ANALYTICAL METHOD VALIDATION PROTOCOL FOR DETERMINATION OF HYDROCORTISONE IN THE CLEANING SAMPLES BY UV SPECTROPHOTOMETER

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1.0 PROTOCOL APPROVAL SHEET:

Prepared By:			
Functional Area	**Name**	**Signature**	**Date**
Quality Control			
Reviewed By:			
Functional Area	**Name**	**Signature**	**Date**
Quality Control			
Quality Assurance			
Approved By:			
Functional Area	**Name**	**Signature**	**Date**
Head Quality Assurance			

ANALYTICAL METHOD VALIDATION PROTOCOL FOR DETERMINATION OF HYDROCORTISONE IN THE CLEANING SAMPLES BY UV SPECTROPHOTOMETER

2.0 OBJECTIVE:

Objective of the protocol is to develop & validate a specific & sensitive analytical method for determination of Hydrocortisone in the cleaning samples by UV Spectrophotometer.

3.0 SCOPE:

The scope of the protocol is to evaluate the acceptability of analytical method to be used for determination of Hydrocortisone in the cleaning samples by UV Spectrophotometer.

It shall define the procedure, documentation, references, acceptance criteria, change control & re-validation criteria to be used for the analytical method to be validated.

4.0 SELECTION OF ANALYTICAL PERFORMANCE PARAMETER:

As per the cGMP guidelines the test method, which are used for assessing the quality of pharmaceutical products with established specification, must be validated for analytical performance parameter viz. Precision, Accuracy, Linearity & Range, Limit of Detection (LOD) & Limit of Quantitation (LOQ).

However, if the method employed is in the Official Pharmacopoeia, Association of Official Analytical Chemists, Books of Methods or in other recognized standard references or is detailed in an Abbreviated New Drug Application (ANDA) and the reference method is not modified, then the method need not be validated for accuracy & reliability parameters but merely verify their suitability under actual conditions of use.

In this study, the method shall be validated for Precision, Accuracy, Linearity & Range, Limit of Detection (LOD) & Limit of Quantitation (LOQ).
Other parameters like specificity, ruggedness & robustness are not taken into account because of the very specific & limited requirement of the method.

References: i) ICH Guideline Q2B : Validation of Analytical Procedures.
ii) USP 28 section 1225 i.e. Validation of Compendial Method.

5.0 DETAILS OF ANALYTICAL METHOD:

5.1 Chromatographic Condition:

Instrument	UV Spectrophotometer
Wavelength	248 nm

5.2 Solution Preparation:

I. Hydrocortisone Solution For Precision study:

a) Stock Solution (1000 ppm): Dissolve 100 mg Hydrocortisone in 100 ml distilled water.

b) 10 ppm: Dilute 1.0 ml of 1000 ppm solution to 100 ml with distilled water.

c) 5 ppm: Dilute 25 ml of 10 ppm solution to 50 ml with distilled water.

II. Solution for Linearity, LOD & LOQ determination:

a) Stock Solution (1000 ppm): Dissolve 100 mg Hydrocortisone in 100 ml distilled water.

b) 12 ppm: Dilute 12 ml of 100 ppm solution to 100 ml with distilled water.

c) 10 ppm: Dilute 1.0 ml of 1000 ppm solution to 100 ml with distilled water.

d) 8 ppm: Dilute 8 ml of 10 ppm solution to 10 ml with distilled water.

e) 5 ppm: Dilute 25 ml of 10 ppm solution to 50 ml with distilled water.

f) 2.5 ppm: Dilute 25 ml of 5 ppm solution to 50 ml with distilled water.

g) 1 ppm: Dilute 10 ml of 10 ppm solution to 100 ml with distilled water.

h) 0.5 ppm: Dilute 10 ml of 5 ppm solution to 100 ml with distilled water.

i) 0.25 ppm: Dilute 10 ml of 2.5 ppm solution to 100 ml with distilled water.

j) 0.1 ppm: Dilute 10 ml of 1 ppm solution to 100 ml with distilled water.

III. Solution For Accuracy study:

a) **Stock Solution (1000 ppm):** Dissolve 100 mg Hydrocortisone in 100 ml distilled water.

b) **10 ppm:** Dilute 1.0 ml of 1000 ppm solution to 100 ml with distilled water.

Flask No.	Volume of 10 ppm solution	Volume of 10 ppm solution added	Make up the volume to	Total conc. (ppm)
Flask 1	2.5 ml	---	10 ml	2.5
Flask 2	2.5 ml	2.5 ml	10 ml	5
Flask 3	2.5 ml	3.5 ml	10 ml	6

6.0 METHOD VALIDATION:

"Validation of a method is the process by which a method is tested by the developer or user for reliability, accuracy and preciseness for its intended purpose."

