Revista Virtual de Química
ISSN 1984-6835

Artigo

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Rev. Virtual Quim., **2014**, *6* (2), 416-431. Data de publicação na Web: 11 de dezembro de 2013

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Interferentes Endócrinos em Águas Naturais: Determinação Voltamétrica de 17α-Metiltestosterona

Resumo: Os hormônios sexuais incluindo o 17α-metiltestosterona (MT) pertencem ao grupo de compostos considerados disruptores endócrinos. No entanto, poucos estudos reportam o hormônio MT em amostras ambientais. Nesse contexto, este trabalho descreve a determinação direta de MT em amostras de águas naturais baseado na electroredução, empregando o eletrodo de gota pendente de mercúrio e a voltametria de onda quadrada.

Palavras-chave: 17α-metiltestosterona; águas naturais; voltametria de onda quadrada; eletrodo de gota.

Abstract

The sex hormones including 17α-methyltestosterone (MT) are within a group of compounds considered as endocrine disruptors. However, few studies report the presence of hormone MT in environmental samples. In this context, this paper describes the direct determination of MT in water samples based on electroredution and employing the electrode hanging mercury drop and square wave voltammetry.

Keywords: 17α-methyltestosterone; natural water; square wave voltammetry; hanging mercury drop electrode.

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DOI: 10.5935/1984-6835.20140029

Volume 6, Número 2 Março-Abril 2014

Revista Virtual de Química
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Endocrine Interfering in Natural Waters: Voltammetric Determination of 17α-methyltestosterone

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Recebido em 19 de agosto de 2013. Aceito para publicação em 22 de novembro de 2013

1. Introduction

The hormone 17α-methyltestosterone (Figure 1) is a synthetic derivative of testosterone and a sex hormone that belongs to the group of anabolic androgenic steroid hormones responsible for sexual characteristics of male mammals. In medicine, it is used to supply the deficiency of testosterone in the human body and to treat symptoms of andropause in men.[1-3]

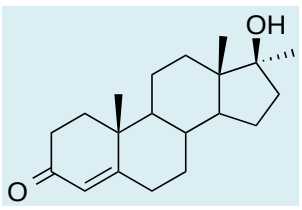

Figure 1. Chemical structure of the hormone 17α-methyltestosterone

It is also widely used in aquaculture for sex reversal in shrimps (*e.g.* Malaysian *Macrobrachium rosenbergii*),[4] fishes (*e.g.* Nile tilapia, *Oreochromis niloticus*),[3,6] trouts (*e.g. Salmo gairdneri)*[7] and ornamental fishes (e.g. zebrafish, *Danio rerio*).[8] Due to this use, the activity of fish culture becomes an important source of discharge of effluents containing 17α-methyltestosterone,[9] justifying the need for monitoring the water bodies in relation to the presence of hormonally active substances. Furthermore, the hormones are within a group of compounds considered endocrine disrupters covering a range of natural and synthetic compounds capable of interfering with the endocrine system in several organisms.[10,11]

Residues of this masculinizing hormone may accumulate in ponds and be slowly released into other aquatic ecosystems contaminating planktonic microorganisms and aquatic ecosystems through accumulation in their tissues and thus affecting other organisms that feed on the former. If the metabolization of hormonal substances in the organisms is low, or absent, the final concentration of this compound may be greater in the upper food chains due to the process of bioaccumulation.[3,10]

The usual analytical methods used to determine 17α-methyltestosterone are based on different chromatographic techniques (gas, liquid and high performance liquid chromatography) in several matrices. The literature discusses work on the quantification of drugs, including hormones, in complex matrices such as waters supply,[11,12,13] river waters,[12,14] in veterinary medicine and foods.[16,17] Some works has addressed the use of modified electrodes for the detection of drugs. Santos et al.[18] used the diamond electrode doped with boron for determining the hormone estriol in women's urine. Goyal et al.[19] determined the testosterone and epitestosterone in human urine using carbon electrode nanotubes. However, few studies have reported on the determination of the hormone 17α-methyltestosterone by electroanalytical techniques. Besides, nothing has been described in scientific literature related to this hormone in environmental matrices. The work of Wang et al.[20], provided preliminary results for the electrochemical reduction of 17α-methyltestosterone at a hanging mercury drop (HMDE) using differential pulse voltammetry and square wave voltametry (SWV).

