Review Article

Supercritical Fluid Technology- An Unlimited Frontier in Herbal Research

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ABSTRACT: Supercritical fluid technology is a process using a supercritical fluid as a solvent. When a fluid is taken above its critical temperature (Tc) and critical pressure (Pc), it exists in a condition called the supercritical fluid state. The physico-chemical properties of a fluid in the supercritical state are in between those of a typical gas and liquid. Due to increasingly stringent environmental regulations, supercritical fluid technology (SFT) has gained wide acceptance in recent years as an alternative to conventional solvent extraction for herbal compounds as well as in many analytical and industrial processes. This article presents the practical aspects of SFT applications in selection of modifiers, on-line offline coupling techniques. SFT can also be used to clean up pesticides from herbal medicines. Carbon dioxide is most commonly used Supercritical Fluid (SF) as it is safe, inexpensive, non-toxic, non-inflammable and inert to most of the materials as well as its solvating strength is adjusted by modifier (methanol). SFT processes can be modeled to acquire useful information for better understanding of the extraction, mechanisms and optimization of the extraction procedures. In recent years, Supercritical Fluid Extraction (SFE) has emerged as a highly promising technology for production of herbal medicines and nutraceuticals with high potency of active ingredients.

Introduction

Medicinal plant extracts are widely used in pharmaceutical and cosmetic products (Samuelsson G. et al., 1992). Supercritical fluid extraction (SFE) is widely perceived as a technique for the extraction of low to moderately polar compounds. With increasing concern over the use of chemical solvents in the manufacture of pharmaceuticals, as well as the need for high quality products, alternatives to energy intensive and costly extraction schemes have been sought. Pharmaceutical compounds are usually polar, non-volatile and the matrices may contain co-extractives. The currently used sample preparation techniques for analysis include solid phase extraction, liquid-solid extraction (Soxhlet extraction), and liquid-liquid extraction. Recent studies have shown that the use of supercritical fluids as an extraction media provides a powerful alternative to traditional extraction methods (Westwood et al., 1993).

Supercritical Fluid

Thermodynamic properties and definitions

The thermodynamic state of a pure component (Rosset et al., 1991) is determined by three variables: the pressure P, the volume V, and the temperature T. The relationship between these three variables is known as the state equation and is represented by a surface in the three-dimensional plotting of P, V, and T. Any pure component, following the value of these three parameters, will be either a solid (S), a liquid (L), or a gas (G). Fig. 1 shows the three states of matter. The point C is known as the critical point, and corresponds to a critical temperature T_C, a critical pressure P_C, and a critical density ρ_c.

At the point C exists an intermediate state between a liquid and a gas called a critical state, or, if sufficiently removed from C, a supercritical state. This state is obtained at temperatures and pressures superior to those of the critical temperature and critical pressure, respectively. If one of these parameters falls under its critical value while the other remains superior to its critical value, we are talking about a subcritical state.

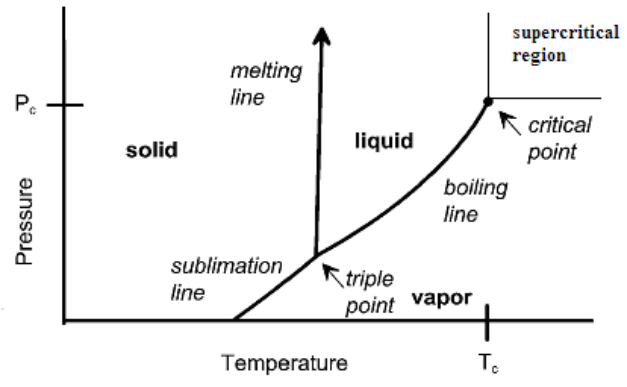

Fig. 1 Schematic phase diagram of a single substance.

