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# RESEARCH ARTICLE

## MEBENDAZOLE SPECTROPHOTOMETRIC DETERMINATION. THEORETICAL AND EXPERIMENTAL STUDY OF THE INTERACTION WITH SODIUM HYDROXIDE

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**ABSTRACT**

USP, EP, and Argentinian Pharmacopoeia proposed HPLC-UV for quantitative quality control of mebendazole (MBZ) tablets. In this work, a spectrophotometric method is proposed. A mebendazole solution was prepared by dissolving the active ingredient in an ethanolic solution of HCl (1: 100) and adding NaOH 3N. It was allowed to stand 10 minutes. Absorbance spectrum was scanned between 350 and 700 nm. Maximum was found at 400 nm. A calibration curve in the range of 0.05 to 0.25 mg / mL, responded to A = (2.2746 ± 0.0224) C + (0.0012 ± 0.0068) with $R^2$ = 0.9999. The RSD% was 0.961 indicating good repeatability for the analytical procedure. Accuracy in recovery experience was found to be 99.2 - 100.6%. Statistical comparison using t -test and F –test indicate that there are no significant differences between HPLC and the spectrophometric methods, whith a 95% confidence level. Specificity and intermediate precision assays were satisfactory. Quantum theoretical chemistry was applied to elucidate the interaction that gives origin to the color, using static approximation and density functional theory, B3LYP and base 6-311G (d, p). Excitation energies B3LYP for MBZ and MBZ product of the interaction with sodium atom, were coincidental with experimental results.



## INTRODUCTION

It is estimated that 50% of the infant population of our country is affected by parasitosis (Espíndola, 2013). In addition, diseases caused by parasitic worms are one of the most important health problems in the world (Hotez*et al.*, 2008). Benzimidazoles are anthelmintics drugs that have a broad spectrum of action against nematode parasites of the intestinal tract (Goodman and Gilman, 2003). Mebendazole is found within this family (Fig. 1), according to IUPAC, Methyl formate [(5-benzoyl-3H-benzimidazole-2-yl) amino]. This drug is one of the most widely used, mainly due to low absorption in the intestine, insignificant level of adverse effects, low cost, good effectiveness and absence of effects on intestinal microbiota (Cañete, 2009). Several techniques have been reported for MBZ determination. Some of them comprise electrochemical methods (Conesa *et al*, 1996; Kumar *et al,* 2007; Ghalkhani *et al*, 2013), liquid chromatography (Mottier *et al*, 2003; Kulik *et al*, 2011; Ulavapalli *et al*, 2011; Santaladchaiyakit *et al*, 2013; Rao *et al*, 2014; Turabi *et al*, 2014), chromatography coupled to mass detectors (Ruyck *et al*, 2003; Chen *et al*, 2011) fluorometric methods (Fattah *et al*, 1983) and Rayleigh spectroscopy (Tian *et al*, 2013). As established by the Argentinian Pharmacopoeia, European Pharmacopoeia and USP, the official methodology for MBZ tablet quantification is HPLC - UV (FA, 2011; EDQM, 2014; USP; 2015). For routine analysis, it is desirable that the technique is simple, rapid and sensitive. The proposed method uses sodium hydroxide solutions which interact with the MBZ molecule and generate a yellow compound with a strong absorption at 400 nm. Due to a lack of bibliographic sources, in order to infer the formation of products that justify this absorption, a theoretical study of design and modeling, using static approximation and density functional theory, B3LYP and base 6-311G (d, p), was performed.

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### Experimental

#### *Reagents and samples*

- Mebendazole, active ingredient: 1010812 Lot-WC, India origin, purity> 99.8%.
- Mebendazole 200 mg tablets produced by PLAMECOR, Lot 039.
- Excipients: white pre-compacted powder, fused silica and talc.

