# Preface

Over the last 15 years, there has been renewed interest in supercritical fluids owing to their unique properties and relatively low environmental impact. Greatest attention has been given to the extraction and separation of organic compounds. Supercritical fluids have also been successfully used for particle production, as reaction media, and for the destruction of toxic waste. Supercritical carbon dioxide has been the most widely used supercritical fluid, mainly because it is cheap, relatively nontoxic, and has convenient critical values. Supercritical fluids have also been used on analytical and preparative scales for many biological and other applications.

Many papers have been published on the use of supercritical fluids. However, few have acted as a detailed instruction manual for those wanting to use the techniques for the first time. We anticipate that this *Methods in Biotechnology* volume, *Supercritical Fluid Methods and Protocols* will satisfy the need for such a book.

Every chapter has been written by experienced workers and should, if closely followed, enable workers with some or no previous experience of supercritical fluids to conduct experiments successfully at the first attempt. The Introduction to each chapter gives the reader all the necessary background information. The Materials and Methods sections describe, in detail, the apparatus and steps needed to complete the protocol quickly, with a minimum of fuss. The Notes section, an acclaimed feature of the *Methods in Biotechnology* series, gives additional information not normally seen in published papers that enable the procedures to be conducted easily. Some of the chapters describe how the procedures can be modified for application to new situations. The first chapter is not a detailed procedure, but a theoretical, general introduction to the area of supercritical fluids intended to instruct novices in this branch of technology.

It is envisaged that *Supercritical Fluid Methods and Protocols* will be useful to both student and experienced research workers in biology and related areas. Our hope is that the experience gained when using these techniques will give these workers the confidence to explore new applications for supercritical fluids.

One can envisage a time in the future when the use of sub- and supercritical carbon dioxide and water becomes very important in laboratory work, with organic solvent use considerably reduced.

Finally, we would like to thank Professor John Walker for allowing us to edit this volume and for his cooperation during the compiling of this book. We would also like to acknowledge Professor E. D. Morgan of Keele University, UK for passing this opportunity on to us. We thank Thomas Lanigan and his colleagues at Humana for their help in seeing our book through press.

***John R. Williams***
***Anthony A. Clifford***

# 2

# Supercritical Fluid Extraction of Caffeine from Instant Coffee

**John R. Dean, Ben Liu, and Edwin Ludkin**

## 1. Introduction

Caffeine—1,3,7-trimethylxanthine—is one of three common alkaloids found in coffee, cola nuts, tea, cacao beans, maté, and other plants. The other two are theophylline and theobromine ***(1)***. The effects of caffeine are commonly reported to be as a stimulant of the central nervous system, cardiac muscle, and the respiratory system. It is also a common diuretic and delays fatigue ***(1)***. It has also been reported ***(1)*** that caffeine in combination with an analgesic, for example, aspirin, can be used in the treatment of headaches. However, there are few data to substantiate its efficacy in this role.

The concept of supercritical fluid extraction (SFE) was introduced in Chapter 1. Extraction with supercritical carbon dioxide ($CO_2$) as the solvent has been used to isolate components from different matrices such as biological and environmental samples ***(2)***. The commercial process of extraction of caffeine from coffee using supercritical $CO_2$ was patented by Zosel in 1964 ***(3)***. The analytical SFE of caffeine from coffee has been reported by other workers using SFE coupled to supercritical fluid chromatography ***(4)***, nuclear magnetic resonance spectroscopy ***(5)***, infrared spectroscopy ***(6)***, and high performance liquid chromatography (HPLC) ***(7)***. However, the use of a nonpolar supercritical fluid, such as $CO_2$, does not exhaustively extract caffeine from instant coffee. As has been reported elsewhere ***(2)***, the polarity of the supercritical fluid can be increased by the addition of a polar organic solvent, for example, methanol. This approach is commonly used for "real" sample analysis.

