Reduction behavior of insecticide azoxystrobin and its voltammetric determination using silver solid amalgam electrode

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Abstract

Electrochemical reduction of systemic fungicide azoxystrobin, used to protect a wide variety of crops, was investigated using cyclic voltammetry with a mercury meniscus modified silver solid amalgam electrode (m-AgSAE). Mechanism of the electrochemical reduction was proposed and supported with high performance liquid chromatography/mass spectrometry analysis of azoxystrobin solutions electrolyzed on mercury pool electrode. An analytical method for the determination of azoxystrobin by differential pulse voltammetry with m-AgSAE was developed. The wide linear dynamic range (2.0×10^{-6}-5.0×10^{-5} mol dm^{-3}) and low limit of detection (7×10^{-7} mol dm^{-3}) were obtained. Accuracy and repeatability of the proposed method was evaluated by the analysis of model solutions with recovery from 96.0 to 104.0 % and relative standard deviation of 5 repeated determinations < 3.5 %. Finally, new voltammetric method using m-

AgSAE was successfully applied for azoxystrobin determination in real samples, concretely in spiked river water and pesticide preparations.

Keywords Pesticides ● Azoxystrobin ● Reduction mechanism ● Voltammetric determination ● Silver solid amalgam electrode

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Introduction

Azoxystrobin (AS, methyl(2E)-2-(2-{[6-(2-cyanophenoxy)pyrimidin-4-yl]oxy}phenyl)-3-methoxyacrylate, CAS: 131860-33-8) belongs to the group of synthetic fungicides strobilurins derived from the active substances produced by wood-destroying fungi [1]. Its structural formula is shown in Fig. 1. AS inhibits mitochondrial respiration of fungi which leads to the oxidative stress in the fungus cells and suppresses their growth [2, 3]. It is used to protect a whole range of crops, such as cereals, vegetables or oilseed rape. Moreover, it can be applied to ornamental plants and conifers [4]. AS also has negative impact on the environment, especially on the aquatic organism. It is toxic for freshwater or marine fish and aquatic invertebrates. In humans, it can cause serious eyes damage, allergic skin reactions or respiratory irritation [5]. Therefore, simple, rapid, sensitive, and selective methods for determining this fungicide should be available.

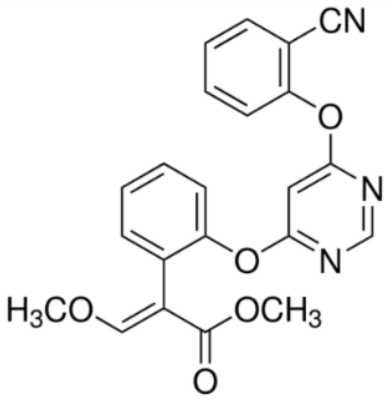


Fig. 1 Structural formula of azoxystrobin

Currently, chromatographic methods are the most commonly used for pesticide determination. Usually, it is necessary to apply some separation procedure before the analysis, most often it is liquid/liquid or liquid/solid phase extraction. High performance liquid chromatography (HPLC) was used for AS determination in combination with diode array [6] or UV spectrophotometric detector [7, 8]. Gas chromatography (GC) is frequently utilized in connection with electron capture detector [9, 10]. Very common in case of AS determination is also connection of chromatography with mass spectrometry [11, 12]. Nevertheless, these methods are quite instrumentally demanding and time-consuming which leads to the development of alternative methods.

Because of AS is electrochemically active molecule, voltammetry seems to be available for its determination. Moreover, voltammetric methods represent simple, fast, and sensitive alternative to the chromatography which can allow also the application in portable analyzers. So far, only one paper dealing with the AS voltammetric determination was published [13]. Pacheco et al. used hanging mercury drop electrode (HMDE) and determined AS via its reduction at the potential about −1000 mV (vs. Ag/AgCl (KCl, 3 mol L^{-1})) in acidic media (0.1 M HCl). These authors did not studied the AS reduction mechanism [13]. Due to the current trend of replacing mercury working electrodes with other ones,

silver solid amalgam electrode (AgSAE) [14] was applied for AS determination in the present work. In addition to analyte determination, its reduction mechanism was also studied. AgSAE represents the most commonly mentioned alternative to HMDE especially due to the exceptional electrochemical properties similar to the mercury electrodes, namely high hydrogen overvoltage and related wide potential window in cathodic area [15]. The great advantage of amalgam electrodes is their versatility. They can be used in analysis of inorganic [14, 16, 17] as well as organic [18-22] compounds or biomolecules as proteins [23-25] and DNA [26-29]. Many papers have also been focused on determining of various pesticides using amalgam electrodes [30-35].

In the present paper, the voltammetric behavior of AS has been studied using mercury meniscus modified (m-) AgSAE and its reduction reaction was investigated. Reduction mechanism was proposed based on HPLC-MS analysis of reduction products generated on mercury pool electrode at controlled potential. Finally, voltammetric method for AS determination was developed and utilized for analysis of spiked natural water as well as a commercially available pesticide preparation.

Results and Discussion

Voltammetric behavior of azoxystrobin in dependence on pH

At the beginning, cyclic voltammogram of 1×10^{-4} mol dm^{-3} AS in solution of 0.1 mol dm^{-3} HCl at scan rate (v) 100 mV s^{-1} was measured on m-AgSAE and the recorded curves are illustrated in Fig. 2A. Initial (E_{in}) and final (E_{fin}) potential was 0 mV and switching potential (E_{switch}) was −1500 mV. The experimental conditions, especially the supporting electrolyte, were taken from literature [13] and the obtained results corresponded with those published by authors of the mentioned paper using HMDE. AS provided one reduction signal on m-AgSAE about the potential (E_p) of −1050 mV (vs. Ag/AgCl (KCl, satur.)). In contrast with HMDE, the obtained signal was strongly influenced by hydrogen evolution due to a bit narrower potential window on m-AgSAE in comparison with mercury electrodes.

The following experiments were focused on voltammetric behavior of AS on m-AgSAE in dependence on pH. Next to 0.1 mol dm^{-3} HCl, Britton-Robinson buffer (BRB) with various pH values (2.0-12.0) served as a supporting electrolyte during this study. Reduction signals were observed only in acidic to slightly acidic medium (pH 1.0-6.0) as it is demonstrated in Fig. 2B. Potential window expanded to more negative potentials with increasing pH. While in media of diluted HCl only one reduction signal was observed, in BRB of pH 2.0-4.0 two cathodic responses were recorded. The first signal was the highest and the best evaluable in BRB of pH 2.0

and then gradually decreased with increasing pH. The second response was registered at pH 2.0 and it reached the maximum at pH 4.0. These results testified about strong dependence of the ongoing elecrode reactions on pH of the electrolyte. Also the significant shifts of both measured signals to the more negative potentials with increasing pH suggested that protons are involved in the electrode process.

Because of the first reduction response of AS was less affected by hydrogen evolution and it was better evaluable in comparison with the second one, it was chosen for analytical application. BRB of pH 2.0 was selected as a suitable supporting electrolyte.

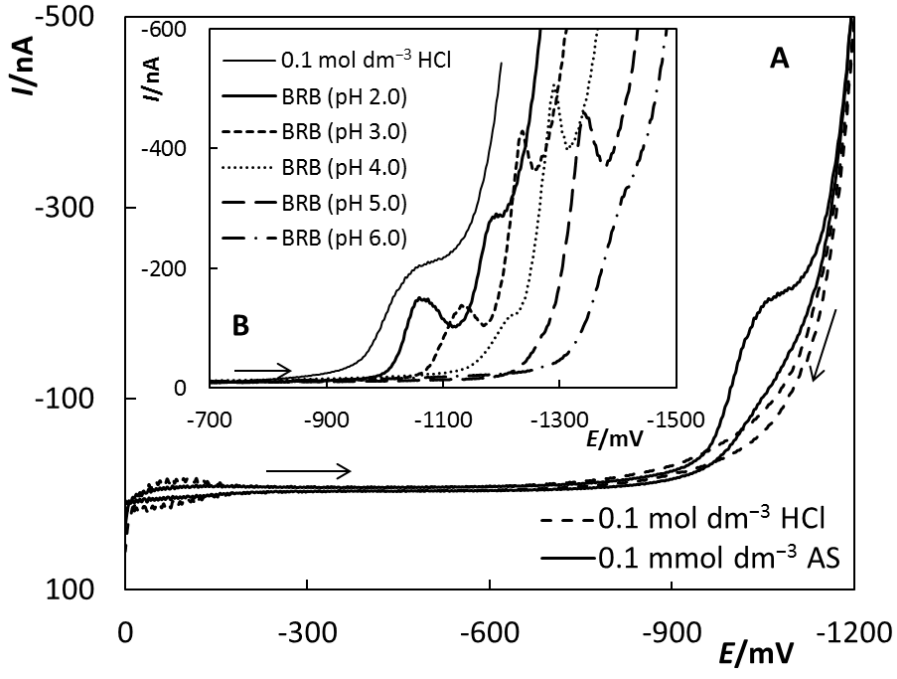


Fig. 2 Cyclic voltammogram of AS in 0.1 mol dm^{-3} HCl (A) and cathodic curves of cyclic voltammograms of AS in 0.1 mol dm^{-3} HCl and BRB (pH

2.0-6.0) obtained on m-AgSAE (B). $E_{in} = E_{fin} = 0$ mV, $E_{switch} = -1500$ mV, $v = 100$ mV s^{-1}, $c_{AS} = 1\times10^{-4}$ mol dm^{-3}.

