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Research article

Development and validation of stability indicating HPLC assay method for Clofazimine capsules

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Abstract

Aim: All the Methods which are available are on UV- visible spectroscopy, but there is interference observed at Clofazimine maxima of Clofazimine Related compound-B. Therefore need to develop method on HPLC. **Method:** To develop a rugged and appropriate HPLC method for determination of assay in different diluents, mobile phases and stationary phases were evaluated. In all trials with other columns poor retention times and unsatisfactory recovery were obtained for the determination of assay of Clofazimine in Clofazimine capsules. **Result:** The estimated percentage difference between unfilter, discarding 3 mL filtrate solution, 5mL filtrate solution and 7 mL filtrate solution is less than 2.0% RSD. Method precision for the Clofazimine is less than 2.0% RSD. Good linearity was observed for the Clofazimine over the concentration range 25ppm to 75 ppm, coefficient of determination r = 0.99. **Conclusion:** A simple, specific, linear, precise, and accurate Assay determination method has been developed and validated for determination of the Clofazimine in Clofazimine Capsules. Method is precise, accurate stability indicating, and rousted which is better than that of methods for the Clofazimine capsule USP monograph.

Keywords: Clofazimine, UV- visible spectroscopy, HPLC, Related compound-B. USP Monograph

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1. Introduction

Clofazimine is a fat-soluble riminophenazine dye used as a combination of Rifampicin and Dapsone (Diaminodiphenyl sulfone-DDS) as a multidrug therapy (MDT) for the treatment of Leprosy, Tuberculosis, and AIDS (Acquire immunodificiency syndrome). It has been used investigational in combination with other anti-mycobacterial drugs to treat Mycobacterium avium infections in AIDS patients and Mycobacterium avium paratuberculosis infection in Crohn's disease patients. Clofazimine also has a marked anti-inflammatory as well as anti-microbial effect and is given to control the leprosy reaction, erythema nodosum leprosum (ENL). The drug is given as an alternative to patients who cannot tolerate the effects of Dapsone for Tuberculosis N,5-bis(4-chlorophenyl)-3-(propan-2-ylimino)-3,5-dihydrophenazin-2-amine. Based on its unique mechanism of action, Clofazimine will provide practitioners with an additional tool in the treatment of Leprosy and anti inflammatory [1-2]. This drug has found diverse use in the treatment of discoid lupus erythematosus, and pyoderma gangrenosum. Clofazimine has acquired new prominence as a component of therapy in the treatment of disseminated Mycobacterium avium intracellulare bacteremia. The exact mechanism of clofazimine action is unknown, but the primary sites of action appear to be the neutrophil and monocyte. Myeloperoxidase is a major constituent of neutrophil proteins and exerts bactericidal properties by catalyzing the oxidation of chloride ion by hydrogen peroxide to hypochlorous acid, the active ingredient of commercial bleach [3].

Hypochlorous acid is a powerful chlorinating agent that inactivates multiple proteins, including bacterial proteins and α-antiproteinase, a major inhibitor of proteolytic enzymes such as collagenaseas and elastases [4]. This lack of inhibition of proteinases may account for local inflammatory effects in sites of neutrophil action. Clofazimine has been found to scavenge hydrochlorous acid, thus reducing the chlorination of protein by

neutrophils [5]. Paradoxically, clofazimine possesses proinflammatory effects. These include stimulation of myeloperoxidase-mediated iodination, phagocytosis, and release of lysosomal enzymes [6].
Chromatographic testing method is not available for determination assay of Clofazimine in Indian Pharmacopoeia 2007 [7]. In recent year simple method was developed for determination of Clofazimine using high performance liquid chromatography (HPLC) [8-10].

