

Analytical Methods

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Journal Name

ARTICLE

Simple and rapid electrochemical sensing method for metribuzin determination in tap and river water samples

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This paper describes the electroanalytical method for determination of metribuzin on thin film mercury electrode using chronopotentiometry. Research included optimization of the most important parameters of chronopotentiometric analysis and the best responses were achieved in Britton–Robinson buffer at pH 5.0, using an initial potential of -0.21 V, ending potential of -1.1 V, and reduction current in range from -4 μ A to -15 μ A. Under these conditions, metribuzin was reduced in an irreversible electrode reaction process with one well defined reduction wave at -0.83 V (vs. Ag/AgCl, KCl, 3.5 mol/dm³). Linear response was observed for three concentration ranges 1-5, 5-15 and 15-30 mg/dm³, with achieved limit of detection of 0.042 mg/dm³. The precision was determined as a function of repeatability and reproducibility, which showed relative standard deviation values not higher than 3.18%. Various interfering substances did not interfere with metribuzin determination. The proposed method was successfully applied to tap and river water samples. Recovery values obtained for spiked river water samples were in good statistical agreement with those obtained by LC-MS/MS method. Accordingly, the proposed chronopotentiometric methodology can be conveniently applied in quality control of environmental water samples as a simple, sensitive, reliable and low cost method.

Introduction

There is a growing concern regarding the risk associated with the exposure of humans to various chemical substances in the nature. Due to the fact that herbicides are environmentally important pollutants, reliable analytical procedures are needed for a systematic control of their contents in agricultural products, food, soil, and water.

Metribuzin (MTZ, 4-amino-6-*tert*-butyl-4,5-dihydro-3-methylthio-1,2,4-triazin-5-one) (Fig. 1) is a triazinone herbicide effectively used for the control of grasses and broad-leaved weeds in soybeans, potatoes, tomatoes, sugar cane, alfalfa, carrots, asparagus, maize and cereals.¹ Efficiency of MTZ against weeds is based on the inhibition of electron transfer in the photosynthesis pathways of plant.²

In spite of enormous benefit for human population due to

increasing yields of various crops, large-scale usage of MTZ has a negative impact on the environment. The pathways of releasing MTZ into the environment are primarily during agricultural spraying operations, or during runoff events in agricultural regions, where it can easily reach lower soil profiles, surface and ground water. Despite the fact that MTZ is slightly soluble in water, there are numerous studies that confirm its presence in surface and ground waters.³⁻¹⁴ The highest MTZ concentration of 940 μ g/dm³ was detected in ground water in Wisconsin.³

According to the World Health Organization, MTZ is considered as moderately toxic (oral LD₅₀ value is 322 mg/kg).¹⁵ By applying EPA's guidelines for assessment of carcinogenic risk, MTZ is classified in Group D: not classifiable as to human carcinogenicity, due to inadequate animal evidence of carcinogenicity.¹⁶

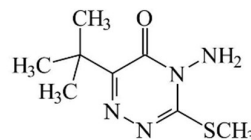


Fig. 1 Molecular structure of metribuzin

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Although toxicological effects of MTZ on humans are weaker than reported for other herbicides, many studies conducted on animals proved that chronic exposure to MTZ can cause serious health effects such as: underfeeding, kidney disfunction, immunological abnormalities, histopathological changes and oxidative stress.¹⁶⁻²⁰ Studies conducted on humans testify to the relationship between certain malignancies, chromosome aberration, immunological abnormalities, oxidative stress and genotoxicity in people who were often subjected to MTZ.^{17,21-23} As a conclusion it can be said that MTZ exhibits low acute toxicity, even though some serious negative effects were observed after chronic exposure. Consequently, the development of simple and selective methods for its determination in environmental samples, especially in water sources is of high interest. Additionally, the determination of pesticide in natural waters is important in the studies of the environmental cycle of pesticide.

Gas and liquid chromatography are the techniques mainly used for the determination of MTZ in environmental water samples.^{4-6,8,24} These methods offer good sensitivity and the possibility for determining several kinds of herbicides simultaneously, but high price of the equipment, complicate compound extraction and clean-up steps make them expensive, tedious and time-consuming. Nowadays, electrochemical methods have attracted more attention for pesticide analysis, due to their advantages of fast response, cheap instrumentation, simple operation, time saving and high sensitivity accompanied by sufficient selectivity, precision, and accuracy.^{25,26} Literature survey has shown a small number of electroanalytical methodologies for quantification of MTZ based on its reduction.²⁷⁻³¹ Hanging mercury drop electrode,^{28,29,31} glassy carbon electrode,³⁰ carbon paste electrodes,³⁰ bismuth film electrode,³² and silver solid amalgam electrodes (AgSAEs)³¹ were among working electrodes employed for MTZ determination in model systems and various samples. Previous works showed the highest sensitivity of mercury electrode towards MTZ determination, but in all investigations hanging mercury drop electrode (HMDE) with many drawbacks were used. In comparison to HMDE, thin film mercury electrode is mechanically more stable than mercury drops, whereas contact and consumption of metallic mercury is minimized,³³ since the preparation of mercury film by electrodeposition requires only solutions of Hg²⁺. Accordingly, by using the thin film mercury electrode it is possible to achieve high sensitivity avoiding most drawbacks of HMDE.

