

Development of a kinetic spectrophotometric method for insecticide diflubenzuron determination in water and baby food samples

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Abstract

A kinetic spectrophotometric method for determining residues of insecticide diflubenzuron 1(4-chlorophenyl)-3-(2,6-difluorophenyl)urea (DFB) has been developed and validated. Kinetic method was based on the inhibitory effect of DFB on the oxidation reaction of sulfanilic acid (SA) by hydrogen peroxide in the presence of Co²⁺ ions in a phosphate buffer, which was monitored at 370 nm. DFB can be measured in the concentration interval 0.102 – 3.40 µg mL⁻¹ and 3.40 – 23.80 µg mL⁻¹. The detection and quantification limits of the method were calculated according to the 3σ criteria and found to be 0.077 µg mL⁻¹ and 0.254 µg mL⁻¹, respectively. The relative standard deviations for five replicate determinations of 0.102, 1.70 and 3.40 µg mL⁻¹ DFB were 2.08, 1.22 and 1.21 %, respectively, for the first concentration interval, and the recovery percentage values were from 94.12 to 97.35 %. HPLC method was used as a parallel method to verify results of the kinetic method. The kinetic method was successfully applied to determine diflubenzuron concentrations in spiked water and baby food samples after solid phase extraction of the samples. The F and t values at 95% confidence level are lower than the theoretical ones, confirming agreement of the developed and the HPLC method.

Keywords: kinetic method; diflubenzuron; HPLC method; SPE; water samples; baby food samples

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1. INTRODUCTION

Diflubenzuron is an odorless, white, crystalline solid. It is almost insoluble in water and poorly soluble in polar organic solvents. In polar to very polar solvents, the solubility is moderate to good, e.g., in acetone it is 6.5 g L⁻¹ at 20 °C. The melting point of DFB ranges from 210-230 °C and the vapor pressure at 25 °C is 1.2x10⁻⁴ mPa. It is relatively stable in acidic and neutral media, but it hydrolyses under alkaline conditions reference.

Diflubenzuron belongs to the group of benzoylurea insecticides (Fig. 1) which are effective as stomach and contact poisons and act by inhibition of chitin synthesis in the insect's cuticle [1]. Due to their low toxicity for mammals and rapid degradation in soil and water, their commercial development and use in agricultural practice has increased. Benzoylureas (BU's) are promising insecticides used for the control of insects attacking a wide range of crops, especially fruits and vegetables.

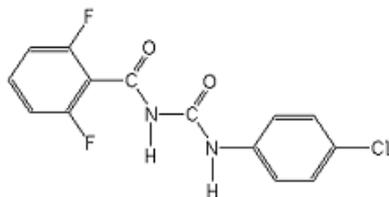


Figure 1. Chemical structure of diflubenzuron

Many analytical methods for determination of diflubenzuron in food, feed and biological matrices have been reported. Due to the high polarity, low volatility and thermolability of diflubenzuron, HPLC has been the method of choice using UV [2-6], FD [7-9], MS [10-14], DAD [15] or fluorescence [16-18] detection. LC-MS/MS has been used for DFB determination in apple, tangerine and honey samples [19,20]. Most protocols include solvent extraction of diflubenzuron from the sample followed by clean-up steps, including solid phase extraction procedures [21,22] and, more recently, the QuEChERS (Quick, Easy, Cheap, Effective,

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Rugged and Safe) approach [23]. Some authors have reported an ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) for DFB determination [24-27]. A direct Laser Photo-Induced Fluorescence (DL-PIF) method has been developed for the determination of DFB in sea and river water [28]. Floated organic drop microextraction (FDME) – high performance liquid chromatography (HPLC) has been developed for extraction and determination of benzoylurea insecticide residues from peach juice drink samples [29]. Diflubenzuron has been determined in fruits by the HPLC method after dispersive liquid-liquid microextraction of samples [30].

Development of new kinetic methods in analytical chemistry can be attributed to the need to analyze very small amounts of a substance, to gain better knowledge of reaction mechanisms, and especially to the great advancement in instrumental techniques, particularly in the field of computerization. Determination of trace elements and compounds in a variety of materials, and also a great importance that small amounts of substances may have in nature, caused the need to develop such analytical methods that allow a more precise determination of their concentrations. Analytical methods that could serve this purpose, need to be highly selective, highly sensitive, rapid, with the extremely low detection limit, rapid analysis rate and should not require using of a complex and expensive apparatus. All these requirements are satisfied by kinetic analysis methods. Application of these methods becomes increasingly important for determination of trace elements based on their catalytic effect in the indicatory reaction. Kinetic analysis methods that are based on homogeneous catalytic reactions for determination of trace amounts of inhibitors have significantly progressed in recent years.

So far, there has been only one report on determination of DFB by a kinetic method. Grahovac *et al.* developed and validated a kinetic method for DFB determination in the range 0.31 – 3.1 $\mu\text{g mL}^{-1}$ [31]. This method was applied for determination of DFB in mushrooms with good recovery.

