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Validation of Emulgel by standard addition method by UV spectroscopy

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Abstract: Pharmaceutical Validation is establishing Documented evidence which provide High degree of assurance that a specific Pharmaceutical Product or Pharmaceutical Dosage Form will consistently produce a product meeting its specification and Quality Characteristics. Validation of Pharmaceutical Product is Play an Important role in authentications, accuracy, Precision & Reproducible data of Pharmaceutical Products. In this research work a simple UV spectroscopic method has been developed for estimation of Nystatin Emulgel (topical Formulation). Nystatin estimated at 305 nm by UV spectrophotometer with the help of lamberts beer's law in Concentration range (1-10 µg/ml). the method is performed as per ICH (International Conference Harmonization) guidelines. In this research work following parameter are performed Accuracy, Precision, Limit of Detection, Limit of Quantification, Range, Linearity, ruggedness Robustness Standard Deviation, Regression Coefficient performed with accurate Procedure respectively. This UV spectroscopic method is Accurate, Cheap, less time Consuming, less Error Techniques. Validation may be applied in form of Standardization of Pharmaceutical Product.

Keywords: Accuracy, Emulgel, Nystatin, Pharmaceutical Validation,

VALIDATION

"Validation of analytical method may be defined as the process by which it established, by laboratory studies, that the performance characteristics of the method meet the requirement for the intended analytical application"

Validation Parameter:

1. Accuracy
2. Precision
3. Specificity
4. Limit of detection
5. Limit of Quantification
6. Linearity

7. Range
8. Ruggedness
9. Robustness

Accuracy:

Accuracy of an analytical method may be defined as "the closeness of true value of test results obtained by that method to the true value. The accuracy should be established across its range." Accuracy assayed as the % recovery & % relative error. Accuracy of an analytical method may be determined by the assay method used on highly pure substances like reference standard and compared it with the same material with a known and established method. This can also further evaluate by addition of known pure substances and assessing the recovery of the added substances or product by spiking with known amount of impurities. This type of spiking techniques can also use for formulated drug product. In case of Quantitative analysis of impurities, accuracy should be assessed on the sample of drug substance Accuracy is calculated on the % of recovery by the assay of the known added amount of analyte in the sample. The ICH recommends that the accuracy should be assessed using a minimum of nine determination over minimum of three concentration levels, covering the specified range.

Procedure:

Standard addition method was employed to determine accuracy**.**

To take 3 different level concentration like (10,20,30 µg/ml) of were prepared. To provide additional support to the accuracy of the developed assay method is called as standard addition method.

A known amount formulation sample and the total concentration was measured at 305nm. The % recovery of added pure drug was calculated using [Ct-Cs/ Ca] ×100,

Where,

Cs: drug concentration of formulation

Ca: added drug concentration

Ct: total drug concentration

Linearity: -

The linearity may be defined as its ability to elicit test that are directly or by a well-defined mathematical transformation proportional to the concentration of analyte in sample within a given range.

The linearity should be established across the range of the analytical procedure. It should be established initially by visual examination of a signal as a function of analyte of contact.

If this appears to be in linear relationship test result should be established by appropriate statistically method like regression analysis, etc.

ICH recommend that for the establishment of linearity, a minimum of 5 concentration normally be used.

Procedure:

It is performed by analyzing standard solution of Nystatin (1-10ug/ml) at 305 nm. Each reading was average of three determinations. Result expressed in correlation coefficient.

Precision / Repeatability / Reproducibility:

It may be defined as the degree of agreement among individual test result whelm method is applied repeatedly to multiple sampling of (homogenous) same sample.

The precision of an analytical method is usually expressed as standard deviation or relative of series measurement.

Precision refers to degree of reproducibility of results. This can be within single laboratory & more than one laboratory.

Precision can also be considered as repeatability of results, which refers to comparison of result of an analysis within a short time by the same analyst same laboratory and same equipment.

