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Determination of Carbendazim in Environmental Samples with Iron(III) and 1,10-Phenanthroline as Reagents

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A simple, rapid and selective spectrophotometric method was developed for the determination of carbendazim in environmental samples. The developed method is based on the oxidation followed by the formation of orange coloured chromogen complex by the reaction of carbendazim with ferric chloride and 1,10-phenanthroline which absorbs maximally at 470 nm. The analytical parameters and their effects on the reported method are investigated. Beer's law was obeyed in the range 5.0-70 μg mL^{-1} with correlation coefficient (r) 0.979. The molar absorptivity, Sandell's sensitivity, detection and quantification limits were also calculated. Interference study was carried for other pesticides and pollutants. The results of the analysis were validated statistically and the method was applied to the determination of carbendazim in various environmental samples.

Keywords: Carbendazim, Ferric chloride, 1,10-Phenanthroline, Environmental samples, Spectrophotometry.

INTRODUCTION

In agriculture plant diseases are inhibited by means of chemical products (fungicides, bactericides *etc*.). Carbendazim (methyl-benzimidazole-2-yl-carbamate) (Fig. 1) is a commonly used fungicide. It belongs to the benzimidazole group of compounds [1,2]. A broad range of diseases on cereals, fruits, cotton, tobacco, turf, ornamentals and vegetables are control by carbendazim pesticide. It is also used in post-harvest food storage and as a seed pre-planting treatment [3,4].

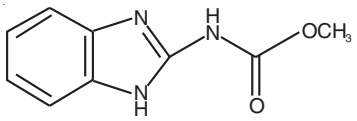


Fig. 1. Carbendazim (methyl-benzimidazole-2-yl-carbamate)

In soil and water carbendazim is quite stable and it is also reported to have an environmental half-life of up to 12 months. The soil persistence and the plant systemic nature of carbendazim can in turn lead to the contamination of water and plant products [5,6]. It can be treated as a dangerous substance. Its mutagenic and teratogenic effects on mammals have also been confirmed even when the substance was applied in a single and relatively small dose [1].

Different sophisticated techniques have been developed in the last few years for the detection of carbendazim such as adsorptive stripping voltammetry [7], liquid chromatography [8], gas chromatography-mass spectrometry [9], high performance liquid chromatography [10,11], infrared spectroscopy [12], fluorescence analysis [13,14], UV-visible spectrophotometric [3,15]. Some of these techniques suffer from poor sensitivity, analyses are limited to laboratory facilities and expensive due to its analytical cost and instability of colour or longer time required for full colour development. To overcome these drawbacks a rapid and sensitive method has been proposed for the determination of carbendazim. Spectrophotometry is considered the most convenient analytical technique because of its inherent simplicity, low cost and wide accessibility in most laboratories.

In the present study a simple, sensitive and cost effective method have been developed for the determination of carbendazim using ferric chloride followed by coupling with 1,10-phenanthroline reagent.

EXPERIMENTAL

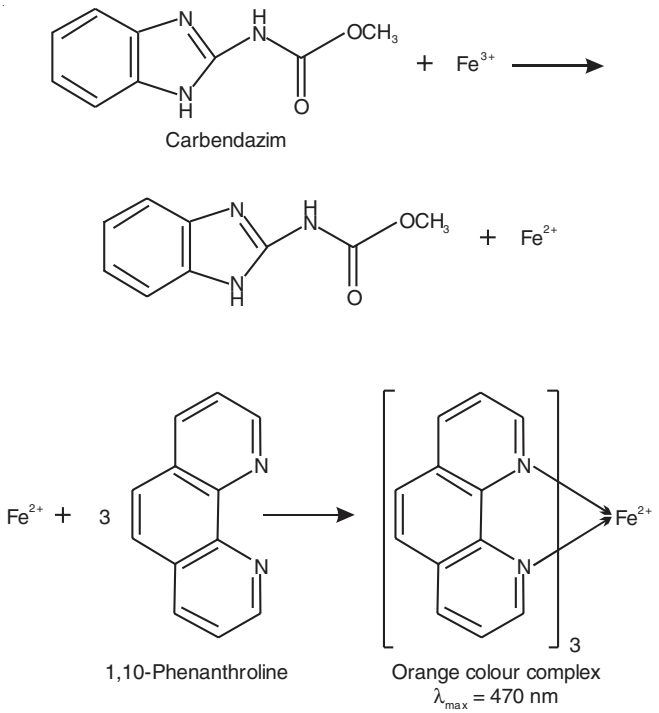
All the spectral measurements were made by a systronic UV-visible spectrophotometer model-104 with 10 mm matched silica glass cell. pH meter model Thermofisher Orion star A211 was used for pH determination. A Remi C-854/4 clinical centrifuge force of 1850 g with permanent swing out rotors was used for centrifugation. Calibrated glassware were