The influence of scan rate on voltammetric behavior of azoxystrobin

In order to determine the controlling process of the AS reduction reactions, dependences on v were measured. In Fig. 3A, obtained cyclic voltammograms of 1×10^{-4} mol dm^{-3} AS in BRB (pH 2) in dependence on v (25-400 mV s^{-1}) are shown. It is evident that the first peak height (I_p) increased with increasing v but this growth was not linear (Fig. 3B). So the adsorption is not controlling process. On the other hand, the dependence of I_p on the square root of v provided linear course (Fig. 3C) which can be described by following equation (1) with the appropriated correlation coefficient. Linear dependence of I_p on $v^{1/2}$ is typical for diffusion-controlled process. The last plot in Fig. 3D represents logarithmic dependence log(I_p)-log(v) that corresponds to the equation (2). From the obtained value of the slope (0.6072±0.0069) close to the 0.5, diffusion as controlling process was confirmed but the theoretical value 0.5 did not lie in the confidence interval of slope which suggested possible influence of adsorption.

$$I_p[\mathrm{nA}] = (-52.69 \pm 0.40)\, v^{1/2}\left[(\mathrm{mV\,s^{-1}})^{1/2}\right] + (98.3 \pm 9.9),$$
$$r = 0.9996 \qquad (1)$$

$$\log(I_p[\mathrm{nA}]) = (0.6072 \pm 0.0069)\ \log(v\ [(\mathrm{mV\ s^{-1}})]) + (1.410 \pm 0.016),$$
$$r = 0.9990 \qquad (2)$$

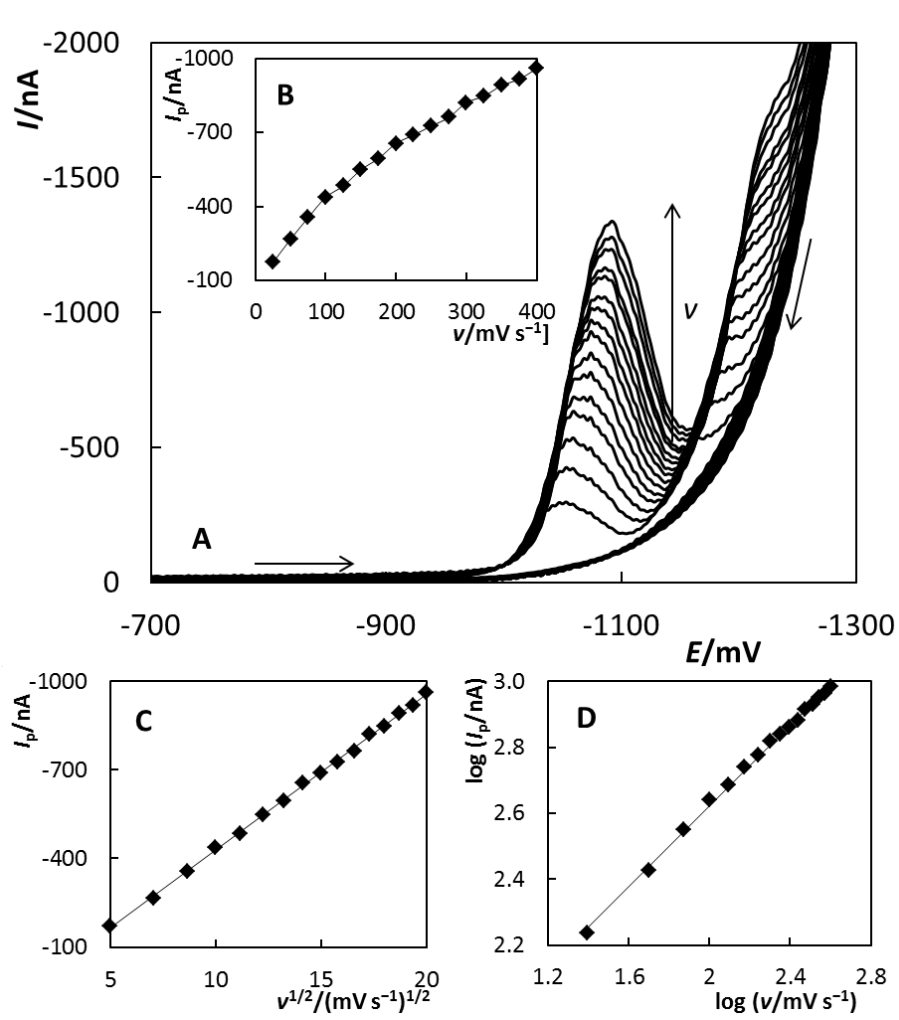


Fig. 3 Cyclic voltammograms of AS obtained on m-AgSAE in dependence on scan rate (A), dependences of I_p on v (B), I_p on $v^{1/2}$ (C) and log(I_p) on log(v) (D). Method: CV, supporting electrolyte: BRB (pH 2.0), $E_{in} = E_{fin}$= 0 mV, E_{switch} = −1500 mV, v = 25-400 mV s^{-1}, c_{AS} = 1×10^{-4} mol dm^{-3}.

Due to the poor evaluability of the second reduction signal of AS in BRB of pH 2, the similar experiments were performed also in BRB of pH 5 where only the second current response of analyte was registered. While the dependence of I_p on v was not linear, the dependence of I_p on $v^{1/2}$ provided linear course and can be chacacterized by equation (3) with the related correlation coefficient. The value of slope of logharitmic dependence (eq. 4) lies between 0.5 and 1 (0.775±0.020) which means that the electrode reaction is probably influenced by both diffusion and adsorption.

$$I_p[\text{nA}] = (-82.2 \pm 1.2)\, v^{1/2}\, \left[(\text{mV s}^{-1})^{1/2}\right] + (266 \pm 17), \quad r = 0.9986 \qquad (3)$$

$$\log\left(I_p[\text{nA}]\right) = (0.755 \pm 0.020)\, \log(v\, [(\text{mV s}^{-1})]) + (1.204 \pm 0.044), \quad r = 0.9951 \qquad (4)$$

Reduction mechanism of azoxystrobin

Electrochemical reduction of AS was studied using controlled potential electrolysis followed by HPLC/MS analysis of generated reaction products. Bulk electrolysis of AS (c = 1×10^{-3} mol dm^{-3}) was performed with mercury pool electrode in supporting electrolyte consisting of 0.2 mol dm^{-3} CH_3COOH and acetonitrile (1/1, v/v) in order to provide acidic medium necessary for electrochemical reaction and to keep AS dissolved. Two

values of constant potential −1200 mV and −1500 mV, corresponding to limiting current of AS cathodic waves, were applied for 1 h on the working electrode. Reference AS solution kept in the electrolytic cell for 1 h at potential 0 V, at which no electrode reaction proceeded, was prepared for analysis to disclose eventual non-electrolytic reactions.

HPLC/MS analysis of the reference AS solution provided a single peak with retention time t_r = 4.11 min, the mass spectrum with ion $[M+H]^+$ at *m*/*z* 404, and the most abundant fragment ion at *m*/*z* 372 (loss of CH_3OH). Chromatograms of the solutions electrolyzed at −1200 mV and −1500 mV were similar and rendered new peaks of reduction products with t_r = 1.57 min, t_r = 1.62 min, and t_r = 2.36 min in positive ionization mode (ESI+) and with t_r = 1.88 min in negative mode (ESI−).

Mass spectrum of the first product with t_r = 1.57 min (Fig. 4A) yielded ion $[M+H]^+$ at *m*/*z* 289 and abundant fragment ions at *m*/*z* 257, 209 and 177 corresponding to neutral losses of CH_3OH, pyrimidine and both of them, respectively. Based on the fragmentation, the structure **5** (Scheme 1) of the reduction product was proposed. The second reduction product with the peak at t_r = 1.62 min provided ion $[M+H]^+$ at *m*/*z* 291 and fragmentation ions at *m*/*z* 259, 209 and 177 (Fig. 4B) formed by losses of CH_3OH, dihydropyrimidine and both of them, respectively. Fragment ion at *m*/*z* 83 appertains to protonated dihydropyrimidine. The difference of two units in

m/*z* of protonated molecules and also in *m*/*z* of the fragment ions (*m*/*z* 257 and *m*/*z* 259) as well as very close retention times proved the second product (**6** in Scheme 1) was formed by dihydrogenation of the first one. The third product with t_r = 1.88 min provided in ESI− a simple mass spectrum with $[M\text{-}H]^-$ at *m*/*z* 118 (Fig. 4C). Odd nominal mass of the corresponding neutral molecule of this product indicates one nitrogen atom in its structure (**4** in Scheme 1). Finally, the fourth product with t_r = 2.36 min and ion $[M+H]^+$ at *m*/*z* 209 provided in its mass spectrum (Fig. 4D) very intensive fragment ion at *m*/*z* 177 corresponding to loss of CH_3OH which is typical for the vinylether moiety of AS. Structure **7** (Scheme 1) was proposed for this product. Besides the four main products mentioned above, several peaks of other side products of the electrochemical reduction of AS were detected in electrolyzed solutions. However, structure of them was not identified for low intensity of signal of its fragmentation ions.

Results of HPLC/MS analysis allowed us to propose a mechanism of the electrochemical reduction of AS (Scheme 1). Electrode reaction starts with the reduction of the pyrimidine moiety. As the voltammetric responses were observed only in the acidic media, the protonation of a nitrogen atom precedes the transfer of the first electron. In the first reduction step the protonated pyrimidine moiety is dihydrogenated to form intermediate (**2**),

which is cleaved into ester (**3**) and 2-hydroxybenzonitrile (**4**). Protonated pyrimidine moiety of intermediate (**3**) is subsequently hydrogenated to dihydro (**5**) and tetrahydro derivative (**6**). Both hydrogenated intermediates tend to eliminate pyrimidine or dihydropyrimidine to get the final product **7**.

