

# Analysis of Wine Aroma by Off-Line and On-Line Supercritical Fluid Extraction-Gas Chromatography

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The applicability of supercritical fluid extraction (SFE) and gas chromatography (GC) for analyzing wine aroma is evaluated by considering three different approaches including (a) SFE off-line, (b) SFE involving the collection of the extracted analytes in the quartz liner of a programmed temperature vaporizer (PTV) placed in a commercial SFE extractor, and (c) direct (on-line) coupling of SFE and GC via PTV. Different solid supports suitable to retain a liquid sample in the extraction chamber are considered, and data concerning the relative standard deviations obtained for the compounds selected under the investigated operation modes are included. Recoveries and detection limits achievable in each analysis are also given. The usefulness of the proposed method is demonstrated by analyzing different wine samples.

**Keywords:** *Wine aroma; isolation and concentration techniques; supercritical fluid extraction (SFE); programmed temperature vaporizer (PTV); capillary gas chromatography*

## INTRODUCTION

The possibility of easily controlling the density of a supercritical fluid with pressure and/or temperature programming is a clear advantage of the use of such a fluid with respect to traditional liquid solvent extraction methods. The wide density range that is available when using a supercritical fluid enables highly selective extractions, and besides this, the solvent strengths, viscosities, and diffusivities of supercritical fluids improve mass transfer (Smith, 1988; White, 1988; King, 1989; Lee and Markides, 1990).

So far, the potential of supercritical fluids has been exploited by means of different approaches including off-line SFE and on-line (or coupled) SFE-GC (Lee and Markides, 1990).

It is generally admitted that off-line SFE is simpler to perform since the optimization of the variables affecting the experimentation is less demanding than on-line optimization (Sandra et al., 1990). However, the on-line SFE-GC approach allows maximum sensitivity due to the quantitative transfer of the extracted analytes to the chromatographic system (Lee and Markides, 1990).

Up to now, different methods have been described to collect and focus the solutes after the SFE depressurization step. Several approaches involve the collection of the extracted analytes in a device external to the GC by depressurizing the SFE effluent either onto a cryogenically cooled surface (Andersen et al., 1989; Liebman et al., 1989; Smith and Burford, 1992) or on a solid support (Miller Schantz and Chesler, 1986; Schneiderman et al., 1987; Saito et al., 1989; Hedrick and Taylor, 1990; Sequeira and Taylor, 1992; Lee and Peart, 1992; Verschuere et al., 1992). The possibility of using either a conventional split-splitless injector (Levy et al., 1987, 1989, 1990; Hawthorne et al., 1990) or a commercial on-column injector has also been reported. In this case, either the stationary phase itself or a retention gap is used to collect and focus the extracted analytes during the SFE step (Hawthorne and Miller, 1987; Wright et

al., 1987; Hawthorne et al., 1988a,b, 1989a,b; Lohleit and Bächmann, 1990).

On the other hand, the use of a programmed temperature vaporizer (PTV) (Poy et al., 1981; Schomburg, 1981) has already proved useful for trace analysis by intermediate trapping of volatile compounds previously removed from the sample matrix by displacing large volumes of gaseous samples (Poy et al., 1982; Nitz et al., 1984; Nitz and Jülich, 1984; Poy and Cobelli, 1985; Villén et al., 1989; Tabera et al., 1991; Blanch et al., 1991; Señoráns et al., 1993). Recently, the use of a fixed restrictor (mostly a capillary restrictor constructed of fused silica tubing, with the dimensions empirically selected to allow adjustment of low mass flow rates at high pressures) directly inserted into the cooled liner of a PTV has also been suggested for on-line SFE-GC coupling (Houben et al., 1990; Huston and Ji, 1991). Also, a modification of a SFE commercial configuration involving the use of a PTV has been previously investigated in our laboratory (Blanch et al., 1994).

To date, most of the work that has been done with SFE has centered on the extraction of analytes from solid matrices. The major problem concerning the extraction from an aqueous sample is matrix isolation, i.e., the retention of water in the extraction cell during the process.

The analysis of wine aroma poses different analytical problems mainly concerning the polarities, volatilities, and concentrations occurring in the mentioned fraction. Moreover, the presence of two major compounds (water and ethyl alcohol) makes difficult the selection of an adequate preconcentration method. So far, different techniques have been proposed for the isolation and concentration of volatile compounds in aroma research (Poole and Schuette, 1983; Jennings and Rapp, 1983; Maarse and Belz, 1985; Cronin and Caplan, 1987; Macku et al., 1988; King and Bott, 1993). Continuous liquid-liquid extraction has been specifically recommended for the analysis of wine aroma (Hardy, 1969; Rapp et al., 1976), although it is a highly time-consuming procedure and demands the use of relatively high volumes of contaminant solvents such as trichlorofluoromethane (Freon 11). In this respect, the development

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of suitable alternatives for the preconcentration of wine aroma is presently of great interest.