The purpose of this chapter is to describe a procedure for the off-line SFE of caffeine from instant coffee granules using supercritical $CO_2$-methanol and to provide an introductory practical/training exercise in the application of SFE. Analysis of the extracts is done by HPLC with ultraviolet detection.

From: *Methods in Biotechnology, Vol. 13: Supercritical Fluid Methods and Protocols*
Edited by: J. R. Williams and A. A. Clifford 

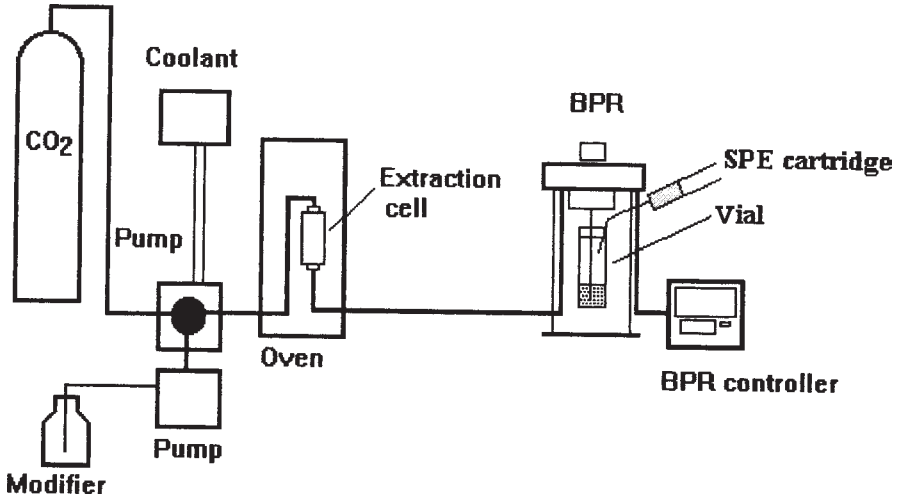


Fig. 1. Schematic diagram of the SFE apparatus.

## 2. Materials

### 2.1. SFE

1. Two reciprocating pumps (*see* **Fig. 1**); one to deliver $CO_2$ and the other to dispense modifier (intelligent HPLC pumps, model PU-980, Jasco Ltd., Great Dunmow, Essex, U.K.).
2. A column oven (Jasco, model 860-CO) which can operate up to 100°C (*see* **Fig. 1**).
3. A back-pressure regulator (see **Fig. 1**) or BPR (Jasco, model 880–81).
4. A recirculating water bath containing an ethylene glycol mixture, which is passed through a jacket that encases the $CO_2$ pump-head only (*see* **Fig. 1**).
5. An extraction cell (*see* **Fig. 1**).
6. Analyte collection occurs during depressurization into a glass collection vial containing a suitable organic solvent (methanol) fitted with a rubber septum through which two holes are pierced (*see* **Fig. 1**). Into one hole passes the connecting tube from the BPR, while the other contains a syringe needle fitted with a solid-phase extraction (SPE) cartridge (C18, Waters Sep-Pak, Millipore Co., Milford, MA). The purpose of the latter is to prevent loss of analyte from the collection vial and to vent the escaping gaseous carbon dioxide.
7. SFE-grade $CO_2$, fitted with a diptube (Air Products Ltd., Sunderland, UK).
8. HPLC-grade methanol.
9. Celite (Celite for GLC, Merck Ltd., Poole, Dorset, U.K.).

### 2.2. HPLC

1. Reciprocating pump (Gilson, model 305, Anachem Ltd., Luton, Beds, UK).
2. Separation column (C18, ODS2, 25 cm × 4.6 mm, Phase Separations Ltd., Clwyd) maintained at a temperature of 35°C.

3. Injection volume, 20 µL.
4. Mobile phase, acetonitrile:water:acetic acid (15:84:1), was pumped at a flow rate of 1 mL/min.
5. An ultraviolet-visible detector (Jasco, UV-975) for monitoring the response at a wavelength of 275 nm.

