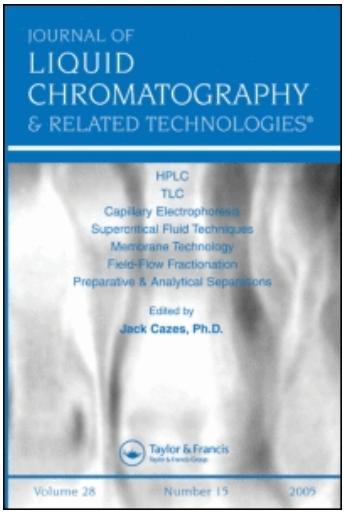

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DETERMINATION OF METRONIDAZOLE BENZOATE IN LIQUID PREPARATIONS BY REVERSED PHASE HPLC

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ABSTRACT

Despite the availability of several commercial suspension formulations of metronidazole benzoate, there is no official method for its analysis. The conventional analytical method requires extensive extraction following each extraction with evaporation then non-aqueous titration with perchloric acid. The method is time-consuming and is not stability-indicating. This proposed HPLC methodology has the advantages of being fast since it requires no chemical manipulation. The assay is stability-indicating being capable of separating the hydrolysis products (metronidazole and benzoic acid) and the common impurity (2-methyl-5-nitroimidazole) from metronidazole benzoate. The method is also specific as it is free of interferences from excipients. The proposed HPLC method was verified for linearity, accuracy and precision and was applied successfully to several commercial suspensions.

INTRODUCTION

Metronidazole benzoate, 2-(2-methyl-5-nitroimidazole-1-yl)-ethyl benzoate (1), is the benzoyl ester of metronidazole, an anti-

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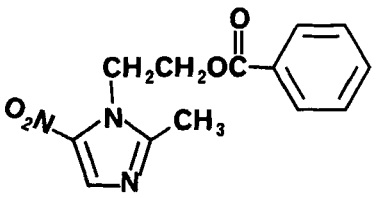
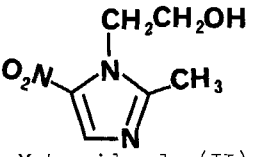
bacterial agent used systemically against a wide variety of anaerobic bacteria and in the treatment of trichomoniasis, amoebiasis and other conditions (1). Metronidazole benzoate is available commercially in the suspension form. The drug hydrolyzes in the gastrointestinal tract to release therapeutic doses of metronidazole over a period of several hours (1).

Several methods have been described for the quantitation of metronidazole in tablets and in biological fluids(2-13). Also, some assays(5,6) have been developed for the analysis of nitro-imidazole compounds. The conventional method for the determination of metronidazole benzoate in pharmaceutical suspensions, requires two steps of extraction with acetone followed by filteration and evaporation of acetone. The residue is then dissolved in acetone and the amount of metronidazole benzoate is determined by non-aqueous titration with perchloric acid using brilliant green as indicator. The method is accurate, but time-consuming and is not stability-indicating. No other method has been reported despite the availability of various commercial suspension formulations of metronidazole benzoate. This paper presents a reversed-phase HPLC method for the determination of metronidazole benzoate (I), its hydrolysis products; metronidazole(II) and benzoic acid(III), and 2-methyl-5-nitro-imidazole(IV) which is usually present as an impurity.

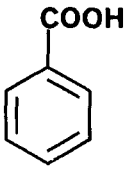
The assay was applied successfully to a number of commercial suspensions and was found free of interferences from excipients normally present in suspension formulations. The elution time was less than 6 min and the detection limit was 10 ng for I . The assay is fast and does not require any sample manipulation.



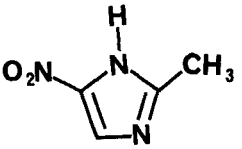
Metronidazole benzoate (I)



Metronidazole (II)



Benzoic acid (III)



2-methyl-5-nitroimidazole(IV)

EXPERIMENTAL

Apparatus

The apparatus employed was Varian 5000 LC HPLC system equipped with a 10-µl manual loop injector (Valco instruments Co., Houston, Texas, U.S.A) connected to spectrophotometric detector (Du Pont Co., Wilmington, DE, U.S.A) and Spectra-Physics 4100 digital integrator (Spectra-Physics, San Jose', CA., U.S.A). A reverse phase column (300 x 4.0 mm) MicroPak MCH-10 (Varian Associates, Inc., Palo Alto, CA, USA) monomeric was used at ambient temperature.