The aim of this paper was to evaluate the usefulness of using supercritical fluids to investigate wine aroma. A comparison of different techniques including off-line SFE and on-line SFE-GC was also intended.

## EXPERIMENTAL PROCEDURES

**Samples.** A test mixture (see Table 2 for composition) consisting of 14 compounds, previously reported as food volatile constituents, was used for the evaluation of the three operation modes investigated. The test composition was established by considering a wide range of polarities and volatilities adequate for the subsequent analysis of real-life samples. A stock solution having about 7.14% of each pure component was stored at  $-30\text{ }^{\circ}\text{C}$  in the dark. The reagents, all of GC grade, were obtained from Merck (Darmstadt, Germany) (isoamyl acetate, ethyl hexanoate, ethyl octanoate, benzaldehyde, diethyl succinate, ethyl dodecanoate, 2-phenylethanol), Aldrich Chemie (Steinheim, Germany) (1-hexanol, linalool,  $\alpha$ -terpineol,  $\gamma$ -decalactone), and Sigma (St. Louis, MO) ( $\beta$ -ionone, ethyl tridecanoate).

The sample volume of the test mixture used for SF extractions was either 1.4 or 1  $\mu\text{L}$  diluted in 1 mL of water ethanol (88:12 v/v), as mentioned below.

**Solid Support.** To prevent liquid sample distribution throughout the extraction system by the effect of the mobile phase, the sample must be retained prior to SFE on a solid support. In this work silanized Volaspher A-2, 80–100 mesh (a siliceous synthetic material from Merck), desilanized Volaspher A-2, 80–100 mesh, glass beads 80–100 mesh, silanized glass wool, and filter paper were tested.

**Experimentation.** SFE experimentation was performed in the three modes described below: (a) off-line supercritical fluid extraction using an original commercial configuration, (b) supercritical fluid extraction-capillary gas chromatography analysis via PTV (i.e., using the quartz liner of a programmed temperature vaporizer for trapping the extracted solutes), and (c) on-line coupling between supercritical fluid extraction and capillary gas chromatography by means of a programmed temperature vaporizer.

**Approach a (Off-Line SFE).** A Hewlett-Packard 7680A extraction module and the available 7.0-mL thick-walled stainless steel thimble were used to perform supercritical fluid extractions. The porous frits contained in the caps at each end of the vessel allow one to hold the sample in place and produce high-pressure seals when the extraction chamber is closed. The mentioned extractor employs a nozzle/trap assembly (variable restrictor) to allow the instant depressurization of the supercritical fluid and, simultaneously, the decoupling of flow and pressure in such a way that the pressure can be controlled independently of the supercritical fluid flow rate. The variable restrictor not only maintains the extraction vessel under pressure but also reduces the risk of plugging typical of fix restrictors and provides the required interface to operate at atmospheric pressure. The SFE extract is collected on a solid-phase trap (held at  $-5\text{ }^{\circ}\text{C}$ ) made of octadecylsilane (ODS) material that is flushed by dichloromethane once the extraction step has been completed. The extract is then collected in a 1-mL vial and subsequently a 2- $\mu\text{L}$  volume is sampled into the gas chromatographic column.

**Approach b (SFE-GC Analysis via PTV).** A modification of the Hewlett-Packard 7680 SFE module previously developed in our laboratory for SFE of solid samples (Blanch et al., 1994) was used. The quartz liner (100 mm  $\times$  1 mm i.d.) of a programmed temperature vaporizer (Perkin-Elmer) is located in place of the analyte trap by using a suitable steel fitting. The extraction time was set at 10 min. The analytes are then trapped in the adsorbent material previously packed in the glass liner (maintained at  $10\text{ }^{\circ}\text{C}$ ), and the chromatographic analysis is carried out just by placing the liner in the injector body of a gas chromatograph. Obviously, this approach does not demand the use of a trap rinse solvent since analyte recovery is achieved by thermal desorption of the retained solutes.

