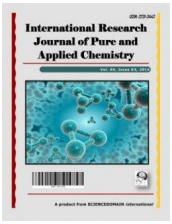

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Micelle-mediating Extraction Combined with Visible Spectrophotometry for the Determination of Ultra Trace Amounts of Bendiocarb Insecticide in Various Matrices after Oxidative Coupling with O-Toluidine

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Authors' contributions

The study was carried out in complete cooperation between the authors. Author ZAAK designed and supervised the work study, wrote the protocol, helped in analyzing the data statistically and wrote the final draft of the manuscript. Author SSA is an MSc student carried out the most experimental works according to the cited plan and managed the literature searches. The authors have been read and approved the final manuscript.

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ABSTRACT

Aims: To develop a new eco-friendly method for the extraction and pre-concentration of Bendiocarb (BC) in real samples by using micelle-mediating extraction (MME) coupled with visible spectrophotometry after oxidative coupling with O-Toluidine.
Study Design: All factors affecting the extraction and determination of BC using micelle-mediation extraction were performed by a classical optimization; in addition the interferences study is also studied.
Place and Duration of Study: Department of Chemistry, College of Science for Women, University of Baghdad, Baghdad, Iraq between May 2015 and September 2015.
Methodology: The developed method is based on an alkaline hydrolysis of BC, and the resultant hydrolyzed phenol is reacted with O-Toluidine in the presence of sodium periodate as an oxidizing

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agent to form yellow colored product which then extracted into micelles of Triton X-114 as a mediated extractant. The extracted product in cloud point layer is separated from the aqueous one by centrifugation for 20 min and dissolved in a minimum amount of ethanol: water (1:1) followed the determination of BC by using spectrophotometry at a wavelength maximum of 430 nm. The established method was applied to the analysis of the spiked vegetables, orange, soil and water samples with appropriate concentrations of BC standard. .

Results: At the established optimized conditions, an enrichment factor of 121.6 fold is obtained leading to achieve the limit of detection of 0.44 ng mL^{-1} with Beer's law concentration range of 2-22 ng mL^{-1}, and the effective optimum linear range of 4.5-21.3 ng mL^{-1} determined by Ringbom's plot. The proposed method gives superior sensitivity in terms of the molar absorptivity of $1.02x10^7$ L mol^{-1} cm^{-1} and extraction efficiency of 97.2%. The average percent recovery of 97.95±1.57%, and a precision (RSD%, n=5) in the range of 0.19-1.56% in real samples are obtained.

Conclusion: The proposed method offers excellent analytical figures of merits such as a limit of detection, high sensitivity, good accuracy and precision and relatively interferences-free compared to the previously reported methods in chemical literatures. It can be considered as an alternative to the other sophisticated techniques such as LC-MS, GC-MS and electrophoresis.

Keywords: Bendiocarb pesticide; O-toluidine; oxidative coupling reaction; cloud point extraction; UV-Vis spectrophotometry.

1. INTRODUCTION

Undoubtedly, unlimited use of pesticides began to increase exponentially, both in agricultural or residential uses in all around the world, because of their importance in boosting crop production yields, and effective in controlling most diseases caused by harmful insects to humans and animals. This increased to use has inevitably paid towards pollution of environmental elements, including soil, water, air, and finally food and thus the risk to our welfare. However, there is considerable efforts by scientists are being made towards using new pesticides characterized by more efficient and less toxic compounds to humans and other organisms. Of these pesticides, bendiocarb is widely used as a carbamate insecticide for agriculture and residential purposes as an insecticide against crawling and flying insects in many countries because it is low toxic and non-carcinogenic pesticide [1]. As with other pesticides, it reversibly inhibits acetyl cholinesterase, an enzyme required for normal transmission of nerve impulses and binds to the active site of this enzyme leading to an accumulation of acetylcholine, which is required for the transmission of nerve impulses, at nerve muscle sites [2]. The oral LD50 in male and female rats ranges from 40 mg/kg to 156 mg/kg [3]. Bendiocarb chemically named by IUAPAC as (2, 2-Dimethyl-1, 3-benzodioxol-4-yl) N-methylcarbamate, is an odourless, a white to light brown powder, has a water solubility of 280 mg L^{-1} at pH 7 and 20ºC, and its chemical structure is depicted in Fig. 1 [4].

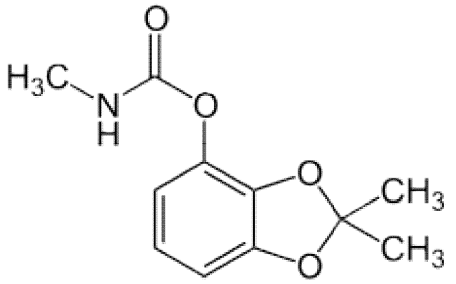

Fig. 1. The chemical structure of Bendiocarb ($C_{11}H_{13}NO_4$, 223.23 g/mol)

Literature survey revealed that bendiocarb can be determined by several methods such as gas chromatography (GC) [5-6] high performance liquid chromatography (HPLC) [7-8], gas chromatography-mass spectrometry(GC-MS) [9], liquid chromatography- mass spectrometry (LC-MS) [10], capillary zone electrophoresis (CZE) [11] flow spectrofluorimetry [12], flow injection analysis [13] and UV-Vis spectrophotometry [14-17]. Although the first four techniques are sophisticated and have a high detection power and sensitivity. But they are relatively expensive and not available in all laboratories. Whilst spectrofluorimetry is difficult because most of the carbamate pesticides are non-fluorescent and need such treatments before detection. In contrast, UV-Vis spectrophotometry is a simple instrument, cheap, easy operated, rapid response time, available in many laboratories and offers acceptable analytical figures of merit and the most commonly used techniques for the determination of pesticides [18]. But due to its

low detection power, extraction and preconcentration procedures are a must which can dramatically improve the detection limit. Also, such chemical pre-treatment to convert the pesticide solution to a colored product enhances the selectivity of the spectrophotometric measurements.

Cloud point extraction(CPE) is now becoming a well-established and accepted as an alternative method of extraction / enrichment methodology of organic pollutants including pesticides in various matrices due to its simplicity, eco-friendly, cheap and relatively high extraction efficiency compared to the conventional liquid-liquid extraction (LLE) and other advanced extraction methods such as solid-phase extraction (SPE) and its developed counterparts, like solid-phase microextraction (SPME), single-drop microextraction (SDME) and liquid phase micro-extraction (LPME) [19-21].

To the best of our knowledge, the application of CPE-Visible spectrophotometry using O-Toluidine as coupling agent for the determination of bendiocarb in complex matrices has not yet been reported. In this work, the effect of several factors which impact the extraction / preconcentration of bendiocarb using CPE are optimized, as well as its determination by molecular spectrophotometry at the absorption maximum of 430 nm in various complex matrices such as vegetables, orange, soil and water.

