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Post-extraction solvent flush of the pressure restrictor in supercritical fluid extraction

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ABSTRACT

When using supercritical fluid extraction to isolate and concentrate pesticide residues from plant tissue samples, extraneous components of the plant matrix are extracted in sufficient quantity to cause intermittent obstruction of the pressure restrictor. In some cases, termination of the extraction occurs due to irreversible plugging of the restrictor. It has been determined that a post-extraction solvent flush of the pressure restrictor is sufficient to prevent restrictor obstruction in almost all cases.

INTRODUCTION

Supercritical fluid extraction (SFE) is increasingly being applied for the isolation of a wide variety of analytes from complex matrices [1]. Many applications involve the coupling of SFE and supercritical fluid chromatography (SFC) for a combined extraction and analysis with a single instrument [2,3]. Other on-line applications involve the interfacing of SFE with mass spectrometry [4] and capillary gas chromatography [5,6]. The coupling of SFE with SFC or other chromatographic modes has merit in many cases [3]. However, such on-line coupling can lead to complications involving undesired extract components which present chromatographic or detector-interference problems or which may be strongly retained on the column, requiring excessive flushing times for removal.

Off-line SFE is also performed whereby the sample extract is collected in a solid phase trap [7] or solvent [8,9] and then further treated to make it suitable for analysis by the appropriate instrumental technique. The use of off-line SFE has several practical advantages. A well-chosen trapping solvent can be evaporated to obtain a more concentrated solution for subsequent analysis. The extract solution can be subjected to chemical treatments such as liquid–liquid extraction or solid phase extraction which, properly chosen, will produce additional selectivity for the analyte of interest. Off-line SFE also provides maximum flexibility in the choice of the analytical technique, allowing optimization of both sensitivity and selectivity for the analyte of interest.

Off-line SFE is not without its problems, however. Potential sample losses due to aerosol formation have led some investigators to the use of liquid nitrogen cold

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traps to ensure complete collection of extracted components [10]. The use of pure carbon dioxide as a supercritical fluid extraction medium has also led to restrictor clogging problems due to the rapid cooling of the fluid which takes place upon expansion to atmospheric pressure [10].

A problem which we have encountered in our use of off-line SFE in the analysis of food crops for pesticide residues is the presence of undesired co-extracted components of the plant tissue matrix. This material collects in the pressure restrictor, causing unpredictable changes in its restrictive ability and intermittent plugging. In many cases, the flow of supercritical extractant has been stopped entirely. This co-extracted material was found to be soluble in organic solvents such as ethanol and isooctane and is most likely plant triglycerides which are known to be extracted from plant tissues at the SFE conditions employed [1,11].

Our interests lie in the determination of "bound" residues, which are incorporated into the plant matrix and thus difficult to extract. In such a situation, a constant flow-rate of supercritical extractant for extended periods of time is crucial for maximum extraction efficiency.

Our solution to this problem of restrictor plugging involves combining a suitable solvent with the supercritical fluid between the extraction cell and the pressure restrictor. This approach eliminates the intermittent plugging of the pressure restrictor due to the accumulation of extracted plant material. The solvent flush is also useful in providing additional enthalpy sufficient to prevent the freezing of the carbon dioxide at the pressure restrictor outlet. Secondary control of the extractant carbon dioxide flow-rate is also provided by varying the solvent flow-rate through the restrictor. This paper describes the apparatus and its operation. Data is presented to illustrate the improvement in consistency of the supercritical carbon dioxide flow-rate during extraction.

EXPERIMENTAL

Chemicals

Isooctane and ethanol were reagent grade and used after filtering through a 0.45-μm glass microfibre filter. 2,4-Dichlorophenol, 99% (Aldrich, Milwaukee, WI, U.S.A) was used without further purification. "Bone dry" welding-grade carbon dioxide was used as the supercritical fluid extractant.

Samples

Plant tissues were straw and seed of triticale and barley. All were stored in glass jars with aluminum foil seals at -20°C. Small amounts of material were ground 10 min in a small grain mill producing 2–4 mm lengths suitable for packing into the extraction cell. The individual portions were combined and thoroughly mixed to ensure a homogeneous sample of each matrix.

