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# Avoid Ghost Peaks in HPLC, GC and MS Chromatograms with Use of Leachable-Free Pipette Tips from Sartorius

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## Abstract

Chemical compounds can leach from common laboratory equipment during sample storage and sample preparation. These compounds can introduce unwanted ghost peaks in HPLC, GC and MS and interfere with sample analysis in a range of activities including analytical quality control. Extraction samples from Sartorius Optifit and Safetyspace® filter tips and their low retention variants were tested for leachables using HPLC, GC-MS and LC-MS. These pipette tips were found to be practically free of leaching compounds and are therefore suitable for use in highly sensitive analytical methods such as HPLC, GC and MS.

## Introduction

While plasticware is widely used in the laboratory setting, low-quality products may affect high precision analyses (1-9). Possible sources of contamination are sample tubes (4, 5), multiwell plates (5), pipette tips (5, 6, 7) and plastic syringes (6) which can leach colorants and plasticizers like phtalates or DiHEMDA (di(2-hydroxyethyl)methyldodecylammonium), resulting in ghost peaks and interfering with analytical results . To avoid ghost peaks, many laboratories have replaced disposable, easy-to-use plastics with glassware that requires cleaning and prerinsing.

In this study, Sartorius Optifit and Safetyspace® filter tips and their low retention variants were analyzed for chemical leachables using high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) following extraction in EtOH and DMSO. These solvents were selected based on their widespread use and aggressiveness compared to milder solvent such as water which might not have resulted in the same level of extraction of potential contaminants.

## Results

GC-MS and LC-MS analyses were performed for Sartorius Optifit tips and Safetyspace® filter tips and their low retention variants. Oleamide was quantified by GC-MS and had a maximum concentration of 0.11 ppm (Limit of Quantification, LOQ 0.01 ppm; fig. 1); erucamide (fig. 2-3) and bDtBPP (bis(2,4-di-tert-butylphenyl)-phosphate; fig. 4) were quantified at a maximum concentration of 0.14 ppm (LOQ 0.001 ppm), and 0.026 ppm (LOQ 0.001 ppm) using LC-MS. Additionally in GC-MS ethyl hexacosanoate was quantified in Low Retention Optifit tips with maximum concentration at 0.25 ppm and an unknown compound with retention time of 8.32 minutes was detected with maximum concentration at 0.13 ppm but its characterization was not possible due to insufficient structural information. In HPLC-UV/VIS only low level of the ubiquitous antioxidant Irgafos 168 (CAS 95906-11-9) was found in Low Retention Optifit tips. No other compounds were detected above their limit of quantification (Table 1).

