METHOD VERIFICATION PROTOCOL FOR (ASSAY) OMEPRAZOLE CAPSULES BP 20 MG BY HIGH PREFORMANCE LIQIUD CHROMATOGRAPHY

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ANALYTICAL METHOD VALIDATION/VERIFICATION PROTOCOL FOR OMEPRAZOLE CAPSULES BP 20 mg

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1.0 Protocol Approval:

This is a specific Protocol for Method Verification of Omeprazole Capsule BP 20 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 Omeprazole Capsule BP 20 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 Omeprazole Capsule BP 20 mg.

4.0 Responsibility:

To conduct the Method Verification for Omeprazole Capsule BP 20 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	R^2 0.99, similar response ratio
3.	Linearity	R^2 0.99, similar response ratio
4.	Range	Concentration where data can be reliably determining (98 to 102 % recovery)
5.	Accuracy	98 to 102 % (in range 50 to 150%)
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

8.3 **Working Standard:**

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

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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	: Octylsilyl silica gel C8 (12.5 x4.6 mm), 5µ or equivalent
Flow Rate	: 1.0 ml /min
Wave Length	: 305 nm
Injection volume	: 40 µl
Temperature	: Ambient.

9.1.2 **Reagent required:**

- Acetonitrile.
- Orthophosphoric acid.
- Water.
- Disodium hydrogen orthophosphate.

9.1.3 **Buffer pH 7.6± 0.05:** Dissolve 1.4 g of disodium hydrogen orthophosphate 900 ml of water, adjust the pH 7.6 ± 0.05 with orthophosphoric acid solution and dilute with water to produce 1000 ml.

9.1.4 **Mobile Phase Preparation:** A mixture of27 volumes of Acetonitrile and 73 volumes of buffer.

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

9.1.6 **Placebo Solution:** Transfer and weight a quantity of the powder about 150mg of Placebo in 200 ml volumetric flask add 150 ml of mobile phase, mix with the aid of ultrasound for 30 minutes, dilute with sufficient mobile phase to produce 200 mL, mix, filter and further dilute 10ml volumes to 100 ml volumes with mobile phase.

9.1.7 **Resolution preparation:** Weigh accurately about 20.0 mg of Omeprazole working standard (BPCRS) and 20 mg of omeprazole Impurity-D-(EPCRS) in to 200ml volumetric flask and add 100 ml mobile phase shake for dissolve and make up to the volume with mobile phase. **(Solution 3).**

9.1.8 **Standard preparation:** Disperse a quantity of Omeprazole BP working standard about 24 mg in 200 mL volumetric flask add 150 ml of mobile phase, mix with the aid of ultrasound for 30 minutes, dilute with sufficient mobile phase to produce 200 mL, mix, filter and further dilute 10ml volumes to 100 ml volumes with mobile phase.

9.1.9 **Test preparation** Disperse a quantity of the powdered of capsule containing about 24 mg Omeprazole in 200 mL volumetric flask add 150 ml of mobile phase, mix with the aid of ultrasound for 30 minutes, dilute with sufficient mobile phase to produce 200 mL, mix and further dilute 10ml volumes to 100 ml volumes with mobile phase and filter with 0.45µ filter paper.

9.1.10 **Procedure:** Separately inject40 µl one injection of blank placebo and resolution, five injections of standard preparation followed by two injections of sample preparation.

9.1.11 **System Suitability:** The resolution factor between the peaks due to impurity D and Omeprazole is greater than 3.0

9.1.12 **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.

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

9.2.1.2 **Resolution preparation:** Weigh accurately about 20.0 mg of Omeprazole working standard (BPCRS)and 20 mg of Omeprazole Impurity-D-(EPCRS) in to 200 ml volumetric flask and add 100 ml mobile phase shake for dissolve and make up to the volume with mobile phase. **(Solution 3).**

9.2.1.3 **Standard preparation:** Disperse a quantity of Omeprazole BP working standard about 24 mg in 200 mL volumetric flask add 150 ml of mobile phase, mix with the aid of ultrasound for 30 minutes, dilute with sufficient mobile phase to produce 200 mL, mix, filter and further dilute 10 ml volumes to 100 ml volumes with mobile phase.

9.2.1.4 **Procedure:** Separately inject 40 µl one injection of blank, six injections of standard preparation.

9.2.1.5 **Acceptance criteria:** Percentage RSD shall be not more than 2.0 % for replicate standard.

9.2.2 **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.

