Getting Started with UltraPerformance Convergence Chromatography

A Practitioner’s Guide for Utilizing UPC$^2$ in the Chromatographic Laboratory
Agenda

- What is UPC²?
- Getting Started
- Important Considerations for UPC²
- UPC² as a Replacement for NPLC
- Summary
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- Getting Started
- Important Considerations for UPC\(^2\)
- UPC\(^2\) as a Replacement for NPLC
- Summary
Convergence Chromatography is a category of separation science that provides orthogonal and increased separation power, compared to liquid or gas chromatography, to solve separation challenges.

UltraPerformance Convergence Chromatography [UPC²] is a holistically designed chromatographic system that utilizes liquid CO₂ as a mobile phase to leverage the chromatographic principles and selectivity of normal phase chromatography while providing the ease-of-use of reversed-phase LC.

The ACQUITY UPC² System is built utilizing proven UPLC® technology to enable scientists the ability to address routine and complex separation challenges while delivering reliability, robustness, sensitivity and throughput never before possible for this analytical technique.
Why is it Called Convergence Chromatography?


In this article Dr. Giddings stated “*One of the most interesting features of ultra high pressure gas chromatography would be convergence with classical liquid chromatography.*”
How Did Convergence Chromatography Evolve?

- GC (Gas Chromatography)
- LC (Liquid Chromatography)
- CC (Convergence Chromatography)
  - GC → Capillary GC
  - HPLC → UPLC
  - SFC → UPC²
How Does Convergence Chromatography Work?

- UPC² is a chromatographic technique similar to HPLC
  - Instead of mobile phase A being aqueous, it is CO₂

- Mobile phases include a supercritical fluid & one (or more) co-solvents
  - CO₂ is the most common supercritical fluid (LC: weak solvent – MP A)
  - Methanol is the most common co-solvent (LC: strong solvent – MP B)
How Does Convergence Chromatography Work?

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  - CO₂ is the most common supercritical fluid (LC: weak solvent – MP A)
  - Methanol is the most common co-solvent (LC: strong solvent – MP B)

- As in LC, additives can be used to improve peak shape and/or manipulate selectivity
  - Common additives: ammonium hydroxide, formic acid, etc.

- **UPC²** provides normal-phase-like selectivities

- **UPC²** is compatible with most popular detection techniques
  - PDA, ELSD, MS, etc.
Agenda

- What is UPC²?
- Getting Started
  - Understanding the Terminology
  - Can My Samples be Analyzed by UPC²?
  - ACQUITY UPC² Columns
  - A Screening Protocol
- Important Considerations for UPC²
- UPC² as a Replacement for NPLC
- Summary
Understanding the Terminology

- Conventional SFC terms such as *solvent*, *co-solvent* and *modifier* ALL refer to the primary liquid component(s) of mobile phase B
  - This *co-solvent* (mobile phase B) is the strong eluting solvent in UPC\(^2\)
  - It is typically methanol but can also be other organic solvents such as ethanol, 2-propanol, acetonitrile, etc. (or combinations)
Understanding the Terminology

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  - This co-solvent (mobile phase B) is the strong eluting solvent in UPC²
  - It is typically methanol but can also be other organic solvents such as ethanol, 2-propanol, acetonitrile, etc. (or combinations)

- An additive is a salt or liquid added to the co-solvent at a low concentration in order to improve peak shape(s) or analyte solubility and may influence selectivity
  - Examples of typical additives include diethyl amine, ammonium hydroxide, formic acid, trifluoroacetic acid, water, etc.
  - Typical additive concentrations are ≤ 2% or 10 mM
Can My Sample Be Analyzed by UPC²?

As with any analytical technique, the more you know about your analyte(s) and sample(s), the better

- What is the solubility of your analyte/sample in various organic solvents (often referred to as Log P)?
- What is its partition coefficient, P (ratio of concentrations of an analyte in a mixture of two immiscible solvents: typically 1-octanol/water)?
Can My Sample Be Analyzed by UPC²?

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  - What is the solubility of your analyte/sample in various organic solvents (often referred to as Log P)?
  - What is its partition coefficient, P (ratio of concentrations of an analyte in a mixture of two immiscible solvents: typically 1-octanol/water)?

- Basically, ANY compound soluble in an organic solvent is a candidate for UPC²
  - Many sample preparation techniques produce samples dissolved in an organic solvent (e.g., liquid/liquid extraction, solid phase extraction, protein precipitation, etc.) which can be injected directly
Can My Sample Be Analyzed by UPC²?

