BIO-ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF CYPERMETHRIN PESTICIDE USING GC-FID METHOD

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Abstract:

Cypermethrin is a synthetic pyrethroid pesticide that is used to treat various fruits and vegetables of pests. Similar to natural pyrethroid, synthetic pyrethroid is generated from pyrethrum flowers (chrysanthemum cinerariaefolium). A very simple Gas chromatography technique was developed which helps in the estimation of Cypermethrin in human plasma. The separation of the drug was achieved on drug column of Zebron GC DB. The retention time was 18.2 mins. This method was validated according to the ICH Guidelines. The linearity was calculated and the regression coefficient was found to be 0.99. The LOD and LOQ were validated. This method was applied for bio analytical with human plasma according to the USFDA guidelines.

Keywords: Gas chromatography, Flame Ionization detector, Cypermethrin toxicity, Blood plasma, ICH guidelines.

Introduction

The pesticide cypermethrin is a synthetic pyrethroid. Similar to natural pyrethroid, synthetic pyrethroid is manufactured from the blooms of pyrethrums (chrysanthemum

cinerariaefolium). [1] It was first synthesized in 1974. CYP is used as a common household insecticide and used to control insects in vegetables, fruits. The insects which come in contact with the CYP get killed and it acts by affecting the CNS of the insects. Cypermethrin is toxic to humans and may cause dizziness, headache and vomiting in low doses and in high doses may cause anaphylaxis and death. [1] The resemblance of Cypermethrin toxicity and organophosphates poisoning is similar and they hospitals are facing a dilemma in the treatment. A case of 30 year old male was reported complaining throat pain, increased salivation, drooling, anxiety, cough and dyspnoea. The person had consumed a liquid, 90 minutes prior to the admission. It was identified as organophosphate poisoning and he was administered with 1.8mg of atropine. Later it found that he consumed Cypermethrin and atropine was stopped and he was given a symptomatic treatment. His condition was improved within 24hrs. [1]

Least methods have been reported for Cypermethrin using GC with Electron Capture Detector (ECD) but no method was reported using Flame Ionization Detector. This method is simple and can be used to find Cypermethrin in plasma.

Materials and Method

Chemical and reagents:

Cypermethrin standard was procured from Sigma Aldrich, India. Cyper25 is a marketed pesticide manufactured by National Pesticides and Cypermethrin was availed from the local market. The chemicals used in the entire study were analytical grade. HPLC grade methanol is used as a diluent for the sample preparation.

Instrumentation:

Gas chromatography GC-2014 Shimadzu with Flame ionization detector (FID) was used. Column used in the study Zebron DB (Length: 30m, Diameter: 0.25 mm and Film: 0.50 µm) and the volume of injection was 2µL. The chromatographic conditions are shown in Table 1.

Analytical method development

Preparation of stock solution

Cypermethrin stock solutions are prepared using 100mg Cypermethrin which is then diluted with 100ml methanol in a volumetric flask which gives a concentration of 1000µg/mL.

Preparation of working standards

Working standards are prepared by using stock solution (1000µg/mL). A series of dilutions (10, 15, 20, 25, and 30µg/mL) were made using the stock solution to find the calibration curve.

Preparation of sample

0.257mL of marketed formula which contains 10% Cypermethrin was measured and made up with HPLC grade methanol which gives 1000µg/mL concentration. From the above solution, 0.1 mL of the solution was transferred to 10ml volumetric flask and make up to the volume with methanol. The solution was filtered through 0.02µm syringe filter.

Bioanalytical method development

Preparation of standard solution

10 mg of Cypermethrin was weighed and dissolved with 10mL of HPLC grade methanol (1000 µg/mL). From the above, prepare 150, 225, 300, 375, 450 µg/mL so the concentration will be 10, 15, 20, 25, 30µg/mL after diluting it with plasma serum and methanol.

Optimized extraction procedure

The protein precipitating extraction method was selected and the protein precipitating agent used was methanol. 100µL of plasma and 100µL of the drug was added to an Eppendorf tube and the mixture was vortexed for a minute and then to the mixture protein precipitation agent (methanol) was added. The entire mixture was vortexed for 60 secs and then centrifuged at 10,000 RPM for 8mins at 4°c. Using a syringe filter the solution was filtered.

