(RESEARCH ARTICLE)

Analytical method development and validation analysis for quantitative assessment of cypermethrin by HPLC procedure

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Abstract

The precise, systematic, definite, particular, linear, exact and robust scientific method was developed and validated for the assay of Cypermethrin in Cypermethrin 25% EC Insecticide for Injection, USP 500 mg/vial. Presently utilized Cypermethrin 25 % EC Insecticide as a working standard having limit was 95% for method development and validation. The quantitative determination bring to accomplished by HPLC- Waters - Alliance 510 system equipped with UV/PDA detector. Methanol, Acetonitrile and water in the ratio (60:20:20 v/v/v) used as mobile phase and flow rate 1.0 ml / min. with 20 minutes run time. The detection was carried at 225 nm with Nucleosil C18 column (250 mm × 4.6 mm x 10 μm) and ambient column temperature was maintained. In this connection, method uses the 20 μl injection volume and diluent as a blank solution. The linearity of this method was found to be linear in the range of 50% to 150% of the working concentration and the range for the analytical method is 25 ppm to 75 ppm. The accuracy and precision of the method were within acceptable. The present developed HPLC method is found to be suitable. The analytical solution was found to be stable up to 48 Hrs at room temperature.

Keywords: Cypermethrin; Robust; Precision; Linearity; Stability

1. Introduction

Cypermethrinis a neurotoxin that swiftly destroys insects by way of attacking their sytemanervosum [1], suspends working ofthe motor nerve fiber, supraesophageal tendon sheath and exasperate affect on respiratory fibril which causes to paralysis and breakdown the coordination with cerebrospinal fiber organs [2], it leads to terminate life of insects. Again in another way, its toxicity could not effect subsurface water or saplings [3], by reason of, this insecticide usually applied to regulate insect near foliage. Technical Cypermethrin varies frm viscous, yellow liquid to semi-solid crystalline mass at ambient temperatures. Experiments articulates aerial implementations of cypermethrin control tsetse fly within a few hours, dead and dying insects were found, epitomize the expeditious break down repercussions of cypermethrin [4]. Insects aftereffect, at the moment that evaluate with funnel traps, came on noticeably increased for 1 day due to spray containing 0.5 g cypermethrin per litre. In cypermethrin along with 140 mg/litre ascorbic acid treatment histopathological disorders were relatively less compared the animals exposed the acute dose of cypermethrin [5]. The structure of Cypermethrin was as follows.

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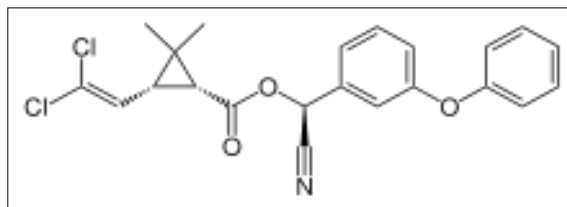
Figure 1 Structure of Cypermethrin

Chemical name: [Cyano-(3-phenoxyphenyl)methyl]3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-Carboxilate acid. Molecular formula: $C_{22}H_{19}Cl_2NO_3$ and Molecular weight is 416.30 g.mol^{-1} [6].

Previous investigations expels that, there was accurate and reliable HPLC method has developed for using stability indicating method for the determination of Cypermethrin spontaneous derferments [7]. Subsequential literature survey, found chromatographic methods of stability validation studies for the resolution of Cypermethrin by using RP-HPLC [8] and for LC-MS for Cypermethrin samples in aquatic insects and fruits [9]. In the same manner cited in the compositions, method was found to be reproducible and convenient for the quantitative analysis of this insecticide [10]. Many authors were investigated the highly sensitive and more productive chromatographic methods for development and validation of different drugs in bulk forms [11].