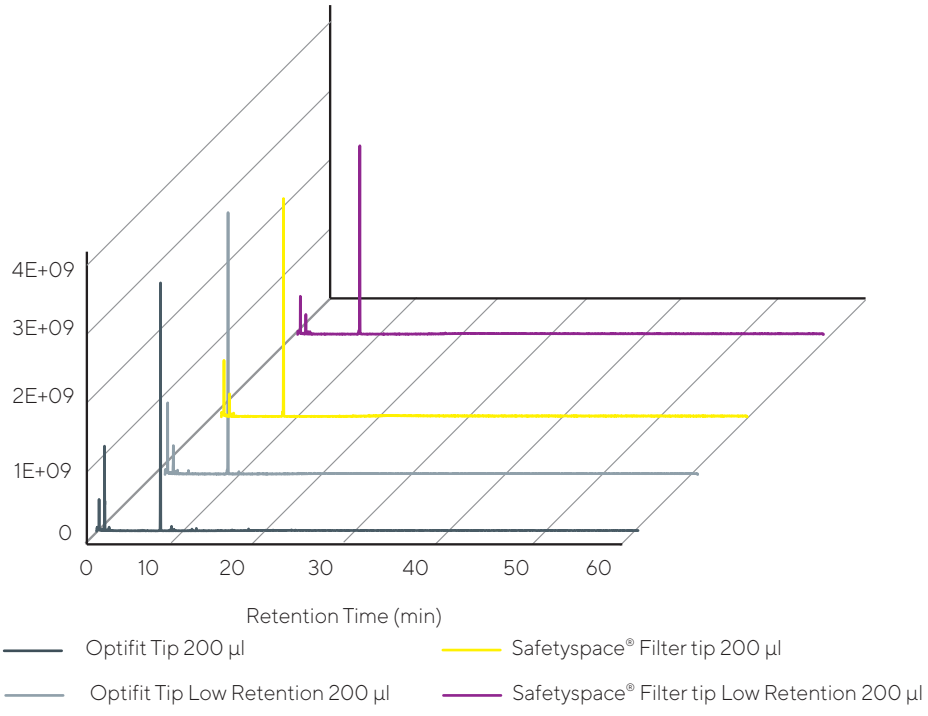


Figure 1. Chemical leachables analysis of Sartorius pipette tip EtOH extracts using GC-MS. Oleamide retention time 19.28 minutes; erucamide, 22.66 minutes; (results below limit of detection); unknown compound, 8.32 minutes; ethyl hexacosanoate, 25.20 minutes; internal standard Irganox 1035, 12.35 minutes.

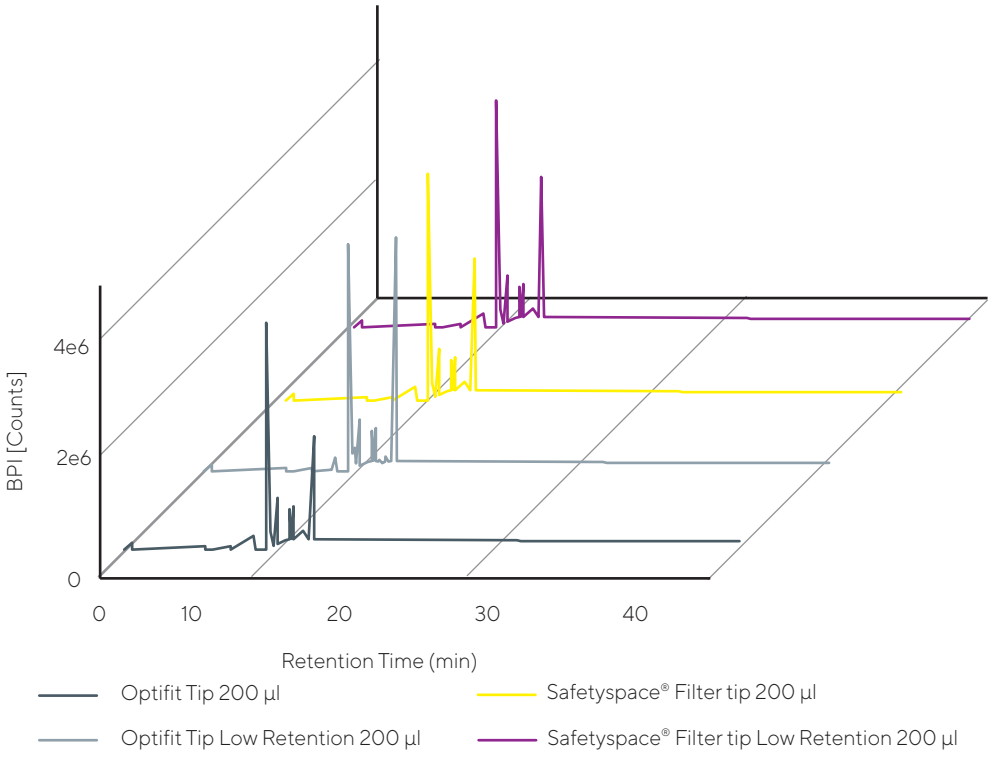


Figure 2. Chemical leachable analysis of Sartorius pipette tip EtOH extracts using LC-HRMS (ESI+). Erucamide retention time was 9.84 minutes; internal standard Irganox 1035, 9.27 minutes.

