Separation of compounds by supercritical fluid chromatography

SFC: a new technology?

The supercritical fluid chromatography (SFC) for the analytical and preparative separation of compounds is one of the different analytical verification procedures, that are possible with the instruments from Brechbühler AG.

Georges Froidevaux[1]

The «Supercritical Fluid Chromatography», better known by the acronym SFC, was first suggested in the year 1962 by Ernst Klesper and colleagues – about six years before the more conventional «High Performance Liquid Chromatography» (HPLC). Back then, Klesper, Corwin and Turner were researching high-pressure gas chromatography via mono- and dichlorodifluoromethane (boiling point > 90 °C for either) for the extraction of a nickel-etioporphyrin II transition metal complex from a stationary poly-ethylene glycol phase. Already at that time, they put forward the idea to collect the fractions at the end of the system – the precursor to preparative SFC.

Althrough SFC-technology was eclipsed by the boom in HPLC at the beginning of the 1970s the, its development did not cease. By now, carbon dioxide is serving as the supercritical fluid, even though other solvents are possible.

What is a supercritical fluid?

The typical states of matter are solid, liquid and gaseous. As one varies the temperature and pressure, under specific conditions one can reach the triple-point (the compound is present as a solid, liquid and gas simultaneously), the critical point (the compound is both liquid and gaseous) or the supercritical area (see image 1), which had been discovered already in the 19. century. This area may be reached only by using suitable control equipment. In 1985 the Japanese companies Jasco and Morinaga & Co. Ltd published the first chromatography- and extraction system based on supercritical fluid. In the same year Jasco introduced the patented back pressure regulator, which made it possible to maintain the supercritical CO2 at constant pressure throughout the instrument and allows preparative application.

Which properties does a supercritical fluid have?

In this state a compound is a kind of hybrid between gas and liquid (image 2, right). It shows the viscosity of a gas, has a density like a liquid, but allows for much more rapid diffusion rates when compared to liquids (table 1).

Which impact does this have on chromatography?
This may be explained by using the Van Deemter equation:

$$H = A + B/u + C * u$$

Where the variable are:
– H the measure for the number of theoretical plate equivalents
– A the Eddy-constant, dependent on the path length of the analyte through the column
– B the longitudinal diffusion coefficient
– C the laminar flow
– u the linear velocity

An efficient separation is reached when the height equivalent to a theoretical plate H (originally a term used for describing distillations) is minimized. This can be achieved by minimizing each factor:

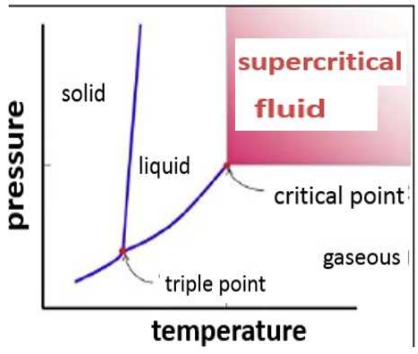

Image 1: Phase diagram

	Diffusion [cm^{2} s^{-1}]	Density [kg m^{-3}]	Viscosity [g cm^{-1} s^{-1}]
gaseous	10^{-1}	1	10^{-4}
supercritical	10^{-3}	100-1000	10^{-4}
liquid	10^{-6}	1000	10^{-2}

Table 1: Comparison of properties

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Image 2: CO2 in different states of matter, from left to right: gaseous/liquid; at supercritical point (Rayleigh-scattering); in supercritial state

– Eddy-diffusion is dependent on the path length of the analyte and depends strongly on the stationary phase (small particle size or Core-Shell-technology like the Kinetex from Phenomenex).

– The longitudinal diffusion describes the peak broadening along the flow direction and depends on the linear velocity u. For example, if the flow is stopped, the diffusion is very high. Conversely: the greater the flow, the better the separation. The flow maximum however is determined by the back pressure and the viscosity.

– The factor C describes the so called mass transfer or the time for the interaction between the stationary (column material) and the mobile (solvent with analytes) phase and is dependent on the viscosity and diffusion of the eluent.

As may be seen from table 1, SFC allows for a higher diffusion and lower viscosity compared to HPLC. Thus, chromatographically speaking, SFC may be regarded as the ideal in the world of GC and HPLC..

Supercritical carbon dioxide has advantages

Of course, other compounds can be used for SFC besides carbon dioxide. Still, CO2 has many benefits. Its critical point lies at 31 °C and 73,8 bar. These are relatively mild conditions: the comparatively low temperature avoids side reactions during extraction, purification or analysis of temperature sensitive compounds. Another benefit is that carbon dioxide at atmospheric pressure is relatively non-toxic and gaseous, which makes it very suitable for preparative purposes.

