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The effect of gamma radiation on the degradation of Salbutamol

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Abstract

The use of ionizing radiation for sterilization of pharmaceuticals is now a well established technology. Degradation of salbutamol was investigated after gamma irradiation using HPLC and ESR spectroscopy. HPLC evidenced the formation of radiolytic products after gamma irradiation. Salbutamol showed a degradation of nearly 2% at 25 kGy. Sterilization of salbutamol in the liquid state appeared not technically feasible. Simulation of the increase of free radicals versus dose was performed using linear and polynomial regression. These radicals could be detected even after a storage period of more than 12 months. © 1997 Elsevier Science S.A.

Keywords: ESR; Free radicals; Gamma irradiation; HPLC; Radiolytic products; Salbutamol

1. Introduction

There is a current interest in the utilization of gamma radiations for the sterilisation of excipients or drugs [1–6]. Prior to the marketing of a drug sterilised by such means, it is necessary to establish that the irradiation treatment does not cause the drug to become unsafe or otherwise unsuitable for use. Accordingly, all drug products sterilised by irradiation are regarded as new drugs.

Radiosterilisation has the following two problems:

- Gamma irradiation produces new radiolytic products; to prove the safety of radiosterilisation, it is important to determine the radiolytic products and elucidate the mechanism of radiolysis. High performance liquid chromatography (HPLC) is the analytical method of choice for the majority of drug stability protocols; it is a selective technique, allowing the separation and possible measurements of degradation products.
- The regulations governing radiosterilisation vary from one country to another. In the international market of the future, there will be a number of drugs that will be irradiated by gamma rays. Thus, it is desirable to establish a method of discrimination between irradiated and unirradiated drugs and to evaluate the dose of irradiation. Electron spin resonance (ESR) appears to be well suited to determine

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and quantify free radicals in complex media [7,8].

The purpose of the present work was to investigate by HPLC and ESR the degradation of Salbutamol (Albuterol) after gamma irradiation.

2. Experimental

2.1. Reagents and solutions

Salbutamol (micronized) was kindly supplied by Laboratoires GLAXO (Evreux, France). Water was de-ionized and double distilled prior to use. All other reagents were of analytical grade and were used as received.

2.2. Irradiation

Samples were irradiated with gamma rays emitted by an IBL 460 (^{60}Co); the dose rate was 1.7 kGy h^{-1}. One unirradiated sample was kept as reference.

2.3. Apparatus

The chromatographic separation was based on that described in the USP XXIII for Albuterol with a slight modification in the methanol/buffer ratio to obtain suitable retention times. The separation was carried out on a Waters μ-Bondapak C18 column (300 × 3.9 mm). The eluent was 5 mM hexanesulfonic acid sodium salt in 1% glacial acetic acid–methanol in a ratio of 80/20 (v/v). The prepared eluent was delivered by a BISCHOFF pump at a flow rate of 1 ml min^{-1}. A KRATOS SPECTROFLOW 783 UV-Visible variable wavelength detector was used (280 nm). Oxidative amperometric measurements were performed using a 400 EC EGG Princeton potentiostat. The potential applied to the glassy carbon electrode was 1000 mV versus silver–silver chloride electrode (SSE). Prior to use, sensitivity and reproducibility were achieved by an electrochemical pretreatment involving cyclic voltammetry where the electrode was cycled between -1 and $+0.6$ V for 5 min at a scan rate of 100 mV s^{-1} [9]. Sample introduction was via a Rheodyne model 7125 injection valve, fitted with a 20 µl loop for direct injection.

ESR spectra were recorded at room temperature using a BRUKER ESP 300 E spectrometer equipped with a variable temperature control apparatus, a data acquisition system and using the following parameters: microwave power: 0.402 mW; modulation amplitude: 0.1 mT; time constant: 40.96 ms; field: 341.3–356.3 mT. All spectra were recorded using the accumulation scan method.

2.4. Samples from the solid state irradiation studies

Before analysis by RP-HPLC, 5 mg of Salbutamol was dissolved in 1 ml of eluent. For the ESR measurements, 10 mg of substance was weighted with an accuracy of 0.2 mg.

2.5. Samples from the liquid state irradiation studies

A 0.5 mg ml^{-1} stock solution was prepared by dissolving 25 mg of Salbutamol in 0.9% NaCl solution. This preparation was acidified with H_2SO_4 to pH 3.5, complete to 50 ml with the saline solution above and desaerated.

Vials (1 ml) were filled with 0.8 ml of this solution and sealed. All samples were triplicated.

