Development and application of kinetic-spectrophotometric method for analysis of diflubenzuron in soil samples using SPE followed by HPLC method

Emilija Pecev-Marinković[1*], Ana Miletić[1], Aleksandra Pavlović[1], Vidoslav Dekić[2]

1-Faculty of Sciences and Mathematics, Department of Chemistry, Višegradska 33, 18000 Niš, Republic of Serbia

2-Faculty of Sciences and Mathematics, Department of Chemistry, Ivo Lole Ribara 29, Kosovska Mitrovica, Republic of Serbia

ABSTRACT

The purpose of this paper is to present a new sensitive and simple kinetic-spectrophotometric method for the determination of the insecticide diflubenzuron (DFB). The kinetic method is based on the inhibition effect of DFB on the oxidation of sulfanilic acid (SA) by potassium periodate in acetate buffer in the presence of Fe(III) ion as a catalyst and 1,10-phenantroline. The reaction was monitored spectrophotometrically by measuring the increase in absorbance with time of the reaction product at 368 nm. Diflubenzuron was determined with linear calibration graph in the interval from 0.0374 to 0.374 $\mu g/cm^3$ and from 0.374 to 26.18 $\mu g/cm^3$. The detection limit and quantification limit of the method with 3σ criteria were 0.0039 $\mu g/cm^3$ and 0.0131 $\mu g/cm^3$, respectively. The relative standard deviations for five replicate determinations of 0.0374, 0.188 and 0.374 $\mu g/cm^3$ DFB were 2.24, 2.11 and 1.10%, respectively. The method was successfully applied to determine DFB residues in soil samples. Solid-phase extraction (SPE) was used for extraction of DFB from soil and samples with Chromabond® (Macherey-Nagel) C18 cartridges. The HPLC method was used as a comparative method to verify the results. The results obtained by two different methods showed good agreement.

Emilija Pecev-Marinković: emilija.pecev@pmf.edu.rs
Ana Miletić: ana.nis.86@hotmail.com
Aleksandra Pavlović: aleksandra.pavlovic@pmf.edu.rs
Vidoslav Dekić: vidoslav.dekic@pr.ac.rs

Introduction

The term pesticide means a various number of compounds like insecticides, germicides, fungicides, herbicides, rodenticides, molluscicides, nematocides, plant growth regulators and acaricides. Several hundred compounds are available for use as pesticides. Pesticides are intensively used in modern agriculture and represent an efficient and economical way to improve the quality and quantity of yields, thus ensuring food safety for a constantly growing population around the world.

Diflubenzuron (Figure 1) is a halogenated benzoylphenylurea pesticide; also, it is an insect growth regulator. It is an effective stomach and contact insecticide acting by inhibition of chitin synthesis and so interfering with the formation of the cuticle. It inhibits chitin synthesis which results in a disruption of the molting process of target pests (Yang et al.,2016; Wang et al., 2018).
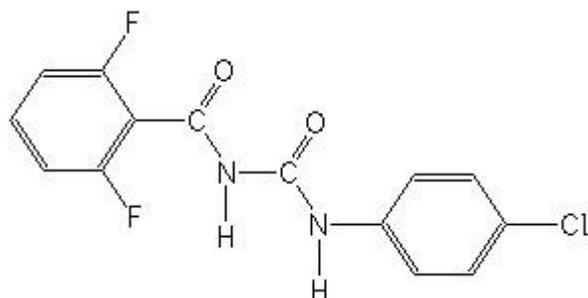
Figure 1. Chemical structure of diflubenzuron

It has been widely used against forest insect pests, and to control dipteran, heteropteran and lepidopteran pests (De Clercq et al., 1995; Erler et al., 2011; Willrich & Boethel, 2001;). Diflubenzuron has shown significant potential in the control of sciarid species (Du et al., 2013). DFB is used in public health applications against mosquitos and noxious fly larvae. It is specified for use as a vector control agent in drinking-water. Specific formulations for control of vectors are specified by WHO/FAO (2020). Difubenzuron was the prototype of all benzoylureas which was firstly discovered in the early 1970's, and in the following 40 years of developing more benzoylureas were prepared and commercialized (Matsmura, 2010; Sun et al., 2015). It is a

direct acting insecticide normally applied directly to plants or water. It is rapidly adsorbed in soil and particles and is immobile in soil. It also rapidly adsorbs to sediments and the sides of vessels and pipes, but it may also partition into the surface film because of its low water solubility. Pesticide analysis is very important because of widespread human exposure to these chemicals. However, with widespread use and accumulation of pesticides over time, the residues of benzoylureas can contaminate water, soil, and food. The pesticides can cause damage effects on human health, such as carcinogenic and allergies, because of long-term toxicity and chronic exposure to these compounds (Olsvik et al., 2013; Wang et al., 2016). The benzoylurea pesticides are not very toxic for numerous marine species such as fish and algae, but due to their specific mode of action they are likely to have adverse effects on non-target species such as the crustaceans and amphipods in the marine environment (Klima, 2011; Macken et al., 2015). Because of these reasons it is important to develop a simple, fast, and sensitive technique for the determination of DFB in different samples.