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Choice of Supercritical Fluids

A number of SFs are available with a range of values for density and critical temperatures. The choice of the solvent is dictated by the physicochemical properties of the compound of interest and role of solvent in the process in terms of solvency or anti-solvency. Although many SFs are suitable for pharmaceutical applications, the most widely used SF is carbon dioxide (Subramanyam et al., 1997). Other SFs generally used are nitrogen oxide, ammonia, chlorofluroethane, trifluromethane and xenon, etc. Although SFs possess very high solvation power or solute capacity at critical points, their solubility can be further modified with regard to a particular solute by incorporating a co-solvent or a co-solute (Luicen et al., 2000). The co-solvents could be a polar or non polar miscible solvent added in small quantities (1%to 5%) to SF, to modify the polarity and solvent strength of the SF. Some examples of co-solvents used are methanol, ethanol, acetone and dimethyl suphoxide. The mechanisms of solubility enhancement by co-solvent/co-solute can be explained by hydrogen bonding, complex formation, dipole interactions and solvent/cosolvent–solute/co-solute interactions. A modifier may be added to SC-CO_2 in order to improve the solubility of the analytes. Normally, the modifier should be a good solvent for the analytes and ethanol, dichloromethane and chloroform have all been used for the extraction of carotenoids and tocopherols from plants and vegetable matrices. Chiu et al described this co-solvent modified supercritical fluid extraction in detail.

Table 1. Comparison of the critical constants for commonly used supercritical fluids
(Skoog et al. 1998)

Fluid	Critical Temperature (°C)	Critical Pressure (atm)
Carbon dioxide (CO_2)	31.1	72.8
Methane (CH_4)	–82.1	45.8
Ethane (C_2H_6)	32.3	48.2
Propane (C_3H_8)	96.7	41.9
Argon (Ar)	–122.3	48.0
Nitrous oxide (N_2O)	36.5	72.5
Water (H_2O)	374.1	218.3

Basis of supercritical fluid technology

SFs are gases/liquids that are at temperatures and pressures above their critical points. At critical points, SFs possess properties of both liquid and gas, with density values similar to those of liquids, and flow properties similar to those of gases, and are thus labelled as fluids. The density values of SFs enable substantial solvation power, and the diffusivity of solutes is higher because of lower viscosity of solutes in SF, thus facilitating mass transfer. The operating conditions of low temperature and pressure make SFs attractive for pharmaceutical research, especially thermolabile materials. The solubilising power of SFs is sensitive to small changes in operating conditions and, hence, fine-tuning of the pressure and temperature can customise the solvent capacity of SFs suited for a particular application (Kakumanu et al., 2005).

Extraction Kinetic

The rate of extraction is due, firstly, to the solubility of the analyte in the supercritical fluid and, secondly, to the transfer of the mass of the solute outside of the matrix. There are four mechanism of mass transfer:

1. The diffusion of the analyte across the internal volume of the sample
2. The desorption from the surface of the sample,
3. The diffusion of the analyte across the boundary layer on the surface of the sample,
4. The transfer in the core of the supercritical phase.

If the most important determining step of the rate is the diffusion across the internal volume of the sample, the rate of extraction will be dependent upon the size of the particles contained in the sample. Pulverization will consequently increase the rate of extraction. The desorption from the surface of the matrix, as well as the diffusion across the boundary layer on the surface of the sample, can be significantly improved by adding a polar modifier such as Methanol. (King et al., 1992)

Carbon dioxide (CO_2) as a supercritical fluid

Among the fluids used in SFE, carbon dioxide is, by far, the most used for the following reasons:

- Non-flammable, exhibits low toxicity
- Low critical temperature (Tc), and low critical pressure (Pc)
- Relatively inert
- No liability owing to residuals following processing
- Safer mixing of hydrogen and oxygen
- Good solvent strength and miscibility with most solvents
- Non-corrosive, odorless and economical
- Safe and environmental friendly

Carbon dioxide is also a slightly polar fluid; therefore at first sight the extraction of solutes strongly linked to a matrix will be difficult. Adding an organic solvent known as a modifier to a supercritical fluid can act significantly on the strength. For example, to extract more polar compounds, it can be interesting to add an alcohol to carbon dioxide.

