ANALYTICAL METHOD VALIDATION REPORT FOR DICYCLOMINE HYDROCHLORIDE AND MEFENAMIC ACID TABLETS

Analytical Method Validation Analysis Records Dicyclomine Hydrochloride and Mefenamic Acid Tablets

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Validation Analysis Records for Dicyclomine Hydrochloride and Mefenamic Acid Tablets

OBJECTIVE: The efficacy & safety of a medicinal product can only be assured by analytical monitoring of its quality.

SCOPE: The scope of analytical validation is to ensure that the procedure under consideration is capable of giving reproducible and reliable results.

Product Name	Mefenamic Acid and Dicyclomine Hydrochloride Tablets
Ingredient	Mefenamic Acid and Dicyclomine Hydrochloride
Label Claim	Each uncoated tablet contain:- Dicyclomine Hydrochloride USP---------20 mg Mefenamic Acid BP----------250 mg
(A)Test Method	By UV Spectrophotometer **Mefenamic Acid**

Specificity (**Diluents Interference**)
Placebo Preparation: :-
A placebo solution was prepared same as the formulation except for the addition of the active ingredients. Here, the product contains no inactive ingredients. So, here the diluents are used as the placebo solution. Absorbance at 350nm, Observation Result: Nil

Conclusion for Specificity:
We observed that at wavelength 350nm there is no significant Absorbance for placebo (Diluents) for Mefenamic Acid assay method. Therefore specificity of the method considered acceptable.

System Accuracy:
The system precision of the above method was carried out by taking Absorbance for six times of the sample preparation.

Serial No.	Absorbance
1.	
2.	
3.	
4.	
5.	
6.	
Mean	
% RSD	

Acceptance Criteria: RSD is not more than 2.0%.

Range:
Definition:
The Range of an analytical method is the interval between the upper & lower level of analyte that has

been demonstrated with precision, accuracy & linearity using the method as written. The Range is normally expressed in same units as test results e.g. Percent or Parts per million, obtained by the analytical method.

Assay:
Mefenamic Acid: Label Claim 250 mg/Tablets
(Limit: 90.0 % to 110.0 % of the labeled amount).

Standard solution :
Accurately weigh ------- mg of Mefenamic Acid WS in 100 ml volumetric flask and make up the volume with 0.1M Methanolic HCl. Filter; take 2 ml and makeup to 100 ml with 0.1M Methanolic HCl.

Sample solution:
Accurately weigh --------mg of sample powdered in 100 ml volumetric flask and make up the volume with 0.1M Methanolic HCl. Filter; take 2 ml and makeup to 100 ml with 0.1M Methanolic HCl.

S.No.	Standards	Absorbance
1.	Standard-1	
2.	Standard-2	
3.	Standard-3	
4.	Standard-4	
5.	Standard-5	
6.	Standard-6	
	Mean	
	% RSD	

S.No.	Sample absorbance	Mean
1.		
2.		

Calculation:

Linearity/ Accuracy:
Definition:
The Linearity of an analytical method is its ability to elicit test results that are directly, or by a well defined mathematical transformation, proportional to the concentration of the analyte in samples within a given range. Linearity is usually expressed in terms of the variance around the Slope of the regression line calculated according to an established mathematical relationship from test results obtained by the analysis of sample with varying concentration of analyte.

Standard solution :
Accurately weigh --------- mg of Mefenamic Acid WS in 100 ml volumetric flask and make up the Volume with 0.1M Methanolic HCl. Filter; take 2 ml and makeup to 100 ml with 0.1M Methanolic HCl.

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Sample solution:

Accurately weigh as required of the sample powdered quantity in 10 ml volumetric flask and make up the volume with methanol. Filter; take 2 ml and makeup to 100 ml with 0.1M Methanolic HCl. Take the absorbance of both the solution standard and test against 0.1M Methanolic HCl as blank at 350nm:

Sr. No.	Standards	Absorbance
1.	Standard-1	
2.	Standard-2	
3.	Standard-3	
4.	Standard-4	
5.	Standard-5	
6.	Standard-6	
	Mean	
	% RSD	

Samples	**Sample absorbance**	**Mean**
Sample-A-01 80.0%		
Sample-A-02 80.0%		
Sample-A-03 80.0%		
Sample-B-01 90.0%		
Sample-B-02 90.0%		
Sample-B-03 90.0%		
Sample-C-01 100.0%		
Sample-C-02 100.0%		
Sample-C-03 100.0%		
Sample-D-01 110.0%		
Sample-D-02 110.0%		
Sample-D-03 110.0%		
Sample-E-01 120.0%		
Sample-E-02 120.0%		
Sample-D-03 120.0%		

Calculation:

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Data Collection:

Concentration (µg/ml)	Corr. Coefficient	Concentration in %	Sample Mean Abs	Recovery%	Corr. Coefficient
		80.0			
		90.0			
		100.0			
		110.0			
		120.0			

From the above results, draw a curve.

