

Scientific paper

A Simple Spectrophotometric Determination of Meptyldinocap by its Hydrolysis

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Abstract

A simple spectrophotometric method is proposed for the determination of meptyldinocap (2,4-dinitro-6-octylphenyl crotonate). The method is based on the hydrolysis of meptyldinocap by hydroxylamine solution in alkaline medium to give 2,4-dinitro-6-octylphenol (2,4-DNOP), having maximum absorption at 380 nm. The reaction is found to be instantaneous in presence of ethanol. Beer's law is valid over the concentration range of 1.2–13 $\mu\text{g mL}^{-1}$ with molar absorptivity and Sandell's sensitivity of $3.22 \times 10^6 \text{ L mol}^{-1} \text{ cm}^{-1}$ and $0.0001 \mu\text{g cm}^{-2}$ respectively. The limit of detection and quantification were 0.0892 and 0.2703 $\mu\text{g mL}^{-1}$, respectively. The tolerance limits of interfering ions are discussed. All variables were studied in order to optimize the reaction conditions. The validity of the method was checked by its simultaneous determination in fruits and water samples and the results were statistically compared with those of a reference method by applying the Student's t-test and F-test.

Keywords: Spectrophotometric, Meptyldinocap, Hydroxylamine

1. Introduction

Meptyldinocap also known as "Karathane" is a contact fungicide with protective and curative action, effective in controlling powdery mildew on grapevine, but is also being developed for use on cucurbits and strawberries.^{1,2} The maximum residue limit (MRL) of meptyldinocap was $0.05 \mu\text{g g}^{-1}$ in mangoes set by the European Union (EC-No 839/2008).³ It is highly toxic to human and warm-blooded animals. It is reported to be carcinogenic, genotoxic and reproductively toxic on long term exposure.⁴

Due to its wide applicability and high toxicity, various methods have been reported for the determination of meptyldinocap such as HPLC,^{5–7} GC/IR and GC/MS,⁸ GC/ECD,⁹ TLC,¹⁰ LC-MS/MS,¹¹ and differential pulse voltametry.¹² Spectroscopic methods by diazotization coupling using reagents NEDA¹³ and by its hydrolysis using ethanolamine¹⁴ also have been reported for its determination.

Due to its toxic nature, it is important to develop some more simple and sensitive methods for the determination of meptyldinocap. The proposed method involves hydrolysis of meptyldinocap (2,4-DNOPC) by alkaline hydroxylamine solution to give 2,4-dinitro-6-octylphenol (2,4-DNOP)^{14–16} having maximum absorbance at 380 nm.

2. Experimental

2. 1. Apparatus

A Systronics Visiscan 167 Spectrophotometer was used for spectral measurements. Systronics pH meter, model 335 was used for pH measurement.

2. 2. Reagents

All the reagents were of analytical grade. Doubly distilled water was used throughout the experiments for preparation and dilution of reagents as well as samples. The experiments were performed using: meptyldinocap (48 % E/C Dow Agro Science Ltd., India), solvents: acetone, ethanol (Merck, Mumbai, India), sodium hydroxide (Merck, Mumbai, India) and hydroxyl ammonium chloride (Merck, Mumbai, India).

Stock solution (1 mg mL^{-1}) of meptyldinocap solution was prepared by dissolving 0.2083g of meptyldinocap in 100 mL acetone and stored in refrigerator. Working standards prepared by appropriate dilution of the stock.

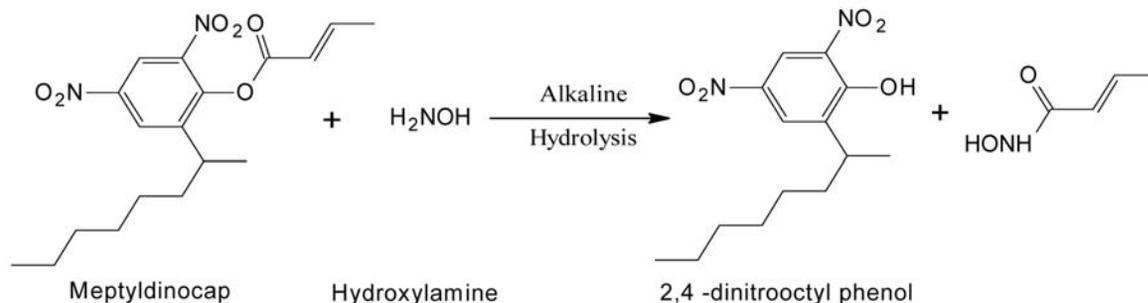
Alkaline hydroxylamine solution was prepared freshly by mixing equal volume of 2 mol L^{-1} hydroxyl ammonium chloride and 3.5 mol L^{-1} sodium hydroxide solutions. The solution kept on ice has been found to be stable for ~3 h.