SFE instrumentation

The SFE apparatus was constructed in-house from available components and is shown in Fig. 1. A standard reciprocating high-performance liquid chromatography (HPLC) pump (Milton Roy MiniPump, Laboratory Data Control, Riviera Beach, FL, U.S.A.) was used to pressurize the carbon dioxide above its critical pressure. The

pump head and check valves were cooled by circulating a −15°C ethylene glycol–water mixture through an aluminium radiator machined to closely fit the pump head. Liquid carbon dioxide was delivered to the pump through 1/8-in. stainless-steel tubing. All other connecting tubing was 1/16-in. stainless-steel.

The extraction cells consisted of empty 5 cm × 4.6 mm HPLC columns (Alltech, Deerfield, IL, U.S.A.) with 2-μm stainless-steel frits on both the inlet and outlet ends. Knurl–Lok fittings (Alltech) with PEEK double-sided ferrules were used to secure the extraction cell in line. A Varian 1400 gas chromatograph oven was used for temperature controle. The extraction cell was preceded by a 10 m coil of 1/16-in. stainless-steel tubing to provide temperature equilibration for the supercritical carbon dioxide. The transfer line exiting the extraction cell was routed through the heated (70–85°C) injector port of the chromatograph oven.

The flushing solvent was pumped by a second MiniPump. Flushing solvent and supercritical carbon dioxide extractant were combined at a stainless-steel tee fitting and routed to the pressure restrictor. The delivery rates of the carbon dioxide and the flush solvent were adjusted independently to maintain a narrow range of extraction pressure.

The pressure restrictor was a 15 cm × 1/16 in. O.D. × 0.020 in. I.D. stainless-steel tubing crimped at the very end. The solvent line into the interfacing tee was replaced with a stainless-steel plug when extract collection without the solvent flush was desired. Collection of the sample extract was performed by placing the tip of the pressure restrictor in a small quantity of solvent contained in a 20 × 150 mm side-arm test tube. A rubber stopper served to seal the side-arm test tube and support the pressure restrictor. In this manner, flow-rates of the gaseous carbon dioxide could be monitored by a bubble flowmeter. A large beaker of tap water (35–40°C) provided

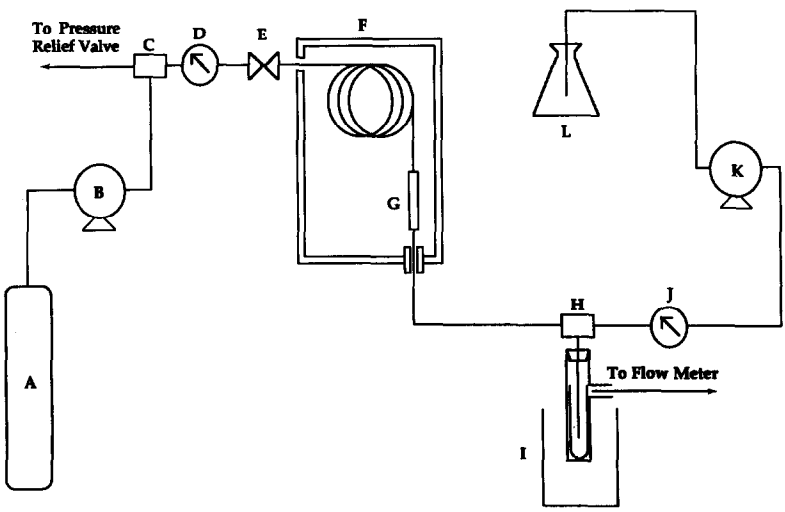

Fig. 1. Supercritical fluid extraction apparatus. See text for additional description. A = Liquid CO_2 cylinder; B = CO_2 pump with cooling assembly; C = tee fitting; D = pressure gauge; E = on/off needle valve; F = oven; G = extraction cell; H = CO_2/solvent interface tee; I = extract collection assembly; J = pressure gauge; K = flush solvent pump; L = solvent reservoir.

thermal mass to the collection apparatus to minimize the freezing of carbon dioxide at the restrictor tip. In cases where analyte was thought to be lost by volatilization from the collection tube, a second side-arm test tube with an impinger was placed in line before the bubble flowmeter.

Extraction

A clean, dry extraction cell was positioned vertically with only the outlet end fitting attached. Silanized glass beads (60–80 mesh, Alltech) were placed into the extraction cell to a depth of about 0.5 cm. This layer of glass beads served as a depth filter, preventing the compaction of the plant tissue sample against the outlet frit. The inlet end fitting was attached loosely to allow the extraction cell and glass beads to be weighed. The cell was opened and a sample of ground plant tissue was packed gently into the extraction cell. The inlet end fitting was replaced and the cell and its contents reweighed. For recovery studies, samples were spiked with 100 μl volumes (Hamilton Microliter syringe) of aqueous 2,4-dichlorophenol standards and then sealed tightly.

