

Sensitive spectrophotometric assessment of carbofuran using dapson e as a new chromogenic reagent in formulations and environmental samples

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Abstract: A simple, sensitive and selective spectrophotometric method for the assessment of carbofuran in various formulations and in environmental water samples is described. The method is based on the coupling of hydrolyzed carbofuran with diazotized dapson e in alkaline medium at 0 – 4° C which gives orange red colored product having the absorption maximum at 480 nm. The product is stable for 48 h. Beer's law is obeyed in the concentration range of 0.1 – 4.0 µg ml⁻¹. The molar absorptivity and Sandell's Sensitivity are 5.0 x 10⁴ L mol⁻¹ cm⁻¹ and 4.4 ng cm⁻² respectively. The method is highly reproducible and is confirmed by RSD values (1.144 %). From the recovery studies it is found that this method is accurate and it can be successfully employed for the determination of carbofuran.

Keywords: carbofuran; dapson e; spectrophotometry; assessment; formulations; environmental.

Introduction

Carbofuran (2, 3-dihydro-2, 2-dimethyl-7-benzofuranol N-methylcarbamate), an anticholinesterase carbamate, is commonly used as an insecticide, nematocid e, and acaricide in agricultural practice throughout the world [1]. It is highly toxic by inhalation and ingestion and moderately toxic by dermal absorption [2]. Risks from exposure to carbofuran are especially high for persons with asthma, diabetes, cardiovascular disease, mechanical obstruction of the gastrointestinal or urogenital tracts [3]. It has also a high potential for groundwater contamination [4]. As a result of its widespread use, carbofuran has been detected in ground, surface, rain waters, in soils, air, foods and wildlife [5, 6]. The formulated granules lead to bird-kill incidents [7]. By considering all these serious impacts on ecosystem it is inevitable to develop a sensitive and cost effective method for the assessment of carbofuran.

Comprehensive literature survey reveals a number of methods for the analysis of carbofuran, which includes LC-MS [8-10], GC- MS [11,12], GC [13-16], GLC [17-21], HPLC [22-24], flow injection spectrophotometric [25] and spectrophotometric techniques [26-41]. Some of these reported methods need costly instruments, laborious procedure and less sensitive, these facts promoted us to develop sensitive and cost effective method.

The survey of literature reveals that there is little spectrophotometric method available for determination of carbofuran. Detection limit of few methods were 0.150 µg/mL [25], 0.250 µg/mL [26], 0.300 µg/mL [28], 0.804 µg/mL [40], 0.062 µg/mL [41], found to be more when compare to proposed having detection limit 0.040 µg/mL. The proposed method is based on the coupling of hydrolyzed carbofuran with diazotized dapson e in alkaline medium giving orange red product and intensity is measured at 480 nm. This method can

be effectively used as an alternative tool for the assessment of carbofuran in both environmental samples and commercial formulations.

Experimental details

Systronics spectrophotometer – 106 Model with 10mm matched quartz cells was used throughout the spectral measurements.

Reagents

Carbofuran (99.9% purity) was supplied by Rallis India Ltd. Bangalore, India. 4-4'-diaminodiphenyl sulfone (dapson) was obtained from Merck (Germany), Sodium nitrite (BDH), Sodium hydroxide (AR), Sulphamic acid, (BDH) were used. All other chemicals used were analytical reagent grade. Double distilled water was used to prepare all solutions and commercial formulations were purchased from local sources and environmental samples were collected from nearby sources.

Sample preparation

A Stock solution of Carbofuran (500 $\mu\text{g mL}^{-1}$) was prepared by dissolving 50mg of pure sample in methanol followed by 5 mL of 2 mol L^{-1} of methanolic sodium hydroxide and after 5 min the solution was made up to the volume in a 100 mL calibrated flask with methanol. Working standards were prepared by suitable dilution. A solution 0.2 % (m/v) dapson was prepared by dissolving 0.2 g of dapson in 5 mL of 1 mol L^{-1} hydrochloric acid and then diluted to 100 mL with water. Aqueous solutions of 0.5 % (m/v) Sodium nitrate, 2% (m/v) Sulphamic acid solution, and 2 mol L^{-1} methanolic sodium hydroxide solutions were prepared.

Sample Preparations.-

Furadan 3G working solution (Furadan 3G granular, Manufactured and Marketed by Rallis India Ltd. Bangalore, India) was prepared by transferring 333.30 mg in 100mL volumetric flask, then dissolved and diluted to the mark with methanol to get 100 $\mu\text{g mL}^{-1}$. Suitable aliquots were analyzed for the estimation of carbofuran using the proposed method.

Carbofuran 10G working solution (Marketed by AIMCO Pesticides Ltd. Mumbai, India)

was prepared by transferring 100.0 mg in 100 mL volumetric flask, then dissolved and diluted to the mark with methanol to get 100 $\mu\text{g mL}^{-1}$. Suitable aliquots were analyzed for the estimation of carbofuran using the proposed method.

