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INDUSTRIAL ANALYTICAL APPLICATIONS: SUPERCRITICAL FLUID EXTRACTION

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By the mid-1980s, routine analysis was accomplished in most industrial laboratories with the help of automated sampling and data-handling systems. The next major bottleneck caused by the burden of large sample loads that existed in many industrial environments was sample preparation and the method development time associated with it. Robots and automated systems were introduced to address these bottlenecks. Supercritical fluid extraction (SFE) was one of the automated systems introduced.

The initial introduction of SFE was unique, in that supercritical fluid chromatography (SFC) was developing at a parallel pace. Both introductions into the analytical community produced scientists who needed to learn the nuances of a totally new fluid, its capabilities, limitations, likeness to and difference from both gases and liquids.

SFE was embraced more readily in the industrial community than SFC. The reasons for this, in its utility as well as its developments, are considered in more detail in this overview.

Actual industrial applications of any analytical technology are sometimes well-guarded secrets. References can come from a variety of sources, not necessarily the published literature. Trends in the applications of SFE are no different. The most use has been in the food industry and the environmental area. Additional applications to a lesser extent are in polymer analysis, consumer products and pharmaceuticals.

Beyond the ability to conduct an analysis previously unachievable, the driving force for the adaptation of a technique into the industrial laboratory depends on a financial benefit. SFE has solved some problems that could not previously be solved. But it has mostly gained an industrial foothold by offering worthwhile cost savings over the already acceptable methodology, such as liquid extraction.

Instrumental Developments

In the development of SFE, industrial applications were the driving force to the advancement of commercial instrumentation. Initial offerings of commercial equipment involved cumbersome sample vessels with a myriad of problems. Not uncommonly, these were placed in single-vessel extraction units. At most, the instrumentation was capable of conducting SFC following the supercritical extraction. Speeds of analysis were typically measured by actual extraction time, and not overall sample handling, operator time or productivity. In this early equipment, productivity was not truly improved and industrial acceptance was slow. This equipment was only viable for those extraction methods that could not be conducted by another extraction solvent, i.e. a liquid or a gas. This led SFC, where both liquid and gas chromatography had a strong hold in the separation field, to be labelled as a niche technique. In the sample preparation field, where alternative automated extraction methods with liquids and gases were not as firmly established, the competition was not as fierce for SFE.

Two paths were chosen. Work ensued to optimize the capabilities of supercritical fluids to encompass those compounds that might be more readily extracted by a liquid or a gas as opposed to a supercritical fluid. In addition, to compete with existing technology, albeit even classical liquid extraction, SFE needed to be able to routinely handle a larger volume of samples than was possible with these early instruments.

The first path necessitated a study of the theory and principles of supercritical fluids in terms of their solubilizing powers, matrix interactions, change with modifier or additive additions and their handling. These are generally considered academic endeavours, but much of this occurred in the industrial environment strictly out of need. Simply, it was cost-beneficial to use SFE over the classical technology. SFE was demonstrated to be a factor of 2–5 times cheaper per analysis but, more importantly, it was demonstrated to reduce classical liquid extraction method development time by 3–5 months. Reducing man-months in the process of reaching a product to market is a critical success factor in business. Not only is labour costly, but timing influences the introduction of product to market, the return of capital investiture and the ability to outpace the competition. This tremendous cost benefit resulted in industrial work on understanding some of the theoretical principles which would have otherwise been more slowly developed in academia.

Beyond the theoretical principles, the biggest single improvement, achieved through the second path, was the incorporation of the sample carousel. Easily adapted from the principle of commercial autosamplers, the carousel not only increased the total number of samples that could be analysed, but enabled unattended method development in the most labour-intensive portion of chemical analysis, the extraction step. Replicates of this have already spilled into other technologies, such as pressurized liquid extraction, but SFE was the first to utilize this feature in sample preparation.