Electroanalytical methods with a mercury electrode have been used in complex matrices, for example to quantify herbicide in natural water,[21] soil, river sediments, and foodstuffs,[22,23] as an alternative to more complex procedures like chromatography. Among these methods are the voltammetrics, that allow the study of the oxidation-reduction processes and provide qualitative and quantitative information of a chemical species from current curves as a function of applied potential between two electrodes, performing a scan at a constant speed in function of time.[24] Additionally, the main advantage of voltammetric techniques is the ability of the measure to be made directly on the sample without prior separation.[25] This fact coupled with the short time in analysis, the low cost of instrumentation compared to chromatographic methods, spectroscopic, spectrometric, among others, made the electroanalytical techniques become increasingly promising for the determination of different compounds in various arrays of interest. However, a discussed drawback to these methodologies is often attributed to the frequent necessity of using a HMDE, whose use is now being limited in analytical procedures due to the hazardous effects of mercury.[26,27] Notwithstanding some organic molecules, such hormones are electroactive only on a mercury surface. So, the use of the modern voltammetric techniques, such as SWV promote a substitution of traditional polarographic techniques, reducing mercury residues and decreasing the time for each analysis.[21,23,24]

The use of solid electrodes for the 17α-methyltestosterone detection is very difficult due to the fact that products of the redox reaction remain strongly adsorbed in the

electrodic surface, making the reproducibility in the voltammetric response difficult. However, the use of HMDE promoted an excellent reproducibility due to the renovation of the electrodic surface after each measurement. A new drop always is automatically formed by nitrogen pressure in the system after dislodging the old drop and extruding more triple distilled mercury. Besides, the use of modern electroanalytical instruments permits a considerable decrease in mercury residues.[23,26,27] Thus the objective of this study was to optimize the electroanalytical procedure for determination of the hormone 17α-methyltestosterone in samples of natural waters, such as fish cultures, through its electroreduction on HMDE using SWV.

2. Experimental

2.1. Materials and Methods

The voltammetric measurements were performed on a PGZ 402 Methrom 757 VA Computrace, coupled to VA Computrace software. A polarographic station (model 757 VA) from Methrom was used, containing an Ag/AgCl/KCl (in 1.0 mol L^{-1}) as the reference electrode, a platinum plate as auxiliary electrode and an hanging mercury drop as the working electrode was used. The HMDE was drop size (0.30 mm^2), and renewed after each measurement. A new drop was automatically formed by nitrogen pressure in the system after dislodging the old drop and extruding more triple-distilled mercury.

A Labmether model pH2–PHf–3B pH-meter equipped with a 3.0 mol L^{-1} Ag/AgCl/KCl-glass combined electrode was used for adjusting pH values. Water, purified by a Human UP-900 system, was used to prepare the solutions. Chemicals were of the analytical reagent grade. 17α-methyltestosterone was provided by DEG Chemical Co. Ltd. (Shangai, China) with a purity of 99.0%. A 9.98×10^{-3} mol L^{-1} stock solution of hormone was prepared using HPLC grade ethanol (Baker). Natural water samples were filtered with a cellulose quantitative filter (0.45 µm-Milipore Millex-HV) using a vacuum pump (Prismatec, model 131B).

Humic acid provided from Sigma Aldrich, with technical grade, was used in the study of interference.

2.2. Optimization of the procedure

The measurements were carried out under ambient conditions. The appropriate solutions were transferred into the electrochemical cell and the optimization of the analytical procedure for SWV was accomplished. To achieve this a systematic study of the experimental parameters that affect the responses, such as the pH of the medium, the pulse potential frequency (*f*) related to total pulse duration, the amplitude of the pulse (*a*) and the height of the potential step (ΔE_s) were evaluated. All parameters were optimized in relation to the maximum value of the peak current and the maximum selectivity (half-peak width).

Before each experiment the solution was purged of oxygen using nitrogen for 4 min, and then for more 30 s after each addition of 17α-methyltestosterone standard solution. A deposition potential of -0.8 V was applied throughout this study, and then the potential swep from -1.0 to -1.7 V, as reported in literature.[20] A known concentration of 17α-methyltestosterone was added to the cell contained 15.0 mL of a supporting electrolyte, after which the experimental and voltammetric parameters were studied. For the actual measurements, several support electrolytes were initially evaluated, with the best results being obtained in 0.1 mol L^{-1} NaOH.

After optimizing the voltammetric parameters, analytical curves were obtained in pure electrolyte solutions by the standard addition method. The standard deviation of

the mean current, measured at the reduction potential of 17α-methyltestosterone for 10 voltammograms obtained by using 10 blank solutions in pure electrolytes (S_b), together with the slope of the straight line of the analytical curves (s) were used in the determination of the quantification and detection limits (*LOQ* and *LOD*, respectively), according to guidelines recommended by IUPAC.[28,29] The recovery experiments were done in order to attest the methodology´s efficiency. These experiments were carried out by adding a known amount of 17α-methyltestosterone to the supporting medium. The measurements were performed in triplicate. The recovery efficiencies (%R) were calculated considering the ratio between the value of the concentration obtained by extrapolating the analytical curves of the corresponding spiked samples and the concentration previously added.[30]

The precision and accuracy of the methodology were tested with different standard solutions of 17α-methyltestosterone and the relative standard deviations (RSD) were calculated, considering in the calculus the standard deviation of the mean current values obtained and the mean peak current values.