6.1 Precision Study:

"The Precision of an analytical method is the degree of repeatability of the results in a series of experiments run during a single session by a single operator with identical reagents and equipment."

The precision of the analytical method is determined by carrying out following analysis for repeatability study: "3 concentrations and 3 replicates of each concentration or a minimum of 6 determinations at 100% of the test concentration."

Perform the Precision study by calculating the percentage relative standard deviation of absorbance of Hydrocortisone at the concentration of 10 ppm solution.

Limit: *%RSD of Absorbance shall be not more than 2.0%.*

Table A: PRECISION STUDY

Hydrocortisone 10 ppm solution	
Solution No.	**Absorbance**
1 of 6	
2 of 6	
3 of 6	
4 of 6	
5 of 6	
6 of 6	
Mean	
Standard Deviation	
%RSD	
Limit	%RSD shall be not more than 2.0%

6.2 Accuracy Study:

"The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often be expressed as percent recovery by the assay of known added amounts of analyte. Accuracy is a measure of the exactness of the analytical method that is true for all practical purposes."

Determine the accuracy of method by spiking various known concentrations of Hydrocortisone (a minimum of 9 determinations i.e. 3 concentration and 3 replicates of each concentration covering the specified range). Calculate the % recovery.

Limit: *90.0% to 110.0%*

Table B: ACCURACY STUDY

S.No.	Volume of 10 ppm solution	Volume of 10 ppm solution added	Make up the volume to	Total conc. (ppm)	Absorbance 1	Absorbance 2	Absorbance 3	Mean	Recovery	% Recovery
1.	2.5 ml	---	10 ml	2.5						
2.	2.5 ml	2.5 ml	10 ml	5						
3.	2.5 ml	3.5 ml	10 ml	6						

$$\textbf{\% recovery} = \frac{\text{Recovery}}{\text{Absorbance of 2.5 ppm}} \times \frac{2.5}{\text{Conc. added}} \times 100$$

6.3 Linearity & Range:

"The Linearity of an analytical method is its ability to elicit test results data directly proportional to the concentration of the analyte in samples within a given range."

"The range of an analytical method is the interval between upper & lower levels of an analyte (including these levels) that has been demonstrated to be determined with precision, accuracy, and linearity using the method as written."

The Linearity & Range of the method has to be studied by taking the absorbance of Hydrocortisone standard solution in the concentration range of 0.1 ppm, 0.25 ppm, 0.5 ppm, 1.0 ppm, 2.5 ppm, 5.0 ppm, 8.0 ppm.

Limit: *The correlation coefficient [r] value shall be not less than 0.95.*

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Table D : LINEARITY & RANGE

S.No.	Conc. (ppm)	Abs 1	Abs 2	Abs 3	Abs 4	Abs 5	Abs 6	Abs 7	Abs 8	Abs 9	Abs 10	Mean	Std. Dev.	% RSD
1.	0.1													
2.	0.25													
3.	0.5													
4.	1													

S.No.	Conc. (ppm)	Abs 1	Abs 2	Abs 3	Mean	Std. Dev.	% RSD
5.	2.5						
6.	5						
7.	8						

6.4 Limit of Detection (LOD):

"Limit of Detection (LOD) is a parameter of the limit test. It is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. Thus, limit test merely substantiate that the analyte concentration is above or below a certain level. The Limit of Detection is usually expressed as the concentration of analyte (e.g. percentage or ppm) in the sample."

The Limit of Detection is the lowest concentration of analyte that can be detected by the given method. It is a limit test that specifies whether or not an analyte is above or below a certain value.

Limit of detection of an analytical method is established by determining signal to noise ratio, usually two- or three-to-one. Incase of non instrumental method it shall be determined by analyzing the sample with known concentration of analyte and by establishing the minimum level at which the analyte can be reliably detected.

From the experimentally observed lowest concentration level, the % RSD of absorbance shall be not more than 33%.

Note:

(i) In case of UV absorbance, any absorbance equal to or less than 0.005 shall be considered as negligible.

(ii) Any concentration with absorbance equal to or less than 0.01 in a set of measurement shall not be considered due to low absorbance value.