The restrictor tip was immersed in the collecting solvent prior to placing the extraction cell on line in the SFE oven. The oven and the carbon dioxide pump were turned on to allow the extraction cell to attain thermal equilibrium while the carbon dioxide pressure built up. When used, the solvent flush pump was turned on once the carbon dioxide pressure and flow were sufficient to prevent backflushing of solvent through the connecting tubing toward the extraction cell. The system generally took less than five min to reach the desired temperature and pressure. Extractions were run for 60 min, unless the carbon dioxide flow was reduced to zero before this time. During the extraction, minor adjustments of both pumps were typically required to maintain pressure within 20–50 p.s.i. of the initial setting.

The restrictor required occasional reconstruction, due to plugging by solid particles or physical damage to the tip. Restrictor calibration was required after reconstruction in order to keep the pump vernier settings approximately the same from one set of runs to the next. For calibration, the carbon dioxide line to the interfacing tee was replaced with a stainless-steel plug allowing only isooctane to be pumped through the restrictor. With an isooctane delivery rate of 0.23 ml/min, the restrictor tip was crimped to yield a back pressure reading of 400–500 p.s.i.

RESULTS AND DISCUSSION

We are exploring the use of off-line supercritical fluid extraction to isolate pesticide residues for subsequent HPLC analysis. Our initial attempts to extract plant tissues used ethanol as a flushing and trapping solvent. Upon dilution with water to provide a solution compatible with our mobile phase, substantial quantities of formerly dissolved plant extract precipitated, making the solution unsuitable for HPLC injection without further clean-up. This precipitated material was found to be soluble in isooctane. However, efforts to remove these components by liquid–liquid extraction with isooctane were unsuccessful due to the formation of a persistent emulsion.

It was suspected that this plant material was also responsible for the inconsistencies in carbon dioxide flow through the pressure restrictor during the course of an extraction. Isooctane was chosen as the flushing and trapping solvent since it retained the water-insoluble extract components while allowing the partitioning of polar and ionized analytes into an aqueous layer without forming an emulsion.

The gaseous carbon dioxide flow-rate during the course of several individual extractions is shown in Fig. 2–5. In each figure, solid symbols indicate extractions run with an isooctane solvent flush, while open symbols indicate no solvent flush. Matrices were extracted in the order in which they are presented. Individual samples follow the order: circle; triangle; square. All twelve samples with solvent flush were completed before the first run without solvent flush. Close operator attention is typically needed to manually adjust conditions at the start of each extraction, precluding immediate measurement of gaseous carbon dioxide flow-rates. The restrictor was reconstructed between the second and third triticale straw samples (Fig. 2) and following each sample without a solvent flush. Even when the restrictor was not changed or was reconstructed and calibrated, initial carbon dioxide flow-rates were seldom identical between sequential runs due to slight variations in cell packing.

As can be seen from the plots of the samples extracted without an isooctane flush in Figs. 2 and 3, a drastic decrease in carbon dioxide flow was observed shortly after the start of the extraction. This behavior was observed to some degree in all straw samples in which an isooctane flush was not used. The decrease in carbon dioxide flow caused by the accumulation of matrix materials in the restrictor without a solvent flush caused a concomitant pressure increase in the extraction cell. This required the operator to continually lower the vernier setting for the pump stroke, thereby maintaining the target pressure and density at the expense of carbon dioxide flow through the extraction cell. Obviously, this is not a desirable situation for efficient extraction. The samples run with a solvent flush exhibit relatively consistent carbon dioxide flow-rates from start to finish of each individual extraction. Without a solvent flush, this consistency is rarely observed. The similarity in the gaseous carbon dioxide flow-rate between samples run without a solvent flush in Fig. 3 is an exception among such extractions. The carbon dioxide flow behavior shown in Fig. 2 is more common.

