

Savillex Technical Note

Irgafos 168 Testing on Purillex[®] PETG Square Media Bottles

Introduction

Irgafos 168 (Tris(2,4-di-tert-butylphenyl)phosphite) is a commonly used antioxidant in plastics, particularly in polyethylene terephthalate glycol (PETG) and other polymeric materials.



Purillex PETG Square Media Bottles

Its primary function is to prevent the oxidative degradation of polymers, extending their shelf life and maintaining their structural integrity. However, the presence of Irgafos 168 in materials used in biopharmaceutical and cell culture applications has raised concerns about its potential impact on cell growth.

Potential Impact on Cell Growth

Leachables and Extractables: In biopharmaceutical processes, single-use systems (SUS) are often utilized, and materials such as PETG, which may contain Irgafos 168, come into direct contact with cell cultures or bioprocessing fluids. Under certain conditions, Irgafos 168 can leach out from the polymer matrix into the surrounding environment. The concern is that if Irgafos 168 or its degradation products migrate into the culture medium, they could potentially inhibit cell growth or interfere with cell metabolism.

Cytotoxicity: Some studies suggest that antioxidants like Irgafos 168 may exhibit cytotoxic effects, particularly at higher concentrations. Cytotoxicity can manifest as reduced cell viability, altered cell morphology, or impaired cellular functions, all of which are detrimental to cell culture processes. The extent of these effects can vary depending on the concentration of Irgafos 168, the specific cell line being cultured, and the duration of exposure.

Regulatory and Safety Concerns: The potential for Irgafos 168 to act as a cell growth inhibitor necessitates thorough testing during the development and qualification of single-use systems. Regulatory bodies require evidence that any leachables from SUS, including Irgafos 168, do not adversely affect the quality and safety of biopharmaceutical products. This involves conducting extractables and leachables (E&L) studies to assess the migration of Irgafos 168 under real-use conditions and evaluating its impact on cell cultures.

Based on industry concerns of the presence of Irgafos 168 and its potential negative impact on cell growth, testing was initiated to characterize and gather baseline information on Savillex Purillex[®] PETG Square Media Bottles via a third party testing facility (Aspen Research, Maple Grove, MN, USA).

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Project Objective

Assess the amount of Irgafos 168 (CSRN 31570-04-4) that migrates from 1 L PETG/HDPE bottles (with HDPE closures) when challenged with 50% ethanol for 21 days at 40°C.

Samples Tested

Sample Description	ARC ID	Date Received
1000 mL Sterile Purillex PETG Square Media Bottle (PN 160-04-1000-2-S; lot 6-4-1L-S-00001; exp 2026-1-23)	55656-1	06/27/2024

Summary Methodology

USP 665 extraction protocol for containers using only extraction solvent C1. BPOG extraction protocol for containers (modified to include only 21-day time point and using only 50% ethanol) Aspen ISO 17025 STM 254 "HPLC-TOFMS Analysis in ESI".

Summary Results

No Irgafos 168 (or its' oxidized form) was detected above the lower limit of quantification (LLOQ) in any of the triplicate samples (LLOQ 10ng/mL). The LLOQ was verified by duplicate spikes of the Irgafos at 10 ng/mL to the 21-day extract of one of the replicates.



Figure 1: Bottles Filled in a Pyrex Dish & Shaker Box in Oven

Method and Results

Extraction: Bottles for testing were supplied to Aspen by Savillex in their ready to use shipping container. Three of the bottles were filled with 1000mL of 50% ethanol (referred to as extraction solvent C1 in USP 665). The bottles were capped with their supplied closure, placed on their side into a Pyrex dish and the whole system put inside a shaker box in an oven set at 40°C (Figure 1).

Blanks were 500mL of the 50% ethanol in a 32oz Qorpak glass jars capped with a Teflon-lined PP lid. The bottle systems were extracted for 21 days with the platform oscillating at 20rpm the entire time. At the conclusion of the 21-day exposure time, the 50% ethanol was transferred from the bottles into pre-cleaned Quorpak jars and held refrigerated until analyzed.