The limits for Assay of Cypermethrin for Injection, USP 500mg/vial, 10 ml are not less than 95.0% and not more than 105.0 % of the labeled amount. This analytical method verification report is intended to summarize the results obtained during the verification of HPLC method for the assay of Cypermethrin in Cypermethrin 25 % EC Insecticide for Injection, USP 500 mg/vial, 10 ml. A High Performance Liquid Chromatography-UV Detection(HPLC- UV/PDA) method for the quantitative determination of analytical method of assay of Cypermethrin in Cypermethrin 25% EC Insecticide for Injection, USP 500mg/vial, 10 ml was developed and validated in the present study.The validation parameters such as Specificity or Selectivity, linearity, Method of precision, Intermediate Precision, Robustness and stability were studied according to the International Conference on Harmonization Guidelines with numbers: Q2A & Q2B of CPMP / ICH / 281 / 95 and non pharmacopoeial Method [12].

2. Materials and method

2.1. Chemicals and reagents

Cypermethrin working standard and Cypermethrin Injection, USP 500 mg/vial, 10 ml was received from reputed local pharmaceutical company. All chemicals and reagents used for present study were of high quality and purity procured from various sources. Acetonitrile, Methanlo-AR were purchased from Merck. Acetonitrile and water (HPLC- Grade) were procured from SD Fine chemicals, India. All the materials used were within the expiry date and stored at recommended storage conditions.

2.2. Preparation of Cypermethrin Standard Solution

Weigh accurately about 50 mg of Cypermethrinworking Standard and transfer to a 50 ml volumetric flask. Add 20 ml of diluent and sonicate to dissolve. Dilute to volume with diluent and mix. Transfer 1.0 ml of solution into a 10 ml of volumetric flask and dilute to volume with the diluent and mix.

(Dilution scheme: 50 mg → 50.0 ml → 1 ml /10.0 ml)

2.3. Preparation of sample Solution

Weigh accurately about 200 mg of sample and transfer to a 50 ml volumetric flask. To dissolve sonicate and augment 20 ml of diluent [13]. Dilute to volume with diluent and mix. Transfer 1.0 ml of solution into a 10 ml of volumetric flask and dilute to volume with the diluent and mix.

(Dilution scheme: 200 mg → 50.0 ml → 1 ml /10.0 ml)

2.4. System Suitability Solution Preparation

 Used Cypermethrin working standard solution as system suitability solution.

2.5. Procedure

Separately inject equal volumes of blank, five replicate injections of system suitability solution (Cypermethrin working standard solution). Subsequently inject two injections of test solution and record the chromatograms. Ignore any peak due to blank in the test solution. Calculate % RSD of five replicate injections of system suitability solution (Cypermethrin working standard solution). Check tailing factor and theoretical plates of the peak in the chromatogram obtained with 5th injection of system suitability solution (Cypermethrin working standard solution).

The limits are as below,
- Theoretical plates should be not less than 2000.
- Tailing factor should be less than 2.0.
- % RSD should be not more than 2.0 %.

No options while fixing limits mention 2 or 3 not 2 and 3. 2 is enough. Everything in same fashion.

2.5.1 Instrumentation and Chromatographic conditions

For the current analysis, the HPLC - Agilent 1100 Series and HPLC- Waters - Alliance 510 pump with UV- 484 detector was used. The Chromeleon software and Data Ace softwares were utilized for data acquirement. Sample injection was done by auto injector which was coupled with instrument itself. System was equipped with HPLC Analytical column- Nucleosil C 18 (250 mm × 4.6 mm x 10-µm dimensions) and column was maintained at ambient temperatures for quantification. Mettler Toledo-B204S as analytical weighing balance was employed for weighing the working substances [14].

2.5.2 Mobile phase preparation

Prepare a mixture of Methanol, Acetonitrile and water in the ratio 60:20:20 respectively used as diluent which was blank sample. Mix well. The rate of flow was 1.0 ml / min. with 20 minutes run time and uses the 20 µl injection volume for testing sample quantity. The detection was carried at 225 nm with ambient chromatographic conditions. Then Filter through 0.2 µm Nylon membrane filter paper and degas prior to use.