Numerous analytical methods have been reported for the determination of DFB in various matrices. Many papers reported determination of DFB by HPLC using different detectors (Ambike and Argekar, 2017; Amelin et al., 2013; Huang et al., 2011; Kim et al., 2013; Ouyang et al., 2015; Tfouni et al., 2013; Yang et al., 2015). GC-MS method is frequently used technique for the analysis and the determination of a different group of pesticides in water samples and in river waters (Chen, 2014; Łozowicka, 2017; Shi et al., 2014; Tian, 2020; Zhang et al., 2014). Some authors reported an ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) for the determination of DFB (Carneiro et al., 2013; Chen et al., 2013; Pengqiang, 2013; Wang et al., 2013; Wang et al., 2014). DFB was determined previously using ultra high-performance liquid chromatography coupled to triple quadrupole tandem mass spectrometry (UHPLC–QqQ-MS/MS) (Martínez-Domínguez et al., 2015). There are various studies on the determination of DFB using Liquid Chromatography-Tandem Mass Spectrometry (LC–MS/MS) (Choi et al., 2015; Irungu et al., 2016; Kim et al., 2015; Lee et al., 2011; Macken et al., 2015; Tran et al., 2012; Zainudin et al., 2015).

Authors from Senegal and France reported a Direct Laser-Photo-Induced Fluorescence (DL-PIF) method for the determination of DFB in river and sea water (Diaw et al., 2013).

Sample preparation plays an important role in the pesticide residues analysis. To date, a large number of methods have been developed and reported for the analysis of benzoylureas in different samples (water, juice, fruit, food, soil samples) such as dispersive liquid–liquid microextraction (Wang et al., 2017), solid-phase extraction (SPE) (Huang et al., 2011), solid-phase microextraction (SPME) (Mei et al., 2015), dispersive solid-phase extraction (DSPE) (Martin Pozo et al., 2019) and magnetic dispersive solid-phase extraction (MDSPE) (Huang et al., 2019a). QuEChERS methods are considered to be a quick, easy, cheap, effective, robust, and safe sample preparation methods. These methods have been used for sample preparation of benzoylurea before LC-MS method (Guimarães de Oliveira et al., 2019; Huang et al., 2019b) or HPLC-MS/MS method (Garsia Melo et al., 2020). Some authors developed multiple monolithic fiber-solid-phase microextraction (MMF-SPME) of samples followed by high performance liquid chromatography with diode array detection (Mei et al., 2014).

The aim of this study was to develop a simple, fast, and sensitive kinetic-spectrophotometric method for the determination of pesticide DFB. Finally, it was proposed the method for the determination of DFB in soil samples after their preparation based on SPE. Kinetic methods of chemical analysis are popular methods for rapid determination of organic species. These methods have some advantages like high sensitivity, low detection limit, good selectivity, rapid analysis and using of inexpensive instrument such as spectrophotometer. In the previous work our laboratory developed and validated two kinetic methods for DFB determination. The first work reported DFB determination in the range $0.31 - 3.10$ μg/cm^3 and the application in mushroom samples (Grahovac et al., 2010). The second kinetic method reported DFB determination in the interval from 0.102 to 3.40 μg/cm^3. This method was applied for the determination of DFB in water and baby food samples (Pecev-Marinković et al., 2018).

In this study we reported a kinetic - spectrophotometric method for DFB determination based on its inhibitory effect on the oxidation of sulfanilic acid by potassium periodate in acetate buffer in the presence of Fe(III) ion as a catalyst and 1,10-phenantroline, which was monitored at 368 nm. The method is simple, sensitive, rapid, precise, and accurate. The limit of detection (LOD) is 0.0039 μg/cm^3.

Experimental

Reagents and chemicals

All chemicals used were analytical reagent grade. Pesticide standard diflubenzuron DFB with a certified purity of 99% was obtained from Dr. Ehrenstorfer (Augsburg, Germany). Standard stock solutions containing 100 μg/cm^3 of DFB were prepared by dissolving the required amounts of the standards in methanol: water (50/50, v/v). They were stored in a refrigerator at 4°C.