Instrumentation

The instrumentation needed to perform a SFT is relatively simple. It should allow for two extraction modes: one dynamic (continuous) and the other static (batch) (Staub et al., 1996; Mohamed et al., 2002; Valle et al., 1999) (Fig: 2 and 3). In the dynamic mode, the extraction vessel is constantly supplied with fresh supercritical fluid, while the recovery container is continually supplied with the solute. In the static mode, the outlet of the extraction vessel is closed and extraction takes place without the regeneration of supercritical fluid. On completion of the extraction, the supercritical fluid rapidly rinses the vessel in order to permit the recuperation of the solute.

There are three ways of recuperating the analyte after depressurization of the supercritical fluid: (1) Thermal trapping, (2) Sorbent trapping (3) Solvent trapping. Thermal trapping is a simple technique because the supercritical fluid is simply depressurized in a cooled recovery container. Unfortunately, this technique is limited to nonvolatile components as high gas flow can lead to the loss of relatively volatile compounds. Even slightly volatile compounds can be led by aerosol formation.

The other two techniques offer increased potential for the quantitative extraction of highly or moderately volatile compounds. Sorbent trapping provides favorable results and is relatively easy to apply. The supercritical fluid is depressurized and then adsorbed on a solid support. Once the supercritical phase extraction has terminated, the analyte is recovered by elution with a small volume (a few milliliters) of solvent liquid. This method has the advantage of being able to increase the selectivity of the extraction by way of the elution solvent. The simplest technique, however, remains that of trapping in a liquid solvent. While this procedure does not offer the selectivity seen in sorbent trapping, it is undoubtedly simpler and quicker.

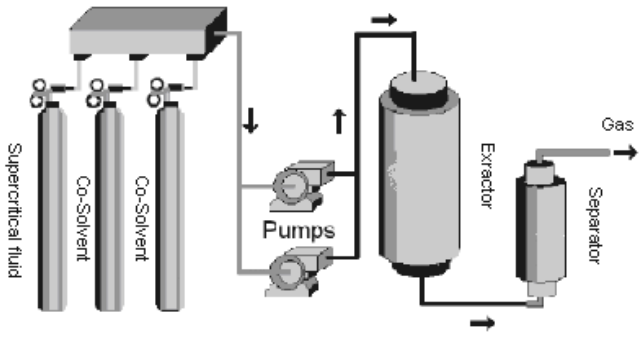

Fig. 2 A schematic diagram of a supercritical fluid batch extraction.

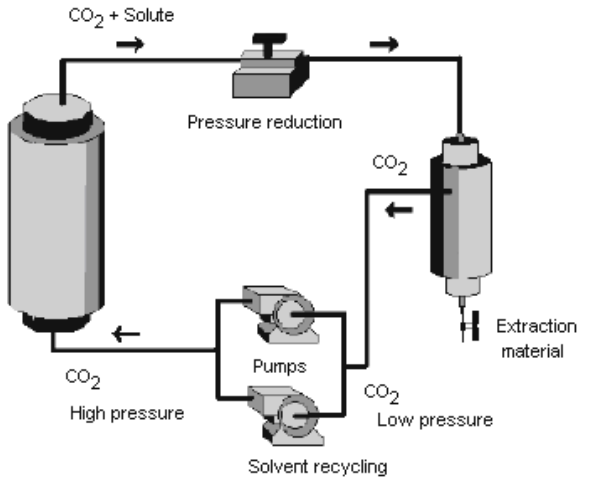

Fig. 3 A schematic diagram of a supercritical fluid continuous extraction.