used after getting cleaned by soaking in acidified solution of potassium dichromate followed by washing with soap water and rinsing two times with distilled water.

All reagents used were of AnalR grade and double distilled water was used during the proposed experiment. A standard stock solution of 1 mg/mL solution of carbendazim (50 % W.P. Gharda Chemicals Ltd. Mumbai) was prepared in double distilled water. Working standard solution was prepared daily by successive dilutions of this stock solution. The aqueous solution of 0.2 % (w/v) ferric chloride was prepared by dissolving 200 mg of chemical in 100 mL of double distilled water and stored in dark bottle. The aqueous solution of 0.4 % (w/v) 1,10-phenanthroline (Merck) was prepared by dissolving 400 mg of chemical in 100 mL ethanol. 10 M hydrochloric acid (Merck) was prepared in double distilled water.

Experimental procedure: Aliquots of standard working solution of carbendazim containing 0.5-70 μg mL^{-1} were transferred into 10 mL volumetric flask. To this 1 mL of 1,10-phenanthroline and 1 mL of 0.2 % ferric chloride solution were added and the resulting solution was heated for 15 min at 70 °C. After heating the solution are cooled at room temperature and finally 1 mL of 10 M hydrochloric acid were added and the volume was made up to 10 mL with distilled water and the absorbance of the orange coloured chromophore was measured at 470 nm against the corresponding reagent blank [3].

Chemical reaction: Iron exhibits variable valency and exists as ferrous (Fe^{II}) and ferric (Fe^{III}) salts acts as a reductant and involved in complex formation with 1,10-phenanthroline which have a tendency to get oxidized. Carbendazim when reacted with known amount of iron(III) undergoes oxidation to give reduced form of ferric ion, *i.e.* Fe^{2+} ion has a tendency to give coloured complex with 1,10-phenanthroline (**Scheme-I**) [3,16].



Scheme-I: Reaction mechanism of carbendazim and 1,10-phenanthroline

RESULTS AND DISCUSSION

Spectral characteristics: The result obtained in the present method was based on the reduction of Fe^{3+} to Fe^{2+} followed by complex formation to form orange coloured chromophore exhibit maximum absorbance at 470 nm (Fig. 2) against the reagent blank. The optical characteristics of the proposed method such as absorption maxima, Beer's law limit (Fig. 3), molar absorptivity, Sandell's sensitivity, correlation coefficient and F-value obtained are presented in Table-1. The regression analysis using the method of least squares was made for the slope and intercept obtained from different concentrations are summarized in Table-1.