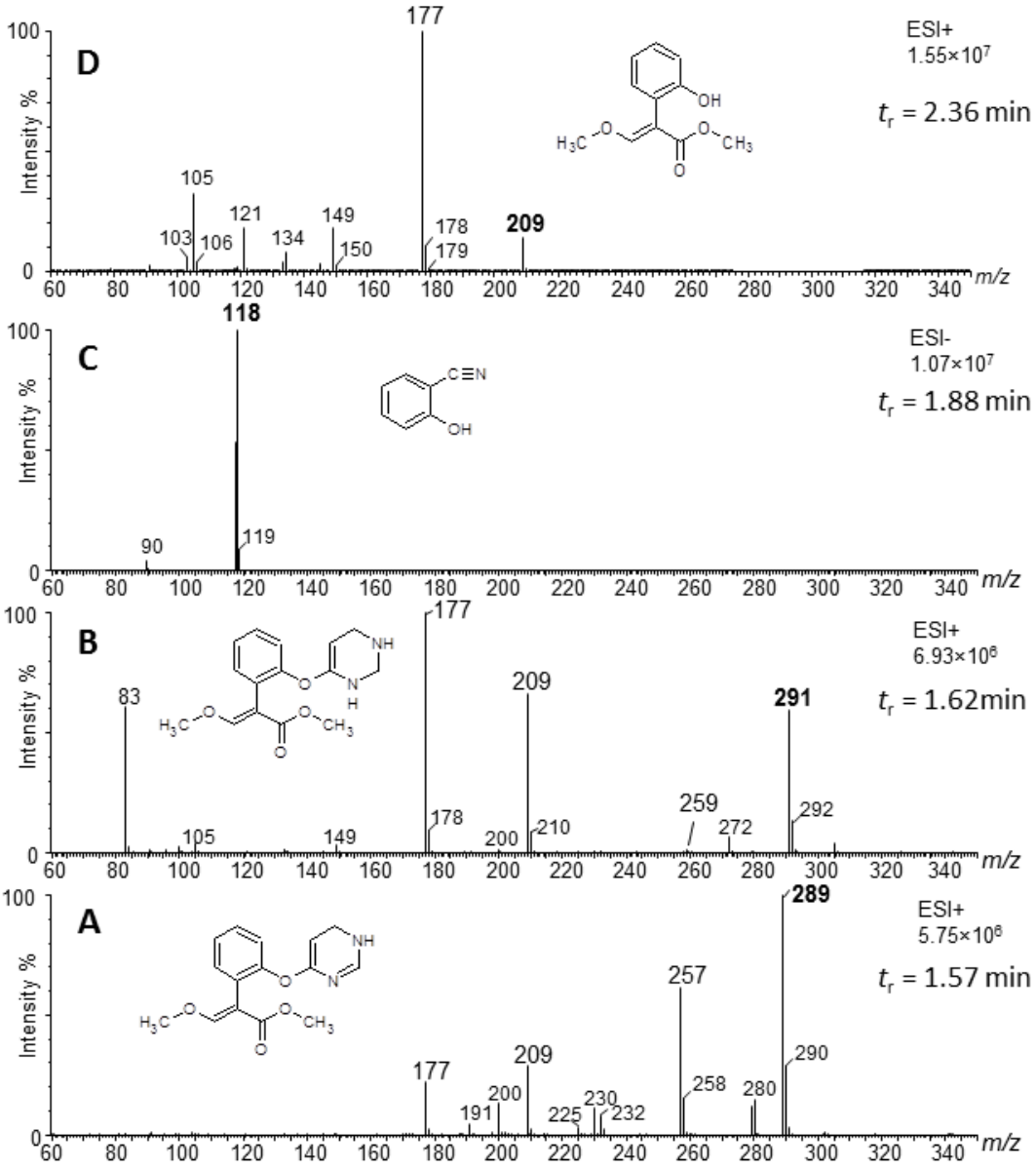
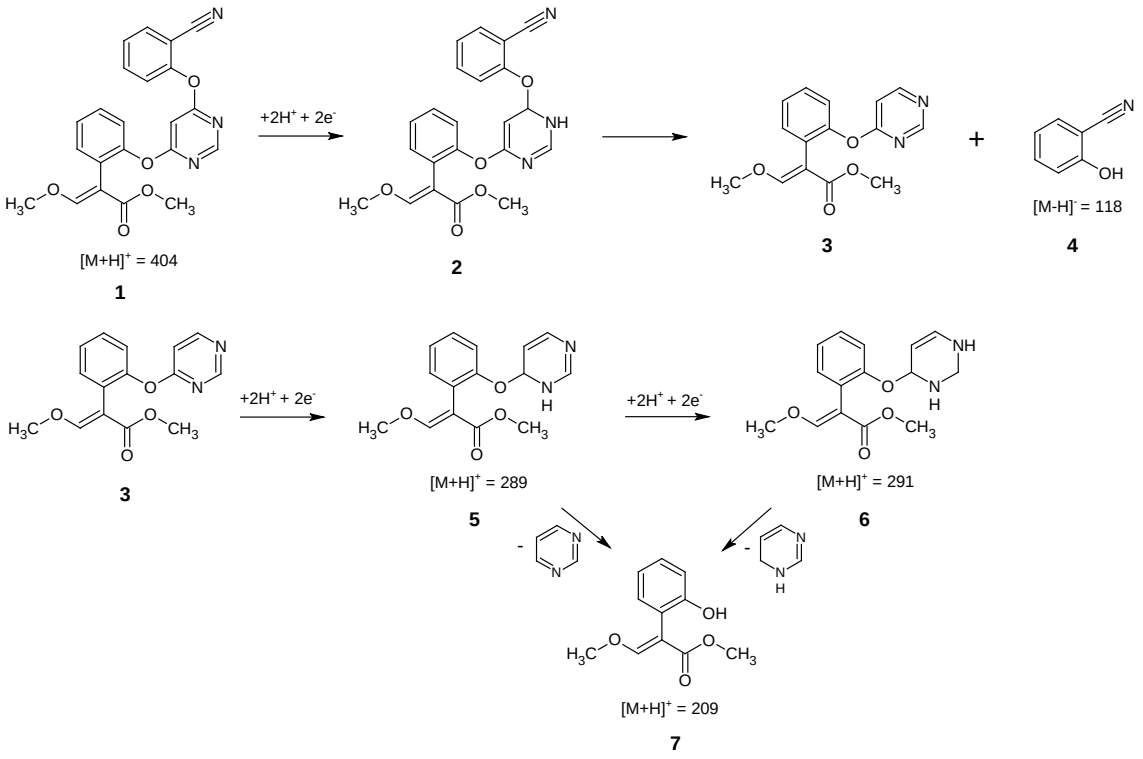


Fig. 4 Mass spectra of extracted ions *m*/*z* 289 (A), *m*/*z* 291 (B), *m*/*z* 118 (C) and *m*/*z* 209 (D) of AS reduction products acquired from AS solution (1×10^{-3} mol dm^{-3}) electrolyzed at −1200 mV (B) and −1500 mV (A, C and D) for 60 min on the mercury pool electrode in the mixture of 0.2 mol dm^{-3} CH_3COOH and acetonitrile 1/1, v/v after chromatographic separation.

Scheme 1



Analytical performance

As already mentioned above, first cathodic signal in BRB of pH 2.0 was utilized for AS determination and differential pulse voltammetry (DPV)

was applied. Basic parameters of DPV as *v*, pulse height, and pulse width were optimized as it is illustrated in Fig. 5. All parameters applied during the optimization experiments are specified in detail at the figure caption under the Fig. 5. AS concentration in voltammetric cell was 2×10^{-5} mol dm^{-3}. It was found that I_p increases with increasing *v* up to 20 mV s^{-1} and then it did not change significantly. At higher rates (> 50 mV s^{-1}) the peak became deformed. 20 mV s^{-1} was set for all further measurements. Pulse height was tested in the range from 10 to 100 mV and the obtained vomtammograms of AS are depicted in Fig. 5A. The inserted dependence of I_p on pulse height shows significant growth of analyte peak between 10 and 30 mV. The further increase in this parameter did not bring any significant improvement of the signal, on the contrary, it deteriorated its shape and the evaluability. Therefore, pulse height of 30 mV was applied for the following experiments. The influence of pulse width on I_p and shape of AS current response was investigated in the range from 10 to 100 ms. Fig. 5D shows that I_p decreases with the increasing pulse width. The value of 20 ms was chosen as suitable for AS determination which practically means 20 ms pulse duration before next 20 ms of current reading.

With respect to the published results [13] dealing with AS determination using HMDE in combination with adsorptive stripping

voltammetry (AdSV), the possibility of analyte accumulation on the surface of m-AgSAE was tested. Various values of accumulation potential (E_{acc}) as well as variously long accumulation time (t_{acc}) were set but without a positive result. AS did not adsorb on the electrode surface, although the previously obtained results proved the influence of adsorption on the ongoing electrode reaction.