**Approach c (On-Line SFE-GC).** In this case, supercritical fluid extractions were performed on a Suprex extractor (Model Prepmaster) which uses a dual piston pump system that does not require external cooling. The system includes a two-position switching valve, the extraction oven, and the extraction vessel. The  $\text{CO}_2$  flows from the pump, through an on/off valve, to the extraction vessel, and into the switching valve. From this valve, the  $\text{CO}_2$  flow is diverted to the PTV injector (Perkin-Elmer) just by directly inserting the "integral" restrictor coming from the extraction cell. This restrictor was fabricated by polishing the closed end (obtained by heating) of a piece of deactivated fused silica capillary (100  $\mu\text{m}$  i.d.) until the desired flow rate was achieved (Guthrie and Schwartz, 1986). The extraction vessel consisted of a piece of tubular stainless steel that is open at one end and contains a frit at the other end which is held in place by a plastic plug. During the extraction time (20 min) the PTV was held at  $-5\text{ }^{\circ}\text{C}$ .

The glass liner used in approaches b and c for collecting the extracted analytes was packed with 15 mg of Thermotrap TA (Chrompack).

Figures 1–3 show detailed schematic diagrams of the configurations used in the three mentioned approaches. As can be seen, the performance of off-line SFE-GC (Figure 1) includes three different steps: supercritical fluid extraction, depressurization and venting of the extraction fluid involving the collection of the extracted analytes, and the chromatographic analysis itself. The off-line SFE-GC via PTV (Figure 2) demands only two steps since the solutes are extracted and collected in a solid support contained in the glass liner of the PTV, as mentioned above. When one is performing on-line SFE-GC via PTV, however, the extraction, collection, and chromatographic analysis can be accomplished in a single step (Figure 3).

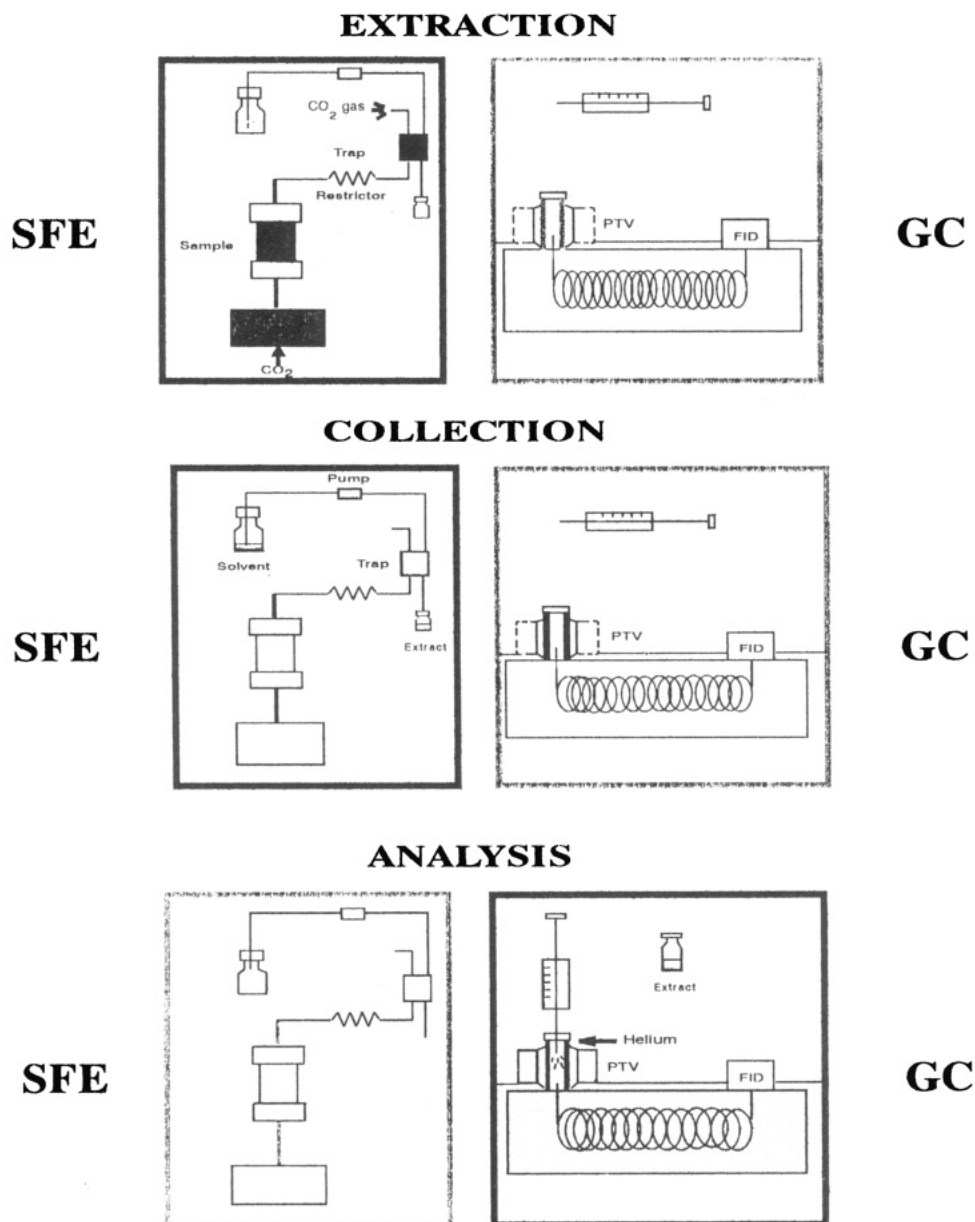
**Gas Chromatographic Analysis.** Separation of the extracted-trapped-recovered analytes was carried out with a Perkin-Elmer 8500 gas chromatograph equipped with a programmed temperature vaporizer injector and a flame ionization detector (FID). The equipment was coupled to a Model 2600 chromatography software system (Nelson Analytical). In all instances, injections were performed in the cold split mode (split ratio, 10:1; septum purge, 5 mL/min) by maintaining the vaporizer at  $30\text{ }^{\circ}\text{C}$  upon sample introduction and subsequent increase at  $14\text{ }^{\circ}\text{C}/\text{s}$  to  $250\text{ }^{\circ}\text{C}$  (5 min). Helium (35 psig) served as carrier gas, and the FID was operated at  $250\text{ }^{\circ}\text{C}$ .

