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Research

Concurrent Determination of Metronidazole And Fenbendazole In Pharmaceutical Dosage Form By Rp-Hplc

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Abstract

A simple, accurate method was developed for the simultaneous quantification of fenbendazole (FBZ) and metronidazole (MTZ) in bulk and tablet formulation. The chromatogram was obtained using a Phenomenex, Luna C18, 250 × 4.6 mm, 5 mm column with mobile phase 10 Mm KH2PO4 (pH 4) and methanol (70:30 v/v), with a flow rate of 0.8 ml/min at 305 nm and 30°C column temperature. The retention times for FBZ and MTZ were 3.689 and 7.232 minutes, respectively, and the regression coefficients for FBZ and MTZ were 0.9923 and 0.9918. The percentage recovery was 101.97% and 100.03% for FBZ and MTZ, respectively, and the LOD and LOQ values obtained from the FBZ and MTZ regression equations were 0.08, 0.25 and 0.04, 0.12 respectively. The proposed method was simple, robust, unique, and profitable, making it suitable for veterinary laboratories.

Keywords: Febendazole, metronidazole, RP-HPLC, Validation

INTRODUCTION

High-Performance Liquid Chromatography (HPLC) is a widely used analytical technique for separating, identifying, and quantifying components in a mixture. It operates on the principle of liquid-phase chromatography, where a liquid mobile phase carries the analytes through a stationary phase (column), leading to differential retention and separation based on chemical properties such as polarity, molecular weight, and interaction with the stationary phase.[1]

Fenbendazole (FBZ) is a broad-spectrum benzimidazole anthelmintic used to treat nematodal infections, primarily in veterinary medicine. It belongs to the carbamate ester, aryl sulfide, and benzimidazole families and has a molecular formula of $C_{15}H_{13}N_3O_2S$ with a molecular mass of 299.3 g/mol. It appears as a solid powder, has low solubility (0.9 µg/mL), and is stored at 25°C.

FBZ acts by binding to tubulin in parasite cells, disrupting microtubule formation and function, which prevents nutrient uptake and leads to parasite death. This mechanism makes it effective against both adult and

larval parasitic worms. Its IUPAC name is *methyl N-(6-phenylsulfanyl-1H-benzimidazol-2-yl)carbamate*, and it is marketed under the brand name Panacur 150mg Vet. [2,3]

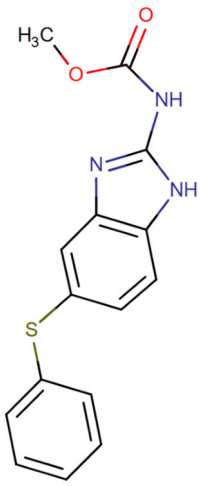

Fig 1: Structure of FBZ

Metronidazole (MTZ) is a nitroimidazole antibiotic used to treat amebiasis, trichomoniasis, giardiasis, and gastrointestinal infections. Unlike typical antibiotics, its antiparasitic properties make it effective against various infections. It is available in topical, tablet, capsule, and suppository forms.

MTZ has a molecular formula of $C_6H_9N_3O_3$, an average molecular weight of 171.154 g/mol, and appears as a solid powder with a solubility of 5.92 mg/mL in water at 25°C. It should be stored at 25°C. Its mechanism of action involves disrupting DNA synthesis in parasites and bacteria, leading to cell death. MTZ is marketed under the brand name Likmez.[4,5]

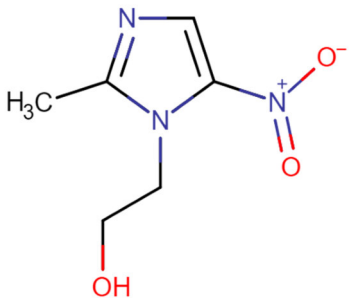

Fig 2: MTZ structure

Several studies have developed analytical methods for MTZ and related drugs. El Bagary et al. introduced a spectrofluorimetric technique using CdS quantum dots for TNZ and MTZ estimation. [6] Patel et al. established an RP-HPLC method for simultaneous quantification of pyrental pamoate, praziquantel, and febantel. [7] Shah et al. developed spectrophotometric and RP-HPLC methods for Fenbendazole and Niclosamide, achieving linearity ($r^2 > 0.995$) and 98-102% accuracy. [8]

