Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Getting Started with UltraPerformance Convergence Chromatography

A Practitioner's Guide for Utilizing UPC^2 in the Chromatographic Laboratory

Agenda

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- What is UPC^2?
- Getting Started
- Important Considerations for UPC^2
- UPC^2 as a Replacement for NPLC
- Summary

Agenda

- **What is UPC²?**
- Getting Started
- Important Considerations for UPC²
- UPC² as a Replacement for NPLC
- Summary

UltraPerformance Convergence Chromatography [UPC²] Defined

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

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 CO_2 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.

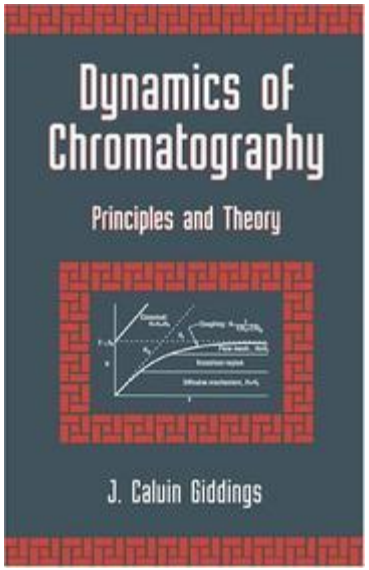
The **ACQUITY UPC² System** is built utilizing proven UPLC® technology to enable scientists the ability to address routine and complex separation challenges while delivering reliability, robustness, sensitivity and throughput never before possible for this analytical technique.

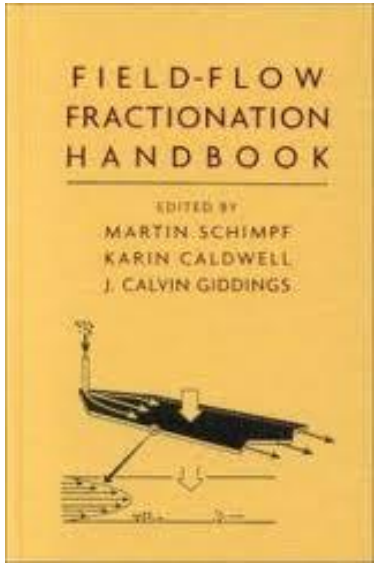
Why is it Called Convergence Chromatography?

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Giddings, J.C. (1965) *A critical evaluation of the theory of gas chromatography*. In *Gas Chromatography*. 1964, edited by A. *Goldup*, p. 3-24. Elsevier, Amsterdam

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.*"

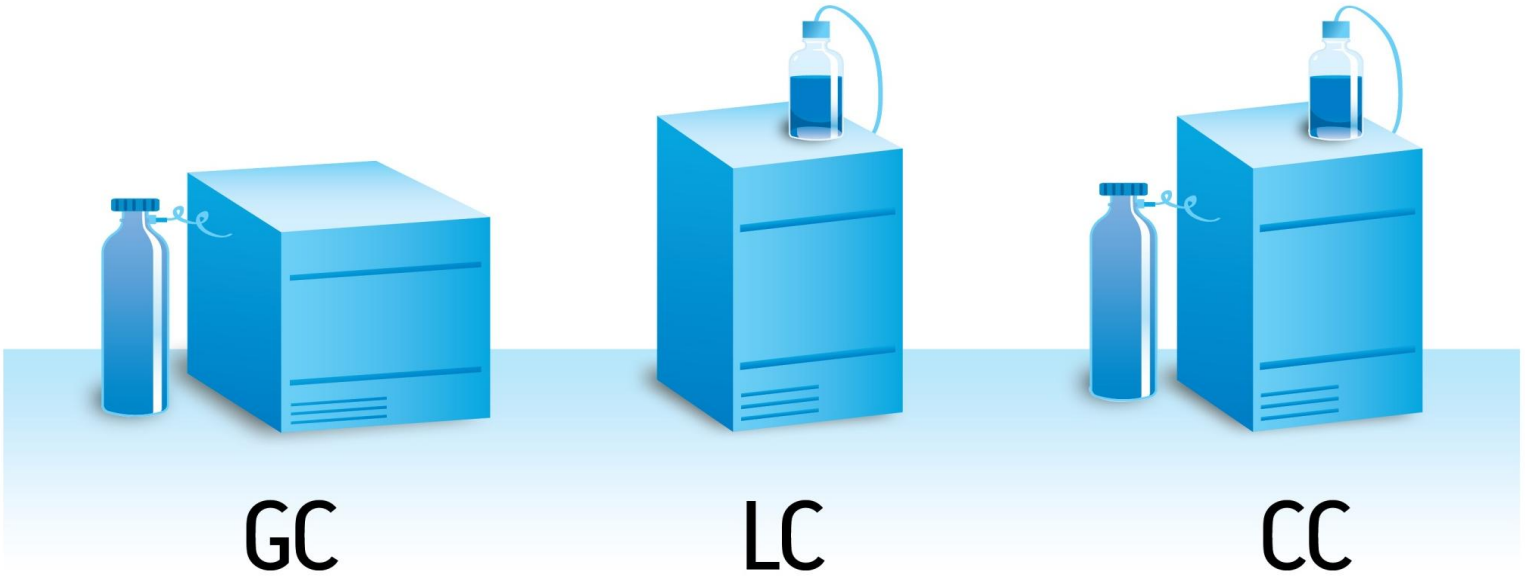




Prof. Calvin Giddings (1930-1996)

How Did Convergence Chromatography Evolve?

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



GC
Gas Chromatography

LC
Liquid Chromatography

CC
Convergence Chromatography

GC → **Capillary GC**

HPLC → **UPLC**

SFC → **UPC²**

How Does Convergence Chromatography Work?

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- UPC^2 is a chromatographic technique similar to HPLC
  - Instead of mobile phase A being aqueous, it is CO_2
- Mobile phases include a supercritical fluid & one (or more) co-solvents
  - CO_2 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?

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- UPC^2 is a chromatographic technique similar to HPLC
  - Instead of mobile phase A being aqueous, it is CO_2
- Mobile phases include a supercritical fluid & one (or more) co-solvents
  - CO_2 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.
- UPC^2 provides normal-phase-like selectivities
- UPC^2 is compatible with most popular detection techniques
  - PDA, ELSD, MS, etc.

Agenda

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- 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

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- 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 ethanol, 2-propanol, acetonitrile, etc. (or combinations)

Understanding the Terminology

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- 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 ethanol, 2-propanol, acetontrile, 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?

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- 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?

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

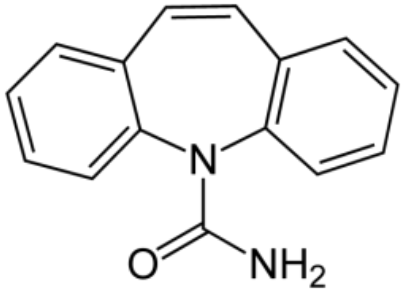
- As with any analytical technique, the more you know about your analyte(s) and sample(s), *the better*
  - What is the solubility of your analyte/sample in various organic solvents (often referred to as Log P)?
  - What is its partition coefficient, P (ratio of concentrations of an analyte in a mixture of two immiscible solvents: typically 1-octanol/water)?

- 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?

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- 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 UPC²

- Understanding analyte solubility is important in UPC²

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

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

Partition Coefficient and UPC²

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- Understanding analyte solubility is important in UPC²
- The 1-octanol/water partition coefficient (P) is a common measure of analyte solubility and is often readily available

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

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

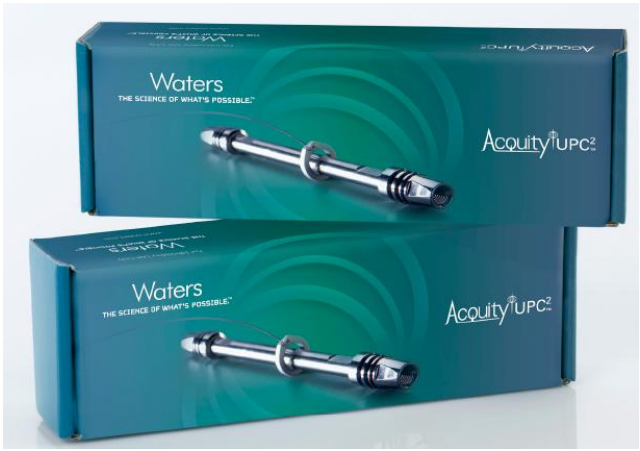
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

UPC² Column Chemistry	Applications Examples
UPC² BEH	OLEDs, polymer additives, pesticides, lipids
UPC² BEH 2-EP	Steroids, pesticides
UPC² CSH Fluoro-Phenyl	Vitamin D metabolites, steroids, natural products
UPC² HSS C18 SB	Fat-soluble vitamins, lipids (free fatty acids)