Tap water, pond water and river water were collected (100 mL each) from Manasagangotri, Kukkarahalli and Kaveri River respectively. These samples were spiked by adding known amounts of carbofuran concentration which lies within the Beer's law range (0.1- 4.0 $\mu\text{g mL}^{-1}$). Using the proposed method, recovery studies was performed.

Recommended procedure:

1.0 mL of 0.2 % (m/v) dapson solution was transferred into each of series of 10 mL calibrated flasks. 1.0 mL of 1.0 % (m/v) aqueous solution of sodium nitrate was added and cooled in ice water bath for 3 min to attain the temperature around 5° C. A 2.0 mL of 2.0 % (m/v) aqueous solution of sulphamic acid was added and reaction mixture was allowed to stand for 2 min with occasional shaking. Aliquot of standard solution of carbofuran (1.0 – 40 μg) was added to the above and resulting solution was made up to the mark with 2 mol L^{-1} methanolic sodium hydroxide and mixed thoroughly. The absorbance of resulting orange red colored solution was measured at 480 nm against the corresponding reagent blank, which has negligible absorption at this wavelength and calibration graph was constructed.

Results and Discussions

Spectral characteristics

The proposed method involves the coupling reaction of diazotized dapson with the hydrolyzed product of carbofuran in sodium hydroxide medium to give orange red colored product having maximum absorption at λ_{max} 480 nm. This wavelength was used for all measurements. The Beer's Law range and molar absorptivity were 0.1-4.0 $\mu\text{g mL}^{-1}$, $5.0 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ respectively. The Sandell's sensitivity of the method was 4.4 ng cm^{-2} . The absorption spectrum of the reaction product formed is shown in the Fig. 1. The corresponding reagent blank has practically negligible absorbance at this wavelength.

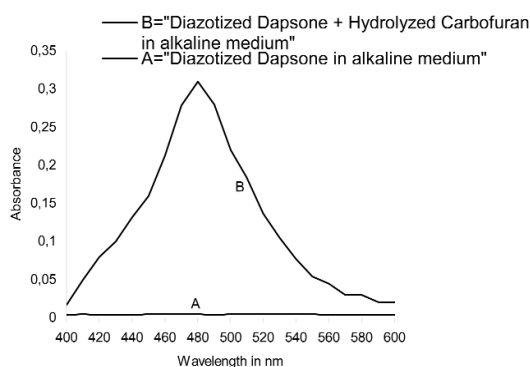


Figure 1. Absorption Spectra of the both Reagent blank and Reaction Product of hydrolyzed Carbofuran and Diazotized Dapsone in the presence of alkaline medium. Final Concentration of Hydrolyzed carbofuran = $2.0 \mu\text{g mL}^{-1}$.

Optimization of the reagent concentration

Various concentration and volume ranges for all the reagents were studied in detail. However, the following are the optimum concentration and volume ranges. For the diazotization coupling reaction, it was found that 1.0 % (m/v) sodium nitrate solution in the range of 1.0 – 3.0 mL, 2.0 % (m/v) sulphamic acid solution in the range of 1.0 – 4.0 mL were optimum to achieve maximum color intensity. Hence, 1.0 mL of sodium nitrate, 2.0 mL of sulphamic acid and 1.0 mL of dapsone were selected for diazotization. The excess of nitrate added during diazotization reaction was removed by the addition of sulphamic acid. An excess of sulphamic acid was found to have no effect on color intensity. Dilution of the coupled product obtained by the interaction of hydrolyzed product of carbofuran and diazotized dapsone was studied water, ethanol, hydrochloric acid, sodium hydroxide and sulphuric acid. Results showed that 2 mol L^{-1} methanolic sodium hydroxide was found to give maximum color intensity and stability of resulting product.

Quantification

Adherence to the Beer's law by the colored product of diazotized dapsone with hydrolyzed carbofuran was determined by measuring the absorbance at 480 nm for a set of solutions containing varying amounts of analyte and specified amounts of reagents against colorless reagent