A Sulfanilic Acid (SA) solution (4×10^{-2} mol/dm^3) was prepared by dissolving a 0.3463 g of SA (Merck) in water in the volumetric flask (50 cm^3).

The initial 1.5×10^{-2} mol/dm^3 solution of potassium periodate was prepared by dissolving 0.075 g KIO$_4$ (Merck) in 50 cm^3 of water.

A solution of Fe (III) 1.0×10^{-3} mol/dm^3 was prepared by dissolving FeCl$_3\times$6H$_2$O (Merck) in 0.1 mol/dm^3 HCl.

A solution of 1,10-phenantroline was prepared by dissolving exact amounts in water.

The acetate buffer pH 4.7 was obtained by mixing solutions of CH$_3$COOH (1 mol/dm^3) and NaOH (1 mol/dm^3). Analytical-reagent grade solvents, methanol (MeOH), acetone ((CH$_3$)$_2$CO), dichlormethane (DCM) and cyclohexane (CHX) were obtained from J. T. Baker (UK). High purity distilled water obtained from Micro Med water purification system TKA Wasseraufbereitungssysteme GmbH was used for solutions preparation.

Soil samples were collected in the period April-May 2011 from different locations (Knez Selo (village), Grdelica (village), Svrljig (town), Nis (town)).

Apparatus

A Perkin-Elmer Lambda UV/Vis spectrophotometer with 10-cm quartz cell pairs was used for recording the absorbance at 368 nm. A water bath thermostat (n-BIOTEK, INC, model NB-301) was employed to control the reaction temperature. A stopwatch was used to record the reaction time.

Chromatographic analyses were performed with an Agilent Technologies, Series 1200 liquid chromatograph, equipped with an Agilent photodiode array detector (DAD), Model 1200 with RFID tracking technology for flow cells and a UV lamp, an automatic injector and Chem Station software. The analytical column was an Agilent – Eclipse XDBC-18 C$_{18}$ column (150×4.6 mm).

A model BÜCHI R-200/205 rotary vacuum evaporator including bath B-490 with a vacuum pump was used to evaporate the extracts.

A solid phase extraction system (J. T. Baker Model SPE-12, UK) with a vacuum pump was used for solid phase extraction of samples. SPE with Chromabond® HR-P cartridges (sorbent mass 200 mg, Macherey Nagel, Germany) were used for extraction of DFB.

Hanna pH-meter instrument was used for checking the pH measurements.

The solutions were thermostated at 25 ± 0.1 C ° before the beginning of the reaction.

In addition, high precision volume micropipettes (Lab Mate$^+$) of 50, 500 and 1000 μL were used for handling or pipetting the solutions.

General procedure

The reaction was performed in a special glass four-compartment reaction vessel-mixer with lapped flap. The reaction was carried out in the following way: in reaction-mixture vessel with four compartments, the solution of SA was placed in one compartment, KIO_4 in the second, buffer solution in the third, Fe (III), *o*-phenantroline and DFB solution were added in the fourth compartment and water was added to the total volume of 10 cm^3. The mixer-vessel was kept for 10 min at temperature of 25 ± 0.1ºC.

The solutions were mixed and homogenized by shaking, and then transferred into 10 cm constant temperature cell of spectrophotometer. The absorption at 368 nm was read over a period of 6 min. The rate of the reactions at different concentrations of reactants was obtained by measuring the slope of the linear part of kinetic curve to the absorbance – time plot. The calibration graphs were obtained by tangent method under the optimum conditions.

Soil sample preparation

10 g of soil samples were measured and then prepared by the addition of appropriate amount of standard stock solution DFB (2 μg/cm^3) and solution of methanol was added until the solvent completely covered the soil particles. The prepared samples were stayed for 1 day. After that, the prepared soil sample was transferred to a separatory funnel and mechanically shaken with acetone/water mixture (80:20, v/v), then it was centrifuged 3 times for 10 min at 3000 rpm. The separated supernatant was transferred into the separating funnel and extracted using DCM:CHX (1:1) using 3 portions of 100 cm^3 solution for 10 min under mechanical shaking. Extracted

solution was put into SPE cartridge which was firstly conditioned. The solution passed through the cartridge under manual positive pressure at flow rate of 1 ml/min. The sample was eluted with 3×1 cm^3 methanol and then extract was collected and evaporated at 60 °C in a rotary vacuum evaporator till dryness. The residue was dissolved with methanol:water (80:20, v/v), transferred into volumetric flask (25 cm^3), and divided into two parts. One part of the solution was filtered through a 0.45-μm microporous nylon membrane (Sigma – Aldrich, USA), then it was transferred into vials for HPLC analysis. For kinetic determination 10 cm^3 of this solution was taken and evaporated at temperature of 60 °C till dryness. The residue was dissolved in methanol and made up with water in 10-cm^3 volumetric flask and used for kinetic determination. SPE with Chromabond$^®$ HR-P cartridge was used for the extraction of DFB. Each sample solution was poured into a Chromabond HR-P C$_{18}$ cartridge which had been conditioned with 3 ml acetone and 2 ml ethanol.

