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Comparison of Supercritical Fluid and Solvent Extraction of Feverfew (*Tanacetum parthenium*)

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High performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC) analyses are used to show that supercritical fluid extraction (SFE) using carbon dioxide is an effective means of extracting a range of components from feverfew samples. It was found that the specific compositions of solvent extracts and SFE extracts are different. The amounts of the presumed active ingredient parthenolide have been confirmed to be very variable. Feverfew seeds were particularly rich in parthenolide, while dried powdered samples contained less and, in one case, none at all. The tunability of SFE has been demonstrated, and it was shown that successive extractions at 100 and 200 atm separate the extracted components into two mutually exclusive groups, with important implications for simplifying subsequent analyses.

Introduction

Feverfew is a traditional herbal remedy for a wide range of medical conditions, including migraine and arthritis[1−4]. The plant contains a large number of natural products, but the active principles probably include one or more of the sesquiterpene lactones known to be present, including parthenolide (Figure 1)[5−6]. Feverfew can be taken in a variety of forms, including the raw leaf or tablets or capsules containing dried leaves or aerial parts of the plant. There can be considerable variations in the content of materials said to be 'feverfew', due to adulteration with other plant species, the use of different parts of the plant, different times of year at which the plant is harvested, and also the known instability of sesquiterpene lactones in storage[5]. There is therefore a need for methods of analysing and assaying feverfew samples, especially those which subject the samples to mild conditions, in order to minimise the chance of sample degradation. Earlier methods for the analysis of feverfew include solvent extraction followed by infrared spectroscopy, which can only indicate total sesquiterpene lactone concentration[7]. An HPLC method with UV detection (following solvent extraction) can give data on individual species present but, because the sesquiterpene lactones lack strong UV chromophores, it is necessary to carry out a derivatisation step prior to HPLC separation[8].

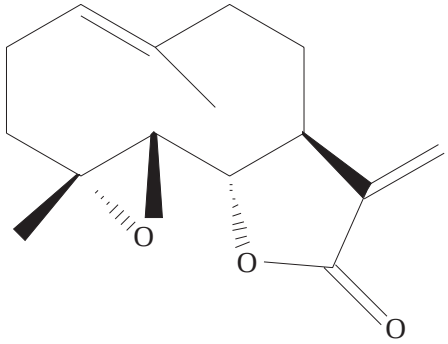

Figure 1. The structure of parthenolide

Supercritical fluid extraction (SFE) has been developed as a technique which can lead to rapid, selective extraction from a variety of matrices under conditions which are much milder than those needed for solvent extraction[4,9−10]. Smith and Burford[10] have reported SFE studies on feverfew samples, with GC analysis of the extracts. These showed that supercritical carbon dioxide could be used to extract sesquiterpene lactones, and they investigated methods of optimising the extraction process. As their GC analysis used flame ionisation detection, however, determination of the separated components of the extract was not possible, although parthenolide itself could be identified by comparison with a standard sample.

Supercritical fluid chromatography (SFC) is particularly useful for the analysis of thermally sensitive materials, and it can also be interfaced to a range of spectroscopic detectors to identify individual members of complex mixtures[9,11−14]. There have been a number of published applications of SFC to the analysis of natural products and foodstuffs, including polyprenols in *Gingko biloba* leaves[15], triglycerides in *Aquilegia vulgaris*[16], an SFC-UV-FTIR-FID study on extracts from an Azeri plant of the genus *Ferula*[17], and triglycerides from a variety of cheeses[18].

We have therefore conducted an extensive study of extractions from feverfew samples of several types (fresh and dried leaves, seeds, and a number of dried powder samples), using both conventional solvent extraction and SFE, and subjected the resultant extracts to HPLC and SFC analysis with UV and FI detection, to obtain much more detailed information about the constituents of the original samples. In the present paper we report a comparison of solvent extraction and SFE (with HPLC or SFC analysis).