- Gather all the information that you can about your target analyte(s)
  - Molecular weight
  - Chemical structure
  - Molecular species (neutral, acid, base)
  - pKa (weak or strong)
  - Log P (for solubility)
  - UV absorbance (for choosing additives)

- Consult literature such as Merck Index, ChemBank, ChEMBL database, Beilstein, Gmelin, peer-reviewed journals, etc.

**Carbamazepine**

Solubility: 2-propanol (1.0 mg/mL), insoluble in water
Species: Neutral
pKa: (weak acid) 13.94
Log P: 1.875

References: Merck Index, ChemBank, ChEMBL database
Partition Coefficient and UPC$^2$

- Understanding analyte solubility is important in UPC$^2$

- The 1-octanol/water partition coefficient (P) is a common measure of analyte solubility and is often readily available

$$\text{Partition Coefficient (P)} = \frac{[\text{Analyte}]_{\text{Organic}}}{[\text{Analyte}]_{\text{Aqueous}}}$$
Partition Coefficient and UPC²

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- The 1-octanol/water partition coefficient (P) is a common measure of analyte solubility and is often readily available

  \[
  \text{Partition Coefficient (P)} = \frac{[\text{Analyte}]_{\text{Organic}}}{[\text{Analyte}]_{\text{Aqueous}}}
  \]

- Log P = log_{10} Partition Coefficient (P)
  - Log P = -2 means 1:100 Organic:Aqueous (100X more soluble in aqueous)
  - Log P = 9 means 10⁹:1 Organic:Aqueous (10⁹X more soluble in organic)

**Rule of Thumb:**
Log P between -2 and 9 means analyte is a potential candidate for UPC²
## UPC² Columns Used for Achiral Applications

<table>
<thead>
<tr>
<th>UPC² Column Chemistry</th>
<th>Applications Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>UPC² BEH</td>
<td>OLEDs, polymer additives, pesticides, lipids</td>
</tr>
<tr>
<td>UPC² BEH 2-EP</td>
<td>Steroids, pesticides</td>
</tr>
<tr>
<td>UPC² CSH Fluoro-Phenyl</td>
<td>Vitamin D metabolites, steroids, natural products</td>
</tr>
<tr>
<td>UPC² HSS C18 SB</td>
<td>Fat-soluble vitamins, lipids (free fatty acids)</td>
</tr>
</tbody>
</table>
- ACQUITY UPC² columns are shipped dry and require at least one hour or 100 column volumes under initial conditions to equilibrate.

- When using additives such (e.g., ammonium hydroxide) equilibration times may be longer.

- Failing to properly equilibrate a UPC² column upon installation can result in irreproducible retention times.
Getting Started: A Recommended Screening Protocol

New sample

Gather compound information: Structure/pKa/MW/Log P

-2 < Log P < 9

Yes

Insoluble

Check solubility in:
- Methanol, Ethanol
- Chlorinated solvents
- THF
- Up to 5% water

Prepare at 0.2 mg/mL

No

Analysis may be difficult

Prepare stock sample (1 to 2 mg/mL) in:
- 90/10 Heptane/2-Propanol (non-polar compounds) or 2-Propanol (polar) or similar

Soluble

Dilute to 0.2 mg/mL in 90/10 Heptane/2-Propanol

No Information Available (Dissolve in Organic Solvent)
# Getting Started: Initial Screening Conditions

<table>
<thead>
<tr>
<th>UPC² Screening Columns: BEH and BEH 2-EP</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd Options: CSH Fluoro-Phenyl &amp; HSS C18 SB</td>
</tr>
</tbody>
</table>

**Gradient:** 2 to 40% Methanol in 5.0 min

**Column(s):** 3.0 x 100 mm, 1.7 μm

**Flow rate:** 2.0 mL/min

**Column Temp:** 35°C - 50°C

**ABPR:** 2000 psi (140 bar)

**Wavelength:** 220 nm (compensated 350-450 nm)

**Weak Needle Wash:** Methanol/2-Propanol (1:1)

**Strong Needle Wash:** Methanol

**Seal Wash:** Methanol

**Co-Solvents**

- B1: Methanol
- B2: Methanol/Acetonitrile (1:1)
- B3: Methanol containing 15 mM NH₄COOH & 2% HCOOH*
- B4: Methanol containing 0.2% NH₄OH* (* - for use with BEH and BEH 2-EP columns only)
Getting Started: Initial Screening Conditions