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Table E: LIMIT OF DETECTION (LOD)

Sr. No.	conc. (ppm)	Abs 1	Abs 2	Abs 3	Abs 4	Abs 5	Abs 6	Abs 7	Abs 8	Abs 9	Abs 10	Mean	Std. Dev.	% RSD
1.	0.1													
2.	0.25													
3.	0.5													
4.	1													

Sr. No.	conc. (ppm)	Abs 1	Abs 2	Abs 3	Mean	Std. Dev.	% RSD
5.	2.5						
6.	5						
7.	8						

6.5 Limit of Quantitation (LOQ):

"Limit of Quantitation (LOQ) is a parameter of quantitative assay for the low levels of compound in sample matrices, such as impurity in bulk drug substances and degradation product in finished pharmaceuticals. It is the lowest concentration of analyte in a sample that can determined with acceptable precision and accuracy under stated experimental condition."

Like LOD, LOQ is expressed as a concentration, with the precision and accuracy of the measurement also reported. Sometimes a signal-to-noise ratio of ten-to-one is used to determine LOQ.

The ICH has recognized the signal-to-noise ratio as typical, and like LOD lists two other options to determine LOQ:

i. Visual non-instrumental method:
   LOQ's determined by techniques such as thin layer chromatography (TLC) or titrations.

ii. Formula to calculate the LOD:
   LOQ's calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOQ according to the formula: LOQ = 10(SD/S). The standard deviation of the response can be determined based on the standard deviation of the blank, on the residual standard deviation of the regression line, or the standard deviation of Y-intercepts of regression lines.

The LOQ shall be determined by serially diluting at lower concentration range. The concentration were selected based on the logic that the method must be enough sensitive to at least quantify less than 5.0 ppm

Determination of solution of Hydrocortisone in the concentration range of 0.1 ppm to 8 ppm were made to establish Limit of Quantitation.
Plot a calibration curve for mean peak Absorbance response against concentration. Calculate a regression coefficient slope & intercept for the curve using the data.

Limit of Quantitation (LOQ) = 10(Standard Deviation of response / Slope)
= 10(SyX / Slope)

The Precision study was carried out by calculating the percentage relative standard deviation of peak Absorbance response of Hydrocortisone at the LOQ concentration.
Limit : %RSD of Absorbance shall be not more than 5.0% for LOQ conc.
One additional detail shall also be considered; LOQ can be affected by the chromatography (peak shape).

Table F : LIMIT OF QUANTITATION (LOQ)

Sr. No.	conc. (ppm)	Abs 1	Abs 2	Abs 3	Abs 4	Abs 5	Abs 6	Abs 7	Abs 8	Abs 9	Abs 10	Mean	Std. Dev.	% RSD
1.	0.1													
2.	0.25													
3.	0.5													
4.	1													

Sr. No.	conc. (ppm)	Abs 1	Abs 2	Abs 3	Mean	Std. Dev.	% RSD
5.	2.5						
6.	5						
7.	8						

Table F: LIMIT OF QUANTITATION (LOQ)

PRECISION FOR LOQ CONCENTRATION	
Solution No.	**Absorbance**
1 of 6	
2 of 6	
3 of 6	
4 of 6	
5 of 6	
6 of 6	
Mean	
Standard Deviation	
%RSD	

7.0 ACCEPTANCE CRITERIA:

S.No.	Performance Parameter	Acceptance Limit
1.	***Precision:*** 10 ppm solution 6 determinations	% RSD of Absorbance shall be not more than 2.0%.
2.	***Accuracy***	Recovery = 90.0% - 110.0%
3.	***Linearity & Range:*** Linearity of 0.1 ppm to 12 ppm solution.	The correlation coefficient [r] value shall be not less than 0.95.
4.	***Limit of Detection (LOD)***	% RSD of Absorbance shall be not more than 33 .0%
5.	***Limit of Quantitation (LOQ)*** *Precision of LOQ concentration*	Based on the response of standard deviation & slope [10(SyX / Slope)]. % RSD of Absorbance shall be not more than 33.0%

8.0 REVALIDATION CRITERIA:

The analytical method shall be re-validated for following changes:

i. Method changes.
ii. Drug substance changes.
iii. Technology changes.
iv. Regulatory requirement updated.

9.0 CHANGE CONTROL:

Any change must be formally requested, documented and accepted by Change Control Committee. The likely impact / risk of the change on the product must be assessed and the need for the extent of re-validation should be determined.

10.0 REVIEW:

The review of the protocol shall be initiated whenever it is experienced that the protocol & its contents need to be changed to update or incorporated latest concept.

11.0 CONCLUSION:

All the analytical parameters studied for the method are well within / not within the acceptance limit set for the method. Therefore, the analytical method of determination of Hydrocortisone content in the cleaning samples is considered as a validated / non-validated method for its intended purpose.