A New Separation Tool for a Broad Range of Analytical Challenges:
UltraPerformance Convergence Chromatography

by Christopher J. Hudalla, Patrick D. McDonald, Waters Corporation, Milford, MA
Web: www.waters.com/upc2

J. Calvin Giddings envisioned that a convergence of the respective benefits of gas chromatography [GC]—higher mobile phase diffusion and efficiency—and liquid chromatography—higher selectivity via orthogonal modes of separation—might be accomplished by using a supercritical fluid as the mobile phase, together with LC stationary phases. Supercritical carbon dioxide (CO_2) is a 'green', non-toxic, inexpensive, highly compressible solvent; its low viscosity may decrease operating pressure while increasing efficiency for a given particle size and linear velocity.[1] By comparison, typical normal- and reversed-phase liquid chromatography (NPLC and RPLC) mobile phases contain toxic, higher viscosity, compression-resistant organic solvents. HPLC-grade solvents, especially those used for RPLC, including water, are very expensive.

Supercritical-fluid chromatography [SFC], with mobile phases containing a small proportion of methanol or another solvent mixed with a preponderance of CO_2, is a form of NPLC that is utilised in separating chiral compounds, especially on a preparative scale. However, unlike the sophisticated solvent delivery apparatus within the latest high- and ultra-performance LC systems (HPLC and UHPLC), the inability to meter supercritical CO_2 reproducibly and reliably, and to control key parameters such as temperature, pressure, and density, has hampered the analytical performance of SFC systems. As a result, SFC has been relegated to niche status in the field of separation science.

Earlier this year, new technology was introduced that may finally enable the realisation of Giddings' vision of convergence chromatography. UltraPerformance Convergence Chromatography™ (UPCC or UPC²) precisely pumps supercritical CO_2 as the predominant mobile phase solvent through small-particle stationary phases offering a wide range of selectivity. UPC² delivers high efficiency, shorter separation times, reduced solvent consumption, streamlined sample preparation, and lower cost of analysis per sample (often a savings of > 95%).

Most significantly, in the last four decades, separation scientists have moved away from NPLC and made RPLC their preferred analytical technique. However, NPLC is

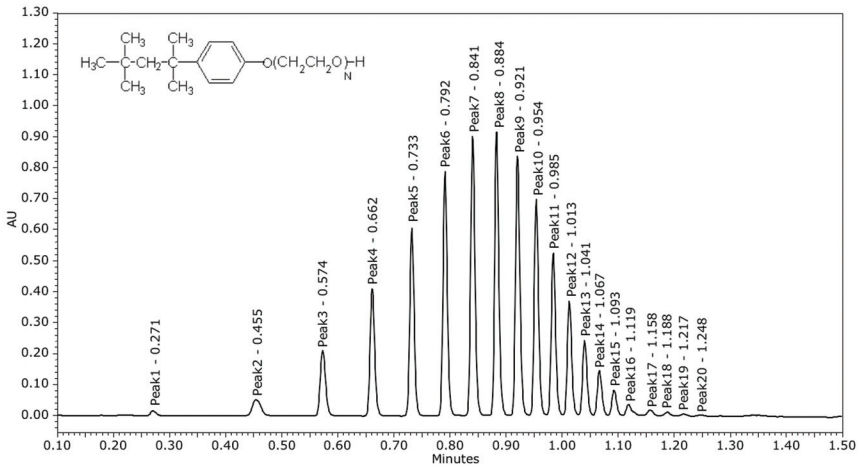

Figure 1: UPC² analysis of Triton-X [10 mg/mL in IPA] on an ACQUITY UPC² BEH column [2.1 x 50mm, 1.7µm] at 40°C [Waters Corporation, Milford, Mass., USA]. Gradient from 2% to 35% MeOH over 1.25 minutes at a flow rate of 2.0mL/min. UV detection at 222 nm. The automated back-pressure regulator [ABPR] is set to 1500 psi.