2.5.3 HPLC Method validation

According to non pharmacopoeial method and the International Conference on Harmonization Guidelines, the method was validated in terms of Specificity or selectivity, linearity, method of precision, intermediate precision, robustnessandstability studies of the samples.

3. Results and discussion

3.1. Specificity /Selectivity

Table 1 System suitability - Selectivity

Sr. No.	Area of Cypermethrin
1	2729.92
2	2765.97
3	2777.66
4	2762.83
5	2786.90
Mean	2764.66
SD (±)	21.65
(%) RSD Standard Deviation	0.78

In accordance of the analytical method the system suitability criteria were detected to converge with the pre-established acceptance criteria. The results of system suitability corresponding selectivity were shown in the Table 1 and standard chromatogram was given in the following Figure 2.

Entire injections were processed at the wavelength furnished in the method. There was no interference observed from diluent blank solution, placebo with Cypermethrin peak. From the Table 1, it was evident that the % of Relative standard lesser than one (0.78).The method is selective.

3.2. Linearity

In the theoretical concentration of preparation of Assay, the linearity evaluation of five standard blends of Cypermethrin were developed in the span of initiating from 50 % to 150 %. As per the protocol the linearity solutions and the system suitability solutions were injected. The linearity graph of concentration in respect of peak performances was plotted and the correlation coefficient was detected.
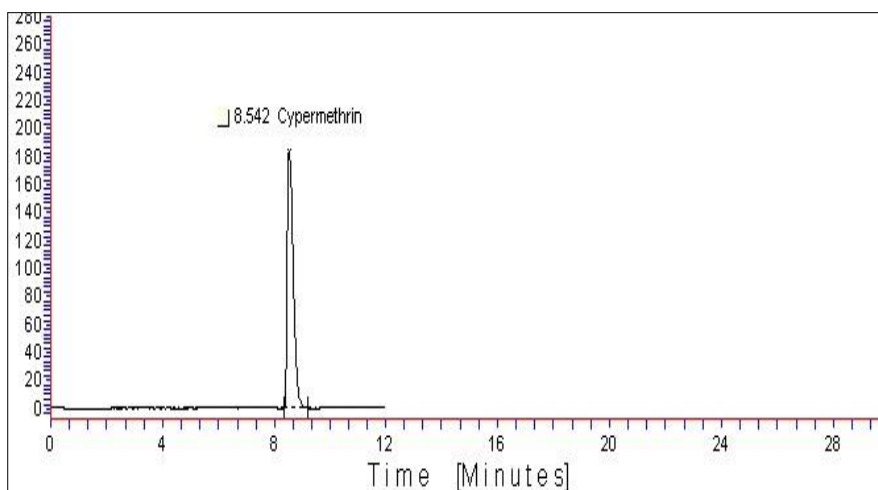
Figure 2 Standard chromatogram of Cypermethrin

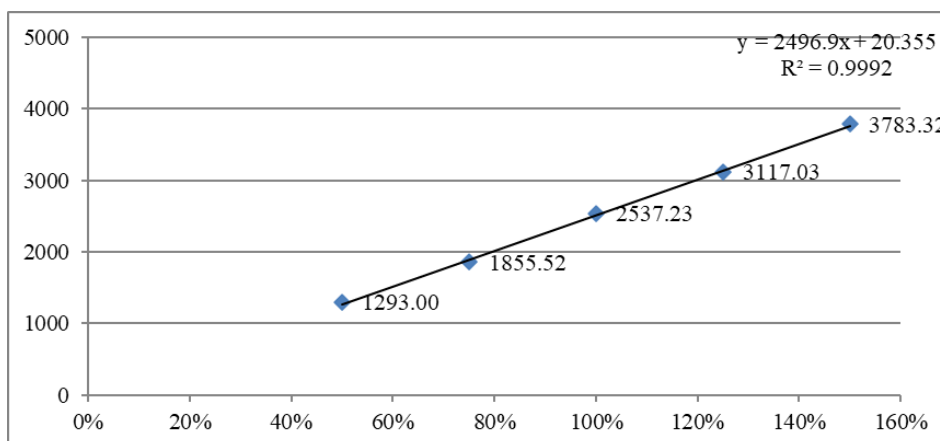| Result-A Table					
Peak No	Ret.Time	Area	Height	Area %	Height %
1	8.542	2762.831	184.573	100	100
Total	8.542	2762.831	184.573	100	100

