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Short communication

Fiber optic-based interface for on-line selective photometric determinations in solid samples by supercritical fluid extraction

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Abstract

A novel windowless fiber optic-based flow-cell constructed from a drilled stainless steel block was used to link a supercritical fluid extractor and a spectrophotometer in order to obtain qualitative and quantitative information from the supercritical extract during the supercritical fluid extraction (SFE) process. The coupled system allows the process to be optimized in terms of extraction rate and extraction yield from the shape of the extraction curve and the area under it, respectively. The SFE of ground coffee samples was monitored spectrophotometrically and the suppression of the contribution of coextracted compounds to the analytical signal was demonstrated from derivative spectra.

Keywords: Interfaces; Extraction methods; Caffeine

1. Introduction

The development of rapid-response analytical tools such as sensors and screening systems is a major goal of analytical chemistry aimed at solving emergent social, industrial and environmental problems. Their main advantage is the reduction of the so-called preliminary operations of the analytical process, which are claimed to be variable, complex, labour-intensive, slow, error-prone, difficult to calibrate and a major source of potential hazards to both laboratory personnel and the environment [1]. These drawbacks hampered their automation, in relation to the other two steps of the analytical process, despite their doubtless significance to the quality of analytical results.

Supercritical fluid extraction (SFE) has lately aroused great interest among analytical chemists as a promising alternative with numerous advantages over traditional methods for sample preparation prior to chromatographic analysis [2,3]. SFE is also receiving attention as a preparative/industrial approach as it allows more concentrated (even solventless) extracts to be obtained and is carried out at moderately low temperatures (thus preserving the integrity of thermolabile compounds and samples) and avoids the release of residual toxic solvents.

The on-line coupling of a supercritical fluid extractor to a detector provides a useful means for optimizing the experimental conditions and maximizing both the extraction rate and the extraction efficiency; also it enables real-time on-line monitoring of the SFE process and sample screening, thus avoiding the need to chromatograph every extract in routine analyses. SFE has thus been used in combination with a wide variety of detection techniques including mass spectrometry (MS), Fourier transform infrared spectrometry (FTIR), atomic absorp-

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tion spectrometry (AAS), UV spectrophotometry and spectrofluorimetry. The combination of a supercritical fluid extractor and a chemical ionization and collision-induced dissociation tandem mass spectrometry detector reported by Kalinoski et al. [4] allowed the rapid identification of mycotoxins in solid samples with a great reduction in sample handling. Miller and Hawthorne [5] proposed the on-line detection by flame ionization detection (FID) and MS in supercritical CO_2 (SC-CO_2) as an alternative to gravimetric determination of solubility. A silica flame-in-tube interface was developed for the on-line AAS detection of several metals in the supercritical fluid extractor eluent [6]. This selective, sensitive coupled system provides subnanogram to low-picogram detection limits and was used to characterize different physical and/or chemical forms of trace metal analytes from differences in their relative rates of mobilization from different matrices, including freeze-dried- and fresh bovine liver. The extractive behaviour of polymer additives under variable sample and extraction conditions has been discussed on the basis of UV traces obtained by using a conventional UV detector connected on-line to a supercritical fluid extractor [7]. Also, textile finishes have been determined by static/dynamic SFE–FTIR [8,9]; the finishes were transfered directly to the FTIR flow cell through a stainless steel line. However, windowless fiber optic-based interfaces [10–12] excel over window high-pressure flow-cells [13,14] in terms of toughness and ease of replacement and cleaning.

In this paper, a novel windowless fiber optic-based flow-cell constructed from a drilled stainless steel block is proposed as an interface between a supercritical fluid extractor and a spectrophotometric detector. The SFE of caffeine is used as a model system. The experimental set-up provides qualitative and quantitative information on real samples (green and roasted coffee). Also, the contribution of interferents to the analytical signal is suppressed by using derivative spectra.