R-squared value (R^2)
The R-squared value, also known as the coefficient of determination, is an indicator that ranges in value from 0 to 1 and reveals how closely the estimated values for the trend line correspond to your actual data. A trend line is most reliable when its R-squared value is at or near 1.

Linearity Equation
Equations for calculating trend line
Calculates the least squares fit for a line represented by the following equation:
y = m x + b
Where m is the slope and b is the intercept.
x = concentration
y = Absorbance Value
Sample

Therefore, from Linearity Equation, **y = mx + b, m** ⟶0.999x
b ⟶0.163

Precision:
The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of a homogeneous sample. The precision of the analytical method is usually expressed as Standard deviation or relative standard deviation (coefficient of variation) of a series measurement. The precision may be measured of either the degree of reproducibility or of repeatability of the analytical method on the normal operating condition.

Precision – Method precision
Mefenamic Acid: Label Claim 250mg/Tablets
(Limit: 90.0 % to 110.0 % of the labeled amount).

Standard solution :
Accurately weigh ------- mg of Mefenamic Acid WS in 100 ml volumetric flask and make up the volume with 0.1M Methanolic HCl. Filter; take 2 ml and makeup to 100 ml with 0.1M Methanolic HCl.

Sample solution:
Accurately weigh eq. to -------mg of Mefenamic acid of sample powdered in 100 ml volumetric flask and

make up the volume with 0.1M Methanolic HCl. Filter; take 2 ml and makeup to 100 ml with 0.1M Methanolic HCl.

Sample Dilutions: By "……..":

(A) Take --------mg of the sample and further proceed as above.

(B) Take ------mg of the sample and further proceed as above.

(C) Take -------mg of the sample and further proceed as above.

(D) Take -------mg of the sample and further proceed as above.

(E) Take -------mg of the sample and further proceed as above.

(F) Take -------mg of the sample and further proceed as above.

Test Data Collection:

Standards	**Absorbance**
Standard 1	
Standard 2	
Standard 3	
Standard 4	
Standard 5	
Standard 6	
Mean	
% RSD	

Samples		Sample Absorbance	Mean
Sample A	T1		
	T2		
Sample B	T1		
	T2		
Sample C	T1		
	T2		
Sample D	T1		
	T2		
Sample E	T1		
	T2		
Sample F	T1		
	T2		

Calculation:

ANALYTICAL METHOD VALIDATION REPORT FOR DICYCLOMINE HYDROCHLORIDE AND MEFENAMIC ACID TABLETS

Table for Six Replicate Assays

Sample Number	Estimated Amount	Mean	Relative Standard Deviation (RSD)
Sample A			
Sample B			
Sample C			
Sample D			
Sample E			
Sample F			

Acceptance Criteria: NMT 2% (% of Relative Standard Deviation)

Intermediate Precision –

(Within laboratory variations such as different days, analyst & equipments):

Analyst: "……":

Standard solution :

Accurately weigh ------- mg of Mefenamic Acid WS in 100 ml volumetric flask and make up the volume with 0.1M Methanolic HCl . Filter; take 2 ml and makeup to 100 ml with 0.1M Methanolic HCl .

Sample solution:

Accurately weigh eq. to ---------mg of Mefenamic acid of sample powdered in 100 ml volumetric flask and make up the volume with 0.1M Methanolic HCl. Filter; take 2 ml and makeup to 100 ml with 0.1M Methanolic HCl.

Sample Dilutions:

By "……":

(A) Take------mg of the sample and further proceed as above.

(B) Take------mg of the sample and further proceed as above.

(C) Take -------mg of the sample and further proceed as above.

(D) Take -------mg of the sample and further proceed as above.

(E) Take --------mg of the sample and further proceed as above.

(F) Take -------mg of the sample and further proceed as above.

Test Data Collection:

Standards	Absorbance
Standard 1	
Standard 2	
Standard 3	
Standard 4	
Standard 5	
Standard 6	
Mean	
% RSD	

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Samples		Sample Absorbance	Mean
Sample A	T1		
	T2		
Sample B	T1		
	T2		
Sample C	T1		
	T2		
Sample D	T1		
	T2		
Sample E	T1		
	T2		
Sample F	T1		
	T2		

Calculation:

Test Data analyst by "……."

Sample Number	Estimated Amount	Mean	Relative Standard Deviation (RSD)
Sample A			
Sample B			
Sample C			
Sample D			
Sample E			
Sample F			

Acceptance Criteria: NMT 2 % (% of Relative Standard Deviation).

