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A Review on Supercritical Fluid Chromatography

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ABSTRACT
The assay of chlorzoxazone (CHL), paracetamol (PCM), and aceclofenac (ACE) in their combination solid dose forms has been established utilising packed-column technology.chromatography using supercritical fluid (SFC). Analytes were clarifiedvia elution with 15% v/v supercritical doped carbon dioxidean ACE 5 Phenyl column with methanol as the modifier (150 34.6 mm, 5 mm). At 215 nm, a detector was used to detect the substance.ultraviolet detector the mobile phase's densities and polaritiesbenefited from pressure, temperature, and modifier effects to be optimized concentration in respect to chromatographic variables like retentiontime, resolution, asymmetry, retention factor, and theoretical plates. It was discovered that the most efficient method foraltering both selectivity and retention. According to the requirements of the International Conference on Harmonization, the developed approach was validated. Comparing the created SFC technique to a published method of high-performance liquid chromatography for estimating Utilizing the Student t-test, compare CHL, PCM, and ACE. In relation to the SFC was found to be superior in terms of speed and the use of organic solvents eco-friendly, too. The newly created SFC approach worked well. for the testing of various commercially available CHL formulations, PCM and ACE both separately and together.

I. CHROMATOGRAPHY

- Chromatography is a laboratory technique for the separation of a mixture into its components.
- The mixture is dissolved in a fluid solvent (gas or liquid) called the mobile phase, which carries it through a system (a column, a capillary tube, a plate, or a sheet) on which a material called the stationary phase is fixed. Because the different constituents of the mixture tend to have different affinities for the stationary phase and are retained for different lengths of time depending on their interactions with its surface sites, the constituents travel at different apparent velocities in the mobile fluid, causing them to separate.
- The separation is based on the differential partitioning between the mobile and the stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus affect the separation.
- The purpose of preparative chromatography is to separate the components of a mixture for later use, and is thus a form of purification.
- This process is associated with higher costs due to its mode of production.
- Analytical chromatography is done normally with smaller amounts of material and is for establishing the presence or measuring the relative proportions of analytes in a mixture. The two types are not mutually exclusive.

Chromatography terms

- **Analyte** – the substance to be separated during chromatography. It is also normally what is needed from the mixture.
- **Analytical chromatography** – the use of chromatography to determine the existence and possibly also the concentration of analyte(s) in a sample.
- **Chromatogram** – the visual output of the chromatograph. In the case of an optimal separation, different peaks or patterns on the chromatogram correspond to different components of the separated mixture. Plotted on the x-axis is the retention time and plotted on the y-axis a signal (for example obtained by a spectrophotometer, mass spectrometer or a variety of other detectors) corresponding to the response created by the analytes exiting

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the system. In the case of an optimal system the signal is proportional to the concentration of the specific analyte separated.

