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

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Market Specific terminology

- **Pharmaceutical packaging**
  - **Extractables** – Compounds that can be extracted from elastomeric, plastic components or coatings of the container and closure system when in the presence of an appropriate solvent(s)
  - **Leachables** – Compounds that leach from elastomeric, plastic components or coatings of the container and closure system as a result of direct contact with the formulation

- **Food packaging**
  - **Intentionally added substances (IAS)** - compounds added to produce the final product
  - **Non-intentionally added substances (NIAS)** - impurities from starting materials, reaction and degradation products formed during manufacturing process
  - **Migrants** - compounds which partition from the packaging into the food
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
Regulated areas for packaging

- Food packaging and contact materials- European guidelines list detailed experimental conditions (simulant, T°, time) based on food type
- Pharma- guidelines exist in Europe and USA
- Cosmetics- only European regulations require testing

=?
Sample Preparation
Major Source of Laboratory Costs

Grinding/Cutting

Soxhlet Extraction

SFE

Microwave

Migration cell
Comparison study of 3 different extraction techniques

- **Microwave**
  - Hexane, Isopropanol

- **Soxhlet**
  - Hexane, Isopropanol

- **Supercritical Fluid Extraction (SFE)**
  - Isopropanol

Multiple solvents to ensure polar and non-polar analytes extracted

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

- High Density Polyethylene pill bottle (HDPE)
- Low Density Polyethylene bottle (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
- 10 mL IPA + stirrer
- 3 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
- 175 mL IPA
- 8 hours
- Dry 15mL Hexane
- Dry 15mL IPA
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 Liquid Like</td>
<td>10⁻⁴ - 10⁻³ Gas Like</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.
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)

- 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.
Extractability Based on Polarity

Increasing Polarity

Non-polars
- Alkanes
- Esters
- Ethers
- Acids
- Amides
- Alcohols
- Amines

Highly polar organics

Inorganic ions

Neat CO₂

SFE

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
Instrumentation

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

Water extraction
- 2g sample
- 20 mL Water
- 72 hours 50°C

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

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
  - 50°C & 300 Bars
  - 2 X
  - 30 min Dynamic
  - 20 min Static
  - 10 min Dynamic
  - Dry
  - 10 mL IPA for PVC
  - 9 mL IPA for HDPE, LDPE, and EVA
- CO₂:IPA 80:20
  - 4 mL/min CO₂
  - 1.0 mL/min IPA
  - 50°C & 300 Bars
  - 2 X
  - 30 min Dynamic
  - 20 min Static
  - 10 min Dynamic
  - Dry
  - 10 mL IPA for PVC
  - 9 mL IPA for HDPE, LDPE, and EVA
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 CO₂ 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
Broad Applicability of SFC Analysis

Polarity limits of chromatographic techniques

- **Lipophilic**
  - Log P > 0
  - Suitable for lipids, liposoluble vitamins, and few plant components.
- **Log P = 0**
  - Suitable for most of the usual drugs.
- **Hydrophilic**
  - Log P < -5
  - Suitable for metabolites, amino acids, nucleotides, and nucleosides.
  - Log P < -10
  - Suitable for antibiotics and polysaccharides.

- **NPLC**
  - Requires a dedicated LC system.
  - Poorly compatible with MS.
  - Hazardous solvent.
- **RPLC**
  - Suitable for limited retention of polar and ionisable compounds.
  - High ACN consumption.
- **HILIC**
  - Suitable till a certain polarity limit.
- **IEX**
  - Requires a dedicated LC system.
  - Poorly compatible with MS.

**SFC**

Same instrument without drastic changes in analytical conditions.

Polarity limit of SFC must be defined.

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

- Mass Spec
- Make-up Pump
- Back Pressure Regulator (Dynamic and Static)
- Splitter
- PDA detector
- Column Manager
- Auxiliary Inject valve
- Inject valve
- Thermo-electric heat exchanger
- CO₂ Supply
- CO₂ Pump
- Modifier Pump
- Modifier
- Waste
For ultimate CC-MS performance, ACQUITY UPC² System coupled with:

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

**Xevo TQ-XS**
- Ultimate sensitivity

**Xevo G2-XS Qtof and Synapt G2-Si**
- 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 UPC² vs. 9.5 min by UPLC
- Orthogonality of techniques demonstrated by change in elution order
- Either of extraction solvents directly injected into UPC²
LDPE, all IPA extracts

High IPA SFE

Low IPA SFE

Soxhlet

Microwave
UV chromatogram of LDPE SFE extract analysed by UPC²

- 3 target compounds identified
Confirmation of identity using MS

Peak in LDPE extract

Peak from Irganox 1076 std
Conclusion

All extraction techniques provided similar extractable 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

- UPC$^2$ gives a fast, high resolution separation and has wide sample diluent compatibility
Thank You!