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ORIGINAL ARTICLE

Determination of secnidazole in tablets and human serum by cathodic adsorptive stripping voltammetry

Gamal O. El-Sayed *, Shalaby A. Yasin, Azza A. El Badawy

Department of Chemistry, Faculty of Science, Benha University, Benha, Egypt

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Abstract The electrochemical reduction of secnidazole was carried out in BR buffer solutions in the pH range 2.0–11.8 by dc polarography. The polarograms exhibited two irreversible reduction waves in acidic media and one wave in alkaline media, corresponding to the reduction of nitro group in the drug. The cathodic adsorptive voltammetric behavior was studied on glassy carbon electrode to optimize an analytical method for determination of secnidazole. The drug was determined in the range between 4.0×10^{-6} and 1.2×10^{-4} mol L^{-1}. The proposed method was successfully applied to the determination of the drug content in tablets with mean recovery and relative standard deviation of 100.91% and 1.82%, respectively. It was also applied to human serum with a good precision and accuracy.

1. Introduction

Secnidazole (Scheme 1) (α,2-dimethyl-5-nitro-1H-imidazole-1-ethanol) is structurally related to the commonly used 5-nitroimidazoles; metronidazole and tinidazole. These drugs share a common spectrum of activity against anaerobic micro-organisms and they appear particularly effective in the treatment of amoebiasis, giardiasis, trichomoniasis and bacterial vaginosis. Secnidazole is rapidly and completely absorbed after oral administration and has a longer terminal elimination half-life than commonly used drugs in this class (Gillis and Wiseman, 1996).

Analytical methods for the determination of secnidazole are very scarce. Some available methods have been found in literature for assaying secnidazole in pharmaceutical preparations by spectrophotometry (Saffaj et al., 2006, 2004). HPLC methods have been also applied for the determination of this drug in pharmaceuticals (El Walily et al., 2000) and human plasma (Ravi et al., 1997). An electron-capture gas chromatographic method has been described for the determination of secnidazole in blood (Bhatia and Shanbhag, 1984).

Although many electrochemical methods have been applied for the determination of drugs derived from 5-nitroimidazole (El-Sayed, 1997; La-Scalea et al., 1999; Brett et al., 1997a,b; Özkan et al., 1998; Abu Zuhri et al., 1999), only one procedure dealing with the voltammetric quantification of secnidazole was found in the literature (Radi and Hassanein, 2000). In this method, secnidazole was determined using adsorptive stripping

* Corresponding author.
E-mail address: gamaloelsayed@yahoo.com (G.O. El-Sayed).

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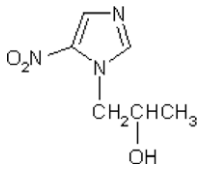


Scheme 1 Structural formula of secnidazole.

voltammetric technique on the surface of hanging mercury drop electrode (HMDE). The monitored adsorptive current was directly proportional to the concentration of secnidazole in the range between 1×10^{-8} and 1×10^{-7} mol L^{-1}, and the method was applied to the analysis of the drug in urine.

The aim of the present work was the study of the electrochemical behavior of secnidazole using dc-polarography and development of a sensitive voltammetric procedure for its determination in dosage form without any extraction procedures. The adsorptive behavior of secnidazole was investigated at the glassy carbon electrode using linear sweep voltammetry. The ability of the developed method to determine the drug in human serum was also demonstrated.

2. Experimental

2.1. Reagents

All reagents were of analytical-reagent grade or better and high-purity water was used throughout. Secnidazole was kindly provided by the Egyptian International Pharmaceutical Industries Co. (EIPICo), 10th of Ramadan City, Egypt. A stock solution of secnidazole (1×10^{-3} mol L^{-1}) in ethanol was prepared and stored in the refrigerator. Working solutions were prepared daily by appropriate dilutions with de-ionized water.

Britton–Robinson buffer solutions (pH 2.0–11.8), used as supporting electrolytes, were prepared by adjusting the pH of a mixture of 0.04 mol L^{-1} acetic acid, 0.04 mol L^{-1} boric acid and 0.04 mol L^{-1} orthophosphoric acid, with appropriate amounts of 0.2 mol L^{-1} sodium hydroxide. Acetate buffer (pH 3.5–5.5; 0.04 mol L^{-1}) was prepared with acetic acid and sodium acetate.

