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A simple stripping voltammetric method for the determination of a new anticancer prodrug in serum

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ABSTRACT

The determination of ethyl [4-oxo-8-(3-chlorophenyl)-4,6,7,8-tetrahydroimidazo[2,1-*c*][1,2,4]triazin-3-yl]acetate (ETTA), a new anticancer prodrug, using adsorptive stripping voltammetry (AdSV) was described for the first time. This method is based on adsorptive/reductive behaviour of ETTA at an in situ plated bismuth film electrode (BiFE) as a sensor. A number of experimental variables (e.g., a composition and pH of the supporting electrolyte, the conditions of bismuth film deposition, an accumulation potential and time, the scan rate, etc.) were thoroughly studied in order to achieve a high sensitivity. Experimental results under optimized conditions revealed an excellent linear correlation between the monitored voltammetric peak current and the ETTA concentration in the range of 2–50 μg L^{-1} following an accumulation time of 300 s. The limit of detection (LOD) for ETTA following 300 s of an accumulation time was 0.4 μg L^{-1}. The proposed facile, sensitive and inexpensive method was successfully applied to the determination of ETTA in serum. The investigated prodrug was extracted from serum using SPE method.

1. Introduction

The ethyl [4-oxo-8-(3-chlorophenyl)-4,6,7,8-tetrahydroimidazo[2,1-*c*][1,2,4]triazin-3-yl]acetate (CAS number 1485351-15-2), namely ETTA (Fig. 1), has been identified as the best drug candidate in a series of innovative fused azaisocytosine-like congeners with significant antiproliferative activities. This small molecular weight abnormal nucleobase, mimicking the ester prodrug, is of great importance with regard to the patented medical application, e.g., antitumour field of relevance. ETTA has previously been reported as a strong and truly selective anticancer prodrug. This agent, useful for treating human cancers, such as multiple myelomas (both resistant (MM1R) and susceptible (MM1S) to thalidomide), cervical cancer (HeLa) and breast cancer (T47D), caused significantly higher necrosis rates in tumour than in normal cells, while having very low cytotoxicity on normal human skin fibroblast (HSF) cells (Sztanke and Sztanke, 2015; Sztanke et al., 2013). ETTA seems to be the most promising anticancer candidate with prospective medical use for further more advanced drug development studies because of its proven effectiveness and selectivity.

Organic compounds, including drugs, have been determined by several methods such as HPLC with a variety of detectors (Malfara et al., 2007; Mohajeria et al., 2010; Urinovska et al., 2012; Hasselstrøm, 2011), capillary electrophoresis (Dixit and Park, 2016), spectrophotometry (Maximiano et al., 2011), chemiluminescence (Bellomarino et al., 2009) and voltammetry (Brycht et al., 2016; Temerk et al., 2016). Voltammetric techniques compared to other methods are more sensitive, inexpensive and do not require the use of large amounts of organic solvents. In stripping voltammetric measurements the lowest limits of detection (LODs) are obtained using mercury electrodes, especially a hanging mercury drop electrode (HMDE). However, due to the reported high toxicity of mercury, there is an on-going quest to replace this one by less toxic materials (Economou and Fielden, 2003; Wang et al., 2000).

Nowadays, toxic mercury is often replaced by less toxic bismuth. Heretofore, bismuth film electrodes (BiFEs) have been widely used for

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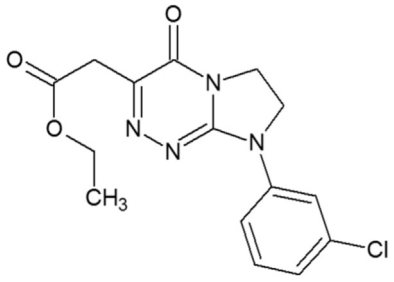

Fig. 1. The structural formula of the ethyl [4-oxo-8-(3-chlorophenyl)-4,6,7,8-tetrahydroimidazo[2,1-*c*][1,2,4]triazin-3-yl]acetate (ETTA) molecule.

the stripping voltammetric determination of metals (Svancara et al., 2010; de Carvalho et al., 2007; Lee et al., 2008; Rutyna and Korolczuk, 2011) and some bioorganic compounds, such as pharmaceutics (Buckova et al., 2005; Nigovic et al., 2009) and pesticides (Figueiredo-Filho et al., 2012; Gerent and Spinelli, 2016; Gerent et al., 2015). BiFEs exhibit several beneficial properties, such as a low toxicity, simple preparation and high sensitivity, and they generate well-defined electrochemical signals (Gerent and Spinelli, 2016).

In voltammetric measurements involving biological samples, the presence of proteins constitutes the major problem, once these compounds can strongly adsorb on the working electrode and so affect the signal recorded. Therefore, an appropriate sample preparation is very important. One of the techniques in bioanalytical chemistry for sample preparation is solid-phase extraction (SPE). This technique, being widely used for isolation of the investigated pharmaceutics from serum samples in chromatographic methods, may also be of assistance in voltammetric measurements.