BEH Technology™

HSS
HIGH STRENGTH SILICA

CSH™ Technology



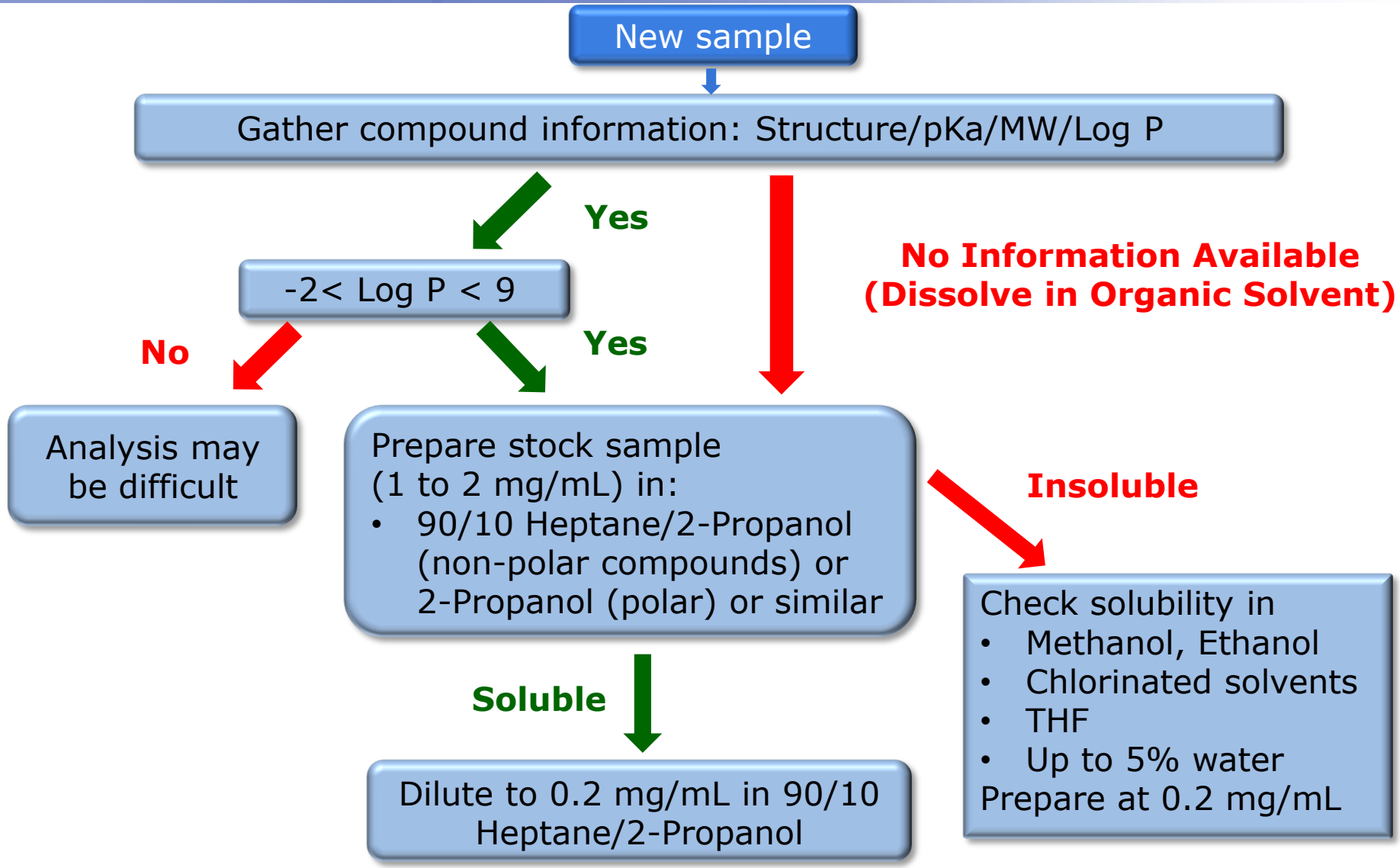
Getting Started: ACQUITY UPC^2 Columns and Equilibration

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- ACQUITY UPC^2 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 UPC^2 column upon installation can result in irreproducible retention times

Getting Started: A Recommended Screening Protocol

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



Getting Started: Initial Screening Conditions

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

UPC² Screening Columns: BEH and BEH 2-EP
2nd Options: CSH Fluoro-Phenyl & HSS C18 SB

Starting Conditions

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 NH_4COOH & 2% HCOOH*
B4: Methanol containing 0.2% NH_4OH*
(*) – for use with BEH and BEH 2-EP columns only

Getting Started: Initial Screening Conditions

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

UPC² Screening Columns: BEH and BEH 2-EP	
2nd Options: CSH Fluoro-Phenyl & HSS C18 SB	

Starting Conditions

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?

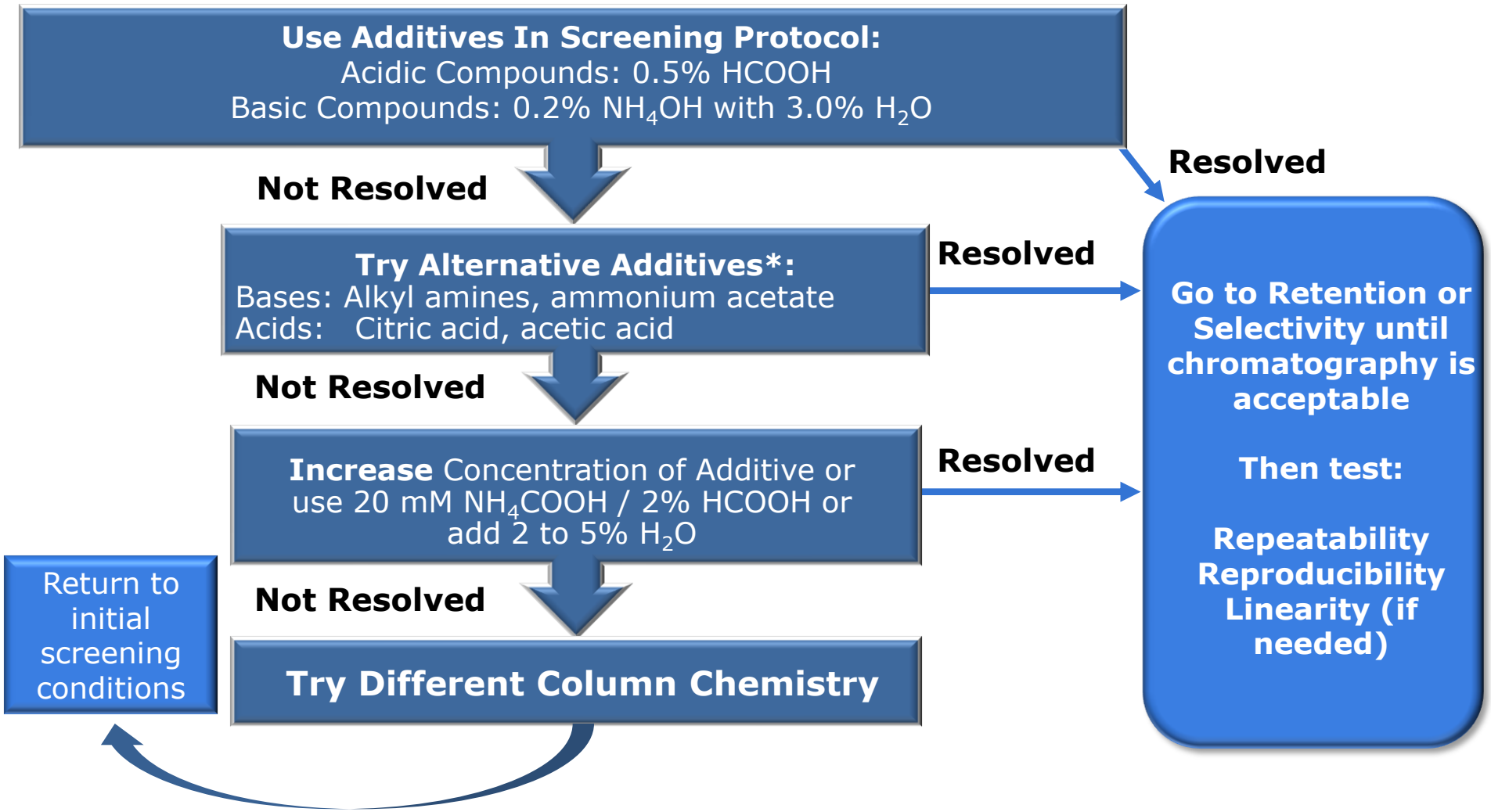
Insufficient Retention?

Inadequate Selectivity?

How can we optimize these parameters?

