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Stability of a Second-Generation Cephalosporin Veterinary Mastitis Formulation After Electron Beam Irradiation

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ABSTRACT

This study focused on the chemical stability of the cephalosporin (6R, 7R)-7-(1-pentafluorophenoxyacetamido)-3-[2-(5-methyl-1,3,4-thiodiazolyl)thiomethyl]- D[3] -cephem-4-carboxylic acid, sodium salt (cephem 1) formulation after electron beam (e-beam) irradiation. The cephem 1 concentrations of samples irradiated at 5, 10, and 15 kilograys for glass vials and low-density polyethylene (LDPE) cannula syringes were not statistically different from the concentrations of the nonirradiated control samples. Samples from each irradiation dose stored in controlled-temperature chambers at 5°C and 30°C for 24 months did not show any concentration changes within statistical limits compared with the nontreated samples. Samples from each irradiation dose stored at 40°C for 12 months also did not show any concentration changes within statistical limits compared with the nontreated samples. The percentage of related substances increased slightly with the increase in e-beam irradiation level and storage temperature, but this increase was within the proposed label claim of 90% to 110% (45-55 mg/g). In conclusion, e-beam sterilization did not affect the chemical stability of cephem 1 intramammary formulation in LDPE cannula syringes, suggesting that e-beam irradiation may be a feasible method for terminal sterilization of this cephem 1 formulation.

Keywords: Cephalosporin, Mastitis, Electron Beam Irradiation, E-beam Irradiation, Chemical Stability

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INTRODUCTION

Cephem 1 (**Figure 1**) is an antibiotic in early development for the treatment of mastitis in lactating dairy cattle to be administered as a single intramammary infusion to each quarter of the udder. The lead intramammary infusion formulation is composed of cephem 1 crystals suspended in a vehicle of microcrystalline wax and peanut oil at a nominal concentration of 50 mg/g cephem 1. The oil suspension formulation is filled into low-density polyethylene (LDPE) cannula syringes for intramammary infusion. As new intramammary products must be sterile in Europe, a method for achieving a sterile product had to be developed. Of the sterilization methods available, terminal sterilization is preferred because it avoids the inherent difficulty of aseptic manufacturing of crystals in an oil suspension and thus is less likely to fail sterility testing.

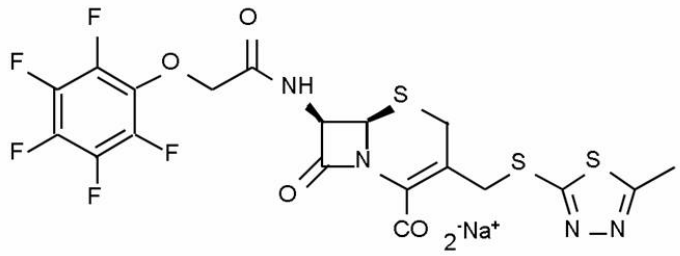

Figure 1. Cephem 1 structure. (6R,7R)-7-(1-pentafluorophenoxyacetamido)-3-[2-(5-methyl-1,3,4-thiodiazolyl)thiomethyl]-D[3]-cephem-4-carboxylic acid, sodium salt.

One form of terminal sterilization technology that has gained recent acceptance in the pharmaceutical

arena is electron beam (e-beam) irradiation. E-beam irradiation has been used in the food industry for several years. In December 1997, the U.S. Food and Drug Administration approved the use of e-beam irradiation of meat, recognizing the safety and effectiveness of irradiation in reducing pathogens in meat products[1].

E-beam irradiation involves the use of high-energy electrons generated by linear or circular accelerators[2]. The high energy levels are required to penetrate products contained in secondary shipping packaging. As the electron beam is scanned through the product, the electrons interact with formulation components (actives and excipients) in the product and create secondary reactive species. These secondary reactive species inactivate the microorganisms by irreparably rupturing the DNA chain, thus preventing reproduction. E-beam sterilization has several advantages over heat and gamma sterilization: It has the shortest process cycle, produces low heat, and does not require an isotopic radiation source. Thus, for the pharmaceutical industry, e-beam irradiation may have a bright future in providing an alternate terminal sterilization method to heat or gamma radiation. This early development study investigated the use of e-beam irradiation on the cephem 1 intramammary formulation to determine whether terminal sterilization using electron beam irradiation was a viable option from the perspective of chemical stability.

