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Ultraviolet Spectrophotometric Method Development and Validation for Estimation of Pesticide Dimethoate in *Brassica oleracea*

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ABSTRACT: Indiscriminate use of pesticides in agriculture is concerned with the health of humans. Accumulation of residues in food and agricultural environment is risking ecological balance. Residues of dimethoate present in locally available variety *Brassica oleracea* commonly called as cauliflower were determined. A simple, sensitive, accurate and economical spectroscopic method has been developed for the estimation dimethoate in *Brassica oleracea*. An absorption maximum was found to be at 240nm with the solvent methanol. Results of the analysis were validated for accuracy, precision, LOD, LOQ and were found to be satisfactory. The proposed method is simple, rapid and suitable for the routine quality control analysis.

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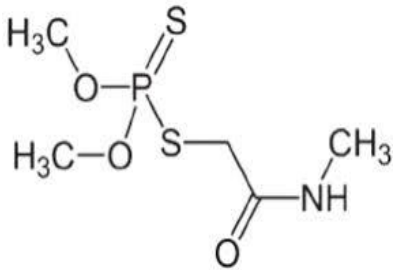
Keywords Dimethoate; *Brassica oleracea*.

INTRODUCTION

Dimethoate [1] is a widely used organophosphate insecticide and acaricide in agriculture. It was patented and introduced in the 1950s by American Cyanamid. Like other organophosphates, dimethoate is an acetylcholinesterase inhibitor which disables cholinesterase, an enzyme essential for central nervous system function. It acts both by contact and through ingestion. It is readily absorbed and distributed throughout plant tissues, and is degraded relatively rapidly. Dimethoate is used for apples, wheat, beans, grapes, pears, pineapples, plums, potatoes, tomatoes, lettuces, carrots, cauliflower .Various methods were available for the determination of dimethoate such as Gas chromatography-Mass spectrometry (GC/MS), HPLC-MS. A cheap, accurate and precise U.V method is developed for the determination of dimethoate in *Brassica oleracea.*



Structure of dimethoate:

Dimethoate IUPAC Name is *O, O*-dimethyl *S*-[2-(methylamino)-2-oxoethyl]dithiophosphate,CAS number is 60-51-5.The Brand name is TAFGOR manufactured by Rallis India limited.

Cauliflower is one of the several vegetables in the species *brassica oleracea* belonging to the family brassicaceae. Cauliflower have been associated with its ability to help in cancer prevention. It provides the support for the antioxidants in the body, detoxification and anti-inflammatory effects. It is rich source of vitamin C and K.

The EPA (Environmental Protection Agency) tolerance level for dimethoate is 0.5 to 2 mg/kg. If this limitation is crossed it may cause adverse effects like numbness, tingling sensations, in coordination, headache, dizziness, tremor, nausea, abdominal cramps, sweating, blurred vision, difficulty in breathing or respiratory depression and slow heart beat. Very high doses may result in unconsciousness and convulsion or fatality. Hence our project aims at detecting and quantifying, pesticide dimethoate present in *brassica oleracea*.

INSTRUMENTATION:

Instrument name	Instrument I.D
U.V/Visible spectrophotometer	LAB INDIA Analytical 2000 U
I.R Spectrophotometer	BRUKER ALPHA ECO-ATR

MATERIALS:

HPLC grade Methanol purchased from Merck (India) life sciences pvt.ltd. , Distilled water procured from Nirmalacollege of pharmacy, Atmakuru, Guntur district. Dimethoate 30% EC (Tafgor) manufactured by Rallis India ltd.

METHOD DEVELOPMENT

Procedure for calibration curve:

1ml of dimethoate solution was pippeted into a 100 ml volumetric flask and diluted with methanol to obtain 300µg/ml solution. From this solution 1ml was taken and make up the volume to 100ml with Methanol. From the above solution aliquots of 0.1, 0.2,0.3, 0.4 , 0.5, 0.6 ml were taken and volume was made up to 10ml with Methanol to obtain concentrations of 30,60,90,120,150 µg/ml. The calibration curve was constructed taking

ASSAY: For the estimation of dimethoate[3] 3gm of sample procured from Mulapadu, Ibrahimpatnam (mandal), krishna dist. was crushed in a mixer separately. The homogenate was extracted with the solvent methanol and centrifuged for 10min at 1000 rpm. The absorbance of sample was measured at 240 nm in U.V spectrophotometer.

Assay result:

The amount of dimethoate was found to be **0.1mg** in 3gm of *brassica oleracea* (cauliflower 1kg) sample procured from and **33.3mg** for the whole weight of *brassica oleracea* (cauliflower). The standard value according to Environmental protection agency (EPA) is 0.5 to 2 mg/kg.