2. MATERIALS AND METHODS

2.1 Apparatus

A Shimadzu double-beam UV-Vis Spectrophotometer model UV-1800 (Kyoto, Japan) working at a wavelength of 190-1100 nm, equipped with 5-mm optical path cell was used for the scanning of the absorption spectra and absorbance measurements for the target analyte throughout this study. The solution pH measurement, a portable pH-meter microprocessor (HANNA, Germany) was used. The shaking water bath SW23 microprocessor with PID temperature control (JULABO GmbH, Germany) was employed during the course of CPE experiments.

2.2 Materials and Reagents

The materials and reagents used in this work with high purity, doubly distilled water were used in the preparation of all solutions and for final rinsing of glass wares. Bendiocarb (BC) insecticide (99.0% purity, mol wt. 223.23 g mol^{-1}) was purchased from Accustandard® (Connecticut, USA). A standard stock solution 1000 µg mL^{-1} of bendiocarb was prepared by dissolving 50 mg of insecticide in a mixture of 3 mL ethanol: water (1:1) in a 50 mL volumetric flask and diluted to mark with water and kept in an amber bottle in the refrigerator. Triton X-114 (purity >99.9%), was purchased from AMRESCO LLC (Solon, USA). A 10% (v/v) of Triton X-114 was prepared by diluting 10 mL in 100 mL water. An O-Toluidine (OT) (99% purity, mol. wt. 107.17 g mol^{-1}) was supplied from Sigma Aldrich (USA). A $1x10^{-3}$ M of O-Toluidine was prepared by dissolving 0.02123 g in 5 mL water and diluted to mark in 100 mL volumetric flask. Sodium periodates (99% purity, mol. wt. 213.91 g mol^{-1}) was obtained from Merck (Germany). A $1x10^{-3}$ M of $NaIO_4$ was prepared by dissolving 0.02139 g in 5 mL water and diluted to mark in 100 mL volumetric flask. Sodium sulphate, sodium acetate and magnesium sulphate 6-hydrate were purchased from Riedel-deHaën AG (Germany). Acetonirile was obtained from BDH (England). Carbograph and an ion-ion exchange (PSA) were purchased from Sigma-Aldrich (USA) and Vertical Chromatography Co., Ltd. (Thailand) respectively.

2.3 Recommended CPE Procedure

To an aliquot of 10 mL of a solution containing known amount of bendiocarb (BC) standard (2-22 ng mL^{-1}) or sample solution were taken into 10 mL graduated centrifugal tubes ; 1.5 mL of 2 M Na_2CO_3 solution was added and kept for 15 min at room temperature for complete BC hydrolysis to phenol. Then, 1.5 mL of 2M HCl was added to neutralize the base followed by adding 1 mL of sodium borate-sodium hydroxide buffer solution (pH=9) . After that, 0.6 mL of $1x10^{-6}$ M O-Toluidine solution , 0.4 mL of $1x10^{-3}$ M sodium periodate and 0.3 mL of 10%(v/v) Triton X-114 were added and diluted to mark with water. The content of each tube was transferred to water bath at 75°C for 30 min to form cloudy solution. The separation of the phases was accelerated by centrifuging at 3500 rpm for 20 min. After decantation of aqueous phase, the surfactant-rich phase that remained adhered to the tube was dissolved with a 1.0 mL of ethanol: water (1:1) and the absorbance of each solution containing BC was measured spectrophotometrically in 5-mm quartz cell at λmax of 430 nm.

2.4 Preparation of Samples

2.4.1 Vegetable and orange

The vegetables (Cucumber and Tomato) and an orange were purchased from local markets in Baghdad, Iraq. The QuEChERS (quick, easy, cheap, effective, rugged, and safe) method used for pesticide residue analysis was adopted with little modification [22] for sample preparation of vegetables and orange. A 0.5 kg of each sample was selected and the edible part was cut into 1-cm pieces and blended using a commercial food mixer for homogenization the sample. A 15 g sample portion was placed in 100 mL conical flask and 20 mL of solvent mixture containing acetic acid and acetonitrile (1:5) was added and the content was shaked vigorously in an electrical shaker for one hr. After shaking, the extract was withdrawn and transferred into 50 mL centrifugal tube and mixed with 10 g sodium sulphate, 4 g magnesium sulphate and 1 g of sodium acetate and centrifuged for 10 min at 250 rpm to separate the phases. The upper layer was taken and mixed with 0.3 g PSA and 0.6 g Carbograph in another centrifugal tube and immediately shaked and filtered. The filtrate solution was evaporated at 50°C on water bath to remove the solvent. The residue was dissolved with water then diluted to 10 mL in standard volumetric flask. Each sample solution was spiked with different concentration of bendiocarb standard and subjected to recommended CPE procedure and BC was determined by spectrophotometry at λ_{max} of 430 nm.

2.4.2 Water

About one liter of drinking and river water samples was randomly collected from the campus of University of Baghdad / Iraq. The river water was first filtered off to remove any suspended materials and all samples were kept in the refrigerator until analyzed. Each sample was spiked with different concentration of bendiocarb standard and subjected to recommended CPE procedure and BC was determined by spectrophotometry at λ_{max} of 430 nm.

2.4.3 Soil

The soil sample was randomly collected from home garden and the soil sample solution was prepared according to the procedure adopted by Pourreza et al. [23] with little modification. The sample was air-dried at room temperature, grinded in agate mortar into small particle size of about 250 µm sieve and stored in closed vessel. 20 g of sample was weighted in 100 mL conical flasks and 40 mL of 0.1 M HCl was added. The content was shaked in a mechanical shaker for one hr., then filtered and the pH of the filtered was adjusted to 7.0 by diluted NaOH. Three potions of the resultant solution were directly spiked with different concentration of bendiocarb standard and subjected to recommended CPE procedure and target analyte was determined by spectrophotometry at λ_{max} of 430 nm.

2.5 Statistical Analysis

Excel 2007 (Microsoft Office®) and Minitab version 14(Minitab Inc., State College, PA, USA) were employed to carry out all statistical calculations such as regression and correlation analysis, ANOVA and significance tests.