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Instrumental Analysis: Samples were analyzed by HPLC/QTOFMS following STM 254. Standards of Irgafos 168 were prepared from the purchased neat material (Aldrich lot MKCH4066, 0.01079g in 10mL acetonitrile, 10ppm) and serial dilutions of the 10ppm stock into acetonitrile. Standards were made encompassing 10ng/mL to 100ng/mL (5 pts). The areas of the Irgafos 168 and the oxidized Irgafos 168 ions were summed and the total Irgafos area plotted against the standard concentration (Table 1). No Irgafos 168 was detected in any of the samples above the signal in the blank (which was well below the 10ppb standard, Table 2).

Table 1: Irgafos 168 Calibration

Sample		Irgafos 168 Results		Oxidized Irgafos 168		
Name	Acq. Date-Time	Conc. (ng/mL)	Area	Area	Area	Sum
ACN	8/1/2024 9:28	0.00		0	0	0
10ppb in ACN	8/1/2024 10:19	10.79	52,832	53,303	0	106,136
25ppb in ACN	8/1/2024 11:11	26.98	137,576	0	0	137,576
50ppb in ACN	8/1/2024 12:02	50.00	298,967	28,800	0	327,767
75ppb in ACN	8/1/2024 12:54	80.93	465,717	17,326	0	483,043
100ppb in ACN	8/1/2024 13:45	107.90	642,754	34,680	16,634	694,068

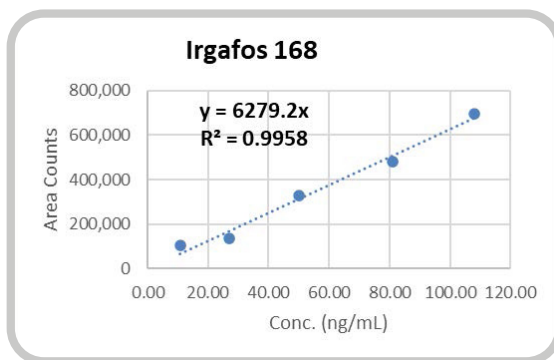


Table 2: Irgafos 168 in the 21-Day 50% Ethanol Extracts

Name	Acq. Date-Time	Area	Area	Area	Sum	Calc. Conc. AS (ng/mL)	Dilution Factor	Calc. Conc. ES (ng/mL)
A56646 Blank Off Top 50% EtOH	8/1/2024 20:37	0	22,592	0	22,592	3.60	1	LLOQ
56646-1A Off Top 50% EtOH	8/1/2024 21:28	0	18,689	0	18,689	2.98	1	LLOQ
56646-1B Off Top 50% EtOH	8/1/2024 22:20	0	25,741	0	25,741	4.10	1	LLOQ
56646-1C Off Top 50% EtOH	8/1/2024 23:11	0	15,731	0	15,731	2.51	1	LLOQ
Chk 25ppb in Acetone	8/2/2024 0:54	157,392	0	0	157,392	25.07	1	LLOQ

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The lowest standard was 10ng/mL (10ppb). The method LLOQ was verified by spiking 1mL of one of the 50% ethanol extracts with 10ng of Irgafos 168 (1µL of a 10ppm Irgafos 168 stock solution). The spikes were quantified against duplicate 10ppb standards and were recovered at 80% and 83% (Table 3) so the method is valid at an LLOQ of 10ppb.

Table 3: Recoveries of 10ppb Spikes

Sample		Irgafos 168 Results		Oxidized Irgafos 168 Results		
Name	Acq. Date-Time	Area	Area	Area	Sum	Rec'y
10ppb in ACN	8/5/2024 12:15	0	0	75,716	75,716	
56646-1A 50%EtOH Spike 10B Irgafos 168	8/5/2024 13:58	0	16,365	44,033	60,398	83%
56646-1A 50%EtOH Spike 10C Irgafos 168	8/5/2024 14:50	0	18,592	39,232	57,824	80%
10ppb in ACN	8/5/2024 15:41	0	0	69,370	69,370	
		mean 10ppb			72,543	

Rec'y = area spike/area mean 10ppb

Conclusion

Following completion of testing as specified in this technical note, no Irgafos 168 (or its' oxidized form) was detected above the lower limit of quantification (LLOQ) in any of the triplicate samples (LLOQ 10ng/mL), affirming the purity of raw materials and manufacturing processes at Savillex.

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