The main purpose of this study was to optimize and develop a simple, sensitive and inexpensive adsorptive stripping voltammetric method for the determination of an innovative anticancer prodrug (ETTA) in serum samples after removal of the interfering matrix components by SPE. This electrochemical method for ETTA determination in the biological fluid is presented for the first time. The electrochemical reduction of ETTA proceeds according to a well-defined mechanism in structurally related 1,2,4-triazinones (Ludvik and Zuman, 2003). In the proposed mechanism (Fig. 2) a two electron reduction of the electroactive –N(2)=C(3) double bond of azomethine-type takes place, resulting in the 2,3-dihydro-derivative of ETTA which contains –NH–CH grouping in the 1,2,4-triazinone template.

2. Material and methods

2.1. Synthesis of ETTA

Ethyl [4-oxo-8-(3-chlorophenyl)-4,6,7,8-tetrahydroimidazo[2,1-*c*][1,2,4]triazin-3-yl]acetate (ETTA) has been synthetized by reacting 1-(3-chlorophenyl)-2-hydrazinylideneimidazolidine hydroiodide with diethyl acetylene dicarboxylate (DEAD) in refluxing *n*-butanol medium containing triethylamine (TEA) or by thermal cyclocondensation of diethyl (2*E*)-2-{(2*E*)-[1-(3-chlorophenyl)imidazolidin-2-ylidene]hydrazono}succinate in boiling organic solvents, as previously patented and published (Sztanke and Sztanke, 2015; Sztanke et al., 2013).

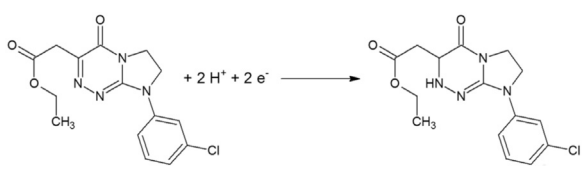

Fig. 2. The proposed electrochemical reduction mechanism of ETTA at the BiFE.

2.2. HPLC-DAD procedure for the identification of ETTA

The Agilent Technologies 1200 HPLC system with a quaternary pump was used for the LC analysis. Analytes were separated using a Synergi Polar RP 150 mm x 4.6 mm column, with 4-μm particle size (Phenomenex, Torrance, CA, USA). The column was thermostated at 22 °C. A mobile phase consisting of three components was employed. These were as follows: acetate buffer (pH 3.5, 0.1 mol L^{-1}) and 0.025 mol L^{-1} diethylamine (DEA) (component A), H_2O and 0.025 mol L^{-1} DEA (component B), acetonitrile and 0.025 mol L^{-1} DEA (component C). An isocratic eluent (20% A, 30% B and 50% C) was used. A mobile phase flow rate was 1 mL min^{-1}. Final samples were injected onto the column using a Rheodyne manual injector with 20 μL loop. The time of analysis was 8 min.

Additionally, an UV–VIS spectra library for the prodrug under investigation was created by collecting ETTA spectra (200–400 nm) from the analysis of individual standards at different concentrations (e.g., 5, 10, 25, 50, 75, 100, 150 and 200 mg L^{-1}). Five different wavelengths (230, 240, 254, 280 and 325 nm) were selected for detection and quantification of the prodrug.

2.3. Apparatus

All measurements were performed using a μAutolab analyser (Eco Chemie, the Netherlands). A glassy carbon electrode (GCE) support was used as the substrate for preparation of a working electrode by deposition of the bismuth film. A platinum wire served as the auxiliary electrode and Ag/AgCl saturated with KCl – as the reference one. A GCE support was polished daily using 0.3 μm alumina slurry on a Buehler polishing pad. A conventional glass cell of volume 10 mL was used for the experiments. For pH measurements an Elmetron pH meter CI-316 was used. Solid-phase extraction (SPE) was carried out using BAKERBOND™ SPE Octadecyl (C18) cartridges (No. 7020-01, 100 mg mL^{-1}) (J.T. Baker, Phillipsburg, USA) on Baker SPE-12G apparatus (Phillipsburg, USA) for sample preparation.

2.4. Reagents

An acetate buffer (used as a supporting electrolyte for the proposed method) was prepared from CH_3COOH and CH_3COONa, which were obtained from Merck (Germany). An ammonium buffer (used for the proposed SPE method) was prepared from ammonium and ammonium chloride, which were purchased from POCh (Poland) and Merck (Germany), respectively. The other reagents were purchased as the highest grade available from Sigma and Merck companies (Germany). All aqueous solutions were prepared with deionized water (Millipore quality).

2.5. Standard procedure of the stripping voltammetric measurements at a bismuth film electrode

In the optimized conditions of measurement, the supporting electrolyte used was acetate buffer (pH=4.2, 0.1 mol L^{-1}). The concentration of $Bi(NO_3)_3$ added to the electrolyte was 50 μmol L^{-1}. During our voltammetric measurements the potential of the electrode was changed in the following sequence: firstly, the electrode was cleaned after the preceding measurement at the potential 0.5 V for 30 s; next a bismuth film was plated on the surface of the electrode at the potential −1.05 V for 20 s; and finally ETTA was accumulated by adsorption on the surface of the preplated BiFE at the potential −0.5 V for 120 s. During all these steps, the solution was mixed using a magnetic stirring bar. Then, after a rest period of 10 s, a square wave voltammogram was recorded at frequency of 50 Hz, while the potential was scanned from −0.5 to −0.85 V. The amplitude and step potential were 25 mV and 4.05 mV, respectively. The measurements were carried out from undeaerated solutions.