DETERMINATION AND QUANITIFICATION OF CYPERMETHRIN PESTICIDE RESIDUE IN CUCUMBER USING RP-HPLC

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ABSTRACT

Pesticides are very important in increasing the crop production but in large quantities they may cause health issues to humans. Cypermethrin is a pyrthroid pesticide. The Cypermethrin was estimated by using an ultra fast liquid chromatography (RP-HPLC) in cucumber. This method is fast, accurate and economical. Column used in the study Phenomenex Luna C18 column (250mm 4.60 mm 5). The mobile phase used in the study was Acetonitrile and methanol in 60:40 ratios. The run time was 6 minutes. The injection volume of the sample was 10 µL. The compound eluted at a wavelength of 235nm. Series of samples in a range of 10, 15, 20, 25 and 30µg/mL were prepared. The regression coefficient was found to be 0.995. The LOD and LOQ were 0.4 and 0.3µg/mL.

KEYWORDS: Pesticide, Cypermethrin, Cucumber, HPLC, Validation, ICH guidelines.

INTRODUCTION:

Cucumber is a regularly used vegetable and they are consumed raw, cooked and processed. [1] cucumbers plants are susceptible to many pests and diseases; these can be controlled only by

using pesticides. [1] The commonly used pesticide in cucumber is pyrethroids. Cypermethrin is a synthetic pyrethroid and is most commonly used as an insecticide. Cypermethrin is used in cucumbers to get rid of insects such as loppers, vegetable weevil and plague thrips. It has eight stereoisomers which are Cis and Trans forms. [3] It causes oxidative stress when regularly exposed. Cypermethrin toxicity may cause serious health issues and in higher doses it may even cause death. To avoid the health risk, the MRL was set by international organizations. The chemicals used in vegetables do not exceed the level (MRLs). The aim of the study is to measure the pesticide residue in the cucumber and to find if it's within the limit. [1] The maximum residue limit for Cypermethrin in cucumbers is set to 0.5mg/kg.

MATERIALS AND METHOD

Instrumentation

HPLC LC-20AD with PDA detector was used. Column used in the study Phenomenex Luna C18 column (250mm 4.60 mm 5). The mobile phase prepared was Methanol and Acetonitrile in 60:40 ratios. The run time was 6 minutes. The sample injection volume was 10 µL. The compound was eluted at a wavelength of 235nm.

Chemical and reagents

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

Analytical method development

Preparation of stock solution

Cypermethrin stock solutions were produced using 100mg Cypermethrin 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). Using stock solution series of dilutions were made to find the calibration curve (30-10µg/mL).

Preparation of sample

0.257ml of marketed formula which contains 10% Cypermethrin was weighed and diluted with HPLC grade methanol which gives 1000µg/mL concentration. From the above solution, 0.1 ml of the solution was added to 10ml volumetric flask which contain methanol and made up to the mark. The resulting solution was passed through 0.02µm syringe filter.

Vegetable sample

Sample collection

Cucumber samples were gathered from a local market of Mysore, Karnataka. These samples were placed in refrigerator and were analysed as early as possible. At the time of working the samples were taken out of the refrigerator and normalised to the room temperature.

Extraction procedure for samples

15g of sample were taken and sliced into small parts and blended using a homogenizer. The sample is added to 45ml of ethyl acetate. The sample was mixed with a magnetic stirrer for 2 minutes and then to the solution adds 20g of anhydrous sodium sulphate and 5g of sodium hydrogen carbonate. Then again place the solution on magnetic stirrer for 15 minutes. The organic layer is separated and left to dry, to remove any moisture. The resulting solution is again made-up with ethyl acetate and later vortexed for 5 minutes and centrifuged for 9 minutes with 10000 RPM. The solution is filtered using 0.4µm syringe filter which was later injected into the HPLC.

Spiked vegetable samples

About 15g of sample is taken and homogeneously chopped and then 1mg of Cypermethrin is and later 40mL of ethyl acetate is added and mixed thoroughly. Then 20g of anhydrous sodium and 5g of sodium hydrogen carbonate is added. The sample is mixed using a magnetic stirrer and made-up with ethyl acetate and the last solution is centrifuged for 8 minutes with 1000 RPM. The sample solution is filtered using a syringe filter and injected into HPLC.