In this paper we reported chronopotentiometric method for MTZ determination by using thin film mercury electrode as a working electrode. Literature search has indicated that there have not been any published papers dealing with the use of chronopotentiometry for determination of MTZ until now, nor by applying thin film mercury as working electrode. The developed method was successfully applied to the

determination of MTZ in spiked tap and river water samples, and obtained results were compared with those obtained by a LC-MS/MS method as an independent method.

Experimental

Instrumentation

Chronopotentiometric measurements were carried out using the M1 analyzer for potentiometric and chronopotentiometric measurements of domestic construction.³⁴ The analyzer was coupled to an Epson LQ-570 printer (Epson, Suwa, Nagano, Japan). The electrochemical cell consisted of a 50 cm³ glass vessel with tapered bottom, and was equipped with an electrical stick stirrer. Experiments were performed in a three electrode system consisting of Ag/AgCl (3.5 mol/dm³ KCl) as a reference electrode, Pt wire ($\phi = 0.7$ mm, $l = 7$ mm) as a counter electrode and a thin film mercury electrode as a working electrode. As an inert support for thin film of mercury, glassy carbon disc electrode was used (total surface area of 7.07 mm²). The potentials reported in this paper were shown versus Ag/AgCl (KCl, 3.5 mol/dm³) reference electrode. All the pH values of solutions were measured using a MA 5705 pH-meter (Iskra, Kranj, Slovenia) equipped with a glass combined electrode, which was calibrated daily with standard buffer solutions.

Chemicals and solutions

The analytical standard of MTZ (purity of 99.5%) was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Standard stock solution of MTZ (0.3 g/dm³) was prepared by dissolution of the appropriate amount of the substance in the ethanol (Zorka Pharma-Hemija, Šabac, Serbia), given that solubility of MTZ in ethanol is much higher than in water (2 g/kg in comparison to 1.2 g/dm³ in water)¹⁶. More diluted solutions were prepared daily by dilution of stock solution with the supporting electrolyte. The studied supporting electrolytes were citrate buffer, phosphate buffer, Britton-Robinson (BR) buffer, acetate buffer and sulfuric acid solution. BR buffer was prepared from equimolar 0.04 mol/dm³ stock solutions of orthophosphoric, boric and acetic acids (Lac-Ner, Brno, Czech Republic). The appropriate pH value of the buffer was adjusted by 0.2 mol/dm³ solution of sodium hydroxide (Donau Chemie, Wien, Austria). All other chemical reagents were Merck PA, and they were used without any further purification. Triply distilled water was used throughout the experiments.

Thin film mercury electrode preparation

Deposition of the thin mercury film was performed potentiostatically from a solution containing 0.11 g/dm³ of Hg²⁺ and 0.02 mol/dm³ of HCl, at the potential of -0.4 V for 240 s. Prior to electro-deposition of mercury, the surface of the glassy carbon was cleaned with filter paper wetted firstly with

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acetone, and then with triply distilled water. During the film deposition, the solution was vigorously stirred. The working electrode can be used for approximately 50 analyses, after which the film was mechanically removed by the filter paper and deposition was repeated in the same way. When the problem with mercury deposition was noticed, the glassy carbon surface was polished with the suspension of aluminium oxide (grain size 0.5 μm) on a special polishing pad. After polishing, the electrode was rinsed with acetone and triply distilled water and sonicated in a mixture of triply distilled water and ethanol (1:1, v/v) for 10 minutes, in order to remove residual polishing particles.

Samples and sample preparations

The river water samples were collected from the River Danube at five different locations on the territory of Novi Sad (Serbia). Tap waters were sampled from our two laboratories. Samples were collected in the plastic bottles, previously washed with nitric acid solution, distilled and triply distilled water and stored in the refrigerator at 4°C in the dark. Before the analysis, 250 cm^3 of each sample was filtrated through membrane syringe filter with pore diameter of 0.45 μm (Chromafil®Xtra PET-45/25, Macherey-Nagel, Düren, Germany). The samples were used to prepare the BR buffer (pH 5). The components of the BR buffer were dissolved in natural water samples and analyzed according to the optimized chronopotentiometric method. In addition, the samples were spiked with certain amount of MTZ and were analyzed in the same way.

