

DOI: <https://dx.doi.org/10.21123/bsj.2023.6785>

## Indirect Flow Injection Spectrophotometric and Chromatographic Methods for the Determination of Mebendazole in Pharmaceutical Formulations

*Idrees F. Al-Momani\** 

*Raghad I. Al Souqi* 

Chemistry Department, Yarmouk University, Irbid, Jordan

\*Corresponding author: [imomani@yu.edu.jo](mailto:imomani@yu.edu.jo)

E-mail address: [raghad.alsouqi94@gmail.com](mailto:raghad.alsouqi94@gmail.com)

Received 25/11/2022, Revised 10/1/2022, Accepted 11/1/2023, Published Online First 20/3/2023



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### Abstract:

Chromatographic and spectrophotometric methods for the estimation of mebendazole in pharmaceutical products were developed. The flow injection method was based on the oxidation of mebendazole by a known excess of sodium hypochlorite at pH=9.5. The excess sodium hypochlorite is then reacted with chloranilic acid (CAA) to bleach out its color. The absorbance of the excess CAA was recorded at 530 nm. The method is fast, simple, selective, and sensitive. The chromatographic method was carried out on a Varian C18 column. The mobile phase was a mixture of acetonitrile (ACN), methanol (MeOH), water and triethylamine (TEA), (56% ACN, 20% MeOH, 23.5% H<sub>2</sub>O, 0.5% TEA, v/v), adjusted to pH = 3.0 with 1.0 M hydrochloric acid. Naphazoline nitrate was used as an internal standard. The absorption of mebendazole was measured using a variable wavelength UV detector at 290 nm. Linearity was obtained in the concentration range of 1-60 and 0.10-3.0 mg/L for the HPLC and FIA, respectively. The methods were applied successfully for the assay of mebendazole in pharmaceutical products and no interferences were observed from the common excipients usually used. The proposed methods were validated for their accuracy and precision.

**Keywords:** FIA, HPLC, Mebendazole, Pharmaceutical products, Spectrophotometry.

### Introduction:

Mebendazole (Fig. 1), chemically known as methyl-5-benzoyl benzimidazole-2-carbamate. It is a white to slightly yellow powder, almost odorless. It belongs to the benzimidazoles family which is known as anthelmintics drugs that work against nematode parasites of the intestinal tract. "It is known to act through irreversible inhibition of glucose uptake in the parasite, leading to depletion of glycogen store"<sup>1</sup>. According to the biopharmaceutical classification system, mebendazole falls under class II which means that it's a drug with low aqueous solubility and high permeability, this leads to convertible absorption of mebendazole. It is official in United States pharmacopeia, Indian pharmacopeia, British pharmacopeia, and European pharmacopeia<sup>1,2</sup>.

Different analytical methods have been reported in the literature for the estimation of mebendazole either in pure form and formulations. These include HPLC<sup>3-5</sup>, spectrophotometry<sup>6-11</sup>, liquid chromatography-mass spectroscopy<sup>12</sup> and fluorimetry<sup>13</sup>. Most of the reported

spectrophotometric methods depend on the formation of complexes which need an extraction step to get the formed colored product. These multi-step batch procedures are tedious, time-consuming, and require large volumes of samples and reagents.

Pharmaceutical analysis is currently concerned with overcoming these disadvantages and automating the traditional methods of analysis. FIA affords a means of automating batch methods, incorporating into them the high speed of analysis and high reproducibility that characterize FIA procedures. Furthermore, FIA has numerous advantages, including flexibility, minimum reagents requirements, and speed. Several methods for "the quantification of several drugs in biological fluid and pharmaceutical products were developed"<sup>14-16</sup>. Most notably, HPLC provides automated, precise, and highly specific quantification of the target analyte in a sample. The literature is still lacking fast and reliable FIA and HPLC methods for the determination of mebendazole. The objectives of the current work are to develop and validate simple,

rapid, and precise HPLC and FIA methods for the analysis of mebendazole in commercial pharmaceutical products.

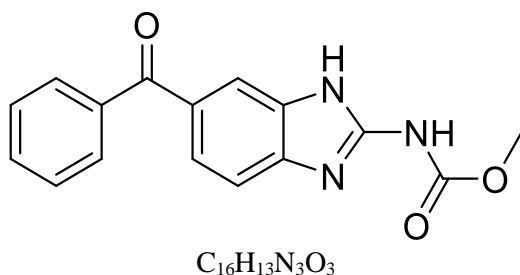


Figure 1. Chemical structure of mebendazole.