MATERIALS AND METHODS

Cephem 1 Mastitis Formulation Manufacture

The cephem 1 (Eli Lilly and Co, Indianapolis, IN) intramammary formulations were prepared with Multiwax ML-445 (Witco Corp, Petrolia, PA) and peanut oil (NF) to test the effect of e-beam irradiation on the stability of cephem 1. The peanut oil/wax vehicle was used to provide a physically stable suspension of the cephem 1 crystals.

The cephem 1 intramammary formulation was made by adding 674.0 g of peanut oil (Sessions Oil Mills, Spencer, IN) to a vessel and heating it to 78°C. While the heated peanut oil was stirred at 350 rpm with an IKA stir motor (IKA® Works, Inc. , Wilmington, NC), 37.5 g of Multiwax ML-445 was added. The stirring rate was increased to 1000 RPM as 38.5 g of cephem 1 was added. The stirring process was completed by using a 2 ½-inch-diameter, high-shear blade in the IKA mixer to homogenize the formulation. The heat was reduced to 60°C, and a magnetic bar stirrer was used to maintain homogeneity in the formulation as the syringes and vials were filled.

Containers

The containers used were clear glass vials (Wheaton 223738, 8 mL) with Teflon (DuPont, Wilmington, DE)-lined stoppers (West 1888 Gray) closed with aluminum crimp seals and De Backer (Hubert De Backer NV, Sint Niklaas, Belgium) LDPE cannula syringes (12 mL T1687 barrel, T1485 plunger, T1631 single cannula cover). Each of the syringes was filled with 6 g of formulation to provide minimal head space in each of the syringes, and the glass vials were filled full to minimize head space. The syringes and vials were appropriately labeled for identification of controls (nonirradiated) and e-beam intensity levels (5, 10, and 15 kilograys [kGy]). All treatment groups, including filled vials and syringes not exposed to e-beam irradiation, were shipped to the e-beam sterilization site. Samples were shipped under ambient temperature conditions (temperature was not controlled).

Irradiation

Electron beam irradiation was performed by Studer AG, Switzerland. A Rhodotron model TT300 (IBA, Inc., Belgium) circular accelerator was used to perform the e-beam irradiation[3]. Samples packaged in groups in secondary paperboard boxes were exposed to 3 different radiation levels: 5, 10, and 15 kGy. The dose rate was 5.3 kGy/sec. The samples labeled as controls were not irradiated. The control samples were shipped to Studer AG along with the samples to be irradiated so that all samples would experience identical shipping conditions. The control and irradiated samples were stored together at all points during the study.

Analytical Methods

The following assays were performed on the samples after irradiation: cephem 1 concentration, degradation

product profile (related substances), and physical observation.

The cephem 1 concentration assay evaluated the stability of cephem 1 after e-beam irradiation and in different temperatures. To perform the cephem 1 concentration assay, duplicate samples were prepared by weighing approximately 1 g of cephem 1 formulation into a 25 mL volumetric flask. The contents of the flask were diluted to volume with 1% trifluoroacetic acid in acetone and mixed. The sample was further diluted by transferring 2 mL of the aforementioned solution using a volumetric pipette into a 50 mL volumetric flask and diluting to volume with 0.1 M phosphate buffer, pH 7. The sample was mixed well and filtered before assay using a Gelman™ GHP Acrodisc (Pall Corporation, East Hills, NY), 0.45 μ m. The final concentration of the samples was approximately 80 μ g/mL. Standards were prepared in 0.1 M phosphate buffer (pH 7) at the following concentrations to bracket the samples: 60, 75, and 125 μ g/mL. The samples were assayed by reverse-phase high-performance liquid chromatography (HPLC) (Beckman Coulter, Fullerton, CA) using a 25 cm x 4.6 mm inside diameter (i.d.) Supelcosil LC-18-DB column (Supelco, Bellefonte, PA), 5 µm packing, and ultraviolet (UV) detection at 273 nm. The flow rate was 1.0 mL/min, and the injection volume was 20 μ L. A 10-minute isocratic run was performed with a mobile phase consisting of 65:35:0.1 (vol/vol/vol) ammonium acetate (0.01 M):acetonitrile:glacial acetic acid.