2. 3. Procedure

An aliquot of working standards containing 30 to 325 μg of meptyldinocap was transferred into a series of 25 mL calibrated flasks, and to each flask 1 mL of 40 % ethanol and 2 mL of alkaline hydroxylamine solution were added, then diluted to mark with distilled water and mixed thoroughly. The yellow color of 2,4-dinitro-6-octylphenol was developed instantaneously at room temperature. The absorbance was measured at 380 nm against reagent blank.

3. Result and Discussion

The hydrolysis of meptyldinocap by alkali to 2,4-dinitro-6-octylphenol is a slow process; it takes several hours depending upon the strength of alkali and the temperature. In the reported method¹¹ meptyldinocap (2,4-dinitro-6-octylphenyl crotonate) was heated at 60 °C in an ultrasonic bath for 1 h to liberate 2,4-DNOP. In the present work 2,4-DNOPC was reacted with alkaline hydroxylamine solution with instantaneous development of colored species, *i.e.* alkaline hydroxylamine solution hydrolyses 2,4-DNOPC to 2,4-DNOP which gives characteristic absorption at 380 nm at room temperature (Scheme 1). The



Scheme 1: Reaction of meptyldinocap with alkaline hydroxylamine

reaction rate is increased when the reaction was performed in the presence of ethanol. The intensity and stability of colored species depends on the concentration of reagent.

3. 1. Effect of Sodium Hydroxide Concentration

The effect of sodium hydroxide concentration on the absorbance value was investigated in the concentration range of 0.5 mol L⁻¹ to 5 mol L⁻¹. Maximum absorbance was obtained at 3.5 mol L⁻¹ further increase in the concentration has no change in absorbance (Fig. 1).

3. 2. Effect of Hydroxylamine Concentration

The effect of hydroxylamine concentration on the absorbance value and reaction rate was studied in the concentration range of 0.5 mol L⁻¹ to 4 mol L⁻¹. Maximum re-

action rate and absorbance was obtained at 2 mol L⁻¹ hydroxylamine further increase in the concentration has no change in absorbance (Fig. 1).

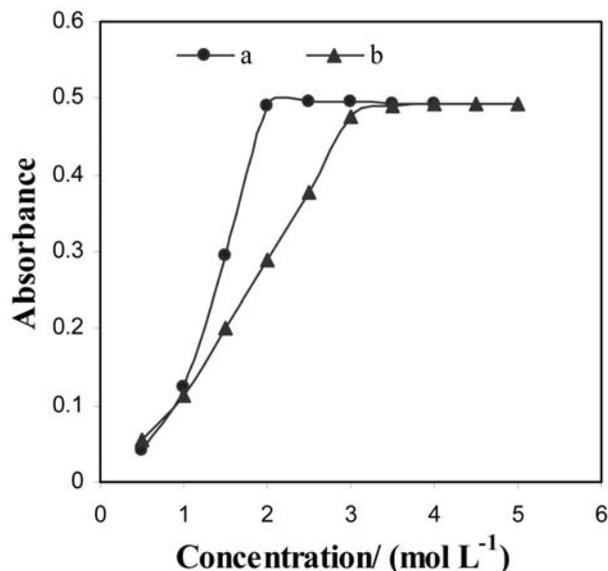


Fig. 1: Effect of concentration on the sensitivity. (a) hydroxylamine, (b) NaOH; Conditions: meptyldinocap: 8 $\mu\text{g mL}^{-1}$; 40 % EtOH; temperature: 25 °C

3. 3. Effect of pH

The effect of pH on the hydrolysis of meptyldinocap was studied in the range of 6 to 12. It has been found that the absorbance and reaction rate of the system increased till pH 10 and remained almost the same in the range 10 to 12 (Fig. 2).

