



DETERMINATION OF METRIBUZIN CONTENT IN PESTICIDE FORMULATIONS USING ELECTROANALYTICAL METHODOLOGY

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The work presents results of the determination of metribuzin content in commercial pesticide formulations by applying chronopotentiometry with thin film mercury electrode as an electrochemical sensor. In the analyzed pesticide formulations, a single well defined reduction peak of metribuzin is observed at the potential around -880 mV. The content of the herbicide in commercial formulations is determined using the calibration curve method, by applying the initial potential of -0.21 V, and the final potential of -1.10 V. Recovery values based on the declared and found content of the active ingredient are in the range from 100.67% to 101.68%, with the values of relative standard deviation lower than 1.00%, indicating high accuracy and precision of the presented method.

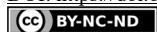
KEY WORDS: metribuzin, chronopotentiometry, thin film mercury electrode, commercial formulation.

INTRODUCTION

Distribution and application of pesticides in agriculture in most cases imply the use of commercial pesticide formulations instead of the active substances. Pesticide formulations are mixtures of one or more active ingredients, and other inert substances, such as solvents, carriers, surfactants, which improve their effectiveness, application, storage, handling, and safety (1). The active ingredient is generally formulated as emulsifiable concentrates, or as solid particles (dust, granules, soluble powder, or wettable powder), and dilution with water is a common procedure before application (2). The content of the active substance in the formulation varies depending on the formulation form, and usually is less than 50% (1, 3). For selection of suitable product and its correct application, the product label information stating the type and amount of the active substances contained in the product is of crucial importance (4).

Metribuzin (4-amino-6-*tert*-butyl-4,5-dihydro-3-methylthio-1,2,4-triazin-5-one, IU-PAC) is a selective triazinone herbicide used as a pre- and post-emergence to discourage the growth of grasses and broad-leaved weeds among various vegetables crops and turf

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grasses (5). The mode of action of this herbicide is based on the inhibition of the photosynthesis, by binding to the quinine-binding protein (D1) of photosynthetic electron transportation and thus blocking it (5, 6). Metribuzin is considered as a highly soluble substance with a low tendency to adsorb to most soils types (7, 8). Releasing of metribuzin in the environment is primarily conducted during agricultural spraying operations, while it may also be released into surface and ground water samples during runoff events (9-12). Inhalation and ingestion during agricultural operations are the primary way of human exposure to metribuzin (12), but exposure may also occur through consumption of contaminated foods or water. Although metribuzin is considered as slightly toxic to non-toxic (depending of the exposure route (12)), some serious adverse health effects were observed during exposure of different kinds of animals to this herbicide (13-15). According to the United States Environmental Protection Agency, metribuzin is classified as D group of chemicals, *not classifiable as to human carcinogenicity* due to the lack of carcinogenicity data in humans. However, the results of Delancey et al. (16) indicated a potential association between the use of metribuzin and certain lymphohematopoietic malignancies among pesticide applicators.

Metribuzin is commercialized as concentrated suspensions, wettable powder, or water dispersible granules at concentration levels from 600.00 g/l in suspensions, to 700.00 g/kg in solid formulations (17). In this study the content of metribuzin was analyzed in the following commercial formulations: *Lord*, *Welton*, and *Sencor Plus*, where this herbicide was the only active substance. *Lord* and *Welton* were in the form of water dispersible granules, while *Sencor Plus* was in the form of concentrated suspension. All three formulations were applied in soybean and potato, while *Lord* and *Welton* were also applied in tomato and alfalfa crops (18-20). The method for the determination of metribuzin recommended by the Collaborative International Pesticides Analytical Council (CIPAC) in pesticide formulations is based on chromatography (21). Generally, chromatography requires several stages before analysis, including laborious and time-consuming clean-up procedure (22-25). Because of a wide variety of plant protection products available on the market nowadays, the current challenge in analytical chemistry is to develop methods for determination of the active substances contained in plant protection products in the shortest possible time, followed by its easy application. In comparison to chromatographic techniques, electroanalytical techniques have several advantages such as low cost, and the possibility of direct analysis, without the need of pre-treatments, thus reducing the waste generation and time required for the analysis.