A technique such as SFE is unlikely to survive in the industrial environment without the development of commercially available instrumentation. In the late 1980s, the market place had several key manufacturers developing and selling suitable equipment. More than a decade later, some of these have merged, some have reduced their efforts, some have retreated and concentrated on more commercially profitable areas. What remains are some well-designed pieces of instrumentation, directed at specific application areas, as in the fat analysers currently offered by Leco and Isco/Suprex. In addition, there are small suppliers whose instrumentation tends to be less expensive but less amenable to adapting to large routine applications.

The development of SFE is by no means complete, nor can the technology be considered mature. Much of the theoretical questions have returned to academia, progress will be slower, but breakthroughs can still be expected. Much of the development in industrial supercritical fluids is currently in process and larger scale and analytical developments will follow these trends.

Industrial Applications

Successful applications have been readily demonstrated in some fields, specifically with environmental and food samples. Other applications have been more difficult to achieve and reproduce. The following applications outline some of the well-known uses of SFE.

Food Industry

Fat analysis Fats, lipids and oils have been extracted from a wide variety of foods from soybeans to oats, corn chips to brownies and meat to meat by-products and fish. Literature reports optimize the pressure and temperature of the extraction, the length of the extraction, the sample weight, the flow rate and the collection mode. Dynamic and static modes of extraction have also been investigated. Percentages of fat have been removed from sample matrices up to the 35% level; typically, gravimetric analysis follows the extraction procedure at these levels. Commercial manufacturers of foodstuffs are required by law to report percentage of fat on the package label. SFE has revolutionized this methodology. An American Society for Testing and Materials (ASTM) method for determining fat in certain selected foodstuffs has been accepted.

The nonpolar nature of fat, lipids and oils makes them extremely amenable to SFE. Liquid extraction of fat from foodstuffs is conducted using hexane solvent. Supercritical carbon dioxide has equivalent solubility properties, i.e. Hildebrand solubility parameter, to liquid hexane at low densities. Total fat analyses as well as the relationship of different lipid classes in foodstuffs have been determined. **Figure 1** shows the dynamic extraction profiles for the removal of triglycerides from 0.5 g of pork loin. As expected, higher densities give higher extraction efficiencies in shorter time periods. Although a time of 100 min may seem excessively long, compared to the classical techniques the time was orders of magnitude less. **Figure 2** compares SFE technology

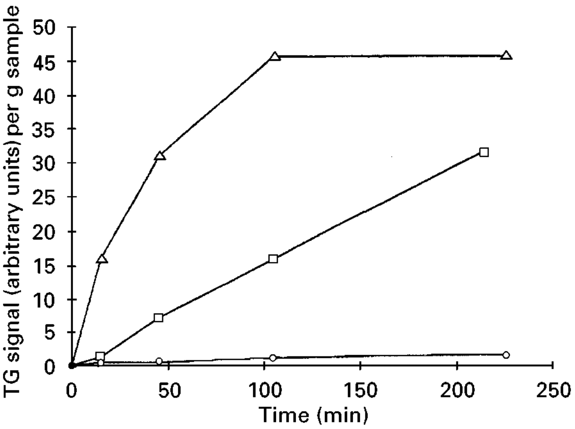

Figure 1 Extraction profiles for different densities. Circles, 105 bar (0.45 g mL^{-1}); squares, 134 bar (0.65 g mL^{-1}); triangles, 370 bar (0.91 g mL^{-1}). Pork loin 0.5 g. Dynamic extraction with pure CO_2. Flow rate: 2 mL min^{-1}, temperature: 50°C, trap: octadecylsilica, trap temperature: 50°C, nozzle temperature: 55°C, rinse solvent: cyclohexane at a flow rate of 1.0 mL min^{-1}, eluted volume: 1.4 mL.

versus the two classical methods for fat analysis: SBR (Schmid, Bondzynski and Rutzlaff) and the popular Bligh & Dyer (B&D) for six different samples. A paired sample *t*-test demonstrated no significant difference at the 95% confidence level for SFE versus the classical methods.