3. 4. Effect of Time and Temperature

The kinetic characteristics of the proposed method were studied. After addition of alkaline hydroxylamine solution in the presence of ethanol, the color intensity of the detection system was recorded as a function of reaction time (Fig. 3). From the figure 3, it can be seen that there was a marked increase in the color intensity within few seconds of addition of reagent and it reaches maximum in about 3 min, after which it remains almost constant. To obtain high sensitivity and reproducible results, a

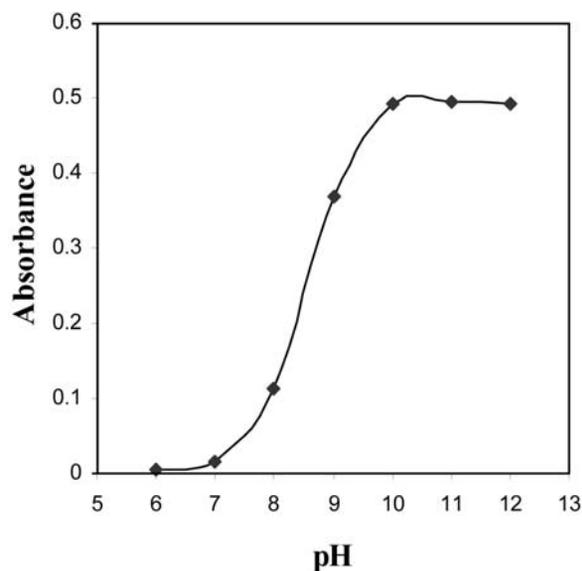


Fig. 2: Effect of pH on the sensitivity. Conditions: hydroxylamine: 2 mol L⁻¹; NaOH: 3.5 mol L⁻¹; 40 % EtOH; temperature: 25 °C

5 min reaction time was selected for further experiments. Further increase in the time there was no change in absorbance. Hence, a 5 min reaction time at room temperature was sufficient for routine analysis. It was also observed that there was no appreciable change in the absorbance if the order of addition of reagents were varied.

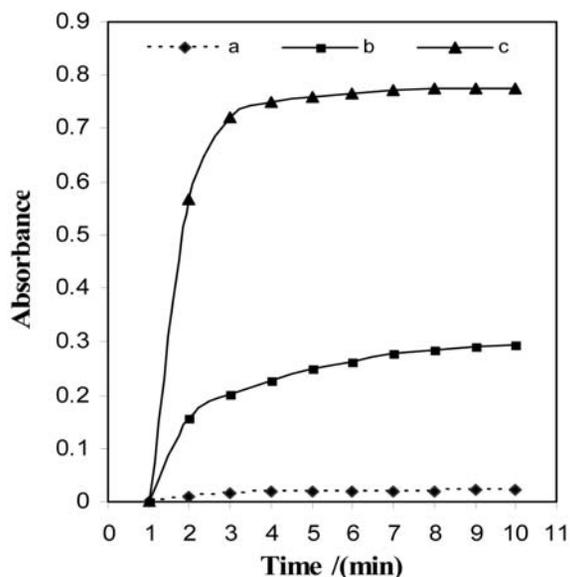


Fig. 3: Kinetic behavior of the proposed reaction. (a) alkaline hydroxylamine, (b) alkaline hydroxylamine + meptyldinocap (12 μg mL⁻¹), (c) alkaline hydroxylamine + meptyldinocap (12 μg mL⁻¹) +40 % EtOH

3. 5. Effect of Ionic Strength

The effect of ionic strength on the sensitivity of reaction was studied. The ionic strength was varied from 0.02 to 0.40 mol L⁻¹ using sodium chloride solution. The

results showed that this factor had no effect on the sensitivity of reaction up to 0.05 mol L⁻¹. A further increase of ionic strength caused a decrease in the sensitivity of reaction.

3. 6. Spectral Characteristics and Analytical Parameters

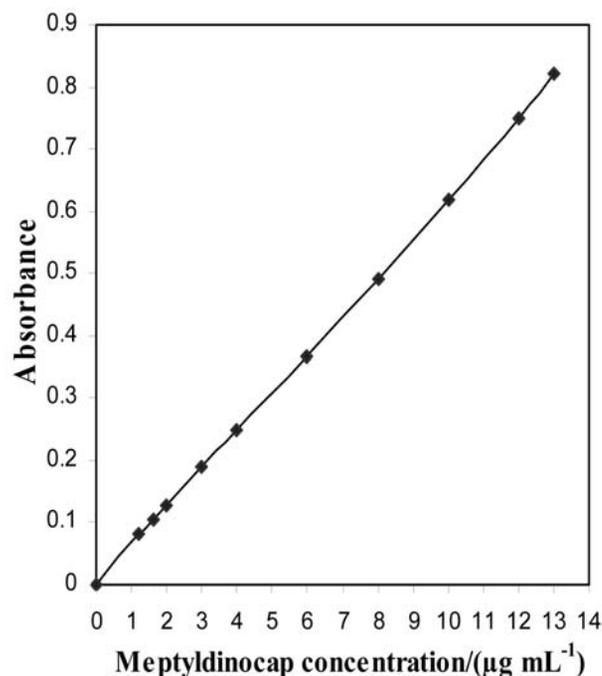


Fig. 4: Calibration line for determination of meptyldinocap. Conditions: hydroxylamine: 2 mol L⁻¹; NaOH: 3.5 mol L⁻¹; 40 % EtOH; temperature: 25 °C

