

Research Report

Validated Gamma Irradiated Trypsin Powder

Douglas R. Purtle, Matthew B. Caffrey, Jeffery A. Doak and Karen J. Etchberger
SAFC Biosciences, Inc., 13804 W. 107th St., Lenexa, Kansas 66215 USA

Abstract

This validation study was conducted to determine the gamma irradiation process parameters that are required to provide a consistent, reproducible and effective method of inactivating potential microbial contaminants while maintaining product performance of dry powder trypsin. This study has led to the establishment of controls over package configurations, process temperatures, process time and radiation dosages.

Porcine parvovirus (PPV), porcine respiratory and reproductive syndrome virus (PRRS) and *Mycoplasma hyorhinis* (mycoplasma) were spiked into a powder and subjected to varying doses of radiation to determine the inactivation kinetics of the individual organisms on a pilot scale level. Dry powder trypsin samples were also exposed to varying levels of radiation to determine the effect of exposure on product performance. Additionally, production scale spiked and non-spiked samples were exposed to a specific range of radiation to determine the effectiveness and the effect of radiation on dry powder trypsin. The mycoplasma and PRRS virus were completely inactivated at 25 kilograys (kGy) while the PPV was reduced by greater than three logs Tissue Culture Infective Dose₅₀/g (TCID₅₀/g) when treated with 43 - 54 kGy. Two lots of trypsin powder were treated at 43 - 54 kGy and compared to non-irradiated controls. Enzymatic activity and dissociation times show that when irradiated under tightly controlled conditions, trypsin powder maintains acceptable levels of performance. Thus, we have demonstrated that gamma irradiation is an effective method to eliminate or reduce potential trypsin contaminants while maintaining product performance.

Introduction

Regulatory pressure is mounting for biological manufacturers to reduce the risks associated with the use of animal-derived components in their processes. The logical approach is to use reduced levels or to totally eliminate these components.

However, with biological processes this is not always feasible. To reduce the risk in using these components some treatment method needs to be used to inactivate potential adventitious agents. Gamma irradiation is one method that has been shown to be effective in significantly reducing microbial contaminants while maintaining product performance. However, to ensure the effectiveness along with the preservation of product efficacy, the irradiation process must be tightly controlled and validated.

Porcine trypsin presents a unique challenge in that it may contain low levels of PPV. Due to PPV's small size, 18 - 25 nm, and its non-enveloped structure, PPV is a very difficult virus to remove or inactivate. Through the validation study, we have demonstrated that the gamma irradiation process can reduce PPV titers by greater than three logs TCID₅₀/g.

The magnitude of the effectiveness of any inactivation process along with the maintenance of product integrity are dependent on the degree of control over the process itself. To establish and validate the gamma irradiated trypsin powder process, we conducted a three-phase study. The first two phases were pilot-scale that determined the minimum and maximum radiation doses; which maintained product performance and provided the highest level of inactivation. The third phase evaluated the chosen radiation dose for performance and effectiveness at the production scale.

Materials and Methods

- Trypsin Powder Porcine 1:250, Catalog No. 85050, SAFC Biosciences, negative for PPV.
- Powder Glucose was used in the inactivation study in place of trypsin due to the destructive nature of trypsin on cell cultures interfering with determining CPE endpoints.
- Cobalt-60 Irradiation facility, Isomedix located in Morton Grove, IL (pilot-scale study) or Libertyville, IL (production-scale study).

