An HPLC Study of Tinidazole Hydrolysis

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Key Words

Column liquid chromatography
Tinidazole
Hydrolysis
Kinetics

Summary

The high-performance liquid chromatographic (HPLC) method herein described allows the simultaneous determination of the hydrolysis kinetics of tinidazole and the formation kinetics of the hydrolysis products. Tinidazole is easily hydrolysed under basic conditions at raised temperature. The rate varies with the pH and the temperature of the solution, and the decomposition follows apparent first-order kinetics. The Arrhenius equation can be used to describe the effect of temperature on the half-life.

Introduction

Tinidazole, 1-[2-(ethylsulfonyl)ethyl]-2-methyl-5-nitroimidazole, belongs to the group of 5-nitroimidazoles, which are used in the chemotherapy of infectious diseases such as Amoebiasis, Giardiasis and Trichomonasis and against anaerobic bacteria. It is commercially available both in solid dosage forms and in solutions for infusion. It is recommended that tinidazole and its formulations be protected from light.

As reported previously [1, 2], tinidazole yields 2-methyl-5-nitroimidazole almost quantitatively during hydrolysis in 0.1 M sodium hydroxide solution, and the 4-nitro isomer of tinidazole is formed, again almost quantitatively, in water containing a catalytic amount of base. There are no reports in the literature describing the hydrolysis kinetics of tinidazole, but the stability of another 5-nitroimidazole, metronidazole, in aqueous solutions and in ointments has been investigated very widely [3–10].

The present paper describes a study on the hydrolysis kinetics of tinidazole in basic solutions. HPLC, which is favoured in the quantitation of tinidazole [11–15], was chosen for the determination of the kinetics, but quantitative high performance thin-layer chromatography (HPTLC) could, perhaps, equally well be used [16]. The HPLC method developed is rapid and sensitive and allows the simultaneous determination of tinidazole and its hydrolysis products.

Experimental

Materials

Tinidazole was kindly supplied by Orion Pharmaceutica (Finland). The isolated hydrolysis products of tinidazole [1], 2-methyl-5-nitroimidazole and the 4-nitro isomer of tinidazole, were used as standards in quantitation. The commercially available 2-methyl-5-nitroimidazole was from Aldrich-Chemie (Germany). The identity and purity of the substances were verified by TLC and HPLC, by UV, IR and by ^{1}H and ^{13}C NMR spectrometry. The internal standard metronidazole was obtained from the Farmos Group (Finland). All other reagents were of analytical grade and the solvents of HPLC grade.

Apparatus

The HPLC analyses were performed on an instrument consisting of an LKB 2150 pump, LKB 2151 variable wavelength monitor, D-2000 chromato-integrator (Hitachi, Merck) and a 20 µl loop injector. Compounds were separated on a reversed phase system based on a HP 79915MO-174 RP-8 (10 µm) 200 × 4.6 mm column. The mobile phase was an isocratic mixture of acetonitrile – 50 mM monopotassium phosphate buffer (pH 3 with o-phosphoric acid) 18:82 (v/v) and the flow rate was 1.0 ml/min. Compounds were monitored at a wavelength of 318 nm.

Hydrolysis of Tinidazole

The hydrolysis of tinidazole was carried out in a thermostated water bath in the temperature range 60–80 °C. Tinidazole solutions (5 mM) were prepared in citrate – phosphate – borate/HCl buffers [17], pH 8–12.

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The samples were taken at time intervals of 10 to 40 minutes depending on the hydrolysis conditions.

Calibration Graphs

A stock solution containing 0.2 mg/ml each of tinidazole and its 4-nitro isomer and 0.1 mg/ml of 2-methyl-5-nitroimidazole, and a second solution containing 0.2 mg/ml of metronidazole for use as an internal standard, were prepared in water. To obtain the calibration graphs, 0.5–5.0 ml of the stock solution and 1.0 ml of the metronidazole solution were diluted to exactly 10 ml in water. The calibration graphs were constructed by plotting the peak-area ratios of tinidazole or its hydrolysis products to the internal standard against the concentration of the compound.

Sample Preparation

For the HPLC analysis, 1.0 ml of the hydrolysed tinidazole solution, 2.0 ml of internal standard stock solution and a sufficient volume of neutralising solution (0.1 M HCl) were diluted accurately to 25 ml.

Results and Discussion

The hydrolysis products of tinidazole have been isolated and their structures verified as 2-methyl-5-nitroimidazole and the 4-nitro isomer of tinidazole [1, 2]. The former is used as a starting material in the synthesis of 5-nitroimidazole drugs, and the latter may be present as an impurity formed in the course of the synthesis. The hydrolysis products were easily separated from each other, as well as from tinidazole and the internal standard metronidazole, by the HPLC method developed (Figure 1). The unhydrolysed tinidazole solution was free from impurities. The behaviour of tinidazole in hydrolysis is completely different to that of metronidazole even though the two compounds are structurally closely related. Metronidazole hydrolyses in extremely basic conditions to ammonia, acetic acid and an amine with an available hydrogen [3, 4].

