



Evaluation of Leachables in TPP TubeSpin® Bioreactors (PP tube) A Migration Study with Methanol, 10% DMSO, and WFI

Recently, several studies have indicated that certain substances, known as leachables, can be released from plastics ^[1]. Leachables are compounds that migrate into process fluids when used as intended. These leachables can adversely impact the quality and safety of products, as they can be bioactive.

To ensure the safety of samples in TPP containers for users, a migration study was conducted with TPP TubeSpin® bioreactors. The study indicated that no leachables were released from the TPP TubeSpin® bioreactors upon exposure to 100% methanol (MeOH), 10% DMSO, or WFI (Water for Injection).

Introduction

In recent years, numerous studies have demonstrated that leachables released from various plastic articles can significantly impact experiments. Items affected by leachables include all types of plastic articles, such as Petri dishes, reaction vessels, pipette tips, and consumables used in cell culture. These leachables can alter the growth of neurons ^[5], lead to anomalies and inconsistent results in enzymatic assays ^[1-3], cause system contamination and sample loss in analytics ^[6], and result in incorrect DNA concentrations ^[7]. Leachables also interfere with cell culture and can negatively affect cell growth ^[8,9].

To rule out any negative impact on cell growth in TPP bioreactors, a migration study was conducted by an external, independent laboratory. The migration solutions chosen were:

1. *10% DMSO*: Used in cell culture as a freezing medium for the cryopreservation of eukaryotic cells.
2. *100% Methanol*: Often used as a cost-effective alternative to ethanol for DNA extraction.
3. *WFI*: Water of particularly high purity, used in the production of parenteral preparations (e.g., infusion or injection solutions).

Both target screening and non-target screening (NTS) were performed on the collected migration samples. Target screening is a targeted investigation for the detection and identification of known substances. In this study, the focus was specifically on detecting DiHEMDA and oleamides.

NTS is an analytical method that captures all substances present in a sample, regardless of whether they are known or not, to discover unknown compounds. NTS is used to analyze the complex compositions of cell culture media, extracts, or samples to identify unknown metabolites or metabolic products. Automated software support enables precise analysis of complex mass spectrometry data, allowing unknown compounds to be quantified and their biological relevance to be understood.



Materials and Methods

The following migration study was conducted with TPP TubeSpin® Bioreactors of 50 ml and 600 ml volumes. Three TPP TubeSpin® Bioreactors (n=3) were filled with 40% of the tube volume with 100% MeOH, 10% DMSO, or WFI. The filled tubes were incubated at room temperature under shaking conditions for up to 8 days. Samples (n=3) were taken at 0, 3, 24, and 192 hours and pooled. The concentration of the target compounds, DiHEMDA and oleamides, was quantified using LC-MS.

For the NTS, a full-scan HPLC-HRMS analysis was performed on the pooled migration samples. The structure elucidation of significant UV and/or mass peaks was examined using various databases.

Results

The results of the quantification of oleamides and DiHEMDA showed no significant migration under the tested conditions (Tables 1 and 2). The measured concentrations in the target analysis were also detected with high signals in the blank samples and were therefore classified as not detectable. All samples were also subjected to an NTS. For WFI, 10% DMSO, and 100% MeOH, no signals were detectable, indicating that no leachables were extractable with the tested solvents.

Table 1: Results of the quantification of oleamides in MeOH, WFI, and DMSO migration experiments. The measured concentrations (*) in the LC-HPLC were also detectable with high signals in the blank samples and are therefore not considered migration compounds of the TPP TubeSpin® 50 and 600.

TubeSpin® Bioreactor	Time point (h)	Oleamides concentration 100% MeOH ng/mL	Oleamides concentration WFI ng/mL	Oleamides concentration 10% DMSO ng/mL
50	0	7,6*	n.a.	12,3*
	3	n.a.	10,6*	5,7*
	24	n.a.	n.a.	n.a.
	192	n.a.	n.a.	n.a.
600	0	7,6*	n.a.	12,3*
	3	5,5*	n.a.	n.a.
	24	n.a.	10,4*	10,5*
	192	n.a.	n.a.	n.a.



Table 2: Results of the Quantification of DiHEMDA in MeOH, WFI, and DMSO Migration Experiments. No concentrations of DiHEMDA were detectable in any of the samples.

TubeSpin® Bioreactor	Time point (h)	DiHEMDA concentration ng/mL	DiHEMDA concentration ng/mL	DiHEMDA concentration ng/mL
50	0	n.a.	n.a.	n.a.
	3	n.a.	n.a.	n.a.
	24	n.a.	n.a.	n.a.
	192	n.a.	n.a.	n.a.
600	0	n.a.	n.a.	n.a.
	3	n.a.	n.a.	n.a.
	24	n.a.	n.a.	n.a.
	192	n.a.	n.a.	n.a.

Conclusion

TPP TubeSpin® bioreactors are manufactured from high-purity polypropylene of USP Class VI. During production, no release agents, plasticizers, biocides, or lubricants are used. This study demonstrates that the use of this high-purity polypropylene, combined with TPP's specialized manufacturing process, prevents the leaching of bioactive substances from the plastic. As a result, users can minimize the risk of unwanted substances inhibiting cell growth, distorting experimental results due to enzymatically active leachables, or compromising the reproducibility of assays.

Glossar

DiHEMDA	di(2-hydroxyethyl)methyldodecylammonium
DMSO	Dimethyl sulfoxide
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
LC	Liquid Chromatography
MeOH	Methanol
n.a.	Not applicable, analyze not found
ng	Nano gram
NTS	Non-Target-Screening
NVOC	non-volatile compound
USP	United States Pharmacopeia
UV	Ultra Violet



Literature

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