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Determination of mebendazole in urine by cathodic stripping voltammetry

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Abstract

A cathodic stripping procedure has been developed for the determination of mebendazole. An 8 min accumulation time on a 0.1 M acetic acid/acetate pH=5 buffer solution gives rise to a detection limit of 3×10^{-11} M. An extraction with ether has been developed for the determination of mebendazole in urine. Concentrations of 5×10^{-9} M in urine can be determined.

Keywords: Mebendazole; Stripping voltammetry

1. Introduction

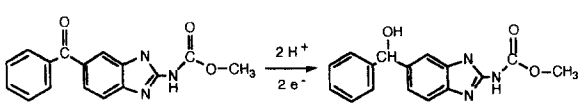
Mebendazole is a broad spectrum anthelmintic widely used against gastrointestinal helminthics in both human and animals. In low concentrations it avoids glucose captivation by nematodes, not affecting glucose metabolism in mammalians even in a high dosage.

Liquid chromatography (LC) with UV detection is the most widely used approach for mebendazole determination [1–4] with limits of detection of about $10\,\mu g\,l^{-1}$. A differential pulse polarographic method for mebendazole determination has been described [5]. It is shown that when performing a cathodic differential pulse (dp) polarogram in a solution containing mebendazole an electrochemical signal due to the reduction of the carbonyl group is obtained (Scheme 1).

This procedure offers a good sensitivity (a $30\,\mu g\,l^{-1}$ detection limit), but it has only been applied to the determination of mebendazole in tablets and in non-biological samples.

If the adsorption of mebendazole on a hanging mercury drop electrode would be possible, then a stripping procedure based on the before mentioned electrochemical reaction could be developed, and an increase in sensitivity can be expected.

Taking into account the acid–base properties of mebendazole [6] ($MBZH^+/MBZ/MBZ^-$ with pk_a 3.5 and 10.2, respectively), when the pH of the measuring solution is set at a value in which mebendazole exists as a neutral molecule (3.5<pH<10.2) adsorp-



Scheme 1.

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tion on a hanging mercury drop electrode might take place.

The aim of this paper is to develop a stripping procedure for mebendazole determination and to apply it for its determination in urine.

2. Experimental

2.1. Apparatus

A METROHM 646 VA Processor equipped with a METROHM 647 VA stand has been used for voltammetric measurements. Measurements of pH were performed with a METROHM 645 pH-meter equipped with a combined glass–Ag/AgCl electrode. All the potentials are reported against Ag/AgCl.

2.2. Reagents

Mebendazole was obtained from Sigma and used as received. Mebendazole standards were prepared by dissolving 29.53 mg of mebendazole in 10 ml of formic acid and diluted to 100 ml with distilled water. For the studies of pH, a Britton–Robinson stock solution composed of 0.5 M phosphoric acid, 0.5 M acetic acid and 0.5 M boric acid, was prepared. Its pH is adjusted with the help of a pH-meter by adding NaOH, and then diluted to the desired concentration. Triply distilled mercury was used. All the reagents employed were of analytical quality grade.

2.3. Procedure

Urine samples are saturated in NaCl (ca. 0.5 g of NaCl for each ml of sample) and the pH is adjusted to 12 with NaOH. 5 ml of urine sample and 10 ml of diethyl ether are shaken in an extraction vessel, the aqueous layer is removed and the ether extract is contacted with 5 ml of 0.1 M Britton–Robinson pH=7, shaken and the aqueous layer removed again. The ether layer is evaporated to dryness, redissolved and rinsed to 50 ml with 0.1 M acetic/acetate buffer pH=5.

This solution is set in the polarographic vessel, purged with nitrogen for 5 min, following an 8 min accumulation while stirring at a −0.2 V potential, a 13 s rest time, and a differential pulse voltammogram from −0.2 to −1.2 V (pulse amplitude=−60 mV, scan rate=10 mV s^{-1}, time step=1.2 s).