Calibration graphs for tinidazole, its 4-nitro isomer and 2-methyl-5-nitroimidazole showed correlation coefficients better than 0.9999. The degradation of trinidazole and the formation of 2-methyl-5-nitroimidazole could be followed simultaneously (Figure 2). Quantitation of the 4-nitro isomer in hydrolysis solutions was not possible because of the very small amounts formed. Tinidazole was hydrolysed in a citrate – phosphate – borate buffer and the pH was brought to neutral with hydrochloric acid before the diluted and filtered sample was injected onto the column. The effects of pH and temperature were studied. All experiments indicated apparent first-order kinetics. Figure 3 illustrates the effect of pH on the decomposition rate of tinidazole. The hydrolysis kinetics of tinidazole was studied only in basic solutions, but it seemed that approaching neutral pH had a positive effect on the stability. Studies on the hydrolysis kinetics of metronidazole over a wide range of pH values have shown that the compound is degraded very fast at basic pH whilst it is highly stable at pH 4–6 [3, 4]. The hydrolysis of tinidazole was speeded up at higher temperature, as shown in Figure 4.

The half-lives of the hydrolysis varied from a few minutes (pH 12, 60 °C) to eight hours (pH 8, 80 °C). The Arrhenius equation is here used to describe the temperature dependence of the half-life. The equation normally uses the rate constant, but half-life can equally be used, as shown in Figure 5 in which the half-

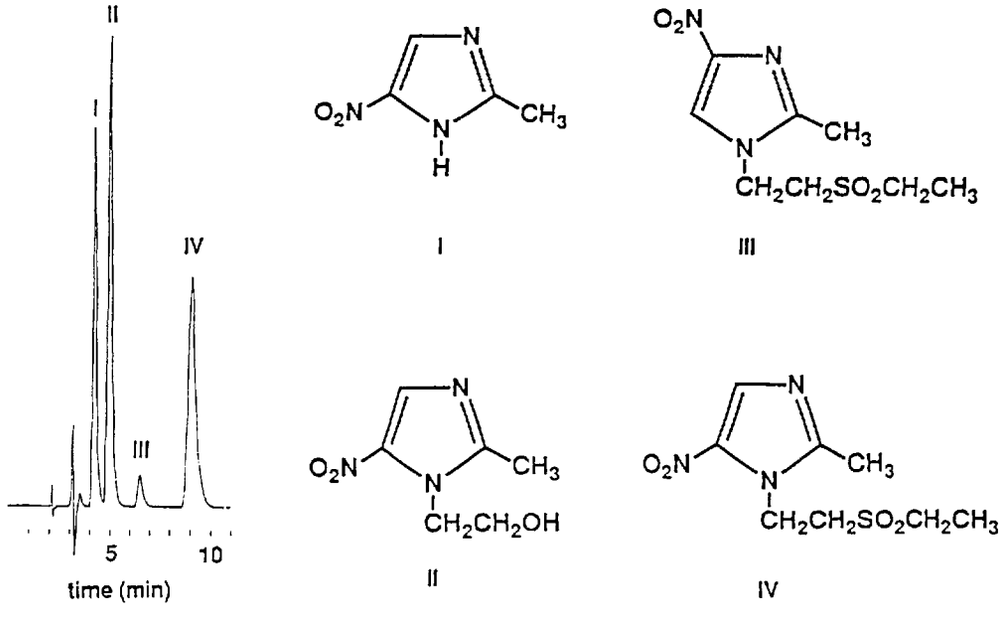

Figure 1

Chromatogram of hydrolysis mixture of tinidazole. **I** = 2-methyl-5-nitroimidazole, **II** = internal standard, **III** = 4-nitro isomer of tinidazole, **IV** = tinidazole.