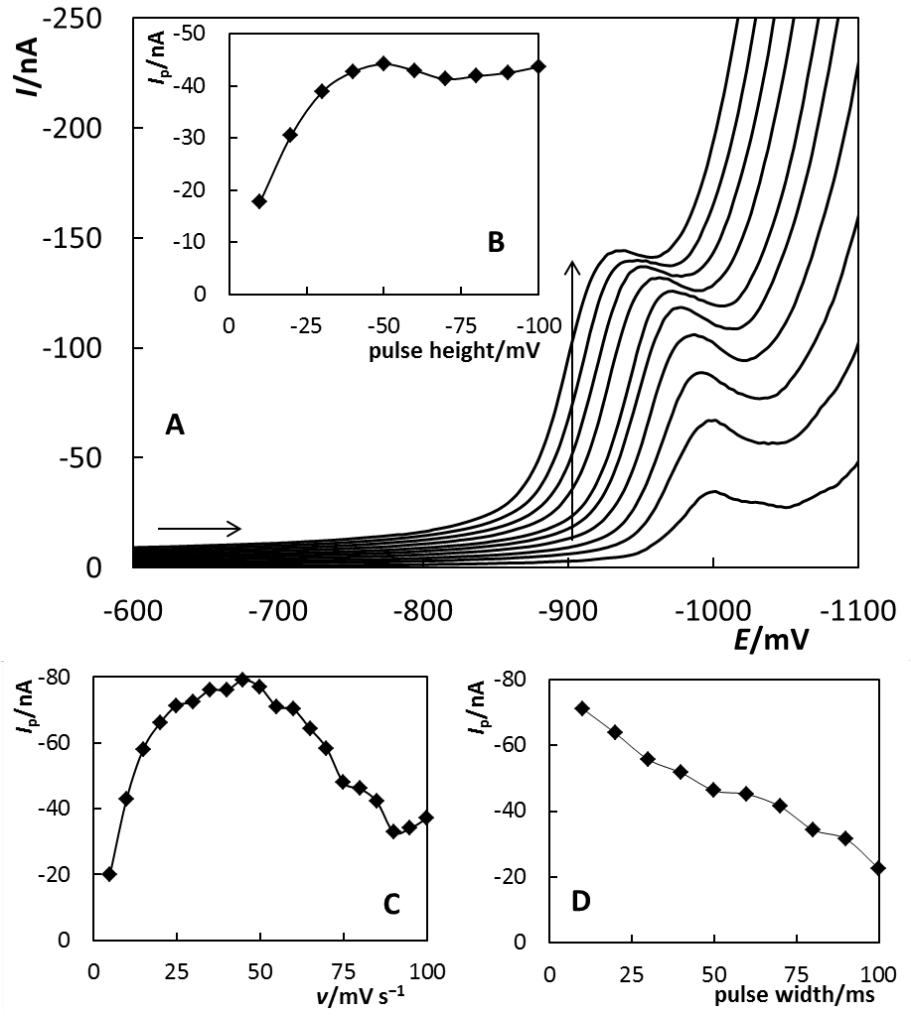


Fig. 5 DP voltammograms of AS obtained on m-AgSAE in dependence on pulse height (A), dependences of I_p on pulse height (B), I_p on scan rate (C)

and I_p on pulse width (D). Method: DPV, supporting electrolyte: BRB (pH 2.0), E_{in} = 0 mV, E_{fin}= −1200 mV, v = 20 mV s^{-1} (A, B, D) and 5-100 mV s^{-1} (C), pulse height = −10-−100 mV (A, B), −50 mV (C), and −30 mV (D), pulse width = 80 ms (A, B, C) and 10-100 ms (D) , c_{AS} = 2×10^{-5} mol dm^{-3}.

At the end of optimization procedure, the conditions of the electrode surface regeneration were investigated. Regeneration was performed directly in analyzed solution before each measurement. Two different approaches were tested. At first, various negative regeneration potential (E_{reg}) values from −1000 to −1400 mV were applied always for regeneration time (t_{reg}) 30 s before measurement of voltammetric curve. Secondly, 30 regeneration cycles consisting of potential jumps between positive (E_{reg1} = 0 mV) and negative (E_{reg2} = −1000-−1400 mV) potential value. Holding time ($t_{reg1, 2}$) for each potential was always 0.3 s. The suitability of regeneration conditions was assessed through the 11 repeated measurements of 2×10^{-5} mol dm^{-3} model solution of AS and calculation of relative standard deviation (*RSD*) of evaluated I_p(AS). The best result (RSD = 2.5 %) was obtained using 30 regeneration cycles with E_{reg1} 0 mV and E_{reg2} −1200 mV. This procedure was applied for all following experiments focused on AS determination in model solutions as well as in real samples.

Determination of azoxystrobin in model solutions

The proposed procedure of DPV with optimized parameters was subsequently applied for analysis of AS model solutions. Various concentration dependences were measured for determination of the basic statistical parameters. In Fig. 6, an example of the recorded voltammograms in concentration range 5.0×10^{-6}-4.5×10^{-5} mol dm^{-3} is presented. The obtained linear dependence of I_p on c_{AS} (Fig. 6B) is described by equation (5) with the appropriated correlation coefficient. Based on these experiments, linear dynamic range (*LDR*) for AS determination using DPV with m-AgSAE was assigned from 2.0×10^{-6} to 5.0×10^{-5} mol dm^{-3}. The limit of detection (*LOD*) was calculated as 7.0×10^{-7} mol dm^{-3} and the limit of quantification (*LOQ*) was 2.3×10^{-6} mol dm^{-3}.

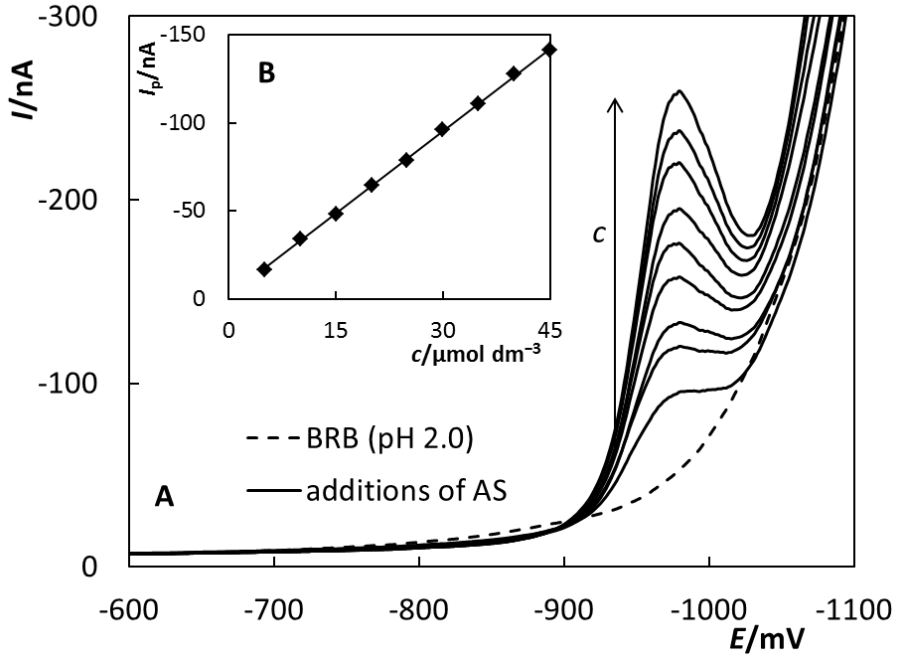


Fig. 6 DP voltammograms of AS obtained on m-AgSAE in dependence on concentration (A) and dependence of I_p on c_{AS} (B). Method: DPV, supporting electrolyte: BRB (pH 2.0), E_{in} = 0 mV, E_{fin}= −1200 mV, v = 20 mV s^{-1}, pulse height = −30 mV, pulse width = 20 ms, c_{AS} = 5.0×10^{-6}-4.5×10^{-5} mol dm^{-3}, regeneration: 20 cycles, E_{reg1} = 0 mV, E_{reg2} = −1200 mV, $t_{reg1,2}$ = 0.3 s.

$$I_p[\text{nA}] = (-3.125 \pm 0.026)\, c\ [\mu\text{mol dm}^{-3}] + (-1.51 \pm 0.73),$$
$$r = 0.9997 \qquad (5)$$

The repeatability of AS determination was verified by analysis of model solutions with analyte content 1.0×10^{-5} and 5.0×10^{-6} mol dm^{-3} using standard addition method. Each determination was 5× repeated and the

relevant statistical parameters, like average values with confidence interval, recovery, and *RSD*, were calculated. An example of specific analysis including the graphical evaluation of standard addition method is depicted in Fig. 7 and the obtained results are summarized in Table 1. It is evident that the proposed method provided correct, accurate, and well repeatable (RSD < 4 %) results which is a condition for application in the analysis of environmental samples and pesticide preparations.

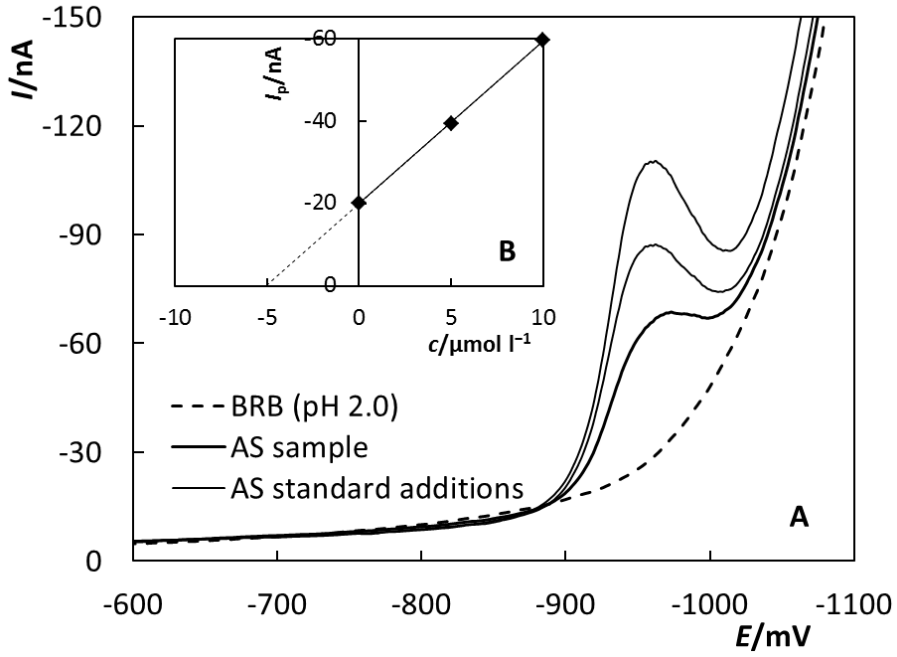


Fig. 7 DP voltammograms of AS determination in model solutions using m-AgSAE and standard addition method (A); graphical evaluation of analysis (B). Method: DPV, supporting electrolyte: BRB (pH 2.0), E_{in} = 0 mV, E_{fin}= −1200 mV, v = 20 mV s^{-1}, pulse height = −30 mV, pulse width = 20 ms, c_{AS} = 5.0×10^{-6} mol dm^{-3}, regeneration: 20 cycles, E_{reg1} = 0 mV,

E_{reg2} = −1200 mV, $t_{reg1,2}$ = 0.3 s, standard additions: V = 75 µl, c = 1.0×10^{-3} mol dm^{-3}.