3. RESULTS AND DISCUSSION

3.1 Preliminary Study

Before proceeding with the study of the influence of the main parameters affecting the cloud point extraction on the colored product, a preliminary study was conducted on the alkaline hydrolysis of bendiocarb. It was reported that carbamate pesticides are easily hydrolyzed in alkaline medium to form phenols [24-25]. Therefore, several alkaline media in different concentration was chosen such NaOH, KOH, NH_3 and Na_2CO_3 to select the best one that give the phenolic product of 0.1 µg mL^{-1} bendiocarb at λ_{max} of 279 nm. Fig. 2 showed that 0.3 M Na_2CO_3 gave the best absorbance compared with other alkaline media at 5 min hydrolysis. Further, the effect of hydrolysis time was also investigated on solution containing 0.1 µg mL^{-1} bendiocarb with 0.3 M Na_2CO_3 and varying time between 0 and 45 min. The results depicted in Fig. 3 have shown that 15 min was a good enough for complete hydrolysis of insecticide. Therefore, 0.3 M Na_2CO_3 and 15 min were selected for subsequent CPE experiments.

3.2 Absorption Spectra

In a series of preliminary attempts to ensure the formation of the colored product between bendiocarb after alkaline hydrolysis and O-Toluidine in presence of sodium periodate at concentration of $1x10^{-4}$ M medium, the colored soluble product was formed giving maximum

absorption around 430 nm when measured against reagent blank. Thereafter, the absorption spectrum of the coupling product between 200-800 nm was recorded after obtaining the optimal conditions listed below via applying the recommended CPE procedure. The spectra of other individual reaction constituents such as bendiocarb, O-Toluidine and sodium periodate solutions were recorded but without adding surfactant. The results are displayed in Fig. 4. It was appeared that the coupling product (violet line) gave the maximum absorption wavelength at 430 nm and bendiocarb alone (red line) gave λ_{max} of 273 nm. The other reagents such as O-Toluidine solution (blue line) gave two absorption maxima of 207 and 287 nm, and sodium periodate solution (green line) gave one absorption maximum at 237 nm. Consequently, the wavelength maximum of 430 nm for the colored coupling product was chosen throughout this study.

3.3 Optimization of CPE Conditions

To establish the most appropriate factors for extraction and preconcentration of bendiocarb via oxidative coupling reaction with O-Toluidine, the following studies were directed towards optimizing the experimental conditions in order to maximize the analytical figures of merit and the extraction efficiency of bendiocarb by using CPE. Therefore, the parameters such as pH, reagent concentration, and sodium periodate concentration, Triton X-144 amount; temperature, time and order of additions were investigated using one factor-at-a-time (OFAT) strategy. Each experiment of the above factors was conducted followed the recommended CPE procedure. The solution pH of is one of the most important parameter to keep of the colored derivative product stable in alkaline solution to prevent its decomposition [26] and subsequent extraction in the micelle-mediating solvent. The influence of pH was conducted by taking 10 mL solution containing 14 ng mL^{-1} of bendiocarb in a series of 10 mL centrifugal tubes, 1.5 mL of 2 M Na_2CO_3 was added and kept the solution stand with occasional shaking for 15 min. After the counteract the alkalinity with dilute HCl, 1 mL of $1x10^{-6}$ M of O-Tolduidine, 0.5 mL of $1x10^{-3}$ M $NaIO_4$, 1 mL of 10% TX-114 were added with varying pH in the range of 7-11 using sodium borate-NaOH buffer solution and followed the recommended CPE procedure. The absorbance of each solution was measured at λ_{max} of 430 nm as displayed in Fig. 5. It can be seen that the absorbance increases gradually up to pH=9 and then suddenly decreases thereafter. Thus, pH of 9 was chosen as optimal for subsequent experiments.

The effect of different concentration O-Toluidine on the absorption signals of 10 mL solution containing 14 ng mL^{-1} of bendiocarb at pH=9 was studied according to the recommended CPE procedure via adding successive increase of $1x10^{-6}$ M with varying volume in the range of 0.1-1.0 mL, while keeping other parameters mentioned in the above section constant. It was shown (Fig. 6) that the maximum absorbance measured at λ_{max} of 430 nm was optimum at $6x10^{-8}$ M (0.6 mL of $1x10^{-6}$ M) of O-Toluidine. Beyond this concentration, any excessive amount of reagent was not necessary. Therefore, 0.6 mL of $1x10^{-6}$ M was used for further experiments.