Chromatographic Conditions

The mobile phase consists of 0.02 M ammonium acetate in acetonitrile/water (47/53) adjusted to pH4.5 using glacial acetic acid. The flow rate was 2.0 ml/min. The detector sensitivity was 0.16 AUFS. The wavelength (λ) was 270 nm. Under these chromatographic conditions, II and IV elute near the solvent front and interfere with

the excipients. Therefore, II and IV were determined by changing the acetonirile/water content in the mobile phase to 12/88. It is important to mention that at this composition, I is retained in the column.

The mobile phase and the sample solutions were filtered using 0.45 µm membrane filter (Gelman Instruments, Ann Arbor, Michigan, U.S.A). The mobile phase was degassed by vacuum prior to use.

Materials

Generally, all chemicals were obtained from commercial sources at the highest available purity and were used without further purification. Acetonitrile, methanol and glacial acetic acid were HPLC-grade obtained from Riedal-Dehaan, Merck and BDH, respectively. Ammonium acetate purum grade was obtained from Fluka. Water was always distilled and deionized.

Standards of I and II were obtained from Ethachem S.A. Their purities were certified as 99.9 and 99.7%, respectively. III(99.9%) was obtained form BDH, and IV (99.0%) was obtained from Aldrich. Misonidazole was obtained from Dept. of Health, Maryland, USA and tinidazole was obtained from Dolder. The internal standard, ethyl paraben, was prepared by refluxing p-hydroxybenzoic acid with ethanol in acid medium.

Excipients usually used in the suspension formulations were supplied by AL-HIKMA pharmaceuticals, Amman/JORDAN.

One of the pharmaceutical suspensions (Nidazole) was supplied by AL-HIKMA pharmaceuticals, other products Flagyl(SPECIA), Elyzol (DUMEX LTD) and Metrozole (JPM-JORDAN) were purchased locally.

Preparation of the Standard Solutions

Internal standard solution - The stock internal standard solution was prepared by dissolving 20 mg ethyl paraben in 100 ml methanol. Solutions for linearity containing 0.075, 0.050, 0.40, 0.025 and 0.01 mg/mL were prepared.

Standard solutions for linearity - Standard solutions of metronidazole benzoate containing 0.75, 0.65, 0.5, 0.35, 0.2 and 0.1 mg/mL were prepared in methanol each containing 0.04 mg/mL ethyl paraben.

Preparation of the Sample Solutions

The sample stock solution was prepared by dissolving accurately weighed amounts of suspensions (equivalent to about 5ml) that contain about 200 mg metronidzole benzoate in 100 ml methanol.

Stability Study

The stability of metronidazole benzoate as a function of pH at 80°C was determined as follows. In screw capped tubes, 0.10 g of metronidazole benzoate was added to 2.5 ml of buffer solutions: pH 8.5 (borate buffer), pH 6.3 (phosphate buffer), pH 5.4 and pH 4.0 (phthalate buffer). The mixtures were placed in a thermostated glycerol-water bath at 80°C. Samples were taken at different time intervals, dissolved in 50 ml methanol, 2.5 ml was added to 2 ml of the stock internal standard solution and was diluted to 10 ml with methanol. The solution was filtered through 4.5 um membrane filter and then subjected to chromatographic analysis.

Percent Recovery Study

The excipients normally added to the metronidazole suspension include microcrystalline cellulose, carboxymethyl cellulose, methyl paraben (V), propyl paraben (VI), sodium citrate, Tween 80, sucrose, sorbitol, glycerin, colors and flavors.

The study was performed by preparing synthetic mixtures identical to the suspension formulations and were spiked with known amounts of metronidazole benzoate (150.8, 177.2, 192.5, 216.5, 237.0 and 250.6 mg) spanning the range of 50-150% of the expected assay values. The resulting mixtures were assayed and the results obtained were compared with the expected results.

Analysis Procedure

2.5 ml of the stock sample solution and 2.0 ml of th stock internal standard solution were diluted to 10 ml with methanol. The resulting solution was filtered and 10 ul injections were made into the HPLC and chromatographed under the conditions described above. The reference standard solution was prepared in the same manner from the stock standard solution (200 mg of I in 100 ml methanol) and chromatographed under the same conditions as the sample. The quantity of each component injected was always within the linearity range.

Calculations

The results were calculated using response rations (RR) relative to internal standard based on peak areas:

$$\text{Percent of the label claim found} = \frac{RR_x}{RR_s} \times 100$$

Where RR_x = sample response ratio; RR_s = standard response ratio.