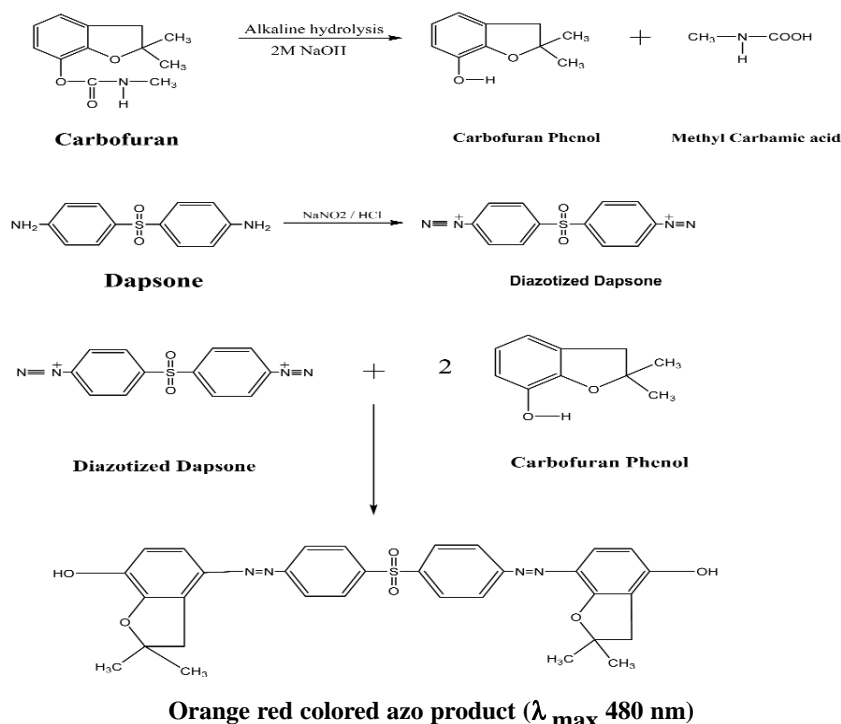
blank. Beer's law was obeyed over the hydrolyzed carbofuran range of $0.1\text{--}4.0 \mu\text{g mL}^{-1}$. Limit of quantification (LOQ) is determined by taking the ratio of standard deviation (σ) of the blank with respect to water and the slope of the calibration curve (a) multiplied by a factor 10. LOQ is approximately 3.3 times the limit of detection (LOD). Naturally, the LOQ slightly crosses the lower limit of the Beer's law range. But LOD is well below the lower limit of the Beer's law range. The upper limit of the Beer-Lambert range is determined by a plot of absorbance against concentration at the value of λ_{max} . Beyond this limit, the correlation results were really affected. Hence, the measurements were excluded above these limits to keep the relationship linear.

Method Validation

The accuracy of the method was established by Recovery studies of hydrolyzed carbofuran at three levels (within the Calibrated graph). The precision was ascertained by calculating the relative standard deviation (RSD) of seven replicate determinations at three levels. The RSD (%) and range of error (%) and values were 1.144 and 1.143 respectively. Which were less than 2 % are indicative of good accuracy and precision of the method. The LOD and LOQ of the method were found to be 0.04 and $0.13 \mu\text{g mL}^{-1}$ respectively. From the regression equation it was observed that correlation coefficient and slope of the curve were 0.9978 and 0.1696 respectively.

Reaction sequence

Carbofuran when dissolved in methanolic sodium hydroxide undergoes hydrolysis to form carbofuran phenol which couples with diazonium cation produced by the diazotization of dapsone to produce the orange red colored product. For the diazotization process, it is expected that the two NH_2 groups in dapsone will be readily diazotized and each diazonium group will then react with two molecule of carbofuran phenol. When the same reaction was carried out with carbofuran dissolved in ethanol or methanol instead of sodium hydroxide does color, this clearly indicates that it is the hydrolysis product of carbofuran which is coupling with diazotized dapsone. The reaction mechanism for the formation of the product is shown in scheme 1.



Scheme1. Proposed Reaction Path of Hydrolyzed Carbofuran with Diazotized Dapsone in the Presence of Alkaline medium.

Effect of temperature and color stability

Under the optimized condition, the orange red colored product was found to be stable for 48 h. Reproducible results were produced in the temperature range of 20 – 55°C. An increase in temperature above 55°C decreased in the absorbance readings indicating the decomposition of the product. However, a temperature of 30° C is recommended for the absorbance measurements.

Effect of Foreign Species

The suitability of the method for application to water samples and other environmental samples were studied by taking various cations, anions and other pesticide species in solutions containing 2.0 $\mu\text{g mL}^{-1}$ carbofuran. The results are given in the *Table 3*. The other pesticides such as parathion ethyl, Malathion, fenitrothion, 2, 4-D, parathion methyl, did not interfere ser-

Table 1. Elemental analysis data of Co(II), Ni(II) and Cu(II) 3-chloro-2-nitrobenzoates

Complex	C / %		H / %		Cl / %		N / %		M / %	
	calcd.	found	calcd.	found	calcd.	found	calcd.	found	calcd.	found
L^- $= C_7H_3O_4NCl$										
$CoL_2 \cdot 2H_2O$	33.87	33.67	2.01	2.00	14.31	14.30	5.64	5.62	11.88	11.80
$NiL_2 \cdot 2H_2O$	33.87	33.58	2.01	2.01	14.37	14.35	5.64	5.63	11.87	11.85
$Cu_2L_4 \cdot 2H_2O$	33.60	33.70	1.60	1.58	14.20	14.21	5.60	5.60	12.68	12.64

iously. The tolerance limits given in *Table 1* are the concentrations of diverse species that cause $\pm 2\%$ error in the determination of carbofuran by this method. The results also showed that a large number of metallic and non-metallic ions did not interfere.