The extraction pressure of 2300 p.s.i. did not produce the same level of co-extracted materials in seed as in straw. Consequently, extraction of seed samples without a solvent flush (Figs. 4 and 5) did not exhibit the same pattern of carbon

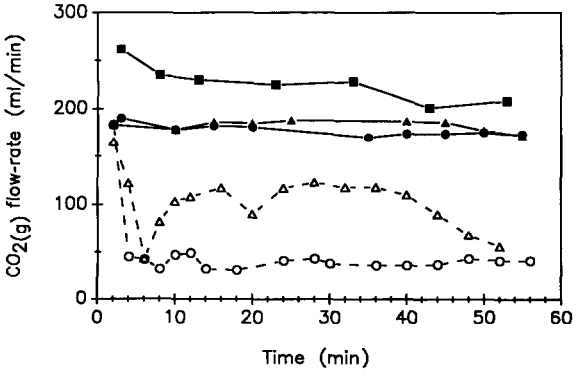

Fig. 2. Flow-rate of gaseous CO_2 for triticale straw extractions. Closed symbols: isooctane solvent flush, 0.23 ml/min (av.). Open symbols: no solvent flush of restrictor. Extraction (CO_2) conditions: 40°C; 2400 p.s.i. (av.), calculated density = 0.801 g/cm^3.

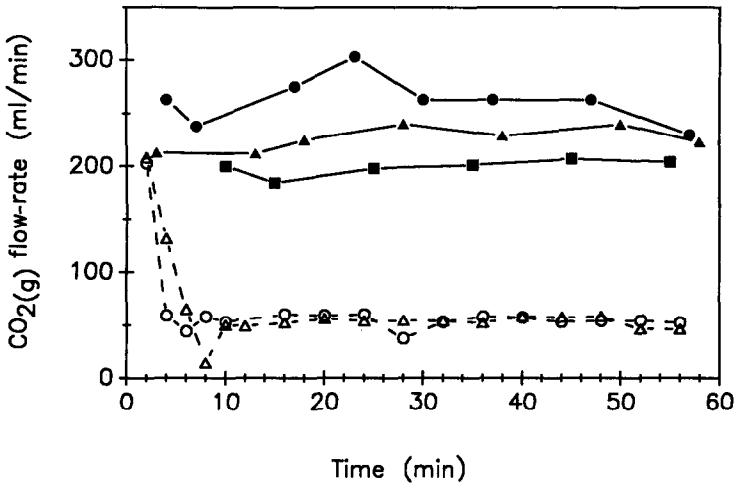

Fig. 3. Flow-rate of gaseous CO_2 for barley straw extractions. Closed symbols: isooctane solvent flush, 0.26 ml/min (av.). Open symbols: no solvent flush of restrictor. Extraction (CO_2) conditions: 40°C, 2400 p.s.i. (av.), calculated density = 0.801 g/cm^3.

dioxide flow. If the seed samples are extracted at a higher density, the extraction behavior with regard to gaseous carbon dioxide flow-rate is similar to that of the straw [12].

An additional problem which results from running extractions without a solvent flush is apparent is Figs. 4 and 5. The initial high flow-rate of carbon dioxide in these experiments was sufficient to evaporate the isooctane trapping solvent from the collection tube, leading to an accumulation of solid carbon dioxide at the restrictor tip and ultimately, to a plugging of the restrictor.

With the solvent flush, this problem was eliminated. At the solvent flush flow-rates used, the solvent added to the collection tube by the flush operation replaced

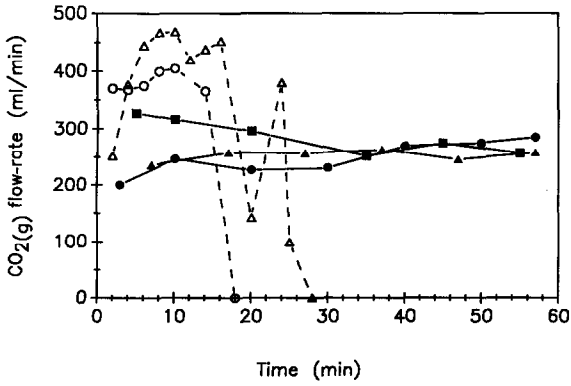

Fig. 4. Flow-rate of gaseous CO_2 for triticale seed extractions. Closed symbols: isooctane solvent flush, 0.28 ml/min (av.). Open symbols: no solvent flush of restrictor. Extraction (CO_2) conditions: 40°C, 2350 p.s.i. (av.), calculated density = 0.790 g/cm^3.