The absorption spectra of final colored product showed a maximum absorbance at 380 nm. The reagent blank has negligible absorbance at this wavelength. Beer's law was obeyed over the concentration range of 1.2–13 μg mL⁻¹ for meptyldinocap (Fig. 4). The molar absorptivity and Sandell's sensitivity¹⁷ of meptyldinocap is given in Table 1. The slope, intercept, and the correlation coefficient were calculated by least square regression analysis (Table 1). The standard deviation¹⁷ and relative standard deviation¹⁷ for seven replicate determinations of 8 μg mL⁻¹ showed that the precision was good for the method (Table 1). Limit of quantification (LOQ) is evaluated by the relation 10 σ/s and the limit of detection is by 3 σ/s, where σ is standard deviation of the blank with respect to water and s is the slope of the calibration curve.

In order to evaluate the analytical applicability of the proposed method to the quantification of meptyldinocap in fruits and water samples, the results obtained by the proposed method was compared to those of the reference method¹³ by applying Student's t-test¹⁷ for accuracy and F-test¹⁷ for precision. The results (Table 1) show that the Student's t-test and F-values at 95 % confidence level are

less than the theoretical values, which confirmed that there is a good agreement between the results obtained by the proposed methods and the reference method with respect to accuracy and precision.

Table 1: Analytical and method validation parameters

Parameter	Dinocap
λ_{\max} (nm)	380
Limit of Beer's Law ($\mu\text{g mL}^{-1}$)	1.2–13
Molar absorptivity ($\text{Lmol}^{-1} \text{cm}^{-1}$)	3.2×10^6
Sandell's sensitivity ($\mu\text{g cm}^{-2}$)	0.0001
Limit of detection ($\mu\text{g mL}^{-1}$)	0.089
Limit of quantification ($\mu\text{g mL}^{-1}$)	0.270
Regression equation ($y = bx + a$)*	
Correlation coefficient (r)	0.999
Slope (b)	0.062
Intercept (a)	0.002
Standard deviation (\pm)	0.004
Relative standard deviation	0.76 %
t^{**}	0.683
F^{**}	1.147

* Concentration in $\mu\text{g mL}^{-1}$; ** The F and t value refer to comparison of the proposed method with the reported method. Theoretical F value at 95 % confidence level is 5.05; Theoretical t value at 95 % confidence level is 2.228.

3. 7. Interference Studies

In order to evaluate the suitability of the proposed method for the determination of meptyldinocap, in various environmental matrices, the effect of common interfering ions and pesticides on the reaction was studied by adding known amount of these compounds and ions to a standard meptyldinocap solution ($8 \mu\text{g mL}^{-1}$) and analyzed, the tolerance limit is given in Table 2.

Table 2: The effect of foreign species on reaction

Interfering ions and pesticides	Tolerance limit ($\mu\text{g mL}^{-1}$) ^a
carbaryl, propoxur	1000
Cd^{2+} , Mg^{2+} , Ca^{2+} , Sn^{2+} , CO_3^{2-} , NO_3^-	500
Fe^{2+b} , NH_4^+	450
Captan	450
moncozeb	400
SO_4^{2-} , CH_3COO^-	250
Cr^{3+} , Na^+ , K^+ , Mn^{2+} , Al^{3+}	200
Atrazine, pyrethroid pesticide	100

^a Causing (\pm)2 % variation in absorbance value; ^b masking by the addition of 2 mL of 0.4 mol L^{-1} EDTA

3. 8. Performance of Proposed Method and Statistical Comparison With Reported Methods

The proposed method was applied for the determination of meptyldinocap by spiking fruits and water sam-

ples with a known quantity of meptyldinocap and recovery studies carried out. The results obtained by the proposed method were confirmed by reported method.¹³ It is evident from Table 3 that there is no significant difference in the results between the spiked amounts and those determined by the proposed and reported method, indicating that the proposed method is an accurate and precise as the reported method.