2. Experimental

2.1. Instruments and apparatus

A Hewlett-Packard HP7680A supercritical fluid extractor equipped with a 7-ml thimble as the extraction cell and a packing of small stainless steel balls as the analyte trap was used. A fiber-optic scanning spectrophotometer (Guided Wave, Model 260) furnished with a 1200 lines/mm grating and an auxiliary ultraviolet deuterium lamp (Model DTL 200), and interfaced to a PC computer, was also used. The supercritical fluid extractor was coupled to the spectrophotometer through a laboratory-made stainless steel flow-cell. As shown in Fig. 1A, the photometric flow-cell (2 cm optical path and ca. 60 μl internal volume) was connected between the prenozzle filter and the pressure isolation valve, so measurements were made prior to depressurisation and the flow-cell was rinsed after each extraction. Commercially available 2 m UV–Vis fiber-optic cables with a black nylon outer jacket and SMA

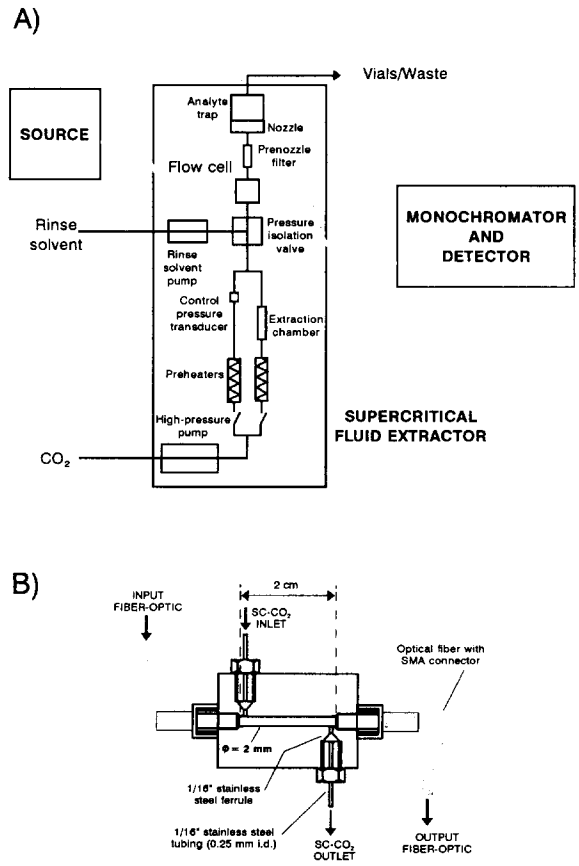

Fig. 1. Schematic diagram of the coupled supercritical fluid extractor–fiber-optic spectrophotometer system (A) and the windowless photometric flow-cell used as the interface (B). For details, see text.

connectors (Oriel Corporation, Stratford, CT, USA) were used to connect the optical devices and the flow-cell. The refractive index of the core and cladding were 1.452 and 1.438, respectively, at 850 nm. The core, cladding and jacket diameters were 600 μm, 750 μm and 1.7 mm, respectively.

The photometric flow-cell, depicted in Fig. 1B, consisted of an appropriately drilled stainless steel block. Fittings for fiber connectors were aligned with a common axis and the SC-CO_2 inlet and outlet were arranged normal to the optical path. Stainless steel tubing (1/16 in. O.D.; 0.25 mm I.D.) and 1/16 in. stainless steel ferrules and screws were used to connect the flow-cell to the extractor components. The unit is robust and can withstand pressures at least up to 350 bar.

An HP 1050 liquid chromatograph and a HP 1040A diode array detector were used for the determination of caffeine in the extracts.

2.2. Reagents and samples

A stock standard solution containing 1 g/ml of caffeine (anhydrous, Sigma) in HPLC-grade acetone (Romil Chemicals) was prepared. Diatomaceous earth (Sigma) was used as a solid support. SFE/SFC-grade CO_2 (Air Products), HPLC-grade methanol and Milli-Q water were also used.

Roasted coffee was purchased locally and green coffee from various countries (Colombia, Brazil, Guatemala, Uganda and Equador) was kindly supplied by a local manufacturer (Cafés Capuchinos, Córdoba, Spain). Coffee beans were ground before extraction.