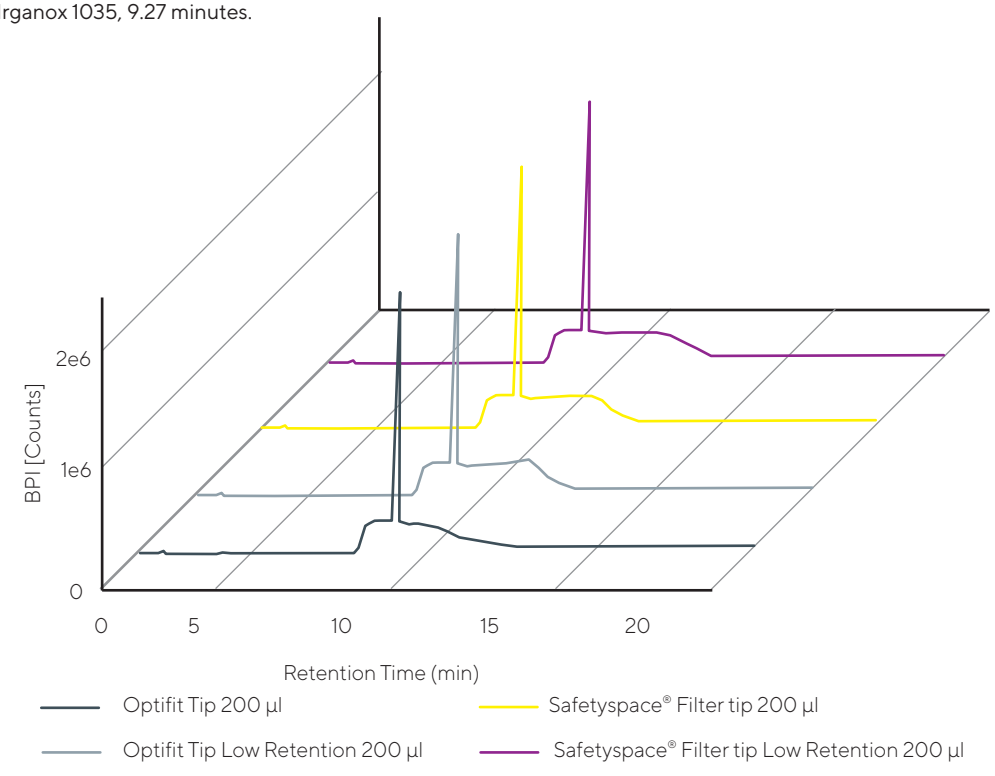


Figure 3. Chemical leachable analysis of Sartorius pipette tip DMSO extracts using LC-HRMS (ESI-). Erucamide retention time was 9.84 minutes; internal standard Irganox 1035, 9.24 minutes.