So far, several electroanalytical methodologies using different types of working electrodes have been reported in the literature for sensitive determination of metribuzin in soil (26) and water samples (27-29). Skopalová et al. studied electrochemical behaviour of metribuzin using polarography and voltammetry at dropping mercury electrode and mercury mini electrode (29). Janíková et al. developed a voltammetric method for determination of metribuzin, employing polished and mercury meniscus-modified silver solid amalgam electrode, and hanging mercury drop electrode (27). Stojanović et al. described chronopotentiometric method for determination of metribuzin using thin film mercury electrode (28). Besides different types of mercury electrodes, L-Norvaline modified glassy carbon electrode was also applied for determination of this herbicide using voltam-



metry (26). While considering the high amount of metribuzin in commercial formulations, the use of extremely sensitive methods does not seem excessively appropriate. Beside the alternative optical methods such as spectrometry (21) and spectrophotometry (30), voltammetry is a unique example of electroanalytical approach for quantification of the herbicide in commercial samples (27, 31).

The aim of this study was to develop a procedure for a fast and accurate determination of metribuzin content in different commercial formulations by chronopotentiometry based on direct measurements, using thin film mercury electrode. The presented method can be used for the quality control of pesticide formulations available on the market as a suitable alternative to the complicated chromatographic methodologies.

EXPERIMENTAL

Chemicals and solutions

Metribuzin, with a purity of 99.50%, was purchased from Dr. Ehrenstorfer (Germany). Metribuzin commercial formulations Welton WG (Galenika Fitofarmacija, Serbia), Lord 700 WG (Willowood, China) and Sencor Plus SC (Bayer CropScience, Germany) were purchased from the local agricultural market. Primary stock solution of metribuzin (0.30 g/dm³) was prepared by dissolving the solid substance in ethanol, and was kept in the freezer. Working solutions were prepared daily by dilution of the primary stock solution with the Britton-Robinson buffer as supporting electrolyte, to give the solution containing metribuzin in the concentration range from 2.00 to 10.00 mg/dm³. Britton-Robinson buffer was prepared from equimolar stock solutions containing 0.04 mol/dm³ orthophosphoric, boric and acetic acid solutions (Lach-Ner, Czech Republic), respectively by adding the appropriate amount of 0.20 mol/dm³ solution of sodium hydroxide (Donau Chemie, Austria) in order to achieve the appropriate pH value. Double distilled water was used throughout the experiments.

Instrumentation

Chronopotentiograms were recorded using the M1 analyzer for potentiometric and chronopotentiometric measurements constructed in our laboratory, coupled with an Epson LQ-570 printer (Suwa, Japan). A three-electrode system consisting of a thin film mercury electrode as a working electrode, an Ag/AgCl (3.50 mol/dm³ KCl) reference electrode, and a platinum wire ($\varphi = 0.70$ mm, $l = 7.00$ mm) as a counter electrode. As an inert support for thin film mercury electrode, glassy carbon disc electrode (total surface area of 7.07 mm²) was used. The potentials were presented with respect to the mentioned reference electrode. The pH measurements were carried out using a pH meter model MA 5705 Iskra (Kranj, Slovenia) with combined glass electrode.

Preparation of thin-film mercury electrode

Thin-film mercury electrode was prepared *ex-situ* from the plating solution containing 0.10 g/dm³ Hg²⁺ ions in 0.02 mol/dm³ hydrochloric acid. A deposition potential of -0.40



V was applied for 4 min, while the solution was vigorously stirred. The working electrode was subsequently rinsed with double distilled water. The working electrode could be used for approximately 50 analyses, after the mercury film is mechanically removed using filter paper wetted firstly with acetone, and then with double distilled water. The deposition of a new thin film of mercury was repeated by the same procedure.

