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Short communication

Extraction of Tanshinone IIA from *Salvia miltiorrhiza bunge* using supercritical fluid extraction and a new extraction technique, phytosol solvent extraction

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Abstract

Supercritical carbon dioxide with and without a methanol modifier has been compared with a new portable cold extraction technique, phytosol solvent extraction (PSE), for the extraction of Tanshinone IIA from Salvia miltiorrhiza bunge. High-performance liquid chromatography was used to analyse the extracts. The results show that the yield extracted by 10% methanol-modified supercritical carbon dioxide was the highest (0.038%, w/w) and the yields extracted by supercritical carbon dioxide at 60°C and 250 kg/cm^2 and phytosol solvents (A,B and D) were similar (0.029–0.032%, w/w). © 1998 Elsevier Science B.V.

Keywords: Supercritical fluid extraction; Phytosol solvent extraction; *Salvia miltiorrhiza*; Tanshinone IIA

1. Introduction

The root and rhizome (Tan-shen) of *Salvia miltiorrhiza bunge* has been commonly used in traditional Chinese medicine for promoting blood circulation to remove blood stasis, clearing away heat, relieving vexation, nourishing blood and tranquillising the mind and cooling the blood to relieve carbuncles. Its components, Tanshinones, have shown some actions of broad-spectrum bactericide, dilating coronary artery and increasing coronary flow [1], cytotoxic activity [2,3] and modulating the effect on mutagenic activity [4].

Supercritical fluid extraction (SFE), which uses predominantly supercritical carbon dioxide as an extraction medium, has been used for a variety of industrial, environmental, food and chemical applications [5]. The application of SFE to natural products, e.g., the extraction of carotenoids, lipids, flavour and fragrance compounds, steroids and triterpenes, alkaloids and mycotoxins, has recently been reviewed [6].

Phytosol solvents are a new range of benign, non-CFC solvents, commercially available for the extraction of analytes from natural products, foods and environmental matrices. The system used in this work is a portable, hand-held extraction unit, capable of extraction sample sizes up to approximately 100 g.

Extraction, using organic solvents, of Tanshinones

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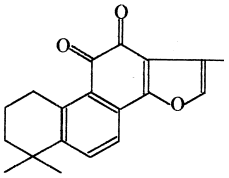

Fig. 1. Structure of Tanshinone IIA.

from Tan-shen has been reported by several workers with subsequent analysis by high-performance liquid chromatography [7] and spectrophotometry [8]. However, no report has been done on the use of SFE for the extraction of Tanshinones from the herbal medicine. The purpose of this study therefore, was to develop an off-line method for SFE of Tanshinone IIA (Fig. 1) from the medicinal plant with analysis by HPLC and to compare SFE with a new portable, cold extraction technique (phytosol solvent extraction, PSE).

2. Experimental

Tan-shen was provided by Wuhan Second Hospital (Wuhan, China) and was ground into powder using a food processor and stored until required. Tanshinone IIA standard was purchased from the China Institute for Drugs and Biological Products Identification (Beijing, China).

Supercritical fluid extraction was undertaken on a Jasco system, which operates with two reciprocating pumps, a master pump (Jasco 880-PU) fitted with a cooling jacket on the pump head and a second pump (slave pump) for the addition of organic modifier, a thermostatically controlled oven (Jasco 860-CO) and an oscillating variable restrictor (back pressure regulator, Jasco 880-81) for depressurisation. The extracted analytes are collected in a screw cap glass vial (25 ml) with a rubber septum at the top. A metal extension to the restrictor is used to pierce the rubber septum and allow collection directly in the collection solvent (methanol). A hypodermic needle with one C_{18} Bond-Elute cartridge attached also pierces the septum. This has two functions, the first to allow the CO_2 to vent to the atmosphere, and the second to remove residual analyte from the venting gaseous CO_2. After each extraction the cartridges are back-flushed with a suitable solvent (methanol) to ensure quantitative recovery of the extracted analyte.

