ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
30 (2002) 279–284

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Voltammetric determination of fenbendazole in veterinarian formulations

Marcelo Firmino de Oliveira *, Nelson Ramos Stradiotto

Instituto de Química, UNESP, 14801-970 Araraquara, SP, Brazil

Received 19 December 2001; received in revised form 5 April 2002; accepted 29 April 2002

Abstract

A versatile voltammetric method for quantitative determination of fenbendazole (FBZ) in commercial tablets has been proposed, where direct dissolution of tablets is carried out in 0.1 mol l^{-1} tetrabutylamoniun tetrafluorborate containing dimethylformamide solutions. Linear sweep (LSV), square wave (SWV) and differential pulse (DPV) voltammetry techniques were applied to study FBZ at a glassy carbon electrode, exhibiting a well defined irreversible oxidation peak at 1.15 V vs. SCE. This methodology allows a precise quantitative determination of FBZ presenting detection limits of 5.2×10^{-5} (LSV), 5.0×10^{-6} (DPV) and 5.0×10^{-5} mol l^{-1} (SWV).

Keywords: Fenbendazole; Anthelmintic; Voltammetric determination

1. Introduction

The treatment of helmintic diseases consists in a public health subject of great importance. In this context, the class of benzimidazole-carbamate compounds has been used in the combat of a broad spectrum of helmintic species in human and veterinary treatments [1], whose primary action mode of this class of compound consists in selective toxicity for helmintic parasites [2].

In this context, two compounds with similar chemical structure of this class can be mentioned, as albendazole (ABZ), a benzimidazole-carbamate with a 5-propillthio group, that has been used effectively in human helminthic diseases and fenbendazole (FBZ), with a 5-phenylthio group which is used against a large variety of helmintic species in veterinary applications [3]. The official method for FBZ assay according to British Pharmacopoeia [4], consists in a potentiometric titration method, where FBZ is dissolved in anhydrous acetic acid and titrated with percloric acid, offering detection limit values around 10^{-4} mol l^{-1}.

Anthelmintic activity, dosage and selectivity of benzimidazole-carbamate compounds have been investigated using traditional methods of analysis as high performance liquid chromatography (HPLC) [5,6], gas chromatography (GC) [7] and spectrophotometry [8].

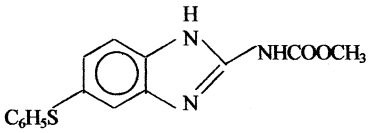
* Corresponding author. Tel.: +55-16-2016-740; fax: +55-16-2227-932

E-mail addresses: marcelex@posgrad.iq.unesp.br (M.F. de Oliveira), nrstradi@iq.unesp.br (N.R. Stradiotto).

0731-7085/02/$ - see front matter
PII: S0731-7085(02)00339-4

Despite the pharmacological importance of benzimidazole-carbamate compounds, there are few electrochemical methods about dosage of these substances [9]. Among these methods, two voltammetric methods can be reported, as ABZ determination by differential pulse adsorptive cathodic stripping voltammetry at a hanging mercury drop electrode [10] and a voltammetric assay of ABZ by direct dissolution in HCl solutions using the techniques of linear, differential pulse (DPV) and square wave voltammetry (SWV) at glassy carbon electrodes [11], being both methodologies applied to ABZ determination in commercially available dosage forms.

According to literature [9,11], electrochemical techniques have demonstrated a large applicability in studies of electrodic reactional mechanisms of pharmaceutical compounds. Additionally, quantitative determination of these substances has been provided by voltammetric methods, presenting results in agreement with usual spectrometric and chromatographic techniques. However, electrochemical studies of FBZ still have not been reported. So, the objective of this work is to investigate the electrodical response of this compound and develop an electrochemical methodology for quantitative determination in pure form and in commercial tablets.