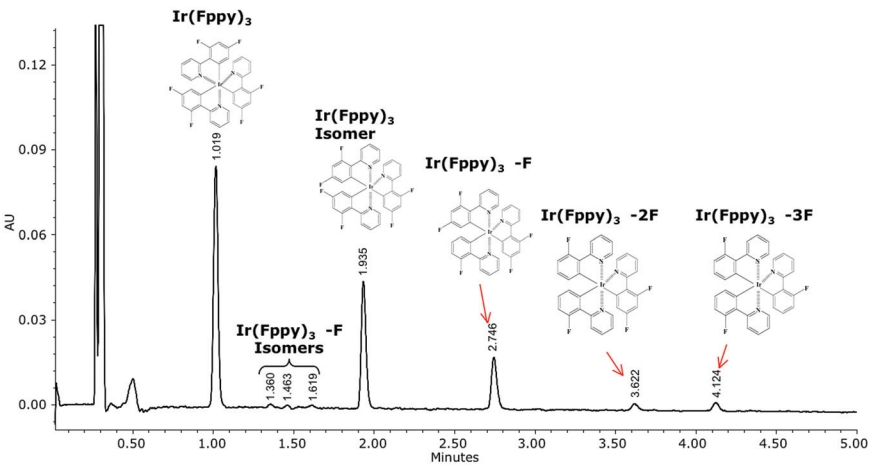

Figure 2: Proposed structure assignments for the unknown impurities found in the Ir[Fppy]$_3$ phosphorescent emitter sample. The separation used an ACQUITY UPC² BEH 2-EP column [3.0 x 100mm, 1.7µm] at 60°C [Waters Corporation, Milford, Mass., USA]. The modifier is 2 mg/mL ammonium formate in methanol with a gradient from 10% to 25% in 5 minutes at a flow rate of 2.0mL/min. The ABPR is set to 1885 psi.

orthogonal to RPLC and has the potential to separate a broader polarity spectrum of analytes. Now, convergence chromatography may harness the power of NPLC, enabling it to become a routine tool in the kit of analytical scientists seeking to solve challenging separation problems. UPC² promises higher throughput and productivity across a wide application space.

High Throughput, Low Cost Analyses

In addition to higher efficiency, one of the greatest advantages of smaller particles is the dramatic reduction in analysis times for chromatographic separations. As an example, consider non-ionic surfactants, mixtures of oligomeric compounds that are typically separated using NPLC, high-temperature GC, or high-temperature SFC, with 20- to 40-minute analysis times. [2,3] Non-ionic surfactants are used in many industrial and household detergent products and emulsifying agents. Compositional characterisation is critical when manufacturing these surfactant mixtures because differences in ethylene oxide chain length affect viscosity, solubility, polarity, and other key product characteristics. Using UPC², scientists analysed and identified 20 oligomers in a Triton-X sample in less than 1.5 minutes with excellent resolution (Figure 1). UPC² is nearly twice as fast as the best traditional method, and the estimated solvent cost per analysis, including only 600 µL of methanol, is $0.03!

A second example is the analysis of raw materials used to manufacture organic light emitting diodes (OLEDs). OLEDs are thin films that luminesce when an electric current is applied and are used in a variety of everyday electronics including televisions, mobile phones, and computer monitors. OLED manufacturing requires high purity raw materials since small amounts of impurities can significantly degrade OLED performance and lifetime. A traditional NPLC separation of the important compounds takes more than 30 minutes. [4,5,6] UPC² Technology performed the separation and characterisation of the primary Ir[Fppy]$_3$ complex, as well as seven structurally-related impurities, in approximately five minutes. Dual detection with tandem UV and mass spectrometry gives additional insight into the identity of the compound of interest as well as the impurities present (Figure 2). Compared to traditional NPLC, UPC² cut the analysis time by more than 80% and reduced cost by 97%, to about $0.05 per analysis.

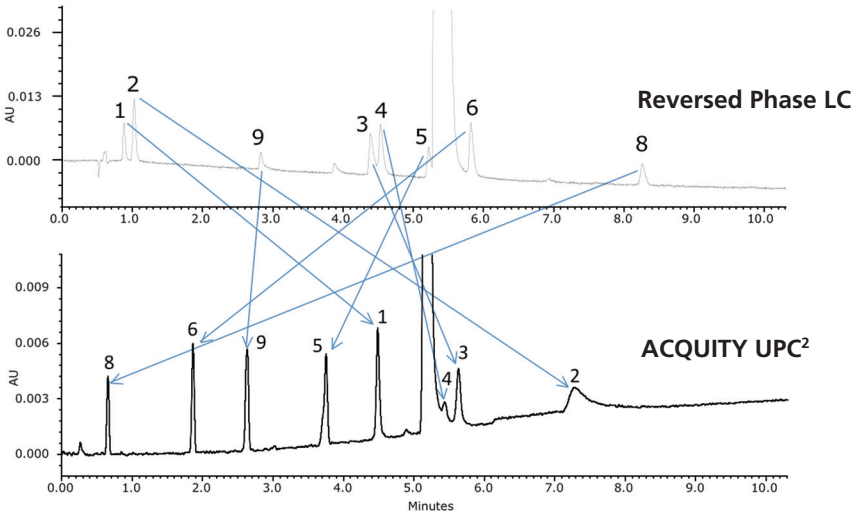

Figure 3: Separations of Metoclopramide under RP-UPLC [top] and UPC² [bottom] conditions. The UPLC method uses an ACQUITY UPLC BEH C_{18} column [2.1 x 100mm, 1.7µm]. Mobile phase A is 0.25% ammonium acetate in water and B is acetonitrile. A 12-minute gradient from 5% to 35% B is used at a flow rate of 0.5mL/min. The UPC² separation uses an ACQUITY UPC² BEH column [3.0 x 100mm, 1.7µm]. A modifier of 2mg/mL ammonium formate in methanol is used with a gradient of 5% to 15% over 12 minutes at a flow rate of 2.5mL/min. The ABPR is set to 1500 psi. UV detection at 275nm for both sets of data.