Figure 1. A typical chromatogram of a 10-µl injection of a synthetic mixture of 2-methyl-5-nitroimidazole (IV)(t_R=2.39), metronidzole (II) (t_R=2.9), misonidazole (t_R=3.69) and tinidazole (t_R=5.88). Chromatographic conditions as discribed in the text.

RESULTS AND DISCUSSION

The chromatogram shown in Figure 1 indicates the possibility of separation and quantitation of II and IV using the 12/88 acetonitrile/water mobile phase. Mezonidazole and tinidazole were included as potential internal standards. Figure 2 shows the specificity of the analysis as demonstrated by the complete separation of I from the most possible excipients which elute on the solvent front. Therefore, the separation is free of interferences.

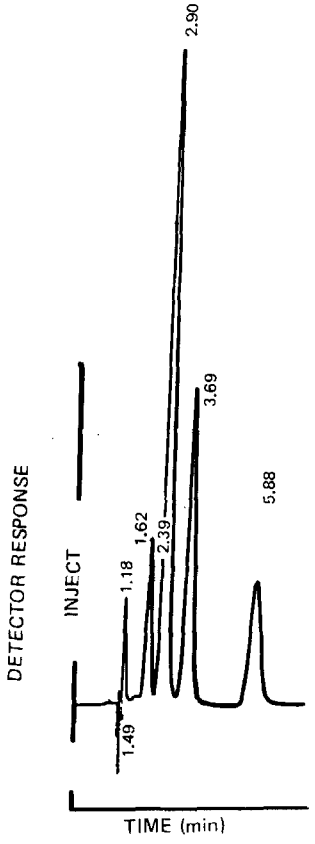


Figure 2. A typical chromatogram of a 10-µl injections of a synthetic mixtue of benzoic acid (III) (t_R=1.95 min), methyl paraben (V) (2.16 min), ethyl paraben (t_R= 2.78 min), propyl paraben (VI) (t_R=3.84 min) and metronidazole benzoate (I) (t_R=5.42 min). Chromatographic condition as described in the text.

Figure 3, a and b shows the chromatograms of one of the pharmaceutical preparations (Nidazole) and its reference standard. This further confirms the specificity of the separation and its applicability to drug analysis.

The linearity of the detector response was determined by preparing calibration standard solutions as decribed in the experimental part. A plot of peak area ratio vs. amount injected for metronidazole benzoate was linear in the range (1.0-7.5 ug) and a plot or peak area vs amount injected for ethyl paraben was linear in the range (0.10 - 0.75 ug) with a correlation coefficient of 0.9995 or better (Figures 4 and 5).

The accuracy of the method was demonstrated by the recovery study, each standard was spiked with a placebo and subjected to HPLC analysis. In all cases satisfactory recoveries and reproducible results of peak area ratios were obtained. A linear regression of the data shows excellent linearity over the analysis srange studied (Table 1).

The detection limit for metronidazole benzoate was 10 ng as determined by diluting the standard solution with methanol and injecting 10 ul into the column.

The results in Table 2 indicate that this HPLC method can be used for the quantitation of I in suspensions. The accuracy of the method was supported by the closeness of the results to the label claim and to those obtained by non-aqueous titration(Table 3). The precision of the HPLC method is supported by the very small relative standard deviation (RSD) based on 6x6 readings. Furthermore, the