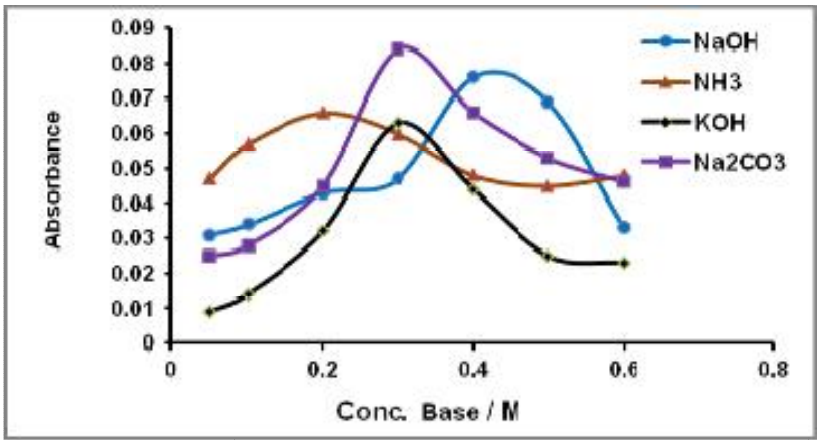

Fig. 2. Effect of different alkaline media on hydrolysis of bendiocarb

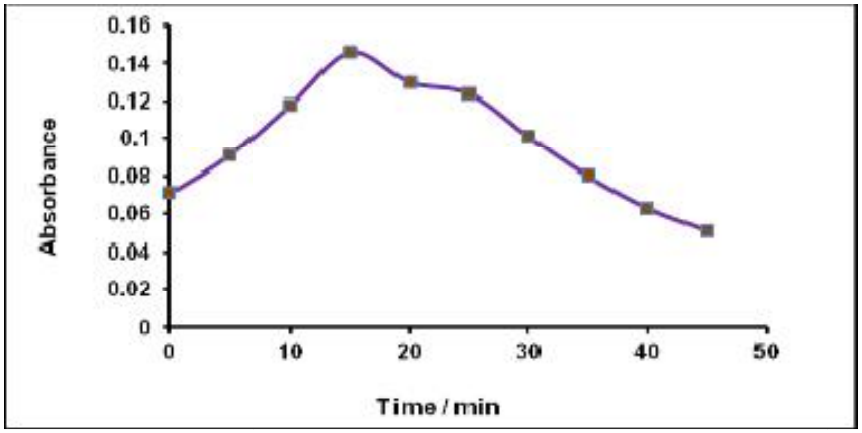

Fig. 3. Effect of alkaline hydrolysis time on bendiocarb with Na_2CO_3

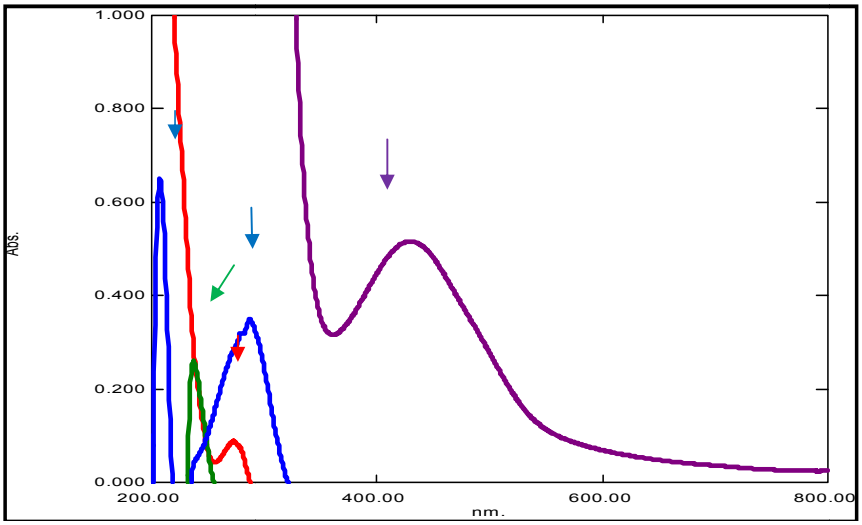

Fig. 4. Absorption spectra (Red) 2.2x10^{-2} M Bendiocarb solution (Blue) 2x10^{-5} M O-Toluidine solution and (Green) 2x10^{-5} M sodium periodate solution (Violet) Bendiocarb-O-Toluidine coupling product by recommended CPE procedure

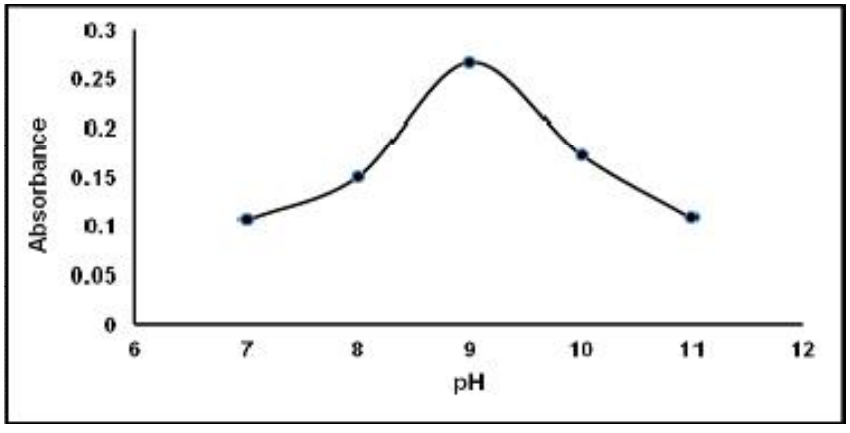

Fig. 5. Effect of pH on the absorbance of derivative product.CPE conditions: equilibration 70°C and incubation time for 40 min

The influence of periodate ion as an oxidizing agent plays an important role in the stage of coupling reaction between the hydrolyzed bendiocarb and O-Toluidine. Therefore, the effect of various concentration of sodium periodate was investigated on the absorbance of 10 mL solution containing 14 ng mL^{-1} of bendiocarb according to the recommended CPE procedure by varying the volume in the range of 0.1-1.0 mL of $1x10^{-3}$ M sodium periodate and keeping the previous optimum parameters constant. The results are shown in Fig. 7. It was noticed that the absorbance signals increased from increasing the concentration of iodate solution up to $4x10^{-5}$ M (i.e. 0.4 mL of $1x10^{-3}$ M in 10 mL solution) and after this concentration the coupling reaction was not significantly influenced. Consequently, a 0.4 mL of $1x10^{-3}$ M of sodium periodates was selected as optimal.

The amount of surfactant as an extracting medium is very important in order to achieve maximum extraction efficiency by minimizing the phase volume ratio (Vs/Va) and therefore improving the preconcentration factor of the method [27]. The variation in absorption signals on the formation of coupling product with Triton X-114 amount was examined within the surfactant volume range of 0.1 – 1.0 mL of 10% (v/v) keeping other optimized parameters constant. As can be seen from Fig. 8, the absorbance of the colored product increased with increases Triton X-114 amount up to 0.3 mL of 10% (v/v) and then slowly decreased at higher amounts. Therefore, a 0.3 mL of 10% (v/v) Triton X-114 was used as the optimum amount to obtain a high preconcentration factor.

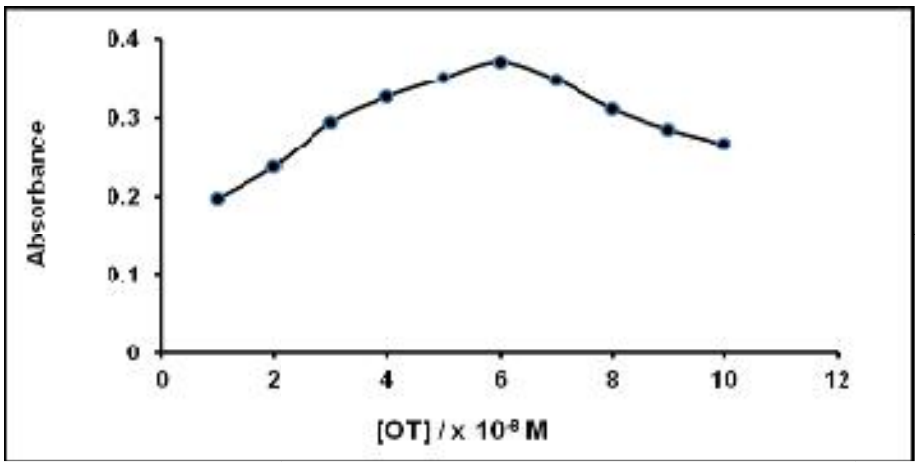

Fig. 6. Effect of O-Toluidine concentration on the absorbance of derivative product by CPE