### 2.3. Sample

1. Instant coffee granules were purchased from local retail outlets in both decaffeinated and caffeinated forms.

## 3. Method

### 3.1. Sample Preparation

1. Grind instant coffee granules into powder using a mortar and pestle, and sieve through a 420-µm filter.
2. Mix one part of the ground instant coffee with one part of Celite (*see* **Note 1**).

### 3.2. SFE

1. Turn on the electrical supply to the SFE system, including the recirculating water bath. Allow 30 min for cooling of the $CO_2$ pump-head.
2. Take an extraction cell (*see* **Note 2**) and tighten, using a wrench, an end-cap on one end only and then weigh the cell.
3. Fill the extraction cell with the coffee/Celite mixture (approx 0.5–0.7 g), and weigh the cell again.
4. Tighten the other end-cap on to the cell with the wrench and insert the capped cell into the oven. Plumb the cell into the SFE system. This requires the use of a wrench to ensure a suitable connection.
5. Connect a glass collection vial containing 2 mL of methanol and fitted with a C18 SPE cartridge to the outlet of the BPR (*see* **Subheading 2.1., step 6**).
6. Set SFE operating parameters: flow rate of liquid carbon dioxide, 1.8 mL/min and methanol, 0.2 mL/min; oven temperature, 60°C; and pressure, 250 kg/cm$^2$. Allow the system to operate for a few minutes to establish a working system. Before the extraction commences, preheat the extraction cell containing the sample to the preset temperature for 10 min (*see* **Note 3**), then undergo a static extraction (no flow of $CO_2$) at the operating conditions for 5 min and, finally, a dynamic extraction (flow of $CO_2$ and methanol) for 1 h.
7. After the allotted extraction time, remove the collection vial from the system and back-flush the C18 SPE cartridge with 2 mL of fresh methanol (*see* **Note 4**).
8. Extract further samples using the stated parameters.

### 3.3. Analysis of Coffee for Caffeine

1. Quantitatively transfer the contents of the collection vial into a 25-mL volumetric flask and adjust to the required volume with a 1:1 water:methanol mixture (for decaffeinated products only). For caffeinated products, pipet 1 mL of

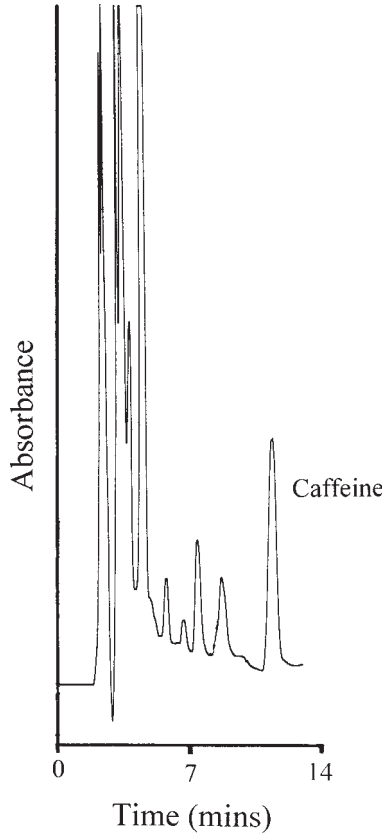


Fig. 2. HPLC chromatogram of caffeine extracted from decaffeinated instant coffee.

the diluted sample solution into another 25-mL volumetric flask and adjust to the required volume with water.

2. Analyze for caffeine using HPLC (*see* **Subheading 2.2.**) by first establishing a calibration graph for caffeine. This entails running a series of 4 to 5 caffeine standards of known concentration in methanol. There should be a linear relationship between absorbance and caffeine concentration over the concentration range of interest. The caffeine peak appears at a retention time of approximately 11 min.
3. Analyze for the unknown levels of caffeine in the coffee extracts.
4. Typical caffeine levels in commercial instant coffees (using four varieties for which decaffeinated and caffeinated were available and a single variety for which only decaffeinated was available) determined by off-line SFE–HPLC ranged from 0.131 ± 0.006% (w/w) to 0.058 ± 0.001% (w/w) for decaffeinated coffee and from 2.373 ± 0.115% (w/w) to 1.811 ± 0.241% (w/w) for caffeinated coffee (*see* **Note 5**). Typical chromatograms obtained for decaffeinated and caffeinated coffee extracts are shown in **Figs. 2** and **3**, respectively.