Table 1 Results of repeated determination of AS in model solutions

Added/mol dm^{-3}	Found/mol dm^{-3}	Recovery/%	RSD_5/%
1.0×10^{-5}	$(1.000\pm0.022)\times10^{-5}$	96.0-104.0	3.39
5.0×10^{-6}	$(4.970\pm0.065)\times10^{-6}$	97.0-102.0	1.97

Determination of azoxystrobin in real samples

Natural water sample was analyzed as it is described in "*Experimental*". Concentration dependence of AS in range from 5.0×10^{-6} to 4.0×10^{-5} mol dm^{-3} was measured in river water which can be described by equations (6). From this equation, statistical parameters for AS determination in river water were calculated as *LOD* 1.18×10^{-6} mol dm^{-3} and *LOQ* 4.0×10^{-6} mol dm^{-3}. These values are quite close to those obtained for model solution of AS in deionized water. Only the value of *RSD* of 11 repeated measurement of 5.0×10^{-6} mol dm^{-3} AS slightly increased in spiked natural water, concretely from 2.5 % for model solutions to 4.1 % for river water. However, all obtained values are < 5 % which corresponds with very good repeatability sufficient for analytical purposes. Proposed method was also verified by repeated determination of AS in spiked river water using standard addition method and the results are shown in Table 2.

$$I_{\mathrm{p}}[\mathrm{nA}] = (-3.298 \pm 0.051)\, c\ [\mu\mathrm{mol\ dm^{-3}}] + (-1.6 \pm 1.3),$$

$$r = 0.9997 \qquad (6)$$

The pesticide preparation was the second type of analyzed practical sample. For this purpose, commercially available preparation Ortiva with the declared AS content 250 g dm^{-3} was purchased. The sample pretreatment and analysis procedures are described in the "*Experimental*". The obtained results are also summarized in Table 2 and they indicate the suitability of the proposed method for analyzing such samples with relatively complicated matrix.

Table 2 Results of repeated determination of AS with m-AgSAE in spiked river water sample and pesticide preparation containing AS

Sample	Added/mol dm^{-3}	Found/mol dm^{-3}	Recovery/%	RSD_5/%
river water	5.0×10^{-6}	$(4.950\pm0.079)\times10^{-6}$	97.4-101.2	2.42
	Declared/g dm^{-3}	Found/g dm^{-3}	Recovery/%	RSD_5/%
Ortiva	250	(249.8±5.2)	95.6-104.0	3.14

Conclusion

Electrochemical reduction of a fungicide azoxystrobin proceeds in only acidic media (pH ≤ 6) proving that protonated form of AS is electroactive. One or two voltammetric responses were observed in dependence on pH.

The reaction mechanism involves reduction of azomethine bonds of protonated pyrimidine moiety and is accompanied by cleavage of 2-hydroxybenzonitrile and pyrimidine or dihydropyrimidine ring to get methyl (2E)-2-(2-hydroxyphenyl)-3-methoxyprop-2-enoate as the final product.

Voltammetric method for AS determination using DPV in connection with m-AgSAE was developed. First cathodic signal in BRB of pH 2 proved to be suitable for analytical purposes. The method provides a wide linear dynamic range (2.0×10^{-6} 5.0×10^{-5} mol dm^{-3}) with limit of detection 7×10^{-7} mol dm^{-3}. The proposed method was verified by successful analysis of spiked river water as well as of pesticide preparations.

Experimental

Chemicals

Unless otherwise indicated, all solutions were prepared in deionized water (conductivity < 0.05 μS cm^{-1}, produced by Milli-Q-Gradient, Millipore, Czech Republic). The standard solution of AS (0.001 mol dm^{-3}) was prepared by dissolution of calculated amount of powder (Sigma-Aldrich, Czech Republic) in acetonitrile (Ing. Petr Švec - PENTA, Czech Republic). This solution was stored in refrigerator in the dark at +4 °C. Analyzed solutions with lower analyte concentrations were diluted daily by BRB.

The supporting electrolyte of 0.1mol dm^{-3} HNO_3 was diluted from 65 % stock solution. BRB of pH values from 2.0 to 12.0 was mixed from acidic component (0.04 mol dm^{-3} solution of H_3PO_4, H_3BO_3, and CH_3COOH) and alkaline component (0.2 mol dm^{-3} NaOH) under the pH-meter. All mentioned chemicals were purchased from Ing. Petr Švec - PENTA, Czech Republic. Acetonitrile (≥99.9%, gradient grade for HPLC, VWR Chemicals), acetic acid (99.7%) and ammonium acetate (for HPLC, both Sigma-Aldrich) were used for controlled potential electrolysis and HPLC/MS analysis. Commercially available pesticide preparation "Ortiva" was produced by Syngenta Czech, Czech Republic.

Instrumentation

Voltammetric measurements were performed on Eco-Tribo Polarograph (Polaro-Sensors, Czech Republic) equipped by software POLAR.PRO 5.1 [36]. Measurements were realized in three-electrode set up with m-AgSAE (Eco-Trend Plus, Czech Republic) as working electrode, saturated silver-silver chloride electrode (Ag/AgCl (KCl, satur.)) as a reference and platinum wire as an auxiliary electrode (both Monokrystaly, Czech Republic). Controlled potential electrolysis was carried out using potentiostat Autolab PGSTAT128N (Metrohm Autolab, Nederland) also in three-electrode arrangement with saturated calomel electrode (SCE) as

reference electrode and platinum auxiliary electrode placed in a space separated by a glass frit (both Metrohm Autolab, Nederland). Mercury pool at the bottom of the polarographic cell served as a working electrode. Electrolyzed solutions were analyzed using Acquity UPLC system (Waters, USA) with PDA detector and mass spectrometric detector (QDA) equipped with heated electrospray ionization (HESI) and quadrupole analyzer.

The pH-meter Accumet AB150 (Fisher Scientific, Czech Republic) was used for measurement of pH values and the ultrasonic bath Bandelin Sonorex (Schalltec GmbH, Germany) served for facilitating dissolution of pesticides.

Procedures

Voltammetric measurements

Before starting the work, m-AgSAE was always activated in solution of KCl (0.2 mol dm^{-3}) at the potential of −2200 mV for 300 s. Regeneration procedure of the electrode surface was inserted before each measurement and it consisted from 30 regeneration cycles of potential jumps between E_{reg1} 0 mV and E_{reg2} −1200 mV. Each potential value was maintained for a $t_{reg1, 2}$ 0.3 s. In everyday measurement, mercury meniscus was renewed approximately once a week by immersion of the electrode into the liquid mercury.

Cyclic voltammetry (CV) was used for investigation of voltammetric behavior of AS on m-AgSAE in dependence on pH and *v*. The applied potential range was from E_{in} 0 mV to E_{switch} −1500 mV and *v* 100 mV s^{-1} for pH study or 25-400 mV s^{-1} for scan rate study. DPV with the following parameters was developed for AS determination: supporting electrolyte BRB (pH 2), E_{in} 0 mV, E_{fin} −1200 mV, *v* 20 mV s^{-1}, pulse height −30 mV, pulse width 20 ms.

I_p was evaluated from the base line inserted as a straight line connecting the minima before and after the peak. The resulting voltammetric curves, their dependencies, and calibration lines were obtained using MS Excel 2010 (Microsoft, USA). Parameters of calibration curves with appropriate confidence intervals at a significance level of $\alpha = 0.05$ were calculated using OriginPro 9 (Origin Lab Corporation, USA). The value of *LOD* was calculated as the 3× standard deviation of the intercept divided by the slope and the *LOQ* as the 10× standard deviation of the intercept divided by the slope.

Bulk electrolysis of AS solution (0.001 mol dm^{-3}) was performed on mercury pool electrode in 0.2 mol dm^{-3} CH_3COOH with acetonitrile (1/1, v/v) at the potentials of -1200 mV and -1500 mV (SCE) for 60 min. Anodic compartment of the electrolytic cell was filled with 0.2 mol dm^{-3} CH_3COOH. Total volume of the electrolyzed sample was 4 mL. Solution

was bubbled and mixed with the nitrogen stream during electrolysis. Electrolyzed samples were directly analyzed using HPLC/MS. As a reference unelectrolyzed sample, solution of AS (0.001 mol dm^{-3}) in 0.2 mol dm^{-3} CH_3COOH with acetonitrile (1/1, v/v) was left for 60 min in electrolytic cell at potential of the working mercury electrode set on 0 V under nitrogen stream. Chromatographic separation was performed on a XSelect HSS T3 column (3 mm × 50 mm, 2.5 µm, Waters) at 25 °C. Mobile phase consisted of 0.01 mol dm^{-3} ammonium acetate in water (solvent A) and a mixture of acetonitrile and water 9/1, v/v (solvent B). Gradient elution was performed: 0-4.5 min (78-12% A), 4.5-5 min (12 % A) with flow rate 0.6 cm^3 min^{-1}. After analysis the column was equilibrated to the initial ratio of both mobile phases for 2 min. The injection volume was 5 mm^3. Mass spectrometric conditions were as follows: positive and negative electrospray mode, capillary voltage 0.8 kV, cone 25 V, source temperature 120 °C and heated probe temperature 600 °C. The acquired mass range was *m*/*z* 60 - 800. Data were processed using MassLynx 4.1 software (Waters).

