

## Erbadays in supercritical fluid chromatography

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## Erbadays in Supercritical Fluid Chromatography

Madonna di Campiglio, Italy, December 13-15, 1988

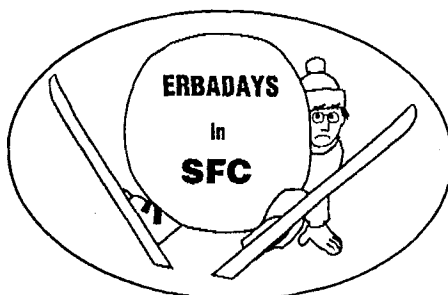
Conference Report by C. Cramers and P. Sandra

Carlo Erba Instruments, supported by its European affiliates and by the Chromatography Group of the Analytical Division of the Italian Chemical Society organized a three-day discussion meeting for European Scientists on Supercritical Fluid Chromatography (SFC). Chairman of the meeting was Dr. S. Trestianu.

The meeting was divided up into 12 serial sessions, dealing with theory, instrumentation, and applications of this revived technique. Attention was paid to hyphenated techniques such as the coupling of SFC to GC, LC, TLC and its combination with FT-IR and MS. Also treated were Supercritical Fluid Extraction, on-line and off-line with CGC and SFC. The implications of using packed, open tubular, and packed capillary columns were intensively discussed.

Present at the meeting were leading members of the European chromatographic community, scientists with relevant experience in the field from industry, and specialists in spectroscopic techniques who are interested in using SFC as a pre-separation technique for FT-IR and MS.

The meeting took place in the Hotel Relais Club des Alpes, providing a superb conference room and excellent accommodation.



Despite competition from the marvellous Dolomites of the famous ski resort of Madonna di Campiglio, all sessions were very well attended. The formula whereby chairmen and discussion starters introduced each subject worked out splendidly. Discussions were so lively that many breaks had to be postponed.

During the different sessions of the meeting, the following comments and opinions were noted.

### **Supercritical Fluid State Equations and Phase Behavior. SFC: Pressure (Density), Temperature, and Composition Programming**

The equations of state, except for pure CO<sub>2</sub>, are not very accurate in describing the relation between pressure, temper-

ature, and density. This is especially the case when modifiers are added. Literature data on phases other than CO<sub>2</sub> and binary mixtures are scarce. Because of the inaccuracy of the equations of state, it was advocated that publications should include data on pressure (-programming), temperature(-programming), modifier(-programming), and if possible on the corresponding densities. Temperature and pressure can be measured relatively easy. Moreover, retention in SFC is a function both of solvation (solubility) and of vapor pressure (volatility). Therefore, for a given supercritical fluid phase, retention must be expressed either by pressure-temperature or by density-temperature. Whereas in the LC-type approach (packed column SFC) retention can often be described by the density alone (low temperatures are used), the situation is different with the GC-type approach (capillary column SFC). Because of the higher temperatures, nowadays up to 200°C, density no longer governs retention. Retention in SFC depends on the stationary phase, on the mobile phase (composition and density, pressure and temperature) and on the temperature. It was felt that the prediction of retention in SFC is not much worse than the description of LC retention behavior.

## The Position of SFC in Separation Sciences and Analytical Chemistry

This discussion session was very lively, and different opinions were advanced. Scientists originating from the GC field investigate SFC either for the analysis of thermolabile compounds and/or high molecular weight compounds not accessible by GC, or to enhance selectivity. LC specialists on the other hand are interested in SFC mainly because of speed of analysis and access to GC detectors. It was striking that chromatography specialists on average were more critical regarding SFC than their spectroscopic colleagues. In general, it was felt that SFC has its own merits in special situations. It was understood that the real challenge to SFC, rather than to perform separations which cannot be achieved by GC or LC is to perform particular applications either faster or more conveniently. Moreover, many scientists from the LC-side stated that method development by SFC is relatively fast. The potential of SFC in combination with hyphenated techniques such as MS, FT-IR, and IP-AES, and in multidimensional chromatography are very promising. The use of supercritical fluids for sample preparation and/or selective extraction was emphasized in the light of fully automated analytical systems such as SFE-CGC-MS, SFE-SFC-MS, etc. . .

