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Optimization of supercritical fluid extraction of volatile constituents from a model plant matrix

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ABSTRACT

α-Cellulose has been used as a model plant matrix to investigate the conditions required to optimize the supercritical fluid extraction of typical plant constituents, limonene, caryophyllene, carvone, eugenol and santonin, using carbon dioxide as the extraction medium. The conditions required for the successful recovery of the analytes were monitored by gas–liquid chromatography. Timed recovery studies enabled differences in the rates of extraction to be determined to ensure that sufficiently long extraction runs were used. Subcritical and supercritical extractions over the ranges − 10 to 80°C and 50–250 bar were examined and 250 bar and 40°C were chosen as the optimum conditions. The effects of the addition of modifiers to the supercritical fluid were also examined. The work also demonstrated that increased selectivity for polar analytes such as lactones could be obtained by trapping the extract on a silica column coupled with selective elution.

INTRODUCTION

Supercritical fluid extraction (SFE) has been used for many years for the extraction of volatile components such as essential oils, aromas and flavours from plant materials on an industrial scale [1,2]. Recently, the application of this technique on an analytical scale has started to attract wide interest [3–5] and a range of examples, including lemon oils [6] and flavour and fragrance components from spices [7–9], have been reported. The supercritical fluid is depressurized to yield the extract, which is then usually analysed chromatographically. Off-line gas–liquid chromatography (GLC) is frequently employed. On-line capillary GLC, in which the fluid is depressurized within the column, can also be used [7–11] but in order to retain volatile constituents cryogenic focusing (less than − 10°C) is usually needed [7,8,10]. Directly coupled SFE–supercritical fluid chromatographic (SFC) systems have also been reported [6,12–14]. A disadvantage of on-line systems is that the sample size may be limited and

the chromatographic column can become contaminated by highly retained components. In addition, with inhomogeneous matrices the small protion analysed may not be representative of the bulk sample.

However, although the extract obtained by SFE often resembles that obtained by other techniques such as extraction with an organic solvent or steam distillation, many of the reported examples on an analytical scale have been qualitative rather than quantitative. Usually only one set of conditions are described and the effects on temperature and density of extraction on the selectivity of the extraction are not reported. However, Hawthorne and co-workers have reported quantitative recoveries of compounds from spiked rosemary samples [7] and have compared the extraction of basil by SFE and solvent extraction [8]. Sandra *et al.* [15] noted that it was easier to establish the optimum conditions for SFE in an off-line mode as the assay step was then independent of the extraction.

This study followed a similar approach and examined in detail the conditions required to give quantitative extraction of a range of test analytes present at known concentrations on a model cellu-

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lose matrix. The work also examined the conditions needed to obtain good recoveries of the analytes on depressurization of the supercritical fluid and the ability of the system to discriminate between different analytes. The work was designed to establish suitable conditions for the extraction of the volatile components from the herbal medicine feverfew, which contains a thermally labile sesquiterpene lactone, parthenolide, and a range of volatile terpenoids [16]. Initial studies of the complex extract obtained by SFE of dried feverfew were difficult to interpret and it was felt desirable to develop a simpler model system to examine the extraction conditions.

EXPERIMENTAL

Chemicals

Eugenol, santonin and α-cellulose were obtained from Sigma (Poole, UK), carvone and anhydrous sodium sulphate from BDH (Poole, UK), limonene and caryophyllene from Koch-Light (Colnbrook, UK), carbon dioxide of industrial grade (99.98%) from BOC (Middlesex, UK) and dichloromethane [high-performance liquid chromatographic (HPLC) grade] and hexane (HPLC grade) from FSA Laboratory Supplies (Loughborough, UK). Water was deionized and scrubbed.

Model plant material

A solution of limonene (60 mg), carvone (60 mg), eugenol (60 mg), caryophyllene (60 mg) and santonin (125 mg) in dichloromethane (50 ml) was added to α-cellulose (25 g). The solvent was allowed to evaporate at room temperature. The model plant material was then stored in a sealed container at −10°C.