3. Procedure for supercritical fluid extraction

A known quantity of Tan-shen (1 g) was placed in the extraction cell. Before the extraction was started, the extraction cell was preheated in the oven for 10 min. The extraction conditions were as follows: extraction time, static extraction for 5 min and then dynamic extraction for 30 min; temperature, 60°C; pressure, 250 kg/cm^2; flow-rate of liquid carbon dioxide, 2 ml/min. The extract was collected in a glass vial containing 4 ml of methanol. After each extraction was completed, the system (excluding the sample cell) was flushed with carbon dioxide (2 ml/min) and methanol (0.5 ml/min) for 5 min. This ensured that any residual Tan-shen was removed from the transfer line. All collected solution was quantitatively transferred to a 25 ml volumetric flask and made up to the mark with methanol. This solution was further diluted 10 times prior to analysis. For methanol-modified supercritical carbon dioxide the above procedure was repeated except that the temperature was 60°C and that in addition to the flow of carbon dioxide (2 ml/min) an additional flow of methanol (0.2 ml/min) was used. This solution was further diluted 10 times prior to analysis.

4. Phytosol solvent extraction

Phytosol solvent is commercially available from ICI plc., Runcorn, Cheshire as 1,1,1,2-tetrafluoroethane. Phytosol solvent A consists of 1,1,1,2-tetrafluoroethane only while butane and dimethylether have been additionally added to phytosol solvents B and D, respectively. A known quantity of powdered Tan-shen (approximately 1.00 g) was placed in the glass extraction vessel and a filter adapter attached. Then phytosol solvent (either A, B or D) (10 ml) was introduced. The assembled extraction cell was then shaken periodically over a period of 30 min at room temperature. After the time had elapsed, the extraction cell was inverted and a glass container containing a pressure valve attached. By careful opening of the tap on the filter adapter, phytosol

solvent containing the Tanshinone IIA was collected in the sealed glass container. The nature of the benign, non-CFC solvent is such that it causes freezing of the glass collector. After a few minutes, in an ambient laboratory temperature the phytosol solvent is removed using a recycling device that preferentially removes the solvent leaving the sample residue in the collector. The extraction procedure was repeated three times per sample. Then the sample residue, from the three extracts, is dissolved in methanol and quantitatively transferred into a volumetric flask and made up to volume (25 ml) using methanol. Prior to analysis the sample was diluted 10 times.

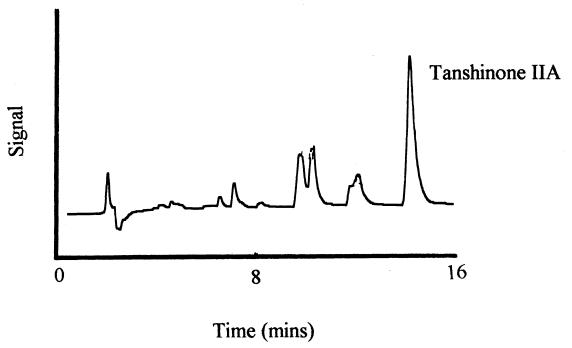

Fig. 2. High-performance liquid chromatogram of Tanshinone IIA using Phytosol A.

5. Analysis of extracts

A high-performance liquid chromatography system was used for analysis of extracts. The system is equipped with a Gilson (Anachem Ltd., Luton, Bedfordshire, UK) reciprocating pump (model 305). Sample and standards were injected (20 μl) onto a separation column (C_{18} ODS2, 25 cm×4.6 mm I.D.) obtained from Phase Separations Ltd., Clwyd, UK, and maintained at a temperature of 35°C. The mobile phase, acetonitrile–water–acetic acid (70:30:1, v/v/v), was pumped at a flow-rate of 1 ml/min. An UV–Vis detector (Jasco, UV-975) was used for monitoring the response at a wavelength of 270 nm. A linear calibration graph was produced for Tan-shen over the concentration range 0.12–12 μg/ml, and gave a correlation coefficient (r) of 0.9999 (n= 5).

6. Results and discussion

Five extraction solvent systems, i.e. phytosol A, phytosol B, phytosol D, supercritical carbon dioxide and 10% methanol-modified supercritical carbon dioxide, were used to extract Tanshinone IIA from powdered Tan-shen in order to evaluate the feasibility of PSE and SFE, and analyse the content of Tanshinone IIA in the sample. Fig. 2 shows an example HPLC chromatogram of an extract using phytosol solvent A. It is possible to identify the Tanshinone IIA peak, which appears at a retention time of approximately 15 min. [Note: All other extracts show similar chromatograms]. It is noted that the extracts using either PSE and supercritical carbon dioxide contain other diterpenes besides Tanshinone IIA as has been reported previously [7].

Fig. 3a,b shows the effect of extraction temperature and pressure on the yield of Tanshinone IIA. It can be seen that at high pressure and low temperature (i.e. high density) a high yield is obtained. However, similar yields are obtained, after 2 h, when the pressure is above 150 kg/cm^2. Also, the maximum yield is obtained in a shorter time period at higher extraction temperature.