### SFC: Packed and Capillary Columns

The present state of the art for fast separations not requiring too many plates (<20,000) favors packed columns with small particle diameters. Capillary columns in this respect will only be competitive when column diameters are reduced to 5-10  $\mu\text{m}$ . This causes numerous instrumental problems yet to be solved. Another advantage of packed columns is the wide variety of commercially available stationary LC phases, contrasting to the limited number of (GC) phases for capillary SFC. This emphasizes the need for new, tailor-made capillary SFC phases.

The effect of modifiers, due to the highly deactivated column wall in capillary SFC, only becomes apparent at high concentrations. In packed columns, on the other hand, the modifier has a much greater effect, mainly due to the deactivation of active silanol sites of the support. Poly-

siloxane-coated silica might diminish the support activity. It was emphasized that, when using appreciable concentrations of modifier, both the initial pressure and temperature have to be increased to maintain supercritical conditions.

Capillary columns, having a high permeability, enable the generation of a large number of theoretical plates. Problems caused by the pressure drop (density!) over the column, *i.e.* the solubility of the analytes along the column, are minor. To enhance the solubility of the analytes in capillary SFC, pressure (density-)programming is mostly applied. To reduce analysis times, columns are operated at ten times the optimum velocity at least. This greatly reduces the number of plates potentially available. When applying density programming, the linear velocity increases. Together with the reduced diffusion coefficients at higher densities, this increases the off-set from optimal conditions even more. The result is a decreased efficiency for later eluting compounds. This problem can theoretically be solved by a programmable restrictor. A

use of GC detectors. The need for pre-detector restrictors, on the other hand, is a disadvantage. A fundamental problem in SFC is the lack of sufficiently polar mobile phases. The use of  $\text{NH}_3$  causes considerable instrumental difficulties. The addition of polar modifiers to  $\text{CO}_2$  helps to solve this problem, especially when using packed columns. A disadvantage of the use of organic modifiers is their response in FT-IR and GC detectors.

Recent advances in mobile phase tailoring include quaternary phases, *e.g.*  $\text{CO}_2$ , water, methanol, butylamine, micelles and reversed micelles and ion pairing additives.

In packed and capillary columns, bare silica is used for group separations. Chiral stationary phases show better selectivity due to the lower separation temperatures in SFC as compared to GC. An important remark concerning packed column SFC was that phases can react with supercritical  $\text{CO}_2$ , *e.g.* aminopropyl silica; other scientists claimed never to have observed this phenomenon.



distinct disadvantage of capillary columns, especially if the diameter is 25 to 50  $\mu\text{m}$ , is their low sample capacity. The use of packed capillary columns was advanced many times, because of their intermediate properties between packed and open tubular columns. The gain in sample capacity, however, is at the cost of accepting an active support material. From a detection point of view, capillary columns in the pressure programming mode offer the advantage of allowing the

### SFC, Instrumental Aspects: Sampling Systems

One of the problems in capillary SFC is still reproducible sampling. It was reported that time splitting with activated valves in this respect offers no advantages over conventional flow splitting. Quantitative repeatability with packed columns is slightly better than with open tubular columns.

Peak focusing in SFC is more difficult to achieve than in capillary GC (solvent effect, stationary phase gradient or cryogenic trapping) or LC (solvent strength). An approach to on-column focusing is to lower the analyte solubility at the column inlet by locally reducing the density. This can be achieved by elevating the temperature and/or by lowering the pressure. For volatile solutes a temperature increase should not be applied. New approaches relate to multi-dimensional techniques: solvent venting or solvent backflush. Both versions pre-separate the analytes from the solvent during sampling.