Chronopotentiometric procedure

A volume of 20 cm³ of the analyzed solution was transferred into the process glass and deoxygenated with high-purity nitrogen for 5 min, while stirring the solution. During the deoxygenation step, in order to prevent the damage of mercury film by nitrogen bubbles, the working electrode was kept in a special glass with doubly distilled water. Afterwards, it was placed in the process glass and the analyzed solution was left quiescent for 10 s, enabling thus diffusive mass transfer throughout the chronopotentiometric measurements. The chronopotentiograms were recorded by scanning the potential in the negative direction from -0.21 V to -1.10 V.

RESULTS AND DISCUSSION

In a study by Ludvík et al. (32), the electrochemical behaviour of metribuzin by using mercury electrode two irreversible reduction signals were observed, corresponding to the reduction of 1,6-azomethine and 2,3-azomethine group of metribuzin (29, 32). A voltammetric method using a mercury electrode based on the first reduction step was applied for determination of metribuzin in water samples (29). In another study the anodic wave observed after electroreduction, associated with the oxidation of methylthiolate generated in the electrolytic process was used to quantify the herbicide content in commercial sample using modified carbon paste electrode (31). Using thin film mercury electrode in this study, the determination of metribuzin was based on its irreversible reduction in the Britton-Robinson buffer, while one well defined reduction peak was observed at the potential of -0.88 V. In accordance with the peak potential, and used electrode material, the obtained reduction peak can be attributed to the reduction of 1,6-azomethine group in the molecule (29). The optimization and validation of chronopotentiometric method for determination of metribuzin was performed by Stojanović et al. (28), and the optimal experimental parameters for performing chronopotentiometry were as follows: initial potential -0.21 V, ending potential -1.10 V, while the Britton-Robinson buffer pH 5 was used as a supporting electrolyte. The method was applied for a sensitive determination of metribuzin content in water samples.

Regarding the high content of metribuzin in commercial formulations, in the first stage of experiments it was necessary to dilute the samples to a concentration range suitable for performing chronopotentiometric measurements. The samples were firstly diluted in ethanol, given that the solubility of metribuzin in ethanol is much higher than in water (12). The proper weight of each pesticide formulation was transferred into the normal vessel of 50 cm³ filled with ethanol, and the concentration of metribuzin was about 7.00 g/dm³. The content was then subjected to ultrasound treatment for 2 min to



support dissolution. The final dissolution (7.00 mg/dm^3) was made in a volumetric flask of 100 cm^3 filled with the supporting electrolyte. A volume of 20 cm^3 of this solution was transferred into the process glass and analyzed by optimized chronopotentiometric procedure using the calibration curve method.

The chronopotentiograms obtained in the Britton-Robinson buffer pH 5 as a blank, and diluted commercial formulations are presented in Figure 1. The reduction peak of metribuzin observed for the analyzed commercial formulations in the potential range from -883 mV to -929 mV coincide with that obtained for the model system. Thus, no interferences from other formulation components were detected in the observed potential range. All measurements were performed in triplicate, and the obtained results are presented in Table 1.

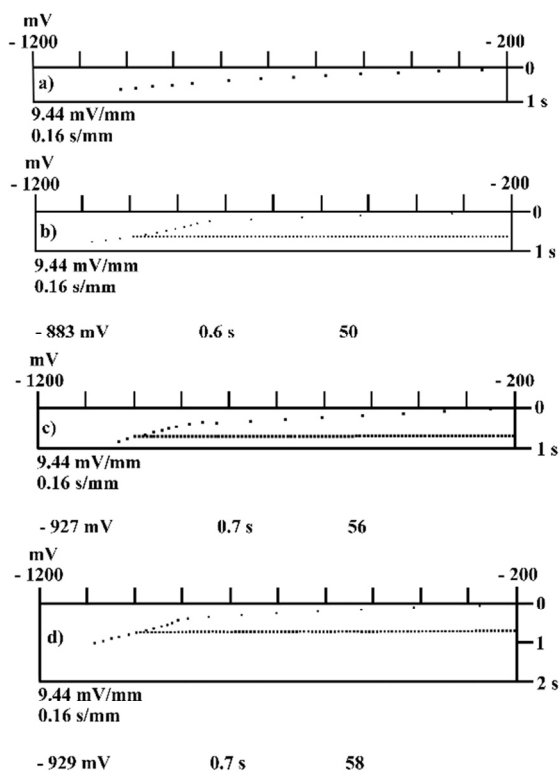
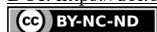


