# Evaluation of matrix influence in extractables and leachables analysis of medical device extracts

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## Plain Language Summary

Accuracy of quantification of extractables and leachables can be reduced by the presence of matrix in the sample. This study is designed to evaluate the extent of the matrix effect in quantifying common polymer additives and explore the feasibility of preparing a suitable matrix matched reference material for the analysis.

## Introduction

**Background**

Extractables and leachables (E&L) are the chemical species that can be released from the device materials to the medium under laboratory and clinical use conditions, respectively. To provide exposure estimate for biocompatibility evaluation, accurate detection and quantification of these E&L are necessary. Liquid chromatography mass spectrometry (LC/MS) is a commonly used analytical technique to identify and semi quantify the nonvolatile extractables in the medium. Matrix effect (ME) occurs when compounds co elute and compete with the ionization of the analyte which can create under/over estimation of the analyte concentration. Occurrence of ME can alter the accuracy, precision and sensitivity of the LC/MS analysis.

**Purpose:**

This study aims to evaluate the matrix effect in LC/MS analysis of device extracts using different polymer materials and extraction conditions. Additionally, the extract stability will be tested to determine the feasibility of developing a matrix matched reference standard.

## Experimental Workflow

**Device Material**

- Acrylonitrile butadiene styrene (ABS)
- USP high density polyethylene (HDPE)
- Low density polyethylene (LDPE)
- Polypropylene (PP)
- Polyurethane with 0.25% zinc dibutyldithiocarbamate (PU).

**Extract the device materials in IPA and EW**

↓ 50C for 24 hours

**Spike the standard mix to the extract/ extract mixture**

↓

**Analyze the spiked extract using LC/UV or LC/MS**

**Spiked standards (2ppm)**

- Bisphenol A (BPA)
- bis(2-ethylhexyl) phthalate (DEHP)
- diethyl phthalate (DEP)
- diphenyl phthalate (DPhP)
- Irganox 1010
- Irganox 1330
- Stearic acid (SA)
- 2,5-Bis(5-tert-butyl-benzoxazol-2-yl)thiophene (BBOT)
- 2,6-Di-tert-butyl-4-methylphenol (BHT)
- 4,4′-Thiobis(2-tert-butyl-5-methylphenol) (STX)
- **Deuterated-DEHP (D4)**
- **Deuterated-BPA (D16)**

} Internal standards

| LC/UV/MS Parameters | |
|---|---|
| Column | Stable Bond $C_{18}$ Poroshell 300 columns with 2.1 mm x 100 mm and 2.7 µm particles |
| LC buffers | • ESI(+): 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B)<br>• ESI(-): 10 mM ammonium acetate in water (A) and acetonitrile (B) |
| Gradient | 40%B from 0.0-2.0 min, 100%B at 10 min and hold until 18.3 min (at 0.55 mL/min) |
| Mass range (*m/z*) | 100-1700 |
| Diode array detector (DAD) | 200-600 nm (280 nm for qualitative analysis) |
| LC/MS libraries | • DSSTox: Extracted from EPA DSSTox (>100,000 compounds consisting of organic acids, carbamic acids, and carbamates)<br>• Agilent E&L PCDL:~1000 compounds polymer additive relevant compounds<br>• Contaminant List:Literature based list of common contaminants (<1000 compounds) |

## Results

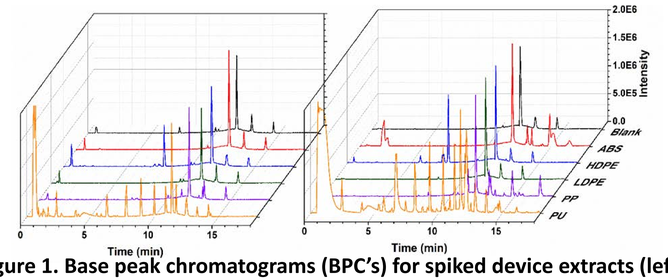


**Figure 1. Base peak chromatograms (BPC's) for spiked device extracts (left) ethanol water (EW) extracts and IPA extracts (right) in ESI(+).**

**Table 1: Identification/quantification of top 5 extractables in the extract mixture (all material extracts mixed at 1:1 ratio) [EW: 1:1 Ethanol:water; IPA: Isopropyl alcohol]**