### Supercritical Fluid Extraction and SFE/SFC, SFE/GC

Supercritical fluid extraction has great potential due to its mild extraction conditions (thermolabile compounds) and the improved mass transfer (thanks to the lower viscosity of a dense gas). SFC can be used to optimize SF extraction. However, one should be aware of the modification of the stationary liquid phase by the supercritical mobile phase. Some other "however's" worth mentioning were also discussed: phase equilibrium measurements are difficult, SFE is strongly dependent on temperature and pressure (high precision is required in order to obtain reproducible results), calculation models are erratic, *etc.*... Moreover, extraction rates (and yields) strongly depend on the particle size and the sample matrix. Modifiers often have to be used in SFE to enhance extraction. Currently, three main approaches to SFE are in use: open loop systems; open loop + consecutive trapping on adsorbents; and closed loop stripping. Off-line SFE systems were discussed. Off-line systems offer more flexibility such as the use of ECD detection in the analysis of pesticide traces (ppb or ppt), PCB's, *etc.*... Problems are encountered due to the poor purity of CO<sub>2</sub>. Very pure CO<sub>2</sub> does not seem to be available in Europe. Preliminary results for the extraction of aqueous samples by supercritical CO<sub>2</sub> were presented. After extraction in an extraction tube, microphase separators are used to accomplish complete phase separation. Several examples of on- and off-line coupling of SFE with GC, SFC, and TLC were presented. In the latter, a modifier was used to improve the trapping efficiency. Preliminary results were shown for a two-dimensional capillary SFC/

capillary SFC system. Selected heartcuts from the first column are trapped on the second column by a slightly higher temperature of the latter.



### SFC, Instrumental Aspects: GC- and LC-Type Detectors

With capillary column SFC, conventional GC-type detectors can be used (FID, NPD, FPD, plasma detectors). In practice, a sensitivity loss is observed compared to GC operation. Obviously, when organic modifiers are used, many advantages of GC detectors are lost. A proposed solution refers to use of very narrow open tubular columns, thus reducing the amount of modifier entering the detector.

In packed column SFC, LC-like detectors are used more commonly (UV, fluorescence, light scattering). Interesting to note is the application of in-column fluorescence, enhancing the sensitivity.

### Hyphenated Techniques: SFC - FT-IR

The main question about SFC - FT-IR is whether to apply off-line or on-line techniques. This implements either off-line removal of the supercritical mobile phase, or the use of a flow cell. The main problem with flow cells is the varying background when applying density programming. Moreover, the spectra as such are influenced by the dielectric constant of the mobile phase, limiting the applicability of vapor phase spectra data bases. Modifiers limit the use of FT-IR because of

the background in specific frequency regions. Xenon appears to be an excellent mobile phase for SFC - FT-IR. Unfortunately, it is very expensive.

The combination of FT-IR with capillary columns requires a compromise between cell volume (spectroscopic requirement) and chromatographic factors (extra-column peak broadening). Packed column SFC is hardly influenced by the volume of the IR-light pipe because of the much higher volumetric flow rates. Off-line removal of CO<sub>2</sub>, *e.g.* by trapping or KBr discs, eliminates many of the aforementioned problems. The trapped spots can be analyzed by IR microscopy.

### Hyphenated Techniques: SFC-MS

Interfaces in use, mainly for packed column SFC, include direct liquid introduction, moving belts, thermospray, and vacuum nebulizer. The advantage of a moving belt system is that clean EI and CI spectra can be obtained. The low temperature needed for CO<sub>2</sub> evaporation makes belt systems eminently suited for thermolabile compounds. In the moving belt, trapping efficiency is superior with modifier-containing mobile phases. The monodispersed aerosol generator interface seems promising for SFC-MS.

For capillary column SFC, restrictor type interfaces are used. Provided the tip of the restrictor is heated very well (480°C was quoted) the coupling offers no difficulties. A disadvantage of the use of CO<sub>2</sub> is that charge-transfer spectra are obtained.

Though similar, these spectra are not completely equivalent to the EI spectra found in large data bases.

### SFC: Industrial Applications

Numerous examples of excellent chromatographic quality were shown. The analysis of polyethylene glycols by SFC and SEC was compared, favoring the SFC approach. Antioxidants, isocyanates, etc. . . seem to be analyzed more easily by SFC than by LC or CGC. SFC is selected in the flavor industries for the analysis of thermolabile sulfur compounds. SFC separations were shown for ionic species, by adding 2-propanol to supercritical CO<sub>2</sub>. The contribution of the modifier is not yet fully understood. Irganox 1425 (a Ca salt) could be analyzed by adding citric acid.