Decaffeination Commercial decaffeination of coffee, tea and cocoa using supercritical carbon dioxide has been the object of huge industrial effort. Patents have been issued since 1974 in this area, to assignees such as General Foods and Société d'Assistance Technique pour Products Nestlé. In Germany, there is a plant able to decaffeinate approximately 27.3 million kg of product per year. There is a substantial effort to make sure the process works and is cost-effective. It is also the first example of a commercial scale supercritical fluid process. Without a doubt, the analytical departments of the commercial operations are examining the effectiveness of supercritical fluids in both analysis and process development, but the work is rarely talked about outside individual commercial enterprises.

Not surprisingly, then, the first example of routine SFE in analysis was in the removal of caffeine from food products. Caffeine, a moderately polar molecule, would not structurally be chosen as likely to have a high solubility in supercritical carbon dioxide. The commercial and analytical scale extraction of caffeine from coffee requires the addition of water as does the extraction of caffeine using methylene chloride. Reports have suggested that there is a chemical bonding of caffeine to the coffee bean or that the moisture instigates a swelling to free the entrapped compound. In either case, this landmark extraction process had opened up the understanding of SFE; overcoming chemical interactions and access to the matrix in total are important features in the investigation of SFE.

Environmental Analysis

Soil analysis Environmental applications of SFE have attracted the largest effort. Soil has received the most attention; it is one of the most difficult matrices and one of the most frequently analysed. Soil types vary widely in both their chemical and physical properties, depending on their geographical origin and their unique history. All extraction methods for soil show wide variability of recoveries and standard deviations of ± 30% are not uncommon. In general, the advent of SFE, which was the first analytical

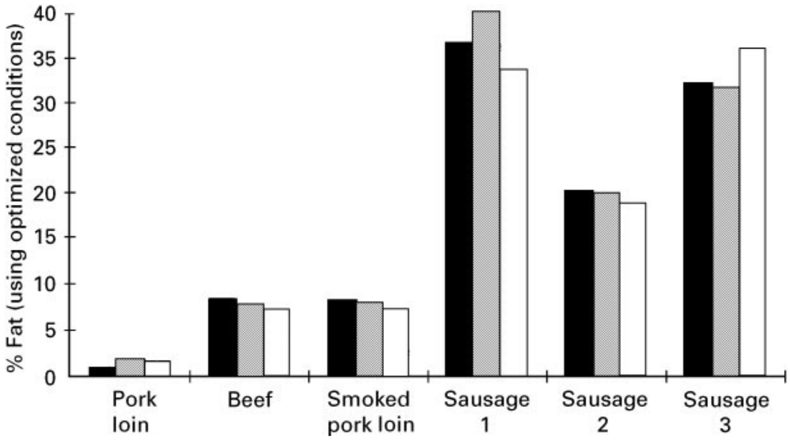

Figure 2 Comparison of total fat determination between SBR (filled columns), SFE (cross-hatched columns) and B&D (open columns). The samples were mixed with Hydromatrix (1 : 2 w/w) and 1 mL cyclohexane was added. Flow rate was 4.0 mL min^{-1}. 8% ethanol was used as modifier; the analytes were collected directly in vials; the rinse solvent pump was operated at 2 mL min^{-1}; other conditions as outlined for Figure 1.

technology for automated soil extraction, reduced this variability to 10–20% within individual soil types. This reduced variability makes differences in extraction from different soils more apparent and gives rise to an understanding of the individual soil parameters controlling extraction.

Analytes of different polarities have been extracted with a wide range of supercritical fluid polarities. Unmodified carbon dioxide showed the best extraction reproducibility, while modified carbon dioxide demonstrated the ability to overcome active sites in the matrix or contribute to desirable matrix swelling. Additives, which show drastic effects in chromatographic retention, also show effects in SFE.

As with other extraction technologies, analytes in spiked samples are easier to recover than are real residues. Bound residues still exist with SFE, but with tighter precision a greater understanding of bound versus available residues has been obtained.

