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Analytical and preparative supercritical fluid extraction of Chamomile flowers and its comparison with conventional methods

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Abstract

Supercritical fluid extraction (SFE) was compared with Soxhlet extraction, steam distillation and maceration for the isolation of the active components present in chamomile flowerheads. The obtained fractions were analysed by GC–MS and reversed-phase HPLC. The yield of essential oil achieved by a 30-min extraction with pure CO_2 at 90 atm and 40°C was 4.4 times higher than that produced by steam distillation performed for 4 h. The recovery of the flavonoid apigenin obtained by supercritical CO_2 after a 30-min extraction at 200 atm and 40°C was 71.4% compared to Soxhlet extraction performed for 6 h and 124.6% compared to maceration performed for 3 days. However, the highly polar flavonoid apigenin-7-glucoside was not extracted by 100% CO_2 (recovery values $< 1.1\%$). Its extraction efficiency was markedly improved by the addition of the polar modifier methanol (5%, v/v) to the CO_2 fluid, yet the obtained recoveries were unsatisfactory (14.6–19.5%). The SFE method was scaled-up for preparative applications using a pilot plant with three separation stages operating in series. Large-scale SFE was technically feasible with pure CO_2 as the extracting fluid. However, the use of CO_2 modified with organic solvents was not effective at the pilot-plant scale.

Keywords: Supercritical fluid extraction; Soxhlet extraction; Steam distillation; Maceration; Chamomile flowerheads

1. Introduction

Medicinal plant extracts are widely used in pharmaceutical and cosmetic products [1–3]. The traditional methods for the extraction of plant materials include steam distillation [2,4,5] and organic solvent extraction using percolation, maceration or Soxhlet techniques [2,6,7]. These procedures, however, have distinct drawbacks such as time-consuming and labour-intensive operations, handling of large volumes of hazardous solvents and extended concentration steps which can result in the loss or degradation of target analytes [2,8,9]. Moreover, there is increasing in-

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terest for alternative extraction technologies consuming less organic solvents, because of the rising solvent acquisition and disposal costs and regulatory restrictions [8,10].

Supercritical fluids have been shown to exhibit several advantages in the extraction of natural products from plant matrices [2,9,11]. The combined liquid-like solvating capabilities and gas-like transport properties of supercritical fluids make them particularly suitable for the extraction of diffusion-controlled matrices such as plant tissues [2,8,9]. Moreover, the solvent strength of a supercritical fluid can be easily tuned by simply changing the applied pressure and/or temperature [2,8]. Carbon dioxide, the most commonly used supercritical fluid, has the additional advantages of being non-flammable, fairly non-toxic, cost-effective and easily removed from the extract following decompression. Finally, due to its relatively low critical temperature (31.1°C), thermal sample decomposition is reduced. Pure CO_2, however, is not an appropriate extraction fluid for polar analytes and retentive matrices. In order to enhance the solvating power of CO_2, the addition of a few percent of a modifier solvent is required [2,8,9].

Chamomile (*Matricaria chamomilla*) flowerheads and extracts are used in the pharmaceutical and especially in the cosmetic industry [12] for their antispasmodic, anti-inflammatory and antimicrobial properties [12–14] and also as natural hair dye and fragrance [13]. A number of studies have reported the supercritical fluid extraction (SFE) of chamomile [15–18]. However, none of these investigations has examined in detail the comparison between SFE and the conventional extraction techniques. Moreover, since the application of SFE has been restricted to the isolation of the essential oil fraction [15–18], the extraction of the other pharmaceutically relevant constituents of chamomile, namely the flavonoids, has not been studied.

To further investigate the potential of SFE for the extraction of active components from aromatic plants, SF extracts of chamomile flowerheads were compared to those obtained using steam distillation, Soxhlet extraction or maceration. The distribution of individual compounds in the extracts produced with the different techniques was determined using GC–MS and HPLC. The scaling-up from analytical to preparative SFE systems was also studied.