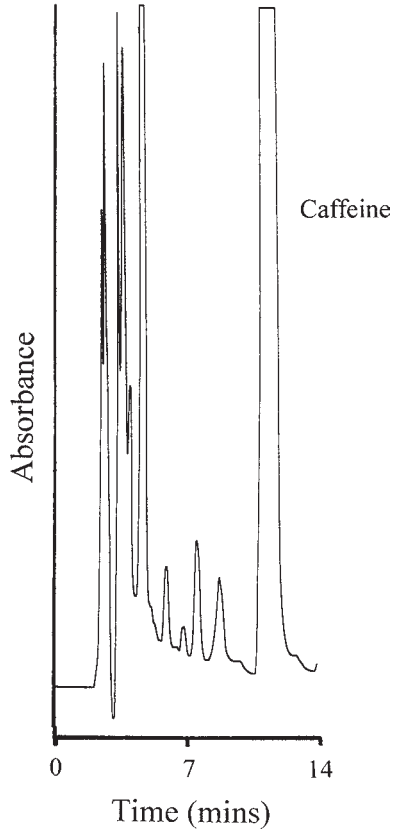


Fig. 3. HPLC chromatogram of caffeine extracted from caffeinated instant coffee.

## 4. Notes

1. Before the ground instant coffee is extracted using 10% methanol-modified supercritical $CO_2$, it should be dispersed with Celite. The grinding and mixing of the coffee with Celite serves to produce a larger surface area for solute–solvent interaction that is, caffeine-$CO_2$/methanol interaction.
2. Ensure the extraction cell is suitable for its purpose, that is, able to withstand high pressure and does not leak.
3. After insertion of the extraction cell into the oven, allow sufficient time for the cell and its contents to reach the preset temperature. Ten minutes was considered to be suitable in this experiment.
4. Back-flush the C18 SPE cartridge with 2 mL methanol after each extraction. This will ensure that quantitative analyses are performed.
5. Under the SFE conditions: pressure, 250 kg/cm$^2$; temperature, 60°C; extraction fluid, 10% methanol-modified $CO_2$; and a flow rate of 2 mL/min, it was possible

to extract approx 83% of caffeine from the ground instant coffee within 1 h, 89% in 2 h and 94% within 3 h (based on the recovery obtained after 5 h).

## References

1. Lopez-Ortiz, A. (1997) Frequently asked questions about coffee and caffeine. internet address: http://www.cs.unb.ca/~alopez-o/caffaq.html
2. Dean, J. R. (1993) Applications of supercritical fluids in industrial analysis. Blackie Academic and Professional, Glasgow, U.K.
3. Zosel, K. (1964) German Patent 1,493,190.
4. Patrick, E., Masanori, Y., Yoshio, Y., and Maneo, S. (1991) Infrared and nuclear magnetic resonance spectrometry of caffeine in roasted coffee beans after separation by preparative supercritical fluid chromatography. *Anal. Sci.* **7,** 427–431.
5. Braumann, U., Handel, H., Albert, K., Ecker, R., and Spraul, M. (1995) On-line monitoring of the supercritical fluid extraction process with proton nuclear magnetic resonance spectroscopy. *Anal. Chem.* **67,** 930–935.
6. Heglund, D. L., Tilotta, D. C., Hawthorne, S. B., and Miller, D. J. (1994) Simple fiber-optic interface for on-line supercritical fluid extraction-Fourier transform infrared spectrometry. *Anal. Chem.* **66,** 3543–3551.
7. Ndiomu, C. F. and Simpson, C. F. (1988) Some applications of supercritical fluid extraction. *Anal. Chim. Acta* **213,** 237–243.