| Name | RT (min) | Extract Mixture | Ionization | Average Concentration (mg/kg) | %RSD |
|---|---|---|---|---|---|
| Oleamide | 9.0 | EW | (+) | 6 | 3 |
| Palmitamide | 10.0 | | (+) | 22 | 2 |
| 20-amino-3,6,9,12,15,18-hexaoxaicosan-1-yl)carbamate | 9.9 | | (+) | 101 | 1 |
| Benzyl[…]carbamate* | 14.2 | | (+) | 103 | 1 |
| Triton X-100 Reduced N5 | 8.0 | | (+) | 187 | 1 |
| Palmitamide | 10.0 | IPA | (+) | 92 | 3 |
| Stearamide | 11.3 | | (+) | 108 | 2 |
| Triton X-100 Reduced N5 | 8.0 | | (+) | 257 | 1 |
| Irgafos 168 Phosphate | 14.2 | | (+) | 656 | 1 |
| Didodecyl 3,3'-thiodipropionate | 14.3 | | (+) | 730 | 6 |
| 2-Hydroxyethane-1-sulfonic acid | 0.5 | EW | (-) | 222 | 2 |
| Carbamic acid, [(trimethoxysilyl)methyl]-, methyl ester | 0.6 | | (-) | 218 | 21 |
| (Acetyloxy)-carbamic acid tert-butyl ester | 0.8 | | (-) | 263 | 1 |
| Carbamic acid, octyl ester | 0.6 | | (-) | 352 | 23 |
| Carbamic acid, dipropylthio-, O-ethyl ester | 11.8 | | (-) | 243 | 7 |
| Octadecyl-4-hydroxyphenylpropionate | 11.0 | IPA | (-) | 2310 | 17 |
| 2-methylidenepentadecanoic acid | 9.2 | | (-) | 2304 | 1 |
| Dodecyl 2-methylacrylate | 8.9 | | (-) | 1856 | 2 |
| Cyanox 1790 | 10.7 | | (-) | 1560 | 2 |
| Carbamic acid, dipropylthio-, O-ethyl ester | 0.5 | | (-) | 1439 | 6 |

*Benzyl [(3S,5S,6S,8S)-8-[(3-amino-2,2-dimethyl-3-oxopropyl)carbamoyl]-6-hydroxy-3-{[4-methoxy-3-(3-methoxypropoxy)phenyl]methyl}-2,9-dimethyldecan-5-yl]carbamate

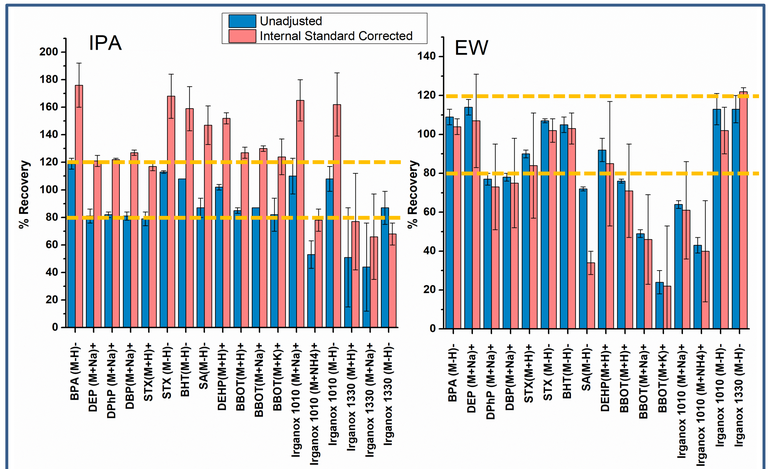


**Figure 2: LC/MS Spike and recovery of standard compound mixture added to the material extract mixture (dashed line indicates the expected recovery)**

**Table 2: LC/UV Spike-and-recovery of standard compound mixture added to the material extract mixture (CE:Co-elution, ND:Not detected)**

| Spiked Analyte | Retention Time (Min) | Signal | IPA | | EW | |
|---|---|---|---|---|---|---|
| | | | Recovery (%) | %RSD | Recovery (%) | %RSD |
| **BPA** | 1.77 | UV (280 nm) | C.E. | C.E. | C.E. | C.E. |
| **DEP** | 2.89 | UV (280 nm) | 114 | 2 | 111 | <1 |
| **DPhP** | 7.29 | UV (280 nm) | 111 | 2 | 110 | 1 |
| **DBP** | 7.86 | UV (280 nm) | 115 | 1 | 88 | 2 |
| **STX** | 8.56 | UV (280 nm) | 105 | <1 | 121 | 1 |
| **BHT** | 8.90 | UV (280 nm) | 106 | 1 | 112 | 4 |
| **SA** | 12.20 | UV (280 nm) | N.D. | N.D. | N.D. | N.D. |
| **DEHP** | 11.57 | UV (280 nm) | C.E. | C.E. | C.E. | C.E. |
| **BBOT** | 11.85 | UV (280 nm) | 103 | <1 | 105 | 1 |
| **Irganox 1010** | 13.34 | UV (280 nm) | 101 | 5 | 97 | 1 |
| **Irganox 1330** | 13.60 | UV (280 nm) | 114 | 8 | 109 | 1 |

## Discussion and Conclusion

- Device materials produced different extractables profiles, which provided a complex matrix when combined
- Multiple databases were used to identify these compounds including DSS-TOX
- LC/UV spike and recovery (accuracy and precision) was improved in the absence of the internal standards. Internal standards did not improve compound recovery in the presence of matrix from the device materials.
- LC/UV was less susceptible to matrix effects compared to LC/MS.
- Depending on the ionization mode and adducts, concentrations of hydrophobic extractables were underestimated by 20 percent or more in matrix.
- Although IPA and EW are sometimes claimed to be equivalent semi-polar solvents, they produced non-equivalent recoveries for the spiked samples.

## Acknowledgements

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