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Spectrophotometric Determination of Mebendazole in Pharmaceutical and Urine Samples by a New Flow Injection with Merging-Zone Technique

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Abstract

To confirm the drug quality and efficacy of treatment in man, it is essential that mebendazole can be accurately determined in pharmaceutical preparations. A new method is, therefore, developed for the determination of the antifungal drug mebendazole using flow injection analysis, combining the properties of merging zones, which is rapid, simple and extremely sensitive for the determination of mebendazole. The reaction employed in this determination depends on the ability of mebendazole to reduce iron (III) to iron (II) in acid and the reduced iron forming an orange-red complex with 1,10-phenanthroline which can be determined spectrophotometrically at 510 nm. The method is shown to be linear with response in the range 25 – 250µg/ml mebendazole and has a LOD and LOQ of 22µg/ml and 25.5µg/ml respectively. The correlation coefficient (r) was 0.9975, indicating excellent linearity. The method was also successfully applied to determine the concentration of mebendazole in tablet formulations, demonstrating its potential for quantitative pharmaceutical analysis. A statistical analysis comparing the results obtained from the newly developed method and established techniques showed no significant discrepancies.

Keywords: Mebendazole, Merging zone, flow injection, Pharmaceuticals, Spectrophotometry

1. Introduction

Mebendazole (shown in Figure 1) is chemically designated methyl-5-benzoyl benzimidazole-2-carbamate. This substance appears as an almost odorless powder which varies in color from white to pale yellow. It is a member of the family of benzimidazoles, which represent a class of anthelmintic agents known for their effect upon nematode intestinal parasites [1-3]. Mebendazole has been woefully neglected for the use in helminthic infestation, but is now being investigated as a potentially useful therapeutic agent in various forms of cancer [4-6]. This agent is supposed to act by producing an irreversible inhibition of glucose uptake by the parasite, thus effecting a depletion of the glycogen reserves of the parasite [7, 8]. According to the Biopharmaceutical Classification System (BCS), mebendazole is considered a Class II drug [9]. This means that it is a drug which is poorly soluble but highly permeable.

Consequently, mebendazole absorption is dissolution rate-limited, with its dissolution behavior expected to strongly influence its bioavailability upon oral administration [10, 11]. Various analytical methods have been reported in the literature for the determination of mebendazole, in pure form, biological and pharmaceutical samples such as high-performance liquid chromatography (HPLC) [12-14], spectrophotometric methods [15-19], liquid

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chromatography-mass spectrometry (LC-MS) [20, 21], HPLC-MS/MS [22] and fluorimetry [23, 24]. Many existing spectrophotometric techniques for analyzing Mebendazole rely on complex formation, necessitating an extraction phase to isolate the colored product [12, 15, 20, 25]. These multi-stage batch processes are laborious, time-intensive, and require substantial samples and reagents [26, 27]. Alternative methods, while available, often prove to be intricate, expensive, and time-consuming. Pharmaceutical analysis is currently focused on addressing the limitations of traditional analytical methods and automating these processes [28-38]. Flow injection analysis (FIA) with a merging zones technique provides a way to automate batch analysis methods while gaining the advantages of high-speed analysis and high reproducibility characteristic of FIA methods [39]. By incorporating merging zones into FIA, batch methods can be automated and performed with the high analytical throughput and reproducibility typical of flow-based systems. A series of active pharmaceutical ingredients have been successfully determined using FIA. The literature currently lacks a fast and reliable FIA method for determining mebendazole. This study aims to develop and validate a simple, rapid, and precise Merging zone FIA method combined with the merging zone technique for analyzing mebendazole in pure and commercial pharmaceutical products.

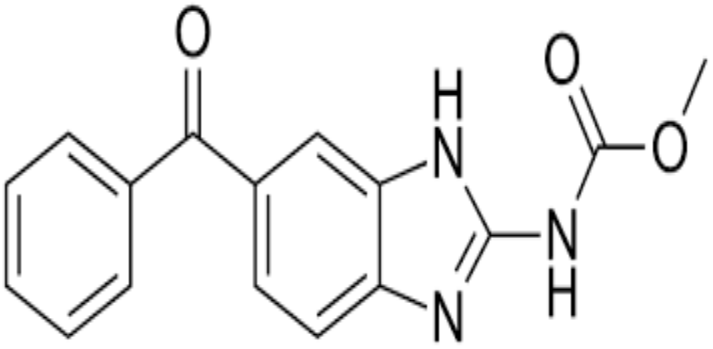

Figure 1: The chemical structure of mebendazole

2. Experiment

2.1. Materials

All reagents used were of analytical grade. The active pharmaceutical ingredient, Mebendazole, was provided by a local manufacturer, Samarra Drug Industry (SDI) in Iraq. The commercial pharmaceutical products analyzed in this study were obtained from local markets. Solvents and additional reagents Fe $(NO_3)_3.9\ H_2O$, 1-10-Phenanthroline, and acetic acid were sourced from Sigma Aldrich.