SFC–SFE equipment

Supercritical extractions and separations were carried out using a Jasco Model 880 pump (Japan Spectroscopic, Tokyo, Japan), fitted with a pump head cooling jacket (−10 to −12°C) attached to a Haake KT2 cooling system, for the delivery of carbon dioxide, and a second Jasco Model 880 pump for the addition of modifier through a Gilson Model 811b dynamic mixer. A Jasco 1-ml extraction vessel was mounted in the sample loop position of a Rheodyne (Cotati, CA, USA) Model 7125 valve

housed in a Jasco Model 815 oven. [For extractions at −10°C, a 1-m length of equilibration tubing and the extraction vessel were placed in a bath of acetone–ice (1:1)]. The eluent was monitored using a Jasco Model 820 absorbance detector at 220 nm. The pressure in the system was maintained using a Jasco Model 812 back-pressure regulator and the extracts were collected in a trap made from a 100-ml round-bottomed flask fitted with a side-arm and cooled in liquid nitrogen.

For the study using a silica trap, a short column (50 × 4.6 mm I.D.) dry packed with Spherisorb S5W (12 μm) (Phase Separations) was placed in the oven between the Rheodyne valve and the detector.

Analytical-scale extraction

A Jasco extraction vessel (1 ml) was packed with spiked cellulose samples (*ca.* 0.5 g) and exposed to various temperatures, pressures and flow-rates of sub- and supercritical carbon dioxide. The eluate from the extraction was trapped in a flask cooled in liquid nitrogen at −170°C. The solidified carbon dioxide was allowed to evaporate at −10°C in a refrigerator and the residual extract was dissolved in dichloromethane, containing safrole as an internal standard, and examined by GLC.

GLC analysis of extract

The GLC analyses were performed using a Carlo Erba Vega 6000 gas chromatograph. Samples (0.5 μl) were injected using a 10-μl syringe in the split injection mode (splitting ratio 20:1) on to a BP1 dimethylpolysiloxane fused-silica column (5 μm film thickness, 12 m × 0.33 mm I.D.) (Scientific Glass Engineering). The injection port was maintained at 180°C and the column oven was programmed from 60 to 300°C at 8°C min^{-1}, then held isothermal at 300°C for 8 min. The analytes were detected using a flame ionization detector and the results were recorded using a Perkin-Elmer Nelson 2600 data system on an Opus III computer. The concentrations of the analytes were calculated from calibration graphs prepared with standard solutions.

RESULTS AND DISCUSSION

Model plant matrix

In order to establish suitable conditions for the

quantitative extraction of terpenoids from plant material, it was decided to examine a model plant material of known composition so that the effects of the conditions on analytes of different structural types could be compared. Spiked matrices, such as polymer resins [17] and glass-wool [18], have been used previously to investigate the solubility of analytes in supercritical fluids. In this study, trial extractions indicated that 500-μm silanized solid glass beads were a poor model for the plant material and typical terpenes were rapidly washed off the surface even under mild extraction conditions. A microparticulate porous silica (Hypersil, 12 μm) matrix was also investigated but this proved too retentive and 5% methanol was needed to extract the simple oxygenated terpenes, whereas most of the components from feverfew were eluted with carbon dioxide at 45°C and 200 bar.

Powdered α-cellulose was found to be a more appropriate model as trial terpenes could be extracted using carbon dioxide at 45°C and 250 bar and it was selected for a more detailed study. As the cellulose is porous the analytes should be distributed throughout the body of the material and they should thus experience sorption and diffusion effects more typical of dried plant material.

A test mixture was prepared which was designed to be representative of the monoterpenes, sesquiterpenes, lactone and plant phenolics found in plant materials. The mixture contained limonene and caryophyllene as non-polar hydrocarbons, carvone as a polar monoterpene, eugenol as a less volatile plant phenolic and santonin to represent a sesquiterpene lactone. The test samples were spiked on to α-cellulose as a dichloromethane solution at similar levels to those of the essential oils in ferverfew. After air drying the treated cellulose, the resulting model plant material was stored in a sealed container at −10°C to prevent any loss of the analytes.

