Original Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF CYPERMETHRIN BY UV SPECTROSCOPIC METHOD

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Abstract

Poisons could be chemicals, pharmaceuticals, solvents, insecticides, metal salts, corrosive liquids (acids, alkalis), natural toxins, or gases like carbon monoxide. Pure chemicals and other complex natural materials can also make up some toxins. The method employed in the study was used to estimate Cypermethrin, an organophosphate compound. Cypermethrin is an agricultural pesticide can cause significant harm to human health if its residue builds up in food. The purpose of this work was to create a UV approach for the quantitative analysis of cypermethrin. This work used ultraviolet-visible spectrophotometry to analyse cypermethrin, having an absorption range of 220–230 nm, which is a powerful tool for elucidating structural details. The methods implemented were linear, specific, and accurate, demonstrating the reliance of the suggested methods. Due to the short time of analysis, the developed methods outperformed other previously established methods.

Keywords: Poisoning, Organophosphates, Pesticides, UV spectroscopy.

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INTRODUCTION

Organophosphates are chemical compounds which are synthesized by reaction between alcohol and phosphoric acid were used as neurotoxins in second world war by German military.1 Organophosphate insecticides such as Cypermethrin, chlorpyrifos, Fonofos and Disulfoton were widely used as pesticides in cultivation and domestic use.2 More than 25000 chemical brands of OPs are available in U.S and the Environmental Protection Agency (EPA) monitors the product use.3

The mechanism of action of organophosphates are by inhibiting acetylcholinesterase irreversibility. The acetylcholinesterase enzyme is found in RBCs, nicotinic and muscarinic receptors in nerve.4 The plasma AchE is present in CNS white matter, heart and pancreas. A reduction in plasma AchE will result in reduction of cholinesterase activity in nervous system.5 The

organophosphates act by phosphorylating the serine hydroxyl group at the active site.6 The bond formed is irreversible which results in deactivation of AchE and build-up of acetylcholine at neuromuscular junction causing continues depolarization of skeletal muscle causing weakness.7 In CNS normal transmission is disturbed and the risk of danger is high if this block is not resolved within 24hrs.8

EXPERIMENTAL DETAILS

Reagents and Chemicals:

Pure Drug for Cypermethrin, used in the study, was supplied by Sigma Aldrich. Methanol was used as the diluent and was obtained from Rankem.

Instrumentation and Spectroscopic Conditions:

UV spectrometer was utilized here to develop and validate a method for estimation of Cypermethrin. The spectrophotometer was manufactured by Shimadzu, Japan. The method was carried out by setting the wavelength at 220 nm using methanol as the diluent.

Preparation of stock solution:

10mg of pure cypermethrin was carefully weighed and taken in to 10ml volumetric flask followed by dissolving and making up the volume using methanol. It was further marked as stock solution A.

Preparation of standard stock solution:

Serial dilutions were performed on the aforementioned stock solution, A, with methanol, to obtain concentrations of 100, 200, 300, 400, and 500 g/ml.

RESULTS:

Linearity

The regression data for cypermethrin showed a definite linear correlation over a calibration range of 100-500 g/ml. The linear regression equation was found to be Y = 0.001x + 0.2528 wherein R2 was found to be 0.9994. The graph obtained for the same is shown in Figure 1. The slope and intercept were observed to be 0.001 and 0.252 respectively. The calibration data obtained has been expressed in Table 1.

Accuracy

Table 2 shows the results of the accuracy studies and shows that the percentage amount detected was between 99.24 and 100.13 percent with a relative standard deviation of less than 2 percent. The specified process was used to evaluate the solutions.

Precision

The accuracy (intraday, Interday, repeatability measurement) results demonstrated outstanding reproducibility with the present relative normal deviation (RSD %) below 2.0 percent. The findings of this indicated an exceptionally exact method as presented in Tables 3 and 4.

Sensitivity

The linearity equation was found to be Y = 0.0318x – 0.0288. The limits of detection and quantitation for cypermethrin were estimated to be 0.502 g and 1.523 g/ml, respectively. The outcome for the same is shown in Table 5.

Repeatability

The six-fold investigation of 15 µg/ml Cypermethrin solution concentration was used to calculate repeatability, and the results showed a percentage range of 98 to 102 percent with an RSD of less than 2 percent. The results have been displayed in Table 6.

Robustness

By evaluating the absorbance when shifting the wavelength range from 220-230nm, the robustness of the method was determined. The outcomes have been enlisted in table 7.

Ruggedness

The absorbance for the various concentration solutions was calculated. The results for both items fall within the expected range. Table 8 lists the outcomes. The results demonstrated that the RSD percentage was under 2 percent.