**Starting Conditions**

<table>
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<tr>
<th>UPC² Screening Columns: BEH and BEH 2-EP</th>
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</table>

**Gradient:** 2 to 40% Methanol in 5.0 min

| Column(s): |
| 3.0 x 100 mm, 1.7 μm |

| Flow rate: |
| 2.0 mL/min |

| Injection volume: |
| 1 μL |

| Temperature: |
| 35°C - 50°C |

| ABPR: |
| 2000 psi (140 bar) |

**Poor Peak Shape?**

**Insufficient Retention?**

**Inadequate Selectivity?**

**How can we optimize these parameters?**
Strategies for Improving Peak Shape

Use Additives In Screening Protocol:
- Acidic Compounds: 0.5% HCOOH
- Basic Compounds: 0.2% NH₄OH with 3.0% H₂O

Try Alternative Additives*:
- Bases: Alkyl amines, ammonium acetate
- Acids: Citric acid, acetic acid

Increase Concentration of Additive or use 20 mM NH₄COOH / 2% HCOOH or add 2 to 5% H₂O

Resolved

Go to Retention or Selectivity until chromatography is acceptable

Then test:
- Repeatability
- Reproducibility
- Linearity (if needed)

Not Resolved

Try Different Column Chemistry

Resolved

Not Resolved

Return to initial screening conditions

(*) – Ensure additive is compatible with mode of detection
Strategies for Increasing Retention

1. Try Additional Co-Solvents: Acetonitrile, 2-Propanol, etc.
   - Resolved

2. Flatten/Reduce Gradient Slope
   - Resolved

3. Try Co-Solvent Mixtures
   - 90/10, 70/30, 50/50
   - Resolved

4. Reduce Density by Decreasing ABPR or Increasing Temperature
   - Resolved

5. Try Different Column Chemistry
   - Not Resolved

   Return to initial screening conditions

Go to Peak Shape or Selectivity until chromatography is acceptable

Then test:
- Repeatability
- Reproducibility
- Linearity (if needed)
Strategies for Changing Selectivity

- Different Co-Solvent and/or Mixtures of Co-Solvents
  - Resolved
  - Not Resolved
    - Different Additive*
      - Resolved
      - Not Resolved
        - Modify Density by Changing ABPR or Temperature
          - Resolved
          - Not Resolved
            - Try Different Column Chemistry
              - Resolved

Go to Peak Shape or Retention until chromatography is acceptable
Then test:
- Repeatability
- Reproducibility
- Linearity (if needed)

(*) – Ensure additive is compatible with mode of detection
Agenda

- What is UPC²?
- Getting Started
- Important Considerations for UPC²
  - Setup Guidelines
  - Co-Solvents
  - Mobile Phase Additives
  - Sample Diluents
  - Pressure and Temperature
- UPC² as a Replacement for NPLC
- Summary
Getting Started: Instrument Setup Guidelines

- Do NOT use Parafilm® to cover bottles (it will dissolve)
  - Use bottle with cap

- Use only Pyrex® (Borosilicate 3.3) bottles or equivalent

- Use highest quality co-solvents and additives

- Use food-grade CO₂ (99.97% pure) or higher

- Keep all co-solvent, needle-wash and seal-wash lines primed

- Contact your local Waters Service Representative with additional questions
Needle wash solvents flush the internal and external portions of the needle to prevent carryover
- The weak and strong washes should contain a co-solvent compatible with your sample
- Starting recommendations:
  - Weak needle wash: methanol/2-propanol (1:1)
  - Strong needle wash: methanol
- Adjust needle wash strengths based upon application requirements

Recommended seal wash is 100% methanol
The Role of Co-Solvents in Convergence Chromatography

- UPC$^2$ with pure CO$_2$ has limited utility due to the poor solvating power of CO$_2$
  - CO$_2$ has the eluting strength of heptane in UPC$^2$
  - Adding an organic co-solvent *increases* the solvating power of CO$_2$

- The co-solvent also affects retentivity and selectivity

- The role of the co-solvent in UPC$^2$ is analogous to that of the strong solvent in liquid chromatography
# Eluotropic (Eluting Strength) Series

