Alternative Solvents for Non-Targeted Extractables Analysis of Blood Contacting Medical Devices

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Plain Language Summary

An important aspect of safety evaluation for medical devices is to perform chemical analysis to understand the identities of chemicals that can be released from devices and their quantities. This work explores alternative solvents to simplify non-targeted extractables analysis of blood contacting medical devices.

Introduction/Purpose

- The standard ISO 10993-18:2020, which describes chemical characterization approaches, includes statement "Based on published research, 40% (by volume) mixture of ethanol/water is considered an appropriate surrogate for blood and blood related substances…";
- However, the published research on 40% ethanol/water approach relies on a very few material-extractable systems. This can be of concern since unreliable extractions systems can underestimate concentrations of extractables, leading to incorrect conclusions or greater uncertainties in toxicological risk assessment.
- The goal of this study was to evaluate considerations for creating and justifying alternative solvents for non-targeted extractables analysis of blood contacting medical devices. We explore two approaches:
  1. *Create simpler simulators of blood with complex protein content that can be used in extractables studies.*
  2. *Create simple solvents that may act like blood in certain extractables studies.*

Materials and Methods

Two UHPLC/UV/MS systems were used (Dionex Ultimate 3000/Agilent 6540B UHD QTOF-MS and Thermo Vanquish Flex/ Q Exactive HF) measuring both UV and MS signals. Also, target specific measurements were done by LC-MS/MS using Agilent 1290/Agilent 6495C QQQ-MS. Current method includes:

- Bis(2-ethylhexyl) phthalate (DEHP- CAS number 117-81-7)
- Mono(2-ethylhexyl) phthalate (MEHP- CAS number 4376-20-9)

Methods are targeted for certain extractables. However, screening is also performed.

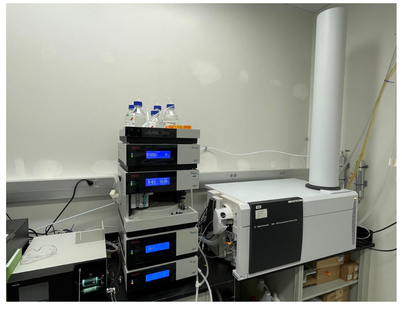




Figure 1: Two of the LC/MS systems used in this work; on the left, Agilent QTOF system; on the right Thermo Q Exactive HF system

Method Verification

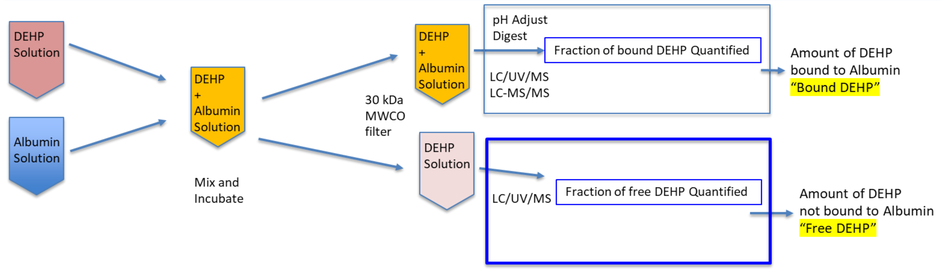


Figure 2: Method Verification Experiment: The purpose is to perform simple analysis to quantify DEHP binding to different types of Albumin (human serum, chicken, bovine serum) and validation of DEHP+MEHP amount quantified in bound Albumin; therefore quantifying the extraction power.
Protein rich solution analysis procedure based on Liu et al 2014 (see reference)

Considerations for extraction and sample processing:

- Albumin concentration in blood: 55 mg/mL;
- Since 55 mg/mL was too concentrated to handle; 10-20 mg/mL albumin was used.
- Albumin stability for long term extraction.
- Albumin may form particulates (especially if higher concentrations used).
- pH Adjustment/digestion makes solutions more difficult to work with in LC/MS settings.
- Low recovery if syringe filters are used.

Reference: "A Novel and Cost Effective Method of Removing Excess Albumin from Plasma/Serum Samples and Its Impacts on LC-MS/MS Bioanalysis of Therapeutic Proteins" Guowen Liu, Yue Zhao, Aida Angeles, Lora L. Hamuro, Mark E. Arnold, and Jim X. Shen, Anal. Chem. 2014, 86, 8336−8343.*

Summary

- Binding of medical device extractables to protein containing solutions such as albumin solution can be measured.
- DEHP present in PVC-Medical grade tubing was determined using different test solution such as albumin, ethanol, hexane and Isopropanol at 37 °C for 24 hours at 100 RPM.
- This ongoing work continues to expand comparison of specified extraction solvents and conditions and verifying with all LC based analytical methods.

Feedback

We appreciate feedback and opportunity to collaborate. We would like to hear from you: please contact at Berk.Oktem@fda.hhs.gov or Kanti.Sapkota@fda.hhs.gov

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Results and Discussion

Table 1: Recoveries in verification experiment using human serum albumin (without syringe filters used; particulates removed by centrifugation). *Calculation assumes 100% recovery. Total incubation time before centrifugation: 2 minutes

Sample name	Concentration of added DEHP (µg/mL)	Measured concentration of Free DEHP (µg/mL)	Calculated* concentration of Bound DEHP (µg/mL)
Solvent Blank	53	53.0	n/a
Albumin 10 µg/mL	53	43.2-45.0	8.0-9.8
Albumin 20 µg/mL	53	44.1-45.0	8.0-8.9

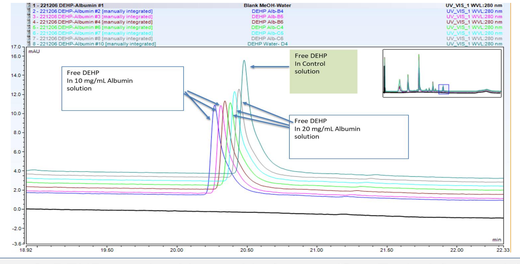


Figure 3: DEHP signal in Method Verification (See Table 1 for details)

Table 2: Recoveries in verification experiment using human serum albumin (with syringe filters used). Total incubation time before centrifugation: 30 minutes

Sample name	Concentration of added DEHP (µg/mL)	Measured Concentration of Free DEHP (µg/mL)	Measured Concentration of Bound DEHP (µg/mL)	Estimated Recovery %
Solvent Blank	10	2.1 - 4.5	<ND	21 to 45
Albumin 10 µg/mL	10	1.3-4.0	2.4-3.1	37 to 71
Albumin 20 µg/mL	10	3.6-5.4	2.7-3.2	63 to 86

Table 3: Determination of DEHP in PVC-Medical grade tubing by LC-MS technique (material was extracted at 37 °C for 24 hours at 100 RPM)

Sample name	Test Solution	Average Calculated amount of DEHP (µg/mL)	Standard Deviation
PVC-Medical grade Tubing	Hexane	80.037	25.584
PVC-Medical grade Tubing	Isopropanol	54.572	6.414
PVC-Medical grade Tubing	40% Ethanol	16.903	9.751