In terms of applications, wide variety of analytes from very nonpolar polychlorinated biphenyls (PCBs) to very polar herbicides have been extracted successfully. Again, optimized conditions show the wide range of polarizabilty that can be obtained with modified carbon dioxide mobile phases. **Table 1** gives a small example of the wide range of compounds that have been extracted from soils and the pertinent conditions. Multi-analyte methods are the norm, and coupled with liquid, gas and SFC, the methods are rugged.

Water analysis Aqueous samples have been extracted using supercritical fluids in several distinct methods. The first method is to place the liquid in the extraction vessel. The supercritical fluid either percolates through the matrix from a bottom entry port to a top exit or, in an opposite fashion, is introduced through the top of the vessel, contacting the sample for a specified period of time before it exits. Sulfonylureas have been extracted from aqueous matrices using a system with a bottom entry and top exit. Flavour components of orange and lemon juice have used the top entry bottom exit format. Alternatively, some workers have passed the liquid either through a filter, pre-coated disc, or a separation cartridge. These trapping materials are then placed separately into extraction cartridges before introduction of the supercritical fluid. Success has been reported with all these techniques for a wide variety of environmental samples.

Agricultural products In this application area, matrices are complex and varied, detection levels are frequently at the parts per billion (p.p.b.) and parts per trillion (p.p.t.) level, and analyte similarity to the matrix is likely. All of these features lead to complex sample preparation and time-consuming method development. Food safety, environmental fate and product stewardship are major concerns for a competitive, consolidating industry. Successful extraction technologies need to demonstrate ease of use on

Table 1 Range of analytes successfully extracted by SFE from soils

Analyte	*Soil*	*Supercritical fluid*	*Temperature*	*Pressure*	*Multi-residue method?*
Total petroleum hydrocarbons (TPHs)	Diesel and gas contaminated	CO_2	80°C	340 atm	Yes
Sulfonylurea herbicides	All types	Methanol and water-modified CO_2	60°C	300 atm	Yes
Ureas	All types	Methanol-modified CO_2	100°C	300 atm	No
Organotin	Marine sediment, clay, topsoil	Methanol-modified CO_2	60°C	450 atm	Yes
Polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs)	Railroad bed, storage dumpster soils and sediment	CO_2	150°C	400 atm	Yes
Nitroaromatic and polycyclic aromatic hydrocarbons (PAHs)	Polluted industrial site and former ammunition plant soils	CO_2 modified with triethylamine and trifluoroacetic acid in toulene	90°C	395 atm	Yes
Nonylphenol polyethoxylates (nonionic surfactants) and carboxylic acid metabolites	Sediment and sewage treatment plant sludge	Water-modified CO_2	80°C	340 atm	Yes

a routine basis, ruggedness, reproducibility, accuracy and commercial availability of equipment. In addition, the current regulatory environment is insistent on advances in multi-residue analysis. These requirements and directives suggest that SFE is an obvious choice.

Herbicide, insecticide and fungicide extraction from soils by SFE has been demonstrated widely; conditions for some of these analytes are listed in Table 1. In addition to the work conducted on soils, plant and raw agricultural commodities have been routinely analysed. Most of the agrochemical classes have been extracted: organophosphates, phthalimides, organochlorines, phenols, carbamates, nitroanilines, oxazolidine, benzimidazoles, triazoles, sulfite esters, pyrethroids, imidazolines and sulfonylureas.

Frequently, sample pretreatment or processing must occur before sample extraction. Although this is time-consuming, this typically also occurs in most classical extraction methodologies. Grains and seeds are ground or milled into powders to expose the internal surface area to the extractant fluid; fruits and vegetables are chopped or diced; straws and hays may be cut or shred; oils may be treated as liquids or deposited on adsorbent surfaces. For some high water content species, drying agents such as Celite 545, Hydromatrix®, magnesium sulfate and molecular sieves have been used to lower the overall water content. They have been added to the extraction vessel before extraction or mixed with the sample during preparation. For very low moisture samples, the opposite sample preparation scheme has been pursued. As in the analysis of coffee beans described above, water has been added to dry samples such as wheat straw to swell the matrix and provide access to interstitial regions.