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

9.2.2.2 **Resolution preparation:** Weigh accurately about 20.0 mg of Omeprazole working standard (BPCRS) and 20 mg of Omeprazole Impurity-D-(EPCRS) in to 200ml volumetric flask and add 100 ml mobile phase shake for dissolve and make up to the volume with mobile phase. **(Solution 3).**

9.2.2.3 **Standard preparation:** Disperse a quantity of Omeprazole BP working standard about 24 mg in 200 mL volumetric flask add 150 ml of mobile phase, mix with the aid of ultrasound for 30 minutes, dilute with sufficient mobile phase to produce 200 mL, mix, filter and further dilute 10 ml volumes to 100 ml volumes with mobile phase.

9.2.2.4 **Test preparation** Disperse a quantity of the powdered of capsule containing about 24 mg Omeprazole in 200 mL volumetric flask add 150 ml of mobile phase, mix with the aid of ultrasound for 30 minutes, dilute with sufficient mobile phase to produce 200 mL, mix, filter and further dilute

10 ml volumes to 100 ml volumes with mobile phase and filter with 0.45µ filter paper. Prepare the six separate samples.

9.2.2.5 **Procedure:** Separately inject 40 µl one injection of blank and resolution, five injections of standard preparation followed by two injections of each sample preparation.

9.2.2.6 **System Suitability & Acceptance criteria:**

- The resolution factor between the peaks due to impurity D and Omeprazole is greater than 3.0
- Percentage RSD shall be not more than 2.0 % for six results.

9.2.2.7 **Calculation:** Calculate the content of Omeprazole (in mg):-

$$\frac{At}{A_S} \times \frac{Ws}{W_{t10}} \times \frac{10}{200} \times \frac{200}{100} \times \frac{100}{100} \times \frac{P}{100} \times \text{Avg fill wt.}$$

Where

A_T : Average area of Omeprazole peak from two injections of test

A_S : Average area of Omeprazole peak from five injections of standard.

W_S : Weight of Omeprazole working standard in mg

W_t : Fill weight of capsule in mg.

P : Potency of working standard used in percent .

9.2.3 **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.

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

9.2.3.2 **Resolution preparation:** Weigh accurately about 20.0 mg of Omeprazole working standard (BPCRS)and 20 mg of Omeprazole Impurity-D-(EPCRS) in to 200ml volumetric flask and add 100 ml mobile phase shake for dissolve and make up to the volume with mobile phase.

9.2.3.3 **Standard preparation:** Disperse a quantity of Omeprazole BP working standard about 24 mg in 200 mL volumetric flask add 150 ml of mobile phase, mix with the aid of ultrasound for 30 minutes, dilute with sufficient mobile phase to produce 200 mL, mix and further dilute 10 ml volumes to 100 ml volumes with mobile phase and filter with 0.45µ filter paper.

9.2.3.4 **Test preparation** Disperse a quantity of the powdered of capsule containing about 24 mg Omeprazole in 200 mL volumetric flask add 150 ml of mobile phase, mix with the aid of ultrasound for 30 minutes, dilute with sufficient mobile phase to produce 200 mL, mix, filter and further dilute

10 ml volumes to 100 ml volumes with mobile phase and filter with 0.45µ filter paper. Prepare the six separate samples.

9.2.3.5 **Procedure:** Separately inject 40 µl one injection of blank and resolution, five injections of standard preparation followed by two injections of each sample preparation.

9.2.3.6 **System Suitability:**

- The resolution factor between the peaks due to impurity D and Omeprazole is greater than 3.0
- Percentage RSD shall be not more than 2.0 % for six results.

9.2.3.7 **Acceptance criteria:**

- 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 %.
- Coefficient of determination (r2) should be greater than 0.99

9.2.3.8 **Calculation:** Calculate the content of Omeprazole (in mg): -

$$\frac{At}{200} \times \frac{Ws}{100} \times \frac{10}{Wt} \times \frac{200}{10} \times \frac{100}{100} \times \frac{P}{1} \times \text{Avg fill wt.}$$

Where

A_T : Average area of Omeprazole peak from two injections of test

A_S : Average area of Omeprazole peak from five injections of standard.

W_S : Weight of Omeprazole working standard in mg

W_t : Fill weight of capsule in mg.

P : Potency of 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:** As per specificity test.

9.3.1.1 **Resolution preparation:** Weigh accurately about 20.0 mg of Omeprazole working standard (BPCRS) and 20 mg of Omeprazole Impurity-D-(EPCRS) in to 200ml volumetric flask and add 100 ml mobile phase shake for dissolve and make up to the volume with mobile phase.