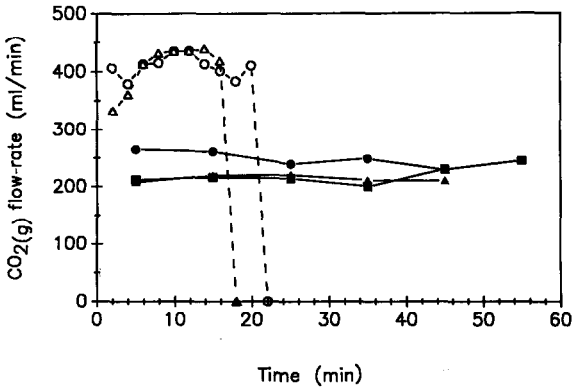

Fig. 5. Flow-rate of gaseous CO_2 for barley seed extractions. Closed symbols: isooctane solvent flush, 0.29 ml/min (av.). Open symbols: no solvent flush of restrictor. Extraction (CO_2) conditions: 40°C, 2280 p.s.i. (av.), calculated density = 0.774 g/cm^3.

solvent lost due to evaporation. Thus, the restrictor tip was never exposed and was always surrounded by a sufficient volume of solvent to prevent accumulation of solid carbon dioxide. In addition, the solvent flush provided additional thermal mass during the passage of extractant through the restrictor. Such benefits have already been noted for extraction co-solvents [10].

It should be emphasized that this solvent flush is not an extraction co-solvent. It is suspected that the flush solvent and the carbon dioxide extractant form a segmented two-phase system in the restrictor. Since this occurs after the extraction cell, the formation of such a two-phase system does not affect extraction efficiency. Indeed, such a situation may increase trapping efficiency of the extracted solutes by providing additional contact with the trapping solvent while the analyte is still being solvated and transported to the restrictor tip by supercritical carbon dioxide. This arrangement may help in minimizing analyte losses due to volatilization or the aerosol formation concomitant with expansion of the carbon dioxide to a gas.

As a consequence of running an extraction with a solvent flush, the total volume of supercritical carbon dioxide in contact with the sample during a complete run is increased, producing higher extraction efficiencies in a finite extraction period. Preliminary results with spiked samples indicate that extraction efficiency of 2,4-dichlorophenol is increased with the solvent flush [12]. However, such spikes are relatively easy to recover in a short period of time, since the spiked solute resides primarily on the surface of the plant matrix.

CONCLUSION

A post-extraction solvent flush of the pressure restrictor has been proven useful in minimizing excessive accumulation of co-extracted material. The choice of flushing solvent can be tailored to the sample matrix without affecting extraction efficiency. This approach should therefore be generally applicable to any supercritical fluid extraction in which the sample matrix yields materials capable of plugging the restrictor.

With isooctane competing for passage through the restrictor, secondary control of the carbon dioxide flow is achieved. The overall gaseous flow-rate can be fine-tuned to a greater degree than is possible by adjustment of the carbon dioxide vernier alone. Only small adjustments of individual pump-stroke vernier settings are required to maintain the desired pressure. Thus, reasonably consistent flow-rates are obtained during the entire course of the extractions.

REFERENCES

1 J. W. King, *J. Chromatogr. Sci.*, 27 (1989) 355.
2 M. E. P. McNally and J. R. Wheeler, *J. Chromatogr.*, 435 (1988) 63.
3 M. R. Andersen, J. T. Swanson, N. L. Porter and B. E. Richter, *J. Chromatogr. Sci.*, 27 (1989) 371.
4 H. T. Kalinoski, H. R. Udseth, B. W. Wright and R. D. Smith, *Anal. Chem.*, 58 (1986) 2421.
5 S. B. Hawthorne, M. S. Kreiger and D. J. Miller, *Anal. Chem.*, 60 (1988) 472.
6 B. W. Wright, S. R. Frye, D. G. McMinn and R. D. Smith, *Anal. Chem.*, 59 (1987) 640.
7 M. Miller Schantz and S. N. Chesler, *J. Chromatogr.*, 363 (1986) 397.
8 S. B. Hawthorne and D. J. Miller, *Anal. Chem.*, 59 (1987) 1705.
9 S. B. Hawthorne and D. J. Miller, *J. Chromatogr. Sci.*, 24 (1986) 258.
10 B. W. Wright, C. W. Wright, R. W. Gale and R. D. Smith, *Anal. Chem.*, 59 (1987) 38.
11 S. T. Schaeffer, L. H. Zalkow and A. S. Teja, in K. P. Johnston and J. M. L. Penninger (Editors), *Supercritical Fluid Science and Technology* (*ACS Symposium Series*, No. 406), American Chemical Society, Washington, DC, 1989, Ch. 26, p. 416.
12 C. A. Thomson and D. J. Chesney, unpublished results.