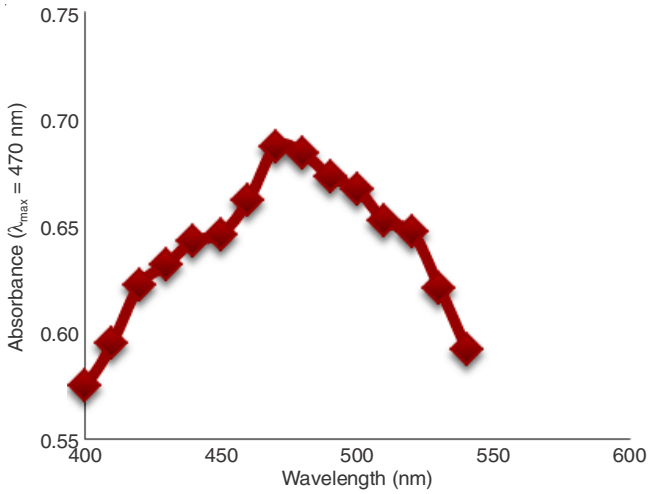


Fig. 2. Absorbance spectra of coloured product

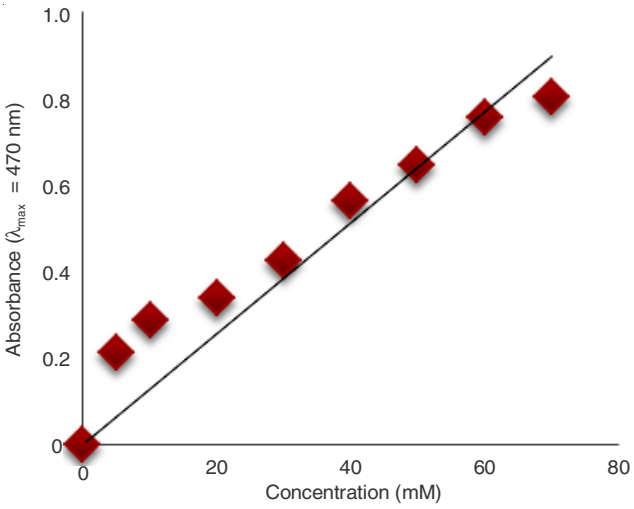


Fig. 3. Calibration curve of carbendazim

Effect of varying reaction conditions

Effect of acid concentration: The effect of different acids (such as sulphuric acid, hydrochloric acid, nitric acid and acetic acid) of same concentration has been studied. The result shows that the optimum acid was 10 M hydrochloric acid.

Effect of 1,10-phenanthroline concentration: In the present method to establish the optimum concentration of the reagent, different volumes of 0.4 % (w/v) solution of 1,10-phenanthroline were used (Fig. 4). The optimum volume used

TABLE-1
OPTICAL CHARACTERISTICS AND STATISTICAL DATA OF THE REGRESSION EQUATION FOR THE REACTION OF CARBENDAZIM WITH 1,10-PHENANTHROLINE

Parameters	Values for the reaction
λ_{max} (nm)	470
Colour	Orange
Beer's law limit (μg mL^{-1})	5.0-70
Molar absorptivity × 10^5 (L mol^{-1} cm^{-1})	3.25
Sandell's sensitivity × 10^{-3} (μg cm^{-2})	0.20
Detection limit (μg mL^{-1})	0.67
Quantitation limit (μg mL^{-1})	2.05
Relative standard deviation (%)	0.562
Regression equation[a]	
Intercept (a)	0.17
Slope (b)	0.0093
Correlation coefficient (r)	0.979
F[b]	1.38

[a]A = a + b C, where C is the concentration in μg mL^{-1}.
[b]Calculated F-values ; (Tabulated F-values for the degrees of freedom and 95 % confidence limits (p = 0.05) are 2.85).

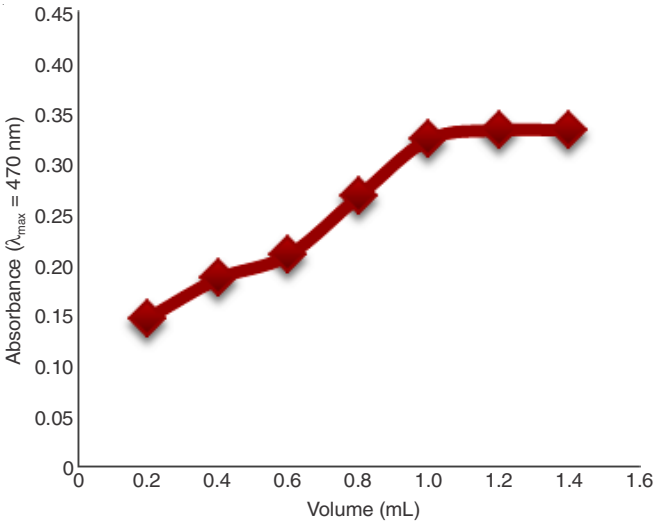


Fig. 4. Effect of reagent on the sensitivity; Conditions: Temperature: 70 °C, Time: 15 min, $FeCl_3$: 0.2 %, HCl: 10 M

for the production of maximum colour intensity is 1 mL with 10 M hydrochloric acid and 0.2 % solution of ferric chloride at 70 °C.