Comparative method

HPLC method was performed with an Agilent Technologies Model 1200 instrument with UV detector, fitted with C$_{18}$ (Zorbax 5 μm, 250 mm x 4.6 mm) analytical column, operating at 25 °C. The mobile phase was methanol-water (80:20, v/v), delivered at a flow rate 1 cm^3/min. The eluate was monitored at wavelength of 254 nm. Injected volume was 10 μl, and the flow rate of the mobile phase was 1 cm^3/min.

Kinetic Procedure

To obtain good mechanical and thermal stability, the instruments were run for 10 min before the first measurement. The reaction was carried out in the following way. In reaction-mixture vessel with four compartments, the solution of SA was placed in one compartment, KIO$_4$ in the second, acetate buffer in the third, Fe(III), *o*-phenantroline and DFB solution were added in the fourth compartment and water was added to the total volume of 10 cm^3. The vessel was thermostated at 25.0 ± 0.1 ºC.

The content was mixed well and then immediately transferred to the spectrophotometric cell with a path length of 10 cm. The change in absorbance was recorded at 368 nm as a function of time every 30 s over a period of 6 min. The rate of the reaction at different concentrations of each of the reactants was obtained by measuring the slope of the linear part of the kinetic curves of the

absorbance-time plot (from Beer's law A=εlc, dA/dt=εl(dc/dt), *slope* = dA/dt, *rate*=dc/dt dc/dt=(dA/dt)/εl). The calibration graph was constructed by plotting the slope of the linear part of the kinetic curve versus concentration of the DFB (c_{DFB}, μg/cm^3).

Results and Discussion

Kinetic studies

The tangent method was used for processing the kinetic data. The rate of the reaction was obtained by measuring the slope of the linear part of the kinetic curves of the absorbance-time plot (*slope*=dA/dt). In order to determine the lowest possible determinable concentration of DFB, working conditions had to be optimized. Therefore, the dependence of the rate of reactions on the concentration of each of the reactants was determined. In Figure 2 the influence of pH on the initial rate in the presence and absence of DFB is shown. The effect of pH on the rate of both reactions, catalytic and inhibited, was studied in the interval pH from 4.0 to 5.0. Reaction rate is increased with increasing pH from 4.0-4.7 for catalytic reaction, and for inhibited reaction is increased from pH 4.0 to 4.5. For further work a pH of 4.7 was used. Catalytic reaction is – 0.75 order in the interval pH 4.0 – 4.70, and the inhibited reaction is –1.2 in the mentioned pH interval.
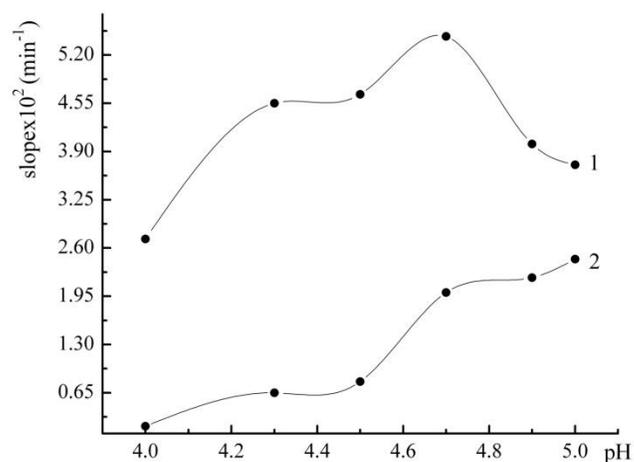
Figure 2. Dependence of the reaction rate on the pH for the catalyzed (1) and inhibited (2) reaction. Initial concentrations: $c(SA) = 4.0 \times 10^{-3}$ mol/dm^3; $c(KIO_4) = 18.0 \times 10^{-4}$ mol/dm^3; $c(Fe(III)) = 3.0 \times 10^{-8}$ mol/dm^3; $c(phen) = 6.0 \times 10^{-5}$ mol/dm^3; $c(DFB) = 26.18$ µg/cm^3; t = $25.0 \pm 0.1°C$.
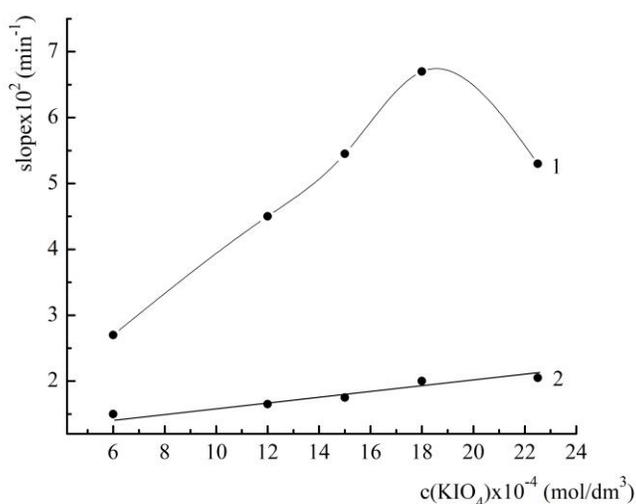
Figure 3. Dependence of the reaction rate on the KIO_4 concentration for the catalyzed (1) and inhibited (2) reaction. Initial concentrations: pH = 4.7; $c(SA) = 4.0 \times 10^{-3}$ mol/dm^3; $c(Fe(III)) = 3.0 \times 10^{-8}$ mol/dm^3; $c(phen) = 6.0 \times 10^{-5}$ mol/dm^3; $c(DFB) = 26.18$ µg/cm^3; t = $25.0 \pm 0.1°C$.