- Ethanol 98%, analytical grade. (Cicarelli, Argentina).
- Hydrochloric acid 38%, analytical grade (Cicarelli, Argentina).
- Sodium hydroxide, analytical grade (Cicarelli, Argentina).
- Potassium phosphate monobasic, analytical grade (Biopack, Argentina).
- Phosphoric acid, analytical grade (Cicarelli, Argentina).
- Methanol, HPLC grade (Biopack, Argentina).
- Formic acid, analytical grade (Cicarelli, Argentina).

**Equipment**

- Boeco UV-Visible Spectrophotometer S26, range 190-900 nm.
- Agilent 1120 HPLC with UV detector of variable wavelength, controlled by EZ Elite Chrome software.
- Acculab precision scale, accuracy ± 0.1 mg.
- Decalab FBR Magnetic Stirrer with hot plate.
- Software Gaussian 09 package Computational Chemistry.

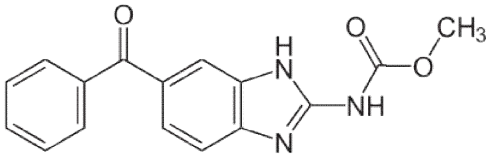


**Fig. 1. Chemical structure of mebendazole ($C_{16}H_{12}N_3O_3$, PM 295.29)**

***Spectrophotometric Procedure***

For the preparation of standard solutions, 50 mg of the active ingredient were dissolved in 50 mL of ethanolic HCl solution (1: 100). Aliquots of 1 to 5 mL of this solution were transferred into 10 mL flasks. An appropriate amount of HCl solution in ethanol was added to each flask. Then 1 mL of 3 M NaOH was added, and diluted to the mark with distilled water. Solutions stood 10 min for full color development. A blank solution, 5 mL of ethanolic HCl solution and 1 mL of 3 M NaOH, brought to 10 mL with distilled water and homogenized, was used. The absorbance at 400 nm was recorded as a function of the concentration of the standards. For sample analysis, 20 tablets were weighed and the average weight of one tablet was calculated (797.2 mg). Afterwards they were pulverized and reduced to a fine powder, yielding a homogeneous mixture. A mass containing the theoretical amount of 50 mg MBZ was dissolved and stirred, for 10 min, into ethanolic HCl solution (1: 100) and carried to final volume of 50 mL. A 10 mL aliquot was centrifuged, for 10 min, at 2000 rpm. The above procedure was applied to an aliquot of 2.5 mL of supernatant. MBZ concentration in the sample was determined by calibration curve.

***Chromatographic conditions (Official method)***

For the mobile phase, a mixture of methanol and 50 mM potassium phosphate monobasic (40:60) was prepared. Was adjusted pH to 5.5 ± 0.1 with 0.1 M KOH or 0.1 M phosphoric acid. For the standard, 25 mg of active ingredient were weighed and dissolved in 10 mL of formic acid. It was carried to a water bath for 15 min, removed, cooled, transferred into 100 mL volumetric flask, diluted to volume with methanol and homogenized. An aliquot of 5 mL of this solution was transferred to 25 mL flask and brought to final volume with mobile phase. The sample was prepared from the homogeneous mixture obtained by pulverizing 20 MBZ tablets. Equivalent mass to an average tablet was dissolved in 50 mL of formic acid, heated in a water bath, stirred, cooled, led to final volume in 100 mL volumetric flask with distilled water and homogenized. An aliquot of 5 mL of this solution was transferred to 100 mL flask, flush with a solution of formic acid in methanol (1: 9). The sample to be injected into the chromatograph was prepared with this last solution. An aliquot of 5 mL of the same was transferred to a 25 mL flask and brought to final volume with mobile phase. For tests RP-18C column 125 x 4.5 mm, mobile phase flow 1.5 mL / min and detection at 247 nm was used. The signal corresponding to MBZ peak, in the standard and sample solutions were recorded. With the peak areas, the concentrations in the injected samples were then determined.

**Analytical Attributes and Validation**

To determine the analytical attributes of the spectrophotometric method and perform validation, the protocol proposed by the current Pharmacopoeias [17-19] was followed.