Results and discussion

Analytical method validation

The validation of the method was performed according to the ICH guidelines. The parameters which were validated are system suitability, linearity, precision, accuracy, system suitability, and robustness, limit of detection and limit of quantification. The Rt (retention time) of Cypermethrin was found to be 18.2 min. The blank chromatogram, standard chromatogram and sample chromatogram was shown in the Figure 2, 3 & 4 respectively.

System suitability

To check the system suitability, the solution of Cypermethrin was six times injected in the HPLC and the data (Tailing factor and theoretical plates) was noted and was given in Table 2.

Calibration curve

A series of concentrations were prepared in a range of 10, 15, 20, 25, 30 µg/mL. The peak area were analysed and plotted against the respective concentrations and the regression coefficient (r) was greater than 0.992. The calibration curve is shown in Figure 5.

Accuracy

The accuracy was done by spiking the formulation with 50%, 100% and 150% of standard Cypermethrin. The percentage recovery is given in the Table 3.

Precision

Intraday (repeatability) and inter day (intermediate) precision of Cypermethrin were analysed by calculating the %RSD for six replicates. Data is given in the Table 4 and Table 5.

Robustness

Robustness is method of its ability to stay unchanged by any minor and intentional in the method parameters for example detector temperature, injection temperature. The results of robustness were given in Table 6.

Bio analytical method validation

The method which is validated was done according to the US-FDA guidelines. The validation parameters performed was linearity, precision, accuracy, specificity. Cypermethrin had a retention time of 18.2 minutes. Figures 5, 6, and 7 depict the blank chromatogram, standard chromatogram, and sample chromatogram, respectively.

Calibration curve

The linearity in plasma was checked in series of concentrations in a range of 10, 15, 20, 25 and 30µg/mL. The peak area were calculated and plotted against the respective concentrations and the regression coefficient (r) was greater than 0.994. The peak area vs. concentration graph is shown in the Figure 8.

Specificity

To check the specificity two separate batches of blood plasma samples were utilised and checked for any interference with the Cypermethrin. There was no interference observed at the same time as that of the Cypermethrin.

Accuracy and Precision

The accuracy and precision for LLOQ, LQC, MQC, HQC were calculated and the data was given in the Table 7 and Table 8

Recovery

The percentage recovery for each concentration was calculated. Mean recovery was 71.3%. Recovery data was given in Table 9.

Stability studies

The stability study of Cypermethrin was conducted in various conditions. LQC and HQC were investigated in different conditions. Bench top was conducted by leaving the solution for 2-12hrs in room temperature. Freeze thaw stability was conducted by placing the solution in -30°C for 12hrs and then placing the solution again in room temperature, repeat the same process and at the end of 3 cycles the samples were analysed. Long term stability was

performed by placing the solution in freezing state for 12 days. The samples were analysed after 12 days. Stability studies are given in Table 10.

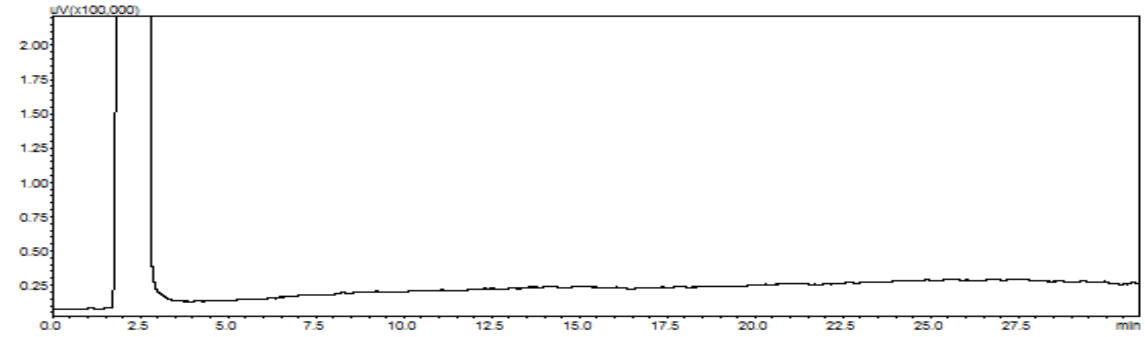

Fig. 1 Blank chromatogram of Cypermethrin.