Phosphate buffers (pH 6.0–8.0; 0.04 mol L^{-1}) were prepared using di-sodium hydrogen phosphate and mono-sodium hydrogen phosphate. Other supporting electrolytes used for stripping quantitative analysis: KCl (0.1 mol L^{-1}), HCl (0.1 mol L^{-1}), $HClO_4$ (0.1 mol L^{-1}) and H_2SO_4 (0.1 mol L^{-1}) were prepared in water as a stock (1 mol L^{-1}) and added to the working solution.

2.2. Apparatus

dc-Polarographic measurements were performed using a Sargent–Welch apparatus model 3001 (USA). A cell with two electrodes: dropping mercury electrode (DME) as working electrode (m = 0.37 mg s^{-1}, t = 3.6 s and h = 60 cm) and a saturated calomel electrode (SCE) as reference, was used.

Voltammetric and stripping measurements were carried out using a potentiostat model 273 A (Advanced Analytics, USA) with a software model HQ 2030. The three-electrode system used consisted of a glassy carbon working electrode (IJ Cambria Scientific Ltd. of a 3 mm diameter), Ag/AgCl (3.5 mol L^{-1} KCl) reference electrode and a Pt auxiliary electrode.

Before each measurement, the glassy carbon electrode was polished with an aqueous slurry of 0.03 μm alumina powder on a piece of cloth until a mirror-like finish was obtained; then it was rinsed with distilled water and dried with a non-abrasive tissue paper. Working solutions were purged with pure nitrogen for 5 min at room temperature, before voltammetric measurements.

The pH values were measured using a pH-meter model HI 8014, Hanna Instruments (Italy).

2.3. Procedure

The general procedure used for dc-polarography and voltammetry measurements was as follows: an aliquot (9.0 mL) of BR buffer of the desired pH value was placed in the working cell and deoxygenated for 5 min, then a negatively directed dc scan was initiated between 0.0 and −2.0 V. After background current was obtained, the run was repeated in the presence of the analyte.

The operational parameters of the linear scan stripping voltammetry were: scan rate 500 mV s^{-1} and step height 5 mV. The analyzed solutions were purged with pure nitrogen gas for 5 min and the solutions were stirred at the same stirring rate during the accumulation step followed by a rest period of 10 s before applying the potential.

2.3.1. Controlled potential electrolysis

Electrolysis of a BR buffer solution (pH 7.5) containing 1.5 mg secnidazole was carried out using a mercury-pool cathode. The electrolysis cell was a 250 mL conical flask, and nitrogen gas was flocculated over the surface of solution during electrolysis. The potential was controlled at −0.7 and −1.4 V (corresponding to the limiting currents of the first and second reduction waves, respectively). The decrease of the current was followed as a function of time and the number of electrons involved in the electrode process was computed from the I–t curve, following the Lingane's procedure (Lingane, 1945).

2.3.2. Pharmaceutical preparations

Secnidazole is commercially available as Secnidazole® (EIPICo, Egypt). Ten tablets of this product (containing 500 mg secnidazole per tablet) were powdered and an amount corresponding to 17.1 mg of secnidazole (corresponding to 1 mmol L^{-1}) were weighed and dissolved in ethanol by sonication for 10 min, transferred to a 100 mL volumetric flask and diluted to the mark with the same solvent and shacked for 5 min.

After the settlement of the non-dissolved excipients, different volumes of the clear supernatant were transferred to a 10-mL volumetric flask and diluted to the mark with BR buffer (pH 5.3). The contents of the measuring flask were then transferred to the working cell and the voltammograms were recorded under the optimum experimental conditions. The amount of secnidazole was calculated by comparison with the standard curve.

2.3.3. Analysis of serum

Human blood samples, obtained from healthy volunteers, were centrifuged at 3000 rpm for 10 min at room temperature.

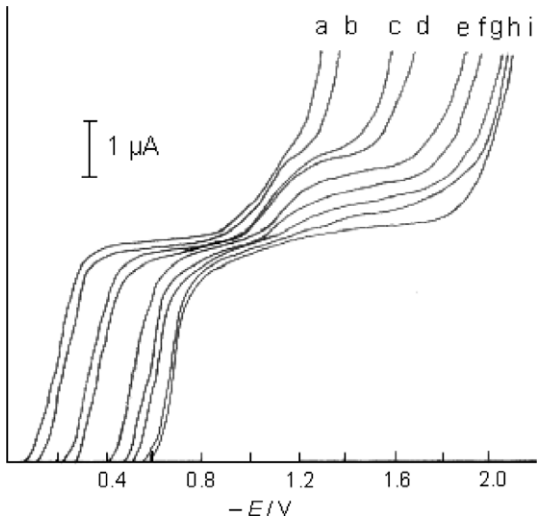


Figure 1 dc-Polarograms of 1×10^{-4} mol L^{-1} secnidazole in BR buffer solutions of different pH values: (a) 2.4; (b) 3.6; (c) 4.5; (d) 5.4; (e) 6.4; (f) 7.3; (g) 8.5; (h) 9.6; (i) 11.8.