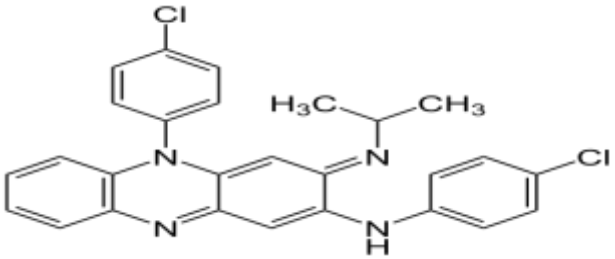

Figure 1: Chemical structure of Clofazimine (N, 5-bis (4-chlorophenyl)-3- (1-methylethylimino)-5H-phenazin-2-amine, Molecular Weight: 473.396 g/mol)

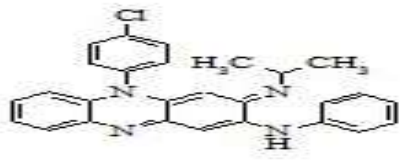

Figure 2: Chemical structure of Clofazimine related compound-B (Molecular Formula: C27H23ClN4, Molecular Weight: 438.96)

2. Materials and methods

Materials: Clofazimine was procured from a [Company], Sodium dodecyl sulfate was procured from Sigma, Mumbai, India. Di-sodium hydrogen phosphate, Ortho-phosphoric acid, and Acetonitrile were procured from Merck, Mumbai, India. Tetra butyl ammonium hydrogen sulfate was procured from Acros, Mumbai, India, and all the chemical ingredient were used as a laboratory grade scale.

Methods:
Method Development and Optimization:

The main objective of this research work was separation and accurate quantification of the clofazimine in Clofazimine Capsules. To develop a rugged and appropriate HPLC method for determination of assay in different diluents, mobile phases and stationary phases were evaluated. In all trials with other columns poor retention times and unsatisfactory recovery were obtained for the determination of assay of Clofazimine in Clofazimine capsules [11].

A number of experiments were conducted to select the best diluents, stationary and mobile phases enabling optimum recovery and retention time for the Clofazimine peak. Placebo interference was observed at different columns by use of different mobile phases. On a 250 mm × 4.6 mm i.d., 5- µm particle, and Inertsil C8-3 column. The mobile phase was a 650:350 mixture of Acetonitrile and 2.25 g of Sodium dodecyl sulphate, 0.85 g of tetra butyl ammonium hydrogen sulphate and 0.885 g of di-sodium hydrogen phosphate in 500mL water adjust the pH of buffer to 3.0 with dilute ortho phosphoric acid. Enhanced chromatographic efficiency and tailing of the Colofazimine, resulting in very good chromatographic method. In the optimized method, the results are indicative of suitability for the purpose intended [12].

Buffer preparation:

Dissolve 2.25 g of Sodium dodecyl sulfate, 0.85 g of Tetra butyl ammonium hydrogen sulfate and 0.885 g of Di-sodium hydrogen phosphate in 500 mL Water. Adjust the pH 3.0 ±0.5 of buffer with dilute ortho phosphoric acid [13].

Preparation Dilute Ortho phosphoric acid:
Transfer 5.0 mL of ortho Phosphoric Acid to a 50mL volumetric flask, and dilute it with water upto 50 mL [14].

Mobile Phase preparation:
Prepare the mixture of Buffer: Acetonitrile (350: 650 v/v) [14].

Diluents preparation:
Use mobile phase as diluent.

Chromatographic parameters:

Column	: Inertsil C8 3 (250 x4.6) mm 5µm or
Wavelength	: 280 nm.
Flow rate	: 1.0 mL/min.
Column temperature	: 30 °C.
Sampler temperature	: 25 °C.
Injection volume	: 20 µL.
Run time	: 25 minutes.

Preparation of Standard solution stock solution:
Transfers about 50.0 mg of Clofazimine working standard into a dry 50 mL volumetric flask add 35 mL dilute (Acetonitrile) sonicate to dissolve and make up with diluents. Further transfer about 5.0 mL of standard stock into a dry 100mL volumetric flask and make up with diluents. Filter through 0.45 µ Nylon filter after discarding 5 mL of filtrate [14].

Preparation System suitability solution:
Transfers about 2.5 mg of Clofazimine for system suitability EP CRS (European pharmacopoeia Chemical Reference Substance) into a dry 5mL volumetric flask add 4 mL dilute sonicate to dissolve and make up with diluents [14].

