Fractionation of Plant Extracts by Supercritical Fluid Extraction and Direct Introduction in Capillary Gas Chromatography using a Programmable Temperature Vaporizer

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Abstract

A simple procedure to improve the sensitivity achievable in the analysis of supercritical fluid extracts of complex matrices is proposed. The method involves the offline coupling of supercritical fluid extraction and gas chromatography (GC) in such a way that the glass liner of a programmed temperature vaporizer is placed after the separation vessels of the extraction module. The subsequent chromatographic analysis is simply achieved by direct introduction of the glass liner into the GC. The proposed procedure is also adequate to perform the fractionation of supercritical fluid extracts into different groups of compounds, thus allowing the performance of highly selective extractions.

Introduction

Supercritical fluid extraction (SFE) has evolved in the last decade as an alternative method for the extraction of organic compounds from a great variety of matrices prior to analysis. In this respect, changes in environmental regulations have contributed to the present worldwide tendency to replace conventionally used solvents with cleaner processes, such as those that utilize supercritical fluid. Moreover, the wide density range available with a supercritical fluid and the possibility of improving mass transfer because of the low viscosities, high diffusivities, and variable solvent strengths of supercritical fluids is very useful to achieve highly selective extractions (1–6).

Specifically, the analysis of foods has benefited from the advantages of SFE for sample preparation, because some of the constituents possess low thermal stabilities or high reactivities, thus demanding the use of mild experimental conditions for sample extraction, such as those provided by supercritical fluids (7–12). Also, the possibility of performing highly selective extractions is especially interesting in food studies, because the complexity of the sample matrix requires the careful optimization of the experimental variables involved in SFE. Keeping in mind the enormous potential of SFE as a method for sample preparation, it should also be considered that in some cases, the overall analysis (i.e., including both SFE and chromatographic steps) is not sensitive enough for some compounds. Specifically, certain species that are of importance for the character of a food or the intensity of its aroma may be present in very low concentrations, thus demanding their effective enrichment prior to the chromatographic analysis. Unfortunately, however, the use of organic solvents may be required to recover the SFE extracts from the vessels in which they are collected, and losses of some compounds by coevaporation with the solvent in the subsequent concentration step are often observed. Moreover, in some cases, experimental conditions do not allow the retention of volatile compounds in the separation vessel and, consequently, their analysis can not be satisfactorily performed.

On the other hand, the use of a programmed temperature vaporizer (PTV) (13–14) has proved useful for performing the internal gas chromatographic (GC) concentration of an extract, thus improving the sensitivity of the analysis (9,15–16).

The aim of this work was to investigate the possibility of improving the sensitivity achievable in the analysis of high-volatile compounds using SFE and subsequent GC by modifying the SFE design in such a way that the glass liner of a PTV is placed after the extraction vessels originally included in the equipment. A further aim of this investigation was to achieve the fractionation of the sample into different groups of compounds. The study was performed using a plant *(Thymus mastichina* L.) that is used as a raw material for flavoring food.

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Experimental

SFE

Extractions were performed using an SFE module designed and manufactured by Iberfluid (Madrid, Spain) that was modified to improve the sensitivity achievable for the high-volatile compounds in the subsequent chromatographic analysis. For this aim, the glass liner of the PTV of the gas chromatograph was placed after the second separation vessel through an adequate steel fitting and sealed by a parafilm (American National Can, Neenah, WI) ferrule, as shown in Figure 1.

The SFE unit consisted of a pump (450 bar), a flowmeter, and a 300-mL extraction vessel connected to two 100-mL separation vessels (Figure 1). The pressure and temperature of the extraction and separation vessels were controlled by metering valves and a thermocouple connected by electronic relays. Carbon dioxide (> 99% purity) was obtained from Carburos Metálicos (Madrid, Spain) and filtered through active charcoal (activated carbon microcolumn). A cooler placed before the pump was used to condense the carbon dioxide.

Procedure

The plants (*Thymus mastichina* L.) were grown in the El Picazo del Júcar area (Cuenca, Spain) and collected at flowering at the middle of June. Each plant was air dried and milled (leaves and flowers), and a 25-g sample was loaded into the extraction vessel. A CO_2 stream (40°C, 350 bar, and 41.7 mL/min measured as liquid flow) was passed through the extractor. The most insoluble and less volatile compounds were collected as a yellow solid from the first separation vessel (F1 in Figure 1), which was maintained at 50°C and 150 bar. A 3-mL volume of *n*-hexane–ethanol (2:1) was used to recover the fraction, which was stored at –18°C until GC analysis.

