by Herbert J. Hedberg and Thomas Ricci

A Novel Centrifugal Fraction Collector for SFC

Introduced in the 1980s as an orthogonal normal-phase method to HPLC, supercritical fluid chromatography (SFC) has demonstrated a number of benefits to the analytical and preparative chromatographer. Because SFC employs green solvents, such as liquid carbon dioxide, the technique greatly reduces the use of hazardous and toxic organic solvents and is gaining more awareness as an environmentally friendly alternative to HPLC.[1–4]

In many applications, SFC demonstrates additional advantages over conventional HPLC separations. Because supercritical fluids possess high diffusivities, the technique often offers enhanced separation speed and resolving power over HPLC—in some applications, by as much as an order of magnitude. Additionally, SFC systems can reequilibrate faster than HPLC systems and therefore can be ready to process other samples in a shorter time frame.

Despite these inherent advantages, SFC has yet to gain widespread acceptance as the separation method of choice, particularly in chemical and pharmaceutical laboratories, where large numbers of high-purity compounds are desired. While HPLC provides a convenient mechanism to isolate and collect sample fractions in an efficient, though costly manner, SFC systems have been limited by their restrictive fraction collection capability. SFC fraction collectors are typically expensive and bulky, requiring valuable space inside a fumehood, and are capable of collecting only a limited number of fractions per separation. Furthermore, SFC fraction collectors do not operate at atmospheric pressure, making the hardware requirements and collection mechanism more complex.

The fraction collector described in this article is designed specifically for SFC,

Figure 1 The CFC-2 fraction collector enables collection of up to 24 fractions while operating at atmospheric pressure, eliminating the need for pressurized equipment and complex methodology required for conventional SFC fraction collectors. The fraction collector can be connected to any SFC system.

delivering HPLC-like convenience to SFC isolation and purification. Based on novel centrifugal technology, the CFC-2 fraction collector (**Modular SFC**, North Attleboro, MA) overcomes the complexities and limitations of conventional SFC fraction collection and allows chromatographers to migrate existing HPLC methods and develop new achiral applications to take advantage of the benefits of SFC chemistries.

SFC fraction collection with HPLC-like convenience

The CFC-2 fraction collector (*Figure 1*) is capable of collecting up to 24 samples (27 mL of modifier solvent per fraction) in the same 20 mm × 150 mm glass tubes used in a conventional HPLC fraction collector. The instrument employs patent-pending centrifugal technology that captures nonvolatile materials entrained in the eluent stream from any SFC system with better than 90% recovery. Fractions are collected at atmospheric pressure, eliminating the complexity and pressurized vessel requirements of typical SFC fraction collectors. The

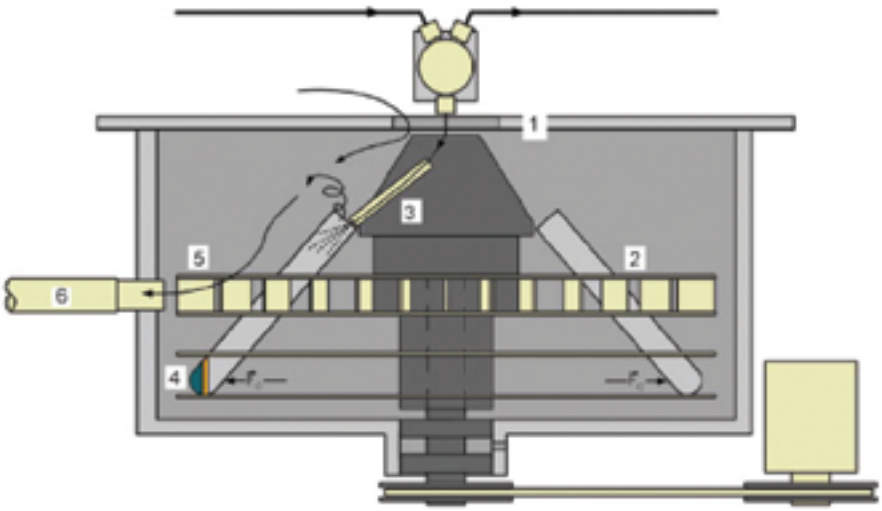

Figure 2 CFC-2 fraction collector enclosure (1) operates at atmospheric pressure and prevents volatile vapors from escaping. A rotor (2), with a capacity of 24 fraction collection tubes, spins at 1500 rpm. A flexible eluent tube (3) directs the flow from the SFC system into each fraction collection tube while the rotor is spinning. Liquids and solids are trapped in the bottom of the collection containers (4) due to the centrifugal force created by the rotor. Volatile gases are expelled from the collection tube and blown by fan blades in the rotor (5) out a flexible exhaust hose (6). The hose is connected to the laboratory venting system, eliminating the need to place the system beneath a fumehood.

