Microchemical Journal 98 (2011) 297–302

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

A new indirect method based on square-wave voltammetry for ceftiofur determination in bovine milk using an alkaline degradation product

Antonio Marcos Jacques Barbosa [a,b], Tatiane Alfonso de Araujo [a], Magno Aparecido Gonçalves Trindade [c], Valdir Souza Ferreira [a,*]

[a] Department of Chemistry, Universidade Federal de Mato Grosso do Sul. Av. Filinto Muller, 1555, Caixa Postal 549, Campo Grande, MS 79074-460, Brazil
[b] Setor de Toxicologia, Laboratório Central de Saúde Pública de Mato Grosso do Sul, Campo Grande, MS 79074-460, Brazil
[c] School of Science and Technology, Universidade Federal da Grande Dourados, Rodovia Dourados-Itahum, Km 12, Dourados, MS 79804-970, Brazil

ARTICLE INFO

Article history:
Received 24 January 2011
Received in revised form 17 February 2011
Accepted 18 February 2011
Available online 26 February 2011

Keywords:
Ceftiofur
Lactams
Milk sample
Hydrolysis
Electroanalysis

ABSTRACT

In this paper we present a new electroanalytical method for determination of ceftiofur based on the hydrolysis of this antibiotic in 0.04 mol L^{-1} Britton–Robinson buffer at pH 10 and 60 °C for 60 min (reduction peak at −0.70 V). Conditions were optimized for complete hydrolysis and quantitative determination of ceftiofur in milk. The method can be successfully used for determination of the antibiotic directly from samples of fluid milk and powder milk spiked to concentrations of 6.0×10^{-8}, 8.0×10^{-8}, and 10×10^{-8} mol L^{-1}, respectively. Recovery test ranged from 98.28% to 100.83%. The limits of detection and quantification were 3.73×10^{-10} and 1.24×10^{-9} mol L^{-1}, respectively. The method has the advantage of eliminating interference from proteins present in the sample, thus obviating the need for exhaustive extraction, which often renders other procedures unfeasible in terms of time and reagent cost. In addition, waste generation was found to be lower than in other methods.

Published by Elsevier B.V.

1. Introduction

Milk productivity is an important contributor to economic dynamics globally. Genetic research, improved nutrition, and close monitoring of animal health are among the measures currently applied to improve the quality of milk production, where milk is a critical source of essential nutrients for children and adults in many societies [1].

From a technological standpoint, residues of antimicrobial agents in milk can lead to considerable losses in the production of fermented derivatives, for instance by inhibiting bacterial fermentation in cheese and yoghurt production [2]. Although consumers' tolerance to antibiotic residues in milk varies widely, the implementation and monitoring of quality assurance programs at every stage of milk handling and processing are now viewed as crucial [3]. Antibiotics present in milk can degrade during pasteurization, generating products with allergenic effects, a problem which cannot be determined by conventional methods. For these reasons, the maximum permitted levels of antibiotic residues in foodstuffs have been gradually lowered [4].

In Brazil, the presence of antibiotics was recently investigated by applying qualitative methods to 210 raw milk samples collected from four dairy production areas. Antibiotic residues were detected in 24 samples (11.4%), suggesting that, as in other countries, antibiotics are a significant chemical hazard in locally produced milk [5].

Ceftiofur is a widely used broad-spectrum third-generation cephalosporin antibiotic approved for treatment of infections in cattle, swine, sheep, goats, turkeys, and chickens. A four-membered ring structure makes β-lactam antibiotics (which include penicillins and cephalosporins) active against gram-positive and gram-negative bacteria by covalently binding to, and interrupting the functioning of, enzymes responsible for bacterial cell wall synthesis [6].

Many countries regulate the use of antibiotics in livestock for food production, establishing maximum residue limits (MRL) and monitoring their occurrence. In Brazil, the National Plan for Residue Control in Animal Origin Products (PNCR), set up by the Ministry of Agriculture, comprises Residue Control Programs for meat, honey, milk, and fish. Product assessment is carried out at private and government-run laboratories capable of applying validated analytical methods for residue identification and quantification [7].