<table>
<thead>
<tr>
<th>Co-Solvent</th>
<th>Eluting Strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentane, Hexane, Heptane</td>
<td>Strongest</td>
</tr>
<tr>
<td>Xylene</td>
<td></td>
</tr>
<tr>
<td>Toluene</td>
<td></td>
</tr>
<tr>
<td>Diethyl ether</td>
<td></td>
</tr>
<tr>
<td>Dichloromethane</td>
<td></td>
</tr>
<tr>
<td>Chloroform</td>
<td></td>
</tr>
<tr>
<td>Acetone</td>
<td></td>
</tr>
<tr>
<td>Dioxane</td>
<td></td>
</tr>
<tr>
<td>THF</td>
<td></td>
</tr>
<tr>
<td>MTBE</td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td></td>
</tr>
<tr>
<td>DMSO</td>
<td></td>
</tr>
<tr>
<td>Acetonitrile</td>
<td></td>
</tr>
<tr>
<td>2-Propanol</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td>Weakest</td>
</tr>
</tbody>
</table>

**CO$_2$ strength**
## Typical Co-Solvents Used in UPC\(^2\)

<table>
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<tbody>
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<td><strong>Weakest</strong></td>
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<tr>
<td>Acetone</td>
<td></td>
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<td></td>
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<td></td>
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<td>Ethyl acetate</td>
<td></td>
</tr>
<tr>
<td>DMSO</td>
<td></td>
</tr>
<tr>
<td><strong>Acetonitrile</strong></td>
<td><strong>Strongest</strong></td>
</tr>
<tr>
<td><strong>2-Propanol</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Ethanol</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Methanol</strong></td>
<td></td>
</tr>
</tbody>
</table>

**CO\(_2\) strength**

**Most commonly used co-solvents**
Co-Solvent Points to Remember in UPC²

- Co-solvents added to CO₂ generally decrease an analyte's retention time. As you increase the co-solvent concentration, the polarity of the mobile phase is changed resulting in decreased retention time(s).

- Different types of co-solvents and co-solvent gradients can be used to alter selectivity and retention times.

<table>
<thead>
<tr>
<th>Co-Solvent Concentration</th>
<th>Polarity</th>
<th>Retention Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑</td>
<td>↑</td>
<td>↓</td>
</tr>
</tbody>
</table>
Effect of Co-Solvent Concentration on Retention

Co-Solvent Concentration → Retention Time

- 17% MeOH
- 15% MeOH
- 13% MeOH
- 11% MeOH
- 9% MeOH
- 7% MeOH

Minutes

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Effect of Co-Solvent Strength on Retention

5% to 40% (5.0 min) gradient using different co-solvents

Co-Solvent Strength ↓ Retention Time ↑
Mixing Co-Solvents in UPC² Metoclopramide and Related Impurities

100% MeOH

90/10 MeOH/ACN

80/20 MeOH/ACN

50/50 MeOH/ACN

30.7 mM NH₄COOH added to all co-solvent mixtures
Additives are used in UPC² to improve the peak shape(s) and/or resolution of the separation

- As in LC, additives can modify the stationary phase surface or act as ion pairs (can change selectivity)
- Varying additive concentration and/or type can improve the separation and/or peak shape(s)
Additives are used in UPC² to improve the peak shape(s) and/or resolution of the separation
- As in LC, additives can modify the stationary phase surface or act as ion pairs (can change selectivity)
- Varying additive concentration and/or type can improve the separation and/or peak shape(s)

- Basic additives can improve peak shape and may slightly change the selectivity of basic compounds
  - Examples: ammonium hydroxide, 2-propylamine, triethylamine, etc.

- Acidic additives can improve peak shape and may slightly change the selectivity of acidic compounds
  - Examples: trifluoroacetic acid, formic acid, acetic acid, etc.
Mobile Phase Additives: Effect of Concentration

Peak shape of acidic compounds *improved with increasing concentration of acidic additive*
Effect of Additives on Strong Bases (β Blockers)

5% to 40% (5.0 min) gradient using methanol & methanol w/different additives

- Methanol
- 0.2% Formic Acid in Methanol
- 20 mM Ammonium Acetate in Methanol
- 0.2% Diethylamine in Methanol

Proper additive selection can improve peak shape
Sample Diluents in UPC\textsuperscript{2}

- Sample diluent can strongly affect peak shape and solubility in UPC\textsuperscript{2} (just like in normal-phase LC, reversed-phase LC, and HILIC)

- Use as weak a sample diluent as possible (balance analyte solubility and peak shape)

- Reduce (or eliminate) water content in sample

- Good generic injection solvent: 90/10 heptane/2-propanol
Sample Diluent Strength and Peak Shape