The main aim of the present work was to develop a simple, selective and sensitive method for determination of DFB at trace levels by a kinetic spectrophotometric method, and also, to apply the new method for DFB determining in water and baby food samples. The method was based on the inhibition effects of DFB on the oxidation reaction of sulfanilic acid with H_2O_2 in phosphate buffer media in the presence of Co^{2+} ions, monitored at 370 nm. The differential variant of tangent method was used for data processing.

2. EXPERIMENTAL

2. 1. Reagents and chemicals

All chemicals used were of analytical reagent grade. Diflubenzuron standard was obtained from Dr. Ehrenstorfer (Augsburg, Germany), while Co^{2+} ($10^{-3} \text{ mol L}^{-1}$) stock standard solution was prepared by dissolving $\text{CoCl}_2 \cdot 2\text{H}_2\text{O}$ (Merck, Germany, *p.a.*) in water. A $4 \times 10^{-2} \text{ mol L}^{-1}$ sulfanilic acid (SA) solution was prepared by dissolving 0.3463 g SA (Merck, Germany, *p.a.*) in 50 mL of water, while hydrogen peroxide solution (2.0 mol L^{-1}) was prepared by diluting a known volume of concentrated solution (Merck, Germany, *p.a.*), standardized permanganometrically. Phosphate buffers [32] were obtained by mixing appropriate volume solutions of KH_2PO_4 (0.067 mol L^{-1}) and Na_2HPO_4 (0.067 mol L^{-1}) (Merck, Germany, *p.a.*), while analytical-reagent grade solvents, methanol (MeOH), acetone ($(\text{CH}_3)_2\text{CO}$), dichloromethane (DCM) and cyclohexane (CHX) were obtained from Baker (UK). High purity deionized water, obtained from Micro Med water purification system TKA Wasseraufbereitungssysteme GmbH (Germany) was used for preparation of solutions.

2. 2. Apparatus

A Perkin-Elmer Lambda UV/Vis spectrophotometer (USA) with 10-cm quartz cell pairs was used for recording the absorbance at 370 nm, whereas a thermostated water bath (n-BIOTEK, INC, model NB-301, Korea) was employed to control the reaction temperature.

Chromatographic analyses were performed using a liquid chromatograph (Series 1200, Agilent Technologies, USA), 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 rotary vacuum evaporator (model BÜCHI R-200/205, Switzerland) including bath B-490 with a vacuum pump was used to evaporate the extracts. An analytical balance (Mettler Toledo, USA) was used to measure the mass. 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 (Macherey Nagel, Germany) were used for extraction of DFB. A pH-meter (Hanna, Germany) was used for pH measurements. A standard benchtop homogenizer (Model PT 2100 Polytron, Fisher Scientific, UK) was used for blending the samples.

2. 3. General procedure

All solutions were thermostated at 25 ± 0.1 °C before the beginning of the reaction. The reaction was performed in a special glass four-compartment reaction vessel-mixer with a lapped flap. An aliquot of SA solution was transferred into the first compartment of the vessel; the second was filled with the phosphate buffer solution; the third with Co^{2+} and DFB, and the fourth with H_2O_2 solution, then completed to the volume of 10 mL with deionized water. The mixer-vessel was kept for 10 min at the temperature of 25 ± 0.1 °C. The solutions were mixed by shaking and then transferred into a 10 cm constant temperature cell of the spectrophotometer. The absorption at 370 nm was read over a period of 6 min at 30 seconds. The reaction rates at different concentrations of reactants were determined from the slopes of linear parts of the kinetic curves (absorbance – time plot). For the kinetic data processing the differential variant of the tangent method was used.

2. 4. Preparation of water samples

Eleven different samples of branded bottled water from various sources in Serbia were purchased in 2016. A stock solution of pesticide DFB standard (100 mg L^{-1}) was prepared by weighing and dissolving the corresponding amounts in MeOH. These standard solutions are stable for the period of at least 3 months. A working standard solution of DFB ($1 \mu\text{g mL}^{-1}$) was prepared by diluting the stock solution in MeOH. Calibration standard solutions were prepared in MeOH: water solutions (80:20, v/v) and then filtered through Millipore membrane Teflon filters ($0.45 \mu\text{m}$ particle size) before being injected into the chromatographic system. 250 mL of water samples were spiked with DFB, then passed through the preconditioned SPE cartridge and finally filtered through Millipore membrane Teflon filters ($0.45 \mu\text{m}$ particle size). Each sample solution was poured into a Chromabond HR-P C_{18} cartridge (sorbent mass 200 mg Macherey-Nagel, Germany) which had been previously conditioned. The first step of the SPE implied conditioning the immunosorbent with 1 column volume of MeOH, and 1 column volume of deionized water. Next, the water samples were filtered through the column and eluted with $3 \times 1 \text{ mL}$ (MeOH-(CH_3) $_2\text{CO}$; 3:2, v/v) and the final amount of solvent in the column was removed under a gentle vacuum, and further dried for 30 min in the gentle vacuum. The filtered extract was collected and then evaporated near dryness in a rotary vacuum evaporator at 60 °C. The residue was dissolved in MeOH and transferred into a volumetric flask filled up with methanol (25 mL). For HPLC determination, an aliquot of this solution was transferred into the vessels.