2.2. Instrument

In addition to the FIA/MZ apparatus (depicted in Figure 2), other key elements include de-ionized water flows; a peristaltic pump; four valves; four coils for mixing; a heat bath for temperature control; and an in-house built, handheld, 510 nm spectrophotometer. The goal of this apparatus is to be able to automatically measure the amount of mebendazole present by introducing the reagents and sample into the apparatus via the valves, mixing the reagents and sample within the coils, and measuring the color intensity of the colored complex that is formed spectrophotometrically. The use of the MZ technique allows for the automation of the traditional batch method of analysis using a high speed analytical instrument and the inherent reproducibility found in flow based instruments. In such a configuration, the drawbacks of conventional analytical methods for mebendazole have been overcome, and it provides a straightforward, rapid, and reliable method for determining mebendazole in different matrices.

2.3. General Procedure

Generally, to investigate an analyte with the flow system set up as shown in Figure 2, valves 1, 2, 3 and 4 were first closed and a rinse of deionised water was passed through the system to give a stable baseline. The sample having the analyte (mebendazole) was sucked into valve 1 during the charging of the necessary reagents [Fe $(NO_3]_3$ solution, 1,10-phenanthroline and acetic acid buffer) into valves 2, 3 and 4. The valves were opened in sequence and each timed, to inject the sample and reagents into the flowing stream of deionised water. The various components passed through mixing coils where they were mixed, the first two coils allowing a time for the first stage of reaction and mixing. The reaction mixture then had logical passage through a heated bath, where temperature control was made for the purpose of optimising the reaction, followed by two further mixing coils to facilitate complete reaction. The reacted sample then arrived at the handmade colourimeter, where the absorbance was measured at 510 nm, which was the wavelength being measured for the concentration of the analyte. The system was thereafter rinsed out with deionised water, preparatory for the next analysis. This procedure was repeated for calibration standards and unknown samples, thus allowing quantitative analysis to be performed, rapidly, automatically and reproducibly, and with less demand on both sample and reagent than is the case with the traditional batch methods.

