PRODUCT REVIEW

SFC: Embraced by industry but spurned by academia

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SFC: Embraced by industry but spurned by academia

Supercritical fluid chromatography has established itself in the pharmaceutical and petrochemical industries but has lost ground in academia.

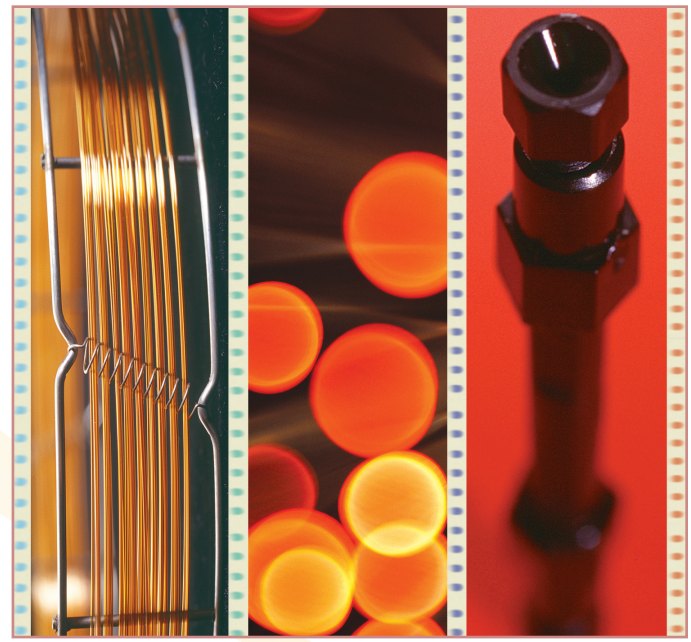
Rajendrani Mukhopadhyay

Supercritical fluid chromatography (SFC) is like a singer who dreams of performing in giant arenas in front of sold-out crowds but can only land small gigs. Since the 1980s, it has made several attempts to get a big break, and when *Analytical Chemistry* last reviewed SFC instruments (**2002**, *74*, 87 A–91 A), the technique was said to be on the verge of a comeback. Six years later, it has yet to share the spotlight with HPLC, but it has been able to gather a small, devoted following in industry.

The lack of attention to SFC, according to experts, can be largely attributed to an ignorance of its capabilities. They say the instrumentation is reasonably mature and the technique works well, especially in the separation of chiral compounds. But SFC struggles to make headlines because it's overshadowed by HPLC. "If you look at articles on a website or in a magazine that talk about methods development or analysis, the articles, by definition, are assuming it's HPLC," says Todd Palcic of Thar Instruments. "A website like separationsNOW has a tab for GC, a tab for IC, a tab for HPLC, but there isn't a tab for SFC. It doesn't have a home."

However, the technique has attracted the pharmaceutical industry. "In the area of pharmaceutical analysis, SFC has probably the best foundation it's ever had," says Larry Taylor of Virginia Tech. "One reason for that is the vendors have really responded to the needs of the pharmaceutical industry." Today, Palcic adds, "If you look at the top 10 pharmaceutical companies in revenue—certainly the top 5—every one of them has between 8 and 50 [SFC] units."

SFC instruments come in two forms. Analytical-scale instruments have flow rates ≥20 mL/min. Preparative instruments are for industrial-scale runs and can be further subdivided. Semipreparative instruments have flow rates from 20 to 200 mL/min; larger preparative systems handle "flow rates at liters per minute with columns measured in inches rather than millimeters." says Jim Gath of Jasco, Inc. "If you ever go into a production process where there is an SFC [system] purifying kilogram quantities for inclusion into product, there may be multiple columns that may be as big as a 6 inch i.d."



Only a handful of vendors sell SFC systems, but one major change will make the future of SFC interesting to follow. In the late 1990s, Berger Instruments had 95% of the global market for these instruments. It was bought out by Mettler-Toledo Autochem in 2000. Meanwhile, a company called Thar Technologies introduced its first analytical SFC product in 2004—until then, the company had only preparative instruments in its product line—and enjoyed brisk sales.

On January 1, 2007, Thar Instruments was spun out of Thar Technologies and acquired the Berger Instruments unit out of Mettler-Toledo Autochem. Palcic explains the rationale for the spin-off and acquisition: "It was clear that without one company with a significant market share exclusively dedicated to SFC, customers would continue to see the technology as a garage company technology." The hope is that the spin-off and extension of Thar Instruments' lineup with the Berger products, all sold under the TharSFC brand, will make SFC a serious contender in the chromatography market.