Molecular structure of FBZ

2. Experimental

2.1. Reagents and solutions

Supporting-electrolyte solution was prepared in 0.1 mol l^{-1} tetrabutylamoniun tetrafluorborate from Merck, containing dimethylformamide, being dehydrated with molecular sieve from Merck. A standard solution 1.0×10^{-2} mol l^{-1} of FBZ was obtained by dissolving the compound with pure reagent from Sigma in a previously supporting-electrolyte solution. The analysis of commercially dosage forms was obtained by direct dissolution of tablets (Panacur®) whose labeled composition was 500 mg of FBZ and 1000 mg of vehicle, being each tablet dissolved in a 250 ml of supporting-electrolyte solution. Additional informations about vehicle composition were not reported in the commercial tablets.

2.2. Apparatus

The voltammetric measurements were obtained in an electrochemical cell was one of the conventional type, having a 2 mm diameter glassy carbon electrode (working electrode), a 0.25 cm^2 platinum plate (auxiliary) and a saturated reference calomel electrode, SCE.

For the controlled potential coulommetry experiments of FBZ, another electrochemical cell was used, being formed by separated compartments with porous glass plate, in order to separate electrogenerated products (catholyte and anolyte). In this step, the electrode system was formed by a 4 cm^2 net platinum pair (working and auxiliary electrodes) and a saturated calomel electrode (reference).

The experiments were carried out with a potentiostat/galvanostat EG&G (PAR), model 173, coupled to a microcomputer.

2.3. Voltammetric procedures

2.3.1. Linear sweep voltammetry (LSV)

A 18 ml of supporting-electrolyte was put in the electrochemical cell, being realized as a nitrogen gas flow for 15 min in order to remove electroative molecular oxygen. A potential sweep was made in order to verify the potential working range. A 2 ml volume of FBZ standard solution was put in this cell, resulting in a 1×10^{-3} mol l^{-1} FBZ solution. This solution received a nitrogen gas flow and a potential sweep was carried out in the work range of pre-obtained potential. The influence of potential scan rate (v) was studied. Afterwards, voltammograms at different concentrations were obtained in order to build a calibration curve and the FBZ sample concentration was obtained by interpolation in this curve,

whose measurements were obtained three times for each solution.

2.3.2. Differential pulse voltammetry

Optimization of operational parameters as potential pulse height (E_p), pulse width (t_p) and scan rate (v), were carried out with 20 ml of a 1×10^{-3} mol l^{-1} deaerated solution of FBZ. Afterwards, the calibration curve for FBZ was obtained. The FBZ sample concentration was obtained by interpolation in the calibration curve, being these measurements obtained three times for each solution.

2.3.3. Square wave voltammetry

According to the previous procedures, a 1×10^{-3} mol l^{-1} deaerated solution of FBZ was used in order to optimize the operational parameters, such as the applied pulse magnitude (E_{sw}), the pulse width (t_p) and the pulse frequency (f), for this technique. Afterwards, the calibration curve for FBZ was obtained with voltammograms at different concentrations and the FBZ sample concentration was also obtained by interpolation in the calibration curve whose measurements were obtained three times for each solution.

2.3.4. Controlled potential coulommetry

The electrolysis of FBZ was obtained by using a 15 ml volume of a 1×10^{-2} mol l^{-1} standard solution at controlled potential in order to determine the number of electrons involved in the reaction. The measurements were obtained in triplicate.

Table 1
Cyclovoltammetric parameters for 1×10^{-3} mol l^{-1} FBZ solution in 0.1 mol l^{-1} tetrabutylamoniun tetrafluorborate in dimethylformamide

v (mV s^{-1})	I_{ap} (μA)	E_{ap} (V)	$E_{ap} - E_{p1/2}$ (mV)
10	8.0	1.16	105
20	13.0	1.16	95
50	23.0	1.18	100
100	31.0	1.19	100
200	48.0	1.20	98
500	79.0	1.22	100

3. Results and discussion

3.1. Oxidation process

Obtained results by cyclic voltammetry have shown that FBZ gives a well defined anodic peak at potential (E_{ap}) of 1.15 V vs. SCE in a 0.1 mol l^{-1} tetrabutylamoniun tetrafluorborate containing dimethylformamide. The absence of a cathodic peak indicates initially an irreversible oxidation process. At this reactional medium, FBZ has presented good solubility, and the stability of FBZ in solution was observed when the same voltammetric answer was obtained after 1 month.

