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Polarographic determination of flubendazole in spiked human urine and plasma

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Abstract

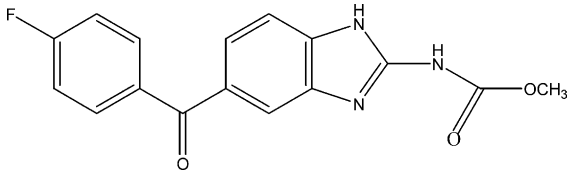
The voltammetric behavior of flubendazole was studied using direct current (DC_t), differential pulse (DPP) and alternating current (AC_t) polarography. The drug manifests a cathodic wave in 20% v/v formic acid solution. The wave was characterized as being irreversible, diffusion-controlled with limited adsorption properties. The diffusion current–concentration relationship was found to be rectilinear over the range 3.2–14.4 μg/ml and 0.1 to 12.8 μg/ml, using DC_t and DPP modes, respectively, with minimum detectability of 0.161 μg/ml (5.14×10^{-7} M) and 0.0.057 μg/ml (1.82×10^{-8} M) using DC_t and DPP modes, respectively. Furthermore, the proposed method was applied to the in-vitro determination of flubendazole in spiked human urine and plasma adopting the DPP technique. The percentage recoveries were 100.20 ± 0.62 and 97.42 ± 0.95, respectively.

Keywords: Flubendazole; Polarography; Voltammetry; Spiked urine; Spiked plasma

1. Introduction

Flubendazole, [5-(4-fluorobenzoyl)-1H-benzimidazol-2-yl]carbamic acid methyl ester, is a broad spectrum anthelmintic widely used against gastrointestinal helminthics in both humans and animals [1]. Several methods have been described for the quantitative determination of flubendazole, including; UV derivative spectrophotometry and differential pulse polarography [2], fluorimetry [3–5], flow injection analysis [6], and HPLC [7–12]. The spectrophotometric method [2] is not sensitive and HPLC methods require sophisticated instrumentation. There is- therefore-a need for a more simple and sensitive method for the determination of flubendazole.

Although chromatographic methods offer high degree of specificity, yet, sample clean up is rather time consuming, which cause certain limitation in their use in routine clinical studies. The voltammetric techniques offer another possibility for the estimation of the drug. Reviewing of the literature revealed that only one report was published for the polarographic determination of flubendazole and it was restricted to dosage forms [2]. In this piece of work we devoted the application of the proposed method to the in-vitro determination of flubendazole in spiked human biological fluids. The most striking feature of the method is that as applied to urine, no prior extraction step is needed. As for analysis of the plasma a simple extraction step was a must.



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2. Experimental

2.1. Apparatus

The polarographic study and DPP measurements were carried out using the Polarecord E 506 Metrohm (Herisau, Switzerland). The drop time of 1 s was electronically controlled using a 663 VA stand from the same company. The polarograms were recorded using a potantial scan rate of 10 mV/s. A three-electrode system composed of a dropping mercury electrode (DME), Ag/AgCl reference electrode, and a graphite rod as the auxiliary electrode, was used. Phase selective AC_t polarograms were recorded using the same instrument; the superimposed alternating voltage being 15 mV at a frequency of 75 Hz and a phase angle of 90°.

Formic acid concentration in the polarographic cell was kept always at 20%. The supporting electrolyte used was 1 M potassium chloride.

2.2. Materials and reagents

All chemicals were of analytical grade

- Flubedazole was purchased from Sigma (St. Louis, MO) and was used as received.
- Plasma was kindly provided by Mansoura University Hospital, and kept frozen until assay after gentle thewing.
- Urine sample was obtained from healthy volunteers (male around 40 years old).
- Formic acid: 98% (w/v) AR grade (Aldrich–Chemie, Germany) 20% (v/v) aqueous solution was prepared.
- Chloroform (Aldrich–Chemie, Germany).
- Potassium chloride (BDH, UK) 1 M aqueous solution.
- Potassium carbonate: (BDH, UK) 0.1 M aqueous solution.

2.3. Standard solutions

A stock solution containing 0.2 mg/ml of flubendazole was prepared in 20% formic acid solution (v/v) and further diluted with the same solvent to give the appropriate concentrations.