compact instrument (20 in. wide × 9 in. high × 26 in. deep) can be placed on a laboratory bench, outside of a hood, because all vapors are directed through an exhaust hose to the nearest laboratory vent system.

Novel centrifugal technology

The fraction collector uses centrifugal force to perform a density separation (effectively separating the gas phase from liquids and solids) upon the spray from the eluent tube. The technology incorporates a rotor, containing up to 24 fraction collection tubes, that spins at 1500 rpm (concentrator speed). The sample collection tubes are standard, off-the-shelf glass containers, which eliminate the need to use pressurized steel collection containers or a pressurized cassette having glass collection containers required by typical SFC fraction collectors (*Figure 2*).

A diverter valve directs the eluent flow from the backpressure regulator of the SFC instrument to either a waste container or to the eluent dispensing tube entering the fraction collector. To prevent cross-contamination among collection tubes in the rotor, the custom valve has the ability to stop the eluent flow for the quarter second that the distributor mechanism is advancing the eluent tube between containers while the rotor continues to spin. This momentary stop flow condition is possible because most of the volume in the tubing to the SFC instrument is compressible CO_2 gas.

The flexible eluent tube fixed to the valve's collect outlet directs the flow from the SFC system into one sample collection container while the rotor is spinning. The eluent tube extends into the fraction vessel and dispenses eluent having volatile and nonvolatile compounds, even solid precipitates that form as the nonpolar CO_2 becomes gaseous and the remaining organic modifier becomes too polar to keep the sample solubilized.

As the eluent spray contacts the wall of the spinning container, the centrifugal$_1$ force causes the highest-density components (liquids and solids) to accumulate in the bottom of the collection container. The CO_2 gas, being the least dense part of the eluent, spills out of the fraction container opening into the rotor housing. Centrifugal fan blades between the rotor disks blow volatile vapors through an exhaust hose to the nearest laboratory vent facility, eliminating the need to locate the fraction collector inside of a fumehood.

To maximize nonvolatile compound yield, the eluent tube is inserted some distance into each fraction container during collection. When the distributor mechanism executes a "next fraction" command, a retraction mechanism withdraws the eluent tube from the current container before advancing to the next container. After the new container is reached, the eluent tube is extended into the container before the diverter valve resumes the eluent flow (*Figure 3*).

Because the rotor spins the fraction containers to generate the required centrifugal force to capture the nonvolatile components from the eluent, collection of consecutive fractions into adjacent fraction tubes would eventually imbalance the rotor. To maintain equal weight distribution during the collection process, the instrument is designed to collect consecutive fractions in containers that are 165° apart from each other on approximately opposite sides of the rotor.

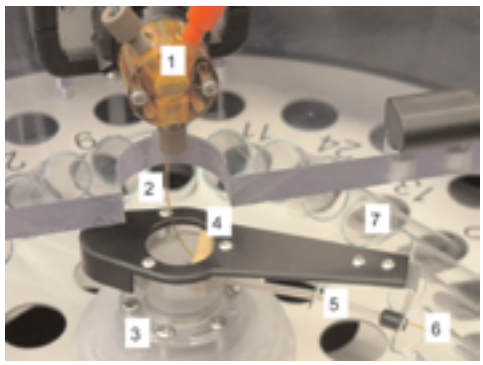

Figure 3 Eluent distribution system: 1) diverter valve for "waste," "collect," and "stop flow" (while switching fractions); 2) fixed end of flexible eluent tube connected to "collect" outlet of valve; 3) indexing mechanism for redirection of eluent tube during fraction change; 4) mechanism to withdraw eluent tube from containers during fraction change; 5) PTFE guide channel supports and directs rotating eluent tube; 6) rotating end of eluent tube inserted into collection container; and 7) one of 24 collection containers in fraction collector rotor.

