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Determination of Pesticide Chlortoluron using HPLC with Amperometric Detection at a Carbon Paste Electrode

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Abstract: Method for the determination of pesticide chlortoluron in environmental samples was developed using HPLC with amperometric detection at the carbon paste electrode based on glassy carbon microparticles. Column LiChroCART® 125-4 Purospher® RP-18 (5 μm) was employed for the separation. Optimum conditions of the determination were mobile phase methanol and Britton-Robinson buffer pH 4.0 (60:40, v/v) and detection potential +1.3 V. Chlortoluron determination was performed in matrices of river water and soil; river water sample was filtered and injected directly; from 2.5 g of soil samples, the analyte was extracted to 5 mL of methanol. This method served well for the separation of the analyte from the matrix interferents and it was possible to determine chlortoluron content above the concentration 0.19 μmol L^{-1} in the case of river water and 0.29 μg g^{-1} in the case of soil.

Keywords: Carbon paste electrode; HPLC; Chlortoluron; Pesticide.

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Introduction

Many herbicides are used in agriculture all over the world. Despite their benefits in increasing agricultural production, herbicides can have a negative impact on the environment and can pose a risk to animals and humans. For these reasons, there is a growing demand for fast and reliable analytical methods for pesticide monitoring in agriculture.

Chlortoluron is a phenylurea herbicide applied against broadleaf weeds and grass in the beet or poppy cultures [1] and against silkybent grass in winter cereals [2]. In some applications, it is combined with other herbicides, particularly from the sulfonylurea family [3]. The mechanism of action is the inhibition of photosynthesis by blocking the electron transfer through the chloroplast membrane [4].

As in the case of many other herbicides, chlortoluron presents a potential danger for the health and environment. Its accute toxicity is low, but it shows carcinogenic properties during the chronic exposition, affecting particularly kidney and liver. Besides, it is directly poisonous to the aquatic organisms [5,6]. It is moderately persistent in soil, with predominant microbial degradation [7] and minor photolytic degradation [8], and it is stable in water [6].

Previous methods for the determination of chlortoluron involve particularly chromatographic techniques, mainly liquid chromatography with spectrophotometric [9-11], fluorescence [12] or electrochemical detection [13]. Gas chromatography is less advantageous due to the necessary derivatization step; nevertheless, the determination method was also developed [2], including the combination with mass spectrometry [14].

Carbon paste electrode (CPE) offers favorable properties in the wide range of measuring techniques, such as wide potential window, low background current and easy surface renewal. Electrode performance can be further enhanced by the addition of modifiers suitable for particular application [15]. Utilization of CPE in flow techniques, such as HPLC, requires the compatibility of the composition of the carbon paste and of the mobile phase. We have proven earlier [16] that CPE based on glassy carbon microparticles is compatible with organic solvents used in HPLC.

The aim of this work is to develop a method for the determination of chlortoluron, using reversed-phase HPLC with amperometric detection at carbon paste electrode in wall-jet arrangement, and to test the possibility of the application of this method for chlortoluron determination in samples of surface water and soil.

Experimental

Chemicals and Reagents

The $1{\cdot}10^{-3}$ mol L^{-1} stock solution of chlortoluron (Fig. 1, analytical standard, Sigma-Aldrich) was prepared by dissolution of 0.0106 g of chlortoluron in 50 mL of methanol (for HPLC, Merck, Germany).

Britton-Robinson (B-R) buffers were prepared by mixing a solution of phosphoric, acetic and boric acid (0.04 mol L^{-1} concentration each) with an appropriate amount of 0.2 mol L^{-1} sodium hydroxide solution (all p.a., Lach-Ner, Czech Republic).

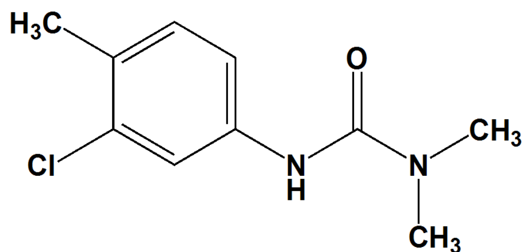


Fig. 1: *Chemical formula of chlortoluron.*

Instrumentation

Chromatographic measurements were performed using high pressure pump HPP 5001 (Laboratorní přístroje Praha, Czech Republic), injector valve with 20 µL loop (Ecom, Czech Republic), and amperometric detector ADLC 2 (Laboratorní přístroje Praha, Czech Republic). LiChroCART® 4-4 Purospher® RP-18 (5 µm) precolumn and LiChroCART® 125-4 Purospher® RP-18E (5 µm) column were used for the separation.

Amperometric detection was carried out in three-electrode system with glassy carbon paste working electrode (GCPE), platinum auxiliary electrode, and Ag/AgCl (3 M KCl) reference electrode. The carbon paste consisted of 250 mg of glassy carbon microparticles (diameter 0.4 – 12 µm, Alfa Aesar) and 100 µL of mineral oil (Fluka) and was packed in Teflon piston driven holders with inner diameter of 2 mm [17]. The working electrode was adjusted in the overflow vessel against the outlet capillary in wall-jet arrangement. All measurements were carried out at laboratory temperature. The mobile phase was degassed by 10min sonication using PSO 2000A Ultrasonic Compact Cleaner (Powersonic, USA) and its flow rate was 1 mL min^{-1}.