2. Experimental

2.1. Materials

Dried chamomile flowerheads (*M. chamomilla*) were obtained from Orbis Flora, Verona, Italy). They had a mean moisture content of 8% on dry basis. Apigenin and apigenin-7-glucoside were purchased from Extrasynthèse (Genay, France). Commercial grade (99.5%) liquid carbon dioxide supplied in cylinders with a dip tube was from SAPIO (Milan, Italy). HPLC-grade acetonitrile and water were supplied by Baker (Phillipsburg, NJ, USA). All other chemicals were of analytical grade (Carlo Erba, Milan, Italy).

2.2. Chromatography

GC–MS analyses were carried out with a Shimadzu QP-5000 GC–MS system (Shimadzu Italia, Milan, Italy) operating in the electron impact mode (70 eV) with transfer line and ion source temperatures maintained at 250°C. A DB-5 fused silica capillary column (30 × 0.25 mm i.d.; J&W Scientific, Folsom, CA, USA) with helium as the carrier gas was used. The GC operating conditions were: injector temperature, 250°C; column temperature programmed between 60 and 240°C at a rate of 3°C/min with initial and final isothermal periods of 2 and 5 min, respectively. The samples (1 μl) were introduced using split injection (split ratio, 10:1). The peaks were identified by comparison of their mass spectra with the US National Institute of Standards and Technology (NIST) and the Essential Oil Components (SZTERP) mass spectra libraries. The percentage composition of individual components was computed from the GC peak areas without any correction for the relative response factors.

The HPLC apparatus consisted of a modular chromatographic system (Model PU-980 pump, Model LG-980-02 gradient unit and Model UV-

975 variable-wavelength UV-detector; Jasco, Tokyo, Japan) linked to an injection valve with a 20-μl sample loop (Rheodyne, Cotati, CA, USA). The detector was set to 335 nm. Data acquisition and processing were accomplished with a personal computer using Borwin software (JBMS Developpements, Le Fontanil, France). Sample injections were effected with a Model 802 RN syringe (10 μl, Hamilton, Bonaduz, Switzerland). Separations were performed on a Zorbax SB-CN column (5-μm particles, 250×4.6 mm i.d.; Rockland Technologies Inc., Newport, Delaware, USA) fitted with a guard column and eluted with a linear gradient (2.5%/min) from 15 to 95% (v/v) acetonitrile in aqueous triethylammonium phosphate (pH 2.8; 0.03M). The eluents were filtered through GV-type filters (0.22-μm; Millipore, Bedford, MA, USA) and deaerated online by a model ERC-3311 automatic solvent degasser (Erma, Tokyo, Japan). Chromatography was performed at ambient temperature, at a flow rate of 1.0 ml/min. Standard solutions of apigenin and apigenin-7-glucoside were prepared in ethanol and analysed by HPLC. The identity of the separated compounds in the chamomile extract was assigned by co-chromatography with the authentic standards. Quantification was carried out by integration of the peak areas using the external standardisation method.

2.3. Analytical-scale SFE

Supercritical fluid extractions were performed with a Spe-ed SFE system (Model 7010/680 atm; Applied Separations, Allentown, PA, USA) which comprises an air-driven pump to deliver the CO_2 to the extraction cell (10-ml stainless steel vessel with 2-μm frits at either ends) housed within a temperature-controlled oven. The CO_2 pump head was cooled to 4°C using a refrigerated circulating bath (Dese Lab, Padua, Italy). The outlet of the extraction cell was connected to a thermally controlled variable restrictor, which maintains supercritical pressure conditions in the system. A reciprocating pump (SSI, LabService Analytica, Bologna, Italy) was used to supply the methanol modifier which was mixed with the pure CO_2 prior to introduction into the extraction vessel. The dried plant material was ground to a powder and loaded (approximately 1 g) directly into the extraction cell. A plug of polypropylene wool was inserted into the cell at both ends. Extractions were carried out at 40 or 45°C, at a pressure of 90 or 200 atm and after a 2-min static period, in the dynamic (continual flow) mode for 30 min. The restrictor was maintained at 70°C and the measured flow rate for the supercritical fluid was 1 l/min of expanded gas. As the CO_2 evaporated at the restrictor outlet due to decompression, the extracted material was collected in a glass vial fitted with a septum and a needle vent and containing ethanol (5–6 ml). The content of the vial was adjusted to a known volume (10 ml) prior to analysis by GC–MS or HPLC.