Trapping studies

Samples of this model plant material (0.5 g, which contained about 1 mg of each terpene and 2.5 mg of santonin) were used to assess the SFE recoveries using a number of different collection techniques. In the initial experiments with carbon dioxide at 40°C and 250 bar, a 15-ml tapered test-tube was placed under the back-pressure regulator to collect the sample. The collected material was dissolved in dichloromethane and the solution was examined using capillary GLC. When the yields of the test compounds were compared with a direct dichloromethane extraction of the model plant material the recoveries of all the compounds were poor (Table I). It appeared that as the carbon dioxide evaporated the test compounds were condensing as a mist in the gas phase, which was not collected but was swept out of the trap by the flow of gaseous carbon dioxide. This problem has also been noted by other workers [17]. Reducing the carbon dioxide flow-rate increased the recovery slightly but greatly extended the extraction time. Collection traps with

TABLE I

COMPARISON OF EFFICIENCIES OF DIFFERENT EXTRACTION AND COLLECTION TECHNIQUES FROM MODEL PLANT MATRIX

Method[a]	Recovery of analyte (%)					
	Limonene	Carvone	Eugenol	Caryophyllene	Santonin	Total
(a) CH$_2$Cl$_2$	46	88	81	95	98	82
(b) 15 ml tube	0	18	13	20	21	18
(c) Methanol	12	27	21	32	35	25
(d) −15°C	2	10	8	16	15	10
(e) −60°C	9	28	34	32	62	33
(f) −170°C	23	86	81	97	93	76

[a] (a) Extraction with dichloromethane overnight at room temperature; (b–f) extraction with carbon dioxide at 40°C and 250 bar; (b) collection in 15-ml tapered test-tube; (c) extract bubbled into methanol, crimped fine-bore HPLC tube; (d) 100-ml collection vessel cooled in methanol–ice (*ca.* −15°C); (e) 100-ml collection vessel cooled in acetone–dry-ice (*ca.* −60°C); (f) 100-ml collection vessel cooled in liquid nitrogen (*ca.* −170°C).

spiral flow paths or increased surface areas, originally designed for preparative GLC, were then tried but with little improvement in recovery rates.

Other workers, particularly Hawthorne and co-workers [7–9], have obtained good recoveries by bubbling the carbon dioxide at a low flow-rate through fused-silica tubing into a solvent, such as dichloromethane. When a similar technique using stainless-steel tubing and a methanol trapping solution was examined with the present samples, the recoveries were low (Table I). The tubing frequently became blocked and caused an erratic flow.

A series of extractions were then carried out using a 100-ml round-bottomed flask fitted with a vent arm as a trap. This was cooled to −15°C (methanol–ice), −60°C (acetone–dry ice) or −170°C (liquid nitrogen) and the extraction yields were compared (Table I). Under the last conditions the carbon dioxide was collected as a solid, which was then allowed to evaporate at −10°C. This method gave recoveries of the test compounds that were similar to those obtained by solvent extraction and was therefore adopted for the extraction study.

The collection vessel can be used for up to 1 h at 1 ml min^{-1} of carbon dioxide. For shorter extractions a smaller collection vessel, such as a test-tube with a side-arm, could be used. The flow of the carbon dioxide into the trap was important and with the 100-ml flask the optimum flow-rate was about 0.8 ml min^{-1}, which was used in all the subsequent studies. At lower flow-rates liquid oxygen condensed in the vessel and at higher flow-rates (1.5 ml min^{-1}) not all the extract was trapped.

In all the extraction studies, including solvent extraction with dichloromethane, the recovery of limonene was very low (0–46%, Table I). It was considered that this was due to the loss of this volatile monoterpene by evaporation during the preparation of the model plant material. However, in reporting the recoveries in the extraction studies it was assumed that the nominal concentration was present.