3. Results and discussion

3.1. Preliminary assays

In order to test the adsorption of mebendazole, the effect of accumulation time before the voltammetric scan was tested. A 10^{-7} M solution of mebendazole at pH=5 was placed in the polarographic cell, and several cathodic dp scans were performed on a hanging mercury drop electrode, with and without the application of an increasing accumulation time

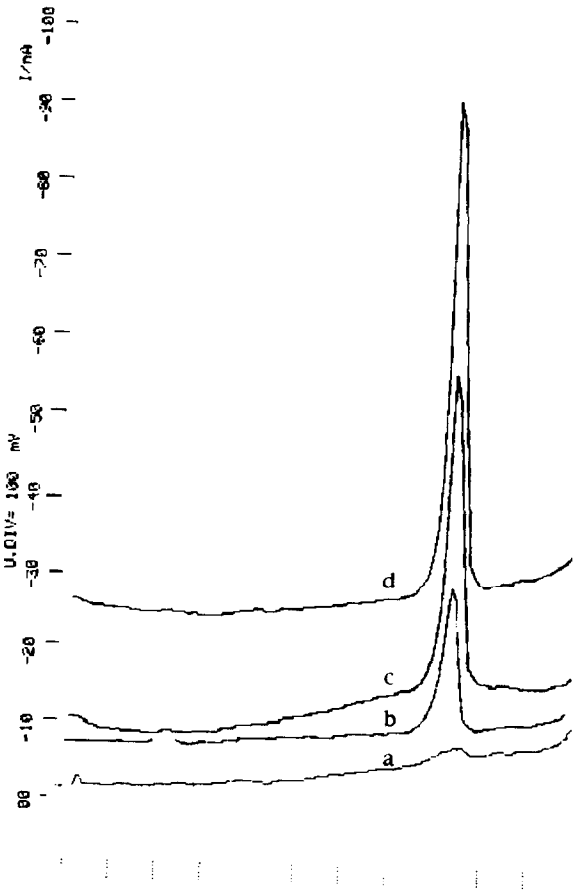


Fig. 1. Polarograms 0.1 M acetic acid–acetate buffer at pH=5 of a blank (a), and 10^{-7} M concentration of mebendazole without accumulation (b) and with 30 s accumulation (c) and 1 min accumulation (d).

(Fig. 1). It was observed that an electrochemical signal that is not present in the blank appears at about −1.0 V. This signal rises proportionally with accumulation time, demonstrating that accumulation of mebendazole by adsorption on the electrode occurs. Subsequently, a stripping procedure can be developed.

3.2. Analytical parameters optimization

3.2.1. Accumulation parameters

Effect of the pH of the supporting electrolyte and accumulation potential: Fig. 2 shows the effect of pH of the measuring cell on peak current for several accumulation potentials. As expected, mebendazole is accumulated at those pH values where it exists as a neutral molecule (3.5<pH<10.2). It was found that the best signal was obtained for a pH=5 and an accumulation potential of −0.2 V.

Effect of the nature and concentration of the supporting electrolyte: Several buffering supporting electrolytes with pK_a close to 5 were tested, and it was found that an acetic acid/acetate buffer gave the greatest peak current. Finally, the effect of the concentration of supporting electrolyte in the measuring cell was investigated (Fig. 3). Peak current increases with decreasing electrolyte concentration down to 0.1 M. When the concentration is lower than 0.1 M, the ionic strength of the solution is not enough for a good electric conduction in the cell, and peak current decreases with concentration. A 0.1 M concentration was found to be optimal.

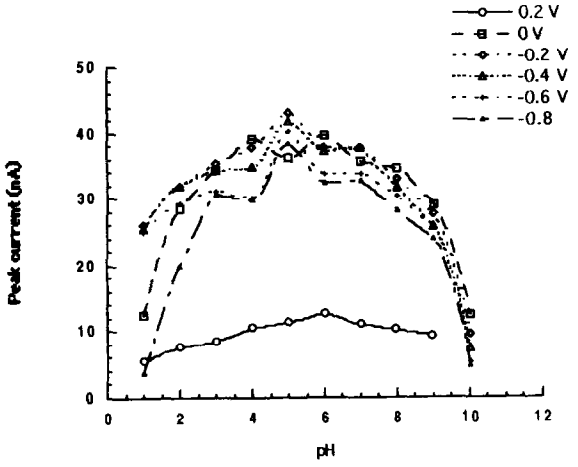


Fig. 2. Effect of the pH of the solution on peak current for several accumulation potentials. 1 min accumulation in 0.4 M Britton–Robinson solutions of 10^{-7} M mebendazole. The represented peak currents are the maximum values obtained for varying accumulation potentials at each pH. The best conditions are pH=5 and −0.2 V accumulation potential.