## Materials and Methods:

### Chemicals

All chemicals were of the highest purity. Mebendazole was gifted by the local pharmaceutical company Al-Hikma (Amman - Jordan). All the studied pharmaceutical products were purchased locally. Chromatographic solvents are purchased from LAB-SCAN. Other reagents and materials are either from Cambrian or Fluca.

### Instrumentation

The proposed flow injection system was constructed using micro-tubes (internal diameter = 0.51 micron). The manifold involves two lines. The first line carries the oxidizing agent, sodium hypochlorite (NaOCl) which reacts with the injected mebendazole. The reaction between mebendazole and NaOCl takes place in the first reaction coil (RC1) and consumes a portion of NaOCl (Fig. 2). The excess sodium hypochlorite mixes with the chloranilic acid (CAA) that is pumped in the second line. The reaction between NaOCl and CAA takes place in the RC2. The decrease in the absorption of the colored dye CAA was recorded at 530 nm using the SPUV-19 UV-Vis instrument. The decrease in the color intensity is proportional to the concentration of mebendazole.

The chromatographic system used in this study was the Knauer model-501 LC system attached to the UV detector. Data were acquired and processed using Eurochrom-2000 software. The separation was achieved using the Varian RP-C18 column (15cm x 4.0 mm ID., 5 $\mu$ m particle size). The mobile phase was a mixture of acetonitrile, methanol, water, and triethylamine (56%, 20%, 23.5%, 0.5%, V: V) adjusted to pH=3 pumped at a flow rate of 1.0 ml/min.

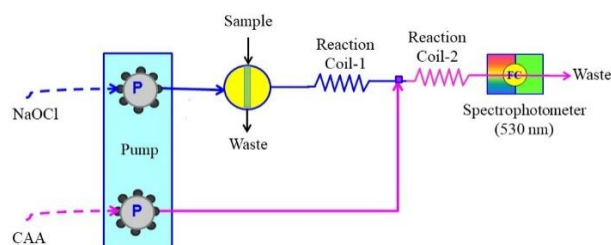


Figure 2. Diagram of the flow injection system.

### Preparation of Solutions for the FIA Method

**Buffer solution (pH=9.5):** Ammonium chloride-ammonium hydroxide buffer solution was prepared by dissolving 2.675 g (0.2 M) of ammonium chloride in about 200 mL distilled water. The pH was adjusted to 9.5 by the addition of 0.2 M NaOH and then completed to 250 ml with distilled water.

**CAA solution:** 2.0 mM of CAA was freshly prepared in ammoniacal buffer (pH=9.5).

**Sodium hypochlorite solution:** 3.0 mM of sodium hypochlorite was prepared in the ammoniacal buffer by dilution of the stock solution.

**Active ingredient standard solution:** a stock solution of 200 mg L<sup>-1</sup> of mebendazole was prepared by dissolving 20 mg of the drug in 10 mL of 10% formic acid then completed to 100 mL with distilled water.

**Mebendazole tablets (Vermox, Vermin):** The contents of ten tablets were pulverized. A quantity equal to one tablet of mebendazole (100 mg mebendazole/tablet) was transferred to a 100 mL volumetric flask, dissolved in 10 mL of 10% formic acid, shaken for 5 min in an ultrasonic shaker, and completed to 100 mL with distilled water. The concentration of mebendazole in this solution is supposed to be 1000 mg L<sup>-1</sup>. Portions of this solution were filtered using 0.2  $\mu$ m syringe filters and used for the preparation of more diluted solutions.

### Preparation of Solutions for the HPLC Method

**Mobile Phase:** One liter was prepared by mixing 560 mL of ACN, 200 mL of methanol, 5 mL of TEA, and about 100 mL of distilled water. The mixture was adjusted to pH= 3 using 1 M HCl and then completed to one liter with distilled water. The final mobile phase composition was (56% ACN: 20% MeOH: 0.5% TEA: 23.5% H<sub>2</sub>O; v/v) adjusted to pH= 3, filtered by Nylon 66 membranes filters (0.45  $\mu$ m \* 47 mm) and finally degassed for 5 min using an ultrasonic shaker.

