Suitable Sterility Methods for Dimethyl Sulfoxide USP, PhEur

September 1, 2020
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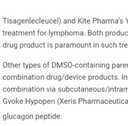
Pharmaceutical Technology, Pharmaceutical Technology-09-01-2020, Volume 2020 Supplement, Issue 4
Page Number: s19–s23

Dimethyl sulfoxide is increasingly used in high-risk parenteral and medical device applications that must be manufactured as sterile products in their finished form. A study evaluated the effects four sterilization techniques have on the product quality of this ingredient.



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Dimethyl sulfoxide (DMSO) is a small-molecule pharmaceutical ingredient with regulated uses as both an excipient and an API (1–2). Its capabilities as a transdermal penetration enhancer and its value as cryopreservation media were first recognized in the early 1960s. In recent years, this versatile substance has been incorporated into pharmaceutical products that require a high degree of microbiological quality.

Recently, DMSO has been used in autologous cell therapies. In 2017, Novartis brought the first FDA-approved gene therapy to market (Kymriah, Tisagenlecleucel) and Kite Pharma's Yescarta (Axicabtagene Ciloleucel) was approved in the United States as a T-cell treatment for lymphoma. Both products contain DMSO and are delivered by intravenous infusion. Sterility of the finished drug product is paramount in such treatments.

Other types of DMSO-containing parenteral products include long-acting injectable (LAI) formulations and certain combination drug/device products. In LAI applications, DMSO usually acts as a solvent to deliver a biopolymer/drug combination via subcutaneous/intramuscular injection. Another drug product in which DMSO sterility is critical is the Gvoke Hypopen (Xeris Pharmaceuticals), which was approved by FDA in 2019 and uses DMSO as a delivery solvent for glucagon peptide.

While excipients are by definition inactive substances, they are potential contributors to a pharmaceutical product's overall bioburden. Hair and other foreign matter may be contributed to the product during filling. There may the potential for cross contamination with materials of animal origin. Additionally, excipients are not chemically inert to every method of sterilization. Even simple small molecules may undergo chemical reaction when irradiated or thermally stressed. An understanding of an excipient's unique chemistry is helpful when developing a sterilization protocol for a finished pharmaceutical formulation.

Procipient (dimethyl sulfoxide *USP, PhEur*), manufactured as a bulk pharmaceutical ingredient by Gaylord Chemical Company, is not supplied as a sterile product. Increasing interest in suitable sterilization techniques compelled the authors to evaluate the impact of established sterilization techniques on the chemical product quality of the ingredient.

Materials and methods

Sterilization methods may be categorized as physical or chemical in nature. Typical chemical techniques may involve programmed exposure to biocidal gases such as ethylene oxide (EO) or treatment with peracetic acid in its liquid phase. DMSO is a readily oxidized substance, known to produce dimethyl sulfone (CAS [67-71-0]) in the presence of oxidants such as hydrogen peroxide. Further, it is not inert to electrophilic substances such as EO and peracids (3). Gases and liquid sterilants are most applicable for the treatment of hard surfaces (medical device components, surgical tools) and are less useful with liquid or solid pharmaceutical formulations. For these reasons, chemical sterilization methods were not evaluated in this study.

Physical methods such as thermal sterilization, irradiation, and filtration are more suitable options for attaining sterility of dimethyl sulfoxide products. DMSO poses technical challenges for these methods, however. It is a strong solvent that can dissolve/degrade polymeric filtration media. DMSO is known to thermally decompose under some conditions. The impact of irradiation on DMSO quality has not been well documented. Another interesting option is the use of extremely high pressure to terminally sterilize pharmaceutical compositions (4). Although this method is likely suitable for use with DMSO from a physicochemical stability standpoint, it is the authors' impression that this technique has not been broadly applied to the terminal sterilization of pharmaceutical finished dosages.

After considering the established and practical options, four sterilization techniques were tested to evaluate their effects on the chemical stability of DMSO. These were e-beam irradiation, gamma irradiation, thermal sterilization using dry heat, and aseptic membrane filtration sterilization. Although DMSO has a relatively high flashpoint (95 °C, open cup), the service provider would not sterilize DMSO samples in an autoclave or using the moist heat method, due to safety concerns.

