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Short communication

Supercritical fluid extraction of diosgenin from tubers of *Dioscorea nipponica*

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Abstract

Supercritical fluid extraction (SFE) was used to extract diosgenin following acid hydrolysis from *Dioscorea nipponica* tuber. Diosgenin determinations were carried out using capillary GC of trifluoroacetate derivatives. Highest diosgenin yields were obtained using 3100 p.s.i. (1 p.s.i. = 6894.76 Pa) pressure for 70 min, but >82% of this yield was extracted by 40 min. Higher recoveries were obtained than by extraction using light petroleum (b.p. 40–60°C) extraction.

1. Introduction

The tuber of *Dioscorea nipponica* is extensively used in China for extraction of diosgenin sapogenin and its glycoside dioscin [1], for use as steroid intermediate in the pharmaceutical industry. The industrial extraction of diosgenin involves mineral acid hydrolysis of chopped tuber, followed by extraction using petrol [2]. This latter stage is expensive and wasteful of solvent; also low diosgenin recovery is achieved.

Analytical-scale extraction of diosgenin is usually carried out using acid hydrolysis and petrol extraction, but again the extraction stage has disadvantages. This time, the light petrol Soxhlet extraction takes much time, particularly when screening large sample numbers. Acid hydrolysis of dioscin is tedious, but essential, either before or after removal from the plant material. No reports have appeared concerning diosgenin extraction using supercritical fluid extraction (SFE), but it was considered that both analytical- and industrial-scale (SFE) extractions would save time and solvents. Previous workers have used SFE for extraction of a wide range of natural products such as oil products [3], essential oils [4], triglycerides [5] and Chinese herbal drugs [6].

2. Experimental

2.1. Materials

Tuber of *Dioscorea nipponica* was collected from natural habitat in the Hubei province, China, and identified by the Hubei Province Institute of Drug Identification. The tuber (8 g) was cut into small pieces and hydrolysed by refluxing with 50 ml of 2 *M* HCl for 3 h. It was next filtered (Whatman No. 1), washed with 5% Na_2CO_3 and water until neutral, and then dried

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at 80°C. Dried, hydrolysed tuber was then powdered prior to extraction.

2.2. Supercritical extraction

The custom-built supercritical fluid extractor [7] was operated at variable temperatures and pressures of CO_2. Samples were extracted in a 2-ml stainless-steel Upchurch short HPLC column (Anachem, Luton, UK) and the CO_2 extraction solvent (BOC) was made supercritical by pumping at 1.5–3.6 ml/min (LC pump T-414, Kontron). Samples of 0.05 g were extracted over a range of temperatures and pressures for differing periods of time. Constant extraction pressures were maintained by modification of flow-rates of the LC pump. Extracts were vented into 2 ml of $CHCl_3$ via a 20 cm × 50 μm I.D. fused-silica capillary restrictor to collect the extracted diosgenin. The extraction cell, restrictor capillary and collection solvent were maintained at constant temperature in an oven. Solvent loss by evaporation was compensated for by periodic addition of $CHCl_3$ to maintain the original volume. Sample extracts (0.5 ml) were derivatised with 0.3 ml of trifluoroacetic anhydride (TFA) (Sigma) at room temperature for 30 min, and then evaporated to dryness in a stream of nitrogen [8]. Sample residues were re-dissolved in a given volume of $CHCl_3$ containing a standard concentration of 1,8-dihydroxyanthraquinone TFA derivative (1 mg/ml) as internal standard. Diosgenin TFA standards were made from commercial diosgenin (Koch-Light) which had been re-crystallised from $(CH_3)_2CO$, and reacted with TFA as for the samples.

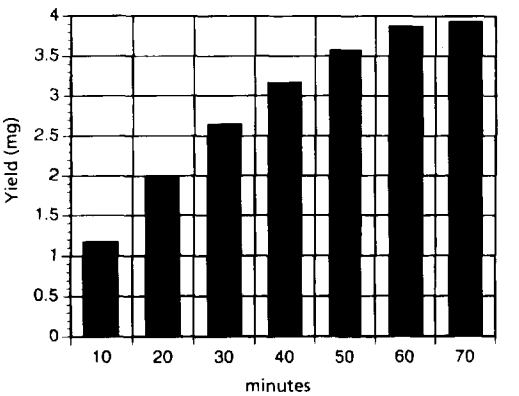

Fig. 1. SFE yields after extraction times up to 70 min (0.05 g sample, 44°C, 3100 p.s.i.).

2.3. Solvent extraction

A 200-mg amount of hydrolysed tuber was extracted in a Soxhlet extractor for 4 h with light petroleum (b.p. 40–60°C) using a 5-ml extraction thimble at a syphon rate of 4 cycles/min.