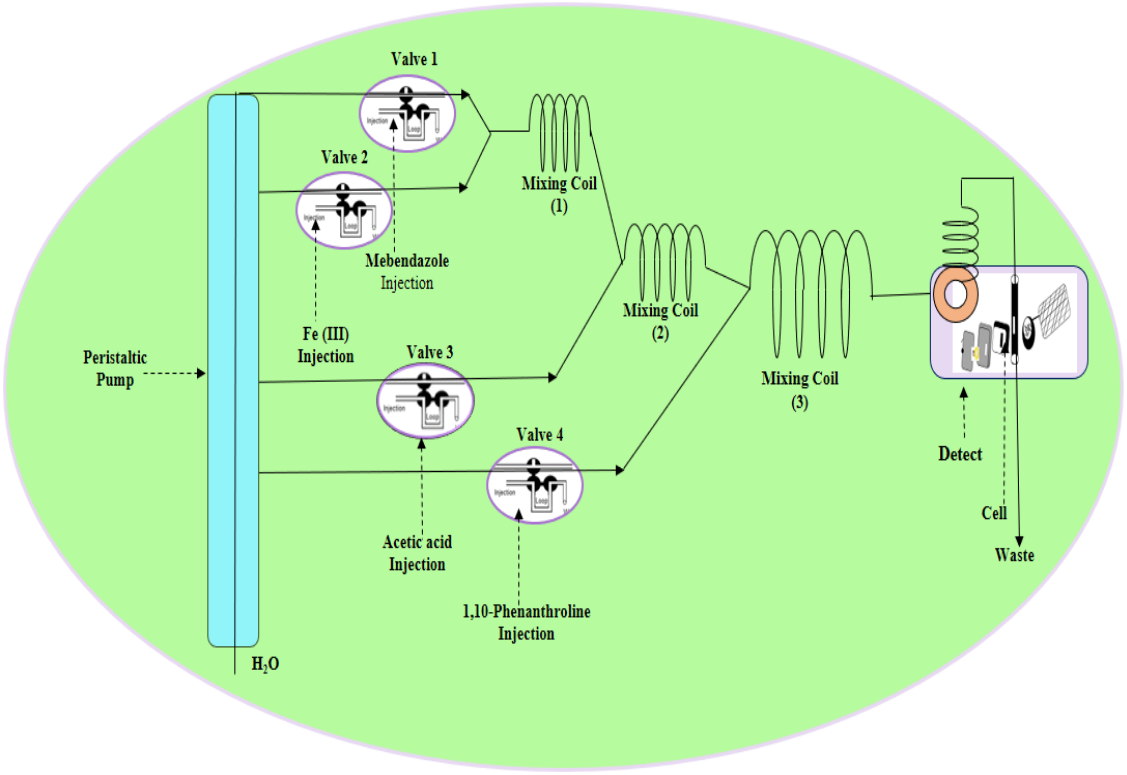

Figure 2: The constructed manifold flow system designed for the determination of mebendazole

2.4. Preparation of solutions

A stock solution of mebendazole with a concentration of 1000 µg/ mL was prepared. This was accomplished by precisely weighing 0.1g of mebendazole and dissolving it in a 100 mL volumetric flask containing double-distilled water as the solvent. The solution was then diluted to the mark with D.W. Additional dilutions of the mebendazole stock solution were carried out to prepare a calibration range of 25-250 µg/ mL mebendazole standard solutions. This was done by dissolving the appropriate volumes of stock solution in distilled water.

2.5. Preparation of commercial tablet

The present study investigated two commercial companies that supply 100 mg of mebendazole. Twenty tablets were weighed, ground into a fine powder, and thoroughly mixed to prepare the sample solutions. An amount of this powder, equivalent to the average weight of one tablet, was accurately weighed and dissolved in double-distilled water. The mixture was mechanically shaken for 20 min to ensure complete dissolution and filtered to remove any insoluble excipients. The filtered residue underwent four rinses with distilled water. Subsequently, it was decanted in a 100 mL volumetric flask and the volume was adjusted up to mark with more distilled water.

3. Results and discussion

3.1. Effect of chemical parameters

To obtain higher absorbance values, which in turn indicates increased sensitivity of the proposed method, different factors under static flow settings were studied. These were the following: 100 µL sample volume, a concentration of mebendazole 100 µg/mL, 1,10-Phenanthroline concentration at Level L5 (0.012 M), acetic acid concentration at Level L3 (2 M), and a flow rate for each mobile phase (water, acid, and reagent) set to 1.0 mL/min. A range of $Fe(NO_3)_3$ concentrations from 3×10^{-4} to 10×10^{-4} M was examined to enhance sensitivity further. It was selected that the 8×10^{-4} M of Fe $(NO_3)_3$ was the best based on these results, and concentrations used in all following assays are presented in Table 1. Further, the ideal concentration of the colorimetric reagent (0.012-0.04 M) was determined using flow conditions of the experimental setup: these conditions included a sample volume of 100 µL, a mebendazole concentration of 100 µg/mL, concentration of $Fe(NO_3)_3$ of 8×10^{-4} M, acetic acid 2 M and flow rates of 1.0 mL/min for all lines (water, acid, and reagent). The optimum concentration of 1,10- Phenanthroline of 0.012 M was determined using the above method, and results are shown in Table 2. Eventually, the developed method was applied to the determination of acetic acid also in the range 1.0-4.0 M using exactly the above experimental conditions: sample volume 100 µL, mebendazole concentration of 100 µg /mL, $Fe(NO_3)_3$ concentration reverse to 8.00×10^{-4} M, or 0.012 M of 1,10-Phenanthroline and all line was exerted at 1 mL/min. (water line excepted choice for flow rate). (Chart: water lines, acid lines, and reagent). According to the results of these experiments, a concentration of 3.0 M of the acid was determined to be optimal and selected for subsequent analyses, Table 3.