Effect of temperature and pressure on extraction

A series of extraction experiments were then carried out on the model plant material using carbon dioxide over a range of temperatures from −10 to 80°C to investigate the effect of changing pressure and temperature on the extraction efficiency. Each
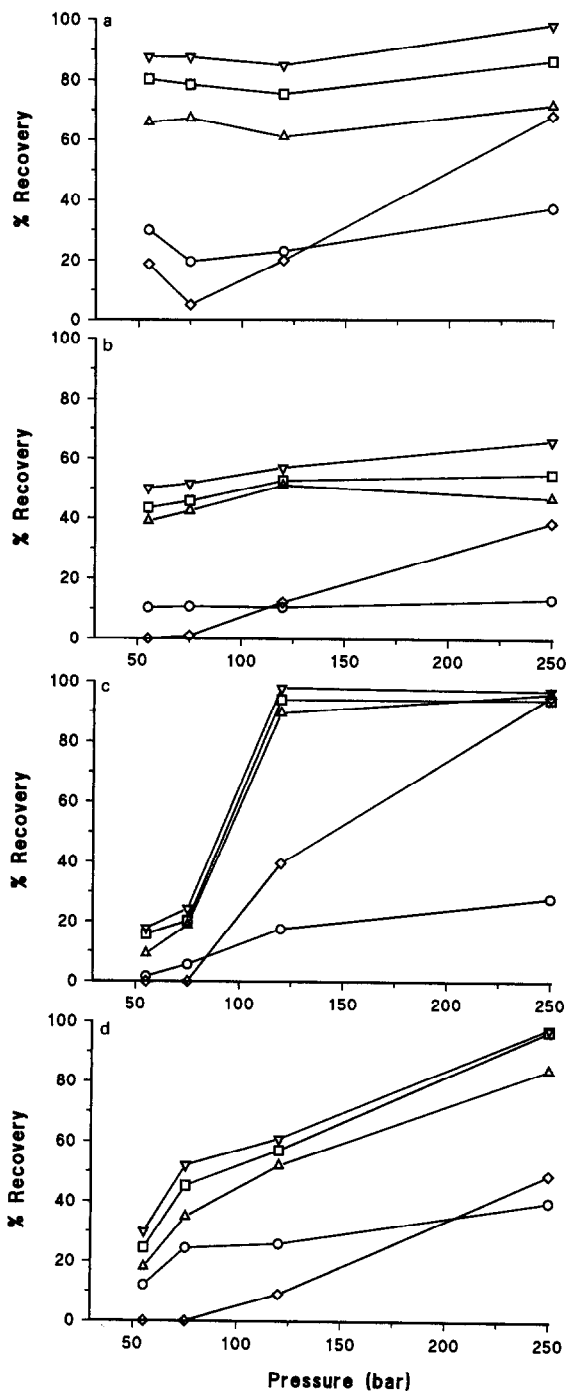

Fig. 1. Recovery yields of essential oils from a spiked cellulose matrix. Analytes: ○ = limonene; □ = carvone; △ = eugenol; ▽ = caryophyllene; ◇ = santonin. Extraction conditions: (a) −10°C, 0.8 ml min^{-1} CO$_2$; (b) 20°C, 0.8 ml min^{-1} CO$_2$; (c) 40°C, 0.8 ml min^{-1} CO$_2$; (d) 80°C, 0.8 ml min^{-1} CO$_2$.
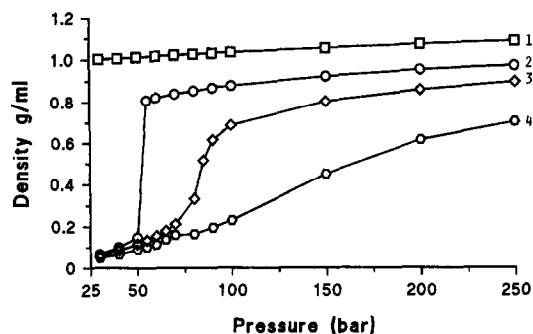
Fig. 2. Density profile of CO_2 (based on ref. 19). Temperature: $1 = -10°C$; $2 = 20°C$; $3 = 40°C$; $4 = 80°C$.

extract was monitored by UV spectrophotometry at 220 nm, which was carried out until the signal returned to the baseline (typically 20–40 min).

On extraction with subcritical liquid carbon dioxide at $-10°C$ the yields of most of the terpene test compounds were independent of extraction pressure (Fig. 1a). This result was expected as the density of carbon dioxide varies very little with pressure at this temperature (Fig. 2, based on ref. 19). However, the yield of santonin increased markedly with increase in pressure.

On increasing the extraction temperature to $20°C$, the yields of each compound were reduced (Fig. 1b), presumably because the density of the liquid carbon dioxide was lower at this temperature. It appeared that the reduced extraction strength of the carbon dioxide was not compensated for by the increase in the volatility of the terpenes.