Sample Preparation

River Water. River water was obtained from Vltava river, Praha Vyton, and was filtered through the MS Nylon Membrane Filter (0.22 µm, Membrane Solutions, USA) Model samples in this matrix were prepared by addition of the the appropriate amount of chlortoluron stock solution to filtered river water.

Soil Samples. Model samples in the matrix of soil were prepared from dry soil (Praha, Modrany; field soil), sieved to the fraction < 120 mesh. Appropriate amount of the stock solution of chlortoluron was added, the soil was homogenized and dried. Analyte was extracted from 2.5 g of soil by 5 mL of methanol. Methanolic solution was mixed with deionized water in the ratio 60:40 (v/v) prior the determination in order to prevent the distortion of the peaks.

Data Processing

All curves were measured in triplicate, unless specified otherwise. The calibration dependences were processed using linear regression method. The quantification limits were calculated as the concentration of an analyte which gave a signal ten times the standard deviation of the signal of lowest evaluable concentration. [18]

Results and Discussion

Optimization of Key Experimental Parameters

The initial composition of the mobile phase for the HPLC of chlortoluron was based on the previous work [1]. The mobile phase consisted of methanol and B-R buffer (60:40, v/v). Optimization of the mobile phase pH was performed in the pH range of the aqueous part from 3.0 to 7.0; peak position and electrode response were observed. Slight increase of retention occurs with pH increase. The retention time is approximately 4 min; this value ensures sufficient separation from potential matrix interferents. Hydrodynamic voltammograms (Fig. 2) show similar behavior of the analyte in media of pH 3.0, 4.0 and 5.0; at pH 7.0, the response is lower.

B-R buffer pH 4.0 and detection potential +1.3 V were selected as optimum conditions, as they offer the maximum response and in the same time the lowest background signal. Under the optimum conditions, signal stability was tested by 20 repeated injections of 20 μL of 100 μmol L^{-1} chlortoluron. The resulting signal is stable with 3.3 % RSD (n = 20). Under the optimum conditions, the concentration dependence was measured in the concentration range from 100 μmol L^{-1} to the determination limit.

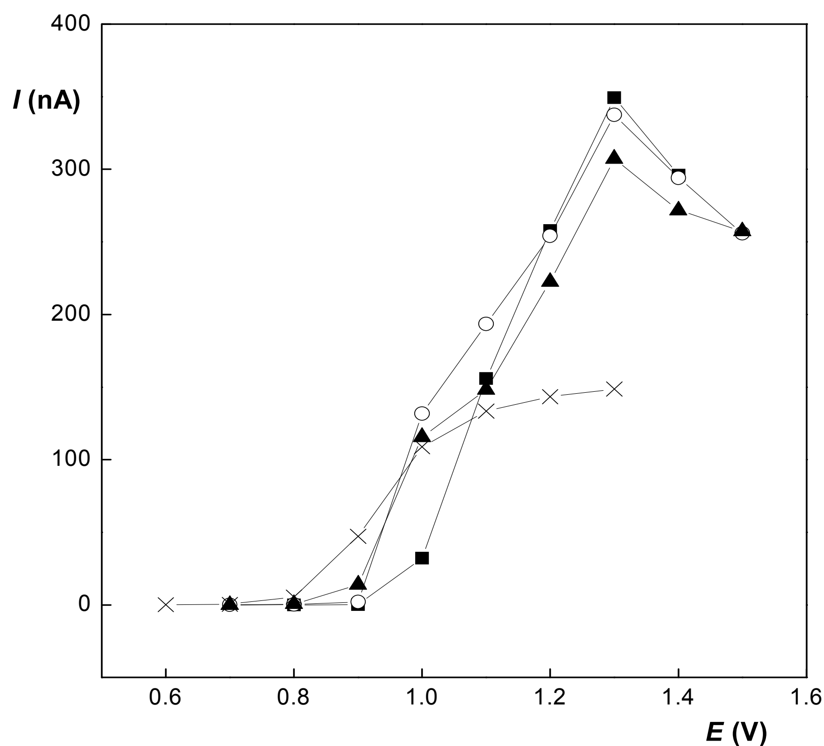
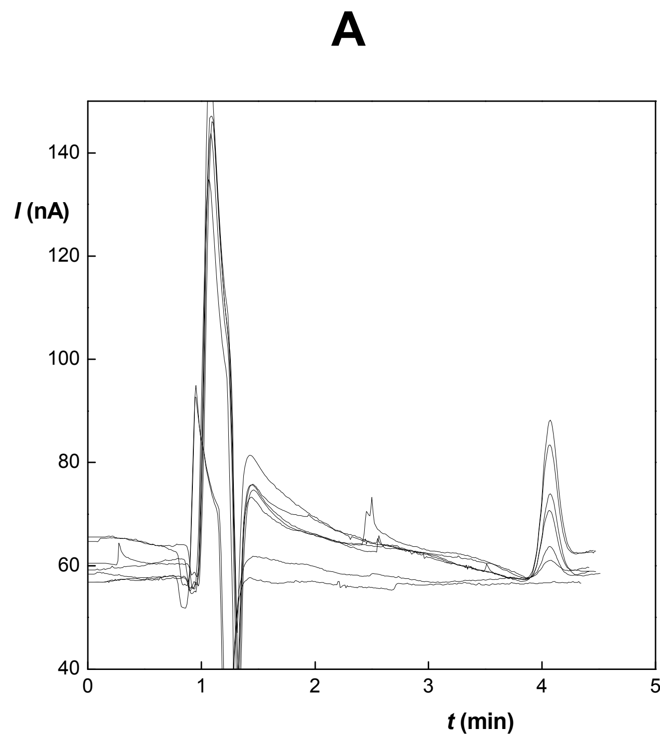