Table 1: The effect of Fe (NO3)3 concentrations using the optimum flow conditions of the proposed method

Conc. of Fe $(NO_3)_3$, M	Abs.	RSD%
3×10^{-4}	0.52	0.2122
5×10^{-4}	0.59	0.3551
8×10^{-4}	0.64	0.5196
10×10^{-4}	0.64	0.0273

Table 2: The effect of 1,10-Phenanthroline concentrations using the optimum flow conditions of the proposed method

Conc. of 1,10-Phenanthroline, M	Abs.	RSD%
0.012	0.63	0.0908
0.02	0.61	0.6907
0.03	0.41	0.2211
0.04	0.32	0.0502

Table 3: The effect of Acetic acid concentrations using the optimum flow conditions of the proposed method

Conc. of Acetic acid, M	Abs.	RSD%
1.0	0.42	0.2121
2.0	0.59	0.3523
3.0	0.64	0.5141
4.0	0.64	0.0252

3.2. Effect of physical parameters

Various physical parameters were inspected to check the proposed method's sensitivity after optimization of all chemical parameters. This investigation aimed to adapt sample volume and flow rates to evaluate their effects on the method's performance. The main objective of these physical parameter optimizations, in addition to the previously established optimal chemical conditions, was to consider a high overall sensitivity and efficiency of the analytical method highlights. Under optimized chemical conditions and other parameters were kept constant, with a sample volume of 200 µL, the influence of flow rate in their analysis was studied. The peristaltic pump was utilized in order to regulate the flow rates through all lines 1.0-5.0 mL/min. Under these constant conditions, besides flow rate, the impact of varying the flow rate parameter was investigated. Based on the results, the 2 mL/ min was chosen as the optimum flow rate and applied for further experiments. Finally, the sample of injected volume was further optimized by employing the previous experimental conditions: 100-300 µL was injected into the manifold system, and the results showed that 200 µL was the volume that given the highest absorbance value, therefore 200 µL was used for further experiments. All the results of the obtained physical parameters are listed in Tables 4 and 5.

Table 4: The effect of applying variable flow rate on the obtained results of the proposed method

Flow rate, mL/min	Abs.	RSD%
1.0	0.42	0.0551
2.0	0.64	0.2241
3.0	0.52	0.3247
4.0	0.52	0.5189
5.0	0.49	0.1251

Table 5: The effect of applying variable sample volumes on the obtained results of the proposed method

Sample volume, µL	Abs.	RSD%
100	0.22	0.0201
150	0.42	0.0512
200	0.64	0.0814
250	0.64	0.0718
300	0.63	0.1222

3.3. Validation of the proposed method

The newly developed analytical methods were subjected to a comprehensive validation process by the International Council for Harmonisation of Technical Requirements for

Pharmaceuticals for Human Use (ICH) guidelines. This validation encompassed several critical parameters, including interferences, linearity, LOD, and LOQ.

3.4. Linearity

Standard solutions of mebendazole at concentrations ranging from 5-400 µg/mL were injected into the optimized flow manifold system. With all operating conditions maintained as previously optimized, the obtained absorbance signals were used to construct a calibration curve relating to mebendazole concentration. Figure 3 shows an excellent linear relationship between absorbance and mebendazole concentration over this range of 25-250 µg/mL was obtained, with very high correlation coefficients. The linear regression, statistical parameters, and correlation coefficients are tabulated in Table 2.