Standard Solutions

By dissolving weighed quantities of clofazimine working standard in the diluents. The final concentrations of the solutions will be approximately 50 ppm for Clofazimine [14].

Sample Solutions

For 50 mg strength 10 intake capsules were taken in beaker along with sufficient quantity of diluents, with the help magnetic stirrer at about 2000 rpm for 30 minutes, diluents is used extracted the drug from capsules. For 100 mg strength 5 intake capsules were taken in beaker along with sufficient quantity of diluents, with the help magnetic stirrer at about 2000 rpm for 30 minutes, diluents is used extracted the drug from capsules. The final concentrations of the solutions will be approximately 50 ppm for Clofazimine [14].

Method Validation

System Suitability

The performance of the method was determined by injecting six replacete of standard solution of 50 ppm Clofazimine. Because to seprate the peaks of the placebo iron pair in the chromatogram is used, quantification criteria were that no placebo interference in Clofazimine peak, the USP plate count for Clofazimine peak is not less than 5000 and the tailing factor no more than 1.5, which ensure baseline separation and symmetrical peak shape [15].

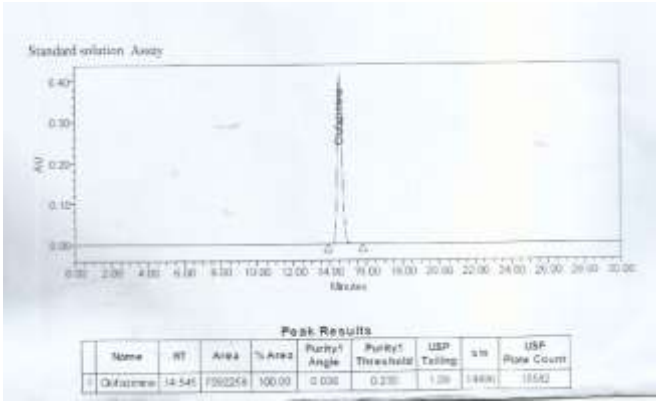

Figure 3: Standard solution assay chromatogram of clofazimine

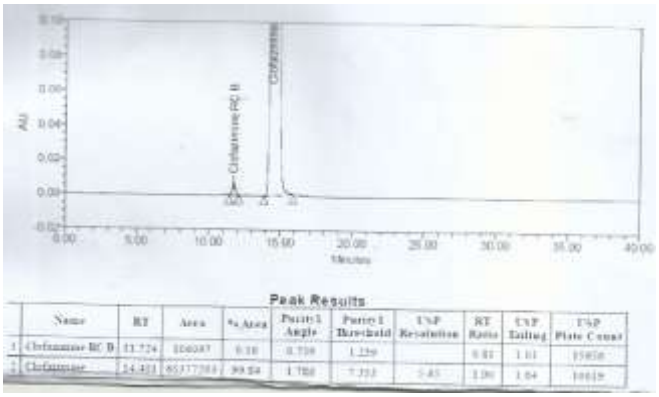

Figure 4 chromatogram of clofazimine related compound-B (RC-B) and Clofazimine

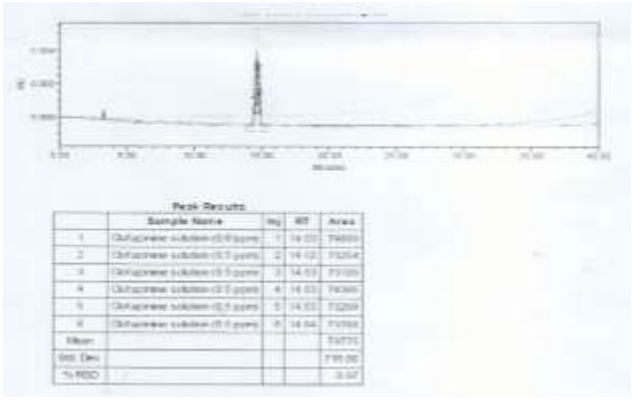

Figure 5 chromatogram of clofazimine

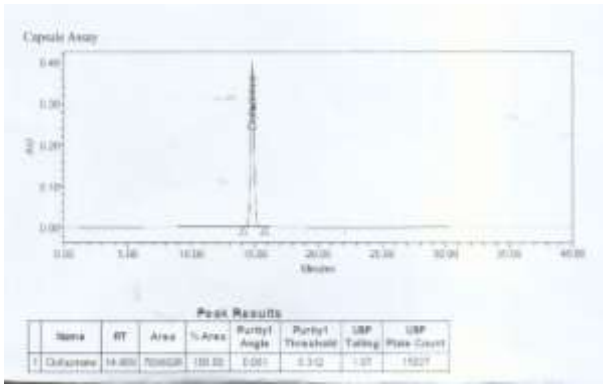

Figure 6 Assay of clofazimine Capsule chromatogram

Linearity

The linearity of an analytical procedure is its ability to furnish responses which are directly proportional to analyte concentration. Linearity for the Clofazimine was determined in the range 25ppm to 75 ppm. The regression equation was obtained by plotting peak area against concentration [14].

Filter compatablity

For assay of Clofazimine in Clofazimine capsules 0.45µ Nylon (Make mdi) was compatable by discarding first 5 mL of filtrate [16].

Accuracy and Recovery

API was addition and recovery experiments were conducted to determine the accuracy of the method for quantification of the Clofazimine in placebo samples. The study was performed in triplicate for amounts of the Clofazimine equivalent to 80, 100, and 120% of the total Clofazimine concentration. Recovery of the Clofazimine was 98.62, 99.07, and 98.85%, respectively [17].