Experimental

Chemicals

Feverfew samples were prepared from locally grown (at Nottingham University) material, together with dried powdered samples from a variety of sources. SFC-grade CO_2 was supplied by Air Products (Rotherham, U.K.). 9-Thiomethylanthracene was prepared by the method described in Reference 8.

Solvent extraction

Samples of feverfew were weighed accurately and extracted by stirring in refluxing chloroform for 2 hours. The extracts were filtered, dried over $MgSO_4$, and evaporated to dryness. The feverfew extracts were kept in a freezer at –15°C prior to analysis.

Supercritical Fluid Extraction

The extraction system used in the present work was a Suprex MPS/225 multipurpose SFE-SFC system (Roth Scientific, Basingstoke, Hampshire, U.K.), employing both off- and on-line SFE modes. A schematic diagram of the SFE-SFC system is given in Figure 2. In all cases, the extraction vessels provided with the system were used. When using the instrument in off-line mode, the extract was collected in a Jasco glass collection vessel, after depressurisation through a 50 μm glass restrictor. In order to minimise problems caused by blockage of the restrictor, the collection unit was placed inside a small ultrasonic bath, of the type used for cleaning glassware. The cavitation caused by the ultrasound prevented the restrictor from clogging with precipitated material, by distrupting any blockage as soon as it formed. The solvent used to collect the sample was dichloromethane.

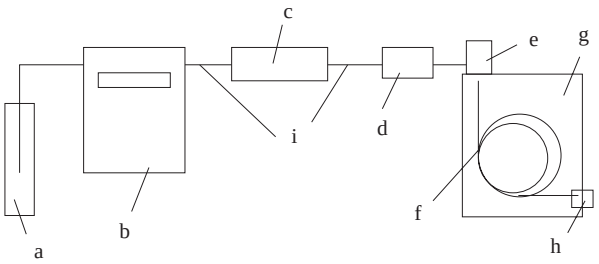

Figure 2. Schematic diagram of on-line SFE-SFC system a) CO_2 cylinder b) pump c) extraction vessel d) cryotrap-heater e) injection port f) chromatography column g) column oven h) detector i) transfer lines.

For on-line SFE-SFC operation, the extract is deposited in the cryotrap which is part of the MPS/225, from where it is automatically transferred to the SFE system-described below.

High Performance Liquid Chromatograph

The HPLC apparatus used was a Waters Associates Model 440 HPLC unit, with a Pye-Unicam LC-UV detector, operating at 369 nm. The column was a 300 mm x 7.8 mm μPorasil (10μm) steel column. The mobile phase was 65% $CHCl_3$ and 35% hexane, with a flow rate of 3 cm^3/min. Sample injections in the range 5-20 μl were used.

Supercritical Fluid Chromatograph

The on-line SFE-SFC experiments used the SFC equipment of the Suprex MPS/225 instrument. This comprises a Keystone 10 cm x 1 mm i.d. packed column, with C_{18} 5μm packing, with a flame ionisation detector.

Results and Discussion

a) Solvent extraction and SFE with HPLC analysis

In this part of the study, solvent ($CHCl_3$) extraction was carried out on fresh feverfew leaves, seeds and a powdered sample. The powder was also extracted by acetone, and by supercritical CO_2 using the Suprex instrument in off-line mode (supercritical CO_2, at 400 atm for 10 minutes at 100°C, in a 3 cm^3 extraction

vessel). All samples were then derivatised by the Michael addition of 9-thiomethylanthracene, as described by Dolman *et al.* (8). Identification of certain sesquiterpene lactones was made by comparison with retention time data on standard samples. These gave the following values (min) under the conditions employed here (Table 1).