A 30 m  $\times$  250  $\mu\text{m}$  i.d. fused silica capillary column coated with a 0.25- $\mu\text{m}$  layer of poly(ethylene glycol) (BTR Cw, Quadrex) was used throughout the experimentation. The column temperature was increased from  $35\text{ }^{\circ}\text{C}$  (3 min) at  $3\text{ }^{\circ}\text{C}/\text{min}$  to  $90\text{ }^{\circ}\text{C}$  (15 min) and then to  $180\text{ }^{\circ}\text{C}$  at  $5\text{ }^{\circ}\text{C}/\text{min}$ .

**Safety Considerations.** Special care must be taken to ensure that the fittings used in SFE experimentation are capable of withstanding the required extraction pressures.

## RESULTS AND DISCUSSION

Initial experimentation was performed to establish the most suitable material to retain the water in the extraction chamber during the process. To this aim, a 1.4- $\mu\text{L}$  volume of the test mixture and the commercial SFE extraction module HP 7680A described under Experimental Procedures were used (approach a). In all cases, the extractions were carried out by using  $\text{CO}_2$  as mobile phase (density, 0.95 g/mL),  $40\text{ }^{\circ}\text{C}$  being the temperature in the extraction chamber, 2 mL/min the  $\text{CO}_2$  medium flow rate, and 1 g the amount of solid support used in each experimental run. At the end of the extraction time (25 min), the extracts obtained were analyzed by GC, and the average recoveries corresponding to each of the different supports (see Experimental Procedures) were comparatively evaluated. The highest recovery values were obtained when using silanized glass wool as solid support, although silanized Volaspher A-2 and filter paper provided very similar values.



**Figure 1.** Schematic diagram of the configuration used for the performance of off-line SFE–GC, including three different steps: supercritical fluid extraction, collection of the extracted analytes, and chromatographic analysis (see text for further details).

However, materials whose surface exhibited a rather high degree of activity (i.e., desilanzated Volaspher A-2 and glass beads) gave low average recoveries (respectively, 69% and 82% lower than those values obtained if silanized glass wool is used). This fact is probably due to the effects of residual adsorption of the components of the mixture under investigation.

Consequently, silanized glass wool was considered to be an adequate material to perform the experimentation in the three approaches already mentioned.