For kinetic determination the water samples were prepared by the same procedure explained in this section, and after evaporation of the extract, the residue was dissolved in 10 mL volumetric flask with 10 % methanol and then used for kinetic determination.

2. 5. Preparation of baby food samples

Eleven commercially available infant baby food items were used for optimization and validation of the analytical method. Baby food items of different brands were purchased in local supermarkets in 2016. Spiked baby food samples (50 g), for recovery determination, were prepared by adding the appropriate amount of the standard DFB stock solution (100 mg L^{-1}) and then the samples were left for a few hours. Each sample was homogenized in 50 mL of acetone, using a laboratory blender for 10 min, and then filtered under vacuum through a sinter glass funnel. Afterwards, the filtrate was mixed with 100 mL CHX:DCM (1:1) and shaken. During the procedure, the two distinct layers were formed; the lower organic layer was transferred to a separating funnel and was decanted through anhydrous Na_2SO_4 . The organic extract was concentrated using the rotary evaporator (at 60 °C) near dryness, transferred to a volumetric flask and filled up with MeOH. This solution was divided into two parts. One part of the solution was used for HPLC analysis and the second one was used for kinetic analysis of samples. The first part of the solution was filtered through a membrane filter Millipore ($0.45 \mu\text{m}$) and transferred into vials for HPLC analysis. The mobile phase was MeOH-water (80:20, v/v) delivered at the flow-rate of 1 mL min^{-1} . The analytical column was an Agilent – Eclipse XDBC-18 C_{18} column ($150 \times 4.6 \text{ mm}$) with diode array detection at 254 nm operating at 25 °C. The second part of the solution was used for kinetic determination, where an aliquot of the solution was transferred into the rotary evaporator and evaporated near dryness (controlled at 60 °C). The residue was dissolved in 10 % methanol.

2. 6. Validation parameters

The proposed method has been validated for linearity, precision, accuracy, recovery and selectivity.

2. 7. Linearity

For evaluation of linearity, determination of DFB was done at ten concentration levels for each calibration curve ($0.102 - 3.40 \mu\text{g mL}^{-1}$ and $3.40 - 23.80 \mu\text{g mL}^{-1}$) and it was assessed by the correlation coefficients. Each measurement was repeated five times.



2. 8. Precision and accuracy

Three concentrations within the linearity range $0.102 - 3.40 \mu\text{g mL}^{-1}$ were selected: 0.102 , 1.70 and $3.40 \mu\text{g mL}^{-1}$. Five solutions of each concentration were prepared and analyzed within one day. Precision of DFB determination, evaluated as repeatability, was calculated in terms of the relative standard deviation (RSD, %). To study the accuracy of the proposed method, recovery experiments were performed by standard addition method. For this, different volumes of DFB standard solution (100 mg L^{-1}) were added to water and baby food samples and spiked samples were prepared by the procedures described in the "Sample preparation part". Accuracy of DFB determination was expressed as percentage difference between the measured and taken concentration (relative error (G)).

2. 9. Limit of detection and limit of quantification

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were evaluated using the following equations [33-36]:

$$\text{LOD} = 3.3 S_0 / b \quad (1)$$

$$\text{LOQ} = 10 S_0 / b \quad (2)$$

where S_0 is the standard deviation of the calibration curve and b is the slope. Both limits were expressed in $\mu\text{g mL}^{-1}$.

2. 10. Statistical analysis

Statistical t- and F- tests have been used to evaluate whether or not there is a significant difference between the performance of the developed and the HPLC method. Both tests were performed using a program in MS Excel. A probability level of $p < 0.05$ was considered statistically significant [37].

3. RESULTS AND DISCUSSION

By a spectrophotometric observation of the absorbance change with time in the system containing SA, H_2O_2 and Co^{2+} ions in phosphate buffer, formation of a yellow colored product was noticed with maximum absorption at 370 nm . By adding the DFB pesticide to the investigated system, it was noticed that the color was formed more slowly, which indicated the inhibitory effect of DFB in the reaction.

The influence of the inhibitors on the rate of catalytic reactions can be explained by various mechanisms. The decrease in the reaction rate under the influence of an inhibitor can be explained by formation of an inactive complex [38]. In the catalytic reaction, the mechanism of reaction is based on the complex formation between a metal ion and a substrate with a specific coordination number of the metal ion. By adding a pesticide to the reaction mixture, a mixed transmission complex substrate – catalyst – inhibitor is formed due to bonding of the metal ion to free electrons of donor atoms in the pesticide molecule (most frequently N, O, S). Depending on the stability of the formed triple complex and the strength of the metal ion-donor atoms of the pesticide, the catalyst activity decreases in the indicator reaction. This means that the reaction takes much more energy to degrade such a complex, resulting in decreased reaction rate, namely the inhibitory effect of the pesticide is expressed. This mechanism cannot be proved by spectrophotometry, although certain investigations recommended the ESR method in order to confirm the presumed reaction mechanism.