FTIR STUDY DISCUSSIONS:

FTIR studies were conducted for pesticide dimethoate and *Brassica oleracea* (Cauliflower). The characteristic peaks were identified. The presence of dimethoate was confirmed by the FTIR studies.

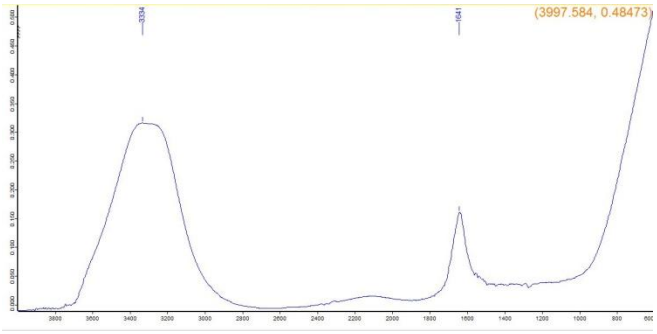


Fig no: 2 FTIR spectra for pesticide dimethoate

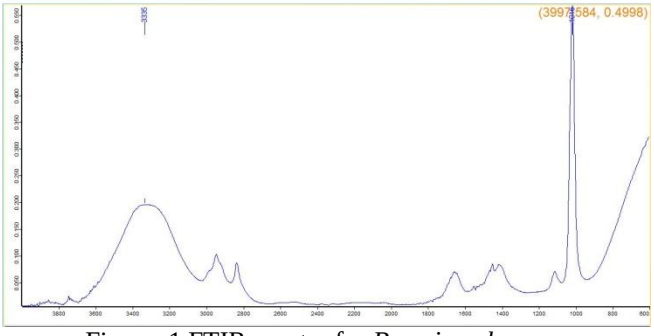


Fig no: 1 FTIR spectra for *Brassica oleracea*

VALIDATION PARAMETERS:

The UV spectrophotometric method was validated in accordance with ICH guidelines.

Linearity:

Fresh aliquots were prepared from stock solution ranging from 15µg to 210µg/ml. The samples were scanned in UV-Visible Spectrophotoeter using methanol as blank. The final concentration of each solution in µg/ml was calculated and plotted against absorbance. The slope, y-intercept were calculated.

Accuracy:

Accuracy of the method confirmed by studying recovery at 3 different concentrations for 50,100 and 150% of these expected, in accordance with ICH guidelines, by replicate analysis. Standard drug solution was added to a pre analysed sample solution and percentage drug content was measured. The results from study of accuracy were reported.

Precision:

The precision of the analytical method was studied by analysis of multiple sampling of homogenous sample. The precision was expressed as standard deviation or relative standard deviation. Six independent test samples of dimethoate were taken and the absorbance was measured at 240nm.

Robustness:

Robustness of the proposed method was determined by analysis of aliquots from homogenous lots by different

physical parameters like pH, wavelength. This is evaluated by carrying out the six replicate samples of dimethoate at 240 +_ 2nm. The relative standard deviation was found within the specified limits.

LOD AND LOQ :

LOD and LOQ were calculated by method based on the standard deviation (σ) and slope of calibration curve using the formula

$$LOD = 3.3\sigma / S$$
$$LOQ = 10\,\sigma / S$$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The LOD and LOQ were calculated as per above formula

Solution stability:

The solution stability of dimethoate in diluents were determined by storing sample solutions in a tightly capped volumetric flask at room temperature for 24 hrs. the amount of dimethoate were measured at different time intervals like initial , 6, 24 and 48 hrs and the results were compared with the freshly prepared dimethoate solution.

FORCED DEGRADATION STUDIES

The specificity of the method was demonstrated through forced degradation studies conducted on the sample using acid, alkaline, oxidative, reductive degradations. The sample was exposed to these conditions and the absorbance was studied thus indicating that the method effectively separated the degraded products from the active pesticide. Regulatory guidelines ICH Q2A, Q2B, Q3B and FDA 21 CFR section 211 requires the development and validation of stability-indicating potency assays.

Acid degradation studies

From the standard stock solution , 5ml was taken in 50 ml volumetric flask, add 1ml of 5N HCL and heated at 70°c for 1hour o a water bath. The flask was removed from the water bath and allowed to cool at room temperature. Add 1ml of 5N NaOH to neutralize the solution cooled at room temperature and diluted to volume with diluent and mixed. The absorbance of the solution was measuredat 240nm.