**IS solution:** 200 mg L<sup>-1</sup> of naphazoline nitrate was prepared in 10% formic acid.

**Active ingredient standard solution:** A stock solution of  $200 \text{ mg L}^{-1}$  mebendazole was prepared using the same procedure of the FIA method. Solutions for the construction of the calibration curve were prepared by proper dilution. A fixed volume of the internal standard solution was added to each flask before completion to volume.

**Mebendazole tablets (Vermox, Vermin):** Mebendazole tablets were dissolved using the same procedure used for the FIA method. Solutions for the construction of the calibration curve were prepared by proper dilution. A fixed volume of the internal standard solution was added to each flask before completion to volume. All sample solutions were filtered through a  $0.2 \mu\text{m}$  cellulose acetate syringe before injection.

### FIA Procedure

The system was operated with the oxidant connected to the injector line and the colored dye (CAA) to the second line. The system operates with the two lines flowing at the same time. Both solutions, the oxidant and the CAA are prepared in ammoniacal buffer (pH = 9.5). A  $60 \mu\text{L}$  of the mebendazole solution is introduced into the oxidant stream ( $3.0 \text{ mM NaOCl}$ ). The oxidant solution is pushed at a  $0.90 \text{ mL/min}$  flow rate. Although mebendazole solutions are prepared in 10% formic acid, the pH of the mixture remains constant at 9.5 because the amount injected is very small ( $60 \mu\text{L}$ ). The colored dye ( $2.0 \text{ mM CAA}$ ), is pumped at a rate of  $0.9 \text{ mL/min}$  to mix with the excess oxidant in RC2.

## Results and Discussion:

### FIA Method

In this work, it was discovered that in an alkaline medium, sodium hypochlorite can oxidize both mebendazole and CAA. Therefore, after the reaction of sodium hypochlorite with mebendazole, the excess oxidant reacts with the dye to fade its color. The absorbance of unreacted CAA was recorded at  $530 \text{ nm}$  (Fig. 3). Many factors affecting oxidation and bleaching reactions have been improved.

The reaction conditions were optimized to achieve maximum sensitivity. The effect of the acidity of the reaction medium was tested by carrying out the reaction using different acidic and basic solutions.

Different solutions including; acetate buffer, phosphate buffer, borate buffer, ammonium chloride buffer, HCl, and NaOH were tested. Based on the information gathered, the basic medium gave the highest signal. When the carrier solution was ammoniacal buffer at pH = 9.5, the analytical signal was at its maximum, because the chlorination of

mebendazole occurs in a slightly basic medium. As a result, this buffer was used as a reaction medium in all following experiments. Similarly, the influence of changing CAA concentration was studied at different concentrations of NaOCl (Fig. 4). The highest absorbance was obtained when the concentration of NaOCl was  $3.0 \text{ mM}$  and the concentration of CAA is  $2.0 \text{ mM}$ . Beyond these levels, there was no substantial increase in the analytical signal.

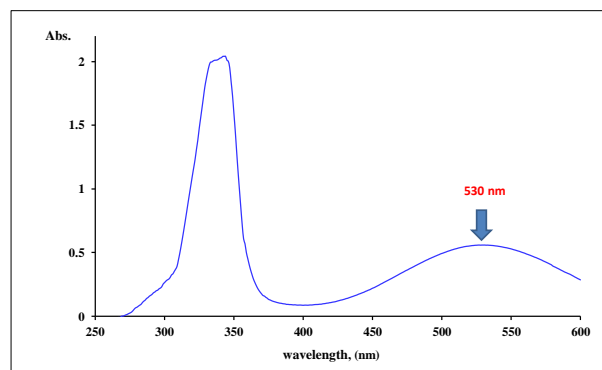


Figure 3. UV-Vis spectra of chloroanillic acid (CAA).

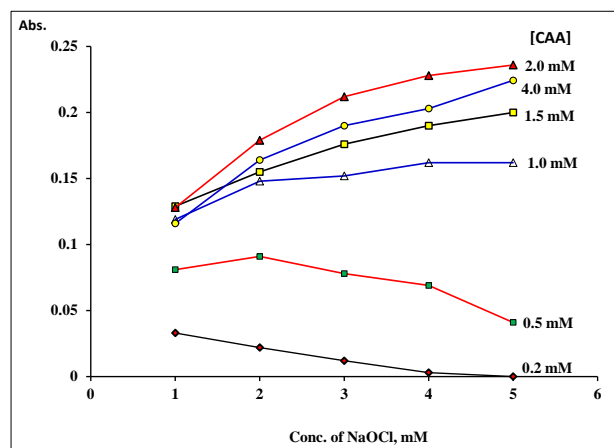


Figure 4. Effect of changing CAA and NaOCl concentrations. [mebendazole] =  $10 \text{ mg L}^{-1}$ . RC1 =  $40 \text{ cm}$ , RC2 =  $60 \text{ cm}$ , sample volume  $60 \mu\text{L}$ , total flow rate =  $1.8 \text{ mL/min}$ , wavelength =  $530 \text{ nm}$ .

