ANALYTICAL METHOD RECORDS OF ANALYSIS

(Cyproheptadine Hydrochloride and Tricholine Citrate Syrup)

ANALYTICAL METHOD VALIDATION REPORT FOR (CYPROHEPTADINE HYDROCHLORIDE AND TRICHOLINE CITRATE SYRUP)

Product Name Cyproheptadine Hydrochloride and Tricholine Citrate Syrup

Ingredient Cyproheptadine Hydrochloride and Tricholine Citrate

Label Claim **Each 5ml contains:**
Cyproheptadine HCl BP------ 2 mg
Tricholine Citrate----------275 mg

(A)Test Method UV-Spectrophotometer.
Cyproheptadine HCl

Specificity (Diluents Interference)

Placebo Preparation:
A placebo solution was prepared same as the formulation except for the addition of the active ingredients. Here used as the placebo solution. Absorbance at 286nm, Observation Result: Nil

Conclusion for Specificity:
We observed that at wavelength 286nm there is no significant Absorbance for placebo (Diluents) for Cyproheptadine HCl 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.

Test Data sheet:

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

Acceptance Criteria: RSD is not more than 2.0%.

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.

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:

Cyproheptadine Hydrochloride (Label Claim 2.0mg / 5.0ml)
(Limit: 90.0 % to 110.0 % of the labeled amount).

Sample Preparation:

Weigh accurately of the sample as required quantity of Cyproheptadine hydrochloride add 20ml of a 1 per cent w/v solution of sodium bicarbonate and extract with two quantities, each of 25ml, of 2, 2, 4-trimethylpentane (iso-octane). Wash the combined 2, 2, 4-trimethylpentane (iso-octane) extract with 5 ml of the sodium bicarbonate solution and discard the washing. Extract the 2,2,4-trimethylpentane(iso-octane) solution with 50 ml of 0.05M sulphuric acid and collect the aqueous extract in a 100 ml volumetric flask. Dilute to volume with 0.05M sulphuric acid and mix. Filter a portion of the first 20 ml of the filtrate. Measure the absorbance of the filtrate at the maximum at about 286 nm, using 0.05M sulphuric acid as the blank. Calculate the content of $C_{21}H_{21}N$, HCl taking 355 as the specific absorbance at 286 nm.

Test Data sheet:

Samples	Sample absorbance	Mean
Sample-A-01 80%		
Sample-A-02 80%		
Sample-A-03 80%		
Sample-B-01 90%		
Sample-B-02 90%		
Sample-B-03 90%		
Sample-C-03 100%		
Sample-C-02 100%		
Sample-C-03 100%		
Sample-D-01 110%		
Sample-D-02 110%		
Sample-D-03 110%		
Sample-E-01 120%		
Sample-E-02 120%		
Sample-E-03 120%		

Calculation:

ANALYTICAL METHOD VALIDATION REPORT FOR (CYPROHEPTADINE HYDROCHLORIDE AND TRICHOLINE CITRATE SYRUP)

Data Collection:

Concentration (µg/ml)	Concentration in %	Corr. Coefficient	Sample Mean Abs	Recovery%	Corr. Coefficient
	80				
	90				
	100				
	110				
	120				

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:

Cyproheptadine Hydrochloride: Label Claim 2.0mg / 5ml

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

Sample Preparation:

Pipette accurately volume of the syrup containing about 2mg of Cyproheptadine hydrochloride add 20ml of a 1 per cent w/v solution of sodium bicarbonate and extract with two quantities, each of 25ml, of 2, 2, 4-trimethylpentane (iso-octane). Wash the combined 2,2,4-trimethylpentane(iso-octane) extract with 5 ml of the sodium bicarbonate solution and discard the washing. Extract the 2,2,4-trimethylpentane(iso-octane) solution with 50 ml of 0.05M sulphuric acid and collect the aqueous extract in a 100 ml volumetric flask. Dilute to volume with 0.05M sulphuric acid and mix. Filter a portion of the first 20 ml of the filtrate. Measure the absorbance of the filtrate at the maximum at about 286 nm, using 0.05M sulphuric acid as the blank. Calculate the content of $C_{21}H_{21}N$, HCl taking 355 as the specific absorbance at 286 nm.

Sample Dilutions:

By "……":

(A) Take 5.0 ml of sample and proceed as per above.

(B) Take 5.0 ml of sample and proceed as per above.

(C) Take 5.0 ml of sample and proceed as per above.

(D) Take 5.0 ml of sample and proceed as per above.

(E) Take 5.0 ml of sample and proceed as per above.

(F) Take 5.0 ml of sample and proceed as per above.

Test Data Collection:

ANALYTICAL METHOD VALIDATION REPORT FOR (CYPROHEPTADINE HYDROCHLORIDE AND TRICHOLINE CITRATE SYRUP)

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		

Calculate the content of $C_{21}H_{21}N$, HCl taking 355 as the specific absorbance at 286 nm.