(B)Test Method **By Titramatric**
Dicyclomine Hydrochloride

Specificity:

Placebo Preparation:

A placebo solution was prepared same as the formulation except for the addition of the active ingredients. Here, the product contains inactive ingredients. So, here the diluents are used as the placebo solution. Observation Result: Nil

Conclusion for Specificity:

We observed that by Titramatric there is no significant volume for placebo for Tranexamic acid assay method. Therefore specificity of the method considered acceptable.

System Accuracy:

The system precision of the above method was carried out by taking volume for six times of the sample preparation of exact weight.

ANALYTICAL METHOD VALIDATION REPORT FOR DICYCLOMINE HYDROCHLORIDE AND MEFENAMIC ACID TABLETS

Serial No.	Titrate Volume
1.	
2.	
3.	
Mean	
% RSD	

Acceptance Criteria: RSD is not more than 2.0%

Range:

Definition:

The Range of an analytical method is the interval between the upper & lower level of analyte that have been demonstrated with precision, accuracy & linearity using the method as written. The Range is normally expressed in same units as test results e.g. Percent or Parts per million, obtained by the analytical method.

Assay:

Dicyclomine Hydrochloride: Label Claim 20mg/Tablets
(Limit: 90.0 % to 110.0 % of the labeled amount).

Reagents:

1. 1.0 M Sulphuric acid
2. Dimethyl yellow solution
3. Chloroform
4. 0.004M sodium dodecyl sulphate

Procedure:

Weigh accurately --------mg of Dicyclomine Hydrochloride WS and --------mg eq. to 20mg of Dicyclomine Hydrochloride of Sample powdered quantity and dissolved in 20 ml of water and shake, add 10 ml of 1M sulphuric acid, 1 ml of dimethyl yellow solution and 40 ml of chloroform, shake and titrate with 0.004M sodium dodecyl sulphate, shaking vigorously and allowing the layers to separate after each addition, until a permanent orange-pink colour is produced in the chloroform layer and calculate the content of Dicyclomine Hydrochloride with standard/sample volume comparison.

Sr. No.	Sample Titrate volume (ml)	Standard Titrate volume (ml)
1.		

Calculation:-

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Linearity/ Accuracy:

Definition:

The Linearity of an analytical method is its ability to elicit test results that are directly, or by a well defined mathematical transformation, proportional to the concentration of the analyte in samples within a given range. Linearity is usually expressed in terms of the variance around the slope of the regression line calculated according to an established mathematical relationship from test results obtained by the analysis of sample with varying concentration of analyte.

Reagents:

1. 1.0 M Sulphuric acid
2. Dimethyl yellow solution
3. Chloroform
4. 0.004M sodium dodecyl sulphate

Procedure:

Weigh accurately Dicyclomine Hydrochloride WS and as required quantity of Sample powdered and dissolved in 20 ml of water and shake, add 10 ml of 1M sulphuric acid, 1 ml of dimethyl yellow solution and 40 ml of chloroform, shake and titrate with 0.004M sodium dodecyl sulphate, shaking vigorously and allowing the layers to separate after each addition, until a permanent orange-pink colour is produced in the chloroform layer and calculate the content of Dicyclomine Hydrochloride with standard/sample volume comparison.

Test Data Collection:-

Serial No.	Standard Titrate Volume
1.	
2.	
3.	
Mean	
% RSD	

Acceptance Criteria: RSD is not more than 2.0%

Sample Concentration	**Titrate Volume (ml)**
Sample-A-01 80%	
Sample-B-01 90%	
Sample-C-01 100%	
Sample-D-01 110%	
Sample-E-01 120%	

ANALYTICAL METHOD VALIDATION REPORT FOR DICYCLOMINE HYDROCHLORIDE AND MEFENAMIC ACID TABLETS

Data Collection:-

Concentration (µg/ml)	Concentration in %	Sample consumed Volume (ml)	Recovery%	Corr. Coefficient
	80.0			
	90.0			
	100.0			
	110.0			
	120.0			

From the above results, draw a curve.

R-squared value ($R^{2)}$

The R-squared value, also known as the coefficient of determination, is an indicator that ranges in value from 0 to 1 and reveals how closely the estimated values for the trend line correspond to your actual data. A trend line is most reliable when its R-squared value is at or near 1.

Linearity Equation:

Equations for calculating trend line
Calculates the least squares fit for a line represented by the following equation:
y = m x + b
Where m is the slope and b is the intercept.
x = concentration
y = volume value
Sample

Therefore, from Linearity Equation, **y = mx + b, m** ⟶ 0.999x
b ⟶ 0.163

Precision:

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of a homogeneous sample. The precision of the analytical method is usually expressed as Standard deviation or relative standard deviation (coefficient of variation) of a series measurement. The precision may be measured of either the degree of reproducibility or of repeatability of the analytical method on the normal operating condition.

