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Analytical method validation for quantitative estimation of chloramben by HPLC for assay of amiben' DS chloramben herbicide

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ABSTRACT

A simple, selective, precise and accurate High Performance liquid Chromatographic method for the analysis of Chloramben in its formulations was developed and validated in the present study. The mobile phase consist a mixture of 0.05% H_3PO_4 solution and acetonitrile in the proportion 20: 80 (v/v). This was found to give sharp peak of Chloramben at a run time of 15 min. HPLC analysis of Chloramben was carried out at a wave length of 225 nm with a flow rate of 1.0mL/ min. The linear regression analysis data for the calibration curve showed a good linear relationship with a regression coefficient 0.999 in the concentration range of 50% to 150%. The linear regression equation was y =3476.7 x - 41.412. The developed method was employed with a high degree of precision and accuracy for the analysis of Chloramben. The method was validated for accuracy, precision, robustness, ruggedness and specificity. The Precision, accuracy, sensitivity, short retention time and composition of the mobile phase indicated that this method is useful for the quantification of Chloramben.

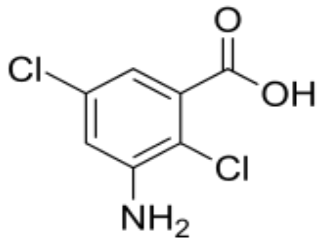
Keywords: Chloramben, HPLC Method, Development and Validation.

INTRODUCTION

One of the reasons for loss in cultivation is using of inefficient pesticides. Determination of Pesticide persistence in Formulations this method is suggestible. Chloramben is a Colorless crystalline solid. Chloramben is a selective herbicide used to control the seedlings of broadleaf weeds and annual grasses. It is mostly used for soybeans, but also for dry beans, peanuts, sunflowers, peppers, cotton, sweet potatoes, squash, hardwood trees, shrubs, and some conifers. Chloramben is considered practically nontoxic. Chloramben dissolves in water, acetone, ethanol, iso propanol. AMIBEN' DS Chloramben Herbicide is a red liquid soluble in water formulation containing 83%. Flow-injection spectrophotometric determination of chloramben[1], Densitometry analysis[2] and several chromatography techniques[3-9] are developed for the determination of Chloramben in various matrices. Most of these methods are complicated, tedious, used large amounts of solvent and time-consuming. Therefore, there is a need for better methods of analysis and this article describes the novel application of a modified QuEChERS method for Chloramben determination in its formulations. The HPLC method described here is simple, sensitive, and reproducible for determination in Formulations with low background interference. An attempt has been made to develop and validate to ensure their accuracy, precision

and other analytical method validation parameters as mentioned in various gradients. For pesticide formulation the proposed method is suitable for their analysis with virtually no interference of the usual additives presented in pesticide formulations.

Structure



Chloramben

Name :3-Amino-2,5-dichlorobenzoic acid
Empirical formula : $C_7H_5Cl_2NO_2$
Molecular weight : 206.02

Instruments required

High performance liquid chromatography, with UV detector, HPLC Analytical column of Ultra Aqueous C18, 150x4.6mm, 5µm, Analytical weighing balance - Mettler Toledo B204S, Millipore Nylon 0.2µm.

Chemicals required

Chloramben working Standard, AMIBEN' DS Chloramben Herbicide, Acetonitrile, Phosphoric Acid, Hydrochloric Acid, Sodium Hydroxide, Millipore Water.

Chromatographic conditions

Column : Ultra Aqueous C18, 150x4.6mm, 5µm.
Mobile Phase : For isocratic system, prepared a mixture of 0.05% H_3PO_4 and acetonitrile 20: 80 proportion respectively. Mix well. Filter through 0.2µ Nylon Membranes filter paper and degas prior to use.
Wavelength : 225 nm.
Flow rate : 1.0 ml / minute.
Injection volume : 10 µl
Run time : 15 minutes
Blank solution : Acetonitrile
Diluent : Acetonitrile

Preparation of Chloramben Standard Solution

Weighed accurately about 20 mg of Chloramben working Standard and transferred to a 20 ml volumetric flask. Added 10 ml of diluent and sonicate to dissolve. Diluted to volume with diluent and mixed. Transfered 1.0 ml of solution into a 10 ml of volumetric flask and diluted to volume with the diluent and mixed.

Preparation of Test Solution

Weighed accurately about 24 mg of Chloramben sample and transferred to a 20 ml volumetric flask. Added 10 ml of diluent and sonicate to dissolve. Diluted to volume with diluent and mixed. Transfered 1.0 ml of solution into a 10 ml of volumetric flask and diluted to volume with the diluent and mixed.