2.4. Gas chromatography

Gas chromatography was carried out using a Hewlett-Packard 5890 GC system fitted with a 5 m × 0.53 mm I.D. OV-1 capillary. Oven temperature was held at 200°C for 2 min, then

Table 1
Comparison of diosgenin yield using light petroleum extraction, with SFE over a range of pressures of CO_2 ($n = 5$ replicate extractions)

	SFE				Light petroleum ($n = 5$)
	CO_2 Pressure (p.s.i.)				
	1890	2250	2700	3100	
Diosgenin (mg/g)	19.33 (S.D. ± 0.36)	30.75 (S.D. ± 1.87)	39.23 (S.D. ± 2.82)	63.16 (S.D. ± 2.29)	47.45 (S.D. ± 5.54)

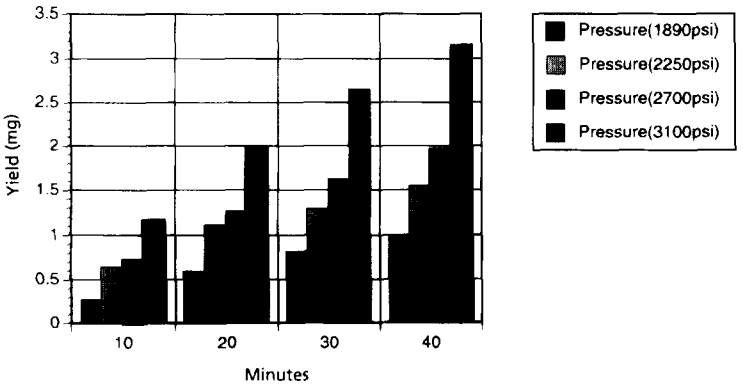

Fig. 2. SFE yields of diosgenin over a range of pressures (0.05 g sample, 44°C).

programmed from 200 to 230°C at 2°C/min; injector and flame ionization detector were kept at 260°C, and eluted components were recorded and integrated using a Hewlett-Packard 3392 A integrator. A linear calibration curve was produced for diosgenin TFA derivative over the concentration range 0.283–1.507 μg/ml, S.D. = 0.9887 ($n = 5$). The identity of diosgenin in sample extracts was confirmed by simultaneous chromatography with standards (diosgenin TFA t_R 14.8 min) and GC–MS (diosgenin TFA chemical ionization MS, M^+ 511 m/z, 397, 282, 139). SFE and solvent extraction both yielded chromatographically pure diosgenin.

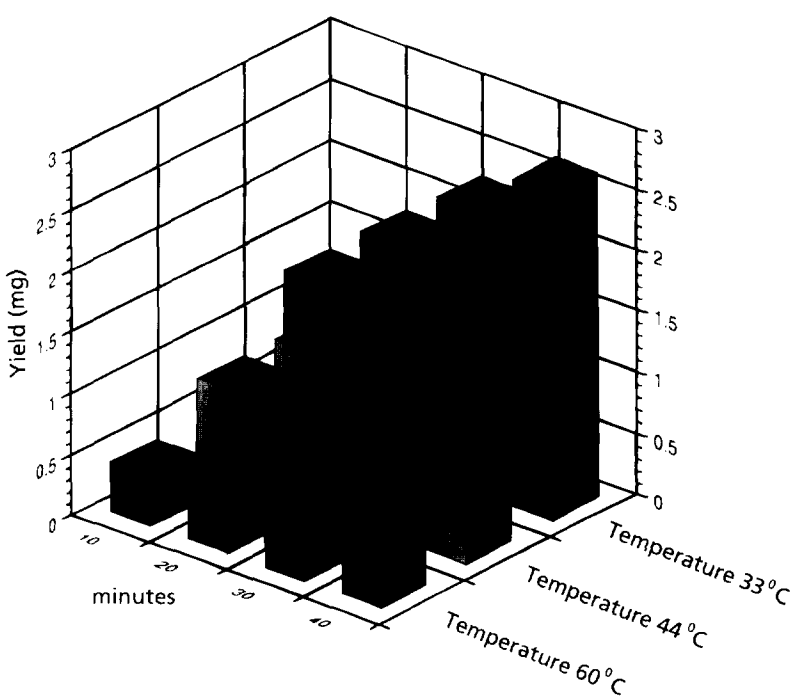

Fig. 3. SFE yields of diosgenin over a range of temperatures (0.05 g sample, 2700 p.s.i.).

3. Results and discussion

Sample amounts of 0.05 g of tuber hydrolysate were extracted over a range of time periods, temperatures, and pressures. Fig. 1 shows the effect of extraction yields over periods extending to 70 min. By 40 min >82% of diosgenin (compared to the yield at 70 min) had been extracted. The effect of extracting hydrolysate for 40 min periods at four different pressures was determined and it can be seen from Fig. 2 that highest yields were obtained at 3100 p.s.i. (1 p.s.i. = 6894.76 Pa). Table 1 shows that extraction with supercritical $C0_2$ at 44°C for 40 min at 3100 p.s.i. produces 33% greater yields than light petroleum (b.p. 40–60°C) extraction.

Fig. 3 shows the effect of extraction temperature on yield of diosgenin. Highest yield occurs at lowest extraction temperature used.

This study shows the possibility that CO_2 SFE could replace organic solvent extraction on at least the analytical scale. It could also be a realistic environmentally sound alternative to petroleum extraction in industry.

Acknowledgements

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