Stability in Solution

The stability at room temperature of solutions of Clofazimine in the diluent was evaluated by injecting the solutions at intervals of 1, 6, 14, 28, and 48 Hr. The overall relative standard deviation of the peak area was calculated

for all the injections. The results showed the solutions were stable for at least 48 h [18-22].

Calculation:

$$\text{Mg/Capsule:} \quad \frac{AT}{AS} \times \frac{DS}{DT} \times \frac{P}{100} \qquad \text{Eq. (1)}$$

$$\text{\% Assay of Clofazimine:} \quad \frac{\text{Mg/ capsule}}{LC} \times 100 \qquad \text{Eq. (2)}$$

Where,

AT: Average area count of two injections of samples

AS: Average area count of five injections of Standard solutions

DS: Dilution factor of Standard solution

DT: Dilution factor of sample solution.

LC: Label clam of capsule

3. Results and Discussion:

Typical retention times Rt for Clofazimine was approximately 14.8 minutes, USP tailing factor is 1.09 and USP plate counts 15582 is shown in Fig.7.6. In the study of repeatability the relative standard deviations of the retention times of Clofazimine were 0.82% for Clofazimine peak. In the study of intermediate precision, results showed that %RSD were of the same order of magnitude as in the repeatability study. Typical chromatogram obtained from Clofazimine Standard and sample 100%, showing the USP Plate counts and USP Tailling. The estimated percentage difference between unfilter, discarding 3 mL filtrate solution, 5mL filtrate solution and 7 mL filtrate solution is less than 2.0% RSD. Method precision for the Clofazimine is less than 2.0% RSD. Good linearity was observed for the Clofazimine over the concentration range 25ppm to 75 ppm, coefficient of determination r = 0.99. Standard addition experiments were conducted in triplicate to determine recovery of the API in Placebo samples at levels of 80, 100, and 120% of the total Colfazimine concentration. Recovery at this level for Clofazimine was 98.62, 99.07, and 98.85%, respectively. And RSD was 0.27%. No significant changes in the concentrations of Clofazimine were observed in diluent during testing of stability in solution. Solutions of Clofazimine were therefore stable for at least 48 h.