The effect of the concentration of KIO$_4$ on the rates is shown in Figure 3. The influence of KIO$_4$ was studied in the range 6.0×10^{-4} – 22.5×10^{-4} mol/dm^3. The reaction rate of both reactions is increased with increasing KIO$_4$ concentration. A KIO$_4$ concentration of 18.0×10^{-4} mol/dm^3 was selected for the further work. Catalytic reaction is the first order in the interval KIO$_4$ 6.0×10^{-4} – 18.0×10^{-4} mol/dm^3, and inhibited reaction is the first order through the whole investigated interval.

The effect of the concentration of SA was studied (Figure 4) in the interval of 1.0×10^{-3} – 6.0×10^{-3} mol/dm^3.
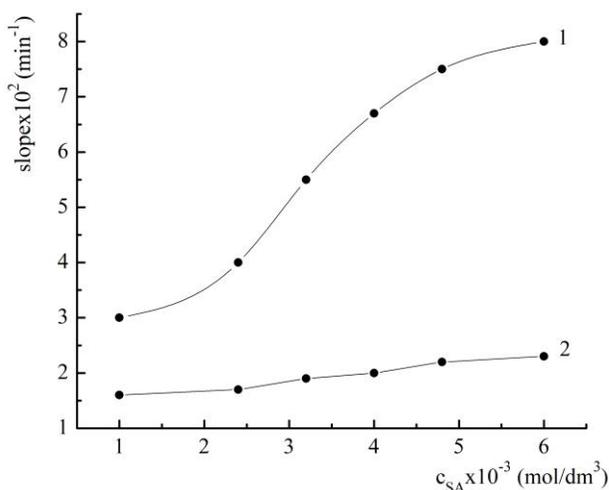
Figure 4. Dependence of the reaction rate on the SA concentration for the catalyzed (1) and inhibited (2) reaction. Initial concentrations: pH = 4.7; c(KIO$_4$) = 18.0×10^{-4} mol/dm^3; c (Fe (III)) = 3.0×10^{-8} mol/dm^3; c(phen) = 6.0×10^{-5} mol/dm^3; c(DFB) = 26.18 μg/cm^3; t = 25.0±0.1°C.

The rate of the catalyzed and inhibited reaction is increased with increasing SA concentration through the whole investigated interval. For further work a concentration of 4.8×10^{-3} mol/dm^3 was selected. Both reactions are the first order in the whole investigated interval.