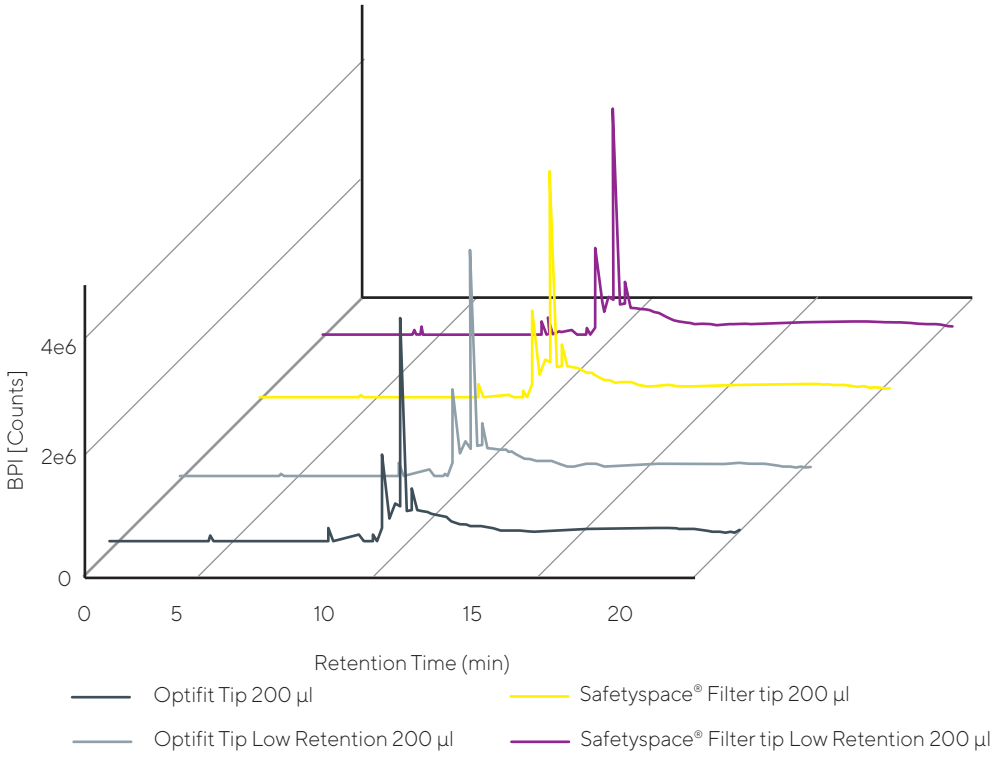


Figure 4. Chemical leachable analysis of Sartorius pipette tip EtOH extracts using LC-MS (ESI-). bDtBPP retention time 6.97 minutes; internal standard Irganox 1035, 9.26 minutes.

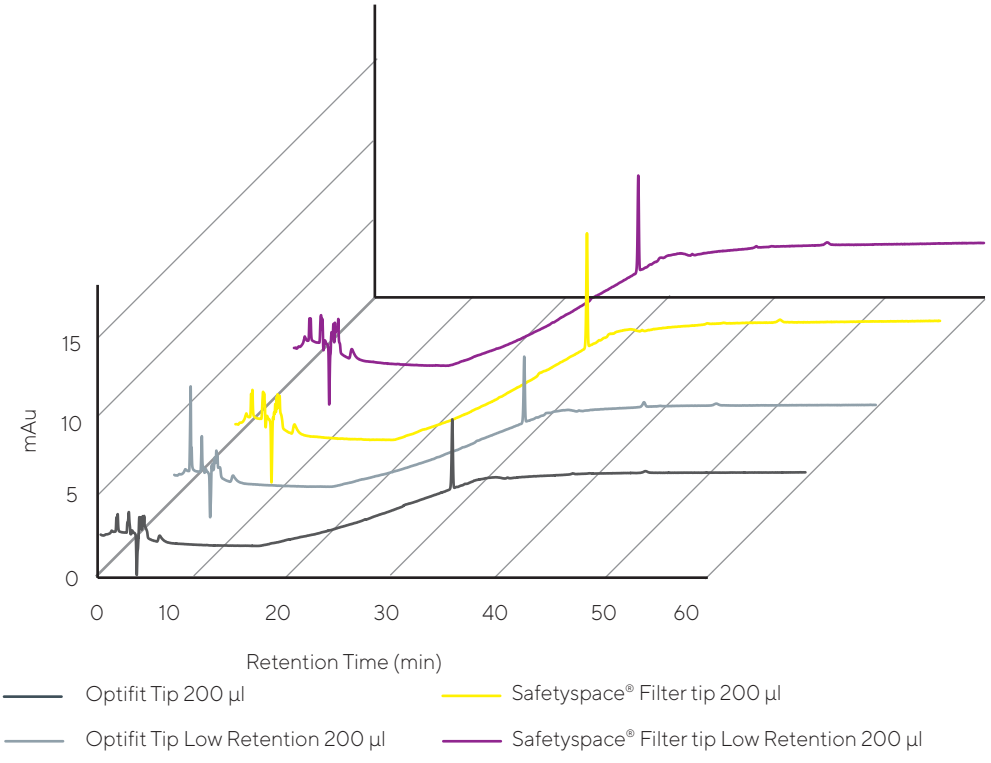


Figure 5. Chemical leachables analysis of Sartorius pipette tip EtOH extracts using HPLC-UV/VIS. Irgafos 168 retention time 40.17 minutes; internal standard Irganox 1035 (Thiodiethylenbis[3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate], CAS 41484-35-9), 30.00 minutes.