Given their simplicity and low cost, microbial assays are routinely applied to milk samples, but can be lengthy (up to several hours) and lack selectivity and sensitivity to reliably identify or quantify residual antibiotics [4]. Sensitive, specific confirmatory tests are therefore still needed. Furthermore, bioanalytical methods require the minimizing of sample preparation procedures, and solid-phase extraction might compromise analyte stability and quantitativity. As a rule of thumb, the simplest preparation procedure is the preferred method [8].

Current methods for analyzing cephalosporins, especially ceftiofur, are based on liquid chromatography [9], electrospray mass spectrometry [10], spectrophotometry [11], or immunochromatography [12]. To our knowledge, no data have been published on voltammetric

* Corresponding author. Tel.: +55 67 33453596; fax: +55 67 33453552.
E-mail address: vsouza@ufms.br (V.S. Ferreira).

0026-265X/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.microc.2011.02.014

determination of ceftiofur based on its hydrolysis product in samples of fluid milk or milk powder.

There is an urgent need for fast, straightforward methods for on-site analysis of antibiotics and other residuals in bovine milk, and electroanalytical techniques have great potential to meet this demand. Electroanalytical instrumentation is portable and compact, yielding results in a matter of minutes without the need for preliminary sample treatment and ensuring selectivity and sensitivity comparable to HPLC methods. The proposed procedure is also less polluting than reference methods. Recently, various electrochemical methods based on adsorptive stripping voltammetry have been published for determination of antibiotics in human urine [13], pharmaceutical formulations, and plasma [14]. Several electroanalytical techniques have been successfully developed by our group for determination of organic compounds in different types of matrices using mercury electrodes [15–17]. Voltammetric techniques using mercury electrodes can be especially valuable analytical tools that in certain cases can successfully compete with modern spectroscopic and separation techniques. The use of mercury as the working electrode has been generally avoided, not only because of toxicity concerns, but also because it complicates the use of portable devices. Nonetheless, mercury is widely acknowledged as the best electrode material, for its easily renewable, atomically smooth surface and large cathodic window. Additionally, mercury provides an extremely useful surface for pre-concentration of analytes before potential scans are performed, as is the case with stripping voltammetry (SV) [18,19]. In this paper we report a simple electroanalytical method in which a single hydrolysis step is followed by direct electrochemical determination of the hydrolysis product, without requiring protein extraction.

2. Experimental

2.1. Instrumentation

An μAutolab Type II device (Eco Chemie) controlled by General Purpose Electrochemical System (GPES) software (Eco Chemie BV) was used to perform the electrochemical measurements. A conventional three-electrode system was employed, which was composed of a hanging mercury drop electrode (HMDE) (area: 0.52 mm^2), an Ag/AgCl reference electrode, and a glassy carbon as auxiliary electrode.

2.2. Reagents and solutions

A ceftiofur analytical standard was prepared daily from Vetranal® (Sigma-Aldrich). Stock solutions (1.0×10^{-3} mol L^{-1}) were prepared daily by dissolving solid ceftiofur in demineralized water from a Milli-Q system (Millipore, Bedford, MA, USA).

Hydrolysis reactions were carried out in a thermostatic bath by placing 10 mL of ceftiofur solution of the appropriate concentration in Britton–Robinson (BR) buffer at pH 10 at 60 °C for 60 min. After being heated for the time required, the solution was cooled in an ice bath and an aliquot of it was removed using a micropipette. The BR buffer solution was prepared by mixing equal volumes of 0.04 mol L^{-1} orthophosphoric acid, acetic acid, and boric acid with an appropriate volume of 0.20 mol L^{-1} sodium hydroxide.

Milk samples were purchased from various markets located in the city of Campo Grande, Mato Grosso do Sul State, in southwestern Brazil, stored at 4 °C after aliquot collection, tested on a microbial assay, and used as blank samples for the application and validation of the new method.

2.3. Milk sample preparation

Aliquots (500 μL) of fluid milk and powder milk spiked with standard drug were heated in a thermostatic bath at 60 °C for 60 min. Aliquots of suitable volume were collected, protected from light in amber vials covered with aluminum foil, and stored at −20 °C until analysis.