Figure 1. Chronopotentiograms obtained for: **a)** Britton-Robinson pH 5 (blank); and diluted commercial formulations **b)** *Lord*; **c)** *Welton*; **d)** *Sencor Plus*; $i_{\text{red}} = -6.4 \mu\text{A}$.

A good correlation between the declared and determined amounts indicate high accuracy, while low values of the relative standard deviation reflect high precision of the chronopotentiometric method. The results obtained by chronopotentiometric measurements were statistically compared with the labelled contents using Student's t-test. Statis-



tical analysis of the results showed no significant differences between the contents. The experimental values at a 95% confidence level did not exceed the theoretical ones ($t = 3.64 < t_{\text{theoretical}} = 4.30$). The results obtained in this study indicate that chronopotentiometry can be used as a routine tool for control of active ingredient content in metribuzin formulations without complicated sample preparation step.

Table 1. Determination of metribuzin content in commercial pesticide formulations by the chronopotentiometric method

Commercial formulation	Labelled content	Determined content ^a	RSD ^b (%)	Recovery (%)
Metribuzin content (g/kg)				
<i>Lord</i>	700.00	711.77	0.97	101.68
<i>Welton</i>	700.00	709.34	0.79	101.33
Metribuzin content (g/l)				
<i>Scencor Plus</i>	600.00	603.99	0.22	100.67

^a Mean value, n=3.

^b Relative standard deviation.

CONCLUSION

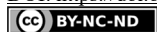
An electroanalytical procedure involving chronopotentiometry was developed for the determination of metribuzin content in commercial pesticide formulations. The main advantage of this procedure is the possibility of direct analysis of herbicide concentration without the need for any preparation step such as extraction or clean-up, which can be time-consuming, and can include the use of large volume of toxic organic solvents. Moreover, the presented method offers more than sufficient sensitivity, accuracy and precision, thus it presents an excellent alternative to chromatographic methods for the fast determination of metribuzin content in pesticide formulations.

Acknowledgements

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant III 46009).

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ОДРЕЂИВАЊЕ САДРЖАЈА МЕТРИБУЗИНА У ФОРМУЛАЦИЈАМА ПЕСТИЦИДА ПРИМЕНОМ ЕЛЕКТРОАНАЛИТИЧКЕ МЕТОДОЛОГИЈЕ

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У овом раду описано је одређивање садржаја метрибузина у комерцијалним формулацијама пестицида уз примену хронопотенциометрије и танкослојне живине електроде као електрохемијског сензора. У анализираним формулацијама пестицида уочен је један добро дефинисан редукциони пик метрибузина при вредности потенцијала од око -880 mV. Садржај хербицида у комерцијалним формулацијама одређен је методом калибрационе криве применом почетног потенцијала од $-0,21$ V и завршног потенцијала од $-1,10$ V. Вредности *recovery* теста засноване на истакнутом и одређеном садржају активне материје биле су у опсегу од 100,67% до 101,68%, уз вредности релативне стандардне девијације ниже од 1.00%, указујући на велику тачност и прецизност приказане методе.

Кључне речи: метрибузин, хронопотенциометрија, танкослојна живина електрода, комерцијална формулација.

Received: 14 March 2018

Accepted: 15 June 2018