The precision of an analytical method is determined by assessing sufficient number of aliquots of a homogenous sample to be able to calculate statically valid estimate of standard deviation. It was determined by repeatability, intraday and intraday reproducibility of the method.

Procedure:

The Precision/repeatability was evaluated by prepare a standard solution 1,5,10 µg/ml, of pure drug and measured absorbance at specific time interval. The intraday and interday data shown in the term of %RSD. Low %RSD method means has good precision. The result of inter and intraday precision were expressed in %RSD.

Limit of Detection:

It may be defined as "the lowest amount of analyte sample that can be detected but not necessarily Quantitated, under the stated experimental conditions"

The LOD is generally expressed as the concentration of analyte or parts per million, etc.

The ICH documents describe a common approach, which is compare measured signals from samples within known low concentration of analytes with those of blank samples.

Limit of Quantification:

It may be defined as "A characteristics of quantitative assay for low levels of compound in sample metrics such as impurities in bulk substances and degradation product in finished pharmaceuticals. It is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions"

LOQ is generally expressed as the concentration of analyte in the sample

The quantitation limit should be subsequently validated by the analysis of suitable numbers of samples known to be near on prepared at the quantitation limit.

Range:

Range may be defined as interval between the upper and lower level of analyte that have been demonstrated to be determined with a suitable level of precision accuracy and linearity using method as writtern. “The range is normally expressed in the same unit as test results obtained by analytical method”

The range of the method validated by verifying that the analytical method provides acceptable precision, accuracy, and linearity when applied to samples containing analyte at the extreme of the range as well as within the range.

Ruggedness:

Ruggedness may be defined as the degree of reproducibility of test result obtained by the same sample under variety of conditions such as different laboratories different analyst different instrument, different lots of reagents, different elapsed assay times, different assayed temperature, different days, etc.

Ruggedness is normally expressed as the lack of influence on test result of operational environment variables of analytical methods. Ruggedness is a measure of reproducibility of test results under the variation in conditions normally expected from laboratory to laboratory & analyst to analyst.

The ruggedness of an analytical method is determined by analysis of aliquots from homogenous lots in different laboratories, by different analysts using operational and environmental condition that may differ but are still within the specified parameter of the assay. The degree of reproducibility of test result in then determined as a function of the assay variables. This reproducibility may to the precision of the assay under normal condition to obtain a measure of ruggedness of the analytical method.

Robustness:

It may be defined as a measure of its capacity to remain unaffected by small but deliberate variation in method parameter and provides an indication of its reliability during normal usage.

Calibration curve of Nystatin

Development and standard curve of drug using UV-spectrophotometer

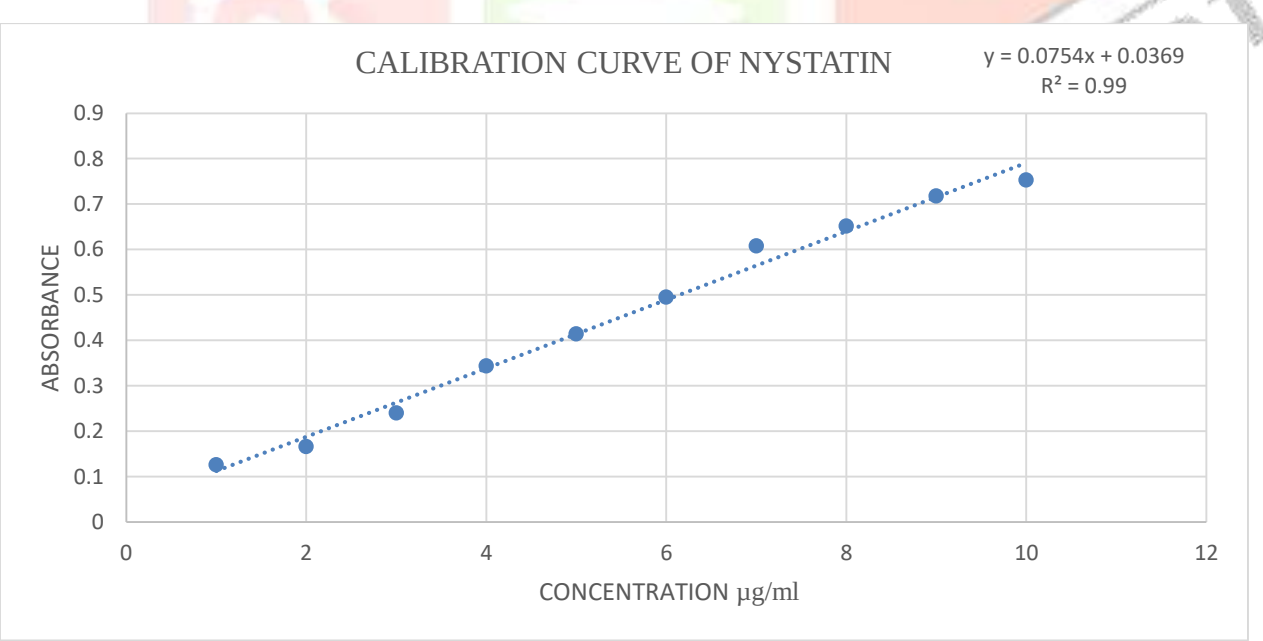
Preparation stock solution of Nystatin:

