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Gamma radiation induced effects on metronidazole

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Abstract

The use of ionizing radiation for sterilization of pharmaceuticals is now a well established technology. In this paper, we have studied the stability of metronidazole after irradiation. Trapped radicals, detectable by electron spin resonance (ESR), appear relatively stable and could be quantified. The formation of radiolytic products was evidenced by high performance liquid chromatography (HPLC).

Keywords: Metronidazole; Gamma irradiation; ESR; HPLC

The application of radiation in pharmaceutical technology has steadily increased during the past few years (Bögl, 1985; Jacobs, 1985; Gopal et al., 1988; Zeegers et al., 1993). The advantages of sterilization by irradiation include high penetrating power, low chemical reactivity, low measurable residues, small temperature rise and the fact that there are fewer variables to control. Thus, the sterilization can be carried out on finally packaged products and is applicable to heat sensitive drugs.

Electron spin resonance (ESR) appears to be well suited for determination of free radicals concentration in complex media. ESR measurements can also be used to detect and distinguish irradiated drugs from non-irradiated ones. High performance liquid chromatography (HPLC) is the analytical method of choice for the majority of drug stability protocols.

The purpose of the present work was to investigate the degradation of metronidazole, antibacterial agent, after gamma irradiation by ESR, HPLC and nitrites determination. Samples (100 mg) were irradiated with gamma rays emitted by a radioactive isotope (^{60}Co); the dose rate was 1.8 kGy/h. One non irradiated sample was kept as a

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reference. Nitrite ion was determined with a method based on that of Barnes and Makohon (1993).

Fig. 1 shows the radical number upon doses at ambient temperature after gamma irradiation. The ESR signal amplitude was the average of five replicates for each dose. The free radicals evolution with dose is quasi-linear from 1 to 50 kGy. The irradiation dose used for the radiosterilization of drugs is usually 25 kGy; thus ESR measurements could be used for the evaluation of irradiation dose. Using the accumulation scan technique, stable paramagnetic centers were detectable at 1 kGy. At room temperature, a significant portion of radicals decayed in several days, whereas 35% of free radicals survived after a storage of 135 days. The shape of the decay curve indicate that free radicals could be detected even after a storage of several months.

The decay of radicals upon storage was simulated using a bi-exponential model (Plonka, 1991):

$$\% \text{ free radical evolution} = A_1 \exp(-k_1 t) + A_2 \exp(-k_2 t)$$

From this result, the decay of free radicals could be divided in two phases:

- the first one corresponding to a fast 'pure' exponential model (coefficients A_1 and k_1);
- the second one corresponding to a slowly 'quasi linear' decay (coefficients A_2 and k_2); this component could be attributed to a solid diffusion mechanism (Duroux et al., in press).

Radiolytic degradation products of metronidazole were analysed by HPLC with UV detection at 314 nm (Fig. 2). The direct analysis revealed the presence of ppm levels of radiolytic products and their rate of formation increased with increasing radiation dose in the range 10–200 kGy. The quantitative data relative to irradiation induced impurities are reported in Table 1. The identification of the degradation peaks of the HPLC chromatogramm has been made by cross-injection and comparison of retention time of reference standards. The identified compound are 2-methyl-5-nitroimidazole and 2-methylimidazole.

At 10 and 25 kGy, the irradiated samples fit with the official requirements fixed by the European Pharmacopeia (Galy, 1995)

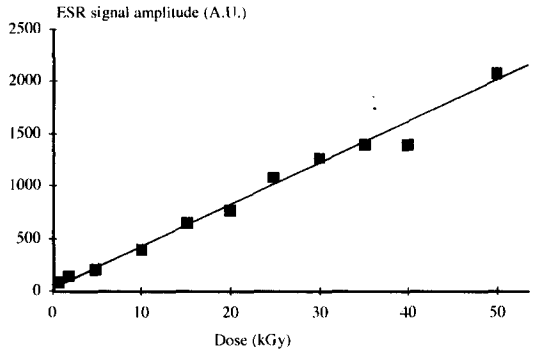

Fig. 1. Free radicals evolution with dose (room temperature). The straight line was obtained by linear regression analysis. Signals were recorded at 0.347 and 0.351 mT. Microwave power: 0.402 mW; modulation amplitude: 0.197 mT; time constant: 40.96 ms.

Nitrite ion levels were determined on unirradiated and irradiated solution of metronidazole (0.5 g w/v) (Table 2). The impact of gamma rays on the nitrite formation appeared to be moderate at 10 and 25 kGy. A level of 20 ppm has been quoted as the maximum acceptable; in the present work, this was not approached for irradiation dose under 25 kGy.

In conclusion, gamma irradiation of metronidazole produces free radicals which appear relatively stable. ESR data could permit the control of the irradiation dose. HPLC measurements evidenced the formation of new degradation products but, at 10 and 25 kGy, the samples fit with the official

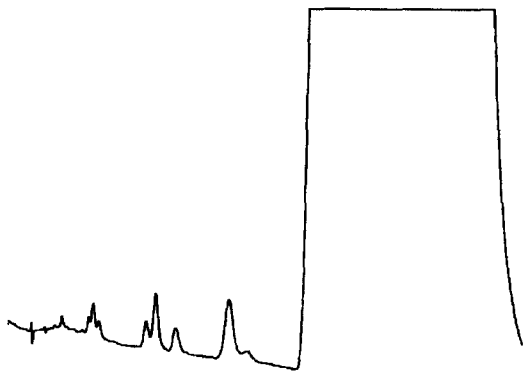

Fig. 2. HPLC chromatogram at 314 nm after irradiation at 25 kGy. Mobile phase: KH_2PO_4 0.05 M/CH_3CN (98:2 v/v). Column Waters μ-Bondapak (300 × 3.9 mm).

Table 1
Effect of radiations on the radiolytic degradation of metronidazole (mean of five replicates)

Dose (kGy)	Degradation products[a] (%)
0	0.03
10	0.04
25	0.07
200	0.24

[a] $= \frac{\Sigma \text{ impurities surface (314 nm)}}{\text{metronidazole surface (314 nm)}}$.

Table 2
Effect of radiations on the formation of NO_2^-

Dose (kGy)	NO_2^- [a] (ppm/100 ml)
0	4.1 (0.4)[b]
10	6.8 (0.9)
25	9.4 (0.9)
200	53.5 (1.1)

[a] Mean of single determination on ten samples.
[b] SD (ppm).

requirements fixed by the European Pharmacopeia.

The identification of the degradation products seems to be fruitful but on a physico-chemical point of view and on the basis of the previous experimental data, the radiosterilization of metronidazole in the solid dry state and in the range 10–25 kGy could be technically feasible.

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