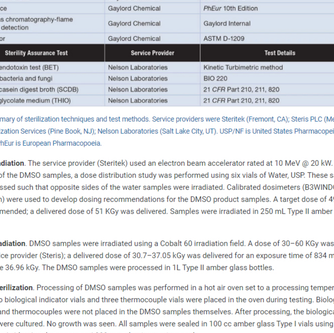

Figure 1. Sampling plan. All figures courtesy of the authors.

Product samples of Procipient (dimethyl sulfoxide *USP, PhEur*) were provided to respected sterilization service providers, which applied the sterilization method to these samples. The treated samples were then returned to the Gaylord lab for chemical analysis, based on the set of stability indicating assays underlying the product's International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use long-term stability program. **Figure 1** diagrams this process. An unopened control sample for subsequent sterility assurance was tested by another service provider, along with the various treated samples. The additional details of the sample plan are given in **Table I**. Additional experimental details follow.

Table I. Summary of sterilization techniques and test methods. Service providers were Steritek (Fremont, CA); Steris PLC (Mentor, OH); Prince Sterilization Services (Pine Book, NJ); Nelson Laboratories (Salt Lake City, UT). *USP/NF* is *United States Pharmacopeia/National Formulary*. *PhEur* is *European Pharmacopoeia*.

Sterilization Technique	Service Provider	Test Details
E beam irradiation	Steri-Tek	51 kGy delivered dose range
Gamma irradiation	Steris Laboratories	^{60}Co; 30.7-37.05 kGy delivered dose; 834 minutes
Dry thermal	Prince Sterilization Services	160 °C, 2 hours
Aseptic filtration	Nelson Laboratories	0.22 µ polytetrafluoroethylene filter; protocol STP0077 Rev 22

Chemical Quality Test	Service Provider	Test Details
Related compounds	Gaylord Chemical	*USP/NF* 42
Water	Gaylord Chemical	*USP/NF* 43
UV Absorbance	Gaylord Chemical	*USP/NF* 44
Non-volatile residue limit	Gaylord Chemical	*PhEur* 10th Edition
Related substances	Gaylord Chemical	*PhEur* 10th Edition
Absorbance	Gaylord Chemical	*PhEur* 10th Edition
Assay, Gas chromatography-flame ionization detection	Gaylord Chemical	Gaylord Internal
APHA color	Gaylord Chemical	ASTM D-1209

Sterility Assurance Test	Service Provider	Test Details
Bacterial endotoxin test (BET)	Nelson Laboratories	Kinetic Turbimetric method
Anerobic bacteria and fungi	Nelson Laboratories	BIO 220
Soybean casein digest broth (SCDB)	Nelson Laboratories	21 *CFR* Part 210, 211, 820
Fluid thioglycolate medium (THIO)	Nelson Laboratories	21 *CFR* Part 210, 211, 820

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E beam irradiation. The service provider (Steritek) used an electron beam accelerator rated at 10 MeV @ 20 kW. Prior to irradiation of the DMSO samples, a dose distribution study was performed using six vials of Water, USP. These samples were processed such that opposite sides of the water samples were irradiated. Calibrated dosimeters (B3WINDOSE, GEX Corporation) were used to develop dosing recommendations for the DMSO product samples. A target dose of 49–53 KGy was recommended; a delivered dose of 51 KGy was delivered. Samples were irradiated in 250 mL Type II amber glass bottles.

Gamma irradiation. DMSO samples were irradiated using a Cobalt 60 irradiation field. A dose of 30–60 KGy was specified by the service provider (Steris); a delivered dose of 30.7–37.05 KGy was delivered for an exposure time of 834 minutes; final dosage 36.96 KGy. The DMSO samples were processed in 1L Type II amber glass bottles.

Dry heat sterilization. Processing of DMSO samples was performed in a hot air oven set to a processing temperature of 160 °C. Two biological indicator vials and three thermocouple vials were placed in the oven during testing. Biological indicators and thermocouples were not placed in the DMSO samples themselves. After processing, the biological indicators were cultured. No growth was seen. All samples were sealed in 100 cc amber glass Type I vials using 20 mm Teflon-coated chlorobutyl stoppers and 200 mm aluminum crimp seals.