2.3. Application to natural water

2.3.1. Collection sites

The suitability of the electroanalytical methodology in measuring 17α-methyltestosterone in natural matrices was tested by spiking water samples from river and pond fish farming environments, located in Guarapuava-Paraná, Brazil. Table 1 shows the location of collection points.

Table 1. Location of collection points for water samples uses in the determination of 17α-methyltestosterone

Sample/ Local	Latitude/ Longitude*
Stream Carro Quebrado – Vila Carli	-25°38’25”33,-51°.48’78”56
Tanks of fish culture – Vale do Jordão	-25°46’86"16,-51°41’15”1
Tank fish-pays 1 – Palmeirinha	-25°.21’25”13,-51°54’12”53
Tank fish-pays 2 - Alto da XV	-25°36’28”16,-51°43’15”3

*Source: Reference 31

Samples of river water and pond fish farming were collected using glass bottles of 250 mL. Prior to use the bottles were washed and left in a bath with solution of HNO_3 10% v/v, and afterwards rinsed with distilled and purified water. Before collecting the sample, bottles were rinsed with water from the collection site (river or pond). Then the samples were taken to the laboratory and stored under refrigeration. Before analysis, the samples were filtered using cellulose filters (0.45 µm). The samples were left without any further pre-treatment or purification steps. Next NaOH was dissolved in a water sample and pH was adjusted (pH 9.0). The NaOH was dissolved in natural water samples and artificially contaminated with 17α-methyltestosterone at predetermined concentrations. The recovery curves were constructed by the standard addition method and the recovery percentage was obtained by the graphical method, in which the abscissa axis refers to the concentration of 17α-methyltestosterone in the electrochemical cell. When the obtained curve was extrapolated to this axis,

the sample concentration was found and the recovery values were calculated according to the previously described method. The recovery curves were constructed using data from three different samples.

3. Results and discussion

3.1. Basic electrochemical investigation

Preliminary cyclic voltammetry experiments, using 6.6×10^{-8} mol L^{-1} of 17α-methyltestosterone with 5.0×10^{-3} mol L^{-1} NaOH (pH 9.0), were conducted to determine the voltammetric responses of 17α-methyltestosterone at the HMDE. Figure 2(A) shows the obtained response. A well-defined reduction peak at -1.3 V attributable to 17α-methyltestosterone was observed. However, no oxidation peak was observed in the reverse potential scan, indicating a totally irreversible redox process. The peak current changed linearly with scan rate in the range 25-300 mV s^{-1}, as shown in Figure 2(B). This behavior suggests that the process is controlled by adsorption of the species to the electrode surface.[32] Additionally, there was a negative shift of the reduction peak potential as the scan was increased, indicative of an irreversible process.[33]

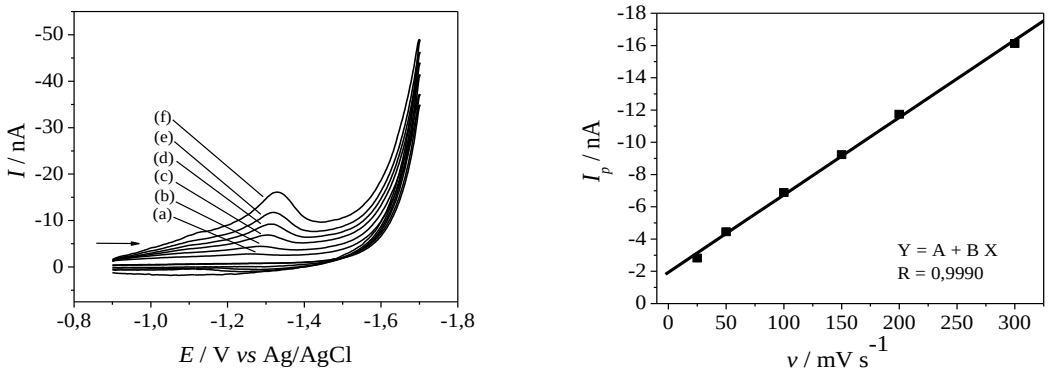

Figure 2. (A) Cyclic voltammograms obtained in the complete determination of hormone 17α-methyltestosterone using CV and HMDE. Conditions: (C_{MT} = 6.62×10^{-8} mol L^{-1}, NaOH solution pH 9.0, potential scan (-0.9 to -1.7 V) E_{dep} (-0.8 V) and Tdep (120 s). (B) Dependence of the intensity of peak current with scan rate for the 17α-methyltestosterone and HMDE using CV (T_{dep} = 120 s)

SWV of 17α-methyltestosterone was carried out next, using the same experimental conditions. A reduction peak in the vicinity of -1.3 V was observed once again. SWV was applied in this study to examine the electrochemical reduction characteristic of the hormone in order to develop a methodology for quantifying the analyte in natural water samples.