2.4. Large-scale SFE

Preparative SFE experiments were performed on a pilot plant (Fedegari Autoclavi, Pavia, Italy) working with a full recycle of the CO_2 downstream the last separator (schematically shown in Fig. 1). It consisted of an 8-dm^3 extraction vessel that can be equipped with two internal baskets of different volumes. A thermostated jacket allowed control of the extraction temperature between 0 and 70°C. A quick clamp closure and crane were used for the fast charging and openin/closure of the extractor. A high-pressure pump (Model EL-1; Lewa Gmbh, Leonberg, Germany) delivered liquid CO_2 flow rates from 5 to 20 kg/h at the maximum operative plant pressure. The apparatus was arranged with three separation stages (Fig. 1) operating in series. At the exit of the second separation vessel the supercritical solution passed through a cyclonic separator where essential oil precipitation was obtained. The cyclonic separator allowed periodical discharge of the extracted material during the SFE process. A differential pressure transducer measured the pressure drop along the extraction vessel. A calibrated Coriolis mass flowmeter was used to measure the liquid flow rate downstream of the pumping unit during the extraction process.

2.5. Conventional extraction methods

Steam distillation was performed on 1-g portion of chamomile flowerheads for 4 h. The extracted essential oil was transferred in a graduate cylinder with chloroform, dehydrated with sodium sulphate and diluted as appropriate for GC–MS analysis.

Soxhlet extraction of the plant material (1 g) was carried out with ethanol for about 6 h. The solution obtained by filtration of the extract was vacuum concentrated prior to analysis by HPLC.

Maceration was performed at ambient temperature in a stoppered container with frequent agitation. The chamomile sample (1 g) was extracted with ethanol for a period of 3 days [7]. The extract was filtered and vacuum concentrated prior to HPLC assay.

3. Results and Discussion

Chamomile contains two main fractions: the essential oil, composed mainly of sesquiterpenes and polyines, and a non-volatile fraction containing flavonoids as major constituents [12,18]. The most characteristic and pharmacologically relevant chamomile compounds (Fig. 2) are the oxygenated sesquiterpenes bisabolol and bisabolol oxides, the sesquiterpene–lactone matricine, the *cis*/*trans*-en-in-dicycloethers and the flavonoids apigenin and apigenin-7-glucoside [12,16,18,19]. For the removal of the essential oil, steam distillation is the most commonly used process [2,5,13] while maceration is generally employed for the isolation of the non-volatile fraction [7].

3.1. Essential oil

The comparison of SFE with steam distillation for the isolation of the chamomile essential oil was carried out with supercritical CO_2 at 90 atm and 40°C (extraction time, 30 min), according to the conditions reported in the literature [18]. Since essential oil components typically have high solubilities in supercritical CO_2 [4], quantitative extractions can be achieved under mild conditions and hence with improved selectivity [2,18]. The GC–MS traces of the SFE and steam distillation extracts from the same batch of chamomile are shown in Fig. 3 (peak identities are given in Table 1), respectively. Qualitatively, the profile of the SFE extract is similar to that of the steam distillation extract, with the exception of some additional late-eluting peaks present in the supercritical fluid extract and which were tentatively identified as waxes. Significant quantitative differences were also apparent (see also Table 1). In particular,