Sevaperuman et al. formulated an RP-HPLC method for Dicyclomine HCl, MET, and Furazolidone in capsules, [9] while Maslarska et al. created an HPLC method for detecting Ofloxacin and MTZ in synthetic combinations. [10] Danao et al. designed an HPLC method for MTZ and Diloxanide Furoate in tablets, [11] and Ghante et al. validated an RP-HPLC method for MTZ and Norfloxacin. [12]

Other studies include Khadabadi et al. using RP-LC for MTZ and Ciprofloxacin, [13] Asma et al. measuring MTZ in porcine corneas, [14] and El Houdary et al. developing a gradient RP-HPLC-DAD for MTZ and other antibiotics.[15]

While there is no existing literature on the combination of FEN and MTZ, studies confirm no drug-drug interactions. Since they have distinct mechanisms of action, their combination reduces antibiotic resistance, supporting the development of a novel antibiotic formulation and an RP-HPLC method for its analysis.

MATERIALS AND METHODS

Chemicals and Solvents

MTZ was procured as a gift sample from Abbott Healthcare Pvt., India and FBZ from Intervet India Pvt. Ltd. HPLC grade Water and Methanol, Formic Acid AR Grade. Tablets with 150mg FBZ and 400mg MTZ were punched in our laboratory.

Instrumentation

HPLC analysis was performed using Shimadzu LC 2030C 3D Plus HPLC (Prominence-i series) with Empower-2 software. The Agilent Zobrax C18 column (250 × 4.6 mm, 5µm) was used for separation.

Preparation of Solutions

The standard solution consists of 400 µg/ml MTZ and 150 µg/ml FBZ. After adding enough diluent, the accurately weighed 150 mg FBZ and 400 mg MTZ APIs were put in a 10 ml volumetric and sonicated for 10 minutes. A 10-milliliter flask was filled with one millilitre of the above-mentioned primary solution, and the remaining volume was filled with diluent. We obtained 15µg/ml FBZ and 40µg/ml MTZ by pipetting 1ml of the aforementioned main solution into a 10ml volumetric flask and mixing the diluent to attain the final volume.

Standard Graph Construction

A solution containing 150 µg/ml FBZ and 400 µg/ml MTZ was divided into aliquots of 1, 2, 3, 4, and 5 ml. After transfer, these aliquots were diluted with a diluent to achieve the desired volume in a 50 ml volumetric flask. The final concentrations for FBZ and MTZ ranged from 5 µg/ml to 60 µg/ml. A volume of 10 µl from each sample was injected three times for each concentration, and a calibration curve was generated by plotting the peak area against the drug concentration.

Assay

Tablets labelled 150 mg of FBZ and 400 mg of MTZ were manufactured in our laboratory. The punched formulation was utilised for the experiment.

Method Validation

System suitability was assessed by injecting standard solutions of FBZ (15µg/ml) and MTZ (40µg/ml) five times to evaluate the tailing factor, area, and USP plate count, with an RSD of less than 2%. Specificity was confirmed as no interfering peaks were observed at the drug retention times. Accuracy and linearity were evaluated by analyzing FBZ and MTZ at concentrations ranging from 10–50 µg/ml, with a calibration curve confirming linearity and a recovery rate of 98–102%. Precision was assessed using spiked solutions at 50%, 100%, and 150% levels. Robustness was tested by varying the flow rate and mobile phase composition, with %RSD remaining within acceptable limits.

RESULTS AND DISCUSSION

The optimized chromatographic conditions included a mobile phase of 10mM KH_2PO_4 and methanol (70:30 v/v) with a flow rate of 0.8 mL/min. Separation was carried out using a Phenomenex Luna C18 column (4.6 × 250 mm, 5 µm), maintained at 30°C. The detector operated at a maximum absorption of 305 nm, with an injection volume of 10 mL and a total run time of 8 minutes. Methanol was used as the diluent, ensuring effective sample preparation and analysis. The retention times was found 3.685 for FBZ and 7.223 for MTZ.