Real samples analysis

River water originated from the river Chrudimka and it was taken in the town Slatiňany, district Chrudim, Czech Republic. Until the time of analysis, the sample was placed in the refrigerator. 13 mL of water sample

spiked by standard solution of AS on the required concentration was transferred into the polarographic cell together with 2 mL of BRB (pH 2.0). Several concentration dependencies have been measured and statistical parameters (*LOD*, *LOQ*) calculated. Repeatability of AS determination was tested at the analyte concentration 5.0×10^{-6} mol dm^{-3} using standard addition method. Standard additions represented 75 µL of AS standard solution with the concentration 0.001 M. Every determination was 5× repeated and the average value with the appropriate confidence interval, recovery and relative standard deviation (*RSD*) were calculated.

Analyzed pesticide preparation "Ortiva" intended especially to protect vegetables against fungal disease is supplied as a suspension concentrate with the declared AS content 250 g dm^{-3}. The solution with the AS concentration about 0.001 M (calculated from the content declared by producer) was prepared by dissolution of the appropriate volume of preparation in acetonitrile applying ultrasonic bath. For the following analysis, 75 µL of the prepared solution was added into the polarographic cell to the 15 mL of BRB (pH 2.0). The analysis was realized and evaluated using standard addition method by the same way as in case of water samples.

Acknowledgements

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Figure Captions

Fig. 1 Structural formula of azoxystrobin

Fig. 2 Cyclic voltammogram of AS in 0.1 mol dm^{-3} HCl (A) and cathodic curves of cyclic voltammograms of AS in 0.1 mol dm^{-3} HCl and BRB (pH 2.0-6.0) obtained on m-AgSAE (B). $E_{in} = E_{fin} = 0$ mV, $E_{switch} = -1500$ mV, $v = 100$ mV s^{-1}, $c_{AS} = 1\times10^{-4}$ mol dm^{-3}.

Fig. 3 Cyclic voltammograms of AS obtained on m-AgSAE in dependence on scan rate (A), dependences of I_p on v (B), I_p on $v^{1/2}$ (C) and log(I_p) on log(v) (D). Method: CV, supporting electrolyte: BRB (pH 2.0), $E_{in} = E_{fin} = 0$ mV, $E_{switch} = -1500$ mV, $v = 25$-400 mV s^{-1}, $c_{AS} = 1\times10^{-4}$ mol dm^{-3}.

Fig. 4 Mass spectra of extracted ions m/z 289 (A), m/z 291 (B), m/z 118 (C) and m/z 209 (D) of AS reduction products acquired from AS solution (1×10^{-3} mol dm^{-3}) electrolyzed at −1200 mV (B) and −1500 mV (A, C and D) for 60 min on the mercury pool electrode in the mixture of 0.2 mol dm^{-3} CH_3COOH and acetonitrile 1/1, v/v after chromatographic separation.

Fig. 5 DP voltammograms of AS obtained on m-AgSAE in dependence on pulse height (A), dependences of I_p on pulse height (B), I_p on scan rate (C)

and I_p on pulse width (D). Method: DPV, supporting electrolyte: BRB (pH 2.0), E_{in} = 0 mV, E_{fin}= −1200 mV, v = 20 mV s^{-1} (A, B, D) and 5-100 mV s^{-1} (C), pulse height = −10-−100 mV (A, B), −50 mV (C), and −30 mV (D), pulse width = 80 ms (A, B, C) and 10-100 ms (D) , c_{AS} = 2×10^{-5} mol dm^{-3}.

Fig. 6 DP voltammograms of AS obtained on m-AgSAE in dependence on concentration (A) and dependence of I_p on c_{AS} (B). Method: DPV, supporting electrolyte: BRB (pH 2.0), E_{in} = 0 mV, E_{fin}= −1200 mV, v = 20 mV s^{-1}, pulse height = −30 mV, pulse width = 20 ms, c_{AS} = 5.0×10^{-6}-4.5×10^{-5} mol dm^{-3}, regeneration: 20 cycles, E_{reg1} = 0 mV, E_{reg2} = −1200 mV, $t_{reg1,2}$ = 0.3 s.

Fig. 7 DP voltammograms of AS determination in model solutions using m-AgSAE and standard addition method (A); graphical evaluation of analysis (B). Method: DPV, supporting electrolyte: BRB (pH 2.0), E_{in} = 0 mV, E_{fin}= −1200 mV, v = 20 mV s^{-1}, pulse height = −30 mV, pulse width = 20 ms, c_{AS} = 5.0×10^{-6} mol dm^{-3}, regeneration: 20 cycles, E_{reg1} = 0 mV, E_{reg2} = −1200 mV, $t_{reg1,2}$ = 0.3 s, standard additions: V = 75 µl, c = 1.0×10^{-3} mol dm^{-3}.

Figures

Figure 1

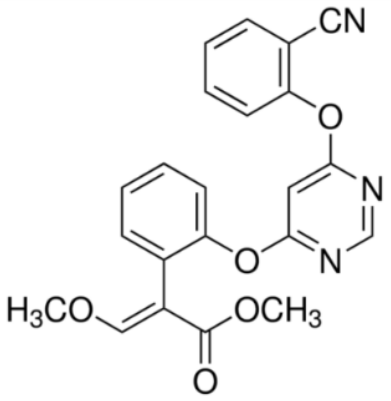
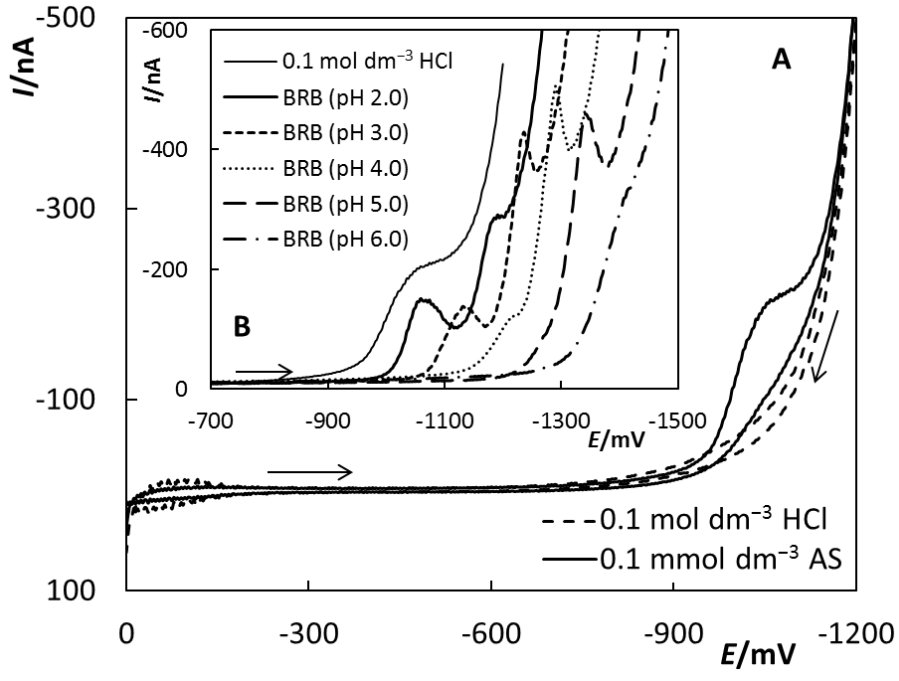


Figure 2



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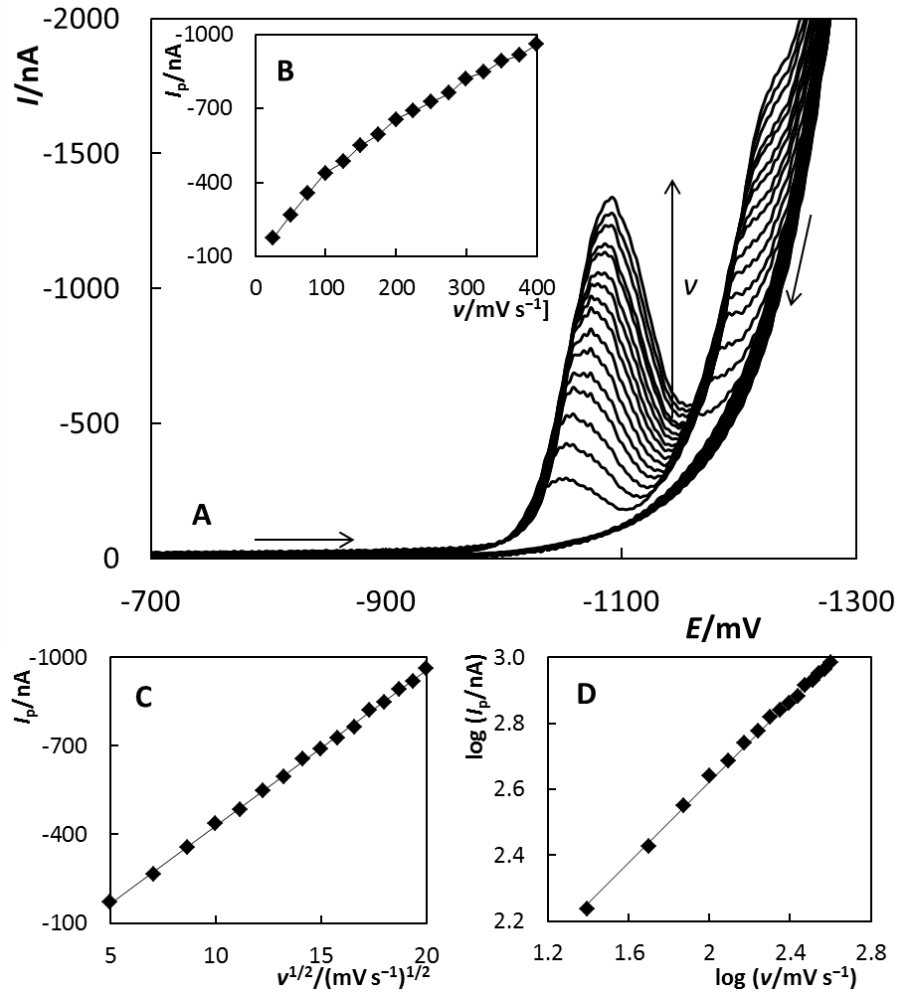