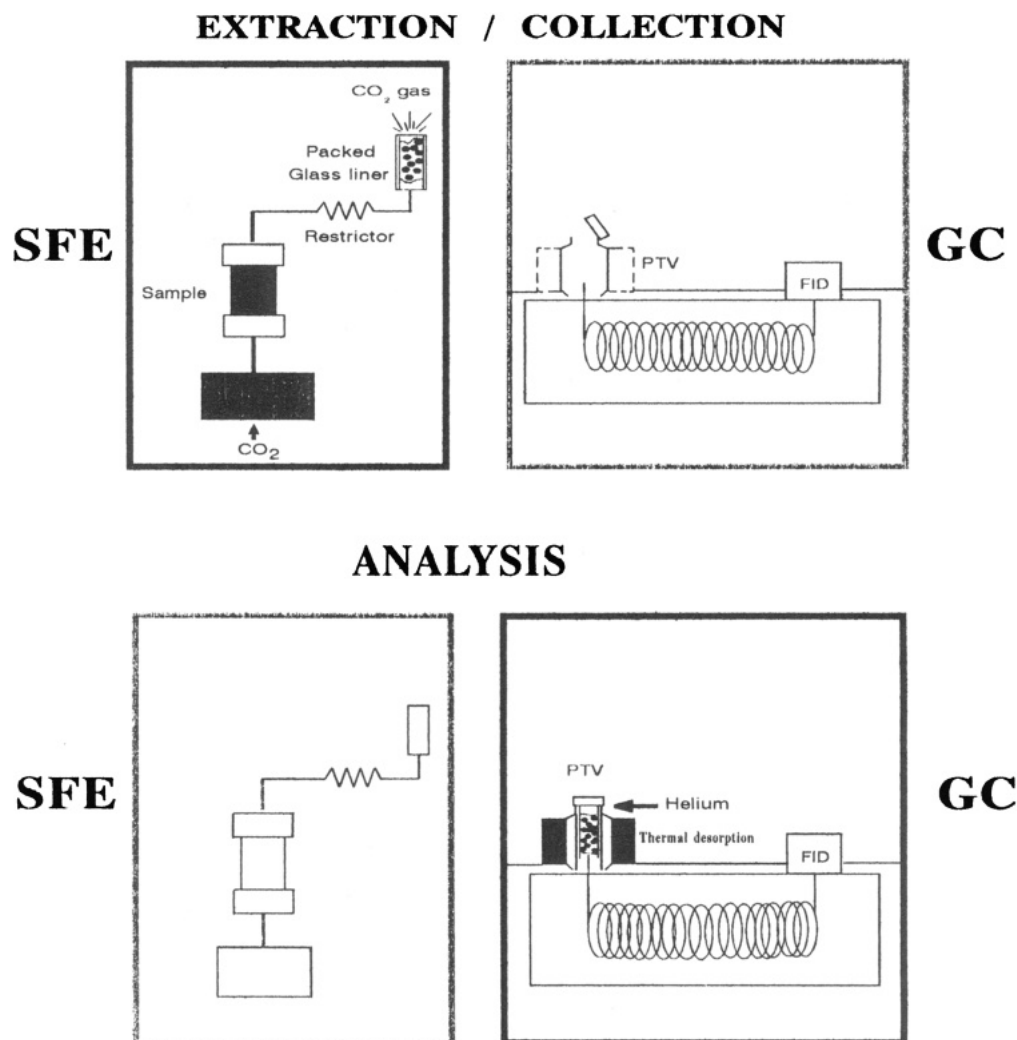
Table 1 gives the experimental values for the variables involved in the extraction step for off-line SFE, off-line SFE–GC via PTV, and on-line SFE–GC via PTV of the test sample. It should be pointed out that the mobile phase density was set at 0.80 g/mL ( $\text{CO}_2$  flow rate, 2 mL/min) instead of the initially selected value (0.95 mg/L) as it provides similar recovery values for the investigated solutes. Moreover, experimentation at  $\text{CO}_2$  density values equal to 0.80 g/mL contributes to simplifying the on-line coupling SFE–GC due to the lower expanded volumes of  $\text{CO}_2$  gas. The extraction

**Table 1. Experimental Conditions of the Extraction Step for Off-Line SFE, Off-Line SFE–GC via PTV, and On-Line SFE–GC via PTV of the Test Sample Used in This Study**

variable	exptl value
sampled volume ( $\mu\text{L}$ )	1.4
amt of support (g) in the extraction chamber	1
extraction chamber vol (mL)	7
mobile phase	$\text{CO}_2$
mobile phase density (g/mL)	0.80
extraction chamber temp ( $^\circ\text{C}$ )	40
mobile phase flow (mL/min)	2

time was established independently, taking into account the characteristics of each of the three considered methods.

Tables 2 and 3 show the relative standard deviations (RSD) obtained, respectively, from the relative (normalized) areas and from the ratio of the absolute peak areas of the considered compounds to that for diethyl succinate, for the three investigated methods. It is clear that the lowest values are generally obtained if the off-line SFE (approaches a and b) is performed, although RSD



**Figure 2.** Schematic diagram of the configuration used for the performance of off-line SFE-GC via PTV experimentation, including the extraction and collection step and the chromatographic analysis (see text for further details).