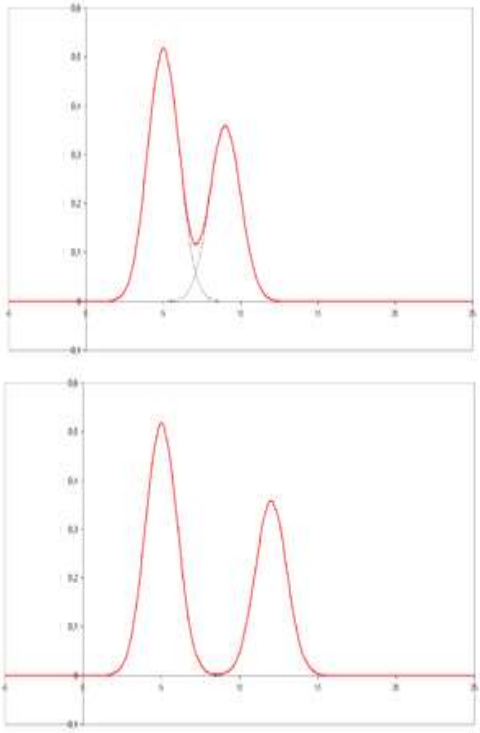

Figure 1l chromatography graph

- **Eluent** (sometimes spelled eluant) – the solvent or solvent fixure used in elution chromatography and is synonymous with mobile phase.
- **Eluate** – the mixture of solute (see Eluite) and solvent (see Eluent) exiting the column.
- **Effluent** – the stream flowing out of a chromatographic column. In practise, it is used synonymously with eluate, but the term more precisely refers to the stream independent of separation taking place.
- **Eluite** – a more precise term for solute or analyte. It is a sample component leaving the chromatographic column.
- **Eluotropic series** – a list of solvents ranked according to their eluting power.
- **Mobile phase** – the phase that moves in a definite direction. It may be a liquid (LC and capillary electrochromatography (CEC)), a gas (GC), or a supercritical fluid (supercritical-fluid chromatography, SFC). The mobile phase consists of the sample being separated/analyzed and the solvent that moves the sample through the column. In the case of HPLC the mobile phase consists of a non-polar solvent(s) such as hexane in normal phase or a polar solvent such as methanol in reverse phase chromatography and the sample being separated. The mobile phase moves through the chromatography column (the stationary phase) where the sample interacts with the stationary phase and is separated.
- **Preparative chromatography** – the use of chromatography to purify sufficient quantities of a substance for further use, rather than analysis.
- **Retention time** – the characteristic time it takes for a particular analyte to pass through the system (from the column inlet to the detector) under set conditions.
- **Sample** – the matter analyzed in chromatography. It may consist of a single component or it may be a mixture of components. When the sample is treated in the course of an analysis, the phase or the phases containing the analytes of interest is/are referred to as the sample whereas everything out of interest separated from the sample before or in the course of the analysis is referred to as waste.
- **Solute** – the sample components in partition chromatography.
- **Solvent** – any substance capable of solubilizing another substance, and especially the liquid mobile phase in liquid chromatography.
- **Stationary phase** – the substance fixed in place for the chromatography procedure. Examples include the silica layer in thin-layer chromatography
- **Detector** – the instrument used for qualitative and quantitative detection of analytes after separation.
- Chromatography is based on the concept of partition coefficient. Any solute partitions between two immiscible solvents. When we make one solvent immobile (by adsorption on a solid support matrix) and another mobile it results in most common applications of chromatography. If the matrix support, or stationary phase, is polar (eg. paper, silica etc.) it is forward phase chromatography, and if it is non-polar (C-18) it is reverse phase.

SFC

- A supercritical fluid, such as carbon dioxide, is used as the mobile phase in supercritical fluid chromatography (SFC), a type of normal phase chromatography. [1][2] It can be used to separate chiral compounds and is utilised for the

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analysis and purification of low to intermediate molecular weight, thermally labile molecules.

- The phase of a substance at its critical temperature and pressure is known as a supercritical fluid. Critical temperature is the temperature below which a gas cannot liquefy in the absence of additional pressure, and critical pressure is the minimal pressure at which a gas can liquefy.

	Gas	**Supercritical fluid**	**Liquid**
Density (g/cm^3)	0.6×10^{-3}-2.0×10^{-3}	0.2-0.5	0.6-2.0
Diffusivity (cm^2/s)	0.1-0.4	10^{-3}-10^{-4}	0.2×10^{-5}-2.0×10^{-5}
Viscosity (cm/s)	1×10^{-4}-3×10^{-4}	1×10^{-4}-3×10^{-4}	0.2×10^{-2}-3.0×10^{-2}

- A dynamic equilibrium leads to the creation of a supercritical fluid. A dynamic equilibrium is produced when a substance is heated to its particular critical temperature in a closed system under constant pressure. In this equilibrium, the same amount of molecules gain energy and lose energy as they transition from the liquid phase to the gas phase and back again. The phase curve between the liquid and gas phases vanishes at this precise location, and supercritical material is revealed.
- A straightforward phase diagram can be utilised to better comprehend the definition of SF. An ideal phase diagram can be seen in Figure. A phase diagram for a pure substance displays the ranges of temperature and pressure at which the substance can exist as a solid, liquid, or gas. The borders of the phase areas are defined by curves where two phases (solid-gas, solid-liquid, and liquid-gas) coexist. For the solid-gas, solid-liquid, and liquid-gas boundaries, respectively, these curves comprise sublimation, melting, and vaporisation. In addition to these binary existence curves, the triple point is a location where all three phases are simultaneously present and in equilibrium (TP).