Calculation:

Table for Six Replicate Assays:

Sample Number	Estimated of % 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):

Sample Preparation:

Pipette accurately volume of the syrup containing about 2mg of Cyproheptadine hydrochloride add 20ml of a 1 per cent w/v solution of sodium bicarbonate and extract with two quantities, each of 25ml, of 2, 2, 4-trimethylpentane (iso-octane). Wash the combined 2,2,4-trimethylpentane(iso-octane) extract with 5 ml of the sodium bicarbonate solution and discard the washing. Extract the 2,2,4-trimethylpentane(iso-octane) solution with 50 ml of 0.05M sulphuric acid and collect the aqueous extract in a 100 ml volumetric flask. Dilute to volume with 0.05M sulphuric acid and mix. Filter a portion of the first 20 ml of the filtrate. Measure the absorbance of the filtrate at the maximum at about 286 nm, using 0.05M sulphuric acid as the blank. Calculate the content of $C_{21}H_{21}N$, HCl taking 355 as the specific absorbance at 286 nm.

Analyst: "……"

(A) Take 5.0 ml of sample and proceed as per above.

ANALYTICAL METHOD VALIDATION REPORT FOR (CYPROHEPTADINE HYDROCHLORIDE AND TRICHOLINE CITRATE SYRUP)

(B) Take 5.0 ml of sample and proceed as per above.

(C) Take 5.0 ml of sample and proceed as per above.

(D) Take 5.0 ml of sample and proceed as per above.

(E) Take 5.0 ml of sample and proceed as per above.

(F) Take 5.0 ml of sample and proceed as per above.

Test Data Collection:

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		

Calculate the content of $C_{21}H_{21}N$, HCl taking 355 as the specific absorbance at 286 nm.

Calculation:

Test Data analyst by "……"

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

(B) Test Method By Liquid Chromatography.

ANALYTICAL METHOD VALIDATION REPORT FOR (CYPROHEPTADINE HYDROCHLORIDE AND TRICHOLINE CITRATE SYRUP)

Tricholine Citrate

Specificity (Diluents Interference):

Placebo Preparation:

A placebo solution was prepared same as the formulation except for the addition of the active ingredients. Here used as the placebo solution. Area at 210 nm, Observation Result: Nil

Conclusion for Specificity:

We observed that at wavelength 220 nm there is no significant area for placebo (Diluents) for Tricholine Citrate assay method. Therefore specificity of the method considered acceptable.

System Accuracy:

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

Test data sheet:

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

Acceptance Criteria: RSD is not more than 2.0%

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.

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.

ANALYTICAL METHOD VALIDATION REPORT FOR (CYPROHEPTADINE HYDROCHLORIDE AND TRICHOLINE CITRATE SYRUP)

Assay:

Tricholine Citrate: Label claim 275mg/5.0ml

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

Chromatographic Condition:-

Wavelength : 210nm

Column : 4.6 mm x 25 cm 5µm C18

Flow Rate : 1.2ml/minute

Injection Volume : 20µl

Buffer:

0.025 M Potassium phosphate buffer solutions, pH 5.7, adjusted with 10 % v/v Orthophosphoric acid solution.

Mobile Phase:

Take 80volumes of buffer, 15 volumes of Acetonitrile and 5.0 volumes of methanol mix well. Then filter and after degassing use for analysis.

Standard preparation:

Weigh to -------- mg of Tricholine Citrate WS add 50 ml of mobile phase, sonicate to dissolve, dilute to 100 ml with mobile phase and filter.

Sample Preparation:

Weigh accurately of the sample as required quantity adds 50 ml of mobile phase, sonicate to dissolve, dilute to 100 ml with mobile phase and filter.

Test data sheet:

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

Samples	Sample Area	Mean
Sample-A-01 80%		
Sample-A-02 80%		
Sample-A-03 80%		
Sample-B-01 90%		
Sample-B-02 90%		
Sample-B-03 90%		
Sample-C-01 100%		

ANALYTICAL METHOD VALIDATION REPORT FOR (CYPROHEPTADINE HYDROCHLORIDE AND TRICHOLINE CITRATE SYRUP)

Sample-C-02 100%		
Sample-C-03 100%		
Sample-D-01 110%		
Sample-D-02 110 %		
Sample-D-03 110 %		
Sample-E-01 120%		
Sample-E-02 120 %		
Sample-E-03 120 %		

Calculation:

Data Collection:-

Concentration (µg/ml)	Concentration in %	Corr. Coefficient	Sample mean area	Recovery%	Corr. Coefficient
	80				
	90				
	100				
	110				
	120				

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:

Tricholine Citrate: label claim 275mg/5.0ml

(Limit: 90.0 % to 110.0 %).

Chromatographic Condition:-

Wavelength : 210nm

Column : 4.6 mm x 25 cm 5µm C18

Flow Rate : 1.2ml/minute

Injection Volume : 20µl

Buffer:

0.025 M Potassium phosphate buffer solutions, pH 5.7, adjusted with 10 % v/v Orthophosphoric acid solution.

Mobile Phase:

Take 80volumes of buffer, 15 volumes of Acetonitrile and 5.0 volumes of methanol mix well. Then filter and after degassing use for analysis.