Table No 1: Method Precision of Clofazimine Capsules

Injection	Standard Area counts of Clofazimine capsule (µv*sec.)
1	7092258
2	7071341
3	7100689
4	7074634
5	7118294
Mean	7091443
SD	19325
% RSD	0.27

Table 1 shows the Standard area counts of Clofazimine capsule mean for five injection solutions is 7091443. There are five injections solution used for Standard Area counts of Clofazimine capsule. The Standard deviation of Clofazimine capsule is 19325

Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Repeatability:

To check the repeatability prepare standard solution and inject five time and check the repeatability with % RSD for RT and area. RSD of standard should less than 2.0%

Table No 2: Method Precision of Clofazimine Capsules (CLO (3899) 013D)

Sample Name	Mean Area	Assay (mg/Capsule)	Assay (% of Claim)
Control Sample-1	7100957	50.3	100.5
Control Sample-2	7085304	50.2	100.3
Control Sample-3	7058520	50.0	99.9
	Mean	50.1	100.2
	SD	0.15	0.31
	% RSD	0.30	0.31

Table 2 shows there are three sample used in this method. % Assay of method precision of sample-1 is 100.5. % Assay of method precision of sample-2 is 100.3. % Assay of method precision of sample-3 is 99.9 Assay of capsule Standard deviation is 0.15 and % RSD is 0.30. %Assay of capsule Standard deviation is 0.31 and % RSD is 0.31.

Table No 3: Method Precision of Clofazimine Capsules CLO (3899)015C

Sample Name	Mean Area	Assay (mg/Capsule)	Assay(% of Claim)
Control Sample-1	7204113	102.0	102.0
Control Sample-2	7169744	101.5	101.5
Control Sample-3	7193636	101.8	101.8
	Mean	101.8	101.8
	SD	0.25	0.25
	% RSD	0.25	0.25

Table 3 shows the method precision there are three samples used in this method. % Assay of method precision of sample-1 is 102.0. % Assay of method precision of sample-2 is 101.5. % Assay of method precision of sample-3 is 101.8. Assay of capsule Standard deviation is 0.25 and %

RSD is 0.25. %Assay of capsule Standard deviation is 0.25 and % RSD is 0.25.

Table No 4: Linearity of Response of Clofazimine Capsules

% linearity	Concentration (µg/mL)	Mean Area Counts(µV*sec)
50	25.24	3420906
80	40.39	5443449
100	50.49	6791812
120	60.59	8190174
150	75.73	10162717
	Slope	133871
	Intercept	42759
	CC	0.99997

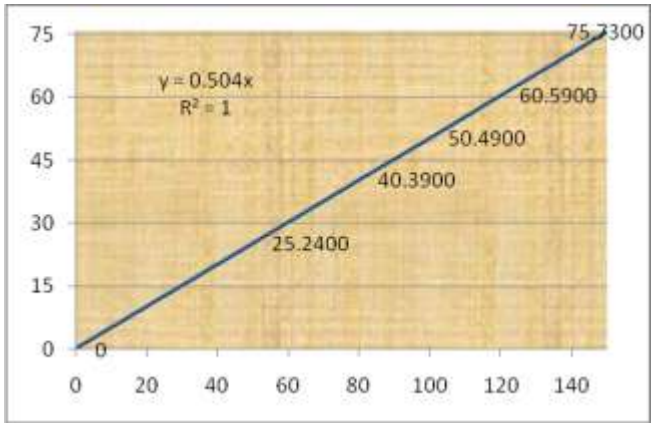

Figure 7: Linearity graph of clofazimine

The linearity coefficient of determination factor was observed 0.504 refer figure 7.7 Hence it is concluded that method is liner and method is suitable shown in Figure 7.

Table No 5: Standard solution Stability in Analytical Solution – RT

Time (min)	Area Counts (µV*esc)	Cumulative %RSD
INITIAL	7092258	-
182	7216749	1.23
217	7202466	0.95
500	7229169	0.88
861	7214171	0.78
1354	7235231	0.74
1834	7220178	0.68
2154	7203735	0.63
2468	7225848	0.60
2684	7202209	0.57
2971	7216925	0.54
HRS	49.5	

Table 5 shows the Standard solution Stability in Analytical Solution – RT total hr used 49.5 Standard area counts difference is near about ±1.0. Cumulative percentage of Standard solution Stability in Analytical Solution – RT is near about ±0.69