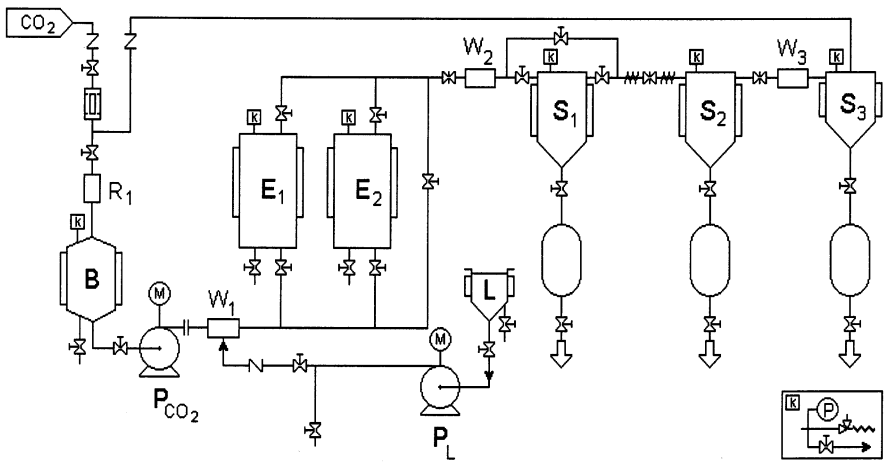

Fig. 1. Schematic drawing of the SFE pilot plant. Legend: CO_2 storage tank (B); extraction vessel (E); safety device (K); liquid storage tank (L); volumetric pump (P_{CO2}, P_L); CO_2 condenser (R_1); separator (S); heat exchanger (W).

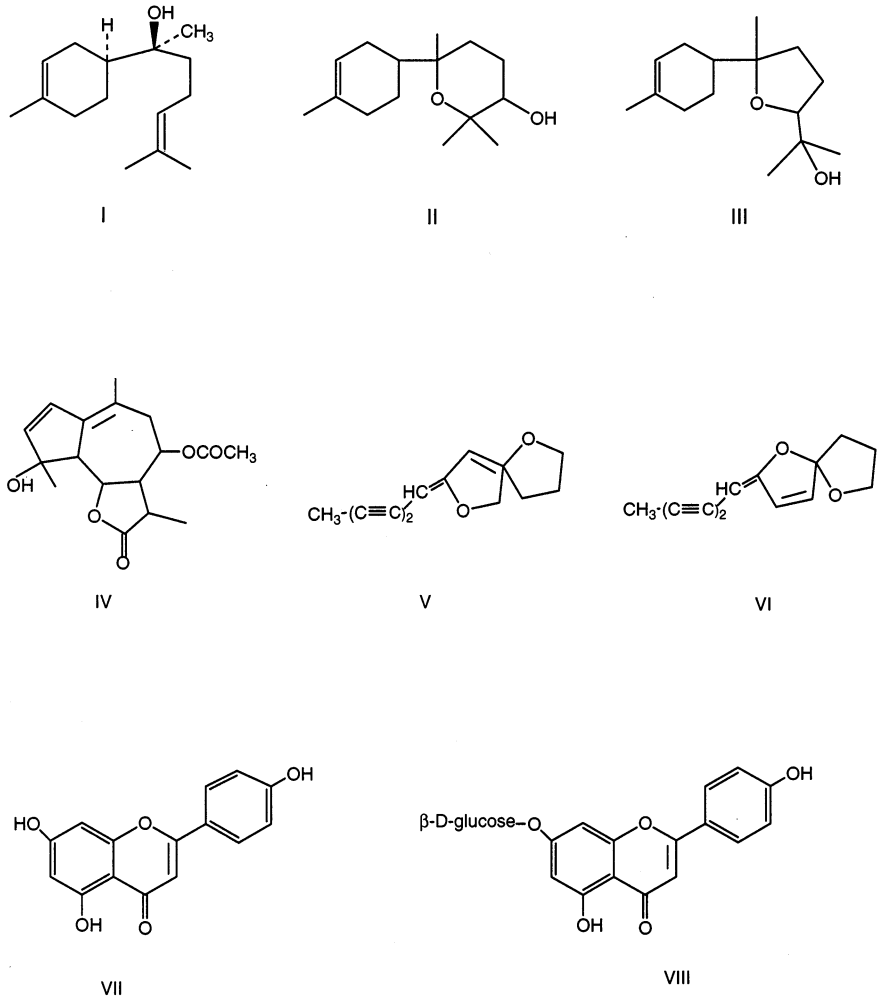

Fig. 2. Chemical structures of chamomile components. I, α-bisabolol; II, α-bisabolol oxide A; III, α-bisabolol oxide B; IV, matricine; V, *cis*-en-in-dicycloether; VI, *trans*-en-in-dicycloether; VII, apigenin; VIII, apigenin-7-glucoside.