Influence of the *o*-phenantroline concentration on reaction rates is shown in Figure 5. It is examined in the interval 2.0×10^{-5} – 8.0×10^{-5} mol/dm^3. The rate of the catalyzed and inhibited reaction was increased with increasing *o*-phenantroline concentration from 2.0×10^{-5} to 6.0×10^{-5} mol/dm^3. For further work a concentration of 6.0×10^{-5} mol/dm^3 was selected.
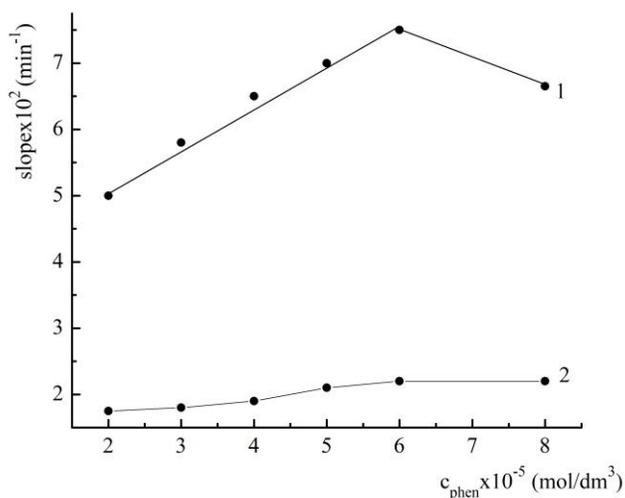
Figure 5. Dependence of the reaction rate on the o-phenantroline concentration for the catalyzed (1) and inhibited (2) reaction. Initial concentrations: pH = 4.7; $c(KIO_4) = 18.0 \times 10^{-4}$ mol/dm^3; $c(SA) = 4.8 \times 10^{-3}$ mol/dm^3; c (Fe (III)) = 3.0×10^{-8} mol/dm^3; $c(DFB) = 26.18$ µg/cm^3; t = $25.0 \pm 0.1°C$.
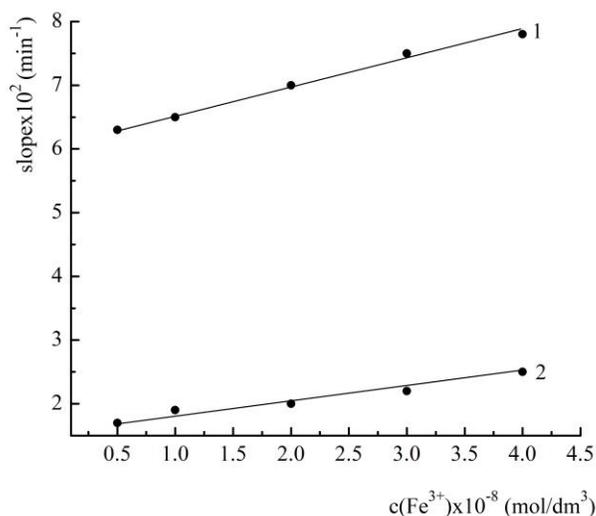
Figure 6. Dependence of the reaction rate on the Fe(III) concentration for the catalyzed (1) and inhibited (2) reaction. Initial concentrations: pH = 4.7; $c(KIO_4) = 18.0 \times 10^{-4}$ mol/dm^3; $c(SA) = 4.8 \times 10^{-3}$ mol/dm^3; c(phen) = 6.0×10^{-5} mol/dm^3; $c(DFB) = 26.18$ µg/cm^3; t = $25.0 \pm 0.1°C$.

The correlation between the slope and the Fe (III) concentration is given in Figure 6. The influence of the concentration of Fe (III) ion on the reaction rates of catalyzed and inhibited reactions was examined in the range 0.5×10^{-8}– 4.0×10^{-8} mol/dm³. A concentration of 3.0×10^{-8} mol/dm³ in the final solution was used throughout the experiments.

Under the optimum reaction conditions: pH=4.7; $c(KIO_4)$ =18.0×10^{-4} mol/dm³; c(SA)=4.8×10^{-3} mol/dm³; c(o-phenantroline)=6.0×10^{-5} mol/dm³; c(Fe(III))=3.0×10^{-8} mol/dm³; t=25.0±0.1°C, the DFB concentration was varied from 0.374 to 26.18 µg/cm³ and from 0.0374 to 0.374 µg/cm³. Figure 7 shows the calibration curve at the temperature of 25°C, which can be used for the determination of the DFB concentration in the interval from 0.0374 to 0.374 µg/cm³. The least squares equation ($y = bx + a$, where b and a are the slope and intercept, respectively) for the calibration graphs and correlation coefficient, r (Miller, 1991) for the determination of DFB in the concentration range 0.0374 to 0.374 µg/cm³ and 0.374 – 26.18 µg/cm³ under the optimal reaction conditions, mentioned above, were calculated:

$$Slope \times 10^2 = -0.1057 \times c_{DFB} + 7.89 \qquad\qquad r = -0.9979 \qquad\qquad (1)$$

$$Slope \times 10^2 = -0.00066 \times c_{DFB} + 3.80 \qquad\qquad r = -0.9927 \qquad\qquad (2)$$

where *slope* is the slope of the linear part of the kinetic curve of the absorbance-time plot (*Slope* = dA/dt = εl (dc/dt)) and c_{DFB} is the DFB concentration expressed in µg/cm³.