Table 1: Calibration Data of Cypermethrin

Sl. No	Concentration (µg/ml)	Absorbance of Cypermethrin
1	100	0.353
2	200	0.461
3	300	0.555
4	400	0.655
5	500	0.764

Table 2: Accuracy Results for cypermethrin by UV Spectroscopy

Level of recovery	Amount of formulation	Amount of Pure drug	Total amount of drug	Absorbance	Difference	%Recovery	Mean Recovery
50%	10	200	210	0.464	0.003	96%	98%
	10	200	210	0.462	0.001	100%	
	10	200	210	0.466	0.005	98%	
100%	10	300	310	0.553	0.002	95%	96%
	10	300	310	0.551	0.004	97%	
	10	300	310	0.552	0.003	96%	
150%	10	400	410	0.659	0.004	97%	98%
	10	400	410	0.661	0.006	98%	
	10	400	410	0.656	0.001	100%	

Table 3: Results for Intraday Precision of Cypermethrin by UV Spectroscopy

Precision	Acceptance criteria	Concentration			Mean Absorbance			%RSD		
Intraday morning	%RSD ≤ 2.0%.	200	300	400	0.449	0.561	0.679	1.648	1.300	1.489
Intraday evening	%RSD ≤ 2.0%.	200	300	400	0.478	0.546	0.645	1.623	1.295	1.456

Table 4: Results for Interday Precision of Cypermethrin by UV Spectroscopy

Precision	Acceptance criteria	Concentration			Mean Absorbance			%RSD		
Interday morning	%RSD ≤ 2.0%.	200	300	400	0.474	0.546	0.659	1.667	1.325	1.422
Interday evening	%RSD ≤ 2.0%.	200	300	400	0.487	0.584	0.668	1.657	1.350	1.435

Table 5: Results for LOD & LOQ Of Cypermethrin by UV Spectroscopy

LOD (µg/ml)	0.133
LOQ (µg/ml)	0.446

Table 6: Results for Repeatability of Cypermethrin by UV Spectroscopy

Sl. No	Concentration (µg/ml)	Absorbance	STDV	% RSD
1	300	0.555	0.0015	0.2883
2	300	0.552		
3	300	0.553		
4	300	0.557		
5	300	0.554		
	Mean	0.555		

Table 7: Results for Robustness of Cypermethrin by UV Spectroscopy

Sl. No	Wavelength (nm)	Absorbance	STDV	% RSD
1	220	0.764	0.614	0.123
2	221	0.752		
3	222	0.731		
4	223	0.701		
5	224	0.655		
6	225	0.641		
7	226	0.623		
8	227	0.555		
9	228	0.528		
10	229	0.461		
11	230	0.353		
	Mean	0.614		

Table 8: Results for Ruggedness of Cypermethrin by UV Spectroscopy

	Concentration µg/ml	Trial 1	Trial 2	Mean	S. D	%RSD
By changing the analyst	100	0.351	0.355	0.353	0.389	0.052
	200	0.463	0.465	0.464	0.427	0.052
	300	0.557	0.554	0.555	0.488	0.095
	400	0.659	0.654	0.656	0.555	0.143
	500	0.758	0.765	0.761	0.625	0.192
By changing the instrument	100	0.358	0.354	0.356	0.355	0.002
	200	0.465	0.469	0.467	0.429	0.053
	300	0.555	0.562	0.558	0.490	0.096
	400	0.652	0.658	0.655	0.554	0.142
	500	0.761	0.769	0.765	0.627	0.194

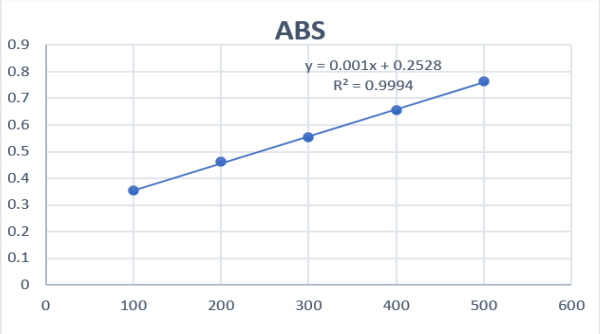

Figure 1-Linearity graph of Cypermethrin

CONCLUSION

For the purpose of detecting cypermethrin, the specified UV approaches have been developed. The methods have been evaluated in accordance with ICH principles. The methods are discovered to be linear, exact, and accurate, demonstrating the dependability of the suggested methods. Due to their quick analysis times, the developed methods outperform other established methods. Thus, cypermethrin may be routinely detected in both human serum and their formulations using the suggested UV techniques.

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