Dry-Heat Sterilization of Parenteral Oil Vehicles

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Abstract

The purpose of this study was to evaluate the effect of temperature and time on the dry-heat sterilization conditions of cottonseed, peanut and sesame-seed oils used as vehicles for parenteral drugs. The three oils were individually spiked with *Bacillus subtilis* spores and exposed to dry heat at four different temperatures (150, 160, 170, and 180°C) for three different time intervals (one, 1.5 and two hours). Following inoculation and dry-heat sterilization, samples were placed in a laminar airflow hood and processed according to <71>, "Sterility Tests" of the USP XXIV/NF 19 using thioglycolate broth and fluid D.[1] The specimens were then placed into an incubator at 30° C and observed for three, five and seven days for bacterial growth. All tests were performed in triplicate. Positive and negative controls were conducted with each group. All three oils were found to be free of viable *Bacillus subtilis* following dry-heat sterilization at 150, 160, 170 and 180°C for one, 1.5 and two hours after incubation for seven days. The positive controls were positive for observed growth and the negative controls had no observed bacterial growth. Dry-heat sterilization of the three oils at 150°C for one hour appeared to be sufficient for time and temperature conditions. However, the authors recommend dry-heat sterilization procedures be validated for each product.

Introduction

Natural edible oils are routinely used as parenteral vehicles for many pharmaceuticals. For use with heat-sensitive sterile compounds, these oils can be presterilized; for heat-stable compounds, terminal sterilization may be used. The purpose of this study was to evaluate the variable of temperature and time for dry-heat sterilization of cottonseed, peanut and sesame-seed oils. The *British Pharmacopoeia* recommends cycles of a minimum of 180°C for not less than 30 minutes, a minimum of 170°C for not less than one hour, or a minimum of 160°C for not less than two hours. However, it also states that ". . .other combinations of time and temperature may be necessary for certain preparations. Whatever combination is selected, the process must be fully validated both for efficacy of sterilization and for product stability."[2] Akers also reports ". . .that the minimal temperature for microbial destruction is generally above 175°C."[3] It is necessary for manufacturers to validate their dry-heat sterilization process when making parenteral products for the pharmaceutical industry.

This study was designed to provide generalized conditions for dry-heat sterilization of parenteral oil vehicles and used a modified USP method to determine the effective temperature and time required to sterilize oils for parenteral use.[1] *Bacillus subtilis* spores were used as a model for determining effective sterilization, as they are very resistant to dry heat.[3] It was important to determine these variables to provide the minimal time and temperature for efficient use of equipment and to minimize high-temperature exposure of active-drug ingredients. The latter is necessary to avoid the degradation of product and/or vehicle while at the same time achieving sterilization.

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Materials and Methods

Materials

The cottonseed oil, sesame-seed oil and peanut oil were supplied by Professional Compounding Centers of America, Inc., Houston, TX. Fluid thioglycolate medium was obtained from Fisher Scientific, St. Louis, MO, and prepared according to the manufacturer's instructions.[4] Fluid D was prepared following USP 24 specifications.[1]

Methods

Preparation of Fluid D – Fluid D was prepared by dissolving 1 g of tryptone and 1 mL of polysorbate 80 in sufficient purified water to make 1000 mL, followed by autoclaving.

Preparation of *Bacillus subtilis* Endospores – *B. subtilis* ATCC No. 6633 was grown on trypticase soy agar plates for three to four days.[5] Endospores were harvested by washing the surface of the plates with sterile saline solution. One milliliter of endospore suspension was heated at 80°C for 30 minutes to inactivate the vegetative cells, and a viable number of the endospores was determined by plate count. The endospore suspension was adjusted to contain 5 x 10^6 endospores/0.2 mL.

Inoculation of Vials with Endospores – Previously sterilized 30-mL amber glass vials were inoculated with 0.2 mL of the spore suspension

Table 1. Effect of Temperature on Viability of *Bacillus subtilis* Spores in the Various Oils.

		Time (hours)			Control	
	Temperature	1	1.5	2	Positive[a]	Negative[b]
Peanut oil	150°C	-	-	-	+	-
	160°C	-	-	-	+	-
	170°C	-	-	-	+	-
	180°C	-	-	-	+	-
Sesame oil						
	150°C	-	-	-	+	-
	160°C	-	-	-	+	-
	170°C	-	-	-	+	-
	180°C	-	-	-	+	-
Cottonseed oil						
	150°C	-	-	-	+	-
	160°C	-	-	-	+	-
	170°C	-	-	-	+	-
	180°C	-	-	-	+	-

a. Vials inoculated with 5 x 10^6 endospores but not subjected to heat treatment
b. Sterilized oils that were not inoculated with endospores

(5 x 10^6 endospores/vial). Inoculated vials were dried in a 37°C incubator. Thirty milliliters of each oil was transferred into the vials containing the dry bacterial endospores and the spores were dispersed by shaking.

Heat Treatment – Nine vials of each oil were placed in an oven set at 150°C. The internal temperature of the vial was monitored with a submerged thermometer in separate containers for each of the oils. The samples were then heated for one, 1.5 and two hours, respectively. The same procedure was then repeated at 160, 170 and 180°C.

Processing of the Heated Oils – After the appropriate heat treatment, the vials were placed in a laminar airflow hood along with the fluid thioglycolate and the fluid D, to minimize the possibility of any outside contamination. The surface of the bottom of the inoculated vials was "scratched" with the tip of a 5-mL pipette to dislodge any settled endospores. The contents of the vials were thoroughly mixed and a 5-mL sample was transferred to the container of fluid D. The fluid D and the sample were thoroughly mixed for uniform distribution. A 10-mL sample of this suspension was transferred into the thioglycolate broth. Inoculated thioglycolate broth was incubated at 30°C for a period of seven days, and the containers were observed on days three, five and seven for the presence of bacterial growth.

Results and Discussion

The thioglycolate broth vials inoculated with experimental and control-oil suspension in fluid D were observed on days three, five and seven for the presence of bacterial growth. Results at the end of seven days' incubation for peanut oil, sesame oil and cottonseed oil are presented in Table 1.

No bacterial growth was observed in any of the three oils treated at 150, 160, 170 and 180°C for one, 1.5 and two hours' duration. All positive-control vials containing oil with endospore inoculations showed bacterial growth. No growth was observed in the negative controls containing oils only.

The results indicate that the application of dry-heat temperatures of 150, 160, 170 and 180°C for one hour was sufficient to achieve oil sterilization. No change was observed in the physical properties of the oils as a result of dry-heat sterilization. We recommend, however, that dry-heat sterilization procedures be validated for each preparation.

References

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