To determine the lowest possible determinable concentration of the insecticide diflubenzuron, working conditions needed to be optimized. Therefore, the dependence of the rate of reactions on the concentration of each of the reactants was determined. For optimal concentration of each reactant with the highest difference in reaction rates of catalyzed and inhibited reaction was chosen as optimal for further investigation. A tangent method was used to process the kinetic data. The reaction rate was obtained by measuring the slope of the linear part of the kinetic curve of the absorbance-time plot (slope = dA/dt).

In Figure 2 the influence of pH on the initial rate at both the presence and absence of DFB is shown. The influence of pH on the reaction rate was studied in the pH interval from 7.0 to 8.0 . The pH of 7.9 was chosen as optimum for further work. Both reactions are of minus first order in the whole investigated pH interval.

Dependence of the initial reaction rate on the H_2O_2 concentration in the interval $0.04 - 0.24 \text{ mol L}^{-1}$ is shown in Figure 3. Inhibited reaction was first order in the whole H_2O_2 concentration interval while the catalyzed reaction was first order in the interval $0.04 - 0.12 \text{ mol L}^{-1}$ and minus first order at concentrations higher than 0.12 mol L^{-1} . The concentration 0.12 mol L^{-1} of H_2O_2 was chosen for further investigation.

Influence of the SA concentration in the interval $1.2 \times 10^{-3} - 4.8 \times 10^{-3} \text{ mol L}^{-1}$ on reaction rates is shown in Figure 4.

Reaction rate of the catalytic reaction was 0.84 in the concentration interval $1.2 \times 10^{-3} - 3.2 \times 10^{-3} \text{ mol L}^{-1}$ while the inhibited reaction was of first order in the whole investigated concentration interval. The concentration of $3.2 \times 10^{-3} \text{ mol L}^{-1}$ of SA was chosen as optimal.

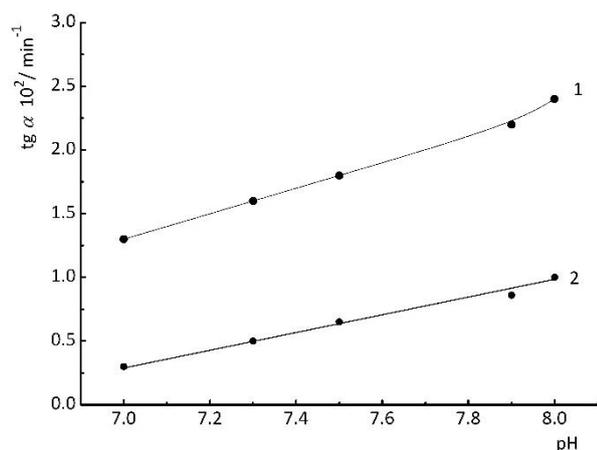


Figure 2. Dependence of the reaction rate on pH for the catalyzed (1) and inhibited (2) reaction. Initial concentrations: $c(\text{SA}) = 4 \times 10^{-3} \text{ mol L}^{-1}$; $c(\text{H}_2\text{O}_2) = 0.2 \text{ mol L}^{-1}$; $c(\text{Co}^{2+}) = 7 \times 10^{-5} \text{ mol L}^{-1}$; $c(\text{DFB}) = 23.80 \mu\text{g mL}^{-1}$; $t = 25.0 \pm 0.1 \text{ }^\circ\text{C}$

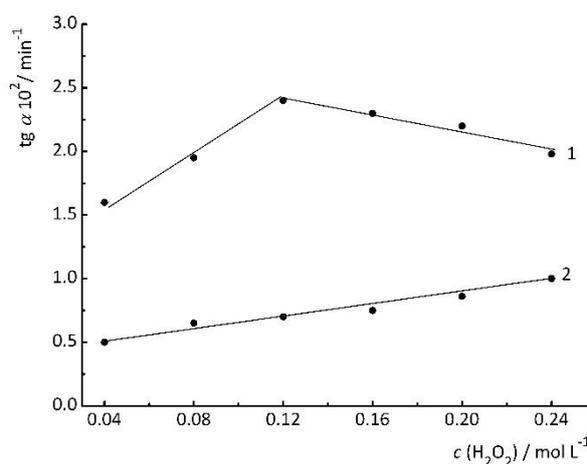


Figure 3. Dependence of the reaction rate on the H_2O_2 concentration for the catalyzed (1) and inhibited (2) reaction. Initial concentrations: $\text{pH} = 7.9$; $c(\text{SA}) = 4 \times 10^{-3} \text{ mol L}^{-1}$; $c(\text{Co}^{2+}) = 7 \times 10^{-5} \text{ mol L}^{-1}$; $c(\text{DFB}) = 23.80 \mu\text{g mL}^{-1}$; $t = 25.0 \pm 0.1 \text{ }^\circ\text{C}$

Correlation between the reaction rates and the Co^{2+} concentration in the range 2×10^{-5} – $10 \times 10^{-5} \text{ mol L}^{-1}$ is presented in Figure 5.

