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STUDY OF MEBENDAZOLE BY DIFFERENTIAL PULSE POLAROGRAPHY

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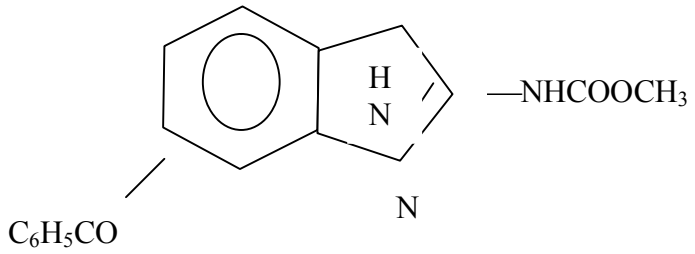
ABSTRACT: A simple and rapid Differential Pulse Polarographic method has been developed for the trace determination of Mebendazole. The studies have been carried out in acetate buffer of pH 4.5. The polarogram showed a well-defined single peak with Ep value of –1.04 V. The detection limit is 0.4 ppm. The electrode process is diffusion controlled and irreversible in nature. The Mebendazole tablets have been analysed by both the calibration method and by standard addition method. Recovery experiments and the mass per tablet are also reported. The statistical data has been calculated to ensure reproducibility, reliability and accuracy of the method.

KEYWORDS: Mebendazole, Differential pulse polarography, drugs

INTRODUCTION:

(5-Benzoyl-1H-benzimidazol-2-yl) carbamic acid methyl ester is commonly known as Mebendazole (MBZ) having molecular weight 295.30 and chemical formula $C_{16}H_{13}N_3O_3$. Its chemical composition is C=65.08%, H=4.44%, N=14.23%, O=16.25%. The crystals of Mebendazole can be obtained from acetic acid and methanol having melting point 288.5^{0}C. It is soluble in Dimethyl formamide (DMF) and formic acid but practically insoluble in water, ethanol, ether and chloroform.[1]

It is an anthelmintic and antinfestive used against hookworm, pinworm, roundworm, tapeworm, threadworm and mixed infestations. It is available in tablet and syrup form. Depending on the type of worm to be treated the dosage varies in adults and children.[2] The following structure represents Mebendazole:



A simple TLC method for identity testing of mebendazole has been reported.[3] The sunlight and UV decomposed samples of mebendazole solutions revealed three spots under UV light by TLC analysis.[4] A simple spectrophotometric method for determination of fenbendazole and mebendazole was based on the reaction with Folin-Ciocalten reagent[5] and using iodobismuthate complex.[6] Hajee et al have presented a liquid chromatographic method for the determination of mebendazole and its metabolites.[7] A simple, fast and precise reverse phase high-performance liquid chromatographic method (RP-HPLC) was developed for the simultaneous determination of purantel pamoate and mebendazole from tablets[8,9] and mebendazole and levanisole tablets.[10] Purantel pamoate and mebendazole have also been simultaneously determined in tablets by high performance TLC.[11,12] A titration method for determination of mebendazole using propanolic potassium hydroxide or perchloric acid as titrants has been described by Bettoni et al.[13] A study has been made of the thermal behaviour of the starting materials to prepare mebendazole, their mixtures and the tablets using a thermobalance.[14]

Literature survey showed that in recent years mebendazole has been studied by Cathodic Stripping Voltammetry in urine[15] but not extensively by DC or Differential Pulse Polarographic techniques. The aim of this study was to develop a simple and rapid method for trace analysis of mebendazole by Differential Pulse Polarography and to quantitate the compound in marketed formulations.

EXPERIMENTAL:

APPARATUS:

Differential Pulse Polarographic studies of Mebendazole were carried out with Metrohm Polarecord E-506 Serie-03 working on 220 volts stabilized AC mains. To it was connected the Metrohm polarography stand E-505. The instrument was kept in an air-conditioned room maintained at 25 + 1^0C and humidity between 50-60%.

The electrode assembly consisted of the dropping mercury electrode (DME) as the working electrode, Ag/AgCl (satu.KCl) electrode as reference electrode and a platinum electrode as an auxiliary electrode. Nitrogen gas was used for deaeration and micropipette (25 µl) was used for addition of Mebendazole solution. Mercury was purified first by aeration method and further distilled under reduced pressure in a mercury distillation unit.