The flow rates of reagents streams were optimized with  $3.0 \text{ mM NaOCl}$  as the oxidizing agent and  $2.0 \text{ mM CAA}$ , both at pH = 9.5. At these concentrations, the maximum signal was obtained as indicated by Fig. 3. Keeping other parameters constant, the influence of the total flow rate was investigated over the range of  $0.60 - 3.2 \text{ mL/min}$ . At higher flow rates, a significant decrease in analytic signals is observed due to incomplete reaction at high mixing speed. At lower flow rates, the given time for the reaction is increased and the peak height is decreased due to the increase in the dispersion of the sample zone. The analytical signal was at its maximum when the overall flow rate for the reagents

was 1.80 mL/min. At this flow rate, one injection takes 30 seconds to analyze and therefore, the analysis rate is expected to be 120 samples/hour. Similarly, the influence of the length of the reaction coils was tested. Various lengths for RC1 and RC2 in the range of 30 to 120 cm were used. The maximum analytical signal was obtained when RC1=40 cm long and RC2=60 cm as these lengths give minimum dispersion and sufficient time for the reaction to complete.

### Development of The HPLC Method

Several mobile phases with different mixing ratios of solvents at different pH values were tested during the chromatographic separation optimization

procedure. In terms of peak symmetry and analysis time, the best separation was achieved when the mobile phase composition was 56% acetonitrile, 20% methanol, 23.5% water, 0.5% triethylamine all adjusted to pH= 3 with 1.0 M HCl. The stationary phase used was a reversed-phase C18 column from Varian. The internal standard (IS) used in this study was naphazoline nitrate, well separated from the mebendazole peak and separated in a suitable time. The retention times for mebendazole and the IS under the selected chromatographic conditions were 3.4 min and 2.70 min, respectively. A typical chromatogram for mebendazole and the internal standard at the selected conditions is shown in Fig. 5

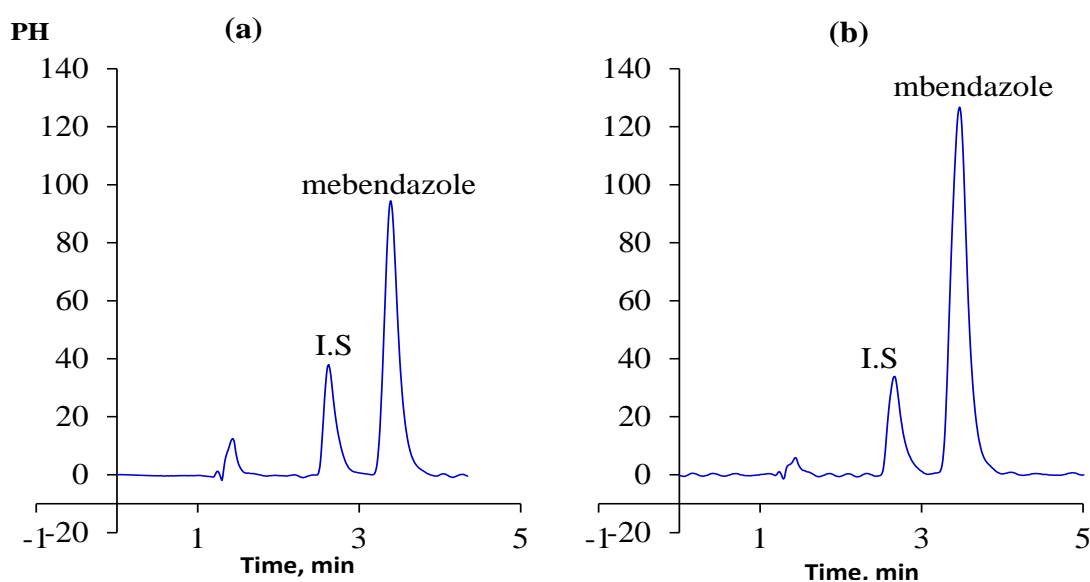


Figure 5. Typical chromatograms for 20 mg L<sup>-1</sup> pure mebendazole (a) and 30 mg L<sup>-1</sup> of mebendazole in the commercial product Vermox (b).