TCP, 3,5,6-trichloro-2-pyridinal, the major product of chlorpyrifos and chlorpyrifos methyl insecticides and trichlopyr herbicide, has been extracted from soil using SFE followed by detection via immunoassay. Effects of modifiers and additives with this extraction were dramatic, as seen in **Table 2**.

Table 2 Effect of a co-solvent on the recovery of TCP from soil with SC-CO_2[a]

Additive	*Recovery (%)*
None	15.4
Methanol	57.4
Methanol and ion-pairing reagent[b]	80.0

[a] 1 mL min^{-1} CO_2 flow rate; 383 bar; 40°C; 30 min extraction; 1 mL methanol, 0.5 mL ion-pairing reagent.

[b] Ion-pairing reagent: 0.1 mol L^{-1} methanolic solution of R-10 camphorsulfonic acid ammonium salt (Aldrich).

Pharmaceutical Applications

Some of the first reports of the use of analytical scale SFE were in the pharmaceutical industry. Specifically, benzoquinones, known as ubiquinones or co-enzyme Q (with a number which represents the number of side chain isoprenoid groups that are present, e.g. Q-6 has six side chains), were extracted from bacterial cell extracts. They have physical properties analogous to synthetic nonionic detergents, in that there is a polar aromatic end-group and a nonpolar oligomeric side chain. Because of the nature of these molecules, their solubility range is large; they are soluble in aqueous and hydrocarbon solvents. This property makes them easily amenable to extraction with either pure carbon dioxide or carbon dioxide systems.

As an example, an experimental human immunodeficiency virus (HIV) protease inhibitor drug (**Figure 3**), has been extracted from animal feed. **Figure 4** shows the effects of extraction temperature and percent ethanol modifier on recovery, while **Figure 5** illustrates the high performance liquid chromatography (HPLC) chromatograms of the animal feed extracts. These chromatograms illustrate the relatively clean background that can be obtained with SF extractions.

Workers from Agriculture and Agri-Food Canada gave an excellent example of a multi-residue methodology in the extraction of 22 organochlorine pesticides from eggs with a programme to screen routinely for these compounds. **Figure 6** shows recoveries of the pesticides using CO_2 with and without methanol. Both solvents yielded adequate extraction results as well as drastic improvements in extraction time and solvent consumption over the existing Soxhlet extraction method. Background was significantly reduced with pure carbon dioxide.

Literature references show that fat- and water-soluble vitamins have been extracted from pharmaceutical formulations as well as food matrices. Traditionally, fat-soluble vitamin analyses require saponification followed by extraction with an organic

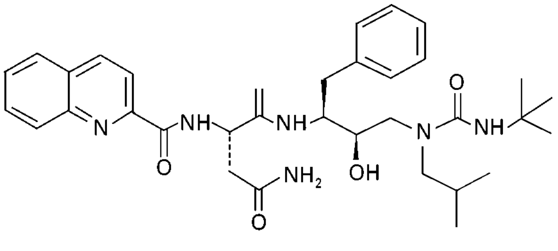

Figure 3 Structure of experimental drug SC-52151 extracted from animal feed using SFE.

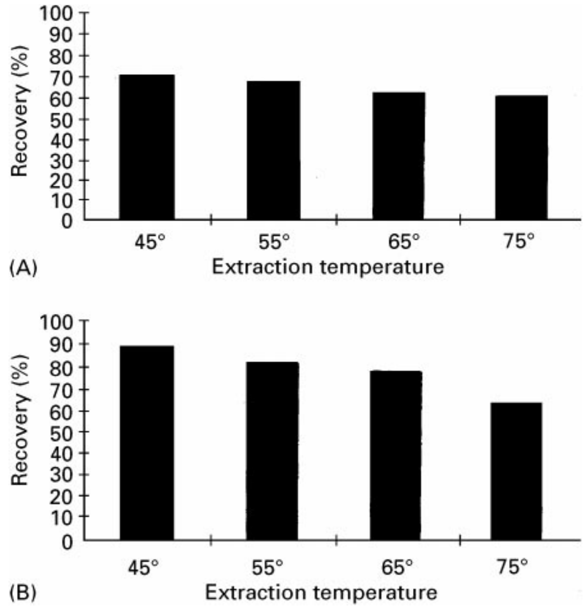

Figure 4 Extraction recoveries for SC-52151 from animal feed spiked at the 0.05% level. (A) 5%; (B) 10% ethanol modifier.

