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SUMMARY **APPLICATION NOTE** No. 417

# Extractables and Leachables in Microcentrifuge Tubes – Extensive HPLC/GC/MS Analysis

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## Abstract

Substances leaching out of plastic consumables can be characterized as a heterogeneous and largely undefined group of chemicals, which may significantly affect experiments and pose a likely source of error in various assay systems. In this Application Note extensive analyses using HPLC, UHPLC, GC and MS methods have identified high amounts of both extractables (up to 1.53 g/kg) and water soluble leachables (up to 31 mg/L), both migrating into samples incubated in microcentrifuge tubes of various manufacturers. Extractables were identified as nucleating agents and anti-oxidants, whereas water-soluble leachables comprised chain-cleaving agents, antistats and nucleating agents/clarifiers.
Samples incubated in Eppendorf Tubes® showed consistently and under all experimental conditions by far the lowest levels of organic substances possibly migrating into samples; thus having the lowest risk to negatively affect assays.

## Introduction

Materials commonly used in medical and life-science lab consumables are typically a blend of base polymers with a broad and heterogenous range of chemicals added during production. These will either help facilitate or accelerate the processability of the polymer, prolong its long-term stability or enhance its performance in the end product in various ways. Increasing scientific evidence indicates, that part of those processing additives may be released (leach) into samples and significantly affect experiments. Ultimately it will pose a likely and largely underestimated source of error in various assay systems [1 - 7].

In this Application Note an extensive analysis using sensitive analytical methods (HPLC, UHPLC, GC, MS) have been applied to comprehensively identify and quantify organic substances leaching from standard microcentrifuge tubes into samples after exhaustive extraction with organic solvent (extractables) and after incubation with water (leachables relevant for life science). The analysis was completed by two ISO 17025 accredited organizations routinely performing material analysis for pharma and life-science sector.

## Materials and Methods

### Extractables

HPLC/MS analysis of samples after exhaustive extraction with dichloromethane (extractables) identified in most of the tubes (A, S, V) high levels of antioxidants/processing stabilizers (AO) and nucleating agents (NA): figure 1.
The total amounts of extractables indicate high to very high levels of organic substances migrating into the samples from microcentrifuge tubes. Large differences between tested tubes were observed: highest levels were found for A tubes (1,525 mg/kg), followed by V tubes (1,320 mg/kg) and S tubes (385 mg/kg). Samples incubated in Eppendorf Tubes showed very low total extractables levels of 255 mg/kg, which represents 16.7% levels observed for A tubes.
The only extractables type detected at low levels in Eppendorf Tubes were branched alkanes.