Effect of diluent on peak shape of butylparaben

<table>
<thead>
<tr>
<th>Injection Volume (μL)</th>
<th>0.5</th>
<th>1.0</th>
<th>1.5</th>
<th>2.0</th>
<th>2.5</th>
<th>3.0</th>
<th>4.0</th>
</tr>
</thead>
</table>

Column: ACQUITY UPC² BEH 2-EP 2.1 x 150 mm
Method: 97/3 CO₂/MeOH; 2 mL/min; 40 C; 2000 psi ABPR; UV@254 nm (350-450 nm compensation)
Effect of Pressure (Density) on UPC² Separations

- Pressure
  - The ABPR backpressure settings affect retention time by changing the density before the release of pressure.
  - As ABPR pressure *increases*, the density *increases* and retention time *decreases*.
Effect of Pressure (Density) on UPC² Separations

- Pressure
  - The ABPR backpressure settings affect retention time by changing the density before the release of pressure
  - As ABPR pressure increases, the density increases and retention time decreases

- Mobile phase composition has a greater effect on retention than pressure or density
  - Pressure/density can be used to optimize/fine-tune your separation
  - Typical operating ABPR range: 1500 – 2200 psi (100 – 150 bar)
Effect of Pressure (Density) on Retention in UPC²

Column: ACQUITY UPC² BEH 2-EP, 3.0 x 100 mm, UV@254 nm,
Temperature: 60°C, Gradient: 10-35% Methanol in 5.0 min
Effect of Column Temperature in UPC²

- Column temperature affects selectivity and retention in UPC²
  - Different analytes are affected to differing degrees
- Like pressure, column temperature affects the mobile phase density in the column
  - As column temperature \textit{increases}, the mobile phase density \textit{decreases}, and retention time \textit{increases} (\textbf{this is the opposite of LC})
Effect of Column Temperature in UPC²

Column: ACQUITY UPC² BEH 2-EP, 3.0 x 100 mm, UV@254,
Gradient: 10-35% Methanol in 5.0 min, ABPR: 1900 psi
<table>
<thead>
<tr>
<th></th>
<th>Peak Shape</th>
<th>Retention</th>
<th>Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stationary Phase</strong></td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><strong>Co-Solvent</strong></td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><strong>Additive</strong></td>
<td>1</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td><strong>Pressure</strong>*</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td><strong>Flow Rate</strong>*</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td><strong>Temperature</strong>*</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><strong>Injection Solvent</strong></td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(*) – Affect density

1 - Greatest effect
4 - Least effect
Agenda

- What is UPC$^2$?
- Getting Started
- Important Considerations for UPC$^2$
- UPC$^2$ as a Replacement for NPLC
- Summary
UPC² as a Replacement for Normal Phase LC

- Normal-Phase LC (NPLC) methods use solvents (aliphatic hydrocarbons and chlorinated solvents) that many laboratories would like to reduce for health, safety, environmental, and cost reasons.

- Since the principles of UPC² are similar to those of NPLC, methods should be able to be converted to UPC²:
  - Reduces solvent usage and disposal
  - Lowers the cost per analysis while enhancing green initiatives.
**UPC² as a Replacement for Normal Phase LC**

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- Since the principles of UPC² are similar to those of NPLC, methods should be able to be converted to UPC²:
  - Reduces solvent usage and disposal
  - Lowers the cost per analysis while enhancing green initiatives

- **UPC² offers significant performance advantages over NPLC**:
  - Better reproducibility
  - Ability to perform gradient separations (most NPLC separations are isocratic)
  - Compatible with mass detection
Replacing NPLC with UPC²: Anthralin USP Drug Substance Assay

- 4.6 x 250 mm silica NPLC column (L3)
- Hexane / Dichloromethane / glacial acetic acid
- 2.0 mL/min

Cost approx: $0.92 per run
Replacing NPLC with UPC²: Anthralin USP Drug Substance Assay

**Normal Phase HPLC**

- 4.6 x 250 mm silica NPLC column (L3)
- Hexane / Dichloromethane / glacial acetic acid
- 2.0 mL/min

**SFC**

- Viridis 2-EP 4.6 x 150 mm
- CO₂ / MeOH / glacial acetic acid
- 3.5 mL/min

**Suitability requirements met with NO change to sample and/or standard preparation**

- Cost approx: $0.92 per run
- Cost approx: $0.04 per run

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Replacing NPLC with UPC²: Reducing Particle Size & Column Dimensions