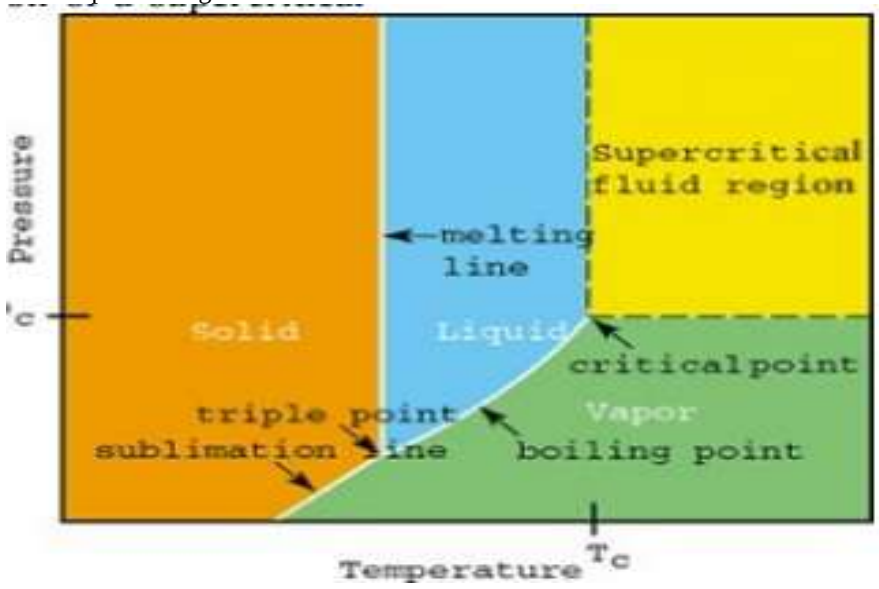

Figure 3 graph of triple point

- The critical point, another distinguishing feature in the phase diagram (CP). At critical pressure (Pc) and critical temperature (Tc), this point is reached (Pc).

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- Theoretically, there are two ways to enter the supercritical region: first, by raising the pressure above the material's Pc value while maintaining a constant temperature, and second, by raising the temperature above the Tc value while maintaining a constant pressure value.
- Increasing the temperature above Tc first, followed by the pressure above Pc.
- As a result of the specific Tc and Pc values for each substance, the critical point is unique to each material.

Supercritical Fluid Physical Characteristics

- As was already mentioned, SF has several characteristics in common with both gases and liquids. This enables us to benefit from a suitable pairing of the features.

Viscosity

- A supercritical fluid's viscosity is roughly 1/10 of that of a liquid, making it essentially identical to that of a gas. Supercritical fluids are hence less resistive to components moving through than liquids. The viscosity of supercritical fluids differs from that of liquids in that temperature has a much greater impact on the viscosity of supercritical fluids than on liquids.

Viscosity, diffusivity, and density all have a connection to one another. They can all be impacted by changes in temperature and pressure in a variety of ways.
For instance, raising pressure causes viscosity to grow, and rising viscosity causes diffusivity to fall.

Chromatography in super fluids (SFC)

- Similar to how supercritical fluids combine the advantages of liquids and gases, SFC combines the benefits and key features of HPLC and GC. When analysing substances that break down at high temperatures with GC and lack functional groups that can be recognised by HPLC detection systems, SFC may be more useful than HPLC and GC.

Column chromatographies have the following three key characteristics:
• Selectivity.
• Efficiency.
• Sensitivity.

- Due to variable mobile phases (particularly within a specific experimental run) and a variety of stationary phases, HPLC typically provides superior selectivity than SFC.
- SFC has good quality in terms of sensitivity and efficiency even if it lacks the selectivity of HPLC. During the chromatographic process, SFC makes it possible to adjust a few characteristics. The analysis can be optimised thanks to this adjusting capability. Additionally, SFC offers a wider variety of detectors than HPLC. SFC outperforms GC for the examination of chemicals that decompose readily; these materials can be employed with SFC because it can operate at lower temperatures than GC.

SFC instrumentation

1.Stationaryphase
2.Mobile phase
3.Pumps
4.Injectors
5.Ovens
6.Detectors

1.Stationary Phase

- The strong solvating abilities of mobile phase in SFC makes the careful selection of stationary phases imperative. Basically, two types of analytical columns are used in SFC, packed and capillary. Earlier work employed absorbents such as alumna, silica or polystyrene or stationary phases insoluble in SC -CO_2. More recent packed column work has involved bonded nonextractable stationary phases such as octadecylsilyl (C_{18}) or aminopropyl bonded silica. SFC columns are pretty similar to HPLC columns in terms of coating materials. there are two types of columns used in sfc.

