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Supercritical fluid extraction and gas chromatographic determination of the sesquiterpene lactone parthenolide in the medicinal herb feverfew (*Tanacetum parthenium*)

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ABSTRACT

Supercritical carbon dioxide was used to extract the sesquiterpene lactone parthenolide, which is reported to be the active component of the medicinal herb feverfew (*Tanacetum parthenium*), from dried plant material for gas–liquid chromatographic analysis. The extracts also contained significant amounts of camphor and chrysanthenol acetate. The pressure and densities required for quantitative extraction and the methods of sample collection were studied. The addition of methanol or acetonitrile as modifiers in the carbon dioxide gave higher yields of parthenolide but the extractions were less selective and considerable amounts of co-extractives were obtained. By employing a trapping column made of cellulose or silica the purity of the extracts could be improved and a practical method for the rapid isolation of parthenolide is described.

INTRODUCTION

Supercritical fluid extraction (SFE) has been used for many years for the preparative-scale isolation of compounds from plant matrices, such as hops, spices and coffee beans [1,2]. In recent years there has also been considerable interest in the application of SFE as an analytical extraction method for sample preparation prior to chromatographic analysis either as an off-line extraction step or on-line with gas chromatography (GC) or other chromatographic systems [3,4]. A major advantage of this technique compared with solvent extraction is that if carbon dioxide is used as the extraction solvent, it can be readily removed from the product by depressurization. As supercritical fluid extractions can be achieved under mild conditions this technique should also reduce the thermal degradation and the poor collection efficiencies of volatile analytes that can sometimes occur during the steam distillation or solvent extraction of essential oils and fragrance components [5]. This advantage has been demonstrated by Stahl and Keller [6] in the isolation of the thermally unstable sesquiterpene β-asarone from *Acorus calamus*.

Previous workers have applied analytical SFE to a wide range of plant materials, including lemon peel [7] and many household spices, such as basil, thyme, rosemary, cloves and oregano [8–11]. A number of these studies included direct coupling of the extraction to a gas chromatograph or a GC–MS system. The quantitative trapping or collection of the volatile extractives was often a problem and has been studied in detail [9,10]. However, many of the reported extractions were qualitative rather than quantitative although comparisons were sometimes made with the chromatograms obtained by alterna-

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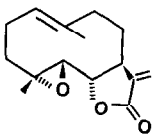

Fig. 1. Structure of parthenolide.

tive extraction techniques, such as solvent extraction [8,10].

Little previous work has been reported on the application of SFE to the examination or isolation of components from medicinal herbs. The herb feverfew [*Tanacetum parthenium* (L.) Schultz Bip.] has recently attracted considerable interest for the treatment of migraine and arthritis [12–14] and contains the sesquiterpene lactone parthenolide (Fig. 1) [15], which is reported to be the active principle [16]. In order to monitor the concentration of the active component in any samples being prepared for medicinal use, assay methods are required. Previous methods for the determination of parthenolide in feverfew have been reported by a number of groups and were usually based on selective solvent extraction followed by infrared spectrometry [17] or extraction and high-performance liquid chromatography (HPLC) [18]. More recently, two further methods based on HPLC determinations have been reported [19, 20]. The isolation of larger amounts of parthenolide has also been carried out using droplet counter-current chromatography [21], but this method is slow.

However, as parthenolide is relatively unstable and degrades on storage [15], there was concern that losses might occur on solvent extraction. In this study we therefore examined the application of SFE followed by capillary GC for the determination of parthenolide from feverfew. We also examined methods for the rapid preparative-scale extraction of feverfew. The extraction conditions and sample collection technique were based the optimum conditions obtained from an initial study of the extraction of a range of terpenoids, including the sesquiterpene lactone santonin, from an α-cellulose model plant matrix [22]. In a subsequent study [23] SFE was also used to obtain the essential oils of feverfew and the related species, tansy and German chamomile, which have been reported as substitutes for commercial feverfew samples.