ANALYTICAL METHOD VALIDATION REPORT FOR (CYPROHEPTADINE HYDROCHLORIDE AND TRICHOLINE CITRATE SYRUP)

Standard preparation:
Weigh to -------- mg. of Tricholine Citrate WS add 50 ml of mobile phase, sonicate to dissolve, dilute to 100 ml With mobile phase and filter.

Sample Preparation:
Pipette accurately of the sample according to requirement add 50 ml of mobile phase, sonicate to dissolve, dilute to 100 ml with mobile phase and filter.

Sample Dilutions:
By ……..:-

(A) Take 5 ml of the sample and proceed as per above.

(B) Take 5 ml of the sample and proceed as per above.

(C) Take 5 ml of the sample and proceed as per above.

(D) Take 5 ml of the sample and proceed as per above.

(E) Take 5 ml of the sample and proceed as per above.

(F) Take 5 ml of the sample and proceed as per above.

Test Data Collection:-

Standards	Area
Standard 1	
Standard 2	
Standard 3	
Standard 4	
Standard 5	
Standard 6	
Mean	
RSD	

Samples		Sample Area	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 (CYPROHEPTADINE HYDROCHLORIDE AND TRICHOLINE CITRATE SYRUP)

Table for Six Replicate Assays

Sample Number	Estimated Amount of Tricholine Citrate	Mean	% 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: ……..-

Chromatographic Condition:-

Wavelength : 210nm

Column : 4.6 mm x 25 cm 5µm C18

Flow Rate : 1.2ml/minute

Injection Volume : 20µl

Buffer:

0.025 M Potassium phosphate buffer solutions, pH 5.7, adjusted with 10 % v/v Orthophosphoric acid solution.

Mobile Phase:

Take 80volumes of buffer, 15 volumes of Acetonitrile and 5.0 volumes of methanol mix well. Then filter and after degassing use for analysis.

Standard preparation:

Weigh to -------- mg. of Tricholine Citrate WS add 50 ml of mobile phase, sonicate to dissolve, dilute to 100 ml With mobile phase and filter.

Sample Preparation:

Pipette accurately of the sample according to requirement add 50 ml of mobile phase, sonicate to dissolve, dilute to 100 ml with mobile phase and filter.

Sample dilution:

(A) Take 5 ml of the sample and proceed as per above.

(B) Take 5 ml of the sample and proceed as per above.

(C) Take 5 ml of the sample and proceed as per above.

(D) Take 5 ml of the sample and proceed as per above.

(E) Take 5 ml of the sample and proceed as per above.

ANALYTICAL METHOD VALIDATION REPORT FOR (CYPROHEPTADINE HYDROCHLORIDE AND TRICHOLINE CITRATE SYRUP)

(F) Take 5 ml of the sample and proceed as per above.

Test Data Collection:-

Standards	Area of Tricholine Citrate
Standard 1	
Standard 2	
Standard 3	
Standard 4	
Standard 5	
Standard 6	
Mean	
RSD	

Samples		Sample Area of Tricholine Citrate	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:

Table for Six Replicate Assays:

Sample Number	Estimated Amount of Tricholine Citrate	Mean	% RSD
Sample A			
Sample B			
Sample C			
Sample D			
Sample E			
Sample F			

Robustness:

To demonstrate the analytical method is capable to yield reproducibility results under; small but deliberate variations in method parameters during normal usage such as composition & Flow rate of mobile phase.

ANALYTICAL METHOD VALIDATION REPORT FOR (CYPROHEPTADINE HYDROCHLORIDE AND TRICHOLINE CITRATE SYRUP)

Procedure:

Perform the robustness study by injecting single of resolution solution & standard solution for six times for the following parameters.

- Change in ratio of the mobile phase. Record the observation in below observation table.
- Change in Flow rate of mobile phase. Record the observation in below observation table.

OBSERVATION TABLE:-

Change ratio in the mobile phase at 210nm						
Mobile phase			Flow rate ml/min	System suitability		
Buffer	ACN	Methanol		Retention time	Theoretical Plates	Tailing Factor
790 ml	155 ml	55 ml	1.2 ml/min.			
800 ml	150 ml	50 ml	1.2 ml/min.			
810 ml	145 ml	45 ml	1.2 ml/min.			

Change in flow rate at 210 nm						
Mobile phase			Flow rate ml/min	System Suitability		
Buffer	ACN	Methanol		Retention time	Theoretical Plates	Tailing Factor
800 ml	150ml	50ml	1.1ml/min.			
800 ml	150ml	50ml	1.2ml/min.			
800 ml	150ml	50ml	1.3ml/min.			

Acceptance criteria:

Analytical method validation shall be robust (i.e. Theoretical Plates is not less than 1000 & tailing factor is not more than 2.0).

Analysed By/On: **Checked By/On:**