ELSEVIER

International Journal of Pharmaceutics 149 (1997) 85–91

international journal of pharmaceutics

Radiosterilization dosimetry by ESR spectroscopy: ritodrine hydrochloride and comparison with other sympathomimetics

J.P. Basly [a,*], M. Bernard [b]

[a] *Laboratoire de Chimie Analytique et Bromatologie, Faculté de Pharmacie, Université de Limoges, 2 rue du docteur Marcland, 87025 Limoges Cedex, France*

[b] *Laboratoire de Biophysique Pharmaceutique, Faculté de Pharmacie, 2 rue du docteur Marcland, 87025 Limoges Cedex, France*

Received 13 September 1996; accepted 14 December 1996

Abstract

The use of ionizing radiation for sterilization of pharmaceuticals is now a well-established technology. The purpose of the present work was to apply the ESR spectroscopy to the irradiation dosimetry of ritodrine hydrochloride. Numerical simulation of the free radicals dependence on dose at ambient temperature was performed using five models. The best fit between experimental and calculated values for a dose range of 0–50 kGy was obtained with a polynomial function and the Hill function. These five models were applied to five other sympathomimetics (terbutaline, fenoterol, orciprenaline, salbutamol and isoproterenol) with the same conclusion. Decay of radicals upon storage was performed using a bi-exponential model. Discrimination from irradiated to unirradiated samples by ESR is possible even after a storage of several months. HPLC evidenced the formation of radiolytic products after gamma-irradiation. Ritodrine hydrochloride showed a degradation of 2.8% at 25 kGy. © 1997 Elsevier Science B.V.

Keywords: Ritodrine hydrochloride; β-Agonist; ESR spectroscopy; Dosimetry; Decay of radicals; HPLC; Radiolytic products

1. Introduction

Radiation sterilization technology and its application in the manufacture of pharmaceuticals and cosmetics are being more actively investigated now than at any other time (Jacobs, 1995; Reid, 1995; Boess and Böegl, 1996; Tilquin and Rollmann, 1996). Research carried out in the early 1970s focused on the treatment of pharmaceuticals with high doses an of radiation. This often resulted in unacceptable colour, odor and viscosity changes, as well as undesirable chemical changes. However, with the advances made in aseptic processing, we now have products and materials which are much cleaner, from a microbiological point of view, and thus are likely to

* Corresponding author.

PII S0378-5173(96)04855-7

require much lower radiation doses to achieve 10^{-6} SAL (Sterility Assurance Levels). It may be the only way to sterilize many biologicals or biologically derived products because of their sensitivity to heat.

The radiosterilization, however, has the following problems:

(a) Gamma irradiation produces new radiolytic products; to prove the safety of radiosterilization, it is important to determine and quantify the radiolytic degradation and elucidate the mechanism. 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.

(b) The regulations of radiosterilization differ among countries. In the international markets 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 to discriminate between irradiated and unirradiated 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 between irradiated drugs and unirradiated ones (Gibella et al., 1993; Miyazaki et al., 1994).

Following previous studies on drugs radiosterilisation (Basly et al., 1996a,b,c,d; Fauconnet et al., 1996), the purpose of the present work was to investigate the possibility of using ESR as dosimetric methods after irradiation of ritodrine hydrochloride. Comparison of this results with those obtained previously for other sympathomimetics were considered (Table 1).

Table 1
Symbols used in this study

Symbols	Compound name	Origin
1	Ritodrine hydrochloride	Solvay Duphar B.V. (NL)
2	Terbutaline sulfate	Astra (F)
3	Fenoterol hydrobromide	Boehringer Ingelheim (F)
4	Orciprenaline sulfate	Boehringer Ingelheim (F)
5	Salbutamol	Glaxo (F)
6	Isoproterenol hydrochloride	Aldrich (USA)

2. Materials and methods

2.1. Reagents and samples

Water was de-ionized and double distilled; all other reagents were of analytical grade and were used as received. Before chromatographic analysis, 5 mg of ritodrine hydrochloride was dissolved in 1 ml of eluent. For the ESR measurements, 10 mg of substance was weighted with accuracy of 0.2 mg.