Figure 3 Linearity of Standard

The average peak area of Cypermethrin peak at each concentration level was determined and the linearity graph was plotted against the sample concentration in percentage. The results of linearity study are as given in Table 3. Above Figure 3 interprets, observation of a linearity graph of the average area at every level against the concentration (%) was plotted and was detected to be a straight line graph. The calibration curve regression equation was Y=2496 x + 20.35 with a correlation coefficient value of R^2=0.999.

- A linearity graph of the average area at each level against the concentration (%) is plotted and is found to be a straight line graph.
- The correlation coefficient is detected to be greater 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.
- The range for the analytical method is 50 ppm to 150 ppm.

Table 2 System suitability Linearity standard of Cypermethrin

Sr. No.	Area of Cypermethrin
1	2484.90
2	2471.81
3	2468.40
4	2393.31
5	2467.01
Mean	2457.08
SD (±)	36.35
(%) RSD	1.48

Table 3 Results of linearity of standard

Linearity Level	Sample Concentration (in ppm)	Sample Concentration(in ppm)	Peak Area	Correlation Coefficient
Level – 1	50	50	1293.00	
Level – 2	75	75	1855.52	
Level – 3	100	100	2537.23	0.999
Level – 4	125	125	3117.03	
Level – 5	150	150	3783.32	

3.3. Precision

3.3.1 System Precision

The system precision performed by injecting ten replicate injections of system suitability solution and the chromatograms are studied for the system suitability criteria. Acceptance criteria: RSD % of peak areas of ten replicate injections of system suitability solution should not be more than 2.0 % and system suitability criteria have to pass as per analytical method. By the inference of analytical method, the system suitability criteria were detected to coincide with the pre-established acceptance criteria.

Table 4 System Suitability-System Precision

Sr. No.	Area of Cypermethrin
1	2620.12
2	2627.75
3	2611.12
4	2624.39
5	2623.43
6	2619.89
7	2625.17
8	2623.31
9	2637.55
10	2627.65
Mean	2624.04
SD (±)	6.76
(%) RSD	0.26

Result: Inference of the above data resolved that the system precision is well established.

3.3.2 Method Precision

Six test solutions of Cypermethrin in CYPERMETHRIN 25 % EC INSECTICIDE and were prepared as per the analytical method. The percentages of RSD and assay of six test solutions was calculated. % RSD concludes, with the results of six test solutions should be accept only less than 2.0%. By the inference of analytical method the system suitability criterion was detected to coincide the pre-established acceptance criteria. The results of assay obtained from six test solutions preparations are presented in Table - 6.

Table 5 System suitability - Method precision Analyst – 1 HPLC No.: EH/R&D/HPLC-024

Sr. No.	Area of Cypermethrin
1	2470.32
2	2468.96
3	2473.88
4	2478.51
5	2476.93
Mean	2473.72
Standard Deviation (±)	4.11
(%) Relative Standard Deviation	0.17

Table 6 Results of method precision

Test Solution	% Assay of Cypermethrinn
1	100.25
2	99.44
3	99.99
4	100.57
5	100.11
6	100.80
Mean	100.19
SD (±)	0.48
(%) RSD	0.47

Remark

The % RSD of the six assay results is detected less than 2.0% and coincide the pre-established acceptance criteria. Hence, it is inferred that the method is precise.