Samples were tested using the HPLC (UV, 273 nm) cephem 1 related-substances method to determine if degradation reactions had occurred either as a function of irradiation, of temperature, or of their combined effects. The related-substances method was a stability-indicating assay capable of resolving related substances and degradation products. Samples were prepared by weighing approximately 1 g of cephem 1 formulation into a 25 mL volumetric flask. The sample was diluted to volume with 1% trifluoroacetic acid in acetone and mixed. The sample was further diluted by transferring 3 mL of the aforementioned solution using a volumetric pipette into a 10 mL volumetric flask and diluting to volume with 0.1 M phosphate buffer, pH 7. The sample was mixed and filtered before assay using a Gelman™ GHP Acrodisc, 0.45 μ m. The approximate concentration of the samples was 600 μ g/mL. The samples were assayed by HPLC using a 25 cm x 4.6 mm i.d. Supelcosil LC-18-DB column and 5 µm packing. The flow rate was 1.0 mL/min, and the injection volume was 20 μ L. A 60-minute gradient run was performed with mobile phase A-ammonium acetate (0.01 M)/0.1% glacial acetic acid-and mobile phase B-acetonitrile/0.1% glacial acetic acid. The initial conditions were 95% mobile phase A and 5% mobile phase B, which were held for 5 minutes. These conditions were changed to 60% B by a linear gradient ramp from 5 to 45 minutes. The conditions were again changed to 100% B by a linear gradient ramp from 45 to 47 minutes. From 47 to 49 minutes, the system was re-equilibrated at 95% A and 5% B. These conditions were held for the remaining time of the run, 60 minutes.

Physical observations were noted for vial/stopper, syringe color changes, formulation color changes, and formulation consistency.

Table 1. Formation of Related Substances in Cephem 1 Formulation.

INITIAL	Nontreated (Percent)	5 kGy (Percent)	10 kGy (Percent)	15 kGy (Percent)
Glass	**0.94**	**0.99**	**0.92**	**0.97**
LDPE	**0.98**	**1.02**	**1.10**	**1.14**

5°C LDPE	Nontreated (Percent)	5 kGy (Percent)	10 kGy (Percent)	15 kGy (Percent)
3 months	**0.91**	**1.16**	**1.22**	**1.33**
12 months	**1.32**	**1.34**	**1.29**	**1.41**
18 months	**1.31**	**1.43**	**1.38**	**1.44**
24 months	**0.90**	**1.01**	**1.04**	**1.15**

30°C LDPE	Nontreated (Percent)	5 kGy (Percent)	10 kGy (Percent)	15 kGy (Percent)
3 months	**0.91**	**1.27**	**1.39**	**1.47**
12 months	**1.32**	**1.26**	**1.59**	**1.65**
18 months	**1.31**	**1.24**	**1.32**	**1.40**
24 months	**0.90**	**1.22**	**1.34**	**1.49**

40°C LDPE	Nontreated (Percent)	5 kGy (Percent)	10 kGy (Percent)	15 kGy (Percent)
3 months	**0.91**	**1.37**	**1.53**	**1.61**
12 months	**1.32**	**1.84**	**1.9**	**2.22**

RESULTS

The results for cephem 1 concentration measured immediately following irradiation and stored at 5, 30, and 40°C for up to 24 months after irradiation are presented in **Table 1**. The concentration was calculated using the slope generated from a linear standard curve bracketing the sample concentrations. The result for each irradiated sample is presented as a percentage of the nonirradiated samples and are graphically displayed in **Figure 2**. The results for cephem 1 related substances measured immediately following irradiation and stored at 5, 30, and 40°C for up to 24 months after irradiation are presented in **Table1** . The total related substances were calculated by adding the area of the individual related substances and dividing by the total peak area (which included the cephem 1 peak).