2.4. Voltammetric determination

Aliquots (10 mL) of BR buffer at pH 10 were transferred to an electrochemical cell and deaerated for 10 min. A selected accumulation potential was then applied to a mercury drop for a uniform accumulation period while the solution was stirred at 1500 rpm. After a 10 s rest period, adsorptive stripping square-wave voltammetry was applied in the negative direction over a voltage range of −0.4 V to −1.85 V vs. Ag/AgCl. After a background voltammogram was recorded, aliquots containing between 60 and 150 μL of ceftiofur stock solution were added to the cell and a square-wave voltammogram was recorded under the same conditions using a new drop. Quantification was performed by the standard addition method under optimized conditions. All measurements were made at room temperature.

3. Results and discussion

Square-wave voltammetry was used to track the degradation of ceftiofur in BR buffer for the pH range of 2.0 to 11.0. At pH 2.5, ceftiofur yielded two peaks, situated at −0.50 and at −1.00 V (labeled **1C** and **2C**, respectively). At pH 5, however, a third peak was detected at −0.70 V (peak **3C**) (Fig. 1), corresponding to the electrochemically reducible product of ceftiofur hydrolysis. The following experiments were performed in cases where ceftiofur hydrolysis was found to be temperature and time-dependent.

The effect of pH on the peak current corresponding to the hydrolysis product (peak **3C**) was investigated by maintaining the solution being degraded at 25 °C for 60 min and at 50 °C for 60 min for each pH value (from 2.0 to 11, in integer increments). As shown in Fig. 2, degradation became significant from pH 9.0 onwards for both temperatures, with maximum current reached at pH 10.

At 50 °C, maximum peak height was observed for degradation at pH 10. At 25 °C, the compound responsible for this peak was not formed in appreciable amounts below pH 11. Fig. 3 shows the effect of degradation temperature on the height of square-wave voltammetric peaks at −0.50 and −1.00 V (peaks **1C** and **2C**, respectively) and −0.70 V (peak **3C**) for 5.0×10^{-7} mol L^{-1} ceftiofur in BR buffer at pH 10.0 after 30 min heating. The first peak increased in height as the

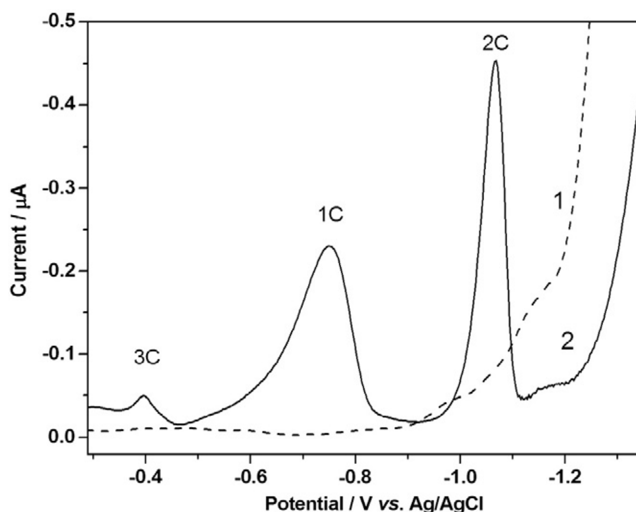

Fig. 1. Square-wave voltammograms for the ceftiofur detection (5.0×10^{-7} mol L^{-1}) in Britton–Robinson (BR) buffer at pH 5.00. Curve A: Supporting electrolyte (BR buffer at pH 5.00); Curve B: 5.0×10^{-7} mol L^{-1} ceftiofur solution after 15 min hydrolysis in BR buffer at pH 5.00 at room temperature. Parameters: frequency (f) = 90 Hz, scan increment (ΔE_s) = 4 mV and pulse amplitude (E_{sw}) = 25 mV.

Download English Version:

https://daneshyari.com/en/article/1228040

Download Persian Version:

https://daneshyari.com/article/1228040

Daneshyari.com