Table 1 lists examples of systems for analytical-scale SFC; Table 2 lists preparative-scale SFC instruments. Note that the tables are meant to be representative, not comprehensive; vendors may offer similar products not included here.

SFC and HPLC

SFC will always be compared with HPLC. "SFC is essentially analogous to normal-phase LC," explains Daniel Armstrong at the University of Texas Arlington. "If you can do the nor-

Table 1. Selected analytical SFC instruments.[1]

Company	Jasco, Inc. 410-822-1220 www.jascoinc.com	TharSFC 412-435-0200 www.tharsfc.com	Selerity Technologies 801-978-2295 http://selerity.com
Product	Jasco Analytical SFC/SFE	SFC Method Station II	Series 4000
Cost (U.S.D.)	55,000–110,000, depending on configuration	64,000–121,000	70,000
Maximum total flow rate (mL/min)	20	15	Pressure-controlled; flow rate is not measured
Maximum operating pressure (bar)	500	400	400
Cosolvent modifier flow rate (mL/min)	≥10	≥10	None
Maximum column size (mm)	10	20	1.0
Maximum temperature (°C)	60	90	200
Other features	Circular dichroism screening and extraction are available; performs standard HPLC	A 10-column analytical 2-Prep Oven with pull-out drawer design and two-valve configuration is included; fraction collection available; autosampler permits two sample trays; software options include Chromeleon, ChemStation, and ChromScope	Uses a high-pressure syringe pump; accommodates capillary columns; specifically tailored for the petrochemical industry

[1]Some companies may offer similar products not listed here. Contact the vendors for their full product lines.

mal-phase LC separation, you could probably do that same separation, or something analogous to it, by SFC. You can't replace reversed-phase [HPLC] with it."

Two differences distinguish SFC from HPLC systems. Because supercritical CO_2 is the mobile phase in SFC, a pump is necessary for the liquid CO_2. This means the system has to have a chilled head. (SFC also uses a second pump, which is the same as an LC pump.) The other difference is that the whole system has to be under pressure to keep the CO_2 in a liquid state until it reaches the UV detector.

SFC has two virtues that HPLC cannot claim. First, the pumping rates are high, because supercritical CO_2 has low viscosity. The high pumping rate saves time. "SFC is like the Formula One of chromatography," says Eric Lang of Novasep, Inc. "You can have very fast separations."

The second advantage is that the compound of interest can be isolated in a relatively small amount of solvent because CO_2 vaporizes away. This is particularly important for preparative applications, in which the volumes can be large. "If those two [advantages] are important to you, which they are in a lot of applications, then it's very useful to go to SFC," says Armstrong.

Garnering attention in industry

The pharmaceutical industry has latched onto SFC because it's "very amenable to preparative separations—large-scale injections, grams per minute, kilograms per day," says Taylor. In particular, pharmaceutical companies use SFC when they require very high purity preparative separations, particularly for chiral compounds. "The pharmaceutical business seems to have gone toward the direction where the synthesis of new drugs must be performed on the pure enantiomeric material, rather than the racemic mixture," he adds.

In addition, Palcic says, "We've seen significant growth in high-purity achiral purifications, where [SFC is] starting to bite into the flash- or normal-phase HPLC preparative market quite a bit."

SFC has its origins in the petrochemical industry and continues to hold its place there. As the Clean Air Act takes more precedence in the U.S., the technique will become more important, says Jody Clark of Selerity Technologies. California has mandated that SFC be the method used for determining air quality, and Clark expects other states to follow suit. "Europe uses an LC method [to test air quality], and there is also a GC method, which is much more complex. SFC is very simple, which is why it won out in California," she says.

However, there are limitations to what SFC can accomplish in industry. Preparative-scale chromatography can handle only so much in quantity. "I would say for amounts >100 kg, it's better to use another technique, like continuous chromatography or HPLC, which are more efficient in terms of operating costs, thanks to solvent recycling," says Lang. "SFC is a very good method for early development, from a couple of milligrams to 100 kg. After that, it's not the best method to use. . . . We generally suggest our customers use SFC in early development and then switch to continuous chromatography or HPLC for much more efficient and productive large-scale separations."

Costs are also an issue at the large preparative scale. "It's pretty easy to [run] an HPLC system with a column containing 1 kg of packing material," says Christopher Welch of Merck Research Laboratories. "But if you're talking about an SFC system with a column that contains 1 kg of packing material, you've got a pretty complicated, expensive piece of equipment because of the pressure situation. The cost of the two pieces of equipment is not comparable as you go to a larger scale."

But at the small preparative scale, Welch explains, the costs are pretty comparable, and there, SFC can beat out HPLC. "You can buy a piece of equipment at around the same cost

of HPLC, but it works a lot better," he says.