3. Results and discussion

3.1. HPLC

3.1.1. Irradiation in the solid state

The impurity profiles were recorded using UV and electrochemical detection and appeared similar. The chromatograms of irradiated samples (25 kGy) are shown in Fig. 1. Other samples (irradiated and non irradiated) were examined and found to be similar in their impurity profiles. The amount of impurities and degradation products was determined at 280 nm. We assumed that the relative molar response factor (RRF) for an impurity was equal to one (i.e. the molar response factor of impurities at 280 nm were equal to the molar response factor of Salbutamol at 280 nm). The predominant impurity was 5. An increase in

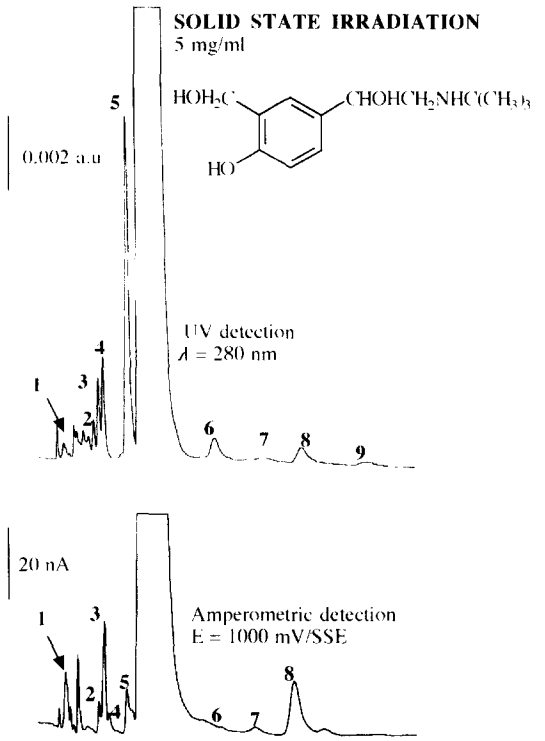

Fig. 1. Typcial HPLC chromatoghram of Salbutamol after radiosterilization (25 kGy).

the irradiation dose caused the amount (%) of impurities to increase (Fig. 2). This curve was modelized by a smooth linear function:

$$\text{impurities}(\%) = 0.158 + 0.0056D \text{ (dose in kGy)}$$

Salbutamol showed a degradation of nearly 2% at

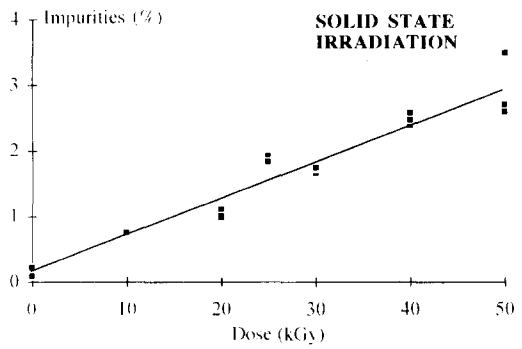

Fig. 2. Influence of radiation dose on the formation of impurities (three replicates).

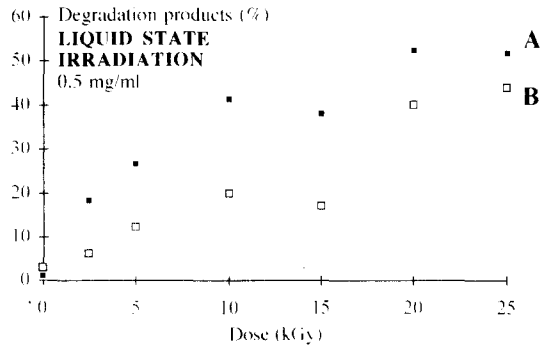

Fig. 3. Influence of radiation dose on the degradation of Salbutamol.

25 kGy. We are currently working to identify impurities using additional means of analysis (e.g. mass spectromery).

3.1.2. Irradiation in the liquid state

The dose dependence of the amount (%) of impurities is represented in Fig. 3. The impurity profiles were similar to those obtained during the irradiation in the solid state.

It can be seen that sterilization of Salbutamol in the liquid state is not technically feasible due to the high level of degradation.

3.2. ESR

A typical ESR powder spectrum after irradiation at 25 kGy is presented in Fig. 4. Using the accumulation scan method, stable paramagnetic centers were detectable even at 1 kGy.