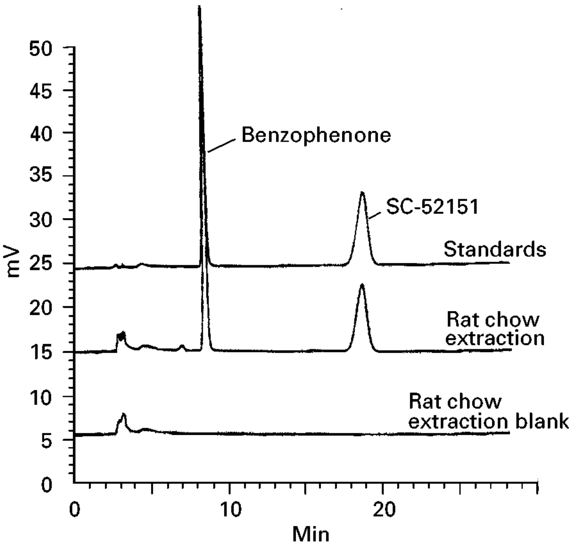

Figure 5 Reversed-phase HPLC traces of SFE animal feed extracts. Chromatographic conditions: column, Supelco C-8 DB (250 × 4.6 mm i.d.; 5 μm particle size); mobile-phase flow rate, 1.0 mL min^{-1}; injection volume, 10 μL, detection wavelength, 205 nm; mobile phase, 0.04 mol L^{-1} sodium pentanesulfonate.

solvent. Solvent reduction and clean-up are generally also needed. SFE eliminates these steps. The water-soluble vitamins are more readily extracted with modified carbon dioxide and minimal clean-up is required. Vitamins A_1, B_6, C, D_2, D_3 and K have all been successfully extracted using SFE.