Faltering in academia

Academic acceptance of SFC was hurt by some early missteps. In the early 1980s, SFC was touted as a way for scientists who performed GC to expand their range of samples. With supercritical fluids, the thinking went, researchers now had a mobile phase with some solvating power. Instead of looking at 8–9% of the compounds in the world by GC, researchers could increase the number of compounds analyzed by using SFC.

But the early instrumentation involved open-tubular columns, which were riddled with problems, and the challenges soon led to disappointment and disillusionment. In the 1990s, Terry Berger, who spun Berger Instruments out of Agilent Technologies (at that time, Hewlett–Packard), pushed the use of LC-type packed columns, and SFC experienced a rebirth. However, problems persisted. The early instruments "weren't as hardy as HPLC" ones, says Armstrong. But "they have gotten better. They still aren't quite as hardy, but they are a lot better than they used to be."

Another reason for SFC stalling in academia is that many of those scientists are accustomed to HPLC and are loath to invest the time and energy to switch. "I still marvel that people do things by LC which today are much easier done by SFC," says Taylor. "I think the inertia to use new techniques is extremely high in analytical chemistry. Methods were developed using reversed-phase chromatography—those methods were good and continue to be used—but most people really don't want to have to develop a new methodology using supercritical fluids."

The inertia holds because SFC's advantage over HPLC isn't earth-shattering. "In SFC, the primary advantage you are going to find is speed," says J. David Pinkston of Procter & Gamble. The improvement in speed is "usually a factor of 3–5. It's not a factor of 10 or 100." He explains that the speed factor might be useful for scientists who deal with thousands or tens of thousands of samples. But for most academic scientists, a factor of 3–5 in increased speed doesn't justify the switch to SFC.

And with SFC, resources can be a problem. "I would have an analytical instrument except for one reason—you need a house source of CO_2," says Armstrong. "You can go through a tank of CO_2 in 2 or 3 days of heavy use."

In the earlier days, SFC instrumentation required high-quality CO_2, and that made many researchers balk at the idea of using the technique. However, manufacturers have worked around the problem, and now "the 'McDonalds'-grade CO_2 is your best choice," says Palcic. "It's very inexpensive. It's already going into your soft drinks. Whether you are in Indonesia, India, or Indiana, you're going to have access to soft-drink-quality CO_2."

For a long time, the range of samples in SFC was limited to relatively nonpolar substances, but that seems to have changed in the past 5 years. Taylor and Pinkston explain that compounds such as large peptides can be separated by SFC. "The range of applicability is getting closer and closer to what I'd say is the range of applicability of reversed-phase HPLC," says Pinkston.

Despite advances in the instrumentation, interest in SFC in academic circles has waned. "That's the big disappointment, as far as I'm concerned, as an academician," says Taylor. "I don't think I'm exaggerating when I say [there are] probably not more than four or five academic laboratories doing methods development or research in SFC." Although supercritical fluids are popular with polymer scientists because different morphologies and materials can be made with them, Taylor says that "for analytical purposes, SFC at the academic level—I don't want to say died—is certainly not very well developed."

The lack of academic interest has its consequences. "We're not training many students in the area, so they will have to

Table 2. Selected preparative SFC instruments.[1]

Company	Jasco, Inc. 410-822-1220 www.jascoinc.com	Novasep, Inc. +33-3-83-49-70-00 www.novasep.com	TharSFC 412-435-0200 www.tharsfc.com
Product	Jasco Prep SFC/SFE	Supersep 30-50	SFC Prep 80 (benchtop)
Cost (U.S.D.)	150,000–250,000, depending on configuration	Contact vendor for quote	179,500
Maximum total flow rate	180 mL/min	350 mL/min	80 g/min (mass flow)
Maximum operating pressure (bar)	350	300	400
Cosolvent modifier flow rate (mL/min)	60	100	35
Maximum column size (mm)	30	50	30
Maximum temperature (°C)	60	60	90
Other features	Extraction available; has 8-fraction collection capability	Patented CO_2 recycling system reduces eluent consumption; Supersep Max, a proprietary dual-column technology, is available as an option to boost the productivity of difficult binary separations; highly customizable; flexible CO_2 supply (gas or liquid)	Takes up only 1 m^2 of bench space but provides the capacity of a much larger LC system; engineered to be simple, fast, and efficient; syringe-pump injection and ≥12 high-pressure cyclone separators provide precision, purity, and ease of use

[1]Some companies may offer similar products not listed here. Contact the vendors for their full product lines.

be trained when they reach their industrial appointment. It means SFC in terms of the fundamentals—developing things for the sake of new knowledge—that's probably not going to happen," says Taylor. "Federal agencies are not going to fund SFC work."