The recovery of Tanshinone IIA from powdered Tan-shen was determined, using the five solvent systems over periods extending to 3 h for phytosol solvents A, B and D, and 2 h for supercritical carbon dioxide (with and without methanol), respectively, and are shown in Fig. 4. The highest recovery was obtained using 10% methanol-modified supercritical carbon dioxide (0.042%, w/w) and the recovery was similar between PSE and supercritical carbon dioxide (0.033–0.036%, w/w). The results for the extraction of Tanshinone IIA from powdered Tan-shen using PSE (1.5 h) and SFE with and without methanol modifier (30 min) are summarised in Table 1.

Considering that the active components in medicinal plants are changeable, depending upon their growing conditions, the season of harvest and the part of the plant utilised, it is necessary to have rapid and efficient methods of extraction and analysis, as demonstrated in this paper. Both extraction tech-

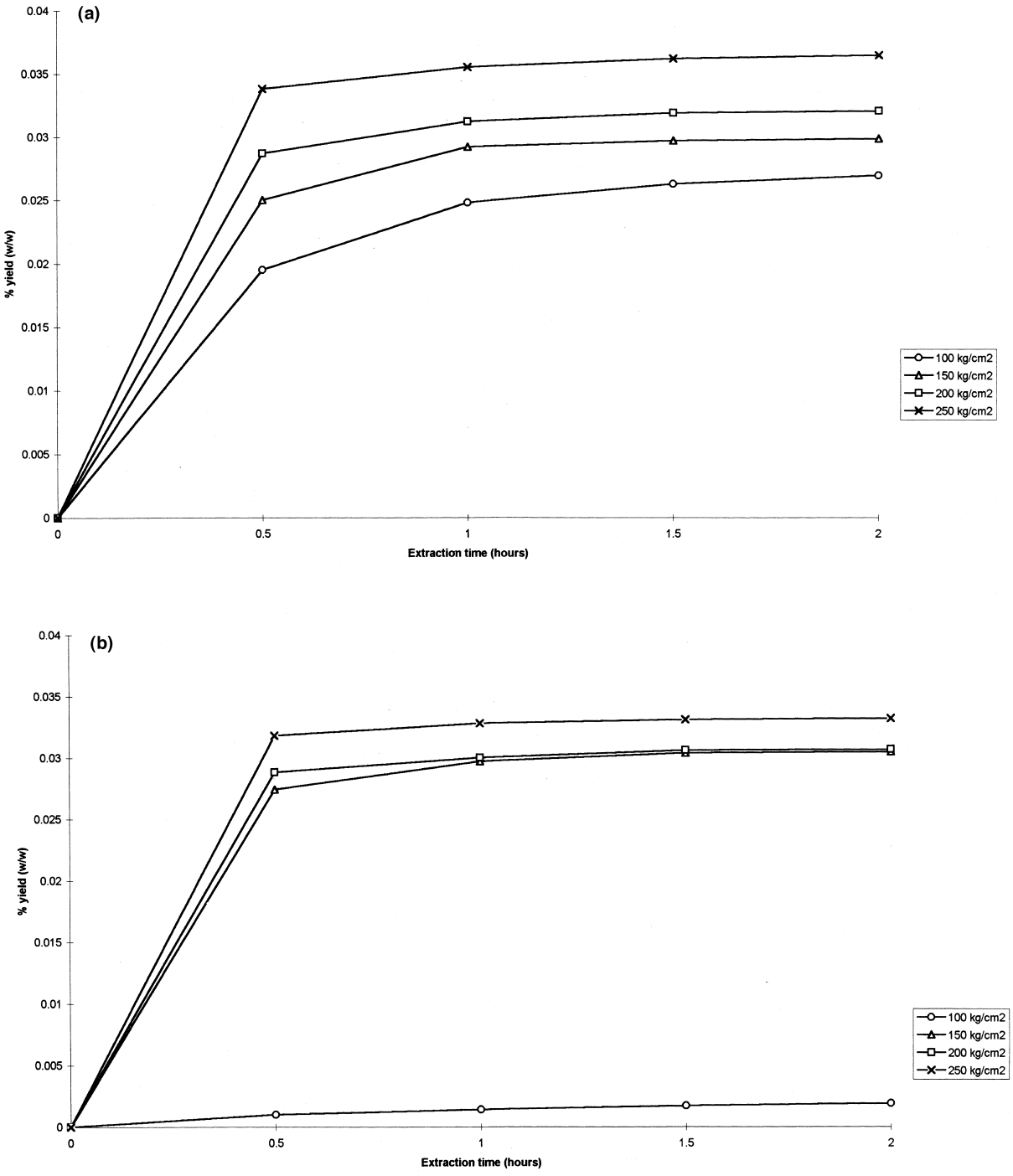

Fig. 3. Influence of pressure on the cumulative percentage yield of Tanshinone IIA extracted by CO_2 only. (a) 40°C and (b) 80°C.