2.4. Procedures

2.4.1. Recommended analytical procedure

Aliquot volumes of flubendazole covering the working range were transferred into 25 ml volumetric flask. 5 ml of formic acid was added and diluted to the mark with distilled water. These solutions were poured into the polarographic cell and nitrogen gas was passed for 5 min. The DC_t and DPP polarograms were recorded. The concentration of flubendazole was calculated using the regression equation.

2.4.2. Assay of flubendazole in biological fluids

2.4.2.1. For spiked urine. A 0.5-ml urine sample spiked with a suitable amount of flubendazole was transferred into 25 ml volumetric flask. Five milliliters of formic acid was added and diluted to the volume with distilled water. Then proceed as described in Section 2.4.1. The concentration of the drug was calculated using the regression equation.

2.4.2.2. For spiked plasma. A 0.5-ml of spiked plasma was transferred into 125 ml separating funnel. One milliliter of 0.1 M potassium carbonate was added. Extraction with 3×5 ml of chloroform was carried out, then the two layers were allowed to separate, the organic layer was passed over anhydrous sodium sulphate. The combined extracts were evaporated under nitrogen gas. The residue was dissolved in 5 ml of formic acid and completed to 25 ml with distilled water. Then proceed as described in Section 2.4.1. The concentration of the drug was calculated from the regression equation.

3. Results and discussion

Fig. 1 shows typical DC_t and DPP polarograms of flubendazole in 20% formic acid. Flubendazole produces a well-defined cathodic wave. Logarithmic analysis of the reduction wave resulted in a straight line. The number of electrons transferred at the rate determining step (αn_a) value was calculated according to the treatment of Meites and Israel [13] and it was found to be 0.78. Assuming that the rate- determining step involves the transfer of two electrons, the value of the slope point out to the completely irreversible nature of the reduction process [13].

3.1. Study of the wave characteristics and electrode reaction

Increasing the mercury height (h) resulted in a corresponding increase in the waveheight (w); a plot of $\sqrt{h}$ versus the waveheight gave a straight line. A plot of $\log h$ versus $\log w$ gave a straight line, the slope of which was 0.88. Changing the ionic strength of the supporting electrolyte by addition of increasing volumes of 1 M KCl resulted in a negligible effect on the waveheight. The effect of drop time was studied over the range 0.4–3 s, and the corresponding waveheight was measured. It was found that increasing the drop time would result in a subsequent increase on the waveheight of flubendazole, however, a drop time of 1 s was chosen as a

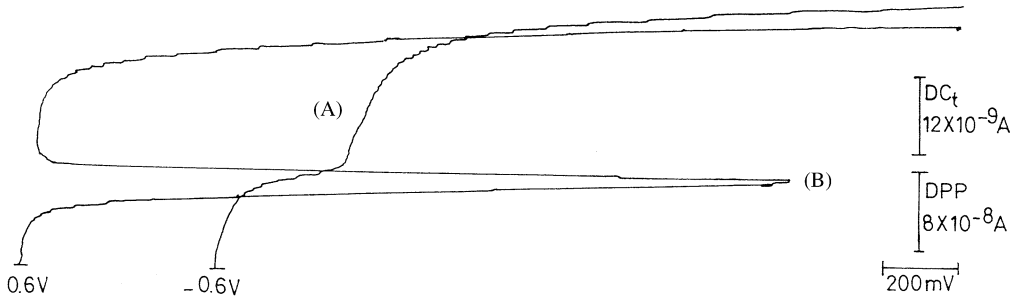

Fig. 1. Typical polarograms of flubendazole (12.8 μg/ml) in 20% formic acid. (A) DC_t mode. (B) DPP mode.

compromise to save time. These characters point out to the diffusion controlled nature of the wave.

The alternating current behavior (AC_t) of flubendazole was studied using a phase-selective angle of 90°. The summit potential was shifted to more negative value by 110 mV than the corresponding $E_{1/2}$ value. It was found that both the reactant and the reduction product are adsorbed to the mercury surface (Fig. 2).

Flubendazole was found to be stable in 20% formic acid for about one and half-hour at room temperature after which its waveheight began to decrease slowly.

The number of electrons consumed during the reaction was determined through comparison of the waveheight of flubendazole with that obtained from an equimolar solution of a previously studied structurally related compound with the same reducible function group (benzoyl group), and of nearly identical value of diffusion-coefficient; namely mebendazole [14]. In 20% formic acid, both compounds gave one wave of the same height, thus it is concluded that two-electrons are involved in the reduction process.