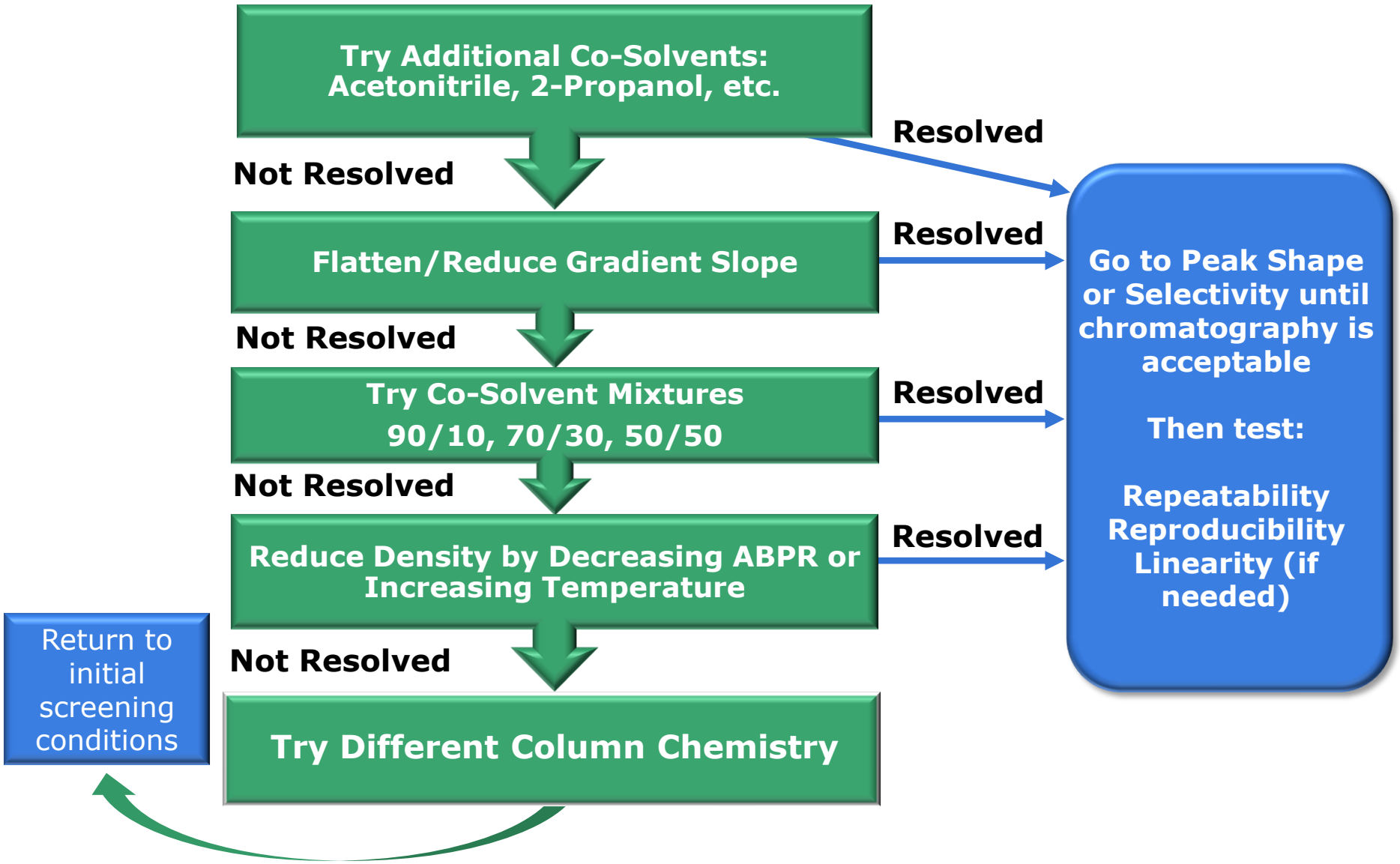
Strategies for Improving Peak Shape

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

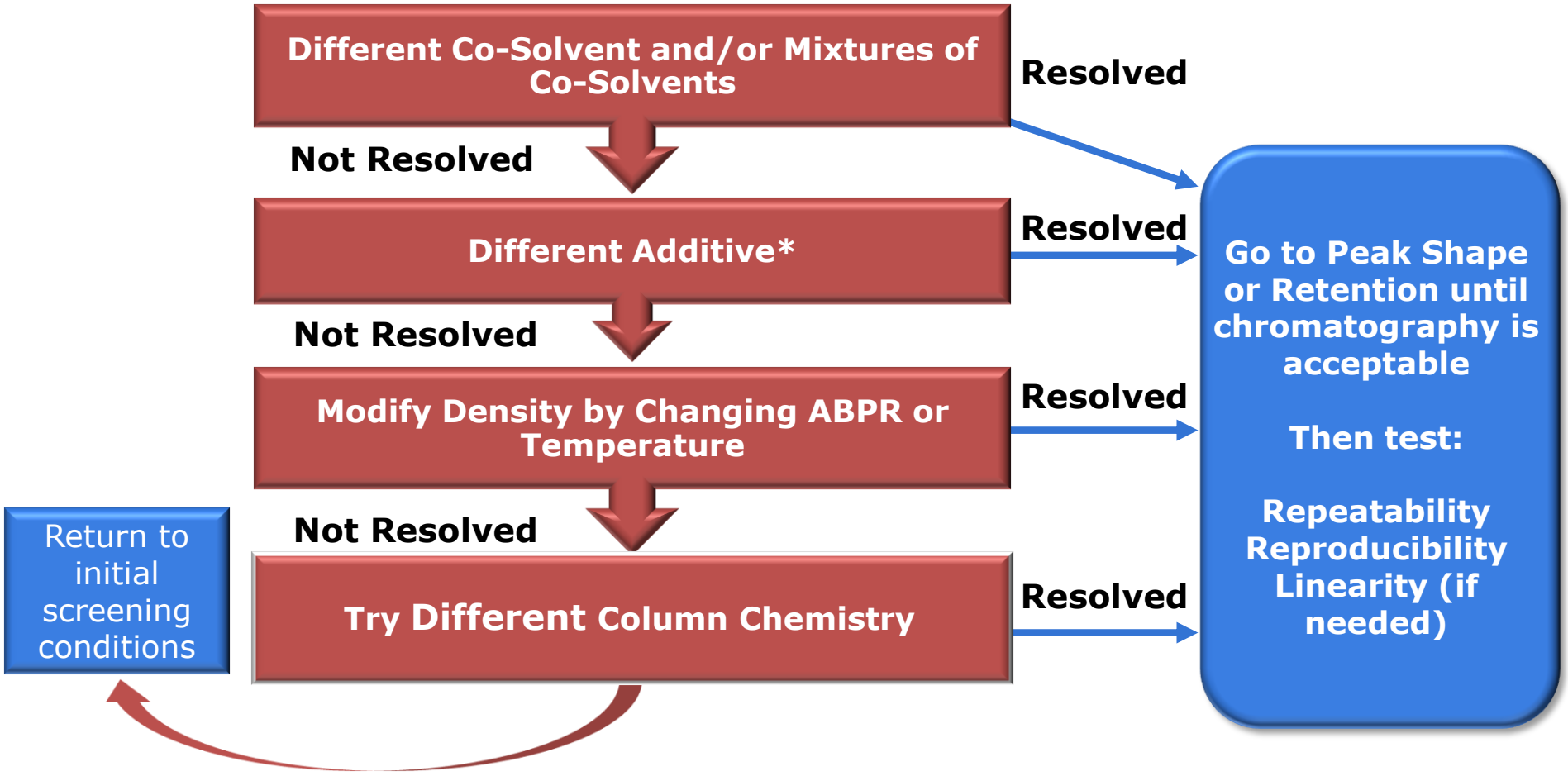


(*) – Ensure additive is compatible with mode of detection

Strategies for Increasing Retention



Strategies for Changing Selectivity



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

Agenda

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- What is UPC^2?
- Getting Started
- **Important Considerations for UPC^2**
  - **Setup Guidelines**
  - **Co-Solvents**
  - **Mobile Phase Additives**
  - **Sample Diluents**
  - **Pressure and Temperature**
- UPC^2 as a Replacement for NPLC
- Summary

Getting Started: Instrument Setup Guidelines

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- 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 CO_2 (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

Getting Started: Needle Wash and Seal Wash Solvents

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- 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

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- 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

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

CO_2 strength →

Co-Solvent	Eluting Strength
Pentane, Hexane, Heptane	**Weakest**
Xylene	
Toluene	
Diethyl ether	
Dichloromethane	
Chloroform	
Acetone	
Dioxane	
THF	
MTBE	
Ethyl acetate	
DMSO	
Acetonitrile	
2-Propanol	
Ethanol	
Methanol	**Strongest**

Typical Co-Solvents Used in UPC²

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

CO_2 strength →

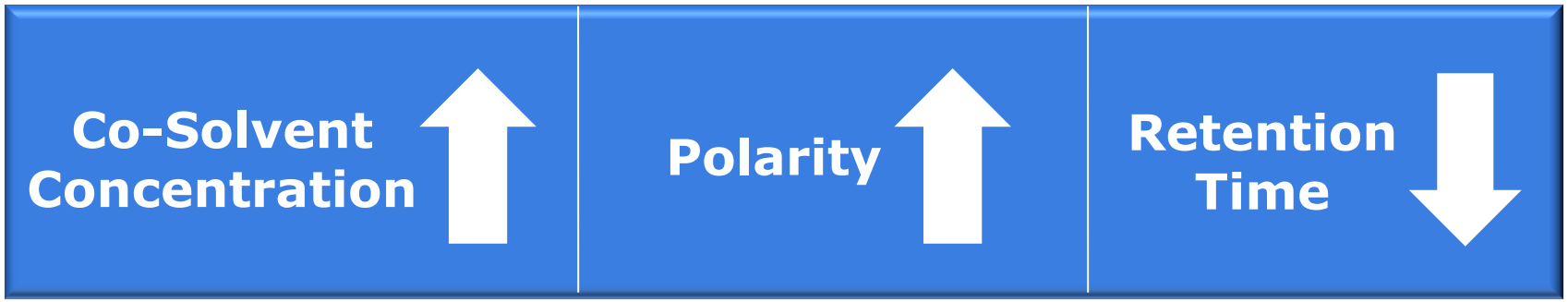
Co-Solvent	Eluting Strength
Pentane, Hexane, Heptane	**Weakest**
Xylene	
Toluene	
Diethyl ether	
Dichloromethane	
Chloroform	
Acetone	
Dioxane	
THF	
MTBE	
Ethyl acetate	
DMSO	
Acetonitrile	
2-Propanol	
Ethanol	
Methanol	**Strongest**

Most commonly used co-solvents

Co-Solvent Points to Remember in UPC²

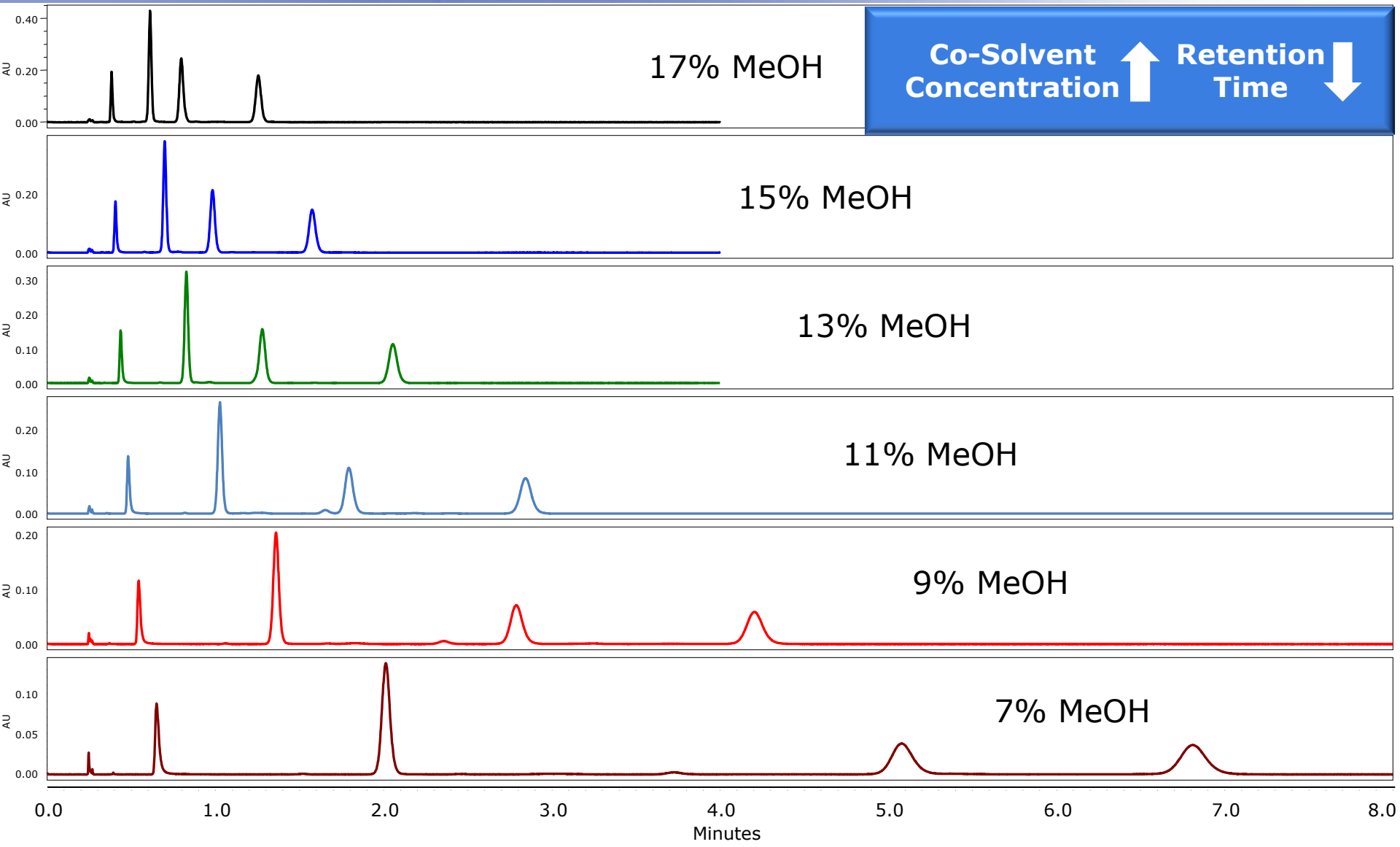
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- Co-solvents added to CO_2 generally decrease an analyte's retention time. As you increase the co-solvent concentration **the polarity of the mobile phase is changed** resulting in decrease retention time(s)
- Different types of co-solvents and co-solvent gradients can be used to alter selectivity and retention times



