UltraPerformance Convergence Chromatography
Supercritical Fluid Separations
With The Power & Performance of ACQUITY
Agenda

- What is a Supercritical Fluid?
- What is Supercritical Fluid Chromatography?
- Introduction To UPC²™
- ACQUITY UPC²™ Performance
- ACQUITY UPC²™ Applications
- Summary
"A **supercritical fluid** is any substance at a temperature and pressure above its critical point, where distinct liquid and gas phases do not exist.

It can effuse through solids like a gas, and dissolve materials like a liquid.

In addition, close to the critical point, small changes in pressure or temperature result in large changes in density, allowing many properties of a supercritical fluid to be "fine-tuned".

Supercritical fluids are suitable as a substitute for organic solvents in a range of industrial and laboratory processes. Carbon dioxide and water are the most commonly used supercritical fluids, being used for decaffeination and power generation, respectively.*

CO2 Phase Diagram

Critical T = 31.1 °C
Critical P = 74 bar/1070 psi

* Adapted from http://en.wikipedia.org/wiki/File:Carbon_dioxide_pressure-temperature_phase_diagram.svg

©2012 Waters Corporation
Diffusivity & Viscosity More Like A Gas
- Diffusivity promotes chromatographic mass transfer/resolving power
- Lower viscosity promotes chromatographic speed

<table>
<thead>
<tr>
<th></th>
<th>Diffusivity (cm²/s)</th>
<th>Viscosity (g/cm x s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gas</strong></td>
<td>10⁻¹</td>
<td>10⁻⁴</td>
</tr>
<tr>
<td><strong>Supercritical Fluid</strong></td>
<td>10⁻⁴ - 10⁻³</td>
<td>10⁻⁴ - 10⁻³</td>
</tr>
<tr>
<td><strong>Liquid</strong></td>
<td>&lt; 10⁻⁵</td>
<td>10⁻²</td>
</tr>
</tbody>
</table>

Solvating Power Like A Liquid
What is Supercritical Fluid Chromatography (SFC)?

- Chromatographic Technique Similar to HPLC
- Mobile Phase = Supercritical Fluid + One or More Co-Solvents
  - $\text{CO}_2$ = most common supercritical fluid
  - MeOH = most common co-solvent
SFC Characteristics

- Normal Phase Technique
  - Polar stationary phase/non-polar mobile phase
  - Elution order from lowest to highest polarity
  - Strong solvent = more polar solvent = MeOH
  - Weak solvent = less polar solvent = CO₂

- Compatible With Common LC Detectors
  - UV-VIS, ELSD, MS

- Utility For Many Industries/Market Segments
  - Applicable to wide range of compounds/varied functionality & polarity
  - Petrochem, pharma, polymer, environmental, food, natural products

- Ideal For Prep Applications
  - Easy removal of CO₂ from fractions

- Method Of Choice For Chiral Separations
SFC vs. GC vs. LC

- Better Mass Transfer Than LC
  - More efficient separations

- Better Solvation Than GC
  - Useful for broader range of analytes
  - Not restricted to volatile analytes

- Easily Manipulated Density
  - For fine tuning separations

Schoenmakers, Uunk 1987
SFC vs. LC
Separation Speed & Efficiency

Greater Diffusivity
- Higher optimal flow rates
- Shorter run times
- Same resolving power

Lower Viscosity
- Longer columns can be used
- Increased separation efficiency/equivalent run times
Which Compounds Work?

- Candidates for SFC
  - Normal phase LC separations
  - Separations that are problematic for LC and GC
  - Compounds in need of derivatization for GC
  - Compounds soluble in methanol or less polar solvents

Data from Actelion Corp. Web Site
Do Small Particle, i.e. < 2 µm, Column Chemistries Provide The Same Benefits To SFC As They Do To Traditional Reversed Phase LC?
SFC Column Efficiency Curves

- 5.0 µm XBridge™ HILIC, 3.0x50 mm
- 3.5 µm XBridge™ HILIC, 3.0x50 mm
- 2.5 µm XBridge™ HILIC, 3.0x50 mm
- 1.7 µm ACQUITY BEH, 3.0x50 mm
SFC Resolution Comparison

Column 1
5 µm Particles

Column 2
1.7 µm Particles

SFC Separations
3.0 x 100 mm Column
2 mL/minute
CO₂/Methanol Gradient
Introduction & Performance
A World Without UPC²

LC

- Versatile, liquid soluble compounds
- Change column and solvent
- Change temperature
- Derivatization

SFC

- Versatile, liquid soluble compounds
- CO₂ mobile phase, higher solvation
- Change column, pressure, temperature
- Add organic modifiers
- No derivatization

GC

- Compounds that can vaporize without degradation
- Change column
- Change temperature
- Derivatization
Why Is SFC A Niche Technique?