The CO_2 leaving the first separation vessel was subsequently passed through the second separation vessel, which was maintained under conditions suitable to retain medium-volatile compounds (25°C and 50 bar). In this case, the collected fraction was recovered with a 2-mL volume of ethanol, then it was stored at –18°C until GC analysis. The fractions obtained from both separations vessels (F1 and F2) were collected through the corresponding sampling valves placed in the bottom of the separators.

The CO_2 stream leaving the second separation vessel was passed through the glass liner of the PTV (Figure 1), which was maintained at atmospheric conditions (temperature and pressure). To retain the solutes of interest, a 22.5-mg plug of Tenax TA was used as adsorbent material inside the glass liner. The extraction time was 2 h, but the glass liner containing the sorbent material was removed upon completion of the first 5 min.

GC analysis

Analyses of the 3 fractions mentioned previously (i.e., F1, F2, and that collected in the glass liner) were performed using a Hewlett-Packard (Wilmington, DE) model 6890 GC provided with a PTV and a flame ionization detector (FID).

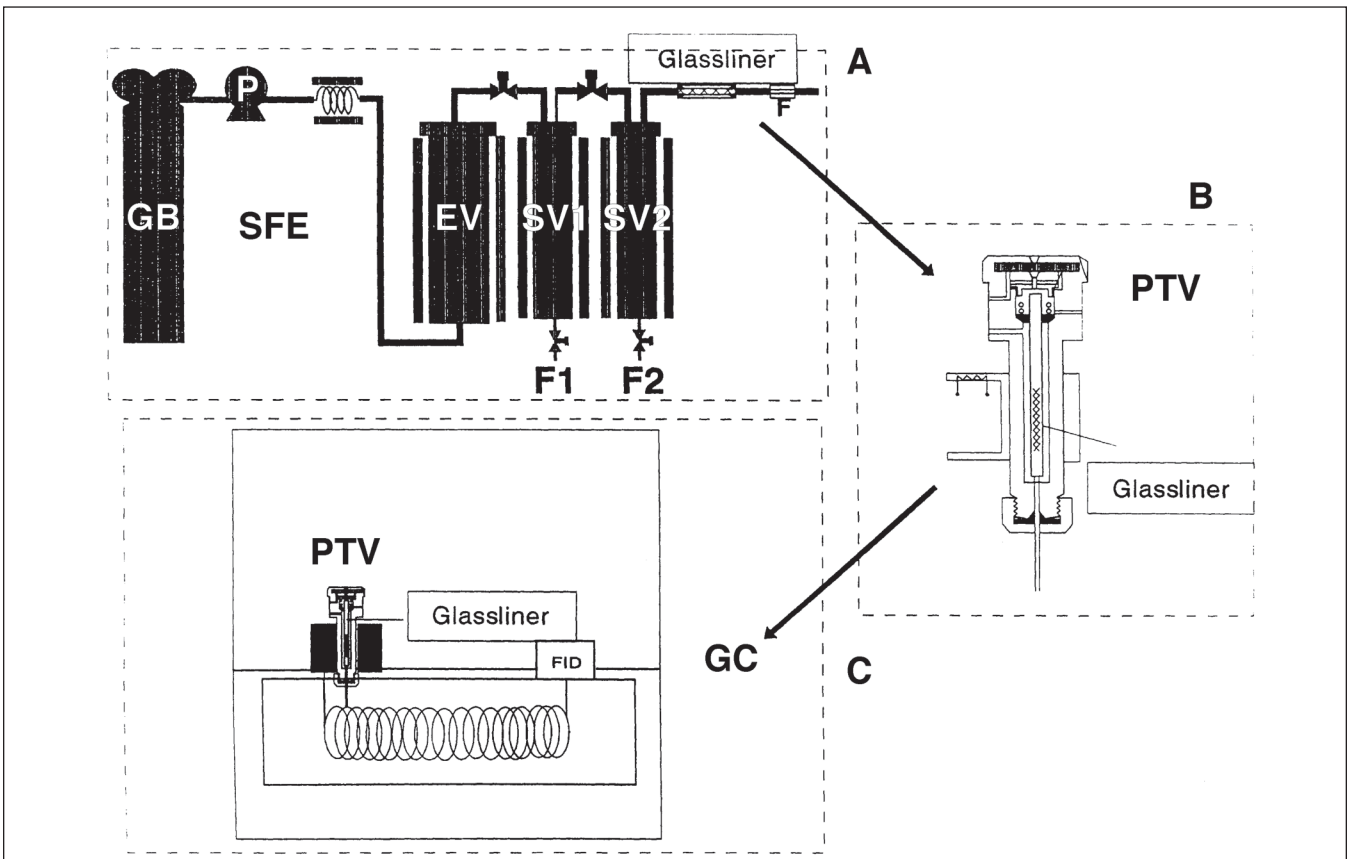