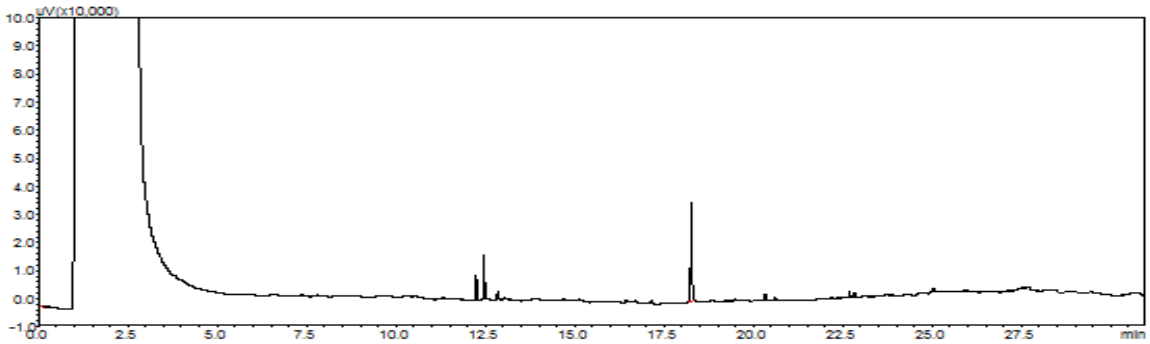

Fig. 2 Standard chromatogram of Cypermethrin.

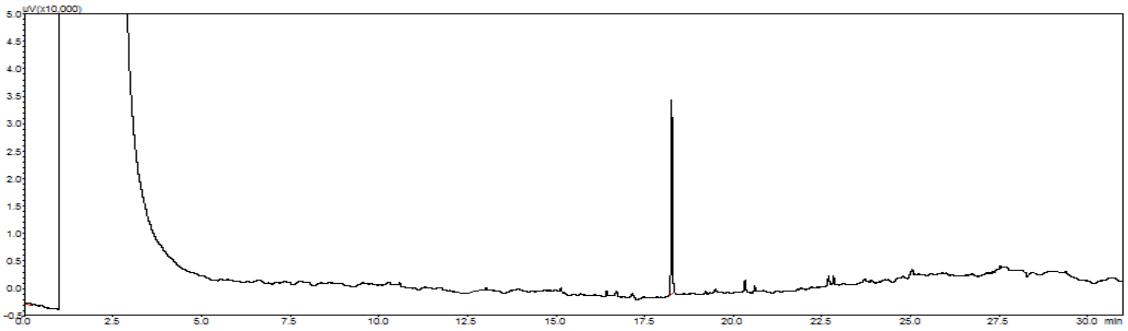

Fig. 3 sample chromatogram of Cypermethrin.

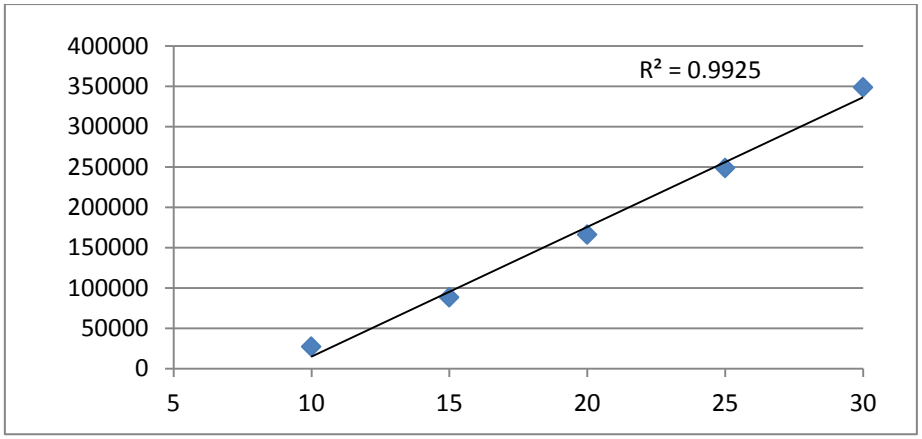

Fig. 4 Calibration curve of Cypermethrin.