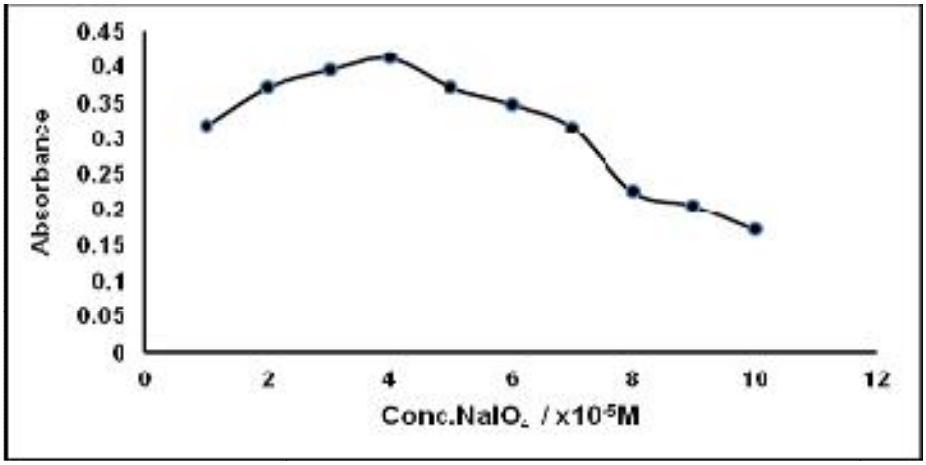

Fig. 7. Effect of sodium periodate on the absorbance of derivative product by CPE

The equilibrium temperature and incubation time are a complementary aspect of the thermodynamic process of micelles agglomeration and the formation of the cloud point layer, which is basically in the extraction operation of CPE. Fig. 9 shows the effect of equilibration temperature on the absorption signal of the derivative product by varying temperature from 25 to 80ºC at incubation time of 40 min. The experimental results showed that the maximum absorption signal for the derivative product was attained 75ºC. Thereafter, the CPE efficiency of the target analyte was decreased by increasing temperature. Thus, an equilibration temperature of 75°C for maximum extraction of the reaction product was chosen as optimal.

The influence of the incubation time was also tested in the range of 5 – 60 min at 75ºC. It was observed that the incubation time of 30 min was sufficient for the maximum absorption signal of the derivative product as displayed in Fig. 10. The effect of centrifugation rate and time was also investigated on extraction efficiency. A centrifuging time of 20 min at 3500 rpm was selected for the entire CPE procedure as being optimum and beyond this time no confirmation was observed for improving analytical response.

3.4 Effect of Order of Additions

The effect of order for additions on the absorption signal of the yellow derivative product was also examined. Table 1 reveals that the best order of addition is number 2 due to giving a highest absorption signal among the others.

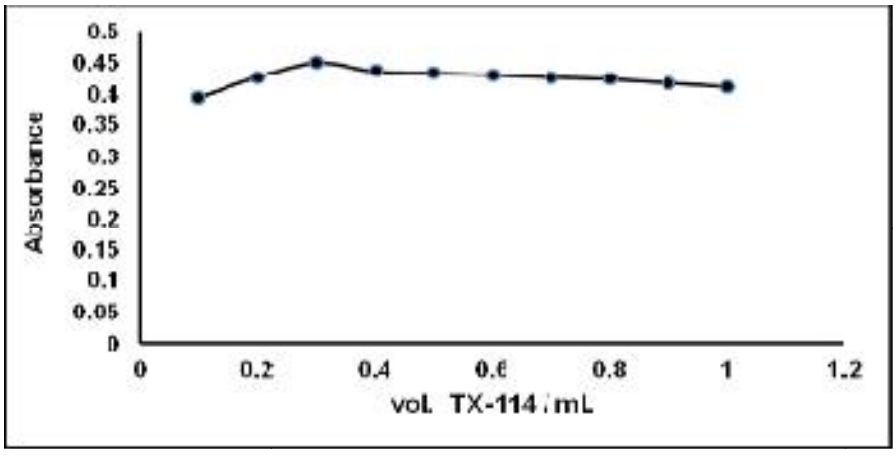

Fig. 8. Influence of Triton X-114 on the absorbance of derivative product by CPE

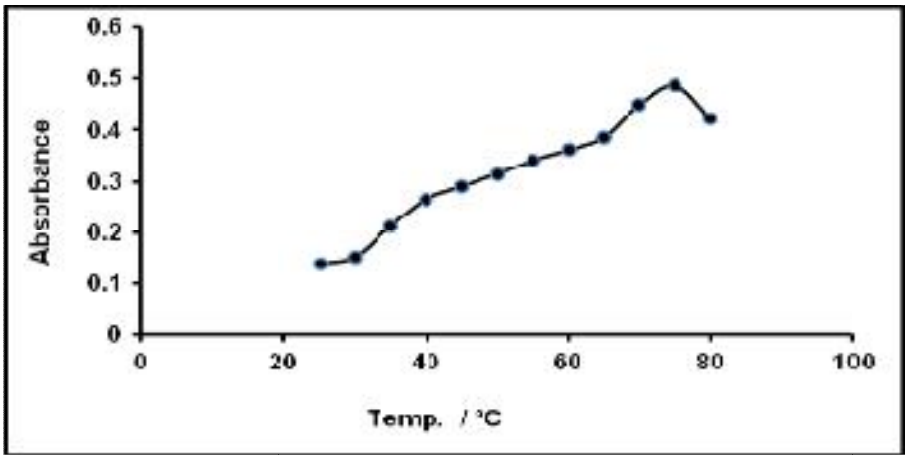

Fig. 9. Effect of equilibration temperature on the absorbance of derivative product by CPE

3.5 Stiochoimetry of the Extracted Derivative Product

The slope analysis method was adopted for the determination of the stiochoimetric amount between the reaction of bendiocarb and O-Toluidine reagent via the analysis of the dependence log D = f (log $C_{Reagent}$) measured under the established optimum conditions [28] and using the order of addition No. 2. Fig. 11 shows the slope for log D as a function of log [OT] is equal to 1.039, indicating the reaction between the insecticide and O-Toluidine in the coupling system to form the colored product of a ratio of 1:1 is extracted into the surfactant-rich phase. The colored product was stable for one day in surfactant-rich phase. Thus the most probable reaction is preceded into two steps to form the yellow product as shown in the Fig. 12.

3.6 Calibration Curve

Under the optimized established conditions, a linear calibration graph for the spectrophotometric detection of bendiocarb was constructed in the range of 2-22 ng mL^{-1} standard solution as shown in Fig. 13. Above this range (> 22 ng mL^{-1}), the Beer's law was not obeyed giving a negative curvature toward the concentration axis (not shown in Figure). This may be due to insufficient reagents to comply with the stiochiomteric amount to produce the yellow derivative product that absorbs at λ_{max} of 430 nm or most probably the formation of other species which compete with coupling product. Despite that, a Ringbom plot is also constructed between log [Bendiocab] and (1-T), where T is transmittance, in order to know the effective optimum concentration range as shown in Fig. 14.