Figure 1. Scheme of the experimental setup of the equipment used for SFE (A), showing the glass liner of the PTV-injector used to collect the most volatile compounds (B) and subsequently to achieve their thermal desorption during the GC analysis (C). GB, gas bottle; P, pump; EV, extraction vessel; SV1 and SV2, separation vessels; F, flowmeter; F1 and F2, first and second fraction; PTV, programmed temperature vaporizer; FID, flame ionization detector; GC, gas chromatograph.

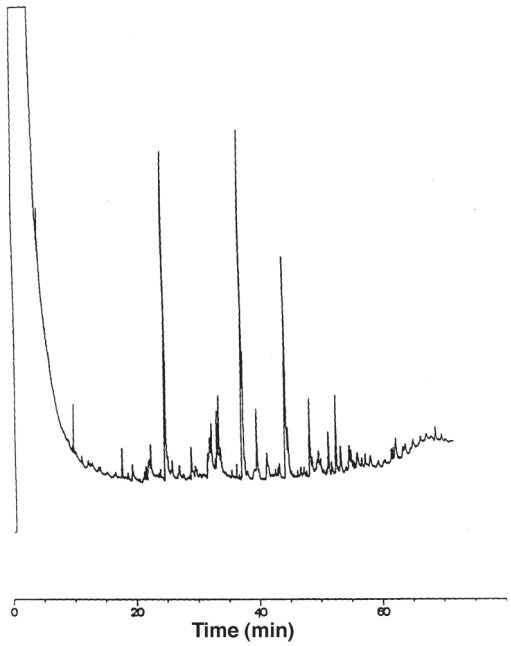

Figure 2. Chromatogram of a 2-µL injection of the SFE extract (first fraction) obtained from *Thymus mastichina* L. The extraction time was 2 h. The column was a fused-silica capillary column (25 m × 0.25-mm i.d.) coated with a 0.25-µm layer of Chirasil-β-Dex. The oven temperature was programmed from 60 to 180°C (2°C/min) and the final temperature was kept for 20 min. Injection mode: splitless. Full scale range was 100 pA.

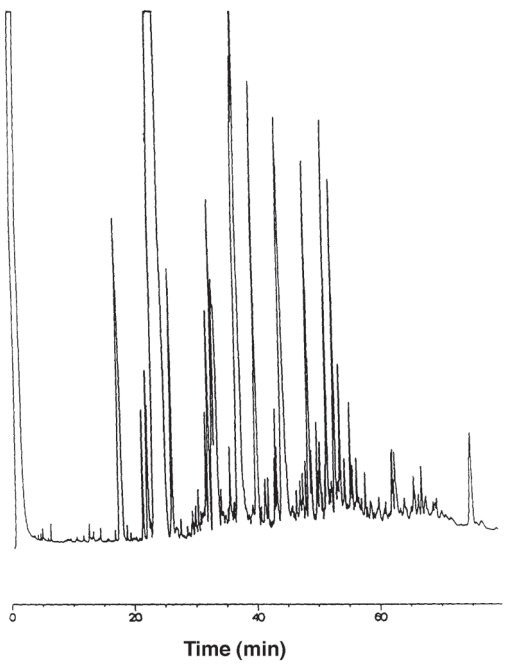

Figure 3. Chromatogram of a 2-µL injection of the SFE extract (second fraction) obtained from *Thymus mastichina* L. The extraction time was 2 h. The column was a fused-silica capillary column (25 m × 0.25-mm i.d.) coated with a 0.25-µm layer of Chirasil-β-Dex. The oven temperature was programmed from 60 to 180°C (2°C/min) and the final temperature was kept for 20 min. Injection mode: splitless. Full scale range was 400 pA. The injection mode was split (split ratio, 10:1).