The potency method was developed to assay several candidate formulations of varying composition. For the wax-based formulation described here, the method achieved an intermediate precision of 4.1% relative standard deviation (RSD) during routine use, which includes multiple days of analysis, the use of several instruments, and the work of various analysts. Within-day variation (repeatability) was less than 2% RSD for triplicate preparations, justifying the routine assay of duplicate preparations. The extraction solvent system

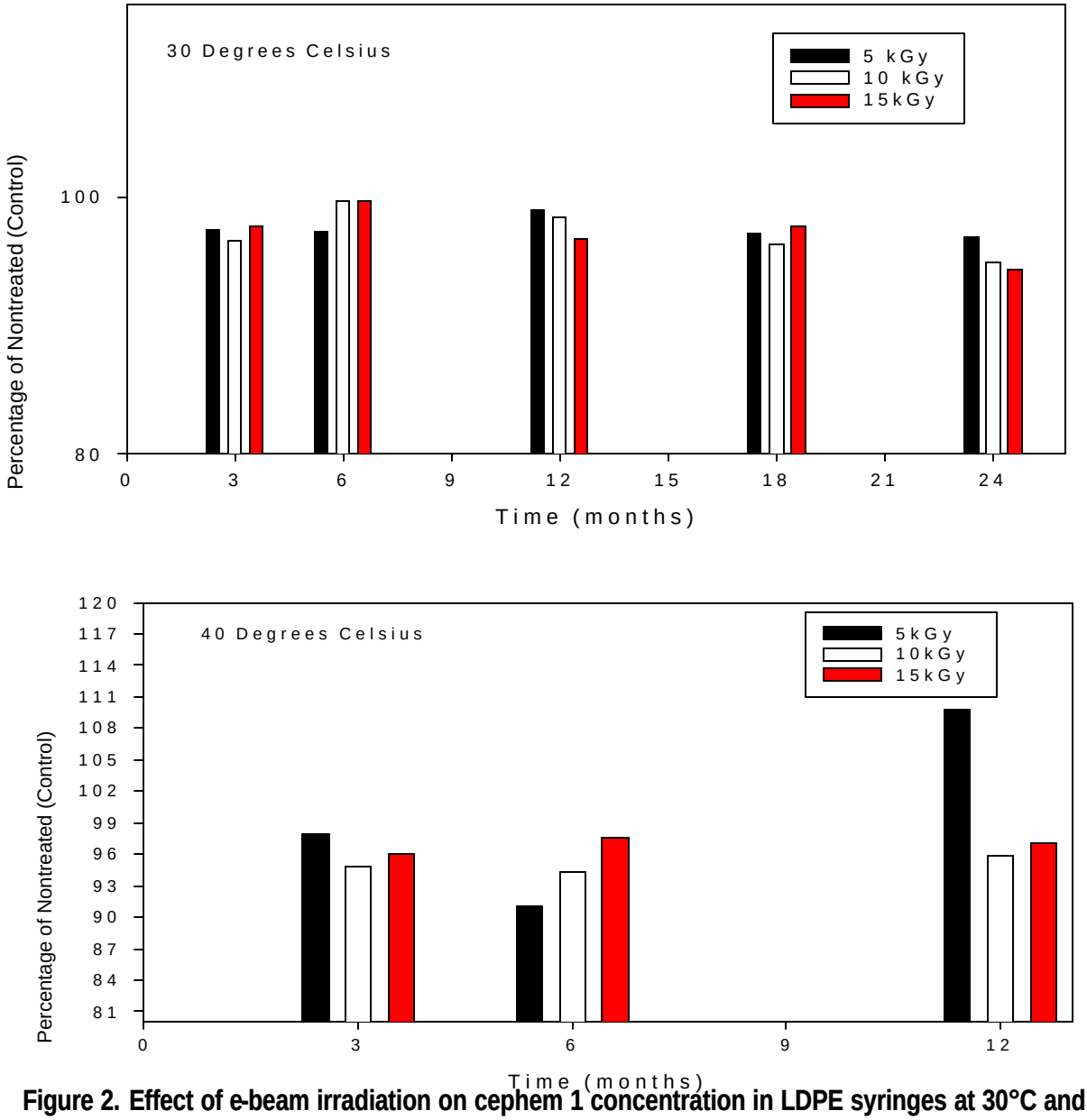

Figure 2. Effect of e-beam irradiation on cephem 1 concentration in LDPE syringes at 30°C and 40°C.

used in the method was shown to recover more than 98% of cephem 1 from peanut oil/wax formulations of the type employed in this study.

The chromatographic conditions used in the assay for potency were capable of resolving related substances and degradation products as determined with stressed samples of cephem 1. Samples were exposed to stress conditions including exposure to heat (37, 52, and 65°C), 0.1 N HCl, pH 8 phosphate buffer, water and peroxide. Although cephem 1 was shown to be stable in bulk form under thermal stress at temperatures up to 65°C for 2 weeks, rapid degradation was detected in other stressed solutions. In water a 7% decrease and in pH 8 phosphate buffer an 11% decrease was detectable in 24 hours at 37°C. In the presence of acid (HCl) or oxidizer (peroxide) at 37°C, greater than 90% degradation was detected over 24 hours. The cephem 1 concentrations of the initial formulation samples irradiated at 5, 10, and 15 kGy in both the glass vials and LDPE cannula syringes were not statistically different