EXPERIMENTAL

Chemicals

Air-dried powdered feverfew was supplied by British Analytical Control (Burton, UK) (Batch no. A3783). Feverfew plants were grown locally from seed. Feverfew varieties came from the medicinal herb collection at the Chelsea Physic Garden, London, UK. The carbon dioxide was of industry grade (99.98%) supplied by BOC (Middlesex, UK). Acetonitrile was of HPLC grade from FSA Scientific Apparatus (Loughborough, UK). Water was scrubbed and deionized. Parthenolide standard was donated by Dr P. J. Hylands of Chelsea College, London, UK.

SFE equipment

Supercritical extractions and separations were carried out using a system consisting of a Jasco Model 880 pump (Japan Spectroscopic, Tokyo, Japan) for the delivery of carbon dioxide, which was fitted with a cooling jacket around the pump head attached to a Haake KT2 cooling system. A second Jasco Model 880 pump for the addition of modifier was linked 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. The eluent was monitored at 220 nm using a Jasco Model 870 absorbance detector fitted with a high-pressure flow cell. The pressure in the system was maintained using a Jasco Model 880-81 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.

The carbon dioxide flow-rate was measured using a rotameter to give a volume flow-rate. The modifier concentration was calculated as percentage by mass of the mobile phase.

For the trapping studies, either a short column (50 × 4.6 mm I.D.) dry packed with Spherisorb S5W (12 μm, Phase Separations) or a 10-ml Jasco extraction vessel packed with α-cellulose (Sigma) was placed in the oven between the Rheodyne valve and the detector.

Analytical scale extraction

The extraction vessel (1 ml) was packed with

plant material (*ca.* 0.5 g) and was extracted with supercritical carbon dioxide at 250 bar and 45°C at 0.85 ml min^{-1}. The eluent 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 residue was dissolved in dichloromethane. The composition of the extraction was determined by GC.

GC analysis of extract

The GC 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 polydimethylsiloxane fused-silica column (5-μm film thickness, 12 m x 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 concentration of the parthenolide was calculated from calibration graphs by comparison with safrole (1 mg ml^{-1}), which was added to the extracts as an internal standard.

Solvent extraction methods

French Pharmacopoeia method [18]. Dried powdered feverfew (10 g) was extracted with methanol (100 ml) at 45°C for 30 min and then with fresh methanol (40 ml) for 40 min. The methanol extracts were combined and evaporated to dryness and the residue was dissolved in methanol for analysis.

Marchand et al. [24] method. Dried feverfew (10 g) was extracted with chloroform (100 ml) at room temperature for 30 min. The solution was filtered and the filtrate evaporated to dryness. The residue was dissolved in methanol (10 ml), filtered, the solvent evaporated and the residue dissolved in methanol for analysis.

Bloszyk et al. [17] method. Dried feverfew (10 g) was extracted with three 200-ml portions of methanol at 40°C. The extracts were combined and evaporated to 10 ml. Water (50 ml) was added and the remaining methanol evaporated under vacuum. Saturated lead acetate solution was added until precipitation ceased and the mixture was left for 1 h then centrifuged. The supernatant was extracted with chloroform (3 × 100 ml) and the extracts were combined, filtered and evaporated. The residue was dissolved in chloroform for analysis.

Govindachari et al. [15] method. Dried feverfew (10 g) was extracted with hexane (2 × 100 ml) and the extracts were combined, filtered and evaporated to 3 ml. The solution was passed through a column of neutral alumina with toluene, the eluate was evaporated to dryness and the residue was dissolved in 2 ml of hexane–dichloromethane (1:1) for analysis.

DISCUSSION AND RESULTS

Initial studies

To evaluate the efficiency of SFE as a method for the determination of parthenolide in feverfew, a trial extraction of a commercial sample of powdered dried feverfew, using carbon dioxide at 250 bar and 40°C, was compared by capillary GC with extracts of the same material obtained using three published methods for the determination of parthenolide [15,17,18] and a general extraction method for sesquiterpene lactones [24].