Precision – Method precision

Dicyclomine Hydrochloride: Label Claim 20mg/Tablets
(Limit: 90.0 % to 110.0 % of the labeled amount).

Reagents:

1. 1.0 M Sulphuric acid
2. Dimethyl yellow solution
3. Chloroform
4. 0.004M sodium dodecyl sulphate

ANALYTICAL METHOD VALIDATION REPORT FOR DICYCLOMINE HYDROCHLORIDE AND MEFENAMIC ACID TABLETS

Procedure:
Weigh accurately Dicyclomine Hydrochloride WS and Sample powdered eq. to 20mg Dicyclomine Hydrochloride and dissolved in 20 ml of water and shake, add 10 ml of 1M sulphuric acid, 1 ml of dimethyl yellow solution and 40 ml of chloroform, shake and titrate with 0.004M sodium dodecyl sulphate, shaking vigorously and allowing the layers to separate after each addition, until a permanent orange-pink colour is produced in the chloroform layer and calculate the content of Dicyclomine Hydrochloride with standard/sample volume comparison.

Sample Dilutions:
By ……..-

Sample (A) Weigh accurately --------mg of sample powdered and further proceed as above.

Sample (B) Weigh accurately--------mg of sample powdered and further proceed as above.

Sample (C) Weigh accurately ---------mg of sample powdered and further proceed as above.

Sample (D) Weigh accurately--------mg of sample powdered and further proceed as above.

Sample (E) Weigh accurately -------mg of sample powdered and further proceed as above.

Sample (F) Weigh accurately-------mg of sample powdered and further proceed as above.

Test Data Collection:-

Serial No.	Standard Titrate Volume
1.	
2.	
3.	
Mean	
% RSD	

Samples	**Titrate Volume (ml)**
Sample A	
Sample B	
Sample C	
Sample D	
Sample E	
Sample F	
Mean	
%RSD	

Acceptance Criteria: NMT 2% (% of Relative Standard Deviation)

Calculation:-

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Table for Six Replicate Assays

Sample Number	Estimated Amount	Mean	Relative Standard Deviation (RSD)
Sample A			
Sample B			
Sample C			
Sample D			
Sample E			
Sample F			

Acceptance Criteria: NMT 2% (% of Relative Standard Deviation)

Intermediate Precision –

(Within laboratory variations such as different days, analyst & equipments):

Analyst: (II) …….-

Dicyclomine Hydrochloride: Label Claim 20mg/Tablets

(Limit: 90.0 % to 110.0 % of the labeled amount).

Reagents:

1. 1.0 M Sulphuric acid
2. Dimethyl yellow solution
3. Chloroform
4. 0.004M sodium dodecyl sulphate

Procedure:

Weigh accurately Dicyclomine Hydrochloride WS and Sample powdered eq. to 20mg Dicyclomine Hydrochloride and dissolved in 20 ml of water and shake, add 10 ml of 1M sulphuric acid, 1 ml of dimethyl yellow solution and 40 ml of chloroform, shake and titrate with 0.004M sodium dodecyl sulphate, shaking vigorously and allowing the layers to separate after each addition, until a permanent orange-pink colour is produced in the chloroform layer and calculate the content of Dicyclomine Hydrochloride with standard/sample volume comparison.

Sample Dilutions:

Sample (A) Weigh accurately --------mg of sample powdered and further proceed as above.

Sample (B) Weigh accurately--------mg of sample powdered and further proceed as above.

Sample (C) Weigh accurately ---------mg of sample powdered and further proceed as above.

Sample (D) Weigh accurately--------mg of sample powdered and further proceed as above.

Sample (E) Weigh accurately -------mg of sample powdered and further proceed as above.

Sample (F) Weigh accurately-------mg of sample powdered and further proceed as above.

ANALYTICAL METHOD VALIDATION REPORT FOR DICYCLOMINE HYDROCHLORIDE AND MEFENAMIC ACID TABLETS

Test Data Collection:-

Serial No.	Standard Titrate Volume
1.	
2.	
3.	
Mean	
% RSD	

Samples	**Titrate Volume (ml)**
Sample A	
Sample B	
Sample C	
Sample D	
Sample E	
Sample F	
Mean	
%RSD	

Acceptance Criteria: NMT 2% (% of Relative Standard Deviation)

Calculation:-

Table for Six Replicate Assays

Sample Number	Estimated Amount	Mean	Relative Standard Deviation (RSD)
Sample A			
Sample B			
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Sample D			
Sample E			
Sample F			

Acceptance Criteria: NMT 2% (% of Relative Standard Deviation)