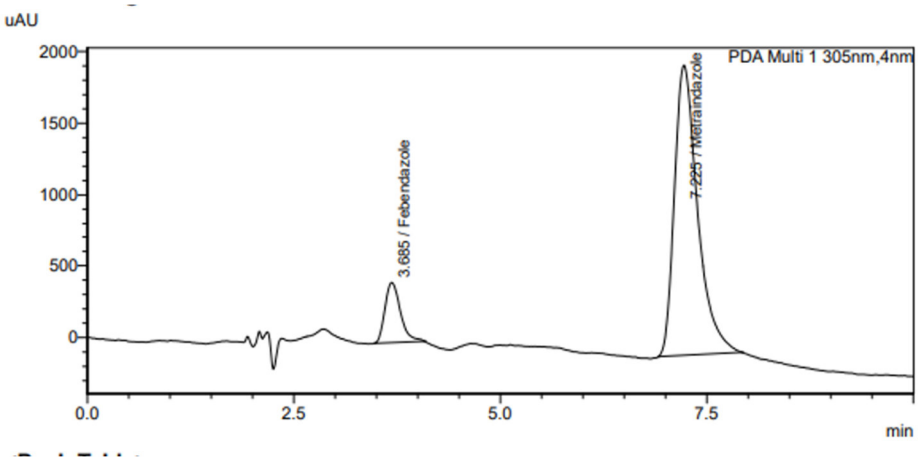

Fig 1: Chromatogram (Optimised)

The following table 1 and Figs. 4 and 5 shows the calibration data of FBZ and MTZ. Correlation coefficients obtained were 0.9923 for FBZ and 0.9918 for MTZ.

Table 1: Linearity data of FBZ and MTZ

Conc (µg/mL)	FBZ (Peak Area)	MTZ (Peak Area)
5	4626	29800
10	5452	39590
20	7387	57733
40	11012	80742
60	13450	106212