Table No 6: For 50 mg Sample solution Stability in Analytical Solution – RT

Time (min)	Area Counts (µV*esc)	Cumulative %RSD
Initial	7098026	-
182	6967579	1.31
217	7015963	0.94
500	6928092	1.04
861	6922908	1.04
1354	6930279	0.99
1834	6974235	0.90
2154	6987249	0.84
2468	7007769	0.80
2684	6942996	0.77
2971	6996443	0.73
HRS	49.5	

Table 6 shows the 50 mg Sample solution Stability in Analytical Solution – RT total hr used 49.5 Standard area counts difference is near about ±1.0. Cumulative percentage of sample solution Stability in Analytical Solution – RT is near about ±0.58

Table No 7: For Standard solution filter study on 0.45µ Nylon

Time (min)	Area Counts (µV*esc)	Cumulative %RSD
Unfilter	7092258	-
By discarding 3mL	6914774	1.79
By discarding 5mL	7115421	1.56
By discarding 7mL	7241685	1.90

For Standard solution Filter study on 0.45µ Nylon Area counts of unfilter is 7092258. For Standard solution Filter study on 0.45µ Nylon Area counts of by discarding 3mL is 7092258. For Standard solution Filter study on 0.45µ Nylon Area counts of by discarding 5mL is 7115421. For Standard solution Filter study on 0.45µ Nylon Area counts of by discarding 7mL is 7241685, shown in Table 7. For Standard solution Filter study on 0.45µ Nylon Cumulative %RSD by discarding 3mL is 1.79 For Standard solution Filter study on 0.45µ Nylon Cumulative %RSD by discarding 5mL is 1.56 For Standard solution Filter study on 0.45µ Nylon Cumulative %RSD by discarding 7mL is 1.90

Table No 8: For 50 mg Sample solution filter study on 0.45µ Nylon

Time (min)	Area Counts (µV*esc)	Cumulative %RSD
Initial	7098026	-
By discarding 3mL	6986967	1.12

By discarding 5mL	6942847	1.14
By discarding 7mL	7105414	1.15

Table 8 shows the 50mg sample solution Filter study on 0.45µ Nylon Area counts of initial is 7098026. For 50mg sample solution Filter study on 0.45µ Nylon Area counts by discarding 3mL are 6986967. For 50mg sample solution Filter study on 0.45µ Nylon Area counts by discarding 5mL are 6942847. For 50mg sample solution Filter study on 0.45µ Nylon Area counts by discarding 7mL are 7105414. For 50mg sample solution Filter study on 0.45µ Nylon Cumulative %RSD by discarding 3mL is 1.12. For 50mg sample solution Filter study on 0.45µ Nylon Cumulative %RSD by discarding 5mL is 1.14. For 50mg sample solution Filter study on 0.45µ Nylon Cumulative %RSD by discarding 7mL is 1.15

Table No 9: Robustness Study of Clofazimine Capsules-Column temperature Variation

Injection	Temperature minus Standard Area counts of Clofazimine capsule (µv*sec)	Temperature plus Standard Area counts of Clofazimine capsule (µv*sec.)
1	7139664	7090349
2	7137707	7112651
3	7120939	7105482
4	7125044	7093305
5	7129107	7125084
Mean	7130492	7105374
SD	8047	14255
% RSD	0.11	0.2

Table 9 shows the standard area counts of Temperature minus mean for five injection solution is 7130492. Standard area counts of Temperature plus mean for five injection solution is 7105374
There are five injections solution used for Standard Area counts of Temperature minus and Standard Area counts of Temperature plus. The Standard deviation of Temperature minus is 8047 and Temperature plus is 14255

Table No 10: Robustness Study of Clofazimine Capsules-Column temperature Variation

Sample	Mean Area	Assay (mg/Capsule)	Assay (% of Claim)
MP-1-Temp Minus	7181899	99.9	99.9
MP-2-Temp minus	7184552	99.9	99.9
MP-3-Temp Minus	7209885	100.3	100.3
	Mean	100.0	100.0
	SD	0.21	0.23
	% RSD	0.21	0.23

Table 10 shows the Robustness Study of Clofazimine Capsules-Column temperature Variation used in this method. % Assay of Column temperature of MP-1-Temp Minus is 99.9. % Assay of Column temperature of MP-2-Temp Minus is 99.9. % Assay of Column temperature of MP-3-Temp Minus is 100.3. Assay of capsule Standard deviation is 0.21 and % RSD is 0.21. %Assay of capsule Standard deviation is 0.23 and % RSD is 0.23.