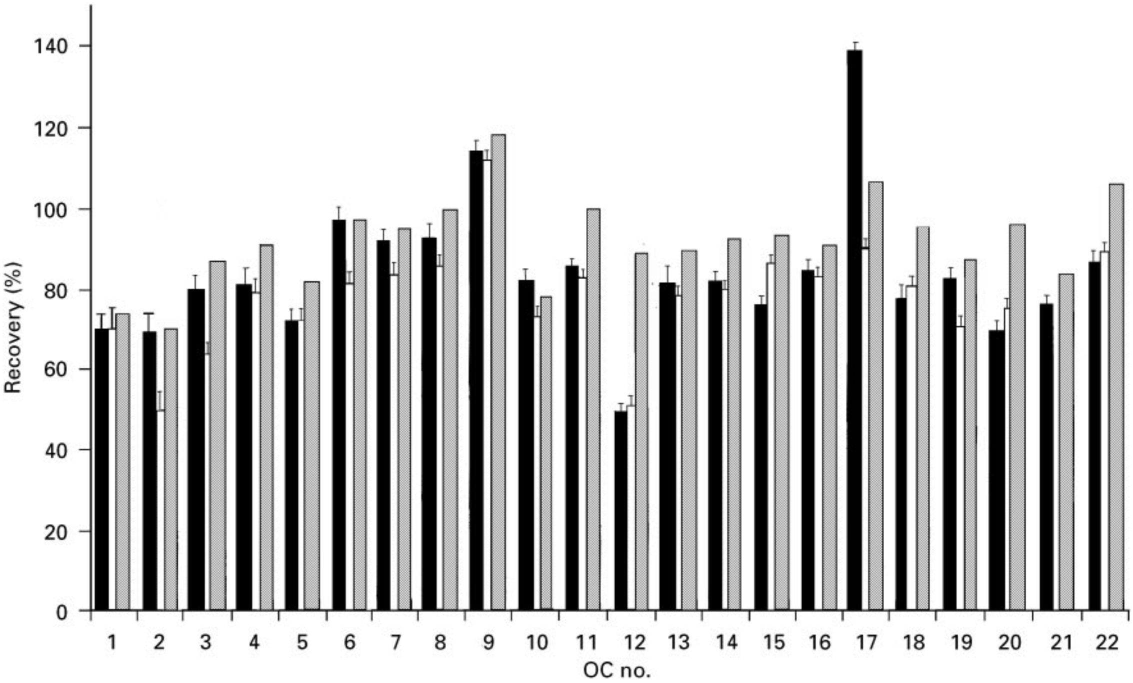

Figure 6 Recoveries of organochlorine pesticides from eggs using SFE with (filled columns) and without methanol (open columns) as modifier. Hatched columns represent spiked blanks. Error bars represent standard deviation of five replicates for methanol-modified extractions at three levels 3, 5 and 10% where concentration showed no significant difference. Organochlorine no. 1, hexachlorobenzene; 2, α-hexachlorobenzene; 3, lindane; 4, heptachlor; 5, aldrin; 6, ronnel; 7, β-hexachlorocyclohexane; 8, chlorpyrifos; 9, dicofol; 10, oxychlordane; 11, heptachlor epoxide; 12, α-endosulfan, 13, *trans*-chlordane; 14, *cis*-chlordane; 15, *p,p′*-DDE, 16, dieldrin; 17, endrin; 18, *o,p′*-DDT; 19, *p,p′*-tetrachlorodiphenylethane; 20, *p,p′* DDT; 21, mirex; 22, methoxychlor.

Herbal medicines have been characterized for industrial marketing in Japan using online SFE/SFC. A typical example is the case for atractylon, a characteristic component of atractylodes rhizome. Rapid oxidation and degradation in UV light make manual methods difficult to execute with acceptable precision. The speed of the analysis by SFE coupled online with SFC make unstable, thermally labile and light-sensitive compounds easier to analyse.

Polymer Applications

The uses of supercritical fluids in industrial polymerization processes are many and varied. They include polymer fibre spinning, polymer-organic solvent-phase separation, fractionation, extraction of low molecular weight oligomers from polymers, high pressure polyethylene polymerization and fractionation and monomer purification. Backbone structure and slight differences in molecular weight can cause changes in polymer solubility properties. These differences are present in commercially prepared polymers and enable fractionation or extraction to be conducted on a large scale.

On the analytical scale, process monitoring of purity, oligomer content, oligomer and antioxidant extraction, additive concentration and removal of impurities have been conducted by SFE. Under supercritical conditions, the polymer matrix has been found to swell, in the same manner as has been described for soils. This has enabled SFE to accomplish analyses that were not previously possible by liquid extraction methods.

The most frequently reported area of SFE applications for polymers is the analysis of additives. Additives influence the physical nature of the polymer and come from a wide variety of classes. They can be low molecular weight and volatile or greater than 1 kDa with solubility in only some liquid solvents. Their analysis generally requires long, tedious multi-step procedures, which may include sample preprocessing, Soxhlet extraction, concentration and clean-up. The low viscosity and high solute diffusivity of supercritical fluids aids in the reduction and, in some cases, the elimination of these steps. The ability to determine the distribution of the additives within the polymeric grid has been demonstrated by several reports along with routine quantitative analysis. Commercial competitive product analysis is standard in industry and SFE has aided in the speed, ease and accuracy of this type of analysis.

The use of supercritical fluids in the polymer industry has opened up an area of knowledge that was previously closed; work continues to be actively conducted.

Additional Industrial Applications

In the fuel industry, stripping organics from minerals and shale rock has been conducted by SFE. Frequently, these methods used classical extraction methods that required several days. Equivalent results have been obtained in rapid extraction times of 15–30 min. Aromatic and aliphatic hydrocarbons (C_{10}–C_{35}), as well as naphthalene and asphaltene, are typical analytes that have been removed.