***Theoretical study of the interaction MBZ-NaOH***

In order to provide a foundation that gives support to the experimental evidence, manifested through MBZ absorption with NaOH in the visible region of the spectrum, with quantitative characteristics, a theoretical study of design and product modeling was carried out. The calculations were performed with the Gaussian 09 (Frisch *et al*, 2004) package Computational Chemistry. Optimization geometries MBZ and more stable products found were performed using the hybrid version of B3LYP (Becke, 1993) and the base assembly 6-311G (d, p) [22]. The minimum more stable conformations were optimized again using the same functional and assembly base, along with the continuous model (PCM) (Cossi*et al*, 2003) to obtain the structure whose energy was minimal in solution using the same experimental solvent. Vertical excitation energy was determined with the most stable structures. A comprehensive treatment was given to the MBZ, product 1 and product 2 electronic excitation spectra using time-dependent density functional theory (TDDFT). It is emphasized that the Kohn–Sham (KS) molecular orbital (MO) method, which is the basis for the TDDFT calculations, affords a MO interpretation of the ground state electronic structure and of the nature of the excitations.. To compare theoretical results with experimental data, only the theoretical energy excitation was considered, with the highest strength of the oscillator.

## RESULTS AND DISCUSSION

***Specificity***

The absorption spectra for reagent solution and reagent plus placebo solution were very similar to each other and showed a very close response to baseline (Fig. 2). Furthermore, the response of the reagent plus sample solution showed a pronounced absorbance maximum at 400 nm. The excipients

accompanying the formulation are chemically inert to the reagents used, this confirms method specificity.

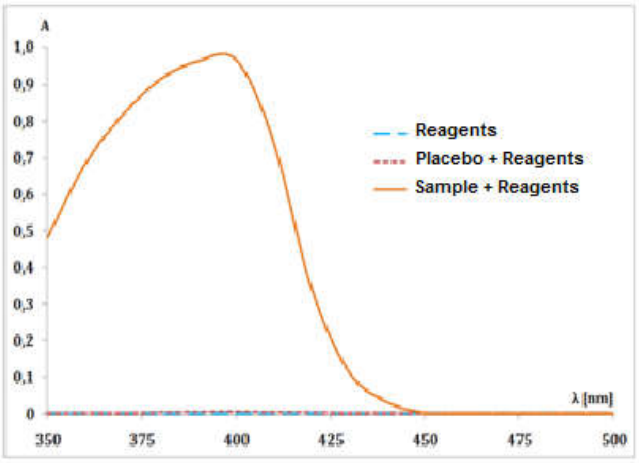


**Fig. 2. Specificity test. Absorption spectra of solutions of reagents, placebo plus reagents and sample plus reagents [MBZ] = 0.3 mg / mL**

### *Linearity*

To evaluate the linearity of the proposed method, a calibration curve with MBZ standard solutions was built. The obtained data were plotted and subjected to linear regression analysis (Table 1). The calibration curve fits the equation A = 2.2746 C + 0.0012. The correlation coefficient of the linear fit ($R^2$) is 0.9999. According to the variance analysis for a 95% confidence level, the intercept includes the point (0, 0) (Table 1).

**Table 1. Variance Analysis of the Linear Regression for MBZ-NaOH method. Confidence level of 95% (p = 0.05)**

| REGRESSION ANALYSIS | |
|---|---|
| Correlation coefficient, $R^2$ | 0.9999 |
| Intercept | 0.0012 ± 0.0068 |
| Slope | 2.2746 ± 0.0224 |

The method was linear in the range of the studied concentrations. It was unnecessary to explore a wider range of responses since this method is proposed for quality control of the active ingredient. The detection limits (LOD) and quantification limits (LOQ), reached values of 0.009 and 0.030 mg / mL respectively. They were calculated as follows:

$LOD = y_A + 3.\ s_A$ Ec. 1

$LOQ = y_A + 10.\ s_A$ Ec. 2

Where:

$y_A$: intercept

$s_A$: deviation of the intercept

### *Repeatability*

Twelve aliquots were analyzed. They were prepared from a pool of tablets belonging to the same lot (Table 2). The method yields a variation coefficient (CV) of 0.961%; less than 2%, required for such analytical determinations [4-6].

