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A High-performance Thin Layer Chromatography Densitometric Method for the assay of Mebendazole Tablets

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A simple, precise and accurate high-performance thin layer chromatographic (HPTLC) method was developed for the assay of mebendazole tablets. The separation was carried out by using HPTLC Silica gel 60 F254, (20 × 10 cm) with 250 µm thickness using ethyl acetate - ammonia 25% solution (25:0.5) as a mobile phase. HPTLC separation of the drug was followed by densitometry measurement at 310 nm. Mebendazole was satisfactorily resolved with retention factor (Rf) values of 0.51 ± 0.02. The method complied with International Conference on Harmonization acceptance criteria for linearity (250 - 600 ng), precision, accuracy and specificity.

Key words: HPTLC, method validation, assay, mebendazole

INTRODUCTION

Mebendazole (Figure 1) is chemically, methyl 5-benzoyl-2-benzimidazole carbamate [1]. It is a highly effective broad spectrum anthelmintic indicated for treatment of nematode infestations, including round worm, whipworm, thread worm and hookworm. It is poorly absorbed with no systemic effects [2-5] and it is practically insoluble in water, dilute mineral acids, alcohol and methylene chloride [6]. Mebendazole works by selectively inhibiting the synthesis of microtubules in parasitic worms and by destroying extant cytoplasmic microtubules in their intestinal cells thereby blocking the uptake of glucose and other nutrients. This results in the gradual immobilization and eventual death of the helminths [7].

Different analytical methods have been described for the assay of mebendazole in dosage forms and biological fluids such as high-performance liquid chromatography (HPLC), spectroscopic techniques and titration [8]. The official method for the assay of mebendazole in dosage forms as described by United States Pharmacopeia (USP) employs HPLC [9].

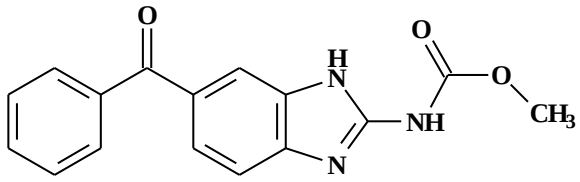

Figure 1: Chemical structure of mebendazole

Even though HPLC is selective and sensitive, it has a number of drawbacks such as utilization of large amounts of solvents and being time consuming, thus making it costly.

On the other hand, high-performance thin layer chromatography (HPTLC) has been employed successfully in the assay of several pharmaceutical dosage forms such as paracetamol tablets, clotrimazole cream and cotrimoxazole tablets [10]. Compared to HPLC,

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HPTLC has a number of advantages such as short run time, capability to analyze multiple samples simultaneously and consume less solvent which reduces analysis costs significantly. In addition, it minimizes exposure risks and significantly reduces disposal problems of organic effluents, thereby limiting environmental pollution [10-14]. Given the merits of HPTLC over HPLC, this work explores its capability in the assay of mebendazole dosage forms.

EXPERIMENTAL

Reagents and solvents

Analytical grade reagents were used during method development and validation. Methanol and glacial acetic acid were manufactured by Scharlau Chemie (Gato Perez, Spain). Ethyl acetate, toluene, and ammonia solution (25%) were from Fisher Scientific (Loughborough, Leicestershire, UK). In-house prepared distilled water was used.

For the determination of specificity, the following excipients were used: microcrystalline cellulose (FMC BioPolymer, Philadelphia, PA, USA); sodium carboxymethyl cellulose and polyvinylpyrrolidone cross-linked (Associate Co. Ltd, Shenzhen, China) and magnesium stearate (Shandong Liaocheng Ehua Medicine, Liaocheng, China). These excipients were selected because of their availability and common use in mebendazole tablets formulations.

Reference standards and samples

Mebendazole reference standard [98% w/w] was obtained from Sigma-Aldrich (Hamburg, Germany). Samples of generic mebendazole tablets (100 mg) manufactured by Elys Chemical Industries (Nairobi, Kenya) were purchased from local pharmacies in Dar Es Salaam region.