3.2. Analytical applications

Polarograms of flubendazole exhibit well-defined cathodic wave. The current is diffusion-controlled and

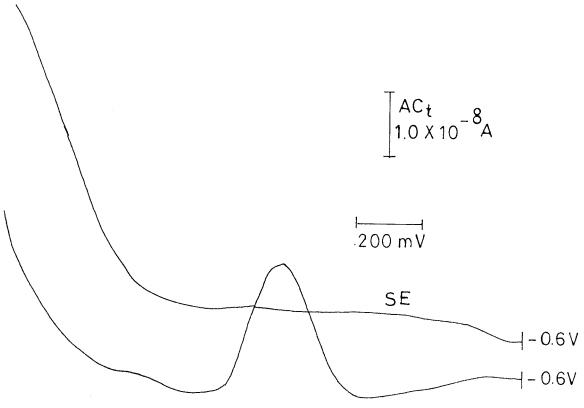

Fig. 2. Alternating current behaviour of flubendazole (12.8 μg/ml) in 20% formic acid. Superimposed alternating voltage: 15 mV; frequency 75 Hz; phase angle 90°. (SE: supporting electrolyte).

is proportional to the concentration of the depolarizer over a convenient range of the concentration. Both DC_t and DPP modes were successfully applied to the assay of flubendazole in pure form. The DPP mode was applied to the assay of flubendazole in spiked biological fluids.

Plots representing the relationship between the concentration of flubendazole and the diffusion current give straight lines over the concentration range of 3.2–14.4 and 0.10–12.80 μg/ml, using DC_t and DPP modes, respectively, with minimum detectability of 0.161 μg/ml (5.14×10^{-7} M) and 0.0.057 μg/ml (1.82×10^{-8} M) using DC_t and DPP modes, respectively.

Linear regression analysis of the data gave the following equations:

$$i_d = -7.143 \times 10^{-5} + 0.028c \quad (r = 0.9999)$$

using DC_t mode and

$$i_p = -2.850 \times 10^{-7} + 0.066c \quad (r = 0.9999)$$

using DPP mode, respectively; where c is the concentration in μg/ml, i_d is the diffusion current (in μA) in the DC_t mode and i_p is the current (in μA) in the DPP mode, respectively.

Statistical evaluation of the regression lines including standard deviation of the residual ($S_{y/x}$), standard deviation of the intercept (S_a) and standard deviation of the slope (S_b) are abridged in Table 1.

Statistical analysis of the results [15] obtained by the proposed and a reference method [3], using the Student's t-test and variance ratio F-test, shows no significant difference between the performance of the two methods regarding the accuracy and precision, respectively (Table 2).

Co-formulated compounds such as, enrofloxacin, oxolonic acid, sulphamethoxazole did not interfer with the assay since they have no reduction peaks under the same conditions, while oxytetracycline interfer and should be removed if present.

3.3. Analysis in biological fluids

Flubendazole, a benzimidazole carbamate anthelmintic, is an analogue of mebendazole and has similar

Table 1
Performance data of the proposed methods

Parameters	DC_t mode	DPP mode
Concentration range (μg/ml)	3.2–14.4	0.10–12.8
Minimum detection limit (M)	(5.14×10^{-7})	(1.82×10^{-8})
Correlation coefficient	0.9999	0.9999
Slope	0.028	0.066
Intercept	-7.143×10^{-5}	-2.850×10^{-7}
$S_{y/x}$ [a]	1.506×10^{-3}	1.253×10^{-3}
S_a [b]	1.189×10^{-3}	8.94×10^{-4}
S_b [c]	8.44×10^{-5}	9.153×10^{-5}
% Error [d]	0.19	0.21
Applications	pure form	pure form, spiked urine and spiked plasma

[a] $S_{y/x}$, standard deviation of the residual.
[b] S_a, standard deviation of the intercept of regression line.
[c] S_b, standard deviation of the slope of regression line.
[d] % Error $= \mathrm{RSD}\%/\sqrt{n}$.