Table 1. HPLC Retention time data of some sesqueterpene lactones

Compound name	Retention time (min)
parthenolide	2.2-2.4
tanaparthin-α-peroxide	3.7-3.8
tanaparthin-β-peroxide	4.5-4.8
3-β-hydroxycostunolide	17.7
canin	23.5

All five of these species were identified in the chromatogram of the fresh leaves, together with a number of other, unidentified components. The seeds contained only parthenolide and tanaparthin-α-peroxide, while the powder showed parthenolide and the tanaparthin peroxides (the latter is only in the organic extracts). By measuring the peak areas corresponding to parthenolide (2.2 min) it was possible to estimate the amount of this component in each of the extracts (Table 2).

Table 2. Parthenolide content of feverfew samples (Dolman HPLC assay)

Sample	Weight % parthenolide
fresh leaves ($CHCl_3$)	0.660
seeds ($CHCl_3$)	>1.134
powder ($CHCl_3$)	0.062
powder (acetone)	0.040
powder (SFE)	0.091

Several conclusions can be drawn from these results

i) The seeds contain very high levels of parthenolide. This may be associated with parthenolide being an insecticide or antibiotic (i.e., fungicide or bacteriocide), concentrated in the seeds to protect them from insect attack prior to germination.

ii) Both the seeds and the fresh leaves contain much more parthenolide than the powdered samples. This conclusion supports that of Smith and Burford[10], who suggest that parthenolide decomposes on standing, but it may also reflect the fact that parthenolide is harder to extract from dried samples.

iii) There is a significant difference between the solvent extraction and SFE results for the powder. Significantly more parthenolide was extracted in 10 minutes by supercritical CO_2 than in 2 hours by $CHCl_3$ or acetone. The extraction by organic solvent is clearly non-quantitative, but there is no guarantee that total extraction has been achieved by SFE. There is a further difference between solvent extraction ($CHCl_3$) and SFE, as shown by comparing the two HPLC traces (Figure 3) (in order to show clearly the minor components, the parthenolide peaks are off-scale). Although the SFE chromatogram shows more parthenolide than the $CHCl_3$ extraction, supercritical CO_2 has extracted a smaller number of sesquiterpene lactones than has $CHCl_3$. The components missing from the SFE extract are presumably the more polar ones, which are not so soluble in supercritical CO_2.

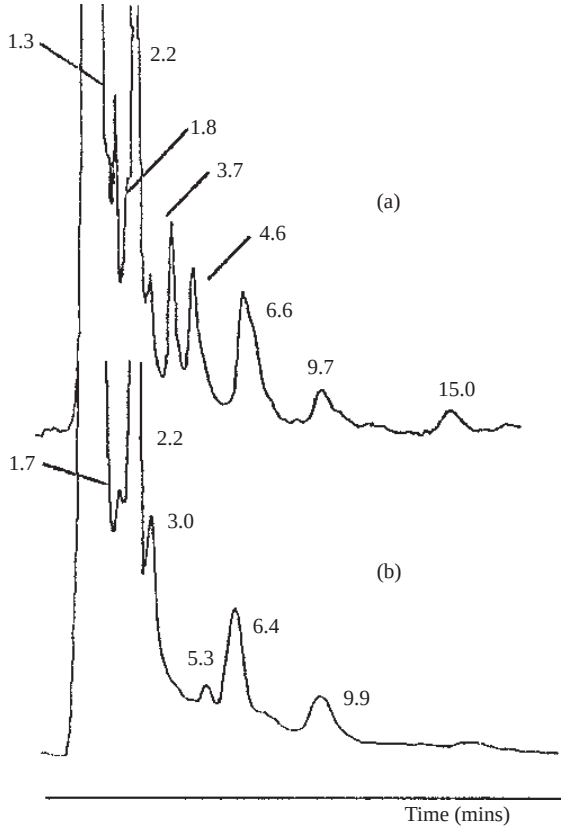

Figure 3. Comparison of the HPLC chromatograms of (a) chloroform and (b) supercritical carbon dioxide extracts of feverfew powder (For conditions see text; retention times in minutes).

b) On-line SFE-packed column SFC

The Suprex MPS/225 system can be used to carry out repeated supercritical fluid extractions, with each one being linked on-line to packed-column SFC. This enables a series of extractions to be carried out under different conditions, to show the tunability of supercritical fluids in extraction. This property is potentially very valuable in simplifying the analysis of complex mixtures, by performing group separations at the extraction stage, and also by choosing conditions to minimise the extraction of unwanted or uninteresting material, both of which are difficult or impossible to achieve by conventional solvent extraction.

