A new sensitive and facile Spectrophotometric Determination of Monocrotophos in Environmental, Biological and Agricultural Samples

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Abstract: - A highly sensitive spectophotometric method is developed for the determination of parts per million levels of widely used organophosphorus pesticide monocrotophos. The method is based on alkaline hydrolysis of monocrotophos to N-methylacetoacetamide followed by coupling with diazotized 2, 4- dinitrophenyl hydrazene in alkaline medium. The absorption maxima of the yellow coloured compound formed is measured at 490 nm. Beer's law is obeyed over the concentration range of 1.5 to 6.0 µg in a final solution volume of 25 ml. The molar absorptivity and Sandell's sensitivity were found to be $5.1x10^5$ (±100) l $mole^{-1}cm^{-1}$ and 0.008 µg cm^{-2} respectively. The standard deviation and relative standard deviation were found to be ± 0.006 and 2.5% respectively. The method is simple sensitive and free from interferences of other pesticides and diverse ions. The method has been satisfactorily applied to the detection/determination of monocrotophos in environmental, agricultural and biological samples.

Introduction:-Monocrotophos is widely used for agricultural practices, because of its high effectivity and relatively low price. Compared with organochlorine pesicides, it demonstrate relatively low environmental persistence, but a high toxicity. Pure monocrotophos is colourless crystals, soluble in water, acetone and aliphatic alcohols and slightly soluble in mineral oils. It has a mild ester odour. It is corrosive to black iron, drum steel, stainless steel and brass. [1-3] **Use: -** Monocrotophos (azodrin, nuvacron)an organophosphorus pesticide (IUPAC name dimethyl (E) 1-methyl-2-methylcarbamoyl) vinyl phosphate), is widely used in agriculture to control sucking , chewing and boring insects on a wide range of crops. It is mainly used against cotton pests, but can also be used on citrus, olives, rice, maize, sorghum, sugar cane, sugar beet, peanuts, potatoes, soya beans, vegetables, ornamentals plants, tobacco, coffee, bananas, melons, green beans, bell peppers, and strawberry. [4-6] MCP persists in soil in the dark for 30 days at neutral pH. It is hydrolyzed in alkaline conditions. Its solubility in water is 100% and hence it may appear in wastewater arising from MCP manufacturing units. When it is sprayed on crops, it may remain as soil residue and also enter water sources such as rainwater and ground water because of seepage through soils. [7-9] It do not have long persistence and are generally found at low levels in food Samples of dwarf beans potatoes, maize, citrus and some of its metabolites have been identified in the milk, muscle, and liver of cows and in the milk of goats following ingestion of this chemical. [10, 11] Monocrotophos is one of the most avian toxic pesticides known. Highly publicized deaths of over 5000 Swainson's Hawks in Argentina during the late 1990s. [12-15].

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The acute oral lethal dose (LD50) for rats is 14 mg/kg. According to WHO, the ingestion of 120 mg monocrotophos can be fatal to humans. Following exposure by any route, other systemic effects may include pallor, nausea, vomiting, diarrhea, abdominal cramps, headache, dizziness, eye pain, blurred vision, constriction or dilation of the pupils, tears, salivation, sweating and confusion. Respiratory failure or cardiac arrest may cause death. [16-25]. The easy availability in the market and high toxicity of monocrotophos makes it a preferred product for those attempting self-harm by consuming insecticides. There is a gross underreporting of poisoning cases in India, close to 20,000 cases in 2007. It also causes paralysis in children in cotton-growing areas in Brazil, Parana-State, Paraguay, Egypt, Indonesia, and Sri Lanka. Monocrotophos is classified as a highly hazardous pesticide by WHO in 2004. [26-35]. Because of the wide applicability and high toxicity of monocrotophos, numerous instrumental methods have been described for the detection/determination of monocrotophos, such as, infrared spectrophotometry,mass spectrometry, liquid chromatography, reversed-phase column liquid chromatography, high performance liquid chromatography, viva test system, biosensor, IPLB-LdFB cells, biochemical alteration, selective solid-phase extraction, modified wall-jet electrode, haemato-biochemical and immuno-pathophysiological studies etc. A few spectrophotomertic methods using sulphanilamide or sulphanilic acid,iron (III) complex,aromatic amine, and sodium iodide-bromide, and p-amino acetophenone have also been reported. [36-51]

In the present method, it is aimed to develop a simple sensitive, cost effective method for detection of monocrotophos.In this method monocrotophos is hydrolysed to give N-methyl acetoacetamide, which is further coupled with diazotized 2, 4-dinitrophenyl hydrazene in alkaline medium. The method has been applied to the determination of monocrotophos in various samples of polluted water, vegetables, fruits, foliages and biological samples

Experimental:- Apparatus: - A Systronics UV-VIS spectrophotometric (model 104) with matched silica cells was used for all spectral measurements. A Systronic pH meter (model 331) was used for pH measurements. A Remi C-854/4 clinical centrifuge force of 1850 g with fixed swing out rotors was used for centrifugation.