System suitability solution

Separately injected equal volumes of blank, five replicate injections of system suitability solution here Chloramben Standard working solution is used as system suitability solution. Then injected two injections of test solution and recorded the chromatograms. Disregard any peak due to blank in the test solution. % RSD of five replicate injections of system suitability solution is calculated and recorded in table-1.

Table - 1: System suitability - Selectivity

Sr. No.	Area of Chloramben
1	2786.71
2	2775.98
3	2781.56
4	2777.41
5	2721.53
Mean	**2768.64**
Standard Deviation (±)	**26.66**
(%) Relative Standard Deviation	**0.96**

Tailing factor and theoretical plates of the peak in the chromatogram obtained with 5th injection of system suitability, Solution checked. The limits are as below,

1. Theoretical plates should be not less than 2000.
2. Tailing factor should be less than 2.0.
3. % RSD should be not more than 2.0%.

VALIDATION PARAMETERS

Specificity / selectivity

Selectivity was performed by injecting the diluent blank solution, system suitability solution, test solution and results are recorded in table- 2.

Table - 2: System - Selectivity

Sr. No.	Area of Chloramben
1(Blank)	Blank
2(Standard 1)	2786.71
3(Standard 2)	2775.98
4(Standard 3)	2781.56
5(Standard 4)	2777.41
6(Standard 5)	2721.53
7(Sample 1)	2727.02
8(Sample 2)	2742.48
Mean	2758.96
Standard Deviation (±)	27.70
(%) Relative Standard Deviation	1.00

FORCED DEGRADATION

The forced degradation studies are performed to establish the stability indicating nature of the assay Method and to observe any degraded compounds. Chloramben working standard and Samples are subjected to stress with 5N HCl, 5N NaOH, Thermal degradation and UV degradation. The stress conditions are followed for degradation mentioned in table-3.

Table – 3: Conditions – Forced Degradation

Sample stress condition	Description of stress condition
Acid degradation	5N HCl heated at about 60°C for 10 min on a water bath.
Alkali degradation	5N NaOH heated at about 60°C for 10 min on a water bath.
Thermal degradation	105ºC for 12 hours
UV degradation	expose to UV-radiation for 7 days

All the above solutions are chromatographed and recorded the chromatograms and results recorded in tables 4 & 5.

Table – 4: System suitability – Forced Degradation

Sr. No.	Area of Chloramben
1	2810.45
2	2849.58
3	2877.40
4	2814.38
5	2857.70
Mean	**2841.90**
Standard Deviation (±)	**28.79**
(%) Relative Standard Deviation	**1.01**

Table - 5: Results - Forced Degradation

Acid Stress	**% Degradation**
Standard	-
Sample	0.039
Alkali Stress	**% Degradation**
Standard	0.003
Sample	0.011
Thermal Stress	**% Degradation**
Standard	-
Sample	0.001
UV Stress	**% Degradation**
Standard	-

Sample	0.065

LINEARITY

Linearity and Range for standard

For the linearity study five standard solutions of Chloramben were prepared from the range starting from 50% to 150% of the theoretical concentration of assay preparation.

The system suitability linearity standard solutions were injected and results are mentioned in table - 6.

Table 6: System suitability - Linearity of standard

Sr. No.	Area of Chloramben
1	2811.99
2	2854.78
3	2812.75
4	2831.09
5	2846.77
Mean	**2831.48**
Standard Deviation (±)	**19.41**
(%) Relative Standard Deviation	**0.69**

The peak area of Chloramben sample at each concentration level was recorded in table-7. The linearity graph of concentration against peak response was plotted shown in Figure – 2 and the correlation coefficient was determined here correlation coefficient should be greater than or equal to 0.999.

Table 7: Results of linearity of sample

Linearity Level	Sample Concentration (in %)	Sample Concentration (in ppm)	Peak Area	Correlation Coefficient
Level – 1	50	50	1666.51	**0.999**
Level – 2	75	75	2566.02	
Level – 3	100	100	3478.56	
Level – 4	125	125	4340.12	
Level – 5	150	150	5125.37	