from those of the nonirradiated control samples. In glass, the percentages of nontreated cephem 1 in the samples irradiated at 5, 10, and 15 kGy were 100.5, 99.5, and 99.0, respectively, relative to a nonirradiated control sample. In LDPE syringes, the percentages of nontreated cephem 1 in the samples irradiated at 5, 10, and 15 kGy were 96.3, 98.4, and 97.5, respectively, relative to a nonirradiated control sample. Thus, the e-beam irradiation did not initially affect the chemical stability of cephem 1.

The samples were stored at 30°C and 40°C in LDPE syringes to determine the shelf-life of the cephem 1 formulation in the final package after e-beam irradiation. Because LDPE syringes are the intended market package, only the LDPE syringes were placed into long-term storage in controlled temperature stability chambers. At 30°C for 24 months, the percentages of nontreated cephem 1 for the samples irradiated at 5, 10, and 15 kGy were 96.9, 94.9, and 94.4, respectively, relative to a nonirradiated control sample. At 40°C for 12 months, the percentages of nontreated cephem 1 for the samples irradiated at 5, 10, and 15 kGy were 109.7, 95.9, and 97.1, respectively, relative to a nonirradiated control sample. Thus, the chemical stability of the cephem 1 molecule was not affected by e-beam irradiation and high temperatures. The recommended shelf-life for this cephem 1 formulation would be 24 months.

Throughout the 24-month stability study, the percentage of related substances in irradiated samples did not increase more than 3% relative to the nonirradiated control samples (**Table 1**). Related-substance profiles are shown in **Figure 3** for 0 and 15 kGy irradiated LDPE syringe samples.