Instrumentation

The instruments used during method development consisted of a densitometer with a TLC scanner 3 operated with Wincats (version 1.4.3) planar chromatograph software for data management and integration, a Linomat 5 automatic sample applicator equipped with a Hamilton syringe (100 µl) for sample application and a rectangular flat bottomed developing tank (Camag, Muttenz, Switzerland). Silica gel 60 F_{254} precoated HPTLC plates (Merck, Darmstadt, Germany) of dimensions 5 × 10 cm and 20 × 10 cm were used for chromatographic separation.

Preparation of stock and working solutions

Two stock solutions were prepared, for mebendazole reference standard and mebendazole tablets respectively. Mebendazole solvent consisting of toluene - ethyl acetate - glacial acetic acid (14:4:4) was used for this purpose.

The reference standard stock solution was prepared by dissolving 10 mg of mebendazole CRS in a 10 ml mebendazole solvent with aid of sonication for 10 minutes.

For the sample stock solution, 20 tablets of mebendazole (100 mg) were weighed and finely powdered using mortar and pestle. Powder equivalent to 10 mg of mebendazole powder was weighed into a 10 ml volumetric flask and dissolved in mebendazole solvent.

Further dilutions of the stock solutions were made using methanol to obtain the required concentrations. For reference, 100% solution was taken as 0.1 mg/ml.

Chromatography

Activated HPTLC plates were labeled and the solvent front marked at 70 mm from the bottom edge. Five µl of sample was applied 8 mm from the bottom using a Linomat 5 applicator. Thereafter, the plates were dried in a hot air chamber for 10 minutes before developing them in a saturated tank charged with mobile phase for 20 minutes. Scanning of the developed plate was performed by using a TLC scanner 3 in reflectance absorbance mode supported by Wincats (version 1.4.3) planar chromatograph software.

Development of assay method

Since mebendazole has solubility issues, this work was started by finding the appropriate

solvent for dissolving mebendazole. Different solvents such as toluene, methanol, ethyl acetate, acetone, glacial acetic acid and formic acid were tested. Different ratios of solvent mixtures of varying polarities were investigated until the best solvent system was obtained. Caution was taken to ensure that the chosen solvent mixture would have no interference problem with the main peak.

Method development for the assay of mebendazole tablets was conducted starting with HPTLC conditions previously reported for metronidazole [10]. This method employed a mobile phase containing a mixture of ethyl acetate - ammonia solution, 25 % (25:0.5 v/v) and a detection wavelength of 313 nm. Further work was done on scanning the appropriate ultraviolet wavelength for detection of mebendazole.

Method Validation

Validation of the analytical method was performed by using the optimized HPTLC conditions according to International Conference on Harmonization guidelines [15]. The parameters validated include specificity, linearity, accuracy and precision.

***Specificity*:** Specificity was determined by spotting three solutions; a blank solution of mebendazole solvent consisting of toluene - ethyl acetate - glacial acetic acid (14:4:4), a solution of excipients (placebo) and 0.1 mg/ml mebendazole solution (reference) using the developed method.

***Linearity*:** Evaluation of linearity for the method was performed by serial dilutions of the standard stock solution to five concentration levels ranging from 50% - 120 % corresponding to 250 - 600 ng/spot. Spots were applied on the plate for each concentration starting with lowest concentration to avoid carryover effect. The procedure was repeated for three days. The results were plotted on charts of peak area of the developed spot versus the concentrations spotted. The coefficient of determination, r^2, was used as a measure of linearity.

***Precision*:** Repeatability and intermediate precision were determined for the assay method in which six replicate samples solutions were prepared independently corresponding to 100 % (0.1 mg/ml) level of the assay concentration. Intermediate precision was done by using two analysts on two different days.

***Accuracy*:** In determining accuracy, five concentration levels containing 50 %, 70 %, 80 %, 100% and 120% of mebendazole reference standard were prepared and spotted. Meanwhile placebo solution was spiked by mebendazole reference standard at concentrations corresponding to 80%, 100% and 120 %. These spiked placebo solutions were prepared independently three times and spotted. This was repeated for three days. The percentage recovery from spiked samples was used to determine accuracy of the HPTLC method.

RESULTS/DISCUSSION

Optimization of solvent and mobile phase

The optimum solvent system was established as toluene - ethyl acetate - glacial acetic acid (14:4:4), referred to as mebendazole solvent. This solvent system was subsequently used to dissolve mebendazole standard and samples preparations. Further dilutions were done in methanol.