RESULTS AND DISCUSSION

Analytical method validation:

According to the ICH guidelines the parameters were validated. The parameters validated were system suitability, linearity, precision, accuracy, system suitability, and robustness, limit of detection and limit of quantification. The RT was 3.4 minutes. The blank chromatogram, standard chromatogram and sample chromatogram was given in the Figure 1, 2 & 3 respectively.

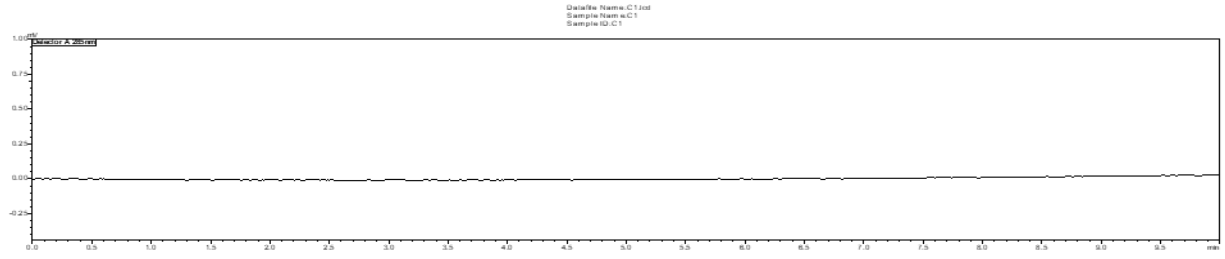

Figure 1: Blank chromatogram of Cypermethrin.

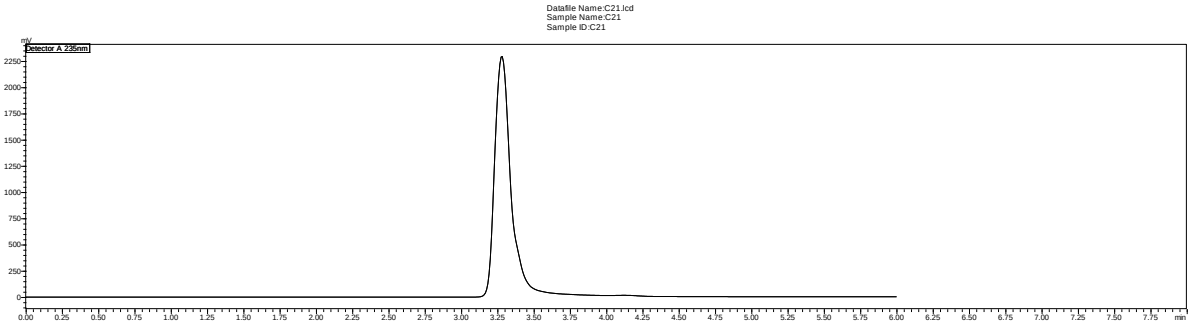

Figure 2: Standard chromatogram of Cypermethrin.

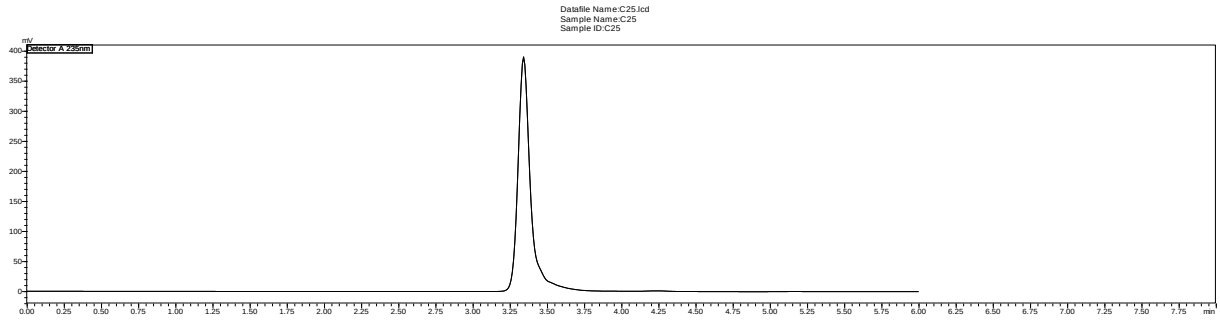

Figure 3: Sample chromatogram of Cypermethrin.