3.2. Optimization of the experimental conditions

3.2.1. Study of preconcentration time

The deposition time of 17α-methyltestosterone on the surface of the mercury drop was obtained by adding to the electrochemical cell 15.0 mL of electrolyte solution and 4.65×10^{-8} mol L^{-1} of hormone. A

potential pulse frequency (*f*) of 140 s^{-1}, a pulse amplitude (*a*) of 50 mV, and a scan increment (ΔE_s) of the 2 mV were used in these experiments. The time of preconcentration was varied from 30 to 230 s. Figure 3 shows the current in function of the variation of preconcentration time.

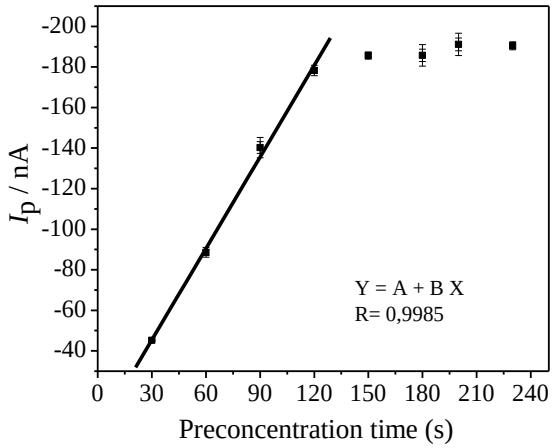

Figure 3. Change of the intensity of the peak current as a function of time by preconcentration of the hormone 17α-methyltestosterone using SWV with the use of HMDE. Conditions: C_{MT} = 4.65×10^{-8} mol L^{-1}, in NaOH solution pH 9.0, preconcentration time (30, 60, 90, 120, 150, 180, 200 e 230 s)

The intensity of the peak current for the hormone 17α-methyltestosterone showed a linear increase over time of pre-concentration until 120 s. Beyond 120 s a leveling off in the current was observed, indicating saturation of 17α-methyltestosterone at the HMDE. It remained constant for longer times, which indicates the saturation of the mercury drop electrode. The time chosen for the pre-concentration of the hormone 17α-methyltestosterone at HMDE was based on this result.

3.2.2. Supporting electrolyte and pH of the medium

Experiments using different supporting electrolytes were also performed in fixed conditions of 17α-methyltestosterone concentration and voltammetric parameters (1.0×10^{-7} mol L^{-1} of 17α-methyltestosterone, frequency of the pulse potential (*f*) of 100 s^{-1}, amplitude of the pulse (*a*) at the 50 mV, scan increment (ΔE_s) of 2 mV and preconcentration time of 120 s), while the supporting electrolyte was changed. Britton-Robinson buffer (pH 9.0), Na_2SO_4 (pH 5.0 and 9.0), and phosphate buffer (pH 5.0 and 9.0) at 0.1 mol L^{-1} and NaOH (pH 9.0) at 5.0×10^{-3} mol L^{-1} were evaluated for this set of experiments.

A well-defined peak was presented in all the electrolytes tested, with different peak potential and peak current values. The observed differences in current and potential peak values are due to the effects of the anionic nature of the supporting electrolyte on the 17α-methyltestosterone reduction process.[34] The best response, measured in terms of the highest analytical signal and optimal reproducibility in the responses, was obtained with 5.0×10^{-3} mol L^{-1} NaOH solution (pH 9.0). NaOH solution was used as the supporting electrolyte, and the effect of its pH value on the electrochemical responses of 17α-methyltestosterone from pH 7.5 to pH 11.0 was evaluated. The pH values were adjusted to the desired value with the addition of appropriate amounts of 0.1 mol L^{-}

[1] NaH_2PO_4 stock solution. The resulting voltammetric responses are illustrated in Figure 4, which shows the increase of the peak current with an increase of pH value.