The starting point for method development utilized a HPTLC method previously reported for metronidazole. The mobile phase consisted of a mixture of ethyl acetate - ammonia solution, 25 % (25:0.5). When this mobile phase was tried as such, it yielded acceptable retention factor (Rf) value of 0.51.

Chamber saturation time was established as 30 minutes while chromatogram development time was 6 minutes at a migration distance of 70 mm under ambient conditions of 23 - 28 °C and 36 - 54% relative humidity. A λmax of 310 nm was found to be optimum and used in all subsequent experiments. Figure 2 represent a typical densitogram of mebendazole obtained under the optimum HPTLC conditions.

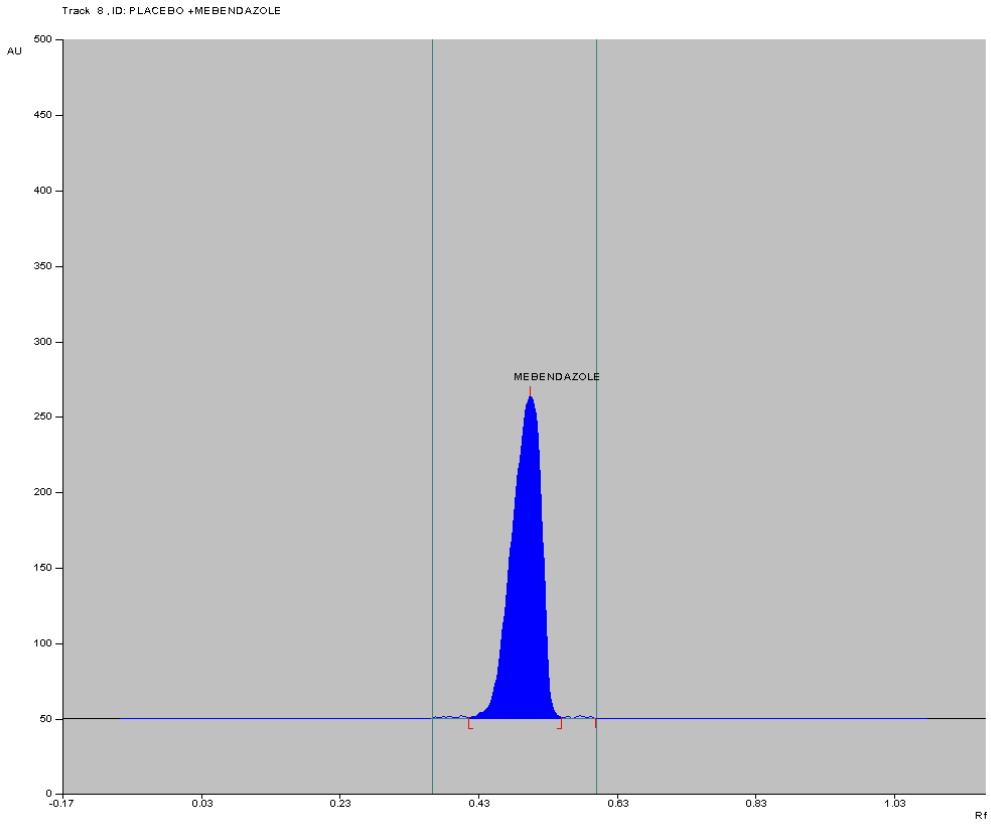

Figure 2: Typical densitogram of mebendazole obtained under the optimized HPTLC conditions.

Concentration - 0.1 mg/ml mebendazole, Mobile phase - ethyl acetate - ammonia solution, 25% (25:0.5 v/v), HPTLC plate dimensions - 5 cm × 10 cm, Application volume 5µL, Detection wavelength - 310 nm.

Method Validation

***Specificity*:** The densitograms obtained (Figure 3) showed the method was specific for the assay and was selective for the active pharmaceutical ingredient since there were no interferences from the blank of solvent or placebo. Hence the method was considered to be specific for routine assay of mebendazole tablets.

***Linearity*:** Table 1 shows intra and inter-day regression coefficient data after analyzing linearity in the given range. The developed method is linear with r^2 for linear regression being 0.994 and polynomial regression at 0.998. In both cases, r^2 was >0.98. Hence, the method complies with the criteria for linearity.