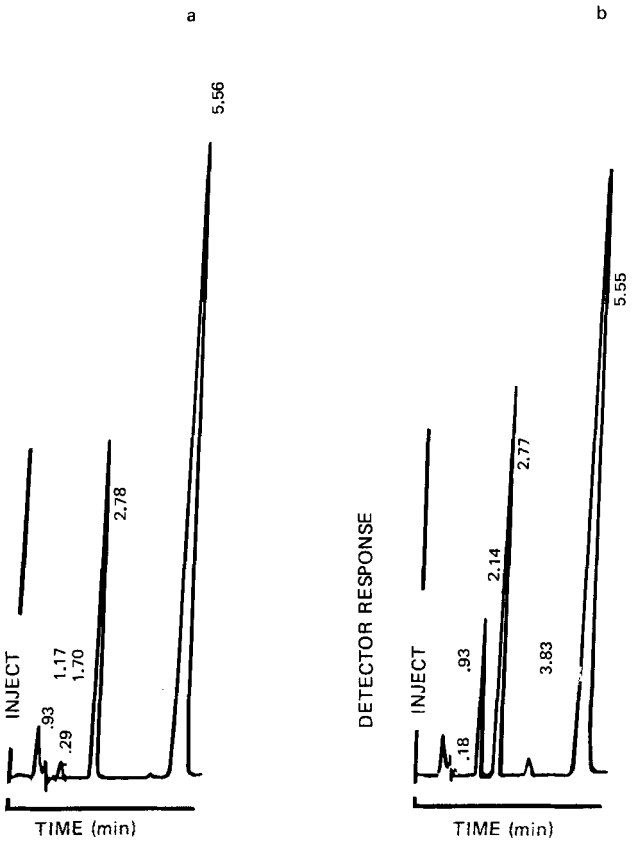


Figure 3. a) A typical chromatogram of a 10-µl injection of a standard containing 5 µg metronidazole benzoate (I) (t_R= 5.56 min) and 0.4 µg ethyl paraben (internal standard) (t_R= 2.78 min).

b. A typical chromatogram of a commercial product (Nidazole). Methyl paraben (V) (t_R=2.14 min), ethyl paraben (t_R=2.77 min), propyl paraben (VI) (t_R=3.83 min), metronidazole benzoate (I) (t_R=5.55 min).

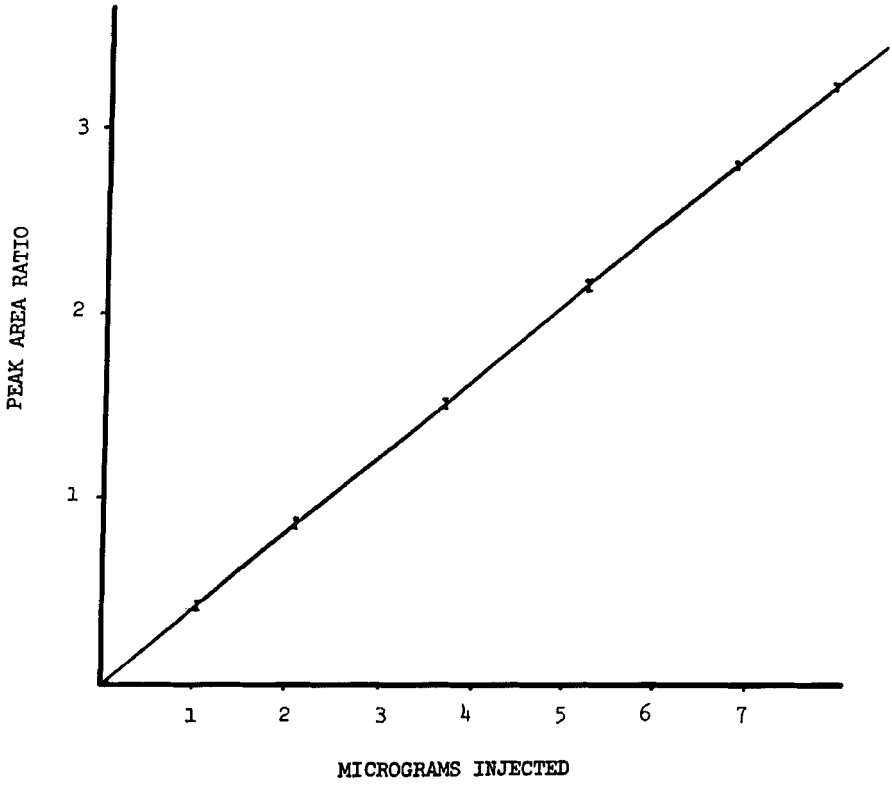


Figure 4. Plot of amount injected of metronidazole benzoate (I) versus peak area ratio.

non-aqueous titrimetric method requires more extensive sample manipulation and is, therefore, time-consuming.

The results of stability study performed on I in duplicate and at different pH values at 80°C are summerized in Table 4. These results show that the maximum stability of I is at pH 4.0.

In conclusion, the HPLC assay described here has been shown to be of general applicability to commercially available products.

TABLE (1)

Recovery Study of Metronidazole Benzoate

mg Added	mg Found[a]	% Recovery[a]
150.8	150.5 ± 0.8	99.8 ± 0.9
177.2	178.5 ± 0.4	100.8 ± 0.4
192.5	193.2 ± 0.6	100.4 ± 0.6
216.5	214.8 ± 0.2	99.2 ± 0.2
237.0	237.4 ± 0.5	100.2 ± 0.5
250.6	250.0 ± 0.8	99.8 ± 0.8

Slope = 0.99; Intercept = 1.78; R= 0.9969

[a]Mean ± RSD for 6 determinations.