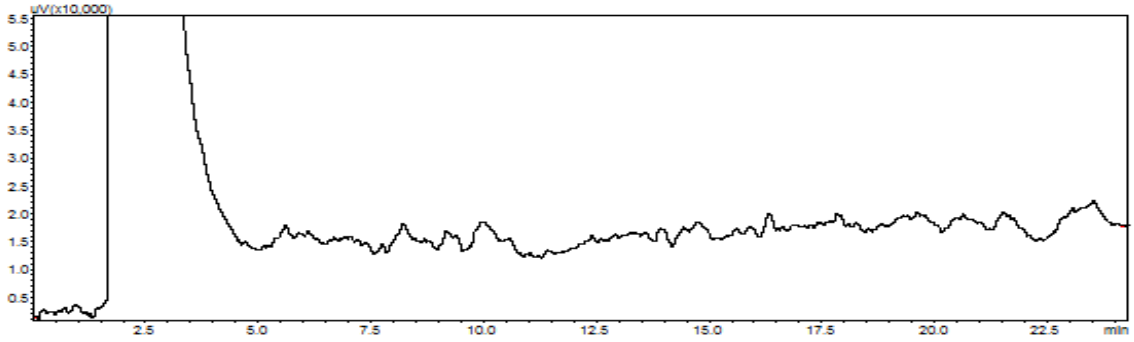

Fig. 5 Blank chromatogram of plasma.

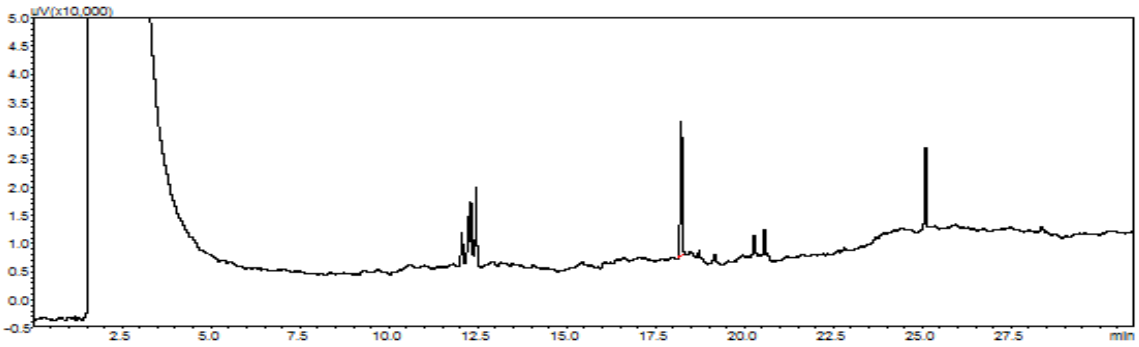

Fig. 6 Standard chromatogram of Cypermethrin in plasma.

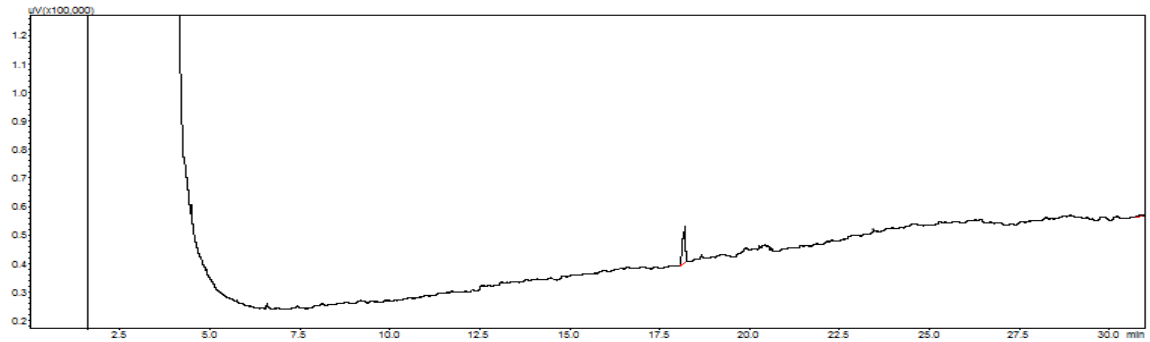

Fig. 7 Sample chromatogram of Cypermethrin in plasma

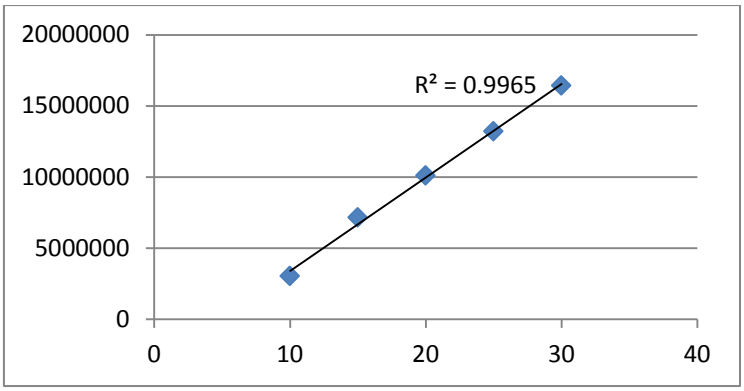

Fig. 8 Calibration curve of Cypermethrin.