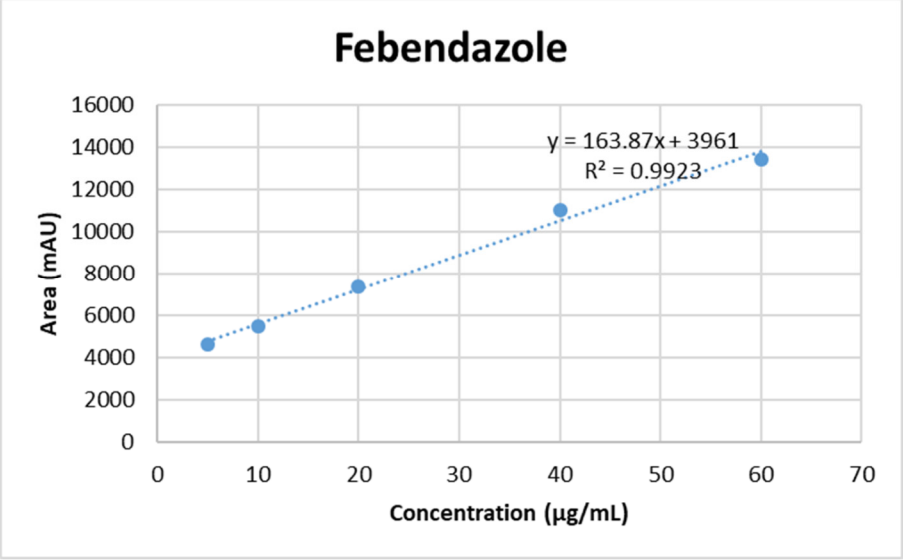

Fig 2: Calibration graph for FBZ

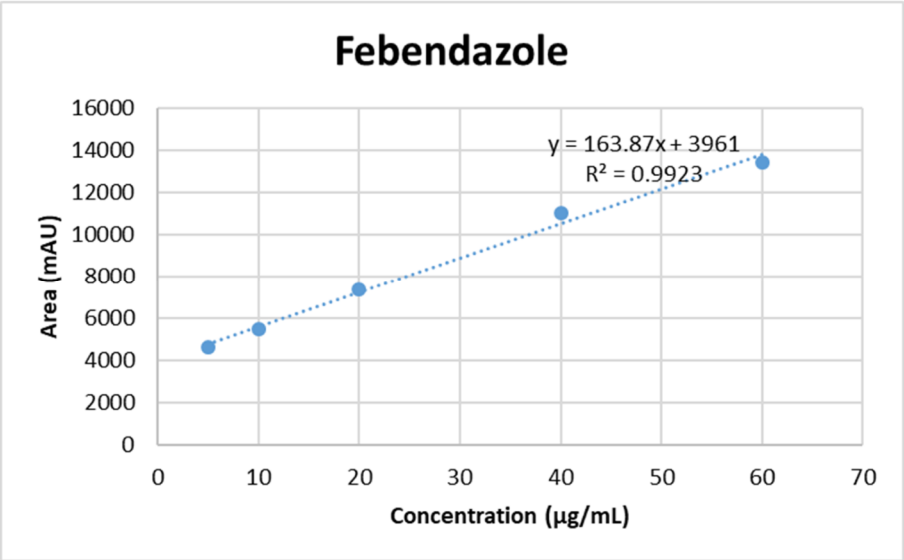

Fig 3: Calibration graph for MTZ

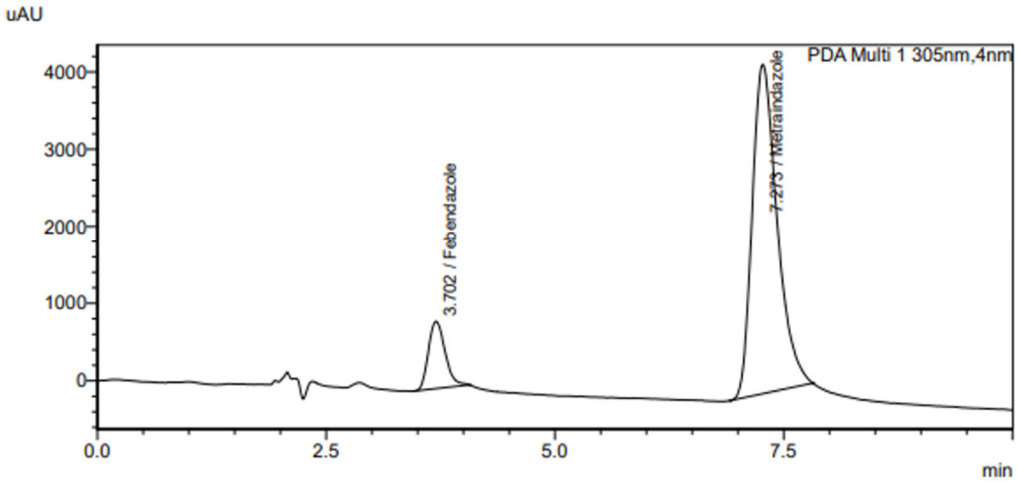

Fig 4: Chromatogram of Assay

The assay results for FBZ showed an average assay value of 99.99%, with a %RSD of 0.6, indicating high accuracy and precision. Similarly, the assay results for MTZ demonstrated an average assay value of 99.92%, with a %RSD of 0.5, confirming consistency and reliability in drug quantification (Fig. 6). The standard and sample areas for both compounds were closely matched, ensuring the method's robustness. The low %RSD values (≤ 1.0%) further validate the precision of the analytical method, making it suitable for routine quality control analysis.

The method validation for Fenbendazole (FBZ) and Metronidazole (MTZ) demonstrated excellent linearity within the 5-60 µg/mL range, with regression coefficients of 0.9923 for FBZ and 0.9918 for MTZ, confirming a strong correlation between concentration and response. The assay results showed 99.99% for FBZ and 99.92% for MTZ, falling within the acceptable 90-110% range, ensuring accurate quantification. Specificity testing confirmed the method's uniqueness, as no interfering peaks were observed.

System precision was validated with %RSD of 0.9% for FBZ and 0.6% for MTZ, while method precision showed 0.9% for FBZ and 0.5% for MTZ, meeting the requirement of %RSD ≤2.0, indicating high reproducibility. Accuracy values were 101.97% for FBZ and 100.03% for MTZ, within the 98-102% range, confirming method reliability.

The limits of detection (LOD) were 0.08 µg/mL for FBZ and 0.04 µg/mL for MTZ, while the limits of quantification (LOQ) were 0.25 µg/mL for FBZ and 0.12 µg/mL for MTZ, meeting the standard S/N ratio

criteria (3:1 for LOD and 10:1 for LOQ). Robustness testing, including variations in flow rate (FM), flow precision (FP), mobile phase composition (MM), and mobile phase precision (MP), resulted in %RSD values below 2.0, confirming the method's stability under minor modifications. These results validate the method as precise, accurate, sensitive, and robust, making it suitable for routine analysis of FBZ and MTZ.

CONCLUSION

An accurate, precise, robust, and cost-effective method for the concurrent estimation of FBZ and MTZ in tablets has been developed. FBZ and MTZ were retained for 3.689 and 7.232 minutes, respectively. The recovery rates obtained for FBZ and MTZ were 101.97% and 100.03%, respectively. LOD and LOQ values of 0.08 and 0.25, and 0.04 and 0.12, respectively, were determined using the regression equation. The regression coefficient for FBZ is 0.9923, whereas for MTZ it is 0.9918. The developed technique was cost-effective and straightforward, rendering it suitable for the analysis of veterinary samples in drug testing laboratories.

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