In comparison, for other solvents to reach the critical point requires much higher temperatures (e.g. water: TC = 374 °C, alcohols: TC >239 °C) or are corrosive (e.g. ammonia is toxic, supercritical water is oxidising). Admittedly, dinitrogen monoxide has values similar to carbon dioxide, however, it is a stronger greenhouse gas than carbon dioxide and acts on the ozone-oxygen cycle. Finally carbon dioxide is very cheap to purchase.

SFC – a revival?

Particularly in recent years has SFC come to the fore again. There are different reasons for this. For example normal phase chromatography and extractions are based in part on volatile, chlorinated, harmful and/or flammable organic solvents (e.g. methylene chloride) and is accordingly not always MS-compatible.

Image 3: Typical analytical SFC-System from Jasco, consisting of CO2-pump, autosampler, column oven, detectors, back pressure regulator and control software

In addition, the latter may pose a risk for health or environment. Recent, more stringent regulations for the determination of the purity of compounds with chiral elements (ChemieXtra reported in the May/2013 edition) require separations, that can be incompatible with the water used in reversed phase chromatography. Supercritical CO2 on the other hand has an elution strength comparable to dichloromethane and is compatible with chiral columns such as the LUX-series from Phenomenex. Typically alcohols are used as modifiers. Image 3 shows an analytical SFC-instrument, in this case from the Jasco SF-2000 series.

Additionally alternatives were sought during the production shortage (and the concomitant scarcity) of acetonitrile, one of the classic eluents in reversed phase chromatography. SFC thereby underwent its «green renaissance», as it was called «green chromatography».

Application examples

Typical applications using supercritical carbon dioxide are common. Examples touching everyday life include the extraction of caffeine from coffee (1964 by K. Zosel patented in Germany) or the «extraction» of dirt from clothing. Furthermore supercritical fluid extraction SFE is used to isolate many polycyclic aromatic hydrocarbons from dust or specific compound classes (alkaloids, vitamins, pesticides etc.) from different matrices such as plants, food or soil.

Analytical SFC is also used in the analysis of different distillation fractions of mineral oil. Peptide may also be separated by SFC, as in the application note published in 2012 by Jasco Inc.: a mixture of five standards from Sigma-Aldrich (G 3502(GLY-TYR), V 8376 (VAL-TYR-VAL) M6638 Methionine Enkephalin Acetate (TYR-GLY-GLY- PHE-MET), L 9133 Leucine Enkephalin (TYR-GLY-GLY-PHE-LEU) and A9522 Angiostensin II Acetate (ASP-ARG -CAL-TYR-ILE-HIS-PRO-PHE) was analysed via HPLC (water/acetonitrile, 1ml/min) as well as via SFC (modifier: 0,2% trifluoroacetic acid in methanol, 2ml/min).

The run time was shortened from 60 to 12 minutes. Simultaneously, two thirds less solvent was used and one third less organic waste was produced.

Also, polar compounds may be separated via SFC and silica-based stationary phase. In the example (see image 4, 5 and 6), three acidic, two basic, one neutral and one amphoteric compound are shown. Via method optimization by addition of different modifiers (here methanol) similar to reversed phase HPLC can lead to success.