Table1: Chromatographic conditions

Column	Zebron phase column
Column temp	280°C
Injector temp	300°C
Injection volume	1µg/ml
Detector	Flame ionization detector
Detector temp	210°C
Run time	31 mins

Table 2: System suitability

Specification	**Approval Specification**	**Outcomes**
Tailing factor	Less than 2.0	1.2
Theoretical plates	Greater than 2000	31092

Table 3: Accuracy data

Level of recovery	**Amount of Formulation (µg/mL)**	**Amount of pure drug (µg/mL)**	**The total amount of drug**	**Peak area**	**Difference**	**%recovery**	**Mean**
50	20	10	30	183823	156894	94.795	96.963
	20	10	30	196420	154396	96.543	
	20	10	30	187543	153285	98.873	
100	20	20	40	322973	156896	99.753	99.974

	20	20	40	328754	158543	98.406	
	20	20	40	368459	196654	101.302	
150	20	30	50	507653	156875	98.980	99.864
	20	30	50	518532	166854	99.098	
	20	30	50	503245	154387	100.957	

Table 4: Inter day precision

Concentration	**Peak area**
20	166076
20	163573
20	166194
20	169042
20	166290
20	164832
Average	165239
Standard deviation	1176.8
%RSD	0.3

Table 5: Intraday precision

Concentration	Peak area
20	164963
20	168930
20	163974
20	162394
20	167903
20	165387
Average	166193.6
Standard deviation	2143.2
%RSD	0.2

Table 6: Robustness data

Detector temperature	**Conc (µg/mL)**	**Peak Area**	**Detector temperature**	**Conc (µg/mL)**	**Peak Area**
205ºC	20	169043	215ºC	20	173484
	20	166345		20	169032
	20	162176		20	179842
	AVG	165854		**AVG**	174119

	STDV	3458		**STDV**	3432.9
	%RSD	0.03		**%RSD**	0.02
Injector temperature	**Conc (µg/mL)**	**Peak Area**	**Injector temperature**	**Conc (µg/mL)**	**Peak Area**
295ºC	20	167643	305ºC	20	165432
	20	163294		20	153834
	20	175432		20	158723
	AVG	168789		**AVG**	159329
	STDV	4387		**STDV**	5398
	%RSD	0.03		**%RSD**	0.03

Table 7: Inter day precision

Concentration	**Peak area**
20	123543
20	120848
20	120879
20	121964
20	127531
20	128329
Average	123849
Standard deviation	3319.677
%RSD	0.02

Table 8: intra precision

Concentration	**Peak area**
20	119754
20	128643
20	120762
20	128421
20	120523
20	129532
Average	136272
Standard deviation	3164.19
%RSD	0.2

Table 9: Results Showing Recovery for Cypermethrin

Standards	**Concentration**	**Analytical Peak Area**	**Bioanalytical Peak area**	**% Recovery**
LLOQ	10	26927.9	18999.8	70.5583
LQC	15	88286.4	60130.6	68.1084
MQC	20	166076.8	120848.4	72.7665
HQC	25	248792.2	186686.1	72.0174
UQC	30	348628.6	256171.6	73.4798

Table 10: Stability studies of Cypermethrin

Stability	**Standards**	**Concentration µg/mL**	**Mean recovered conc (µg/mL)**	**%Accuracy**	**SD**	**%CV**
Bench-top	LQC	10	43.53	96.5	0.567	1.45
	HQC	30	48.96	93.9	0.367	1.24
Freeze thaw	LQC	10	38.18	92.7	0.743	1.85
	HQC	30	40.48	93.05	0.643	1.35
Long term	LQC	10	38.96	95.85	0.854	1.64
	HQC	30	42.65	92.63	0.645	1.74

Conclusion

The bioanalytical method developed was simple, accurate and the parameters are within the acceptance level as per USFDA guidelines. It is implied from the findings that this method is suitable for the detection of Cypermethrin in biological fluids.

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