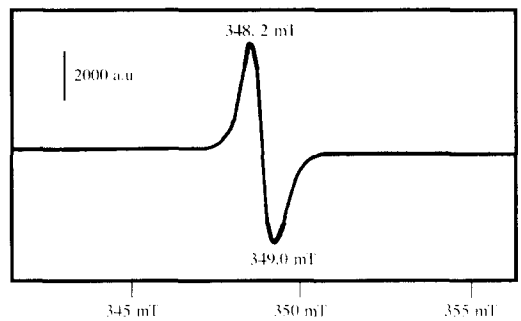

Fig. 4. ESR spectrum (25 kGy).

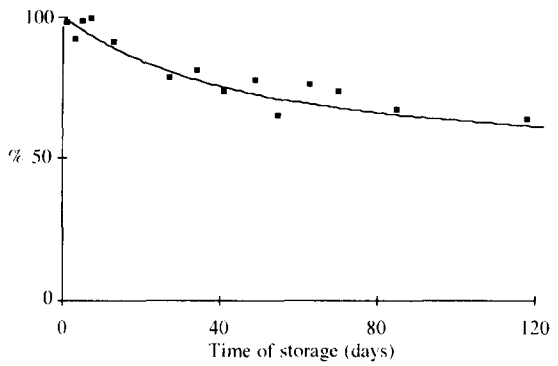

Fig. 5. Decay of radicals upon storage.

From Fig. 4, changes in the ESR signal was followed by monitoring the maximum height (peak to peak) of the spectra at 348.2 and 349.0 mT.

Tests were carried out to investigate wether storage had an effect on the free radicals concentration. Storage at ambient temperature in a sealed quartz tube over several weeks (120 days) showed a variation of the level of free radicals. The decay was simulated using a bi-exponential model [10] (Fig. 5):

level of free radicals (%)

$$= 26.99 \exp(-0.0323x) + 73.01 \exp(-0.0012x)$$

where x is the storage time in days

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

- the first one corresponding to a fast 'pure' exponential model (coefficients 26.99 and 0.0323);
- the second corresponding to a slowly 'linear' decay (coefficients 73.01 and 0.0012). This component could be attributed to a solid diffusion mechanism [11].

From the results above, discrimination of irradiated drugs from non-irradiated ones could be possible even after storage longer than 12 months.

Fig. 6 shows the free radical dependence on dose at ambient temperature after gamma irradiation. Numerical simulation of the results were performed using linear regression (A) and polynomial regression (B) analysis:

(A) level of free radicals (a.u) $= 762.0 + 299D$

(B) level of free radicals (a.u) $= 542.0 + 369.5D - 2.47D^2$ where D is the irradiation dose (kGy); $-2.47D^2$ was introduced as a corrective term.

From this results, two points can be discussed:

- evaluation of the irradiation dose by post-irradiation using linear regression (A) could be possible for doses lower than 15–20 kGy;
- for a dose of 25 kGy (dose currently used for radiosterilisation), estimation of the dose could be considered using the polynomial regression (B).

4. Conclusion

Gamma irradiation causes some degradation in the solid state and unacceptable levels in solution.Some additional means of analysis is required for the validation of sterilization by gamma irradiation (modification of the chromatographic conditions [12], structure of the impurities).

Gamma irradiation produces free radicals which appear to be relatively stable; ESR measurements can be used for detection and discrimination of irradiated drugs from unirradiated ones. The shape of decay curve upon storage indicate that the free radicals could be detected even after a storage of several months. The increase in the ESR signal versus dose was simulated using linear and polynomial regression analysis. Estimation of the irradiation dose could be possible if:

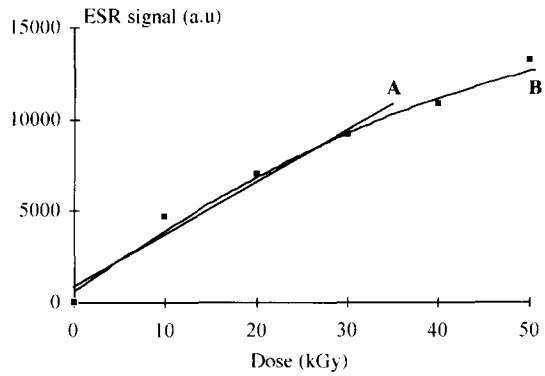

Fig. 6. Free radicals dependence on dose.

- the decay curve of the radicals upon time is measured;
- if the date of irradiation is known, the dose could be evaluated by post-irradiation using a polynomial regression.

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