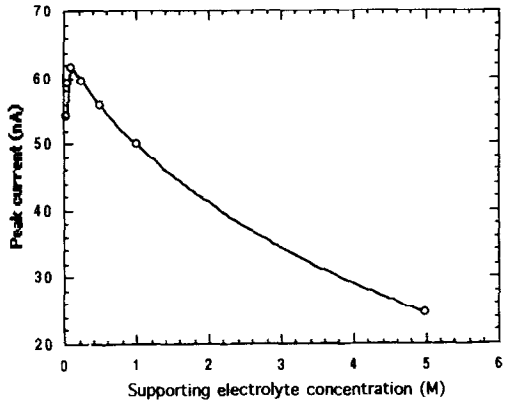


Fig. 3. Effect of the supporting electrolyte concentration on peak current for 1 min accumulation in 10^{-7} M mebendazole at pH=5 acetic acid/acetate buffers. Maximum peak current is obtained for a 0.1 M supporting electrolyte concentration.

Effect of the accumulation time: The effect of varying accumulation time on peak current was studied for three concentration values (Fig. 4), and a virtually linear increase of peak current with accumulation time was found until the saturation of the electrode is reached. This saturation occurs, for a 10^{-7} M mebendazole concentration, at an accumulation time of 8 min, but is not reached in the studied time for the other two concentrations investigated

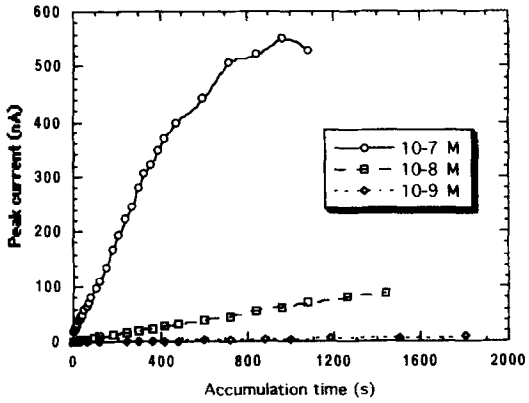


Fig. 4. Influence of accumulation time on peak current at three mebendazole concentrations (10^{-7}, 10^{-8} and 10^{-9} M) accumulations on pH=5 0.1 M acetic acid/acetate.

(10^{-8} and 10^{-9} M). An 8 min accumulation time was chosen.

Rest time: It was found that a lower standard deviation and a higher signal was obtained when a 13 s rest time was applied before the voltammetric scan.

Effect of the drop size: A linear increase of the peak current with drop area was found. A 1.3×10^{-3} cm^2 area (the largest drop supplied by the employed mercury stand) was chosen.

3.2.2. Instrumental parameters

The parameters inherent to dp polarography were optimized. A −60 mV pulse amplitude, a 1.2 s pulse period and a 10 mV s^{-1} were found to be the optimal conditions.

3.2.3. Calibration plots

Calibration plots were obtained in the optimal conditions. A detection limit of 3×10^{-11} M and a determination limit of 1.4×10^{-10} M were found. The response is linear up to 10^{-7} M. Values of correlation coefficient r>0.999 were obtained.

3.2.4. Precision and accuracy

Table 1 shows precision and accuracy at several concentration values, obtained from series of four replicated measurements. Relative errors and relative standard deviations are smaller than 3.5% and 2.8%, respectively.

3.3. Determination of mebendazole in urine

Direct determination of mebendazole in urine is not possible, so a preceding clean-up procedure must be employed. Two kinds of interferents can be expected: (i) substances that are present in urine and provide an electroactive signal similar to that of mebendazole, and (ii) surface active compounds that can competitively adsorb on the electrode surface giving rise to a decrease in the response of mebendazole. A SEP-pack C_{18} cartridge clean-up procedure employed successfully in LC [2] was tested, but good results were not obtained because of the effect (ii), that does not occur in a chromatographic system. Then, an extraction procedure with diethyl ether was tested.

Table 1
Precision and accuracy for the determination of mebendazole standard solutions at several concentrations

Concentration (M)	4×10^{-10}	10^{-9}	5×10^{-9}	10^{-8}	5×10^{-8}
Relative error (%)	−0.25	−3.5	+0.8	−2.6	+0.29
Relative standard deviation (%)	2.0	2.8	1.7	1.8	1.5

3.3.1. Development of an extraction procedure

To optimize a method of extraction, an initial general procedure is followed, and some parameters of interest were investigated.