**Table 2. Repeatability test for MBZ proposed method**

| # | Mass recovered [mg / tablet] | $M_{Average}$ [mg / tablet] | S [mg] | CV% |
|---|---|---|---|---|
| 1 | 198.87 | 200.79 | 1.93 | 0.961 |
| 2 | 199.81 | | | |
| 3 | 202.13 | | | |
| 4 | 201.64 | | | |
| 5 | 204.71 | | | |
| 6 | 200.96 | | | |
| 7 | 199.37 | | | |
| 8 | 197.56 | | | |
| 9 | 200.14 | | | |
| 10 | 203.16 | | | |
| 11 | 200.66 | | | |
| 12 | 200.47 | | | |

### *Intermediate Precision*

The study was performed at three concentration levels, by adding solutions of the active ingredient over placebo. Different sample volumes were taken and the spectrophotometric method proposed for MBZ tablet was applied. Each analysis was performed by triplicate. Results are shown in Table 3, and are expressed as MBZ mass recovered per tablet. The overall coefficient of variation obtained in the intermediate precision test was less than twice the coefficient for repeatability (CV% GLOBAL <2 x CV% Repeatability). The evaluated precision showed satisfactory results [10-12].

### Recovery Test

The assay was performed in triplicate and at three concentration levels. Placebo solution enriched with different volumes of 1.0 mg / mL MBZ stock solution (Table 4) was used. The recovery is between 99.2 and 100.6%, values fall within the allowed ranges by current Pharmacopeia (98.0 to 102.0%) [17-19].

### *Comparison between the official (HPLC – UV) and the proposed method*

The results obtained for six determinations using the official method are shown in Table 5. The proposed spectrophotometric method was compared with the official using Student's t test and Fisher F (Table 6). Statistically, no significant differences were found between the results obtained by both methods in determining MBZ into tablets with a 95% confidence level.

### *Theoretical study results*

The UV-visible absorption spectra obtained in the laboratory, shown in Fig. 3, were used to compare the experimental results with theoretical results. The most probable interactions that arise from the comparison of theoretical study with experimental evidence are summarized in Table 7. For MBZ, theoretical wave lengths proposed by modeling are 290 nm and 240 nm, are very close to their experimental values (285 and 235 nm, respectively). The products of interaction with sodium hydroxide that showed better agreement with experience are shown in Fig. 4. Product 1, corresponding to the interaction of the sodium atom with two nitrogen atoms of the MBZ molecule, has absorption peaks at 398 nm (transition from orbital 82 → 84, HOMO → LUMO + 1) and 270 nm (transition 82 → 85 orbital, LUMO HOMO → + 2).

**Table 3. Reproducibility test for MBZ-NaOH method performed over 3 levels of concentration by two different analysts on two different days. Acronyms: $V_{MBZ}$, volume of MBZ; $M_{Global}$, average mass per tablet; S, standard deviation; and CV% percent variation coefficient**