Calibration curve

A series of concentrations were prepared 10, 15, 20, 25, 30 µg/mL. the peak areas were analysed and plotted against the concentrations and the regression coefficient (r) was obtained which was greater than 0.99. The calibration curve is shown in Figure 4.

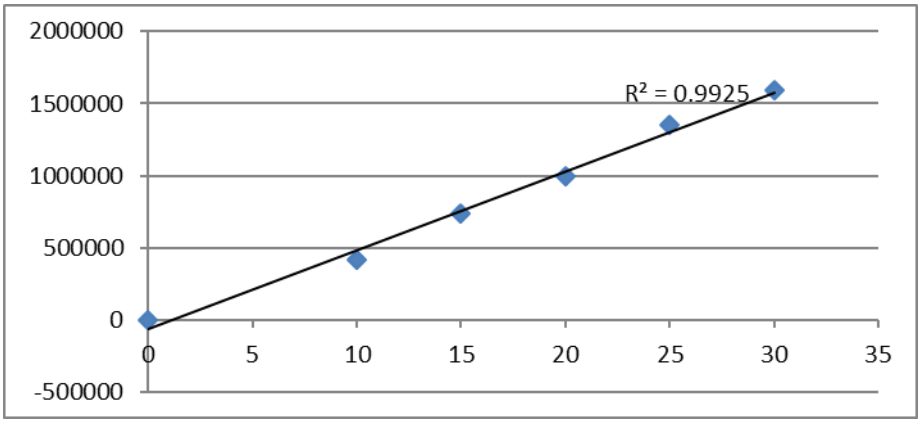

Figure 4: Calibration curve of Cypermethrin

System suitability

The system suitability was establised by injected the solution six times and the data has been collected. The system suitability data is given in Table 1.

Table 1: system suitability

Specification	**Approval Specification**	**Outcomes**
Tailing factor	Less than 2.0	1.1
Theoretical plates	Greater than 2000	43218

Accuracy

The accuracy was determined by spiking the formulation with 50%, 100% and 150% of standard Cypermethrin. Accuracy studies were given in the Table 2.

Table 2: Accuracy data

Level of recovery	**%recovery**	**Mean**
50	94.795	96.963
	96.543	
	98.873	
100	99.753	99.974
	98.406	
	101.302	
150	98.980	99.864
	99.098	
	100.957	

Precision

Precision of Cypermethrin was determined and the %RSD was analysed for six replicates. The intraday (repeatability) and inter day (intermediate) precision were shown in Table 3 and Table 4. **Table 3:** Inter day precision

Concentration (µg/mL)	**Peak area**
20	965342
20	970322
20	987543
20	996543
20	954873
20	987132
Average	976959.2
Standard deviation	1453.8

%RSD	0.4

Table 4: Intraday precision

Concentration (µg/mL)	Peak area
20	998289
20	965329
20	976523
20	964320
20	954302
20	997257
Average	976003.3
Standard deviation	1668.9
%RSD	0.2

Robustness

Robustness is a parameter of analytical method validation which is the measure of its ability to remain unaffected by small and deliberate changes of the system. Robustness studies were given in the Table 5.