•Open tubular columns
•packed columns

- Open tubular Ones are preferred more and they have similarities to HPLC fused-silica columns. This type of columns contains internal coating of cross-linked siloxane material as stationary phase. The thickness of that coatings can be 0,05-1 µ m. The length of that columns can be in the range of 10-20 m. Packed Columns Similar to HPLC columns (10, 5, or 3 µm porous particles) Silica based chemically bonded phases Typically 10 cm long X 4.6 mm.

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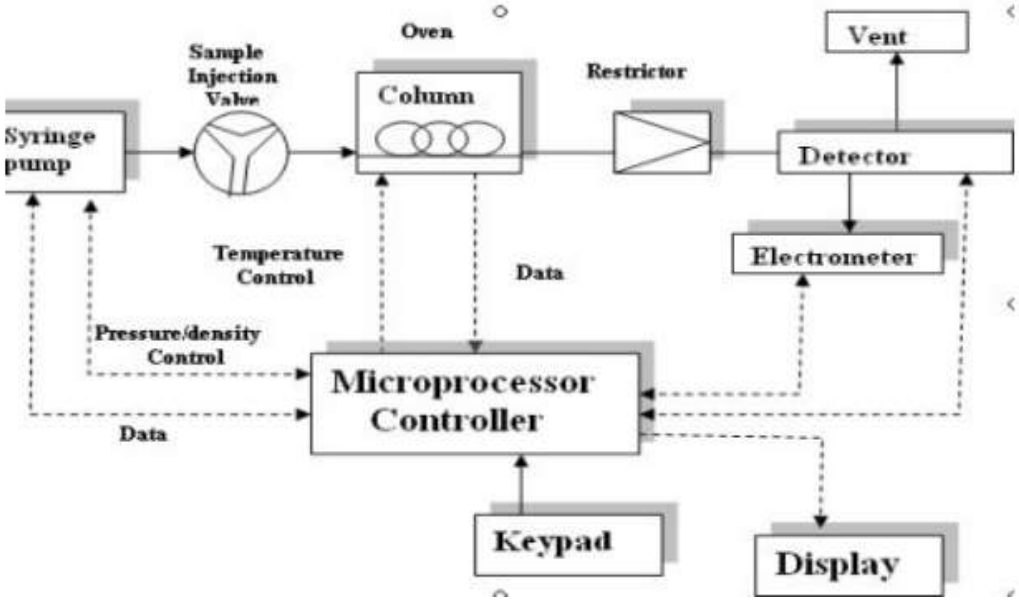

Figure 4 instrumentation

2.Mobile Phases

- The mobile phase is composed primarily of super critical carbon dioxide, but since CO_2 on its own is too non-polar to effectively elute many analytes, co-solvents are added to modify the mobile phase polarity. Co-solvents are typically simple alcohols like methanol, ethanol or isopropyl alcohol. Other solvents such as acetonitrile, chloroform or ethyl acetate can be used as modifiers.

Solvent	**Critical Temperature (°C)**	**Critical Pressure (bar)**
Carbon dioxide (CO_2)	31.1	72
Nitrous oxide (N_2O)	36.5	70.6
Ammonia (NH_3)	132.5	109.8
Ethane (C_2H_6)	32.3	47.6
n-Butane (C_4H_{10})	152	70.6
Diethyl ether (Et_2O)	193.6	63.8
Tetrahydrofuran (THF, C_4H_8O)	267	50.5
Dichlorodifluoromethane (CCl_2F_2)	111.7	109.8

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3.Pumps

- Here mainly flow control is necessary so syringe pumps are used for capillary SFC for consistent pressure and for packed columns for easier blending of the mobile phase or introduction of modifier fluids reciprocating pumps are used.

4.Injectors

- In capillary SFC small sample should be quickly injected into the column and so pneumatically driven valves are used. For packed SFC a typical injectionvalve is commonly used.

5.Ovens

- Ovens Conventional GAS chromatography & liquid chromatography ovens are used.