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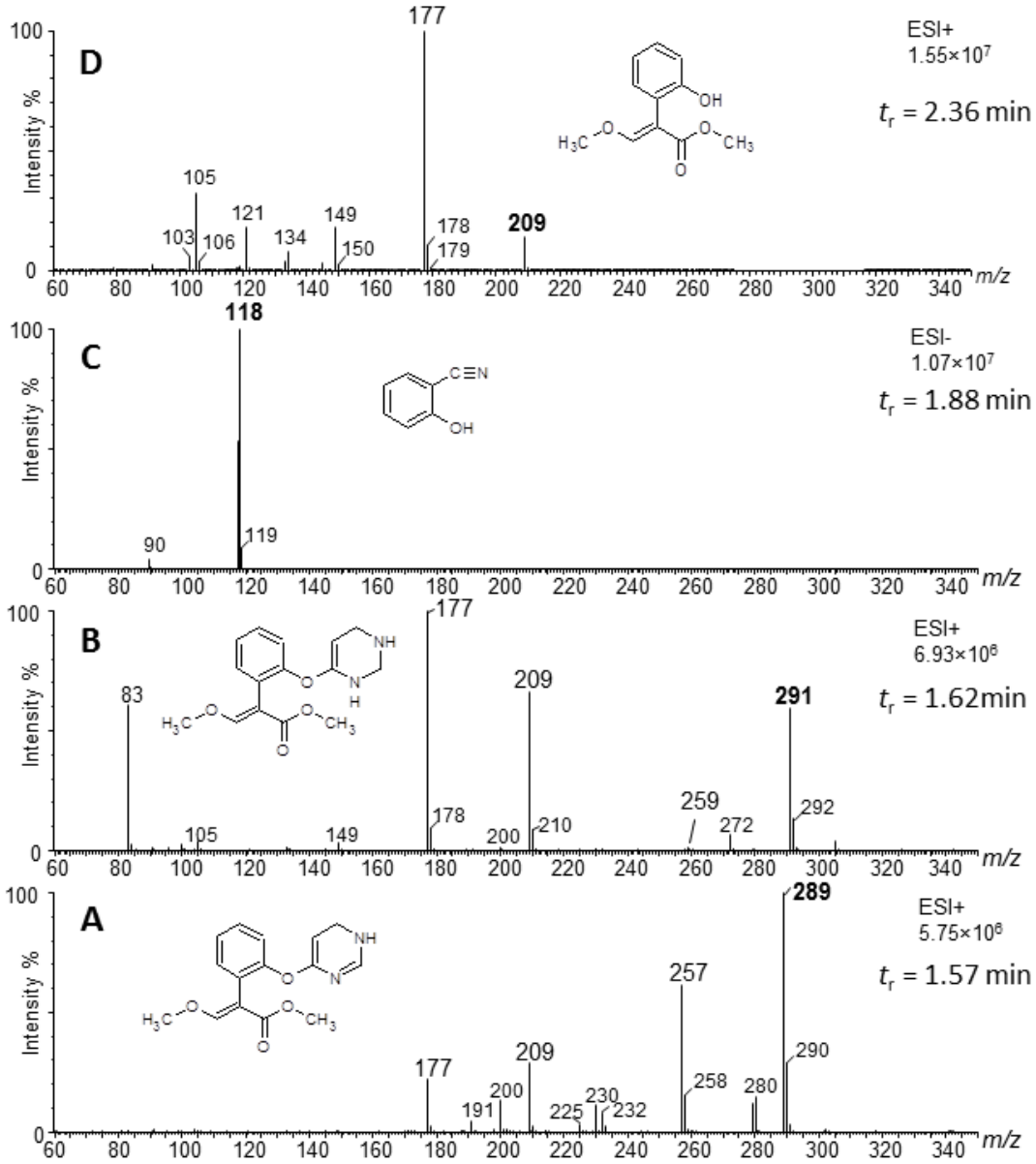
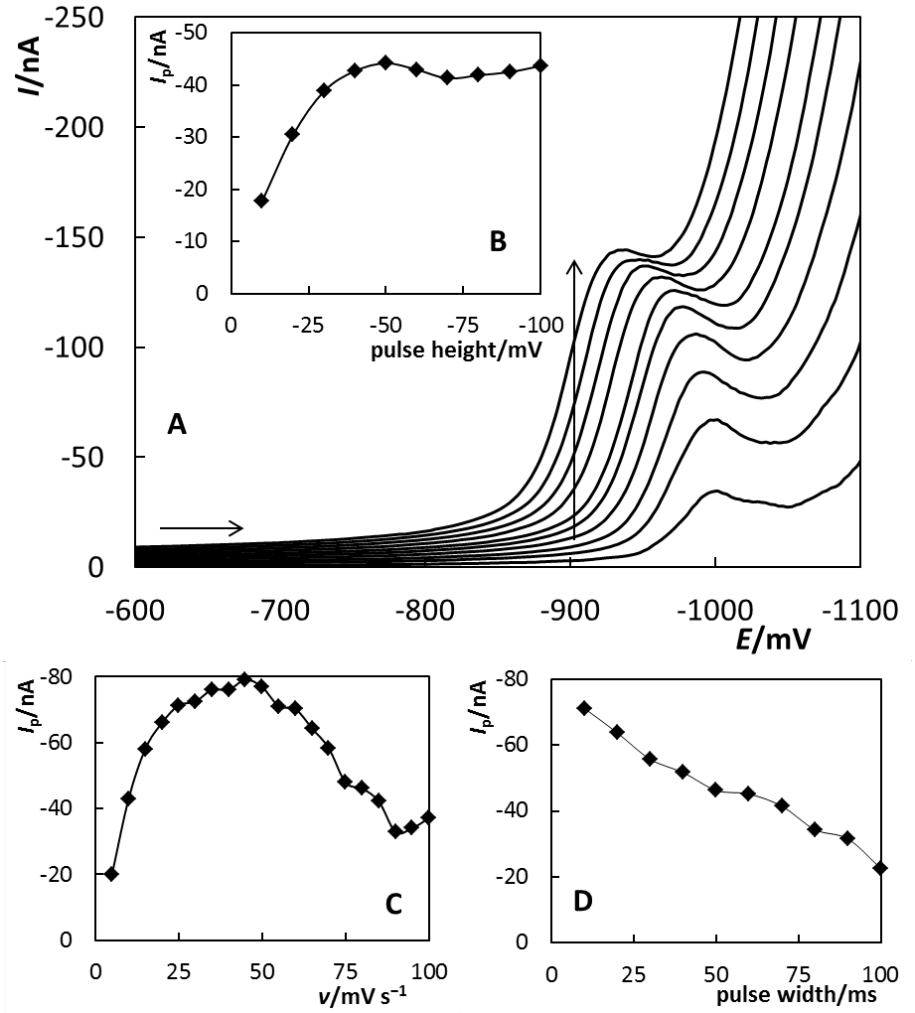
Figure 4


D
ESI+
1.55×10^7
t_r = 2.36 min
177
209
105
121
149
103
106
134
150
178
179
C
ESI-
1.07×10^7
t_r = 1.88 min
118
90
119
B
ESI+
6.93×10^6
t_r = 1.62min
177
209
291
83
105
149
178
200
210
259
272
292
A
ESI+
5.75×10^6
t_r = 1.57 min
289
257
209
177
200
191
225
230
232
258
280
290
Intensity %
m/z

1 *Figure 5*



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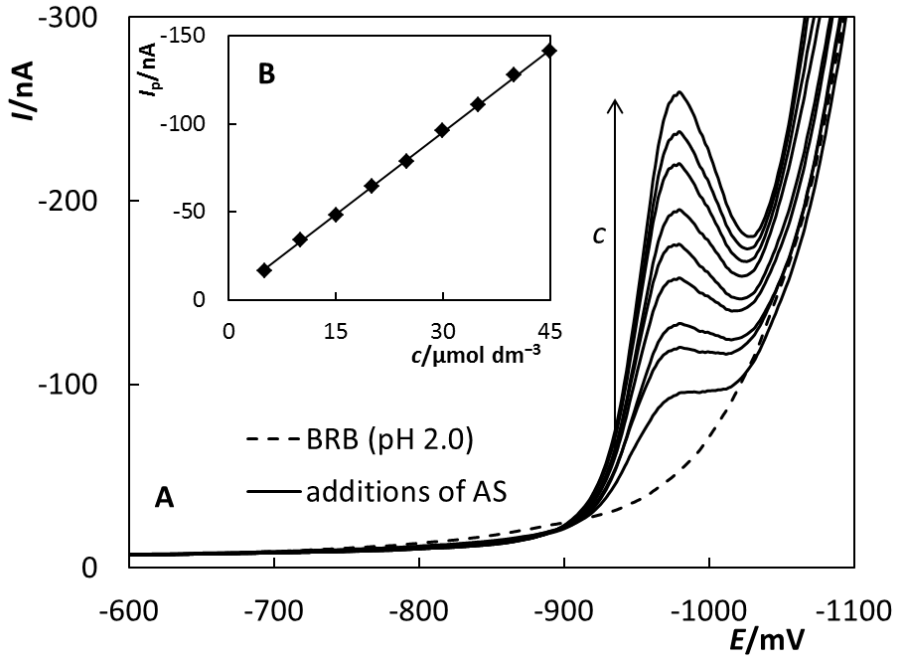
1 *Figure 6*



