Unified Chromatography: How the History of SFC brings Separation Methods Together

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The preceding articles in this issue of *Chromatography Today* illustrate how chromatography with a supercritical fluid as mobile phase is alive and well as a versatile separation method with key applications in numerous areas of pharmaceutical, industrial, environmental and industrial analytical chemistry. We seek to show, though, that the unique properties of supercritical fluids have had a broader impact across the wider field of separation science, largely, we suggest, through the development of so-called unified chromatography (UC).

How does UC work? The underlying principle is that there are no theoretical boundaries between mobile phases, as was pointed by Calvin Giddings [1] over half a century ago, and UC was invented in 1987 making use of this and the observation that as the column diameter decreases so do the differences between chromatographic techniques. Since then there has been much ingenious if sporadic, activity, but recent advances make it an idea whose time has now come; much contemporary chromatography bears the imprint of UC largely through the principles and practice of SFC.

The history of SFC (see *Table 1*) itself began in the 1800s when the supercritical state was characterised for CO_2 by Andrews [2] working in Belfast. Supercritical CO_2 was then used in separation science in the 1900s, prefacing SFE. But it was James Lovelock [3] who first suggested in 1958 its use as a chromatographic mobile phase. Just as the Nobel Prize winning inventors of liquid-liquid partition chromatography, Martin and Synge, had proposed that the mobile phase could also be a gas, Lovelock realised the high liquid- like density, high gas-like diffusivity and low viscosity of fluids above the critical point would extend both GC and LC. In confirmation, packed-column SFC was demonstrated in 1962 and then quickly developed through the 60s with new detection and pressure programming methods, but it was SFC on capillary columns, invented by Novotny and Lee in 1981 [4] which led to an exponential growth in SFC applications. It must be said, however, that the practicalities of capillary SFC, in particular the very small dead volumes made necessary by the small column diameters, have acted as something of a brake on progress and more recently packed column SFC has revived and its scope increased.

How UC relates to SFC arose from the combination of the practical variants of chromatography, which are usually defined from the viewpoint of the mobile phase. The chromatographic family includes:

- Gas chromatography (GC)
- Supercritical fluid chromatography (SFC)
- Subcritical fluid chromatography (SubCFC)
- Enhanced fluidity chromatography (EFC)
- Elevated temperature liquid chromatography (ETLC)
- Liquid chromatography (HPLC) and UltraHPLC (UHPLC)
- Capillary electrochromatography (CEC)

Historically, each variant of chromatography has been carried out on a dedicated instrument, but as the column diameter decreases the three main variants become more similar, and the question arises as to whether separate instruments are required. In UC two or more of the different variants above are carried out on a single hybrid chromatograph – an instrument first

Table 1: The Early SFC Timeline

Andrews	1879	Characterisation of critical point
Lovelock	1958	Suggestion of SFC
Klesper	1962	Packed column SFC demonstrated
Sie and Rijnders	1966	Use of FID in SFC
Sie and Rijnders	1967	First use of term 'SFC'. UV/Visible detector in SFC
Jentift and Gouw	1978	Pressure programming in SFC
Randall and Wahrhaftig	1978	Coupling of SFC to MS
Novotny and Lee	1981	Invention of capillary SFC
	1982 onwards	Commercial SFC chromatographs

demonstrated for GC, SFC and HPLC by Ishii et al. in 1987 [5].

Since then many hybrid instruments have been described e.g. the microcolumn GC / SFC / HPLC system of Tong et al. (1995) [6] culminating in the modern commercial (Agilent) SFC / UHPLC hybrid system which allows completely flexible switching between UHPLC and SFC without equipment changes. Hybrid capillary column UC was also demonstrated and shown to be particularly effective for wide boiling mixtures from the energy industries. Here the process was sequential GC (with helium mobile phase) followed by SFC with supercritical CO_2. A less obvious but still real example of a hybrid separation system is pressure assisted capillary electrochromatography, in which the liquid mobile phase or charged analyte flow through a packed capillary bed containing HPLC stationary phase is driven by either pressure or by electro-osmosis.

But how has SFC influenced the actual practice of the other chromatographic variants? High-pressure GC and solvating GC (SGC) show obvious affinities with SFC (in fact CO_2 is often the mobile phase in SGC) but in the case of capillary GC the answer has two aspects: the first lies in the column revolution in the 1980s which saw the development of durable fused silica columns for GC in place of the fragile glass columns, which were the only option available until then. This proceeded in parallel with the increased demand and availability of columns for capillary SFC, particularly those with so-called 'designer' stationary phases such as phenyl and cyanopropyl. The small diameter (50 and 100 µm) SFC columns were just those required for so-called fast GC, where speed of analysis increases with decreasing column radius.