9.3.2 **Standard solution stock:** Disperse a quantity of Omeprazole BP working standard about 24 mg in 200 mL volumetric flask add 150 ml of mobile phase, mix with the aid of ultrasound for 30 minutes, dilute with sufficient mobile phase to produce 200 ml.

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

9.3.4 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.

9.3.4.1 **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.

9.3.4.2 **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.

9.3.4.3 **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.

9.3.4.4 **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.

9.3.4.5 **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.

9.3.5 **Procedure:** Separately inject40 µl one injection of blank and resolution, five injections of standard preparation followed by one injections of each concentration sample solution.

9.3.6 **System Suitability:** The resolution factor between the peaks due to impurity D and Omeprazole is greater than 3.0

9.3.7 **Acceptance criteria:**

9.3.7.1 Percentage RSD shall be Not more than 2.0 %.

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

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 40 µl one injection of blank and resolution, 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 each concentration absorbance.

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:** As per specificity test.

9.5.1.1 **Resolution preparation:** Weigh accurately about 20.0 mg of Omeprazole working standard (BPCRS)and 20 mg of Omeprazole Impurity-D-(EPCRS) in to 200 ml volumetric flask and add 100 ml mobile phase shake for dissolve and make up to the volume with mobile phase.

9.5.1.2 **Standard preparation:** Disperse a quantity of Omeprazole BP working standard about 20 mg in 200 mL volumetric flask add 150 ml of mobile phase, mix with the aid of ultrasound for 30 minutes, dilute with sufficient mobile phase to produce 200 mL, mix, filter and further dilute 10 ml volumes to 100 ml volumes with mobile phase.

9.5.2 **Test preparation**

9.5.2.1 **Recovery for 80 % level:** Transfer and weight a quantity of the powder about 150 mg of Placebo in 100 ml volumetric flask and spiked a quantity about 16 mg Omeprazole in 200 mL volumetric flask add 150 ml of mobile phase, mix with the aid of ultrasound for 30 minutes, dilute with sufficient mobile phase to produce 200 ml, mix, filter and further dilute 8 ml volumes to 100 ml volumes with mobile phase and filter with 0.45µ filter paper.**(Test solution: 8 PPM).**

9.5.3 **Recovery for 100 % level:** Transfer and weight a quantity of the powder about 150 mg of Placebo in 100ml volumetric flask and spiked about 20 mg Omeprazole in 200 mL volumetric flask add 150 ml of mobile phase, mix with the aid of ultrasound for 30 minutes, dilute with sufficient mobile phase to produce 200 ml, mix, filter and further dilute 10 ml volumes to 100 ml volumes with mobile phase and filter with 0.45µ filter paper.**(Test solution: 10 PPM).**

9.5.4 **Recovery for 120 % level:** Transfer and weight a quantity of the powder about 150 mg of Placebo in 100ml volumetric flask and spiked a quantity about 24 mg Omeprazole in 200 mL volumetric flask add 150 ml of mobile phase, mix with the aid of ultrasound for 30 minutes, dilute with sufficient mobile phase to produce 200 ml, mix, filter and further dilute 8 ml volumes to 100 ml volumes with mobile phase and filter with 0.45µ filter paper.**(Test solution: 12 PPM).**

9.5.5 **Procedure:** Separately inject 40 µl one injection of blank and placebo, five injections of standard preparation followed by triplicate injections of each sample solution.

9.5.6 **Calculation:**

Recovery in (mg):

At Wt. 10 100 100 P
----------X---------X--------X-------X---------X--------X 1
As 200 100 110100

Recovery in (mg)

Recovery in (%): ----------X 100

Spiked amount in(mg)

9.5.7 **Acceptance criteria:**

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

9.5.7.2 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, resolution and 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 40 µl one injection of blank and resolution, five injections of fresh standard, one injection of initial standard and 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 that 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 Omeprazole (in mg):-

At	Ws10	200	100	P	
---------X	---------X	----------X	---------X	---------X	---------X Avg fill wt.
200	100	Wt10	100		

Where

A_T: Average area of Omeprazole standard peak from two injections of test.

A_S: Average area of Omeprazole peak from five injections of standard.

W_S : Weight of Omeprazole BP working standard in mg.

W_t: Weight of sample in mg.

P: Potency of Omeprazole BP standard 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 BP / ICH/ IHS.

9.13 **Abbreviations:**

QA	Quality Assurance
QC	Quality Control
SOP	Standard Operating Procedure
No	Number
Sr.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