Table 2
ESR parameters

Compound	1	2	3	4	5	6
Sweep field (mT)	341.3–356.3	341.3–356.3	341.3–356.3	341.3–356.3	341.3–356.3	341.2–346.2
Frequency (GHz)	9.78	9.78	9.78	9.78	9.78	9.65
Microwave power (mW)	0.4	0.4	0.4	0.4	0.4	10
Modulation	100	100	100	100	100	100
Frequency (kHz) gain	63 000	63 000	63 000	63 000	63 000	3200
Modulation amplitude (mT)	0.1	0.1	0.1	0.1	0.1	0.2
Time constant (ms)	41	41	41	41	41	164
Accumulation scan	25	25	25	25	25	No
Sweep time (min)	2.1	2.1	2.1	2.1	2.1	2.7
Peak to peak height determination (mT)	347.4	348.0	347.0	346.8	348.2	342.9
	348.7	348.9	349.9	349.6	349.0	344.7

Table 3
Mobile phases used in this study

Compound	Mobile phase (methanol/acetic acid/water % ratio)	Ion pairing agent
1	30.0/0.7/69.3	Na hexanesulfonate 3.5 mM
2	20.0/0.8/79.2	Na hexanesulfonate 4 mM
3	20.0/0.8/79.2	Na hexanesulfonate 4 mM
4	10.0/0.9/89.1	Na hexanesulfonate 4 mM
5	20.0/0.8/79.2	Na hexanesulfonate 4.5 mM
6	10.0/0.9/89.1	Na heptanesulfonate 4.5 mM

2.2. Irradiation

Samples (30 mg) were irradiated with gamma rays emitted by an IBL 460 (^{60}Co); the dose rate was 1.6 kGy/h. One unirradiated sample was kept as reference.

2.3. Apparatus

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 parameters described in Table 2.

Changes in the ESR signal was followed by monitoring the maximum height (peak to peak) of

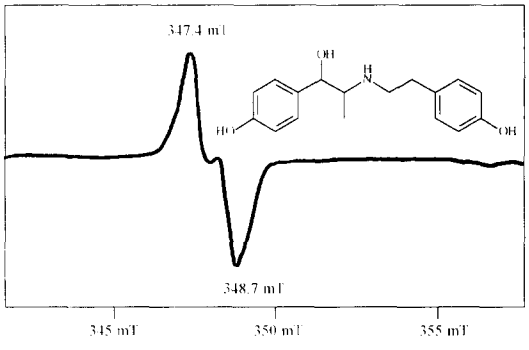

Fig. 1. ESR spectrum of ritodrine hydrochloride after gamma irradiation.

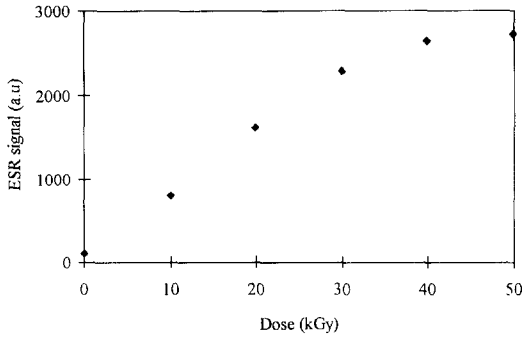

Fig. 2. Free radicals dependence on dose.

the spectra at 347.4 and 348.7 mT. Numerical simulation of the results were performed using WINREG software.

The chromatographic separation was based on that described in the USP XXIII for albuterol (salbutamol) 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 mobile phases used in this study are summarized in Table 3. The eluent was delivered by a Bishoff pump at a flow rate of 1 ml/min. A Kratos Spectroflow 783 UV-visible variable wavelengh detector was used (280 nm). Oxidative amperometric measurements were performed using a 400 EC EGG Princeton potentiostat. The potential applied to the glassy electrode was 1000 mV vs. silver–silver chloride electrode (SSE). Sample introduction was via a Rheodyne model 7125 injection valve, fitted with a 20-μl loop for direct injection.