The advance of open-tubular column technology was not, however, the only major influence of capillary SFC on GC. The second came from the competition as the most suitable technique for the analysis of mixtures of increasingly high molecular weight compounds in petroleum- derived and oligomer samples. This acted as a driver for high temperature GC. New thermostable GC columns became available capable of eluting and separating the mixtures to which capillary SFC was applied, but high temperature GC and capillary SFC should not be regarded as competitors, however, since each has its own application areas. A fundamental consideration which has to be taken into account is that HTGC affords higher efficiency in the shortest time as a consequence of more rapid solute diffusion in gases in comparison with supercritical fluids.

While SFC has had a substantial impact on chromatography in the gas phase, this has been even more profound for liquid phase separations. The conventional view is that packed column SFC is only suitable for non-polar analytes but following on from Terry Berger's work [7, 8], Caroline West [9] showed recently in *Chromatography Today* how many polar compounds can be chromatographed if a modifier such as water is added to the CO_2. Similar experience with sub- and supercritical CO_2 both alone and in mixtures, with additives in the mobile phase, led Susan Olesik [10-13] and her group to explore in the 1990s the concept of enhanced – fluidity chromatography, to bridge the gap between SFC and HPLC. Of course it was well known that liquid CO_2 is a low cost solvent which can be used as a substitute for a non-polar mobile phase in normal phase HPLC separations such as the aromatics in fuels. But in EFC the mobile phase is a mixture of a liquid solvent combined with liquid CO_2. This has properties approaching those of a supercritical fluid – low viscosity (leading to reduced column pressure drops and hence faster flow rates), and increased solute diffusion coefficients (increased efficiency), but retains the solvent strength of a liquid.

A suitable mobile phase for normal phase EFC is n-hexane + CO_2, for reverse phase EFC, methanol, water + CO_2 and for size exclusion chromatography tetrahydrofuran + CO_2. The only restriction on mobile phase composition, explored by Tom Chester in the 1990s [14, 15], is imposed by the extent of the one-phase region of the phase diagram for mobile phase mixtures. The substitution in EFC of water and CO_2 for organic solvent then has outstanding environmental and cost saving consequences. If 'exotic' mobile phases are employed only very small volumes are required, while the CO_2 eluent is easily removed from fractions separated by preparative EFC.

The advantages of EFC are also conferred on chromatographic separation at elevated temperature (ETLC), as Tony Edge [16] has discussed in a recent issue of *Chromatography Today*. Solubility in water generally increases markedly with temperature while the viscosity falls and diffusion rate also increases. The net result is an increase in the range of analytes which can be separated with less organic solvent, and with fast analysis times and improved column efficiencies. The large change in density with pressure near the critical temperature can bring about highly selective separations - for example resolution of the required pair by changing the CO_2 mobile phase temperature shown in Figure 1. The chromatographic advantages of using enhanced fluidity mixtures are comparable to those of ETLC.

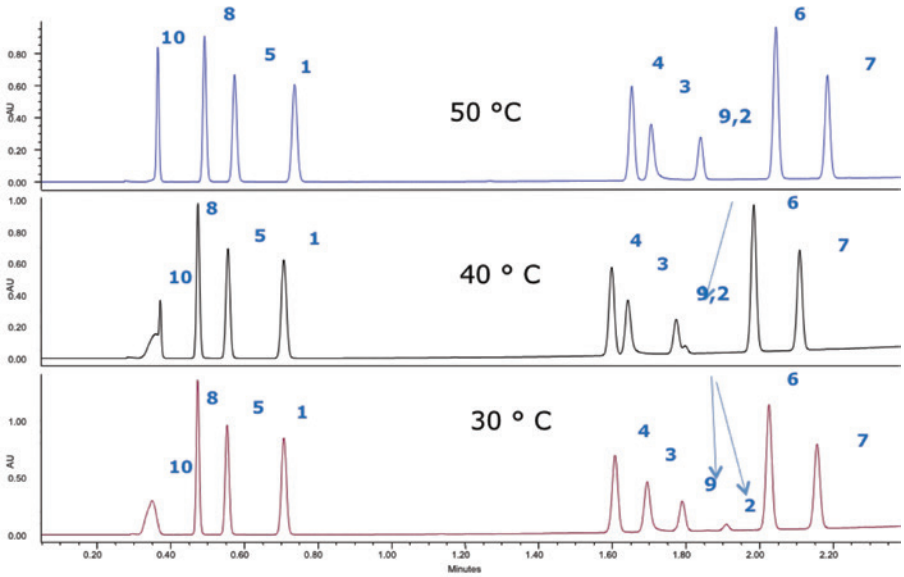

Figure 1
Effect of temperature on the UPC2 separation of flavour compounds with CO_2 mobile phase. (Courtesy of the Waters Corporation).
1-vanillin, 2-4-hydroxybenzalcohol, 3- 3,4-dihydroxybenzaldehyde, 4-vanillic acid, 5-ethyl vanillin, 6-4-hydroxybenzoic acid, 7-coumaric acid, 8-coumarin, 9-ferulic acid, 10-piperonal
Column: 2-EP 3.0 x 100 mm, 1.7 µm; Wavelength: 260 nm-Compensated