### Validation of the FIA and HPLC Methods

Calibration graphs for the proposed FIA and HPLC methods were constructed by injecting standard solutions of mebendazole. Regression equations for the absorbance-concentration plots were obtained by least-squares analysis. Calibration details for the new methods are given in Table. 1. The plots for the proposed FIA and HPLC methods were found to be linear over the concentration ranges presented in the table. The excellent linearity of the calibration graphs is reflected by the high values of the correlation coefficients.

The limit of detection (LOD) was estimated as the concentration results in a signal equal to three times the standard error in the y-intercept of the calibration curve (3SE/slope). Likewise, the limit of quantitation (LOQ) was determined as 10SE/slope. Results are presented in Table. 1. The intraday (within-day) precision was estimated by replicate

analysis of mebendazole at various periods within the same day. Likewise, the inter-day precision was estimated on several days. In all cases, recoveries obtained for both methods were satisfactory, and the percent RSD values were below 5%.

Table 1. Calibration parameters for the proposed FIA and HPLC methods.

Parameter	FIA method	HPLC method
Linearity range (mg L <sup>-1</sup> )	0.10 – 3.00	1.0 – 60
Intercept (b)*	0.002	0.056
Slope (a)*	0.088	0.149
Corr. Coeff. (r <sup>2</sup> )	0.9988	0.9996
LOD(mg L <sup>-1</sup> )	0.058	0.949
LOQ(mg L <sup>-1</sup> )	0.192	3.162

### Applicability of The HPLC Method

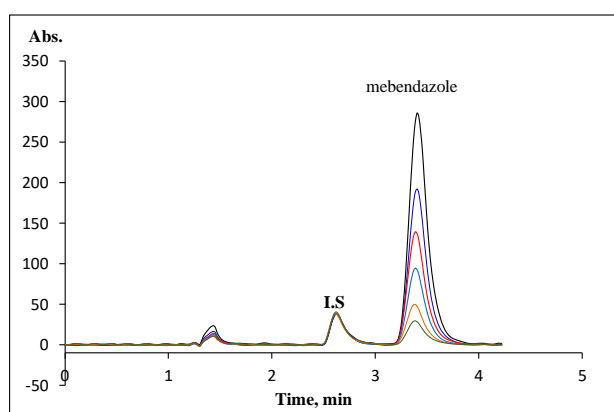
The intraday and inter-day precisions were evaluated by replicating the analysis of mebendazole within the linearity range at different time intervals within the day and at different days. Five measurements at different time intervals injected each time. The results indicated high precision, as the percent RSD was < 2%. Recovery studies were carried out to determine the correctness of the proposed approaches using known concentrations of mebendazole in synthetic mixtures. Common excipients including magnesium stearate, maize starch, lactose, microcrystalline cellulose, and colloidal anhydrous silica have been added in over 100-fold amounts to each solution. To avoid obstruction of the flow system, insoluble materials

were removed by filtration through membrane filters. Table. 2, shows the comparison of the obtained results with the expected values. The data showed no significant differences, and the recoveries were satisfactory at all concentrations.

Moreover, commercial pharmaceutical products were analyzed by the proposed methods. Two commercial products were analyzed in this study. Both methodologies had high recoveries (> 95%), as indicated in Table. 2. The accuracy of the suggested procedures is supported by the proximity of the results to the acceptable values. These findings confirm the applicability of these methods in pharmaceutical analysis. Moreover, there were no additive-induced interferences in any of the analyzed samples (Fig. 6).

**Table 2. FIA and HPLC recovery results from synthetic mixtures containing common excipients and two commercial products.**