Aliquot volumes of a sample were fortified with 0.1 mL perchloric acid (0.1 mol L^{-1}) and different volumes of secnidazole solution dissolved in ethanol to achieve final drug concentrations ranging from 10 to 50 μg mL^{-1}. Then, the protein precipitated was discarded and 100 μL aliquot from each solution was completed with BR buffer (pH 5.3). Then transferred to the working cell and the voltammetric procedure was continued as for pure drug. The standard addition method was used to determine the drug concentration.

3. Results and discussion

3.1. Polarographic behavior of secnidazole

The dc polarographic behavior of 1×10^{-4} mol L^{-1} secnidazole was examined in BR buffer (pH 2.0–11.8). Two reduction waves were produced in acidic solutions, the height of the second wave equals to the half height of the first one. In alkaline solutions, the height of the second wave decreased with increasing the pH till it vanished at pH > 9, Fig. 1.

The half-wave potentials ($E_{1/2}$) of both waves are dependant on pH and shifted towards more negative potential on increasing the pH of the medium. This behavior indicates that protonation takes place before first electron uptake (Meites, 1965). The height of the first wave was found to be pH-independent, whereas the height of the second wave decreased with increasing the pH in the entire pH range. The heights of the waves were found to be limited by diffusion as evidenced by the linearity of plots of i_d vs $\sqrt{h}$ and i_d vs depolarizer concentration.

The logarithmic analysis of the polarograms confirmed the irreversible nature of the electrode process (Zuman, 1969). The plots of E_{de} vs $\log(i/i_d - i)$ for the reduction wave at different pH values gave straight lines of slope values S_1 mV ($S_1 = 59/\alpha n_a$). The estimated values of α (transfer coefficient) and n_a (number of electrons participating in the rate-determining step) are listed in Table 1. The plots of $E_{1/2}$ vs pH are linear with slopes (S_2) 60.5 and 140 mV pH^{-1}, for the first and second waves, respectively.

The number of hydrogen ions (Z_H^+) participated in the rate-determining step was calculated using the relation:

$$Z_H^+ = S_2/S_1$$

The values of the transfer coefficient α estimated either from S_1 ($\alpha = 59/S_1 n_a$) or S_2 ($\alpha = (59/S_2)(Z_H^+/n_a)$) are listed in Table 1. It is clear from the data obtained that one proton participated in the rate-determining step for the two waves, whereas two electrons or one electron participated in the rate-determining step for the first and the second waves, respectively.

3.2. Coulometric analysis

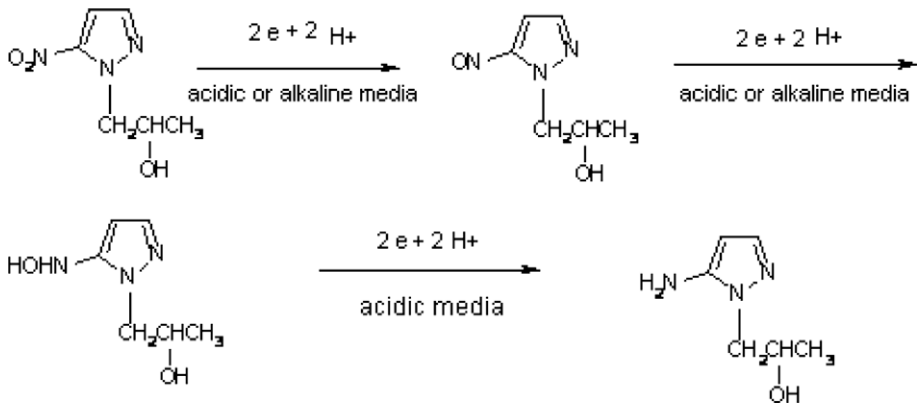
From coulometric analysis, the number of electrons involved in an acidic media is six while four electrons involved in the reduction process in an alkaline media. A mechanism can be proposed for the reduction of this compound which is analogous for the reduction of aromatic nitro derivatives (Rubinstein, 1985; Tallec et al., 2000; Radi and El Ries, 1999; Fotouhi and Faramarzi, 2004), Scheme 2.