When analyzing the first two SFE fractions, a 2-cm plug of sylanized glass wool was placed in the glass liner of the PTV injector, which was maintained at 45°C upon sample introduction. Analysis of the third fraction above mentioned (i.e., that collected into the glass liner) was simply performed by thermal desorption. In all cases, desorption of the retained material and transfer to the capillary column was achieved by raising the injector temperature (at approximately 12°C/s) to 350°C. The final temperature was held for 11 min. A fused-silica column (25 m × 0.25-mm i.d., 0.25-µm film thickness:) of Chirasil-β-Dex was used (17), and helium served as the carrier gas (25 psig). The column temperature was started at 60°C, then raised 2°C/min to 180°C, and maintained at 180°C for 20 min. Data acquisition from the FID was performed using an HP ChemStation (Hewlett-Packard).

Results and Discussion

Figures 2 and 3 show the chromatograms resulting from a 2-µL injection of the first and second fraction, respectively, of the SFE extract obtained from *Thymus mastichina* L. Figure 4 is the chromatogram obtained by the thermal desorption of the material previously retained in the glass liner of the PTV when placed into the SFE equipment as shown in Figure 1.

As it is well known, low density values of supercritical CO_2 provide high selectivity but not efficient extraction. Therefore, in order to achieve good performance in a reasonable extraction time, sufficient solvating power is needed, although the extraction selectivity will be reduced. In this case, pressure and temperature modifications in the separation vessels allowed the

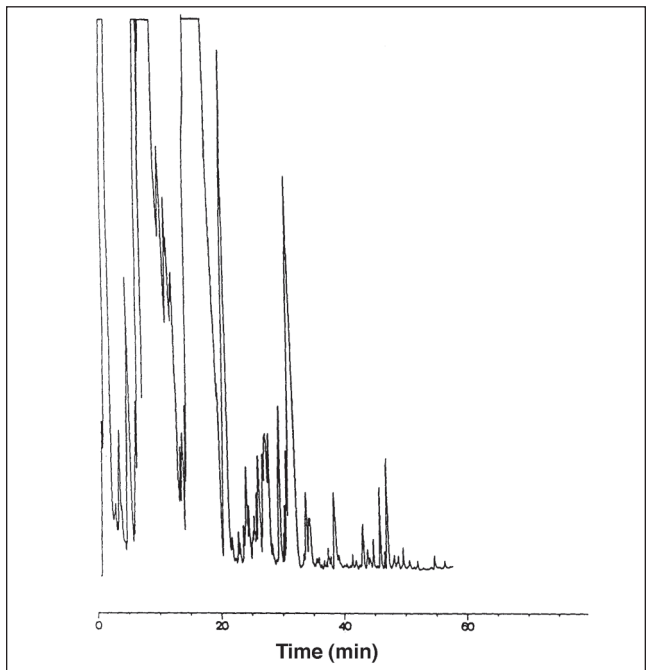

Figure 4. Chromatogram obtained by the thermal desorption of the SFE extract from *Thymus mastichina* L. adsorbed in the glass liner of a PTV. The extraction time was 5 min. Full scale range was 1600 pA. The injection mode was splitless.

fractionation. As previously mentioned, experimental conditions were established to make possible the separation of the low- and medium-volatile compounds in the first and second separation vessel, respectively, whereas the most volatile compounds were retained in the packing material placed inside the glass liner.

In the first separation vessel, the less volatile compounds were precipitated by decreasing the pressure, and those nonsoluble compounds which had reached their vapor pressure were swept by increasing the temperature. In the second separation vessel, the pressure and the temperature were decreased until the CO_2 became a gas in order to separate the medium-volatile compounds from the high-volatile compounds, which finally reach the glass liner.

It should be emphasized that the collection of the first two fractions is achieved by means of organic solvents that are not necessary for the third fraction; the extracted analytes are spread out over the packing material used in the glass liner of the PTV, and the subsequent chromatographic analysis is carried out by just placing the liner in the injector body of the chromatograph. Analyte recovery is thus achieved by thermal desorption of the retained solutes, benefiting from the ability to transfer all of the extracted analytes to the chromatographic system. A further advantage of the proposed procedure is the reduction of the potential for analyte loss and degradation.