REAGENTS AND SOLUTIONS:

All chemicals used were of AR grade. The solutions were prepared in double distilled water. A pure sample of Mebendazole was obtained from Maharashtra Antibiotics Ltd. (Nagpur). Since mebendazole is insoluble in water the original stock solution of Mebendazole was prepared by dissolving 0.1g Mebendazole in 100 ml DMF. Mebendazole gets precipitated easily when mixed with water. Hence, a certain amount of DMF has to be added to maintain it in solution. For all the analysis not more that 8 ppm of mebendazole was added after which the results obtained were not reproducible.

BUFFER SYSTEMS:

The various buffers in which the mebendazole system was studied were Britton-Robinson buffer (pH range 3.0-10.0), Acetate buffer (pH range 4.0-6.0), Borate buffer (pH range 7.5-11.0), Mcllavaine buffer (pH range 3.5-6.5), Tetra methyl ammonium iodide (TMAI)/ Tetra ethyl ammonium bromide (TEAB) and Clark-Lubs buffer (pH range 5.0-10.0).[16]

GENERAL PROCEDURE:

For each polarographic determination the total volume of the solution was maintained at 25 ml which contained 15 ml of the selected buffer (Acetate buffer pH 4.5), 1 ml DMF and 0.5 ml 0.2 % Triton-X-100. Varying concentrations of Mebendazole was added from the stock solution using a micro pipette. In all cases a blank recording was first performed with the base electrolyte solution and suitable blank correction, if necessary, was applied in the calculations. The experiments were repeated a number of times to ensure reproducibility of results. The studies of Mebendazole were carried out with recorder settings as given below:

Starting potential : -0.6 V

Potential range : -1.5 V

Paper speed : 60 mm/min

Pulse amplitude : 80 mV

Scan rate : 6 mV/sec

Drop time : 2 secs

Sensitivity : 4 x 10^{-9} A/mm

Mode : DPP

Total volume : 25 ml

Deaeration time : 20 mins.

A typical polarogram obtained for 5 ppm of Mebendazole is shown in Figure 1

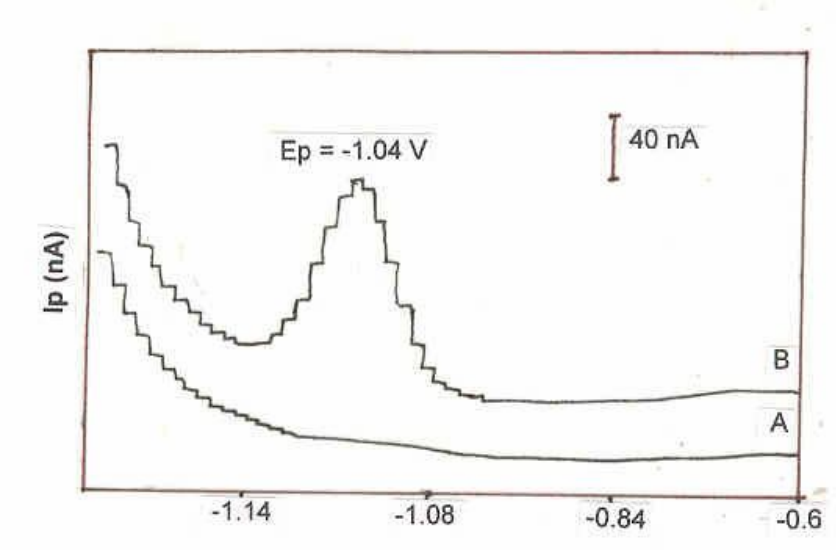


Figure 1: Polarogram of acetate buffer (pH 4.5) containing 0.5ml 0.1% Triton-X-100 and 1 ml DMF(A) and 5 ppm MEB (B)

RESULTS AND DISCUSSION:

The polarograms of Mebendazole were recorded in different buffer systems. Mebendazole gave a peak only in acidic medium and with the increase in pH, the Ep values shift towards negative direction. In Britton-Robinson buffer the peak is symmetrical from pH3.0 to 5.5 and on further increasing the pH the peak becomes highly unsymmetrical. The change in the Ep value in this pH range was from –0.8V to –1.05V. In acetate buffer the Ep value shifted from –0.92V to –1.04V in the pH range 4.0 to 5.5. In 0.1 M TMAI a symmetrical peak was obtained with Ep value –0.92V while in clark-lubs buffer only pH 5.0 gave a symmetrical peak with Ep value –1.00V. In borate buffer and TEAB the peak obtained is not symmetrical. Acetate buffer pH 4.5 was selected because it gave a narrow symmetrical peak and further studies were carried out using this solution.