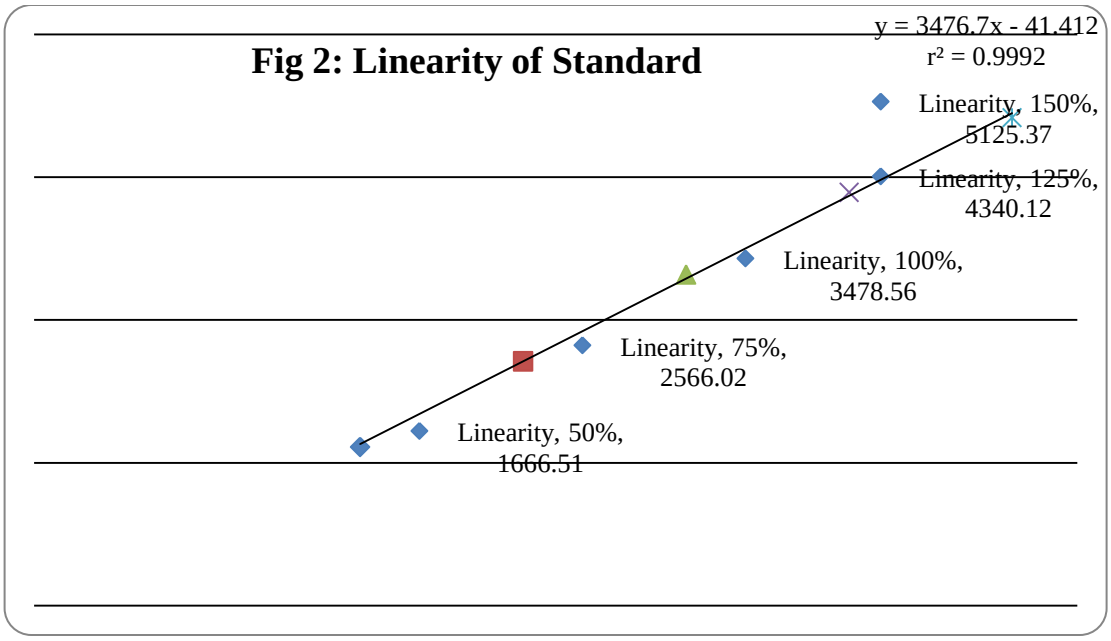


Figure 2: Linearity graph of Chloramben standard

PRECISION

System precision

The system precision was performed by injecting 10 replicate injections of system suitability solution and the results are recorded in table -8

Table 8: System precision

Sr. No.	Area of Chloramben
1	2789.81
2	2722.70
3	2772.05
4	2778.71
5	2782.76
6	2780.41
7	2728.07
8	2734.32
9	2712.27
10	2705.04
Mean	**2750.61**
Standard Deviation (±)	**33.01**
(%)Relative Standard Deviation	**1.20**

Method precision

Six test solutions of Chloramben in AMIBEN' DS Chloramben Herbicide was prepared as per the analytical Method. The % RSD and % assay of six test solutions was calculated. The results are noted in table 9 & 10.

Table - 9: System suitability - Method precision

Analyst – 1 HPLC No.: EH/R&D/HPLC-024

Sr. No.	Area of Chloramben
1	2892.77
2	2891.25
3	2862.03
4	2874.48
5	2862.68
Mean	**2876.64**
Standard Deviation (±)	**14.89**
(%) Relative Standard Deviation	**0.52**

Table - 10: Results of Method precision

Test Solution	% Assay of Chloramben
1	101.59
2	100.68
3	99.99
4	102.39
5	102.55
6	100.41
Mean	**101.27**
Standard Deviation (±)	**1.07**
(%) Relative Standard Deviation	**1.06**

Intermediate precision

Six test solutions of AMIBEN' DS Chloramben Herbicide was prepared as per the analytical Method on different day. These test solutions were analyzed by a different analyst using different HPLC column of same make but having different serial number and different HPLC system. The % RSD and % assay results of six samples from Method precision and six samples from intermediate precision was calculated. Results are noted in table - 11, 12 & 13.

Table - 11: System suitability - Intermediate precision

Analyst – 2	**HPLC No.: EH/R&D/HPLC-023**
Sr. No.	**Area of Chloramben**
1	4650.94
2	4681.44
3	4686.42
4	4642.67
5	4632.96
Mean	**4658.89**
Standard Deviation (±)	**23.80**
(%) Relative Standard Deviation	**0.51**

Table - 12: Results of Intermediate precision

Test Solution	**% Assay of Chloramben**
1	99.80
2	101.23
3	98.44
4	102.71
5	99.85
6	100.84
Mean	**100.48**
Standard Deviation (±)	**1.46**
(%) Relative Standard Deviation	**1.45**

Table - 13: Results of twelve test solutions of Chloramben in AMIBEN' DS Chloramben Herbicide (six of Method precision & six of intermediate precision)