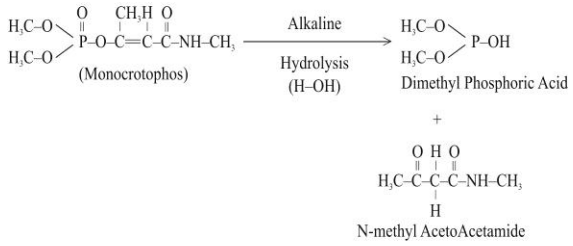
Reagent:-All reagents used were of Anal R grade or of the best available quality. Double distilled demineralized water was used throughout. A stock solution of monocrotophos (Hindustan Ciba-Geigy Bombay, India),was prepared by dissolving 1µg ml^{-1} in ethanol. A working standard solution was prepared by suitable dilution of the standard solution, as and when required. 1% m/v sodium nitrite solution was prepared in 10% v/v hydrochloric acid. 1.0 mol l^{-1} sodium hydroxide aqueous solution was used. 0.2% m/v solution of 2,4 Dinitrophenylhydrazene (Sigma-Aldrich) is prepared by dissolving appropriate weight in 250 ml 1M HCl solution.

Procedure: - An aliquot of test solution containing 1.5 - 6.5 mg of monocrotophos was taken in a 25 ml graduated tube, and then 1.0ml of 1M sodium hydroxide was added. The solution was kept in cool water (5-10^{0}C) for 30 min, then 1.0 ml of diazotzed 2, 4-dinitrophenylhydrazene were added to each. The solution was kept 15min for full colour development. It turns to yellow coloured species .The solution was then diluted to the mark with water and absorbance was measured at 490 nm against a reagent blank.

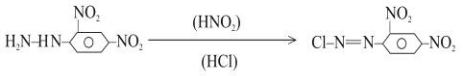
RESULTS AND DISCUSSION

Proposed Reaction Mechanism :-

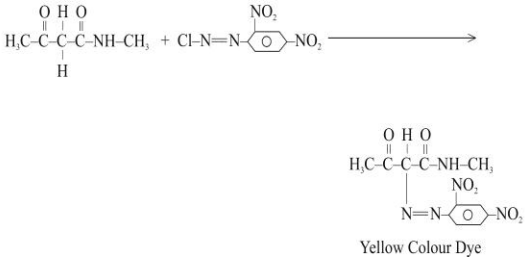
(1) Hydrolysis :-



(2) Diazotisation of 2,4 Dinitrophenyl Hydrazene



(3) Coupling Reaction



Absorbance Spectra:-The absorption maxima of the yellow coloured compound formed is measured at 490 nm. Beer's law is obeyed over the concentration range of 1.5 to 6.0 µg in a final solution volume of 25 ml. The molar absorptivity and Sandell's sensitivity were found to be 5.1x10^5 (±100) l $mole^{-1}cm^{-1}$ and 0.008 µg cm^{-2} respectively. (Fig.-1).

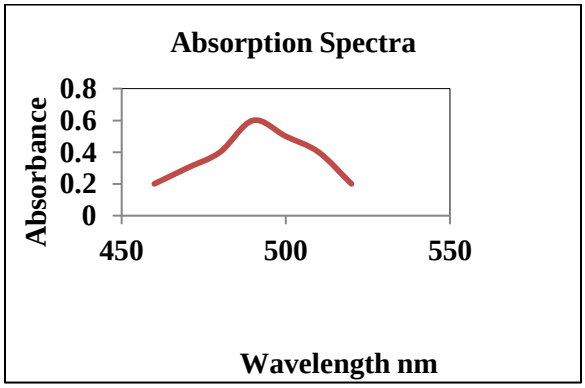


Figure1:-Absorption Spectra between monocrotophos and 2,4dinitrophenylhydrazene.

Effect of Time and Temperature: Hydrolysis of monocrotophos to N-methylacetoacetamide was studied at different temperatures and it was observed that maximum hydrolysis was observed with 1.0 mol L^{-1} sodium hydroxide at temperature range of 25-30°C.The time necessary for the full colour development was found to be 30 to 90 minutes. (Fig-2).