SFE produced an enrichment of the active components matricine (evaluated as chamazulene) and the *cis*/*trans*-en-in-dicycloethers in the extract.

The yield (expressed as weight of extract divided by the weight of the starting material) obtained by SFE (0.97%) after 30-min extraction was 4.4 times higher than that produced by steam distillation (yield, 0.22%) performed for 4 h. Moreover, in accordance with a previous study [18], the SFE extract was dark yellow indicating that no thermal degradation of the naturally occurring matricine to chamazulene had occurred. In fact, the latter compound is responsible for the blue colour of the steam distillate [12].

The scaling-up from the laboratory-based instrument (sample size, ca. 1 g) to the preparative-scale (sample size, multi-kg) was performed using the system illustrated in Fig. 1 under the same conditions described above (90 atm, 40°C) but with a 120 min extraction time. Compared to the analytical apparatus, the large-scale SFE plant exhibited several advantages including: (i) the cost-saving factor and the virtually zero environmental impact due to the recycling of CO_2 and (ii) the enhanced selectivity produced by the fractionation of the supercritical CO_2 extract in the sepa-

ration vessels. No distinct difference was found between analytical- (yield, 0.97%) and preparative- (yield, 0.92%) scale SFE in terms of oil yield and gas chromatographic patterns. However, none of the peaks eluting at retention times >46 min was detected in the extract obtained by preparative SFE. This demonstrated the efficient removal of waxes by precipitation in the first separator (Fig. 1). Moreover, also in the case of large-scale SFE, no conversion of matricine to chamazulene was observed as indicated by the yellow color of the obtained product.

3.2. Flavonoids

Although several researchers have reported the SFE of chamomile [15–18], the extraction of the flavonoid components has not been investigated.