3.3.3 Intermediate Precision

Table 7 System suitability Intermediate precisionAnalyst – 2HPLC No.: EH/R&D/HPLC-023

Sr. No.	Area of Cypermethrin
1	2082.46
2	2088.89
3	2078.90
4	2077.89
5	2004.76
Mean	2066.58
SD (±)	34.83
(%) RSD	1.69

Table 8 Results of intermediate precision

Test Solution	% Assay of Cypermethrin
1	98.55
2	98.77
3	99.05
4	99.98
5	99.20
6	100.09
Mean	99.27
S D (±)	0.63
(%) RSD	0.64

Six test solutions of CYPERMETHRIN 25 % EC INSECTICIDE 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 of % assay results of twelve test solutions (six samples from method precision and six samples from intermediate precision) was calculated. % RSD of the results of twelve test solutions (six of method precision and six of intermediate precision) should not be more than 2.0 %.

The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table 7 for system suitability results). % RSD of assay results from method precision and intermediate precision (12 results) are presented in Table 9.

Table 9 Results of twelve test solutions of Cypermethrin in (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 Cypermethrin**
1	100.25
2	99.44
3	99.99
4	100.57
5	100.11
6	100.80
Analysis performed during intermediate precision study By Analyst 2 on system 2 and on column 2 on day 2	
Column sr. no.	015132560136 02
Test Solution	% Assay of Cypermethrin
7	98.55
8	98.77
9	99.05
10	99.98
11	99.20
12	100.09
Mean of twelve samples	99.73
Standard Deviation ($\pm$)	0.72
(%) Relative Standard Deviation	0.72

Result

The analysis was carried out on six test solutions of the same lot of the drug product 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 (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.

3.4. Robustness

3.4.1 Change in Column Lot

(Experimental Condition: Nucleosil C 18 - 250 mm × 4.6 mm x 10-µm)

The analytical method represents that the system suitability criteria were detected to coincide the pre-established acceptance criteria.

Table - 11 represents change in Column Lot results.

Table 10 System suitability of Assay - Robustness with change in Column

Sr. No.	Area of Cypermethrin	
	Same column	Different column
1	2470.32	2009.66
2	2468.96	2012.61
Mean	2469.64	2011.13
SD (±)	0.96	2.09
(%) RSD	0.04	0.10

The assay results were obtained with different flow rate conditions are as given in Table 11.

Table 11 Results of change column Lot

Flow rate →	Same column	Different column
Sample	% Assay	
Test solution	100.25	99.75
Average assay result from method precision	100.19	100.19
Mean	100.22	99.97
Standard Deviation (±)	0.04	0.31
(%) Relative Standard Deviation	0.04	0.31

3.4.2 Change in Flow Rate (± 0.2 mL/minute)

(Normal Experimental Condition: 1.0 ml/minute)

The analytical method represents that system suitability criteria were detected to coincide the pre-established acceptance criteria.

Table 12 System suitability - Robustness along with change in flow rate

Sr. No.	Area of Cypermethrin	
	0.8 mL/minute	1.2 mL/minute
1	2422.57	2544.97
2	2415.66	2552.71
Mean	2419.12	2548.84
Standard Deviation(±)	4.89	5.48
(%) RSD	0.20	0.21

The assay results obtained with different flow rate conditions are as given in Table 13.

Table 13 Results for change in flow rate

Flow rate →	0.8 mL/minute	1.2 mL/minute
Sample	% Assay	
Test solution	101.11	99.57
Average assay result from method precision	100.19	100.19
Mean	100.65	99.88
Standard Deviation (±)	0.65	0.44
(%) Relative Standard Deviation	0.65	0.44

3.4.3 Change in Wavelength (± 2 nm)

(Normal Experimental Condition: 225 nm)

The analytical method represents that the system suitability criteria were detected to coincide the pre-established acceptance criteria.

Table 14 System suitability - Robustness with change in wavelength

Sr. No.	Area of Cypermethrin	
	223 nm	227 nm
1	2537.92	2549.59
2	2550.01	2557.83
Mean	2543.96	2553.71
Standard Deviation (±)	8.55	5.83
(%) Relative Standard Deviation	0.34	0.23

The assay results obtained with different wavelength conditions are given in Table 15.