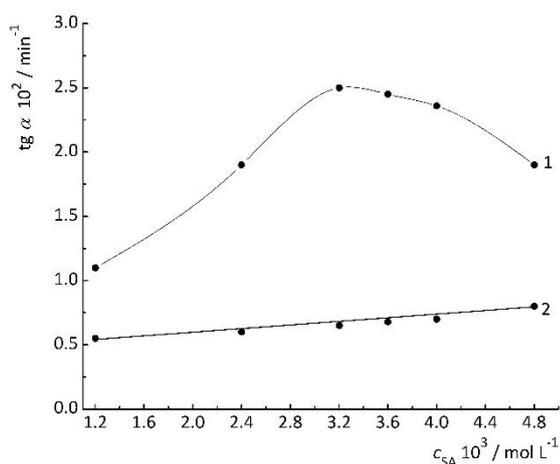


Figure 4. Dependence of the reaction rate on the SA concentration for the catalyzed (1) and inhibited (2) reaction. Initial concentrations: $\text{pH} = 7.9$; $c(\text{H}_2\text{O}_2) = 0.12 \text{ mol L}^{-1}$; $c(\text{Co}^{2+}) = 7 \times 10^{-5} \text{ mol L}^{-1}$; $c(\text{DFB}) = 23.80 \mu\text{g mL}^{-1}$; $t = 25.0 \pm 0.1 \text{ }^\circ\text{C}$

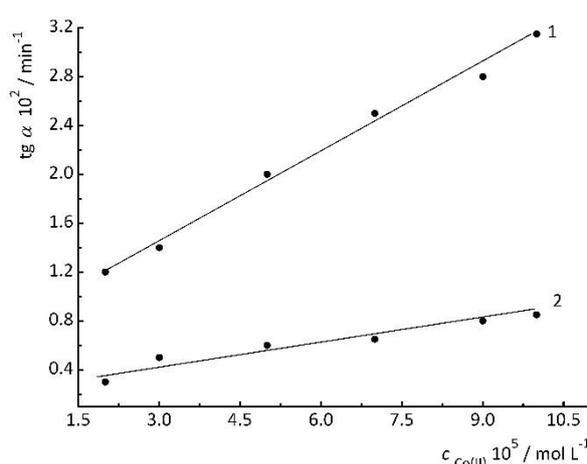


Figure 5. Dependence of the reaction rate on the Co^{2+} concentration for the catalyzed (1) and inhibited (2) reaction. Initial concentrations: $\text{pH} = 7.9$; $c(\text{H}_2\text{O}_2) = 0.12 \text{ mol L}^{-1}$; $c(\text{SA}) = 3.2 \times 10^{-3} \text{ mol L}^{-1}$; $c(\text{DFB}) = 23.80 \mu\text{g mL}^{-1}$; $t = 25.0 \pm 0.1 \text{ }^\circ\text{C}$

Both reactions were of first order in the whole investigated concentration interval of Co^{2+} and $7 \times 10^{-5} \text{ mol L}^{-1}$ was selected as the optimal concentration.

Investigation of the effects of reactant concentrations on the rates of the catalyzed and inhibited reactions, optimal experimental conditions were determined as: $\text{pH} = 7.9$; $c(\text{H}_2\text{O}_2) = 0.12 \text{ mol L}^{-1}$; $c(\text{SA}) = 3.2 \times 10^{-3} \text{ mol L}^{-1}$; $c(\text{Co}^{2+}) = 7 \times 10^{-5} \text{ mol L}^{-1}$; $t = 25.0 \pm 0.1 \text{ }^\circ\text{C}$.

Under these conditions, the influence of DFB concentration on the reaction rate was investigated, and two calibration curves with linearity intervals of $0.102 - 3.40 \mu\text{g mL}^{-1}$ and $3.40 - 23.80 \mu\text{g mL}^{-1}$ were obtained.

The equations of the calibration curves were:

$$\text{Slope } 10^2 = -0.39 \cdot c_{\text{DFB}} + 2.42 \quad r = -0.9979 \quad (3)$$

$$\text{Slope } 10^2 = -0.226 \cdot c_{\text{DFB}} + 1.19 \quad r = -0.9967 \quad (4)$$

where c_{DFB} is the concentration of diflubenzuron expressed in $\mu\text{g mL}^{-1}$.

The obtained calibration curves at the temperature of $25.0 \pm 0.1 \text{ }^\circ\text{C}$ can be used for determination of DFB concentrations in the interval $0.102 - 3.40 \mu\text{g mL}^{-1}$ (Fig. 6) and $3.40 - 23.80 \mu\text{g mL}^{-1}$ (Fig. 7), respectively.

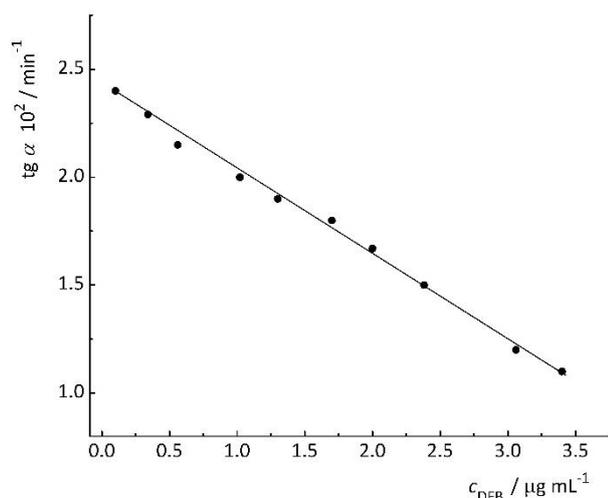


Figure 6. Dependence of the reaction rate on the DFB concentration in the interval 0.102-3.40 µg mL⁻¹. Initial concentrations: pH 7.9; c(H₂O₂) = 0.12 mol L⁻¹; c(SA)=3.2×10⁻³ mol L⁻¹; c(Co²⁺)=7×10⁻⁵ mol L⁻¹; t = 25.0±0.1 °C