Fig. 2: *Hydrodynamic voltammograms of chlortoluron at CPE in mobile phase with different pH (pH 3.0 (■); pH 4.0 (○); pH 5.0 (▲); pH 7.0 (×)).* Column LiChroCART® 125-4 Purospher® RP-18 (5 μm), mobile phase B-R buffer of selected pH and methanol (40:60, v/v), flow rate 1.0 mL min^{-1}, injected 20 μL of 100 μmol L^{-1} chlortoluron solution.

Parameters of the dependence are summarized in Table I and selected chromatograms are shown in Fig. 3 overleaf. Obtained dependences are linear in the whole concentration range and have negligible intercept. Determination limit was calculated as 0.11 μmol L^{-1}.

Table I: *Parameters of the concentration dependences of chlortoluron in various matrices.*

Matrix	Linear dynamic range (μmol L^{-1})	Slope [mA mol^{-1} L]	Intercept [nA]	Correlation coefficient	Determination limit [μmol L^{-1}]
Pure solvents	0.2 – 100	3.5	-0.42	0.9991	0.11
River water	0.2 – 100	3.4	-0.28	0.9972	0.19
Matrix	Linear dynamic range (μg g^{-1})	Slope [nA $μg^{-1}$ g]	Intercept [nA]	Correlation coefficient	Determination limit [μg g^{-1}]
Soil	0.1 – 100	4.7	5.5	0.9962	0.29



B

140
I (nA)
120
100
80
60
0 1 2 3 4 5
t (min)

Fig. 3: *Chromatograms of chlortoluron samples of various concentrations in matrices of river water (A) and soil (B).* Column LiChroCART® 125-4 Purospher® RP-18 (5 µm), mobile phase B-R buffer of pH 4.0 and methanol (40:60, v/v), flow rate 1.0 mL min^{-1}, E_{DET} = +1.3 V, chlortoluron concentration (A) 10; 8; 6; 4; 2; 1; 0 µmol L^{-1}, (B) 10; 7; 4; 2; 1; 0 mg g^{-1}.

Determination in Real Matrices

The applicability of the determination in the environmental matrices was tested by the determination in the model samples of river water and soil. River water was filtered and injected directly. Soil samples were extracted by sonification and shaking with methanol. (For the detailed sample preparation, see Experimental.) By the comparison with the response of standard solutions of the corresponding concentration, it was confirmed that the recovery of the extraction step is sufficient, reaching 96 % for chlortoluron concentration of 7 $\mu g\ g^{-1}$ and 93 % for 40 $\mu g\ g^{-1}$.

As the last part of the work, applicable concentration range for chlortoluron determination in the real samples was investigated. In the case of the determination in river water, substantial matrix peak appeared at the dead time. This peak was not observed in the soil extracts; apparently, the extraction step ensures almost clean sample. Nevertheless, concentration dependences in both matrices (see Table I overleaf) are linear, with the determination limits 0.19 $\mu mol\ L^{-1}$ and 0.29 $\mu g \cdot g^{-1}$. This sensitivity allows one the determination of chlortoluron concentrations expected in the environmental samples.

Conclusions

A method for the determination of chlortoluron was developed using reversed-phase HPLC with amperometric detection at GCPE in wall-jet arrangement. The optimum conditions for the determination are LiChroCART® 125-4 Purospher® RP-18 (5 μm) column, mobile phase methanol and BR buffer pH 4.0 (60:40, v/v), and detection potential +1.3 V. The applicability of the method was confirmed by the determination of chlortoluron in model samples of river water and soil, in the latter case after the extraction by methanol. Determination limits reach values 0.11 $\mu mol\ L^{-1}$, 0.19 $\mu mol\ L^{-1}$ and 0.29 $\mu g\ g^{-1}$ in pure solvents, river water, and soil, respectively, which is in compliance with the sensitivity needed for analysis of real samples.

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