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Short Communication

FLUORIMETRIC DETERMINATION OF MEBENDAZOLE AND FLUBENDAZOLE IN PHARMACEUTICAL DOSAGE FORMS AFTER ALKALINE HYDROLYSIS

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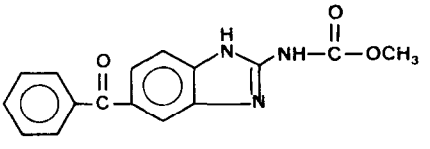
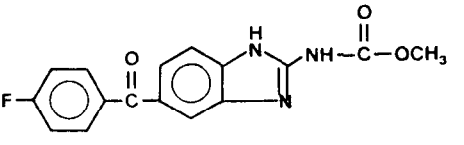
Summary. The selective and very sensitive fluorimetric determination of mebendazole and flubendazole is based on alkaline hydrolysis and adsorption on Whatman 42 filter paper. Limits of detection are 0.1 μg ml^{-1} and 0.5 μg ml^{-1}, respectively, with linear response up to 10 μg ml^{-1} and 50 μg ml^{-1} The fluorescence produced is very stable (λ_{em} = 460 nm) and the method is applicable to anthelmintic pharmaceutical preparations.

Mebendazole [I; (5-benzoyl-1H-benzimidazol-2-yl)-carbamic acid methyl ester] and its monofluorinated derivative, flubendazole {II; [5-(4-fluorobenzoyl)-1H-benzimidazol-2-yl]-carbamic acid methyl ester}, are frequently used as anthelmintic drugs. Most benzimidazole derivatives show intense



(I)



(II)

luminescence characteristics [1—3] (π—π* fluorescence and n—π* phosphorescence). Longworth et al. [4] reported that high fluorescence intensities were observed for benzimidazole and 5,6-dimethylbenzimidazole in various organic solvents. Mebendazole and flubendazole show little or no fluorescence at pH 1—13 in various organic solvents [5], probably because of fluorescence quenching by the carbonyl group. It was thought that the fluorescence behaviour of these compounds might be improved to some extent after hydrolysis with sodium hydroxide. In this communication, practical data are reported for the fluorimetric determination of small amounts of mebendazole and flubendazole, i.e., excitation and emission

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maxima, relative fluorescence intensities, optimum measuring conditions, linear ranges and detection limits. The proposed method is applied in the analysis of some anthelmintic preparations.

Experimental

Chemicals. Mebendazole and flubendazole, generously donated by Janssen Pharmaceutica (Beerse, Belgium), were used as received. The pharmaceutical formulations (Vermox and Flumoxal tablets and suspensions) were obtained from the same manufacturer.

All reagents and solvents used were of analytical grade. Deionized water was used throughout; its quality was checked by fluorimetric scanning at the highest instrumental sensitivity settings.

Apparatus. Fluorescence was measured by using a PMQ-3 Zeiss densitometer in the fluorescence mode; the instrument was equipped with a mercury lamp and a Spectra-Physics Minigrator. The wavelengths for excitation and emission were 365 nm and 460 nm, respectively. The filter paper (Whatman 42) was fixed between two glass plates on the stage of the scanner. The operating instrumental parameters were as follows: scanning speed 50 mm min^{-1}, peak width 1, slope sensitivity 200, baseline 5, tailing peak 60, and minimum area 200. An Aminco-Bowman t.l.c. scanner was unsatisfactory for measuring the fluorescence emission of the dried spots on a filter paper fixed to a specially made sample holder because of stray light. The reflection mode in the Zeiss scanner is important for accurate measurements.

A Camag u.v. lamp (type 29000) was used at 254 nm and at 366 nm for viewing solutions and solid supports. A Nanomat (Camag) apparatus was used to spot 100—200-nl portions of the solutions.

Procedures. Mebendazole or flubendazole (2—5 mg) was dissolved in 10 ml of 1 M sodium hydroxide and the solution was heated in a boiling water-bath for 45—60 min in order to obtain complete hydrolysis.

In order to obtain emission spectra and quantitative results, the hydrolysates were spotted on Whatman 42 filter paper after dilution with methanol—1 M sodium hydroxide (98 + 2, v/v). Spotting was done in lines 1.5 cm apart; the filter paper was dried under an infrared lamp at 65°C for 5—10 min, allowed to cool to room temperature in a desiccator, attached to clean glass plates with tape, and carefully placed on the stage of the Zeiss scanner. The fluorescence signals were recorded and peak heights measured.