Table No 11: Robustness Study of Clofazimine Capsules-Column temperature Variation (CLO (3899)015C)

Sample	Mean Area	Assay (mg/Capsule)	Assay(% of Claim)
MP-1-Temp Minus	7125753	99.2	99.2
MP-2-Temp minus	7138146	99.3	99.3
MP-3-Temp Minus	7140306	99.4	99.4
	Mean	99.3	99.3
	SD	0.11	0.10
	% RSD	0.11	0.10

Table 11 shows the Robustness Study of Clofazimine Capsules-Column temperature Variation used in this method. % Assay of Column temperature of MP-1-Temp Minus is 99.2. % Assay of Column temperature of MP-2-Temp Minus is 99.3. % Assay of Column temperature of MP-3-Temp Minus is 99.4. Assay of capsule Standard deviation is 0.11 and % RSD is 0.11. %Assay of capsule Standard deviation is 0.10 and % RSD is 0.10.

Table No 12: Robustness Study of Clofazimine Capsules-Flow Variation

Injection	Flow Minus Standard Area counts of Clofazimine Capsule (µv*sec.)	Flow plus Standard Area counts of Clofazimine Capsule (µv*sec.)
1	7880247	6449706
2	7897203	6433925
3	7908651	6438677
4	7875805	6406479
5	7901860	6488599
Mean	7892753	6443477
SD	14134	29824
% RSD	0.18	0.46

Table 12 shows the Standard area counts of flow minus mean for five injection solution is 7892753. Standard area counts of flow plus mean for five injection solution is 6443477
There are five injections solution used for Standard Area counts of flow minus and Standard Area counts of flow

plus. The Standard deviation of flow minus is 14134 and flow plus is 29824

Table No 13: Robustness Study of Clofazimine Capsules-Flow Variation CLO (3899)015C

Sample	Mean Area	Assay (mg/Capsule)	Assay (% of Claim)
MP-1-Flow Minus	7976205	100.1	100.1
MP-2-Flow minus	7978075	100.1	100.1
MP-3-Flow Minus	8036213	100.8	100.8
	Mean	100.4	100.3
	SD	0.43	0.40
	% RSD	0.43	0.40

Table 13 shows the Robustness Study of Clofazimine Capsules-Column flow Variation used in this method. % Assay of flow variation of MP-1-flow Minus is 100.1. % Assay of flow variation of MP-2-flow Minus is 100.1. % Assay of flow variation of MP-3-flow Minus is 100.8

Assay of capsule Standard deviation is 0.43 and % RSD is 0.43. %Assay of capsule Standard deviation is 0.40 and % RSD is 0.40.

Table No 14: Robustness Study of Clofazimine Capsules-Flow Variation Robustness

Sample	Mean Area	Assay (mg/Capsule)	Assay (% of Claim)
MP-1-Flow Minus	6510146	99.5	99.5
MP-2-Flow minus	6518415	99.6	99.6
MP-3-Flow Minus	6546603	100.0	100.0
	Mean	99.7	99.7
	SD	0.29	0.26
	% RSD	0.29	0.26

Table 14 shows the Robustness Study of Clofazimine Capsules-Column flow Variation used in this method. % Assay of flow variation of MP-1-flow Minus is 99.5. % Assay of flow variation of MP-2-flow Minus is 99.6. % Assay of flow variation of MP-3-flow Minus is 100.0

Assay of capsule Standard deviation is 0.29 and % RSD is 0.29. %Assay of capsule Standard deviation is 0.26 and % RSD is 0.26.