6.Columns

- Two types of analytical columns are used in SFC ie. packed and capillary. Packed columns contain small deactivated substances to which the stationary phase adheres. These are conventionally stainless steel. Capillary columns are open tubular columns made of fused silica which have small internal diameter.

7.Detectors

- Flame ionization detectors and flame photometry detector, liquid-phase detectors like refractive index detector, ultraviolet-visible spectro - photometric detectors and light scattering detectors have been employed for SFC.

Advantages

- Due to the decreased viscosity of supercritical fluids, which make SFC a faster procedure than HPLC, the SFC technique can combine the best features of GC and HPLC. This is made possible by the physical qualities of supercritical fluids, which exist between liquids and gases. Low viscosity promotes rapid flow of the mobile phase.
- SFC can be used to investigate some delicate materials that are susceptible to high temperatures because of the critical pressure of supercritical fluids. These substances could be molecules that break down at high temperatures or substances with low vapour pressure or volatility, including polymers and big biological molecules. High pressure situations give the opportunity to work at lower temperatures than are often required. Therefore, SFC analysis of the temperature-sensitive components is possible.

DISADVANTAGES:

- SFC is pressure operating conditions. High-pressure vessels are expensive and bulky. Maintaining pressure in SFC is difficult. supercritical fluids are highly compressible and their physical properties change with pressure. Cleaning will be time consuming.

Types of Chromatography

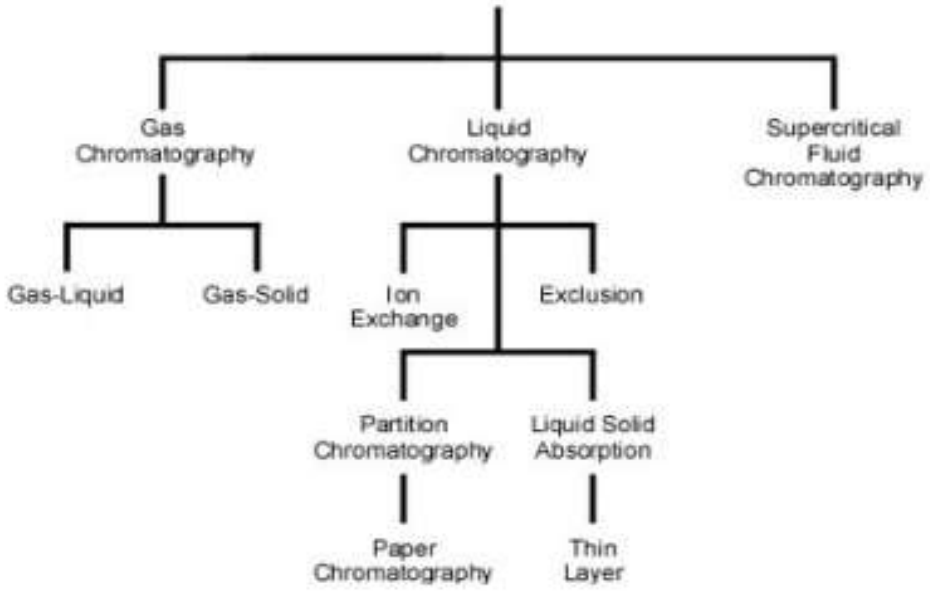

Figure 2 type of chromatography

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Applications of Chromatography

- The technique of chromatography is extensively employed in the pharmaceutical industry in order to analyze and identify the presence of any trace amounts of chemicals and elements in a given sample.
- Another vital application of chromatography in this sector is in the separation of certain chemical compounds based on their molecular masses (and sometimes on the basis of the elements that constitute them).
- The technique of chromatography also plays a crucial role in the development of new drugs. For example, the presence of impurities and other unknown compounds can be detected in the drug sample with the help of chromatography. Furthermore, the purity of the drug sample can also be analyzed with the help of this technique.
- Chromatography plays a vital role in the chemical industry for the testing of water samples for purity.
- The testing of air samples for their purity is also accomplished by chromatographic techniques in the chemical industry.
- The presence of toxic contaminants in oils and pesticides (the most notable of which being polychlorinated biphenyls, often abbreviated to PCBs) can be determined with the help of specialized chromatographic techniques such as GC and HPLC.
- It can also be noted that chromatography also has many applications in the life sciences.
- In the field of molecular biology, the study of proteomics and metabolomics often involve the use of various hyphenated chromatographic techniques (the most notable of which being EC-LC-MS).
- Nucleic acid research is also known to make extensive use of such chromatographic techniques.
- A specific type of chromatography known as HPLC is widely used in protein separation applications. This type of chromatography is also useful in enzyme purification, plasma fractionation, and insulin purification.