Aseptic filtration. Sterilization by aseptic filtration is the most commonly used method of sterilization for DMSO. It has been proven in the industry to be an acceptable sterilization method for solutions or liquids that may not be sterilized in a final container (5). Aseptic filtration of DMSO is widely used in research, clinical, and regulated products. Because this is a widely used and an industry standard, the authors deemed it suitable to test one sample lot for comparative purposes.

Sterility was performed in compliance with FDA regulations 21 *Code of Federal Regulations* Parts 210, 211 and 820, following Nelson Laboratories Standard Test Protocol: STP0077 Rev 22. DMSO samples were processed through a closed-membrane filtration system in a cleanroom environment; an industry standard (6). DMSO product was passed through a 0.22 µm polytetrafluoroethylene (PTFE) membrane and filter housings that are DMSO compatible. Following filtration, 100 mL of product was incubated for 14 days; incubation temperature of 20–25 °C soybean casein digest broth (SCDB)/30–35 °C fluid thioglycolate medium (THIO). This test verifies the product has been successfully filtered sterilized.

Sterility assurance

Bioburden and endotoxin tests were performed to validate the effectiveness of the different sterility methods. Even though Procipient is not supplied as a sterile product, the manufacturer's historical data has shown no adverse changes in both bacterial endotoxin tests (BET) and bioburden tests performed internally. Results for BET and bioburden were compared with the non-sterilized samples; no growth was shown in any of the processed samples. The BET utilized was the kinetic turbimetric method (Nelson Labs LAL110); for bioburden both aerobic bacteria and fungi (Nelson Labs BIO220) and a sample suitability per organism (Nelson Labs BIO930) was performed. Results of all samples submitted were <0.2 EU/mL (1:40 dilution) for BET and <1 Anaerobic/<1 fungal colony forming units/1 mL for bioburden.

A basic sterility assurance test was performed by incubating for 14 days at 20–25 °C SCDB/30–35 °C THIO. No growth was detected in both mediums.

Results

Of the four sterilization methods evaluated, only one (aseptic filtration) met all chemical quality requirements. Clearly, E-beam irradiation, gamma irradiation, and dry heat sterilization negatively impact the quality of DMSO. A summary of the chemical stability results is given in **Table II**.

Table II. Summary of results, dimethyl sulfoxide chemical stability following sterilization treatment. APHA is American Public Health Association. USP is United States Pharmacopeia. PhEur is European Pharmacopoeia.

The results from sterility assurance testing on all samples were consistent, and positive across all four sterility test methods. For all samples, no growth was reported in either soybean casein digest broth (SCBD) or fluid thioglycolate medium. For BET testing, < 0.2 EU/mL was reported for sterility test methods, at a 1:40 dilution. Bioburden testing reported < 1 Anaerobic and < 1 fungal colony forming units (CFU)/1 mL.

Discussion

Based on the above, the preferred method of the sterilization of DMSO is sterile filtration. The chemical quality data on the DMSO sample that had been sterilized by this method were indistinguishable from the untreated (and analytically pure) material. Aseptic filtration is, in fact, a proven DMSO sterilization technique in use today; there are "DMSO-compatible" filter media, filter housings, etc., available from the manufacturers of filtration products.

For many simple applications (i.e., sterilization of DMSO for use as cryomedia), aseptic filtration is ideal. As mentioned above, the materials of construction of the filter, peristaltic pump tubing, O-rings, etc., must be scrutinized. Unless verified through proper leachables/extractables testing, the use of specific perfluorinated materials (PTFE, Teflon, Kalrez) and some polyolefins (high density polyethylene, high density polypropylene) are prescribed. When pure DMSO is being processed, some polymers used in medical applications should be scrutinized, notably polyvinylchloride, with other inactive ingredient, the formulation's package, and the drug substance itself. The authors hope this work will serve as useful a starting point for pharmaceutical formulators and medical device designers as they consider the impact that a sterilization technique may have on the DMSO component of their products.

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Article Details

Pharmaceutical Technology
Supplement: *APIs, Excipients, and Manufacturing*
September 2020
Pages: s19–s23

Citation