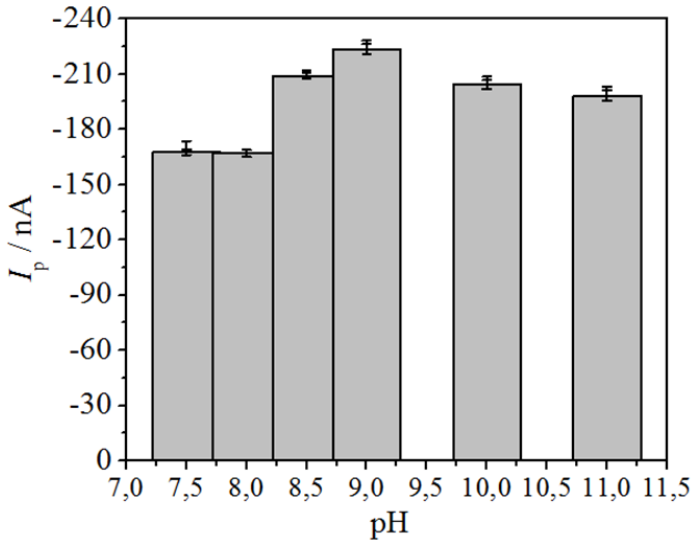

Figure 4. The relationship between the pH values and peak current of the NaOH solutions of 5.0×10^{-3} mol L^{-1}, at different of pH values 7.5 to 11

3.2.3. Optimization of the voltammetric parameters

All voltammetric parameters were optimized to obtain the best analytical signal, in terms of peak current (I_p) and peak potential (E_p). In addition, frequency *(f)*, amplitude (a) and scan increment (ΔE_s) were individually studied. The f value was varied from 10 to 140 s^{-1}. The results showed that an increase in f was accompanied by an increase in I_p, until an f value of 140 s^{-1}. Additionally, a linear relationship between the I_p and the f value was observed (Figure 5(A), which, according to the theoretical model proposed by Lovric and co-workers for SWV,[35,36] may indicate processes controlled by irreversible adsorption of the species on the electrode surface, agreeing with the cyclic voltammetric result for 17α-methyltestosterone shown in Figure 2 (A).

The influence of a on the intensities of I_p was considered for values of a ranging from 10 to 50 mV. The results demonstrated that the increase in a values promoted an increase in I_p until 50 mV, as shown in Figure 5(B). Additionally, the I_p showed no variation as a function of a.

An increase in ΔE_s promotes an increase in the scan rates of the voltammetric experiments and consequently, for some cases will also increase the signal and the sensitivity of the technique. The ΔE_s value was evaluated from 2 to 10 mV and the results indicated that an increase in ΔE_s promotes an increase in I_p values for voltammetric peaks, as shown in Figure 5(C).

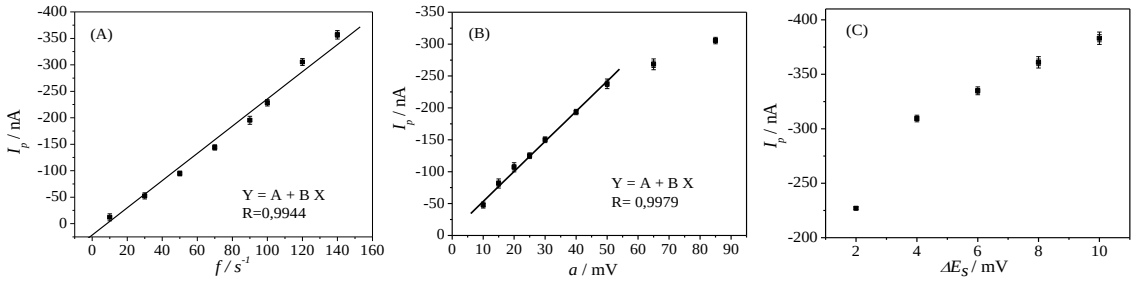

Figure 5. Relationship between the peak currents and frequency of pulse potential (A), pulse amplitude (B) scanning increment (C) square wave voltammograms obtained of 1.0×10^{-7} mol L^{-1} 17α- methyltestosterone in NaOH 5.0×10^{-3} mol L^{-1}, pH 9.0, with ΔE_s = 2 mV, a = 50 mV and f = 100 s^{-1}, on HMDE with variation of each parameter

3.3. Analytical performance

An examination of the relationship between values of I_p and the voltammetric parameter, as presented in Figure 6, indicates that the voltammetric parameter values that can be employed for the analytical determination of 17α-methyltestosterone on HMDE were ΔE_s = 2 mV, a = 50 mV and f = 140 s^{-1}.

The calibration plots based on the peak current of 17α-methyltestosterone at an HMDE, after a 120 s preconcentration time, in the presence of NaOH as supporting electrolyte were constructed next. The voltammograms obtained and the linear relationships between the I_p values and the added concentrations are presented in Figure 6(A). Figure 6(B) represents the mean values between the three constructed curves.