For LC-MS/MS analysis, extraction and pre-concentration was performed by solid phase extraction (SPE) with Supelco, Supel™ - Select HLB cartridges (200 mg, 6 cm^3), preconditioned with 5 cm^3 of methanol and 5 cm^3 of triply distilled water. Blank or spiked water samples were loaded on the cartridges at the rate of 5 cm^3/min by using water vacuum pump. After passing the sample, the cartridges were washed with 10 cm^3 of triply distilled water, air dried for 10 minutes, and the analyte was eluted with 5 cm^3 of methanol. The eluate was brought to dryness under a gentle nitrogen stream. The residue was dissolved in the 0.25 cm^3 of initial mobile phase, and amount of 10 mm^3 was injected into LC-MS/MS system. Quantification of MTZ was performed by means of the calibration curve method.

Procedures

Electrochemical measurement. All electrochemical measurements were carried out at the ambient temperature of the laboratory (23-25°C). For all experiments, electrochemical cell was filled with 20 cm^3 of the analyzed solution. As analyzed solution, model system or real water samples were used. Model system represented supporting electrolyte spiked with known amount of MTZ standard. It was

used for optimization and validation processes. In order to remove dissolved oxygen and to provide inert atmosphere inside the electrochemical cell, before electrochemical measurement a stream of ultrapure N_2 was passed through the stirred solution for 5 min in the first cycle and 15 s for each successive cycle. In order to prevent the damage of mercury film by nitrogen bubbles, the working electrode was kept in a special glass with triply distilled water during the deoxygenation step. Then, it was placed in the measuring cell and the solution was left quiescent for 10 s in order to enable uniform distribution of the substance in the vicinity of the electrode surface and the condition of diffusive mass transfer throughout chronopotentiometric measurements. The chronopotentiograms were recorded by scanning the potential towards the negative direction. Reduction currents were chosen in accordance with the analyzed or expected concentration. All measurements were performed in a triplicate.

Optimization and validation processes. The optimization of electroanalytical chronopotentiometric method for determination of MTZ was carried out by a systematic study of the experimental parameters that affect the response of analyte, including type and pH of the supporting electrolyte, initial and ending potential and reduction current. All parameters were thoroughly optimized in relation to the height of analytical signal and its reproducibility. Experimental parameter that provided the highest, well-defined, reproducible and sharp MTZ analytical signal was chosen as optimal.

After the optimization of experimental parameters, analytical procedure for quantitation of MTZ by chronopotentiometry was validated with the respect to linearity, limit of detection (LOD), limit of quantification (LOQ), precision, robustness, selectivity and accuracy, according to ICH guidelines.³⁵ Linearity range was examined by standard addition methods by plotting the reduction time against concentration of MTZ for three concentration ranges: 1-5 mg/dm^3 , 5-15 mg/dm^3 and 15-30 mg/dm^3 . Applied reduction currents were -4.4, -8.3 and -14 μA , respectively. Calibration graphs were constructed using data from three consecutive measurements for each addition of standard, and they were evaluated by the least-squares linear regression method. The relevant results (slope and intercept) were reported with 95% confidence level. The slope (b) and standard deviation of the intercept (Sa) of the calibration curve defined for concentration range (1-5 mg/dm^3) was used in the determination of limit of detection ($\text{LOD}=3.3\text{Sa}/\text{b}$) and limit of quantitation ($\text{LOQ}=10\text{Sa}/\text{b}$).³⁵ Precision was estimated by means of repeatability (intra-day precision) and reproducibility (inter-day precision) at two concentrations of MTZ, 1 and 5 mg/dm^3 . Repeatability was assessed as the relative standard deviation RSD (%) of five identically prepared and measured MTZ standard solutions

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within the same day. For the reproducibility evaluation, relative standard deviation RSD (%) was calculated considering average results of experiments conducted in five different days. Repeatability was estimated using the same working electrode, while for the reproducibility assessment, every day new working electrode was prepared. Reduction current applied was $-5 \mu\text{A}$ for both MTZ contents.

The suitability of the electroanalytical methodology in measuring MTZ in real sample matrices was tested by analyses of tap and river water samples. In order to verify the accuracy of the method, recovery assays at three different concentrations were carried out by adding known amounts of MTZ to the water samples. Recovery test was performed for each water sample in three replicates. Quantification of MTZ in the samples was performed by means of the multiple standard additions methods. The percentage analytical recovery values were calculated considering the ratio between the value of the concentration obtained from the spiked samples and actual added concentration. For additional check of the accuracy of the developed method, parallel analyses of river water samples were done by means of LC-MS/MS analyses.