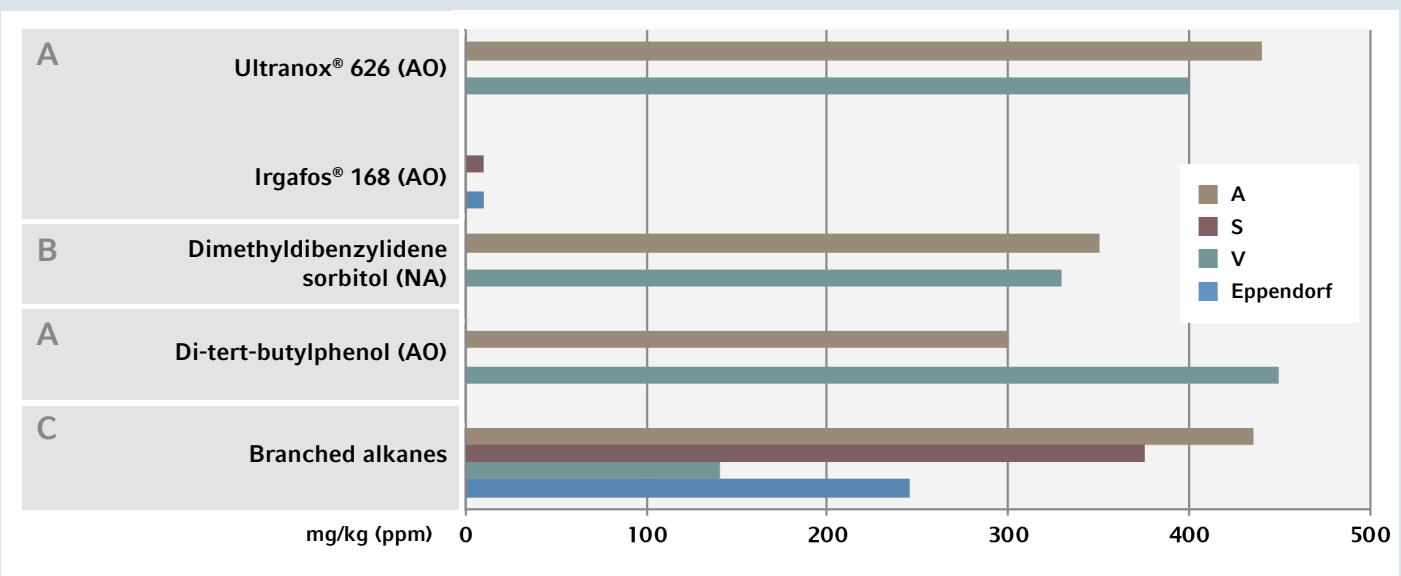


**Figure 1:** Identification and quantification of extractables in samples incubated in microcentrifuge tubes.
**A)** Antioxidants/processing stabilizers (AO): widely used to stabilize structure of polymer; several were reported to affect various assay systems and rated as critical [5, 8, 9].
**B)** Nucleating agents (NA): used to provide property enhancement, improved molding productivity and increased transparency (clarifiers). Several NAs were reported to interfere with biological assays and rated as potentially critical [5, 8, 10].
**C)** branched alkanes: polymerization by-products, generally regarded as uncritical.

**Water soluble leachables – volatile organic compounds (VOC)**

GC/MS analysis of volatile organic compounds (VOC) in water samples revealed middle levels (up to ca. 42 µg/kg) of various classes of organic substances predominantly in tubes A and S (fig. 2). Detailed description of identified VOC is provided in Application Note No. 417.

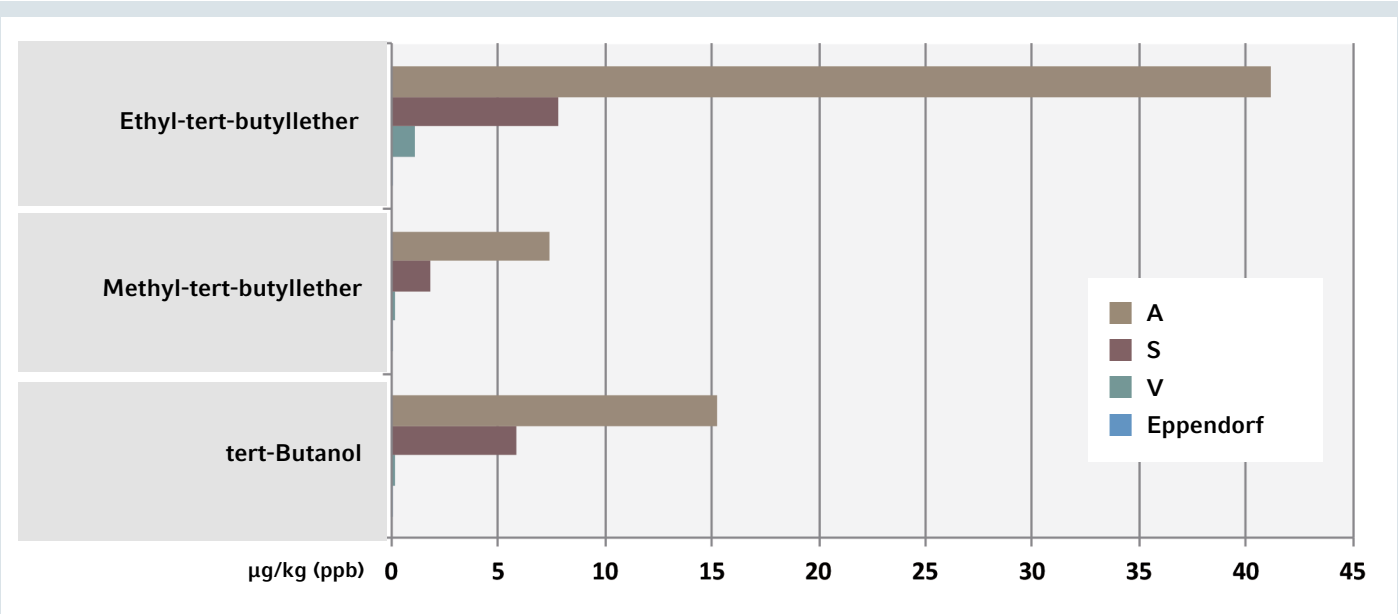


**Figure 2:** Identification and quantification of volatile organic compounds in water samples incubated in microcentrifuge tubes. Majority of VOC identified are chain-cleaving reagents (typical process additives) used to reduce chain length and increase the melt flow.

**Water soluble leachables – non-volatile organic compounds (NVOC)**

The analysis of non-volatile organic compounds (NVOC) has been further narrowed to tubes showing intermediate extractable levels: V tubes and Eppendorf Tubes (showing lowest levels): The detailed UPLC/MS analysis revealed four different compounds in water samples incubated in V tubes. The amounts range from 1,400 to 23,000 µg/L (ppb) and the substances belong to two classes of polypropylene additives: antistatic agents and nucleating agents/clarifiers (fig. 3).