| $V_{MBZ}$ [mL] | Analyst 1 | | Analyst 2 | | Reproducibility | |
|---|---|---|---|---|---|---|
| | DAY 1 | DAY 2 | DAY 1 | DAY 2 | | |
| *1.0 mL* | 198.15 | 204.22 | 202.66 | 198.69 | | |
| | 199.23 | 202.36 | 197.63 | 200.15 | | |
| | 202.56 | 200.63 | 198.55 | 201.94 | n | 12 |
| X [mg] | 199.98 | 202.4 | 199.61 | 200.26 | X [mg] | 200.56 |
| S [mg] | 2.3 | 1.795 | 2.678 | 1.628 | S [mg] | 2.149 |
| CV% | 1.15 | 0.89 | 1.34 | 0.81 | CV% | 1.07 |
| *2.0 mL* | *198.64* | 203.98 | 202.75 | 203.36 | | |
| | *203.87* | 200.54 | 200.67 | 201.46 | | |
| | *201.34* | 201.01 | 197.96 | 199.64 | n | 12 |
| M [mg] | 201.28 | 201.84 | 200.46 | 201.49 | X [mg] | 201.27 |
| S [mg] | 2.615 | 1.865 | 2.402 | 1.86 | S [mg] | 1.959 |
| CV% | 1.3 | 0.92 | 1.2 | 0.92 | CV% | 0.97 |
| *3.0 mL* | 199.68 | 201.48 | 197.64 | 198.64 | | |
| | 201.67 | 202.63 | 199.82 | 203.64 | | |
| | 203.93 | 198.69 | 202.63 | 201.69 | n | 12 |
| M [mg] | 201.76 | 200.93 | 200.03 | 201.32 | X [mg] | 201.01 |
| S [mg] | 2.126 | 2.026 | 2.502 | 2.52 | S [mg] | 2.075 |
| CV% | 1.05 | 1.01 | 1.25 | 1.25 | CV% | 1.03 |
| $M_{GLOBAL}$ [mg] | | | | 200.95 | | |
| $S_{GLOBAL}$ [mg] | | | | 2.024 | | |
| CV% $_{GLOBAL}$ | | | | 1.01% | | |

**Table 4. Recovery test for MBZ active ingredient over placebo. Acronyms: $M_{MBZadded}$, MBZ mass added; $M_{MBZrecov}$, MBZ mass recovered; % R, percent recovery; X, average mass; S, standard deviation; and CV% percent variation coefficient**

| $M_{MBZadded}$ | $M_{MBZrecov}$ | %R | % R | Precision | |
|---|---|---|---|---|---|
| [mg] | [mg] | | average | Parameter | Value |
| 1 | 0.995 | 99.5 | 99.2 | X [mg] = | 0.992 |
| | 0.988 | 98.8 | | S [mg] = | 0.004 |
| | 0.992 | 99.2 | | %CV = | 0.403 |
| 2 | 2.036 | 101.8 | 100.6 | X [mg] = | 2.011 |
| | 2,021 | 101.1 | | S [mg]= | 0.031 |
| | 1.977 | 98.9 | | %CV = | 1.541 |
| 3 | 2.965 | 98.8 | 99.9 | X [mg] = | 2.997 |
| | 3.009 | 100.3 | | S [mg]= | 0.028 |
| | 3.016 | 100.5 | | %CV = | 0.934 |

**Table 5. Determination of MBZ in tablets by HPLC-UV. Acronyms: $M_{MBZrecov}$, MBZ mass recovered; $M_{AverageRecov}$, MBZ average mass recovered; S, standard deviation; and CV% percent variation coefficient**

| Run | Signal | Concentration | $M_{MBZrecov}$ | $M_{AverageRecov}$ | S | CV% |
|---|---|---|---|---|---|---|
| Nº | [area] | [mg/mL] | [mg/tablet] | [mg/tablet] | [mg] | |
| ST | 2641.9 | 1.000 | - | - | - | - |
| 1 | 2624.4 | 0.993 | 198.67 | 200.24 | 2.045 | 1.021 |
| 2 | 2630.8 | 0.996 | 199.15 | | | |
| 3 | 2662.6 | 1.008 | 201.56 | | | |
| 4 | 2675.8 | 1.013 | 202.56 | | | |
| 5 | 2667.9 | 1.010 | 201.96 | | | |
| 6 | 2609.8 | 0.988 | 197.56 | | | |

**Table 6. ANOVA test results between the official method (HPLC-UV) and MBZ - NaOH spectrophotometric method. Confidence level of 95% (p = 0.05). Values in parentheses represent the respective critical values**

| Parameter | Method | | |
|---|---|---|---|
| | Spectrophotometric | | HPLC - UV |
| $M_{Average} \pm S$ [mg] | 200.46 ± 1,31 | | 200.24 ± 2.05 |
| CV% | 0.776 | | 1.225 |
| $S^2$ | 1.714 | | 4.184 |
| Test F | | 2.441 (5.050) | |
| Test t | | 0.222 (2.228) | |