Effect of Co-Solvent *Concentration* on Retention

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



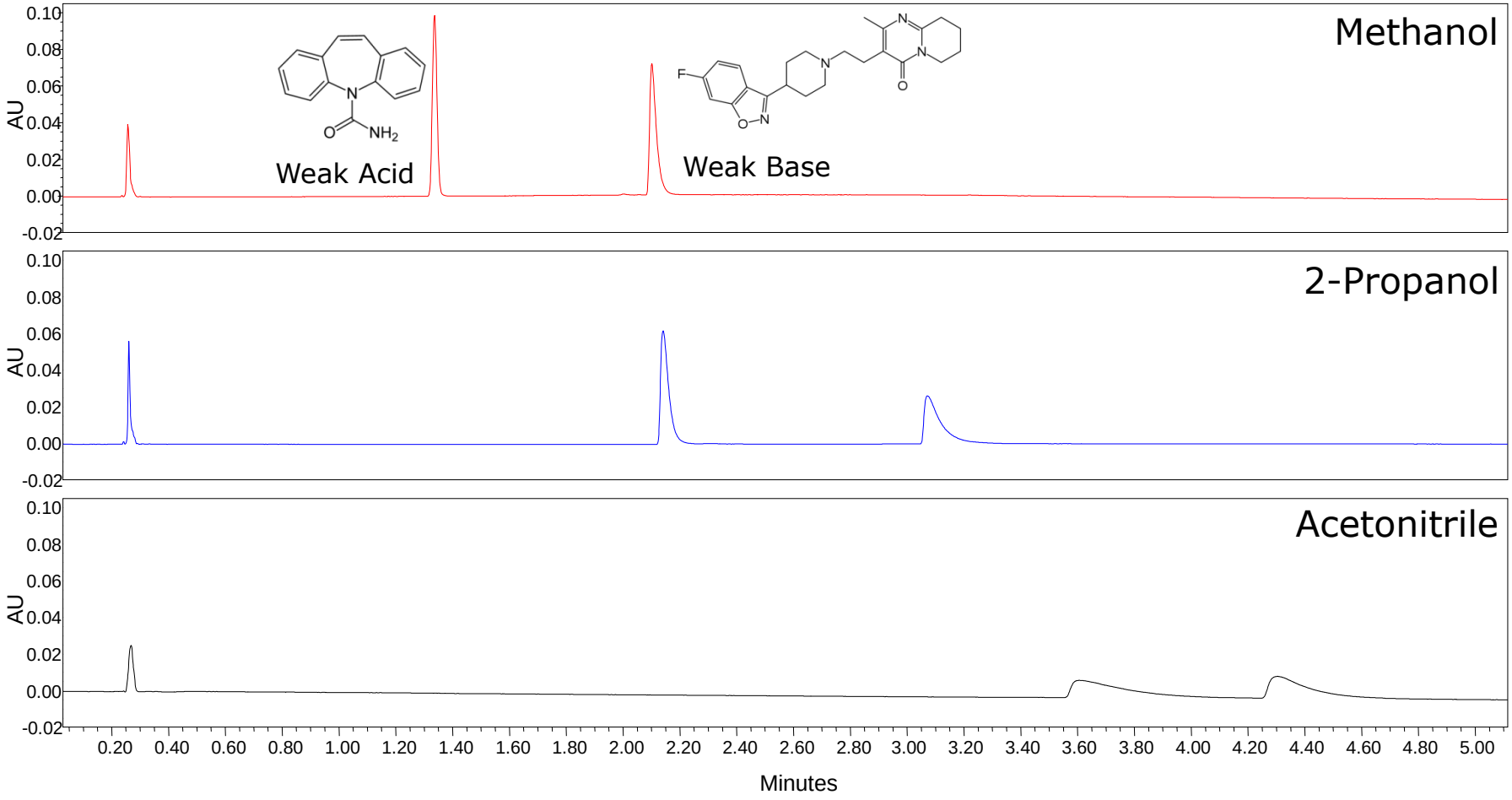
Co-Solvent Concentration ↑ Retention Time ↓

Effect of Co-Solvent *Strength* on Retention

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

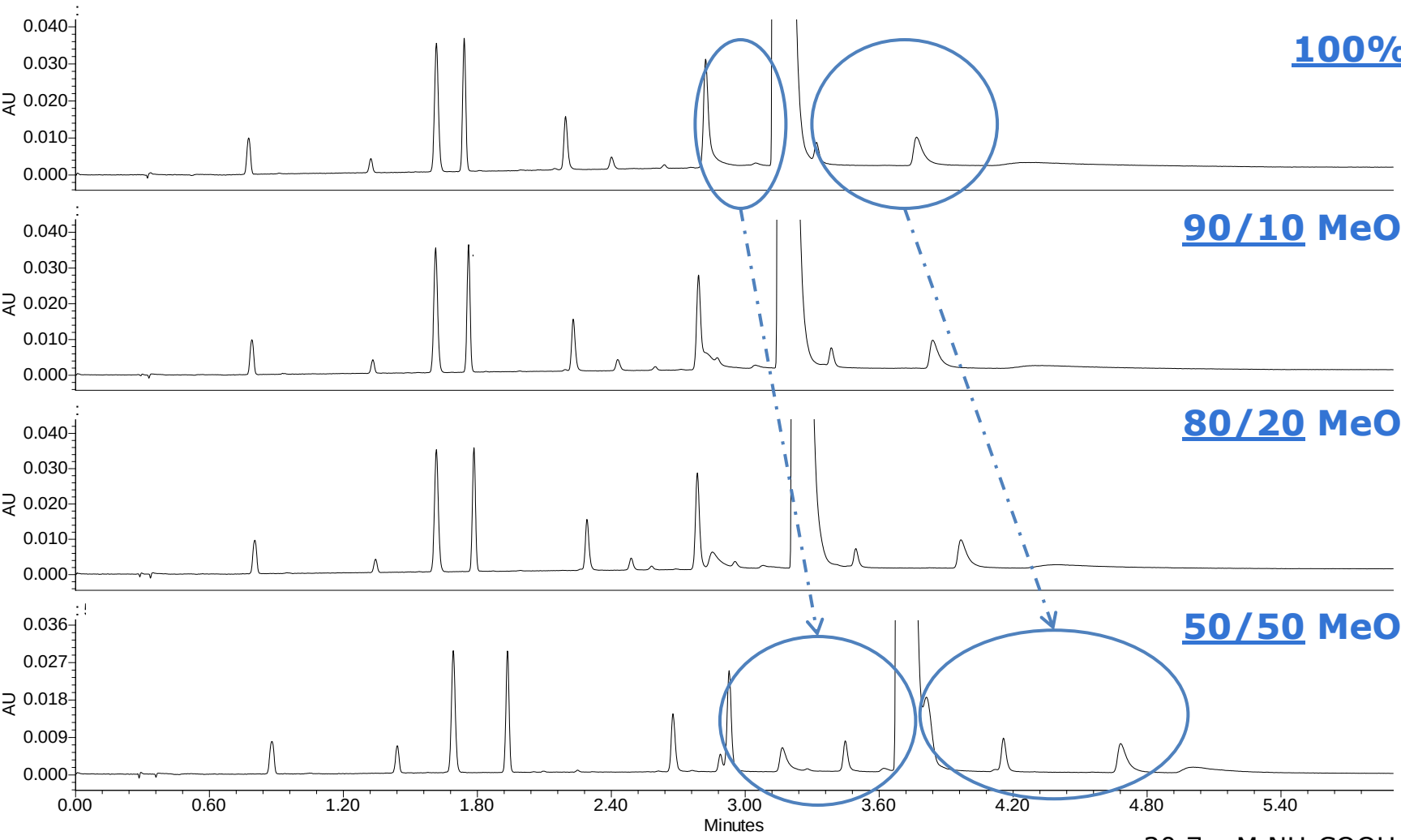
5% to 40% (5.0 min) gradient using different co-solvents

Co-Solvent Strength ↓ Retention Time ↑



Mixing Co-Solvents in UPC²
Metoclopramide and Related Impurities

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



30.7 mM NH_4COOH added to all co-solvent mixtures

Using Mobile Phase Additives in UPC^2

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- Additives are used in UPC^2 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)

Using Mobile Phase Additives in UPC²

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

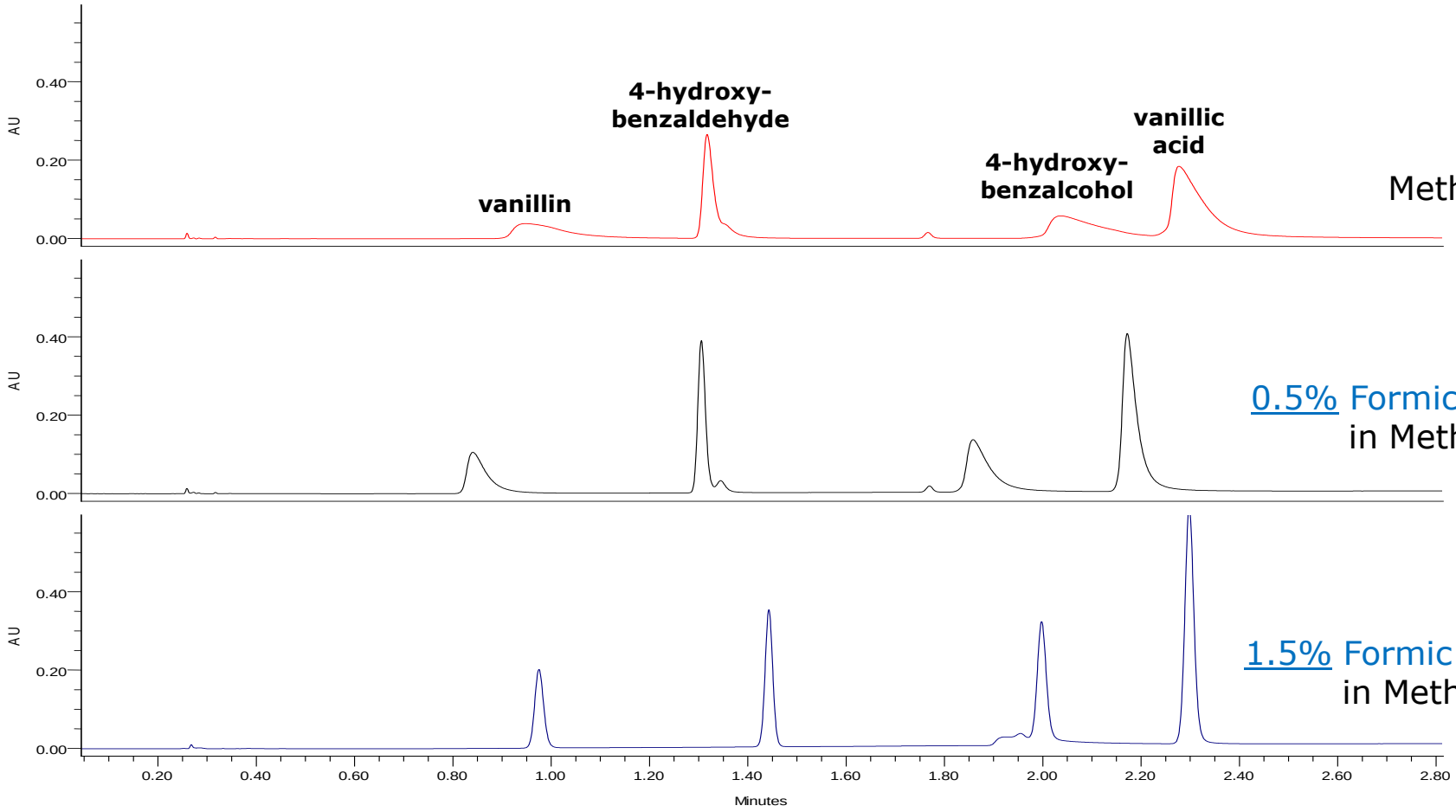
- Additives are used in UPC² to improve the peak shape(s) and/or resolution of the separation
  - As in LC, additives can modify the stationary phase surface or act as ion pairs (can change selectivity)
  - Varying additive *concentration* and/or *type* can improve the separation and/or peak shape(s)