LC-MS/MS analysis

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For validation study, in addition to the chronopotentiometric technique used in this work, the LC-MS/MS method was also handled to quantify MTZ content in river water samples. LC-MS/MS analysis was performed by liquid chromatograph Agilent 1200 Series (Agilent Technologies Inc., USA) equipped with triple-quad mass spectrometer Agilent 6410 (Agilent Technologies Inc., USA). Separation was achieved using XBridge C18 column (150 x 3 mm) with $3.5 \mu\text{m}$ particle size (Waters, Milford, USA) maintained at 40°C . The mobile phase consisted of 0.1% (v/v) formic acid in methanol (A) and 0.1% (v/v) formic acid in water (B), with a flow rate of $0.5 \text{ cm}^3/\text{min}$. The gradient used started with 70% of mobile phase B during 2 minutes hold constant, followed by a linear gradient reaching 50% B after 15 minutes, kept constant for 4 minutes, and finally decreased to 30% B after 20 minutes and kept on 30% for 6 minutes. Mass spectrometer was operated in multiple reactions monitoring (MRM) mode for mass analysis of positive ions generated by electrospray ionization (ESI). The operating parameters for the mass spectrometer were as follows: heater gas temperature of 350°C and vaporization temperature of 250°C . Nitrogen was used as a nebulizer gas at 50 psi and flow rate of $5 \text{ dm}^3/\text{min}$, capillary voltage of 3500 V and charging voltage of 2000 V. For quantification of MTZ two precursor-to-product ion transitions were chosen 215.1-187.1 and 215.1-131.0. MassHunter Workstation software (Agilent Technologies, USA) was used for the control of equipment, data acquisition and analysis.

Results and discussion

Effect of different supporting electrolytes

In the electroanalytical method development, the selection of the supporting electrolyte is of great importance since it can affect electrode reaction by modifying the thermodynamics and kinetics of electrochemical processes and charge transfer on the electrode surface.³⁶ In our case, preliminary experiments were carried out in various supporting electrolytes in order to assess their impact on the monitored electroanalytical signal. Experiments included $0.1 \text{ mol}/\text{dm}^3$ acetate, citrate, phosphate and BR buffers and $0.025 \text{ mol}/\text{dm}^3$ sulfuric acid. In order to choose appropriate supporting electrolyte, chronopotentiograms in the blank and in solutions containing different concentrations of MTZ (1, 5 and $10 \text{ mg}/\text{dm}^3$) were recorded. Chronopotentiograms were recorded from initial potential of -0.21 V to ending potential of -1.1 V . Applied currents were -4 , -7 and $-9 \mu\text{A}$ for concentration of MTZ 1, 5 and $10 \text{ mg}/\text{dm}^3$, respectively. The criterion for selection of adequate supporting electrolyte was the height of the analytical signal accompanied with the sharp and non-extended chronopotentiogram. Analytical signal of MTZ was detected in all examined electrolytes and the chronopotentiograms exhibited a single well-defined reduction wave in potential range from -0.690 V to -0.915 V in various electrolytes. In reversible potential scan no corresponding signal was recorded indicating that the electrode process can be regarded as electrochemically irreversible. Among the supporting electrolytes studied, BR buffer showed the best performance regarding the height and sharpness of the MTZ analytical signal, as well as background chronopotentiograms. Variations of ionic strengths were also investigated using 0.02, 0.04, 0.06, 0.08 and $0.1 \text{ mol}/\text{dm}^3$ BR buffer at pH 5.0. It was observed that changing the strength of BR buffer showed no distinct effect on the reduction time and potential. Since a slightly more reproducible signal was observed in $0.04 \text{ mol}/\text{dm}^3$, it was chosen for all further measurements.

The effect of pH of the BR buffer on reduction time and potential was investigated in the pH range of 2-10. A well-defined wave corresponding to MTZ electrochemical reduction was observed within the pH interval from 4 to 10. Within this pH range, MTZ analytical signal increased and reached maximum value at pH 5 and then decreased (Fig. 2). The wave potential shifted towards more negative values as the pH of the supporting electrolyte was increased (Fig. 2) indicating that the mechanism of the electrode reaction is dependent on pH. Below the pH 4, no signal of MTZ was observed since the ending potential was not reached due to the problems with hydrogen bubble formation and electrode area blockage. As optimal value of BR buffer pH 5 was chosen. Chronopotentiograms recorded in BR buffer pH 5 before and after addition of $1 \text{ mg}/\text{dm}^3$ MTZ is shown on Fig. 3.