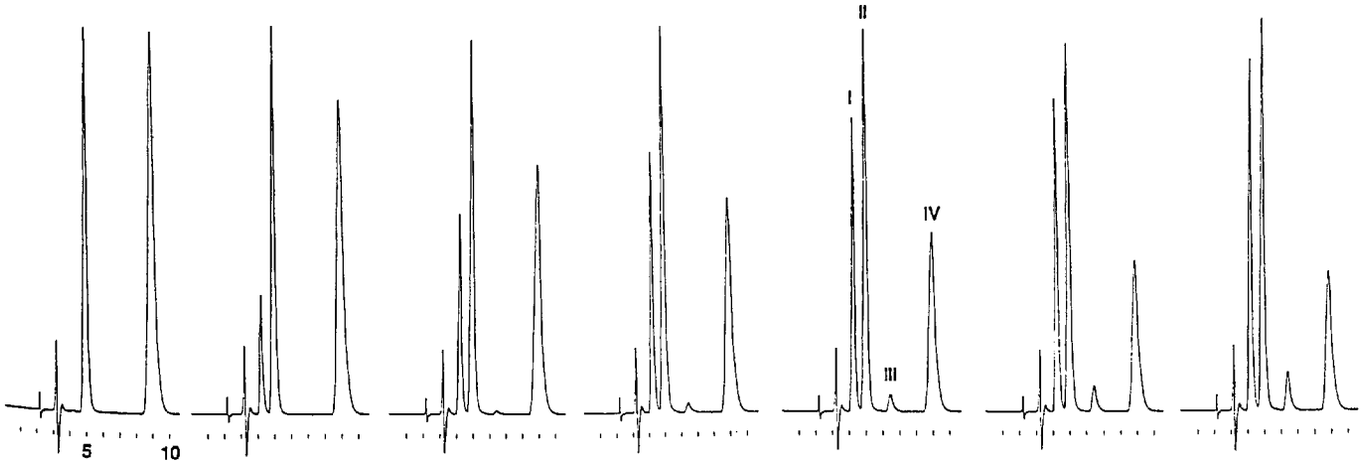

Figure 2

Chromatograms of hydrolysis of tinidazole (5 mM) in citrate – phosphate – borate buffer pH 10.0 at 70 °C after (left to right) 0, 15, 30, 45, 60, 75 and 90 minutes.

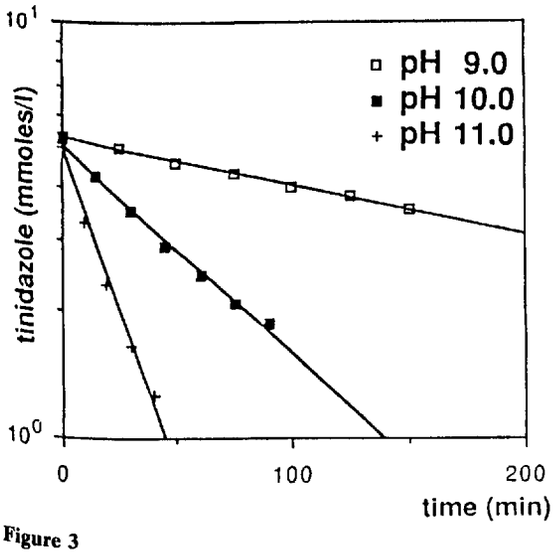

Figure 3

Effect of pH on the hydrolysis of a 5 mM solution of tinidazole at 70 °C.

life is plotted against 1/T. The least-squares line, also shown in the figure, has a correlation coefficient of 0.9994. The activation energy derived from the slope has a value of 130.9 kJ/mole, or 31.3 kcal/mole. For the hydrolysis of metronidazole the activation energy, for a 0.05 % solution in a 50 mM phosphate buffer at pH 8, is 26.6 kcal/mole [3]. According to the Arrhenius equation, the half-life of tinidazole at pH 10.0 at 25 °C would be approximately 42 days and at 20 °C 104 days. This data, combined with the results shown in Figure 3, can be taken as indirect proof of the good stability of a tinidazole infusion solution at room temperature if protected from light.

Of the two hydrolysis products, the formation of 2-methyl-5-nitroimidazole was not logarithmic, apparently because of the small amounts of the 4-nitro isomer formed at the same time (Figure 6). It may be noted that the decrease in the concentration of 2-methyl-5-nitroimidazole at 80 °C is compensated by further

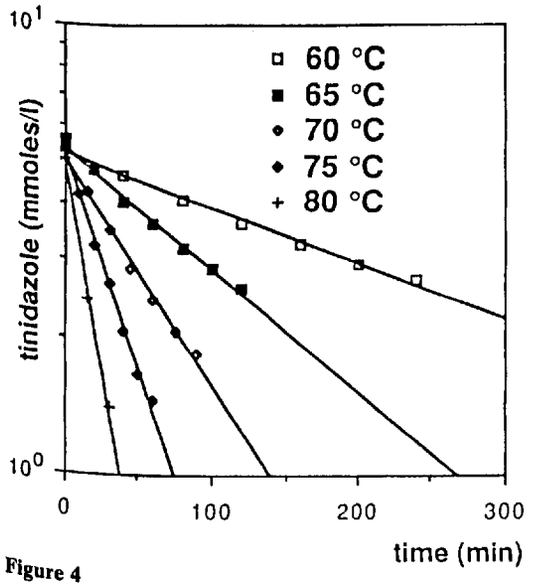

Figure 4

Effect of temperature on the hydrolysis of a 5 mM solution of tinidazole in citrate – phosphate – borate buffer, pH 10.0.