The French Pharmacopoeia method of extraction with methanol (which is normally assayed by HPLC) [18] gave a dark green extract and a yield of 0.04% of parthenolide. The method of Bloszyk *et al.* [17], in which methanol extraction was followed by treatment with lead acetate and re-extraction into chloroform, gave a cleaner yellow extract and a 0.02% yield of parthenolide. A general method for the extraction of sesquiterpene lactones devised by Marchand *et al.* [24], in which the sample is extracted with chloroform, yielded only a small amount of parthenolide (0.006%) but a larger amount of a closely related material. However, extraction of feverfew with hexane followed by chromatography on alumina as reported by Govindachari *et al.* [15], as a source of parthenolide yielded only monoterpenes. The SFE and collection of the extract at room temperature gave a yellow orange oil and a yield of 0.03% of parthenolide. This method could be completed in only 20 min, rather than the several hours for the other methods, and required no solvent evaporation stages.

SFE extraction of feverfew

A detailed study was therefore undertaken to improve the yield and selectivity of the SFE method. Because of the complexity of the feverfew extract and problems with the instability of parthenolide, initially a model plant matrix prepared from α-cellulose, spiked with mono- and sesquiterpenes and the sesquiterpene lactone santonin, was examined [22]. The optimum extraction conditions for this system were determined to be carbon dioxide at 250 bar and 40°C for 15–20 min at 0.8 ml min^{-1}. To avoid losses of volatile components, the extract was collected as solid carbon dioxide using a trap cooled in liquid nitrogen at −170°C followed by slow evaporation of the carbon dioxide in a refrigerator.

In comparative extractions there were significant differences in the overall parthenolide content between older commercial samples (used in the trial studies), freshly dried plant material and stored dried plant material (which might be up to 2 years old). These differences could result both from differences in the original parthenolide content of the plants and from the degradation of parthenolide with time. Consequently, when this method was evaluated for the extraction of dried feverfew samples care had to be taken in comparing the results of experiments carried out at different times. However, comparisons on a single source of plant material could be made within a short series of experiments such as changing the conditions used for extraction.

Extraction of a new sample of recently dried feverfew leaves with carbon dioxide at 250 bar and 45°C at 0.85 ml min^{-1} and trapping in liquid nitrogen gave 0.07% of parthenolide in a pale yellow extract. Milder extraction conditions using a pressure of only 120 bar gave a very similar yield (0.08%). The chromatogram (Fig. 2) of the extract at 250 bar showed that more monoterpenes had been collected than in the trial study, confirming the improvement in the trapping efficiency. The other major peaks were subsequently identified, by GC–MS [23] and a comparison with standards, as chrysanthenol acetate (0.10%) and camphor (0.08%), both of which have been reported previously from feverfew [25]. A minor peak with a similar retention to parthenolide was identified as dihydroparthenolide.

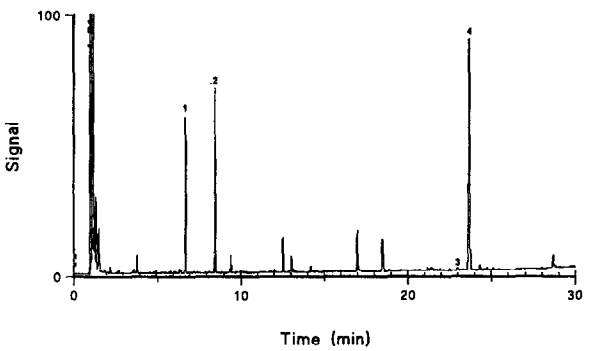

Fig. 2. Gas chromatogram of an extract of freshly dried feverfew obtained with carbon dioxide at 250 bar and 45°C at 0.85 ml min^{-1}. Conditions: column, BP1 (25 m × 0.25 mm I.D.); temperature, increased from 60 to 300°C at 8°C min^{-1}, then isothermal at 300°C for 8 min. Peaks: 1 = camphor; 2 = chrysanthenol acetate; 3 = dihydroparthenolide; 4 = parthenolide.