2.3. SFE procedure

The CO_2 was aspirated from a cylinder furnished with a dip tube at flow-rates ranging from 1.0 to 4.0 ml/min (liquid) using a dual-piston pump and was passed through the extraction cell, which contained the material to be extracted. Extraction was started after the extraction vessel attained the working conditions (temperature 40–100°C; pressure 90–383 bar, depending on the particular experiment). After an equilibration time of 0.5–10 min (static SFE), the supercritical fluid was passed through the sample for 15 min, leached the analyte and drove it to a stainless steel bead trap through a variable restrictor that avoided plugging to a great extent and provided a constant flow-rate during the extraction process. The latter was mandatory in order to obtain reproducible results in the on-line monitoring of the SC extraction cell eluent. In a subsequent step, following system depressurisation, a methanol–water (40:60, v/v) stream at a flow-rate of 0.5 ml/min was pumped through the flow-cell and trap by means of a syringe pump. In this way, the flow-cell was cleaned after each extraction and the analyte solution was collected in a 2-ml vial. The trap was maintained at 35°C and the nozzle was maintained at 45 and 35°C during the extraction- and rinsing steps, respectively.

2.4. On-line photometric monitoring of the SC extract

Absorbance measurements were made at a fixed wavelength (Pointer mode) or over a wavelength interval (Scanner mode). Data were collected only during dynamic extraction. Fig. 2 shows typical recordings obtained in both modes. SC-CO_2 at a specific pressure, temperature and flow-rate was passed through an empty extraction vessel in order to establish a reference value (100% transmittance). Absorbance data at a fixed wavelength were acquired every 0.1 min in the pointer mode; on the other hand, data acquisition and storage over the range 260–320 nm ($\Delta\lambda$=1 nm) required 0.167 min with scanner monitoring, so the throughput was ca. 6 scans/min. Data were processed using BASIC software [15]. The area under the SFE curve was calculated using Simpson's rule-based software. Smoothed, first- and second-derivative spectra, and the SFE curve at a fixed wavelength, were also obtained from the scan set obtained in the scanner mode.

2.5. Chromatographic determination

The determination of caffeine in the liquid extract was performed on an Ultrabase-C_{18} (250×4.6 mm, 5 μm) column. The injected volume was 20 μl and the flow-rate of the methanol–water (40:60, v/v) mobile phase was 1 ml/min. The chromatogram was recorded at 274 nm, the maximum absorbance wavelength for caffeine, and the analyte was quantified from the peak area.

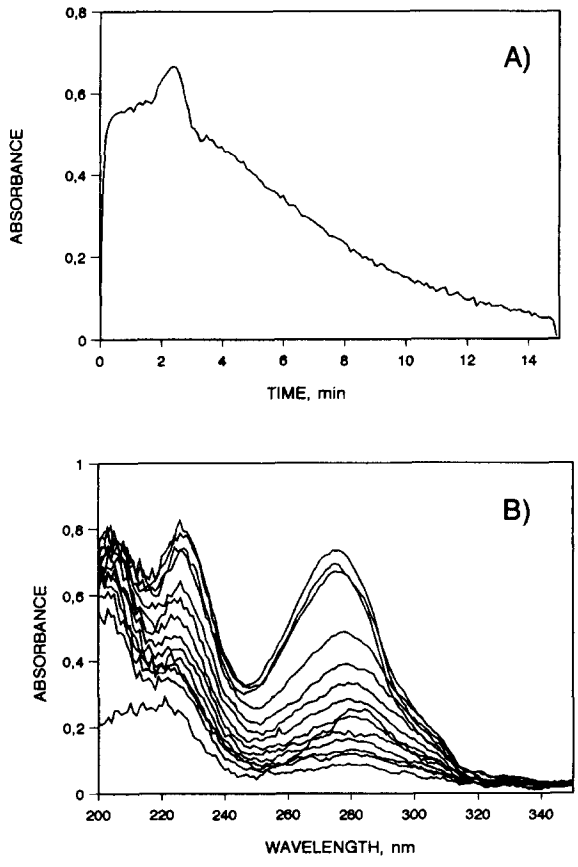

Fig. 2. Absorbance recording at 275 nm (A) and successive scans (B) obtained during the extraction of a roasted coffee sample (25 mg) with SC-CO_2. Time between scans: 1 min.

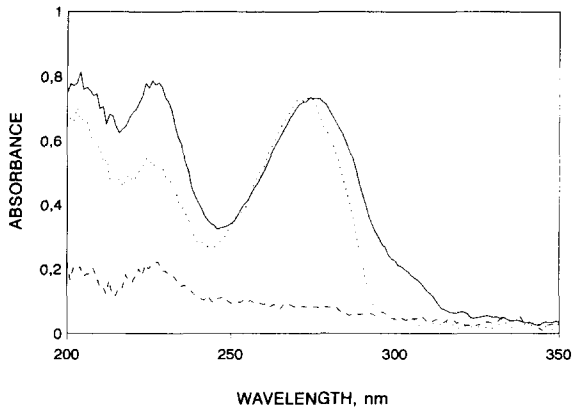

Fig. 3. Spectra obtained during the SFE of roasted coffee (—) and pure caffeine spiked with diatomaceous earth (·····). Blank (—): diatomaceous earth spiked with pure acetone and dried.

