

Design and construction of a simple supercritical fluid extraction system with semi-preparative and preparative capabilities for application to natural products

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Abstract

The construction of a simple and versatile preparative-scale supercritical fluid extraction (SFE) system is described. The system has several options for the extraction cell and avoids the need for a special pump for fluid pressurization, one of the most expensive parts of a typical SFE system. The extracts obtained using this system are similar to those obtained by steam distillation under less favourable experimental conditions.

1. Introduction

Soxhlet extraction is the most widely used method for the extraction of vegetable material with solvents of different polarities, for both the usual extraction and selective extraction. However, this technique is time consuming and does not always produce the desired results. Comparative studies of these techniques and supercritical fluid extraction (SFE) have shown that SFE gives better yields, requires less solvent, has a shorter extraction time and is able to extract labile compounds sensitive to thermal decomposition [1–3].

Carbon dioxide is the most popular supercritical solvent for SFE because of its favourable characteristics, including mild critical conditions [$T_c = 31^\circ\text{C}$ and $P_c = 72.9$ atm (1 atm = 101 325

Pa)], high volatility and diffusibility, low viscosity, non-toxicity, non-reactivity, non-flammability, easy and ready availability and relative cheapness. These characteristics make it the ideal solvent for supercritical extraction, mainly of thermally unstable materials [4–6].

Although there are extensive experimental data on the solubility and extractivity of natural products, including steroids, alkaloids, anti-tumor compounds, essential oils and caffeine, in both pure and modified supercritical CO_2 , there are few publications describing the construction and adaptation of preparative-scale SFE systems optimized for phytochemical work that allow the direct extraction of vegetable material [7–10].

Most of the published work involved complex and expensive SFE systems. In this paper we describe an SFE system for preparative- and semi-preparative-scale use with natural products that is simple and inexpensive in construction

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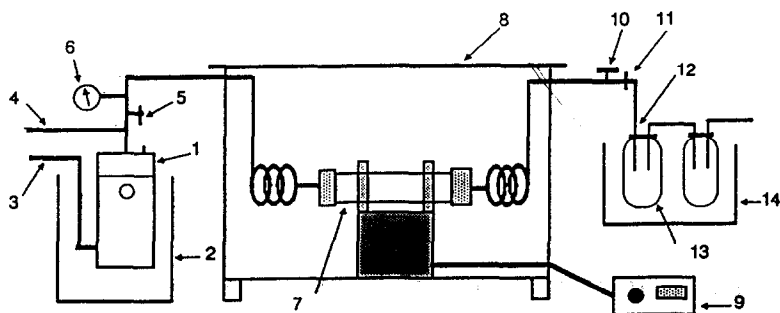


Fig. 1. Supercritical fluid extraction system with both analytical and semi-preparative capabilities. 1 = Vessel; 2, 14 = cryogenics; 3 = CO₂; 4 = nitrogen; 5 = valve 1; 6 = gauge; 7 = cell; 8 = oven; 9 = heater; 10 = valve 2; 11 = filter; 12 = restrictor; 13 = collectors.

and versatile to use. The application of this system to the extraction of vegetable material, *Cymbopogon citratus*, in both dynamic and sequential extraction modes, using modified supercritical CO₂, is also described.

2. Experimental

2.1. Construction of the preparative SFE system

The complete system, as illustrated in Fig. 1, was constructed of stainless steel. It consists of a pressurization vessel (1) with two inlets, one for carbon dioxide (3) and the other for nitrogen (4), which is used to pressurize the system. Valve 1 (5) installed before the gauge (6) allows the use of a static mode when closed along with valve 2 (10). The temperature of the extraction

cell (7), installed in an oven (8), is controlled through an electrical heater (9). The second valve (10) controls the exit of the extract to a filter (11) and to a tapered stainless-steel (0.03 mm) restrictor (12). The extract is collected in the sample collector vessel (13) with cryogenic capabilities when desired (14). The dimensions of the major components of the designed SFE system are listed in Table 1.