Online and Offline SFE Techniques

SFE comprises two integrated parts: extraction of the analytes from the sample matrix and subsequent collection (trapping) of the analytes. The collection can be achieved either on-line into a chromatographic instrument such as GC or SFC, or off-line by depressurizing the supercritical fluid (SF) into a collection device. The collection device can be an empty vessel, a vessel containing a small volume of organic solvent, a solid-phase trap, or acryogenically cooled capillary. Hence, there are many possibilities for achieving collection in SFE, and they all have their advantages and disadvantages, as well as different parameters to optimize (Skoog et al., 1998).

Off Line SFE technique:

Off line SFE allows direct collection of the extracted analytes either in a solvent or passing the SCF through a column packed with Chromatography adsorbent or can be collected in a cryogenic vessel for subsequent analysis. Precaution must be taken in collection methods to avoid sample losses. Off line SFE is simpler to perform and after extraction the extract can be analyzed by any appropriate analytical method. In off line SFE loss can occur during collection or during subsequent analysis (Sewram et al., 1998).

On line SFE technique:

It is comprised of extraction and analysis and has been used by many researchers as an analytical tool for the study of medicinal plants. The extracted components are transferred directly from the SFE cell into the GC column via an on-column injector. SFE flame ionization detector (SFE/FID) and mass spectroscopy (SFE/MS) are used as detectors. SFE can be directly coupled with HPLC too with appropriate detector depending upon the type of analysis to be carried out. Online SFE requires an understanding of

SFE and chromatographic conditions and the sample extract is not available for analysis for different analytical methods. However, sample handling between extraction and chromatographic analysis is eliminated by the on line analysis. On line extraction mostly can be coupled with capillary GC (SFE/GC) and both packed and capillary SFE (SFE/SFC) (Sewram et al., 1998).

Advantages Over Other Methods

The traditional methods for the extraction of plant materials include steam distillation and organic solvent extraction using percolation, maceration or Soxhlet techniques (Harborne et al, 1973; Nairn et al., 1990). These procedures, however, have distinct drawbacks such as time-consuming and labour-intensive operations, handling of large volumes of hazardous solvents and extended concentration steps which can result in the loss or degradation of target analytes. Moreover, there is increasing interest for alternative extraction technologies consuming less organic solvents, because of the rising solvent acquisition and disposal costs and regulatory restrictions (Harborne et al., 1973). Supercritical fluids have been shown to exhibit several advantages in the extraction of natural products from plant matrices. The combined liquid-like solvating capabilities and gas like transport properties of supercritical fluids make them particularly suitable for the extraction of diffusion controlled matrices such as plant tissues. Moreover, the solvent strength of a supercritical fluid can be easily tuned by simply changing the applied pressure and/or temperature (Harborne et al., 1973).

Applications of S.F.T.

Applications in Plant material extraction:

- Potential of SFE for extracting drugs, such as quinine, from plant material (Ndiomu et al., 1988).
- SFE of taxanes as anticancer drugs from dried needles of the English yew tree *Taxus baccata* (Heaton et al., 1993).
- Extraction of volatile components in Chinese herbal medicines. Three kinds of herbs, frankincense, myrrh and *Evodia rutaecarpa* were extracted using supercritical CO_2 (Ma et al., 1991).
- Application of SFE to extract diosgenin used as steroid intermediate, following acid hydrolysis from tubers of *Dioscorea nipponica* (Liu et al., 1995).
- Quantitative extraction of taxol and baccatin III from the needles of Taxus *cuspidate* (Chun et al., 1994).
- *Zingiber zerumbet* rhizomes used in Indonesian traditional medicine, were extracted using supercritical CO_2 at 60°C and 200 bar for 120 min (Ahmad et al., 1994).

Applications in chemical analysis

- Analysis of kavalactones from *Piper methysticum* (kava-kava) (Anna et al., 2004).
- Tocopherol measurement in edible products of vegetable origin (Diego 2004).
- Determination of lycopene in food by on-line SFE coupled to HPLC using a single monolithic column for trapping and separation (Jaroslav et al., 2004).
- Chemical analysis of *Ginkgo biloba* leaves and extracts (Teris et al., 2002).
- Separation of rosemary antioxidant compounds by supercritical fluid chromatography on coated packed capillary columns (Pilar et al., 2004).
- Preparative purification of the major anti-inflammatory triterpenoid esters from Marigold (*Calendula officinalis*) (Hamburgera et al., 2003).
- Coupling SFE to uterotonic bioassay: an on-line approach to analyzing medicinal plants (Sewram et al., 1998).

