Spectrophotometric Method for the Determination of Carboxin in its Formulations and Environmental Samples

C.SWARNA, K.PURUSHOTHAM NAIDU,
G.NAGENDRUDU, N.V.S.NAIDU[*] and K.SARASWATHI

Department of Chemistry
S.V.University, Tirupathi-517502, A.P., India
swarnalahari@gmail.com

Abstract: Simple, precise, rapid, sensitive and accurate spectrophotometric methods have been developed for the estimation of carboxin in pure form and in its formulations. The first method is based on oxidative coupling of carboxin with 1,10-phenonthrolin in presence of ferric chloride to form orange colored product with λ_{max} of 510 nm.The product obeyed Beer's law in the concentration range 1-10 mL (10 to 100 µg/mL) with molar absorptivity of 1.1425×10^3 Sandell's sensitivity 0.2061. The second method is based on the reaction of carboxin with 2,2′-bipyridine to form orange red colored product exhibiting λ_{max} of 522 nm with molar absorptivity, Sandell's sensitivity 2.2605×10^3, 0.1041 respectively. It obeys Beer's law in the concentration range of 0.5-50 mL (5 to 50 µg/mL). The assay of results was found to be in good agreement with label claim.

Keywords: Spectrophotometry, Carboxin, 1,10-Phenonthroline, 2,2′-Bipyridine, Ferric chloride, Orthophosphoric acid

Introduction

Carboxin (2,3-dihydro-6-methyl-oxathiin-5-carboxanilide, vitavax) is one of the several systemic fungicide (Figure 1) used in agriculture to control pathogenic fungi. Pesticides are widely used to protect the crops from a variety of pests. The use of pesticides benefits in increasing agricultural production but the repeated and indiscriminated use of certain pesticides have led to their accumulation in plants, animals, solid and sediments this effecting widespread contamination of the environment[1]. Such application of pesticides has the drawback of pesticide residues which remain in fruits and vegetables, constituting a potential risk to consumers. Fruits and vegetables are the foods that receive the highest doses of pesticides[2].
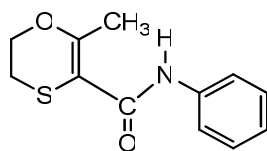
Figure 1. Structure of carboxin

Carboxin is anilide fungicide and intensively applied at various stages of cultivation and during post harvest storage to provide protection against rotting[3]. Although it has low mammalian toxicity, fungicide residues levels in food stuffs are generally legislated to minimize the exposure of consumers to the harmful or unnecessary intake of pesticides[3].

Most analytical methods for pesticide analysis are based on chromatographic techniques by both gas chromatography (GC) and high performance liquid chromatography (HPLC). HPLC is obviously preferred approach for polar less volatile and thermally labile pesticides such as carbamate. HPLC is an effective method for separating and analyzing the various carbamate pesticides employing different detectors[4,5]. Phototransformation of carboxin takes place when it is dissolved in water and it causes aquatic animals. Toxicity of the pesticide and its sulfoxide to aquatic organisms are reported[6]. The determination of pesticides applied in soya cultivation by using C8 co-column and subsequent chromatographic analysis by HPLC-DAD was developed. It proved that good recuperation for carboxin in soya cultivation[7]. Mechanism of action of carboxin and the development of resistance in yeast has already reported. Carboxin prevents the growth of yeast by inhibiting protein synthesis[8]. Carboxin and oxycarboxin undergo photolytic reactions in the presence of organic and inorganic soil components. Humic and fulvic acids in aqueous solution lead to enhanced photodegradation of carboxin[9]. The extraction of carboxin from cabbage samples using florisil sorbent solid phase extraction following with HPLC-UV analysis has been used as a reliable tool in residue analysis. The carboxin residues found in the cabbage sample with the safety label are likely to be lowerlevel than those in the sample without safety label[10]. All the above methods are expensive require long separation times for analysis. No spectrophotometric method is reported so far, for the determination of carboxin. In this work we adopted two simple spectrophotometric methods for the determination of carboxin in its food grains and water samples, formulations. These methods are based on the oxidation of carboxin with ferric chloride and subsequent chelation of the resulting iron(II) with 1,10-phenonthroline (1st method) and with 2,2′-bipyridine (2nd method). The resulting colored products were measured at 510&522 nms against the corresponding reagent blank respectively.

Experimental

A Shimadzu UV visible spectrophotometer (UV-2450) with 1 cm quartz cells was used for all the spectral measurements. All the chemicals used were of A.R. grade.

Chemicals and reagent

Methanol, 1,10-phenanthroline, ferric chloride, orthophosphoric acid, 2,2′-bipyridine is obtained from sigma aldrich and SD fine chemicals India. Sample of pesticide and internal standard were obtained from dhanuka agro chemicals limited Haryana, India respectively. Double distilled water was used to prepare all solutions for the method.