The simplicity of the proposed procedure should be noted, because sample handling between the extraction of the third fraction and its chromatographic analysis only demands the removal of the glass liner of the SFE module and its insertion into the body injector assembly of the GC.

It is interesting to emphasize that the proposed method can also be adequate in performing the fractionation of supercritical fluid extracts of different foodstuffs, even though a careful optimization of the experimental variables should previously be made. Because of the increasing demand of natural compounds in the food industry caused by the consumer objection towards artificial components, the extracts obtained by fractionation could have interesting commercial possibilities.

Acknowledgments

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References

1. *Supercritical Fluid Chromatography*, R.M. Smith, Ed. Royal Society of Chemistry, London, England, 1988.
2. *Modern Supercritical Fluid Chromatography,* C.M. White, Ed. Hüthig, Heidelberg, Germany, 1988.
3. J.W. King. Fundamentals and applications of supercritical fluid extraction in chromatographic science. *J. Chromatogr. Sci.* **27:** 355–64 (1989).
4. Analytical Supercritical Fluid Chromatography and Extraction, M.L. Lee and K.E. Markides, Eds. Chromatography Conferences, Provo, UT, 1990.
5. T.L. Chester, J.D. Pinkston, and D.E. Raynie. Supercritical fluid chromatography and extraction. *Anal. Chem.* **68:** 487–514 (1996).
6. M.D. Luque de Castro and M.T. Tena. Strategies for supercritical fluid extraction of polar and ionic compounds. Tren. *Anal. Chem.* **15:** 32–37 (1996).
7. M.T.G. Hierro and G. Santa-María. Supercritical fluid extraction of vegetable and animal fats with CO_2. A mini review. *Food Chem.* **45:** 189–92 (1992).
8. *Extraction of Natural Products Using Near-critical Solvents,* M.B. King and T.R. Bott, Eds. Chapman & Hall, Glasgow, Scotland, 1993.
9. G.P. Blanch, E. Ibáñez, M. Herraiz, and G. Reglero. Use of a programmed temperature vaporizer for off-line SFE/GC analysis in food composition studies. *Anal. Chem.* **66:** 888–92 (1994).
10. G.P. Blanch, G. Reglero, and M. Herraiz. Analysis of wine aroma by off-line and on-line supercritical fluid extraction–gas chromatography. *J. Agric. Food Chem.* **43:** 1251–58 (1995).
11. *Supercritical Fluid Processing of Food and Biomaterials,* S.S.H. Rizvi, Ed. Blackie Academic & Professional, London, England, 1994.
12. M.T.G. Hierro, P. Ruiz-Sala, L. Alonso, and G. Santa-María. Extraction of ewe's milk cream with supercritical carbon dioxide. *Z. Lebensm. Unters. Forsch.* **200:** 297–300 (1995).
13. W. Vogt, K. Jacob, and H.W. Obwexer. Sampling method in capillary column gas–liquid chromatography allowing injections of up to 250 µL. *J. Chromatogr.* **174:** 437–39 (1979).
14. W. Vogt, K. Jacob, A.B. Ohnesorge, and H.W. Obwexer. Capillary gas-chromatography injection system for large sample volumes. *J. Chromatogr.* **186:** 197–205 (1979).
15. J. Villén, F.J. Señoráns, G. Reglero, and M. Herraiz. Analysis of volatile components by direct injection of real-life samples by using a programmed-temperature vaporizer. *Z. Lebensm. Unters. Forsch.* **202:** 270–74 (1996).
16. J. Villén, G.P. Blanch, M.L. Ruiz del Castillo, and M. Herraiz. Rapid and simultaneous analysis of free sterols, tocopherols and squalene in edible oils by coupled reversed-phase liquid chromatography–gas chromatography. *J. Agric. Food Chem.* **46:** 1419–22 (1998).
17. V. Schurig, D. Schmalzing, U. Mühleck, M. Jung, M. Schleimer, P. Mussche, C. Duvekot, and J.C. Buyten. Gas chromatographic enantiomer separation on polysiloxane–anchored permethyl cyclodextrin (Chirasil-Dex). *High Resolut. Chromatogr. Chromatogr. Comm.* **13**: 713–17 (1990).

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