The effect of variation in the volume of DMF was studied by increasing the amount of DMF added to the buffer solution to keep the substance in solution state as well as maintaining the symmetry of the peak. Varying concentration of DMF (0.5, 1, 2, 3, 4 ml) were taken and the polarograms were recorded. It was found that as the volume of DMF increases the peak becomes more and more unsymmetrical as well as there was a decrease in the diffusion current. It was observed that 1 ml of DMF in 25 ml of the total buffer solution was necessary for mebendazole to remain in solution state along with maintaining the clarity and symmetry of the peak. The maximum concentration of mebendazole which could be reproducibly determined with 4% of DMF was 12 ppm.

The effect of maxima suppressor was studied using Triton-X-100, Gelatin, Methylene blue and bromophenol blue. It was observed that with 0.5 ml of 0.2% Triton-X-100 a narrow symmetrical peak was obtained and with the increase in concentration of Triton-X-100 there was a decrease in the peak height. An unsymmetrical peak was obtained (Ep –0.96V) with 0.5 ml 0.1% Methylene blue, 0.5 ml 0.1% Bromophenol blue, 0.5 ml 0.1% gelatin and when no maxima suppressor was added. Hence, 0.5 ml of 0.2% triton-X-100 was selected as optimum concentration for carrying out further studies of mebendazole.

The lowest determinable limit of Mebendazole was found to be 0.4 ppm. At a sensitivity of 4 x 10^{-10} A/mm,10 μg of mebendazole in 25 ml gave a distinct peak of Ip = 2.8 nA. On varying the drop time it was observed that the diffusion current increases with the increase in drop time.[17] With the increase in pulse amplitude also the diffusion current showed a linear increase.

A drop time of 2 secs was selected because maximum diffusion current was observed at this value. The variation of diffusion current with pulse amplitude was found to be linear till 80 mV after which a deviation was observed hence 80 mV was chosen for further studies.

The graph between E vs log (i/id-i) from a DC polarogram showed that it was diffusion controlled process. A series of DC polarograms were recorded at varying concentrations of mebendazole and $E_{1/4}$-$E_{3/4}$ was calculated which was found to be greater than 56 mV. Also the value of slope calculated from the graph of E vs log (i/id-i) was greater than 59.2 mV. The graph of id Vs $\nu^{1/2}$ (ν- scan rate) did not pass through the origin and the value of Ep also showed a change with a change in the drop time. Thus, implying that the reaction is irreversible.[18,19]

A calibration plot was prepared by taking varying concentrations of mebendazole (1-8 ppm). A straight line was obtained passing through the origin. The linearity of the graph was observed from 0.4 ppm to 12 ppm. The diffusion current obtained at different concentrations and the calibration plot is shown in Figure 2.

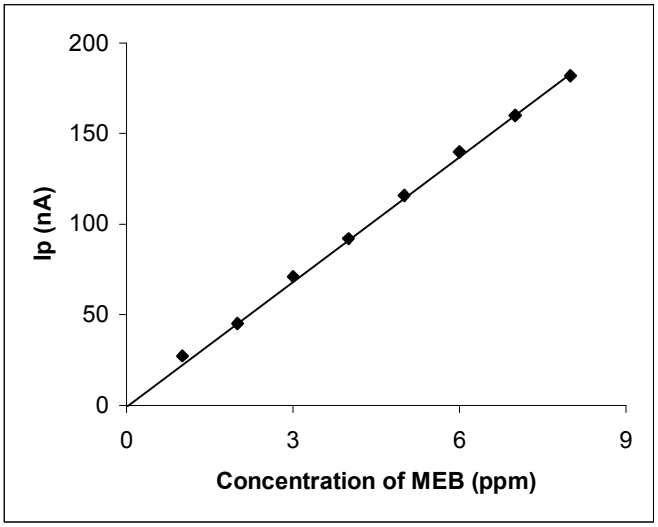


Figure 2: Calibration plot

The utility of the method developed was seen by its application to the determination of Mebendazole in two marketed formulations namely 'Mebendazole tablet I.P.'(MBZ-IP) and 'Mebex'. Ten tablets of each formulation were weighed and powered. Powder equivalent to 100 mg of Mebendazole was weighed accurately and dissolved in 50 ml of DMF. The solution was filtered and the volume was made upto 100 ml with DMF. This stock solution was used for further studies.

Increasing concentrations of MBZ-IP from the stock solution was added in micro quantities of 25 µl and the polarograms were recorded. The actual concentration was determined with the help of calibration graph. The same procedure was repeated with the stock solution of Mebex tablet. The concentration obtained by this method as a replicate of three results is as shown in table 1.

Table 1: Determination of amount of MBZ by calibration curve method.