Table 15 Results for change in wavelength

Wavelength→	223 nm	227 nm
Sample	% Assay	
Test solution	100.19	100.43
Average assay result from method precision	100.19	100.19
Mean	100.19	100.31
Standard Deviation (±)	0.00	0.17
(%) RSD	0.00	0.17

3.4.4 Change in composition of mobile phase

(Normal Experimental Condition: Methanol: Acetonitrile : water = 600 ml : 200 ml : 200 ml)

The system suitability criteria were detected to coincide the pre-established acceptance criteria as per the analytical method.

Table 16 System suitability - Robustness with change in mobile phase composition

Sr. No.	Area of Cypermethrin	
	58 MeOH:21 ACN:21 W	62 MeOH:19 ACN:19 W
1	2602.51	2691.28
2	2616.26	2698.71
Mean	2609.39	2694.99
SD (±)	9.73	5.25
(%) Relative Standard Deviation	0.37	0.19

The assay results obtained with change in mobile phase composition are as given in Table - 17.

Table 17 Results change in composition of mobile phase

Mobile phase composition	58 MeOH:21 ACN:21 W	62 MeOH:19 ACN:19 W
Sample	% Assay	
Test solution	99.80	99.07
Average assay result from method precision	100.19	100.19
Mean	100.00	99.63
Standard Deviation (±)	0.28	0.79
(%) RSD	0.28	0.79

Results

- The analysis of the same lot of CYPERMETHRIN 25 % EC Insecticide was carried out at different conditions of column lot, flow rate, wave length and change in composition of mobile phase.
- The system suitability was detected to coincide the pre-established criteria at all the stipulations and the % RSD is not more than 2.0 % in between results obtained with modified stipulation and average result of Method precision.
- The analytical Method meets the pre-established acceptance criteria for robustness study as per protocol. Thus, the Method is robust.

3.5. Stability of Analytical Solution

System suitability solution and test solution of Cypermethrin in CYPERMETHR IN 25 % EC INSECTICIDE brought to developed on session 0th, 12th, 24th, 36th and 48th hour of experiment and stored these solutions at normal storage temperature for every time period up to 48 hrs and analyzed these solutions on 48 hrs with newly prepared test solution.

Results for Solution Stability shown in the above Table-18. During the analysis the system suitability solution was prepared afreshly. The assay of CYPERMETHRIN 25 % EC INSECTICIDE in the sample was calculated. The system suitability was detected to coincide the pre-established criteria and the % RSD between assay results obtained for afreshly prepared test solution and the stored test solutions is less than 2.0 %. The Assay level observes there is no significant change up to 48 Hrs of test solution at room temperature. Hence, consequently it can be concluded that the solution is stable up to 48 Hrs at room temperature.

Table 18 Results for Solution Stability

% Assay results computed against the newly prepared system suitability standard	
Sample	% Assay of Cypermethrin
0th hr	100.04
12th hr	100.22
24th hr	99.79
36th hr	100.52
48th hr	98.92
Mean	99.90
SD ($\pm$)	0.61
(%) RSD	0.61

4. Conclusion

The HPLC-UV/PDA method for determination of Cypermethrin for Injection, USP 500 mg/vial, 10 ml was completely validated by using specificity or selectivity, linearity, method of precision, intermediate precision, robustness and stability parameters. The approach was validated in accordance with ICH and non pharmacopeia standards. A simple economic HPLC method has been developed for the quantitative estimation of Cypermethrin injection with good precision, linearity, and robust. The prepared method was detected to be specific and accurate for the assay of Cypermethrin. A system suitability test was established and recorded for the Cypermethrin injection. The analyte was considered stable if there is no significant change in % assay. Hence the solution was found to be stable up to 48 Hours at room temperature. For these reasons, hence, it is concluded that the analytical method was validated, can be used for routine analysis and for stability study. Consequently, the suggested method can be easily used for the quantitative quality control in agro industries, and future research also.

Compliance with ethical standards

Acknowledgments

Disclosure of conflict of interest

No Conflict of interest exists among the authors.

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