3.3. Cyclic voltammetry

The nature of the electrochemical process was studied by cyclic voltammetry. A typical cyclic voltammogram of 1×10^{-4} mol L^{-1} secnidazole at glassy carbon electrode in BR buffer at pH 7.34 without accumulation is shown in Fig. 2. One cathodic peak was observed over the entire pH range used (2.0–11.8), its height decreased with increasing pH. On the

Table 1 Polarographic data for 1×10^{-4} mol L^{-1} secnidazole in BR buffer solutions of different pH values.

pH	S_1 (mV)	αn_a	α	S_2 (mV)	Z_H^+	α
First wave			($n_a = 2$)			($Z_H^+/\alpha_a = 0.5$)
2.44	78.7	0.74	0.37	60.55	0.77	0.37
5.42	78.1	0.75	0.38		0.77	
7.34	69.2	0.85	0.42		0.87	
8.24	65.3	0.90	0.45		0.93	
Second wave			($n_a = 1$)			($Z_H^+/\alpha_a = 1$)
2.44	147	0.40	0.40	140.00	0.95	0.42
5.42	127	0.46	0.46		1.10	
7.34	152	0.39	0.39		0.92	
8.24	163	0.36	0.36		0.85	



Scheme 2 Electrode reduction mechanism of secnidazole at different pH values.

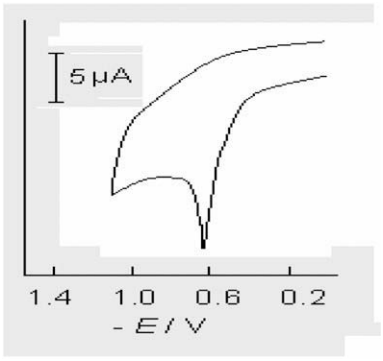


Figure 2 Cyclic voltammogram of 1×10^{-4} mol L^{-1} secnidazole in BR buffer at pH 7.34 at scan rate of 500 mV s^{-1}.

reverse scan, no anodic peak was observed, indicating that the reduction of secnidazole at GCE is an irreversible process.

The effect of sweep rate (t) on the cyclic voltammograms was examined from 25 to 500 mV s^{-1} at a fixed concentration of 1×10^{-4} mol L^{-1} secnidazole. The peak potential (E_p) shifted cathodically, as expected for irreversible reduction process. The peak current (i_p) increased steadily with increasing (t) and a straight line was observed when (i_p) was plotted against ($t^{1/2}$), revealing that the cathodic reduction is mainly controlled by diffusion.

A straight line was obtained from E_p–log t relationship; described by the equation: $E_p = 0.1443 \log t + 0.5016$ ($n = 5$, $r = 0.994$) at pH 3.10. From the slope of the straight line, αn_a value was calculated by applying the equation, $\Delta E/\Delta \log t = 30/\alpha n_a$ (Sawyer et al., 1995). The value of α was found to be 0.32, confirming the irreversible nature of the reduction reaction.

A plot of log i_p vs log t gave a straight line with slope of 0.63, indicating that the reaction is controlled mainly by diffusion with slight adsorption contribution (Laviron et al., 1980).

3.4. Cathodic adsorptive stripping voltammetry

3.4.1. Effect of supporting electrolyte and pH

The nature of the supporting electrolyte and pH of the working solution are important factors which influence the adsorptive reduction process of an electrolyte on the electrode surface. The effect of the supporting electrolyte nature on the shape and height of the reduction peak was examined using different electrolytes: BR buffer, acetate buffer, phosphate buffer, KCl, HCl, H_2SO_4 and $HClO_4$.

The voltammetric measurements were carried out for a standard solution of the investigated drug (1×10^{-4} mol L^{-1}) applying linear sweep mode without accumulation. Well-defined reduction peaks were obtained with BR buffer of pH 5.30.

3.4.2. Effect of accumulation potential

The effect of the accumulation potential (E_{acc}) on the adsorptive stripping current was evaluated over the range 0.0 to −0.4 V for 5×10^{-5} mol L^{-1} secnidazole in BR buffer of pH 5.3, for an accumulation period of 360 s, Fig. 3. The results obtained shown that a maximum i_p value was obtained at accumulation potential −0.4 V.

