



## Evaluation of Leachables in TPP TubeSpin® Bioreactors: A Migration Study with Methanol, 10% DMSO, and WFI



### Abstract

In recent years, several studies have demonstrated that certain substances, known as leachables, can be released from plastic labware <sup>[1]</sup>. Leachables are compounds that migrate into process fluids during intended use. These leachables can potentially have undesirable effects on product quality and safety, as they may be bioactive.

To ensure the safety of samples in TPP vessels for the user, a migration study was conducted using TPP TubeSpin® Bioreactors. The study demonstrated that no leachables were released from the TPP TubeSpin® Bioreactors under the influence of 100% methanol, 10% DMSO, or WFI (Water for Injection).

### Introduction To

Numerous studies have shown that leachables emanating from various plastic items can significantly impact experiments. All plastic labware can be affected, including petri dishes, reaction vessels, pipette tips, and cell culture consumables. These substances can alter neuron growth <sup>[5]</sup>, lead to anomalies and inconsistent results in enzymatic assays <sup>[1-3]</sup>, cause system contamination and sample loss in analytics <sup>[6]</sup>, and result in erroneous DNA concentration measurements <sup>[7]</sup>. Leachables also interfere with cell culture and can negatively affect cell growth <sup>[8,9]</sup>.

rule out negative impacts on cell growth in TPP bioreactors, a migration study was performed by an external, independent laboratory. The following migration solutions were selected:

- **10 % DMSO:** Used in cell culture within freezing media for the cryopreservation of eukaryotic cells.
- **Methanol:** Frequently used as a cost-effective alternative to ethanol for DNA extraction.
- **WFI:** High-purity water, used for the production of parenteral (e.g., infusion or injection solutions).

Both target screening and non-target screening (NTS) were performed on the collected migration samples. Target screening refers to the specific search for known substances; in this study, the focus was on DiHEMDA and oleamides. NTS is an analytical method where all substances contained in a sample—whether known or unknown—are recorded to discover unknown compounds. NTS is used to analyze complex compositions of cell culture media, extracts, or samples to identify unknown metabolites. 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 using 50 mL and 600 mL TPP TubeSpin® Bioreactors. Three TPP TubeSpin® Bioreactors of each size (n=3) were filled to 40% of the tube volume with 100% methanol, 10% DMSO, or WFI. The filled tubes were incubated at room temperature for up to 8 days with shaking. Samples (n=3) were taken after 0, 3, 24, and 192 hours and pooled. The concentration of the target compounds DiHEMDA and oleamides was quantified using LC-MS (Liquid Chromatography-Mass Spectrometry).



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

## Results

Quantification results for oleamides and DiHEMDA show no significant migration under the tested experimental conditions (Tables 1 and 2). Concentrations measured in the target analysis were also detected in the wash blanks with high signals and were therefore classified as non-detectable. All samples were also subjected to NTS. No signals were detectable for WFI, 10% DMSO, or 100% methanol, indicating that no leachables were extractable with the solvents tested.

**Table 1: Quantification of oleamides in MeOH, WFI, and DMSO migration experiments.**

Measured concentrations (\*) in LC-HPLC were also detectable in wash blanks with high signals and are therefore not classified as migration compounds from the TPP TubeSpin® 50 and 600.

TubeSpin Bioreactor	Timepoint (h)	Oleamide concentration 100 % MeOH [ng / mL]	Oleamide concentration WFI [ng / mL]	Oleamide 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: Quantification of DiHEMDA in MeOH, WFI, and DMSO migration experiments.**

No concentrations of DiHEMDA were detectable in any 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. No release agents, plasticizers, biocides, or lubricants are used during production. This study demonstrates that the use of high-purity USP Class VI polypropylene and TPP's specialized manufacturing process prevents the leaching of bioactive substances from plastic chemicals. Consequently, the risk of experimental results being distorted by enzymatically active leachables or assays becoming non-reproducible due to growth-inhibiting substances is reduced for the user.

## Glossar

DiHEMA	di(2-hydroxyethyl)methyl dodecyl ammonium
DMSO	Dimethylsulfoxid
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
LC	Liquid Chromatography
MeOH	Methanol
n/a	Not Applicable, analyte not found
ng	Nanogram
NTS	Non-Target-Screening
NVOC	Non-Volatile Compound
USP	United States Pharmacopeia
UV	Ultraviolet

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## Literature

- [1] McDonald, G Reid et al. "Bioactive contaminants leach from disposable laboratory plasticware." *Science (New York, N.Y.)* vol. 322,5903 (2008): 917.  
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