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Radiation sterilization dose calculation for heparin and aprotinin based on ISO Method 1

A.M. Dám [a], L.G. Gazsó [a], S. Kaewpila [a,1], I. Maschek [b]

[a] *'Frédéric Joliot-Curie' National Research Institute for Radiobiology and Radiohygiene, P.O. Box 101, Budapest 1775, Hungary*
[b] *Richter Gedeon Pharmaceutical Factory, Budapest, Hungary*

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Abstract

Using bioburden information, the radiation sterilizing doses were found to be 11.0 kGy for aprotinin solution, and 24.7 kGy for heparin Na, respectively. The calculations were performed on the basis of ISO recommendations.

Keywords: Radiation sterilization; Pharmaceutical; Dose calculation; ISO recommendations

Ionizing radiation is widely used for the sterilization of disposable medical products and supplies. This use has raised the possibility of the radiation sterilization of pharmaceuticals as well, especially when conventional sterilizing processes have proved inadequate. One of the most important points is the dose of irradiation, since the wide-scale application of radiation treatment cannot be introduced for pharmaceuticals unless the applied dose is reduced. The difficulty is that certain undesirable chemical and physical changes may accompany the treatment of pharmaceuticals by irradiation, especially on fulfilling the requirements of the 'general sterilizing dose' of 25 kGy and occasionally even higher. The choice of sterilization dose depends on three parameters, namely, the initial microbiological contamination (bioburden), the radiosensitivity of the microorganisms and the sterility assurance level (SAL) required. SAL is derived mathematically and defines the probability of non-sterility for each individual item. It is the expected probability of a surviving microorganism on each individual product after exposure to a valid sterilization process. SAL is expressed as 10^{-n}

Adapting the recommendations of the International Organization for Standardization (ISO) 'Sterilization of Health Care Products – Methods for Validation and Routine Control – Gamma and Electron Beam Radiation Sterilization' (International Standards Organization, 1991) offers a real possibility of choosing the most appropriate sterilizing dose for pharmaceuticals as well. To introduce these procedures for pharmaceuticals, ISO Method 1, i.e., 'Dose setting using bioburden information', was applied for heparin Na and aprotinin samples.

[1] Present address: Office of Atomic Energy for Peace, Bangkok, Thailand.

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Table 1
Calculation to determine sterilizing dose for heparin Na based on ISO/DIS 11137: Method 1

Parameter	Value	Definition for parameter determinations
SAL	10^{-6}	regarding that the product must be sterile, it requires an SAL of 10^{-6}
Bioburden	830/g	average bioburden for sample tested was 830
SIP [a]	0.1	1/10 portion was selected for resistance group verification
SIP bioburden	83	product bioburden of 830-times the SIP value of 0.1
Resistance group verification dose	7.8 kGy	sublethal verification dose for an SAL of 10^{-2} and an average SIP bioburden of 83 are found in ISO Table C2 (as a bioburden of 83 is not listed in the table, the next largest bioburden of 88.67 is used)
Sterility results	no positives at 7.8 kGy	delivered dose was within the specified dose range (6.8–8.8 kGy) and the sterility test results were acceptable (i.e., ≤ 2 positives); therefore, verification is accepted
Sterilizing dose for 10^{-6} SAL	24.7 kGy	10^{-6} SAL minimum sterilization dose for an average bioburden of 830 is 24.7 kGy

[a] Sample item proportion (SIP): the portion of the medical device or unit that is irradiated and tested for dose-setting procedures.

Heparin Na and aprotinin samples were kindly provided by Richter Gedeon Pharmaceutical Factory (Hungary) for experimental purposes. The initial contamination was determined by solving the samples (1 g heparin, 1 ml aprotinin) in physiological saline. After the appropriate dilution 0.2 ml of solution were spread on tryptone-glucose-yeast extract agar plates (Plate count agar,

Table 2
Calculation to determine sterilizing dose for aprotinin solution based on ISO/DIS 11137: Method 1

Parameter	Value	Definition for parameter determinations
SAL	10^{-6}	regarding that the product must be sterile, it requires an SAL of 10^{-6}
Bioburden	0.1/ml	average bioburden for samples tested was 0.1
SIP	1	1 ml of sample was selected for resistance group verification
SIP bioburden	0.1	product bioburden of 0.1-times the SIP value of 1
Resistance group verification dose	1.3 kGy	sublethal verification dose for an SAL of 10^{-2} and an average SIP bioburden of 0.1 are found in ISO Table C2
Sterility results	no positives at 1.3 kGy	delivered dose was within the specified dose range (0.3–2.3 kGy) and the sterility test results were acceptable (i.e., ≤ 2 positives); therefore, verification is accepted
Sterilizing dose for 10^{-6} SAL	11.0 kGy	10^{-6} SAL minimum sterilization dose for an average bioburden of 0.1 is 11.0 kGy