STANDARD ADDITION METHOD:

A polarogram of an unknown concentration of MBZ-IP was recorded to which 1 ppm and 2 ppm of standard Mebendazole was added and the polarograms were recorded again. The concentration of the unknown solution was then calculated. The same procedure was repeated with the stock solution of Mebex tablet. The polarograms obtained before and after standard addition are shown in Fig. 4. The results obtained are as shown in table 2.

Table 2: Determination of amount of MBZ by standard addition method.

RECOVERY EXPERIMENT

To determine the percentage recovery of Mebendazole, a fixed quantity of mebendazole sample solution was taken and to it three different (25,50,75 µl) levels of working standard mebendazole was added. At each level the polarograms were recorded 7 times and the amount of mebendazole computed using the formula:

Percentage Recovery

$$= \frac{N(\Sigma XY) - (\Sigma X)(\Sigma Y)}{N(\Sigma X^2) - (\Sigma X)^2} \times 100$$

Where,

N = No. of observations

X = Amount of drug added

Y = Amount of drug obtained

The same procedure was adopted for both the marketed samples of mebendazole at two different initial concentrations. The average recovery for MBZ-IP was 100.97 % and for Mebex was 101.48 %.

ANALYSIS OF TABLET:

Five tablets of MBZ-IP were crushed and weighed and the average mass per tablet was determined. This was dissolved in 500 ml of double distilled water and labelled as solution A. 0.1 ml and 0.2 ml was pipetted out from solution A into two separate volumetric flasks and 30 ml of acetate buffer pH 4.5, 1 ml of 0.2% Triton-X-100 and 2 ml of DMF was added and the volume was made upto 50 ml and labelled as solution B and C respectively. From the solution B, 20 ml was pipetted out and the polarograms were recorded following the usual procedure. After recording the polarogram, 1 ppm of standard solution of Mebendazole was added to the cell, deaerated for 1 min and the polarograms were recorded again under the same conditions. Again 25 ml of solution B was pipetted out and the polarograms were recorded as given above. The same procedure was repeated with solution C. The wave height h and H before and after standard addition were

obtained and the mass of Mebendazole per tablet was calculated using the formula:

Mass of MBZ in mg per tablet =

$$= \frac{a\ b\ h \times 1000}{(H - h)\ W}$$

where,
a = amount of MBZ reference standard added (mg)
b = Average mass of tablet (g)
W = Mass of sample taken for polarographic determination (mg)
h = wave height before standard addition
H = wave height after standard addition

The above steps were repeated by taking 10 tablets and recording the polarograms followed by calculations. The mass of Mebex tablet was also determined in the same manner. The results revealed that the mass of Mebendazole per tablet in MBZ-IP was 100.46 mg and 100.13 mg in Mebex.

The statistical results show a relative mean deviation of 2.04%. The standard deviation was calculated to be 2.5 with coefficient of variation as 2.82, which showed that the procedure is reproducible, reliable and accurate. The method is simple to carry out and less time consuming. The set conditions can be applied to study marketed formulations. Hence, an easy, simple and rapid method has been proposed for the study of mebendazole introducing polarography as a new and advanced technique.

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Table 1: Determination of amount of MBZ by calibration curve method.

Drug sample	Volume of drug sample added (µl)	Ip (n A)	Amount of MBZ (ppm)	
			Expected	Observed
Mebendazole Tablet I.P.	25	24	1.0	1.0
	50	44	2.0	1.9
	75	68	3.0	3.0
	100	92	4.0	4.0
	125	114	5.0	5.0
	150	136	6.0	6.0
	175	160	7.0	7.0
	200	180	8.0	7.9
Mebex	25	24	1.0	1.0
	50	46	2.0	2.0
	75	68	3.0	3.0
	100	92	4.0	4.0
	125	112	5.0	4.9
	150	136	6.0	6.0
	175	160	7.0	7.0
	200	182	8.0	8.0

Table 2: Determination of amount of MBZ by standard addition method.

Drug sample	Volume of Unknown solution (µl)	Volume of standard solution (µl)	Ip (nA)	Amount of MBZ (ppm)	
				Expected	Observed
Mebendazole Tablet I.P.	50	-	48	-	-
	50	25	72	2.0	2.0
	50	50	96	2.0	2.0
	75	-	72	-	-
	75	25	96	3.0	3.0
	75	50	120	3.0	3.0
Mebex	50	-	0.04	-	-
	50	25	0.08	2.0	2.0
	50	50	0.12	2.0	2.0
	100	-	0.115	-	-
	100	25	0.155	4.0	3.92
	100	50	0.19	4.0	3.92

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