2.2. Chromatographic conditions

Chromatograms were obtained with a Hewlett-Packard (HP) Model 5890 Series II system, employing a 0.33- μ m HP-1 fused-silica capillary column (12 m \times 0.2 mm I.D.) with hydrogen as the carrier gas and the following temperature programme: 100°C for 2 min, increased at 3°C/min to 150°C and then at 6°C/min to 280°C, held

Table 1
Dimensions of the major components of the SFE system

| Parameter | Pressurization vessel (mm) | | Extraction cell (mm) | | | | Oven (mm) |
|-------------------|----------------------------|---------|----------------------|-----|-------|-------|-----------|
| | 1 | 2 | 1 | 2 | 3 | 4 | |
| External diameter | 100 | 137 | 0.4 | 0.9 | 45 | 49 | |
| Internal diameter | 65 | 102 | 0.2 | 0.7 | 30 | 34 | |
| External length | 175 | 150 | 222 | 217 | 439 | 345 | 700 |
| Internal length | 153 | 140 | 210 | 205 | 424 | 330 | |
| Thickness | 18 | 17 | 0.1 | 0.1 | 8 | 8 | 1.5 |
| Width | | | | | | | 300 |
| Height | | | | | | | 300 |
| Total volume (ml) | 507.44 | 1143.39 | 0.7 | 7.9 | 299.5 | 299.5 | 63 000 |

for 20 min. Volumes of 1.5 μ l of a dichloromethane solution (8 mg/ml) were injected using the split mode (1:28). Flame ionization detection (FID) was applied.

2.3. Plant material

Leaves of *Cymbopogon citratus* were collected in the UNAERP (Universidade de Ribeirão Preto) vegetable biotechnology facilities. The plant was dried, ground and stored before use.

2.4. Calibration and supercritical fluid extraction

Calibration of the SFE system was done by measuring the extraction time [the total time that all the supercritical fluid contained in the pressurization vessel (500 ml) took in traversing the system]. The temperature and the loading of the extraction cell were fixed (75°C and 80 g of plant material, respectively). Three different pressures (80, 100 and 120 atm) were used for supercritical CO₂ and for CO₂ modified with 10% of hexane, ethyl acetate or methanol.

The SFE of *C. citratus* was performed in the SFE system shown in Fig. 1. The extraction was done with CO₂ modified with 10% of hexane, followed by sequential extraction of the same sample with CO₂ modified by 10% of acetone. Amounts of 45 g of plant material were used for these extractions. The SFE conditions were temperature 75°C, pressure 80 atm and volume of supercritical fluid for each extraction 1000 ml. Each extraction was completed in 90 min. A conventional extraction of the essential oils was done by steam distillation in a conventional distillation system with 45 g of plant material and 1000 ml of distillate water at its boiling point over 4 h. After a clean-up, these extracts were chromatographed by high-resolution GC using the described conditions.

3. Results and discussion

The extraction system developed in this work (Fig. 1 and Table 1) is versatile as it allows the

use of different extraction cells and a wide variety of solvents and modifiers for CO₂. In addition, it allows the use of several extraction techniques, including static extraction, when both valves 1 and 2 are closed after loading the extraction cell, or dynamic extraction when both valves 1 and 2 are open. Having the same matrix in a dynamic extraction and changing the supercritical fluid, a sequential extraction, such as that presented in this paper, is carried out.

This system has the additional advantage of being less expensive than most commercially available systems and can be constructed very simply in an ordinary workshop. Its limited operational conditions of pressure (nitrogen) and temperature (about 100°C) are suitable for the extraction of most natural products, where there are many thermally unstable products of great interest. This limitation is compensated for by the possibility of using different modifiers and the ease of operation.

The use of a variety of modifiers during the system calibration (Fig. 2 and Table 2) showed that the extraction time is directly proportional to the modifier density, *i.e.*, the higher the density the longer is the extraction time. This is well exemplified by ethyl acetate, which is denser than any of the other modifiers studied. This suggests that the extraction capability and the selectivity depend on the fluid density and can be changed with the type of the modifier. For pure fluids (exemplified by CO₂ in the dense gas state) an increase in temperature will produce an increase in viscosity. As a consequence, an increase in the extraction time will be observed.