Extraction using modified carbon dioxide

Because the yields of parthenolide were lower than the average content of 0.42% reported by Jessup [26] for freshly dried material, the effect of modifiers in the extraction fluid was examined. These often increase the recoveries of relative polar analytes from complex matrices [27, 28]. The addition of either 4% of methanol or 4% of acetonitrile at 250 bar and 45°C gave a waxy green extract from dried feverfew and the yield of parthenolide virtually doubled from 0.07% to 0.16% or 0.14%, respectively. In each instance the yields of the more volatile and less polar monoterpenes were virtually unchanged, which suggested that they had been fully extracted with carbon dioxide alone. Water saturated carbon dioxide (produced by bubbling the liquid carbon dioxide through a water trap at room temperature) was also examined but although it gave a yield of 0.14% of parthenolide, it gave a slightly reduced yield of the less polar essential oils (chrysanthenol acetate was reduced from 0.10% to 0.06% and camphor from 0.08% to 0.04%).

All the modifiers had the disadvantage that they were less selective than carbon dioxide and increased the amount of extraneous material that was extracted. For example, methanol or acetonitrile modifiers gave a dark green waxy material with a spectrum corresponding to chlorophyll (characteristic peaks at 214, 322, 436 and 665 nm). In contrast, carbon dioxide alone gave a yellow–orange extract, the colour of which could be attributed to carotenoids (the UV spectrum contained three

characteristic bands around 446 nm). The presence of carotenoids was expected, as Favati *et al.* [29] have reported that at 300 bar and 40°C, 96% of the carotene and 30% of lutein could be extracted from plant samples by SFE with carbon dioxide.

The solubility of the parthenolide in carbon dioxide was not thought to be the cause of its low extraction with the unmodified fluid, as oxygenated sesquiterpenes have been reported to have good solubility in supercritical carbon dioxide [30]. It was also possible to extract readily the related sesquiterpene lactone santonin from the cellulose model sample matrix with carbon dioxide alone [22]. It therefore appeared that parthenolide may be present at different sites on the plant matrix, from some of which it can be readily extracted, but it is more tightly held to others and would require the addition of a polar modifier to be released. This idea of an analyte being present as a "free" and "bound" form has also been put forward by King *et al.* [31], who investigated the extraction of rape seed with liquid carbon dioxide.

An alternative idea was that parthenolide may be microencapsulated physically within the plant by means of the glandular trichomes, which are present on the leaves of feverfew [32]. The effect of extraction on these trichomes was investigated by using scanning electron microscopy (SEM) to examine air-dried feverfew leaves exposed to various conditions. Before extraction the glandular trichomes were intact but after extraction the trichomes were ruptured and empty. Sugiyama and Saito [7] have reported that supercritical carbon dioxide could rupture similar oil-containing structures present on lemon peel. However, physical entrapment is unlikely to be the cause of the limited recovery as the trichomes were ruptured with carbon dioxide alone.

The feverfew samples were also exposed to supercritical carbon dioxide plus 10% of methanol but the glandular trichomes underwent a different physical change, "collapsing" on exposure to the solvent. Blakeman and Atkinson [32] discovered a similar phenomenon with the feverfew glandular trichomes when using conventional liquid extraction with chloroform.

Timed extractions from plant material

To determine if parthenolide was being only slowly released from the plant material, extraction with carbon dioxide at 250 bar and 45°C was monitored by collecting extracts at 1-min intervals. Almost all of the "free" parthenolide was collected in the second min and the extraction was apparently complete within 4 min. This contrasted with the extraction of santonin from the α-cellulose model matrix, which took 20 min using similar extraction conditions [22]. This rapid but incomplete extraction from the feverfew plant material suggested that only surface or "unbound" oils were being extracted under these conditions.