- Considered Exotic
  - Not taught as part of analytical chemistry curriculum

- No Major Chromatography Company Presence
  - "Frankensystems" comprised of modules from multiple vendors

- Problems With Robustness
  - Poor precision
  - Baseline noise/poor sensitivity
  - Unsuitable for regulated environments

- Systems Don't Provide A Complete Solution
  - Hardware/Software/Chemistry/Support
The ACQUITY UPC$^2$

- Designed Holistically For Optimum Performance
- Built Upon Proven UPLC Technology
  - Quantifiable increases in productivity
- SFC Capability With LC Familiarity
- Reproducible & Robust
  - Can withstand rigors of method validation
- Complete Solution
  - Hardware, software, chemistry, service & support
ACQUITY UPC² System Features

- **UPC² Binary Solvent Manager**
  - Blends & delivers CO² & co-solvent
  - 4 selectable co-solvents
  - 0.010 to 4.000 mL/min flow rate range

- **UPC² Fixed Loop Sample Manager**
  - Partial or full loop injection modes

- **ACQUITY Column Manager**
  - Multi column selector for selectivity screening

- **UPC² Convergence Manager**
  - Incoming CO² management & system pressure control
  - Key to precision, ruggedness & reducing baseline noise

- **Empower™ & MassLynx™ Compatible**
UPC²™ Compound Diversity

- Gradient Separation Diverse Polarity Mix
  - 18 components,
  - Amines, vitamins, dyes, steroids, antibacterials
- Exceptional Resolution
- Exceptional Precision
  - Retention time repeatability < 0.4 % RSD, N = 6

Column: ACQUITY UPC² Hybrid, 2.1 x 150 mm, 1.7µm
Flow: 1.2mL/min
Col Temp: 45 C
Detection: UV 230nm
Inj Vol: 2µL
Gradient: 2 – 16% MeOH in 7 minutes
ABPR: 130 bar
**UPC²™ Gradient Performance**

Overlays Of 10 Injections

0.5% Difference in Solvent Composition

- Precise & Accurate Co-Solvent Addition
  - Essential to the separation of closely related compounds, e.g. isomers, plant extracts, & essential oils of natural products

**Specifications**

- BEH 2-Biphenyl, 3 x 100 mm, 1.7 µm
- 2.00 mL/min, 55 ºC, 1958 psi (135 Bar)
- Injection Volume = 3 µL, 10 repeated injections
- PDA 280 nm, monitored (500-600 nm) 20 Hz, TC = 0.2 sec
UPC²™ Injector Linearity

Excellent Partial Loop Linearity
- 1 - 10 μL

R² = 0.999962
Y = 1.50e+005 X + 5.96e+003

Flow rate: 4 mL/min
Mobile phase: CO₂:methanol 70:30
Back pressure: 120 bar
Temp: 40 °C
UPC²™ Detector Sensitivity

Low Noise For Enhanced Sensitivity

| EP Imp. B | 0.985 | 613 | 0.02 |
| EP Imp. D | 1.549 | 1118 | 0.04 |
| EP Imp. A | 1.752 | 1681 | 0.07 |
| EP Imp. C | 2.136 | 870 | 0.03 |
| Metoclopr. | 2.278 | 2518759 | 99.73 |
| EP Imp. G | 2.459 | 1290 | 0.05 |

*Spectral integrity maintained for low level impurities for related compound confirmation*
UPC²™ Column Chemistries

- BEH
- BEH 2-Ethylpyridine
- CSH Fluoro-Phenyl
- HSS SB

1. Coumarin
2. Flavone
3. Caffeine
4. Thymine
5. Papaverine
6. Sulfamethoxazole
7. Cytosine
8. Sulfamethizole

Column: 4.6 x 150 mm, 5 μm 5-40% MeOH without additive 3.0 mL/min at 40 °C, 150 bar Injection volume: 3 μL
Applications
Lipids
- Rapid Screening Separation
- No Derivatization
- Direct Injection of Extraction Solvent
**UPC² & Lipidomics**

*Mouse Heart Extract/MS Detection*

---

**Glycerolipids**

(NH₄⁺ adducts)

- **SM**
- **PC**
- **PE**
- **LPE**
- **MAG**
- **TAG**
- **DAG**
- **Cardiolipins**
- **PGPS**
- **LPC**

---

©2012 Waters Corporation
Applications
Mixed Tocopherols
**UPC²™ Mixed Tocopherols**

**Column:** ACQUITY UPC² BEH  
3.0 x 100 mm, 1.7 μM  
**Flow Rate:** 2.5 mL/min  
**Column Temp:** 50 °C  
**Detection:** UV 293 nm (compensated 500 - 600 nm)  
**Inj Vol:** 1 μL  
**ABPR:** 1885 psi  
**Gradient:** 2 – 5 % Methanol in 1.5 minutes
Applications
Organic Light Emitting Diodes
**UPC²™ Organic Light Emitting Diodes**

- **Ir(fppy)₃**
  - Area, 0.4% RSD
  - RT, 0.1% RSD

- **α-NHP**
  - Area, 0.4% RSD
  - RT, 0.1% RSD

- **TCTA**
  - Area, 1.1% RSD
  - RT, 0.1% RSD

- **Balq (a)**
  - Area, 1.1% RSD
  - RT, 0.1% RSD

- 7 min UPC² Analysis
  - 10x reduction vs. NPLC

- 4 Components/7 Impurities
  - Resolves NPLC co-elution
UPC²™ OLEDS
Ir(fppy)₃ Degraded Sample Via MS

Ir(fppy)₃

Ir(fppy)₃ – F

Ir(fppy)₃ – F?