Oxoid CM 325). The plates were incubated for 3 days at 32° C and the colony forming units were scored. According to ISO Method 1 recommendation, 100–100 samples (0.1 g or 1 ml) were irradiated with the verification dose and sterility testing was carried out. The irradiated samples were put into sterile tryptone-soya-broth media (tryptone-soya-broth media, Oxoid CM 129) and incubated for 14 days at 32° C.

The radiation facility was an RH-γ-30 ^{60}Co apparatus with a dose rate ranging from 34.74 to 33.16 Gy/min. The dose rate was measured by ferrous sulfate dosimetry. The biological activity of the samples was assessed in the Laboratory of the Richter Pharmaceutical Factory according to FIP and USP XXII directives (Federation Internationale Pharmaceutique, 1968; US Pharmacopeia XXII, 1990).

The initial contamination of samples was found to amount to 830 microorganisms/g for heparin and 0.1 microorganisms/ml for aprotinin. The calculation of the sterilizing dose was conducted according to ISO Method 1 (see Tables 1 and 2). The recommendation states that a so-called verification dose must be chosen (International Standards Organization, 1991; Table C2) to achieve a given sterility assurance level (10^{-6}) related to the bioburden. The verification doses were found to be 7.8 and 1.3 kGy, for heparin and aprotinin, respectively. 100–100 samples were irradiated with the verification doses and sterility tests were performed. The results of the sterility test were acceptable, because none of the 100 samples showed positive growth of microorganisms. Regarding the required SAL (10^{-6}), the sterilizing dose for heparin was found to be 24.7 kGy and for aprotinin 11.0 kGy. The biological activity of the irradiated samples was determined according to FIP directives and USP XXII.

Two production lots of heparin Ca and three of heparin Na were irradiated with the calculated sterilizing dose for analytical measurements. Generally, the activity of treated samples was found to be lower than that of the unirradiated ones but within the acceptable range. The activity of samples from two production lots (heparin Na lot I and heparin Na lot III) was found to be lower than the acceptable level (Table 3).

Table 3
Activity of non-irradiated and irradiated samples

Samples	Unirradiated (IU/mg)	Irradiated (IU/mg)
Heparin Ca lot I	155.48	153.30
Heparin Ca lot II	155.54	152.80
Heparin Na lot I	142.00	133.60
Heparin Na lot II	177.00	173.30
Heparin Na lot III	139.60	133.60
Criteria of activity ≥ 140 I.U./mg		
	KIE/ml	KIE/ml
Aprotinin s.c. lot I	93864	88636
Aprotinin s.c. lot II	90059	87553
Aprotinin s.c. lot III	80878	78540
Criteria of activity minimum 75000 KIE/ml		

Three production lots of irradiated aprotinin were compared to the non-irradiated ones. Some reduction (3–6%) was found in the activity, but all of the irradiated samples conform to the acceptable limit.

According to the generally accepted regulations, 25 kGy is the minimum radiation sterilization dose, if there is no information about the bioburden. In the case of pharmaceuticals which are different in chemical structure, difficulties arise with the traditionally applied dose of 25 kGy, due to radiation damage of irradiated material (Schuttler et al., 1978). Irradiation with a low dose has the advantage of minimizing damage to the products (Gopal, 1978). While useful methods that can offer reliable microbiological control for radiation sterilization have been proposed by many authors (Davis et al., 1984; Doolan et al., 1985; Dwyer et al., 1985; Fitch et al., 1985; Dabord and Laizier, 1987) for single use medical devices, there is no generally accepted method for pharmaceuticals.

In accordance with the prescription of the USP XXII recommendations, the ISO method can also be used as a dose-setting procedure for pharmaceuticals. We investigated two different products: heparin in solid form and aprotinin solution. Based on the bioburden data, the sterilizing doses were calculated for heparin and aprotinin according to ISO standards (Tables 1 and 2) and were found to be 24.7 and 11.0 kGy, respectively. The calculated doses could not cause any

significant loss of activity in samples (Table 3). Our results indicate that there is a real possibility of reducing the sterilizing dose and/or evaluating the product-specific doses for these two materials.

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