Alkali degradation studies

From the standard stock solution, 5ml was taken in 50 ml volumetric flask, add 1ml of 5N NaOH and heated at 70°c for 1 hour on a water bath. The flak was removed from the water bath and allowed to cool at room temperature. Add 1ml of 5N HCL to neutralize the solution,cooled at room temperature and diluted to volume with the diluent and mixed up to the volume. The absorbance of the solution was measured at 240nm.

Oxidation

From the standard stock solution 5ml was taken in 50 ml volumetric flask, add 1ml of 30% H_2O_2 and heated at 70°c for 1hr on water bath. The flask was removed from the water bath and allowed to cool at room temperature and diluted to volume with diluent and mixed. The absorbance of the solution was measured at 240nm.

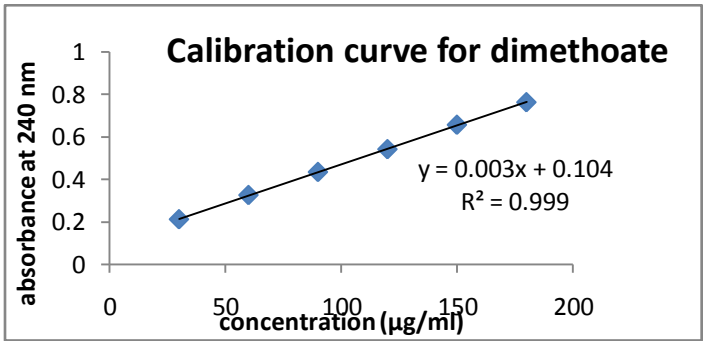
Reduction

From the standard stock solution 5ml was taken in 50ml volumetric flask , add 1ml of 10% sodium bisulphate and heated at 70°c for 1hr on water bath. The flask was removed from the water bath and allowed to cool at room temperature and diluted to volume with diluent and mixed. The absorbance of the solution was measured at 240nm.

RESULTS AND DISCUSSIONS

Calibration: Table 1:

S.No	Concentration (µg/ml)	Absorbance at 240nm
01	30	0.2128
02	60	0.3267
03	90	0.4352
04	120	0.5425
05	150	0.6573
06	180	0.7635



Calibration curve for dimethoate

Validation parameters:

Linearity:

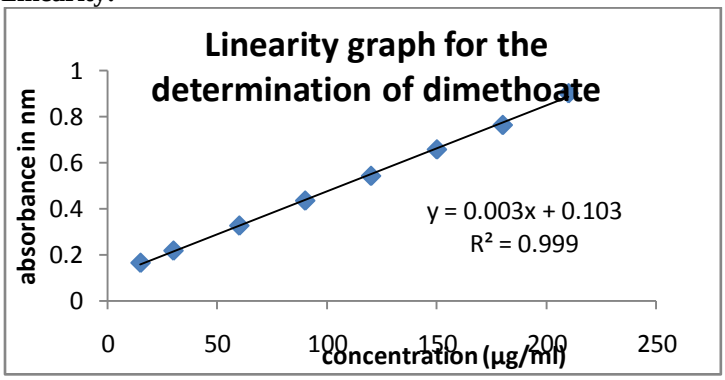


Fig no : 3 **Linearity graph for the determination of dimethoate**

S.NO	PARAMETER	RESULTS	
01	Linearity Correlation coefficient	15 ppm – 210 ppm R^2 = 0.999	
02	Accuracy	% Recovery = 100.13%	
03	Precision	Mean = 0.5474 S.D = 0.006 % R.S.D = 1.09%	
04	Robustness		
	(i) pH	5.0	5.4
		Mean = 0.4687 S.D = 0.0050 % R.S.D = 1.066%	Mean = 0.4524 S.D = 0.00047 % R.S.D = 0.103%
	(ii)Wavelength	238nm	242nm
		Mean = 0.6963 S.D = 0.0101 % R.S.D = 1.45%	Mean = 0.6759 S.D = 0.00860 %R.S.D = 1.272%
05	LOD & LOQ	185.4 & 561	
06	Solution stability	Stable upto 48 hrs	
07	Acid degradation	Mean = 2.8156 S.D = 0.0260 % R.S.D =0.923%	
08	Alkali degradation	Mean = 2.8448 S.D = 0.0111 % R.S.D = 0.390%	
09	Oxidation & Reduction	Not stable with oxidizing and reducing agents	

CONCLUSION

In the present investigation, new analytical method was developed for the estimation of dimethoate in *Brassica oleracea* using U.V Spectrophotometer. The presence of dimethoate was confirmed by FTIR studies. A simple, precise and robust method was developed for the estimation of pesticide dimethoate. It was concluded that high amounts of dimethoate residues were present in *Brassica oleracea.*

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