The results of the oxidation of FBZ have shown diffusional control, being the anodic peak current dependence in relation to the square root of scan rate linear over the studied range (Table 1). The increment of E_{ap} values with the increase of scan rate indicates the characteristic behavior of irreversible processes [12]. Obtained media value for the $|E_{ap} - E_{p/2}|$ parameter was found to be 99 mV, being constant with scan rate increase, indicating a possible EE electronic transfer type [13]. FBZ electrolysis indicated that two electrons are involved ($n = 1.81 \pm 0.14$).

The observed anodic current is due to the oxidation of sulfur atom present in the molecule. At solid electrodes at room temperature (25 °C) and in aprotic solutions, aliphatic or aromatic sulfides are promptly oxidized to their corresponding dication forms [9] in a two-electron process, being these species able to participate in further reactions, as dimerization or sulfonium formation.

$$C_{15}H_{13}N_3O_2S \rightarrow C_{15}H_{13}N_3O_2S^{+2} + 2e^- \quad (1)$$

Possible interferents for these techniques are organic sulfide species as ABZ, due to its similar structure containing sulfide group. However, due to its applicability in human helmintiasis, ABZ is not expected in veterinarian formulations. Other benzimidazole compounds of veterinarian usage as oxifendazole [3] do not present available sulfide groups for oxidation at studied conditions in this work.

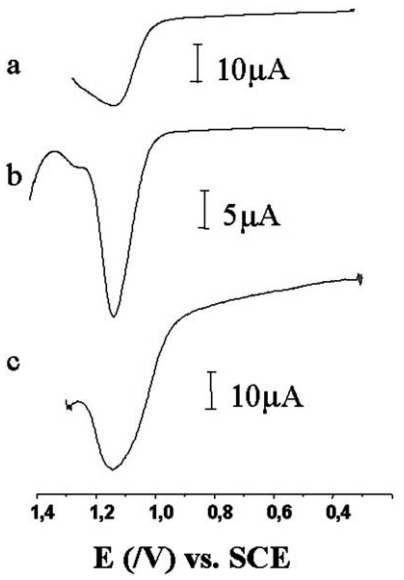


Fig. 1. Voltammetric behavior for 1×10^{-3} mol l^{-1} FBZ solution in 0.1 mol l^{-1} tetrabutylamoniun tetrafluorborate in dimethylformamide. (a) LSV: $v = 100$ mV s^{-1}; (b) DPV: $v = 10$ mV s^{-1}, $E_p = 100$ mV and $t_p = 10$ ms; (c) SWV: $f = 200$ Hz and $E_{sw} = 35$ mV.

3.2. Voltammetric behavior

Voltammograms for FBZ were obtained by LSV (Fig. 1; curve a) at different concentrations in the range 2×10^{-4}–3×10^{-3} mol l^{-1}.

The anodic peak current dependence in relation to concentration is linear (Fig. 2; curve a), giving a linear correlation coefficient of 0.995, a calibration curve slope of 3.0×10^4 μA l mol^{-1}, detection and quantification limits [14,15] of 5.24×10^{-5} and 8.73×10^{-5} mol l^{-1}.

In the optimization studies of operational parameters for DPV (Fig. 1; curve b), a potential pulse height of 100 mV, a pulse width of 10 ms and a scan rate of 5 mV s^{-1} were adopted. These parameters are in good agreement with typical conditions of use [16].

The obtained voltammograms by DPV at different concentrations were used to build a calibration curve, whose concentration range was from 5.0×10^{-5} to 5.0×10^{-4} mol l^{-1}. The anodic peak current dependence in relation to concentration is linear (Fig. 2; curve b), exhibiting a linear correlation coefficient of 0.998, a calibration slope equal to 2.5×10^4 μA l mol^{-1} and detection and quantification limits of 5.02×10^{-6} and 8.36×10^{-6} mol l^{-1}.

