Selective Extraction And Analysis of Chemical Migrants from Packaging Material using a Supercritical Fluids (SFE)

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Regulatory aspects

- The pharmaceutical drug companies have to prove that nothing harmful gets transferred from the packaging into drug product. Or that whatever does leach into sample is below the thresholds established by the guidelines.

- For food industry only certain chemicals must be checked/monitored e.g. bisphenols A, B and E, phthalates. But other have to be determined as well.

- Under the Federal Food, Drug, and Cosmetic Act, cosmetic products and ingredients do not require FDA approval before they go on the market. The exception is color additives.

- FDA periodically buys cosmetics and analyzes them, especially if aware of a potential problem. The information obtained can be used to alert consumers, support regulatory actions, or issue guidance for industry. FDA does not have the resources to sample and analyze all cosmetics on the market.
Typical extractables & leachables

- Chemical additives, plasticizers, antioxidants and contaminants present in individual polymers
- Monomers and oligomers from incomplete polymerization reactions
- Volatile compounds from the secondary packaging such as inks and adhesives
- Residual compounds from the surfaces of the molding equipment, antistatics etc
Sample Preparation
Major Source of Laboratory Costs

- Sample preparation is the most often cited area of improvement to save time and operating costs

- Most sample preparation involves being in an organic phase
  - Liquid/Liquid, PPT, Soxhlet, Distillation, Evaporation and Reconstitution

- Many matrices will respond best to organic phases (gels, blisters, ointments, synthesis solvents, etc.)
Comparison study of 4 different extractions techniques

- Compare 4 different extraction techniques of 4 common packaging materials:
  
  - **Liquid extraction**
    - Water
  
  - **Microwave**
    - Hexane, Isopropanol
  
  - **Soxhlet**
    - Hexane, Isopropanol
  
  - **Supercritical Fluid Extraction (SFE)**
    - Isopropanol

Compare extraction profiles of the same packaging materials by using UPC² (SFC)
Samples

- High Density Polyethylene bottle (HDPE)
- Low Density Polyethylene container (LDPE)
- Ethylene Vinyl Acetate plasma bag (EVA)
- Polyvinyl Chloride blister pack (PVC)

Analytes:
- Irgafos 168, 5-chloro-2-hydroxy-4-methylbenzophenone (5-Cl-2-OH-4-methyl BP), 4-hydroxy-2-octyloxybenzophenone (4-OH-2-octyloxy BP), Irganox 245, Lowinox 44B25, Naugard 445, Diphenyl phthalate, Tinuvin 328, Uvitex OB
Extractions conditions

**Microwave extraction**
- 2g sample
- 1 cm² pieces
- Teflon Vessel
- 10 mL Hexane
  - + stirrer and heating element
  - 3 hours
  - 50°C
- 10 mL IPA
  - + stirrer
  - 3 hours
  - 50°C

**Water extraction**
- 2g sample
- 1 cm² pieces
- 20 mL
- Headspace Vial
- 20 mL Water
  - 72 hours
  - 50°C

**Soxhlet**
- 3 g of PVC
- 5 g of HDPE, LDPE, and EVA
  - 1 cm² pieces
  - Whatman 33 mm x 94mm cellulose extraction thimble
  - 175 mL Hexane
  - 8 hours
  - Dry
  - 15mL Hexane
  - Dry
  - 15mL IPA

- 175 mL IPA
  - 8 hours
  - Dry
  - 15mL IPA
Supercritical Fluid Extraction (SFE)
What Is Supercritical Fluid Extraction

- Supercritical Fluid Extraction is the process of separating one or multiple components (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent.

- Extraction is usually from a solid matrix

- SFE can be used as:
  - a sample preparation step for analytical purposes
  - or on a larger scale to either strip unwanted material from a product (e.g. decaffeination) or collect a desired product (e.g. essential oils).