Analysis performed during Method precision study By Analyst 1 on system 1 and on column 1 on day 1	
Same column	**% Assay of Chloramben**
1	101.59
2	100.68
3	99.99
4	102.39
5	102.55

6	100.41
Analysis performed during intermediate precision study By Analyst 2 on system 2 and on column 2 on day 2	
Column sr. no.	**015337030136 01**
Test Solution	**% Assay of Chloramben**
7	99.80
8	101.23
9	98.44
10	102.71
11	99.85
12	100.84
Mean of twelve samples	**100.87**
Standard Deviation (±)	**1.29**
(%) Relative Standard Deviation	**1.28**

Robustness

Two test solutions of the same lot of Chloramben in AMIBEN' DS Chloramben Herbicide as per analytical Method Prepared and Injected this solution along with diluent blank solution and system suitability solution along different chromatographic conditions as shown below:

Change in column lot (same make, different serial no.)

Change in flow rate (± 0.2 ml/minute)

Change in wavelength (± 2 nm)

Change in composition of mobile phase (± 20ml)

Change in Column Lot

Normal Experimental Condition is Ultra Aqueous C18, 150x4.6mm, 5µm

The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical Method. Results recorded in Table - 14 for system suitability.

Table - 14: System suitability - Robustness with Change in Column Lot

Sr. No.	**Area of Chloramben**	
	Same column	**Diff column**
1	2822.44	2844.37
2	2821.56	2815.71
Mean	**2822.00**	**2830.04**
Standard Deviation (±)	**0.62**	**20.27**
(%) Relative Standard Deviation	**0.02**	**0.72**

The assay results obtained with different flow rate conditions are as given in Table - 15.

Table - 15: Results for Change in Column Lot

Flow rate →	**Same column**	**Diff column**
Sample	**% Assay**	
Test solution	101.60	101.18
Average assay result from Method precision	101.27	101.27
Mean	**101.44**	**101.23**
Standard Deviation (±)	**0.23**	**0.06**
(%) Relative Standard Deviation	**0.23**	**0.06**

Change in Flow Rate (± 0.2 ml/minute)

Normal Experimental Condition is 1.0ml/minute. The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical Method. Results recorded in Table - 16 for system suitability.

Table - 16: System suitability - Robustness with change in flow rate

Sr. No.	**Area of Chloramben**	
	0.8mL/minute	**1.2 mL/minute**
1	2684.89	2652.85
2	2637.13	2631.87
Mean	**2661.01**	**2642.36**
Standard Deviation (±)	**33.77**	**14.84**
(%) Relative Standard Deviation	**1.27**	**0.56**

The assay results obtained with different flow rate conditions are as given in Table 17.

Table - 17: Results for change in flow rate

Flow rate →	**0.8 mL/minute**	**1.2 mL/minute**
Sample	**% Assay**	
Test solution	99.94	100.45
Average assay result from Method precision	101.27	101.27
Mean	**100.61**	**100.86**
Standard Deviation (±)	**0.94**	**0.58**
(%) Relative Standard Deviation	**0.93**	**0.57**

Change in Wavelength (± 2 nm)

Normal Experimental Condition is 225nm. The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical Method. Results recorded in Table - 18 for system suitability.

Table - 18: System suitability - Robustness with change in wavelength

Sr. No.	Area of Chloramben	
	223 nm	**227 nm**
1	2681.66	2654.78
2	2640.22	2638.10
Mean	**2660.94**	**2646.44**
Standard Deviation (±)	**29.30**	**11.79**
(%) Relative Standard Deviation	**1.10**	**0.45**

The assay results obtained with different wavelength conditions are as given in Table - 19.

Table - 19: Results for change in wavelength

Wavelength →	**223 nm**	**227 nm**
Sample	**% Assay**	
Test solution	100.14	100.59
Average assay result from Method precision	101.27	101.27
Mean	**100.71**	**100.93**
Standard Deviation (±)	**0.80**	**0.48**
(%) Relative Standard Deviation	**0.79**	**0.48**

Change in composition of Mobile Phase

Normal Experimental Condition: Buffer is Acetonitrile = 20ml: 80ml.The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical Method. Results recorded in Table - 20 for system suitability.

Table - 20: System suitability - Robustness with change in composition of mobile phase

Sr. No.	Area of Chloramben	
	22ml:78ml	**18ml:82ml**
1	2669.76	2672.11
2	2676.25	2646.17
Mean	**2673.01**	**2659.14**
Standard Deviation (±)	**4.59**	**18.34**
(%) Relative Standard Deviation	**0.17**	**0.69**

The assay results obtained with change in composition of mobile phase are as given in Table - 21.