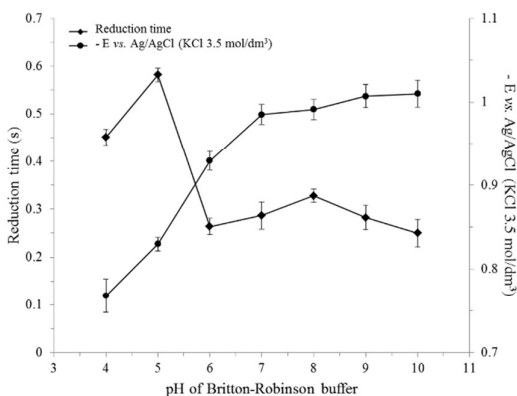


Fig. 2 Effect of pH on the reduction time (♦) and reduction potential (•) of 1 mg/dm³ MTZ in BR buffer (mean value ± 2SD, n = 3), $i = -4 \mu\text{A}$

Optimization of chronopotentiometry operating parameters

The optimization of operating parameters of chronopotentiometry influencing the response signal of analyte is an important step in the development methodology. Hence, the instrumental parameters such as an initial potential, final potential and current range were investigated in order to optimize the instrumental set-up for determination of MTZ. All experiments were carried out at 2 mg/dm³ and 10 mg/dm³ of MTZ in BR buffer at pH 5. For each operational parameter value, experiments were performed in triplicate.

Influence of the initial and ending potential. Influence of the initial potential on the MTZ reduction signal was examined in the range from 0.16 V to -0.92 V. Applied reduction current was -5.2 μA , and the value of the final potential was -1.1 V. Well defined signals of MTZ were detected when applied initial potential was in the range from -0.21 V to -0.80 V.

More positive values of initial potential brought to a permanent damage of mercury film, while at values of initial potential lower than -0.8 V, the analyte could not be detected. Obtained results as well as reproducibility of the analytical signals (mean ± 2SD, n = 3) are shown on Fig. 4. Since sufficient intensity of analytical signal of MTZ accompanied with high reproducibility (RSD = 0.02%, n = 3) was obtained at the initial potential value of -0.21 V, it was accepted as optimal for all subsequent measurements. Regarding the value of the final potential, as optimal value -1.1 V was chosen. At more negative ending potential, chronopotentiograms were outstretched, and these conditions led to fouling of the thin film mercury electrode.

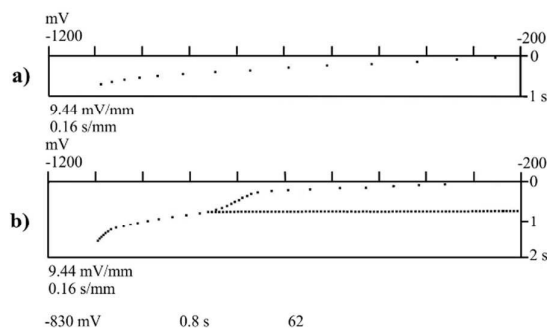


Fig. 3 Chronopotentiograms of (a) 0 mg/dm³ and (b) 1 mg/dm³ of MTZ in BR buffer at pH 5 on thin film mercury electrode, $i = -4 \mu\text{A}$

Influence of the reduction current. In chronopotentiometry, the choice of the optimal value of reduction current depends on the concentration of the analyte. Generally, for lower concentrations lower values of the reduction current should be applied, and *vice versa*. The studied ranges of reduction current for solutions containing 2 mg/dm³ and 10 mg/dm³ of MTZ were from -2 μA to -21.1 μA , and from -6 μA to -32 μA , respectively. Reduction time of MTZ exponentially decreased with more negative values of reduction current for both lower ($\tau_{\text{red}} = 1.8482e^{0.1419i}$, $r = 0.9940$) and higher ($\tau_{\text{red}} = 1.5762e^{0.0873i}$, $r = 0.9927$) concentration of MTZ. Additionally, lower currents led to deformation of chronopotentiograms and decrease of the reproducibility, while higher currents caused the decrease of the sensitivity. Dependence of the reduction time on the reduction current defined in solution containing 2 mg/dm³ of MTZ is shown on Fig. 5. Each value of the reduction time is presented as a mean of three analyses, while the reproducibility for investigated current value is shown as interval around each value (2SD). Reduction potential of MTZ did not vary significantly with the currents applied and was appearing at a potential range from -0.80 V to -0.90 V (RSD = 4.84%, n = 30).