Three successive 10 minute extractions of a dried, powdered sample of feverfew were carried out at a pressure of 100 atm, at 100°C in 0.5 cm^3 Suprex extraction cell, with a sample of 0.04g, and with the cryotrap at 0°C. The SFC conditions were as follows: pressure programme 100-450 atm at 10 atm/min, with 5 min at 450 atm; temperature 100°C; FID temperature 375°C. The resultant chromatograms are shown in Figure 4. The sample was then subjected to three further extractions, with all conditions identical except for the extraction pressure of 200 atm, with the chromatograms shown in Figure 5. Further extractions, this time at 300 atm, gave chromatograms which were very similar to those at 200 atm, but much weaker (Figure 6).

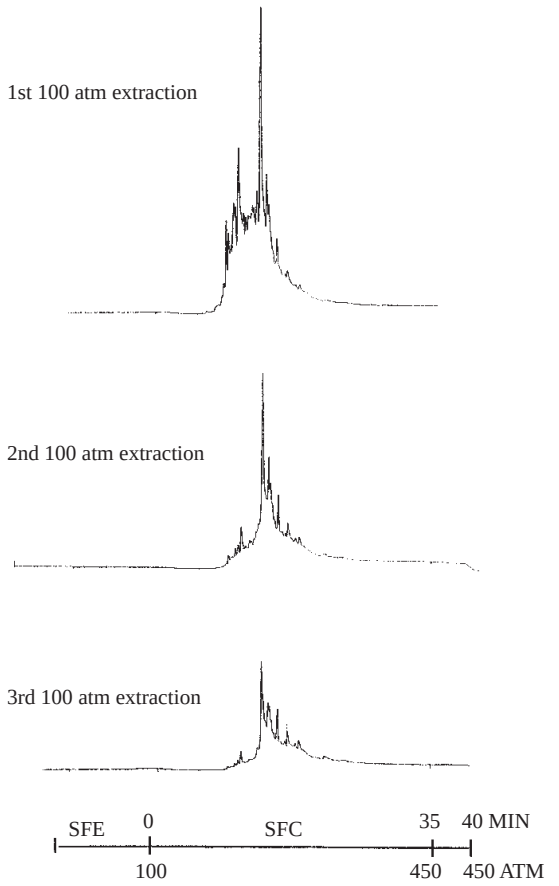

Figure 4. On-line SFE-packed SFC of dried powdered feverfew, with repeat extractions at 100 atm (For conditions see text).