In the optimization studies of operational parameters for SWV (Fig. 1; curve c), a half potential pulse magnitude (E_{sw}) of 35 mV and pulse frequency (f) of 225 Hz were adopted in good accordance with typical conditions of use [16]. A calibration curve was obtained for the concentration range from 1.0×10^{-4} to 1.4×10^{-3} mol l^{-1}.

The anodic peak current dependence in relation to concentration is linear (Fig. 2; curve c), exhibiting a linear correlation coefficient of 0.998, a slope of calibration curve equal to 3.5×10^4 μA l mol^{-1} and detection and quantification limits of 5.03×10^{-5} and 8.38×10^{-5} mol l^{-1}.

According to the obtained calibration curves for FBZ reported in Fig. 2, it was possible to describe the linear dependence of i_{ap} against FBZ

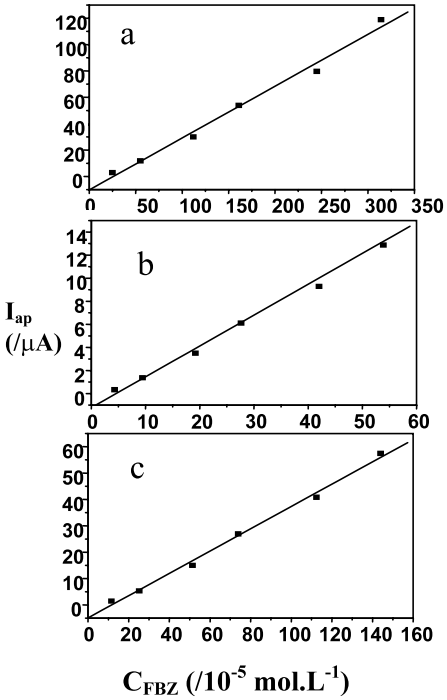


Fig. 2. Linear anodic peak current dependence in relation to FBZ concentration for the three voltammetric techniques: (a) LSV; (b) DPV; and (c) SWV.

Table 2
Determination of commercial sample FBZ concentration by different voltammetric techniques

Technique	C_{FBZ} (mg)	Error (%)	Detection limit (10^{-6} mol l^{-1})	Quantification limit (10^{-6} mol l^{-1})	Sensibility (10^{4} μA l mol^{-1})
LSV	481.4	3.72	52.4	87.3	3.01
DPV	482.1	3.63	5.02	8.36	2.54
SWV	479.0	4.35	50.3	83.8	3.52

Concentration of FBZ according to the labeled amount (500 mg). LSV, linear scan voltammetry; DPV, differential pulse voltammetry; and SWV, square wave voltammetry.

concentration for the three voltammetric techniques, as follows:

LSV:

$$Y = -(1.40 \pm 0.059) + (3.01 \pm 0.13) \times 10^4 X$$

DPV:

$$Y = -(0.34 \pm 0.01) + (2.54 \pm 0.08) \times 10^4 X$$

SWV:

$$Y = -(6.28 \pm 0.02) + (3.52 \pm 0.13) \times 10^4 X$$

These linear and angular coefficients are reported in μA and μA l mol^{-1}, respectively.

3.3. Pharmaceutical dosage forms

Obtained results for the determination of FBZ concentration at commercial samples by the three voltammetric methods were reported in Table 2, whose measurements were obtained in triplicate.

The FBZ concentration in the sample was determined by LSV as being 481.4 ± 18 mg, exhibiting an error of 3.72% in relation to the labeled amount. The analysis of the obtained voltammograms with pure FBZ and these of a commercial sample indicates that other compounds present in the sample do not interfere in the voltammetric determination of FBZ.

The determination of FBZ concentration in the sample by differential pulse sweep voltammetry was obtained as being 482.1 ± 17 mg, showing an error of 3.63% in relation to the indicated value by the labeled amount.