Normal Phase LC USP Method
Chromatographic Assay of Tolbutamide

4.0 x 300 mm, silica column (L3)
1.5 mL/min

Hexane, water-saturated-hexane, THF, alcohol, and glacial acetic acid (475:475:20:15:9)

Solvent cost per run ~ $1.40

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Replacing NPLC with UPC²: Reducing Particle Size & Column Dimensions

Normal Phase LC USP Method
Chromatographic Assay of Tolbutamide

- 4.0 x 300 mm, silica column (L3)
- 1.5 mL/min

- Hexane, water-saturated-hexane, THF, alcohol, and glacial acetic acid (475:475:20:15:9)

Solvent cost per run ~ $1.40

UPC² Method

- ACQUITY UPC² BEH, 3.0 x 100 mm, 1.7 µm
- 2.5 mL/min

- CO₂ / MeOH / IPA (95/2.5/2.5) containing 0.2% TFA

Solvent cost per run ~ $0.01

UPC² is 10X faster
Replacing NPLC with UPC\textsuperscript{2}: Low Level Impurity Analyses by UPC\textsuperscript{2}

USP Method: Chromatographic Purity of Estradiol

Normal Phase HPLC: Cost per run \(\sim\) $5.89

4.6  250 mm silica column
2,2,4-trimethylpentane / n-butyl chloride / MeOH, 2.0 mL/min

<table>
<thead>
<tr>
<th>Compound</th>
<th>RT</th>
<th>%Area</th>
<th>S/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unk. Impurity</td>
<td>6.24</td>
<td>0.006</td>
<td>2.9</td>
</tr>
<tr>
<td>Unk. Impurity</td>
<td>Not Found</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Unk. Impurity</td>
<td>10.86</td>
<td>0.01</td>
<td>2.7</td>
</tr>
<tr>
<td>Unk. Impurity</td>
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<td>36.81</td>
<td>0.077</td>
<td>9.2</td>
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Replacing NPLC with UPC²: Low Level Impurity Analyses by UPC²

USP Method: Chromatographic Purity of Estradiol

Normal Phase HPLC: Cost per run ~ $5.89

4.6 250 mm silica column
2,2,4-trimethylpentane / n-butyl chloride / MeOH, 2.0 mL/min

<table>
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<th>RT</th>
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<th>S/N</th>
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Transferred UPC² Method

2.1 x 150 mm ACQUITY UPC² BEH, 1.7 µm CO₂ / MeOH

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Agenda

- What is UPC²?
- Getting Started
- Important Considerations for UPC²
- UPC² as a Replacement for NPLC
- Summary
# Summary: UPC² Applications Examples

<table>
<thead>
<tr>
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<th>GC (Gas Chromatography)</th>
<th>LC (Liquid Chromatography)</th>
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UltraPerformance Convergence Chromatography is a powerful analytical technique that utilizes CO$_2$ and co-solvent(s) as mobile phases.

UPC$^2$ streamlines laboratory workflow with the ability to retain and separate any compound soluble in an organic solvent.
Summary

- UltraPerformance Convergence Chromatography is a powerful analytical technique that utilizes CO$_2$ and co-solvent(s) as mobile phases.
- UPC$^2$ streamlines laboratory workflow with the ability to retain and separate any compound soluble in an organic solvent.
- Peak shape, retention and selectivity can be improved and manipulated by varying and understanding the roles of co-solvent, additive, sample diluent, pressure, temperature and stationary phase.
- UPC$^2$ is a sustainable (green) chromatographic technique that offers significant advantages over normal-phase LC including lower cost per analysis, superior reproducibility, and compatibility with modern detection techniques such as mass spectrometry.
Acknowledgements

- Paula Hong
- Mark Baynham
- Helene Boiteux
- Baiba Cabovska
- Jacob Fairchild
- Chris Hudalla
- Kenneth Fountain
- Andy Aubin
- Mike Jones
- Doug McCabe
Thank You For Your Time and Attention

For more information please visit: http://www.waters.com/UPC2
For More Information

- Instrumentation information available on the ACQUITY UPC² documentation CD (PN 715002482) and at www.waters.com:
  - ACQUITY UPC² System Guide
  - ACQUITY UPC² Operator’s Overview & Maintenance Information Guides:
    - ACQUITY UPC² Binary Solvent Manager
    - ACQUITY UPC² Convergence Manager
    - ACQUITY UPC² Photodiode Array Detector
    - ACQUITY UPC² Column Compartments

- ACQUITY UPC² Columns Care & Use Manual (PN 720004349EN)