The yields obtained by steam distillation, SFE with CO₂-10% hexane and SFE with CO₂-10% acetone extraction are 0.2066, 0.0217 and 0.0074 g (0.46, 0.05 and 0.02%), respectively.

The chromatographic profiles obtained by high-resolution GC (Fig. 3) were analysed and show that, in region I, the extracts obtained using CO₂ modified with 10% of hexane and steam distillation contain the same compounds, although with a visible intensification of some peaks, such as 4, 14 and 15. A further supercritical fluid extraction, with CO₂ modified with 10% of acetone, on the same matrix extracted differ-

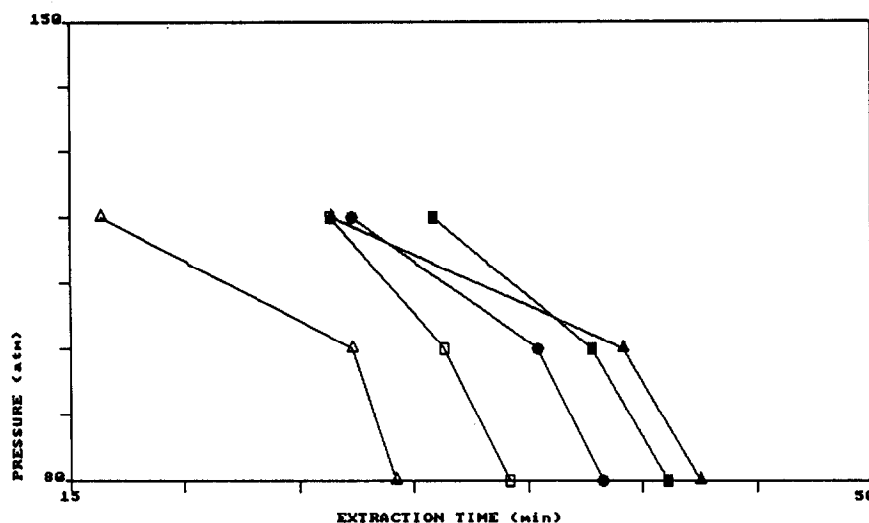


Fig. 2. Calibration graph for the designed system showing pressure vs. extraction time for several fluids. Δ = CO_2 (75°C); \blacktriangle = CO_2 (95°C); \square = CO_2 -10% hexane (75°C); \bullet = CO_2 -10% methanol (75°C); \blacksquare = CO_2 -10% ethyl acetate (75°C).

ent compounds, such as 9, 12 and 13, not present in the other extracts. In region II, several compounds can be observed that are not present in the steam distillation extract (Fig. 3a) and were extracted only by modified CO_2 . In region III, the CO_2 -acetone extract shows enhancement of several compounds while compounds 20, 21 and 22 were not extracted with the other solvents.

4. Conclusions

The system described shows sufficient versatility to be used in several extraction modes, allows the use of several modifiers, either gas or liquid,

is inexpensive in construction and easy to operate and maintain. It also presents both analytical and semi-preparative capabilities.

The results for natural products obtained with this system indicate that SFE using modified CO_2 can be selectively tuned by choosing the type and percentage of the modifier utilized. Sequential extraction can be extremely useful when there is a need to extract selected compounds, to enhance the extraction yields or to remove compounds that will interfere in subsequent analysis.

This contribution may open up new perspectives in the study of CO_2 modifiers, mainly in studies with plants and their volatile compounds.

Table 2
Extraction time obtained in the SFE system calibration

| Pressure (atm) | Extraction ^a time (min) | | | | |
|----------------|------------------------------------|------|------|------|------|
| | 1 | 2 | 3 | 4 | 5 |
| 80 | 42.5 | 29.2 | 34.2 | 41.1 | 38.3 |
| 100 | 39.2 | 27.3 | 31.3 | 37.8 | 35.4 |
| 120 | 26.4 | 16.4 | 26.4 | 30.9 | 27.3 |

^a 1 = CO_2 (95°C); 2 = CO_2 (75°C); 3 = CO_2 -10% hexane (75°C); 4 = CO_2 -10% ethyl acetate (75°C); 5 = CO_2 -10% methanol (75°C).