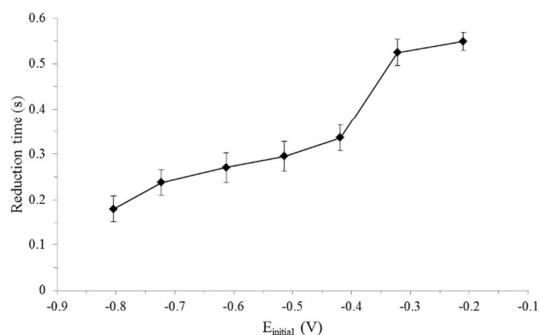


Fig. 4 Influence of the initial potential on the MTZ analytical signal. $c_{\text{MTZ}} = 2 \text{ mg/dm}^3$; $i = -5.2 \mu\text{A}$

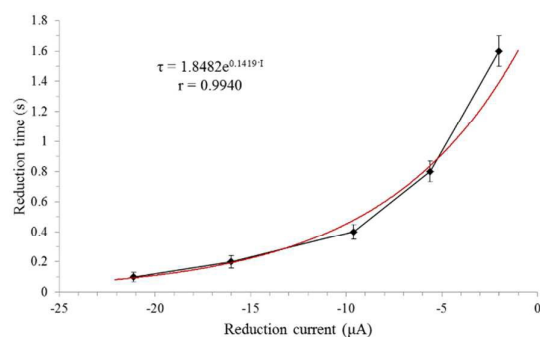


Fig. 5 Influence of the reduction current on the MTZ analytical signal. $C_{\text{MTZ}} = 2 \text{ mg/dm}^3$

According to the criteria of rectilinear sequence of the dependence $I \cdot \tau_{\text{red}}^{1/2} = f(I)$, appropriate interval of reduction current that should be applied to investigated concentrations is from -4 to -15 μA . With respect to the required sensitivity, particular value of cathodic current should be chosen from the given range.

Method validation

Linearity. Using the experimental conditions selected for the determination of MTZ, analytical figures of merit were obtained. Table 1 summarizes the characteristics for the calibration plots calculated using the least-squares method, as well as the standard deviations of the intercept (Sa) and slope (Sb) of the defined linear dependences. The dependence of the transition time on MTZ concentration showed a good linearity for all examined concentration ranges ($r = 0.995 - 0.998$).

LOD and LOQ. The range of applicability of the developed chronopotentiometric method was enclosed within the limit of detection (LOD) and the limit of quantification (LOQ). Calculated values of LOD and LOQ were $42.15 \mu\text{g/dm}^3$ and $127.72 \mu\text{g/dm}^3$, respectively.

Table 1 Regression and statistical parameters of calibration curves of MTZ using the proposed chronopotentiometric method

Concentration range [mg/dm^3]	Slope	Intercept	Sb ^a	Sa ^b	r
1-5	0.368	-0.312	0.009	0.005	0.995
5-15	0.078	-0.099	0.001	0.004	0.998
15-30	0.061	-0.590	0.001	0.024	0.996

^aSb represents the standard deviation of slope in $\text{s-dm}^3/\text{mg}$, $n = 3$.

^bSa represents the standard deviation of intercept in s, $n = 3$.

SPE would be necessary as pre-concentration step for determination of lower MTZ concentrations. By using SPE in analysis of water samples, as it was described for LC-MS/MS analysis, limit of detection can be improved up to $3.37 \mu\text{g/dm}^3$. Comparison of the LOD of our developed method and other previously published electroanalytical techniques for MTZ determination is given in Table 2. It is evident that mercury electrodes possess the highest sensitivity regarding MTZ determination.^{28,29,31} Polished silver and mercury meniscus modified silver solid amalgam electrodes (p-AgSAE and m-AgSAE) have also proved to be very sensitive toward MTZ.³¹ In comparison to the reported electrochemical applications which used bismuth film electrode (BiFE),³² or solid electrodes based on carbon materials,³⁰ the developed method offered a remarkably improved sensitivity. When mercury electrodes are in question, higher sensitivity of HMDE can be explained due to the much higher active surface of electrode, in comparison to thin film mercury electrode. Even HMDE provides slightly more improved sensitivity towards MTZ, it has a lot of disadvantages in comparison to thin film mercury electrode. HMDE requires a mercury reservoir and regular maintenance of the capillary, and incorporates complicated electronics and mechanics for precise drop generation and disposal.³³ The use of metallic mercury is connected with potential risk of poisoning and contamination, and disposal problem. Moreover, due to mechanical instability, it is not particularly suitable for on-site analysis or for application in flow-through systems. On the other hand, by using thin film mercury electrode, most of disadvantages of HMDE can be overcome. Namely, the minimal contact, exposure and consumption of metallic mercury and much better mechanical stability are evident advantages of using thin film mercury electrode. Lower detection limits achieved by applying the aforementioned electrodes can be also attributed to the high sensitivity of voltammetric techniques, especially in combination with stripping techniques with preconcentration step (Table 2). Yet, chronopotentiometry is distinguished by a simpler instrumentation, shorter analysis time in comparison with voltammetric techniques, and much easier optimization parameters procedures. In addition, simplicity of performing chronopotentiometric analysis with thin film mercury electrode favors this method for practical uses and applications for in situ analyses.