3. Results and discussion

3.1. Preliminary experiments

An inert solid support (diatomaceous earth) containing caffeine was prepared in order to obtain the spectrum of pure caffeine. A solution of caffeine in acetone was added to diatomaceous earth for this purpose. Once dry, the solid was extracted with pure SC-CO_2 at 40°C and 281 bar (equivalent to a density of 0.9 g/ml) and a flow-rate of 2 ml/min. The spectrum for caffeine in SC-CO_2 is shown in Fig. 3. The blank was obtained from the extraction of diatomaceous earth spiked with pure acetone. The spectrum obtained from the SF extract of roasted coffee under the same conditions was similar, but not exactly identical with that for pure caffeine. The absorbance at 300 nm revealed that compounds from coffee were also extracted. A lipid extract was obtained when the trap was rinsed with acetone, the spectrum of which exhibited a maximun at 286 nm in hexane.

3.2. Influence of experimental variables

The coupled SC extractor–fiber optic spectrophotometer system was used to continuously monitor the extraction process in order to study the influence of experimental variables. In fact, the system can be used to evaluate both the extraction kinetics and the extraction yield and hence to identify the more influential variables on the extraction rate and/or efficiency. Caffeine extraction from spiked diatomaceous earth gave rise to absorbance–time recordings with highly variable shapes. In contrast, recordings for roasted coffee were quite reproducible. The influence of the analyte distribution in the solid on the extraction kinetics is obvious. Extractions from spiked supports were more rapid, as a result of surface distribution, than those from ground coffee beans, where caffeine is expected to be homogeneously distributed within the matrix. The relative standard deviation of the area

under the extraction curve was less than 20% for roasted coffee.

All extractions were carried out using 25 mg of ground roasted coffee and were monitored at 275 nm. Too large sample sizes caused trap overloading and gave rise to absorbance values that were higher than unity. The area under the absorbance–time curves obtained during extraction was related to the amount of caffeine extracted (provided the flow-rate was constant), while the shape of the curves was related to the extraction kinetics. The extractions were carried out at densities above 0.7 g/ml in order to avoid the pitfalls associated with the variation of the solute molar absorptivity in SC-CO_2 and the spectrum shifts with CO_2 density, as those density-dependent changes in solute molar absortivities become negligible at high densities [16]. A relatively small amount of sample (25 mg) was used in these experiments and the large void volume of the extraction vessel (7 ml) was markedly influential on the shape of the curves.

The areas under extraction curves, obtained at a variable CO_2 flow-rate, are not directly correlated with the amount of caffeine extracted because the residence time in the detection zone increased with decreasing flow-rate. Thus, the area must be multiplied by the flow-rate value when areas at different flow-rates are to be compared. Removal of extracted caffeine from the extraction vessel was expedited by increasing the flow-rate, but the amount extracted remained constant. Flow-rates above 3 ml/min gave rise to noisy, irreproducible extraction curves, as the likely result of too high a flow-rate resulting in pressure oscillations in the flow-cell, which was very close to the nozzle. It should be noted that the caffeine concentration in the CO_2 eluent of the extraction cell remained roughly constant during the first few minutes of extraction, even though increasing the flow-rate also increased the dilution. Once surface caffeine had been extracted during static SFE, the amount of caffeine that passed from sample to extractant should have been independent of the flow-rate if the extraction process had been governed by caffeine diffusion through the matrix. However, the absorbance signals decayed at 7, 3.5 and 2.3 min at a flow-rate of 1, 2 and 3 ml/min, respectively, thus revealing the effect of flow-rate in sweeping caffeine-containing CO_2 in the void volume out of the extraction cell. For samples limited primarily by the desorption/kinetic step, grinding the sample, addition of modifier or increasing the extraction temperature are more effective approaches for increasing the extraction rate and extraction efficiency than increasing the flow-rate, extraction time or using smaller sample amounts [17,18].