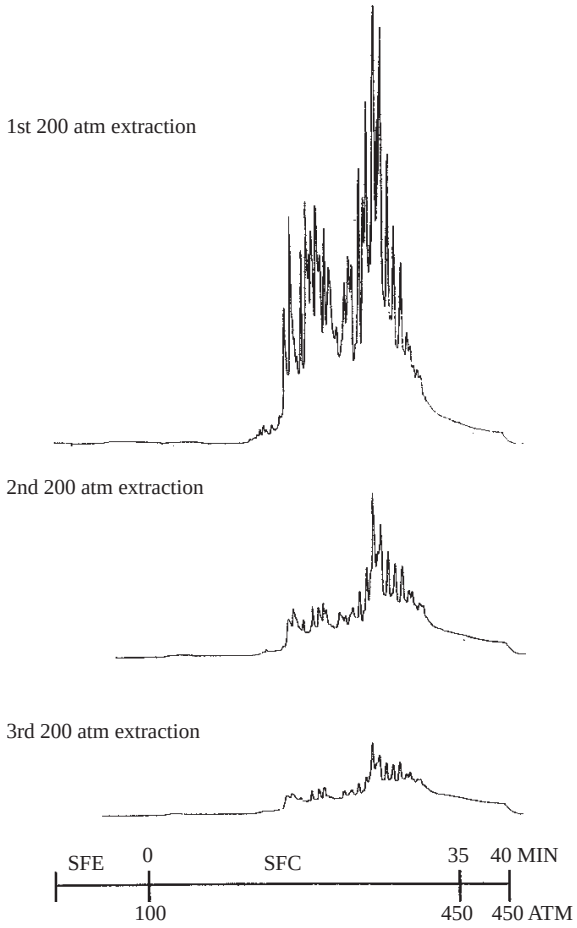

Figure 5. On-line SFE-packed SFC of dried powdered feverfew, with repeat extractions at 200 atm (For conditions see text).

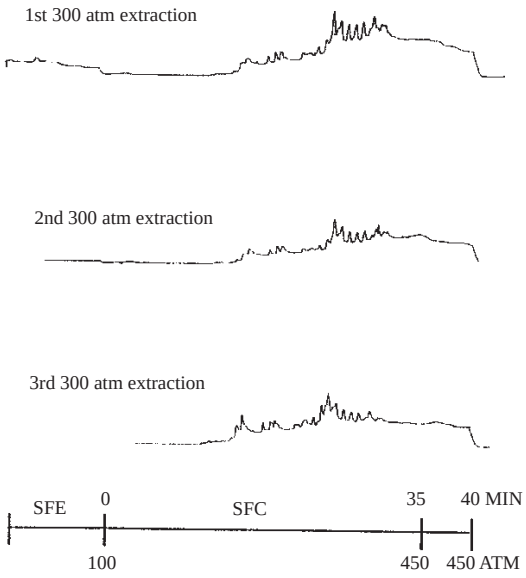

Figure 6. On-line SFE-packed SFC of dried powdered feverfew, with repeat extractions at 300 atm (For conditions see text).

From Figures 4 and 5 one can draw several conclusions. Thus the supercritical extract of dried feverfew powder is a complex multicomponent mixture, as already suggested above, and more detailed work with spectroscopic detectors will be necessary to identify the components. The successive extractions at 100 atm show that the early-eluting components are extracted more rapidly. Later-eluting components are still being extracted on the third 100 atm extraction, whereas the early-eluting components can only just be detected in the third extract (Figure 4). A very important observation is that different compounds are extracted in the 100 and 200 atm extractions; Figure 7 shows that comparing the first extraction at each pressure, there is virtually no overlap, i.e., none of the components extracted at 200 atm can ever be extracted at a lower pressure. This result has important implications for sample pre-treatment, demonstrating the use of the tunability of SFE in providing a simpler set of extracts for analysis. Increasing the pressure from 200 to 300 atm has little effect on the extraction. Comparison of the third 200 atm extraction (Figure 5) with the first 300 atm extraction (Figure 6) shows that the chromatograms are almost identical. This could be due to the fact that such an increase in pressure leads to only a small increase in mobile phase density and hence solvating power, or that there are no further components which can be solubilised by density increase. Figure 6 shows also that replicate extractions at 300 atm show only small decreases in the peak sizes, indicating a slow extraction rate compared to those at 100 and 200 atm Quantitative removal of extractable material from powdered feverfew, even by SFE, is therefore likely to be a lengthy process.