Table 5: Robustness data

Wavelength (nm)	**Conc(µg/mL)**	**Peak Area**	**Wavelength (nm)**	**Conc(µg/mL)**	**Peak Area**
234	20	978989	236	20	943207
	20	954371		20	964276
	20	976523		20	985420
	AVG	969961		**AVG**	964301
	STDV	1106.7		**STDV**	1723.9
	%RSD	0.1		**%RSD**	0.01
Mobile phase	**Conc(µg/mL)**	**Peak Area**	**Mobile phase**	**Conc(µg/mL)**	**Peak Area**
Acn: MeOH (58:38 v/v)	20	967538	Acn: MeOH (62:42v/v)	20	974632
	20	987423		20	963262
	20	952864		20	957352
	AVG	969275		**AVG**	965082
	STDV	1734.9		**STDV**	8782.5
	%RSD	0.4		**%RSD**	0.3
Flow Rate	**Conc (µg/ml)**	**Peak Area**	**Flow Rate**	**Conc (µg/mL)**	**Peak Area**
	20	953753		20	974628

	20	987353		20	936395
1.1mL/min	20	963538	0.9mL/min	20	949859
	AVG	968214.7		**AVG**	953627.3
	STDV	1728.3		**STDV**	1939.06
	%RSD	0.2		**%RSD**	0.4

Limit of detection and limit of quantification

The LOD and LOQ of Cypermethrin were found to be 0.4 and 0.3 µg/mL.

Vegetable sample

The spiked cucumber sample and cucumber extract chromatograms are shown in figure 5 and 6 respectively.

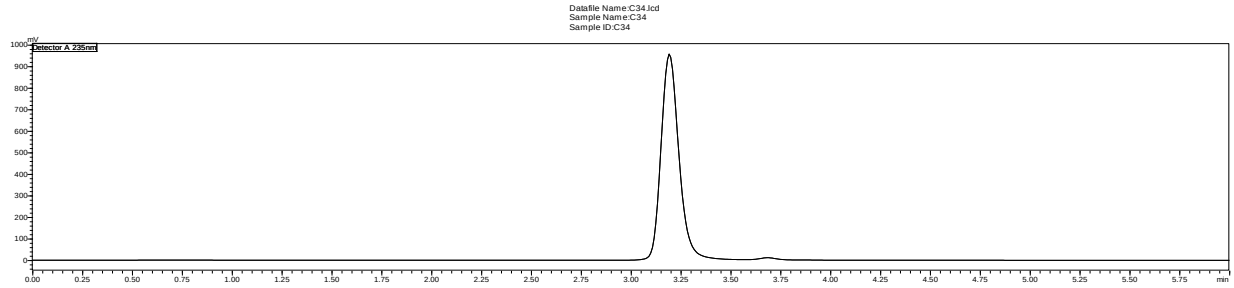

Figure 5: Chromatogram of spiked cucumber.

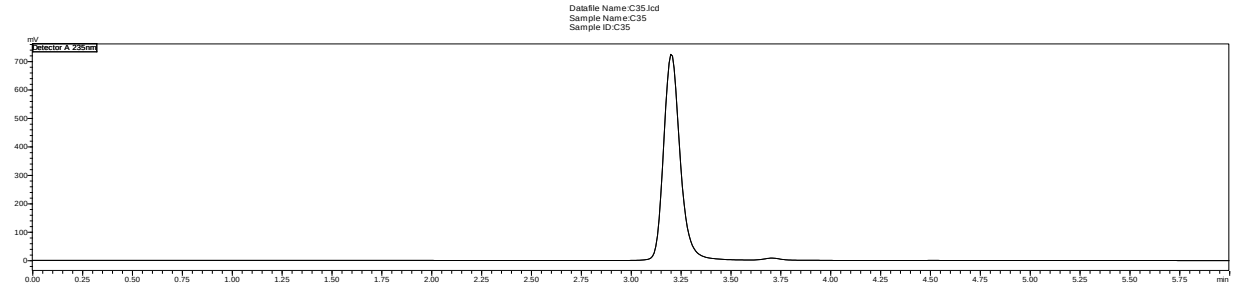

Figure 6: Chromatogram of cucumber extract

System suitability

The solution of Cypermethrin was injected six times and the data is given in Table 6.

Table 6: system suitability

Specification	**Approval Specification**	**Outcomes**
Tailing factor	Less than 2.0	1.3
Theoretical plates	Greater than 2000	45655

Calibration curve

A concentration range of 10, 15, 20, 25, and 30µg/mL were prepared. Calibration curve was determined and plotted against respective concentrations and the r^2 was calculated and it was found to be 0.9946. The calibration curve is shown in the figure 7.