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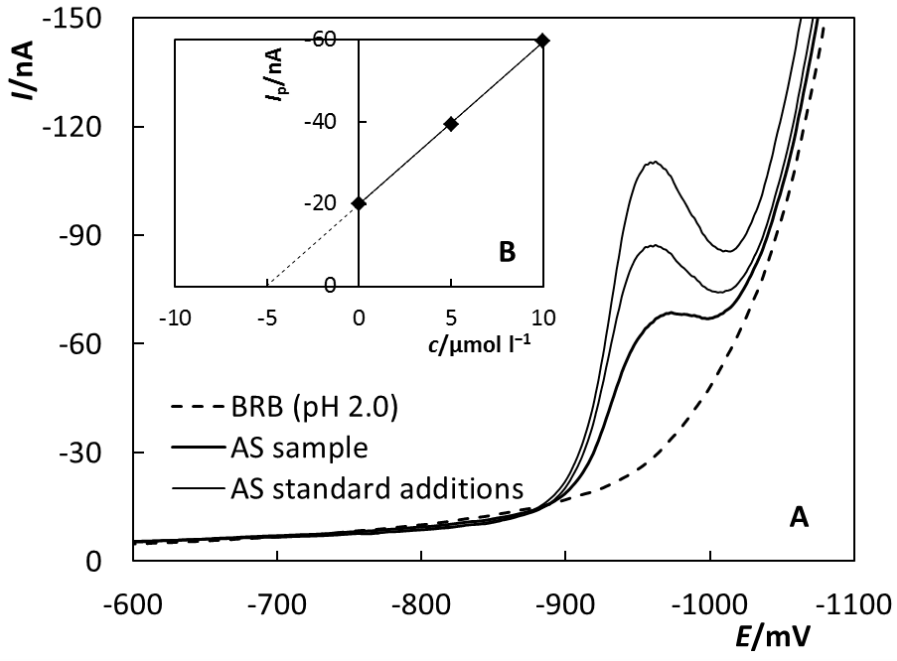
4 *Figure 7*



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Tables

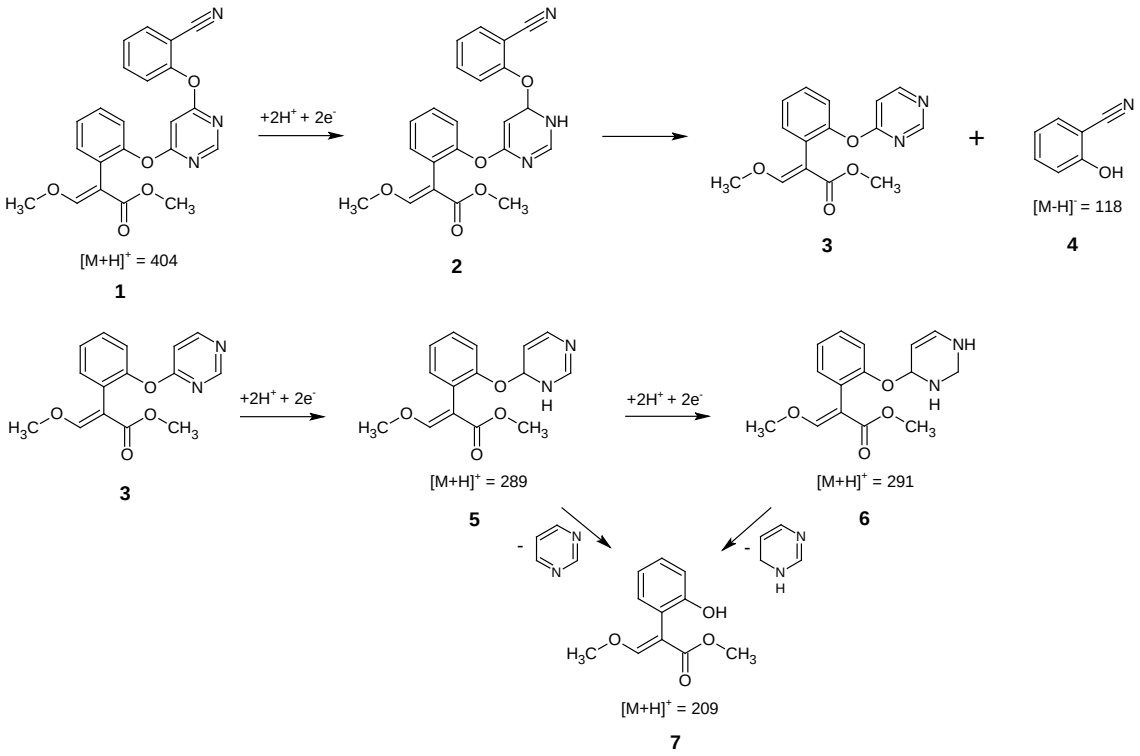
Table 1 Results of repeated determination of AS in model solutions

Added/mol dm^{-3}	Found/mol dm^{-3}	Recovery/%	RSD_5/%
1.0×10^{-5}	$(1.000\pm0.022)\times10^{-5}$	96.0-104.0	3.39
5.0×10^{-6}	$(4.970\pm0.065)\times10^{-6}$	97.0-102.0	1.97

Table 2 Results of repeated determination of AS with m-AgSAE in spiked river water sample and pesticide preparation containing AS

Sample	Added/mol dm^{-3}	Found/mol dm^{-3}	Recovery/%	RSD_5/%
river water	5.0×10^{-6}	$(4.950\pm0.079)\times10^{-6}$	97.4-101.2	2.42
	Declared/g dm^{-3}	Found/g dm^{-3}	Recovery/%	RSD_5/%
Ortiva	250	(249.8±5.2)	95.6-104.0	3.14

1 *Scheme 1*



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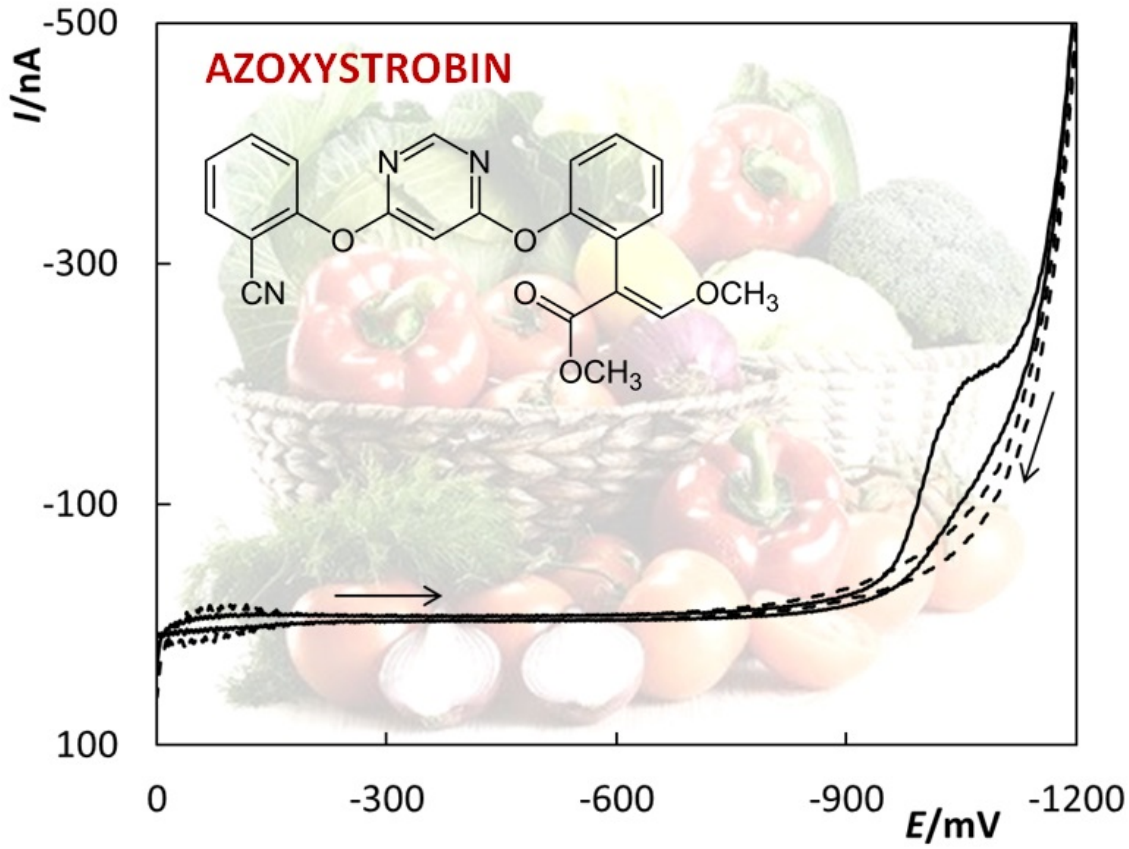
1 Graphical abstract

2



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