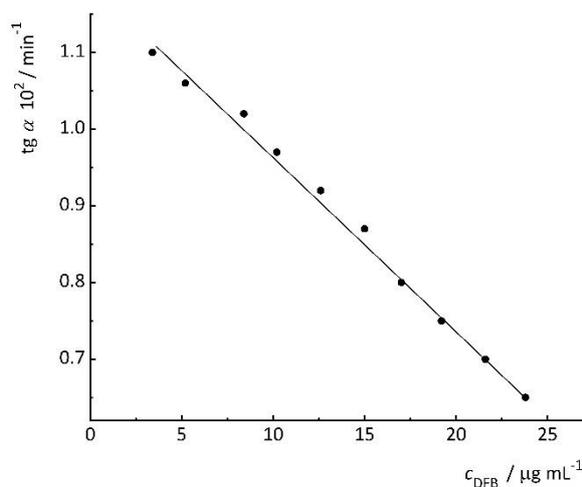


Figure 7. Dependence of the reaction rate on the DFB concentration in the interval 3.40-28.30 µg mL⁻¹. Initial concentrations: pH 7.9; c(H₂O₂) = 0.12 mol L⁻¹; c(SA)=3.2×10⁻³ mol L⁻¹; c(Co²⁺)=7×10⁻⁵ mol L⁻¹; t = 25.0±0.1 °C

Quantitative parameters of the analysis are given in Table 1.

Table 1. Quantitative parameters of the analysis

Calibration range, µg mL ⁻¹	0.102-3.40 n = 10
Regression equation	Slope×100= - 0.39·c _{DFB} + 2.42
(Slope ± SD)×10 ²	-0.39 ± 0.01
(Intercept ± SD)×10 ²	2.42 ± 0.02
Correlation coefficient (r)	-0.9979
Variance (S ₀ ²)	9·10 ⁻⁸
Detection limit, µg mL ⁻¹	0.077
Quantification limit, µg mL ⁻¹	0.254

Low values of variance confirmed negligible scattering of the experimental data points around the line of regression and good sensitivity of the proposed method.

The kinetic equations for the catalyzed and inhibited reaction were deduced according to the obtained graphic correlations:

$$\text{Rate}_I = k c_{\text{H}^+}^{-1} c_{\text{H}_2\text{O}_2} c_{\text{SA}}^{0.84} c_{\text{Co(II)}} \quad (5)$$

$$\text{Rate}_{II} = k_1 c_{\text{H}^+}^{-1} c_{\text{H}_2\text{O}_2} c_{\text{SA}} c_{\text{Co(II)}} c_{\text{DFB}}^{-1} \quad (6)$$

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

3. 1. Accuracy and precision

In order to evaluate the accuracy and precision of the method, three concentrations of DFB from the calibration curve were selected. Reaction rates for chosen concentrations were measured in five replicates. Standard solutions of 0.102, 1.70, and 3.40 µg mL⁻¹ of DFB were analyzed using the recommended procedure. Five replicate determinations of each concentration gave relative standard deviations (RSDs) of 2.08 %, 1.22 %, and 1.21 %, respectively. The results obtained on five DFB determination replicates, standard deviations, percent error and quantitative recoveries obtained from linear regression equations are listed in Table 2. The results were reproducible with low SD and RSD. Recoveries can be also considered to be very satisfactory.

Table 2. Accuracy and precision of diflubenzuron determination

Taken DFB, µg mL ⁻¹	Found DFB ^a , µg mL ⁻¹ ±SD,	RSD, % ^b	G, %	Recovery, %
0.102	0.096±0.002	2.08	-5.88	94.12
1.70	1.64±0.02	1.22	-3.53	96.47
3.40	3.31±0.04	1.21	-2.65	97.35

^aMean and standard deviation for five determinations at the 95 % confidence level; ^brelative standard deviation; G- relative error; number of replicates was five.

3. 2. Selectivity of the method

In order to investigate selectivity of the proposed method, the effects of various species on determination of 15.23 µg mL⁻¹ DFB were studied (Table 3).