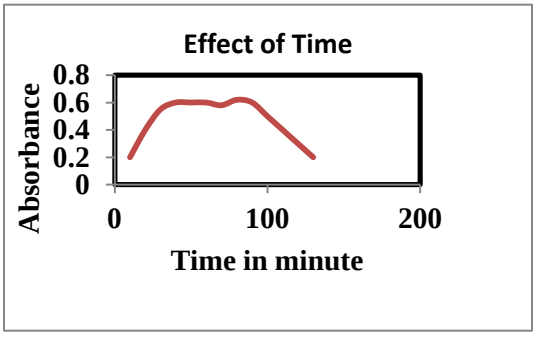


Figure 2:- Effect of Time on absorbance.

Effect of pH: The pH range of 8.0-10.0 was found to be the best for formation of yellow coloured dye and no buffer solution was used to stabilize the colour. Increase of pH above 10 severely affected the stability and sensitivity of the dye. Below pH 8.0, not suitable for yellow colour formation. (Fig-3).

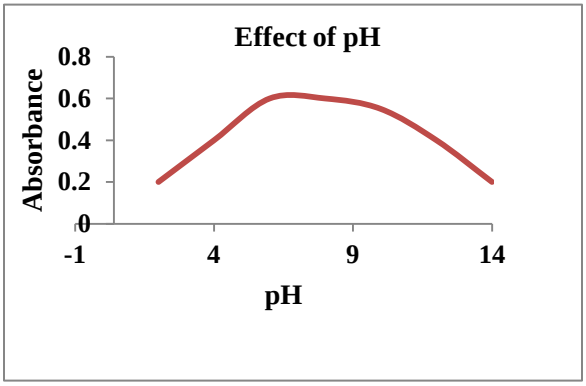


Figure 3:-Effect of pH on absorbance.

Effect of the Reagents Concentration:

A. Effect of 2, 4-dinitrophenylhydrazene (DNPH):-It was observed that 1 ml of diazotized 2, 4, dinitrophenylhydrazene was sufficient for complete colour reaction (Fig -4).

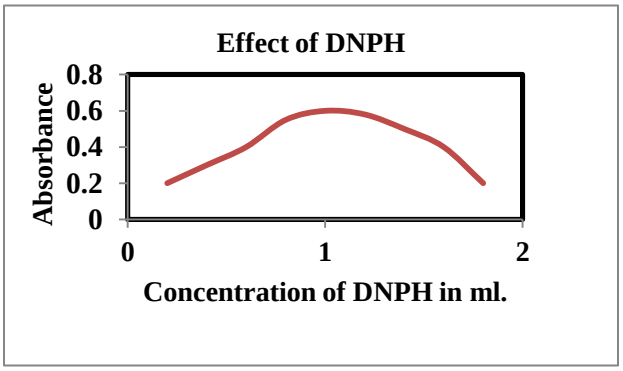


Figure 4:- Effect of the concentration of DNPH on absorbance.

B. Effect of the concentration of monocrotophos: - The constant and maximum absorbance of the yellow coloured compound formed is found at the concentration range of 5.0 µg in a final solution volume of 25 ml. (Fig-5).

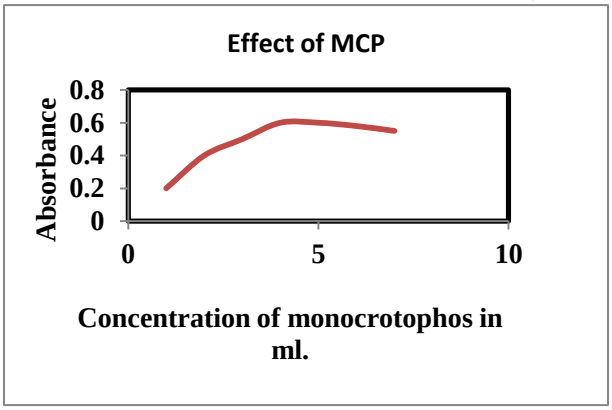


Figure 5:- Effect of the concentration of MCP on absorbance.

C. Effect of Sodium Hydroxide:- It was observed that maximum hydrolysis was observed with 1.0

mol L^{-1} sodium hydroxide at temperature range of 25-30°C as it gave maximum absorbance values, good stability and quantitative results. (Figure-6).

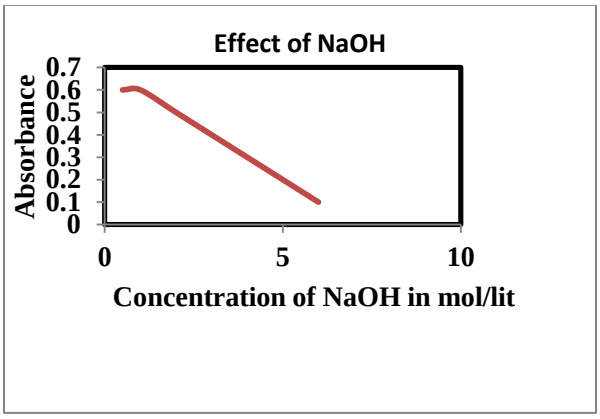


Figure 6:- Effect of the concentration of NaOH on absorbance.