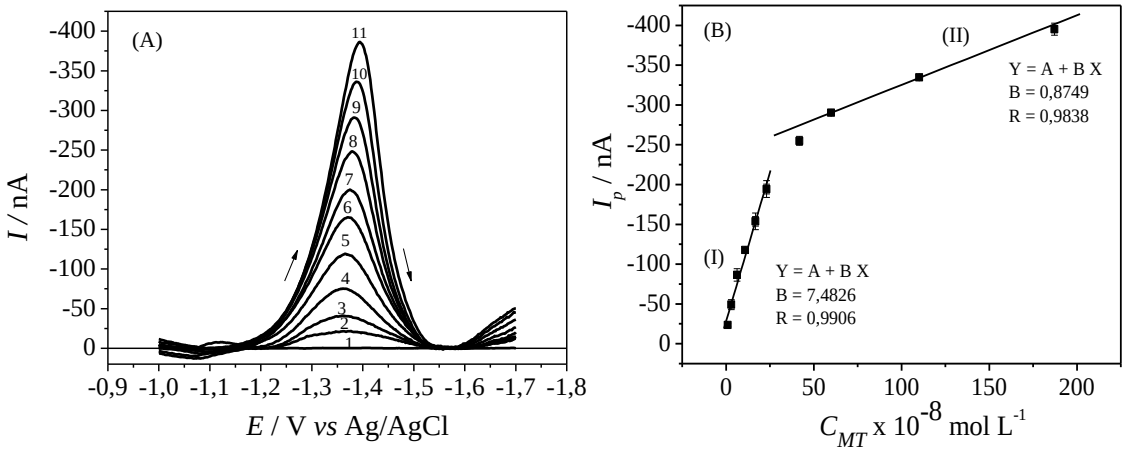

Figure 6. (A) square wave voltammograms obtained with HMDE and addition of standard solution of 17α-methyltestosterone, with f = 140 s^{-1}, a = 50 mV, ΔE_s = 2 mV, NaOH $5{,}0\times10^{-3}$ mol L^{-1}, pH 9,0. Concentration: (1) 0, (2) 9.99×10^{-9}, (3) 2.99×10^{-8}, (4) 6.29×10^{-8}, (5) 1.09×10^{-7}, (6) 1.67×10^{-7}, (7) 2.31×10^{-7}, (8) 4.18×10^{-7}, (9) 5.98×10^{-7}, (10) 1.10×10^{-6}, (11) 1.87×10^{-6} mol L^{-1}. (B) Curve of standard addition of hormone 17α-methyltestosterone on HMDE at different concentrations

According to Figure 6(B), there are two linear ranges: between 1.0×10^{-8} to 2.3×10^{-7} mol L^{-1} and between 4.2×10^{-7} to 1.9×10^{-6} to mol L^{-1}. This suggests an adsorption process. For low concentrations the adsorption process did not alter the kinetics of the electrode surface. For higher concentrations the peak current intensity decreased due to

saturation of the electrode surface by the electroactive species.

The calibration equation can be represented by the expressions (1) for low concentrations of 17α-methyltestosterone (2) to concentration greater than 2.3×10^{-7} mol L^{-1}:

$$I_p = (28.10 \pm 6.62)\times10^{-7}\ (A) + (7.5 \pm 0.51)\ C\ (A/mol\ L^{-1}) \quad (1)$$

At concentrations of 17α-methyltestosterone higher than 2.3×10^{-7} mol L^{-1}, the corresponding expression is:

$$I_p = (237.76 \pm 2.76)\times10^{-7}\ (A) + (0.87 \pm 0.044)\ C\ (A/mol\ L^{-1}) \quad (2)$$

Using the slope (1) as an estimate for sensitivity, the former range clearly shows a greater sensitivity.

Table 2 shows the detection limits for 17α-methyltestosterone, which were obtained using differential pulse voltammetry,[20] high performance liquid chromatography (HPLC)-ultraviolet (UV),[16] HPLC-mass spectrometry (MS/MS)[15] and HPLC/ Detector Diode Array (DAD),[38] that are the typical methodologies employed to determine 17α-methyltestosterone levels in different samples. Although the LOD of the proposed method is larger than those described by other researchers, we emphasize that our method is fast because it eliminates steps as extraction and pre-concentration of the analyte necessary for chromatographic techniques. [15,16] The only reported work in the literature for the 17α-methyltestosterone determination was developed by Wang et al.[20] employing the mercury electrode and adsorptive voltammetry technic. However, their method was optimized with preconcentration time of 15 min and aplied for drug concentration, while in the present study accumulation time was 2 min and applied in environmental samples (section 3.2.1). There is hitherto no legislated limit for 17α-methyltestosterone and therefore it is necessary to periodically monitor its concentration in water resources. The *LOD* values observed in the present study are similar to those previously published using other electroanalytical methodologies to determine steroid sex hormones, including the 17α-methyltestosterone.[37,38]

Recovery studies by standard addition were performed by the standard addition method to validate the method, minimizing the matrix effect. Known concentrations of MT (smaller range and higher value) were added in water samples using the optimized parameters. The recovery percentages were used to evaluate and quantify 17α-methyltestosterone that was added. The recovery efficiency (*%R*) of the methodology could thus be determined. Recovery percentages are tabulated in Table 3.