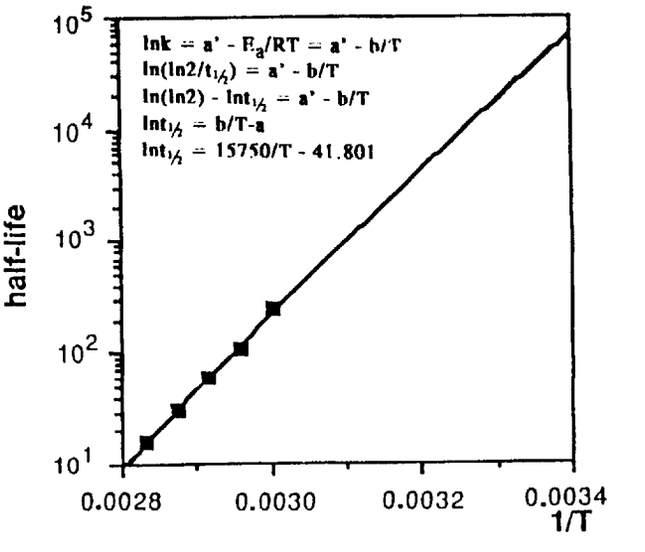

Figure 5

The Arrhenius plot for tinidazole at pH 10.0.

increase in the amount of 4-nitro isomer in the reaction mixture. According to the results obtained here and by Rao et al. [2], the elimination of the N^1 alkyl side chain of tinidazole is the primary reaction in hydrolysis; this is followed by N-realkylation on the other nitrogen atom (N^4).

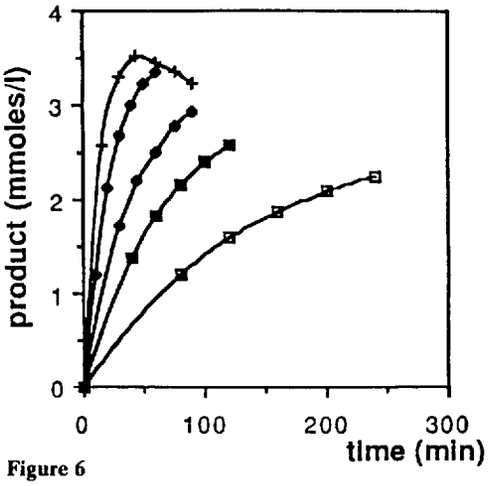

Figure 6

The formation of 2-methyl-5-nitroimidazole at different temperatures at pH 10.0. Codes as in Figure 4.

References

[1] *H.Salomies,* Acta Pharm. Nord. **3**, 211 (1991).
[2] *A.K.S.B. Rao, R. S. Prasad, C. G. Rao, B. B. Singh,* J. Chem. Soc. Perkin Trans. **1**, 1352 (1989).
[3] *S. K. Baveja, A. V. R. Rao,* Indian J. Technol. **11**, 311 (1973).
[4] *S. K. Baveja, H. K. Khosla,* Indian J. Technol. **13**, 528 (1975).
[5] *H. Theuer,* Pharm. Zeit **128**, 2919 (1983).
[6] *C. Bannert, H. Hehenberger, P.-V. Kraus, W. Messerschmidt, R. Pantze, O. Ruckriegel,* Krankenhauspharmazie **4**, 1 (1983).
[7] *T. Visser, A. B. M. van Veen, Th. Vos,* Ziekenhuisfarmacie **5**, 1 (1989).
[8] *S. Ebel, M. Ledermann, B. Mümmler,* Arch. Pharm. **323**, 195 (1990).
[9] *J. J. A. M. Kraus, P. Vermeij,* Pharm. Weekbl. **116**, 840 (1981).
[10] *C. DeMuynck, J. P. Remon,* Drug Dev. Ind. Pharm. **13**, 1483 (1987).
[11] *J. Nachbaur, H. Joly,* J. Chromatogr. **145**, 325 (1978).
[12] *K. B. Alton, J. E. Patrick,* J. Pharm. Sci. **68**, 599 (1979).
[13] *M. Menouer, S. Guermouche, M. H. Guermouche,* J. Pharm. Belg. **42**, 243 (1987).
[14] *S. Ray,* East. Pharm. **32**, 125 (1989).
[15] *S. K. Pant,* East. Pharm. **33**, 137 (1990).
[16] *H. Salomies,* J. Planar Chromatorgr. **5**, 291 (1992).
[17] *T. Teorell, E. Stenhagen,* Biochem. Z. **299**, 416 (1938).

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