### SFC: Pharmaceutical and Biomedical Applications

The analysis of pharmaceutical formulations by CSFC and packed column SFC has been illustrated. Method development by SFC was experienced to be much faster than by LC. It was also emphasized that derivatization in SFC is of utmost importance. The main objective of derivatization is to enhance the solubility of the analytes in supercritical CO<sub>2</sub>. A typical example is the analysis of phospholipids. The separation of enantiomers by subcritical and supercritical fluid chromatography was discussed, and high enantioselectivity was demonstrated. The importance of the total analytical scheme in monitoring drugs by SFC-MS was also stressed.

### Conclusion

It only remains to say that we gratefully acknowledge the opportunity Carlo Erba created for European scientists to discuss supercritical fluid chromatography in such a magnificent environment. The Italian organization and hospitality was perfect, the cooking too good for weight watchers.

If it is accepted that "*Heaven is where the police are British, cooks French, mechanics German, lovers Italian and everything is organized by the Swiss*", and that "*Hell is where the cooks are British, mechanics French, lovers Swiss, police German and everything is organized by the Italians*" (Dr. Henriquez in his opening address), we are looking forward to returning to hell.

## Letters to the Editors

### Column Size in Analytical Packed Column Liquid Chromatography

Sirs:

Packed column size in liquid chromatography (LC) should be determined by the sample size that has to be handled. In this regard the following facts should be considered for analytical purposes:

— analytical detection (UV, fluorescence, GC, MS) is at its best with amounts of compound in the 1-10 ng range. For a mass-sensitive detector this is quite straightforward. For concentration-sensitive LC detectors the cell volume is somewhere between 30 nl and 8  $\mu$ l (factor of 266). This would imply tremendous sample size differences, far exceeding the 1-10 ng range. However, the cell path length also has to be considered and this varies between 0.1 to 10 mm (factor of 100 in opposite direction). Therefore, for concentration-measuring detectors too, even with greatly different cell volumes, the 1-10 ng range is applicable.

— LC columns can, without showing efficiency loss, handle samples that are 10<sup>5</sup>-10<sup>6</sup> times smaller than their mass of stationary phase. This has been demonstrated many times, both for normal phase and for reversed phase LC in stu-

dies relating sample mass to column efficiency. Sample size affecting efficiency varies of course with the nature of the stationary phase and with that of the sample, but all phases will handle 1  $\mu$ g sample per g of phase easily, and most will indeed not be affected by 10  $\mu$ g/g.

Packed columns for purely analytical purposes should therefore contain between: 1-100 ng  $\times$  10<sup>5</sup> or 0.1-10 mg of stationary phase. At an apparent density of about 0.5 for current silica gel-based stationary phases, this is equivalent to a total column volume of 0.2-20  $\mu$ l. Over a column length of 10 to 30 cm, the most applicable column length, the larger volume implies an i.d. of about 250-320  $\mu$ m. This is the size of current packed fused silica capillary columns, which accordingly seem most appropriate for analytical LC. In practice however, at the present time, analytical LC columns have much larger i.d., in the range of 2-6 mm or about 100 times larger volume. This has much to do with packing and operating problems. It is – or it was – easier to pack the larger i.d. columns efficiently than smaller capillary columns. This is no longer the case. Smaller i.d. columns, with

excellent efficiency, can now be produced routinely and the problems related to column stability have also been overcome. Recent papers on miniaturized packed column LC in this and in other Journals provide ample proof of this. The time seems ripe for instrument manufacturers to do something about the remaining "operating" problem and to devise really appropriate instrumentation.

Micro-LC or on LC packed columns of 250-320  $\mu$ m (made of fused silica or something else?) is probably one of the coming things in LC. There could be many reasons for being attracted to Micro-LC: high efficiency, mass sensitivity, inertness, life expectancy, spent solvent problems, column to column reproducibility, coupling to hyphenated techniques, LC-GC and LC-LC, and still others. One of them could be that it is the most natural size for analytical packed column LC as argued above.

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Gent