**Table 2. Relative Standard Deviations ( $n = 5$ ) Obtained from the Relative (Normalized) Peak Areas for Off-Line SFE-GC and On-Line SFE-GC**

compound	off-line SFE	off-line SFE-GC via PTV	on-line SFE-GC via PTV
isoamyl acetate	6.8	<i>a</i>	1.9
ethyl hexanoate	2.7	9.8	7.8
terpinolene	4.3	9.8	5.3
1-hexanol	5.9	11.6	3.1
ethyl octanoate	8.8	9.4	7.1
benzaldehyde	3.2	7.9	9.8
linalool	3.8	5.5	8.5
diethyl succinate	5.9	5.4	6.8
$\alpha$ -terpineol	5.5	2.5	8.8
ethyl dodecanoate	0.1	6.5	9.0
2-phenylethanol	1.0	6.6	8.1
$\beta$ -ionone	2.2	5.8	10.8
ethyl tetradecanoate	4.0	12.6	2.9
$\gamma$ -decalactone	6.7	3.5	19.1

<sup>a</sup> Nondetected compound.

values lower than 20% are always obtained when on-line SFE-GC via PTV is carried out (approach c).

Table 4 gives the average recoveries obtained for the studied compounds. It is evident that the on-line coupling provides, in general, the highest values, but also high recoveries can be achieved for most compounds if off-line SFE is performed. Recoveries obtained for the most volatile compounds by off-line SFE-GC via PTV

**Table 3. Relative Standard Deviations ( $n = 5$ ) for the Ratio of the Absolute Peak Areas of the Considered Compounds to That for Diethyl Succinate for Off-Line SFE-GC and On-Line SFE-GC**

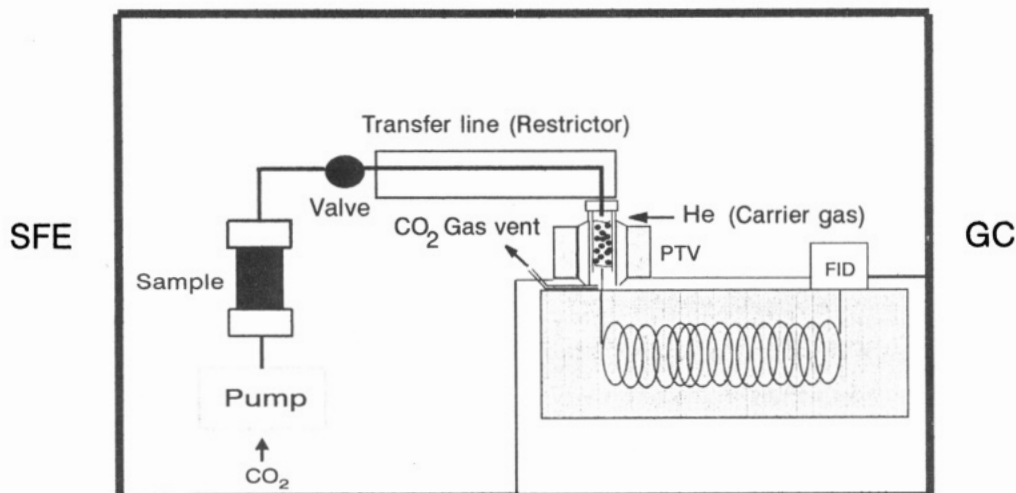
compound	off-line SFE	off-line SFE-GC via PTV	on-line SFE-GC via PTV
isoamyl acetate	0.8	<i>a</i>	15.7
ethyl hexanoate	6.1	9.2	12.6
terpinolene	7.2	8.8	11.9
1-hexanol	6.6	10.7	8.1
ethyl octanoate	8.0	7.4	0.9
benzaldehyde	8.1	8.0	11.9
linalool	2.2	4.9	8.6
diethyl succinate			
$\alpha$ -terpineol	0.8	7.5	6.3
ethyl dodecanoate	0.5	8.7	2.4
2-phenylethanol	1.6	4.3	1.3
$\beta$ -ionone	5.4	19.8	15.5
ethyl tetradecanoate	2.0	5.0	9.5
$\gamma$ -decalactone	3.6	8.5	18.4

<sup>a</sup> Nondetected compound.

are, however, quite low, although values higher than 79% are achieved for eight compounds. Anyway, it should be expected that the careful optimization (i.e., by using an adequate experimental design) of those variables affecting the experimentation allows an improvement of the mentioned recovery values.