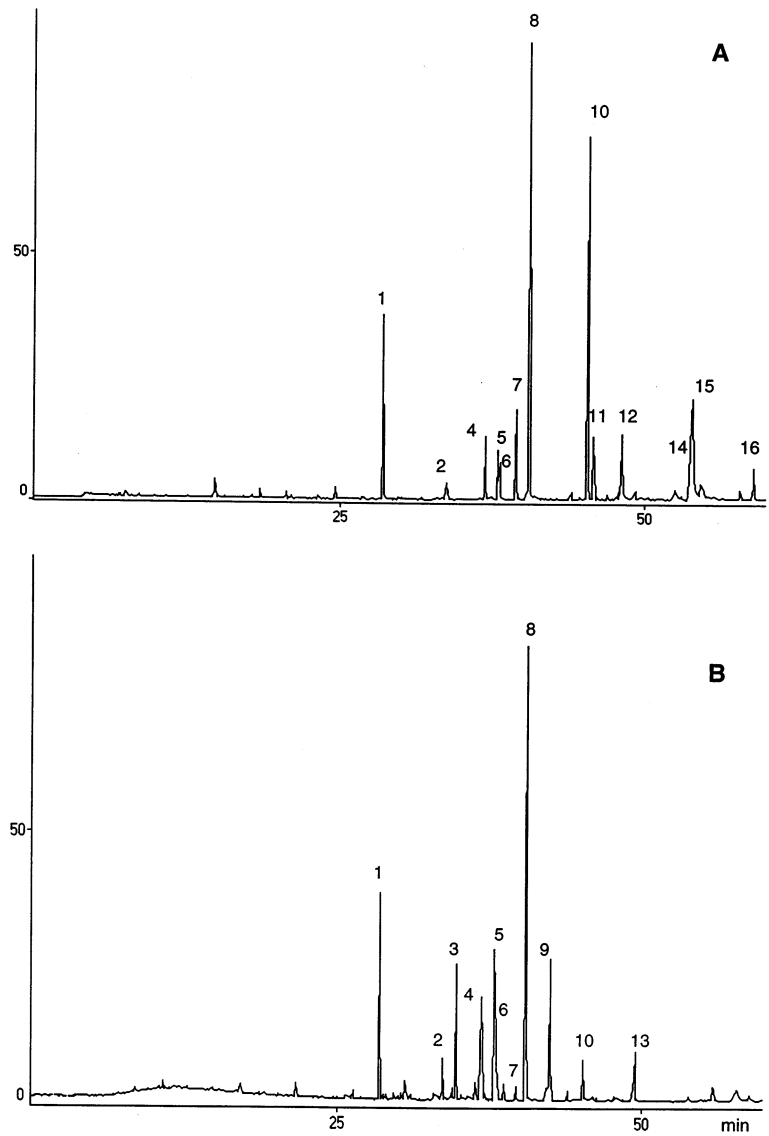

Fig. 3. GC–MS chromatograms of the SFE (A) and steam distillation (B) extracts of chamomile. Individual peaks are identified in Table 1. Operating conditions as described in Section 2.

Table 1
Major peaks in the GC–MS traces of the chamomile extracts

Peak No.[a]	Compound	% Relative abundance[b]	
		SFE[c]	Steam distillation
1	β-Farnesene	9.6	12.8
2	Spathulenol	1.0	2.6
3	Unidentified	–	9.2
4	α-Bisabolol oxide B	4.6	7.8
5	α-Bisabolone oxide	2.5	9.2
6	α-Bisabolol	2.3	3.6
7	Matricine (chamazulene)	7.3	<0.5
8	α-Bisabolol oxide A	28.5	36.6
9	Unidentified	–	9.6
10	*cis*-en-in-dicycloether	25.9	2.7
11	*trans*-en-in-dicycloether	3.9	–
12	Wax[d]	4.8	–
13	Unidentified	–	3.3
14	Wax[d]	1.7	–
15	Wax[d]	4.8	–
16	Wax[d]	1.9	–

[a] Peak numbers refer to the chromatograms in Fig. 3.
[b] GC peak area percentage. Each value is the mean of triplicates.
[c] SFE conditions: p, 90 atm; extraction *T*, 40°C; extraction time, 30 min; restrictor *T*, 70°C; flow-rate, 1 l/min gaseous CO_2.
[d] Tentative identification based on MS.

This is surprising given their broad pharmacological properties (i.e. antiphlogistic, antioxidant and radical scavenging activities) and the considerable levels present in the chamomile flowerheads [12,20].

The initial development of the conditions for analytical SFE of the flavonoid fraction was performed in the pressure range of 200–400 atm and at temperatures between 40 and 50°C. In order to determine the optimum operating parameters, the SFE recoveries from the chamomile matrix were compared to those obtained by conventional procedures, including Soxhlet extraction and maceration. The former was selected because it is recognised as the reference technique [21] whereas the latter represents the most common method for the large-scale production of non-volatile plant extracts [7]. The efficiency of the different techniques was evaluated by HPLC assay of the most active [12] flavonoids (i.e. apigenin and apigenin-7-glucoside) present in the obtained extracts.

The first objective of this study was to estimate the effect of the extraction pressure and hence density on recovery. Extraction for 30 min with supercritical CO_2 at 200 atm and 40°C recovered 0.8 and 71.4% of the Soxhlet extractable apigenin-7-glucoside and apigenin, respectively (Table 2). Compared to the yield obtained by maceration, 1.1% of apigenin-7-glucoside and 124.6% of apigenin were recovered by SFE (Table 2). These results indicate that the extraction efficiencies achieved by Soxhlet are higher than those attainable by maceration. Nevertheless, the former technique is not applicable to the large-scale production of plant extracts. Representative HPLC traces of the chamomile extracts obtained with the different methods examined in this study are illustrated in Fig. 4A–C. The higher complexity of the chromatographic patterns produced by Soxhlet (Fig. 4A) and maceration (Fig. 4B) indicates that SFE affords enhanced extraction selectivity compared to the classical techniques.

Increasing the supercritical CO_2 pressure from 200 to 400 atm did not achieve any significant improvement in the recoveries of the two flavonoids which appeared to level off at the increased density.