Orthogonality, Wide Applicability, and Streamlined Sample Preparation

Analytical characterisation of a sample often requires complementary techniques to assess the specificity of any given method. For chromatographic method development, an orthogonal method may be used to identify molecules that may co-elute, or not elute at all, in another method. This is critical for pharmaceutical development where precise determination of impurities and degradation products is required to meet regulatory guidelines for identification, reporting, and toxicological qualification. Metoclopramide is an antiemetic often used to treat nausea and vomiting. A comparison of its analysis under reversed-phase UltraPerformance LC® and UPC², respectively, demonstrates the orthogonality of these two separation modes (shown in Figure 3).

As compared to RPLC, UPC² derives its orthogonality from interaction with polar moieties, even in predominantly hydrophobic molecules. This means that UPC² may be used to analyse a broad range of analytes with wide-ranging chemical properties, including hydrophobicity (log P). Consider the separation of vitamin E isomers (tocopherols), whose important biological functions relate especially to their antioxidant activities.[7] Because of their high log P values (~ 10.0), typical NPLC analysis time of tocopherol mixtures is about 20 minutes. With UPC², four tocopherol isomers are separated to baseline in 30 seconds (Figure 4). An additional benefit of using

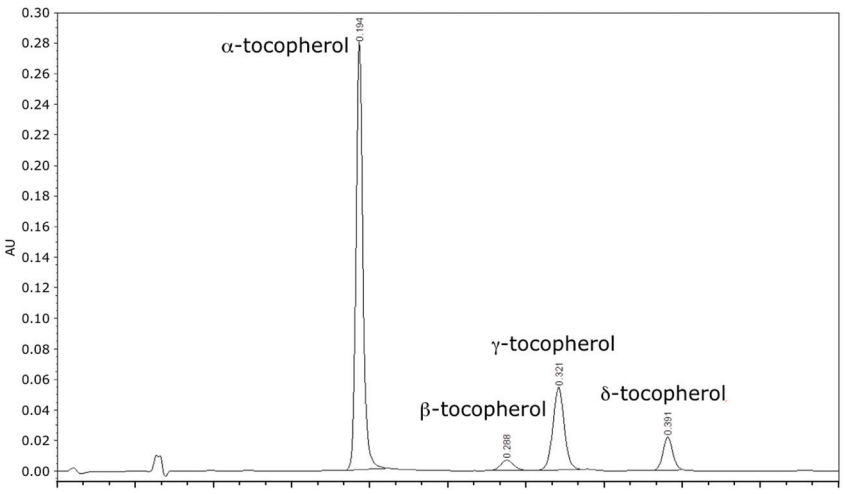

Figure 4: UPC² analysis of Vitamin E [tocopherol] isomers on an ACQUITY UPC² BEH column [2.1 x 50mm, 1.7µm] at 40°C [Waters Corporation, Milford, Mass., USA]. Gradient from 1% to 4% MeOH over 24 seconds at a flow rate of 3.0mL/min. UV detection at 293nm. The ABPR is set to 1885 psi.

UPC² to analyse high log P analytes is the compatibility of UPC² mobile phases with common extraction solvents (e.g., hexane) used for such non-polar compounds. RPLC requires that such a non-polar organic extract be evaporated and reconstituted in the aqueous/organic mobile phase or another mobile-phase compatible solvent. In contrast, the ability to inject a non-polar extract directly into a UPC² separation system streamlines sample preparation, saving time and money, while increasing throughput.

Conclusions

The ACQUITY UPC² System is a powerful separations tool for many important applications spanning areas as diverse as consumer products, pharmaceuticals, industrial chemicals, food, and nutrition. When compared to traditional RP-HPLC and SFC, a holistically designed ACQUITY UPC² System, with advanced supercritical-fluid metering capability, appropriate injection and detection means, and narrower columns packed with smaller, selective particles, enables orthogonal separations, higher efficiencies, lower solvent consumption (sometimes from mL to µL), streamlined sample preparation, and reduced cost per sample analysis. For many laboratories, the powerful orthogonal separation capability and cumulative savings of resources will provide significant scientific and financial incentives for the broad adoption of UPC² Technology as a routine analytical tool.

References

[1] Thiebaut, D., et al.: Second International Conference on Packed Column SFC, Oct 1-2 [2008]

[2] Asmussen, C., et. al., J High Res Chrom, 21[11]: 597–604 [1998]

[3] Fytianos, K., et. al., Chemosphere, 35[7]: 1423–1429 [1997]

[4] Sivasubramaniam,V., et al., Cent. Eur. J. Chemistry, 7[4]: 836–845 [2009]

[5] Baranoff, E., et al., Inorg. Chem., 47: 6575–6577 [2008]

[6] Kondakov, D., et al., J. Appl. Physics, 101: 024512 [2007]

[7] Burton, G.W. and Ingold, K.U., J. Am. Chem. Soc., 103: 6472–6477 [1981]