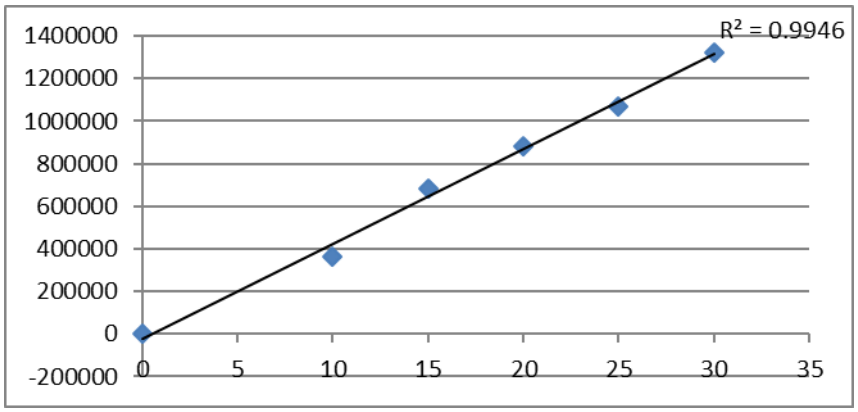

Figure 7: Calibration curve of Cypermethrin in Cucumber.

Accuracy

It is established by spiking the extract of cucumber with 50%, 100% and 150% of standard extract of cucumber which is spiked with Cypermethrin. They analysed the data was given in the Table 7.

Table 7: Accuracy data

Level of recovery	%recovery	Mean
50	97.34	97.02
	96.2	
	97.540	
100	98.17	98.73
	98.07	
	99.87	

150	98.98 100.7 98.84	99.56

Precision

The samples are repeated injected for about 6 times, the inter day (repeatability) and intraday (intermediate) was analysed and the % RSD is calculated. Data is given in Table 8 and 9.

Table 8: Inter day precision

Concentration (µg/mL)	**Peak area**
20	886363
20	876346
20	836383
20	896379
20	863629
20	892927
Average	875337.8
Standard deviation	2249.14
%RSD	0.2

Table 9: Intraday precision

Concentration (µg/mL)	**Peak area**
20	853535
20	836838
20	836363
20	873563
20	835353

20	893738
Average	854898.3
Standard deviation	2412.76
%RSD	0.1

Robustness

The ability of a sample to remain unchanged by any minor and intention changes in the parameters. It is checked by changing the flow rate and wavelength. The data is analysed and shown in the Table 10.

Table 10: Robustness data

Wavelength (nm)	**Conc(µg/mL)**	**Peak Area**	**Wavelength (nm)**	**Conc(µg/mL)**	**Peak Area**
234	20	837639	236	20	813873
	20	837373		20	829292
	20	836376		20	823737
	AVG	837129.3		**AVG**	822300.7
	STDV	665.82		**STDV**	7809.20
	%RSD	0.795		**%RSD**	0.9497
Mobile phase	**Conc(µg/mL)**	**Peak Area**	**Mobile phase**	**Conc(µg/mL)**	**Peak Area**
Acn: MeoH (58:42 v/v)	20	797594	Acn: MeoH (62:38 v/v)	20	783479
	20	769595		20	748979
	20	758478		20	794844
	AVG	775222.3		**AVG**	775767.3
	STDV	2016.5		**STDV**	2388.18
	%RSD	0.8575		**%RSD**	0.9482
Flow Rate	**Conc (µg/ml)**	**Peak Area**	**Flow Rate**	**Conc (µg/mL)**	**Peak Area**
1.1mL/min	20	849438	0.9mL/min	20	869699
	20	837384		20	868675
	20	849874		20	878595
	AVG	845565.3		**AVG**	872323
	STDV	7088.6		**STDV**	5455.789
	%RSD	0.5769		**%RSD**	0.8685

CONCLUSION

The method is easy, accurate and is validated according to ICH guidelines which include system suitability, linearity, precision, accuracy, LOD and LOQ and robustness. This approach can be used to find the pesticide Cypermethrin in vegetables like cucumber and we can determine if the vegetable can be safely consumed.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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