When the extractions were carried out at $40°C$ a different extraction profile with pressure was obtained (Fig. 1c). At low pressures the yields of all the test compounds were low, reflecting the low carbon dioxide density (Fig. 2). The yields remained largely unchanged when the pressure was increased up to 70 bar, but then improved markedly with further increases in pressure and were nearly quantitative for carvone, eugenol and caryophyllene at 120 bar. A higher pressure of 250 bar was required to achieve a similar recovery for santonin. These changes in the yields of the lower molecular weight compounds closely mirrored the changes in the eluent density with pressure. However, at 120 and 250 bar the carbon dioxide density is lower than at

$20°C$ so that the volatility of the analytes must now play a role in giving the higher extraction yields at the higher temperature. The lower viscosity of the supercritical carbon dioxide will also assist mass transfer of the analytes from the matrix. The increased extraction strength may also be due to the formation of solvent clusters which are considered to be prevalent near the critical point in the supercritical phase [20]. Thus, at 120 bar, the local solvent density about the solute may be higher than the bulk density [21].

On further increasing the temperature to $80°C$, the extraction profile changed again (Fig. 1d). At 75 bar there was an small increase in the recovery yield compared with $40°C$ even though the corresponding density was smaller. This change can be related to significant increases in the vapour pressure of the analytes (except for santonin) [18]. At higher pressures the recovery was lower than at $40°C$, which was attributed to a lower eluent density at the higher temperature (Fig. 2). Thus, at 75 bar the volatility of the analyte appears to dominate the extraction, but at 120 bar the eluent density is the more important factor. As the pressure (and hence eluent density) was increased further to 250 bar most of the test compounds, with the exception of santonin, were efficiently extracted. These competing effects have also been demonstrated for similar essential oils by Stahl and Gerard [18], who reported comparable solubility isotherms for essential oil components coated on silanized glass beads.

Rate of extraction

As the time required for a quantitative extraction may limit the sample throughout of an analytical extraction system, the extraction profiles of the different test compounds with time were examined. Under subcritical dense gas conditions ($-10°C$ and 250 bar), the extraction of the less polar compounds was initially rapid but santonin was only slowly extracted and levelled off at less than 60% (Fig. 3a).

At a higher temperature but lower pressure ($40°C$ and 55 bar) the extraction was again rapid for the less polar terpenes carvone and caryophyllene, but levelled off at a 55% yield (Fig. 3b). The recovery of eugenol appeared to be increasing with time but the signal from the spectroscopic monitor returned to the baseline after 30 min. Very little of the more polar lactone santonin was obtained. On raising the
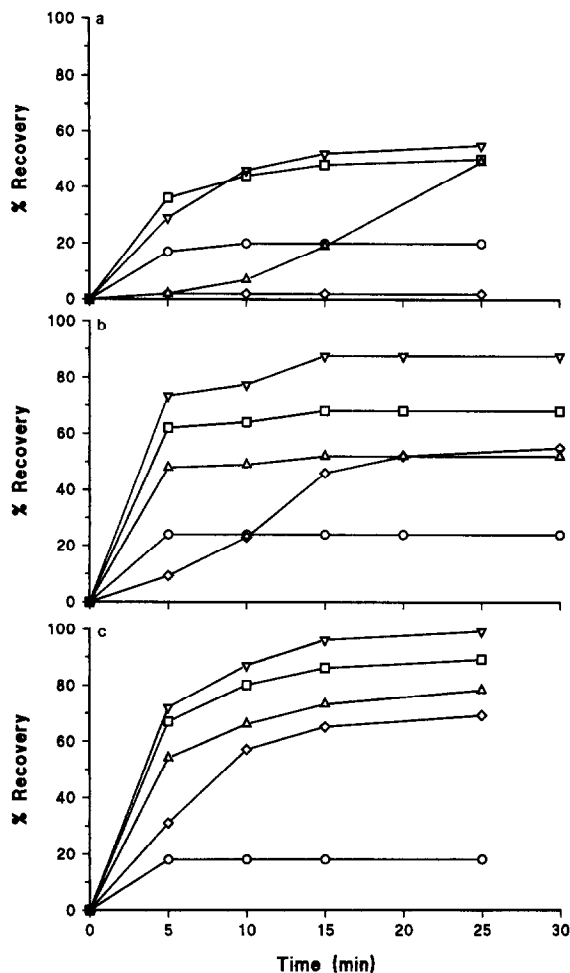
Fig. 3. Extraction profiles for the extraction of model plant matrix. Analytes as in Fig. 1. Extraction conditions: (a) 55 bar, 40°C, 0.8 ml min^{-1} CO$_2$; (b) 250 bar, −10°C, 0.8 ml min^{-1} CO$_2$; (c) 250 bar, 40°C, 0.8 ml min^{-1} CO$_2$.