100 mg pure drug of Nystatin was accurately weighed was transformed to 100 ml volumetric flask. The drug was dissolved in 5 ml Glacial acetic acid as co solvent. Then the volume was make up to 100 ml with Methanol, to get a 1000 µg/ml stock solution, further stock solution was diluted suitably to get a 10ug/ml solution used as working solution, Then take 1,2,3,4,5,6,7,8,9, and 10ml of the from working solution in 10 ml volumetric flask, Then the volume made up to 10 ml using Methanol, which was analyzed at spectrum measurement by UV-visible double beam spectrophotometer (400-200). The absorbance of above solutions was measured at 305nm and calculated.

The standard calibration curve is prepared by 100 mg of Nystatin is dissolved in (5 ml) glacial acetic acid as cosolvent and keep sonicate for 30 minutes further dilute up to 100 ml with analytical Methanol to form 1000 µg/ml stock solution. Make suitable dilution from stock solution to prepare 1,2,3,4,5,6,7,8,9,10 mcg/ml solution. All of the above solution measure absorbance with UV visible spectrophotometer at 305 nm. The standard plot of absorbance against concentration plotted as show graph no. 1

Table no 1: Calibration curve of Nystatin

Concentration (µg/ml)	Absorbance at 305 nm
01	0.126
02	0.166
03	0.241
04	0.344
05	0.414
06	0.495
07	0.608
08	0.652
09	0.718
10	0.753



Graph no. 1 calibration curve of Nystatin

RESULTS: The Results of Validation Parameter shown in Table Respectively.

SR. NO.	Validation Parameter	Nystatin
1	Wavelength	305
2	Regression co efficient	0.0754x+0.0369
3	Slope	0.0754
4	Intercept	0.0369
5	Range	1-10 µg/ml
6	Correlation co efficient	0.99
7	Limit of Detection	1.2 µg/ml
8	Limit of Quantification	2.6 µg/ml
9	Standard deviation	0.028

Precision

Drug in Formulation	Pure drug added	Total drug in sample	Total drug found	%Recovery
10	10	20	21.1	105.5
20	20	40	39.1	97.75
30	30	60	61.6	102.8

Repeatability Interday

Concentration	30 min	60 min	90 min	120 min
1 µg/ml	0.122	0.128	0.126	0.123
5 µg/ml	0.410	0.412	0.411	0.410
10 µg/ml	0.753	0.756	0.754	0.754

Repeatability Intraday

Concentration	Day 1	Day 2	Day 3
1 µg/ml	0.122	0.120	0.122
5 µg/ml	0.410	0.410	0.408
10 µg/ml	0.753	0.752	0.750

Conclusion: The all Validation Parameter was found to be in acceptable range

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