Use of an in-line cellulose or silica trap

Because of the colour and extraneous material in the extract, the use of on-line traps was investigated to try to obtain a cleaner extract. A second extraction vessel (10 ml) packed with powdered α-cellulose was placed in the column oven between the sample extraction vessel and the detector. On extraction of feverfew with carbon dioxide, at 250 bar and 45°C most of the plant pigments were retained on the cellulose to give a pale yellow extract. The gas chromatogram showed that virtually none (<5%) of the volatile components had been retained and this was confirmed by subsequently washing the cellulose with methanol-modified carbon dioxide (5:95). With carbon dioxide extractions this trap would retain the pigments for about 2 h of extraction before a significant colour started to appear in the extracts.

To obtain greater discrimination, the cellulose trap was replaced by a short chromatographic column packed with Spherisorb S5W silica. This combination had been successfully tried during the studies with the model plant system and enabled a discrimination to be obtained between the terpene hydrocarbons and the sesquiterpene lactone santonin [22]. On extraction of feverfew with carbon dioxide at 250 bar and 45°C a series of six bands were detected by the UV monitor and collected separately. On analysis by GC these showed only poor discrimination between the volatile terpenes. Both chrysanthenol acetate and camphor were present in fractions C, D and E (Fig. 3). When no more components were being eluted from the column, the extraction vessel was switched out of the eluent stream. The silica column was then eluted with methanol–carbon dioxide (10:90) to give a final

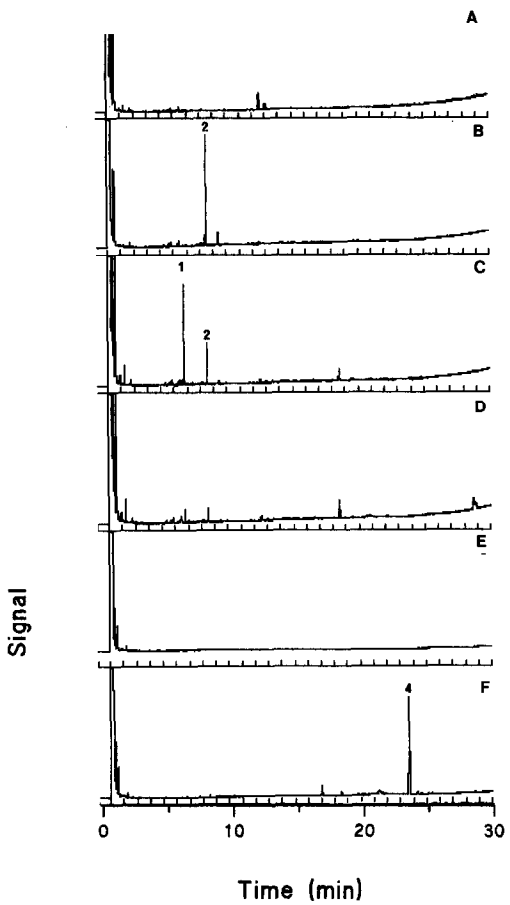

Fig. 3. Selective extraction of feverfew using an on-line silica trap. Chromatograms of successive fractions from freshly dried feverfew with a silica trap in-line. Eluent: carbon dioxide at 250 bar and 45°C at 0.85 ml min^{-1} to give fractions A–E then 10% methanol in carbon dioxide with the extraction vessel switched out of the eluent flow to give fraction F. GC conditions and peaks as in Fig. 2.

fraction which contained about 80% pure parthenolide in a yield of 0.18% (Fig. 3F). Because the modified eluent was only used after the extraction vessel had been switched out of the system, no extraction of the more polar materials or pigments occurred from the plant material.