Initial general procedure: Urine from untreated persons is spiked with mebendazole 10^{-6} M. The ionic strength of the urine samples was fixed saturating them with NaCl (0.5 g of NaCl for each ml of sample). After shaking 5 ml of urine and 10 ml of diethyl ether in an extraction vessel, the urine layer was removed. The ether layer is then evaporated with the help of a stream of nitrogen. A few ml of pH=5 0.1 M acetic/acetate buffer can be added to the ether to avoid losses of mebendazole when trying to dissolve it again. The residue of evaporation is rinsed to 50 ml with acetic/acetate buffer and placed in the polarographic cell.

Effect of the pH of the urine: The influence of urine pH on peak height was investigated (Fig. 5, circles).

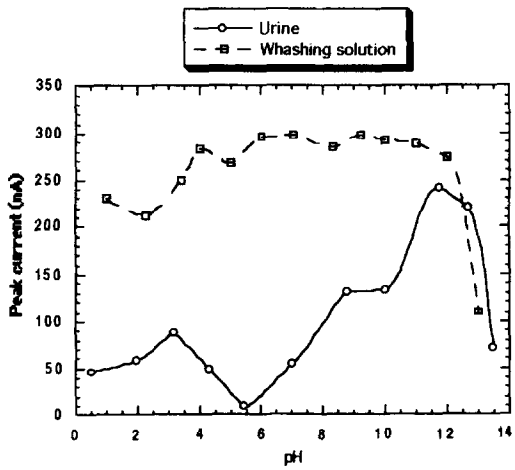


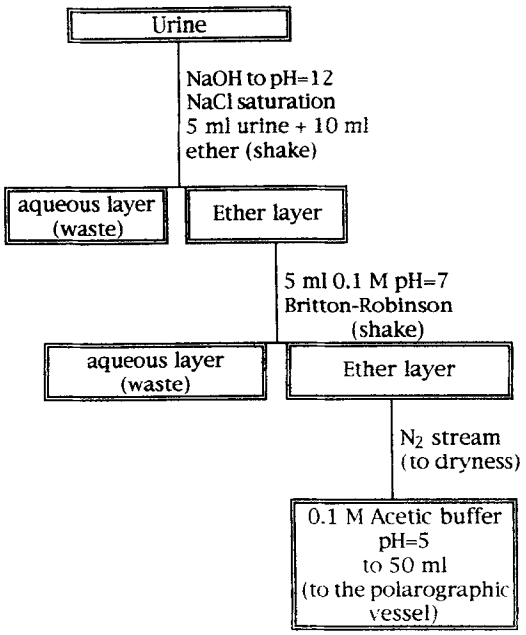
Fig. 5. Effect of extracting 10^{-6} M mebendazole in urine with diethyl ether at several pH values (circles) on peak current and effect of washing the ether extract with 0.1 M Britton–Robinson solutions of different pH (squares) when urine is extracted at pH=12. Urine pH is adjusted with NaOH or HCl and ionic strength is fixed by saturating it with NaCl.

Urine pH was adjusted using a pH-meter and adding NaOH or HCl to the urine samples. At the pH values in which mebendazole exists as neutral molecules, peak heights are the smallest. This does not mean that the yield of mebendazole extraction is worst, but a great amount of interferents can be extracted with it, resulting in a decrease of the adsorption capacity for mebendazole. The best results were obtained at pH values between 10 and 13. At this pH, mebendazole exists as anionic molecules, and it might be extracted as an ion pair with Na^+, but this has not been investigated. A pH=12 was chosen.

At this point, the yield of the procedure was 50% for a 10^{-6} M concentration of mebendazole in urine. This extraction procedure was tested in water and a 96% yield was found, so the losses of yield are due to competitive adsorption. To improve the results the ether extract was washed to eliminate some interferents.

Effect of washing the ether extract with different pH solutions: After the extraction of a pH=12 urine sample, when the urine layer had been removed, the ether layer was contacted with 0.1 M Britton–Robinson buffer solutions at different pH and shaken again (Fig. 5, squares). After that, the aqueous layer is removed and the procedure mentioned above is continued. It can be seen that washing the extract with $4<pH<12$ solutions improves the results due to the elimination of some interferents. It also demonstrates that peak height is not a good measure of the yield of the extraction, because when the extract is washed with solutions of neutral pH results are improved, while extraction at those pH values gave the worst results. A pH=7 was chosen.

Scheme 2 summarizes the steps in the extraction procedure



Scheme 2. Extraction procedure.