3.4.3. Effect of accumulation time

The dependence of peak current on accumulation time (t_{acc}) was studied at concentration 5×10^{-5} mol L^{-1} secnidazole. The peak current increased with increasing accumulation time from 0.0 to 60 s, then it decreased with increasing time (Fig. 4). Hence, an accumulation time of 60 s was chosen to evaluate the best work conditions to the proposed method.

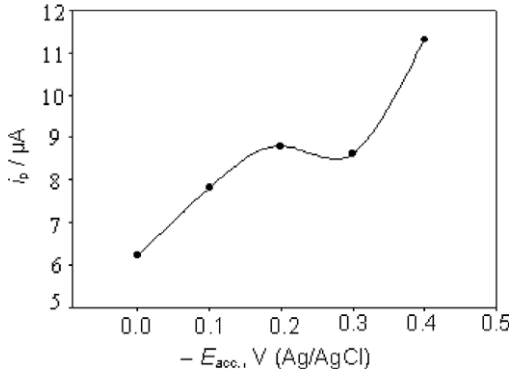


Figure 3 Effect of deposition potential on 5×10^{-5} mol L^{-1} secnidazole in BR buffer solution of pH 5.3 at $t_{acc} = 60$ s, $t = 500$ mV s^{-1} and step height = 5 mV.

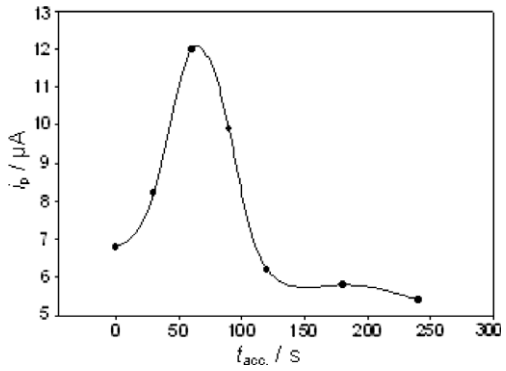


Figure 4 Effect of deposition time on 5×10^{-5} mol L^{-1} secnidazole in BR buffer solution of pH 5.3 at $E_{acc} = -0.4$ V, $t = 500$ mV s^{-1} and step height = 5 mV.

3.5. Instrumental parameters

Fig. 5 displays the resulting peak current vs scan rate (t) for 5×10^{-5} mol L^{-1} secnidazole. Maximum response was obtained at a scan rate of 500 mV s^{-1}, so this value of scan rate was chosen for analytical purposes. The dependence of peak current on the step height was examined in the range 5–30 mV. The peak current increased slightly with increasing step height, but a well-defined peak was observed at a step height of 5 mV, so this value was the best value for quantitative applications.

3.6. Calibration plots

Using the optimized experimental conditions for the determination of secnidazole, the linearity of the i_p response to different analyte concentrations was evaluated. Linear sweep adsorptive voltammograms were recorded at scan rate 500 mV s^{-1} using an accumulation potential of −0.4 V for an accumulation time of 60 s, for solutions containing increasing concentrations of secnidazole. The results showed that the peak current increased linearly with the analyte concentration in the range 4.0×10^{-6}–1.2×10^{-4} mol L^{-1} (0.66–20.4 μg mL^{-1}). The peak current is related to the concentration of secnidazole (C) by the following equation:

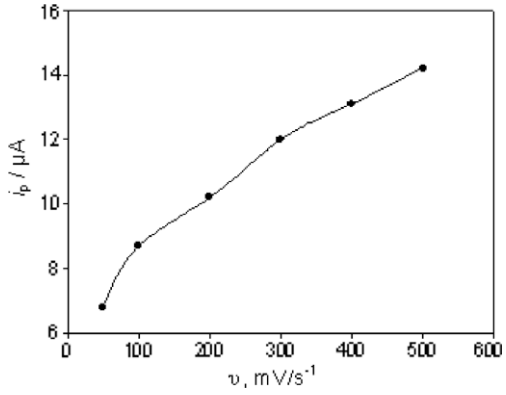


Figure 5 Effect of scan rate on 5×10^{-5} mol L^{-1} secnidazole in BR buffer solution of pH 5.3 at $E_{acc} = -0.4$ V, $t_{acc} = 30$ s and step height = 5 mV.