Effect of ferric chloride: The influence of $FeCl_3$ concentration on the sensitivity was studied with 10 M hydrochloric acid and 0.4 % (w/v) of 1,10-phenanthroline at 70 °C. The result shows that 1 mL of $FeCl_3$ is sufficient for full colour development (Fig. 5).

Effect of temperature and time: The effect of temperature on the reaction was also studied in the range of 30 to 90 °C with the optimum of the reagent concentration. The reaction does not proceed at room temperature and the reaction is catalyzed by heat and is maximum at 70 °C. The time required to complete the reaction is 15 min.

Effect of interferents: Method validity was assessed by investigating tolerance limit value of different foreign species and other pesticides in a solution containing 20 μg mL^{-1} of carbendazim. In this experiment solutions containing 20 μg mL^{-1} of carbendazim and various amounts of different foreign species and other pesticides were treated according to the

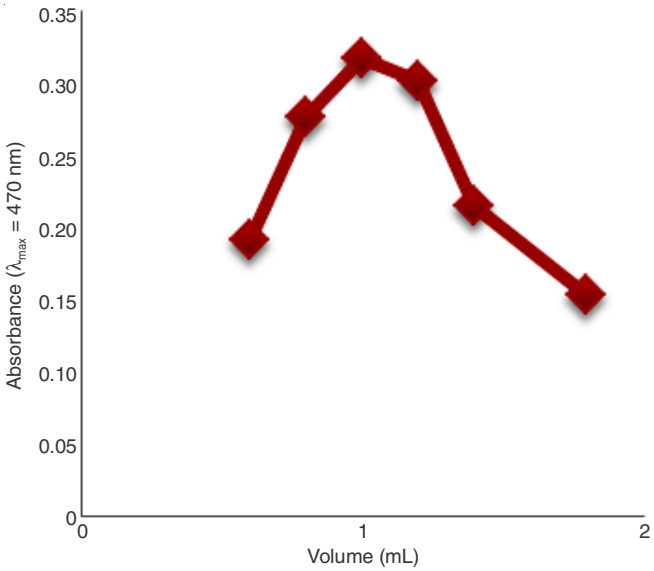


Fig. 5. Effect of $FeCl_3$ conc. on the sensitivity; Conditions: Temperature: 70 °C, Time: 15 min, Dye: 0.4 %, HCl: 10 M

recommended procedure. An error of ± 2 % in the absorbance reading was considered tolerable. It was found that the foreign species and other pesticides do not interfere with the proposed method (Table-2).

TABLE-2
TOLERANCE LIMIT OF FOREIGN SPECIES ON DETERMINATION OF 20 μg CARBENDAZIM

Foreign species	Tolerance limit* (μg mL^{-1})	Foreign species	Tolerance limit* (μg mL^{-1})
Cypermethrin	150	Ca^{2+}	850
Fenvalerate	200	Zn^{2+}	700
Isoprothiolane	50	SO_4^{2-}	500
Ethion	130	Mn^{2+}	400
Thiochlorprid	300	Al^{3+}	900
Prophenophos	450	Ba^{2+}	600
Bagon	100	Cu^{2+}	450

*Tolerance limit is the amount of foreign species that causes an error of ± 2 % in absorbance value.

Method validation

Linearity: By given experimental conditions for carbendazim determination, standard calibration curves were prepared by plotting an increase in absorbance *vs.* concentration. The statistical parameters were given in the regression equation calculated from the calibrated graph (Table-1). The linearity of calibration graph was prepared by high value of correlation coefficient (r^2) and small value of y-intercept of the regression equation. The molar absorptivity and relative standard deviation were also calculated and recorded in Table-1 [17].