TABLE (2)

HPLC Results of Metronidazole Benzoate Suspensions

(%) Lable Claim ± RSD

Sample	Nidazole	Metroazole	Elyzol	Flagyl
1	100.0 ± 0.8	98.6 ± 1.0	99.8 ± 0.4	99.4 ± 0.6
2	99.3 ± 0.6	99.3 ± 0.7	100.5 ± 0.4	100.6 ± 0.5
3	99.8 ± 0.6	99.3 ± 0.6	99.2 ± 0.5	99.9 ± 0.2
4	100.2 ± 1.1	100.3 ± 0.6	99.3 ± 0.7	99.3 ± 0.4
5	100.6 ± 0.9	100.8 ± 0.2	100.2 ± 0.4	100.1 ± 0.7
6	100.6 ± 0.9	99.6 ± 1.2	99.6 ± 0.6	99.8 ± 0.6
Mean ± RSD (n = 6 x 6)	100.0 ± 0.5	99.7 ± 0.8	99.8 ± 0.5	99.9 ± 0.5

TABLE (3)

Non-Aqueous Titration Results of Metronidazole Benzoate Suspensions (%) Lable Claim ± RSD

Sample	Nidazole	Metrozole	Elyzole	Flagyl
1	97.8	99.4	97.6	98.9
2	97.9	101.3	98.9	100.3
3	98.7	100.2	98.8	99.1
4	97.9	100.6	98.8	100.7
5	98.6	102.7	96.8	101.0
6	98.9	100.9	98.5	101.6
Mean ± RSD (n = 6)	98.3 + 0.5	100.8 ± 1.1	98.2 ± 0.9	100.3 ± 1.1

TABLE (4)

Stability of Metronidazole Benzoate at 80°C

pH	Storage Period (Day)	% Found
8.50	28	71.5
6.25	28	76.4
6.35	35	74.6
4.00	35	84.0

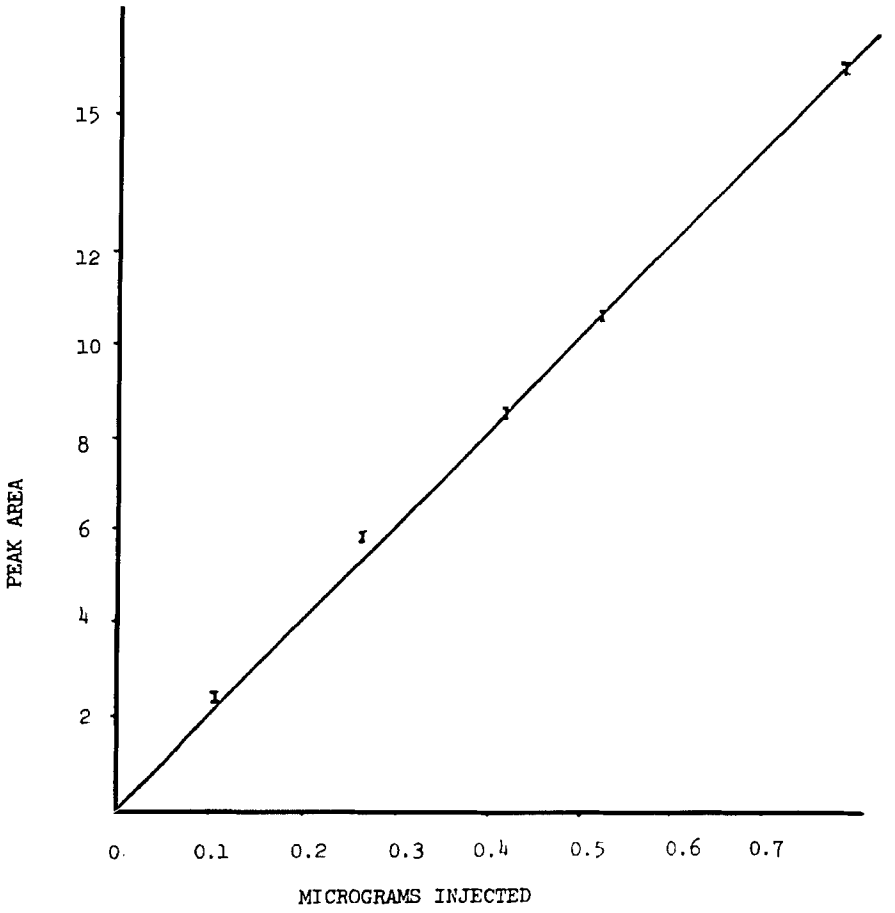


Figure 5. Plot of amount injected of ethyl paraben versus peak area.

The method is accurate, precise, rapid and easy to perform. It can be easily applied for the determination of hydrolysis products and commonly found impurities.

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