The following kinetic equations for the catalyzed and inhibited reaction were deduced based on the obtained graphic correlations:

$$Rate_I = k_1 \cdot c_{H^+}^{-0.75} \cdot c_{KIO_4} \cdot c_{SA} \cdot c_{phen} \cdot c_{Fe(III)} \qquad\qquad (3)$$

$$Rate_{II} = k_2 \cdot c_{H^+}^{-1.2} \cdot c_{KIO_4} \cdot c_{SA} \cdot c_{Fe(III)} \cdot c_{DFB}^{-1} \qquad\qquad (4)$$

,where k_1 and k_2 are constant proportional to the rate constant of the catalyzed and inhibited reaction, respectively.

The equations are valid for the following concentrations: acetate buffer pH 4.0 – 4.7 for catalytic reaction and from 4.5 to 5.0 for inhibited reaction; $c(KIO_4)$ = 6.0×10^{-4} – 18.0×10^{-4} mol/dm³; c(SA) = 1.0×10^{-3}– 6.0×10^{-3} mol/dm³; c(o-phen) = 5.0×10^{-5} – 8.0×10^{-5} mol/dm³; c(Fe(III)) = 0.5×10^{-8} – 4.0×10^{-8} mol/dm³ and c(DFB) = 0.0374 – 26.8 µg/cm³.
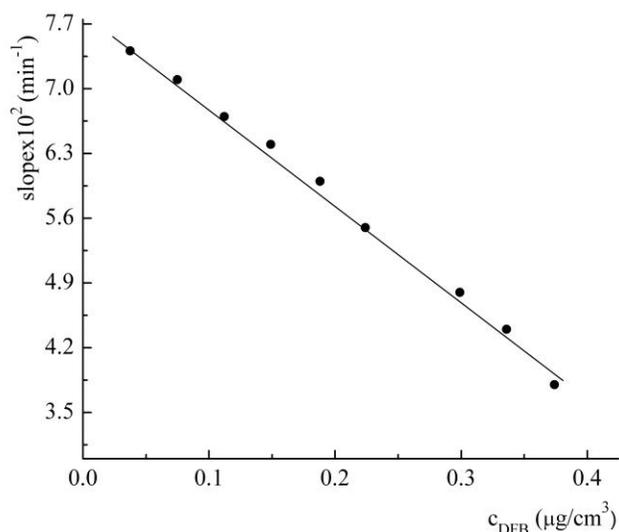
Figure 7. Dependence of the reaction rate on the DFB concentration in the interval 0.0374-0.374 μg/cm^3. Initial concentrations: pH = 4.7; c(KIO$_4$) = 18.0×10^{-4} mol/dm^3; c(SA) = 4.8×10^{-3} mol/dm^3; c(*o*-phen) = 6.0×10^{-5} mol/dm^3; c (Fe (III)) = 3.0×10^{-8} mol/dm^3; t = 25.0±0.1°C.

The Limit of Detection (LOD) and Quantification (LOQ) were evaluated using the following equations (Bendito and Silva, 1988; Motolla, 1988; Prichard and Barwick, 2007): LOD = 3.3S$_0$/b and LOQ = 10S$_0$/b, where S_0 is the standard deviation of the calibration line and b is the slope. They were found to be 0.0039 μg/cm^3 and 0.0131 μg/cm^3, respectively.

The precision and accuracy of the system were studied by performing the experiment 5 times for different concentration of DFB. The results of accuracy and precision of the recommended procedure are presented in Table 1.

Table 1. Accuracy and precision of DFB determination

Added (μg/cm^3)	Determined[a] $\bar{x}$ ±SD (μg/cm^3)	n	RSD (%)	G (%)	$\dfrac{\bar{x}-\mu}{\mu}\cdot 100$ (%)[b]	Recovery (%)
0.0374	0.0366±0.0008		2.22	2.78	-2.14	97.86
0.188	0.193±0.004	5	2.11	2.63	2.66	102.65
0.374	0.382±0.005		1.30	1.35	2.13	102.10

[a] Mean and standard deviation of five determinations at the 95 % confidence level; n- number of replicates; RSD - relative standard deviation; G- relative error; [b] accuracy of the method

Interference studies

To assess the selectivity of the method, the interference due to several cations and anions was studied in detail. Different amounts of ionic species were added to the DFB solution. Table 2 gives the tolerance limits (expressed as v/v ratios), for the species studied in the determination of 3.74 $\mu g/cm^3$ of DFB. The maximum tolerated level was taken as that causing a difference in the rate of the inhibited reaction not larger than 5%. It may be seen that Cu^{2+} to DFB interferes with reaction. The other investigated ions have practically no influence on the determination of DFB by this method. And it means that selectivity of the method is good.