Good to be green?

Given that SFC relies mostly on CO_2 with only modest amounts of organic solvent for its mobile phase, one would think the technique would be the first to get on the "environmentally friendly" bandwagon. But it turns out the "green" part isn't SFC's primary selling point. Being green, the experts say, is the icing on the cake but not the cake itself. "It all comes down to dollars, which means speed and the ease of collecting product," states Armstrong. "The fact that it can be considered to be somewhat green is fine, and certainly not a bad thing, but that's not why most people are going for it. It's speed."

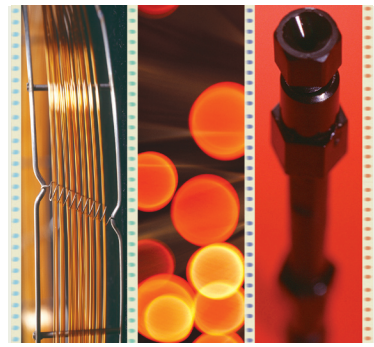
But vendors say the green factor is worth pushing as SFC generates very little organic waste compared with HPLC. "In process-scale HPLC, you can recycle your solvents. But at a small preparative scale, you don't do it," says Lang. "So at the small scale, if HPLC is the most versatile technology, SFC is the quickest and greenest chromatography technique."

But the consequences of the waste can be immaterial to the researchers. "Folks in the lab generating the waste, say from [an] HPLC system, don't really know what it costs to dispose [of] the waste. The disposal cost comes from a different pot [of money] they often don't see. They know how much it costs to buy the solvent, but to get rid of it, they just put it in a drum or container and somebody comes by and picks it up. They don't have to worry about it," explains Pinkston. "If waste costs became a bigger part of the equation, then a system like a SFC system might be more important."

Vendors are optimistic that other factors will underscore SFC's green quality and sway users in their favor. "In the U.S.—and Europe has already moved in this direction—people are switching from hexane to *n*-heptane. Pharmaceutical companies will have to buy [a] heck of a lot more expensive solvents, because heptane is two to four times more expensive," points out Palcic. "The oil prices are also affecting the hydrocarbon solvent prices. It's going way up."

In contrast, CO_2 is inexpensive. Gath notes that CO_2 is a major waste product in many industrial processes. Gas companies come in to recapture the released CO_2, which is then sold to other industrial users.

And vendors are confident that industry will want to become more politically correct and accept SFC on the basis of its environmental friendliness. Palcic cites a recent case: "A group of scientists put in a request for six instruments, and the only one that got approved was called 'a green chromatograph'. . . . It was the only one that got funded, because it was green."



Current SFC instrumentation needs a makeover. The instrument should be miniaturized and combined with other separation methods.

Makeover needed

Experts say SFC has established itself as the preferred way of doing chiral analysis on both analytical and preparative scales, and it will continue to hold strong in that arena. They also say SFC will become the norm for small-scale purifications, and there's a chance it will be used for high-throughput pharmaceutical purifications. "That's a significant market," says Welch. "Tens of thousands of molecules are made that are all purified with reversed-phase chromatography, right now, in a high-throughput, automated mode. SFC is making some pretty good inroads to displace that."

But change is expected to be slow. "I don't see a dramatic revolution or an explosion. I see a gradual evolution," says Pinkston. "I just think it will be a slow progression in pharma as it catches on and moves into other companies interested in saving a dollar here and a dollar there."

Experts say that the fundamentals of SFC need revisiting. "The current instrumentation hasn't been modified very much in 20 years. It's really ripe for a tune-up," says Welch. With SFC, an analysis might take just 30 seconds. "But then your autosampler takes minutes to queue up the next sample. There's no technological reason why that has to be the case. It's just ancient technology," states Welch. "A redesign of the instrumentation is really [required] at this time to maximize the speed advantage of SFC."

New partnerships should be forged. "I think multidimensional chromatography is certainly a place some effort can be had," says Taylor. "SFC can be coupled rather nicely with LC and GC. LC is probably a more important interface, because one of the difficulties in SFC is [that] the mobile phase doesn't have extremely high solvating power."

The other instrumentation makeover Welch wants to see is miniaturization. By downsizing the columns and reducing the amounts of solvents needed, Welch points out, SFC can become even greener. Miniaturization, in turn, can lead to multiplexing of several columns. Welch says, "There's an opportunity for somebody who can do that."

Rajendrani Mukhopadhyay is a senior associate editor of Analytical Chemistry.