As far as the detection limits are concerned (Table 5), it should be emphasized that maximum sensitivity

## EXTRACTION / COLLECTION / ANALYSIS



**Figure 3.** Schematic diagram of on-line SFE-GC coupling using a programmed temperature vaporizer as interface. The extraction, collection, and chromatographic analysis can be accomplished in a single step (see text for further details).

**Table 4.** Average Recoveries Obtained for the Studied Compounds in the Three Investigated Approaches

compound	off-line SFE	off-line SFE-GC via PTV	on-line SFE-GC via PTV
isoamyl acetate	96.4	<i>a</i>	109.2
ethyl hexanoate	94.0	17.3	96.6
terpinolene	89.8	11.4	97.0
1-hexanol	99.9	7.2	101.9
ethyl octanoate	95.8	92.6	96.2
benzaldehyde	99.3	5.3	91.4
linalool	99.8	92.0	91.4
diethyl succinate	97.4	87.5	98.1
$\alpha$ -terpineol	97.9	93.4	97.4
ethyl dodecanoate	77.6	89.9	99.2
2-phenylethanol	103.2	95.5	100.4
$\beta$ -ionone	94.2	87.1	97.1
ethyl tetradecanoate	86.8	35.8	57.6
$\gamma$ -decalactone	99.7	79.7	63.0

<sup>a</sup> Nondetected compound.

is achieved if on-line SFE-GC via PTV is performed. Off-line SFE-GC via PTV also provides quite satisfactory values, whereas, as was expected, definitely lower sensitivities (higher detection limits) are obtained in off-line SFE since the dilution of the extracted solutes with the solvent used for flushing the trap results in a significant decrease of the overall sensitivity of the process.

To illustrate the applicability of the on-line SFE-GC method considered in this work for the analysis of real-life samples, Figure 4 shows the chromatograms obtained from two white wines, two rosé wines, and two red wines (alcoholic content, 12% v/v). It should be pointed out that in spite of the high sensitivity achievable with the mentioned technique, a relatively high volume of wine (1 mL) is required to efficiently perform its extraction and analysis. Due to this fact, the solid support to be used in the extraction chamber must be selected by taking into account its specific surface area and its capability for samples having a high content in ethanol. Among the solid supports used in this study (see Experimental Procedures), silanized Volaspher A-2 and filter paper showed the highest specific surface, their chemical nature and the activity of their surface being adequate to perform the analysis of wine samples. Moreover, similar average recovery values were achieved

**Table 5.** Detection Limits ( $D_L$ )<sup>a</sup> of the Considered Compounds for the Three Investigated Methods

compound	off-line SFE $D_L$ ( $\mu\text{g}$ )	off-line SFE-GC via PTV $D_L(10^4)$ ( $\mu\text{g}$ )	on-line SFE-GC via PTV $D_L(10^4)$ ( $\mu\text{g}$ )
isoamyl acetate	0.9	<i>b</i>	1.3
ethyl hexanoate	1.0	8.0	1.4
terpinolene	4.5	28.7	3.4
1-hexanol	1.1	29.6	2.1
ethyl octanoate	1.1	1.6	1.5
benzaldehyde	1.1	21.9	1.3
linalool	0.8	2.6	2.6
diethyl succinate	0.5	3.0	2.7
$\alpha$ -terpineol	0.2	0.8	0.8
ethyl dodecanoate	0.8	2.0	1.8
2-phenylethanol	0.3	1.0	0.9
$\beta$ -ionone	0.8	2.1	1.9
ethyl tetradecanoate	0.8	2.7	1.7
$\gamma$ -decalactone	0.4	1.0	1.3