Applications

Determination of carbofuran in Commercial Formulations. -

The method was applied to determine carbofuran in different carbofuran formulations. Here, two different commercial formulations [Furadan 3G (granular) and 10G (granular)] were prepared by appropriate dilution with methanol (within the Beer's Law range 0.1- 4.0 $\mu\text{g mL}^{-1}$) and analyzed by the recommended procedure. The results are represented in *Table 2*. Statistical data showed that this method can be effectively em-

ployed for the determination of carbofuran in various environmental and commercial formulations.

Recovery of the carbofuran residues from spiked water samples.

Water samples (100mL) were spiked with known amount of carbofuran (0.1-4.0 $\mu\text{g mL}^{-1}$) in methanol. The mixture was transferred into a separating funnel and insecticide was extracted using approximately 15 mL of Chloroform in each occasion till complete extraction. The combined extracts were washed with 2mL of 0.1 mol L⁻¹ potassium carbonate to break any emulsion formed during the extraction. The extracted insecticide was dried over anhydrous sodium sulphate. Chloroform was evaporated on a water bath and residue was dissolved in methanol. The amount of carbofuran was determined using the proposed procedure and data are shown in table 3.

Table 2. Wavenumbers (cm^{-1}) of COO^- bands in 3-chloro-2-nitrobenzoates of Co(II), Ni(II), Cu(II) and Na and of the COOH

Complex L^- = $\text{C}_7\text{H}_3\text{O}_4\text{NCl}$	$\nu_{\text{C=O}}$	ν_{asOCO}	ν_{sOCO}	$\Delta\nu_{\text{OCO}}$	$\nu_{\text{C-Cl}}$	$\nu_{\text{M-O}}$
$\text{CoL}_2 \cdot 2\text{H}_2\text{O}$	—	1590	1400	190	740	500
$\text{NiL}_2 \cdot 2\text{H}_2\text{O}$	—	1590	1410	180	740	480
$\text{Cu}_2\text{L}_4 \cdot 2\text{H}_2\text{O}$		1610	1410	200	750	530
HL	1710	—	—	—	—	—
NaL	—	1625	1400	225	760	440

Table 3. Average Recovery of Carbofuran from Spiked Water Samples using Proposed Method

Tap Water ^b			Pond Water ^c		River Water ^d	
Carbofuran Fortified in $\mu\text{g mL}^{-1}$	Carbofuran Found in $\mu\text{g mL}^{-1}$	Recovery % [RSD] ^a	Carbofuran Found in $\mu\text{g mL}^{-1}$	Recovery % [RSD] ^a	Carbofuran Found in $\mu\text{g mL}^{-1}$	Recovery % [RSD] ^a
0.75	0.71	95.26 \pm 0.06	0.70	96.99 \pm 0.89	0.72	95.69 \pm 1.44
1.50	1.44	96.01 \pm 0.80	1.43	95.22 \pm 0.55	1.45	96.88 \pm 1.09
2.25	2.16	95.89 \pm 0.1	2.12	94.15 \pm 0.67	2.16	96.19 \pm 0.78
3.0	2.93	97.50 \pm 0.89	2.80	93.38 \pm 1.09	2.91	97.00 \pm 0.69
3.5	3.38	96.68 \pm 0.72	3.33	95.21 \pm 1.11	3.38	96.65 \pm 0.28
4.0	3.88	97.09 \pm 1.04	3.75	93.85 \pm 1.18	3.80	95.01 \pm 1.20

^a n = Average values of seven determinations, Water samples (100 mL each) were collected from Manasagangotri^b, University of Mysore Kukkarahalli ^c, Mysore District, Karnataka, India. Kaveri River ^d, Mandya District, Karnataka, India

Conclusions

The proposed spectrophotometric method is simple to perform, cost effective. The sensitivity, simplicity, temperature independence and stability of the colored product are the advantages of this method. The diazotizing reagent used here is colorless, highly water soluble and cheaper when compared to the other reagents used in reported methods. This method can be used to detect $0.040 \mu\text{g mL}^{-1}$ and quantitatively detect $0.130 \mu\text{g mL}^{-1}$ of carbofuran and does not involve extraction step and the use of carcinogenic solvents. The proposed method does not entail any stringent experimental variables which affect the reliability of the results. From the recovery studies, it reveals that this method can serve as an alternative method for determining carbofuran residues in various environmental samples.

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