pressure to 250 bar the rate and overall recovery of all the test compounds increased (Fig. 3c) and from the spectroscopic profile the extraction was effectively complete in less than 15 min. In each of these experiments the final results were similar to those of the single-step extraction shown earlier.

As all the test compounds were present at low concentrations in the model plant material, it was expected that the concentrations of the analytes in the supercritical extraction fluid should be below their solubility limits [18]. Sample solubility should

not be the limiting factor in the extraction process. The extraction of the test compounds must therefore depend on their distribution between the carbon dioxide and sorptive sites on the sample matrix as well as diffusion from the matrix into the supercritical fluid.

Bartle *et al.* [22] proposed that in extractions two mass transfer steps may be present, an initial rapid surface extraction then a slower diffusion-limited step of extraction out of the matrix. However, insufficient data were available here to permit a similar analysis.

From these studies, 250 bar and 40°C were selected as suitable starting conditions for the study of the extraction of the essential oils from feverfew [23].

Modified extraction eluents

Because of the slow and sometimes incomplete extraction of santonin, the possible application of the addition of polar modifiers to the carbon dioxide extraction fluid was examined. Under mild conditions of 120 bar and 40°C, only 38% of santonin could be obtained from a spiked cellulose matrix with carbon dioxide alone. The recovery increased to 85% with the addition of 4% of acetonitrile to the carbon dioxide. If the carbon dioxide was bubbled through a water-filled trap to give a saturated solution the recovery of santonin was 92%. However, the addition of 4% of methanol or 4% of chloroform had no effect on the recovery. In each instance a second extraction at 250 bar and 40°C, using just carbon dioxide, confirmed that the santonin which had not had been extracted by the modified eluent had remained on the matrix and could be recovered by the more severe conditions.

It was surprising that methanol had little effect as it has been widely used to deactivate adsorption sites on stationary phases in SFC [24,25]. However, apart from acetonitrile, none of the organic modifiers could match the yield achieved using unmodified carbon dioxide at 250 bar.

Use of a silica "trap" to obtain selectivity

It is useful to be able to use differences in extraction conditions to achieve selective separations, as there can often simplify subsequent chromatographic separations. However, in this study stepwise changes in the extraction pressure or temper-

ature were insufficiently discriminating to resolve any of the present test compounds.

Differences in extraction rates caused by polarity differences can be enhanced by using a selective trap of a polar material to give a greater discriminating power than the original sample matrix. A short silica column was therefore placed in the oven between the extraction vessel and the detector. A sample of the model plant material was extracted under the optimum conditions of carbon dioxide at 250 bar at 40°C, which should extract all the components of the test mixture. The eluent flow was passed through the silica column and an on-line spectroscopic detector showed a series of four broad peaks, which were each collected and examined by GLC. These fractions contained, respectively, predominately limonene and caryophyllene, carvone and finally eugenol. No santonin was eluted from the column and it appeared to have been completely retained on the silica.

The extraction vessel was then switched out of the carbon dioxide flow and carbon dioxide containing 12% of methanol was passed directly through the silica trap. This yielded a santonin fraction free from the less polar components. However, the santonin was released only slowly from the silica column and to obtain a good recovery a higher than usual flow-rate of the eluent (2.5 ml min^{-1}) was needed. This fractionation technique has subsequently been used with feverfew to give a highly purified sesquiterpene lactone fraction containing the active principle parthenolide, free from the less polar terpenes [23].

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