The results for the recovery of 17α-methyltestosterone in water, aqueous standard and natural samples ranged from 100.4% to 115.0%. These results were presented as appropriate coefficient of variation (CV) of 2.16%, 9.82% and 1.54%, respectively. Ribani et al.[39] described that compounds in trace levels are supported up to 20% CV considering the complexity of the sample. This indicates that the current methodology can be successfully applied to the analytical determination of 17α-methyltestosterone, even in complex samples such as natural water.

The calculated values for LOD and LOQ were respectively, 1.0×10^{-8} mol L^{-1} (3.1 μg L^{-1}) and 3.6×10^{-8} mol L^{-1} (10.78 μg L^{-1}).

The precision and accuracy of this methodology were evaluated by examining the reproducibility and repeatability over many experimental trials. The reproducibility was determined on different days, using five different solutions containing 1.9×10^{-7} mol L^{-1} of 17α-methyltestosterone. The *RSD* values obtained for the I_p value was 14.91%. The repeatability was determined by measuring in five separate times the same solution

containing 1.9×10^{-7} mol L^{-1} of 17α-methyltestosterone. The *RSD* value obtained was 2.85%. These results are a strong indicator that the proposed methodology may provide a suitable precision and accuracy in the analytical determination of 17α-methyltestosterone.

As the calculated values for *LOD*, *LOQ*, the recovery efficiencies and the reproducibility are all suitable for use in the determination of low levels of 17α-methyltestosterone, the proposed methodology was employed to determine hormone residue levels in natural water.

Table 2. Analytical parameters obtained for 17α-methyltestosterone determination using different analytical procedures

	Remarks	LOD	Recovery	Ref.
This work SWV	NaOH 5×10^{-3} mol L^{-1} (pH 9.0)	1.0×10^{-8} mol L^{-1} (3.1 µg/L)	100.4 % in purified water, 108.8 % in water fish tank	-
DP and SMDE	NaOH 5×10^{-3} mol L^{-1}	3.3×10^{-10} mol L^{-1} (0.1 µg/L)	89 ± 7% in tablets	[18]
HPLC-UV	Liquid-liquid extraction and solid phase	1.3 mg Kg^{-1}	91.5 – 100.3 % in fish feed	[16]
HPLC-MS/ MS	Extraction in solid phase	< 0.30 ng/ L	91-110 % in water Danube River	[15]
HPLC/ DAD	Extraction in solid phase	0.38 µg/L	27.5 ± 8.9 % in urine matrix	[30]

DP = Differential pulse; SMDE = static mercury drop electrode; HPLC = High-performance liquid chromatography.

Table 3. Addition and recovery of 17α-methyltestosterone (MT) in the water samples

Sample	**MT**	**Concentration**	
		mol L^{-1}	**µg L^{-1}**
Purified water	Added	5.31×10^{-8}	16.06
	Measured	$5.33\times10^{-8} \pm 1.15\times10^{-9}$	16.12 ± 0.35
	Recovery	100.38%	-
Fish culture	Added	4.65×10^{-8}	14.06
	Measured	$5.07\times10^{-8} \pm 4.98\times10^{-9}$	15.34 ± 1.51
	Recovery	108.79 %	-

3.4. Study of interferents

After obtaining the limits of detection, quantification and study of recovery, some possible interferents in water samples were investigated. In this work, we have considered humic acid (HA) for being one of the main components present in natural waters.[40,41]

Thus the study of HA interferent was carried out with the addition of aliquots of HA from a stock solution of 1.5 g L^{-1}. Studies

with different levels of HA were evaluated, varying from 4.9×10^{-6} to 3.8×10^{-2} g L^{-1}. For low concentrations of HA (4.9×10^{-6} at 2.9×10^{-5} g L^{-1}) no interference was observed. However, with the addition of a more concentrated solution of HA, the peak current decreases to 64 % on the initial peak current to the concentration of 1.9×10^{-7} mol L^{-1} 17α-methyltestosterone. In higher quantities of HA the solution within the cell becomes brown. In the water samples, despite of being colorless, a significant interference was observed when carrying out the 17α-methyltestosterone determinations.

Additionally, we investigated the effect of matrix comparing the measurements with ultra pure water and natural water samples, which were made successive additions of 17α-methyltestosterone. The Figure 7 shows the variation of the intensity of the peak current as a function of the 17α-methyltestosterone concentration in purified water and tank fish culture water. The concentration of 17α-methyltestosterone added was 4.6×10^{-8} to 4.2×10^{-7} mol L^{-1}. It can be observed that the slopes of the showed a difference of approximately 50 %, indicating a negative interference in natural water samples.