Both caffeine and coextractives (lipid material) contributed to the area under the extraction curve at 275 nm. However, only coextractives are monitored at 305 nm, so the area under the extraction curve at 305 nm can be used to select the conditions for minimal coextractive recovery.

The amount of caffeine extracted and the areas under the extraction curves at 275 and 305 nm, obtained under five different sets of working conditions, are shown in Fig. 4. Increasing pressure gave rise to increased areas at 275 nm, even though the amount of caffeine extracted remained almost constant (as determined by HPLC). High pressures were more favourable for the extraction of coextractives than for that of caffeine. The influence of the extraction temperature was more marked: Increased amounts of caffeine were extracted by the increasing temperature; also, the area at 305 nm only increased substantially above 70°C. Thus, a low density (0.7

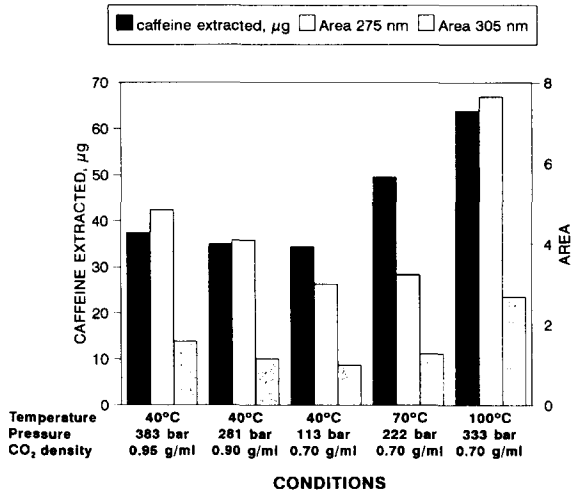

Fig. 4. Influence of the experimental conditions on the amount of caffeine extracted (determined by HPLC), area under the extraction curve at 305 nm (monitoring of coextractives) and at 275 (monitoring of both caffeine and coextractive).

g/ml) and an intermediate temperature (70°C) enabled more selective SFE.

3.3. Selective determinations

The performance of the coupled system was tested by using it to monitor the SFE of coffee samples of different origins. Extractions were carried out under the conditions given in Table 1 and were monitored in the scanner mode. SFE kinetic curves at different wavelengths were obtained from the scan set provided by the coupled system. The area under the extraction curves at 275 nm, obtained in this way for the different coffee samples, was plotted against the amount of caffeine determined by HPLC. The results are shown in Fig. 5A. The points are arranged along two different linear correlation segments. The lack of proportionality between the area at 275 nm and the caffeine extracted was the result of the contribution of coextractants to the absorbance and to the expected differences in coextractive composition between coffee samples of different origin.

Interference removal using derivative spectra was attempted. As shown in Fig. 6, the contribution of coextractives to the analytical signal was suppressed by the use of first-derivative spectra at 284 nm. The spectra set (raw data) were derived. Then, the extraction curve at 284 nm was obtained from the new set of first-derivative spectra. Finally, the area under the extraction curve thus obtained was calculated and plotted against the amount of caffeine extracted. Since the derivative at 284 nm was negative, the absolute value of the area was used. The correlation graph is shown in Fig. 5B. Thus, the derivative signal allows the interferences to be minimized because the signals obtained by using the proposed system show a good correlation (r=0.975) with the HPLC results for different concentrations of caffeine in several coffee samples.