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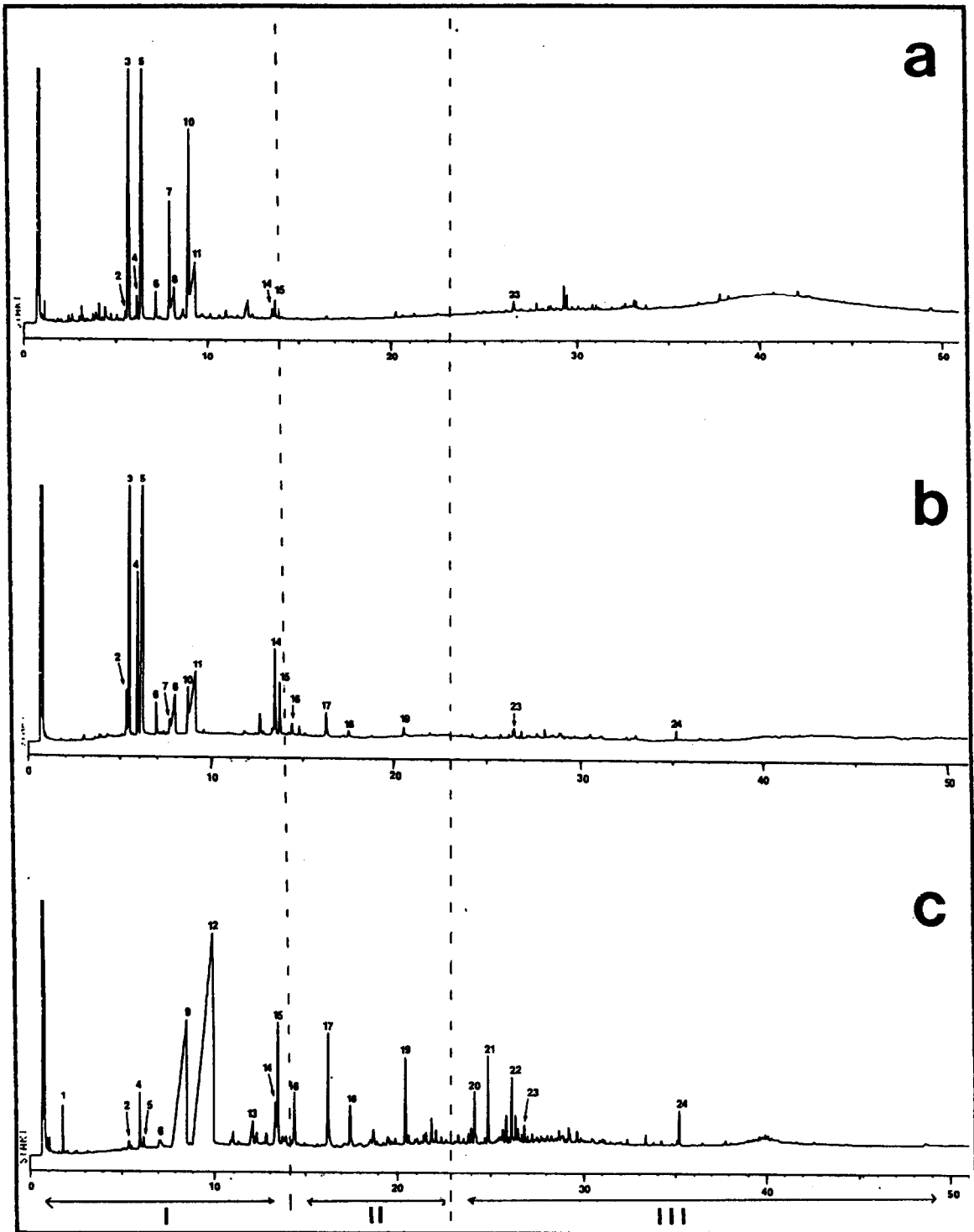


Fig. 3. Chromatograms obtained by high-resolution GC-FID of extracts from *C. citratus* obtained by (a) steam distillation, (b) SFE with CO_2 -10% hexane and (c) SFE with CO_2 -10% acetone. Time scale in min.

6. References

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