Precision. The precision of the developed method, as the measure of intra-day repeatability was 1.57% for concentration of MTZ of 1 mg/dm^3 , while in solutions containing 5 mg/dm^3 the value of relative standard deviation was 2.01%. Inter-day relative standard deviations of five average assay results, as a measure of reproducibility, were 2.78% and 3.18%, for lower and higher MTZ content, respectively. These results indicate that the proposed method possess excellent precision for determination of MTZ.

Table 2 Comparison of the sensitivity of the proposed method with previously reported electroanalytical methods for MTZ determination

Technique	Electrode	LOD [$\mu\text{g}/\text{dm}^3$]	Reference
Differential pulse adsorptive stripping voltammetry	HMDE ^a	0.27 ($t_{\text{acc}} = 10 \text{ s}$)	[28]
Fast scan differential pulse voltammetry	HMDE	4.6	
Linear sweep voltammetry	HMDE	8.57	
Elimination voltammetry with linear scan	HMDE	21.43	[29]
Square-wave stripping voltammetry	CPE/Castor oil ^b	267.42	
	CPE/Nujol oil ^c	803.98	[30]
Square-wave voltammetry	GCE ^d	24856.48	
	BiFE ^e	1285.68	[32]
	GCE	4714.16	
Differential pulse voltammetry	HMDE	4.07	
	m-AgSAE ^f	12.86	[31]
	p-AgSAE ^g	16.07	
Chronopotentiometry	Thin film Hg electrode	42.15	This study

^a hanging mercury drop electrode, ^b carbon paste electrode prepared with castor oil, ^c carbon paste electrode prepared with nujol oil, ^d glassy carbon electrode, ^e bismuth film electrode, ^f mercury meniscus modified silver solid amalgam electrode, ^g polished modified silver solid amalgam electrode

Robustness. The robustness of an analytical procedure is a measure of its ability to remain unaffected by minor, but intentional variations in method parameters. The studied variables included the change in pH (± 0.2), the initial potential ($\pm 0.05 \text{ V}$) and reduction current ($\pm 0.3 \mu\text{A}$) during the analysis of $5 \text{ mg}/\text{dm}^3$ of MTZ in BR buffer at pH 5. The robustness of the proposed method was demonstrated by constancy of the transition time with deliberate small changes in the experimental parameters.

Selectivity. In order to evaluate the selectivity of the proposed method for MTZ determination, the influence of some inorganic ions and other pesticides which may accompany MTZ in real water samples was examined by analyzing synthetic sample solution containing 2, 5, and $10 \text{ mg}/\text{dm}^3$ of MTZ and various excess amounts of some interfering substances. Considering the precision of the method, the tolerance limit was defined as the maximum concentration of interfering compound that caused a variation of MTZ analytical signal less than 5%. Majority of investigated substances in 100-fold excess (K^+ , Na^+ , Ca^{2+} , Mg^{2+} , SO_4^{2-} , HCO_3^- , and NO_3^-), and 50-fold excess (Pb^{2+} and imidacloprid) provoked slightly reduced MTZ analytical signal, while 100-fold excess of Cl^- and Fe^{2+} and 50-fold excess of Zn^{2+} ions produced a minor increase in transition time. Variation of analytical signal in the presence of these interfering substances did not exceed 5% ($\text{RSD} < 4.98\%$), while somewhat higher variation ($\text{RSD} < 6.8\%$) was observed in the presence of 10-fold excess of metamiltron and 2-fold excess of Cd^{2+} . Thus, it can be concluded that proposed chronopotentiometric method is selective enough for MTZ determination.

Accuracy. Estimation of the proposed method accuracy was done by means of calculating the recoveries in real water samples. The proposed method was applied in the analysis of tap water samples. The original samples did not indicate the presence of MTZ above the LOD of the method. Thus, the samples were spiked with the analyte at three different concentration levels (4.80 , 6.50 and $12.40 \text{ mg}/\text{dm}^3$). Measurements were performed in triplicate and the determinations were carried out by multiple standard additions method. Obtained results for recovery are shown in Table 3. Satisfactory results were obtained in all cases indicated on good accuracy of the proposed method.

Table 3 Recoveries of metribuzin in spiked tap water samples

Sample	Added [mg/dm^3]	Found [mg/dm^3]	Recovery [%]
TW1	0.00	nd ^a	
	4.80	4.67 ± 0.21^b	97.3 ^c
	6.50	6.46 ± 0.18	99.4
	12.40	11.99 ± 0.23	96.7
TW2	0.00	nd	
	4.80	4.86 ± 0.24	101.3
	6.5	6.38 ± 0.27	98.1
	12.4	12.02 ± 0.34	96.9

^a nd – not detected.

^b mean value \pm 2SD, $n = 3$.

^c mean recovery, $n = 3$.