The considerations above form the basis for the development of new instruments of great versatility which use the advantages of SFC to bridge the gap between GC and HPLC. They use CO_2 as primary liquid phase and allow simultaneous programming of the mobile phase composition from pure CO_2, (liquid, or sub or supercritical) to pure organic solvent, along with pressure and temperature programming. These instruments permit maximum selectivity and resolution and minimum analysis time while being as 'green' as can be currently achieved. The technique has been termed UltraPerformance Convergence Chromatography™ or UPC² (Waters Corporation) [17]. An example of the application of UPC² is the rapid low-solvent volume separation of steroids with a methanol/ CO_2 mobile phase (Figure 2).

UPC² also permits normal and reverse phase separations in the same run, as well as chiral and achiral separations; oligomers can also be resolved. As in SFC, by adding low concentrations of polar compounds to the mobile phase peak shapes can be improved e.g. those of basic analytes if ammonia is added. The overall result means that UPC² is especially applicable to the rapid (often sub-minute) and highly efficient analysis of numerous pharmaceutical compounds with the consumption of very small amounts of organic solvent.

As an analytical technique, chromatography is preeminent. Recently, the medicinal chemist and *Chemistry World* columnist Derek Lowe [18] pointed out its supreme value in the characterisation of the organic chemist's mixtures, 'Chromatography' he asserted 'hasn't just made organic chemistry easier; in most cases, it makes it possible'. For our part, we would propose that chromatography with supercritical fluids has had a vital role both directly and indirectly in making this come about.

Acknowledgements

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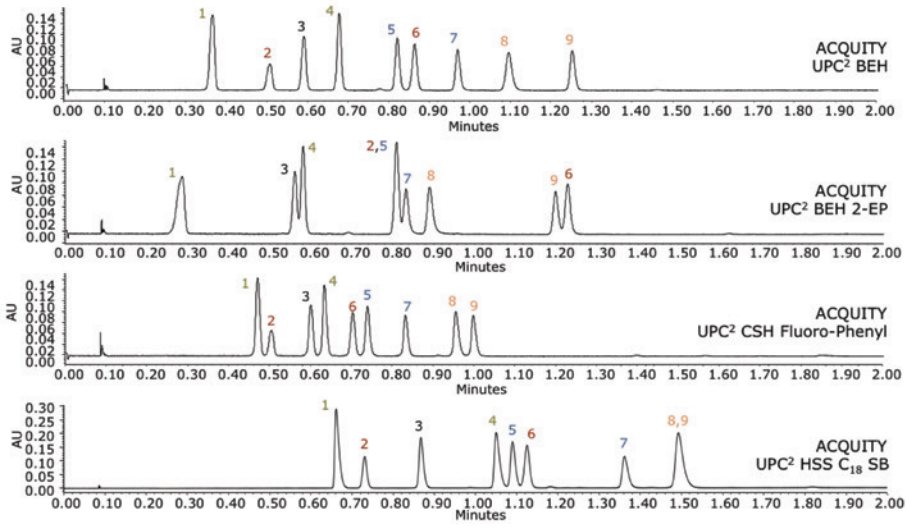

Figure 2
Development of a method for the separation of steroids. A 2-minute screening gradient was used to evaluate different columns. (Courtesy of the Waters Corporation)
Experimental conditions: 3.0 x 50 mm column with 2-17% MeOH in CO_2 in 2 minutes at 3.65 mL/min, 40°C
Steroids: 1. Androstenedione, 2. Estrone, 3.17a-Hydroxyprogesterone, 4. Testosterone, 5.11-Deoxycortisol, 6. Estradiol, 7. Corticosterone, 8. Aldosterone, 9. Cortisol

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Aspects of the Applications of Chromatography-Mass Spectrometry to Environmental Water Analysis

The applications of Chromatography – Mass spectrometry are extremely wide ranging, none more so than in the analysis of environmental water matrices. Depending on the sophistication of the mass spectrometry as an analysing tool sample preparation is either of vital importance or may be largely unnecessary. The purpose of the analysis is to characterise the sample as fully as possible or to measure discrete components with high precision?

This meeting is designed to draw attention to the methodology employed to analyse environmental water and related matrices containing target compounds with a range of physico-chemical properties. The analysis may be intentionally non-targeted, for example to screen for many classes of substance without chemical bias.

The Environmental Mass Spectrometry Special Interest Group of the BMSS will be presenting a free to enter seminar at WWEM The International Conference and exhibition for Water, Wastewater and Environmental Analysis on November 5th at the Telford International Centre in Telford.

For details of the full programme and free entry please log onto www.wwem.uk.com.