All the results given are based on 5—10 independent samples. The precision of measurements was evaluated by repeated spotting and measurement of the same solution.

Results and discussion

Hydrolysis of compounds I and II with 1 M sodium hydroxide produced yellow solutions. These alkaline solutions did not exhibit fluorescence on excitation at 254 nm or 366 nm, nor did their extracts into various organic solvents fluoresce, even when extraction was done from the neutralized or

acidified hydrolysates. Unexpectedly, when the alkaline solutions were spotted on filter paper and placed under u.v. irradiation, strong bluish-white fluorescence was obtained, and when liquid nitrogen was poured over the spots, a very intense bluish-white fluorescence was followed by a long-lasting greenish phosphorescence. These luminescence phenomena were visible even for nanogram amounts per spot, 1 ng for mebendazole and 5 ng for flubendazole. These observations prompted more detailed investigation on the qualitative and quantitative aspects of the active fluorophors.

The most probable hydrolysis reaction for compounds I and II is splitting of the carbamate ester group to form an amino group. The resulting compound is very polar and so is likely to be attached strongly to filter paper through hydrogen bonding to the cellulose hydroxyl groups. This semi-rigidity leads to higher fluorescence yields [6]. It was observed that the intense bluish-white fluorescence of the alkaline spot decreased on acidification (1 M HCl), probably because of protonation of the free amino group formed in the hydrolysis.

The methanolic sodium hydroxide solution used for dilution of the hydrolysates provided good spotting properties (low viscosity) and good solvent properties for the fluorophor. Many commercially available solid supports were tested (Schleicher and Schüll No. 604, Schleicher and Schüll No. 589[3], Whatman 1PS, Whatman No. 5 or 42 and Toyo No. 131 filter papers, silica gel 60 t.l.c. plates, silica reversed-phase (RP-18F 254 HPTLC plates, alumina plates and cellulose plates). Whatman 42 filter paper yielded the highest signal-to-background ratio and the most reproducible readings. Preliminary drying of the spotted plates seems to be necessary for obtaining highest fluorescence signals, as the rigidity of the molecules plays an important role in their luminescence. The fluorescence signal increased by about 10% when air-drying was replaced by infrared drying (65°C) for 5—10 min.

Under the above conditions, the reproducibility of the signals was quite good. The peak heights for spots obtained for mebendazole (8 ng/spot) showed a relative standard deviation of 9.5% (n = 19). The fluorescence spectra of the products from both mebendazole and flubendazole (20 μg ml^{-1}) on the filters showed broad emission bands covering 450—500 nm with the maximum at about 460 nm. The calibration graphs for mebendazole and flubendazole were linear from the detection limits of 0.1 and 0.5 $\mu g\ ml^{-1}$ up to 10 and 50 $\mu g\ ml^{-1}$ in the solution spotted, with correlation coefficients of 0.998 and 0.999, respectively. Table 1 shows the results obtained from the fluorimetric determination of the pharmaceutical preparations.

In contrast to other methods that require preliminary extraction procedures, the proposed method allows direct and rapid estimation of both anthelmintics. No interference was observed from any of the adjuvants in the formulations examined. It is of interest that pure mebendazole in ethanol produced about 1% of the relative fluorescence intensity of the fluorescent derivative resulting from its alkaline hydrolysis. This clearly indicates a possibility of determining the fluorophor if it is present as a decomposition

TABLE 1

Determination of mebendazole and flubendazole in some pharmaceutical preparations by fluorescence scanning on filter paper after alkaline hydrolysis

Compound	Preparation	Average recovery (%)	Standard deviation[a]	Relative standard deviation (%)
Mebendazole	Vermox tablets	99.71	2.34	2.35
	Vermox suspension	100.54	2.32	2.30
Flubendazole	Flumoxal tablets	99.04	2.25	2.27
	Flumoxal suspension	100.56	2.99	2.97

[a] $n \geq 6$.

or metabolic product [7]. A full description of the isolation and structure elucidation of the intensely fluorescing mebendazole derivative will be reported in a later paper.

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