D. Effect of the Foreign Species: The effect of common foreign species and pesticides was studied to assess the validity of the method. *Tolerance limit is amount of the foreign species that causes an error of ±2% in absorbance values.

Known amounts of foreign species and pesticide were added to the standard solution containind 5 of monocrotophos prior to hydrolysis and the solution was analysed by the proposed method .the method was found to be free from interference of most of the foreign species and pesticides. (Table -1).

Table1: - Effect of Foreign species and pesticides (Monocrotophos -5mg/25 ml.).

Foreign spe.	Tol. limit*	Foreign spe	Tol. limit*
Methanol	4500	Fe^{3+} Fe^{2+}	600
D.D.T, BHC	750	Ni^{2+}, Pb^{2+}	
Formaldehyde	700	SO_4^{2-}	450
Malathion, Parathion	500		350
Phorate,	500	Zn^{2+}	
Quinolphos Benzene	350	Co^{2+}	100
Toluene, Xylene	350	Cu^{2+}	100
	300		100
	100		

APPLICATIONS The proposed method has been applied satisfactorily to the determination of monocrotophos in various samples of polluted water, vegetable, fruits, foliages and biological fluids. **Determination of monocrotophos in polluted water:** Kharun River (Raipur) water sample, which received run off water from agricultural field, was collected. These samples were filtrated through a Whatman No. 40 filter paper. Aliquots of water samples were taken to 25-ml graduated tube and sodium hydroxide was added. They were kept in a hot water bath analysed as described above. (Table-2)

Table 2;-Determination of Monocrotophos in water and vegetable samples.

Samples	MCP originally found * (a)	MCP added (µg) (b)	Total MCP found by proposed method (c)	Difference (c-a)	Recovery % (c-a) -------- ×100 (b)
Polluted water**	2.23 2.72	2.5 4.0	4.61 6.48	2.38 3.76	95.2 94.0
Tomato***	4.10 2.73	2.5 4.0	6.50 6.51	2.40 3.78	96.0 94.5
Apple***	3.8 2.57	2.5 4.0	6.00 6.48	2.20 3.91	88.0 97.7

*Mean of three replicate analyses.

**Water sample 25 ml; after treatment 1 ml aliquot was analyzed.

*** Sample 25 gm (taken from agriculture field, 1 ml aliquot of sample was analyzed after treatment as described in procedure section).

Determination of monocrotophos in vegetable and fruits: Various sample of vegetable, fruits and foliages each of 50gm, were taken collected from agricultural field and local market. The samples were macerated with two 20 ml portions of ethanol-demineralized water (1+1), filtered though a thin cotton cloth and filtrate was centrifuged at 1850g for 10 min. Filtrate were diluted in 50ml flask by ethanol. In a 25 ml graduated tube 1ml.of this solution, 1.0 ml of 1.0 mol l^{-1} sodium hydroxide and 1ml. of diazotized2,4-dinitrophenylhydrazene were added. Then shaken thoroughly and kept at 0–5^0 C for 15 min for full colour development. (Table-2)

Determination of monocrotophos in biological samples: The method has been applied for the determination of monocrotophos in biological samples as the presence of monocrotophos in blood and urine has been reported in detectable concentration. [52-53] Synthetic samples were prepared by adding known amounts of monocrotophos to these samples and than analysed after removal of protein with trichloroacetic acid [54-55] as described above.
(Table-3:-Determination of monocrotophos in biological samples.

Samples	Amount of monocrotophos added (µg)	Monocrotophos found**(µg)	Recovery %
Urine*	2.5 5.0	2.42 4.77	96.8 95.4
Blood*	2.5 5.0	2.41 4.86	96.4 97.2

* Amount of biological samples =1ml, after treatment as described in procedure section.
** Mean of three replicate analyses.

Conclusion: - The reported reagent was selective for monocrotophos; The color of derivatives of monocrotophos with 2, 4-dinitro phenylhydrazine is stable at room temperature. The proposed method is simple, rapid and can be used for the determination of monocrotophos in trace amounts. The method has been successfully applied for the determination of monocrotophos in biological samples. The proposed method has been also applied satisfactorily to the determination of monocrotophos in various samples of polluted water, vegetable, fruits and foliages. The recoveries, of monocrotophos were added to

various samples of vegetables, fruits foliages and biological fluids and then analyzed by the proposed as well as the reported methods. [51] (Table-2). The recoveries were found to be 88 to 98%.

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