Applications of SFC

- SFC has a variety of uses in the food, environmental, and pharmaceutical industries. In this way, classes of substances such as pesticides, herbicides, polymers, explosives, and fossil fuels can all be studied.
- Antibiotics, prostaglandins, steroids, taxol, vitamins, barbiturates, non-steroidal anti-inflammatory drugs, and many more pharmacological components can all be analysed using SFC.
- Numerous medicinal substances can be separated chirally.
- SFC is employed in the petroleum sector.
- For the separations hydrocarbon
- Determination of total aromatic content analysis.
- Antioxidant such as polyphenols, carotenoids and tocopherols (vitamin E), which are found in a wide variety of fruits and vegetables (beta carotene in carrots, lycopene in tomato...) can be selectively extracted using supercritical CO_2. In addition, the textures of the extracts obtained (thyme, rosemary, lavender, chamomile...) are free from traces of organic solvents.
- Among the spectrum of the extracts obtained from plants, diterpenes (antioxidants), triterpenes (phytosterols), or even the tetraterpenes (carotenes) which may be of interest to the pharmaceutical sector, can be easily extracted. Supercritical fluids may also be utilised for the production of fine powders, in particular for the formulation of active principles. Pierre Fabre laboratories (France) which have developed the production of fine powders by supercritical CO_2, were awarded the Pierre Potier 2009 "Innovation in chemistry for sustainable development" for its Formulplex ® process.
- This process uses supercritcal fluids to increase the bioavailability of active principles. Very recently, Critical Pharmaceuticles (United Kingdom) offers biocompatible and biodegradable polymers (synthesized using environment friendly supercritical CO_2) for medical and pharmaceutical applications.

II. CONCLUSION

- In overall ranking of chromatographic techniques, it can be judges that SFC falls somewhere between HPLC and GC.
- In field of pharmaceutical chemistry and bioanalytical applications SFC gained its applications.
- The current work shows that the established SFC approach could be used to analyse all commercially available formulations that contained CHL, PCM, and ACE in both solo and combination forms. dosage styles. The time-consuming mobile phase setup Selectivity can obviate the requirement for HPLC. be optimised by adjusting variables such as temperature, pressure, wavelength of detection and

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modifier concentration. an SFC is an environmentally favourable method because the mobile phase utilised is dioxide of carbon. the capacity to separate objects more quickly to investigate thermolabile materials without the use of organic solvents and nonpolar molecules, which distinguish SFC from HPLC. respectively, GC.Due to its relative affordability and speed, the SFC approach could be employed as a substitute method for analysing pharmaceutical dosage forms and bulk pharmaceuticals.and less expensive than traditional HPLC techniques.

III. SUMMARY

- SFC has been shown to be a replacement technology for normal phase chiral HPLC, with vastly superior speed, safety, equally wide applicability, and significant savings in solvent cost. At the semiprep level, the small fraction sizes significantly reduce the dry-down time and cost. The non-flammable nature of the mobile phase means that many jobs that were outsourced in the past can now be performed in a normal laboratory environment.
- SFC has been growing rapidly, particularly in drug discovery in the pharmaceutical industry. However, many researchers in the field remain unfamiliar with the technology or its advantages. Surprisingly, the vast majority of chiral separations and purifications continue to be performed using very expensive, flammable organic solvents, producing slow, inefficient separations, with much larger than necessary fractions, in an environment-unfriendly manner. The lack of significant penetration of SFC into these and related fields may be due to the lack of academic training in the field. Few universities have SFC equipment, probably due to the historically higher entry cost to practice SFC versus HPLC. Another factor may have been the historically poor sensitivity of analytical-scale SFC, which prevented its use in validated trace analysis. These limitations have now been overcome. The future of SFC seems very bright, since it appears to solve many of the limitations of HPLC and fulfill many of the separation requirements moving forward.

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