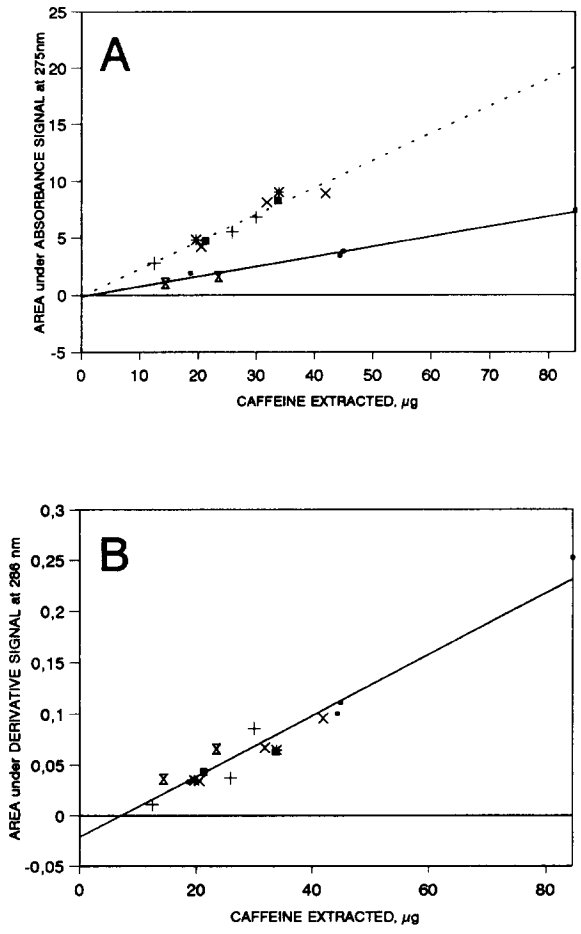

Fig. 5. Correlation between the area under the kinetic extraction curve and the amount of caffeine determined by HPLC from different types of coffee. Analytical signal: absorbance at 275 nm (A) and derivative at 286 nm (B). Symbols: roasted (□); Colombia (+); Brazil (*); Ecuador (■); Guatemala (×) and Uganda (☆).

Table 1
Working conditions

SFE conditions	
Pressure (bar)	113
Temperature (°C)	40
CO_2 density (g/ml)	0.70
CO_2 flow-rate (liq.) (ml/min)	2.0
Extraction time (min)	15
Equilibration time (min)	0.5
Nozzle temperature (°C)	45 (a) 35 (b)
Trap temperature (°C)	35
Scanner settings	
Wavelength range (nm)	260–320
Wavelength step (nm)	1
Scan time (min)	0.167
Point averages	1
Number of scans per sample	90

(a) Extraction and (b) rinsing step.

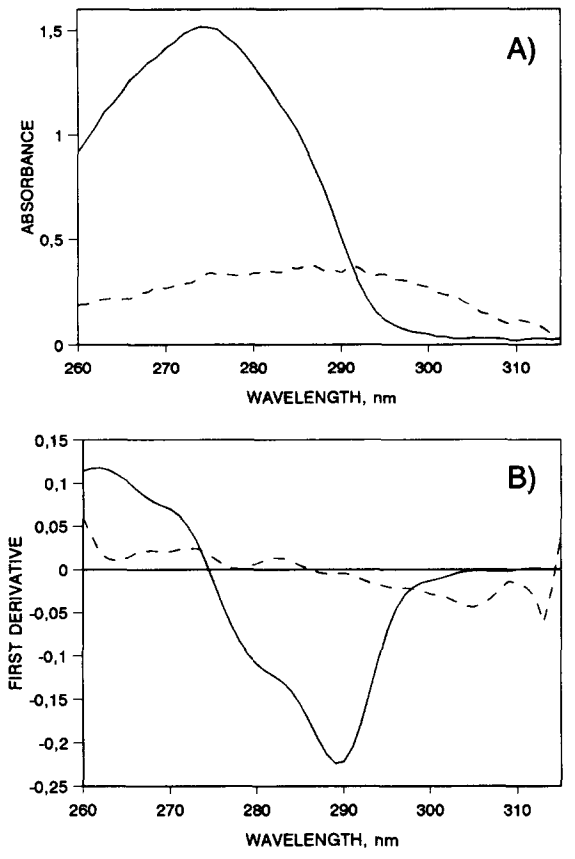

Fig. 6. Absorbance (A) and first-derivative spectra (B) in SC-CO_2 for caffeine (—) and coextractives (– – –).

4. Conclusions

An inexpensive, robust fiber optic-based interface for coupling a supercritical fluid extractor to a spectrophotometer was developed. The coupled system allows the on-line spectrophotometric monitoring of the supercritical fluid extraction process. Measurements are made in supercritical medium prior to depressurization and analyte collection, even though the system also provides a final liquid extract that can be analysed by HPLC. The proposed experimental assembly is a useful means for studying SFE mechanisms and for validating theoretical SFE models. Monitoring at a wavelength where only lipid coextractives absorb enables selection of appropriate conditions for minimal coextractive recovery. The contribution of coextractives to the analytical signal must be suppressed by deriving spectra in order to ensure good correlation between the area under the extraction curve and the amount of caffeine extracted.

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