4. Application

4. 1. Determination of Meptyldinocap in Water

To check the applicability of the method, the determination of meptyldinocap in polluted water from agriculture fields was carried out. The samples were found to be free from meptyldinocap. Therefore, known amount of meptyldinocap were added to polluted water samples. Aliquot of spiked water samples was extracted with 2×20 mL of benzene from 100 mL of sample. The benzene extract was evaporated off under reduced pressure to obtain the solid residue containing meptyldinocap. This residue was dissolved in ethanol and analyzed by the proposed as well as the reported method¹³ and the result is shown in Table 3.

4. 2. Determination of Meptyldinocap in Fruits

Fruits samples were collected from fields were meptyldinocap was sprayed. These samples were washed well with 2×20 mL of ethanol. The washings were analyzed by the proposed method. The fruits were found to have negligible amount of meptyldinocap. Therefore known amount of meptyldinocap was spiked to 25 g of mangoes and grapes and left for 24 h. The mango and grapes sample were chopped and macerated with 50 mL of acetone: methanol: 4 N HCl (100:10:5; v/v/v). The sample was centrifuged, and the supernatants were transferred to a clean bottle via a glass wool. An aliquot 30 mL of acetone: methanol: 4 N HCl (100:10:5; v/v/v) was added to the residue and macerated as previously. The sample was centrifuged, and the supernatants were combined. The total volume was adjusted to 100 mL using the extraction solvent. An aliquot of supernatants was evaporated off under reduced pressure and then residue is dissolved in ethanol and analyzed by proposed as well as the reported method¹³ and the result are shown in Table 3.

5. Conclusion

The proposed method is simple, rapid and sensitive as compared with other spectrophotometric method for

Table 3: Determination of meptyldinocap in various samples

Sample	Meptyldinocap added (μg)	Proposed method			Reported method		
		Total meptyldinocap recovery found ^a (μg)	SD ^b	%	Total meptyldinocap recovery found ^a (μg)	SD ^b	%
Water	50	47.40	± 0.03	94.80	46.20	± 0.04	92.40
	100	96.74	± 0.02	96.74	94.80	± 0.03	94.80
	200	198.80	± 0.02	99.40	196.68	± 0.03	98.34
Mangoes	50	47.87	± 0.04	95.74	47.16	± 0.04	94.32
	100	98.70	± 0.03	98.70	97.52	± 0.03	97.52
	200	199.20	± 0.03	99.60	197.60	± 0.03	98.80
Grapes	50	47.81	± 0.03	95.62	46.76	± 0.03	93.72
	100	97.82	± 0.03	97.82	96.80	± 0.03	96.80
	200	198.01	± 0.04	99.01	197.40	± 0.04	98.70

^a Mean of three observations. ^b Standard deviation. ^c The reported method involves the reduction of nitro group of meptyldinocap to amine and coupled with NEDA by diazotization coupling.

determination of meptyldinocap. The color development was rapid and involves less stringent control of experimental conditions including duration of time analysis and pH control, which make this method easy and more practical as far as the procedure is concerned. In addition, the proposed method neither involves solvent extraction nor employs sophisticated instruments. The statistical parameter and recovery study data clearly indicates the precision and accuracy of the method. Thus, it can be concluded that this method can be used as a chemical sensor for determination of meptyldinocap in water and fruits samples.

6. Acknowledgement

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Povzetek

Predlagamo preprosto spektrofotometrično metodo za določitev meptildinokapa (2,4-dinitro-6-oktilfenil krotonat). Metoda je osnovana na hidrolizi meptildinokapa z raztopino hidroksilamina v alkalnem, pri čemer nastane 2,4-dinitro-6-oktilfenol (2,4-DNOP), ki ima absorpcijski maksimum pri 380 nm. Reakcija poteče takoj v prisotnosti etanola. Beerov zakon velja v koncentracijskem območju $1,2\text{--}13\ \mu\text{g mL}^{-1}$, molska absorptivnost je $3,22 \times 10^6\ \text{L mol}^{-1}\ \text{cm}^{-1}$ in Sandellova občutljivost je $0,0001\ \mu\text{g cm}^{-2}$. Meja zaznave je $0,0892\ \mu\text{g mL}^{-1}$, meja določanja pa $0,2703\ \mu\text{g mL}^{-1}$. Razpravljamo tudi o mejah tolerance za moteče ione.

Preučevali smo vse spremenljivke z namenom optimizacije reakcijskih pogojev. Uspešnost metode smo preverili s hkratno določitvijo v vzorcih sadja in vode, rezultate pa smo statistično primerjali z rezultati referenčne metode; uporabili smo Studentov t test in F test.