Preparative-scale SFE

In order to prepare a larger amount of parthenolide, a new sample of dried feverfew (10 g) was extracted with carbon dioxide at 250 bar, 45°C and 0.85 ml min^{-1} to give an orange-yellow extract. This was separated on a silica gel TLC plate using chloroform–acetone (95:5) to give parthenolide (14.3 mg, 98% pure by GC), whose structure was confirmed by comparison using IR, ^{1}H and ^{13}C NMR spectroscopy and mass spectrometry with literature values and with an authentic sample.

Effect of sample preparation

Because many of the components of feverfew were volatile or unstable and might be lost on drying or storage, extracts of freshly dried leaves and stored dried samples were compared. With carbon dioxide a sample of freshly dried leaves (4 h at 50°C) gave a yield of parthenolide of 0.38%, which decreased on storage at room temperature for 1 year to 0.31% and after 2 years to 0.18%. A number of additional peaks were present in the extracts of the older samples and can be attributed to degradation products (Fig. 4). Commercial feverfew samples which were 4 years old yielded only 0.002% of parthenolide. The monoterpenes had also decreased markedly and were virtually absent after 4 years.

Differences of more than 20% in the initial parthenolide levels were observed between freshly dried materials from individual plants, whether collected in the wild or cultivated. Triplicate samples were therefore taken from a single plant and examined by SFE and GC. The variation in the parthenolide content was about 14% R.S.D., similar to that obtained by Hawthorne *et al.* [10] in a study of basil (R.S.D. 6–17%).

As it has been reported that the trichomes on the

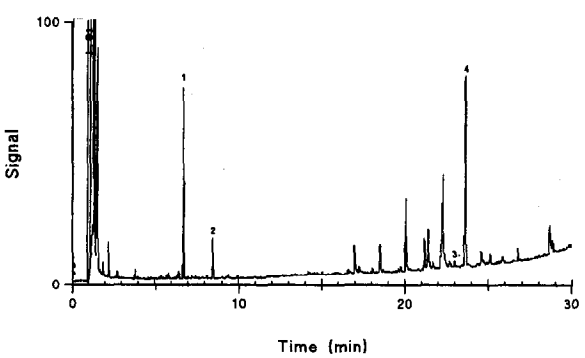

Fig. 4. Gas chromatogram of an extract of a 1-year-old sample of dried feverfew from the same plant as Fig. 2. Extraction, analysis and peaks as in Fig. 2.

leaves were the source of parthenolide [32], parts of two plants grown from seeds obtained from different suppliers were compared but the levels of parthenolide in the leaves and in the flowering heads were 0.26% and 0.21%, respectively for the first plant, and 0.18% and 0.16% for the second. In each instance the monoterpenes patterns were very similar.

Varieties

A number of samples of leaves were taken from different varieties of feverfew grown in the Chelsea Physic Garden. Two-year-old dried plant material was examined and the most common variety Schultz Bip. contained 0.13% of parthenolide. Similar levels were present in Balkan Peninsula (0.13%) and Schneeball varieties (0.12%) but lower levels were found in Golden Veis (0.05%), Boule de Neige (0.05%), and Flore Pleno (0.01%) varieties. The Boule de Neige and Schneeball plants were thought to be the same variety from different original locations. These differences may not be very significant, as Hylands [33] found a similar variation in parthenolide content during a season and between seasons.

These differences emphasize a probable need to assay and control the level of parthenolide present in commercial herbal products as plant mass alone is not a good indication of the amount of the lactone. A brief survey of a range of tablets and capsules marketed as containing feverfew, which had been stored for 2 years, suggested that many contained little or no parthenolide, with many of the tablets having less than 10% of the expected level from the stated content of plant material. This result agrees with a study of the biological activity of commercial samples which found levels much lower than claimed [34]. These results emphasize a need for levels of parthenolide in herbal medicines to be controlled and for the probable necessity to indicate an effective shelf life and storage conditions.

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