The plot has sigmoid shaped with a linear segment of intermediate absorbance values (0.171- 0.784) and concentration values (5.0–21.3 ng mL^{-1}). This range achieve the greatest photometric accuracy because the measurement should be within 15%-65% T or 0.19-0.82 A. However, the best fit was obtained for a first order equation (Table 2 and Fig. 13) with correlation coefficient of 0.9997 and coefficient of determination (R^2) was 99.95% which suggests statistically valid fit. This was supported by ANOVA analysis (Table not shown) giving that MS_{reg}/MS_{error} =19579.53 of the target analyte for 1 and 9 dof, larger than critical value (F^1_9= 5.21 at 95% CI) and confirmed by the normal probability plot (Fig. 15) which revealed that an ideal linear trend, indicative of normality of absorbance response being acceptable and statistically valid [29]. We use this fitted linear calibration model to estimate the Bendiocarb concentration on all analyzed samples which appear justified, on statistical basis.

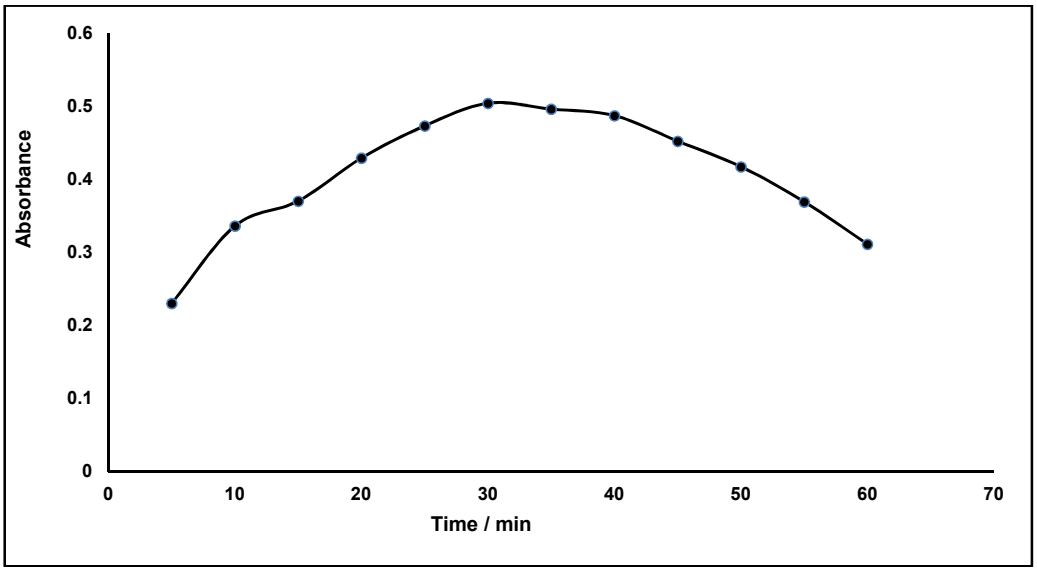

Fig. 10. Effect of incubation time on the absorbance of derivative product by CPE

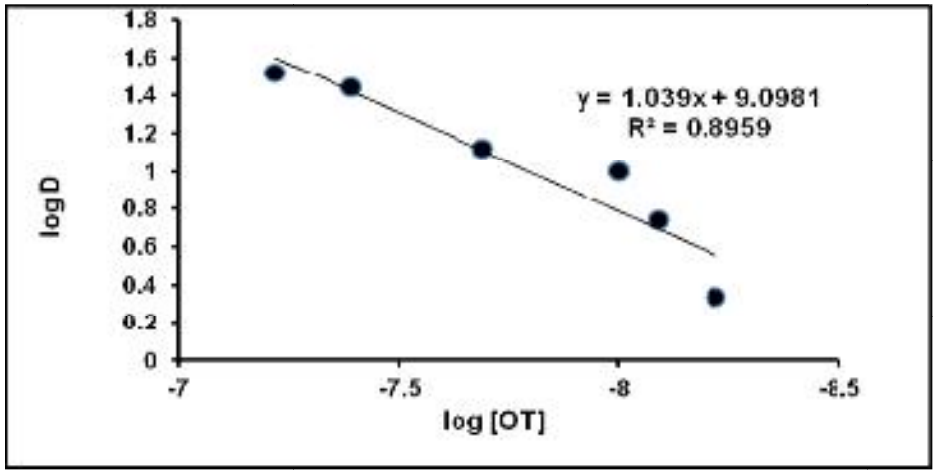

Fig.11. Slope analysis graph for the determination the composition of coupling product