In spite of showing a lower detection limit, the differential pulse technique needs more time for analysis, due to the necessity of lower scan rate values (among 5–10 mV s^{-1}) against 100 mV s^{-1} that is traditionally utilized in LSV.

For reversible processes, the obtained detection limit with DPV can reach 10^{-8} mol l^{-1} and for irreversible processes, this limit is almost 10^{-6} mol l^{-1} [17]. In this work, the lowest detection limit was found to be 5.02×10^{-6} mol l^{-1} when DPV is utilized.

The obtained FBZ concentration in the sample by square wave sweep voltammetry was found to be 479.0 ± 21 mg, showing a deviation of 4.35% in relation to the indicated value on the sample label.

In relation to the three utilized voltammetric methods, SWV required a lower analysis time, among 0.5 and 1 s in comparison to 100 s interview of differential pulse technique.

4. Conclusions

The proposed methodology of FBZ analysis that was developed in this work allows quantitative determination of this compound in pharmaceutical dosage forms. The direct dissolution of pharmaceutical tablets in supporting electrolyte containing dimethylformamide solutions allows a fast, economical and reproducible determination of this compound with no interference of additional substrates of pharmaceutical tablets. Obtained detection limits for the three voltammetric techniques were obtained among 10^{-6}–10^{-5} mol l^{-1}, presenting good advantage in relation to the official potentiometric method.

Acknowledgements

The authors gratefully acknowledge the financial support of CAPES and FAPESP, Brazil.

References

[1] D.S. Nakos, N.A. Botsoglou, I.E. Psomas, J. Liq. Chromatogr. 19 (1994) 4145–4155.
[2] E. Lacey, Int. Parasitol. 18 (1988) 885–936.
[3] G. Morovján, P. Csokán, L. Makranszki, E.A. Nagy, K. Tóth, J. Chromatogr. A 797 (1998) 237–244.
[4] British Pharmacopoeia (Veterinary), Stationery Office, London, UK, 2001, pp. 50–51.
[5] J. Markus, J. Sherma, J. A.O.A.C. Int. 75 (1992) 1129–1134.
[6] J.A. Bogan, S. Marriner, J. Pharm. Sci. 69 (1980) 422–423.
[7] R.T. Wilson, J.M. Groneck, A.C. Henry, L.D. Rowe, J. A.O.A.C. 74 (1991) 56–67.
[8] C.S.P. Sastry, V.A.N. Sarma, U.V. Prasad, C.S.R. Lakshimi, Ind. J. Pharm. Sci. 4 (1997) 161–169.
[9] A.J. Bard, H. Lund, Encyclopedia of Electrochemistry of the Elements, vol. XII, Marcel Decker, Inc, New York, 1978, pp. 453–455.
[10] A.Z.A. Zuhri, A.I. Hussein, M. Musmar, S. Yaish, Anal. Lett. 32 (1999) 2965–2975.
[11] M.F. Oliveira, N.R. Stradiotto, Anal. Lett. 34 (2001) 377–387.
[12] A.J. Bard, L.R. Faulkner, Electrochemical Methods: Fundamentals and Applications, John Wiley & Sons Inc, New York, 1980, pp. 213–232.
[13] G. Bontempelli, F. Magno, G.A. Mazzocchin, R. Seeber, Annali di Chim. Rome 79 (1989) 103–216.
[14] M.R. Smyth, J.G. Osteryoung, Anal. Chem. 50 (1978) 1632–1637.
[15] H.D. Ruyck, R.V. Renterghem, H.D. Ridder, D.D. Brabander, Food Control 11 (2000) 165–173.
[16] D.T. Sawyer, W.R. Heineman, J.M. Beebe, Chemistry Experiments for Instrumental Methods, John Wiley & Sons Inc, New York, 1984, pp. 104–108.
[17] A.M. Bond, Modern Polarographic Methods in Analytical Chemistry, Marcel Decker Inc, New York, 1980, pp. 112–119.