Ir(fppy)₃ -2F?
Applications
Chiral Separations
(R) & (S) Benzyl Mandelate
0.20 mg/mL each

"Pure" (R) Benzyl Mandelate
2.0 mg/mL

0.02% Impurity

$R_S = 7.28$
Normal Phase LC Method Conversion & GC Alternative
NPLC To UPC²™ Method Conversion  
USP Impurity Analysis For Estradiol

- **UPC²**
  - 3x reduction in run time
  - 2 more impurities found
  - >10x reduction in solvent cost
  - No halogenated solvent waste

### ACQUITY UPC²
Solvent Cost/Run ~ $0.05

### Normal Phase HPLC
Solvent Cost/Run ~ $5.89

<table>
<thead>
<tr>
<th>Compound</th>
<th>UPC² RT</th>
<th>%Area</th>
<th>S/N</th>
<th>Normal phase HPLC RT</th>
<th>%Area</th>
<th>S/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unk. impurity</td>
<td>2.26</td>
<td>0.012</td>
<td>3.4</td>
<td>6.24</td>
<td>0.006</td>
<td>2.9</td>
</tr>
<tr>
<td>Unk. impurity</td>
<td>2.59</td>
<td>0.004</td>
<td>1.9</td>
<td>Not Found</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unk. impurity</td>
<td>3.34</td>
<td>0.010</td>
<td>3.1</td>
<td>10.86</td>
<td>0.010</td>
<td>2.7</td>
</tr>
<tr>
<td>Unk. impurity</td>
<td>5.66</td>
<td>0.006</td>
<td>1.7</td>
<td>Not Found</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unk. impurity</td>
<td>6.15</td>
<td>0.016</td>
<td>5.5</td>
<td>20.85</td>
<td>0.018</td>
<td>3.0</td>
</tr>
<tr>
<td>Unk. impurity</td>
<td>8.13</td>
<td>0.013</td>
<td>3.1</td>
<td>26.63</td>
<td>0.021</td>
<td>3.2</td>
</tr>
<tr>
<td>Estradiol</td>
<td>8.81</td>
<td>99.890</td>
<td>–</td>
<td>30.86</td>
<td>99.87</td>
<td>–</td>
</tr>
<tr>
<td>Main impurity</td>
<td>9.99</td>
<td>0.046</td>
<td>16.0</td>
<td>36.81</td>
<td>0.077</td>
<td>9.2</td>
</tr>
</tbody>
</table>
**Biodiesel Glyceride Analysis**

**GC-FID**
*Derivatized Biodiesel standard*

- Petroleum Diesel Hydrocarbons
- FAMEs
- Monoglycerides
- Diglycerides
- Triglycerides

**ACQUITY UPC² ELSD**
*Underivatized Biodiesel standard*

- Underivatization
- 2.5x reduction in analysis time
- Glycerides well separated from FAMES

**UPC²**
- No derivatization
- 2.5x reduction in analysis time
- Glycerides well separated from FAMES
Summary
Analyze a Diverse Range of Compounds

Hydrophilic
- Volatile carboxylic acids
  - Aldehydes
  - Ketones

Sulfonamides
- Nitriles
- Organophosphorous pesticides

Hydrophobic
- C_{2}/C_{6} hydrocarbons

Polarity
- Nitrosamine
- TMS derivative of sugars

GC
- Volatile
- Volatility
- Nonvolatile

HPLC
- Synthetic food dyes
- Amino acids
- PG, OG, DG phenols
- Enzymes
- Aflatoxins
- Enzymes
- Antibiotics
- Flavonoids
- Natural food dyes
- Fat soluble vitamins
- Phospholipids
Green Cost Effective Solution

$UPC^2 CO_2$

Cost Effective, Avoids Toxic and Volatile Solvents.

Reducing Overall Cost of Operation.
ACQUITY UPC²™

- Merges Proven UPLC Technology With SFC
- Improves Resolution, Sensitivity & Speed
- Direct Replacement For NP LC
- Method Of Choice For Chiral Separations
- Reduces Cost Of Operation
- Reduces Use Of Toxic Solvents
- Complete System Solution
  - Hardware, software, chemistry support