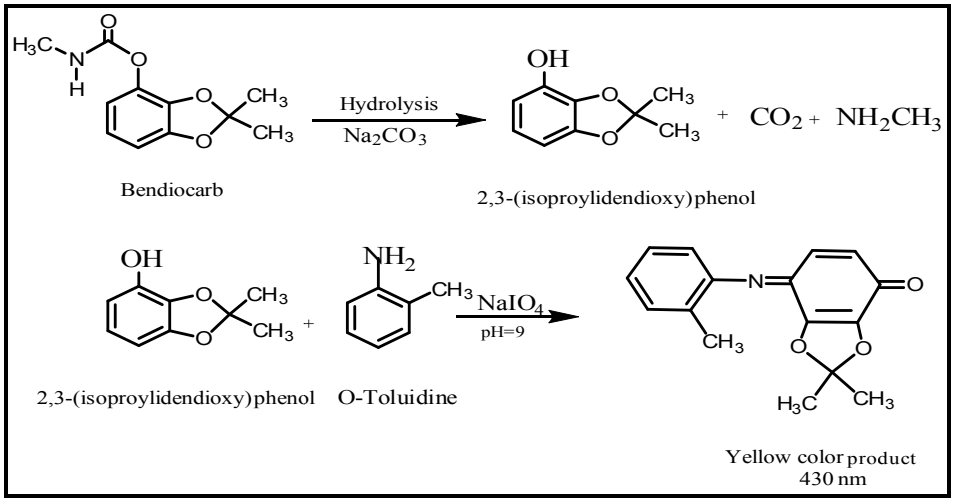

Fig. 12. The most probable coupling reaction path

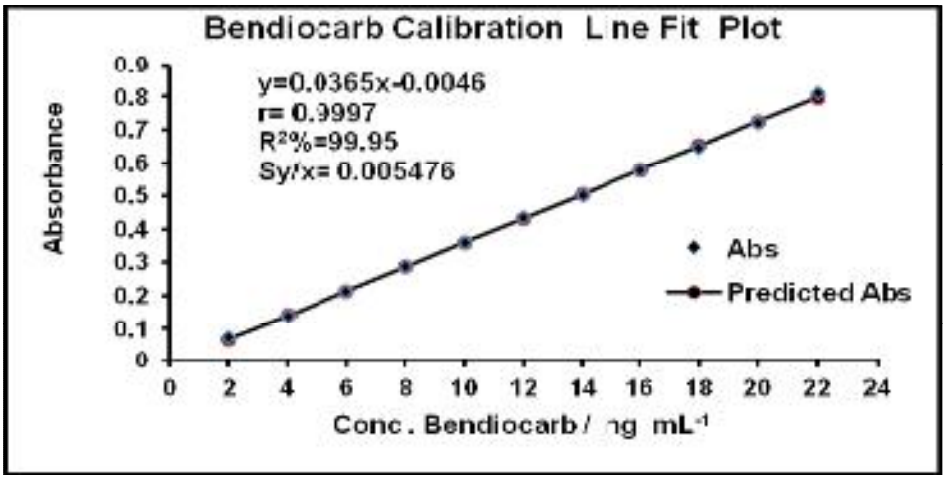

Fig. 13. Calibration curve for bendiocarb by the proposed method

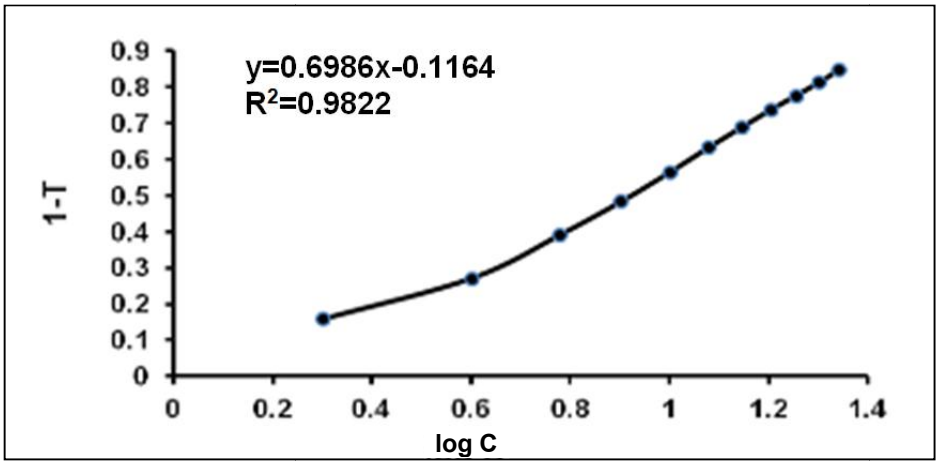

Fig.14. Ringbom plot of the product by the proposed method

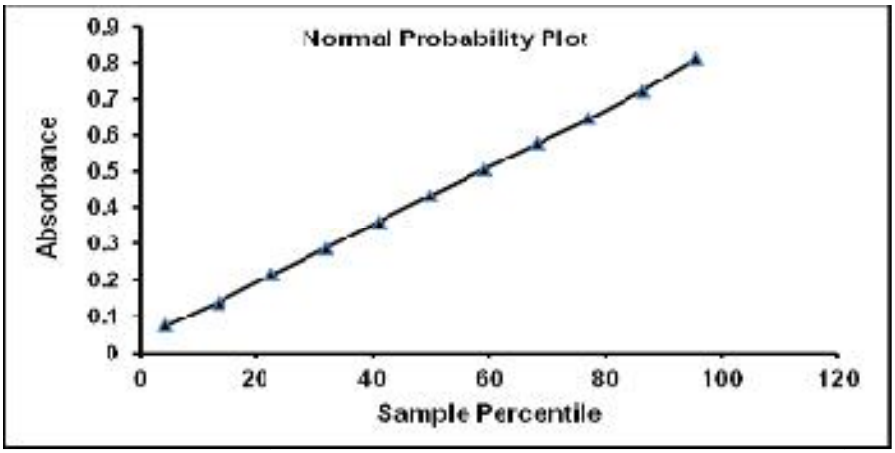

Fig.15. Normal probability plot of absorbance

Table 1. Effect of order of addition

No.	Order of addition	Abs, at λ_{max}(430nm)
1	BC+ $NaIO_4$+ OTD+ Buffer solution+TX-114	0.321
2	BC+ OTD+ $NaIO_4$+TX-114+ Buffer solution	0.504
3	BC+ Buffer solution +$NaIO_4$+ OTD+TX-114	0.279
4	BC+ Buffer solution + OTD+$NaIO_4$+TX-144	0.195

It can be seen that a high enrichment factor (Table 2) of about 121 fold led to achieve superb limit of detection (LOD= 0.44 ng mL^{-1}) and limit of quantitation (LOQ= 1.49 ng mL^{-1}) for bendiocarb by developed CPE-Spectrophotometry. LOD and LOQ are based on the standard deviation of the response and the slope of the calibration curve using the following equations; LOD = $3\sigma_B/s$; LOQ = 10 σ_B/s, where (σ_B) is the standard deviation from the regression line and (σ) its slope. This in turn led to obtain an excellent sensitivity in terms of molar absorptivity which found to be of $1.02x10^7$ $L.mol^{-1}.cm^{-1}$. Concerning the detection limit, our finding was between 200-1200 times better than that obtained Dhaher et al [16] that used diazotized coupling of bendiocarb with p-amino phenol, Alvarez-Rodríguez et al. [17] used a coupling with diazotized trimethylaniline (TMA) in a sodium dodecyl sulphate and Kumar and Rekha [19] used three different azo dyes coupling reagents, by spectrophotomtic detection without CPE, which were of 0.265 µg mL^{-1} ,

0.2-2.0 μg mL^{-1} and 0.285-0.564 μg mL^{-1} respectively. But, it was about 8-17 times worse than that obtained by Faber and Scholer [6] whom employed a gas chromatography after flash-heater methylation with trimethylsulfonium hydroxide, and about 100 worse than that obtained by Lin et al. [11] who used CZE with pre-column hydrolysis and amperometric detection, which were of 0.025-0.05 ng mL^{-1} and 0.004 ng mL^{-1} respectively. However, the LOD of the method was also calculated and found to be 0.0029 mg kg^{-1} for bendiocarb, by considering a limit of detection of 0.44 μg L^{-1} in aqueous solution and 15 g of vegetable and fruit samples in 10 mL solution. This finding has encouraged us to apply the proposed method in the estimation of bendiocarb in real samples such as vegetables, fruits and environmental samples to test its applicability and reliability. In fact, the developed method of this work may achieve the requirements of the international standards in terms of the maximum residue limits (MRL) of bendiocarb insecticide in different types of foods set by FAO/WHO that is in the range of 0.1-0.05 mg kg^{-1} [30].

3.7 Accuracy Test

Since the certified reference materials (CRM's) that specify the exact quantity of bendiocarb are not available, the accuracy of the proposed method was performed by assessing the recovery percentage by the spiking river and soil samples with 4, 10 and 16 ng mL^{-1} standard bendiocarb followed the recommended CPE procedure. The results are summarized in Table 3. It can be noticed that a good accuracy in terms of percent recoveries can be achieved within the range of 97.95±1.57%. This confirmed that the systematic errors are almost absent, concluding the presence of matrix constituents of these samples have no effect on the determination of bendiocarb Meanwhile, each spiked sample was repeated five times for precision testing in term of %RSD and found in the range of 0.19-1.56, indicative a good precision.