Table 2. Effect of the foreign species on the determination of 3.74 $\mu g/cm^3$ of DFB

Foreign species	Tolerance level ($c_{Interferent}/c_{DFB}$)	
Li^+, Na^+, K^+, Mg^{2+}, NH_4^+, NO_3^-, F^-, Cl^-	10^3	5-10
Mg^{2+}, Ca^{2+}, SO_4^{2-}, As^{3+}, $S_2O_3^{2-}$	10^2	5-10
I^-, NO_2^-	10	<5
Ba^{2+}, SO_3^{2-}, CO_3^{2-}, Mn^{2+}, Ni^{2+}, $Zn^{2+}Al^{3+}$	1	<5
Co^{2+}, Fe^{2+}	0.1	5-10
Cu^{2+}		interfere

Applicability of the proposed method

The proposed method was applied to the determination of DFB in soil samples using the direct calibration curve. The results were compared with parallel HPLC method using a point hypothesis test (Hartman et al., 1995; Skoog et al., 1996). Detection of DFB under the optimum conditions (methanol-water (80:20, v/v)) delivered at a flowrate of 1 mL/min, and detection at a constant wavelength of 254 nm gave satisfactory results for the sensitivity of all spiked samples. They were treated as described in the Experimental section. As can be seen from Table 3, the results obtained for this method are in accordance with the parallel HPLC method. Therefore, the proposed method could be used for the determination of DFB in real samples after SPE. Table 3 shows that F and t values at 95% confidence level are less than the theoretical ones, confirming no significant differences between the performance of the proposed and HPLC method.

Table 3. Determination of diflubenzuron in soil samples by kinetic and HPLC method

Soil sample	Added DFB (μg/cm^3)	Found by kinetic method[a] $\bar{x} \pm SD$ (μg/cm^3)	RSD[a] (%)	$\dfrac{\bar{x} - \mu}{\mu} \cdot 100$	Recovery[a] (%)	Found by HPLC[a] $\bar{x} \pm SD$ (μg/cm^3)	Recovery[a] (%)	F value[b]	t value[b]
S1	0.10	0.093±0.003	3.20	-7.0	93.00	0.094±0.002	94.00	1.12	0.63
S2	0.20	0.195±0.01	5.10	-2.5	97.50	0.193±0.01	96.50	2.56	1.33
S3	0.26	0.249±0.006	2.40	-4.23	95.70	0.25±0.002	96.15	2.45	1.05
S4	0.18	0.17±0.003	1.76	-5.55	94.40	0.178±0.002	98.80	1.86	0.74
S5	0.08	0.085±0.001	1.17	6.25	106.25	0.087±0.001	108.75	1.09	0.25
S6	0.05	0.047±0.007	6.40	-6.0	94.00	0.049±0.001	98.00	1.17	0.74
S7	0.60	0.56±0.02	3.57	-6.66	95.00	0.58±0.03	96.66	2.86	1.25
S8	0.90	0.86±0.05	5.80	-4.44	95.50	0.85±0.05	94.44	2.92	2.14
S9	1.70	1.73±0.03	1.73	1.76	101.00	1.72±0.02	101.17	1.03	0.71
S10	3.60	3.57±0.2	5.60	-0.83	99.16	3.58±0.1	99.45	1.78	0.65
S11	7.00	7.05±0.3	4.25	0.71	100.00	7.02±0.3	100.30	2.59	1.05
S12	10.20	10.13±0.5	4.93	-0.68	99.30	10.19±0.5	99.90	1.37	0.96

[a] Data are based on the average obtained from five determinations.

[b] Theoretical F-value ($v1=4$, $v2=4$) and t-value ($v=8$) at 95 % confidence level are 6.39 and 2.306, respectively

Conclusion

The new kinetic-spectrophotometric method for the determination of diflubenzuron proposed in this paper is simple, rapid, inexpensive, and thus, it is very appropriate for routine quality control analysis of DFB in real samples. Spectrophotometry is the technique of choice even today due to its inherent simplicity. It is frequently used in the laboratories of the developing countries to overcome a variety of analytical problems. For the most laboratories for kinetic evaluations, spectrophotometer is available, and it is not an expensive apparatus. Advantage of the proposed method is simplicity owing to the elimination of some experimental steps such as extraction, and derivatization prior to absorbance measurements. The simple and not expensive chemicals are used. The procedure is easy to execute and requires less sample handling than some other methods currently described in the literature. Statistical comparison of the results with parallel HPLC method showed good agreement and indicates no significant difference in accuracy and precision. Reliable recovery data were found at various concentrations, after spiking samples, and good limits of quantification were attained. Therefore, the proposed method could be used for the determination of DFB in soil samples after extraction.

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Conflict-of-Interest Statement

None.

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