Application in bioassay chemical analysis

Bioassays are a common adjunct to chemical analysis. Traditionally, samples to be used for bioassay have been prepared by solvent extraction, vacuum distillation, membrane processes, lyophilization, etc (Wolfe et al., 1994 and 1992). Although there have been reports of SFE coupled to immunoassay analysis, these studies have been performed using off-line collection of the extracted analytes. Online approaches provide potential for combined sample preparation and analysis and the potential to transfer every extracted analyte molecule to the detection system thereby increasing sensitivity. Methanol has been by far the most commonly used modifier in a wide range of sample matrices.

Table 2. Applications of Supercritical Fluid Technology.

APPLICATIONS
EXTRACTION
Volatile oil from *Eucalyptus camaldulensis var. brevirostris* leaves (Chen et al., 2000)
Extraction of Caffiene from green tea *Camellia sinensis*
Artemisinin, Artemisinic acid from *Artemisia anuua L*
Cocoa butter from *Brazilian cocoa* (Saldana et al., 2000)
Ginkgolide A, B, C, J and bilobalide from Ginkgolide standard extracts Podophyllotoxin from *Dysosma pleiantha* roots
Steviol glycosides from *Stevia rebaudiana*
Hyoscyamine and Scopolamine salts from *Scopolia japonica*
SEPARATION AND REFINING
Fractionation of essential oils
Fractionation of wax
REMOVAL OF PESTICIDES AND HERBICIDES
Organochlorine pesticides from vegetable sample
FOOD TECHNOLOGY
Cholesterol free food products (Mohamed et al., 1998)
Decaffeination of coffee and tea (Saldana et al., 2002)

Conclusion

In view of India's rich botanical and marine resources, SFT has high potential in producing nutraceuticals, foods, flavors, fragrances, cosmetics, and biologically active principles and thereby achieving a significant value addition to its traditional export of raw natural materials. Thus, use of the Supercritical fluid technology can help Indian Herbal industry to gain its share in rapidly growing international market for high quality, value added herbal products.

References

Anna Rita Bilia, Luca Scalise, Maria Camilla Bergonzi, Analysis of kavalactones from *Piper methysticum* (kava-kava) Franco F. Vincieri. *J. Chromatogr.B* **812:** 203-214 (2004).

Chen YT and Ling YC. An overview of supercritical fluid extraction in Chinese herbal medicine: from preparation to analysis. *J. Food Drug Anal.* **8:** 235-247 (2000).

Chiu KL, Cheng YC, Chen JH, Chang CJ, Yang PW. Supercritical fluids extraction of Ginkgo ginkgolides and flavonoids, *J. Supercrit. Fluids* **24:** 77-87 (2002).

Diego JM, Gomez-Coronadoa, Elena Iba nezb F. Javier Rupereza, Coral Barbasa. Tocopherol measurement in edible products of vegetable origin *J. Chromatogr. A.* **1054:** 227-233 (2004).

Harborne JB, Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis Chapman and Hall. London pp: 6 (1973).

Kakumanu VK, Bansal AK. Supercritical fluid technology in pharmaceutical research. Business Briefing: future drug discovery, reference section 1-4 (2005).

King JW, France JE. Basic principles of analytical supercritical fluid extraction. In Analysis with Supercritical Fluids: Extraction and Chromatography B Wenclaviak (Ed.). Springer-Verlag. Berlin 1992: 32-57.

Luicen FP, Foster NR. Solubilities of solid mixtures in supercritical carbondioxide: a review. *J. Sup. Fliuds.* **17:** 111-134 (2000).