In addition, results of recovery experiments showed that other components present in tap water did not interfere with the MTZ analytical response which confirmed previous results regarding the selectivity of the chronopotentiometric method for MTZ determination.

For additional verification of the accuracy of the developed method, LC-MS/MS parallel analyses of river water samples were done. Obtained results are presented in next section.

Application to tap and river water samples

Application of the proposed method in the real sample analysis is one of the primary requirements for its validation. The proposed method was used for determination of MTZ in river

water samples and determinations were performed by multiple standard addition method.

The results of the proposed chronopotentiometric method were evaluated statistically in comparison to the LC-MS/MS method (Table 4). In all analyzed samples, electrochemical measurements showed no response that can be assigned to MTZ whereas the chronopotentiogram profile indicated the absence of interfering electroactive compounds. These results suggested that MTZ was not present in the river waters at the detection limit of the proposed method.

Analyses of spiked river water samples resulted in the high recoveries confirming that the proposed method is applicable for analysis and correct and accurate determination of MTZ even in very complicated matrices like river waters. According to the results of t- and F-tests, the variances between the two methods were found to be insignificant at 95% probability level, indicating that no significant differences existed between the performances of the two methods regarding their accuracy and precision. Accuracy of the proposed method was accompanied with high reproducibility of the results (RSD =

4.05%). Recovery ranged from 92.5% to 102.5%. These values of recovery were within the calculated acceptable range,³⁷ indicating that the method is suitable for MTZ determination in real sample.

Conclusions

In this study chronopotentiometry was applied for the first time for MTZ determination in combination with thin film mercury electrode. The method was based on irreversible reduction of the analyte in BR buffer at pH 5.0. Achieved detection limit of 42.15 $\mu\text{g}/\text{dm}^3$ is comparable to values reported in the literature for other electrochemical methods. Minor influence of certain interfering substances proved the selectivity of the proposed method. The analytical utility of the proposed method was assessed by applying it to the determination of MTZ to spiked tap and river water samples, without complex sample pretreatment. Results obtained by chronopotentiometry were in good agreement with those obtained by reference LC-MS/MS method, confirming thus accuracy of developed method. In comparison to LC-MS/MS method, chronopotentiometry showed to be an uncomplicated, fast and cheap technique with satisfactory results regarding precision and accuracy, which allows easy determination of MTZ in real samples.

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Table 4 Results obtained for the analysis of river water samples by the proposed chronopotentiometric method and reference LC-MS/MS method

Sample	MTZ added [mg/dm^3]	Chronopotentiometry [mg/dm^3]	LC-MS/MS [mg/dm^3]
RW1	0.00	nd ^a	nd
	0.40	0.41 ± 0.01^b (102.5) ^c	0.38 ± 0.09 (95.0)
RW2	0.00	nd	nd
	0.40	0.38 ± 0.01 (95.0)	0.42 ± 0.08 (105.0)
RW3	0.00	nd	nd
	0.40	0.37 ± 0.02 (92.5)	0.42 ± 0.09 (105.0)
RW4	0.00	nd	nd
	0.40	0.38 ± 0.01 (95.0)	0.43 ± 0.06 (107.5)
RW5	0.00	nd	nd
	0.40	0.37 ± 0.03 (92.5)	0.38 ± 0.03 (95.0)

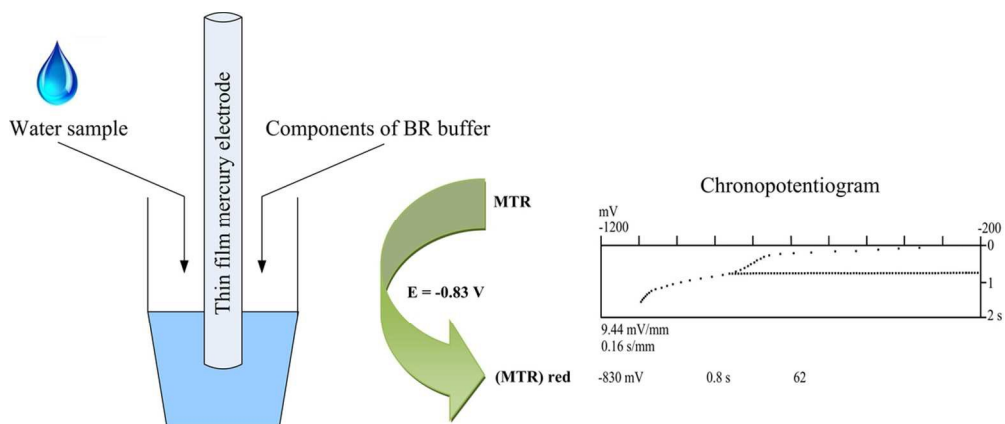
^and – not detected.

^bmean value \pm 2SD, n = 3.

^cmean recovery, n = 3.

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