product	FIA			HPLC		
	Taken (mg L <sup>-1</sup> )	Conc. found ± SD	%Recovery ± %RSD	Taken (mg L <sup>-1</sup> )	Conc found ± SD	% Recovery ± RSD
synthetic	0.50	0.52 ± 0.04	103.03 ± 1.92	10.00	10.37 ± 0.17	103.67 ± 1.65
	1.00	1.02 ± 0.02	102.46 ± 0.58	30.00	30.61 ± 0.56	102.03 ± 1.82
	1.50	1.50 ± 0.03	100.00 ± 0.87	60.00	59.98 ± 0.97	99.97 ± 1.62
Vermin	0.50	0.48 ± 0.03	96.00 ± 1.46	10.00	9.62 ± 0.10	96.20 ± 1.00
	1.00	0.97 ± 0.02	97.00 ± 1.24	30.00	30.02 ± 0.18	100.07 ± 0.61
	2.00	2.04 ± 0.03	102.30 ± 0.82	60.00	60.02 ± 0.84	100.04 ± 1.41
Vermox	0.50	0.48 ± 0.03	96.50 ± 1.46	10.00	10.25 ± 0.05	102.45 ± 0.53
	1.00	0.97 ± 0.01	97.50 ± 0.72	30.00	31.09 ± 0.85	103.63 ± 1.45
	2.00	2.01 ± 0.04	100.50 ± 0.62	60.00	58.89 ± 0.85	98.15 ± 1.45



**Figure 6. Typical chromatograms for mebendazole in one of the pharmaceutical products (Vermox) (5, 10, 20, 30, 40 and 60 mg L<sup>-1</sup>). The mobile phase was 56% ACN: 20% MeOH: 23.5% H<sub>2</sub>O: 0.5% TEA all adjusted to pH= 3 by 1.0 M HCl.**

### Conclusion:

Results of this study indicated that FIA can be used to assess the concentration of mebendazole in different products with an RSD of < 5% at a speed

of 120 samples.h<sup>-1</sup>. The developed method is sensitive, reliable, reproducible, fast, and cost-effective. The developed method can be used for the analysis of mebendazole in different formulations without any interferences.

The suggested HPLC method has also been employed for the estimation of mebendazole in commercial pharmaceutical formulations. The results indicate that the method is accurate, specific, and precise. The analysis time in the suggested HPLC method is about 4 min for the complete separation of mebendazole from the common excipients generally used in pharmaceutical products. When the recoveries of the FIA and HPLC methods were statistically compared, no significant difference was found between them ( $\alpha > 0.05$ ).

### Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours,

have been given the permission for re-publication attached with the manuscript.

Ethical Clearance: The project was approved by the local ethical committee in Yarmouk University, Jordan.

#### Authors' contributions statement:

I F. AM: corresponding author, methodology design, conceptualization, supervision, writing and editing the manuscript. R. A S: samples analysis, writing the first manuscript and data analysis. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Deanship of Scientific Research and Graduate Studies at Yarmouk University, Jordan.

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## تقدير الميبيندازول في المستحضرات الصيدلانية باستخدام التحليل بالحقن الجرياني والكروماتوغرافيا السائلة

رغد إبراهيم السوقي

ادريس فالح المومني

قسم الكيمياء، كلية العلوم، جامعة اليرموك، اربد، الأردن

### الخلاصة:

تم تطوير طرق كروماتوغرافية وطيفية للتقدير الكمي للميبيندازول في المنتجات الصيدلانية. تعتمد طريقة الحقن الجرياني على أكسدة الميبيندازول بفانض من هيبوكلوريت الصوديوم عند درجة الحموضة = 9.5 ، يليه تفاعل المؤكسد الزائد مع حمض الكلورانيليك (CAA) لإزالة لونه الأرجواني. تمت مراقبة امتصاص CAA المتبقي عند 530 نانومتر. الطريقة سريعة وبسيطة وانتقائية وحساسة. تم تنفيذ الطريقة الكروماتوغرافية على عمود غير قطبي C18. كان الطور المتحرك عبارة عن خليط من أسيتونيتريل (ACN) ، ميثانول (MeOH) ، ماء وثلاثي إيثيل أمين (TEA) ، 56% (CAN) ، 20% (MeOH) ، 23.5% (H<sub>2</sub>O) ، 0.5% (TEA) ، حجم / حجم ، عند درجة الحموضة = 3.0. تم استخدام نترات النفازولين كمعيار داخلي. تم إجراء الكشف عند 290 نانومتر باستخدام كاشف الأشعة فوق البنفسجية. تم تطبيق الطرق بنجاح لتقدير الميبيندازول في المنتجات الصيدلانية ولم يلاحظ أي تداخلات من المواد الشائعة المستخدمة عادة في الصناعة الدوائية.

**الكلمات المفتاحية:** الحقن الجرياني، الكروماتوغرافيا السائلة، ميبيندازول، منتجات صيدلانية، قياس الطيف الضوئي.