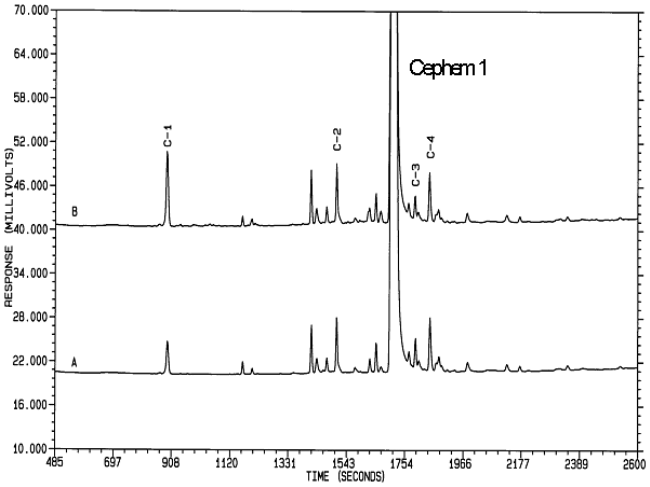

Figure 3. Chromatograms of related substances of cephem 1 in LDPE syringes after 0 kGy irradiation (A) and 15 kGy irradiation (B).

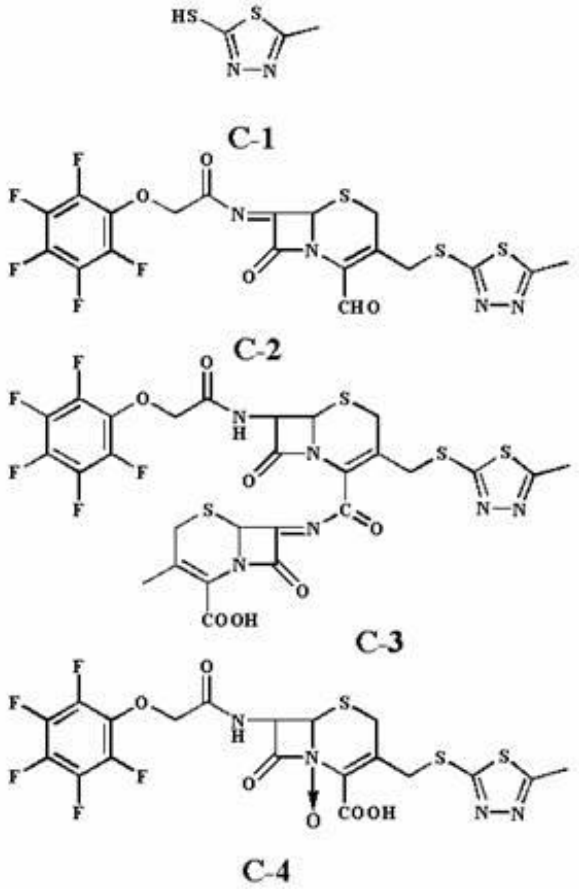

Figure 4. Cephem 1 related substances identified in the bulk drug.

The related substances that were identified during initial characterization of the bulk drug lot prior to the

initiation of this study are shown in **Figure 4**, with the letter designations corresponding to the labeled peaks in **Figure 3**. These related substances were identified using a combination of authentic standards, diode array spectroscopy, and Liquid Chromatography/ Mass Spectrometry (LC/MS). The 3-cephem nucleus and the pentafluorophenoxy acetic acid sidechain (detected at 225 nm) were also identified in the lot, but at low levels of 0.01%.

The irradiation of the LDPE syringe samples did not result in significant changes to the related-substance profiles other than an upward trend in the thiodiazole-related substance C-1 from 0.16% for the nontreated LDPE syringe to 0.25%, 0.31%, and 0.36% for the 5, 10, and 15 kGy doses, respectively. A general trend noted was that the related substances increased with dose under storage at elevated temperatures of 30°C or 40°C. **Figure 5** shows the related-substance chromatographic profile obtained on the LDPE syringe samples irradiated at 15 kGy and stored at 30°C for 2 years. Related-substance data from the 6-month stability time point were discarded from the study owing to suspect data attributed to a system malfunction.