Noteworthy, clarifier Millad 3988 and related compounds exhibits a strong absorption signals resembling nucleic acids. This may partially be linked to UV-absorbing leachables spectra observed using standard spectrophotometric methods. The total amount of detected NVOC in the sample incubated in V tubes was very high: 31,050 µg/L. No compounds could be identified in samples incubated in Eppendorf Tubes (fig. 3). For a detailed description of identified NVOC refer to Application Note No. 417.

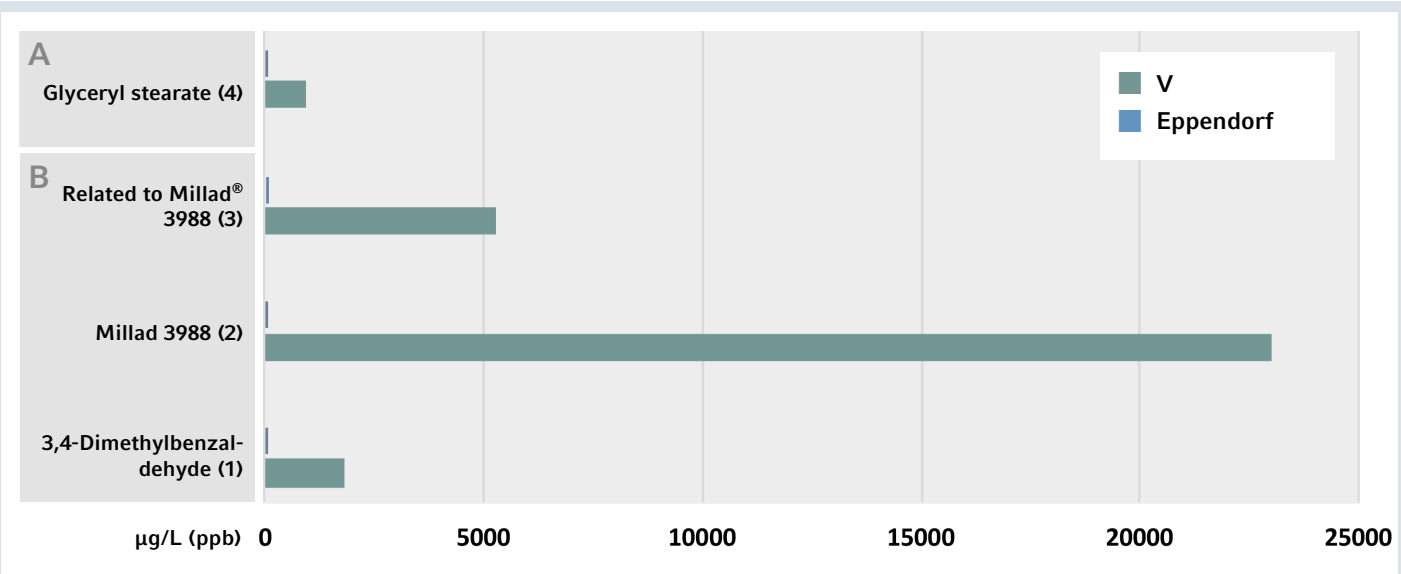


**Figure 3:** Identification and quantification of non-volatile organic compounds in water samples incubated in microcentrifuge tubes.
**A)** antistatic agents: common polypropylene additives; may interfere with biological assays and are potentially critical [5, 8, 10, 11, 12].
**B)** nucleating agents/clarifiers: used to provide property enhancement, improved molding productivity and increased transparency (clarifiers).

## Summary and conclusions

The extensive analysis of extractables and water soluble leachables identified high amounts of organic substances migrating into samples incubated in standard microcentrifuge tubes of most manufacturers tested.
The analysis of samples after exhaustive extraction (extractables) revealed high levels (1,525 mg/kg, tubes A) of typical polypropylene additives: anti-oxidants/processing stabilizers and nucleating agents. Samples incubated in Eppendorf Tubes showed very low total extractables levels of 255 mg/kg and consisted of uncritical polymerization by-products: branched alkanes.

Water-soluble leachables could be identified as typical processing and polymerization additives: chain-cleaving agents, antistatic agents and nucleating agents/clarifiers. All of the identified extractables and water soluble leachables belong to commonly used polypropylene additives. As shown in literature [1 - 5], these may have negative effects on various assay systems. In addition, identified nucleating agents/clarifiers: 3,4-dimethylbenzaldehyde, dimethyl-dibenzylidene sorbitol (Millad 3988) and related compounds exhibit strong absorption spectrum resembling nucleic acids. This may be associated to UV-absorbing leachables spectra observed using standard spectrophotometric methods.

Noteworthy, samples incubated in Eppendorf Tubes showed under all experimental conditions by far the lowest levels of extractables and water-soluble compounds migrating into samples. The only class of detected extractables were low levels of branched alkanes which are typical polypropylene polymerization by-products and are generally regarded as uncritical. No critical polymerization or production additives were detected whatsoever, indicating that irrespectively of experimental conditions (water, organic solvents) Eppendorf tubes will provide lowest risk for sample contamination and assay interference.

## Literature

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