- Carbon dioxide (CO₂) is the most used supercritical fluid, sometimes modified by co-solvents such as ethanol or methanol.
  - > 31°C and 74 bar (1073 psi)

- It is based on the principle that solubility in a supercritical fluid increase dramatically with increasing density, and different solutes have different solubility at the same condition.
**Why A Supercritical Fluid?**

Why do Supercritical fluids make good mobile phases for chromatography?

<table>
<thead>
<tr>
<th></th>
<th>Diffusivity (cm²/s)</th>
<th>Viscosity (g/cm x s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gas</td>
<td>10⁻¹</td>
<td>10⁻⁴</td>
</tr>
<tr>
<td>Supercritical Fluid</td>
<td>10⁻⁴ - 10⁻³</td>
<td>10⁻⁴ - 10⁻³</td>
</tr>
<tr>
<td>Liquid</td>
<td>&lt; 10⁻⁵</td>
<td>10⁻²</td>
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</tbody>
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Diffusivity describes the rate at which one substance can move through another.

Viscosity is resistance to flow.

High diffusivity, and low viscosity combine in SFC to give **fast, efficient chromatography**.
Supercritical Fluid Applications

Chromatography (SFC)
- Analysis & Purification
- Chiral Separation
- Normal Phase

Extraction (SFE)
- Bioactive compounds
- Nutraceuticals
- Spices and aromatic
- Decaffeination
- Decontamination or cleaning
- Valuable molecules from waste

Reaction
- Hydrogenation
- Hydroformylation
- Carboxylation

SF Particle Design (RESS & SAS)
- Polymerization
- Cristallization
- Impregnation
Why A Supercritical Fluid?
Extractability Based on Polarity

Increasing Polarity

- Non-polars (Alkanes)
- Ethers
- Alcohols
- Acids
- Amines
- Highly polar organics
- Inorganic ions

- Neat CO₂
- CO₂ + modifier
- CO₂ + modifier + ternary additives
- CO₂ + modifier + ternary additives + water

Liquid – based extraction methods

Small molecules | Peptides | Large proteins

Increasing Molecular Weight

One of the largest advantages of SFE: Selectivity
Advantages of SFE:

- **Increased selectivity and specificity**
  - Fine tune the extraction with changes in co-solvents (Method Dev)

- **Decreased cost** per sample
  - Minimal procurement or disposal cost of CO$_2$ in comparison to organic solvents
  - *Improves extraction efficiency and reduces extraction time* vs. other sample preparation techniques

- **Minimize exposure** to organic solvents
  - Lack of residual organic solvents
  - Is environmentally friendly

- **Accelerate** the extraction process
  - Extract analytes faster than comparative techniques
  - Eliminate cumbersome traditional solid/liquid extraction (ie. Sohxlet or solvent soak)

- **Ability to handle** thermally labile compounds
  - Operates at lower temperatures than PSE, MAE and soxhlet
An extraction technique **complementary/alternative to Soxhlet or liquid/liquid extraction**

- CO$_2$ in combination with an organic solvent, most commonly alcohols, is used as the extraction solvent
Extraction Modes

- Extractions are done in dynamic, static, or combination modes.

- In a **dynamic extraction** the supercritical fluid **continuously flows** through the sample in the extraction vessel and out the restrictor to the trapping vessel.

- In **static mode** the supercritical fluid is **held in the extraction vessel** for some period of time before being released through the restrictor to the trapping vessel.

- In **combination mode**, a static extraction is performed for some period of time, followed by a dynamic extraction.
SFE conditions

**Microwave extraction**
- 2 g sample
- 10 mL Hexane
- 10 mL IPA
- 3 hours 50°C

**Water extraction**
- 2 g sample
- 10 mL Hexane
- 10 mL IPA
- 20 mL Water
- 3 hours 50°C
- 72 hours 50°C

**Soxhlet**
- 3 g of PVC
- 5 g of HDPE, LDPE, and EVA
- 175 mL Hexane
- 175 mL IPA
- 8 hours
- 8 hours
- Dry 15 mL Hexane
- Dry 15 mL IPA