3.8 Interferences Study

In order to study the selectivity of the proposed method of the determination of bendiocarb, the effect of several foreign species expected to be in the selected analysed samples was studied by addition of 10, 25, 100 μg of each interferent species to the standard solution containing 14 ng mL^{-1} followed the recommended CPE procedure. The results are presented in Table 4. It is agreed that an extraneous species deemed to interfere seriously when it gives a relative error percent more than ±10% [31]. It can be seen that there is no appreciable effect of most foreign species in

Table 2. The statistical data and analytical figures of merits for Bendiocarb using by CPE- Spectrophotometry

Parameter	Value
Colored product	yellow
λ_{max}(nm)	430
Regression equation (11 point)	y = 0.0365x-0.0056
Correlation coefficient(r)	0.9997
Correlation of Determination (R^2%)	99.95
Std. dev. of regression line ($s_{y/x}$)	0.005476
C.L. for the slope (b± ts_b) at 95%	0.0365±0.00018
C.L.for the intercept(a± ts_a) at 95%	-0.0056±0.00242
Beer's law range (ng mL^{-1})	2-22
Ringbom plot (ng mL^{-1})	4.5-21.3
Limit of Detection (ng mL^{-1})	0.44
Limit of Quantitation (ng mL^{-1})	1.49
Sandell's sensitivity (μg cm^{-2})x10^{-3}	0.024
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	1.02× 10^7
Composition of product* (BC:OTD)	1:1
RSD% (n=5) % at 6 ng mL^{-1}	0.97
RSD% (n=5) % at 12 ng mL^{-1}	0.24
Preconcentration factor	55.5
Enrichment factor**	121.6
Extraction efficiency (%)***	97.2

**Slope analysis method ** Calculated as the ratio of slope of calibration curve obtained by CPE to that obtained without pre-concentration ***calculated from formula cited in Reference [25]*

the determination of bendiocarb (%E_{rel} less than ±5%), except of vitamin C which caused severe interferences on the recovery of the colored product. Therefore, this interfering species should be removed or masked before determination of bendiocarb, or added to standard bendiocarb solutions before the construction of the calibration curve.

Table 3. The accuracy and precision of the proposed method for the determination of Bendiocarb

Sample	Amount BC added (ng mL^{-1})	Amount BC found (ng mL^{-1})	Recovery (%)	E_{rel} (%)	RSD (%) n=5
River water	4	3.98	99.5	-0.5	1.56
	10	9.78	97.8	-2.2	1.12
	16	15.80	98.8	-1.3	0.83
Soil	4	3.83	95.8	-4.3	1.03
	10	9.64	96.4	-3.6	0.57
	16	15.91	99.4	-0.6	0.19

Table 4. Effect of diver's species on the percent recovery of bendiocarb by the proposed method

Foreign species	Recovery %			Recovery % mean±SD
	10 µg	25 µg	100 µg	
K^+	99.78	100.70	100.7	100.39±0.53
Ca^{2+}	98.78	101.14	101.78	100.57±1.57
Mg^{2+}	99.57	100.90	99.40	99.95±0.82
Fe^{2+}	99.14	101.57	101.38	100.36±1.13
Co^{2+}	98.41	101.35	101.78	100.51±1.83
Vitamin B	98.80	101.60	99.90	100.10±1.41
Vitamin C	82.14	75.40	60.32	72.62±11.17
Glucose	98.57	100.78	101.35	100.23±1.47
Fructose	100.35	100.78	101.58	100.90±0.64
Protein	95.00	101.78	96.50	97.76±3.56

Table 5. Analytical results of bendiocarb in different samples by proposed method

Sample	Bendiocarb added (ngml^{-1})	Bendiocarb found (ng mL^{-1}±SD)	Recovery% (mean±SD)	RSD% n=5
River water	8	7.81±0.07	97.62±0.83	0.85
	12	11.63±0.05	96.91±0.49	0.41
	16	15.49±0.05	96.81±0.29	0.30
Tap water	8	8.01±0.11	100.1±1.32	1.32
	12	11.84±0.14	98.67±0.86	1.15
	16	15.92±0.15	99.50±0.94	0.94
Soil	8	7.65±0.05	95.63±0.59	0.62
	12	11.66±0.02	97.17±0.17	0.18
	16	15.34±0.05	95.88±0.28	0.30
Cucumber	8	7.70±0.18	96.25±2.26	2.35
	12	11.70±0.21	97.50±1.79	1.84
	16	15.50±0.24	96.87±1.50	1.55
Tomato	8	7.92±0.17	99.00±2.09	2.12
	12	11.61±0.21	96.75±1.83	1.89
	16	15.58±0.05	97.40±0.31	0.32
Orange	8	7.86±0.13	98.25±1.64	1.67
	12	11.48±0.14	95.67±1.15	1.20
	16	15.61±0.14	97.56±0.91	0.93

3.9 Application

In a bid of the preliminary tests, it was found that all the selected samples of this study do not have any residue of bendiocarb insecticide. Therefore, water, soil, cucumber, tomato and orange samples solutions were spiked with bendiocarb standard at concentration of 8, 12 and 16 ng mL^{-1}. The spiked samples were treated according to the recommended CPE procedure in five replicates measurements and bendiocarb concentration in each spiked sample was measured spectrophotometrically at λ_{max} of 430 nm. The results are summarized in Table 5 above. It was observed that the average recoveries of the spiked bendiocarb standard were of 95.63-101.10% with average RSDs of 0.28- 2.26%. By comparing our findings with the reported method [1] in terms of recovery% (95.57-97.28%) in tap water analysis, the F-test revealed that there is no significant difference in precision between the two methods at 95% C.I, but t-test has shown a significant difference (t_{cal} =4.29 > t_{cri} =2.77 at α=0.05, df=4) in accuracy in terms of percent recovery. This may be ascribed to low recoveries of bendiocab obtained in tap water of the reported method.

4. CONCLUSION

A green method was developed for the extraction and preconcentration of bendiocarb insecticide in a variety of samples via oxidative coupling with O-Toluidine to form a colored product which can be separated in one step by CPE procedure and determined spectrophotometrically at absorption maximum of 430 nm. The proposed method offers excellent analytical figures of merits such as high sensitivity, good accuracy and precision and relatively interferences-free compared to the previously reported methods in chemical literatures. Due to its superb detection limit, it can be easily used as a simple and rapid screening method for the detection of bendiocarb residue in various real samples. In addition, the established method can be considered as an alternative to the other sophisticated techniques such as LC-MS, GC-MS and electrophoresis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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