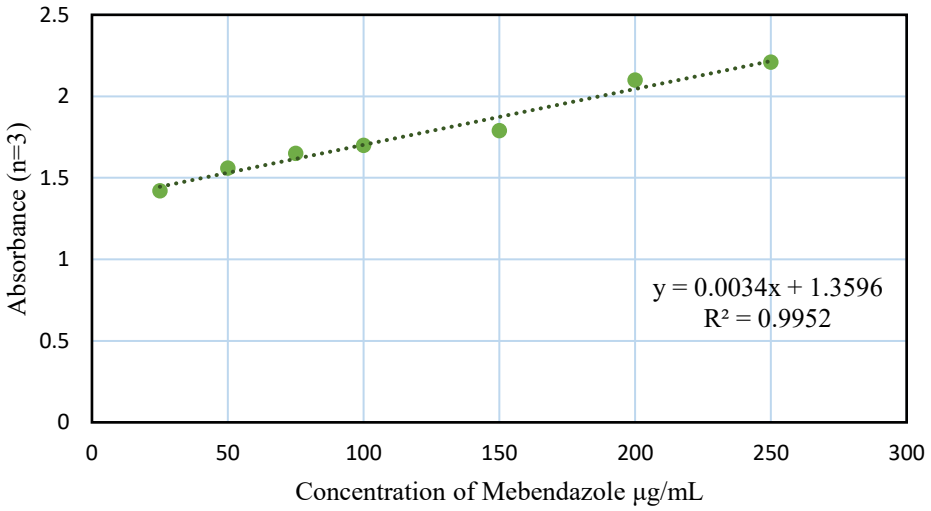

Figure 3: The calibration curve of the proposed method (FIA)

3.5. Limit of detection and quantification

A critical component of pharmaceutical research and development is the creation of analytical methods that exhibit high precision, accuracy, robustness, and linearity.
Therefore, the quantitative analytical methods' limit of detection (LOD) was determined. The LOD can be calculated using the standard error (SE) of the y-intercepts from the regression lines. For this particular analytical approach, all samples must be analyzed within the concentration range defined by the LOD and LOQ. To determine the LOD, which represents the lowest concentration of analyte that can be reliably detected, the following calculations are typically performed:
LOD = 3* (slope/SD)
LOQ = 10* (slope/SD)
In which SE represents the standard error of the response.
All the obtained data of linear equations for proposed and reference methods are listed in Table 6.

Table 6: A statistical comparison of the linear equations defined in the proposed and previous methods

Parameter	Proposed method	Reference method
Linear range, µg/mL	25-250	30-85
Coefficient of determination, R^2	0.9952	0.9957
Linear equation	0.0034x + 1.3596	0.0529x +0.8953
Intercept	1.3456	0.8953
slope	0.0034	0.0529
LOQ, µg/mL	22	28
LOD, µg/mL	25.5	35

3.6. Effect of interferences

Since the mebendazole tablets contain the active pharmaceutical ingredient (API) and common excipients, an investigation of potential interference from excipients was conducted to quantify 100 mg/tablet mebendazole. Table 7 summarizes the results examining the effects of excipients when determining the labeled tablet strength of 100 mg/Tablet mebendazole using the developed method. The analysis revealed that tablet excipients did not interfere with the drug's detection in various formulations. Therefore, the developed method was an appropriate assay for quantitatively determined mebendazole in commercial tablets containing different excipients.

Table 7 : The effect of interference on the proposed method using 10 µg/mL of drug

Excipients	Added, µg/mL	Found, µg/mL
Gelatin	10	30.12
Starch	10	30.15
Sucrose	10	29.98
Lactose	10	30.22
Magnesium stearate	10	29.97

3.7. Application

Two distinct commercial pharmaceutical formulations, each containing 100 mg of the active ingredient per tablet, were analyzed to evaluate the efficacy of the newly developed analytical method. The quantitative analysis utilized a flow injection procedure with the method of ion-pair association reaction. In order to determine the precision of the method, three analyses of the same sample were made. The results from this replicate are shown in Table 8. The data from the triplicate analysis have been presented so that the method may be evaluated for repeatability.

Recovery percentages (Rec %) were also determined by using the standard addition method to spike predetermined amounts of mebendazole reference standard into solutions of the prepared tablet samples. The Rec % obtained for the samples were in excellent agreement with the label claims. A statistical analysis t-test was conducted between the results obtained from the proposed method and those from the reference method [16], and the results showed no significant difference between the two methods.

Table 8: Implementing the developed method for quantifying mebendazole in tablet formulations

Drug	Claimed dosage mg	Found		t-test
		Proposed method (FIA)	Reference method (UV)	
Vermox	100	100.21	99.98	1.25
Mebex	100	99.98	98.89	2.35

Conclusion

The analytical method developed in this study represents a significant innovation in pharmaceutical analysis. The scientific literature has not reported any procedure combining flow injection analysis with merging zone techniques for determining mebendazole in tablet formulations. The optimized operational conditions have allowed for the achievement of good linearity curves with excellent recovery percentages when analysing spiked samples via the standard addition method. The results obtained from applying the developed method demonstrate that a flow injection manifold system comprising a single detector with a merging zone is a suitable analytical technique, offering simplicity, accuracy, sensitivity, selectivity, cost-effectiveness, and precision for determining mebendazole content in standard and tablet formulations.

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