"Waste/collect" and "next fraction" controls are enabled by contact closure outputs on the SFC instrument. The CFC-2 system is also internally programmable to operate these functions by time or by detecting peak height thresholds generated by an analog output from the SFC system's detector. A chart mark contact closure is available to document when transitions are made between collection containers. Future versions will allow mass-directed fractionation capability.

The question is often asked, "Doesn't centripetal, not centrifugal, force capture the nonvolatile compounds in the collection containers?" The answer is that it all depends on the observer's frame of reference. When taking a hard right while driving, physics describes the car applying a "real" centripetal force to the driver to make the driver change direction. Alternatively, the apparent feeling that the driver's shoulder is pushing outwards on the car door is the "fictional" centrifugal force.

The bottom line is that 500 years of instrument-naming convention has resulted in calling the laboratory equipment that performs density separations by spinning containers a centrifuge. Therefore, centrifugal fraction collector is what everyone would expect the CFC-2 instrument to be called. Any other name would be a distraction.

Enabling SFC applications

The CFC-2 fraction collector allows collection of a large number of fractions during a broad polarity separation by SFC.[5–7] The convenience and capabilities afforded by multiple fraction collections simplify the migration of traditional HPLC applications to SFC for complex mixtures requiring many fractions or multiple samples requiring only a few fractions each. Examples follow.

1. *High-purity chiral separations*. Chiral compounds are a common artifact in medicinal and other synthetic chemistry. The retention tuning attributes of SFC drive its application to these particularly difficult purifications of chiral compounds—both intermediates and final products—for a wide variety of chemotypes. Typically, chemists have barely enough fraction capacity to collect the two enantiomers following the SFC separation. Ideally, three fraction cuts should be made of each chiral peak. With the high fraction counts of the CFC-2 fraction collector, chiral chemists can now use SFC as a high-throughput purification tool. For example, in a purification of four enantiomeric mixtures, the instrument enables collection of six fractions each before taking time to replace a new set of test tubes, thus speeding the medicinal chemistry process.
2. *Isolation of impurities from reaction products*. During drug development, analytical procedures are required for the detection and quantization of degradation and reaction products. Analytical methods are designed to reveal impurities and degradation compounds in the drug product. The resolution of SFC has proven useful in isolating these impurities, and the instrument's ability to conveniently collect up to 24 fractions increases the productivity of SFC in this fraction-intensive area.
3. *Fractionation of natural products and other complex mixtures*. In working with natural products and other complex mixtures, preparative SFC has demonstrated superior speed and sample loading for the prescreening purification of drug-like molecules. By greatly reducing the variety of compounds in a potential screening sample and perhaps even beginning to accumulate some characteristic data about individual fractions, correlations among hit samples become more apparent earlier in the screening campaign. The CFC-2 brings the convenience and familiarity of HPLC fractionation to the attractive productivity gains and economies offered by SFC.
4. *Isolation of metabolites and biomarkers from biological fluids*. The emerging fields of metabonomics and metabolomics are rapidly gaining widespread use in the pharmaceutical, health-care, and agrochemical industries. Metabonomics enables the rapid identification of endogenous metabolites and biomarkers in various biofluids as a result of a disease state, toxicity, genetic modification, or environmental factors. SFC has demonstrated better separation efficiency and less solvent consumption than conventional approaches, but has not gained widespread adoption due to the limitations of fraction collection. The CFC-2 overcomes these prior limitations of low numbers of fractions and time-consuming collection container washing protocols designed to prevent cross-contamination.
5. *Flash SFC*. Flash liquid chromatography (Flash LC) has proven to be a useful technique for the rapid separation and cleanup of excess reactants and reaction products in synthetic organic chemistry, particularly in medicinal chemistry departments of the pharmaceutical industry. Due to the limitations and complexity of collecting fractions with SFC, the concept of a quick and effective cleanup tool using supercritical CO_2 has not been feasible. However, with the CFC-2 fraction collector, Flash SFC becomes a viable concept, offering inherent speed, resolution, and loading capabilities, in addition to greatly reduced organic solvent consumption and virtually dry fractions immediately following the Flash SFC cleanup step.

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