Mansoori GA, Suwono A, Sitompul JP, Samudro HM Ratnawati. In: Proceedings of the international Conference on Fluid and thermal energy pp. 573 (1997).

Mohamed RS, Neves GBM, Kieckbusch TG. Reduction in cholesterol and fractionation of butter oil using supercritical CO_2 with adsorption on alumina. *I. J. Food Sci. Tech.* **33:** 445-454, (1998).

Mohamed RS, Mansoori GA. The Use of Supercritical Fluid Extraction Technology in Food Processing. Featured Article - Food Technology Magazine June 2002.

Nairn J G, in: Gennaro A R (Ed.). Remington's Pharmaceutical Sciences 18th edition. Mack. Easton. PA. pp1543 (1990).

Park HS, Choi HK, Lee SJ, Park KW, Choi SG, Kim KH. Effect of mass transfer on the removal of caffeine from green tea by supercritical carbon dioxide. J.Supercrit Fluids **42:** 205-211(2007).

Prokopczyk B, Hoffmann D, Cox JE, Djordevic MV & Brunnemann KD. Supercritical fluid extraction in the determination of tobacco-specific *N*-nitrosamines in smokeless tobacco. *Chem. Res. Toxicol.*, **5:** 336-340(1992).

Rosset R, Caude M, Jardy A. Chromatographies en Phases Liquides et Superctitiques. Masson. Paris. 815-907 (1991).

Saldana MDA, Mohamed RS, Mazzafera P. Supercritical carbon dioxide extraction of methylxanthines from maté tea leaves. *Brazilian J. Chem. Eng.* **17:** 251-259, (2000).

Saldana MDA, Zetzl C, Mohamed RS, Brunner G. Decaffeination of guaraná seeds in a micro-extraction column using water-saturated CO_2. *J. Supercrti. Fluids A*, **22:** 119-127, (2002).

Samuelsson G. *Drugs of Natural Origin*. Swedish Pharmaceutical Press, Stockholm, 1992.

Sewram V, Raynor M W, Raidoo D M, Mulholland D A., Coupling SFE to uterotonic bioassay: an on-line approach to analyzing medicinal plants *J. Pharm. Biomed. Anal* **18:** 305-318 (1998).

Skoog DA, Holler FJ, Nieman TA. Principles of Instrumental Analysis. 5^{th} edition, 1998. Eastern Press Banglore. pp. 768-777.

Staub C, Edder P, Veuthey J L. Importance of Supercritical Fluid Extraction in Hair Analysis. In Drug Testing in Hair P. Kintz (Ed.) CRC Press 1996: 121-149.

Stearman GK, Wells MJM, Adkisson SM, Ridgill TE. Supercritical fluid extraction coupled with enzyme immunoassay anaylysis of soil herbicides Analyst **120:** 2617-2621 (1995).

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Subramanyam B, Rajeswski R, Snavely K. Pharmaceutical processing with supercritical carbon dioxide *J. Pharm. Sci.* **86:** 885–890 (1997).

Teris A van Beek, Chemical analysis of *Ginkgo biloba* leaves and extracts *J. Chromatogr. A* **967:** 21–55 (2002).

Tzeng TC, Lin YL, Jong TT, Chang CMJ. Ethanol modified supercritical fluids extraction of scopoletin and artemisinin from Artemisia annua L. Sep. Purifi. Tech. **56:**18–24 (2007).

Valle J M del, Aguilera JM. High pressure CO_2 extraction: fundamentals and applications in the food industry. *Int. J. Food Sci. Tech.* **5:** 1-24 (1999).

Westwood SA. Supercritical fluid extraction and its use in chromatographic sample preparation. Blackie. Glasgow. (1993).

Wolfe M F, Seiber J N, Hinton D E. Proceedings of the 5th International Symposium on Supercritical Fluid Chromatography and Extraction Baltimore USA, 20-21 (1994), 403-409 (1992).