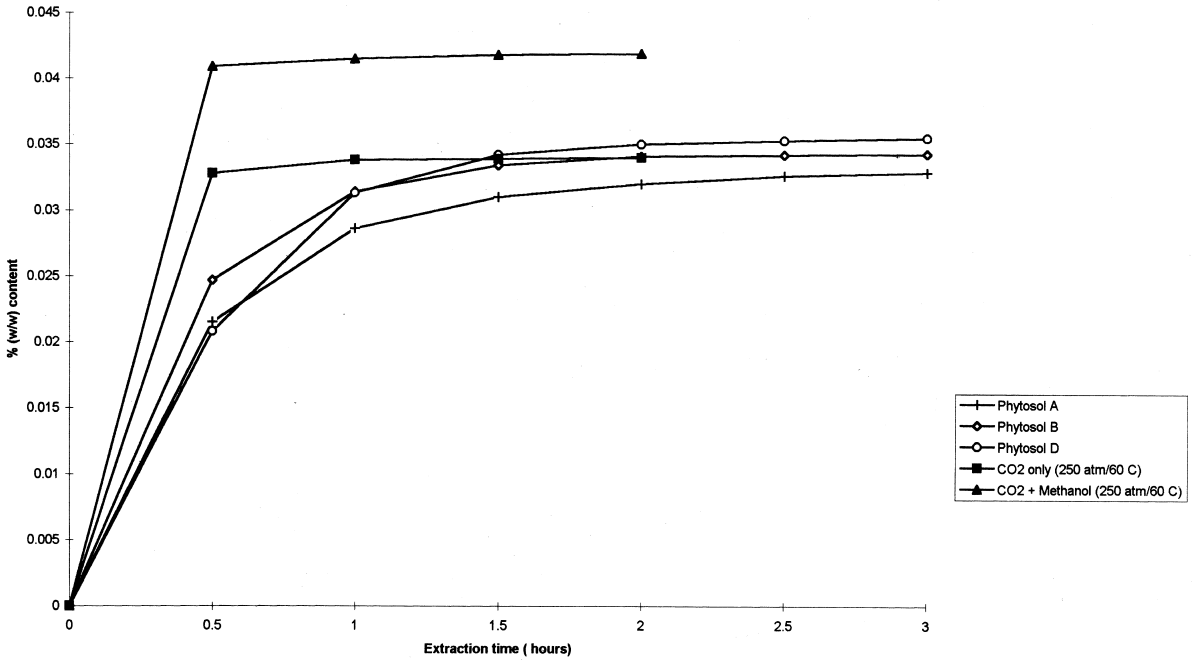

Fig. 4. Cumulative extraction yield of Tanshinone IIA from *Salvia miltiorrhiza bunge*, % (w/w). Influence of extractant solvent.

Table 1
Percentage (w/w) extraction yield of Tanshinone IIA from salvia miltiorrhiza bunge

Extractant	Extraction conditions	Percentage yield (w/w) mean±S.D. (n=5)
Phytosol A	10 ml of solvent for 30 min, repeated three times. Room temperature	0.031±0.003
Phytosol B	10 ml of solvent for 30 min, repeated three times. Room temperature	0.029±0.004
Phytosol D	10 ml of solvent for 30 min, repeated three times. Room temperature	0.033±0.002
Supercritical carbon dioxide	Temperature, 60°C; pressure, 250 kg/cm^2; flow-rate, 2 ml/min; dynamic extraction time, 30 min	0.031±0.002
10% Methanol-modified supercritical carbon dioxide	Temperature, 60°C; pressure, 250 kg/cm^2; flow-rate, 2 ml/min CO_2 and 0.2 ml/min methanol; dynamic extraction time, 30 min	0.038±0.002

niques are also available in pilot plant form for larger scale extraction.

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