The next SFE parameter examined was temperature. No improvement in the extraction efficiency was observed when the temperature was raised from 40 to 50°C at 200 atm. Higher temperatures were not investigated, because this approach would limit the advantages offered by SFE for the isolation of the thermally labile flavonoids [22].

In order to increase the solubility and extractability of apigenin-7-glucoside and apigenin in supercritical CO_2, the addition of the polar modifier methanol to the extracting fluid was examined at 200 atm and 45°C. The HPLC assay demonstrated that the relative extraction efficiencies obtained by 5% (v/v) methanol-modified CO_2 compared to the yield from Soxhlet and macera-

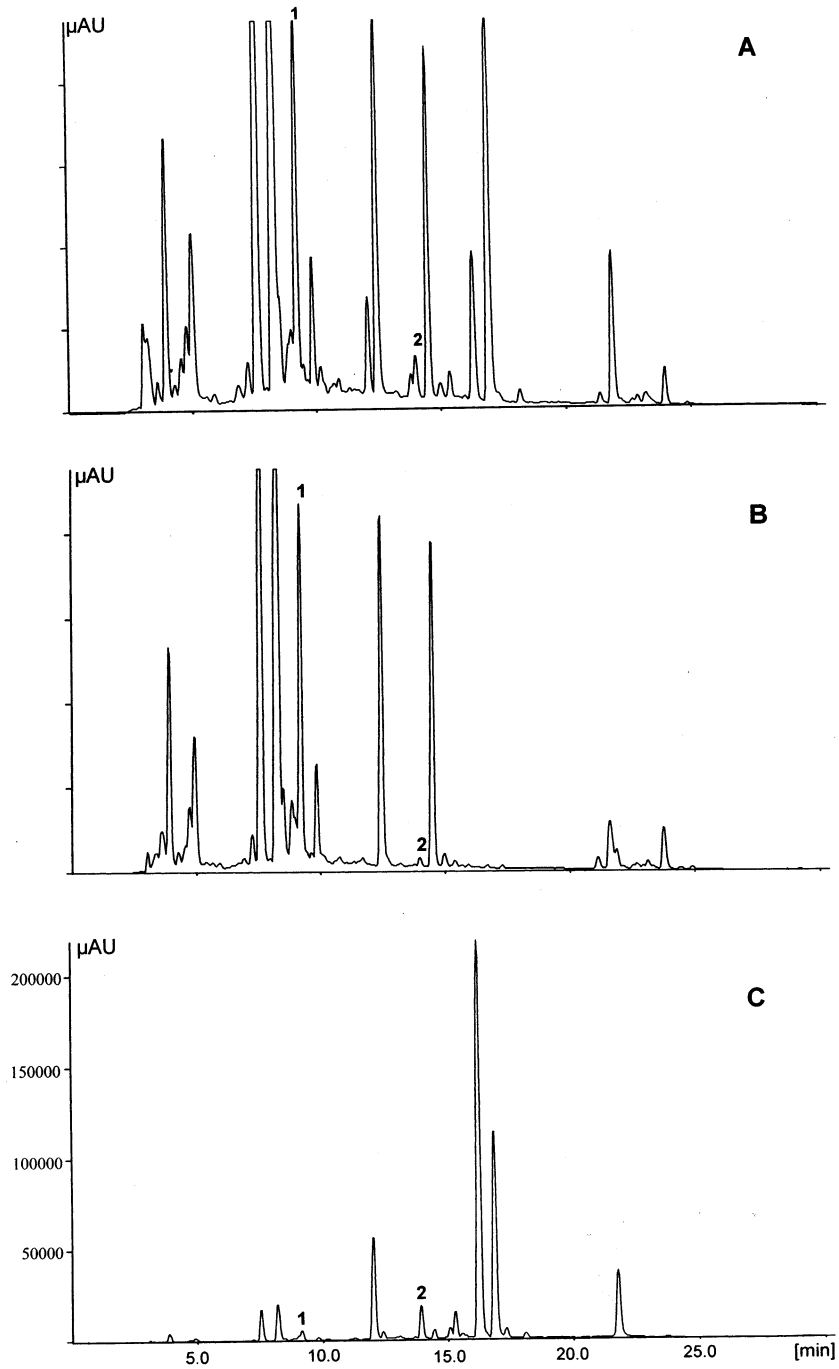

Fig. 4. Comparison of the HPLC chromatograms of chamomile extracts obtained by different methods: A, Soxhlet extraction; B, maceration; C, SFE. Peaks: 1, apigenin-7-glucoside; 2, apigenin. Operating conditions as described in Section 2.