Table 3. Effects of the foreign species on determination of 15.23 µg mL⁻¹ of diflubenzuron

Foreign species	Tolerance level ($C_{\text{interferent}}/C_{\text{DFB}}$)
Li ⁺ , Na ⁺ , K ⁺ , Cl ⁻ , SO ₃ ²⁻ , SO ₄ ²⁻	10 ³
C ₂ O ₄ ²⁻ , Mg ²⁺ , NO ₃ ⁻ , NO ₂ ⁻ , F ⁻	10 ²
Ca ²⁺ , Ni ²⁺ , CH ₃ COO ⁻ , Pb ²⁺	10
Ba ²⁺ , Zn ²⁺ , Mn ²⁺ , NH ₄ ⁺ , Fe ²⁺ , Fe ³⁺	1
Cu ²⁺	Interfere in all concentrations

The maximum tolerable level was taken as that causing the difference in the inhibited reaction rate not larger than 5%. The obtained results show that most cations and anions did not interfere even when present in 1000-fold greater concentration than DFB. It can be seen that Cu²⁺ has shown the catalytic effect.

3.3. Analysis of real samples

To evaluate the analytical applicability of the proposed method, DFB concentration was determined in spiked water samples and baby food samples. The samples were prepared by solid phase extraction (Experimental section). The results are presented in Tables 4 and 5, respectively.

Table 4. Determination of DFB in water samples by kinetic and HPLC methods

	Added DFB, µg mL ⁻¹	Determined DFB by kinetic method ^a , µg mL ⁻¹	Recovery ^a , %	RSD, %	Determined DFB by HPLC method ^a , µg mL ⁻¹	Recovery ^a , %	t value ^b	F value ^b
Jana	2.04	2.10±0.06	102.94	2.85	2.21±0.01	108.33	1.91	1.33
Heba	2.40	2.29±0.02	95.42	0.87	2.32±0.03	96.67	1.26	2.54
Rosa	2.88	2.78±0.02	96.53	0.72	2.72±0.01	94.44	2.30	4.77
AquaViva	1.20	1.08±0.06	90.00	5.55	1.07±0.03	89.17	0.10	6.20
Voda Voda	2.40	2.18±0.07	90.83	3.21	2.29±0.02	95.42	2.08	2.85
Prolom	1.32	1.39±0.03	105.30	2.16	1.39±0.03	105.30	1.95	5.02
Minaqua	3.12	2.92±0.06	93.59	2.05	2.99±0.01	95.83	1.85	1.16
Mivela	2.23	2.34±0.07	104.93	3.0	2.19±0.01	98.21	0.90	2.50
Drinking water	1.80	1.7±0.1	94.44	5.88	1.73±0.03	96.11	2.29	6.01
Zlatibor water	2.50	2.45±0.04	98.00	1.63	2.47±0.01	98.80	1.46	2.63
Knjaz Milos	1.80	1.71±0.02	95.00	1.17	1.75±0.02	97.22	1.12	2.43

^aData are based on the average obtained from five determinations

^bTheoretical F-value ($v_1=4$, $v_2=4$) and t-value ($v=8$) at 95 % confidence level are 6.39 and 2.306, respectively.

Table 5. Determination of DFB in baby food samples by kinetic and HPLC method

	Added DFB, µg mL ⁻¹	Determined DFB by kinetic method ^a , µg mL ⁻¹	Recovery ^a , %	RSD, %	Determined DFB by HPLC method ^a , µg mL ⁻¹	Recovery ^a , %	t value ^b	F value ^b
Apple Frutek	1.00	0.90±0.01	90.00	1.11	0.92±0.01	92.00	2.19	4.32
Pear Frutek	0.53	0.48±0.01	90.56	2.08	0.49±0.02	92.45	1.69	2.86
Apple Juvitana	0.28	0.25±0.01	89.29	4.00	0.26±0.02	92.86	1.95	5.62
Pear Juvitana	0.19	0.17±0.01	89.47	5.88	0.18±0.01	94.74	1.20	3.50
Plum Frutek	0.15	0.15±0.01	100.00	6.67	0.15±0.03	100.0	1.96	4.29
Apple Fruit baby	0.21	0.22±0.01	104.76	4.54	0.21±0.02	100.0	1.89	4.69
Carrots Fruit baby	0.25	0.26±0.02	104.00	7.69	0.25±0.01	96.15	1.96	3.58
Apple Hipp	0.12	0.11±0.03	91.67	2.73	0.12±0.03	100.0	1.39	3.67
Pear Hipp	1.20	1.22±0.04	101.67	3.28	1.21±0.01	100.83	1.85	3.62
Carrot Frutek	1.60	1.54±0.05	96.25	3.25	1.56±0.03	97.50	2.06	3.24
Plum Hipp	1.80	1.76±0.08	97.78	4.54	1.78±0.02	98.89	1.95	4.35

^aData are based on the average obtained from five determinations;

^bTheoretical F-value ($v_1=4$, $v_2=4$) and t-value ($v=8$) at 95% confidence level are 6.39 and 2.306, respectively

Figures 8a and 8b show chromatograms for DFB determination in spiked water samples and baby food samples, respectively, at DFB concentration of 3.12 µg mL⁻¹ (a) and 1.80 µg mL⁻¹ (b) under optimal conditions (MeOH-water,

80:20, v/v; at the flow rate of 1 mL min⁻¹, and wavelength of 254 nm at the temperature of 25 °C). Detection at the wavelength of 254 nm gave satisfactory sensitivity results for all spiked samples.

