Towards a better awareness of the advantages of supercritical-fluid chromatography

F. Vérillon

Gilson S.A., B.P. 45, 72 rue Gambetta, 95400 Villiers le Bel, France

Isn't the reality portrayed in Escher's lithography *Sky and Water I* (1938) strange and at the same time comprehensible? Isn't it a "view cell" given by the artist to scientists, particularly those interested in this dossier? The air and water creatures that inhabit Escher's lithography evoke the physical properties of the fluid state, gas and liquid being its extreme forms. Escher is able to overlap them in the same environment, showing no boundary between sky and water and creating a milieu where birds and fish are able to cohabit, combine their attributes, increase their ability to occupy the space, even going beyond the frame. Similarly, pressure raised above the atmospheric value can generate supercritical fluid (discovered in 1822), eliminate the boundaries between gas and liquid, combine the best properties of these common forms, broadening their application range (perhaps beyond what we may imagine today), particularly in the field of chromatography where this "general" fluid state was implemented for the first time in 1962.

In contrast with the predominant chromatographic techniques (GC and LC), supercritical-fluid chromatography (SFC) requires pressure at the column outlet to create this intermediate state of high internal energy, which offers a gas-like mass transfer and liquid-like solvating properties. This opens a broad range of tridimensional operating conditions by varying pressure in addition to composition (LC dimension) and temperature (GC dimension) [1]. With a unique array of physical, economical and ecological properties, carbon dioxide (CO_2) is the prominent component of SFC mobile phases. In this role, CO_2 is mixed under pressure with a polar modifier, generally a common solvent containing the secondary additives classically required in LC conditions. Available worldwide since 1992, modern commercial instruments control pressure independently of flow rate. SFC requires open-tubular or packed columns. Packed columns have clearly enhanced the dynamism of applications since 1996. Thus, both materials and methods used in SFC are now presented as advanced forms of those used in high-performance LC (HPLC). Consequently, some SFC systems offered today perform both SFC and HPLC for analytical as well as preparative work, at a reasonable extra cost compared to that of HPLC workstations. This approach should significantly broaden the worldwide installed base presently estimated at one thousand units for lab-scale SFC systems operating with CO_2 and standard HPLC columns. In other respects, "green" processes based on SF-CO_2 replacing organic solvents are increasingly moving out of the lab towards chemical industries electing to implement SFs for "sustainable" technology as well as for short-term real economic benefits [2-4].

The purpose of this dossier is to present examples of how, why, and where SFC can be used to implement fast, highly efficient and cost-effective separation methods for industrial needs. Whether analytical or preparative, whether unique, complementary, or competing with existing methods, SFC contributes to the ongoing rationalization efforts in the field of molecular separations. Seven original articles by eminent authors from industry and academia in countries at the cutting edge of drug discovery, all point in the same direction: highlighting the advantages and new possibilities opened up by SFC.

Starting with sample injection, Tyge Greibrokk (University of Oslo, Norway) and Tom Chester (Procter & Gamble, Cincinnati, OH, USA) explain the formation of mixtures with the mobile phase and the phase transitions the sample may experience at the column inlet, as being two keys to understanding this first stage. In the light of their explanations with phase diagrams for several modifier solvents forming "type-I" mixtures with CO_2 (i.e. CO_2 and modifier are miscible as liquids), very useful rules can be deduced. For instance: at any pressure higher than about 17 MPa, regardless of temperature, CO_2 is miscible in all proportions (i.e. liquid-vapor phase separation can never occur) with at least thirteen common modifier solvents.

For the petrochemical industry, Didier Thiébaut and Éric Robert (ESPCI, Paris, France) present a review of recent advances in SFC of petroleum fractions. They demonstrate that SFC with flame-ionization detector (FID), or, better, with dual UV/FID detection mode, especially fits the need for two major applications: simulated distillation, extending the range of this technique from C_{80} using GC up to C_{140} residues, and group-type separation (saturates, olefins, aromatics, resins, asphaltenes, plus subgroups, etc.) for which the advantages of SFC over LC have been recognized by an ASTM method for the analysis of diesel fuels.