- *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

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

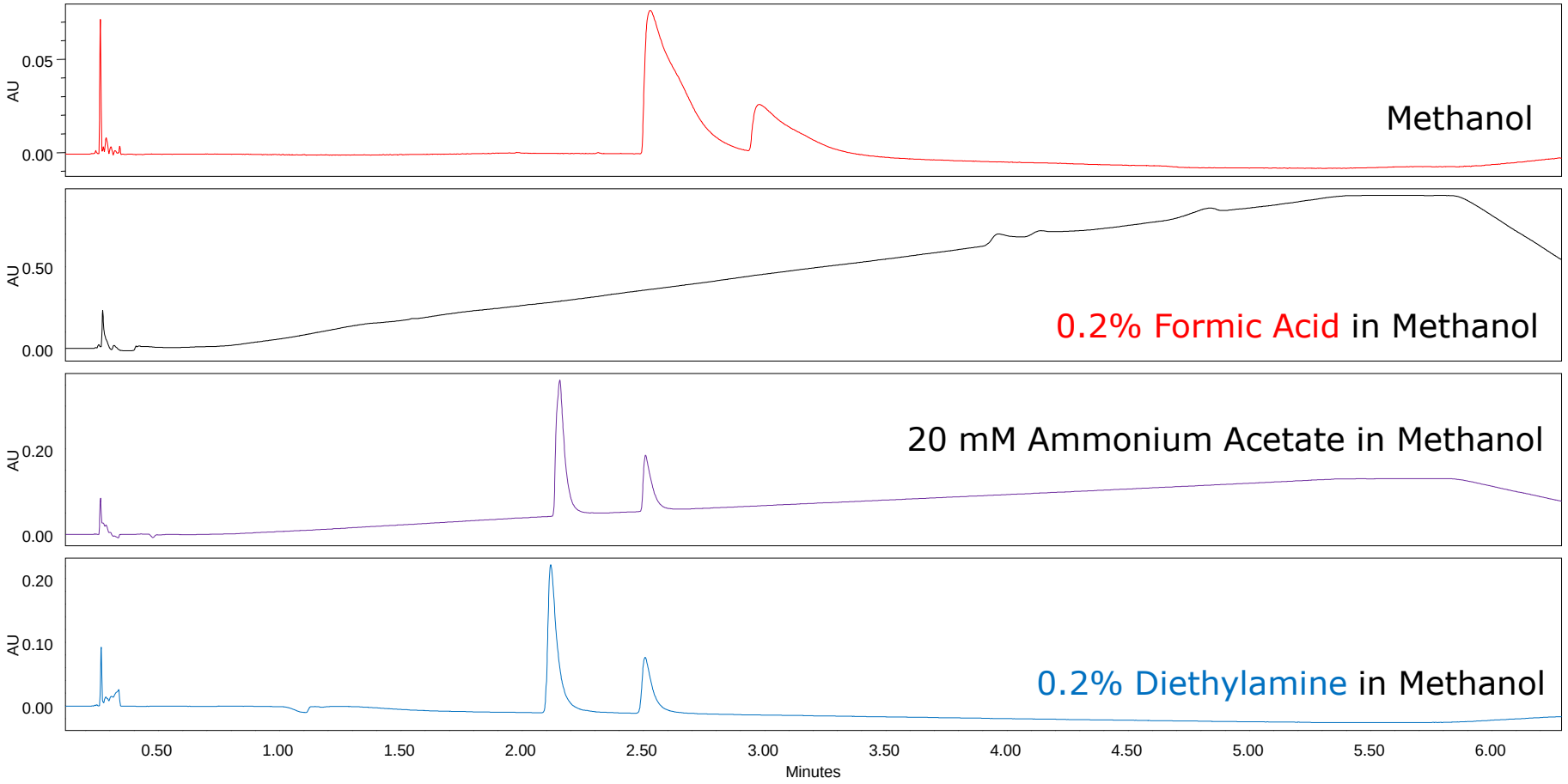


Peak shape of acidic compounds *improved* with *increasing* concentration of acidic additive

Effect of Additives on Strong Bases (β Blockers)

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

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

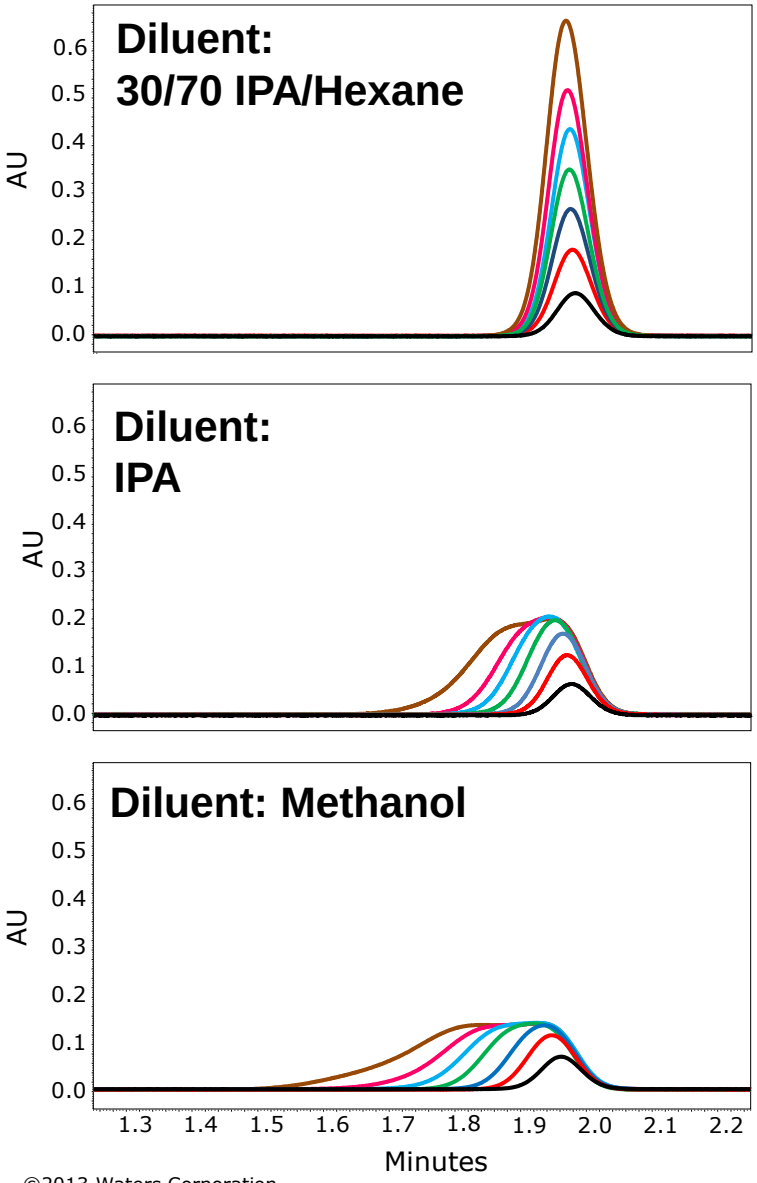


Proper additive selection can improve peak shape

Sample Diluents in UPC^2

- Sample diluent can strongly affect peak shape and solubility in UPC^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

Injection Volume (µL)
0.5
1.0
1.5
2.0
2.5
3.0
4.0

Column: ACQUITY UPC^2 BEH 2-EP 2.1 x 150 mm

Method: 97/3 CO_2/MeOH; 2 mL/min; 40 C; 2000 psi ABPR; UV@254 nm (350-450 nm compensation)

Effect of Pressure (Density) on UPC^2 Separations

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- 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