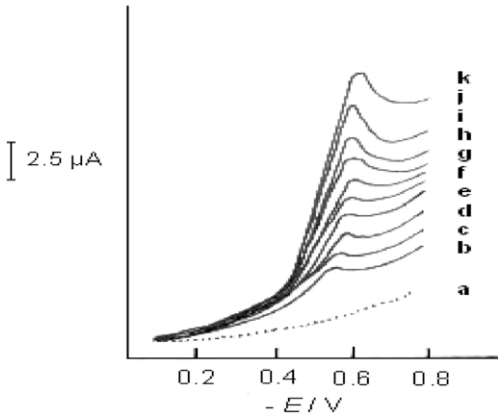


Figure 6 CAdS voltammograms for different concentrations of secnidazole at optimal conditions: (a) background; (b) 4×10^{-6}; (c) 6×10^{-6}; (d) 8×10^{-6}; (e) 1×10^{-5}; (f) 2×10^{-5}; (g) 4×10^{-5}; (h) 6×10^{-5}; (i) 8×10^{-5}; (j) 1×10^{-4}; (k) 1.2×10^{-4} mol L^{-1}.

$$i_p\ (\mu A) = 3.35 + 9.025 \times 10^7 C\ (\text{mol L}^{-1})$$

The linearity of this relationship was evaluated from the correlation coefficient ($r = 0.9996$) at $n = 10$. The detection limit of the proposed method is 1.2×10^{-6} mol L^{-1}, which was calculated as the concentration that gives a signal to background noise ratio equal to 3 (Househam et al., 1987). The validity of the method was supported by the constancy of i_p/C. Fig. 6 shows the dependence of the cathodic adsorptive peak current on secnidazole concentration under optimal conditions.

3.7. Precision and accuracy

In order to assess the precision, as percentage relative standard deviation (RSD%) and the accuracy, as percentage relative error (E_r%) of the proposed method, solutions containing five different concentrations of secnidazole were prepared and analyzed in five replicates. The data obtained are summarized in Table 2.

3.8. Analysis of pharmaceutical formulations

The validity of the proposed voltammetric method was investigated by assaying Secnidazole® tablets (each is labeled to contain 500 mg secnidazole per tablet). The recoveries were calculated with reference to the calibration graph. The statistical calculations for the assay results show a good precision of the proposed method, Table 3.

Table 2 Precision and accuracy for the adsorptive cathodic determination of secnidazole in pure form.

Nominal value (μg mL^{-1})	Found[a] ± SD	RSD (%)	E_r (%)
2	1.99 ± 0.03	1.5	−0.5
5	5.11 ± 0.03	0.6	2.2
12	12.10 ± 0.15	1.2	0.8
15	15.16 ± 0.17	1.1	1.1
20	20.06 ± 0.26	1.3	0.3

[a] Average of five determinations.

Table 3 Assay results for analysis of Secnidazole® tablets.

Amount taken (μg mL^{-1})	Found[a]	Recovery (%)	RSD (%)
1.6	1.61	100.60	2.3
3.2	3.27	102.30	2.3
6.4	6.48	101.25	2.1
8.0	7.98	99.75	1.4
12.0	12.08	100.66	1.0
	Mean	100.91	1.82

[a] Average of five determinations.

Table 4 Analytical results of secnidazole in serum using sample the proposed procedure.

Added (μg L^{-1})	Found[a] (μg L^{-1})	Recovery (%) ± SD
1	1.05	101.0 ± 1.3
2	2.08	103.0 ± 2.5
3	3.17	103.2 ± 1.1
4	3.96	99.0 ± 2.3
5	5.05	101.1 ± 2.0

[a] Average of four determinations.

3.9. Analysis of serum

The proposed method was applied to the determination of secnidazole in spiked human serum using the standard addition method. The direct determination of secnidazole in human serum was found to be possible after dilution of the sample with the supporting electrolyte. The percentage recovery of the drug in serum, based on the average of four replicate measurements, is listed in Table 4. The values obtained for recovery are acceptable for biological fluids.

4. Conclusion

The proposed method provides a sensitive and simple approach for determination of secnidazole in tablets and serum samples. The cathodic adsorptive technique is rapid and simple in relation to other conventional methods, such as spectrophotometry and chromatography. A recently published spectrophotometric method (Saffaj et al., 2006) is based on the reduction of the drug with zinc and hydrochloric acid followed by diazotization and coupling with β-naphthol, which is a time-consuming method. The sample preparation procedure in the proposed method is very rapid since there is no need to eliminate excipients during the analysis of pharmaceutical formulations. So it could be used for quality control of secnidazole preparations.

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