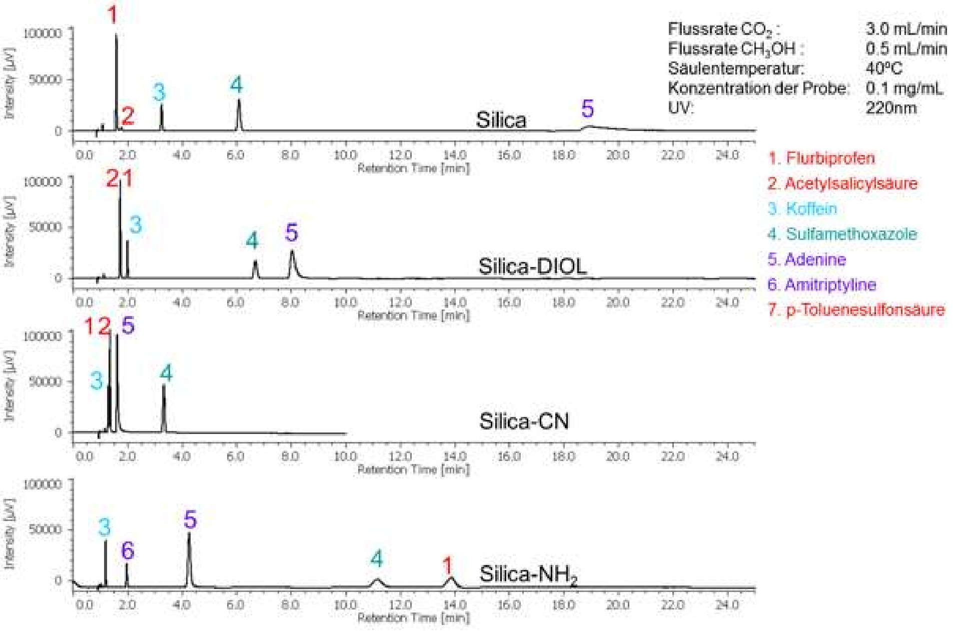

Image 4: SFC-separation on different stationary phases, no additives

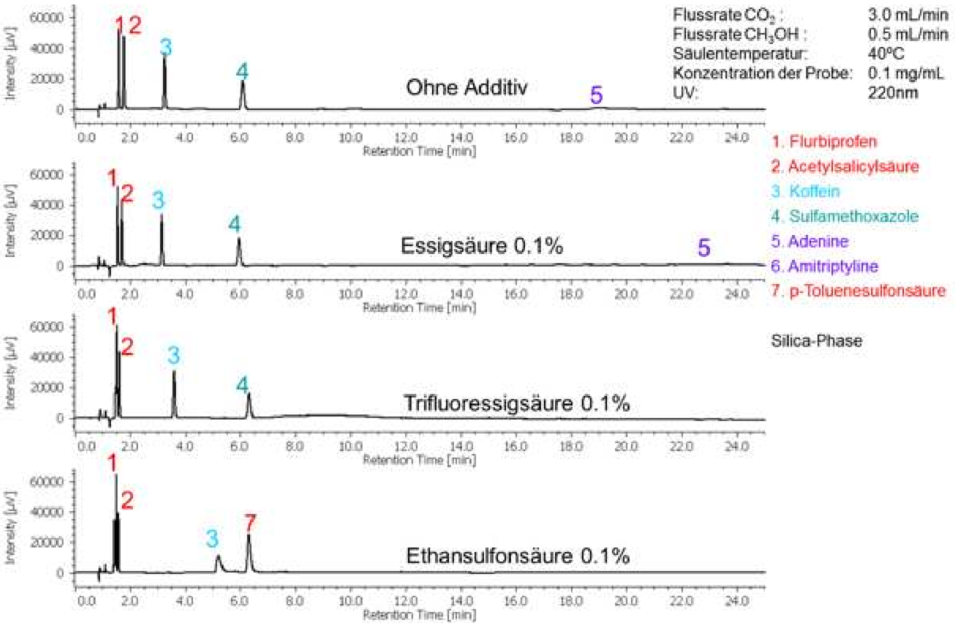

Image 5: separation on silica phase with different acidic additives

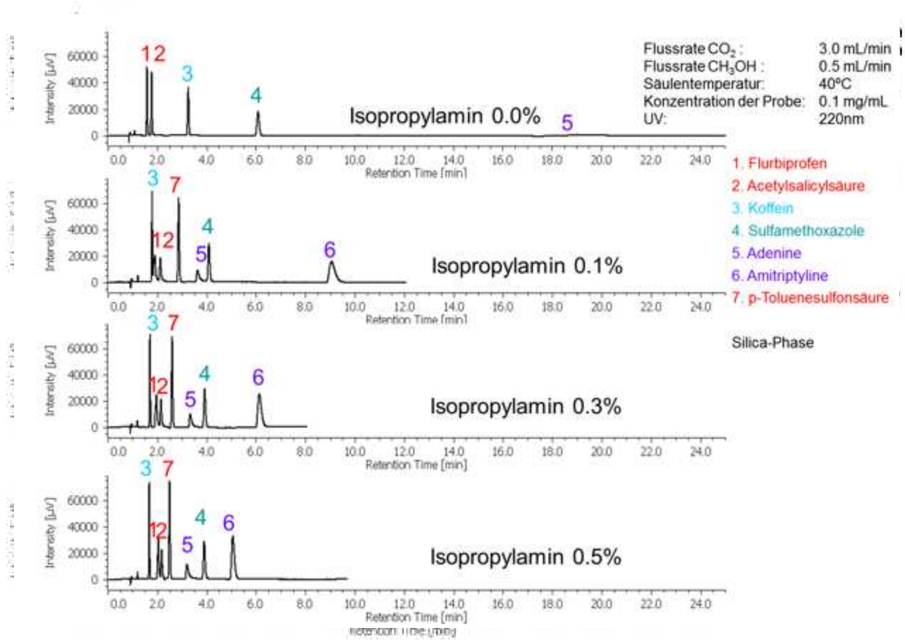

Image 6: Separation on silica-phase with basic additive in different concentrations

In image 6 it is apparent that after the different measurements, the addition of 0.5 percent isopropylamine is the best choice. The presented possibilities offered by supercritical chromatography and extraction systems with the selected applications demonstrate only a small part of the potential of this technology. Besides SFC systems, Brechbühler AG also offers HPLC and X-LC (UHPLC)-systems as well as a wide selection of corresponding columns..

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