Table 2
Polarographic analysis of flubendazole in pure form using DC_t and DPP modes

Parameters	DC_t mode	DPP mode	Reference method [3]
No. of experiments	8	12	3
Mean found (%) ± SD	100.02 ± 0.55	99.81 ± 0.72	100.2 ± 0.41
Variance	0.30	0.52	0.17
Studen's t-value	0.52 (2.26)	0.89 (2.16)	
Variance ratio F-test	1.76 (4.74)	3.06 (3.98)	

Figures in parentheses are the tabulated t and F values, respectively, at $P = 0.05$ [15].

actions and uses. The oral dose of flubendazole is 100 mg as a single dose, reapeted if necessary after two or 3 weeks [1]. For ascariasis, hook worm infections, and trichuriasis 100 mg is given twice daily for 3 days, this dose gives a final free drug plasma concentration of about 2 μg/ml, [16] which lies within the working concentration range of the proposed method.

The DPP mode could be successfully applied to the determination of flubendazole in spiked urine (Fig. 3) and plasma over the specific concentration range for flubendazole. In case of spiked urine no prior treatments are necessary, while in case of spiked plasma, the method involved extraction of the drug using chloroform. The extraction procedure described by Osterhuis et al. [7] was adopted.The results are abridged in Table 3. The mean percentage recoveries for flubendazole in

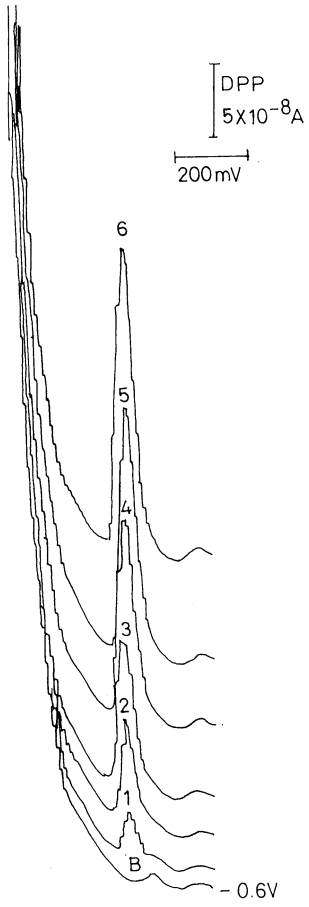

Fig. 3. Differential pulse polarographic waves of flubendazole in spiked human urine: (1) 0.5 μg/ml; (2) 1.0 μg/ml; (3) 1.5 μg/ml; (4) 2.0 μg/ml; (5) 2.5 μg/ml; (6) 3.0 μg/ml; B: blank.

Table 3
Polarographic determination of flubendazole in spiked urine and plasma using DPP mode

Sample	Amount added (μg)	% Recovery
1-a-Urine (intra-day precision)	1.00	100.00
	1.00	100.90
	1.00	99.70
Mean ± SD		100.20
		0.62
1-b-Urine (inter-day precision)	1.0	98.71
	1.0	99.74
	1.0	100.00
Mean ± SD		99.48
		0.68
2-Plasma (intra-day precision)	1.0	97.73
	1.0	96.36
	1.0	98.18
Mean ± SD		97.42
		0.95

spiked urine and plasma are 100.20 ± 0.62 and 97.42 ± 0.95, respectively using DPP mode. In the proposed method the extraction step can be excluded for spiked

urine using DPP mode resulting in increased sensitivity and saved time, together with elimination of consumption of expensive chemicals.

3.4. Precision

The within-day precision was evaluated through replicate analysis of urine sample spiked with 1 μg/ml of flubendazole. The percentage recoveries based on the average of three separate determinations are 100.20 ± 0.62, thus indicating the high precision of the method (Table 3). The inter-day precision was evaluated through replicate analysis of urine sample spiked with 1 μg/ml of flubendazole. The percentage recoveries based on the average of three separate determinations are 99.48 ± 0.68, thus indicating the high accuracy of the method (Table 3) and the low standard deviation indicates the high precision of the proposed method.

4. Conclusion

Simple, rapid and highly sensitive method was developed for the determination of flubendazole in spiked human urine and plasma. It has distinct advantages over the other existing methods regarding sensitivity, time saving and minimum detectability, moreover, it can be applied to the determination of flubendazole in spiked urine without prior treatment. In addition it can be applied for routine analysis and no sophisticated instrumentation is required.

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