The only negative effect of the e-beam irradiation process was that the cephem 1 formulation darkened as the irradiation dose was increased. This did not affect the cephem 1 concentration. The color of the formulation in glass containers and LDPE syringes changed from cream to light tan. The color of the glass containers changed from colorless to a smoky gray tint, but there was no effect on the color of the LDPE syringes. The darkening of glass is characteristic of gamma or e-beam irradiation and has been observed in previous studies[4]. The paperboard secondary package did not affect the e-beam penetration capability; this is evidenced by the color change in the glass vials. It was also observed that as the e-beam dose was increased from 5 kGy to 15 kGy, the color of the formulation became darker as the dose was increased. No irradiation dose level resulted in any visually observable change in the consistency or flow characteristics of the formulation when it was extruded from the syringe.

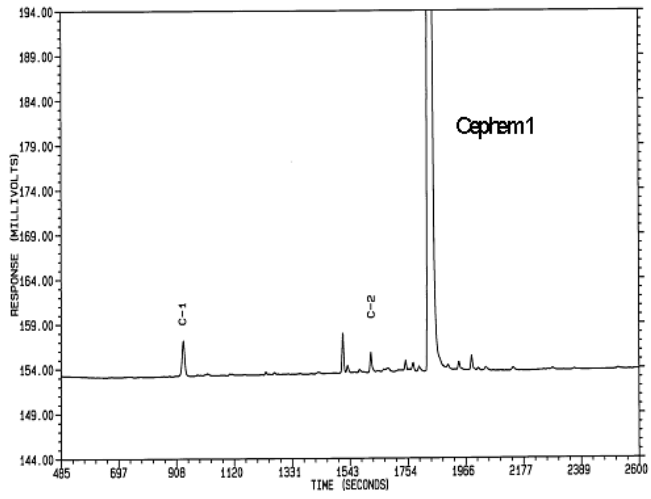

Figure 5. Chromatograms of related substances of cephem 1 in LDPE syringes after 15 kGy irradiation and 2 years' storage at 30°C.

DISCUSSION

Relatively few reports on the chemical stability of cephalosporin antibiotics after e-beam irradiation have been published[5]. Cephem 1 is a fluorinated analogue of cefazolin. The stability of cefazolin upon gamma beam irradiation has been studied by Jacobs[6]. Jacobs found that cefazolin bulk drug was stable after being irradiated with doses up to 50 kGy. These findings are consistent with the results of the current study and provide further evidence that cefazolin and analogues in this class are relatively resistant to chemical degradation upon irradiation with doses in the range expected to be used for sterilization of a pharmaceutical product.

Sterility was not examined in this early-phase development study. Current international guidelines require proof of product sterility if the dose is lower than 25 kGy, which is the accepted dose for achieving a Sterility Assurance Level (SAL) of 10^{-6} [7]. The use of doses in the range of 5 to 15 kGy would be anticipated for pharmaceuticals, which may be more susceptible to irradiation-induced degradation at higher energy doses. Sterility is normally readily achievable in this dose range if the initial bioburden is low and the product is manufactured under Good Manufacturing Practices (GMP) conditions. A brief description of the intended sterile manufacturing process will serve for illustrative purposes. This formulation would use sterile crystalline cephem 1. The bioburden levels of the excipients would be

closely monitored and kept at a minimum. The peanut oil/wax mixture would be sterile filtered. The crystalline cephem 1 would then be added to the sterile filtrate in a sterile operation. This material would then be subjected to terminal sterilization by e-beam irradiation. In the food-processing arena, which is closely related to pharmaceutics, a high dose (10 kGy or greater) of e-beam irradiation ensures complete sterility[8]. Lencioni and coworkers have also demonstrated, using a model pharmaceutical preparation, that bacterial sterilization is achievable using e-beam irradiation doses below 25 kGy[9].

CONCLUSION

Electron beam sterilization processing did not affect the chemical stability of the cephem 1 intramammary formulation in LDPE cannula syringes. Additional studies are required to determine if the e-beam irradiated product is sterile, as this was not a focus of the present investigation.

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