Table - 21: Results for change in composition of mobile phase

Composition of Buffer : Acetonitrile	**22ml:78ml**	**18ml:82ml**
Sample	**% Assay**	
Test solution	99.39	100.64
Average assay result from Method precision	101.27	101.27
Mean	**100.33**	**100.96**
Standard Deviation (±)	**1.33**	**0.45**
(%) Relative Standard Deviation	**1.32**	**0.44**

Stability of Analytical Solution

System suitability solution and test solution of AMIBEN' DS Chloramben Herbicide were prepared on 0^{th}, 12^{th}, 24^{th}, 36^{th} and 48^{th} hour of experiment and stored these solutions at room temperature for every time interval up to 48 hours and analyzed these solutions on 48 hours with freshly prepared test solution. The system suitability solution was prepared freshly at the time of analysis. The results are recorded in table – 22.

Table - 22: Results of Analytical solution Stability

TIME	**Standard Area**	**Average standard area**	**Sample area**	**Average Sample area**
0^{th} hr	2853.22	2835.53	2849.53	2867.365
	2817.84		2885.20	
12^{th} hr	2892.77	2889.07	2856.28	2864.685
	2885.37		2873.09	
24 hr	2801.84	2809.865	2817.43	2823.485
	2817.89		2829.54	
36 hr	2865.18	2861.935	2836.73	2784.095
	2858.69		2731.46	
48 hr	2837.81	2829.505	2829.72	2842.775
	2821.20		2855.83	
Mean	**2845.18**	**2845.18**	**2836.48**	**2836.48**
Standard Deviation (±)	**30.78**	**30.79**	**42.34**	**34.29**
(%) Relative Standard Deviation	**1.08**	**1.08**	**1.49**	**1.21**

The assay results obtained during solution stability experiment are as given in Table- 23

Table - 23: Results for solution stability

% Assay results calculated against the freshly prepared system suitability standard	
Sample	**% Assay of Chloramben**
0^{th} hr	102.11
12^{th} hr	100.13
24 hr	101.47
36 hr	98.23
48 hr	101.45
Mean	100.68
Standard Deviation (±)	1.55
(%) Relative Standard Deviation	1.54

Results and Discussion

System selectivity

All the injections were processed at the wavelength provided in the Method. There was no interference observed from diluents blank solution, excipients blend solution with Chloramben peak. The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical Method. Hence this Method is selective.

Forced degradation

There is no interference between the peaks obtained for the chromatograms of degradation preparations. The degradation peaks under forced degradation are well separated from each other. The peak purity for Chloramben peak is passing. Hence, the Method is very precise, selective and specific to the estimation of Assay of Chloramben in AMIBEN' DS Chloramben Herbicide by HPLC and the same Method is stability indicating, as the degraded products are well separated from Chloramben and as well from each adjacent peaks.

Linearity

Linearity graph of the average area at each level against the concentration in v/v% is plotted and is found to be a straight line graph. The correlation coefficient is found to be more than 0.999.Hence it is concluded that the Method is found to be linear in the range of 50% to 150% of the working concentration.

Precision

The analysis was carried out on six test solutions of the same lot of the pesticide by two different analysts using two different equipments within the same laboratory using two different columns of the same make but having different serial numbers on two different days. The % RSD of the twelve assay results which six of Method precision and six from intermediate precision is found to be less than 2.0%. Thus, the Method is found to be rugged and precise.

Robustness

The analysis of the same lot of AMIBEN' DS Chloramben Herbicide was carried out at different conditions of column lot, flow rate, wavelength, and change in composition of mobile phase. The % RSD between results obtained with changed condition and average result of Method precision is not more than 2.0%.The analytical Method meets the pre-established acceptance criteria for robustness study. Thus, the Method is robust.

Stability of Analytical solution

The % RSD between assay results obtained for freshly prepared test solution and the stored test solutions is less than 2.0%. There is no significant change in assay level observed up to 48 hours for test solution at room temperature. The system suitability was found to meet the pre-established criteria and it can be concluded that the solution is stable upto 48hours at room temperature.

Summary and conclusion

The above summary and the validation data summarized in this paper shows that the analytical Method of assay of Chloramben in AMIBEN' DS Chloramben Herbicide by HPLC is found to be suitable, selective, specific, precise, linear, accurate and robust. The analytical solution is found to be stable up to 48 hours at room temperature.

Hence, it is concluded that the analytical Method is validated and can be used for routine analysis and for stability study.

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