In the fibre industry, treatments with UV-stabilizers, removal and quantitation of residual dye components, and characterization of coatings, binders and adhesives have been conducted with the aid of SFE. Subsequent quantitative analysis either with SFC, LC, GC or mass spectrometry have all been successfully reported. The suggested procedures appear to be rugged enough to use for routine sample analysis.

Future Developments

The amount of research on SFE in the industrial environment is cyclical. Currently more work is being conducted on a larger scale in the areas of high temperature reactions, heterogeneous catalysis, reaction/separation schemes, antisolvent recrystallization and microencapsulation. However, research at the analytical scale continues. Without a doubt success with larger scale industrial processing leads to process monitoring and analysis. Analysis schemes, of necessity, frequently mimic the larger scale. This is one of the areas where SFE, and most likely supercritical chromatography, will continue to evolve with the pace controlled by need and economics. SFE, though novel in some locations, is no longer a new technology.

Low waste generation in SFE was originally touted as a huge payoff for exploiting the technique. Reduction in waste has been achieved with all automated sample preparation technologies, although the analytical laboratory has not been pushed to reduce solvent waste to the point that large scale processes have. The ability of a supercritical fluid to conduct the extraction of an analyte and leave no organic waste is a feature that can be exploited. The potential that it can be cleaned and recycled with minimum energy input makes it still more desirable. This is an area which could force the acceptance of supercritical technologies, including extraction, where it has been slow to take off.

See also: **II/Extraction:** Supercritical Fluid Extraction.

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INKS: FORENSIC ANALYSIS BY THIN-LAYER (PLANAR) CHROMATOGRAPHY

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Introduction

Because of the immense number of documents written, printed and copied each year, suspect documents generated by these methods have become common subjects for forensic examinations.

The forensic analysis of inks is performed to determine if inks are similar or different; to establish the authenticity of a document; to establish whether a document could have been produced on the purported date; or to determine the origin of a document. Although the chromatographic analysis of writing and stamp pad inks have been extensively documented, little has been written on the analysis of ink jet inks (jet inks) or photocopier/laser printer/LED printer toners, despite the volumes of such evidential documents under investigation.

All inks are composed of colorants in a vehicle. Today's inks include: writing, typewriting, stamp, printing, printer, jet and toner. The consistency of the vehicle ranges from liquids, such as those used in writing and jet inks; to paste, characteristic of some printing inks; to solids, such as those used in typewriting ribbons and toner.

This article only deals with inks whose colorants are separable by thin-layer chromatography (TLC). Such colorants include dyes and pigments that have some solubility in the extraction solvent. Since most printing inks use insoluble pigments as colorants, and therefore are not analysable by TLC, such inks are not considered here. Additionally, the jet inks and toner under consideration are those used in full colour systems (YMCK).

The TLC separation of soluble inks involves six steps: sampling, extraction, spotting, developing, visualization/detection and interpretation.

A TLC method successfully used for the comparison of writing inks has translated well for the analysis of today's modern imaging media – jet ink and toner. Although colour toners are very different from classically defined 'ink', they are made up of separable dyes and TLC has proved useful in their analysis.

TLC is not an identification method unless used for comparison with a complete collection of standards. TLC is used in conjunction with a reference specimen library to match the manufacturer of an ink. These reference collections must contain samples of all inks manufactured throughout the world. Deficiencies in the library weaken the interpretation of a match and increase the number of nonmatches.

The most important criterion in the application of TLC to matching inks with library standard inks is that the inks under investigation and library inks be chromatographed under identical conditions using identical methods. A match becomes an identity only if the match is known to be unique and/or the library is complete.

Described in this article are procedures for analysing inks. The first step in an ink analysis is the identification of the ink type. This is best determined microscopically (**Figures 1–5**). This determination is critical because different chromatographic methods are used for different inks.

Once an ink is identified, the manufacturer may provide crucial information regarding the ink's formulation, earliest possible production date, and distribution.