**Table 7. Results of the comparison between theoretical study and experimental data**

| Molecule | $\lambda_{exp}^{max}$ | $\lambda_{teor}^{excit}$ | MO | Assignment |
|---|---|---|---|---|
| | [nm] | [nm] | | |
| MBZ | 285 | 290 | 82→83 | $\pi \rightarrow \pi^*$ |
| | 235 | 240 | 82→84 | $\pi \rightarrow \pi^*$ |
| Product 1 | 400 | 398 | 82→84 | $\pi \rightarrow \pi^*$ |
| | 260 | 270 | 82→85 | $\pi \rightarrow \pi^*$ |
| Product 2 | 400 | 345 | 82→84 | $\pi \rightarrow \pi^*$ |
| | 260 | 279 | 82→85 | $\pi \rightarrow \pi^*$ |

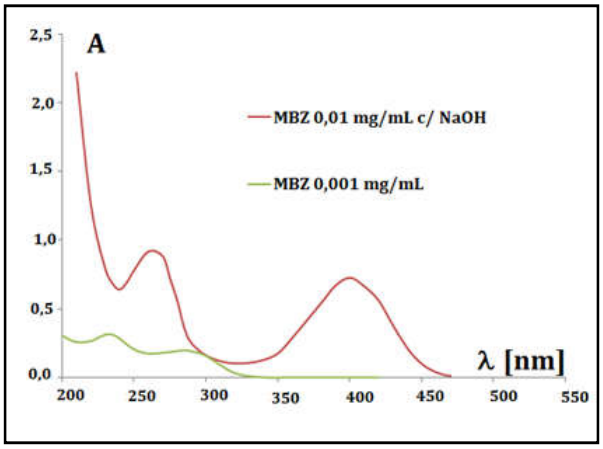


**Figure 3. UV-visible absorption spectra of MBZ and its interaction product with NaOH**

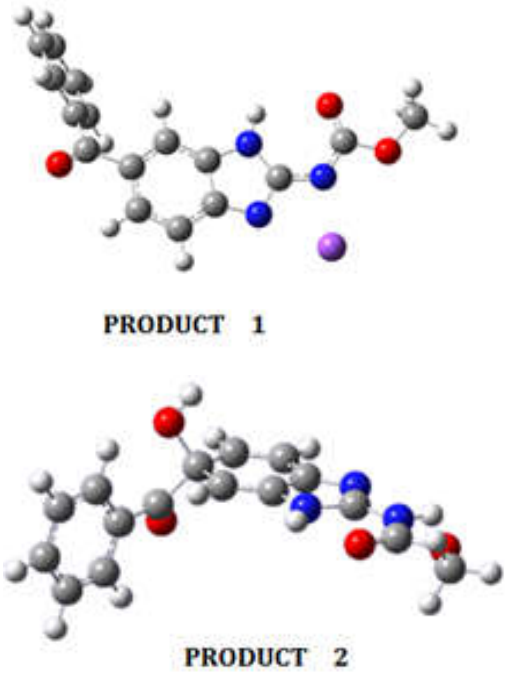


**Fig. 4. Interaction Products between MBZ and NaOH**

Meanwhile, Product 2 which arises from the interaction of hydroxyl with the MBZ benzene ring, has absorption peaks at 345 nm (transition from orbital 82 → 84, HOMO → LUMO + 1) and 279 nm (transition from orbital 82 → 85, HOMO → LUMO + 2).

## Conclusions

The results allow the application of this spectrophotometric methodology to quantify the MBZ active ingredient in pharmaceutical tablets, with precision and accuracy comparable with the official method, without interference from the excipients, with clear advantages in terms of costs and speed of analysis, and complying with the requirements established by the current Pharmacopeia. Absorbance measurements were complemented with the application of theoretical chemistry, using Density functional theory, B3LYP and the basis 6-311G (d, p). The energies of excitations B3LYP for the MBZ and MBZ product interaction with sodium agree with the experimental results.

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