Table 4
Functions used in numerical simulations

Model	Function
α (linear function)	ESR signal = $a + bD$
β (polynomial function)	ESR signal = $c + dD - eD^2$
γ	ESR signal = fD^g
δ (Michaelis function)	ESR signal = $\frac{h}{(1 + i/D)}$
ε (Hill function)	ESR signal = $\frac{j}{1 + (k/D)^l}$

Table 5
Coefficients of numerical simulations

	1[a]	2	3	4	5	6
a	90.00	−135.0	−10.00	225.0	762.0	−142.8
b	74.20	1122	240.0	330.0	299.0	961.5
c	38.00	−616.8	37.64	136.4	542.0	−335.9
d	104.2	1365	278.8	412.6	369.5	1057
e	1.015	14.26	2.223	3.967	2.477	3.900
f	213.1	3211	5584	1125	1000	1254
g	0.659	0.604	0.697	0.590	0.655	0.905
h	5669	59 325	18 717	19 410	26 767	246 604
i	49.18	37.59	60.65	37.42	54.67	237.2
j	3282	36 093	11 880	15 133	13 260	94 339
k	19.29	16.17	27.09	23.24	16.35	58.92
l	1.783	1.969	1.387	1.269	1.392	1.261

[a] 1, 2, 3, 4, 5, 6 are, respectively, ritodrine hydrochloride, terbutaline sulfate, fenoterol hydrobromide, orciprenaline sulfate, salbutamol, and isoproterenol hydrochloride.

3. Results and discussion

3.1. ESR

ESR can be used as an identification test if:
The radicals are quite stable with regard to the maximum time of storage
the relative signals must be clearly distinguishable from those of the reference samples
the signal must be strictly constant if we also require an estimation of the initial dose.

ESR powder spectrum of ritodrine hydrochloride after irradiation (25 kGy) is presented in Fig. 1.

Table 6
Estimation, for ritodrine hydrochloride, of the errors[a] (%) on the ESR signal using the five models described in text

Dose (kGy)	10	20	30	40	50
Model α	3.3	3.4	1.1	15.8	39.7
Model β	21.6	5.3	1.7	2.2	0.4
Model γ	20.8	5.7	12.4	8.1	3.4
Model δ	19.1	0.6	6.2	3.7	5.0
Model ε	3.6	3.9	1.5	2.3	2.0

[a]

$$\text{error (\%)} = \left| \frac{\text{ESR signal (calc.)} - \text{ESR signal (exp.)}}{\text{ESR signal (exp.)}} \right| \times 100$$

3.1.1. Dosimetry

Fig. 2 shows the free radical dependence on dose at ambient temperature after gamma irradiation. Numerical simulation of the results were performed using five models (Table 4). The coefficients of this five models for ritodrine, terbutaline, fenoterol, orciprenaline, salbutamol and isoproterenol are given in Table 5.

Estimation of the errors on the ESR signals, performed using models α-ε is given in Table 6 for ritodrine hydrochloride and Table 7 for sympathomimetics 2–6.

The best fit between experimental and calculated values for dose ranging 0–50 kGy was obtained with polynomial function (model β) and Hill function (model ε).

From results of Tables 6 and 7, estimation of the irradiation dose by post-irradiation using a linear function could generally be performed for dose ranging 0–15 kGy. Since the irradiation dose currently used for radiosterilization is 25 kGy, evaluation of a dose higher than 15 kGy by post-irradation could be considered using a more sophisticated model (polynomial function or Hill function).

Limit of detection (LOD) of the free radicals (~ limit of discrimination between irradiated and unirradiated samples), defined as the mean blank value plus three times the standard deviation of the blank (Mehta, 1989), is 1 kGy.