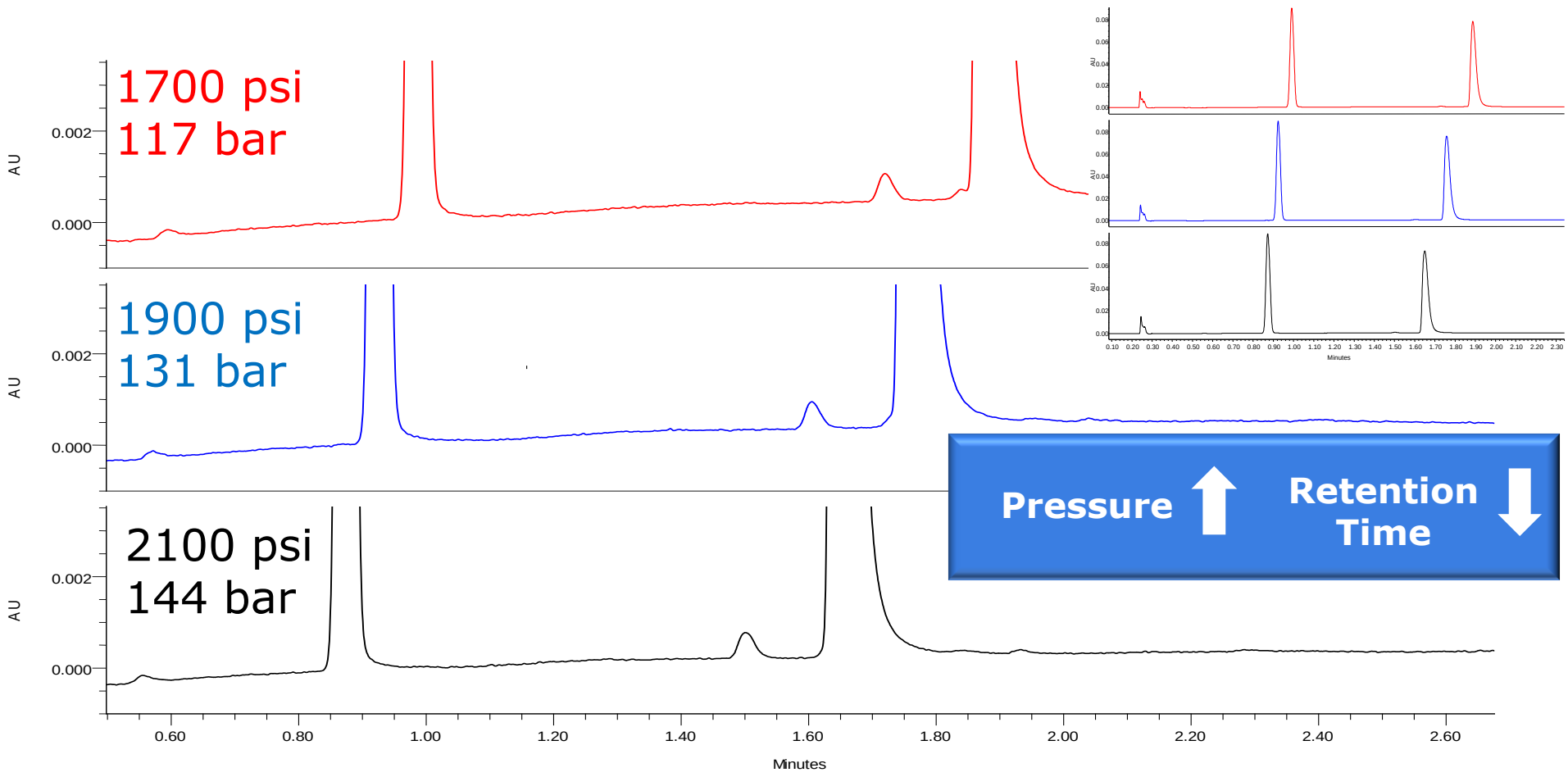
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- 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

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

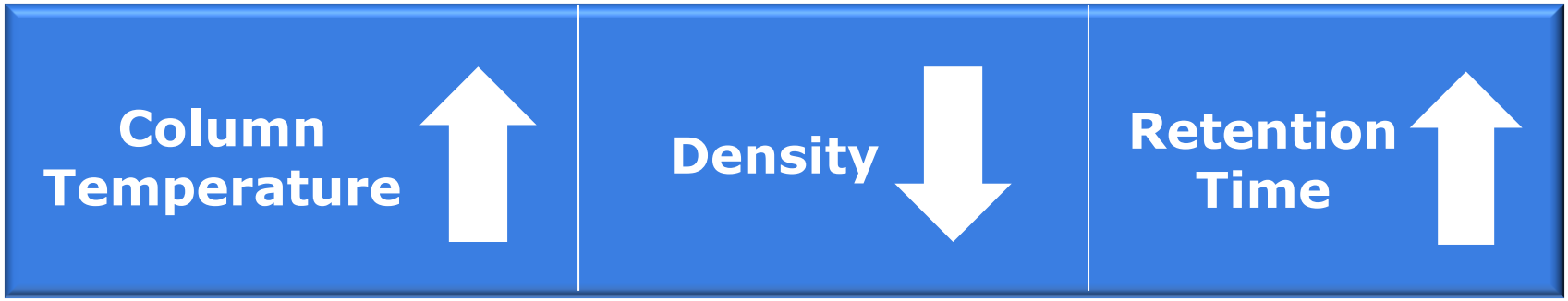


Column: ACQUITY UPC² BEH 2-EP, 3.0 x 100 mm, UV@254 nm,
Temperature: 60°C, Gradient: 10-35% Methanol in 5.0 min

Effect of Column Temperature in UPC^2

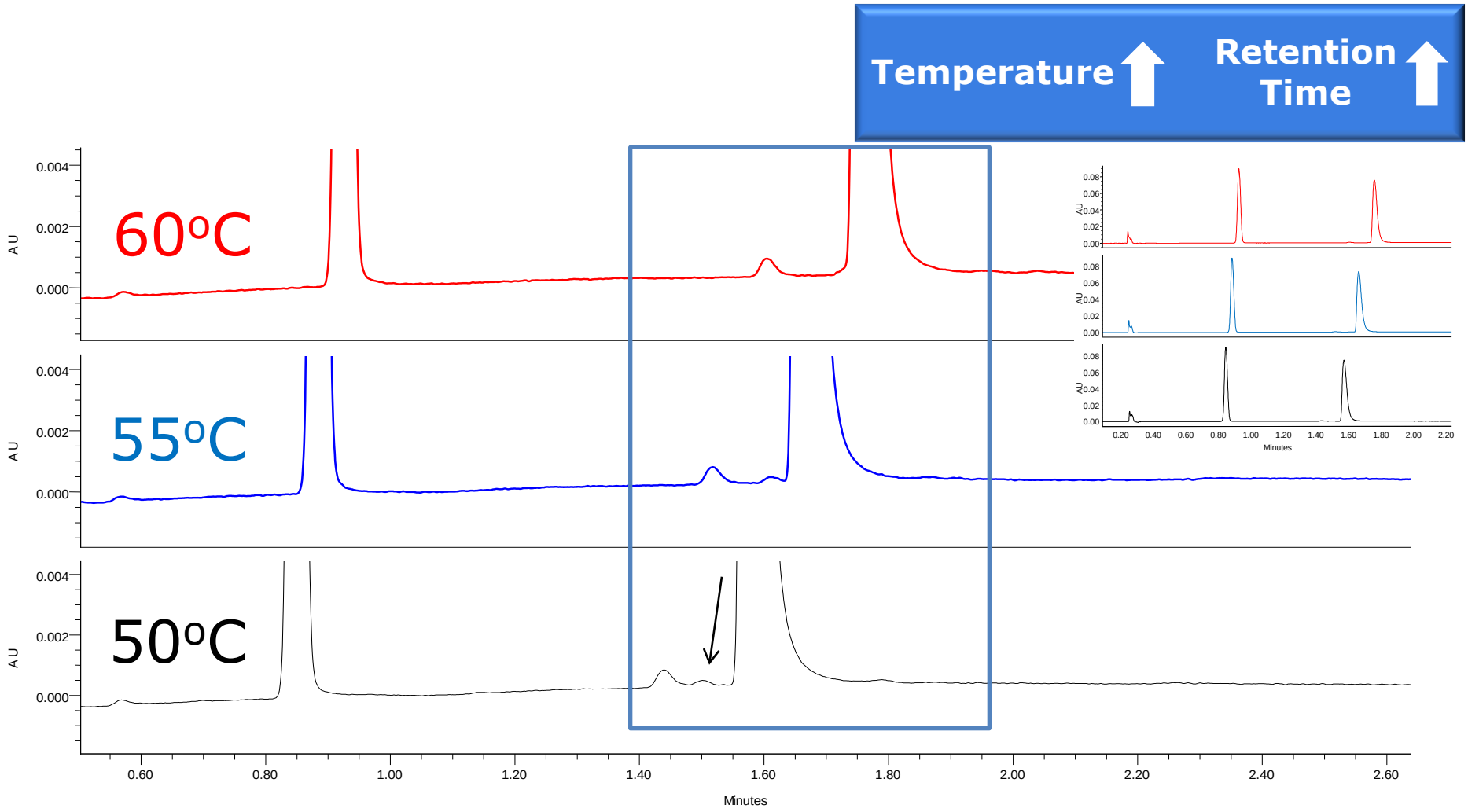
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- Column temperature affects selectivity and retention in UPC^2
  - Different analytes are affected to differing degrees
- Like pressure, column temperature affects the mobile phase density in the column
  - As column temperature *increases*, the mobile phase density *decreases*, and retention time *increases* **(this is the opposite of LC)**



Effect of Column Temperature in UPC²

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



Column: ACQUITY UPC² BEH 2-EP, 3.0 x 100 mm, UV@254,
Gradient: 10-35% Methanol in 5.0 min, ABPR: 1900 psi

Summary: Optimizing Separations in UPC²

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

	Peak Shape	Retention	Selectivity
Stationary Phase	4	2	1
Co-Solvent	3	1	2
Additive	1	4	3
Pressure*		3	4
Flow Rate*		3	4
Temperature*		3	3
Injection Solvent	2		

(*) – 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

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- 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

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- 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

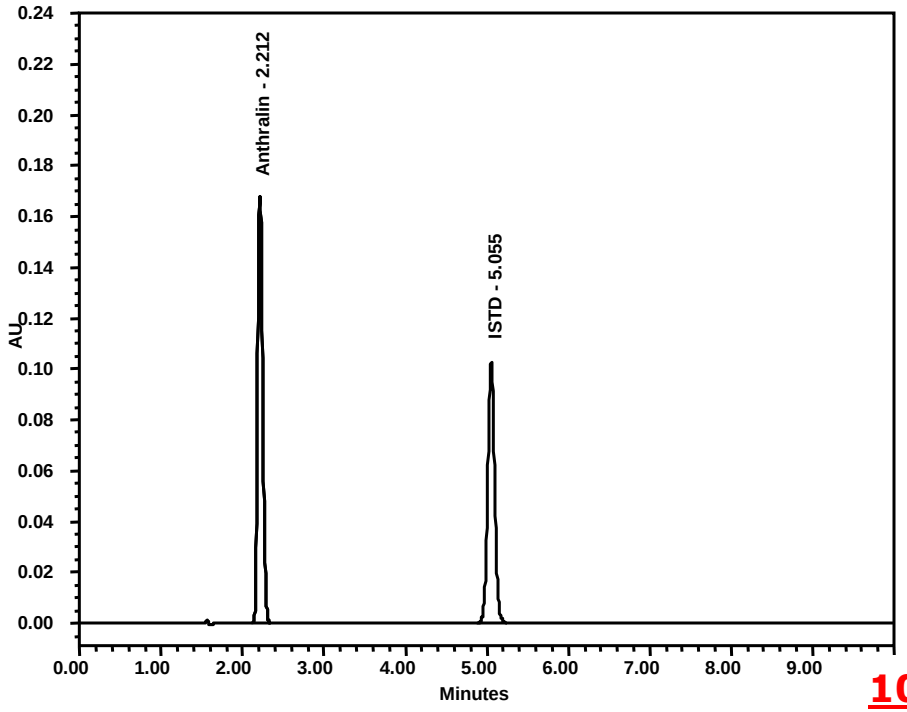
- Since the principles of UPC² are similar to those of NPLC, methods should be able to be converted to UPC²
  - Reduces solvent usage and disposal
  - Lowers the cost per analysis while enhancing green initiatives