Preparation of the standard solution

About 100 mg of carboxin pure or equivalent formulation powder was accurately weighed and dissolved in 20 mL of methanol filtered by whatman filter paper No 41 and to remove the insoluble matter. The residue was washed with 10 mL portions of methanol three times and the total volume of the filtrate was made up to 100 mL with methanol. The final working standard solution carboxin containing 1 µg/mL was prepared by using further dilutions. 0.2% $FeCl_3$, 0.2 M 1,10-PTL, 0.2 M 2,2-BPL, 0.1 M H_3PO_4 were prepared for analysis.

General procedure

Method A

Aliquots of standard pesticide solutions of carboxin ranging from 1-10 mL (10 to 100 µg/mL) were transferred into a series of 10 mL of graduated tubes. To each tube 1 mL of 0.2 M 1,10-phenonthroline solution was added followed by 1 mL of (0.2%) ferric chloride solution and resulting solution was heated for 15 min at 100 °C and finally 1 mL (0.1 M) of orthophosphoric acid solution was added. The volume was made up to 10 mL with distilled water and the absorbance of the orange colored product was measured at 510 nm against reagent blank. The amount of carboxin present in the sample solution was computed from beer's law plot shown in Figure 1.
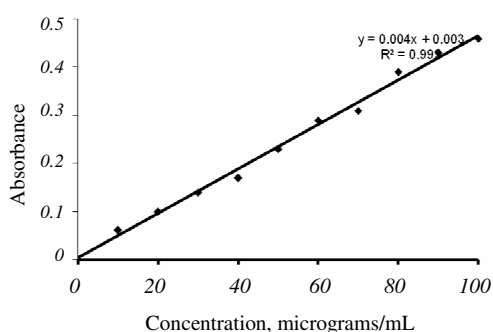

Figure 1. Calibration curve (Method A)

Method B

Aliquots of standard pesticide solutions of carboxin ranging from 0.5 to 50 mL (5 to 50 µg/mL) were transferred into a series of 10 mL graduated tubes. To each tube 1 mL of 2,2′-bi pyridine solution was added followed by 1 mL (0.2%) of ferric chloride solution and the resulting solution was heated for 15 min at 100 c and finally 1 mL (0.1 M) of orthophosphoric acid was added. The volume was made up to 10 mL with distilled water and the absorbance of the orange red colored product was measured at 522 nm against reagent blank. The amount of carboxin was computed from beer's law plot shown in Figure 2.
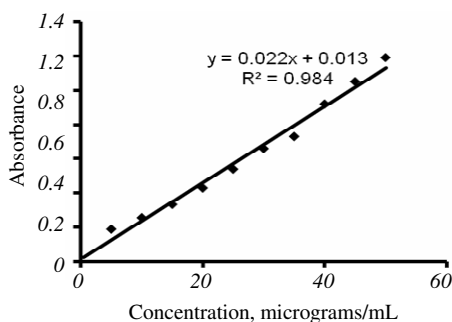

Figure 2. Calibration curve (Method B)

Recovery of carboxin from spiked grains

100 g of each grain (Soybeans and Wheat) were spiked with 200 mL chloroform for 5 min. The samples were fortified with different concentrations of carboxin in methanol and blended

for 3 min. Chloroform was filtered into 250 mL standard flask through Whatman No. 1 filter paper and the residue was retained. The residue was washed twice with 10 mL chloroform and blended for 2 min. Chloroform extracts were combined and made up to the mark. Known aliquots of the chloroform extracts were used for color development after evaporating chloroform on a steam bath. The residue was dissolved in methanol and the amount was determined spectrophotometrically.

Determination of recovery in fortified water samples

After collection of the water sample (minimum volume one litre) the pH values were adjusted below 4 with 20% H_2SO_4. Then fortified with different concentrations of carboxin dissolved in methanol. Next extract each sample in a 250 mL separating funnel with 100 mL chloroform. The chloroform extract were transferred into a funnel and re-extracted the aqueous phase twice with further 50 mL of chloroform. The second chloroform extract was added to the first and washed the combined extract with 0.1 M potassium carbonate solution, then dried the chloroform by passing it through anhydrous sodium sulphate in a filter funnel and collected the extract in a 250 mL flask. The chloroform extract was reduced to 100 mL.

Results and Discussion

The proposed methods involve the oxidation of carboxin with ferric chloride and subsequent complexation of resulting Fe^{2+} with 1,10-PTL in method A and with 2,2′-bipyridyl in method B. Fe^{3+} (from $FeCl_3$) oxidizes with carboxin and the produced Fe^{2+} forms a orange red colored complex, ferroin with 1,10-phenontheoline in method A which exhibits and absorption maximum at 510 nm and in method B the resulting orange red colored due to the formation of Fe^{2+}. Bipy complex absorbs maximally at 522 nm. In both methods the absorbance of the colored solution increases linearly with an increase in concentration of the carboxin.