Table 7
Estimation, for sympathomimetics 2–6, of the errors (%) on the ESR signal using the five models described in text

Dose (kGy)	10	20	30	40	50
Model α					
2	8.8	1.9	24.9	39.7	72.1
3	0.8	0.2	17.5	23.4	45.9
4	11.3	3.4	13.7	29.9	54.8
5	20.9	4.3	5.8	16.8	20.4
6	17.4	5.8	1.5	13.4	10.3
Model β					
2	17.1	6.5	3.3	2.1	1.1
3	10.0	1.3	5.0	1.2	3.3
4	2.7	3.2	0.6	0.3	0.5
5	15.8	1.4	2.3	4.5	3.2
6	22.0	5.0	4.4	5.7	1.6
Model γ					
2	30.2	12.6	5.8	6.3	5.5
3	17.3	6.2	2.3	6.0	3.8
4	10.2	0.1	5.9	3.9	4.8
5	4.8	1.0	0.9	2.9	2.3
6	24.9	6.9	6.5	1.3	0.5
Model δ					
2	25.8	8.2	1.0	3.9	4.7
3	17.3	6.1	2.3	6.0	3.8
4	3.1	2.6	2.9	2.8	2.9
5	12.7	1.8	3.2	3.9	3.6
6	23.6	5.4	5.0	5.3	1.2
Model ε					
2	1.9	3.0	4.6	2.9	0.7
3	0.6	1.9	3.9	3.3	1.2
4	2.7	3.9	1.2	2.4	1.8
5	6.2	7.3	0.9	5.4	17.4
6	12.7	5.1	3.2	6.2	2.6

3.1.2. Decay of radicals upon storage

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) was performed. Fig. 3 plots the evolution of the percentage of free radicals versus storage. The decay was simulated using a bi-exponential model.

ESR signal (%) $= 69.52 \exp(-0.004x) + 30.48 \exp(-0.0955x)$ where x was the storage time in days.

The decay could be divided in two phases:
a fast exponential decay (coefficients 30.48 and 0.0955)
a slowly 'quasi-linear' decay (coefficients 69.52 and 0.004).

After 30 days of storage, the exponential component became negligible and the decay appeared linear; during, this time, 38% of free radicals diseappered. This simulation of the free radicals decay by a bi-exponential model was applied to β-agonists 2–6; coefficients of the bi-exponential models, storage time (t) corresponding to elimination of the exponential component and percentage of free radicals which disappeared during this time are given in Table 8.

From the results above, discrimination from irrradiated and unirradiated samples could be possible even after a storage longer than 12 months.

3.2. HPLC

The impurity profiles were recorded using in-pair chromatography (IPC) and appeared similar using UV detection and electrochemical detection. The chromatograms of irradiated samples (25 kGy) are shown in Fig. 4. Other samples (irradiated and unirradiated) 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 reponse factor of impurities at 280 nm were equal to the molar response of ritodrine at 280 nm). The in-

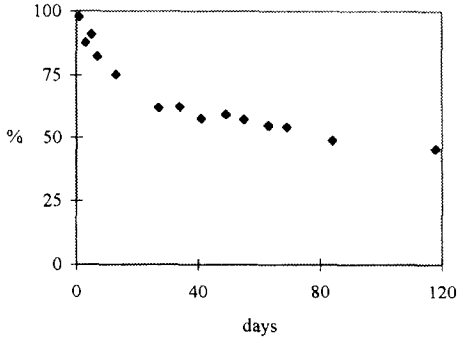

Fig. 3. Decay of radicals upon storage.

Table 8
Free radicals evolution as function of storage time

	2	3	4	5	6
A	20.78	ESR signal quasi-constant	42.20	26.98	ESR signal quasi-constant
a	0.1177		0.1868	0.0323	
B	79.22		57.80	73.02	
b	0.0001		0.0002	0.0012	
t (days)	13		20	40	
%	20		38	23	

ESR signal (%) = A $\exp(-ax)$ + B $\exp(-bx)$, where x is time of storage (days).

creasing of the irradiation dose caused the amount (%) of impurities to increase (Fig. 5). Ritodrine hydrochloride showed a degradation of 2.8% at 25 kGy.

