ASSAY OF PHOTOLYSED METRONIDAZOLE SOLUTION

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Exposure of the nitro-aromatic drug chloramphenicol to light leads to the formation of products which cause interference with ultraviolet spectrophotometric assay for the drug (Mubarak et al, 1982). The present study examines the validity of ultraviolet spectroscopy as a means of assay for the nitroimidazole drug metronidazole (Fig.1.) in photodegraded solution.

A 0.5% aqueous solution of metronidazole (Sigma Chemical Co.) was deaerated by bubbling with nitrogen and photolysed with simulated sunlight by the method of Evans et al (1975). HPLC examination of the solution was carried out at intervals, using a 15cm C_{18} reversed-phase column with 10% methanol in water as mobile phase, flow rate 2ml/min., sample volume 20 microlitres and ultraviolet detection at 240 nm. Elution of photolysed metronidazole solution showed the presence of numerous unresolved peaks with retention times between 1.3 and 6.0min. Metronidazole eluted as a fully resolved peak at 7.0 min. No combination of methanol, acetonitrile or water was capable of resolving any of the degradation products. The degradation products could not be observed with detection at 310 nm. Metronidazole was determined after 1:25 dilution with water by comparison with a standard curve using the above conditions with detection at 310 nm. The drug was destroyed during photolysis by zero-order kinetics (slope = -1.834, r = 0.996).

The ultraviolet spectroscopic assay was carried out in duplicate by diluting samples 1:500 with 0.05M aqueous sulphuric acid (10%) in methanol, and measuring the absorbance at the maximum at 310 nm in 1cm cells against solvent blank. The difference between duplicate determinations was less than 1% in each case. The maximum decreased in intensity during photolysis (Fig 2) and no new peaks appeared. The gradient was not significantly different to that found using HPLC assay ($t = 0.603$, $t_{0.005(2)15} = 2.131$) and it is concluded that the photodegradation products do not interfere with ultraviolet assay of metronidazole.

Electrolytic or xanthine oxidase reduction of metronidazole's nitro group causes fragmentation of the imidazole ring and production of numerous polar aliphatic products (Knox et al, 1983; Chrystal et al, 1980). The ultraviolet spectral characteristics and HPLC behaviour of metronidazole's photo-degradation products suggests photoreduction of its nitro group leads to a similar fragmentation.

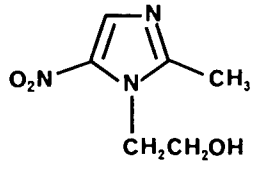

Fig 1. Metronidazole

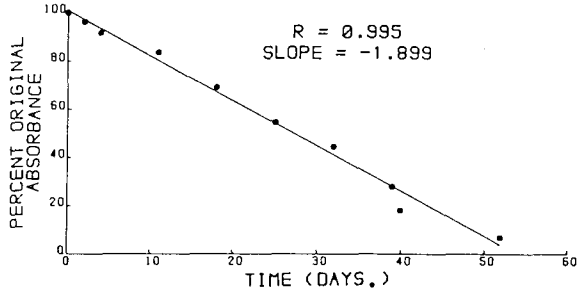

Fig 2. Decrease in absorbance at 310 nm during photolysis (Points are the mean of two replicates)

Chrystal, E.J.T. et al (1980) Mol. Pharmacol. 18: 105-111
Evans, P.G.E. et al (1975) Pharm. Acta Helv. 50: 94-99.
Knox, R.J. et al (1983), Biochem. Pharmacol. 32: 2149-2156.
Mubarak, S.I.M. et al (1982), Pharm. Acta Helv. 57: 338-344.