In the pharmaceutical industry, SFC was introduced in the Swiss pharmacopoeia in 1994 and is therefore officially recognized as an appropriate method for the analysis of drug substances and products. While this recognition is slower in Europe and in the USA, it has however recently reached the stage of public inquiry for Europe [5]. In the context of current Good Manufacturing Practice (cGMP) for the pharmaceutical industry, today the method of choice for assay and

Article available at http://analusis.edpsciences.org or http://dx.doi.org/10.1051/analusis:1999270671

impurity analysis during the development and production of drugs is reversed-phase HPLC (RP-HPLC). Nevertheless, Klaus Anton and Christoph Siffrin (Novartis, Basel, Switzerland) emphasize that orthogonal separation techniques are essential in the registration process to show that RP-HPLC gives reliable results. They demonstrate that SFC with UV detection, as a normal-phase separation technique, not only meets this requirements, but is also a suitable tool for drug substance and drug product analysis. They present qualification parameters: selectivity, linearity, precision, limit of detection and limit of quantitation along with methods for their determination as well as specifications in the light of ICH Guidelines for industry.

As a technique for elucidating structure, the success of first GC-MS and now of LC-MS will most probably be followed by that of SFC-MS. Two articles deal with this topic. The ultimate goal of Tim Baker and David Pinkston (Procter & Gamble, Cincinnati, OH, USA) is to investigate the role of SF-CO_2 in nebulization in view of improving sensitivity for the analysis of pharmaceutical compounds with HPLC/MS/MS. They present the results of their preliminary study on SF-CO_2-assisted nebulization in HPLC/electrospray MS. For example, using a 0.5 ml/min flow-rate (85 % aqueous), they observe a seven-fold increase in peak height and area with SF-assistance. Also for enhanced response in SFC with evaporative light-scattering detection (ELSD), as well as with atmospheric-pressure chemical-ionization MS (APCI-MS), Bernard Herbreteau, Arnaud Salvador, Michel Lafosse and Michel Dreux (Université d'Orléans, France) describe an interface, with restrictor inside the column oven, that lead to a signal-to-noise ratio enhanced by a factor of ten to thirty for the analysis of methylated glucose.

For developing safer drugs, chiral separation is an area where SFC has already been widely adopted for analytical and preparative purposes in industrial laboratories. Anne Thienpont and Guy Félix (ENSCPB, Bordeaux, France), together with Joseph Gal and Christine Aeschlimann (University of Colorado, Denver, USA), present the stereochemistry of two azole antifungal drugs, one of which has three chiral centers and is in fact a mixture of two racemates, i.e. four stereoisomers. Using an optical-rotation detector and a column packed with a regioselectively derivatized polysaccharide, they also conclude that SFC allows analyses in at least half the time, while maintaining the resolution obtained by HPLC with the same column.

Last but not least, to cope with the large number of samples generated by combinatorial and medicinal chemists, Keith Coleman (Anachem Ltd., Luton, UK) proposes a generic preparative SFC method. Using a 10-mm bore column, crude sample injections of about 200 µl, and modifier gradient of 7-min cycle time, Keith obtains a success rate estimated as higher than 90 % with peak purity higher than 85 %. Thus he demonstrates that preparative SFC can be an effective means of rapid routine purification that provides high-purity analytes at the tens-of-milligram levels in a concentrated liquid form.

Without being exhaustive, this dossier reflects the diversity and vitality of SFC solutions for industry. It is my pleasure to acknowledge the cooperation and support of the authors as well as that of the editorial staff of *Analusis*.

References

1. Chester, T. L. Chromatography from the mobile-phase perspective, *Anal. Chem News & Features*, **1997**, 165A-169A.
2. McCoy, M. Industry intrigued by CO_2 as solvent, *Chemical & Engineering News*, June 14, **1999**, 11-13.
3. Eckert, C. A.; Teja, A. 5th International Symposium on Supercritical Fluids (ISSF 2000), Atlanta, GE, USA), April 8-12, 2000.
4. H. Engelhardt, 9th International symposium on supercritical-fluid chromatography and extraction, 4th European symposium on supercritical-fluid chromatography and extraction, Munich, Germany, April 13-14, 2000.
5. Pharmeuropa, Supercritical-fluid chromatography, 10, 2, June 1998, 244-247.