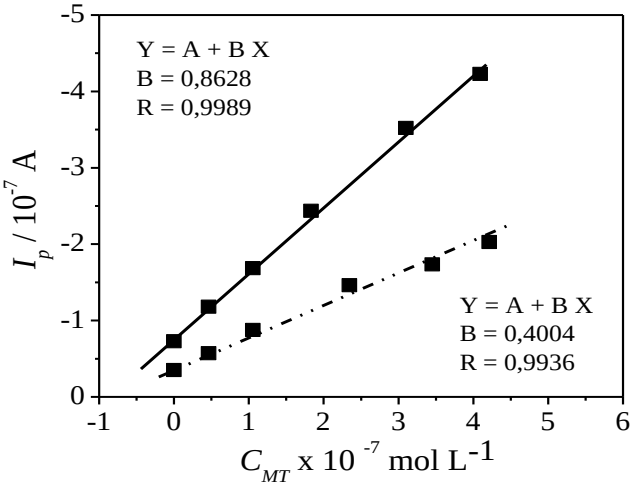

Figure 7. Relationship between the intensity peak current and the concentration of 17α-methyltestosterone in relation to the matrix in ultrapure water (__) and water fish tank (----), at the concentrations 4.6×10^{-8} to 4.2×10^{-7} mol L^{-1}

The slopes were 0.8628 A/ mol L^{-1} and 0.4004 A/ mol L^{-1} with regard to purified water and to water tank fish culture, respectively. This suggests that substances other than HA may be causing a decrease in the analytical signal of 17α-methyltestosterone in the samples. Thus for suppressing interference in the quantification of 17α-methyltestosterone, samples were diluted before the determinations. Aliquots of 5.0 mL of the sample solution containing the electrolyte NaOH was added to the measurement cell with 10.0 mL of electrolyte prepared in ultrapure water. Thus the interfering substances were also diluted, thereby minimizing interference.

3.5. Application to natural water

The methodology was applied to quantify the hormone 17α-methyltestosterone in water samples collected in fish ponds and the river. The NaOH supporting electrolyte was prepared in these matrices. 10.0 mL of supporting electrolyte and 5 mL of sample were added to the electrochemical cell and then the solution was degassed by 4 min to purge of oxygen present. Next the

measurement was performed with standard addition procedure. The intensity of the peak current of the 17α-methyltestosterone in the samples was measured at potential -1.35 V. The measurements were performed in triplicate.

The Results indicated the presence of the hormone 17α-methyltestosterone in natural samples from ponds of fish culture, despite of not being employed in these sites 17α-methyltestosterone on sex reversal processes (Table 4). However, the highest levels of the hormone 17α-methyltestosterone were found in the tanks of fish culture in the "Vale do Jordão". A possible source of the 17α-methyltestosterone is the fish food, as manufacturers produce food without addition of 17α-methyltestosterone, as well as tilapia food, which may contain this hormone. Additionally, carcasses and viscera of fish can be re-used as inputs in food manufacturing.[9]

Table 4. Determination of 17α-methyltestosterone (MT) in water samples

Sample	*Concentration of MT	
	mol L^{-1}	μg L^{-1}
Tank 01 – Vale de Jordão	$1.8\times10^{-7} \pm 9.2\times10^{-9}$	54.4 ± 2.8
Tank 04 – Vale de Jordão	$4.7\times10^{-7} \pm 5.13\times10^{-8}$	142.2 ± 15.5
Tank 06 – Vale de Jordão	$2.5\times10^{-7} \pm 2.4\times10^{-9}$	75.6 ± 0.7
Tank Palmeirinha	$2.2\times10^{-7} \pm 1.0\times10^{-8}$	66.5 ± 3.0
Tank 01 Alto da XV	$1.6\times10^{-7} \pm 1.8\times10^{-8}$	48.4 ± 5.4
Tank 02 Alto da XV	$1.7\times10^{-7} \pm 2.4\times10^{-8}$	51.4 ± 7.3
Arroyo Carro Quebrado	< LOQ*	-

* 3.6×10^{-8} mol L^{-1} (10.78 μg L^{-1}).

4. Conclusion

The present study shows how the use of SWV allied to HMDE makes it possible to determine 17α-methyltestosterone in level at detection limits to trace elements that are similar to those published in the literature using chromatographic methodologies, with the advantage that it does not need steps of pre-treatment or purification. Matrices effects in the samples were minimized by diluting the sample without the need of prior treatment. This procedure can be used to investigate environmental impacts resulting from the abusive use of these compounds, and the contamination of groundwater resources.

Acknowledgements

The authors wish to thank CAPES for scholarships, and FINEP, CNPq and UNICENTRO, Brazil, for support and infrastructure.

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