3.3.2. Limit of detection, recovery and precision

Fig. 6 shows the voltammograms of a urine blank and a 10^{-8} M mebendazole in urine obtained following the proposed procedure. No electrochemical signal appears in the blank at the potential where reduction of mebendazole occurs.

For the calculation of detection and determination limits, a urine sample from a non-treated person was taken. Four blanks were recorded following the general procedure described in Section 2.3, and the signal of the blank was measured. After that, several aliquots of this urine sample were spiked with mebendazole to 4×10^{-9}, 10^{-8}, 10^{-7} and 10^{-6} M concentrations, and they were measured following the procedure described in Section 2.3 in order to obtain a calibration plot. Limits of detection and determination were calculated as the concentrations, obtained from the calibration plot, that provide a signal equal to the average signal of the blank plus 3 and 10 times the standard deviation obtained from four replicated measures of a 10^{-8} M sample, and were found to be 3.7×10^{-9} and 5.3×10^{-9} M, respectively. The repeatability, based on four extractions of a 10^{-8} M sample, was 2.3% (relative standard deviation).

Recovery was calculated comparing the signal provided by a doped urine sample from non-treated persons after following the procedure in Section 2.3 with the direct measure of a mebendazole standard of the same theoretical concentration in acetic–acetate buffer. As can be seen in Table 2, recovery varies between 75–80% for concentrations over the limit of

Table 2
Recovery of mebendazole in urine at several concentrations

Concentration (M)	4×10^{-9}	10^{-8}	10^{-7}	10^{-6}
Recovery (%)	65.8	78.5	79.1	75.9

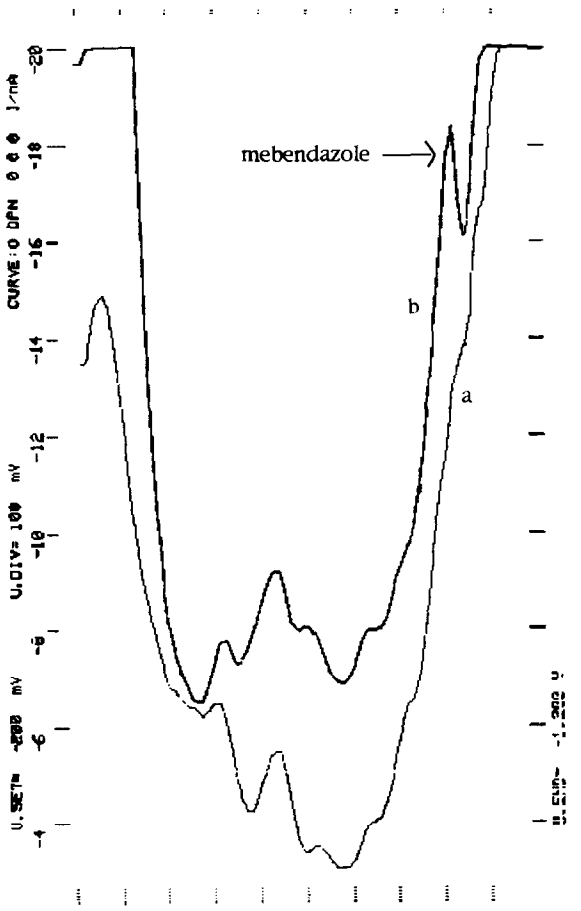


Fig. 6. Polarograms obtained from (a) an urine blank, and (b) 10^{-8}M mebendazole in urine, after following the general procedure described in Section 2.3.

determination. So, if a direct calibration with standards is employed, a 20–25% of error in defect will be obtained.

3.4. Standard addition procedure

The relatively low recovery makes it advisable to employ a standard addition procedure for the determination of mebendazole in urine rather than the employment of a calibration with standards. Samples can be determined using the following standard addition procedure: a sample is first treated following the general procedure described in Section 2.3 and after that an aliquot of the sample is spiked with a known amount of mebendazole and the general procedure is followed again. The increase on peak current can be used to determine the amount of mebendazole present in the sample. Determinations of a 10^{-7}M sample based on this procedure, showed errors between 6% and 15%.

4. Conclusions

A cathodic stripping polarographic procedure for the determination of mebendazole has been developed. Sensitivity has been improved by three orders of magnitude with respect to the dp procedure previously described [5]. The detection limit for mebendazole determination in urine has been improved in one order of magnitude with respect to typical LC procedures.

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