United States

SAFC Biosciences, Inc.
13804 W. 107th Street
Lenexa, Kansas 66215
USA
Phone +1 913-469-5580
Toll free-USA 1 800-255-6032
Fax +1 913-469-5584
E-mail info-na@sial.com

Europe

SAFC Biosciences Ltd.
Smeaton Road, West Portway
Andover, Hampshire SP10 3LF
UNITED KINGDOM
Phone +44 (0)1264-333311
Fax +44 (0)1264-332412
E-mail info-eu@sial.com

Asia Pacific

SAFC Biosciences Pty. Ltd.
18-20 Export Drive
Brooklyn, Victoria 3025
AUSTRALIA
Phone +61 (0)3-9362-4500
Toll free-AUS 1 800-200-404
Fax +61 (0)3-9315-1656
E-mail info-ap@sial.com

Lyophilized Microorganisms

- All media and reagents were supplied by SAFC Biosciences unless otherwise specified.
- Porcine parvovirus (PPV) Strain Tennessee, Lot No. 95071677PPV, American Biosciences. The virus was produced on Swine Testicular cells, ATCC No. CCL 33 in Minimal Essential Medium (MEM) with 5% horse serum. The virus culture fluids were harvested and lyophilized with a stabilizer in 50 mL glass vials.
- Porcine reproductive and respiratory syndrome virus (PRRS), field isolate. The virus was produced on a proprietary PRRS susceptible cell line using medium supplemented with serum. The culture fluids were harvested and lyophilized with a stabilizer in 50 mL glass vials.
- *Mycoplasma hyorhinis*, was obtained from Dr. Roth, Iowa State University, Ames, Iowa. The mycoplasma was grown in pleuropneumonia-like organism broth (PPLO). After harvesting, the mycoplasma cultures were lyophilized in 50 mL glass vials.

Spiked Sample Preparation

The lyophilized organisms were “diluted” into glucose (PPV and PRRS) or trypsin (mycoplasma) by slowly grinding the organisms into the powder using sterile mortars and pestles.

Pilot-scale Study

Duplicate 10 gram samples of trypsin were irradiated at 25, 35, 45 and 55 kGy ($\pm 10\%$ kGy). The samples were evaluated for trypsin activity by the Chromozym™TRY assay and the dissociation time was determined for a 0.25% trypsin solution on fibroblastic and epithelial-like cells.

The 10 gram samples of spiked glucose were titered on appropriate cell lines or in PPLO broth (mycoplasma). If the sample was negative, the supernatant was passaged three times to enhance any possible low levels of contamination.

Production-Scale

One kilogram samples of trypsin were irradiated at a specified dose range as determined by the pilot-scale study (43 - 54 kGy). The trypsin was then evaluated for trypsin activity and dissociation time at a 0.25% concentration in Hanks' Balanced Salt Solution (HBSS). The one kilogram spiked glucose samples were titered with the negative samples being passaged three times to enhance any possible low levels of contamination.

Process Controls

Delivered Dose — Through extensive dose mapping and a proprietary model, the radiation dose was monitored, controlled and delivered throughout the trypsin powder.

Shipment and Handling — Each shipping container was packed to a standardized configuration and shipped on a SAFC Biosciences' truck to the irradiation facility.

Results

Pilot-Scale

Table 1 shows the effects on performance at the various radiation doses. The table represents the dissociation time with both fibroblast (3T3) and epithelial-like (MDBK) cells. The third column of the table shows the trypsin activity per milligram of powder as measured by the Chromozym™TRY assay.

Table 1 Pilot-Scale Performance			
Irradiation Dose Range (kGy)	3T3 Cells (minutes)*	MDBK Cells (minutes)*	Trypsin Activity Unit/mg
Control 0 kGy	2:10	24:56	600
26.0 - 26.3	2:31	24:15	504
35.7 - 37.4	2:35	23:14	492
42.3 - 43.1	2:30	20:55	528
51.2 - 52.5	2:40	21:35	552

*0.25% trypsin in HBSS without EDTA

The results of the pilot-scale inactivation study are shown in Table 2. Pilot-scale inactivation study used glucose as grinding medium due to the destructive nature of trypsin on cells. The inactivation of the mycoplasma was evaluated in both glucose and trypsin.