- **UPC² 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

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Normal Phase HPLC



10.0

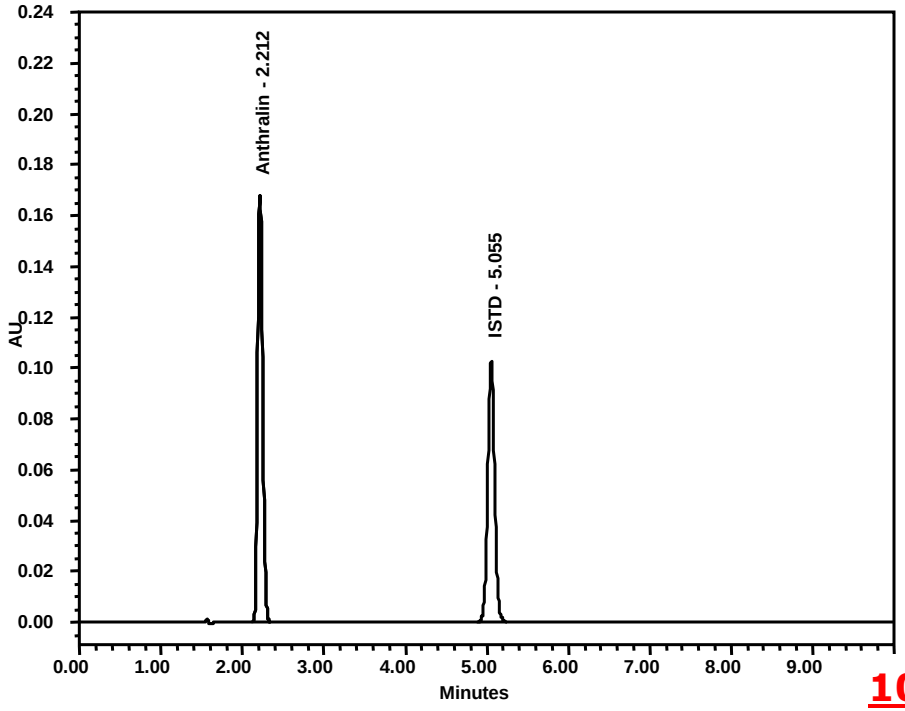
- 4.6 x 250 mm silica NPLC column (L3)
- Hexane / Dichloromethane / glacial acetic acid
- 2.0 mL/min

Cost approx: $0.92 per run

Replacing NPLC with UPC²: Anthralin USP Drug Substance Assay

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Normal Phase HPLC

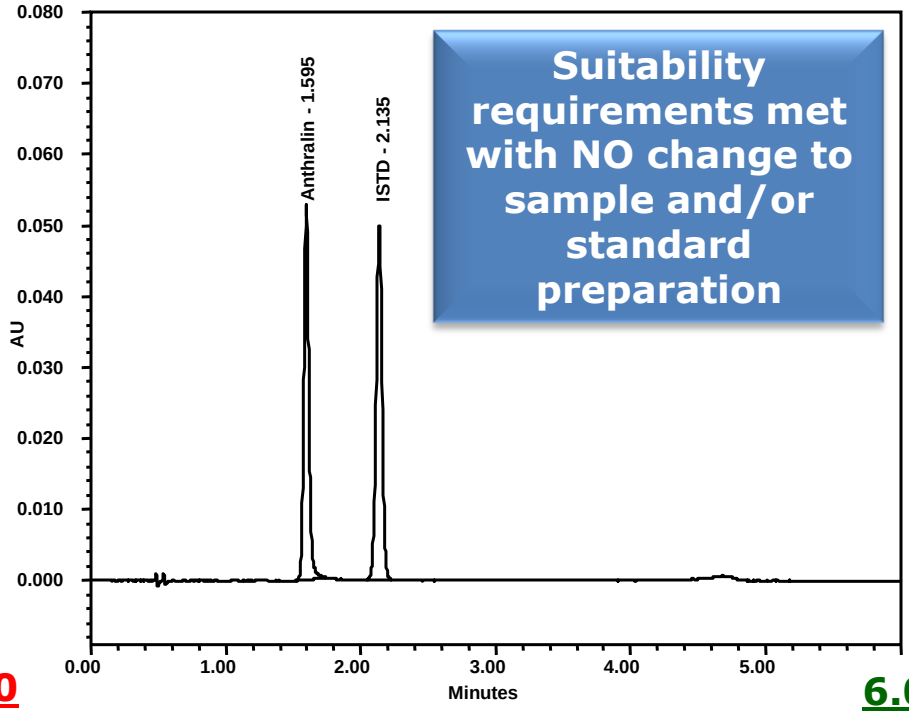


10.0

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

Cost approx: $0.92 per run

SFC



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

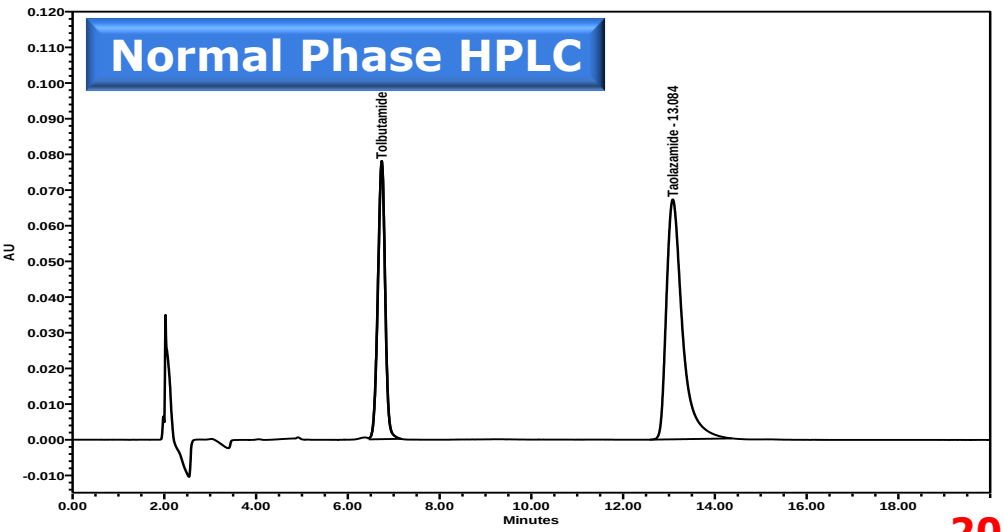
6.0

- Viridis 2-EP 4.6 x 150 mm
- CO_2 / MeOH / glacial acetic acid
- 3.5 mL/min

Cost approx: $0.04 per run

Replacing NPLC with UPC²: Reducing Particle Size & Column Dimensions

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



20.0

Normal Phase LC USP Method

Chromatographic Assay of **Tolbutamide**

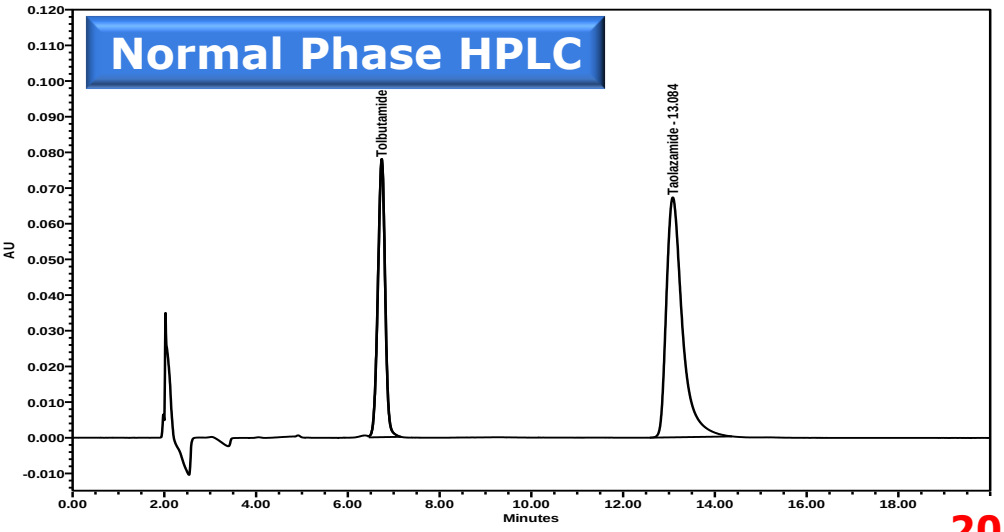
4.0 x 300 mm, silica column (L3)
1.5 mL/min