Limit of detection (LOD) and limit of quantitation (LOQ): Limit of detection (LOD) is evaluated by the relation 3.3 σ/s and the limit of quantitation (LOQ) is by 10 σ/s, where σ is the standard deviation and s is the slope of the calibration curve (Table-1) [16].

Accuracy and precision: The accuracy and precision of the proposed spectrophotometric method was determined at two concentration levels of carbendazim by analyzing 3 replicate samples of each concentration. The relative standard

deviation (RSD) for the result does not exceed 2 % proving the high reproducibility of results and precision of the method.

Robustness and ruggedness: For the evaluation of method robustness, some parameters were interchanged like temperature, time, dye concentration, standard pH, *etc*. In these experiments, one parameter was changed whereas the others were kept unchanged and the recovery percentage was calculated each time. The results showed that small variation in the method variables did not affect the recovery values as shown in Table-3 [18].

TABLE-3
ROBUSTNESS OF THE PROPOSED SPECTROPHOTOMETRIC METHOD

Recommended condition	Carbendazim	Recovery % ± SD*
Standard pH	1.0	92.6 ± 0.505
	1.2	90.3 ± 0.529
Temperature (°C)	70	95.4 ± 0.948
	65	91.1 ± 0.880
Reaction time (min)	15	97.1 ± 0.529
	17	97.1 ± 0.911

*Values are mean of three determinations.

Determination of carbendazim in polluted water: Water samples from rivers receiving run off from various agricultural fields, where carbendazim were sprayed are collected. Then these samples are filtered through Whatman No. 40 filter paper. Now the water is evaporated to dryness and the residue was dissolved in 50 mL of ethanol. Aliquot of water samples were taken in 10 mL volumetric flask, followed by the addition of 1 mL of 1,10-phenanthroline and 1 mL of 0.2 % ferric chloride solution and analyzed as described above. Synthetic sample were prepared by adding known amount of carbendazim and kept for 3-4 h and analyzed as described above. The results are summarized in Table-4.

Determination of carbendazim in different vegetables, fruits, grains and soil: Various samples of vegetables, fruits and soil (each 5 g) were collected from agricultural fields, where carbendazim had been sprayed as a fungicide. The samples were macerated with 20 mL portions of ethanol:double distilled water (1:1) filtered through a Whatman filter paper No. 40 and the filtrate was centrifuged at 1850 rpm for 10 min. In case of vegetables, grains and fruits, the filtrate was quantitatively transferred into 50 mL calibrated flask and made up to the mark with distilled water. 5 mL aliquot were taken in a 10 mL volumetric flask, added 1 mL of 1,10-phenanthroline and 1 mL of 0.2 % ferric chloride solution and analyzed as described above. Synthetic sample were prepared by adding known amount of carbendazim and kept for 3-4 h and analyzed as described above. The recoveries range from 87.8-97.1 % and the results are summarized in Table-4.

Conclusion

The proposed method is simple, sensitive, rapid and can be used for the determination of carbendazim in trace amounts in different environmental samples. Additional advantages of this method are that colour develops instantaneously. Furthermore, this method does not involve the elaborate clean up procedures and this method is good alternative to some reported costly instrumental method. The technique also been statistically evaluated and free from the interferences by excipients and the results obtained are accurate and precise as indicated by good recoveries of the pesticide and low RSD values.