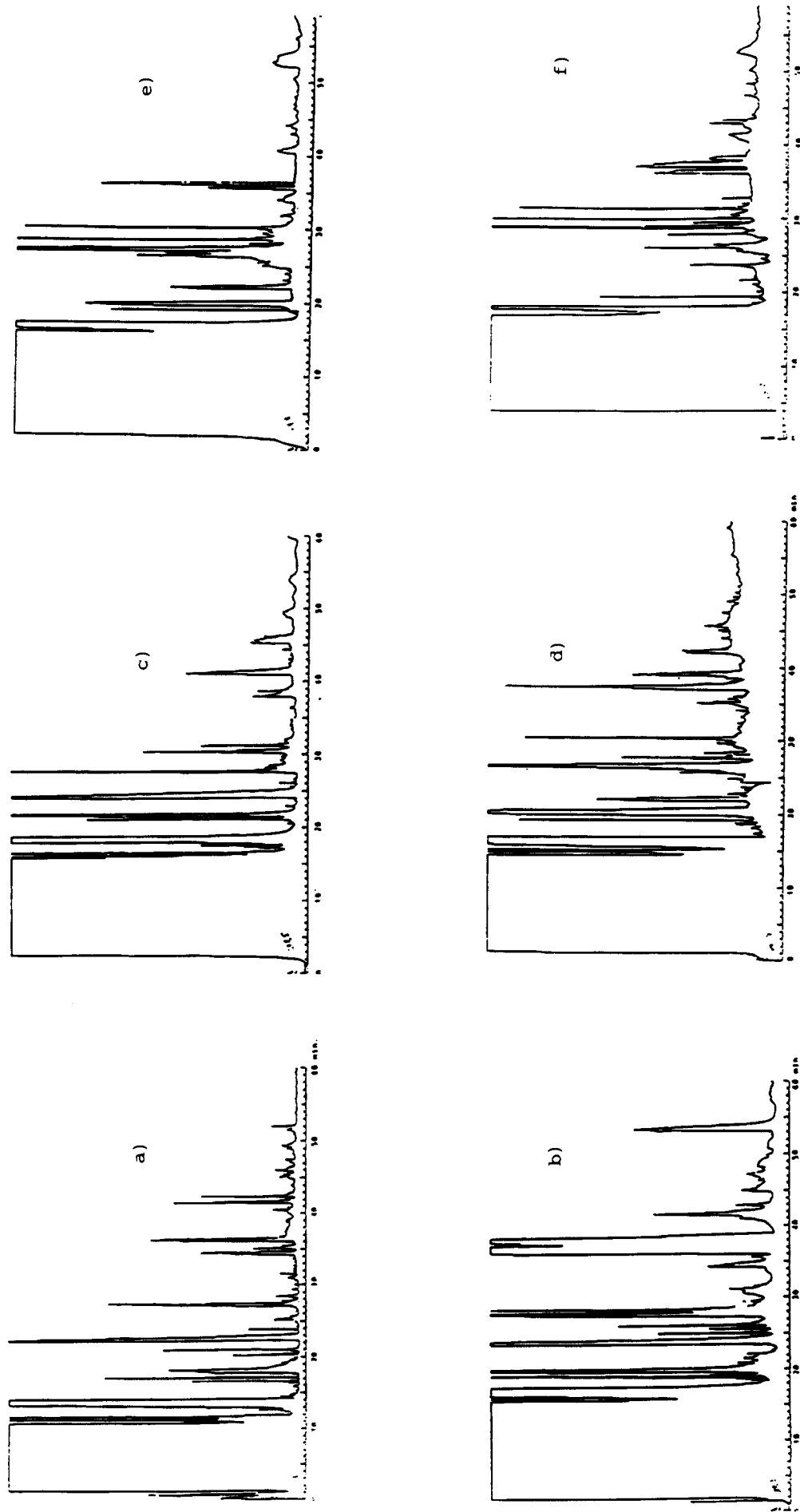
<sup>a</sup> Calculated from a signal equal to twice the detector base line noise. <sup>b</sup> Nondetected compound.

using the two mentioned supports when the SF extracts were obtained from a 1- $\mu\text{L}$  volume of the test sample diluted in 1 mL of water/ethanol (88:12 v/v), in the experimental conditions given in Table 1.

Chromatograms shown in Figure 4 were obtained by using filter paper to retain a 1-mL volume of the wine sample analyzed. It is evident, however, that other adsorbents may exhibit the general characteristics required to efficiently perform the mentioned analysis (i.e., microcrystalline cellulose may be an interesting alternative to the use of filter paper).

## CONCLUSIONS

On-line coupling between supercritical fluid extraction and capillary gas chromatography using a programmed temperature vaporizer as interface seems to be a good option for performing the extraction, collection, and chromatographic analysis of wine aroma in a single step. Relative standard deviations ranging from 0.9% to 19.1% were achieved for the test mixture compounds, whereas the recoveries obtained varied from 57.6% to 109.2%, detection limits ranging between 0.08 and 0.34 ng. However, several problems can be derived from the



**Figure 4.** Chromatograms obtained from two white wines (a and b), two rosé wines (c and d), and two red wines (e and f) by using the on-line SFE-GC via PTV approach.



use of a fixed restrictor. Off-line SFE-GC can be used for the analysis of wine samples as it reduces the risk of plugging in the restrictor and also provides satisfactory relative standard deviations (0.1–8.8%) and acceptable recoveries (77–103%), although detection limits are clearly higher (0.2–4.5 µg). Off-line SFE-GC via PTV seems to be a good alternative for the analysis of wine samples, though the quantitation of the most volatile compounds requires further optimization.

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