Table 2 Pilot-Scale Inactivation					
Organism	Control 0 kGy	22.7 - 25.1 kGy	31.5 - 34.3 kGy	43.7 - 46.4 kGy	53.7 - 57.9 kGy
PPV	$10^{6.75}$ TCID ₅₀ /g	$10^{4.79}$	$10^{4.33}$	$10^{3.87}$	$10^{3.17}$
PRRS	$10^{4.2}$ TCID ₅₀ /g	$< 10^{2.2}$	$< 10^{2.2}$	$< 10^{2.2}$	$< 10^{2.2}$
Mycoplasma (glucose)	2.9×10^5 CFU*/g	0	0	0	0
Mycoplasma (trypsin)	6.1×10^5 CFU*/g	0	0	0	0

*Colony Forming Units (CFU)

PPV titers were reduced by greater than three logs at radiation doses above 43 kGy. Both the PRRS and mycoplasma were negative at all radiation doses. To further show complete inactivation the PRRS and mycoplasma samples were passaged three times to amplify any possible low levels of viable organisms. Both organism samples were negative after three passages at all radiation levels.

Production-Scale

One kilogram samples of trypsin were irradiated at 43 - 54 kGy. After irradiation, the samples were rehydrated in HBSS at 0.25% with and without 0.1% EDTA and the dissociation time was determined using three different cell lines, Table 3.

Table 3 Production-Scale Performance			
Sample	3T3 Cells (minutes)	MDBK Cells (minutes)	VERO Cells (minutes)
Non-irradiated LOT A (without EDTA)	2:37	NT	12:30
Irradiated LOT A (without EDTA)	2:34	NT	12:53
Non-irradiated LOT A (with EDTA)	3:00	8:00	NT
Irradiated LOT A (with EDTA)	3:00	8:00	NT
Irradiated LOT B (with EDTA)	4:00 98% viable	6:00 98% viable	NT

NT= Not Tested

Table 4 shows the results of the production-scale inactivation of the three organisms spiked into one kilogram samples of glucose. After irradiation the samples were rehydrated in sterile deionized water and titered on appropriate cell lines or in PPLO broth (mycoplasma).

Table 4 Production-Scale Inactivation		
Organism	0 kGy	43 - 54 kGy
PPV	$10^{6.95}$ TCID ₅₀ /g	$10^{3.89}$ TCID ₅₀ /g
PRRS	$10^{4.83}$ TCID ₅₀ /g	0
Mycoplasma	2.54×10^5 CFU/g	0

PPV was reduced by 3.06 logs while the PRRS and mycoplasma were reduced by greater than 4.83 and 5.0 logs, respectively. The PRRS and mycoplasma spiked samples were further passaged three times and evaluated for the presence of either cytopathic effect (CPE) or colony growth. Both organisms were negative after three passages.

Conclusions

Treatment with gamma radiation at 43 - 54 kGy significantly reduces the level of three common porcine microorganisms, PPV, PRRS and *Mycoplasma hyorhinis*.

Product performance is not affected by the exposure to radiation. The dissociation time of radiated trypsin with EDTA showed no difference when compared to non-radiated trypsin.

In conjunction with extensive prescreening of raw material which includes 9CFR 113.53 testing, gamma irradiation of trypsin increases the level of assurance and "safety" of using an animal-derived product.

While no inactivation process is 100% effective, gamma irradiation conserves product performance and is minimally intrusive, efficient, controllable and reproducible in providing a material free from contaminating organisms.

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United States

SAFC Biosciences, Inc.
13804 W. 107th Street
Lenexa, Kansas 66215
USA
Phone +1 913-469-5580
Toll free-USA 1 800-255-6032
Fax +1 913-469-5584
E-mail info-na@sial.com

Europe

SAFC Biosciences Ltd.
Smeaton Road, West Portway
Andover, Hampshire SP10 3LF
UNITED KINGDOM
Phone +44 (0)1264-333311
Fax +44 (0)1264-332412
E-mail info-eu@sial.com

Asia Pacific

SAFC Biosciences Pty. Ltd.
18-20 Export Drive
Brooklyn, Victoria 3025
AUSTRALIA
Phone +61 (0)3-9362-4500
Toll free-AUS 1 800-200-404
Fax +61 (0)3-9315-1656
E-mail info-ap@sial.com