New Separation Tool for a Broad Range of Analytical Challenges

UltraPerformance Convergence Chromatography (UPC²)

Convergence chromatography is a new separations technique that uses small particle sizes of 3.5 to 1.7 μm, with carbon dioxide as the primary mobile phase component to achieve high chromatographic efficiencies and fast optimum linear velocities [1]. Based on reliable instrumentation optimized for low dispersion and high sensitivity, UltraPerformance Convergence Chromatography (UPC²) addresses challenging separations yielding speed, sensitivity, and resolution along with unique selectivity for chiral and achiral LC applications.

With CO₂ as the primary mobile phase, replacing environmentally harmful normal phase solvents results in a significant reduction in the amount of toxic waste generated. In addition, UPC² provides orthogonal selectivity to reversed phase liquid chromatography (RPLC) for compounds encompassing a large range of hydrophobicities and chemical properties, further expanding beyond the chiral and normal-phase application space typically reserved for SFC applications. The versatility of this technique allows it to address challenging analyses from many application areas while increasing throughput and productivity. At the same time, the decrease in analysis time and solvent consumption results in a significant decrease in the cost of analysis per sample, often greater than 95% compared to alternative chromatographic techniques. Here, we present how UPC² can be used as a routine tool in the analytical laboratory to address challenging or problematic separations from several different application areas.

Better Resource Utilization

In addition to more efficient separations, another advantage to using smaller particles is the reduction in analysis times. One example is for the separation of non-ionic surfactants, which are typically characterized using normal phase LC, high temperature GC, or high temperature SFC, with analysis times ranging from 20 to 40 min [2,3]. Non-ionic surfactants are used in many industrial and household detergent products and emulsifying agents. Compositional characterization of these surfactants is critical for manufacturing because the differences in ethoxy chain length affect the viscosity, solubility, polarity, and other characteristics of the mixture. The application of the presented technology to the analysis of Triton-X results in the characterization of a Triton sample in less than 1.5 min, with excellent resolution and identification of 20 oligomers (fig. 1). The reduction of analysis time compared to traditional methods is more than 90%, and the method only uses 600 µl of methanol, which equates to an estimated solvent cost of $0.03 per analysis.

An additional example is the analysis of organic light emitting diodes (OLEDs), which are typically characterized by microscopy in addition to LC techniques, and generally require greater than 30 minutes for analysis time [4-6]. OLED’s are thin films that exhibit electroluminescence when an electric current is applied...
plied. They are used in a variety of everyday electronics including televisions, mobile phones, and computer monitors. The use of high purity raw materials is critical for the OLED manufacturing process as small amounts of impurities can significantly degrade OLED performance and lifetime. The application of UPC² technology to this problem results in the characterization and identification of the Ir(Fppy)₃ complex in addition to seven structurally-related impurities in approximately 5 min. Dual detection using UV and mass spectrometry provides additional information into the identity of the compound of interest as well as the impurities present (fig. 2). This method results in a reduction of analysis time compared to traditional methods by more than 80 % while cutting analysis cost down to approximately $0.05 per analysis.

**Orthogonality and Wide LogP Range**

Analytical characterization of a sample often requires complimentary methods to assess the specificity of any given method. For chromatographic method development, orthogonal methods may be used to identify molecular moieties that may appear as co-elutions or non-elutions in another method. This is particularly critical for pharmaceutical development where the precise determination of impurities and degradation products is required to meet regulatory guidelines for identification, reporting and toxicological qualification. The pharmaceutical compound Metoclopramide is an antiemetic often used to treat nausea and vomiting. A comparison of the separation under RP-UPLC conditions to data collected using UPC² demonstrates the orthogonality of the method (shown in fig. 3).

In addition to providing orthogonal selectivity to RPLC, UltraPerformance Convergence Chromatography can be used to analyze a diverse set of analytes with a wide range of chemical properties, most especially compounds with a wide range of hydrophobicity (log P). An example is the separation of the Vitamin E tocopherol isomers, which play an important role in many biological functions, most notably relating to their antioxidant activities [7]. Because of the high log P values for the tocopherols (~10.0), typical analysis time using normal phase LC is about 20 minutes. With UPC², the four tocopherol isomers are baseline separated in 30 seconds (fig. 4). An additional benefit is the compatibility with common extraction solvents used for these types of analytes (i.e., hexane, isopropanol). While techniques such as RPLC require evaporation and reconstitution in an LC compatible diluent, extracts can be injected directly, which saves significant time and cost, and increases throughput.

**Conclusions**

The ability to characterize compounds with a large range of chemical properties broadens the scope of viable applications that can be addressed by UPC². These benefits can be realized for many application areas including consumer products, pharmaceuticals, industrial chemicals and food and nutrition. The high efficiency and sensitivity results in savings in time and solvent consumption, both of which provide additional benefits in the form of cost reduction for analyses. The cumulative savings on all resources provides significant financial incentive for broad adoption of this technique as a routine analytical laboratory tool.

**References** are available from the authors.

**Contact**
Christopher J Hudalla  
Jeff Bieszki  
Andrew Aubin  
Michael D Jones  
Rui Chen and Kenneth J Fountain  
Waters Corporation, Milford, MA  
www.waters.com/upc2

![Fig. 3: Separations of Metoclopramide under RP-UPLC (top) and UPC² (bottom) conditions. The UPLC method uses an Acquity UPLC BEH C₁₈ column (2.1 x 100 mm, 1.7 µm). 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 0.5 ml/min. The UPC² separation uses an Acquity UPC² BEH column (3.0 x 100 mm, 1.7 µm). A modifier of 2 mg/ml ammonium formate in methanol is used with a gradient of 5 % to 15 % over 12 minutes at a flow rate of 2.5 ml/min. The ABPR is set to 1500 psi. UV detection at 275 nm for both sets of data.](image)

![Fig. 4: Analysis of Vitamin E tocopherol isomers on an Acquity UPC² BEH column (2.1 x 50 mm, 1.7 µm) at 40 °C (Waters). Gradient from 1 % to 4 % MeOH over 24 seconds at a flow rate of 3.0 ml/min. UV detection at 293 nm. The ABPR is set to 1885 psi.](image)