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

Solvent cost per run ~ $1.40

Replacing NPLC with UPC²: Reducing Particle Size & Column Dimensions

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



20.0

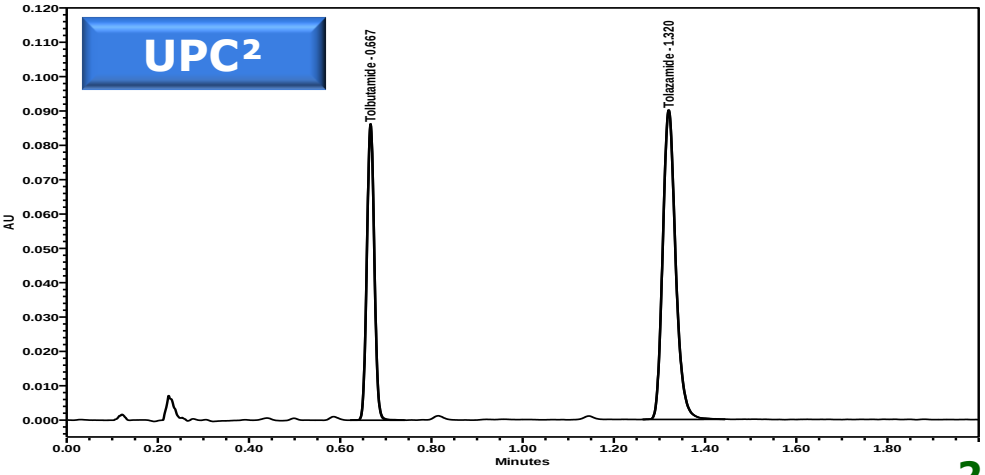
Normal Phase LC USP Method

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



2.00

UPC² Method

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

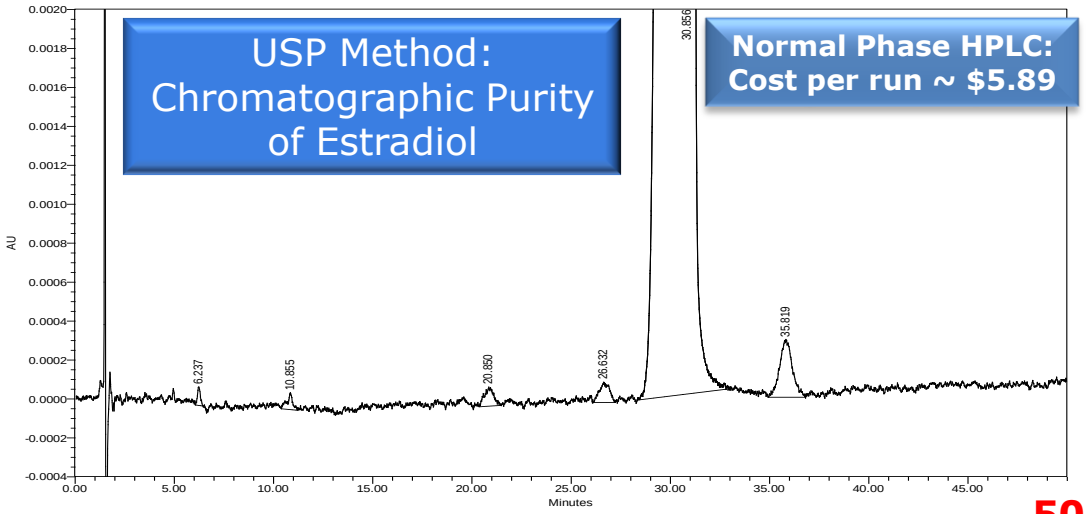
CO_2 / MeOH / IPA (95/2.5/2.5) containing 0.2% TFA

Solvent cost per run ~ $0.01

UPC² is 10X faster

Replacing NPLC with UPC²: Low Level Impurity Analyses by UPC²

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



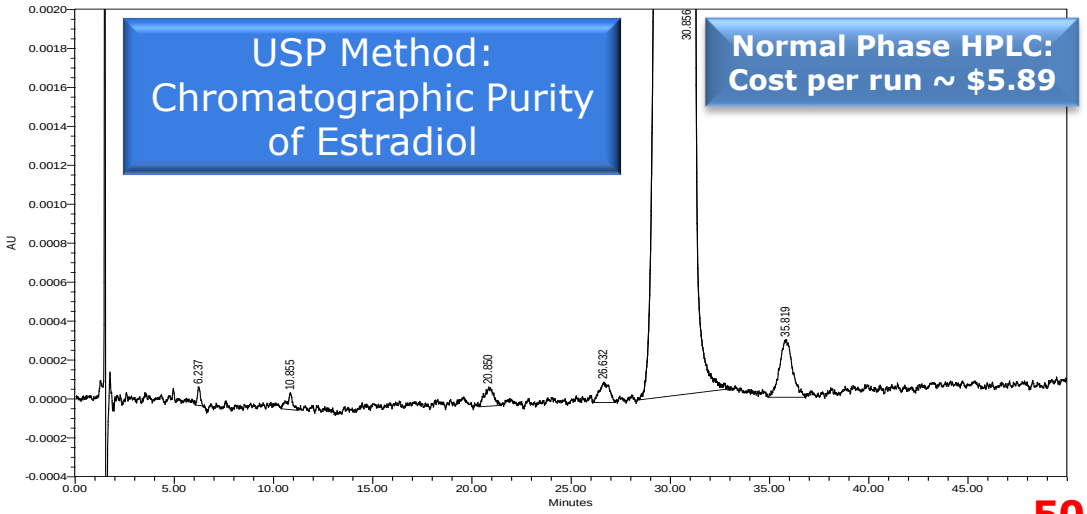
50.0

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

Compound	RT	%Area	S/N
Unk. Impurity	6.24	0.006	2.9
Unk. Impurity	Not Found	---	---
Unk. Impurity	10.86	0.01	2.7
Unk. Impurity	Not Found	---	---
Unk. Impurity	20.85	0.018	3
Unk. Impurity	26.63	0.021	3.2
Estradiol	30.86	**99.87**	---
Main Impurity	36.81	0.077	9.2

Replacing NPLC with UPC²: Low Level Impurity Analyses by UPC²

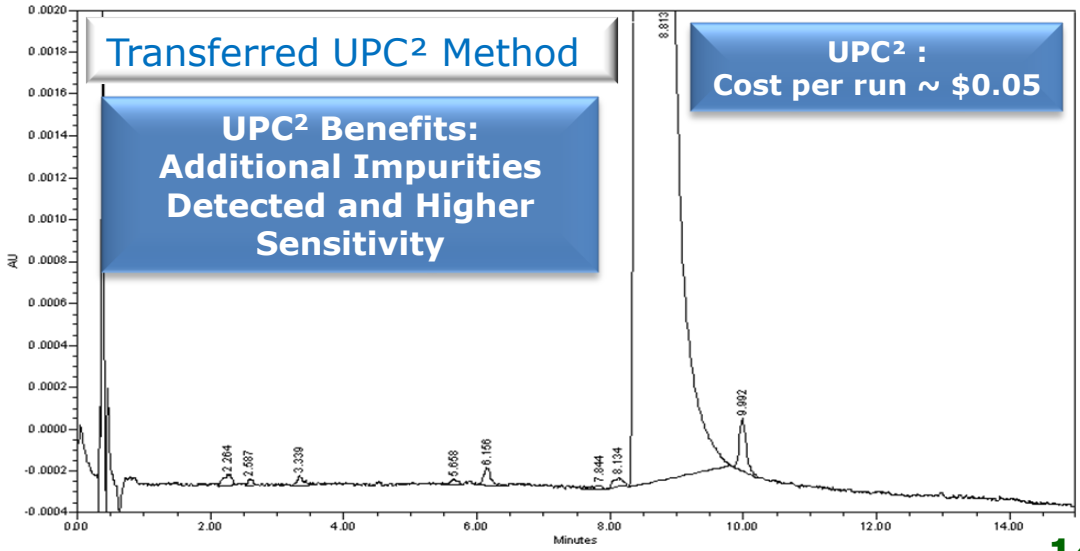
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



50.0

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

Compound	RT	%Area	S/N
Unk. Impurity	6.24	0.006	2.9
Unk. Impurity	Not Found	---	---
Unk. Impurity	10.86	0.01	2.7
Unk. Impurity	Not Found	---	---
Unk. Impurity	20.85	0.018	3
Unk. Impurity	26.63	0.021	3.2
Estradiol	30.86	**99.87**	---
Main Impurity	36.81	0.077	9.2



14.0

2.1 x 150 mm ACQUITY UPC² BEH, 1.7 µm
CO_2 / MeOH

Compound	RT	%Area	S/N
Unk. Impurity	2.26	0.012	3.4
Unk. Impurity	2.59	0.004	1.9
Unk. Impurity	3.34	0.01	3.1
Unk. Impurity	5.66	0.006	1.7
Unk. Impurity	6.15	0.016	5.5
Unk. Impurity	8.13	0.013	3.1
Estradiol	8.81	**99.89**	---
Main Impurity	9.99	0.046	16

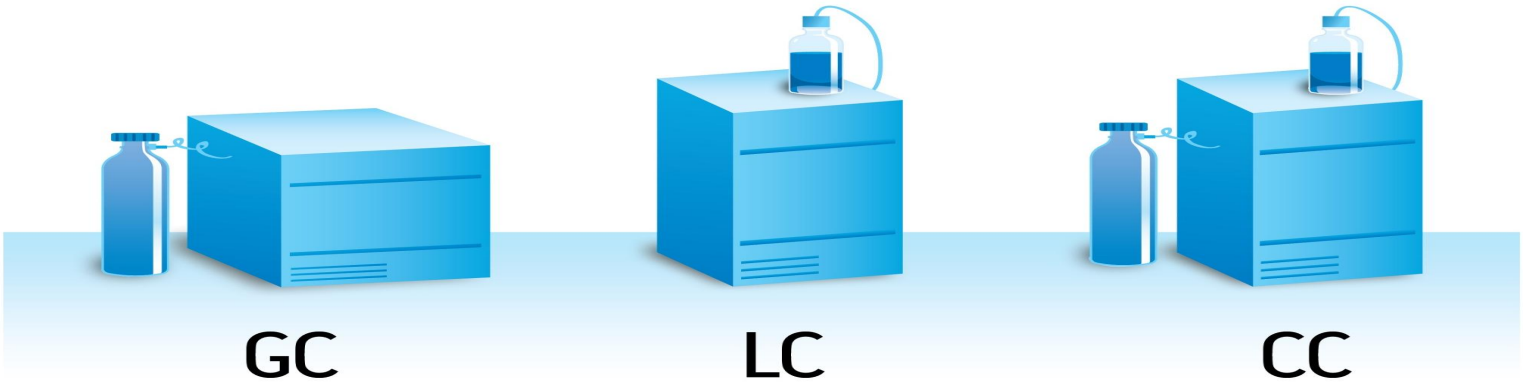
Agenda

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- What is UPC^2?
- Getting Started
- Important Considerations for UPC^2
- UPC^2 as a Replacement for NPLC
- **Summary**

Summary: UPC² Applications Examples

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



	GC Gas Chromatography	LC Liquid Chromatography	CC Convergence Chromatography
Azo dyes		✓	✓
Explosives	✓	✓	✓
Bile acid profiling	✓	✓	✓
Lipids	✓	✓	✓
Natural products	✓	✓	✓
Agrochemicals		✓	✓
OLEDs		✓	✓
Extractables	✓	✓	✓
Fat-soluble vitamins		✓	✓
Steroids/estrogens	✓	✓	✓
Positional isomers	✓	✓	✓
Vitamin D metabolites		✓	✓

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

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

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

Thank You **For Your Time and Attention**

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

For More Information

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- Instrumentation information available on the ACQUITY UPC^2 documentation CD (PN 715002482) and at www.waters.com:
  - ACQUITY UPC^2 System Guide
  - ACQUITY UPC^2 Operator's Overview & Maintenance Information Guides:
    - ACQUITY UPC^2 Binary Solvent Manager
    - ACQUITY UPC^2 Convergence Manager
    - ACQUITY UPC^2 Photodiode Array Detector
    - ACQUITY UPC^2 Column Compartments
- ACQUITY UPC^2 Columns Care & Use Manual (PN 720004349EN)