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TABLE-4
DETERMINATION AND RECOVERY OF CARBENDAZIM IN VARIOUS ENVIRONMENTAL SAMPLES BY PROPOSED METHOD

Sample	Carbendazim originally found: Proposed method (µg/mL)	Carbendazim added (µg/mL)	Total carbendazim found: Proposed method (µg/mL)	Recovery (% ± R.S.D)*
Water**	2.3	20	21.76	97.3 ± 0.612
	1.72	10	11.07	93.5 ± 0.801
Soil***	3.6	20	22.12	92.6 ± 0.544
	2.59	10	11.62	90.3 ± 0.698
Cotton***	1.21	20	20.53	96.6 ± 0.343
	0.62	10	10.05	94.3 ± 0.636
Rice***	2.86	20	19.04	95.2 ± 0.474
	1.13	10	9.43	94.3 ± 0.530
Ladyfinger***	1.78	20	20.87	95.4 ± 0.874
	0.92	10	10.03	91.1 ± 0.905
Cabbage***	3.09	20	22.17	95.4 ± 0.745
	2.29	10	11.07	87.8 ± 0.904
Potato***	2.63	20	21.62	94.95 ± 0.930
	1.09	10	9.08	90.8 ± 0.841
Beans***	3.47	20	22.89	97.1 ± 0.780
	2.19	10	11.90	97.1 ± 0.781
Tomato***	0.96	20	19.62	93.3 ± 0.855
	0.38	10	9.87	94.9 ± 0.634

*Recovery was calculated as the amount found/amount added × 100. Values are mean ± R.S.D. for three determinations; **Sample taken = 20 mL, ***Sample taken = 5 g.

REFERENCES

1. P.C. García J.M. Ruiz, R.M. Rivero L.R. López-Lefebre, E. Sánchez and L. Romero, *J. Agric. Food Chem.*, **50**, 279 (2002).
2. A. Kalwasinska, J. Kesy, W. Donderski and E. Lalke-Porczyk, *Pol. J. Environ. Stud.*, **17**, 515 (2008).
3. K.P. Naidu, T. Niranjan and N.V. S. Naidu, *Int. J. ChemTech. Res.*, **3**, 1728 (2011).
4. N. Pourreza, S. Rastegarzadeh and A. Larki, *Talanta*, **134**, 24 (2015).
5. X. Zhang, Y. Huangn, P.R. Harvey, H. Li, Y. Ren, J. Li, J. Wang and H. Yang, *PLoS ONE*, **8**, e74810 (2013).
6. H. Fang, Y. Wang, C. Gao, H. Yan, B. Dong and Y. Yu, *Biodegradation*, **21**, 939 (2010).
7. A.M. Ashrafi, J. Dordevic, V. Guzsvany, I. Svancara and T. Trtic-Petrovic, *Int. J. Electrochem. Sci.*, **7**, 9719 (2012).
8. E. Mallet, D. Barcelo and R. Tauler, *Chromatographia*, **46**, 342 (1997).
9. R. Paranthaman, A. Sudha and S. Kumaravel, *Am. J. Biochem. Biotechnol.*, **8**, 1 (2012).
10. L. Lin, C. Yang, Z. Peng and M. Wang, *Adv. Mater. Res.*, **781-784**, 491 (2013).
11. X.S. Liu, Z.F. Tong and L. Zheng, *Chin. J. Anal. Lab.*, **25,** 74 (2006).
12. G. Meszlenyi, J. Kortvelyessy, E. Juhasz and M. Lelkes, *Analyst*, **115**, 1491 (1990).
13. X.T. Jiang and M. Ding, *Chin. J. Anal. Chem.*, **17**, 823 (1989).
14. S.H. Zhu, H.L. Wu, B.R. Li, A.L. Xia, Q.-J. Han, Y. Zhang, Y.-C. Bian and R.-Q. Yu, *Anal. Chim. Acta*, **619**, 165 (2008).
15. Q. Li andW.X. Li, *Deciduous Fruits*, **3,** 47 (2007).
16. B.M. Gurupadayya, M.N. Trinath and K. Shilpa, *Indian J. Chem. Technol.*, **20**, 111 (2013).
17. G. Ayman, A. Alaa, E.-S. Ragaa and A. Magda, *Chem. Ind. Chem. Eng. Q.*, **16**, 1 (2010).
18. S.M.A. Ahmed, A.A. El-Zomrawy, A.S.N. Al-Kamali and K.A.S. Ghaleb, *Arab. J. Chem.*, **8**, 500 (2015).