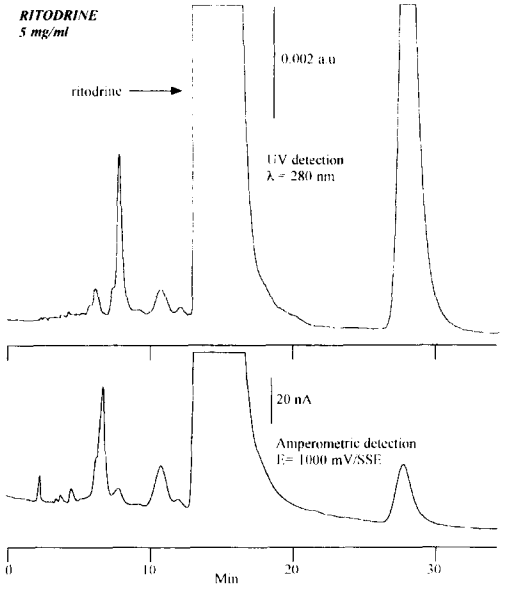

Fig. 4. Typical HPLC chromatograms of ritodrine after radiosterilization.

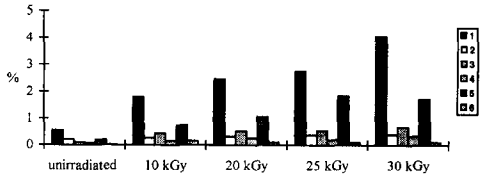

Fig. 5. Evolution of the percentage of impurities with the irradiation dose.

4. Conclusion

Estimation of the irradiation dose could be possible if:

the decay curve of radicals upon time is measured

if the date of irradiation is known, the dose could be evaluated by post-irradiation using linear regression for dose ranging 0–15 kGy and a more sophisticated model (polynomial function or Hill function) for dose higher than 15 kGy.

Degradation of ritodrine hydrochloride is important after irradiation in solid state. The results suggest that radiosterilization of ritodrine hydrochloride may not prove to be technically feasible especially at high doses.

References

Basly, J.P., Duroux, J.L. and Bernard, M., Gamma-radiation-induced effects on metronidazole. *Int. J. Pharm.*, 139 (1996a) 219–221.

Basly, J.P., Duroux, J.L. and Bernard, M., Gamma-irradiation sterilisation of orciprenaline and fenoterol. *Int. J. Pharm.*, 142 (1996b) 125–128.

Basly, J.P., Duroux, J.L. and Bernard, M., Radiosterilisation dosimetry by ESR spectroscopy: application to terbutaline. *Int. J. Pharm.*, 142 (1996c) 247–249.

Basly, J.P., Duroux, J.L. and Bernard, M., The effect of gamma radiation on the degradation of salbutamol. *J. Pharm. Biomed. Appl.*, (1996d) submitted for publication.

Boess, C. and Böegl, K.W., Influence of radiation treatment on pharmaceuticals: a review. Alkaloids, morphine derivatives and antiobiotics. *Drug Dev. Ind. Pharm.*, 22 (1996) 495–529.

Fauconnet, A.L., Barly, J.P. and Bernard, M., Gamma radiation induced effects on isoproterenol. *Int. J. Pharm.*, 144 (1996) 123–125.

Gibella, M., Crucq, A.S. and Tilquin, B., Détection RPE de l'irradiation des médicaments. *J. Chim. Phys.*, 90 (1993) 1041–1053.

Jacobs, G.P., A review of the effects of gamma-radiation on pharmaceutical materials. *J. Biomed. Appl.*, 10 (1995) 59–96.

Mehta, A.C., The validation criteria for analytical methods used in pharmacy practice research. *J. Clin. Pharm. Ther.*, 14 (1989) 465–473.

Miyazaki, T., Kaneko, T., Yoshimura, T., Crucq, A.S. and Tilquin, B., Electron spin resonance study of radiosterilization of antibiotics: ceftazidime. *J. Pharm. Sci.*, 83 (1994) 68–71.

Reid, B.D., Gamma processing technology: an alternative technology for terminal sterilization of parenterals. *PDA J. Pharm. Sci. Technol.*, 49 (1995) 83–89.

Tilquin, B. and Rollmann, B., Recherches à conseiller pour l'application de la stérilisation ionisante des médicaments. *J. Chim. Phys.*, 93 (1996) 224–230.