# Conclusions

Proper technique and use of the appropriate laboratory ware can help ensure the reproducibility and reliability of analytical experiments by minimizing the possibility of ghost peaks. The results obtained from our analyses show that Sartorius pipette tips release extremely low levels of a limited number of chemicals (Table 1). The levels quantified were below the lowest biologically relevant interfering concentrations reported in the literature for erucamide (0.338 ppm, (8)), bDtBpp (0.035 ppm, (3)) and oleamide (100 ppm, (9)). Additionally, no plasticizers were detected in samples from Sartorius pipette tips.

High quality Sartorius Optifit and Safetyspace® filter tips and their low retention variants can therefore be used in HPLC, GC-MS and LC-MS sample preparation without the risk of introducing interfering ghost peaks into the resulting chromatograms.

Table 1. Chemical leachables from Sartorius pipette tips

| Compound | CAS registry number | Quantified amount (ppm) | LOQ (ppm) |
|---|---|---|---|
| Erucamide | CAS 112-84-5 | 0.009-0.14 | 0.001 |
| bDtBPP | CAS 69284-93-1 | 0.02-0.026 | 0.001 |
| Oleamide | CAS 301-02-0 | <0.01*-0.11 | 0.01 |
| BBP, Benzyl butyl phthalate | CAS 85-68-7 | -† | 0.01 |
| Bis(methylglycol) phthalate | CAS 117-82-8 | -† | 0.01 |
| DBP, Dibutyl phthalate | CAS 84-74-2 | -† | 0.1 |
| DEHP, Bis(2-ethylhexyl) phthalate | CAS 117-81-7 | -† | 0.1 |
| DiHEMDA | CAS 22340-01-8 | -† | Undetermined‡ |

*Below limit of quantification (LOQ)
†Below limit of detection (LOD)
‡No sample was positive for DiHEMDA therefore no LOQ was determined

# Methods

### Laboratory Equipment-related Leachable Testing

A variety of pipette tips (200 µl Optifit tip (Product Number 790200), Safetyspace® Filter tip (Product Number 790201F) and low retention variants (Product Numbers LH-L790200 and LH-LF790201)) were rinsed using 100 µl of 1) ethanol or 2) DMSO by aspirating the solvent into the pipette tip, holding for five seconds and dispensing directly into sample tubes. Several pipette tips of the same type (5 pipette tips for a total of 500 µL, pooled sample) were used to generate a test volume sufficient for laboratory equipment-related leachable analysis by HPLC-UV/VIS, GC-MS or LC-HRMS.

### HPLC-UV/VIS

Ethanol extracts were injected into Agilent 1200 infinity (Agilent Technologies Inc., California, USA) HPLC system equipped with a Nucleosil C18 (5 µm x 250 mm x 4.6 mm) column and an ultraviolet/visible (UV/VIS) detector. Flow rate: 1 ml/min; wavelength: 220 nm; Injection volume: 20 µl; Temperature: 40 °C; Mobile phases: A) Acetonitrile | B) Water

### GC-MS

Ethanol and water extracts were prepared by liquid-liquid extraction and injected into the Clarus 600GC—Clarus 600T MS Turbo (PerkinElmer Inc., Massachusetts, USA) with C18 Elite-5MS (60 m × 0.25 mm × 0.25 µm) column. Semi-quantification was performed by using the internal standards 2-fluorobiphenyl (10 µg/ml) for liquid injection and toluene-d8 (0.1 µg/ml) for Headspace injection.

LC-MS

Ethanol and DMSO extracts were injected directly into the Waters ACQUITY UPLC I-Class—Waters Xevo G2-XS QTof (Waters Corp, Massachusetts, USA) LC-MS system with BEH C18 (1.7 µm, 2.1 × 100 mm) column (Flow rate: 0.5 ml/min; Injection volume: 1 µl; Temperature: 40 °C; Mobile phases: A) Acetonitrile | B) Water with 10 mmol NH4CH3COO, Gradient: 0 – 0.5 min 5% A, 0.5-9 min 5% A, 9-39.5 min 99% A, 39.5-40 min 5% A). Screening was performed by visual comparison of the base peak ion (BPI) chromatogram of the sample blank with the sample(s) and the UV/VIS chromatogram of the sample blank with the sample(s), respectively.

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