tion were, respectively, 14.6 and 19.5% for apigenin-7-glucoside, and 143.3 and 187.7% for apigenin (Table 2). As expected, the extraction recovery increased with the addition of the cosolvent (5%, v/v), the greatest improvement being observed for apigenin-7-glucoside. Higher percentages of methanol modifier were not employed because this approach nullifies in part the aim of minimising the volume of hazardous solvents used.

The large-scale SFE of the flavonoid fraction was carried out with pure CO_2 at 200 atm and 40°C. The results obtained are consistent with those produced by analytical-scale SFE using 100% CO_2, although the recovery values achieved by preparative SFE are lower (Table 2). The addition of the methanol modifier to the supercritical CO_2 was not attempted on the pilot plant because of its high toxicity. Consequently, propylene glycol and ethanol, which are widely used for the liquid extraction of plant materials, were examined as alternative cosolvents (5% v/v) in the large-scale SFE of chamomile. However, the former exhibited scarce miscibility with supercritical CO_2, whereas the latter did not produce any significant improvement in the flavonoid extraction yields. Therefore, it is apparent that the SFE technology based on the use of organic solvent-modified CO_2 although feasible at the analytical scale, presents difficulties at the preparative scale.

4. Conclusions

SFE both at the analytical- and preparative-scale offers considerable advantages over the traditional method of steam distillation for isolating the essential oil from chamomile flowerheads. Using supercritical CO_2, extractions can be performed in a shorter time and under mild conditions, thus minimising degradation of thermolabile components (e.g. matricine) and increasing the yield of volatile analytes. Regarding the flavonoid fraction, SFE provides a rapid and quantitative method for the isolation of the moderately polar apigenin, producing cleaner extracts than those obtained with the conventional methods (Soxhlet and maceration). However, the disadvantages of supercritical CO_2 for the extraction of the very polar apigenin-7-glucoside are also apparent. The SFE technique with unmodified CO_2 can be scaled-up for preparative isolation work, whereas difficulties were encountered at the pilot-plant scale when the addition of cosolvent was required. However, the possibility of produc-

Table 2
Relative recoveries for apigenin-7-glucoside and apigenin from chamomile using SFE compared with conventional extraction methods

Compound	% Recovery[a,b]		
	Analytical SFE[c] (CO_2)	Analytical SFE[d] (5% methanol in CO_2)	Preparative SFE[e] (CO_2)
Apigenin-7-glucoside	0.8[a]; 1.1[b]	14.6[a]; 19.5[b]	0.5[a]; 0.7[b]
Apigenin	71.4[a]; 124.6[b]	143.3[a]; 187.7[b]	56.1[a]; 105.7[b]

[a] Percentage recovery based on the amount extracted by Soxhlet extraction. Each value is the mean of triplicates with RSD < 16.8%.

[b] Percentage recovery based on the amount extracted by maceration. Each value is the mean of triplicates with RSD < 16.8%.

[c] SFE conditions: p, 200 atm; extraction T, 40°C; extraction time, 30 min; restrictor T, 70°C; flow-rate, 1 l/min gaseous CO_2.

[d] SFE conditions: p, 200 atm; extraction T, 45°C; extraction time, 30 min; restrictor T, 70°C; flow-rate, 1 l/min gaseous CO_2.

[e] SFE conditions: p, 200 atm; extraction T, 40°C; extraction time, 120 min; flow-rate, 17 kg/h liquid CO_2; first separator: $p = 200$ atm, $T = 0$°C; second separator: $p = 80$ atm, $T = 40$°C; third separator: $p = 40$ atm, $T = 25$°C.

ing plant extracts without any contact with conventional organic solvents and thus directly usable, makes the SFE technique an attractive alternative to the currently used methods.

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