**Supercritical Fluid Extraction**
- 2 g of PVC
- 3 g of HDPE, LDPE, and EVA
- 1 cm² pieces
- CO₂:IPA 98:2
  - 4.9 mL/min CO₂
  - 0.1 mL/min IPA
- CO₂:IPA 80:20
  - 4 mL/min CO₂
  - 1.0 mL/min IPA
- 50°C & 300 Bars
  - 30 min Dynamic
  - 20 min Static
  - 10 min Dynamic
- 2 X
- Dry
  - 10 mL IPA for PVC
  - 9 mL IPA for HDPE, LDPE, and EVA

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What About The Analysis?
Supercritical Fluid Chromatography

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Broad Applicability

Polarity limits of chromatographic techniques

**Lipophilic**

- **10**
  - Lipids (triglycerides)
  - Liposoluble vitamins
  - Few plant components

- **5**
  - Most of usual drugs

**Log P**

- **0**
  - Metabolites
  - Amino acids/peptides
  - Nucleotides/Nucleosides

**Hydrophilic**

- **-5**
  - Antibiotics
  - Polysaccharides

- **-10**

**NPLC**

Requires a dedicated LC system. Poorly MS compatible. Hazardous solvent.

**RPLC**

Limited retention of polar and ionisable compounds.

**SFC**

Same instrument without drastic changes in analytical conditions

**HILIC**

Suitable till a certain polarity limit. High ACN consumption.

**IEX**

Requires a dedicated LC system. Poorly MS compatible.

**?**

Polarity limit of SFC must be defined.

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Courtesy of A. Grand-Guillaume Perrenoud, D. Guillarme, Pr J-L. Veuthey, University of Geneva
The ACQUITY UPC²

- Mass Spec
- Make-up Pump
- PDA detector
- Column Manager
- Back Pressure Regulator (Dynamic and Static)
- Auxiliary Inject valve
- Inject valve
- Thermo-electric heat exchanger
- mixer
- CO₂ Pump
- Modifier Pump
- CO₂ Supply
- Modifier
- CO₂ Supply
- Waste

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UPC$^2$: Compatibility with all MS Technologies

For ultimate CC-MS performance, ACQUITY UPC$^2$ System coupled with:

- **ACQUITY QDa**
  - Single quadrupole detector for robust and routine performance

- **Xevo TQ-S**
  - Ultimate sensitivity

- **Xevo G2-S Qtof and Synapt G2-S**
  - Qualitative and quantitative results from a single platform
Workflow Benefit of ACQUITY UPC² for the Analysis of Polymer Extracts

- **Polar Solvent Extraction**
  - Inject direct on LC
  - Back-extract with a non-polar solvent for GC injection

- **Non-Polar Solvent Extraction**
  - Inject direct on GC
  - Evaporate and reconstitute in a more polar solvent for LC injection

- **Polar or Non-Polar Extraction**
  - Inject direct On UPC2
Chromatographic separations

4 min separation by UPC\(^2\) vs. 9.5 min by UPLC
UPC² results - Microwave and Water extraction profiles for LDPE

Hexane

IPA

Water
Soxhlet and Microwave IPA extractions

**Soxhlet**
- PVC
- EVA
- LDPE
- HDPE

**Microwave**
- PVC
- EVA
- LDPE
- HDPE
SFE extracts

**Low IPA**

- PVC
- EVA
- LDPE
- HDPE

**High IPA**

- PVC
- EVA
- LDPE
- HDPE
LDPE, all IPA extracts

High IPA SFE

Low IPA SFE

Soxhlet

Microwave
UV chromatogram of LDPE SFE extract analysed by UPC$^2$
Confirmation of identity using MS

Peak in LDPE extract

Peak from Irganox 1076 std
Conclusion

All extraction techniques provided similar extractables profiles, but...

- SFE consumes much less solvent and is quicker than Soxhlet extraction.

- The MV-10 SFE System has automated method development and extractions on 10 samples to simplify the process.

- UPC² gives a fast, high resolution separation and has wide sample diluent compatibility.
Thank You!