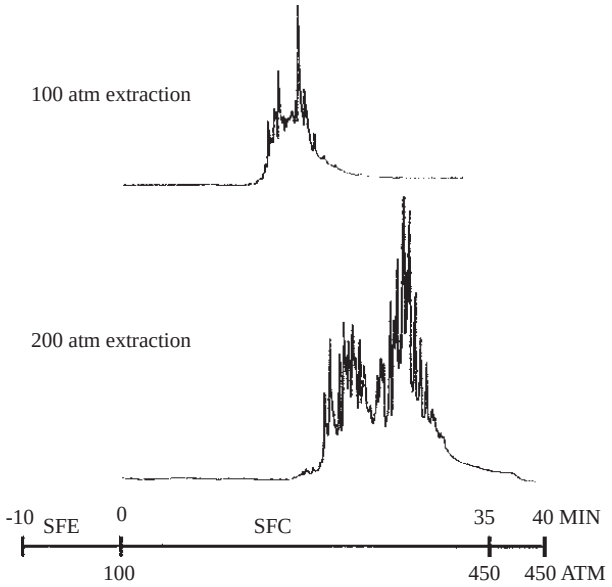

Figure 7. On-line SFE-packed SFC of dried powdered feverfew, comparing first extractions at 100 and 200 atm.

Conclusions

We have shown that the assay of Dolman *et al.* is a valid method for estimating the amount of parthenolide in feverfew samples, and that SFE can give good extractions in quite short times. However, the material extracted by solvent extraction and SFE is not the same, and total extraction by SFE is still a time-consuming process. It is clear that the amount of parthenolide in feverfew samples is very variable. The tunability of SFE has been amply demonstrated by extracting feverfew at different pressures.

References

1. M. I. Berry, **Pharm. J.**, (1984) 611.
2. E. S. Johnson, N. P. Kadam, D. M. Hylands, **Br. Med. J., 291** (1985).
3. W. A. Groenewegen and S. Heptinstall, **J. Pharm. Pharmacol.**, **42** (1990) 553.
4. A. M. G. Brown, C. M. Edwards, M. R. Davey, J. B. Power, K. C. Lowe, **J. Pharm. Pharmacol.**, **49**, 558 (1997).
5. T. R. Govindachari, B. S. Joshi and V. N. Kamat, **Tetrahedron**, **21** (1965) 1509.
6. W. A. Groenewegen D. W. Knight and S. Heptinstall, **J. Pharm. Pharmacol.**, **38** (1986) 709.
7. E. Bloszyk, B. Geppert and B. Drozdz, **Planta Med.**, **34** (1987) 79.
8. D. M. Dolman, D. W. Knight, U. Salan and D. Toplis, **Phytochem. Anal.**, **3** (1992) 26.
9. T. L. Chester, J. D. Pinkston and D. E. Raynie, **Anal. Chem.**, **66** (1994) 106R.
10. R. M. Smith and M. D. Burford, **J. Chromatogr.**, **627**, 255 (1992).
11. R. M. Smith (Ed.), **Supercritical Fluid Chromatography,** Royal Society of Chemistry, London, (1988).
12. K. Jinno (Ed.), **Hyphenated Techniques in Supercritical Fluid Chromatography and Extraction**, Elsevier, Amsterdam, (1992).
13. T. J. Jenkins, M. Kaplan, M. R. Simmonds, G. Davidson, M. A. Healy and M. Poliakoff, **Analyst**, **116**, 1305 (1991).
14. T. J. Jenkins, M. Kaplan, G. Davidson, M. A. Healy and M. Poliakoff **J. Chromatogr.**, **626**, 53 (1992).
15. H. Huh, E. Staba and J. Singh, **J. Chromatogr.**, **600**, 364 (1992).
16. M. Demirbuker, L. G. Blomberg, N. Urban Olsson, M. Bergqvist, B. G. Hersloef and F. Alvarado Jacobs, **Lipids**, **27**, 436 (1992).
17. T. J. Jenkins, M. Kaplan, G. Davidson , **J. High-Res. Chromatogr.**, **17**, 160 (1994).
18. M. Kaplan, G. Davidson, M. Poliakoff, **J. Chromatogr., A, 673,** 231 (1994) .
19. R. M. Smith, **LC-GC**, **13**, 930 (1995).