***Precision*:** Table 2 shows precision data obtained after analyzing mebendazole tablets. It can be observed that the percentage relative standard deviations (% RSD) values were found to be 1.5 and 1.6 for repeatability and intermediate precision, respectively. Hence, it can be inferred that the method is adequately precise.

***Accuracy*:** The percentage recovery results obtained are shown in Table 3. In all the three levels examined the percent accuracy range of 99 – 100.2 % which falls within the acceptable criteria for HPTLC methods. Furthermore, the method was applied to perform the assay of commercial mebendazole 100 mg tablet. Results obtained are within the USP limits of 98% to 102%. Hence, it can be concluded that the method is accurate.

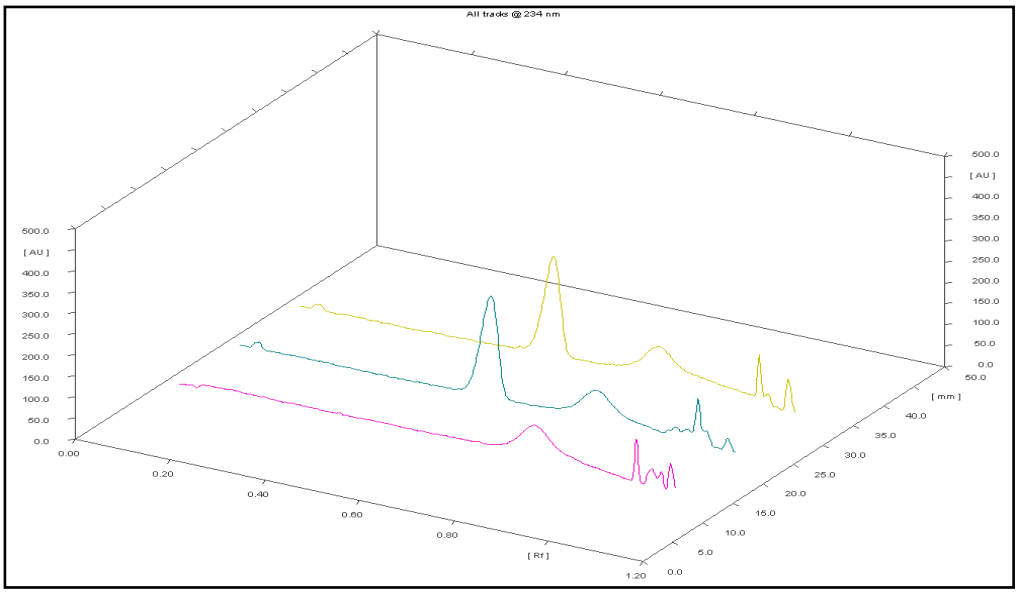

Figure 3: Typical densitograms obtained for blank solution and mebendazole solution.

Mobile phase; ethyl acetate - ammonia solution, 25% (25:0.5 v/v), detection: 310 nm.
Track 1 - blank, track 2 - sample reference standard solution, track 3 - sample solution

Table 1: Regression coefficients for linearity

Parameter	Linear regression	Polynomial regression
Concentration range (ng/spot)	250 - 600	250 - 600
x^2-coefficient	-	-0.0062
Slope	10.488	-
y-intercept	2979	1944
r^2	0.994	0.998

Table 2: Precision parameters

Parameter	Day 1	Day 2	Average
Mean peak area	9777.27	10492.47	10134.87
SD	144.70	176.86	160.78
% RSD	1.5	1.7	1.6
n	6	6	12

n - number of replicate samples, SD - standard deviation, RSD - relative standard deviation

Table 3: Accuracy results of the method

Concentration levels	80%	100%	120%
% Recovery	99.0	99.0	100.8
SD	80.7	234.4	72.6
% RSD	1.58	2.8	0.6
n	9	9	9

n - number of replicate samples, SD - standard deviation, RSD - relative standard deviation

CONCLUSIONS

A simple HPTLC method for the assay of mebendazole tablets was developed. A solvent system for dissolving mebendazole was also optimized. The HPTLC method was validated according to ICH guidelines and proved to be specific, linear, precise and accurate. This method can be used for routine assay of mebendazole tablets.

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