The optical characteristics such as beer's law limits, molar absorptivity, Sandell's sensitivity, the regression analysis were made for the slope, intercept correlation and the results are shown in Table 1.

Table 1. Optical characteristics and validation data of spectrophotometric methods

Parameters	Method A	Method B
λ_{max} nm	510	522
Beer's Law limit, µg mL^{-1}	1.0-10	0.05-5.0
Molar absorptivity, L mL^{-1} cm^{-1}	1.1425×10^3	2.2605×10^3
Specific absorptivity	0.00485	0.009606
Sand ell's sensitivity, µg mL^{-1}	0.2061	0.1041
Regression equation[**] (y=bx +c)		
Slope (b)	0.004	0.224
Intercept (C)	0.003	0.013
Correlation coefficient (r)	0.999	0.984
Relative Standard Deviation (R. S. D %)[*]	1.24	0.73
Colour	Orange red	Orange red

Average of five determinations, [**]Y=bx+c, Where Y is the absorbance, x is the concentration of the pesticide µg mL^{-1}

The proposed methods for the determination of carboxin were simple, accurate, linear, precise and reproducible and hence the methods can be successfully applied for the routine analysis of carboxin in its formulations, fortified water samples and food grains. The percentage results were shown in Tables 2, 3 & 4.

Table 2. Determination of carboxin in formulations

Formulation	Formulation, %	Amount found in, µg/mL		Recovery, %	
		Method A	Method B	Method A	Method B
Vitavax	10	9.86	9.84	98.6	98.4
	20	19.89	19.68	99.45	98.40
	30	29.78	29.88	99.26	99.60
	40	39.92	39.89	99.80	99.70
	50	49.86	49.84	99.72	99.68

Table 3. Recovery of carboxin from fortified water samples

S.No.	Fortification level, µg/mL	Tap Water			
		Method A		Method B	
		Amount of found, µg/mL	Recovery, %	Amount of found, µg/mL	Recovery, %
1	0.5	0.49	98.0	0.48	96.0
2	1.0	0.98	98.0	0.96	96.0
3	1.5	1.49	99.3	1.47	98.0
4	2.0	1.98	99.0	1.97	98.5
5	2.5	2.48	99.2	2.46	98.4

Table 4. Recoveries of carboxin added to (food grains) soyabeans and wheat

S.No	Amount of Carboxin added µg/mL	Average amount found, µg/mL				Recovery, %			
		Method A		Method B		Method A		Method B	
		Wheat	Soyabean	Wheat	Soyabean	Wheat	Soyabean	Wheat	Soyabean
1	2	1.92	1.93	1.96	1.99	97.0	96.5	98.0	99.0
2	4	3.86	3.84	3.92	3.94	96.5	96.0	98.0	98.5
3	6	5.92	5.94	5.89	5.89	98.6	99.0	97.0	98.1
4	8	7.88	7.90	7.89	7.92	98.5	98.7	98.6	99.0
5	10	9.92	9.95	9.88	9.94	99.2	99.5	98.8	99.4

Conclusion

The spectrophotometric methods developed for the determination of carboxin was readily available and inexpensive. The methods are selective, sensitive and reproducible. Both methods are based on well characterized complexion reactions. Hence the proposed methods can be adopted for the routine analysis of the purity of the commercial formulations and environmental samples.

Acknowledgment

References

1.   Dalvi R R and Salunkhe D K, *J Toxicol.,* 1975, **3(3),** 269-285.
2.   Torres C M, Pico Y and Manes J, *J Chromatogr A,* 1996, **754**, 301-331.
3.   Ana J G, Jordi M, Gurllermina F and Pico Y, *J Chromatogr A,* 2004, **1050,** 119-127.
4.   Wu J, Tragas C, Lord H and Pawliszyn J, *J Chromatogr A,* 2002, **976,** 357-367.
5.   Brian A B, Practical HPLC Methodology and Applications. John Wiley and Sons., New York, 1992.
6.   Marina Dellagreca, Maria Rosaria lesce, Flavio Cermola, Maria Rubino and Marina lsidori, *J Agric Food Chem.,* 2004, **52(20),** 6228-6232.
7.   Liane Maldaner, Cesar C Santana, Isabel C S F Jardim, *J Liq Chromatogr Relat Technol.*, 2008, **31(7),** 972-983.
8.   Gunasekaran P and Tauro P, *J Biosci.,* 1982, **4(2)**, 219-225.
9.   Hustert K, Moza P N and Kettrup A, *Chemosphere*, 1999, **38**, 3423-3429.
10. Khin L, Zan and Somporn Chantara, *Chiang Mai J Sci.,* 2007, **34(2),** 227-234.