Table No 15: Recovery Calculations for Clofazimine Capsule

Injection	Standard Area counts of Clofazimine capsule (µv*sec.)
1	7092258
2	7071341
3	7100689
4	7074634
5	7118294
Mean	7091443
SD	19325
% RSD	0.27

Table 15 shows the Standard area counts of Clofazimine capsule mean for five injection solutions is 7091443. There are five injections solution used for Standard Area counts of Clofazimine capsule. The Standard deviation of Clofazimine capsule is 19325

Table No 16: Recovery Calculations for Clofazimine Capsule

Sample	Amt Recovered	Actual Amt Added	% Recovery	Mean of % recovery
*Rec-1 80% of 50mg	393.85	399.94	98.48	
Rec-2 80% of 50mg	386.77	395.00	97.92	98.62
Rec-3 80% of 50mg	396.92	399.10	99.45	
Rec-1 100% of 100mg	492.98	502.43	98.12	
Rec-2 100% of 100mg	500.02	503.74	99.26	99.07
Rec-3 100% of 100mg	500.95	501.85	99.82	
Rec-1 100% of 120mg	589.99	602.24	97.97	
Rec-2 100% of 120mg	601.10	601.84	99.88	99.85
Rec-3 100% of 120mg	611.15	600.85	101.71	
		Mean	99.18	
		SD	1.227	
		% RSD	1.24	

Table 16 shows the % Mean of recovery Rec-2 80% of 50 mg is 98.62. % Mean of recovery Rec-2 100% of 100 mg is 99.07. % Mean of recovery Rec-2 100% of 120 mg is 99.85 Recovery Calculations for Clofazimine Capsule. % Recovery mean is 99.18. % Recovery standard deviation is 1.227. % Recovery %R standard deviation is 1.24

Robustness

Robustness was performed on standard solution and sample, on Flow (+) i.e. 1.1 mL/ min, Flow (-) i.e. 0.9 mL/ min, Column temperature (+) i.e. 35 °C and Column temperature (-) i.e. 25 °C.

Final Method conclusion

Recent studies have shown that few numbers of existing methods are available for assay of Clofazimine Capsule. For valid separation of enantiomeric structure from the API this method has been developed. Following are the results showed this method is validated; Typical retention times Rt for Clofazimine was approximately 14.8 minutes, USP tailing factor is 1.09 and USP plate counts 15582. In the study of repeatability the relative standard deviations of the retention times of Clofazimine were 0.82% for Clofazimine peak. In the study of intermediate precision, results showed that %RSD were of the same order of magnitude as in the repeatability study. Typical chromatogram obtained from Clofazimine Standard and sample 100%, showing the USP Plate counts and USP Tailling. The estimated percentage difference between unfilter, discarding 3 mL filtrate solution, 5mL filtrate solution and 7 mL filtrate solution is less than 2.0% RSD. Method precision for the Clofazimine is less than 2.0% RSD. Good linearity was observed for the Clofazimine over the concentration range 25 ppm to 75 ppm, coefficient of determination r = 0.99. A simple, specific, linear, precise, and accurate Assay determination method has been developed and validated for determination of the Clofazimine in Clofazimine Capsules. Method is precise, accurate stability indicating, and rousted which is better than that of methods for the Clofazimine capsule USP monograph.

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Abbreviation:

AIDS= Acquire immune-dificiency syndrome,
API= Active pharmaceutical ingredient,
AU= Absorbance unit,
BP= British pharmacopoeia,
C= concentration,
cc= Concentration curve,
DDS= Diaminodiphenyl sulfone,
ENL= Erythema nodosum leprosum,
EPCRS= European pharmacopoeia Chemical Reference Substance,
FDA= Food and Drug Administration,
HPLC= High Performance Liquid Chromatography,
ICH= International Conference on Harmonization,
Imp=Impurity,
IP= Indian pharmacopoeia,
l= Path length,
LOD= Limit of detection,
LOQ= Limit of quantification,
MDT= Multidrug therapy,
MP= Method precision,
ppm= Parts per million,
RSD= Relative standard deviation,
RT= Retention time,
SD= Standard deviation,
TF= Tailing Factor
USP= United State pharmacopoeia,
US=United States,
UV= Ultra Violet detector,
WHO= Word Heath Organization.