3.1 Percentage RSD shall be not more than 2.0 % for replicate standard and tailing factor not more than 2.

9.4.3.2 Coefficient of determination (r2) should be greater than 0.99

9.5 **Accuracy:**Accuracy expresses the closeness of agreement between the value found and the value that is accepted as either a conventional true value or an accepted reference value. It may often be expressed as the recovery by the assay of known, added amounts of analyte. Samples (spiked placebos) are prepared normally covering 80% ,100%& 120% of the nominal sample preparation concentration each in triplicate. These samples are analyzed and the recoveries of each are calculated.

9.5.1 **Blank Preparation & Chromatographic Conditions:**As per specificity test.

9.5.2 **Standard preparation:**Dissolve and transfer about equivalent to 100 mgAzithromycin working standard USP in 100 ml volumetric flask add 75 ml mobile phase shake and sonicate and make up the volume with mobile phase.

9.5.3 **Test preparation:**

9.5.3.1 **Recovery for 80 % level:**Transfer and weight a quantity of the powder about 8 mg of Placebo in 100 ml volumetric flask and spiked a quantity about 80 mg Azithromycin working standard USP in 100 mL volumetric flask add 50 ml of mobile phase, mix with the aid of ultrasound dilute with sufficient mobile phase to produce 100 ml, mix and filter with 0.45µ filter paper.**(Test solution: 800 PPM).**

9.5.3.2 **Recovery for 100 % level:** Transfer and weight a quantity of the powder about 8 mg of Placebo in 100 ml volumetric flask and spiked a quantity about 100 mg Azithromycin working standard USP in 100 mL volumetric flask add 50 ml of mobile phase, mix with the aid of ultrasound dilute with sufficient mobile phase to produce 100 ml, mix and filter with 0.45µ filter paper.**(Test solution: 1000 PPM).**

9.5.3.3 **Recovery for 120 % level:** Transfer and weight a quantity of the powder about 8 mg of Placebo in 100 ml volumetric flask and spiked a quantity about 120 mg Azithromycin working standard USP in 100 mL volumetric flask add 50 ml of mobile phase, mix with the aid of ultrasound dilute with sufficient mobile phase to produce 100 ml, mix and filter with 0.45µ filter paper.**(Test solution: 1200 PPM).**

9.5.4 **Procedure:** Separately inject 100 µl one injection of blank, five injections of standard preparation followed by triplicate injections of each sample solution.

9.5.5 **Calculation:**

Recovery in (mg):

$$\frac{At}{As} \times \frac{Ws}{100} \times \frac{100}{Wt} \times \frac{P}{100} \times \frac{748.98}{785.02} \times 1$$

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$$\textbf{Recovery in (\%):} \quad \frac{\text{Recovery in (mg)}}{\text{Spiked amount in(mg)}} \text{X } 100$$

9.5.6 **SystemSuitability:** Percentage RSD is not more than 2.0 % for replicate standard and tailing factor not more than 2.

9.5.7 **Acceptance criteria:**

9.5.7.1 The percent recovery of the spikedstandard should be within 100 ± 2 % for the average of each set of three weights.

9.5.7.2 Each individual sample recovery should lie within the range of 98% to 102%.

9.5.7.3 Percentage RSD shall be Not more than 2.0 %

9.6 Robustness: Robustness is the measure of the ability of an analytical method to remain unaffected by small but deliberate variations in method parameters.

9.6.1 **For Wavelength of UV-Visible Detector:**The procedure shall be used to verify that error in the detector wavelength at most, ±2 nm and check the system suitability parameters.

9.6.2 **For Flow rate:**The procedure shall be used to verify that error in the flow rate at most, ± 0.2 ml/min and check the system suitability parameters.

9.6.3 **For Column temperature:**The procedure shall be used to verify that error in the Column temperature at most, ± 0.5°Cand check the system suitability parameters.

9.7 Solution stability: Stability is determined by comparing the response and impurity profile from aged standards or samples to that of a freshly prepared standard and to its own response from earlier time points.

9.7.1 Prepare fresh blank, resolutionand standard as per the test method.

9.7.2 Analyze these solutions as per the test method.

9.7.3 Analyze these sample versus fresh standard with initial,4,8 ,12 and 24 hours.

9.7.4 Calculate the percent recoveries calculated for all solutions.

9.7.5 **Procedure:**Separately inject 100 µl one injection of blank, five injections of fresh standard, one injection ofinitial standardand preparation followed by two injections of sample solution.

9.7.6 **Acceptance Criteria:**

- For assay level standards, the fresh standard and the verification standard should not differ more than 2.0%.
- For the assay level, the standard and sample solutions are considered sufficiently stable over time if the recovery value does not vary more than 2.0 % from the initial result.

9.7.7 **Calculation:** Calculate the content of Azithromycin (in mg): -

$$\frac{At}{As} \times \frac{Ws}{100} \times \frac{100}{Wt} \times \frac{P}{100} \times \frac{748.98}{785.02} \times \text{Average fill Wt.}$$

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W_S : Weight of Azithromycin working standard in mg

W_t : Weight of test in mg.

P : Potency of Azithromycin working standard used in percent.

9.8 Deviations: State the impact of the variation or deviation on the ability of the experiment to be suitable to Verification.

9.9 Recommendations: Indicate any changes that need to be made to the Test Method before it should be approved. These changes should be a result of the robustness testing outcome and may include modifying or supplementing the System Suitability section of the Test Method and/or adding caution statements about requirements for analyst control of experimental parameters.

9.10 Attachments: Calibrated equipment list, signature log of executors, copies of pertinent training records, data tables, chromatograms or printouts from equipment, figures as defined by results presentation and appropriate notebook references or pages.

9.11 Conclusion: Summarize the results of the Verification Study and conclude whether or not the Test Method is appropriate for its intended use base on the Verification results given in this report and the acceptance criteria set forth in the Verification Protocol.

9.12 Reference:

9.12.1 SOP No.....

9.12.2 USP / ICH/ IHS.

9.13 Abbreviations:

QA	Quality Assurance
QC	Quality Control
SOP	Standard Operating Procedure
No	Number
S.No.	Serial Number
SPE	Specification
USP	United states pharmacopeia
IHS	In -House
ICH	International conference on Harmonization
RSD	Relative standard deviation
M	Molar

mg	Milligram
nm	Nanometre
%	Percent

9.14 Revision History:

S.No.	Revision No	Details of Changes	Reason for Revision
1.	00	NA	New