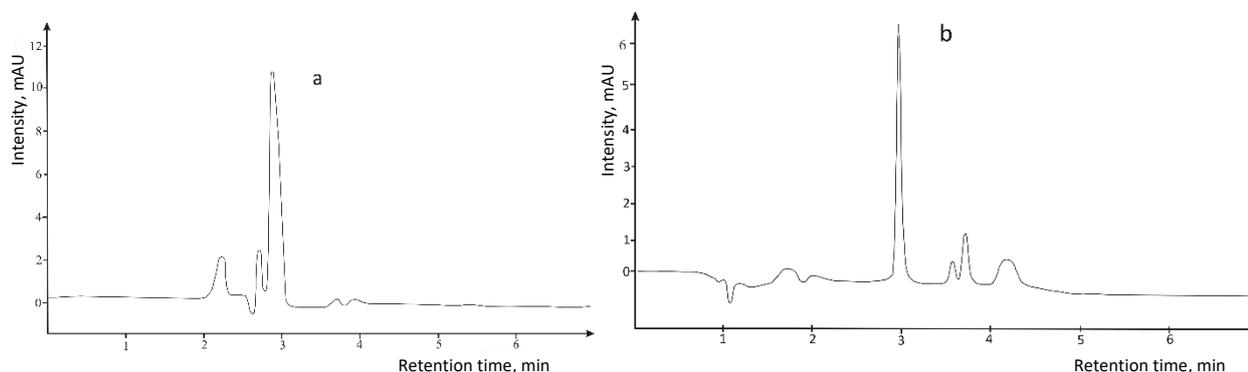


Figure 8. HPLC chromatograms of spiked water sample with 3.12 µg mL⁻¹ DFB (a) and spiked baby food sample with 1.80 µg mL⁻¹ DFB (b). Column C₁₈ (Zorbax, 5µm, 250 mm × 4.6 mm). Mobile phase: MeOH-water, 80:20 (v/v); at a flow rate of 1 mL min⁻¹. Detection: spectrophotometer at 254 nm

Calculated recoveries of DFB show that the proposed method is applicable and valid for analysis of the investigated samples. Results obtained by kinetic method are in accordance with those obtained by the HPLC method.

Tables 4 and 5 show that F and t values at 95 % confidence level are lower than the theoretical ones, confirming insignificant difference between the performance of the developed and HPLC methods. Both recovery percentages and relative standard deviations (RSD) were satisfactory and indicated good performance of the proposed method for analysis of diflubenzuron in water and baby food samples.

4. CONCLUSION

A new reaction system for kinetic spectrophotometric determination of DFB was suggested along with application for analysis of water and baby food samples. This method offers several distinct advantages namely, high selectivity and sensitivity, cheap reagents, simple and inexpensive instruments, ease of operation and rapidity. Statistical comparison of the obtained results with results of the HPLC method showed good agreement and indicated insignificant differences in accuracy and precision. Reliable recovery data were found at various concentrations, after spiking water and baby food samples. Good quantification limits were also attained. Overall, this method provided satisfactory results in the analysis of water samples and baby food samples.

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SAŽETAK

Razvoj kinetičko-spektrofotometrijske metode za određivanje insekticida diflubenzurona u uzorcima voda i kašicama za ishranu beba

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U radu je opisan razvoj kinetičko-spektrofotometrijske metode za određivanje tragova insekticida diflubenzurona. Kinetička metoda se zasniva na reakciji oksidacije sulfanilne kiseline vodonik-peroksidom u fosfatnom puferu u prisustvu Co²⁺ jona kao katalizatora. Dodatkom diflubenzurona u pomenuti sistem, uočen je inhibitorni efekat na brzinu indicator-ske reakcije. Ovakav efekat diflubenzurona na brzinu reakcije iskorišćen je za njegovo određivanje. Brzina reakcije praćena je spektrofotometrijski na talasnoj dužini od 370 nm. Ispitan je uticaj svih reaktanata na brzinu katalitičke i inhibitorne reakcije i određeni su optimalni eksperimentalni uslovi odigravanja reakcije. Kalibracione prave, date kao zavisnost brzine reakcije od koncentracije diflubenzurona, su linearne u opsegu dva koncentraciona intervala: 0,102-3,40 µg mL⁻¹ i 3,40 – 23,80 µg mL⁻¹. Izračunate granice detekcije i granice određivanja, prema 3σ kriterijumu, iznose redom 0,077 µg mL⁻¹ i 0,254 µg mL⁻¹. Određena je tačnost i reproduktivnost metode za tri koncentracije diflubenzurona (0,102, 1,70 i 3,40 µg mL⁻¹) u pet ponavljanja pri čemu su relativne standardne devijacije bile 2,08, 1,22 i 1,21 %. Uzorci voda i bebi kašica su pripremani ekstrakcijom na čvrstoj fazi (Solid Phase Extraction), a potom su koncentracije diflubenzurona određivane kinetičkom i HPLC metodom. HPLC metoda je korišćena kao uporedna metoda za potvrdu rezultata dobijenih kinetičkom metodom. Primena t-testa i F-testa pokazala je dobro slaganje rezultata dobijenih dvama metodama.

Ključne reči: kinetička metoda, diflubenzuron, HPLC, SPE, uzorci voda, hrana za bebe